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$_3^-$.

Comment: The sediment column (B2) integrated activities would also imply a whole sediment column integrated chemolithothrophic 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 NH4+.

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 NH4+ concentration measurements (Holmes et al., 1999) as well as a more detailed description of when NO2− was removed by sulfamic acid addition for NO3− 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 O2 as the result of low metabolic rates. We have now added the parenthetical “(e.g., organisms adapted to respiration under low O2 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.
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) \(\text{N}_2\) 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 \(\text{N}_2\) fixation. Since it is a rather novel observation, some further supporting discussion seems to be warranted: 1) The low values of \(\delta^{15}\text{N}_{\text{NTR}}\) imply that a large fraction of organic nitrogen oxidized to nitrate originates from \(\text{N}_2\) fixation, particularly at the sites where the lowest \(\delta^{15}\text{N}_{\text{NTR}}\) is calculated. It would be instructive to provide the readers with some further quantitative assessment of what fraction of N oxidized comes from \(\text{N}_2\) fixed (assuming the exported \(\delta^{15}\text{N}\) 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 \(\text{N}_2\) fixation discussion, it would be helpful to have at least some idea of what \(\delta^{15}\text{N}\) 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 \(\delta^{15}\text{N}\) 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 NH₄⁺ was below detection using the OPA fluorescence method (detection limit ~20nM).

Comment: 2) The reported rates should be compared to other published rates of N₂ 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 N₂ 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 (N₂ 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 δ¹⁵N_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 δ¹⁵N_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 δ¹⁵N_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 N₂ fixation can be supported by previously reported rates of H₂ 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 H₂ 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 H₂ production as a source of electrons supporting the subsurface microbial community. The radiolytic H₂ production rates calculated for the SPG subsurface study are several orders of magnitude lower than the H₂ production rate that would correspond to our predicted N-fixation rates at NP. While H₂ does not generally ‘leak out’ of N fixing bacteria – a small efflux of H₂ 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 δ¹⁵N and δ¹⁸O, 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 O2 profiles” with “steeper O2 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.


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 ($^{15}\epsilon_{DNF}$) 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.
Nitrogen cycling in the subsurface biosphere: Nitrate isotopes in porewaters underlying the oligotrophic North Atlantic

Scott D. Wankel¹,*, Carolyn Buchwald¹, Wiebke Ziebis², Christine B. Wenk³,^ and Moritz F. Lehmann³

¹Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, 266 Woods Hole Rd., Woods Hole, Massachusetts, USA 02543
²University of Southern California, Department of Biological Sciences, Allan Hancock Foundation Building, Los Angeles, California, USA 90089
³University of Basel, Department of Environmental Science, Bernoullistrasse 32, Basel, Switzerland CH-4056
^now at Weizmann Institute of Science, Department of Earth and Planetary Sciences, Rehovot, Israel 76100

Running Title: Nitrogen cycling in deep-sea porewaters

Keywords: Nitrate, porewater, isotopes, nitrification, denitrification, nitrogen fixation, North Pond, oligotrophic, North Atlantic, IODP

*Correspondence:
Dr. Scott D. Wankel
Woods Hole Oceanographic Institution
Department of Marine Chemistry and Geochemistry
266 Woods Hole Rd., MS 25
Woods Hole, MA 02543
sdwankel@whoi.edu
**ABSTRACT**

Nitrogen (N) is a key component of fundamental biomolecules. Hence, the cycling and availability of N is a central factor governing the extent of ecosystems across the Earth. In the organic-lean sediment porewaters underlying the oligotrophic ocean, where low levels of microbial activity persist despite limited organic matter delivery from overlying water, the extent and modes of nitrogen transformations have not been widely investigated. Here we use the N and oxygen (O) isotopic composition of porewater nitrate (NO$_3^-$) from a site in the oligotrophic North Atlantic (IODP) to determine the extent and magnitude of microbial nitrate production (via nitrification) and consumption (via denitrification). We find that NO$_3^-$ accumulates far above bottom seawater concentrations (~21 µM) throughout the sediment column (up to ~50 µM) down to the oceanic basement as deep as 90 mbsf, reflecting the predominance of aerobic nitrification/remineralization within the deep marine sediments. Large changes in the δ$^{15}$N and δ$^{18}$O of nitrate, however, reveal variable influence of nitrate respiration across the three sites. We use an inverse porewater diffusion-reaction model, constrained by the N and O isotope systematics of nitrification and denitrification and the porewater NO$_3^-$ isotopic composition, to estimate rates of nitrification and denitrification throughout the sediment column. Results indicate variability of reaction rates across and within the three boreholes that are generally consistent with the differential distribution of dissolved oxygen at this site, though not necessarily with the canonical view of how redox thresholds separate nitrate regeneration from dissimilative consumption spatially. That is, we provide stable isotope evidence for expanded zones of occurring nitrification and denitrification. The isotope biogeochemical modeling also yielded estimates for the δ$^{15}$N and δ$^{18}$O of newly produced nitrate (δ$^{15}$N$_{NTR}$ and δ$^{18}$O$_{NTR}$), as well as the isotope effect for denitrification (ε$_{DNF}$), parameters with high relevance to global ocean models of N cycling. Estimated values of δ$^{15}$N$_{NTR}$ were generally lower than previously reported δ$^{15}$N values for sinking PON in this region. We suggest that these values may be, in part, related to sedimentary N-fixation and remineralization of the newly fixed organic N. Values of δ$^{18}$O$_{NTR}$ generally ranged between -2.8 and 0.0‰, consistent with recent estimates based on lab cultures of nitrifying bacteria. Notably, some δ$^{18}$O$_{NTR}$ values were elevated, suggesting incorporation of 18O-enriched dissolved oxygen during nitrification, and possibly indicating a tight coupling of NH$_4^+$ and NO$_3^-$ oxidation in this metabolically sluggish environment. Our findings indicate that
the production of organic matter by in situ autotrophy (e.g., nitrification, nitrogen fixation) supplies a large fraction of the biomass and organic substrate for heterotrophy in these sediments, supplementing the small organic matter pool derived from the overlying euphotic zone. This work sheds new light on an active nitrogen cycle operating, despite exceedingly low carbon inputs, in the deep sedimentary biosphere.
1. INTRODUCTION

Below the surface ocean, the dark ocean, including environments above and below the seafloor, hosts the largest habitable environment on the planet and is home to a wide range of globally relevant biogeochemical processes (Orcutt et al., 2011). While significant progress has been made in recent years toward characterizing the geological, chemical, and ecological composition of a variety of subsurface environments (Orcutt et al., 2011; Edwards et al., 2011; Edwards et al., 2012b), the potential for impact of these systems on global biogeochemical cycles remains poorly understood. Most of our knowledge about subseafloor microbial activity stems from research focusing on productive continental margins, where relatively high fluxes of organic matter from surface primary productivity support a large heterotrophic and mostly anaerobic microbial community, (e.g., (Blair and Aller, 2012)). By comparison, vast areas of the seafloor, in particular those underlying ocean gyres, characterized by low primary productivity and low organic matter flux to the sea floor, have received far less attention (D’Hondt et al., 2009; Mason et al., 2010; Fischer et al., 2009). In contrast to well-studied ocean margin sediments, oxygen (O$_2$) and nitrate (NO$_3$), two powerful oxidants of organic carbon, penetrate deeply into the sediment underlying oligotrophic ocean waters (D’Hondt et al., 2009; Murray and Grundmanis, 1980; Rutgers van der Loeff et al., 1990; Sachs et al., 2009; D’Hondt et al., 2015; Røy et al., 2012; Fischer et al., 2009). Furthermore, in the sediments overlying relatively young and permeably ocean crust, O$_2$ and NO$_3$ are also supplied via upward diffusion from oxic and nitrate-replete fluids flowing through basaltic basement as has been shown for the North Pond site, which is located on the western flank of the Mid-Atlantic Ridge (Orcutt et al., 2013; Ziebis et al., 2012). At North Pond, where the sediment cover is thin (< ~ 25 m), O$_2$ penetrates the entire sediment column; where sediment thickness is elevated, conditions become anoxic at mid-depths. Aerobic heterotrophic respiration likely dominates organic carbon oxidation in the upper sediment column. However, as organic carbon becomes limiting at greater depths, autotrophic processes (e.g., nitrification) are likely to gain relative importance. Further, there is evidence that the upward supply of oxidants from the basaltic basement supports increased microbial activity (Picard and Ferdeman, 2011). However, a fundamental understanding of the relative importance of specific metabolic activities that drive and sustain subsurface communities is lacking. Because central ocean gyres cover roughly half of the global seafloor, understanding the nature of the biosphere hosted within these sediments may provide important insights into its role in global
marine, nitrogen and carbon cycling. Here we focus specifically on elucidating subsurface nitrogen cycling and its role in supporting heterotrophic and autotrophic processes in oligotrophic deep-ocean sediments underlying the North Atlantic Gyre, at North Pond (22°45′N, 46°05′W).

IODP Expedition 336 (Mid-Atlantic Ridge Microbiology, Sept. 16 – Nov. 16, 2011) aimed to directly address the nature of microbial communities in both ocean crust and sediments at North Pond, a characteristic sediment-filled 70 km² depression surrounded by high relief topography common to the western flank of the Mid-Atlantic Ridge (Becker et al., 2001; Langseth et al., 1992). While a majority of seafloor subsurface biosphere research has focused on aspects of sedimentary carbon, sulfur and iron cycles, the potential role of N in supporting subsurface microbial activity has been largely unexplored. Despite exceedingly oligotrophic conditions, life persists and evidence for active heterotrophic and autotrophic microbial communities in North Pond sediments is mounting (Ziebis et al., 2012; Picard and Ferdelman, 2011; Orcutt et al., 2013).

