

## ***Interactive comment on “Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium” by C. H. Frame and K. L. Casciotti***

**Anonymous Referee #2**

Received and published: 18 June 2010

General comments: Frahm and Casciotti present a conclusive dataset on regulation of nitrous oxide production by an ammonia oxidizer, depending on culturing conditions such as oxygen supply and cell density in the culture media. They aim to gauge bulk N<sub>2</sub>O production under culture conditions that are closer to natural conditions than in previous studies. Using measurements of d18-ON<sub>2</sub>O and site preference in N<sub>2</sub>O molecules, they aim to unravel the relative contribution of two possible pathways of N<sub>2</sub>O production in culture experiments, depending on culturing conditions. The study provides new insights into the regulation of nitrification and according nitrous oxide production. The data presented are certainly relevant and also helpful for interpretation of environmental datasets, and the manuscript in total is well-written and should be

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suitable for publication in Biogeosciences after minor to moderate revision.

Specific comments: One drawback in structure is the merging of results and discussion section. I would very much appreciate it if these were split up into 2 separate sections – as it is now, I find it difficult to see the results of the different experiments as a whole. I would also suggest to include a schematic figure showing potential incorporation of O<sub>2</sub> and H<sub>2</sub>O during the two discussed N<sub>2</sub>O production pathways – this would help to guide the reader through the discussion. Furthermore, I have a number of specific comments on parts that may be changed for the sake of clarity – although separation of discussion and results may be the biggest help there.

p. 3025, l 3: Please give a reference.

Methods section: I do not find any record of experiments with enriched water here, which is somewhat puzzling once one reaches the results that refer to these (p. 3037)

p. 3028, l 25 “15.6 +- 0.2 mV-s” – I would prefer a unit like nmol here.

p. 3031, l 12 to15: As NO apparently does induce the transcription, as I read this section, I find this expression somewhat unnecessarily complicated.

p. 3031 l 20 to l 2, p. 3032: high-density cultures produce lower N<sub>2</sub>O yields, but did not have time to adjust their enzyme activity – the discussion of mechanisms is somewhat confusing. N<sub>2</sub>O is not necessarily a desired product, and the given doubling times also do not really fit the preceding statements. This needs some clarification.

p. 3033, l 15/16: is there an explanation for this increased production? via NH<sub>2</sub>OH?

p. 3035, l 6: Please define M. Furthermore, what was assumed for M(NH<sub>2</sub>OH), as mentioned in line 7?

p. 3039, lines 24/25: I assume this finding can be related to the results of figure 1? It would be nice to show the influence of cell density also in figure 3 (possibly by increasing symbol size and some shading).

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p. 3042, lines 19-23: I find the reference to archaea somewhat out of place, because it did up to this point not play a role in the manuscript. Would be somewhat appropriate the discussion, but what can be concluded about archaea from this study?

Supplements: I find the sensitivity analysis (both text on p. 3040 and figures) somewhat difficult to grasp. I would prefer if this was either extended so it was an integral part of the manuscript, or omitted.

Appendix A: reference to the “Casotti MS” should be replaced by something more meaningful

Figure 1: should the label on the y-axis be yield per N-NH<sub>3</sub> (instead of N-NO<sub>2</sub>), as mentioned in the text p. 3029, lines 20-22?

Figures 3 and 4 would definitely profit from increased symbol and caption size. As stated above, I would find it appropriate to include cell density effects in Fig. 3

Technical corrections: p. 3034, l. 4 “the NO<sub>2</sub>- produced” should this be nitrate? see mentioning of nitrite in line 9.

p. 3035, l. 22 “providing a minimum” should this be maximum?

p. 3040, lines 17-19: awkward sentence

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Interactive comment on Biogeosciences Discuss., 7, 3019, 2010.

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