

## ***Interactive comment on “Direct O<sub>2</sub> control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments” by Adeline N. Y. Cojean et al.***

**Anonymous Referee #2**

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Cojean and Co workers investigate the partition between DNRA and denitrification at different oxygen concentrations in slurries prepared from lake sediments. From their experimental data they conclude that that NO<sub>3</sub> reduction rates (here DNRA and denitrification rates) are generally reduced in the presence of oxygen as compared to rates measured at anoxic conditions, but that nitrate reduction is still going on at oxygen concentrations ranging from 0.8 to 78.6 μM. The authors further conclude from their experimental data that the partition of nitrate reduction between denitrification and DNRA differs at different oxygen concentrations, so that the relative importance of DNRA increases with increasing oxygen concentrations. In my classical (and perhaps narrow minded?) view the conclusion on aerobic nitrate reduction and on the partition between

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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).

1. The experiments were performed as slurry incubations in serum bottles to which 15NO<sub>3</sub> were added. What was the resulting 15NO<sub>3</sub> concentration in the slurries? 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, through 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. 3. The rates of DNRA and denitrification were calculated from the accumulation of 15N<sub>2</sub> and 15NH<sub>4</sub> 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.

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. Please report those statistical data (eventually in table 1). I would also recommend the use of a statistical method that investigate if the parti-

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

5. A comment to the statement I.185. It is stated that the high background of  $^{14}\text{NH}_4$  prevent the  $^{15}\text{NH}_4$  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 S<sub>35</sub> based studies of sulfate reduction or <sup>14</sup>C 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.

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