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.

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. Fe^{2+}, H_2S) in regulating the partitioning between denitrification and DNRA. As Fe^{2+} and H_2S levels, as well as the presence of NO_x itself, are redox-dependent, O_2 may indirectly affect denitrification and DNRA rates simply by affecting the Fe^{2+}, H_2S or NO_x 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 Fe^{2+}/H_2S-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 O_2 control on DNRA (e.g. continuous control of the O_2 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 O_2 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 (~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 N_2 atmosphere to avoid any O_2 contamination. Also, before incubations, the slurries were preincubated overnight to remove any potential traces of O_2. Additionally, N_2 contamination was avoided by flushing the gas-tight syringe several times with He prior to sampling. The procedures and precautions to avoid O_2 or N_2 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 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 NO_3^- conc. in slurry was 120 ± 2 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 ^{15}N-N_2 measurement through mass spectrometry. Initially, the incubation lasted for 24 h, however, the precise monitoring of O_2 conc. over such a long time was quite difficult and NO_3^- 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 to obtain clear and robust ^{15}N-N_2 signals and control of O_2 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 N_2 atmosphere to prevent O_2 contamination, and additional contamination of N_2 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 O_2 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 O_2 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/ NO_3^-, where DNRA is favored at high OC/ NO_3^- (e.g. NO_3^--limiting) ratios, and denitrification at lower OC/NO_3^- 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 O_2 treatments (e.g. higher OM remineralization rates at higher O_2 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 ≥ 510 mg/L; Cojean et al., in prep.), suggesting that OC was never limiting over the 10 h incubation period (8°C). Similarly, NO_3^- was always present in excess during the entire incubation experiment (maximum decrease in NO_3^- conc. of about 8.1 ± 1.5 µM from T0 to Tend). Hence, as neither NO_3^- nor OC were limiting, it is likely that the partitioning between denitrification and DNRA in the different O_2 treatments, as compared to control incubations, was not so much affected by changes in the OC/ NO_3^- but rather by O_2.

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- NO_3^- 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 ^{15}N-N_2 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 NO_x. Nitrate is consumed quantitatively within the first mm, and there is not the slightest indication for net production of NO_x, e.g. indicated by a local NO_3^- 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 NH_4^+ adsorption to the sediment, which we consider negligible. Yet, we cannot exclude completely the biotic immobilization of NO_3^- in NO_3^--storing microorganisms, especially since we observed the presence of *Beggiatoa* sp., which are able to store significant amounts of NO_3^- 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 O_2 concentrations in the near-bottom waters. While there will be a more or less direct link between the bottom water O_2 concentrations and the O_2 penetration within sediments, we can only speculate about what the O_2 concentrations will be right at and below the sediment-water-interface over the annual cycle. In any case, independent of the O_2 in bottom waters, O_2 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 O_2 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 O_2 conc. measured over time in the supplementary 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 O_2 measurement, and between O_2 readings, the bottles were placed on a shaker (80 rpm). As mentioned above, we did not observe any dramatic decline of O_2 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 eliminating SO4 presence or its formation somehow?

Reply: We measured free H_2S 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 O_2 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 O_2 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 O_2 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 O_2 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 O_2 microprofiling do not match because the microprofiling was not performed under in situ conditions but under aerated conditions (and higher temperature). Thus, the O_2 profiles reflect the potential biological oxygen demand of the two sampling stations, and the ex-situ O_2 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 O_2 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.

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 NO_3^- conc. in slurry was 120 ± 2 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 O_2 concentration over the incubation time will be included in the supplementary information.

Yes, targeted O_2 concentrations were as much as possible maintained during the course of the experiments by injection of pure O_2 whenever necessary. The measured O_2 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 $^{15}N\text{-}N_2$ conc. over time will be included in the supplementary information. Essentially in all incubations the concentration of produced $^{15}N\text{-}N_2$ exhibited a linear increase over the incubation time (4 time points), which was confirmed by the linear regression analysis (mean of $R^2 > 0.86$ for all experiments). However, concerning the production of $^{15}NH_4^+$, the concentration of $^{15}NH_4^+$ 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 $^{15}NH_4^+$ 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 O_2 concentration did not drop that much below target levels and b) at least for the $^{15}N\text{-}N_2$ production, but most likely also for $^{15}N\text{-}NH^{4+}$, 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 analysis, where you investigate for significant (positive/negative) correlation, between e.g. DNRA/(DNRA+Denitrification) and the oxygen concentration.

Reply: We thank the reviewer for his valuable inputs regarding the statistical analysis. The t-test results showing the significant difference between denitrification/DNRA rates in the different O_2 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 NO_3^- reduction (%) and the increase of O_2 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.

R5: We thank the reviewer for her/his valuable input and the literature provided. In the revised manuscript, we will delete the statement.

List of relevant changes

Adjustments according to our responses to comments by anonymous referee #1

Line 86: “ventilated” changed to “oxygenated” (Ref. 1, R1)
Line 101: Information about the temperature added (Ref.1, R2)
Line 103: Additional information about the core sectioning procedure included (Ref. 1, R3)
Line 108: The reference for the artificial lake water included (Ref. 1, R4)
Line 113-115: Additional information about the potential influence of He purging on the in situ conditions provided in the Materials and Methods section 4.3 (Ref. 1, R5)
Line 116: Details provided about the NO_3^- added in slurry experiments (in the Materials and Methods section 4.3) (Ref. 1, R6)
Line 118-120: Additional information concerning the design of the experimental set-up (e.g. duration of the experiments) added in the Materials and Methods section 2.3 (Ref. 1, R7)
Line 139 & 285: A table showing the O_2 concentrations during the incubation experiments added in the Supplementary Information Table SI.1 (Ref.1, R8)
Line 145: Additional details about the detection limit of the NO_x-analyser included in the Materials and Methods section 2.5 (Ref. 1, R9)
Line 153: Additional information concerning the calculation of ^{15}N-N_2 production rates added in the Materials and Methods section 2.6 (Ref. 1, R10)
Line 195-202: Additional information about the error of the percentage numbers provided (Ref. 1, R11)
Line 214: We replaced “reduced” with “consumed (Ref. 1, R12)
Line 289: Statement about the greater resistance of benthic microbial communities than pelagic ones at high O_2 concentration deleted (Ref. 1, R13).
Line 321: Additional information concerning the H_2S concentrations in slurries provided (Ref. 1, R14)
Line 321/325/330/350/353: Parts of the discussion that were quite speculative rephrased (Ref. 1, R15)
Line 422: Statement about “Nar versus Nap” deleted (Ref. 1, R16)

Figure 3: Statistical analysis of the data included in the figure (Ref. 1, R17)
References/Figures: several minor adjustments of graphs according to the referee’s remarks

Adjustments according to our responses to comments by anonymous referee #2

Line 116: Detail about the NO_3^- amendments provided in the Materials and Methods section 4.3 (Ref. 2, R1)
Line 139 & 285: A table showing the O_2 concentration trends during the incubation experiments added in the Supplementary Information Table SI.1 (Ref.2, R2)
Line 154: An exemplary graph showing the evolution of ^{15}N-N_2 production over incubation time (and regression analyses) for two O2 levels included in the supplementary information (Ref. 2, R3)
Figure 3 & line 203: Statistical analysis of the data included in Figure 3, in the results section 3.3 and in the Supplementary Information Fig. SI.2 (Ref. 2, R4)
Line 187: Statement suggesting that, due to high $^{14}NH_4^+$ background, the portion of $^{15}NH_4^+$ being nitrified was negligible, deleted (Ref. 2, R5)

