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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.

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Response to comments by Anonymous Referee #1

I have several methodological and interpretation questions. Also I think the data could be used to obtain more information in the processes studied. many of the questions left open are supposed to be in another article in prep (Cojean in prep). Maybe the authors should consider to include this data here.

Reply: We thank the reviewer for her/his valuable inputs. We will make changes in the revised manuscript accordingly. In particular, we will consider mentioning some of

the results from the companion paper, which we plan to submit soon. In any case, the scope of the two articles is very different, and the main focus of the other paper is on the role of inorganic electron donors (e.g. Fe2+, H2S) in regulating the partitioning between denitrification and DNRA. As Fe2+ and H2S levels, as well as the presence of NOx itself, are redox-dependent, O2 may indirectly affect denitrification and DNRA rates simply by affecting the Fe2+, H2S or NOx concentrations. We will stress this point in the revised manuscript, but we prefer not to discuss these aspects in greater detail, as they will be part of the paper on Fe2+/H2S-modulation.

L74. "aquatic", you mean limnetic?

Reply: No, we actually meant aquatic in general. To our knowledge, there are no experimental studies on the systematic O2 control on DNRA (e.g. continuous control of the O2 concentration over time) with samples from an aquatic environment (e.g. marine or freshwater). Most of the time, in previous research, measured DNRA rates were simply correlated with the in situ O2 concentrations.

L89. "ventilated", waters are oxygenated but not ventilated

Reply: We will change that.

L97-99. How do authors explain the presence of nitrate under anoxic conditions?

Reply: Nitrate diffuses down to bottom waters, where it is consumed by denitrification after the onset of denitrification in June. Thereafter, concentrations decrease, but nitrate is not used up completely, simply because the duration of anoxia until spring mixing is not long enough. In the ocean, the presence of nitrate in oxygen-free water column zone is the norm rather than the exception.

L102. At which temperature was the profiling made?

Reply: At room temperature ($\sim$20°C).

L104. How were the cores sectioned ? Under N2 or normal atmosphere? Please add

information on time, temperature and xg of centrifugation.

Reply: Cores were sectioned under normal atmosphere. Sediment sections were centrifuged for 10 min at 4700 rpm (room temperature). This information will be included in the revised manuscript.

L109. I understand that all manipulations were performed under normal atmosphere.

Reply: No, sampling of gas and liquid samples were performed in a glovebox under N2 atmosphere to avoid any O2 contamination. Also, before incubations, the slurries were preincubated overnight to remove any potential traces of O2. Additionally, N2 contamination was avoided by flushing the gas-tight syringe several times with He prior to sampling. The procedures and precautions to avoid O2 or N2 contamination are routine in our labs.

L111. Can you provide a reference for the artificial lake water?

Reply: Yes, this will be included in the revised manuscript. The reference is: Smith E.J., Davison W. and Hamilton-Taylor J. (2002) Methods for preparing synthetic freshwaters. Wat. Res. 36, 1286-1296.

L115. Flushing and preincubating overnight have an impact on the presence of other gases (N2O, CO2 etc) as well as on the availability of labile organic matter, both dissolved and particulate. A comment on these limitations of sediment slurries should be added in my opinion.

Reply: We are not sure whether purging will significantly affect the DOC pool, which consists mostly of longer carbon-chain molecules rather than volatiles. But certainly, the interference with in situ conditions in general is non-negligible. For example, larger aggregates in the sediments might have been disrupted altering microbe-particle interactions. Also, the pH may have been changed slightly. We will mention this aspect in the revised manuscript.

L117. No information on how much nitrate was added is given. How does it compare

to the natural concentrations? If the concentrations used are not saturating for the enzymes in question, relative rates can be dependent on the enzymatic kinetics of each process.

Reply: This information will be included in the revised manuscript. The final NO3- conc. in slurry was $\sim$ 120 μmol L-1. The experimental concentration was thus higher than the in situ one and significantly higher than the half saturation concentrations for either of the nitrate-reducing processes.

