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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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General Comments: [Referee] The authors have presented a well-designed and well-executed experiment on N2O production in Chesapeake Bay. While the experiments and data are worthy of publication, the manuscript itself needs major revisions before it is accepted for publication. In reading the manuscript the introduction and methods were written clearly and concisely, but the results and discussion needs a substantial reworking. The paragraphs jumped from one topic to the next and often I found the subtitled headings were not appropriate for the range of topics covered in the text below. In the abstract the authors suggest two potential impacts of what reducing N

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to the bay will have on N2O production. I think the paper is missing a more detailed explanation of how the experiments done lead to the conclusions they make, and they should expand on the specific details of these impacts that they suggest. I also think the authors could elaborate on the limitations of their study and what could be done in the future.

[Response] The reviewer's major criticism concerns the structure of results and discussion, which we will explain later. The main objective of this work is to examine the control of water column N2O production via denitrification in the Bay. The experiments were designed to suit our objective, by increasing the availability of oxygen and dissolved inorganic nitrogen through manipulation, as well as measuring N2O production in different seasons. In doing so we concluded that increasing DIN availability and decreasing oxygen availability could stimulate denitrification during summertime anoxia. In fall and spring the N2O production rates via denitrification were low, probably due to lack of anoxic conditions and concomitant low abundance of denitrifiers.

The major limitation of our study, like other tracer incubation studies in aquatic environments, is that the N2O production rates measured here should be treated as potential rates because the experimental conditions were not 100% identical to in situ environments. Despite these artifacts, the conclusions that DIN and oxygen availabilities control N2O production during anoxic events are robust. The current work could be complemented by these future research directions, (1) measurement of nitrification rates in oxic waters and associated N2O production and nitrification genes; (2) measurement of N2O reduction rates within anoxic layer and characterization of N2O dynamics in the Chesapeake Bay; (3) Elucidation of intracellular nitrite exchange during nitrate reduction in natural waters; (4) Investigation of the physical, chemical and biological controls of N2O emission in the Chesapeake Bay. A revised "Conclusion and outlook" section will incorporate the above in the next version.

Minor Comments: Abstract: [Referee] Pg1 line15: "N2O production was positively correlated with the ratio of nitrate to nitrite concentrations." Please clarify if this was in

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situ concentrations or the nitrate and nitrite added to the incubation.

[Response] This statement describes N2O production rates under manipulated nitrate and nitrite concentrations. All of rates reported in the manuscript were measured using 15N tracers, meaning the substrate concentrations (nitrate and nitrite) were higher than in situ levels. We revised the sentence in the new version.

[Referee] Pg1 line18-19: What do you mean by nitrogen deficiency? What lengths of time are your referring to when you write “short term” and “in the long-run”, can you approximate the time scale?

[Response] The term “nitrogen deficiency” refers to nitrate and nitrite concentrations below detection. This occurs during 4 months of summer from June to September. We use “short-term” to describe a time scale of months, and “long-run” of years. We will revise the sentence in the next version to improve clarity.

Methods: [Referee] Pg4 line15: Why do you add 20 nM N2O before the incubation? If it is so you can have enough N2O to measure the N2O production, could you add it after the incubation is killed? Can you clarify here in the text? Do you think adding it before, could affect the production of N2O?

[Response] It is an issue of detection limit of mass spectrometer, which requires > 2 nmol of N. We agree that the added tracer concentration is slightly higher than in situ (6 – 12 nM). It is our intention to add the N2O before the samples were preserved so as to have a more similar condition to in situ. The effect of N2O concentration on the rate of N2O production should be investigated.

[Referee] Pg4 line17: What was the total concentration of nitrite+nitrate of tracer added?

[Response] Here is the description of DIN manipulation experiment, which was conducted in July 2016 only. The total concentrations of nitrate and nitrite were 5 μM each, and all the substrate concentrations of every experiments conducted are listed

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in table 1.

[Referee] Pg5 line8: How does 5uM of tracer compare to the in situ amounts of nitrite and nitrate.

[Response] This was stated in page 7. In July 2016, in situ nitrate and nitrite concentrations were below detection (0.02 μM), and the experimental conditions are in no way representing the in situ. Hence we treated the rates as potential rates. In November 2016, in situ nitrate and nitrite concentrations at the depth of experiment (17 m) were 5.0 and 0.4 μM, respectively.

Section 3.2 Active N2O production by denitrification [Referee] Pg8 line3: In the title you suggest all N2O is from denitrification, it would be good discuss why it is not from nitrification, if you are using that as the section title.

[Response] Indeed. We only measured N2O production via denitrification. Unfortunately we did not measure N2O production via nitrification. We will change the title to "Active N2O production in the water column" to minimize confusion.

[Referee] Pg8 line16: I think it's good you say here they are potential rates of N2O production, because you are removing all the oxygen, even if that's not the in situ value. I am curious why there are no potential rates in May. Can you add some discussion on why removing the oxygen does not stimulate N2O production in May versus November?

