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# *Interactive comment on* "Isotopomeric characterization of nitrous oxide produced by reaction of enzymes extracted from nitrifying and denitrifying bacteria" *by* T. Yamazaki et al.

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The authors present pioneering data on the isotopomeric composition of nitrous oxide generated directly from purified enzymes. To the best of my knowledge this has not yet been conducted and the data is very insightful into our interpretations of the isotopic composition of nitrous oxide generated by microbial activity. There are, however, a number of analytical considerations that prevent me from recommending publication of this manuscript in its present form. In particular these relate to generation and storage of the NO enriched solution and to the lack of control of oxygen levels during the microbial culture experiments. Overall, the data is quite compelling and well worthy



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of publication in this journal as long as these concerns can be addressed.

Specific comments: Line 22: Details of enzyme purification missing. Page 27, line 4: How do the authors explain 151% production of N2O from NO? Line 13: Greatest N2O production under highest O2 level? Not what I would have expected. Line 23: Need for improved writing I believe these were “batch” cultures which means that the O2 level likely dropped during the course of the experiments. This might explain why there was more N2O production with 21% O2; O2 may have rapidly been exhausted at the lower O2 level and declined to low levels in the culture at 21% O2. Thus this experiment is not likely an accurate representation of N2O production as a function of O2. Page 28, Lines 17 and 20. I believe you mean to indicate “preservation” and not “reservation”. Line 21. I am not certain if solubility constants for NO in ethanol are known but it is well known that cold solutions hold more gas than warm solutions. Thus the authors’ speculation that colder temperatures might have resulted in a higher NO concentration in solution than expected is reasonable. However, there would have to have been a source of NO in storage for the ethanol to obtain a new and higher equilibrium concentration. As NO is very reactive this seems unlikely. The only reasonable possibility would be if they stored the ethanol with NO in the headspace but this also seems unlikely to me. The authors need a better explanation of these results.

Page 29, Line 5: In Ostrom and Ostrom (2011), we suggested that the SP for N2O production is more likely controlled by the specific enzyme involved rather than by the “species”. Do the authors have any indication that the specific enzyme or enzyme functional group (metal co-factor) differed between the species? Line 7-8: As written, the sentence suggests that N2O can be produced directly from nitrite via NIR. The authors should indicate that N2O is produced from nitrite by the sequential reduction of nitrite to NO (using NIR) followed by reduction of NO (NOR).

Page 29, Lines 6-23: In all actuality, N2O production by nitrifiers is enhanced under low O2 conditions not complete anaerobic conditions (see Poth and Focht, 1980). N2O production by nitrifiers decreases markedly at no O2. It is very likely that the production of



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N2O as O2 levels drop in cultures of nitrifiers is the result of nitrifier-denitrification. The authors should describe N2O production during nitirification more accurately. What is most surprising, however, is that the greatest degree of N2O production is at 21% O2; which is in contrast to prior studies of nitrifiers in culture which show little N2O production at these O2 levels (Poth and Focht, 1980 for example but there are others). I think it is very likely that as the O2 levels in the headspace of these experiments dropped over the course of the experiment. If the O2 levels were not maintained, or at least monitored over the course of the incubations, then the authors cannot relate N2O production or SP to the O2 level. This is a significant detriment to the study. Further, I find the relationships based on only three levels of O2 are not very strong. Lastly, if O2 levels were not controlled there is little point to quantifying the relative contribution of hydroxylamine oxidation and nitrifier-denitrification to N2O production on the basis of SP. Thus the statement that even under aerobic conditions nitrifier-denitrification occurred cannot be supported. It is quite likely that O2 levels dropped over the course of the experiment. Given this, the calculations of the relative importance of N2O production by the two processes (Eq. 4) are not valid. Overall, this was too simplistic approach.

Page 31: This is a very simplistic explanation of production mechanisms and difficult to follow. A diagram or figure would be quite helpful.

I find the structure of Table 3 confusing; why average by day? If letters indicate different days why average across days.

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Interactive comment on Biogeosciences Discuss., 10, 16615, 2013.