L118. Details should be added on how the duration of the experiments and time points were determined. Was it based on the oxygen evolution? On some previous experiments? How did the authors know in advance that the incubation should last approx 10 h?

Reply: This information will be added in the revised manuscript. Preliminary tests were performed in order to assess the minimal incubation time required to obtain a measurable signal for 15N-N2 measurement through mass spectrometry. Initially, the incubation lasted for 24 h, however, the precise monitoring of O2 conc. over such a long time was quite difficult and NO3- conc. dropped quite rapidly, which may have affected the partitioning between the two processes. Therefore, we tested several incubation periods (15 h, 10 h), and ultimately decided to go with 10 h incubations. This way, we were able obtain clear and robust 15N-N2 signals and control of O2 conc. in slurries in parallel experiments was easier to manage.

L121. Any particular reason for the difference in the compensation method? If transfer of liquids was performed under normal atmosphere how did the authors account for any oxygen contamination? How were samples poisoned to prevent microbial activity?

Reply: We decided to use 2 different compensation methods for T0 and other time points in order to avoid, as much as possible, the dilution of the residual nutrient and gas pools by milli-Q and He addition, respectively. At T0, He was used because we collected a greater liquid volume (6 mL) in order to measure nutrient concentrations and

DNRA rates, while at T1 and T2, only a 2 mL gas sample was taken in exchange with anoxic milli-Q instead of He. At T3, the incubation was stopped and we did not need to compensate for pressure changes inside the vials anymore, therefore no liquid/gas was added. As mentioned above, all liquid and gas samples were performed in a glovebox under N2 atmosphere to prevent O2 contamination, and additional contamination of N2 was prevented by flushing the syringe several times with He prior to sampling. Samples were not poisoned. Liquid samples were filtered (0.2 μm) and stored at -20°C.

L125. Monitoring of O2 concentrations was possible with the optode spots. It is important to know the degree of the "marked decline" was observed and have an estimate of the possible effect on the rates (by looking at the corresponding N species concentrations).

Reply: A table showing the exact O2 concentration measured in slurry at different time points during the incubation will be included in the supplementary information. Oxygen concentration did never drop below 85% of the initial targeted O2 concentration. We will therefore replace “marked decline” by “decline” in the revised manuscript.

L132. Gordon reference is missing.

Reply: Thanks, it will be added.

L140. Did the authors measure organic carbon content which is an important factor in the partitioning between DN and DNRA?

Reply: We did not measure the organic carbon (OC) content during these incubation experiments, but we do not think that changes in OC content over the incubation time played an important role in regulating the partitioning between the two processes in this particular case. In general, the most important factor to consider is the ratio OC/ NO3-, where DNRA is favored at high OC/ NO3- (e.g. NO3–limiting) ratios, and denitrification at lower OC/NO3- ratios (e.g OC-limiting; van den Berg et al., 2016). We agree that changes in OC content over the incubation time likely varied among the different

O2 treatments (e.g. higher OM remineralization rates at higher O2 conc.), however, OC concentration measurement in slurry incubation experiments within the frame of another study using sediments from the same setting displayed relatively high OC concentrations (initial OC concentration $\geq$ 510 mg/L; Cojean et al., in prep.), suggesting that OC was never limiting over the 10 h incubation period (8°C). Similarly, NO3- was always present in excess during the entire incubation experiment (maximum decrease in NO3- conc. of about 8 μmol L-1 from T0 to Tend). Hence, as neither NO3- nor OC were limiting, it is likely that the partitioning between denitrification and DNRA in the different O2 treatments, as compared to control incubations, was not so much affected by changes in the OC/ NO3- but rather by O2. van den Berg E.M., Boleij M., Kuenen J.G., Kleerebezem R. and van Loosdrecht M.C.M. (2016) DNRA and denitrification coexist over a broad range of acetate/N- NO3- ratios in a chemostat enrichment culture.

L147. Rates were determined based on initial and final data? or regression over time?

