

## ***Interactive comment on “Nitrous oxide (N<sub>2</sub>O) production in axenic *Chlorella vulgaris* cultures: evidence, putative pathways, and potential environmental impacts” by B. Guieysse et al.***

**Anonymous Referee #1**

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In the paper by Guieysse, et al., the authors present a study on the production of nitrous oxide (N<sub>2</sub>O) in pure cultures of *Chlorella vulgaris*. The dataset comprises molecular and chemical measurements and a set of incubation data; it concludes in presenting a potential production pathway, which, however, could not be verified by genetic data (although at least two complete *Chlorella* genomes are available). Comparably high N<sub>2</sub>O production from incubation measurements is presented using various setups and incubation conditions (e.g. light manipulations). The authors identified two major factors for N<sub>2</sub>O production, which are light and nitrite. An antibiotic inhibitor experiment was used to shut down bacterial N<sub>2</sub>O production. Altogether, the presented study might be suitable for Biogeosciences rather than for any other journal. However, the study

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is in some points not completely convincing as some details are not clearly presented and the discussion is partially too speculative and lacks some comprehensiveness. However, I would like to give the authors the chance to convince with their study (as the presented findings really challenge the picture of N<sub>2</sub>O formation) by resubmitting a revised version considering the following points:

General comments

My major concerns are first that an exclusion of other formation pathways is not fully convincing. The authors are shutting down the bacterial producers by using antibiotics, which is fine. However, what about archaea (and here not only the AOA might be taken into account as there are also denitrifiers among them as reviewed by Cabello and colleagues, 2004). At this point, a production from nitrite in AOA cannot be fully excluded at that point, you absolutely need to consider, that your culture might contain AOA, which are able to -at least -contribute to N<sub>2</sub>O production, here. Did you ever check for archaea by 16SrDNA PCRs at the endpoints of your experiments? (This should not be too difficult to do, as you already have the DNA extracted.)

Another critical point for me is that in Fig. 2, N<sub>2</sub>O formation is detectable, independent of antibiotic treatment; however, more N<sub>2</sub>O is formed in the presence of antibiotics. Fig. 2B shows that the algae growth is more or less not impacted by antibiotics (the authors stated that the algae is impacted by antibiotics, but this is not visible from the figure). I don't understand the point, the authors try to make in the text, here. Moreover, the comparison to the experiments of Löscher et al. is just not correct. It is misleading to compare rates from an experiment performed under conditions specifically chosen for one marine archaeon (*N. maritimus*) with an experiment performed under completely different conditions. It is not necessarily expected that archaea would behave similarly in your experiment.

Second, without any hint from the genome for a putative enzyme capable of N<sub>2</sub>O formation, the suggestions on the formation pathways are just speculative. As two

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genomes are available, one could at least try to identify possible gene candidates. Otherwise a deduction from fungi or *Synechocystis* is just not plausible. Alternatively, a study using nitrite isotopes could be used to identify the production pathway. But without any additional hint or dataset, the respective paragraph is just speculative.

Third, without any information on ammonia and nitrite behavior during the experiments, any suggestion on the production pathway of N<sub>2</sub>O is just a hypothesis. It is also not understandable, how denitrification enzymes can be active in an oxic environment.

Specific comments

All species names should be in italics.

Abstract: Phrases like 'might significantly contribute' would have more power when supplied with a real number.

Introduction: Please introduce the phylum *Chlorella* and its meaning in/for the environment to strengthen the meaning of your study for the Biogeosciences readers. Which strain was used?

Material and Methods: 12mM is a pretty high concentration for nitrite, in how far are the results transferable to anything in the environment, then?

e.g. p. 9741, l.20: cite the original studies of Santoro et al. and Löscher et al. as these are not findings by Hatzenpichler, who reviewed the topic.

p.9744, l.5: I guess, not everyone is aware of the protocol of Fragerstone et al., thus, it could be helpful to introduce it with one sentence.

p.9744, l.9: Did you mean Eppendorf?

p.9745, l.12: Did you mean gas chromatography?

p.9751, l. 15ff: The production pathway in AOA is not that unclear anymore, it seems to be similar to the bacterial pathway (see Vajrala, et al. PNAS, 2013).

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p.9752,l. 9: NO detoxification is a feature of denitrification, not of nitrification

Fig. S2-1: Present this as a table.

Fig. S3-1: I would propose to put this figure into the main text, from my point of view, this figure is very impressive and makes a strong point with regard to the potential production pathway.

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