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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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[Referee] The manuscript by Qixing Ji and colleagues investigates the controls on nitrous oxide concentrations in Chesapeake Bay. It is a topic that the authors are very familiar with and this expertise is reflected in the experimental design investigating the effect of nutrients and oxygen on nitrous oxide. The datasets are useful and should be published, but I recommend restructuring the manuscript to focus on the strengths of the work and dealing with the issues raised.

Major comments [Referee] As can happen with studies which conduct repeated experiments at different time intervals with varying measurements, it is difficult at times to

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track all the activity. I believe there is a discrepancy between the Methods and Results, as Figure 2 shows N2O production rates for July, November, and May, yet in the Methods you state that the experiments were only conducted in July. Instances like this make it very difficult for the reader to follow.

[Response] The N2O production rates shown in figure 2 were measured in control incubations, which were performed on all three sampling dates. In control incubations, samples received 5 μmol L-1 15N-nitrate or 15N-nitrite, respectively; and oxygen was removed from samples by helium flushing. The DIN manipulation experiments were only conducted in July. We explained this in page 5 and revised table 1 to minimize confusion.

[Referee] I had a hard time relating the experiments with the estuarine environment. If you want to measure nitrous oxide emissions from Chesapeake Bay, the work conducted in this study is not what needs to be conducted. You would need high resolution surveys of key hydrographic parameters coupled with nitrous oxide measurements, either continuously or at targeted times. I recommend the authors focus more on the experiments as a means to better understand the controls on nitrous oxide production and use Chesapeake Bay as the contextual background, rather than attempting to explain nitrous oxide dynamics in this estuary.

[Response] We agree with the reviewer's suggestion, and this focus on control is exactly what we intended, with the title stating explicitly that this manuscript is about examination of the control of N2O production, rather than emissions, from the Chesapeake Bay. We mention briefly that water column N2O the may be emitted due to disruption of water column stratification (page 13), which is a motivation for the research and points to further research directions. We'll revise accordingly in the next version.

[Referee] Two examples of the mis-match between the datasets reported and the contextual description provided in the Introduction are (1) The abstract talks about intense

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N2O efflux from estuaries, but the results show nitrous oxide concentrations close to air-saturation values. (2) The title mentions seasonal anoxia, but this is not shown in the results.

[Response] For (1), this is a more of a “general” statement that estuaries can be sites of intense N2O efflux, and previous studies have shown that the Chesapeake Bay is a N2O source. This context motivated our research. The measurements and experiments showed that despite the fact that the water column is close to N2O saturation level, N2O production can occur. We further explain in page 11. For (2) The oxygen profiles obtained from this study (and many others) show that water column anoxia occurs in summer. In spring and autumn, the water column is oxygenated. These results demonstrate the water column anoxia is a seasonal event, as clearly shown in the two papers by Lee et al., which are cited in the manuscript. A revised abstract will be presented in the next version to minimize confusion to the readers.

[Referee] I am not sure if the May 2017 dataset is necessary in Figure 1. It is helpful in Figure 2 only.

[Response] The May dataset illustrates seasonal variation in the usual parameters, and thus supports the seasonal nature of N2O production and consumption. In May 2017, low oxygen condition was forming, along with higher availability of nitrate. These conditions are becoming favorable for denitrification in the water column.

[Referee] You need to include a description of how you calculate N2O production in the Methods section.

[Response] The rate calculation is presented in section 2.4, equation 1

[Referee] The N2O profiles puzzle me in the context of the other results. It looks like July 2016 is the only profile which has significant differences with depth, decreasing in concentration between 3 and 13 m. However, this time point is associated with the highest rates of N2O production (Figure 2). Therefore, N2O consumption is very

important yet hardly mentioned in the manuscript. In context of your comment that estuaries are emitting large quantities of N2O, the consumption processes deserve more attention.

[Response] N2O consumption is indeed important; however it was not quantified nor addressed directly in the current manuscript. The work here focuses on the controls of N2O production via denitrification. One of the future research direction is to examine the N2O reduction during anoxic events. The fact that significant N2O production was detected in the month when N2O concentration was lowest implies that N2O consumption was also occurring and probably minimizing efflux to the atmosphere.

