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Biogeosciences Discussions

Interactive comment on "High production of nitrous oxide (N_2O), methane (CH_4) and dimethylsulphoniopropionate (DMSP) in a massive marine phytoplankton culture" *by* L. Florez-Leiva et al.

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Interactive comment on "High production of nitrous oxide (N2O), methane (CH4) and dimethylsulphoniopropionate (DMSP) in a massive marine phytoplankton culture" by Florez-Leiva et al.,

Dear Editor, We want to thank the reviewer for all their comments and suggestions. We have carefully read them and have consequently made note of certain mistakes in the text. We have therefore made the necessary changes in this revision. The most

important change is related to explaining the experimental setup, since in the first version of our manuscript we didn't specify important experimental details which caused the reader to misunderstand the experimental setup. We didn't carry out a specific experiment but our measurements were taken from a real open pond currently used for feeding fish and mollusk species. Therefore we didn't have replicate pools or control experiments. Also we weren't able to manipulate environmental variables such as the kind of water used in the pool or a cover to protect it from rain or sunshine. Therefore, the main goal of our research was to evaluate the real contribution of aquaculture (and fish farmer) to greenhouse gas emissions. We believe that the changes made to the text have improved the manuscript. Please find the responses to the overall and specific comments enclosed. Sincerely, Lenin Florez-Leiva

Major revisions: General comments: Nannochloris (this should be written in italic): it is not mentioned which species or strain was used for the experiment. Nannochloris was changed to Nannochloris sp. The fertilization was made before cell inoculation. We added the dates as marked in Figure 1 in order to clarify your doubts. The impact of aquaculture (particularly the cultivation of microalgae) and certain environmental interpretations were rephrase. Also, it is not described how the organisms were grown before using them, here. The inoculation of concentrated algae and dilution was done in filtered and enriched marine water. Incubation took places in a mesocosm, which is not reported to be run in replicates or with a control without the culture. We monitored gases and tracers in an open pool that is frequently used by fish farmers, so we did not have the chance to select the kind of massive culture system, neither the culture media nor the target cultivated algae.

Also, the mesocosm was filled with seawater which was not characterized regarding nutrient background, DOM, or planktonic or microbial community structure.The production of methane and nitrous oxide is assumed to take place exclusively by microbes. Consequently, it is of highest importance to clarify, whether the commonly known producers are present or absent, e.g. by 16S rDNA analysis, any key gene assay or

isotopic studies. In general, it is doubtful to use an open pond of this size without protecting it from environmental influences such as rain, which can also influence the whole ecosystem in the tank and bias the community structure. Despite some inconvenience, this kind of open pool is one of the most used systems in the world. We want to clarify that this study was not designed by us; we simply sampled several physical and chemical variables (including gases and tracers). This work was conducted within a regular culture system. This work can be extrapolated to other systems in the world where cultivation is practiced. So, we believe that the term “mesocosm” used here is an inappropriate word. A parallel or control study (i.e. without Nannochloris sp) as suggested by the referee was not possible. But, the first five days (without fertilization and inoculation) can be considered as a control. As it was observed, the pond has nutrient and gas content similar to the adjacent ocean.

Additionally, the sampling interval (at least between day 20 and day 30) seems to be insufficient.

It is unclear, which connection is there between the production of N2O in phase II and the presence of Nannochloris sp... The observed formation could also be interpreted as the classical dependency between N2O being produced by nitrification when oxygen decreases. We agree with the reviewer that this is a very relevant point. Our data shows green algae growth associated with N2O production. However, we are not sure about the biogeochemical mechanisms by which N2O is being recycling. The culture is not axenic, but we can assure the reviewer that there is not meio- or macrozooplankton or faecal pellet that could be acting in the gas production.

To me, it is not clear, why a certain phytoplankton species (or genus) should be responsible for N2O production or uptake, especially, when the bloom already broke down. It is likely, that a consecutive bloom of micro organisms accounts for the biological formation, here. Previous research and new advances in the functional and phylogenetic diversity of marine microorganisms, has shown that algae are not only phototrophs. Studies by Weathers, (1984) shows N2O production in an axenic culture of several

green algae grown on nitrite. The process was stimulated by glucose, but was not affected by the presence of acetylene or the photosynthetically produced oxygen. We have extended this discussion in the ms. In addition, we restructured the results, describing gas production rates and effluxes, in order to emphasize our main contribution. In fact, we want to draw attention to the potential effect in terms of gas production and concomitant efflux towards the atmosphere. Weathers, P. N2O evolution by green algae, Appl. Environ. Microb., 48(6),1251-1253,1984. ÂňÂňÂňÂňÂňÂň

Specific comments:

Concentrations mentioned in the abstract are not consistent with those shown in the results part. We reorganized the abstract and results.

p.6760, l.15: the time unit is missing This is confusing, we do not know which page referred to and that means.

p.6707, l.12: cost efficient We have changed the text as was suggested.

p.6707, l.20: global trace gas production Table 2 is not mentioned in the results part, don’t introduce new results in the discussion part.

We restructured the ms, moving results from the discussion to results section.