Direct O_2 control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments

Adeline N.Y. Cojean[1, 2], Jakob Zopfi[1], Alan Gerster[1], Claudia Frey[1], Fabio Lepori[3], Moritz F. Lehmann[1]

[1]Aquatic and Stable Isotope Biogeochemistry, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland
[2]Now at: Centre for Hydrogeology and Geothermics (CHYN), University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland
[3]Institute of Earth Science, Scuola Universitaria Professionale della Svizzera Italiana (SUPSI), Trevano, CH-6952 Canobbio, Switzerland

Correspondence to: Adeline N.Y. Cojean (adeline.cojean@unine.ch)

Abstract. Lacustrine sediments are important sites of fixed nitrogen (N) elimination through the reduction of nitrate to N_2 by denitrifying bacteria, and are thus critical for the mitigation of anthropogenic loading of fixed N in lakes. In contrast, dissimilatory nitrate reduction to ammonium (DNRA) retains bioavailable N within the system, promoting internal eutrophication. Both processes are thought to occur under oxygen-depleted conditions, but the exact O_2 thresholds particularly of DNRA inhibition are uncertain. In O_2-manipulation laboratory experiments with dilute sediment slurries and $^{15}NO_3^-$ additions at low- to sub-micromolar O_2 levels, we investigated how, and to what extent, oxygen controls the balance between DNRA and denitrification in lake sediments. In all O_2-amended treatments, oxygen significantly inhibited both denitrification and DNRA compared to anoxic controls, but even at relatively high O_2 concentrations ($\geq$ 70 µmol L^{-1}), nitrate reduction by both denitrification and DNRA was observed, suggesting a relatively high O_2 tolerance. Nevertheless, differential O_2 control and inhibition effects were observed for denitrification versus DNRA in the sediment slurries. Below 1 µmol L^{-1} O_2, denitrification was favored over DNRA, while DNRA was systematically more important than denitrification at higher O_2 levels. Our results thus demonstrate that O_2 is an important regulator of the partitioning between N-loss and N-recycling in sediments. In natural environments, where O_2 concentrations change in near bottom waters on an annual scale (e.g., overturning lakes with seasonal anoxia), a marked seasonality with regards to internal N eutrophication versus efficient benthic fixed N elimination can be expected.

1 Introduction

Over the last decades, intensive human activities have dramatically affected the nitrogen (N) cycle in aquatic systems through elevated inputs of reactive (biologically available) N. In some lakes, external N loading can lead to excessive algal blooms in the upper water column, and the subsequent decomposition of the sinking algal biomass is often associated with O_2 depletion in the deeper water column, and possibly, seasonal or permanent anoxia (e.g. Blees et al., 2014; Lehmann et al., 2004, 2015). Depending on the O_2-concentrations in the water column, and the reactivity of the sediment organic matter, the oxygen

penetration depth within lacustrine sediments can vary (e.g. Lehmann et al., 2009), and so will the transition zone that separates aerobic from anaerobic biogeochemical reactions. Under oxygen-depleted conditions, both in the water column and in sediments, anaerobic N-transformation processes such as denitrification, anammox and/or dissimilatory nitrate reduction to ammonium (DNRA) become important. While denitrification and anammox can mitigate excessive N loading (eutrophication) by converting reactive nitrogen (NO_3^-, NO_2^-, NH_4^+) to N_2, which subsequently returns to the atmosphere, DNRA retains a bioavailable form of nitrogen within the ecosystem, fostering internal eutrophication of lakes (Tiedje, 1988).

The biogeochemical conditions that regulate the partitioning between these different N-transforming processes (and others) in benthic environments remain uncertain, but links to other biogeochemical cycles are likely an important factor. It is commonly accepted, that when organic matter and nitrate concentrations are relatively high, nitrate is mostly reduced to N_2 by denitrifying bacteria (Gruber, 2008; Seitzinger et al., 2006; Seitzinger, 1988). In contrast, DNRA seems to be favoured in sediments with an excess of electron donors (total organic carbon (TOC), H_2S, Fe^{2+}) relative to nitrate (Brunet and Garcia-Gil, 1996; Roberts et al., 2014). In organic matter-rich lake sediments, the contribution of anammox to the total fixed-N transformation fluxes across the sediment-water interface is most likely minor relative to both denitrification and DNRA, since anammox seems to occur primarily in sediments with low organic matter content (Babbin et al., 2014; Thamdrup and Dalsgaard, 2002).

Changes in the redox zonation may have profound impact on the benthic N cycle (Otte et al., 1996). Seasonal cycles of water column mixing and stagnation can modulate the penetration of redox boundaries into the sediments, potentially changing the redox environments of, for example, nitrifiers, denitrifiers, anammox, and DNRA bacteria. Oxygen can hence be considered a key regulator of benthic N exchange (Glud, 2008; Tiedje, 1988), and its effects are multifold. On one side, increasing O_2 concentrations may expand the oxic/suboxic zone where nitrification can supply NO_3^- and NO_2^- for denitrification and anammox, enhancing the overall fixed-N loss (Lehmann et al. 2015). On the other hand, O_2 can inhibit nitrate/nitrite reduction. When surface sediments are oxygenated, the facultative anaerobic microbes will preferably use oxygen, as the heterotrophic respiration with O_2 yields more energy to cells for growth than with other oxidants (i.e., NO_3^-, NO_2^-; Froelich et al., 1979; Payne et al., 2009; Thauer et al., 1977). Moreover, under oxygenated conditions, the synthesis and/or the activity of the key enzymes involved in nitrate/nitrite reduction may be suppressed (Körner and Zumft 1989, Baumann et al. 1996, Dalsgaard et al., 2014). Existing reports on O_2 tolerance and inhibition of denitrification and anammox in environments differ quite significantly. Inhibition may occur already at very low (nanomolar) O_2 concentrations (Dalsgaard et al., 2014), but experimental studies also revealed that relatively high O_2 levels may be required (up to 16% saturation levels) to induce a 50% inhibition of anammox (Jensen et al., 2008; Kalvelage et al., 2011). The apparent persistence of denitrification at relatively high O_2 concentration levels led to a revision of the classical paradigm regarding the absolute O_2 inhibition of nitrate reduction in nature (Tiedje et al., 1988), with important implications regarding the total volume of hypoxic zones in the ocean or in lakes that hosts microbial N_2 production (Paulmier and Ruiz-Pino, 2009).

While oxygen inhibition/tolerance of denitrification and anammox has been studied previously in the ocean water column (Jensen et al. 2008, Kalvelage et al. 2011, Babbin et al. 2014, Dalsgaard et al. 2014), investigations into the O_2 control on benthic N-reduction are rather rare, and limited to sandy and low organic matter marine sediments (Gao et al., 2010; Jäntti and Hietanen, 2012; Rao et al., 2007). Despite intensified research, the exact O_2 thresholds with regards to the direct inhibition of benthic N reduction are still poorly constrained. This is particularly true for DNRA. Recent work has highlighted the significance of DNRA even in the presence of relatively high O_2 concentrations (i.e., at hypoxic levels (i.e., 10-62 µmol L^{-1}), or concentrations even greater than 62 µmol L^{-1}) in estuarine sediments (Roberts et al., 2012, 2014) and marine sediments (Jäntti and Hietanen, 2012), but a systematic investigation of how DNRA is impacted at low micromolar O_2 levels in aquatic sediments (and how in turn the balance between denitrification and DNRA is affected), does to our knowledge not exist.

