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

L. Farías et al.

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Received and published: 20 October 2009

Chile, Concepción, October 7th, 2009

Dear Dr. Lavik,

Thank you very much for your suggestions, that without a doubt, resulted in an improved version of our ms entitled “**Chemolithoautotrophic production mediating the cycling of the greenhouses gases N₂O and CH₄ in an upwelling ecosystem**” by Laura Farías and collaborators. We herein explain how we responded to each individual observation:

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GC# 1. It is not at all easy to treat such a huge complex dataset which is probably the reason why scientists so seldom try to use oceanographic monitoring data to upscale the results from their experiments. In total there are 7 main figures with a total of 21 panels and 5 Tables with upto 325 data cells each, dealing with physical and chemical oceanographic parameters, inventory calculation for the fixed nitrogen species, CH₄ and N₂O gas analysis on both experiments as well as monitoring, air-sea flux calculations of CH₄ and N₂O, 13DIC assimilation experiments and natural abundance 13C isotopes on bulk POM. Although the graphic presentation is good and most numbers presented in the tables seems okay, it took me a lot of effort time to overview the dataset and partly because of the size of the tables it takes a lot of time to read and follow the data references in the result and discussion part. Still after reading it partly 3 times I am not sure if I see the same in the tables as what they describe and discuss.

GC# 2. The rather large amount of abbreviations (O-L, B-L, S-L, B-Layer, S-Layer, additional to the more common ones like AAO, AMO, CA, ATU, OM, POC, GC7,) does not ease the understanding, i.e. is there a difference between B-L and B Layer and does n.d. means no data or not detected. In the end most of the information in all of these data get lost and in the summary there are rather vague statements without any specific numbers or estimates (more like the abstract), whereby the most specific conclusion I cannot really see in the data (see below). I think this is a very important study based on an impressive dataset, but needs to be restructured where the most important data gets more visible and the statements in the conclusions/summary more defined.

R: In relation with your main concern regarding the understanding and fluidity of the text, we consider non-viable to re-structure the manuscript at this stage. Doubtlessly our contribution or interpretation of the obtained results comes from the division of the system in three layers. Thus most of the analyses and the presentation of the data are based on this division. However, we tried to be much clearer in the presentation of results for each layer based on the proposed biogeochemical and physical criteria.

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Some of the general improvements to the revised manuscript include:

-Definitively we agree with you that N_2O and CH_4 fluxes across the air-sea interface do not contribute to the conclusion and general idea of this ms. They represent complementary data, that can be more useful if they are analyzed in the oceanographic context (wind pattern, upwelling index, etc). We removed these data from the text and, therefore, table 1 was reduced.

-Additionally, we removed as much acronyms as possible along the text.

-Also, we now present data of gas and nutrients content in the water column in the same temporal window, it is from April 2007 to March 2009. We are including new data as well, from February and March of 2009, in order to complete the two years observation period.

-We re-arranged the tables and figures and also we have eliminated from a table (ex table 2) the calculations of percentage of nutrient inventories for each layer, since these calculations can be reproduced from the basic data.

GC# 3. The statements in the abstract - The conclusion about the importance of dark assimilation of inorganic Carbon, chemolithoautotrophic assimilation, in an upwelling area is well founded. However, is the mentioned 27 % the average of the percentage dark fixation or the percentage of the integrated total assimilation (i.e. if it makes up 88-98% when total production is 0.5 mg/d/m² and 2-4% when production is 50mg/d/m², the average % can be about 27% but the total contribution of dark fixation might be 5%). This is a very important finding and needs to be clarified and probably both numbers should be in.

R: We understand your argument regarding the percentage of chemolithotrophic production in the study area, and agree that making an average from percentages can produce problems according to the type of distribution of the data. In order to avoid confusions, we put the rank of rate (total autotrophic and total chemoautotrophic pro-

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duction) and rank of the percentage of total integrated rates of the chemosynthesis versus the total autotrophic (photo + chemoautotrophy). These calculations are displayed in the abstract and reproduced on the discussions and conclusion; they are based on estimations from Table 3. Also we include in the discussion section a figure with the distribution by layers and periods of total autotrophic and chemoautotrophic production.

GC# 4. The argument about the chemoautotrophic assimilation causing the lighter $d^{13}C$ values at the oxycline (O-Layer or O-L) is interesting, but should not then this be the zone of the highest dark fixation? According to the dark assimilation rates in Table 4 then the higher rates are found in the bottom waters (B-L, B-Layer).

R: The results of natural C isotopic ratio of particulate organic carbon ($\delta^{13}POC$) and the rates of dark carbon assimilation are our most direct evidences that chemolithoautotrophic activities are occurring in this system. Regarding whether dark carbon assimilation is concentrated in the oxycline or bottom layer (and excluding surface layer) we argue that our system is very dynamic due to active a relaxed upwelling events. In table 4, we show results from specific experiments performed only at two depth (which of course limit the vertical resolution of our data) although in January of the 2009 rate of dark CA was higher in the bottom water whereas in September 2009 the rate was greater in the oxycline. The activity surely will depend as much on the existence of electron acceptor and donor, as well as on oxygen and other electro donors that could be limiting. A further effort will be dedicated in an upcoming manuscript to deepen our comprehension of the regulation of these processes.