Nitrogen plays a central role as a limiting nutrient in many regions of the sunlit surface ocean (Rabalais, 2002), as nearly 90% of the biologically available fixed N in the ocean resides below the euphotic zone in the deep ocean NO₃⁻ reservoir (Gruber, 2008). Globally, sediments (especially continental shelves) are considered a net sink of fixed nitrogen through reductive anaerobic processes including denitrification and anaerobic ammonium oxidation (Christensen et al., 1987; Devol, 1991; Prokopenko et al., 2013). For example, coupled nitrification (the chemolithotrophic oxidation of NH₄⁺ to NO₃⁻) and denitrification (the typically heterotrophic reduction of NO₃⁻ to N₂) have been shown to be important in N budgets in sediments of continental shelves, ocean margins and estuaries (Risgaard-Petersen, 2003; Granger et al., 2011; Lehmann et al., 2004; Lehmann et al., 2005; Wankel et al., 2009). There is abundant evidence demonstrating the importance of both oxidative and reductive N cycling processes (and their tight coupling) operating in sediment environments. In contrast to sediments on continental shelves, however, data from sediments underlying large swaths of the oligotrophic ocean suggest an entirely different framework. For example, NO₃⁻ concentration data from North Pond demonstrate the accumulation of NO₃⁻ with depth (Ziebis et al., 2012) implicating the role of in situ production, supported by the autotrophic oxidation of ammonium and nitrite (e.g.,...
nitrification). To what degree this NO$_3^-$ pool supports other subsurface microbial communities as an electron acceptor source, however, remains unclear. In addition, the supply of dissolved substrates (O$_2$, NO$_3^-$, DOC) from the underlying crustal aquifer may play a primary role in supporting these deep sediment communities. At a global scale, this geochemical exchange among crust, ocean and sediments across vast reaches of the seafloor, and its link to subsurface microbial activity, may well be important for global biogeochemical cycles.

Dual isotopes of NO$_3^-$ represent a powerful tool for disentangling the combined activities of multiple N cycling processes (Casciotti et al., 2008; Lehmann et al., 2005; Sigman et al., 2005; Wankel et al., 2007; Marconi et al., 2015; Fawcett et al., 2015). Nitrate-removal processes (whether assimilatory or dissimilatory) have been shown to impart linearly coupled increases in both N and O isotope ratios of the remaining NO$_3^-$ pool (Karsh et al., 2012; Kritee et al., 2012; Granger et al., 2008; Granger et al., 2004). In contrast, however, nitrification, the two-step oxidation of NH$_4^+$ to NO$_2^-$ followed by NO$_2^-$ oxidation to NO$_3^-$, represents a decoupling of the N and O isotope systems in the resulting nitrate (Casciotti and McIlvin, 2007; Buchwald and Casciotti, 2013; Wankel et al., 2007). Whereas the N atoms derive from the NH$_4^+$ (which can be assumed to originate from the sedimentary organic nitrogen pool), the oxygen atoms derive, to varying degrees, from both water and dissolved O$_2$ (Buchwald and Casciotti, 2010; Buchwald et al., 2012; Casciotti et al., 2010). Thus, by combining isotopic mass balances of both N and O in the NO$_3^-$ system, along with our understanding of organism-level constraints on the isotope systematics of these transformations, we can deduce the relative roles of multiple N cycling processes (e.g., Wankel et al., 2009, Lehmann et al. 2004; Bourbonnais et al. 2009). Here we use the dual isotopic composition of nitrate (N and O isotopes) as a record of microbial processes occurring in the low-carbon sediments of North Pond underlying the oligotrophic North Atlantic gyre. By combining the N and O isotope mass balance with an inverse reaction-diffusion model approach, we use these data to estimate rates of nitrification and denitrification, and to provide new constraints on some isotope parameters for these processes.

2. MATERIAL AND METHODS

2.1 Sediment and porewater collection
Sediment cores were collected at three sites in the North Pond Basin (Figure 1) as part of the IODP Leg 336 expedition and have been described extensively elsewhere (Expedition-336-Scientists, 2012a). Four boreholes were drilled (U1382B, U1383D, U1383E and U1384A, referred to hereafter as ‘2B’, ‘3D’, ‘3E’ and ‘4A’). Sites 3D and 3E were next to each other and as drilling logs indicated that the core from 3E showed excessive signs of disturbance upon retrieval and potential contamination by seawater, it was excluded from our study. Sediment cores were retrieved using the Advanced Piston Corer, which penetrated the seafloor sediments until contact with basement, followed by extended core barrel coring of the upper section of basement rock. Site 2B (~90m sediment thickness, depth to basement) is located in the deeper part of the pond, approximately 25m away from DSDP ‘legacy hole’ 395A, which was instrumented as a CORK observatory (Davis et al., 1992). Site 3D (~42m sediment thickness, depth to basement) lies in the northeastern region towards the edge of North Pond (~5.9km away from U1382A), whereas site 4A (~95m sediment thickness, depth to basement) is located on the northwest side in a deeper part of the basin, approximately 3.9 and 6.2km distance from U1383 and U1382, respectively (Figure 1). Two decades of temperature and flow records revealed vigorous subsurface flow (Becker et al., 1984; Gable et al., 1992), with geothermal surveys indicating that recharge is from the southeastern side of the basin (near 2B and 3D) flowing to the northwest (towards 4A) (Figure 1). All sediments were comprised of light-brown to brown nannofossil ooze with intercalations of foraminifer sand. In the lowest few meters close to the sediment/basement contact, sediments exhibited a darker brown color and sometimes rust-colored clay-rich zones (Edwards et al., 2012a; Expedition-336-Scientists, 2012b).

Porewater samples for concentration and stable isotope analyses were collected either directly from cores on the shipboard catwalk immediately after core retrieval (and stored at -80°C until analysis), from whole core rounds (~10cm core sections) that had been preserved at -80°C for ~1 year until thawing and porewater extraction, or from subsampled sediments collected shipboard and stored at -20°C for ~9 months. Porewaters from whole core rounds (~40ml) and subsampled sediments (~3ml), were extracted using rhizon samplers (0.2µm) (Seeberg-Elverfeldt et al., 2005) and stored frozen (-20°C) until analysis.

2.2 Concentration measurements
Concentrations of NO\textsubscript{3} (i.e., NO\textsubscript{3}~\textsuperscript{-} plus NO\textsubscript{2}~\textsuperscript{-}) from shipboard-extracted porewaters were measured via ion chromatography ~3 months after collection, while concentrations from home-laboratory extracted porewaters were measured by chemiluminescence after reduction in a hot acidic vanadyl sulfate solution on a NO\textsubscript{x} analyzer (Braman and Hendrix, 1989) (detection limit <0.5\textmu M). Concentrations of NO\textsubscript{2}~\textsuperscript{-} were quantified by using the Griess-Ilosvay method followed by measuring absorption at 540nm (Grasshoff et al., 2007) or by chemiluminescence in a sodium iodide solution on a NO\textsubscript{x} analyzer (Garsside, 1982;Cox, 1980). NO\textsubscript{3}~\textsuperscript{-} was quantified by difference between NO\textsubscript{x} and NO\textsubscript{2}~\textsuperscript{-} (Grasshoff et al., 2007). Ammonium concentrations were measured using the orthophthalaldehyde fluorescence method with a detection limit of 20nM (Holmes et al., 1999).

2.3 Nitrate stable isotope composition

Nitrate N and O isotopic composition were measured using the denitrifier method (Casciotti et al., 2002;Sigman et al., 2001), in which sample NO\textsubscript{3}~\textsuperscript{-} is quantitatively converted to N\textsubscript{2}O using a lab-grown denitrifying bacterium before being extracted and purified on a purge-and-trap system similar to that previously described (McIlvin and Casciotti, 2010). Where detected (O\textsuperscript{18}-depleted zones of 2B), NO\textsubscript{3}~\textsuperscript{-} was removed by sulfamic acid addition (Granger and Sigman, 2009) prior to isotopic analysis of the NO\textsubscript{3}~\textsuperscript{-}. Isotopic analysis of shipboard extracted samples (16) were conducted at the University of Basel using a Delta V Advantage (Thermo Inc.), while all other samples (29) were measured on an IsoPrime100 (Elementar, Inc.). Corrections for drift, size and fractionation of O isotopes during bacterial conversion were carried out as previously described using NO\textsubscript{3}~\textsuperscript{-} standards USGS 32, USGS 34 and USGS 35 (Casciotti et al., 2002;McIlvin and Casciotti, 2011), with a typical reproducibility of 0.2‰ and 0.4‰ for \delta\textsuperscript{15}N and \delta\textsuperscript{18}O, respectively.

3. RESULTS

3.1 Previous measurements of dissolved oxygen and organic matter content at North Pond

Oxygen penetration depths, which have been discussed previously (Orcutt et al., 2013), vary distinctly among the three sites at North Pond indicating much greater respiratory consumption in 2B than in the profiles of other two sites, 3D and 4A (Figure 2). In 2B, dissolved oxygen levels are drawn down to near detection by a depth of about 10mbsf (although low levels
of dissolved O_2 seem to persist as deep as 30 mbsf. In contrast, at site 3D, dissolved O_2 levels are
drawn down close to the detection limit (~5 μM) for an interval of only ~3m between a depth of
~30 to ~33 mbsf and in 4A, zero-level O_2 concentrations were observed over the interval between
32 and 54m. At North Pond, O_2 (and NO_3^-) is also supplied via diffusion from the underlying
basaltic crustal aquifer (Figure 2) (Orcutt et al., 2013; Ziebis et al., 2012). Although not measured
during this work, sediment organic carbon and nitrogen content was measured on several of the
piston cores collected during the site-survey, averaging 0.15% and 0.02%, respectively (Ziebis et
al., 2012). Although quantification of such low organic-N and -C levels is challenging and is
afflicted with relatively large uncertainties, no discernable OM-elemental differences among the
sites were noted (Ziebis et al., 2012). Similarly, no differences in organic phosphorus content
were noted among the three sites (Defforey and Paytan, 2015).

