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# *Interactive comment on* "The fate of fixed nitrogen in oligotrophic marine sediments: an in situ study" *by* Stefano Bonaglia et al.

**Stefano Bonaglia et al.**

stefano.bonaglia@gmail.com

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Here we present our answers (marked AC) below the original referees' comments (RC).

Anonymous Referee #1

RC - General Comments The authors present a study in which they quantified the fate of fixed nitrogen in sediments of a cold, oligotrophic system. The authors used 15N tracers and a combination of in situ incubations using a benthic lander and ex situ sediment core and slurry incubations. The authors are the first to simultaneously measure rates of denitrification, anammox, and DNRA in oligotrophic sediments. They accomplish this using in situ lander incubations, which are logistically difficult to perform, but may actually provide more accurate estimates of in situ rates than traditional core or slurry incubations. The authors found that denitrification dominated N2 production, but



anammox bacteria were also active, accounting for 18-26% of N2 production. The authors also measured detectable DNRA and found that DNRA rates were highest, and comparable to denitrification rates, at the shallow coastal station. A sediment nitrogen budget was constructed and indicated that, despite the N2 production measured at the stations, the primary fate of sediment organic nitrogen in the summer is recycling and efflux as TDN back into the overlying water. Lastly, this study compared concentrations of ladderane lipids, a biomarker for anammox bacteria, to anammox rates and found no correlation between the two. These datasets are sparse in the literature, so this is an informative contribution to the scientific community studying anammox. Overall, I think the authors addressed important questions related to sediment nitrogen cycling that will be of interest to many readers of this journal. The paper is very well written and organized clearly. I am comfortable with the conclusions and support publication of this manuscript with minor edits, as detailed below.

AC - Thank you for the very detailed and insightful analysis of our manuscript and for the positive reception of our work. We really appreciate the efforts and the time the referee invested in improving the manuscript.

RC - Specific Comments p.1, line 12 insert "the" before "global" p.2, line 5 delete "to" before "âĹij45%" p.2, line 8 define the abbreviation "DNRA" the first time it's used in the text body p.2, line 13 insert "the" before "electron" p.2, lines 23-24 It would be helpful if you mention briefly the link between Mn and anammox, since it is related to your hypotheses and your interpretation of your results. p.2, line 28 define the abbreviation "GOB" the first time it's used p.3, line 1 I suggest replacing "happen" with "occur"

AC – We will be glad to consider these specific comments in the revised manuscript.

RC - p. 3, line 7 suggested change: ". . ..we hypothesize that we will measure low benthic N cycling rates. . ."

AC – In our opinion it does not sound correct to use the future tense in this context. We therefore propose the following compromise, at the infinitive form: "...we hypothesize

to measure. . .". We will then change all the verbs of this last paragraph to the infinitive form or present tense for consistency.

RC - p.3, line 9 change to "porewater," (one word) to be consistent with the rest of the text p.4, lines 27-28 It would be helpful here if you could define what the average (or range of) water height(s) above the sediment surface was for the lander incubations. No need to list it for every incubation, just give the reader an idea of how much water volume was involved in these incubations. p.6, line 28 Is the 75uM concentration for the sum of 15NH4+ + 14NO3- or for each of the N species?

AC – We will address these minor edits in the revised manuscript.

RC - p.7, lines 18-20 For clarity, I suggest you present the r-IPT equations from Risgaard-Petersen et al. (2003) so that readers who are unfamiliar with them can understand how you get from p29N2 and p30N2 and ra to p14. This will also give you a chance to define p14 explicitly, and describe how it represents N2 produced without the 15N addition, i.e., actual N2 production. Many unfamiliar with IPT think that the added 15NO3- will stimulate denitrification and that those rates are included in your results, when in actuality the IPT approach allows one to separate p14 (actual) from total N2 production from 15N and 14N (potential).

AC - The r-IPT equations from Risgaard-Petersen et al. (2003) will be added to the text in order to explain how, for example, p14lan was calculated. For conciseness, however, we won't present the calculations also for p14wc and p14sl as the reader will have sufficient information to understand that they were calculated in the same fashion as for p14lan but from sediment core incubations (water phase and slurry phase, respectively).

RC - Eqn. 2 Somewhere here in the text describing eqn. 2 you should state clearly that p14sl includes both water and sediment p14.

AC - We will add in the text above Eq. 2 that slurried phase means water plus sediment.



RC - p. 7, lines 25-26 I understand why you have to use the same Fwc measured in 2014 for the 2013 calculationsâA ËŸTyou don't have the sediment core incubations from 2013. ËĞI'm just not convinced that the Fwc values would be consistent from 2013 to 2014. Your rates (denitrification, anammox, O2, TDN, etc.) as well as OPD show year-to-year variability, so it would not be surprising to me if the Fwc values were variable. Perhaps here (or elsewhere) you could defend this assumption in a bit more detail and discuss the potential implications for your calculated rates?ÂÍ

AC - We acknowledge the referee for this adequate comment. We cannot indeed exclude that Fwc in 2013 could have been slightly lower than those we measured in 2014 because the oxygen penetration depths were higher in 2013 than in 2014 (Table 1), suggesting that N2 production happened deeper in the sediment. A low underestimation of in situ N2 production in 2013 cannot be excluded. We will add this argument in the Results when we present our measured Fwc values.