In this study, we provide first experimental evidence for direct O_2 control on the fate of reactive N in lacustrine sediments with high organic matter content. Through slurry incubation experiments with sediment from a eutrophic lake in Switzerland (Lake Lugano), ^{15}N-labelled substrates and manipulated O_2 concentrations, we investigated the functional response of benthic N-reducing processes to changing O_2 levels. We demonstrate that denitrification and DNRA are differentially sensitive towards O_2, which has important implications for fixed N removal in environments that undergo short- and longer-term O_2 changes, such as seasonally stratified (anoxic) lakes or other aquatic environments with expanding volumes of hypoxia/anoxia.

2 Sampling site, materials and methods

2.1 Sampling location

Sediment sampling took place in the south basin of Lake Lugano, a natural alpine lake situated at the border between Switzerland and Italy. Between April and January, the water column of the basin is stratified, with bottom-water suboxia/anoxia starting in late spring/early summer (e.g., Lehmann et al. 2004; Lehmann et al. 2015). During winter (February/March) the lake turns over and bottom waters are oxygenated until the water column re-stratifies in spring, and bottom-water O_2 concentrations decrease again (Fig. 1). Water column O_2 and N-compound (NO_3^-, NO_2^-, NH_4^+) concentrations were measured as part of a long-term monitoring campaign promoted by the International commission for the protection of Italian-Swiss waters (CIPAIS; Commissione Internazionale per la Protezione delle Acque Italiano-Svizzere) and run by SUPSI (University of Applied Sciences and Arts of Southern Switzerland) on behalf of the Admnistration of Canton Ticino. Sediment cores were taken at two locations, Figino (8°53'37"E, 45°57'31"N, 94 m depth) and Melide (8°57'29"E, 45°56'22"N, 85 m depth) in October 2017, using a small gravity corer (inner diameter 6.2 cm). Figure 1 displays representative seasonal trends in the deep south basin. During oxygenation of the bottom waters, nitrate concentrations in the water 2 m above the sediments reach about 75 µmol L^{-1}, and even during water column anoxia, near-sediment nitrate concentrations rarely dropped below 15 and 5 µmol L^{-1} at Figino and Melide, respectively (Fig. 1; SUPSI data), so that the sediments are constantly exposed to nitrate-

Commenté [MOU1]: Ref. 1, R1

containing bottom waters. Ammonium concentrations in bottom water were relatively high (~ 30-140 µmol L^{-1}) during anoxia and close to the detection limit during months when the water column was mixed.

2.2 Porewater sampling

Porewater oxygen microprofiles were generated using an O_2 microsensor (Unisense) with a tip diameter of 100 µm in duplicate cores from both sites. The overlying water was gently stirred (without disturbing the sediment-water interface) and aerated to determine the O_2 penetration depth under oxygenated conditions at room temperature (~20 °C). Porewater samples for the analysis of dissolved inorganic nitrogen concentrations were obtained by sectioning of a separate set of cores from the same sites at 1 cm-interval under normal atmosphere and at room temperature (~20 °C), and centrifuging of the samples (4700 rpm, 10 min).

Commenté [MOU2]: Ref. 1, R2

Commenté [MOU3]: Ref. 1, R3

2.3 N-transformation incubation experiments

In a first step, incubations to measure potential denitrification and DNRA rates under control (i.e., anoxic) conditions were performed. At each site, fresh surface sediments (upper 2 cm) from duplicate sediment cores were homogenized to prepare dilute sediment slurries. Aliquots of 1 g sediment and 70 mL anoxic artificial lake water (NO_3^-, NO_2^-, NH_4^+-free; Smith et al., 2002) were transferred into 120 mL serum bottles. The use of dissolved-NO_x-free artificial water is important to avoid any potential underestimation of N-transformation process rates due to $^{28}N_2$ production from ambient NO_3^- or NO_2^-- present in bottom waters. Serum bottles were sealed and crimped using blue butyl rubber stoppers and aluminum caps. The sediment slurries (generally in triplicates, Table 1) were He-flushed for 10 min to lower the atmospheric N_2 and O_2 backgrounds, and placed overnight on a shaker (80 rpm) at 8 °C in the dark to remove any residual O_2. It needs to be noted that this He-flushing step, although crucial in our experimental set-up, may have interfered with in situ conditions by altering microbe-particle interactions through disruption of larger aggregates in the sediments or by slightly changing the pH in the sediment slurries. Labeled ^{15}N substrate (i.e., $Na^{15}NO_3^-$, 99% ^{15}N, Cambridge Isotopes Laboratories, 120 ± 2 µmol L^{-1} final conc.) was added in order to quantify potential rates of denitrification and DNRA. During the incubation period (ca. 10 hours), anoxic sediment slurries were kept in an incubator on an orbital shaker (80 rpm; 8 °C). Preliminary tests were performed in order to assess the minimal incubation time required to obtain clear and robust ^{15}N-N_2 production trends, and during which it was feasible to maintain a more or less constant O_2 concentration in parallel slurry experiments. For subsampling of gas and liquid samples, the incubation vials were transferred to an anaerobic chamber with N_2-atmosphere. There, 2-mL gas samples were taken at four successive time points (T_0, T_1, T_2, T_3) for N_2 isotope measurement, in exchange with 2 mL He (T_0) or anoxic Milli-Q water (T_1 to T_3) in order to compensate for any pressure decrease inside the vials. Gas samples were then transferred into 3 mL exetainers (Labco), prefilled with anoxic water, and stored upside down until isotope analysis. Liquid samples (6 mL) were taken at T_0 and T_3 for the quantification of DNRA rates through N-NH_4^+ isotope analysis (see below) and for the assessment of nutrient (NO_3^-, NO_2^-, NH_4^+) concentrations.

Commenté [MOU4]: Ref. 1, R4

Commenté [MOU5]: Ref. 1, R5

Commenté [MOU6]: Ref. 1, R6; Ref. 2, R1

Commenté [MOU7]: Ref. 1, R7

2.4 O_2 manipulation experiments

For the O_2 manipulation experiments, serum bottles were equipped with TRACE Oxygen Sensor Spots (TROXSP5, detection limit = 6 nmol L^{-1} O_2, Pyroscience, Germany), allowing non-invasive, contactless monitoring of dissolved O_2 concentrations in the dilute slurry. The sensor spots were fixed at the inner side of the glass wall with silicone glue and the sensor signal was read out from outside using a Piccolo2 fiber-optic oxygen meter (PyroScience). Different volumes of pure O_2 (99,995%) were injected into the headspace of pre-conditioned and $^{15}NO_3^-$-amended slurries using a glass syringe (Hamilton). For each treatment, the gas volume required to reach the targeted O_2 equilibrium concentration (0.8, 1.2, 2, …, 78.6 µmol L^{-1}) was calculated based on the headspace versus slurry volumes, salinity, and temperature (Garcia and Gordon, 1992). Measured O_2 concentrations in slurries after injection of the respective O_2 gas volumes were always close to the ones calculated (the first measurement was performed 30 minutes after injection to ensure gas equilibration between the gas and the liquid phase). Oxygen concentrations in the slurry incubations were monitored with the fiber-optic oxygen meter every 30 minutes and, in case of a decline in dissolved O_2 due to microbial consumption, O_2 was added in order to return to the initial target O_2 concentrations (Supp. Table SI.1). In addition to continuous agitation on the shaking table, the dilute slurries were vigorously shaken by hand every 30 minutes to avoid the formation of anoxic microniches, which may act to increase rates of anaerobic N-transformation processes (Kalvelage et al., 2011).

