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Interactive comment on "Vertical segregation among pathways mediating nitrogen-loss (N_2 and N_2O production) across the oxygen gradient in a coastal upwelling ecosystem" *by* Alexander Galán et al.

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This is a well-written paper, which presents results from 15N-label experiments to gain insights into a number of N-cycling processes (production of dinitrogen, nitrous oxide, nitrite and ammonium) at a coastal upwelling system. The water column oxygen of this system fluctuates seasonally at large magnitude, making this system unique. The authors also examined the seasonal and vertical differences of N-cycling rates that are ultimately dictated by the physical forcing. This dataset is worthy of publication in Biogeosciences if the authors improve the data interpretation and manuscript clarity. A

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number of points are raised below.

1. Issues concerning scientific quality

One of the findings claimed by the authors is the activity of canonical denitrification and anammox in the oxygenated depths. Also, the authors detected nitrification activity under anoxic conditions (Table 3). These interesting findings may reflect the lack of knowledge about central Chilean coastal upwelling system, but they could also be the result of experimental artifacts. Upon careful examination of the oxygen concentrations during the 15N incubation experiments, the validity of these claims and results remain questionable.

The use of inhibitors is one of the highlights of this manuscript. ATU and GC-7 were used to distinguish the nitrification activity contributed from bacteria and archaea, respectively. I think the experiments were successful, as demonstrated in the results of Jan. 2014 (Figure 5b). The sum of nitrite production rates of ATU- and GC7-treatments matches (within the scale of error bars) the rate using 15-ammonium without inhibitor. So it is strange for the authors to claim that GC7 was not effective during the incubation experiment (page 15 line 30-32).

It is confusing that the use of acetylene was to quantify N2O production rate from nitrite reduction (page 7, line 11-13). More appropriately, acetylene addition in 15N-nitrite incubation is to quantify the rate of N2O consumption (equivalent to N2 production), which is the difference in N2O production rates with and without acetylene. Thus, I find it inconsistent in the denitrification data presented in figure 3 and N2O production data in figure 4. If canonical denitrification was active in the oxycline (25 m) as demonstrated in figure 3b and 3d, why was there no N2O production rate at 25 m with acetylene addition (figure 4b and 4d)? The 85 m sample from Jan. 2014 showed high N2O production rate with acetylene, and very low N2O production rate without acetylene (Figure 4d). Why was there no canonical denitrification detected at the same depth? (figure 3d).

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In-depth interpretation of natural nitrate isotope data is needed. The author claimed that nitrate consumption (phytoplankton uptake and denitrification) increased surface delta 15N value in September. However, in January, lower surface delta 15N value (5 permil lower than September) and lower surface nitrate concentration were measured under increased denitrification rate (and potentially increased phytoplankton uptake due to higher Chl-a). The author argued that nitrogen fixation was responsible for lowering the delta 15N. If this is really the case, will nitrogen fixation be the major N source in this region, and will argue against the conclusion that upwelled nitrate is the main fuel for this system (page 17, line16 - 19)? The authors can strengthen the arguments by providing simple calculation to show how much of nitrogen fixation is needed to lower the delta 15N of nitrate by 5 permil.

2. Issues concerning presentation quality

The authors did not provide any molecular data about the microbial community throughout this manuscript. So the sentence starts in line 36 page 2 should be revised to accurately describe the data.

The naming of season and/or sampling times can be easier for readers. When referring to sampling period, which happens in a short time scale, I suggest using “September” and “January”. When discussing seasonal features on a longer time scale, please use “spring” and “summer” There are some details in the “Methods” section that need to be addressed and clarified (1) Page 6, line 23. How long were the 250 ml bottles kept before processing in the laboratory? (2) Table 2, provide the in situ oxygen concentrations for all sampling dates and depths. (3) Page 6, line 30. Provide the volume of water in the 12.6 ml vials because it significantly changes the oxygen concentration in the water phase. Also, how was oxygen (and potentially nitrogen) contamination avoided during water samples transfer from 250 ml bottles to 12.6 ml vials? (4) For January, 25 m samples, it could be technically challenging to measure 15N in N2 if ambient N2 was not removed from the 12.6 ml vials. (5) Page 7, section 2.7. Provide the relative standard error for nitrate isotope analysis. (6) Page 8, line 5. For N2O production, the

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total rates of N2O production is different from the sum of 45- and 46-N2O production because of different fraction labeled of substrate. Please clarify in the main text and in table 2.

The presentation style of the figures can be modified. Overall I hope the authors solve the following issues in the resubmitted manuscript. (1) Figure 1 (a), an enlarged color bar can be placed outside the Chl-a maps. Figure 1 (b), please point out the seasons on the bar plot to help readers navigate. (2) I find the figure 2 difficult to read. Firstly, the panels should be increased to 8 or 10 (4-5 panels each sampling time), so that physical and chemical parameters can be plotted on different panels without confusing x-axis. Secondly, use dashed lines to connect individual measurements to help readers navigate. Thirdly, please emphasize that the "delta 15N" refers to nitrogen isotopic composition of nitrate. (3) It is odd to use bar plots in figure 3, 4 and 5. Firstly, space is wasted when only two depths are presented, and many of the rates are low. Secondly, bar plots can be confusing because they are sometimes misplaced along the depth-axis. On fig 3c the bars are spread between 20 to 30 m but in fact they all represent rates at 25 m. Thirdly, the greyscale color scheme makes many of the bars indistinguishable even on a computer screen (e.g. figure 4d). Fourthly, the fonts of the numbers alongside the axis are different, some are very small, some are ok. In figure 4 the numbers for depth are missing. Lastly, if the value of each rate is shown, there's no need to have a separate zoom-in plot.

The discussion section 4.2 is not well organized and it makes me difficult to grasp authors' main ideas. The first 5 paragraphs (page 12 line 10 to page 14 line 15) are about N2 production. The following two paragraphs (page 14 line 17 to page 15 line 10) are for N2O production. The last two paragraphs (page 15 line 11 to 32, page 15 line 34 to page 16 line 18) are for nitrification and DNRA, respectively. I suggest breaking down section 4.2 into multiple sections (or subsections) to help readers navigate.

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