3.2 NO_3^- and NO_2^- concentration profiles

Bottom seawater NO_3^- concentration at North Pond is approximately 21.6 μM (Ziebis et
al., 2012). At all depths in all three profiles, porewater NO_3^- concentrations exceeded bottom
water NO_3^- concentrations, reflecting the production of NO_3^- by nitrification and the net flux of
NO_3^- to the overlying water from this site of ~4.6 μmoles m^-2 d^-1 (Ziebis et al., 2012), consistent
with other studies of NO_3^- fluxes in pelagic deep-sea sediments (Berelson et al., 1990; Goloway
and Bender, 1982; Hammond et al., 1996; Jahnke et al., 1982; Grundmanis and Murray, 1982).
More precisely, below the sediment-water interface, NO_3^- concentrations increased significantly
with depth (Figure 2), before decreasing again with proximity to the basement/sediment contact.
Mid-profile NO_3^- concentration maxima reached 38.2, 42.2 and 49.1 μM at depths of 19.1, 23.0
and 56.3 mbsf in the cores from sites 2B, 3D and 4A, respectively, depths that generally
coincided with O_2 concentrations below 10 μM. Nitrite was below detection at sites 3D and 4A
and was only detected at anoxic depths in site 2B (Figure 2), where concentrations of up to 6.0
and 6.6 μM were observed at depths of 36 and 59m, respectively.

3.3 NO_3^- N and O isotopic composition

Down-core changes in δ^{15}N and δ^{18}O varied markedly among the three cores (Figure 2).
The most prominent changes in both δ^{15}N and δ^{18}O were observed at site 2B (which had the
most extensive O_2 depleted zone), in which δ^{15}N increased with depth from a value of +5.4‰
(bottom seawater) to a maximum of \(+23.3\%o\) at a depth of 59.2 mbsf and \(\delta^{18}O\) increased from a bottom seawater value of \(+1.8\%o\) to a maximum of \(+23.8\%o\) at a much shallower depth of 32.1 mbsf. Isotopic maxima generally coincided with depths of lowest \(O_2\) concentrations, except in 3D, where the maximum was observed at slightly greater depth than the \(O_2\) minimum (Figure 2).

Substantial N and O isotopic shifts were also observed at site 3D, in which increases above bottom water \(NO_3^-\) values to \(\delta^{15}N\) of \(+11.8\%o\) and a \(\delta^{18}O\) of \(+21.7\%o\) were observed, with both maxima occurring at a depth of 37 mbsf. In contrast, site 4A exhibited only very modest changes relative to bottom seawater, with a maximum \(\delta^{15}N\) value of \(+7.0\%o\) at a depth of 38.8 mbsf and a maximum \(\delta^{18}O\) value of \(+6.3\%o\) at a depth of 44.1 mbsf. Strong correlations were also observed between \(\delta^{15}N_{NO_3^-}\) and \(\delta^{18}O_{NO_3^-}\) (Figure 3), with \(\delta^{18}O_{NO_3^-}\) values always increasing more than the corresponding \(\delta^{15}N_{NO_3^-}\). The relationship between \(\delta^{18}O_{NO_3^-}\) and \(\delta^{15}N_{NO_3^-}\) exhibited a slope of 1.8 for the upper portion of the 2B profile, 3.0 for the 3D profile and 2.4 for the 4A profile – consistently exceeding the 1:1 relationship expected from \(NO_3^-\) consumption alone. In 2B, sampling points near the most \(O_2\) depleted depths and the lower portion of the profile fell closer to the expected 1:1 line for \(NO_3^-\) consumption by denitrification (Figure 3).

4. DISCUSSION

The distribution of porewater nitrate in deep-sea sediments is controlled by the combined influence of diffusion as well as several biologically catalyzed diagenetic processes including nitrification (ammonia and nitrite oxidation to nitrate) and denitrification (loss of N via nitrate reduction to gaseous products, \(NO, N_2O\) or \(N_2\)). Here we use the concentration and dual N and O stable isotope composition of porewater \(NO_3^-\) to gain insight into the magnitude and distribution of N transformation processes. In comparison to models that predict the rates of these processes based solely on concentration profiles of \(NO_3^-\) and \(O_2\), for example, our approach estimates rates using the added constraints provided by recent studies of N and O isotope systematics of nitrification and denitrification (Granger et al., 2008; Buchwald and Casciotti, 2010; Casciotti et al., 2010; Buchwald et al., 2012). In particular, while there are strong and related N and O isotope effects during denitrification (Granger et al., 2008), the isotopic transformations of N and O are decoupled due to differently sourced N and O atoms during nitrate production (Buchwald and Casciotti, 2010; Casciotti et al., 2010; Buchwald et al., 2012). Thus, changes in N and O isotopic composition between intervals of any one depth are the combined result of both diffusion of...
NO$_3^-$ to/from overlying (and underlying) seawater, together with microbially mediated production and/or consumption of NO$_3^-$ within porewaters. Under low oxygen, NO$_3^-$ respiration by denitrification leads to a well-characterized increase in both $\delta^{15}$N and $\delta^{18}$O in conjunction with decreasing NO$_3^-$ concentrations. In contrast, nitrification produces NO$_3^-$ with a $\delta^{15}$N equal to the starting NH$_4^+$ (when accumulation of NH$_4^+$ and NO$_2^-$ is negligible), while the $\delta^{18}$O of the newly produced NO$_3^-$ is set by the $\delta^{18}$O of ambient H$_2$O and O$_2$, as well as kinetic and equilibrium isotope effects associated with the stepwise oxidation of NH$_4^+$ to NO$_3^-$. While elevated NO$_3^-$ concentrations indicate nitrification, extensive zones of low O$_2$ (and NO$_3^-$ replete) porewaters also suggest a high potential for denitrification, which can be verified using nitrate dual isotope measurements. In this way, our modeling approach provides an assessment of the distribution of these N transformations, as well as some additional insights on the nature of N and O source atoms to NO$_3^-$ in these energy-lean systems. Specifically, we show below that the profiles of $\delta^{15}$N$_{NO3}$ and $\delta^{18}$O$_{NO3}$ can be explained by variations in the magnitude of nitrification and denitrification occurring throughout the sediment column, including substantial zones of overlap of these aerobic/anaerobic processes. Finally, we use our model to predict the $\delta^{18}$O and $\delta^{15}$N stemming from nitrate production by nitrification, offering insights into both the nature of processes setting the O isotopic composition of oceanic NO$_3^-$, as well as the sources of N and/or the isotopic partitioning of available N sources in global ocean sediments.

4.1 Diffusion-Reaction Model

The diffusion-reaction inverse modeling approach used here is conceptually similar to other early diagenetic models that simulate porewater profiles of dissolved species through a sediment column harboring both oxic and anoxic organic matter remineralization (Christensen and Rowe, 1984; Goloway and Bender, 1982; Jahnke et al., 1982). It is an inverse modeling approach adapted to distinguish between heavy and light nitrate isotopologues (e.g., Lehmann et al., 2007). Specifically, we use the model to estimate rates of nitrification and denitrification required to fit the concentration profiles of each isotopologue, $^{14}$N$^{16}$O$_2^-$, $^{15}$N$^{16}$O$_2^-$, and $^{14}$N$^{16}$O$^{18}$O$_2^-$ (and, thus, $\delta^{15}$N$_{NO3}$ and $\delta^{18}$O$_{NO3}$ values) under the assumption of steady-state diffusion and microbial production (by nitrification) and/or consumption (by denitrification). Rates of nitrification and denitrification in each porewater sampling interval (e.g., defined as the distance between the lower and upper midpoints between sampling depths) were estimated...
numerically by least squares fitting of the system of equations describing the distribution of each isotopologue (using a genetic algorithm included in the Solver package of Microsoft Excel 2011). This approach involves determination of a non-unique solution using numerical iteration and optimization, and is repeatedly iterated to evaluate robustness of model fits. Certain parameters are allowed to be optimizable by the model, including both the magnitude of, and connection between, the N and O isotope effects for denitrification ($^{15}\varepsilon_{\text{DNF}}$ and $^{18}\varepsilon_{\text{DNF}}$, respectively), as well as the N and O isotopic composition of new NO$_3^-$ produced by nitrification ($\delta^{15}\varepsilon_{\text{DNF}}$ and $\delta^{18}\varepsilon_{\text{DNF}}$, respectively). Uncertainty in model estimates is expressed as the standard error of 10 model-run estimates (Table 1). Conditions at the uppermost part of the sediment column were constrained by measured concentrations and isotope ratios in bottom seawater. Measured concentrations of the NO$_3^-$ isotopologues within each interval, together with the diffusive fluxes defined by the concentration gradients between the over/underlying intervals, were used for model fitting by least-squares optimization of microbial rates of nitrification and/or denitrification.

As measurable NH$_4^+$ was not observed at any depths, it is not included in the model. NO$_3^-$ is the only dissolved N species included in the model and we assume that all NH$_4^+$ generated by remineralization is completely oxidized to NO$_3^-$ (see below). To minimize complexity, other diagenetic reactions that may be important in many sedimentary environments, including anaerobic NH$_4^+$ oxidation, removal of N species through interactions with Fe or Mn and the adsorption and retention of NH$_4^+$ by clay minerals are not specifically addressed. We also neglect effects of compaction as well as potential changes in organic matter reactivity with depth. No difference in the diffusivity among NO$_3^-$ isotopologues was included, since these differences are considered to be very small (Clark and Fritz, 1997).