Commenté [MOU8]: Ref. 1, R8; Ref. 2, R2

2.5 Hydrochemical analyses

Nitrite concentrations were determined colorimetrically using sulfanilamide and Griess-reagent, according to Hansen and Koroleff (1999). Total NO_x (i.e., NO_3^- + NO_2^-) concentrations were measured using a NOx-analyser (Antek Model 745, detection limit = 0.02 µmol L^{-1}), by reduction to nitric oxide (NO) in an acidic V^{3+} solution, and quantification of the generated NO by chemiluminescence detection (Braman and Hendrix, 1989). Nitrate concentrations were then calculated from the difference between NO_x and NO_2^- concentrations. Ammonium was measured by suppression-ion chromatography with conductivity detection (940 Professional IC Vario, Metrohm, Switzerland).

Commenté [MOU9]: Ref. 1, R9

2.6 ^{15}N-based rate measurements

For the determination of denitrification rates, gas samples from the ^{15}N-isotope enrichment experiments were analysed for $^{15}N/^{14}N$ isotope ratios in the N_2 using a Delta V Advantage isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific) with a ConFlo IV interface (Thermo Fisher Scientific). Denitrification (and negligible anammox) rates were calculated based on the quantification of ^{15}N in the N_2 gas in excess of the natural abundance, i.e. by calculating the linear regression of $^{30}N_2$ concentrations (and to a minor extent $^{29}N_2$) over incubation time (Nielsen, 1992; Thamdrup and Dalsgaard, 2002; Supp. Fig. SI.1). DNRA rates were quantified using the isotope-pairing method described by Risgaard-Petersen et al. (1995). Briefly, 2 mL liquid samples were transferred into 6 mL exetainers (Labco) and closed with plastic screw septum caps. The headspace was flushed with He for 2 min to reduce the $^{28}N_2$ background, and 25 µL mL^{-1} of alkaline (16 mol NaOH) hypobromite iodine

Commenté [MOU10]: Ref. 1, R10

Commenté [MOU11]: Ref. 2, R3

solution (3.3 mol L^{-1}) were added through the septum to convert NH_4^+ to N_2 (Robertson et al., 2016). Exetainers were stored upside down and placed on a shaker (100 rpm) for 24 h at room temperature. The produced N_2 was then analysed by IRMS as described above. DNRA rates were determined based on the $^{15}NH_4^+$ production with time, as calculated from the total ^{15}N-N_2 in the hypobromite-treated samples (i.e., calculated from the excess $^{29}N_2$ and $^{30}N_2$ signals). The recovery of ^{15}N-label from identically treated standards was >95%.

2.7 Statistics

Results are presented as the mean and standard error of n replicate experiments (Table 1). Correlation analyses were performed using Microsoft Excel software, and significant differences between results were verified using a Student's *t*-test ($P < 0.05$).

Commenté [MOU12]: Ref. 2, R4

3 Results

3.1 Porewater hydrochemistry

The O_2 microsensor profiles revealed that the O_2 penetration at the two sites under aerated conditions ranged between 2.4 mm (Melide) and 3.7 mm (Figino, Fig. 2). The relatively low oxygen penetration depth is consistent with a high organic carbon content (~8%, data not shown). According to the observed O_2 concentration gradients at the two stations, the potential O_2 flux into sediments was greater at Melide suggesting a higher reactivity of the sedimentary organic matter. In contrast to the microsensor profiling, the sectioning-centrifuging technique was not sufficient to resolve the exact porewater nitrate concentration gradient, yet the observed nitrate concentration data across the sediment-water interface (Fig. 2) clearly indicate that the sediments at both sites represent a sink for water-column nitrate, and that nitrate is consumed to completion already within the top centimeter of the sediments. In contrast, ammonium concentrations just below the sediment-water interface at Figino and Melide increased steeply from 830 and 600 µmol L^{-1} NH_4^+ to 1.7 and 1.2 mmol L^{-1}, respectively.

3.2 N-transformations in control experiments

Potential rates of denitrification and DNRA under true anoxic conditions were quantified at both sampling sites in October 2017. Anammox rates were measured in a previous study at different times of the year, and its contribution to the total fixed-N removal was always less than 1%, thus negligible with respect to other processes (Cojean et al., in prep.). Indeed, in all experiments, denitrification and DNRA were the main benthic N-transformation processes with an essentially equal contribution to the total nitrate reduction (≈0.1 µmol N g^{-1} wet sed. d^{-1}; Table 1 caption). We ensured that measured DNRA rates were not underestimated due to $^{15}NH_4^+$ loss through adsorption on mineral surfaces. Previous results (Cojean et al., in prep.) demonstrate that adsorption of ambient or tracer ammonium does not occur at detectable levels in the dilute sediment slurries. Ammonium consumption by nitrifiers in presence of O_2, however, might slightly affect quantification of DNRA rates.

Indeed, oxic slurry incubation experiments (≥ 73 µmol L^{-1} O_2) revealed that at least at high O_2 concentrations net NO_3^- production occurs (≤ 1 µmol N g^{-1} wet sed. d^{-1}).

3.3 Impact of O_2 on NO_3^- reduction in sediments

The O_2 sensitivity of denitrification and DNRA and inhibition kinetics were investigated through slurry incubation experiments under O_2-controlled conditions. At both sites, potential denitrification and DNRA rates consistently decreased with increasing O_2 concentration (Fig. 3). While the general pattern was systematic for both processes (i.e., an exponential attenuation of both denitrification and DNRA rates with increasing O_2), the response of denitrifiers versus nitrate ammonifiers towards manipulated O_2 differed across sites and treatments. We compared O_2-addition experiments to the anoxic controls to estimate the inhibition of nitrate reduction by O_2. At the lowest O_2 concentration (~ 1 µmol L^{-1} O_2), denitrification was less inhibited than DNRA at Figino (29 ± 20 % and 51 ± 7 % inhibition, respectively) while the suppression was almost equivalent at Melide (43 ± 8 % and 37 ± 9 % inhibition of denitrification and DNRA respectively, Table 1). At O_2 concentrations around 2 ± 0.2 µmol L^{-1}, both denitrification and DNRA rates were more than 50% inhibited compared to the anoxic control (Table 1, Fig. 3). At O_2 concentration ≥ 2 µmol L^{-1}, DNRA rates were generally higher than those of denitrification (with one exception, i.e., 16 µmol L^{-1} O_2 at Figino; Fig. 3), indicating that denitrification was more sensitive than DNRA to elevated O_2 levels. Oxygen concentrations higher than 73 µmol L^{-1} resulted in almost complete inhibition of denitrification at both sites (96 ± 1 % and 93 ± 2 % at Figino and Melide, respectively, Table 1). Oxygen inhibition thresholds for DNRA were even higher, as DNRA rates were significantly less impaired compared to denitrification at these elevated O_2 levels (79 ± 5 % and 75 ± 4 % inhibition compared to the anoxic controls at Figino and Melide, respectively; Table 1). A correlation analysis between the relative contribution of DNRA to the total NO_3^- reduction (%) and the increase of O_2 concentration displayed a positive correlation coefficient of 0.57 and 0.91 for Figino and Melide, respectively (Supp. Fig. SI.2). Hence, the relative contribution of the two processes to total nitrate reduction was significantly affected by changing O_2 concentrations. At anoxic and submicromolar levels of O_2 (≤ 1 ± 0.2 µmol L^{-1} O_2), denitrification rates were higher than those of DNRA, while at higher O_2 concentration the ratio between denitrification and DNRA was shifted in favour of the nitrate ammonifiers (Fig. 4).

