

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 #1

Received and published: 8 June 2010

General Comments:

Result of this research is useful to analyze distribution of N₂O concentration and also isotopomeric composition of N₂O in the oceans. Important results of this work are as follows:

1. They cultured a marine nitrifier under low ammonium concentration (50 micro-mol/L) and estimated yield of N₂O. They cultured the nitrifier under three different O₂ conditions (20%, 2%, or 0.5%) and suggested that the increase of N₂O yield under low O₂ conditions is not as high as those of previous works. Species could be one of the causes of the difference. Previous works used bacteria that are often found in waters

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with high ammonium concentration (e.g. *Nitrosomonas europaea*). Result of this work seems more reliable to apply the yield of N₂O during nitrification in the oceans.

2. This paper shows outstanding results to analyze N₂O cycle in the oceans based on N₂O isotopomer ratios. This paper estimated fractionation factors of $\delta^{18}\text{O}$ and ^{15}N -site preference ($\delta^{15}\text{N}_{\alpha} - \delta^{15}\text{N}_{\beta}$) for NH₂OH oxidation and nitrifier denitrification, individually. The idea to use ^{18}O -labeled H₂O and reduce errors in the estimates of fractionation factors is smart and effective. They could use the idea because they have ability to measure isotopomeric composition of N₂O and also d^{18}O -nitrite. A few laboratories have abilities to measure these isotopic compositions. The fractionation factors estimated in this paper will be applied to analyze N₂O isotope and isotopomer ratios of N₂O in the oceans and possibly in the soils or aquatic environments. They also showed a new explanation to the correlations between ^{15}N -site preference and $\delta^{18}\text{O}$ in N₂O, which are often observed in the field.

This research used only one species of marine ammonia oxidizer. Other species should be examined to know representative fractionation factors for N₂O production by nitrifiers in future. Oxygen exchange with H₂O during NH₂OH oxidation should also be addressed in the future. But the result of this paper indicates that the rate of oxygen exchange should be low.

This paper is worthwhile to publish in Biogeosciences.

Specific Comments:

SC1, p.3021, Line 7-8: "... and nearly one-third of this may come from the oceans (Nevison et al., 1995)." Recent reports show that the oceans account for about 20% of the global N₂O emission, although there are wide range of uncertainties. Reference: Table 7.7 in Chapter 7 of IPCC 2007 (p546, Climate change 2007 The physical science basis)

SC2, p.3032, Line 21-22: "There was no detectable loss of dissolved N from the com-

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bined NH_4^+ and NO_2^- pools.” Please add the value and standard deviation of the difference of the sum of NH_4^+ and NO_2^- before and after incubation.

SC3, p.3040, Line 12-15: “Equation (6) assumes that the oxygen atoms in N_2O produced by NH_2OH decomposition come only from O_2 . If a fraction of this oxygen actually comes from H_2O then the model value of epsilon- NH_2OH should be too low for data from experiments in unlabeled H_2O ($18\text{O}-\text{H}_2\text{O} < 18\text{O}-\text{O}_2$) and too high for data from labeled H_2O ($18\text{O}-\text{H}_2\text{O} > 18\text{O}-\text{O}_2$).” Please show an example if you can estimate. For example if 20% of oxygen comes from H_2O then how much epsilon- NH_2OH changed? Casciotti et al. (2010) (reference in the manuscript) estimated 23% of oxygen is exchanged with H_2O during NO_2^- production by the same nitrifier, *N. marina* C-113a. There is possibility that oxygen in N_2O is exchanged with H_2O during NH_2OH oxidation, either.

SC4, p3040, Line 16: “However, this pattern was not apparent in the residuals of epsilon- NH_2OH from labeled versus unlabeled experiments.” How much the difference of 18-epsilon- NH_2OH when you use data of only labeled or unlabeled experiments? Is there a possibility that this difference is attributed to the oxygen exchange with H_2O or just error in estimation? If you do experiments using several different 18O -labeled H_2O , you may estimate how much ratio of oxygen comes from water during N_2O production by NH_2OH oxidation. But this is another work. I don’t intend to ask you to do further experiments.