Resolving the vertical dimension only, the mass balance differential equations are as follows:

\[
\frac{\partial C_{\text{NO3}}}{\partial t} = \frac{\partial}{\partial x} \left(D_{\text{NO3}} \frac{\partial C_{\text{NO3}}}{\partial x} \right) - \text{DNF}_{14N} + \text{NTR}_{14N} \tag{1}
\]

\[
\frac{\partial C_{\text{NO3}}}{\partial t} = \frac{\partial}{\partial x} \left(D_{\text{NO3}} \frac{\partial C_{\text{NO3}}}{\partial x} \right) - \text{DNF}_{15N} + \text{NTR}_{15N} \tag{2}
\]

\[
\frac{\partial C_{\text{NO3}}}{\partial t} = \frac{\partial}{\partial x} \left(D_{\text{NO3}} \frac{\partial C_{\text{NO3}}}{\partial x} \right) - \text{DNF}_{160} + \text{NTR}_{160} \tag{3}
\]
1 \[ \frac{\partial C_{\delta^{15}O}}{\partial t} = \frac{\partial}{\partial x} \left( D_{\delta^{15}O} \frac{\partial C_{\delta^{15}O}}{\partial x} \right) - DNF_{18O} + NTR_{18O} \] (4)

such that for denitrification (DNF):

3 \[ DNF_{14N} = C_{14NO3} k_{DNF} \] (5a)

4 \[ DNF_{15N} = C_{15NO3} k_{DNF}/\alpha_{DNF} \] (5b)

5 \[ DNF_{16O} = C_{16NO3} k_{DNF} \] (5c)

6 \[ DNF_{18O} = C_{18NO3} k_{DNF} \left[ 1 + ((\alpha_{DNF} - 1) \times (18\epsilon_{15\epsilon_{DNF}})) \right] \] (5d)

7 \[ DNF = DNF_{14N} + DNF_{15N} = DNF_{16O} + DNF_{18O} \] (6)

where D refers to the molecular diffusion coefficient for NO$_3^-$ adjusted for porosity, DNF and NTR refers to the reaction rate of denitrification or nitrification, respectively (in mass volume$^{-1}$ time$^{-1}$), C refers to the concentration of each isotopologue (in mass volume$^{-1}$) and k refers to the first order rate constant (time$^{-1}$). The fractionation factor, \( \alpha \), is defined as the ratio of rate constants for the light isotope over the heavy isotope (e.g., $^{15}\alpha = ^{14}k/^{15}k$) for a given process, alternatively expressed in terms of epsilon where $\epsilon = (\alpha - 1)*1000$ in units of permil (‰). The term $^{18}\epsilon_{15\epsilon_{DNF}}$ refers to the degree of coupling between the N and O isotope fractionation during denitrification, with a value of 1 indicating that the two isotope effects are identical.

For the $\delta^{15}N$ and $\delta^{18}O$ of nitrification (NTR)

17 \[ NTR_{14N} = NTR f_{14NNTR} \] (7a)

18 \[ NTR_{15N} = NTR f_{15NNTR} \] (7b)

19 \[ NTR_{16O} = NTR f_{16ONTR} \] (7c)

20 \[ NTR_{18O} = NTR f_{18ONTR} \] (7d)

where f refers to the fractional abundance of a particular isotopologue and

22 \[ NTR = NTR_{14N} + NTR_{15N} = NTR_{16O} + NTR_{18O} \] (8)

23 \[ f_{14NNTR} = 1/(1 + ^{15}R_{NTR}) \] (9a)
\[ f_{15\text{NTR}} = 1 - f_{14\text{NTR}} \quad (9b) \]

\[ ^{15}\text{R}_{\text{NTR}} = [^{15}\text{N}/^{14}\text{N}]_{\text{NTR}} \quad (9c) \]

and

\[ f_{16\text{ONTR}} = 1/(1+^{18}\text{R}_{\text{NTR}}) \quad (10a) \]

\[ f_{18\text{ONTR}} = 1 - f_{16\text{ONTR}} \quad (10b) \]

\[ ^{18}\text{R}_{\text{NTR}} = [^{18}\text{O}/^{16}\text{O}]_{\text{NTR}} \quad (10c) \]

and \(^{15}\text{R}_{\text{NTR}}\) and \(^{18}\text{R}_{\text{NTR}}\) are used to calculate the \(\delta^{15}\text{N}_{\text{NTR}}\) and \(\delta^{18}\text{O}_{\text{NTR}}\), respectively.

For parameterizing diffusion, we use a porewater diffusion coefficient \(D_s\) based on the molecular diffusion coefficient \(D_m\) at 5 °C for NO\(_3^–\) of 1.05 *10\(^{-5}\) cm\(^2\) s\(^{-1}\) (Li and Gregory, 1974) adjusted for an average porosity \(\phi\) of North Pond sediments of 64% (Expedition-336-Scientists, 2012a), where \(D_s = \phi D_m\) and \(k\) is an empirically derived factor (we use 2.6) accounting for tortuosity of pore space (Hammond et al., 1996; McManus et al., 1995).

Compared with contemporaneous profiles of O\(_2\) and Sr (Orcutt et al., 2013) and other dissolved ions, the NO\(_3^–\) concentration profiles suffer from some apparent analytical noise. The nature of the heterogeneity for NO\(_3^–\) concentration measurements was unclear. However, it is unlikely that this heterogeneity is environmental and we attribute it to small amounts of evaporation during freezer storage of the sediments, which is supported by the apparent smoothness of the isotopic measurements (evaporation would change the apparent concentrations without influencing the isotopic composition of solutes such as NO\(_3^–\)). As such, for the purpose of the diffusion-reaction model, we applied a 5-point weighted triangular smoothing to the concentration data to eliminate outliers and unrealistically sharp gradients (Figure 2). Given the relatively smooth and contiguous vertical profiles of \(\delta^{15}\text{N}_{\text{NO3}}\) and \(\delta^{18}\text{O}_{\text{NO3}}\), only very minor smoothing to these data was required using a similar approach (Figure 2).

Within this model architecture, we explore the influence of four key parameters that could affect the estimation of nitrification and denitrification rates by this isotope mass balance approach, specifically, \(\varepsilon_{\text{EDNF}}, ^{15}\varepsilon_{\text{EDNF}}, \delta^{15}\text{N}_{\text{NTR}}\) and \(\delta^{18}\text{O}_{\text{NTR}}\). Specifically, the expression of the...
full enzymatic level isotope effect ($^{15}\epsilon_{\text{DNF}}$) for denitrification (27‰) can be influenced by electron donor, carbon substrate quality, denitrification rate and metabolic activity (Kritee et al., 2012). Moreover, although the relationship between the kinetic isotope effects for $^{18}$O and $^{15}$N during respiratory consumption of NO$_3^-$ by denitrification (e.g., $^{18}$\epsilon:15$\epsilon_{\text{DNF}}$) has been shown to remain consistent at 1:1, the potential influence of nitrate reduction by periplasmic nitrate reductase (Nap), which imparts a lower $^{18}$\epsilon:15$\epsilon_{\text{DNF}}$ value of 0.6 (Granger et al., 2008; Frey et al., 2014), could play a role in the dual isotope trajectory of NO$_3^-$ consumption (Wenk et al., 2014).

Further, in the absence of NH$_4^+$ accumulation in these sediments, the $\delta^{15}$N$_{\text{NTR}}$ is equal to the source of NH$_4^+$ being oxidized to NO$_3^-$, which is related to the $\delta^{15}$N of the organic matter being remineralized. The $\delta^{18}$O$_{\text{NTR}}$ stems from a combination of factors including the $\delta^{18}$O of the water and dissolved O$_2$ as well as the expression of kinetic isotope effects associated with the incorporation of O atoms from these pools (Buchwald and Casciotti, 2010; Casciotti et al., 2010; Andersson and Hooper, 1983). Below, we use the model to optimize and predict these values and to explore the sensitivity of rate estimates to $^{15}\epsilon_{\text{DNF}}$.

The model contains more parameters than can be explicitly estimated from the small number of data points measured. To minimize the number of variables as much as possible (and maximize the utility of the approach for constraining other variables), we adapt the model implementation for three different O$_2$ regimes: 1) ‘oxic intervals’ where O$_2$ is poised as the more energy-yielding oxidant with respect to NO$_3^-$ (here generally O$_2$ $>$ 40µM) and in which only nitrification is allowed to occur, 2) ‘transitional intervals’ in which both denitrification and nitrification may occur (O$_2$ between $\sim$40µM and 2µM) and 3) ‘anoxic intervals’ where O$_2$ is $<$2µM and in which only denitrification is allowed to occur. We choose 40µM as a boundary for the onset of denitrification based on estimates of thermodynamic energy yield under these conditions for aerobic vs denitrification-based respiration (Brewer et al., 2014). In the oxic intervals – the model is used for parameter estimation of both $\delta^{15}$N$_{\text{NTR}}$ and $\delta^{18}$O$_{\text{NTR}}$ (in addition to nitrification rate), while in the anoxic intervals the model is used to estimate $^{15}\epsilon_{\text{DNF}}$ and $^{18}$\epsilon:15$\epsilon_{\text{DNF}}$ (in addition to denitrification rate). In transitional intervals, $^{15}\epsilon_{\text{DNF}}$ and $^{18}$\epsilon:15$\epsilon_{\text{DNF}}$ are held constant at 25% and 1, respectively, and the parameter $\delta^{15}$N$_{\text{NTR}}$ and $\delta^{18}$O$_{\text{NTR}}$ are estimated through model fitting, together with rates of both nitrification and denitrification. In accordance with previous experimental work (Buchwald and Casciotti, 2010; Buchwald et al., 2012; Casciotti...
et al., 2010), the allowed range of values for δ^{15}N_{NTR} and δ^{18}O_{NTR} were set to -5 to +10‰ and -5 to +20‰, respectively. When ε_{DNF} and ε:15ε_{DNF} were determined by model fitting, parameter estimates were allowed to range between 0 and 30‰ and between 0.6 and 1.2, respectively.

