

Interactive comment on “Chemolithoautotrophic production mediating the cycling of the greenhouses gases N₂O and CH₄ in an upwelling ecosystem” by L. Farías et al.

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Dear Dr. Taylor,

Please find attached our response to the observation done to our manuscript entitled “**Chemolithoautotrophic production mediating the cycling of the greenhouses gases N₂O and CH₄ in an upwelling ecosystem**” by Farías and collaborators for resubmission to Biogeosciences. We really appreciate your comments and suggestions. Once again, thank you for your time and consideration with this ms. we have dealt with the main comments and suggestions made:

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The first concern being total disregard for the potential importance of anammox in dark carbon fixation in both the experimental design and discussion of the results. If the authors have valid justifications for ignoring anammox, then they should explicitly say so in the Introduction. Otherwise, they must acknowledge limitations to their interpretation.

R: According to our previous experience with measurements of anammox in water column and sediments in the study area (Galan unpublished data), anammox could not be detected in water column but this process was active in sediment. Thus, it could not explain Dark CA assimilation in the water column, not at such a high rate. Another important point is that anammox is not involved in N₂O cycling. In the revised version of the manuscript we completed the introduction section with all processes responsible for N₂O and CH₄ cycling (following your suggestion) and separated them in producing and consuming gas cycling processes. We explicitly state that anammox is not responsible for N₂O cycling.

The second concern is the attribution of all observed dark carbon fixation to chemolithoautotrophy. I'm sure the authors are aware that a fraction of measured CO₂ incorporation is due to anaplerotic reactions carried out by all ecophysiotypes. So especially in the photic zone where biomasses are highest, a significant fraction of their 13CO₂ signal may not be chemolithoautotrophy at all. Authors should estimate what this contribution may be.

R: Sincerely, we do not have in mind or accounted for these anaplerotic reactions, but, of course they can be acting in our study area, particularly in the photic zone as the reviewer mentioned. So, we discuss anaplerotic carbon dioxide fixation in the discussion section and suggest that production of oxaloacetate from pyruvate is probably the most physiologically important component of this reaction in this case. However, we also state that we are not able to discern methodologically the relative importance of these reactions and their capacity to support dark carbon assimilation. This certainly remains an open question and should be assessed in future research.

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Thirdly, I believe that referring to their measurements of N₂O and CH₄ as metrics of “cycling” is misleading. What the authors have measured are net fluxes of these gases into headspaces of closed vessels, which will depend upon balance between production and consumption as well as gas solubility. Without directly measuring either production or consumption in addition to net flux, one can say very little about “cycling”, i.e., theoretically the same net flux could be measured if N₂O or CH₄ cycling slowly or rapidly.

R: We tried to be clearer in the introduction and methodology sections respect to gas cycling concept: we mentioned “net rates” because the experimental approach includes both, N₂O and CH₄ production and consume processes and the estimates reflects the dominant contribution of one process over the other. Thus, N₂O and CH₄ concentrations during incubation time (experiment) are measured by the analysis in closed vials by the headspace technique. These measurements are used to calculate the N₂O and CH₄ cycling (net accumulation or depletion). However, the analysis is made in natural incubations (to measure the net rates expressed as Mass Volume⁻¹ Time⁻¹) and in incubations with different inhibitors (to estimate production and consumption). For CH₄ cycling experiments, three experiments were made: naturals, with ATU (to inhibit ammonium oxidation and maybe methane oxidation) and GC7 (to inhibit archaea activity), while for natural N₂O cycling, acetylene and ATU (to inhibit nitrification) experiments were used. CH₄ concentration was measured at 0, 2 and 6 hours. N₂O concentration was measured at 0, 2, 6 and 19 hours. These inhibitors allow us the identification of several processes involved in the cycle of both gases. On the other hand, to monitor these concentrations at different times allow us to determine the rates of production or consumption of these gases by different processes.

We considered all minor comment and we tried to rephrase all sentences that were not well written.

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Best regards,
Laura Farias

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