Consistent with the observed decline in denitrification and DNRA rates based on the ^{15}N-N_2 and $^{15}NH_4^+$ measurements in the ^{15}N-label incubations, nitrate consumption in slurries decreased with increasing O_2 concentration at both stations (Table 1). Similarly, maximum ammonium accumulation was observed in the anoxic controls, whereas at higher O_2 levels ammonium underwent net consumption, indicating the concomitant decrease of DNRA and the increasing importance of nitrification under more oxic conditions, particularly at Melide. In incubations where nitrate concentrations decreased, the ratio of $(NO_3^-)_{consumed}$: $(N\text{-}N_2 + {}^{15}NH_4^+)_{produced}$ was always significantly higher (>5:1) than expected (1:1). This observation is consistent with previous work in the Lake Lugano South Basin (Wenk et al., 2014). Here, whole-core flow-through incubations also revealed that NO_3^- fluxes into the sediments significantly exceeded benthic N_2 production, an imbalance, which could neither be explained by nitrate reduction to ammonium nor incomplete reduction to N_2O. As it is not the scope of this study, we will not

Commenté [MOU13]: Ref. 2, R5

Supprimé: Thanks to the comparatively large background pool of ammonium (~ 42 µmol L^{-1} derived from the porewater of the wet sediment and from organic matter N remineralization), the little $^{15}NH_4^+$ generated by DNRA during the incubation (< 0.1 µmol L^{-1}) is strongly diluted, preventing that a significant fraction of the DNRA-derived $^{15}NH_4^+$ is consumed by nitrifying microorganisms.

Commenté [MOU14]: Ref. 1, R11

Commenté [MOU15]: Ref. 2, R4

Commenté [MOU16]: Ref. 1, R12

discuss this puzzling discrepancy further, but we speculate that excess NO_3^- consumption may be linked to bacterial and algal uptake (Bowles et al., 2012). Biotic immobilization of NO_3^- in marine sediments has been attributed previously to the intracellular storage of nitrate by filamentous bacteria (Prokopenko et al., 2013; Zopfi et al., 2001) and/or diatoms (Kamp et al., 2011), but we do not know yet whether such nitrate sinks are important also in Lake Lugano sediments.

4 Discussion

4.1 Anaerobic N-cycling in the South Basin of Lake Lugano

Benthic denitrification and DNRA were the predominant anaerobic N-transformation processes at the two studied stations. Interestingly, the contribution of DNRA was systematically higher than observed in flow-through whole-core incubations performed with sediment from the same basin. Wenk et al. (2014) reported a maximum DNRA contribution to NO_3^- reduction of not more than 12%, but also argued that their DNRA rate measurements must be considered conservative, because they did not account for the production of $^{14}NH_4^+$ from ambient natural-abundance nitrate. The reason for such a discrepancy is unclear, but there seems to be a tendency for slurry incubations to yield higher DNRA rates compared to denitrification (Kaspar, 1983), implying biasing methodological effects. The observed discrepancies may also be related to natural sediment heterogeneity and/or seasonal/interannual fluctuations in benthic N transformation rates. As for the latter, in 2016, the annual water overturn and bottom-water ventilation was exceptionally suspended and sediments remained anoxic for more than a year. In contrast, in 2017, the water column mixed in January and surface sediments were oxygenated throughout June. Our O_2 manipulation experiments revealed that redox conditions have a marked impact on the partitioning between the two nitrate reduction pathways, and consistent with the slurry incubation data, the extended O_2 exposure of microbes at the sediment-water interface in 2017 compared to the preceding year may have favoured nitrate ammonifiers over denitrifiers. Independent of any possible spatio-temporal variability, in this study, DNRA rates were equal, or even higher, than denitrification. Such a partitioning of the two nitrate reducing processes is not implausible and was similarly observed in a wide range of environments, particularly in more reduced sediments with high organic matter content and comparatively low nitrate levels (Brunet and Garcia-Gil, 1996; Dong et al., 2011; Papaspyrou et al., 2014). More generally, substrate-availability changes induced by O_2 fluctuations may be important drivers of the partitioning between denitrification and DNRA (Cojean et al., in prep.), and environmental conditions that favour DNRA over denitrification may be quite common. However, to our knowledge, experimental evidence for the direct O_2 control on the balance between these two nitrate-reducing processes is still lacking.

4.2 O_2 inhibition thresholds of benthic nitrate reduction

Our study shows that submicromolar O_2 levels significantly lowered both, denitrification and DNRA rates. Denitrification and DNRA were inhibited by about 30-50% at 1 µmol L^{-1} O_2, while in previous studies that investigated O_2 effects on fixed-N elimination in the water column, denitrification was almost completely suppressed at this O_2 level already. For example, by conducting incubation experiments using samples from oxygen minimum zones in the Eastern Tropical Pacific, a 50%