[Response] We explained in the following paragraph. This is likely due to low denitrifier abundance (indicated by nirS gene abundance) in May, during which the anoxic condition had not yet developed. In addition, our anoxic incubation lasted around 2 hours; it is unlikely for denitrifiers to reactivate within such a short time scale. We'll add the above information in the next version.

[Referee] Pg9 lines1-13: This section seems disjointed from the paragraph above and the title of the section. This paragraph refers more to removal of fixed nitrogen from the bay rather than N2O production.

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[Response] Indeed. We will remove this paragraph in the next version.

Section 3.3 N2O production pathways regulated by availability of nitrogen substrate

[Referee] Pg9 line16: I was confused by this term “NO2- (NO3-), is this synonymous with “NO2- or NO3- ”? If so, I would suggest changing it to the latter.

[Response] This is simply a combination of two sentences in one: “. . . increasing nitrite availability favors N2O production from nitrite reduction” and “. . . increasing nitrate availability favors N2O production from nitrate reduction.” We change the sentence to “This suggests increasing NO2- or NO3- availability favors N2O production from the reduction of respective substrate.”

[Referee] Pg10 lines1-21, pg 11 lines1-8: This could use a different subheading? Could you reformat the equations to be easier to read? Also in general this section could use a little more description, as is, it is a little hard to follow. I would start off the section with the punch line (on pg 11) and then describe why the calculations back up that statement.

[Response] We think another sub-heading is probably not necessary but we will reorganize the section in the next version. We’ll add the conclusion “the exchange between intracellular and ambient nitrite during nitrate reduction to N2O is limited” in the beginning of the section. The calculation is to find the 15N fraction label of N2O when nitrite is fully exchanged, and we think it is rather straightforward. We’ll use a different font and size to better illustrate the calculation in the next version.

Section 3.4 Oxygen inhibits N2O production by denitrification

[Referee] Title: I suggest changing the title to something more detailed.

[Response] The title states the major conclusion of this section and seems pretty specific.

[Referee] Pg11 lines11-19: These sentences seem to belong to the other section about

N influx to the bay. They could be moved or deleted during restructuring of discussion sections because it is somewhat repetitive from before.

[Response] Indeed. This paragraph will be organized in the “conclusion and outlook” section.

[Referee] Pg12 lines9-15: The result that there is a different oxygen tolerance for nitrite vs. nitrate reduction is really interesting. I think here is where you should expand on more why you get this result. I know you touch on it more in a later section, but it might be best to put it all together (maybe in it’s own section). Could it be from nitrifier denitrification? How would nitrite oxidation affect the production rate? At low levels of oxygen I would suspect there would be some nitrite oxidation to nitrate.

[Response] It is possible that nitrifier denitrification is responsible for part of N2O production via nitrite reduction. This is because increasing oxygen availability will inhibit the activity of denitrifiers, but not nitrifiers. It is not possible to have a complete explanation with the data presented here. We’ll add the above in the next version.

We did not measure nitrite oxidation rates in the samples but agree that it is likely nitrite oxidation was also occurring. We expect nitrite oxidation to have a negligible effect on N2O production rates, however, since N2O is not a product or intermediate in that reaction and expected rates of nitrite oxidation would not significantly affect the concentration of the substrates directly involved in N2O production. (Nitrite oxidations rates in nM/d would not affect the substrate concentrations, which were in the micromolar range.)

[Referee] Pg12 lines16-19: This paragraph seems out of place? Again, would you suspect some nitrite oxidation in these nitrate reduction to nitrite measurements? How would that affect results?

[Response] See above.

[Referee] Pg13 lines3-4: This line should go with the section on differences of nitrite-

stimulated production vs. nitrate-stimulated N2O production. Pg13 lines10-18: This paragraph is out of place. Pg13 lines19-22: Should this be in the conclusion and outlook?

[Response] The section 3.4 will be re-organized in the next version to improve clarity and make tighter connections between the data and conclusions.

[Referee]Section 4 Conclusion and outlook Pg14 lines14-19: I would reorder this section and put these lines first. Conclusion and outlook section as a whole: Could you add something connecting the two pathways of management (nitrogen influx vs. oxygenation)? Also, could you quantify how each change inhibits N2O production?

[Response] Indeed. The current work will be complemented by these future research directions, (1) measurement of nitrification rates in oxic waters and associated N2O production and nitrification genes; (2) measurement of N2O reduction rates within anoxic layer and characterization of N2O dynamics in the Chesapeake Bay; (3) Elucidation of intracellular nitrite exchange during nitrate reduction in natural waters; (4) Investigation of the physical, chemical and biological controls of N2O emission in the Chesapeake Bay. A revised “Conclusion and outlook” section will incorporate the above in the next version.

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