4.2 Model Results and Implications

4.2.1 Model Estimated Rates of Nitrification and Denitrification

Profiles of sedimentary porewater solutes reflect the combined influence of many processes including diagenetic reactions, which are intimately related to the availability, abundance and quality of organic carbon. In particular, the distribution of dissolved substrates that are available as electron acceptors for microbial respiration of organic carbon, generally reflect stepwise consumption by the most thermodynamically (and kinetically) favorable metabolic processes (e.g., O_2 consumption precedes NO_3^- consumption, which precedes sulfate reduction, etc.). While in organic rich estuarine and continental shelf sediments, dissolved O_2 and NO_3^- are typically consumed within a few cm or mm below the sediment-seawater interface, sediments underlying large areas of the oligotrophic ocean are characterized by very deep penetration of O_2, in some cases even penetrating to the underlying ocean crust (D’Hondt et al., 2015; D’Hondt et al., 2009; Orcutt et al., 2013; Ziebis et al., 2012). In connection with this deep penetration of O_2, deep-sea sediment porewaters also often exhibit extensive accumulation of NO_3^- above ambient seawater concentrations, associated with the oxidation of NH_4^+ released by aerobic remineralization of sediment organic matter (and linked to the consumption of O_2 through Redfield stoichiometry) (Berelson et al., 1990; Christensen and Rowe, 1984; D’Hondt et al., 2009; Goloway and Bender, 1982; Seitzinger et al., 1984). In organic-rich sediments, NO_3^- concentration profiles may exhibit maxima only a few mm or cm below the sediment/water interface. In contrast, in the deep-sea sediments underlying the oligotrophic regions of the ocean, the sedimentary zone where NO_3^- accumulates to 10 to 30µM above bottom seawater concentrations can extend over a much larger vertical extent and nitrate maxima can be found tens of meters below the seafloor. In effect, the redox zonation of O_2 respiration, NH_4^+ oxidation, NO_2^- oxidation and NO_3^- reduction extends over larger depth ranges, and, depending on sediment
thickness and organic carbon content – the redox state of these sediments may never reach the potential for NO$_3^-$ reduction to play a role as a thermodynamically viable metabolic pathway.

While it is not necessarily apparent whether any NO$_3^-$ respiration is occurring based on North Pond concentration profiles alone, dramatic increases in the δ$^{15}$N and δ$^{18}$O of NO$_3^-$ with depth into the anoxic sediment intervals were observed in both 2B and 3D (Figure 2) – indicating isotope fractionation by microbial denitrification. The highest δ$^{15}$N and δ$^{18}$O values in 2B (+22.2‰ and +21.8‰, respectively) generally coincide with the lowest dissolved O$_2$, while highest δ$^{15}$N and δ$^{18}$O values in 3D (+11.8‰ and +19.7‰, respectively) fall just below the depth of lowest O$_2$. In stark contrast, 4A porewaters exhibit only a minor increase in δ$^{18}$O of ~2.7‰ within the anoxic interval, while δ$^{15}$N increased by only 0.8‰ (Figure 2). The marked distinction between 2B/3D and 4A notwithstanding, the dual isotopic composition and concentrations of NO$_3^-$ in these porewaters reveal active nitrogen cycling within all three sites.

Using these changes in dual NO$_3^-$ isotopic composition and concentration, we calculated the rates of nitrification and denitrification necessary to produce the observed patterns within each interval (in the transitional intervals, here we prescribe a value of 25‰ for $^{15}$EDNF, but explore the model sensitivity to this value later). Rates of nitrification and denitrification varied with depth, as well as across the three sites (Figure 4; Table 1). Estimated rates of nitrification in the oxic and transitional intervals were up to 871 nmol cm$^{-3}$ yr$^{-1}$, while rates of denitrification in the anoxic and transitional intervals reached up to 579 nmol cm$^{-3}$ yr$^{-1}$ (Figure 4, Table 1). By comparison, maximum rates of nitrification estimated from O$_2$ and NO$_3^-$ profiles in sediments below the South Pacific Gyre, perhaps the most organic matter depleted seafloor sediments in the world, were predicted to be only as high as 18 – 74 nmol cm$^{-3}$ yr$^{-1}$ (D'Hondt et al., 2009). The estimated nitrification rates at North Pond are still orders of magnitude lower than those typically measured in coastal and estuarine sediments (Rysgaard et al., 1993; Usui et al., 2001; Wankel et al., 2011), and are, thus, consistent with lower total cell abundances and lower abundance of functional genes involved in nitrification recently measured in North Pond sediments (Zhao and Jørgensen, in review) as compared to typical estuarine sediments (Mosier and Francis, 2008; Wankel et al., 2011).
In general, nitrification rates in the oxic intervals near the seafloor were comparable to rates in the oxic layers near the underlying crust. The highest denitrification rates typically coincided with depths where lowest O$_2$ were observed, with the exception of rather low rates near the central anoxic zone of core 2B. Nitrification, which requires O$_2$, is observed as deep as 28m in 2B and all the way to the underlying crust at the other two sites. Interestingly, modeled rates of nitrification were typically highest at comparatively low levels of dissolved O$_2$ (~15µM in 2B, ~10µM in 3D and ~35µM in 4A) – suggesting an important role for micro-aerophilic nitrification (e.g., organisms adapted to respiration under low O$_2$ conditions). The depths of maximum denitrification rates generally coincided with the onset of O$_2$ levels below ~2µM. In 2B, denitrification rates were highest at depths of ~28m and ~72m (Figure 4). In 3D, maximum denitrification rates were observed at 21m – with similar rates between 20 and 25m. Interestingly, the model indicated no denitrification within the 3m anoxic zone of this core – possibly indicating limitation by organic substrate availability. In contrast, rates of denitrification were only estimated to occur within the anoxic zone of site 4A (e.g., not within the transitional intervals, although this was somewhat sensitive to prescribed $^{15}$O$_{DNF}$, see below), with the highest rate of 24 nmol cm$^{-3}$ yr$^{-1}$ at a depth of 44m. Overall, rates of both nitrification and denitrification were highest at site 2B and lowest in 4A, consistent with a greater amount of microbial activity revealed by the steeper O$_2$ gradients in 2B and recent functional gene quantification in NP sediments (Zhao and Jørgensen, in review). More precisely, the abundance of 16S rRNA, and several functional genes, including those for both archaeal and bacterial ammonia oxidation, nitrite oxidation, and denitrification, were all significantly lower in 4A than in the other cores (Zhao and Jørgensen, in review). In all three cores, maximum rates of nitrification exceeded those of denitrification, consistent with the net accumulation of NO$_3^-$ throughout the sediment column. Even in 2B, where O$_2$ is below 2µM over an interval of ~40m, the NO$_3^-$ concentration profile exhibits no obvious influence by NO$_3^-$ reduction (Figure 7).

Finally, the model suggests the co-occurrence of nitrification and denitrification (Figure 4) in the transition zones of our model (depths at which O$_2$ is between 2 and 40 µM). Although denitrification rates generally did not exceed those of nitrification where the two processes are co-occurring (i.e., no net nitrate consumption), the increasing $^{15}$N and $^{18}$O of NO$_3^-$ clearly reflects the influence of NO$_3^-$ loss via denitrification. Indeed, similar inferences have also been
made about such overlap of nitrification and denitrification, albeit in the much deeper and
organic-rich hadopelagic sediments of the Ogasawara Trench (Nunoura et al., 2013). This
observation illustrates the exceptionally extended vertical redox zonation of these sediments –
and highlights the potential interaction between nitrogen transformations that are classically
considered spatially explicit.

4.2.2 Model-predicted values of δ¹⁵N_{NTR}: Implications for N sources and processes in
oligotrophic sediments

In general, where NH₄⁺ from remineralization does not accumulate, it is expected that the
δ¹⁵N of NO₃⁻ produced by NH₄⁺ oxidation will be equivalent to the δ¹⁵N of the NH₄⁺ deriving
from remineralization of organic nitrogen. The δ¹⁵N of organic N of the North Pond sediments
was not quantified in this study (N content as measured by Ziebis et al. (2012) within the upper
8 m was <0.03%). Yet, the average model-predicted δ¹⁵N of newly produced NO₃⁻ (δ¹⁵N_{NTR})
(Figure 5) ranged from -3.1 to +1.1‰ (standard error typically ±0.3 to 0.4‰), generally lower
than that expected based on the δ¹⁵N of sinking organic matter from the surface ocean of
~+3.7‰ (Altabet, 1988; Altabet, 1989; Knapp et al., 2005; Ren et al., 2012). Values of δ¹⁵N_{NTR} >
+1‰ were only observed just above the O₂ minimum at sites 2B and 3D (Figure 5).

Confronted with this difference, we turn to other factors that might play a role in setting
the δ¹⁵N_{NTR}. The lower δ¹⁵N values of newly produced NO₃⁻ can potentially be explained by a
number of possible processes including: 1) isotopic fractionation during remineralization, 2)
competitive branching between NH₄⁺ oxidation (whether anaerobic or aerobic) and NH₄⁺
asimilation or 3) contribution of low δ¹⁵N through organic matter derived from sedimentary N
fixation.