Technical corrections:

TC1, p.3020, Line 2: “greenhouse warming of the atmosphere” is not adequate expression. Possible expressions are “. . . a trace gas that is one of the greenhouse gases . . .” or “. . . a trace gas that contributes to global warming . . .”. Example: Nitrous oxide (N_2O) is a trace gas that is one of the greenhouse gases and contributes to stratospheric ozone depletion.

TC2, P3021, Line 20-p3022, Line 21: Order of sentences is not good.

BGD

7, C1298–C1302, 2010

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Line 20-27: You listed three possibilities. Fourth possibility should be the sentence in P3022 Line 15-17: “N₂O yields based on regressions of oceanographic data are also strongly influenced by mixing gradients, making them unreliable gauges for biological N₂O production (Nevison et al., 2003).”

P3022, Line 9-15: “Stoichiometric relationships among N₂O production, NO₃⁻ regeneration, and AOU are also a convenient tool for converting oceanographic nutrient and O₂ data to estimates of N₂O production (e.g., Codispoti and Christensen, 1985; Fuhrman and Capone, 1991; Jin and Gruber, 2003; Suntharalingam and Sarmiento, 2000) or using N₂O concentration data to calculate nitrification rates (e.g., Law and Ling, 2001). However, there is not a universal AOU:N₂O ratio; open-ocean AOU:N₂O ratios differ from low-O₂ environments (Cohen and Gordon, 1979).”

Previous works assume relationships among N₂O production, NO₃⁻ regeneration, and O₂ consumption because correlations between N₂O and NO₃⁻ concentrations, and AOU found in the oceans. But these correlations will be formed when there is mixing of waters.

These sentences could be replaced by the following: “Stoichiometric relationships among N₂O and NO₃⁻ concentrations, and AOU were used to convert oceanographic nutrient and O₂ data to estimates of N₂O production (e.g., Codispoti and Christensen, 1985; Fuhrman and Capone, 1991; Jin and Gruber, 2003; Suntharalingam and Sarmiento, 2000) or using N₂O concentration data to calculate nitrification rates (e.g., Law and Ling, 2001). However, there is not a universal relationships among N₂O and NO₃⁻ production and O₂ consumption ; open-ocean AOU:N₂O ratios differ from low-O₂ environments (Cohen and Gordon, 1979).”

TC3, Equations 5 in p.3038 and Eq. 6 in p. 3039 Please differentiate short hyphen and minus signs carefully to prevent misunderstanding. Please use short hyphen sign for d18O-N₂O, d18O-NO₂⁻, and d18O-O₂.

TC 4, p.3040 Line 17-19: “Furthermore, if oxygen atoms exchanged between H₂O and

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NH₂OH is occurring, there was too much scatter in the data to resolve it by including an exchange term in Eq. (6).” The sentence is not clear. The sentence can be replaced by the following: When we assumed that oxygen atom in NH₂OH was exchanged with that of H₂O and included an exchange term in Eq. (6), there was too much scatter in the resolved parameters.

TC5, p.3043, Appendix A: Please use superscript for mass numbers in “³¹NO/³⁰NO”.

TC6, P3043, Line 8: Tpyo: these ratio ratios → these ratios

TC7, p.3055, Figures 1-4: Please enlarge fonts of characters in the labels and legends of figures to think the layout of papers. Figures will be allocated in one or two columns in a page.

TC8 P3057: Add “(NOB)” after “nitrite-oxidizing bacteria” in the caption of Fig. 2b.

TC9, P3058: Add units in x and y axis in Fig.3

TC10, P3059, Fig.3: Please show the R² value instead of “R=0.88” for the regression line for labeled H₂O for consistency.

TC11, P3059, Fig.4, x-axis: Replace “Site Preference permil” with “Site Preference, permil” or “Site Preference (permil)” y-axis: Replace “d18O N₂O permil” with “d18O-N₂O (permil)” or “d18O-N₂O, permil” (Here I use character instead of symbol for “permil” because I use text format for submission.)

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

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