inhibition of denitrification was noticed already at 0.2 µmol L^{-1} O_2, and complete suppression at 1.5-3 µmol L^{-1} O_2 (Dalsgaard et al., 2014, Babbin et al., 2014). Similarly, incubation experiments with samples from a Danish fjord exhibited full inhibition of denitrification at 8-15 µmol L^{-1} O_2 (Jensen et al., 2009). In marine sediments, in contrast, denitrification was occurring even at O_2 concentrations greater than 60 µmol L^{-1} (Gao et al., 2010, Rao et al., 2007). This is in agreement with our results showing that at higher O_2 levels (≥73 µmol L^{-1}) denitrification was still active although at very low rates compared to the anoxic control (≥ 93% inhibition). Similarly, DNRA was still occurring, and was less impaired by the elevated O_2 concentration compared to denitrification (≥ 75% inhibition relative to the anoxic control). An increase of DNRA relative to denitrification rates under oxic conditions (> 100 µmol L^{-1} O_2) was also observed in estuarine sediments, though N-removal remained predominant (Roberts et al., 2012, 2014). In brackish sediments in the Gulf of Finland in the Baltic Sea, at elevated O_2 concentrations (from 50 to 110 µmol L^{-1} in bottom waters), benthic DNRA rates were generally higher than denitrification rates (Jäntti and Hietanen, 2012), further supporting our findings. Yet, in contrast to our study, their observations suggest a higher O_2 sensitivity (i.e., greater inhibition) of DNRA compared to denitrification in sediments with higher bottom water O_2 concentrations (> 110 µmol L^{-1}). Given the paucity and discrepancy of existing data in this context, it is premature to conclude that DNRA microbes are generally less or more oxygen-tolerant than denitrifiers. A direct comparison of DNRA O_2 inhibition thresholds in this study and in the study of Jäntti and Hietanen (2012) is difficult because of the differing methodological approaches. There, nitrate reduction rates were determined in whole-core incubations, without manipulating (and measuring) the O_2 concentrations at the sediment depth where nitrate is actually reduced. And although the O_2 penetration depth and porewater O_2 concentrations will respond to a certain degree to the O_2 content in the bottom water, deducing the actual O_2 concentrations for the active nitrate reduction zone within the sediment from O_2 concentrations in the overlying water is problematic. Here, we tested the oxygen sensitivity of a microbial community in suspension, directly exposed to defined O_2 conditions. These incubation data indicate that DNRA is less inhibited than denitrification at O_2 concentrations ≥ 73 µmol L^{-1} and, at the same time, imply that anoxia per se is not a strict requirement for DNRA, as previous ecosystem-scale work has also suggested (Burgin and Hamilton, 2007). Our results also are consistent with observations made in soil microcosms showing that DNRA is less sensitive to increasing O_2 partial pressures than denitrification within the range of 0-2% O_2 v/v (Fazzolari et al., 1998; Morley and Baggs, 2010).

The observed O_2 inhibition thresholds for nitrate reduction are significantly higher than reported from most incubation studies with water column samples (Dalsgaard et al., 2014, Babbin et al., 2014, Jensen et al., 2008). Elevated O_2 tolerance in prior studies was often attributed to the formation of anoxic microniches that may foster anaerobic N-reduction (Kalvelage et al., 2011). It is unlikely that such microniches formed during our incubation experiments since slurries were heavily diluted (1 g sediment in 70 mL water) and vigorously shaken by hand every 30 min, in addition to the continuous agitation on a shaking table during the incubation. Also, experiments were replicated 2-3 times for some O_2-amended treatments, and measured rates were very similar between replicates. If anoxic microniches had formed, we would have expected that their formation is more variable, resulting in a lower reproducibility of the determined rates.

Commenté [MOU17]: Ref. 1, R8

Commenté [MOU18]: Ref. 1, R13

Supprimé: One might speculate that benthic nitrate-reducing microbial communities are more tolerant towards higher O_2 levels than pelagic ones.

The existence of aerobic denitrifiers (e.g. microbes that reduce NO_3^-/NO_2^- to N_2 in presence of O_2) in soils and sediments has been confirmed through isolation of bacterial strains (e.g. Robertson et al., 1995), and it was suggested that they contribute to the total fixed N loss in marine sediments (Carter et al., 1995; Patureau et al., 2000; Zehr and Ward, 2002). Recent studies of permeable marine sediments (Gao et al., 2010) and soils (Bateman and Baggs, 2005; Morley et al., 2008) also observed significant N_2 production in the presence of O_2 and attributed it to aerobic denitrification

4.3 DNRA favoured under less reducing conditions

It is generally assumed that strongly reducing conditions favour DNRA over denitrification, yet in our study, particularly at elevated O_2 concentrations, DNRA rates were higher than those of denitrification. That DNRA often seems to be more important under true anoxic conditions may therefore not be linked directly to the absence of O_2 and differential O_2 inhibition levels of the two nitrate-reducing processes. Indirect mechanisms are likely to be important. For instance, H_2S accumulation, which often accompanies prolonged anoxia, can inhibit denitrification and simultaneously enhance DNRA (An and Gardner, 2002; Rysgaard et al., 1996). Another indirect, redox-dependent factor may be the availability of nitrate. Higher DNRA rates were observed under more NO_3^--limiting conditions induced by prolonged anoxia, probably because nitrate ammonifiers are able to gain more energy per NO_3^- reduced than denitrifiers (Dong et al., 2011). As nitrate concentrations are generally much lower under oxygen-free conditions, it appears plausible that anoxia-associated nitrate and nitrite depletion is conducive to higher DNRA/denitrification rates. While these examples seem to support that DNRA is favoured under true anoxic conditions, results of other studies are more consistent with our observation of higher DNRA than denitrification rates at elevated O_2 concentrations. For example, in estuarine sediments, DNRA was stimulated relative to denitrification under more oxidizing conditions (Roberts et al., 2014, 2012). The authors argued that DNRA is enhanced by increasing Fe^{2+} availability at the oxic-anoxic sediment layer during more oxygenated conditions. These studies highlight the importance of redox conditions in regulating the balance between dentrification and DNRA, however, to what extent O_2 directly controls the partitioning between the two nitrate-reducing processes at the enzyme levels remains, to our knowledge, still unknown. Apparent contradictions with regards to how changing O_2 levels may impact nitrate reduction may simply be due to the counteracting and variable influence of direct versus indirect effects of the variable O_2 concentrations.

We cannot fully exclude that through O_2 manipulation in this study, we partly affected nitrate-reduction indirectly through its control of H_2S or Fe^{2+}. Yet, we set up the experiments in a way that indirect effects should be minimized (e.g., no free sulfide measured in any of the incubations, same organic matter content, same excess NO_3^- concentrations), and this study can thus be considered an investigation into the direct O_2 effect on the partitioning between N-loss by denitrification and N-recycling by DNRA in aquatic sediments. The fact that in our experiments we can essentially exclude the effects of redox-related parameter changes (i.e., H_2S, NO_3^-, and Fe^{2+}) leads us to the conclusion that in the studied sediments from Lake Lugano, O_2

Commenté [MOU19]: Ref. 1, R14

Commenté [MOU20]: Ref. 1, R15

likely controls the balance between denitrification and DNRA at the organism-level, and that denitrification is in fact more sensitive towards increasing O_2 concentrations than DNRA.

4.4 Direct O_2 control on benthic NO_3^- reduction

It has been previously reported that O_2 can either suppress the synthesis of enzymes involved (Baumann et al., 1996) or the enzyme activity itself (Dalsgaard et al., 2014). The observed DIN concentration trends (i.e. decreasing nitrate consumption) with increasing O_2 concentrations suggest that the overall activity is modulated mainly at the nitrate reduction step. Without conclusive information on enzyme activities in hand, we can only speculate at this point about any real difference in O_2-dependent response of the enzymes involved in denitrification versus DNRA. The differential response of denitrifiers and nitrate ammonifiers may, however, suggest a distinct O_2 sensitivity of the nitrate reductase enzymes involved. Denitrifiers and nitrate ammonifiers utilize the same nitrate reductase enzymes (Nar, Nap), and while a differential O_2 sensitivity of the same type of enzyme is difficult to explain, it is certainly possible for different enzymes. Indeed, the membrane-bound (Nar) and the periplasmic (Nap) nitrate reductases have distinct affinities towards NO_3^- and O_2 tolerance (Mohan and Cole, 2007). Periplasmic nitrate reduction is almost exclusively found in the Proteobacteria and many of the organisms possess both Nar and Nap systems, whose production is regulated in response to ambient NO_3^- and O_2 concentrations (Simon and Klotz, 2013). When NO_3^- is scarce, Nap provides a high-affinity (for NO_3^-) but low-activity pathway that does not require NO_3^- transport into the cell cytoplasm (Mohan and Cole, 2007). In presence of oxygen, nitrate transport across the cell membrane is repressed, preventing nitrate reduction by the membrane-bound enzyme Nar with its cytoplasm-facing active site (Moir and Wood, 2001). In contrast, nitrate reduction in the periplasm is less O_2 sensitive, so that microbes possessing and relying on Nap are likely to have an ecological advantage in environments that are subject to O_2 fluctuation (Carter et al., 1995). In nature, nitrate reduction by denitrifiers is assumed to be catalysed primarily by Nar (Richardson et al., 2007), while most nitrate ammonifiers seem to use Nap (Mohan and Cole, 2007).