Nitrogen isotope fractionation during organic matter remineralization has been reported
(Altabet, 1988; Altabet et al., 1999; Estep and Macko, 1984; Lehmann et al., 2002), whereby the
preferential remineralization of low δ¹⁵N organic matter leads to production of low δ¹⁵N NH₄⁺
(which could feed into the production of low δ¹⁵N NO₃⁻). The influence of this phenomenon is
more likely, however, during remineralization of fresh organic matter and where the
heterotrophic community has abundant access to highly labile proteinaceous organic matter.
Competitive branching of NH$_4^+$ supporting simultaneous nutritional supply (as an N source) and energy supply (via autotrophic ammonia oxidation) has been used to explain NO$_3^-$ dual isotopic signatures in N-rich surface waters (Wankel et al., 2007). Because N isotope fractionation during ammonia oxidation is generally thought to be stronger than that of NH$_4^+$ assimilation by phytoplankton under surface water conditions, it was argued that this competitive branching can lead to a shunt of low δ$^{15}$N into the NO$_3^-$ pool via ammonia oxidation (Wankel et al., 2007). In contrast to the high-nutrient, sunlit, productive waters of Monterey Bay, however, under the energy-limited, and extremely low NH$_4^+$ production conditions in North Pond porewaters, it is not clear whether the same mechanisms of NH$_4^+$ partitioning are operating.

Although North Pond porewaters contain abundant NO$_3^-$, assimilation of NO$_3^-$ as a nutritional N source requires an associated metabolic energy for reduction of NO$_3^-$ (via an assimilatory nitrate reductase), a costly process in this energy poor environment. On the other hand, although NH$_4^+$ is more easily assimilated by most microbes, its exceedingly low abundance (<20nM) in North Pond porewaters reflect its scarcity as a source of N required for cell growth. Based on the estimated values of the N isotope effect for NO$_3^-$ consumption (by denitrification) in the porewaters of 2B that average ~20‰ (Table 2), there is little suggestion of NO$_3^-$ assimilation, which would lead to much lower estimated values of $^{15}$NDN (Granger et al., 2010). Thus, it is more likely that nutritional N originates from a reduced form such as NH$_4^+$ and/or organic N. If the N isotope effect for ammonia oxidation is much larger than that of ammonia assimilation, then such a low δ$^{15}$NTR. Although under such low concentrations, it is likely that microbial acquisition of NH$_4^+$ (whether for assimilation or for oxidation) is diffusion-limited, conditions under which the N isotope effect is expected to be near 0‰ (Hoch et al., 1992), a strong difference in the isotope effects for assimilation and oxidation of NH$_4^+$ could still contribute to the production of low δ$^{15}$N nitrate.

A final possibility for the low values of δ$^{15}$NTR could reflect the relative importance of benthic N-fixation operating in North Pond sediments. Bacterial N-fixation is generally thought to be at very low levels.
to result in biomass having a $\delta^{15}N$ between -2 and 0‰ (Delwiche et al., 1979; Meador et al., 2007; Minigawa and Wada, 1986), which could effectively introduce new N and decrease the bulk $\delta^{15}N$ available for oxidation. Benthic N-fixation is not generally considered to be an important enough contributor to the total sediment organic matter to influence the bulk $\delta^{15}N$ values of sediment organic matter. However, given the low organic matter flux to these sediments from the overlying oligotrophic surface waters, a proportionately smaller amount of N-fixation would be required to significantly impact the sediment organic $\delta^{15}N$ value. While N-fixation is an energetically costly metabolism and might seem an unlikely strategy given the abundant porewater NO$_3^-$ pool, it has been recently acknowledged that N-fixation in benthic environments may be widely underestimated, despite high levels of porewater DIN including NO$_3^-$ and/or NH$_4^+$ (Knapp, 2012).

In fact, N-fixation could be ecologically favored in organic-lean sediments like those at North Pond owing to the formation of H$_2$ as an end product, which might afford some community level. Although primarily recycled by highly efficient hydrogenases in N-fixing bacteria (Bothe et al., 2010), a small efflux of H$_2$ could help to fuel other autotrophic metabolisms including both NO$_3^-$ reduction (Nakagawa et al., 2005) or the Knallgas reaction (H$_2$ + O$_2$), perhaps providing some mutualistic benefit. Hydrogen-based metabolism has been proposed as a significant contributor to subsurface autotrophy underlying the oligotrophic South Pacific Gyre (D’Hondt et al., 2009). Although the involvement of so-called alternative nitrogenases (the Fe and V forms), which have been shown to display an even larger kinetic isotope effect (-6 to -7‰) than the Mo-bearing form (Zhang et al., 2014), could offer even greater leverage on lowering of the bulk $\delta^{15}N$ (and source of N for nitrification), their involvement in non-sulfidic marine systems, where Mo is replete and soluble Fe and V is scarce, is expected to be minimal (Zhang et al., 2014). A similar argument was also made for the cryptic involvement of N fixation as source of low $\delta^{15}N$ and explanation for dual NO$_3^-$ isotopic patterns in the large oxygen minimum zone of eastern tropical North Pacific (Sigman et al., 2005). Thus, we conclude that the low predicted values of $\delta^{15}N_{NTR}$ provide compelling evidence for an important role of in situ N-fixation in these organic lean sediments.

Finally, there is a conspicuous increase in the predicted $\delta^{15}N_{NTR}$ at sites 2B and 3D (up to +1.8 and +1.1‰, respectively) between 15-35m. Although we have no compelling explanation
for these observations, it is interesting that these values coincide with the transitional intervals over which $\delta^{18}O_{\text{NO}_3}$ values increase much more rapidly than $\delta^{15}N_{\text{NO}_3}$. While it is possible that our model is insufficient for constraining the isotope dynamics at NP, it may also be that these suboxic depths support differential amounts of in situ nitrogen fixation leading to shifts in the bulk $\delta^{15}N$ available for oxidation by nitrification.

The contribution of competitive branching during NH$_4^+$ consumption notwithstanding, the predicted $\delta^{15}N_{\text{NTR}}$ values can serve as an index of the degree of organic matter deriving from in situ N-fixation versus delivery from the overlying water ($\delta^{15}N \sim +3.7\%o$). Indeed, using a value of -2%o to represent organic matter derived from biological N fixation (Delwiche et al., 1979; Meador et al., 2007; Minigawa and Wada, 1986), our results suggest that between 25 and 100% (with an average of 80 ± 20%) of the organic nitrogen supply in these sediments derives from biological nitrogen fixation, representing a potentially enormous relative role for in situ autotrophy in sustaining these microbial communities. Under an assumption of steady state, and ignoring a potential contribution of competitive branching in NH$_4^+$ consumption, rates of N-fixation were estimated as a fraction of nitrification and ranged up to 690 nmol cm$^{-3}$ yr$^{-1}$ (Table 1). The distribution of N-fixation rates were generally similar to nitrification – with much lower rates (16 – 89 nmol cm$^{-3}$ yr$^{-1}$) at site 4A – again consistent with overall lower cell abundance (Zhao and Jørgensen, in review). These rates are 2-3 orders of magnitude lower than rates measured in coastal and estuarine sediments (e.g., Bertics et al., 2010; Rao and Charette, 2012; Joye and Paerl, 1993), although still much higher than rates measured in studies of N fixation in oligotrophic water column (e.g., Montoya et al., 2004; Capone et al., 2005). Overall, North Pond sediments appear to harbor a spectrum of microbially mediated N transformations, with rates lower than those found in most sedimentary systems, yet still generally higher than those observed in overlying oligotrophic waters. Thus, while the influence of both sediment hosted N fixation and competitive branching during NH$_4^+$ consumption may not be mutually exclusive, our analysis places important upper limits on the nature of autotrophic lifestyles (including N fixation) and the nitrogen economy in the deep subsurface.

4.2.3 Model predicted values of the $\delta^{18}O$ of nitrification ($\delta^{18}O_{\text{NTR}}$)
We also observed variation in estimated values of the $\delta^{18}$O of newly produced NO$_3^-$ ($\delta^{18}$O$_{NTR}$), ranging from -2.8 to as high as +4.1‰ (at the O$_2$ minimum in 3D), which may offer some insight into the nature of nitrification in these sediments and the deep ocean in general. The oxygen isotope composition of newly produced NO$_3^-$ reflects the combination of several complex factors including 1) the $\delta^{18}$O of the ambient water and dissolved O$_2$, 2) kinetic isotope effects during the enzymatically catalyzed incorporation of O atoms during oxidation of NH$_4^+$ and NO$_2^-$, as well as 3) the potential influence of oxygen isotope equilibration between water and NO$_2^-$ (both abiotic and/or that catalyzed by activity of microbial nitrifying bacteria) (Casciotti et al., 2010).

In the upper profile of site 2B and throughout the profile of site 4A predicted values of $\delta^{18}$O$_{NTR}$ clustered between -2.8 and 0.0‰ with no clear trends related to down core concentrations of O$_2$ or NO$_3^-$ (Figure 6). Although slightly lower, this range of values agrees remarkably well with values predicted by experiments using a co-culture of NH$_4^+$ and NO$_2^-$ oxidizing bacteria, which ranged from -1.5 to +1.3‰ (Buchwald et al., 2012). In systems where NH$_4^+$ and NO$_2^-$ oxidizing bacteria co-exist and are not substrate-limited, NO$_2^-$ does not generally accumulate and the importance of oxygen isotope equilibration between NO$_2^-$ and water can be considered minor (~3%) (Buchwald et al., 2012). In this case, the $\delta^{18}$O$_{NTR}$ is primarily set by the $\delta^{18}$O of water (seawater $\delta^{18}$O ~0‰) and dissolved O$_2$ (~+26.4‰ for the deep N. Atlantic; Kroopnick et al., 1972) and the three kinetic isotope effects during the sequential oxidation of NH$_4^+$ to NO$_3^-$ (Buchwald et al., 2012;Casciotti et al., 2010). The resulting $\delta^{18}$O$_{NTR}$ can be described as:

$$\delta^{18}O_{NTR} = \frac{1}{3}\left[\delta^{18}O_{O2} - (^{18}e_{O2})\right] + \frac{1}{3}\left[\delta^{18}O_{water} - (^{18}e_{H2O,1})\right] + \frac{1}{3}\left[\delta^{18}O_{water} - (^{18}e_{H2O,2})\right]$$  \[11\]

where $^{18}e$ is the kinetic isotope effect of O atom incorporation from O$_2$ during NH$_4^+$ oxidation to NH$_2$OH ($^{18}e_{O2}$), and from water during NH$_2$OH oxidation to NO$_2^-$ ($^{18}e_{H2O,1}$) and NO$_3^-$ oxidation to NO$_3^-$ ($^{18}e_{H2O,2}$) (Buchwald et al., 2012).