Is Nannochloris sp. known to produce algal blooms in the environment? Please discuss. Yes. Nannochloris sp. can be adversely affected by other organisms such as dinoflagellates through substances elaborated by the same algae (Derby et al., 2003). Also, the presence of organic nitrogen compounds and the low ratio of nitrogen to phosphorus in the pollutants favor the growth of Nannochloris sp (Rythers, 1954). Derby, N.L., Galliano, N., Krzanowski, J.J., and Martin, D.F. Studies of the effect of Ψ- APONI from Nannochloris sp. on the Florida red tide organism Karenia brevis, Toxicon., 41, 245-249, 2003. Ryther, J.H. The ecology of phytoplankton blooms in Moriches bay and Great South Bay, Long Island, New York, Bio Bull., 106, 198-209,1954.

p6711, l.7: A clear association of methane and Chla is not shown, here. Methane

shows a peak, before Chla reaches a maximum. Additionally, the possibility of CH4 being formed from DMSP was not discussed, here (Damm,E. et al., 2010, Methane production in aerobic oligotrophic surface water in the central Arctic Ocean, BGS); even though it could be a possible pathway, here. We clarified this point (correlation CH4/Ch-a) and provide more information of methanogenesis in the text.

p6711, l.10: The maximum of N2O occurred in phase II and not in phase I as it is said here. Rewrite this paragraph; it is not consistent with your figures and your introduction. We clarified this point by rephrasing the sentence, which now reads "The N2O concentration during the study period increased from 10 μmol m-3 (111% saturation) to 580 μmol m-3 (5500% saturation). The maximum N2O concentration occurred after the first few days of phase II, reaching levels up to 580 μmol m-3, then N2O levels progressively decreased to near seawater background levels"

The overall impact of aquacultures on trace gas production is not shown or discussed sufficiently, here. Additionally, the discussion about environmental trace gas fluxes does not really refer to the topic. Please clarify. A comparison to Williamson & Crutzen, 2010, Nitrous oxide from aquaculture, Nature Geosciences, is missing, here.

We restructured the results, describing gas production rates and effluxes in the results section, with the intention of emphasizing what is our main contribution. In fact, we want to draw attention to the potential effects in terms of gas production and concomitant efflux to the atmosphere. Our data is a direct report of the specific effect of Nannochloris sp cultivation; work by Williamson & Crutzen's, 2010 colleagues is the first theoretical estimation of the emission of N2O in aquaculture, mainly focused on protein production. Also, we included a comparison and discussion with the reference mentioned here.

p.6714, l.5ff.: The conclusion of N2O and CH4 being produced in dependency of or connectivity to a Nannochloris bloom or any other phytoplankton bloom could not be shown here. Production could also be due to (i) microbial formation due to fertilization

in case of N2O, (ii) a consecutive zooplankton bloom and production by archaea in anoxic or suboxic micro- environments (e.g. faecal pellets) in case of methane

We only can affirm that associate to the exponential growth of green algae N2O accumulations takes place, but we are not sure about the biogeochemical mechanisms by which N2O is being recycled. If we pay attention to the antecedents and new advances with respect to the functional and phylogenetic diversity of marine microorganisms, we see that the algae are not just phototrophs. See discussion of Weathers, (19849). Therefore, we extended this discussion in the ms.

p.6714, l.24: citation: Wheathers et al.: please correct in the text and in the list of references p.6714, l.24. It was done as was suggested. p.6715, l.14. This is an interesting point. However, it has been known for a long time that some algae can carry out nitrification and induction of nitrate reductase. (See early works by Morris and Syrett, 1965;1963; Kessler and Oesterheld,1970; Weathers, 1984).

Morris, I and Syrett, P. J., Arch. Mikrobiol., 47, 32, (1963). Morris, I and Syrett, P. J., J. Gen. Microbiol., 38, 21, (1965). Kessler, E and Oesterheld, H., Nature., 228(17), 1970.

Minor revisions The spelling and the use of the English language is not appropriate.

We work with a native to the final version of ms.

p. 6707, line 25: Please paraphrase this sentence, it should be about production and not about recycling of this gas; additionally, Bange, 2005 is not an ideal citation, here (better: Bange et al., 2010, Marine pathways to nitrous oxide (N2O), in "Nitrous Oxide and Climate Change", edited by K.A. Smith). p. 6707, line 25: This was done as was suggested. The fertilization with urea is not very common, why was this used here, instead of ammonia? Please mention the exact composition of fertilizers and the corresponding concentrations.

The fertilization with urea is not very common. It is a fertilizer used in agriculture, but has given good yields in some aquaculture systems. Composition and concentration

of nutrient salts was included.

p6708, l.22: How are representative water samples characterized? Took the sampling place at day or night times?

p6708, l.22. Samples were taken every day during the morning at the same time (10 am). p6709, l.20: the description of nutrient measurements is not clear to me: did you prefilter those samples before freezing?

p6709, l.20. Yes, our samples of nutrients were previously filtered with a 0.75 μm GF/F filter before freezing. p6709, l.23: Did you only measure the cell abundance of Nannochloris sp.? It is unclear, if this method excludes bacterial and archaeal cells.

p6709, l.23. We only measured cell abundance using a haemocytometer. It cannot discern between bacteria or archea cells, in contrast to the cytometer. However, given the monospecific dominance of Nannochloris sp., the determination of cell abundance was considered acceptable. p6710, l.2: tracers and: p6710, l.4: rephrase tracer

p6710, l.2 and p6710, l.4. This was done as suggested. p6711, l.3: the maximum in DMSPd is not so pronounced regarding the error bars, here.

p6711, l.3. We disagree on this point. You can see that increasing DMSPt coincidentally is obtained in the same point, which may suggest that all DMSPt is DMSPd. Also, when monitoring high values, only the senescence phase was noted for both fraction (DMSPt and DMSPd). p.6715, l.11: Rephrase this sentence. p.6715, l.11. We followed the suggestion and rephrase the sentence. Thank you again for your input, all suggestions and comments were considered and included in the new version of the ms. We are waiting for others reviewers in order to work in a final version of first round version, if were the case.

Interactive comment on Biogeosciences Discuss., 7, 6705, 2010.