Reply: Rates were determined through linear regression of 15N-N2 concentrations versus time. We will mention it in the revised manuscript.

L162. Maybe I missed it somewhere but I did not find any statistical analysis of the data.

Reply: We will include results of the statistical analyses in the revised manuscript.

The profiles do indeed indicate that the surface sediment of the Figino is less at the surface than further below. Any reason why? However, the profiles of nitrate do not indicate directly that the sediment acts as a sink given that nitrate and nitrite microprofiles occur same as for oxygen at a mm scale.

Reply: Less what? It is unclear to us what the reviewer refers to. Anyway, the sediments represent a strong sink for NOx. Nitrate is consumed quantitatively within the first mm, and there is not the slightest indication for net production of NOx, e.g. indicated by a local NO3- maximum.

L180. A treatment with ATU to block nitrification would have clarified many of the issues mentioned here.

Reply: We agree that this would have been a good idea. Nevertheless, we believe that our data set is very much meaningful.

L185 & table 1. Rates of 1.1 umol N g d are very low and probably close to the detection limit of the methods used. Data on detection limits should be added in M&M. This is critical when standard error of 6 replicates is 130% of the mean value.

Reply: The detection of the method is on the order of 0.02 μmol L-1. We will mention this in the M&M section.

L192. The authors could take advantage of their data and provide kinetic rate data calculations to allow for easier comparisons with other studies. Also given the big dispersion of the data it would also provide more quantitative information on the differences between sites and processes. Error of percentages should also be provided L196.

Reply: Given the interference with in situ condition, and the addition of substrates in excess, we do not think that the absolute rates, which must be considered potential rates, matter in the context of this study. We specifically look at the relative rates, relative to controls, and relative with regards to the ratio of denitrification and DNRA. As mentioned above, we will provide information on the error of the percentage numbers.

L214. reduced should be consumed. There is a strong discrepancy as the authors mention between the budget and the order of N species transformations based on stable isotopes and total rates.

Reply: We will replace “reduced” with “consumed”.

L217 authors claim that biotic immobilization can be an explanation, however, previously they mention that N species exchangeable fraction was insignificant.

Reply: We referred to the abiotic exchangeable fraction through NH4+ adsorption to the sediment, which we consider negligible. Yet, we cannot exclude completely the biotic immobilization of NO3- in NO3–storing microorganisms, especially since we observed the presence of Beggiatoa sp., which are able to store significant amounts of NO3- in the studied sediments (Cojean et al., in prep.).

L224. Authors should try to put into quantitative perspective their findings given that they have oxygen concentration data for bottom water.

Reply: It is tempting to extrapolate our experimental results to the situation in the water column, and thus assess, for example, expected changes in the denitrification/DNRA ratio with changing O2 concentrations in the near-bottom waters. While there will be a more or less direct link between the bottom water O2 concentrations and the O2 penetration within sediments, we can only speculate about what the O2 concentrations will be right at and below the sediment-water-interface over the annual cycle. In any case, independent of the O2 in bottom waters, O2 will be consumed to completion by aerobic respiration and the oxidation of reduced substances within the first mm of the sediment column.

L237. The effect of oxygen concentration on the partitioning between nitrate reduction will only have an impact on a um scale.

Reply: See last comment. We agree that O2 will only regulate the partitioning between the two nitrate reduction processes in the surficial sediments. Upscaling it to the entire lake-basin is problematic.

L280. Author should show the time evolution of oxygen in the slurries to prove no microniches were created in the slurries. Just mentioning that it is unlikely they were formed is speculation. Where was the spot placed? How many per bottle? Was it always under water? Was any sort of beads were included in the bottles?

Reply: We will upload a table with the O2 conc. measured over time in the supplemen-

tary information. Oxygen spots were placed on the glass wall of each serum bottle and the spot remained under water during measurement. No beads were included, but we shook the bottles manually every 30 minutes, prior to O2 measurement, and between O2 readings, the bottles were placed on a shaker (80 rpm). As mentioned above, we did not observe any dramatic decline of O2 conc. in any of the experiments and as the slurries were strongly diluted, we think that microniches did not form.

