

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.

Anonymous Referee #3

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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

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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

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.

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.

Minor Comments

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

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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.

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

Pg 4 Ln 1: course not courses

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

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?

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

Pg 7 Ln 25: “positively correlates” – yes, but this is difficult to defend statistically with n=3.

Pg 13 Ln 2: I would suggest “microbial groups” instead of microbial communities (which may imply the ‘greater community’ – not just N cycling organisms ?).

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?

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