RC - p.8, line 1 Since you use the term "ra" here, and it's a widely used term to describe the contribution of anammox to total N2 production, I suggest you use it throughout the rest of the text and tables/figures.

AC – We will make this edit throughout the text and in Fig. 6.

RC - p.8, lines 15-16 I have read this section multiple times, and I still am unsure what this sentence means. I think you're saying that you have to use the Fwc calculated from the p14 values for this NH4+ calculation. If p15NH4+ was not detected in just one of the incubations (GOB1-3), why couldn't you use the p15NH4+ fluxes from all of the other incubations? At least they're still related to the parameter you're working with (NH4+). How will using the Fwc derived from the p14 values affect the calculated NH4+ rates?

AC - As we stated it in the Results, DNRA could not be determined in sediment core incubations because p15NH4+ was not detectable in our time courses at three (GOB1, GOB2, GOB3) out of four stations. In sediment cores incubations, p15NH4+ could

only be detected at station RA2. Using the Fwc from p15NH4+ at RA2 for all the other stations would result in much higher, and probably unrealistic, DNRA rates than those we estimated now by using Fwc from whole core's p14. However, we agree with the referee that using Fwc from p14 may not be 100% representative of the in situ, actual rate. Thus, in the Discussion, we will further highlight the fact that our up-scaled DNRA rate may represent an underestimation of the actual DNRA rates.

RC - p. 10, line 13 Insert "from" after "ranged" p. 10, line 24 The sentence "Between the four stations. . ..>GOB3." reads awkwardly. I suggest changing to "Downcore NH4+ concentrations were greatest in RA2, followed by GOB2 >= GOB1>GOB3." p.11, line 5 Replace "GOB3" with "GOB2" p. 11, lines 13-15 The sentence "The facts that . . .supported by DON." is awkwardly worded, making it difficult to understand its meaning. p.11, lines 17-18 reword to ". . .with Fwc values of 0.26, 0.23. . ." p.11, line 22 At the end of this paragraph, I suggest you present the ra values from the slurry incubations (also include in Table 2), since that's really the main point of doing the slurries. It's fine to keep the data in Figure 6 since it's relevant to the discussion of the other NRPs. But I think the data should be first introduced here to make it clear where that data come from. p.12, lines 19-22 The sentence "The 15N isotope pairing technique. . .is low.." is awkwardly worded, making it difficult to follow. p. 13, line 7 Delete "for" before "potential" p. 13, line 18 Replace "Alike" with "Like"

AC – We will gladly address these minor edits in the revised manuscript.

RC - p.13, line 32 Why did you pool all of the data from 0-4cm for the ladderane concentrations to compare to the rate values? The values are highly variable from surface to 4cm. Did you try just using data from anoxic sediments, where anammox may have been occurring? Or depths where NO2- and NH4+ were present? I wonder if you would have seen a better correlation between the ladderane concentrations and the rates. It would be helpful to include some discussion of this.

AC - We pooled the ladderane data from 0-4 cm because this is the approach that is



often used in literature. However, we agree with the referee that this is not the best approach when values are highly variable, as in our situation. We have now correlated the potential anammox rates with (1) the average ladderane abundances and (2) with the ladderane abundances in the anoxic sediments layer (1.5-3.5 cm), which coincides with the layer sampled for anoxic slurry experiments for anammox potential. Yet, we cannot see any significant correlations. We will add this part to the Results. In the Discussion we will explain that these non-significant correlations might be due to the low number of observations.

RC - p. 14, lines 17-18 You mention here that H2S was never detected in the sediment porewater, but you do not present that data anywhere. I suggest you mention it briefly in the results section since you took the time to describe the microsensor method. p. 15, line 5 Replace "process" with "proceeds" p. 15, line 7 Replace "being" with "at" p. 15, line 13 Delete "eventually" p. 15, line 16 Replace "upscale" with "scale up" p. 15, lines 15-17 I'm unsure what conservative method you are referring to. It would be helpful to explain briefly here since it's important enough to bring up in your discussion. p. 16, line 3 Reword to "The removal rate and the recycling rate were constrained by. . ." p. 16, line 10 Replace "prove" with "suggest" p. 16, line 14 Replace "basin-wise" with "basin-wide" p. 16, lines 17-19 You briefly mention the contribution of DNRA to the TDN flux here, but I think it would be helpful to present the data in Fig. 7 so that the reader can get a feel of interstataion variability. Figure 2 Make sure to note which symbols are N vs. C (black vs. white). Figure 6 (c) The y-axis labeled "AAO contribution" should be changed to "ra", as discussed above. Also, the caption for panel (a) should replace "Shaded" with "Hatched" so as not to be confused with the gray shaded bars (2014). Figure 7 In the caption, replace "nitrogen cycling" with "TDN efflux" since that's more accurate.

AC – We will consider these specific comments in the revised manuscript. Your input was very much appreciated.