L285. This makes no sense as pelagic communities are more easily exposed to high oxygen concentrations and thus should be more tolerant, rather than the opposite.

Reply: Good point, and we agree that this is speculation. We will delete the statement.

L292. These sections (4.3-4.5) are purely based on speculations and are not based on any data from this study. Actually, these sections just highlight the limitations of the study.

Reply: We do not agree. In 4.3, for example, we provide conclusive experimental data that indicate that, in relative terms, DNRA is favored over denitrification under less reducing conditions. Going one step further, true, we speculate about the reasons for our observational data, comparing our experimental data with observations from other natural environments. This way we test plausibilities. These sections are part of an honest scientific discussion, where we try to explain the observations we made. Yes, there is a certain degree of speculation involved, and we cannot always provide final answers. Fair enough. But speculative parts are clearly highlighted as such, and it seems appropriate to discuss the potential implications of our results, even if we cannot provide conclusive evidence for all the statements. We agree that our study has limitations, and we do not hide them. And these limitations may become most evident in these speculative sections the reviewer refers to. But we opt to leave them in, maybe rephrased even more cautiously, as they are intended to stimulate future work that will verify/falsify the putative regulating mechanisms proposed.

L314. How did the authors ensure lack of H2S in the slurries forming? is is by elimi-

nating SO4 presence or its formation somehow?

Reply: We measured free H2S concentrations and they were below or close to the detection limit. We will mention this point in the revised manuscript.

L320. Authors have no data to discuss the partitioning between the activity of NAP and NAR enzymes.

Reply: See comment above. Yes, this is speculative, and we will rephrase this part more carefully, so that it is clear that we speculate. But, in the attempt to explain the results, we find, there is some room for speculation. We observe clear experimental evidence for direct O2 control of denitrification versus DNRA. The most obvious “corner” to look for plausible explanation would be at the enzymatic level, and not mentioning this (even if we cannot provide molecular data) would seem unmindful to us. The differential response of denitrifiers and nitrate ammonifiers at least suggests a distinct O2 sensitivity of the nitrate reductase enzymes involved. Unfortunately, we do not have information on the activity of NAP versus NAR, but in line with what we mentioned above, we would like to propose plausible hypothesis that can then be tested by future work.

L412. The data do not differentiate in any way between NAR and NAP.

Reply: Agreed. Since we do not have any solid data in this regard we will delete “(NAR versus NAP)”. But we would like to stick to our more general conclusion that the balance between DNRA and denitrification is modulated by O2 at the nitrate-reducing enzyme level.

Fig 2. Are data single measurements or mean of several replicates? Oxygen in water column of panel b is missing. Y axis scale is not "oxygen penetration depth" but just depth, same as in the first y axis. In the fully mixed lake conditions, oxygen concentrations does not increase above 150 uM yet in the microprofiles O2 is 250 uM.

Reply: The results show the mean downcore concentrations measured in duplicate and triplicate cores for N species and O2 conc., respectively. The second x-axis label

is indeed depth at a different scale, and we will correct it. Concentrations measured in bottom water and overlying water during O2 microprofiling do not match because the microprofiling was not performed under in situ conditions but under aerated conditions (and higher temperature). Thus the O2 profiles reflect the potential biological oxygen demand of the two sampling stations, and the ex-situ O2 penetration depth was meant to serve the biogeochemical characterization of the sediments.

Fig 3. I thin it is better to show the rate data of the same site in the same panel as in Fig 4. Also use different colours to be able to differentiate between thte two.

Reply: We would like to keep the original graph. Indeed, we aimed to show that, independently of the studied site, we observed similar patterns regarding the absolute O2 control on denitrification and DNRA rates. We will, nevertheless, use different colors to differentiate between the two processes.

Fig 4. Mean values can be represented as points with their appropriate error bars plus a statistical analysis to confirm significant differences.

Reply: The statistical results will be included in the manuscript and adequately presented in the figures.

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