[Referee] You should explain to the reader why you focused on the nirS gene and not other relevant genes

[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).

Minor comments [Referee] Page 1 Line 16 Change reducing to decreasing

[Response] Corrected.

[Referee] Page 2 Line 11 Agriculture such as paddy fields?

[Response] Is the reviewer referring to “agricultural land” in line 13? If so, paddy fields are not common in the Chesapeake Bay watershed. Agriculture is the main land use in this region and major agricultural activities in the area include livestock farming, greenhouse and nursery products (flowers, ornamental shrubs, and young fruit trees), corn and soybeans, all of which depend heavily on industrial fertilizers.

[Referee] Page 4 Oxidized nitrogen. I think this refers to NO3 and NO2. I recommend you write NO3 and NO2 if this is correct, as it avoids NO or NOx. Also on Page 7, Line

[Response] Corrected.

[Referee] Page 3 Line 21 It would help orientate the reader if you provide a short explanation for why you chose these 3 depths. For example, why did you only sample below the oxycline in anoxic waters and out of curiosity, why did you not compare anoxic and oxic?

[Response] As stated in the introduction, the anoxic events in the Chesapeake Bay is of great environmental and economic concerns. One of the motivations of this work is to examine the control of N2O production under anoxic condition. We sampled oxygenated condition in May and November because anoxic conditions were not detected at the depths where they would have been expected during the seasonal anoxia, thus the oxic conditions were sampled for comparison and, not surprisingly, had lower rates of denitrification. It is likely that nitrification is important during oxygenated conditions. Future work more focus more on the oxic waters.

[Referee] Page 4 Line 14 Why do you inject N2O to detect N2O production? Is it an issue of detection limit? Or were you looking for N2O consumption?

[Response] It is an issue of detection limit of mass spectrometer, which requires > 2 nmol of nitrogen.

[Referee] Page 4 Line 14 If you inject 1.2 nmol, how do you get 20 nmol L-1? Presumably you verified these target concentrations on a few bottles and you should state the final concentrations achieved.

[Response] N2O concentration in the incubation bottles was estimated as follows: The incubation bottle is 60 ml in volume. Considering only 3 ml of headspace in the bottle, 90 – 95 % will dissolve in water phase (57 ml, 0.057 L) under experimentally relevant conditions. Therefore, 1.2 nmol $\times$ 0.9 / 0.057 L $\approx$ 20.0 nmol L-1. N2O concentration was directly measured in the time course samples on the mass spec, so no assump-

tions were necessary in the actual rate calculations.

[Referee] Page 5 Line 1. Again, presumably you checked the final concentrations of oxygen against your target concentrations. I suspect you also did the air-equilibration at a single temperature which you should state.

[Response] Oxygen saturated site water was made by vigorously shaking an open-capped bottle with water collected at depth. Concentration was calculated using formula from Gordon and Garcia, 1992, at measured temperature (< 0.5 degC difference of in situ) and salinity. Oxygen concentrations were not measured directly in these experiments. We have, however, used an optical sensor to measure concentrations directly in the same kinds of bottles in similar experiments and the agreement between estimated target concentration and measured is excellent. Thus it is not necessary to measure every bottle every time.

[Referee] Page 5, Line 19 The alternative to injecting N2O standards into crimp-sealed vials is to air-equilibrate at controlled temperatures. This might be easier?

[Response] The measurement of N2O is using purge-and-trap technique, which analyze all of N2O contained in the vials by a mass spectrometer. Thus, the total amount of N2O is important, even the N2O standards may not be in equilibrium between the water and headspace.

[Referee] Page 7, Line 13 Where is the intense efflux that you mentioned in the abstract?

[Response] As explained earlier, this refers to a previous study documenting the Chesapeake Bay as a N2O source. Conditions in estuaries are highly variable, in both time and space, which is one of the motivations for investigating the control mechanisms on N2O production. Failure to detect efflux at this time does not mean that intense efflux does not occur at this site at other times, or in other parts of the Bay.

[Referee] Page 7, Line 13 If you are going to talk about saturation, you have to provide

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the saturation value for each of the three sampling occasions.

[Response] The surface N2O saturation values in July, November and May are: 6.6, 10.4 and 12.0 nmol L-1, respectively. These values have been included in the revised text.

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