Clearly, more fundamental research is required in environmentally relevant non-model microorganisms or mixed communities, to understand better the combined effects of O_2 on the nitrogen-transforming metabolic pathways and their regulation. Additional O_2 inhibitory effects at one of the down-cascade enzyme levels (Nir, Nrf, Nor, Nos) are likely to exhibit variable O_2 sensitivities (Baumann et al., 1996, 1997; Körner H. and Zumft, 1989; Poock et al., 2002). While we are aware that the treatment above is speculative, we argue that our observations of higher DNRA/denitrification ratios at higher O_2 provides at least putative evidence that microorganisms performing DNRA using Nap may be more O_2-tolerant than denitrifiers using Nar, a hypothesis that requires further testing.

4.5 Implication for N-elimination versus N-recycling in lakes with fluctuating O_2 conditions

The redox-sensitive partitioning of nitrate elimination (through N_2 production by denitrification) versus fixed-N recycling (by nitrate ammonification) has likely important ecosystem-scale consequences. The annual water column turnover of holomictic

Commenté [MOU21]: Ref. 1, R15

Commenté [MOU22]: Ref. 1, R15

Commenté [MOU23]: Ref. 1, R15

Commenté [MOU24]: Ref. 1, R15

lake basins such as the south basin of Lake Lugano plays an important role in regulating the contribution of N-removal and N-recycling in the water column (Lehmann et al. 2004; Wenk et al, 2014). To which extent O_2 fluctuations affect N transformation reactions within the sediments remains uncertain. Winter water column turnover ventilates the bottom waters and re-oxygenates surface sediments that were anoxic for several months. Hence, at least in the top millimeters of the sediment column, we can expect changes in the benthic N cycling. Based on our incubation experiments, the O_2 inhibition threshold was lower for denitrification than for DNRA, possibly reflecting differential adaption of the in situ microbial community of denitrifiers and nitrate ammonifiers to fluctuating O_2 conditions of bottom waters. Indeed, many nitrate ammonifiers possess both nitrate reductase enzymes (Nap and Nar) and can switch between the two respiratory systems providing them with an ecological advantage over denitrifiers when substrates become limiting (i.e., with regards to the primary reductant used in energy metabolism; Mohan and Cole, 2007). During oxygenated bottom-water conditions, within the benthic redox transition zone, nitrate-reducing microbes at the sediment-water interface will be exposed to elevated O_2 concentrations, similar to the ones tested here. Our experimental data imply that then, at least in the uppermost sediments, DNRA is favoured over denitrification. We may even expect an O_2-regulated zonation of DNRA and denitrification. As a consequence, when denitrification-driven nitrate-reduction is pushed down, it is possible that NO_3^- will be partially consumed through DNRA before it gets to the "denitrification layer", as nitrate ammonifiers are less O_2 sensitive than denitrifiers. In contrast, denitrification is likely to be a more important nitrate-reducing process compared to DNRA during water column stratification (suboxia/anoxia of bottom waters), when the sediments are fully anoxic.

In the discussion thus far, we implicitly assume that the main control O_2 exerts on the absolute and relative rates of denitrification and DNRA is due to its inhibitory effects at the organism-level, yet the effect of O_2 on the coupling of nitrification and nitrate reduction by either denitrification or DNRA remained unaddressed. Oxygen fluctuations in the natural environment will affect nitrate regeneration by nitrification, and hence determine how much nitrate is available for microbial reduction. It has been shown previously that through oxygenation events (e.g., the increase in bottom water O_2 concentrations during episodic mixing/ventilation), the overall benthic N elimination in lakes may be enhanced through coupled nitrification-denitrification, at least transiently (Hietanen and Lukkari, 2007; Lehmann et al., 2015). So, while the direct effect of elevated O_2 would be to hamper fixed N elimination by denitrification at the organism-level, the oxygenation of previously ammonium-laden but nitrate free (pore-) waters would help to better exploit the benthic nitrate-reduction potential by increasing the nitrate availability for nitrate-reducing microbes within the sediments, so that the overall nitrate reduction may be stimulated (Lehmann et al. 2015). Yet, as shown in the present study, oxygenation of the water column and the upper surface sediments may also act to shift the balance between denitrification and DNRA towards DNRA, thus promoting N-recycling rather than fixed-N elimination through denitrification. Total nitrification rates were not measured in this study, but nitrate concentration changes in sediment slurries suggest that at elevated O_2 levels there is at least some production of nitrate. There is no obvious reason to assume that O_2-stimulation of the coupling of nitrification and denitrification on the one hand, and of nitrification and DNRA on the other would per se be different. Yet, as demonstrated here, DNRA appears to be less O_2 sensitive compared

to denitrification. It is thus reasonable to expect a higher coupling of nitrification with DNRA than with denitrification during oxygenated bottom-water conditions. Indeed, there is putative evidence for such an indirect link between O_2 and elevated coupled nitrification-DNRA. In a recent study with estuarine sediments, stronger stimulation of DNRA compared to denitrification was observed during oxygenation of bottom waters, in parts attributed to the coupling to nitrification (Roberts et al., 2012). Additional experimental work is required to better understand the role of nitrification in regulating the balance between benthic denitrification and DNRA during oxygenation of bottom waters.

It is important to understand that in the natural environment, O_2 will not be the only regulator of the balance between denitrification and DNRA. As previously mentioned, the partitioning of the two nitrate-reducing processes can also be modulated by the substrate (e.g., NO_3^-, NO_2^-, TOC, H_2S, Fe^{2+}) availability. The latter may be redox controlled or not. Such regulation may be linked to the differential substrate affinity of the two processes when competing for the same electron acceptor (e.g., nitrate/nitrite) providing selective pressure that can drive communities either towards denitrification or DNRA (Kraft et al. 2014), or simply due to differing substrate requirements in the case of chemolithotrophic versus organotrophic nitrate reduction.

