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Biogeosciences
Discussions

Interactive comment on "Direct O_2 control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments" *by* Adeline N. Y. Cojean et al.

Adeline N. Y. Cojean et al.

adeline.cojean@unine.ch

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Response to comments by Anonymous Referee #2

General comments: In my classical (and perhaps narrow-minded?) view the conclusion on aerobic nitrate reduction and on the partition between DNRA and denitrification in relation to oxygen is still a bit controversial. Therefore I would like the authors to present more information about the actual conditions of their experiments, and apply stronger statistics, as well as present the data from their statistical analyses (it is mentioned that t-tests were used, but no results (p values etc) are presented.

Reply: We thank the anonymous reviewer for her/his insightful comments and questions, which we will address point-by-point below. Indeed, we will, present more information on the experimental conditions and we will include results of the statistical analyses in the revised manuscript.

1. The experiments were performed as slurry incubations in serum bottles to which 15no3 were added. What was the resulting 15NO3 concentration in the slurries?

Reply: The final NO3- conc. in slurry was $\sim$ 120 μmol L-1. This information will be included in the revised manuscript.

2.Oxygen was added to the headspace in the slurries and monitored with Oxygen Sensor Spots during the course of the experiments. Please present the data on oxygen concentrations in the serum bottles, trough out the course of the experiment. Is the oxygen concentration constant during the course of the experiment for all treatments or does the concentrations drops to critical levels in some of those? Present those data eventually in the supplementary information.

Reply: The data on O2 concentration over the incubation time will be included in the supplementary information. Yes, targeted O2 concentrations were as much as possible maintained during the course of the experiments by injection of pure O2 whenever necessary. The measured O2 concentration never dropped below 85 % of the initial targeted concentration in all vials.

3.The rates of DNRA and denitrification were calculated from the accumulation of 15N2 and 15NH4 over the course of an incubation period of 10 hours. Please present data that shows how the concentration of the isotopes changed during the course of the experiment. Do you see a linear increase in the concentration of the isotopes as function of time during the entire incubation period? Can you document such an increase eventually through linear regression analysis? Point 2 and 3 are mandatory for a reliable interpretation of the data. The optimal situation is a) The oxygen concentration does not drop significantly (or not critically) during the course of the experiment. b) There is

a linear increase in the concentration of the isotopes as function of time. An eventual derivation from this situation can compromise the validity of the experiment and the conclusions that can be drawn from the data.

Reply: A representative, exemplary graph showing the evolution of 15N-N2 conc. over time will be included in the supplementary information. Essentially in all incubations the concentration of produced 15N-N2 exhibited a linear increase over the incubation time (4 time points), which was confirmed by the linear regression analysis (mean of R2 > 0.86 for all experiments). However, concerning the production of 15NH4+, the concentration of 15NH4+ was unfortunately measured only at the beginning and at the end of the incubation only (to avoid dilution during sampling of liquid samples). Yet, preliminary tests of the method with 4 time points displayed a linear increase also of 15NH4+ over time and we can thus expect a similar response in all experiments. We agree with the reviewer that point 2 and 3 are very important for a reliable interpretation. But as stated above, a) the O2 concentration did not drop that much below target levels and b) at least for the 15N-N2 production, but most likely also for 15N-NH4+, linear behavior without any significant lack phase or obvious changes in the transformation rates can be confirmed.

4.Statistics is a strong tool for getting sound scientific statements. It is mentioned that t-tests were used to test for differences DNRA and denitrification rates, but no data from these tests are shown. Pleases report those statistical data (eventually in table 1). I would also recommend the use of a statistical method that investigate if the partition of DNRA and denitrification, differs significantly at different oxygen concentrations. Use eventually an ANOVA analysis. You might e.g. use DNRA/(DNRA+Denitrification) i.e. the proportion of DNRA to the measured nitrate reduction rate, as test variable. I understand that the experiment was performed, with replicates and that both rates of denitrification and DNRA were measured simultaneously in the same serum bottle. So it should be possible do a sound statistical analysis. Such an analysis can only strength your interpretation and conclusions. Alternatively use a correlation anal-

ysis, where you investigate for significant (positive/negative) correlation, between e.g. DNRA/(DNRA+Denitrification) and the oxygen concentration.

Reply: We thank the reviewer for her/his valuable inputs regarding the statistical analysis. The t-test results showing the significant difference between denitrification/DNRA rates in the different O2 treatments versus those in the control experiments will be included in the revised manuscript. And yes, we also performed a correlation analysis between the relative contribution of DNRA to the total NO3- reduction (%) and the increase of O2 concentration, and the results displayed a positive correlation coefficient of 0.6 and 0.9 for Figino and Melide, respectively. We will include those results in the text of the revised manuscript.

5. A comment to the statement l.185. It is stated that the high background of 14NH4 prevent the 15NH4 from becoming nitrified, and that the isotope derived rate of DNRA, therefore is not underestimated due to nitrification. I do not think that this argument is valid. The problem is the same as for other tracer studies like S35 based studies of sulfate reduction or 14C based studies of e.g. methane turnover, where you have production and consumption occurring simultaneously. Moeslund et al. (1994) showed with experimental data that Sulfate reduction rates as measured with radiotracers, added to the experimental system at very low concentrations was underestimated if sulfide oxidation was present. Xiao et al. (2018) showed from modeling of a tracer study that the degree of underestimation of rates of methane production was proportional to the incubation period in systems with methane production and methane oxidation. I suggest therefore that you delete this statement. Note that if your overall conclusions regarding DNRA and denitrification and oxygen are correct, an eventual underestimation of DNRA at high oxygen concentrations, would not compromise that conclusion. Moeslund, L., B. Thamdrup, and B. Barker Jørgensen. 1994. Sulfur and iron cycling in a coastal sediment: Radiotracer studies and seasonal dynamics. Biogeochemistry 27: 129-152. Xiao, K.-Q., F. Beulig, H. Røy, B. B. Jørgensen, and N. Risgaard-Petersen. 2018. Methylotrophic methanogenesis fuels cryptic methane cycling in marine surface

sediment. Limnol. Oceanogr. 63: 1519-1527.

Reply: We thank the reviewer for her/his valuable input and the literature provided. In the revised manuscript, we will delete the statement.

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