GC# 5. In short the authors state that: Dark CA was reduced by 27-48% by ATU (AAO inhibitor) and 24-76% by GC7 (archaea inhibitor)! By looking at the results presented in Table 4 I do not see this, i.e. 3.53 ± 1.4 is not significantly different from 2.54 (no error range) and even using the average values I would get lower % values. There is a significant difference by the addition of GC7 which is interesting but looking further down the table the addition of GC7 increases the CH_4 consumption considerably, which

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is not contradicting the statement that the inhibited Archaea are gaining their energy from CH₄ oxidation (AMO)

R: The second point important to us was to identify reactions/processes responsible for dark carbon assimilation in our system. To do so we have worked with ATU and GC7 (see results in table 3), two already described inhibitors for aerobic ammonium oxidation and archaean activity. We have included the errors of the rates of Dark CA (see Table 4), but unfortunately the number of independent experiments done in each opportunity has been different, so statistic comparisons do not have major significance. We are therefore only showing tendencies and indeed, both inhibitors showed significant differences in most of the cases (t-student test).

Regarding your question of why GC7 significantly increase methane consumption, we consider this a very good point. The net rates of cycling mean that producing and consuming processes exist at the same time. Thus negative rates mean that consumption is higher than production, or that the later is inhibited, or both. Since it is expected that this inhibitor also affects microorganism that produces methane (which can be mainly archaeas), the net obtained rate is negative.

CG # 6. A similar problem occurs with the statement that AAO produced N₂O at a rate of 8.88-43.85 nMd⁻¹. The lower estimate (8.88) I can find as the net N₂O production without any inhibitor and the max (43.85) is not appearing in the table. I do not see why the net rate is taken as AAO activity, because in most of the experiments the N₂O production does not significantly change by the addition of ATU. Why is all other

sources of N₂O excluded? In the detailed discussion about this (p29 line 1-4) aerobic denitrification (Kuenen et al) as well as potential N₂O production by the anammox reaction (Kartal et al) is not included. Moreover, using the authors CA attributed to AAO (27-48% of dark CA) to calculate the Amonium consumption would give ammonium oxidation rates in the range of 0.2-3_M NH₄ d⁻¹ using an conservative conversion

factor of 1mgC /mol NH₄ (Feliatra and Bianchini 1993, Buttinini et al 2000), with an

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6, C2582–C2588, 2009

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average ~ 1 M/day which is well above the values from Lipschulz et al (1990), Ward and Lipschulz (1989) and Lam et al (2008) from Peru. The above mentioned concerns need to be addressed properly before the statement that NH_4 and CH_4 oxidation is the main driver of the Dark CA or not. At the moment this is not convincing and the dark CA in it self as well as the flux of the climate gases N_2O and CH_4 might be two more interesting results to focus on Suggestions:

R: We present results of N_2O and CH_4 cycling on Table 4. They showed that except for Jan 2008 and Sept 2008 (80 m) all net N_2O cycling rates were reduced with ATU. We insist again on the fact that we are showing tendencies. In order to determinate N_2O cycling rates through nitrification (N_2O_n) and denitrification (N_2O_d), a combination of 15% (v/v) acetylene 0.2 mM Allylthiourea, ATU and anoxia were used in several previous and in this studies (Castro and Farias, 2004, Farias et al., 2009). In this ms. we only presented data of N_2O and CH_4 cycling with ATU. ATU and anoxia, both ammonium oxidation inhibitors, were used to evaluate net N_2O cycling of denitrification (net N_2O_d), which equaled production minus consumption by denitrifiers-, under *in situ* O_2 levels and anoxia, respectively. The acetylene, an inhibitor of N_2O reductase, and ammonium monooxygenase (AMO) prevented N_2O production by nitrification and its reduction by denitrification. Therefore, the rate of N_2O produced by denitrification (**N_2O_d**) in all the experiments corresponded to the rate of N_2O accumulated after inhibition with acetylene. Net cycling rate (without any inhibitor) represents N_2O (net **N_2O_d** + N_2O_n). Thus, the difference between control vs. ATU treatment is de N_2O produced by nitrification (ammonium oxidation). Consequently, we are confident in our initial statement that part of N_2O accumulation in the water column came from ammonium oxidation and maintain such statement in the revised version of the manuscript. Besides, the preformed correlations between nutrients and gases confirmed that AO occurred.

Regarding if ammonium oxidation would correspond to the carbon fixation. This estimation was introduced as an illustration in the discussion section. As you suggested,

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we included an estimation of $N/C = 8.3 \text{ mol mol}^{-1}$. But note that several ratios have been described with a high level of variability in previous studies (e.g. Ward et al 1989 :0.43-3.5, Feliatra-Bianchi 1993-1999:10.5-15.8 and Brion 1997:10-25).

We have estimated the contribution of ammonium oxidation by nitrifiers based on the assumption that 1 mg of carbon fixed is equivalent to 1.5 mmol of ammonium oxidized.

In that case, if 100% of dark carbon is fuelled by aerobic AO and considering dark carbon fixation values between 0.4 and 1.5 mg C $\text{m}^{-3} \text{d}^{-1}$ (see table 3), equivalent to 0.4 $\mu\text{g L}^{-1} \text{d}^{-1}$ (see new or old? table 3), the estimated ammonium oxidation is 0.6 to 2.25 $\text{mmol m}^{-3} \text{d}^{-1}$ (or 0.6-2.2 $\mu\text{mol L}^{-1} \text{d}^{-1}$). Considering that 40% of dark carbon assimilation corresponds to AAO, ammonium oxidation rate should be 0.24-0.88 $\mu\text{mol L}^{-1} \text{d}^{-1}$. This range of values is very reasonable for the study area and others, and is supported by estimations of the turnover time of ammonium pool, and reported rates of ammonium oxidation measured in the study area by chemical assays (Molina et al submitted) and isotopic techniques (Fernandez et al in prep).

Observation 9: I agree that the conclusion and abstract did not reflect the main results, we modified it.

All minor comments were considered.

Thanks again for your input!

Sincerely,

Laura Farías
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University of Concepción

Interactive comment on Biogeosciences Discuss., 6, 6205, 2009.

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