While the value of seawater $\delta^{18}$O can be considered to be relatively constant (~0‰) in North Pond porewaters, the respiratory consumption of O$_2$, as evident in the observed concentration profiles, imparts a relatively strong isotopic fractionation (Bender, 2010).
Using a separate reaction-diffusion model (not shown) we estimated the $\delta^{18}\text{O}_{\text{O}_2}$ to be as high as +70‰ where concentrations of O$_2$ have been drawn down $>$95% of the level found in bottom seawater. Incorporation of this highly $^{18}\text{O}$-enriched O$_2$ by nitrification in these low O$_2$ intervals may contribute to observed increases in $\delta^{18}\text{O}_{\text{NTR}}$ predicted by our model. In particular, in the low O$_2$ intervals of 2B and 3D, $\delta^{18}\text{O}_{\text{NTR}}$ values as high as +4.1‰ at the four intervals coinciding with the maximum O$_2$ drawdown (Figure 6), and thus may point to incorporation of high-$\delta^{18}\text{O}$ O$_2$. For example, assuming a $\delta^{18}\text{O}$ value of 0‰ for seawater, using Eq. 11 and a combined isotope effect of 18‰ for the two steps of NH$_4^+$ oxidation to NO$_2^-$ ($^{18}\text{O}_{\text{O}_2} + ^{18}\text{H}_2\text{O},1$; the two have not yet been resolved from one another; (Casciotti et al., 2010)) and a value of 15‰ for $^{15}\text{H}_2\text{O},2$ (O atom incorporation during NO$_2^-$ oxidation to NO$_3^-$, (Buchwald et al., 2012)), $\delta^{18}\text{O}_{\text{NTR}}$ values of -2‰, +2‰ or +6‰ would imply incorporation of O from an O$_2$ pool with a value of $\sim$+45‰, +57‰ or +69‰, respectively.

If these higher values are indeed the result of enriched O$_2$ incorporation, then they also provide indirect information on the degree of oxygen isotope equilibration occurring between NO$_2^-$ and water. Specifically, if some proportion of an elevated $\delta^{18}\text{O}_{\text{O}_2}$ signal is propagated into the NO$_3^-$ pool, then this suggests that the intermediate NO$_2^-$ pool did not completely equilibrate with ambient water (which would effectively erase all signs of precursor molecule $\delta^{18}\text{O}$). Within these low O$_2$ transitional intervals in 2B and 3D, it appears that the turnover of the very small NO$_2^-$ intermediate pool may be faster than the time required for complete equilibration between NO$_2^-$ and water (Buchwald and Casciotti, 2013). In contrast, the low O$_2$ interval from site 4A does not exhibit elevated $\delta^{18}\text{O}_{\text{NTR}}$ values near the oxygen minimum, perhaps suggesting that the turnover of NO$_2^-$ here is slower (allowing complete equilibration) or that equilibration is biologically catalyzed (e.g., enhanced by enzymatic activity (Buchwald et al., 2012)). Although the concentrations of the NO$_2^-$ pool were generally below detection, making the accurate determination of its turnover time impossible (via $\delta^{18}\text{O}$), the use of NO$_2^-$ oxygen isotopes as an independent measure of metabolic processes where concentrations persist at measurable levels may be a potentially powerful indicator of biological turnover of NO$_2^-$ ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO$_2^-$ in 2B, where NO$_2^-$ was detected at two depths, were not determined as part of this study). Future
studies should target this pool as a complementary dimension for constraining subsurface biosphere metabolic rates.

4.2.4 Model sensitivity to prescribed $^{15} \varepsilon_{DNF}$ in transitional intervals

In the transitional intervals where both nitrification and denitrification are allowed to co-occur, the model is underdetermined and requires some variables to be prescribed. We chose to prescribe a value for the kinetic isotope effect of denitrification ($^{15} \varepsilon_{DNF}$) and here examine the sensitivity of estimated rates nitrification and denitrification, as well as predicted values of $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$. Given the rather tightly confined range of determined values for $^{15} \varepsilon_{DNF}$ in the anoxic zone of site 2B, averaging 20 ± 1.8‰, for illustration, we bracket our prescribed $^{15} \varepsilon_{DNF}$ in transitional intervals with values of 15 and 25‰ (rate estimates where the prescribed $^{15} \varepsilon_{DNF}$ is as low as 5‰ are given in Table 1). Overall, the model-predicted rates of nitrification and denitrification, as well as values of $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$ were largely insensitive to changes in the prescribed strength of the isotope effect for denitrification ($^{15} \varepsilon_{DNF}$) (Figure 4).

Specifically, when the prescribed value of $^{15} \varepsilon_{DNF}$ decreased from 25‰ to 15‰, changes in the predicted values of $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$ were generally small (Figures 5 and 6), varying by a maximum of 0.9‰ (average 0.5‰) and 2.3‰ (difference 0.6‰), respectively. An exception to this are the intervals bracketing the anoxic zone of the profile at site 2B (at depths of 27.9m and 70.8, 72.9m), which yielded predicted $\delta^{15}N_{NTR}$ that were either 2.1‰ lower (at 27.9m) or ~5‰ higher (at 70.8m and 72.9m). Predicted $\delta^{18}O_{NTR}$ values were also quite sensitive to $^{15} \varepsilon_{DNF}$ in this interval with values that were 3.8‰ (at 27.9m) and 7.2-8.0‰ higher (at 70.8m and 72.9m). While we cannot rule out the potential influence of changes in physiological expression of isotope effects, the sensitivity of $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$ to $^{15} \varepsilon_{DNF}$ at these depths may point to an unresolvable artifact of this model approach. Further work being indicated, incorporation of dual nitrite isotopes could certainly aid in resolving this apparent sensitivity. However, this sensitivity was not observed in the other transitional intervals of 2B, 3D or 4A and conclusions regarding $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$ still appear robust.

Finally, although literature values of $^{15} \varepsilon_{DNF}$ almost uniformly fall between values of 13 and 30‰, values of $^{15} \varepsilon_{DNF}$ as low as 2-5‰ have been observed occasionally in culture studies
(Granger et al., 2008; Wada et al., 1975). While we have no direct evidence that such low values would be relevant in our study, we report the sensitivity of rate estimates and $\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$ (Table 1). In short, a prescribed value for $^{15}\varepsilon_{DNF}$ of 5‰ leads to increased estimates of $\delta^{14}N_{NTR}$ and $\delta^{18}O_{NTR}$ by an average of 2.3‰ and 1.4‰, respectively (Figures 5 and 6). These higher estimates of $\delta^{14}N_{NTR}$ would implicitly require a lower contribution of N-fixation derived nitrogen as argued for above, though not eliminate its role completely, especially in 4A where $\delta^{13}N_{NTR}$ values remain between -1 to +1‰ (Figure 5). While rates of nitrification were less sensitive (somewhat higher in the upper layers of 4A), this very low prescribed value of $^{15}\varepsilon_{DNF}$ often lead to dramatically increased estimates of denitrification rates – in particular in the upper transitional layers of profiles at 2B and 3D where ~10-20 fold higher maximum denitrification rates are required to reconcile nitrate concentration and isotope data (Table 1).

### 4.2.5 Model-predicted values of $^{18}\varepsilon$:$^{15}\varepsilon_{DNF}$ and $^{15}\varepsilon_{DNF}$

In the anoxic intervals, estimated values of $^{18}\varepsilon$:$^{15}\varepsilon_{DNF}$ ranged from 0.83 to 1.11 with an average value of 0.99±0.1 (Table 2), consistent with a prominent role of respiratory nitrate reductase (Nar), which imparts a $^{18}\varepsilon$:$^{15}\varepsilon_{DNF}$ of -0.96±0.01 (Granger et al., 2008). Notably, however, the lower values of 0.86 and 0.83 observed near the top and the core of the anoxic zone in site 2B could suggest influence of nitrate reduction by periplasmic nitrate reductase (NAP) (Granger et al., 2008) and chemolithotrophic NO$_3^-$ reduction (Frey et al., 2014; Wenk et al., 2014), which has been shown to impart a lower $^{18}\varepsilon$:$^{15}\varepsilon_{DNF}$ closer to 0.6. In this particular interval, this could suggest that as much as 43% of nitrate reduction is chemolithotrophic and perhaps metabolically linked to the oxidation of inorganic substrates such as reduced iron or sulfur species. Although outside the scope of this study, interrogation of genetic markers of respiratory and periplasmic nitrate reductase could shed more light on the role nitrate use by subsurface microbial communities.

As discussed above, the model-estimated values of $^{15}\varepsilon_{DNF}$ (averaging 20.0‰ ± 1.8‰; Table 2) at site 2B are quite consistent with values from a wide range of studies (Granger et al., 2008), and references therein). Notably however, a different pattern emerges from the two anoxic intervals of site 4A. Although model-estimated values of $^{15}\varepsilon_{DNF}$ were unresolvable at 38.8m (likely because the changes in $\delta^{15}N$ and $\delta^{18}O$ were too small for reliable model fits), estimated
$^{15}\text{ENF}$ values at 44.1m were –8.1 ± 0.4‰, much lower than observed in 2B. In general, the values observed in 2B are consistent with observations from other environments hosting denitrification (Granger et al., 2008), and suggest that denitrifying organisms may be adapted to low levels of carbon (here <0.2% sediment organic carbon) and that their physiological poise may be similar to those found in other anaerobic environments (albeit adapted to grow at exceedingly slow nitrate reduction rates). However, the lower estimated $^{15}\text{ENF}$ values in 4A might also reflect something else. Given the apparent low reactivity of the sediments of site 4A, it is also possible that these particularly low $^{15}\text{ENF}$ values stem from denitrification operating under extreme physiological energy limitation – as discussed below.

