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Biogeosciences Discussions

Interactive comment on "Nitrogen and oxygen availabilities control water column nitrous oxide production during seasonal anoxia in the Chesapeake Bay" *by* Qixing Ji et al.

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Summary The authors present an examination of N2O dynamics studied in the Chesapeake Bay during three samplings. Since the Chesapeake Bay exhibits a strong seasonal shift in water column redox state, the study focused on trying to link these shifts with N2O production mechanisms at and below the oxic/anoxic interface. The authors bring a range of chemical, molecular and isotopic tools to bear on these dynamics, with an emphasis on elucidating N2O producing processes occurring in the bottom waters and the primary controls on them. This contribution is timely – as coastal and estuarine systems are dynamic and generally understudied with respect to their place in

the global N2O budget. Overall the data appear to be of high quality. The manuscript is generally well written, though parts could benefit from some reorganization. I have some questions about the data interpretation as outlined below. Overall I think this work is worthy of publication, but that the manuscript could be improved through some more careful consideration of clarifying some sections.

Major Comments [Referee] Pg 9 Ln 15: I appreciate the use of targeted assays for NO2- and NO3- reduction, though it is unclear whether this was designed to constrain/target nitrifier denitrification specifically or explicit nitrite reducing denitrifiers (???). Clearly denitrifying organisms also use NO2- in their electron transport chain. In Section 3.3 the authors attempt to tackle this – and I appreciate the argument that they are making about NO2- transport across the membrane – but I feel that this section is confusing as written. Using calculations laid out here, and making a few key assumptions, the authors conclude that since the level of 15N label in the N2O pool is much higher than if there had been full exchange, then exchange between the cellular and ambient NO2- is minimal. I acknowledge that this is a difficult aspect of N cycling to track, but I am not overly convinced that they have proven that this type of exchange is 'minimal.' Their calculation demonstrates that high levels of exchange are not occurring, but whether modest levels might be influencing the results is unclear. Perhaps this argument could be streamlined and clarified.

[Response] The reviewer acknowledges the difficulty of our attempt to estimate intracellular nitrite exchange; we appreciate it. The hypothesis is: nitrite is fully (100%) exchanged during nitrate reduction to N2O. And the calculation result shows that 15N-fraction labeled of N2O from the calculation does not match our measurements. Therefore, we reject the hypothesis. Yes, it is possible that some level of exchange might occur, but they would be undetectable by this argument. We think it is impossible, and beyond the scope of this paper, to quantify the actual percentage of nitrite using the data presented here. More elaborate experiments can be conducted in the future to tackle this question.

[Referee] Additionally, perhaps the introduction needs some sort of clearer description of the types of metabolisms being targeted by the study (complete denitrifiers, nitrite denitrifiers, nitrifier denitrification). These classifications of microbes and processes are confusing even to those who regularly study N cycling.

[Response] We focus only the reductive pathways of N2O production under anoxic conditions. Given the limitation of 15N tracer study, only the denitrification pathways "NO2- → N2O" and "NO3- → N2O" can be quantified. It is therefore very difficult to attribute the pathway to certain groups of microbes. To minimize confusion, we generalize the term "N2O production during denitrification" throughout the text. The current data set does not support in-depth discussion of functional microbial groups responsible for N2O production because we cannot differentiate among the different kinds of microbes that can perform nitrite reduction to nitrous oxide using tracer experiments.

Minor Comments [Referee] Pg 1 Ln15: I believe nitrate and nitrite are reversed here (and many other times throughout – leading to some frustration/confusion).

[Response] We did not follow the reviewer's suggestion for this sentence. The experimental data shows that, higher nitrate or nitrite availability positively correlates with N2O production rates from respective substrates. The sentence itself is correct.

[Referee] Pg 1 Ln17: Since the field data demonstrate that there is no net flux to the atmosphere– it seems odd to emphasize N2O efflux here.

[Response] To minimize confusion, we changed the word "efflux" to "production"

[Referee] Pg 3 Ln 19: Please clarify whether a headspace was left in the incubation bottle or not.

[Response] The headspace (3 mL) was left throughout the incubation.

[Referee] Pg 4 Ln 1: course not courses

[Response] Thanks for catching that error – it was in line 21.

[Referee] Pg 5 Ln 3: Please indicate whether oxygen concentrations were measured or calculated?

[Response] The concentrations were calculated, as stated later in the sentence.

[Referee] Pg 6 Ln 1: Why were no other functional gene assays performed? I think this is justified later, but given that nirS only reflects denitrification – its linkage with N2O from this pathway is clear, yet reveals little about the dynamics of the other pathways investigated as I understand. Why not include nirK? Or norB?

[Response] Good point. The nirS gene encodes the genetic material for nitrite reductase, the enzyme responsible for nitrite reduction to nitric oxide. NirS is often used as a proxy for the abundance and diversity of denitrifying bacteria (which was our application here) and is the gene in the denitrification sequence that is most reliably associated with a complete denitrification pathway (Graf et al. 2014).

[Referee] Pg 7 Ln 19: I believe nitrate and nitrite are reversed here again.

[Response] Corrected.

[Referee] Pg 7 Ln 25: "positively correlates" – yes, but this is difficult to defend statistically with n=3.

[Response] The sentence is changed to ". . .nirS abundance increases with increasing measured rates of N2O production."

[Referee] Pg 13 Ln 2: I would suggest "microbial groups" instead of microbial communities (which may imply the 'greater community' – not just N cycling organisms ?).

[Response] The word "groups" has replaced "commnuities".

[Referee] Pg 14 Ln 5: It seems that if nitrifier denitrification and ammonia oxidation are implicated in N2O production as discussed – then the nitrifier community dynamics would also play an important role and should be acknowledged?

[Response] Indeed. The reviewer points out one of the future research direction, that is to examine the link between nitrifying community and N2O production via nitrification. We'll add the above in the next version of the manuscript.

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