While a number of studies have shown that the apparent N isotopic effect for nitrate reduction by denitrification can vary from 5 to 30‰ (e.g., Barford et al., 1999; Delwiche and Steyn, 1970; Granger et al., 2008), recent evidence suggests these variations are largely regulated by changes in the combination of cellular uptake and efflux of NO$_3^-$ leading to the expression (or repression) of the enzyme level isotope effect outside the cell (Granger et al., 2008; Kritee et al., 2012; Needoba et al., 2004). For example, at low extracellular NO$_3^-$ concentrations – low $^{15}\text{ENF}$ values suggest that nitrate transport (having a low $^{15}\epsilon$) becomes the rate-limiting step (Granger et al., 2008; Lehmann et al., 2007; Shearer et al., 1991). In North Pond porewaters, however, at depths where O$_2$ is low enough for denitrification to occur, NO$_3^-$ concentrations remain well above 30µM, a threshold well above the $K_m$ for NO$_3^-$ transporters (2-18µM; Parsonage et al., 1985; Murray et al., 1989; Zumft, 1997), suggesting that low $^{15}\text{ENF}$ due to transport limitation of NO$_3^-$ reduction is unlikely.

In general, greater expression of the intrinsic enzymatic isotope effect (e.g., higher observed $^{15}\text{ENF}$) should occur under conditions in which there is a higher efflux of intracellular NO$_3^-$ relative to NO$_3^-$ uptake (Kritee et al., 2012). Interestingly, this efflux/uptake ratio appears to be linked to nitrate reduction rates in denitrifying bacteria, with lower cell-specific nitrate reduction rates leading to lower efflux/uptake ratios and lower observed cellular level $^{15}\text{ENF}$ (Kritee et al., 2012). Indeed, evidence seems to indicate that this efflux/uptake ratio in denitrifying bacteria is highly regulated and that NO$_3^-$ uptake is sensitive to cellular level energy supply. For example, under conditions in which organisms are required to maintain a careful
regulation of energetically costly metabolic processes, it is logical that there would be a lower
density of NO$_3^-$ transporters and that intracellular NO$_3^-$ concentrations would be maintained at or
near optimal levels for reduction by nitrate reductase. Similarly, growth under energy-poor
carbon substrate supply may also lead to lower observed $^{15}$ε$_{\text{DNF}}$, due to an energy-driven decrease
in NO$_3^-$ uptake, lower intracellular NO$_3^-$ concentrations and a lower efflux/uptake ratio.

We suggest that the difference between the lower $^{15}$ε$_{\text{DNF}}$ value estimated from the anoxic
interval of 4A and the more ‘conventional’ values from deeper within anoxic intervals of 2B
could stem from physiological-level controls on the cellular level expression of $^{15}$ε$_{\text{DNF}}$.
Specifically, as all porewater evidence from site 4A (O$_2$, NO$_3^-$, N and O isotopes) indicates
substantially lower levels of microbial activity, denitrification may actually be more energy-
limited by carbon (compared to denitrification in the deeper intervals of 2B). This suggests that
the operation of denitrification under extremely carbon-poor environments (4A) may lead to
conditions where the enzyme-level N isotope fractionation of denitrification is under-expressed
on both the cellular, and ecosystem levels, and $^{15}$ε$_{\text{DNF}}$ values are much lower than commonly
encountered under even just slightly more energy-replete conditions (e.g., 2B).

5. SUMMARY

In summary, the porewater nitrate isotopic composition reflects the active redox cycling
of nitrogen by the subsurface microbial community – including both oxidative and reductive
transformations. The variations in reaction rates across and within the three North Pond sites are
generally consistent with the distribution of dissolved oxygen, but not necessarily with the
canonical view of how redox thresholds act to spatially separate nitrate regeneration from
dissimilatory consumption (e.g., denitrification). The incorporation of nitrate dual isotopes into
an inverse reaction-diffusion model provides evidence for extensive zones of overlap where O$_2$
and NO$_3^-$ respiration (nitrification and denitrification) co-occur. The isotope modeling also
yielded estimates for the $^\delta^{15}$N and $^\delta^{18}$O of newly produced nitrate ($^\delta^{15}$N$_{\text{NTR}}$ and $^\delta^{18}$O$_{\text{NTR}}$), as well
as the isotope effect for denitrification ($^{15}$ε$_{\text{DNF}}$), parameters with high relevance to global ocean
models of N cycling (Sigman et al., 2009). Estimated values of $^\delta^{15}$N$_{\text{NTR}}$ were generally lower
than previously reported $^\delta^{15}$N values for sinking PON in this region, suggesting the potential
influence of sedimentary N-fixation and remineralization/oxidation of the newly fixed organic N.
Model estimated values of $\delta^{18}$O$_{NTR}$ generally ranged between -2.8 and 0.0‰, consistent with lab studies of nitrifying bacteria cultures. Notably, however, some $\delta^{18}$O$_{NTR}$ values were elevated, suggesting incorporation of $^{18}$O-enriched dissolved oxygen during the nitrification process, and implying relatively rapid rates of nitrite turnover in environments supporting nitrification. In contrast, the accumulation of NO$_2^-$ under denitrifying conditions likely reflects limitation of NO$_2^-$ reduction by organic matter availability and generally low rates of N-based heterotrophic respiration. Importantly, our findings indicate that the production of organic matter by in situ autotrophy (e.g., nitrification and nitrogen fixation) must supply a substantial fraction of the biomass and organic substrate for heterotrophy in these sediments, supplementing the small organic matter pool derived from the overlying euphotic zone. Thus, despite exceedingly low exogenous organic matter input, this work sheds new light on an active nitrogen cycle in the deep sedimentary biosphere underlying half of the global ocean.
FIGURE CAPTIONS

Figure 1. Map of North Pond study site (created using the default Multi-Resolution Topography Synthesis base map in GeoMapApp ver. 3.5.1). Color scale reflects water depth in meters with contour intervals of 100m.

Figure 2. Depth profiles from IODP sites U1382B, U1383D and U1384A at North Pond of porewater concentrations of O₂ (from Orcutt et al., 2013) and NO₃⁻ as well as the N and O isotopic composition of NO₃⁻ (δ¹⁵N_{NO₃} and δ¹⁸O_{NO₃}). The red circle at the top of the profiles denotes the bottom seawater NO₃⁻ concentration of 21.6µM (Ziebis et al., 2012). Horizontal black lines indicate depth of contact with ocean crust. Gray boxes indicate ‘transitional’ zones in which a reaction-diffusion model is used to calculate co-occurring nitrification and denitrification (see text for details). Strong increases in δ¹⁵N and δ¹⁸O in U1382B coincide with depths having the lowest O₂ concentration and are indicative of the influence of denitrification. While the NO₃⁻ concentrations profiles of U1383D and U1384A appear similar, stark differences in the nitrate dual isotopic composition reflect the generally low level of microbial activity in U1384A.

Figure 3. Dual isotope plot illustrating the relationship between δ¹⁵N_{NO₃} and δ¹⁸O_{NO₃} in North Pond porewaters. The diagonal line, rooted at a value for bottom seawater (δ¹⁵N of +5.5‰ and δ¹⁸O of +1.8‰), depicts a 1:1 slope representative of the expected change in δ¹⁵N and δ¹⁸O by the process of denitrification alone. Trends falling well above this 1:1 line, together with the concentration profiles reflect the combined role of nitrification in these porewaters.

Figure 4. Model estimated rates of denitrification and nitrification (nmol cm⁻³ yr⁻¹) based on fitting of nitrate concentration and N and O isotopic composition. Estimates within transitional intervals are calculated using a value for ¹⁵ε_{DNF} of either 15‰ (dashed) or 25‰ (solid). Error bars are shown for the ¹⁵ε_{DNF} = 25‰ only and indicate the standard error of 10 model runs (see text). Note the different scales for rates among the three profiles.

Figure 5. Model estimated values for the N isotopic composition of new nitrate produced by nitrification (δ¹⁵N_{NTR}) occurring within porewaters. Values are calculated for depths at which O₂ concentrations were >2µM (e.g., oxic and transitional intervals). Sensitivity of δ¹⁵N_{NTR} to
prescribed values of the isotope effect for denitrification ($^{15}$ε$_{DNF}$) in transitional intervals, where both nitrification and denitrification can co-occur, is also indicated.

Figure 6. Model estimated values for the O isotopic composition of new nitrate produced by nitrification ($δ^{18}$O$_{NTR}$) occurring within porewaters. Values are calculated for depths at which O$_2$ concentrations were >2µM (e.g., oxic and transitional intervals). Sensitivity of $δ^{18}$O$_{NTR}$ to prescribed values of the isotope effect for denitrification ($^{15}$ε$_{DNF}$) in transitional intervals, where both nitrification and denitrification can co-occur, is also indicated.
AUTHOR CONTRIBUTIONS

SW, WZ, CW, and ML conceived of the study. SW and WZ procured funding to carry out the presented work. CW and WZ participated in the IODP Expedition 336 as shore-based scientists, received and analyzed samples. SW collected samples from frozen archives. SW analyzed the samples, developed the model, and interpreted the model results with assistance from CB and ML. SW wrote the paper with input from CB, WZ, CW and ML. All authors discussed the paper and commented on the final manuscript.
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