For example, nitrate concentrations in the water column of the lake sampled in this study (Lake Lugano) varied significantly over the year, with very low NO_3^- concentrations during the stagnation period (during anoxia; Fig. 1). As a consequence, it is reasonable to assume that the relative partitioning between denitrification and DNRA in a natural environment is affected by the fluctuating nitrate concentrations (e.g., Tiedje et al., 1988, Dong et al., 2011). Similarly, Fe^{2+} levels in near-bottom waters and sediment porewaters in Lake Lugano are greater during the anoxia/stratification period (Lazzaretti et al., 1992). At least in environments where chemolithotrophic processes contribute to the overall nitrate reduction, such redox-dependent Fe^{2+} concentration changes (or changes of other electron donors such as HS^-) may affect the balance between DNRA and denitrification (e.g., Robertson et al. 2015). Hence, in addition to the direct regulating effects of O_2 on the partitioning between denitrification and DNRA, which we have demonstrated here experimentally, O_2 can act as indirect regulator of fixed N elimination versus regeneration. The ultimate ecosystem-scale DNRA/denitrification ratio in environments that are subject to O_2 fluctuating conditions is difficult to predict, because direct and indirect O_2 regulation may act concomitantly and in opposite ways.

5 Conclusion

The presented results broaden the range of O_2 inhibition thresholds of benthic denitrification at micromolar O_2 levels, demonstrating that benthic denitrification may resist full inhibition up to almost 80 µM O_2. Similarly, sedimentary DNRA does not necessarily require true anoxia, and was even less sensitive than denitrification to higher O_2 levels. Our data suggest that the balance between DNRA and denitrification is modulated by O_2 at the nitrate-reducing enzyme level. However, more

Commenté [MOU25]: Ref. 1, R16

Supprimé: (Nar versus Nap)

in-depth investigations on the exact role of oxygen in regulating other denitrification and/or nitrate-ammonification enzymes in microbial pure culture experiments are needed. The differential tolerance of denitrifiers versus nitrate ammonifiers towards O_2 has important implications for natural environments with fluctuating O_2 conditions. Based on our results, one might argue that DNRA may be more important during phases of bottom-water oxygenation, while anoxic conditions during the stratification period may favour full denitrification to dinitrogen. Whether and when fixed nitrogen is preserved in a lake or eliminated by denitrification is, however, difficult to predict, as this will depend also on multiple indirect effects of changing O_2 levels. For example, nitrification and the redox-dependent modulation of substrates that may be relevant for denitrification or DNRA (such as nitrite, the substrate at the branching point between the two processes, and/or sulfide as potential inhibitor of denitrification and stimulator of chemolithotrophic DNRA) will play an important role both with regards to the overall nitrate reduction rate, as well as the balance between different nitrate reducing processes. Internal eutrophication from N in high-productivity lakes is generally less of a concern than from P. Nevertheless, it needs to be considered that oxygenation may reduce the overall fixed N-elimination capacity of the bottom sediments by impairing denitrification more than DNRA, partially counteracting the generally positive effects of hypolimnetic ventilation in the context of benthic nutrient retention/elimination, and with implications on the nutrient status in the water column.

Data availability

Data can be accessed upon request to the corresponding author.

Author contribution

JZ and MFL initiated the project. ANYC performed all sample collection and conducted the experimental work with help from AG. FL provided the water column chemistry profiles. ANYC, JZ and MFL performed data analysis and interpretation. ANYC and MFL prepared the manuscript with input from all co-authors.

Competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank Thomas Kuhn for technical support in the laboratory and Stefano Beatrizotti, Maciej Bartosiewicz, Guangyi Su and Jana Tischer for assistance during sampling on the lake. We also thank Elizabeth Robertson and Bo Thamdrup for their help during the development of the method for slurry incubation experiments. The study was funded by the Swiss National Science Foundation (SNF) project 153055, granted to Jakob Zopfi and Moritz F. Lehmann. We are also grateful to the Freiwillige

Akademische Gesellschaft (FAG) Basel that also financially supported the study.

References

An, S. and Gardner, W. S.: Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas), Mar. Ecol. Prog. Ser., 237, 41–50, doi:10.3354/meps237041, 2002.

Babbin, A. R., Keil, R. G., Devol, A. H. and Ward, B. B.: Organic matter stoichiometry, flux, and oxygen control nitrogen loss in the ocean., Science, 344, 406–408, doi:10.1126/science.1248364, 2014.

Bateman, E. J. and Baggs, E. . M.: Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space, Biol. Fertil. Soils, 41, 379–388, doi:10.1007/s00374-005-0858-3, 2005.

Baumann, B., Snozzi, M., Zehnder, A. J. B. and van der Meer, J. R.: Dynamics of denitrification activity of *Paracoccus denitrificans* in continuous culture during aerobic-anaerobic changes, J. Bacteriol., 178(15), 4367–4374, doi:10.1128/jb.178.15.4367-4374.1996, 1996.

Baumann, B., Snozzi, M., van der Meer, J. R. and Zehnder, A. J. B.: Development of stable denitrifying cultures during repeated aerobic-anaerobic transient periods, Water Res., 31(8), 1947–1954, doi:10.1016/S0043-1354(97)00053-5, 1997.

Blees, J., Niemann, H., Wenk, C. B., Zopfi, J., Schubert, C. J., Jenzer, J. S., Veronesi, M. and Lehmann, M. F.: Bacterial methanotrophs drive the formation of a seasonal anoxic benthic nepheloid layer in an alpine lake, Limnol. Oceanogr., 59(4), 1410–1420, doi:10.4319/lo.2014.59.4.1410, 2014.

Bowles, M. W., Nigro, L. M., Teske, A. P. and Joye, S. B.: Denitrification and environmental factors influencing nitrate removal in Guaymas Basin hydrothermally altered sediments, Front. Microbiol., 3, 1–11, doi:10.3389/fmicb.2012.00377, 2012.

Braman, R. S. and Hendrix, S. A.: Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection, Anal. Chem., 61(24), 2715–2718, doi:10.1021/ac00199a007, 1989.

Brunet, R. C. and Garcia-Gil, L. J.: Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments, FEMS Microbiol. Ecol., 21(2), 131–138, doi:10.1016/0168-6496(96)00051-7, 1996.

Burgin, A. J. and Hamilton, S. K.: Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways, Front. Ecol. Environ., 5(2), 89–96, 2007.

Carter, J. P., Hsiao Y.A., Spiro, S. and Richardson, D. J.: Soil and sediment bacteria capable of aerobic nitrate respiration, Appl. Environ. Microbiol., 61(8), 2852–2858, 1995.

Cojean, A.N.Y., Lehmann, M.F., Robertson, E.K., Thamdrup, B., Zopfi, J.: Controls of inorganic substrates (H_2S, Fe^{2+}, Mn^{2+}) on NO_3^--reducing processes in surface sediments of an eutrophic lake, in preparation.

Dalsgaard, T., Stewart, F. J., Thamdrup, B., De Brabandere, L., Revsbech, N. P., Ulloa, O., Canfield, D. E. and DeLong, E.

F.: Oxygen at nanomolar levels reversibly suppresses process rates and gene expression in anammox and denitrification in the oxygen miminum zone off northern Chile, MBio, 5(6), e01966-14, doi:10.1128/mBio.01966-14, 2014.

Dong, L. F., Sobey, M. N., Smith, C. J., Rusmana, I., Phillips, W., Stott, A., Osborn, A. M. and Nedwell, D. B.: Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries, Limnol. Oceanogr., 56(1), 279–291, 2011.

Fazzolari, É., Nicolardot, B. and Germon, J. C.: Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory nitrate reduction to ammonium in repacked soil cores, Eur. J. Soil Biol., 34(1), 47–52, doi:10.1016/S1164-5563(99)80006-5, 1998.

Froelich, P. N., Klinkhammer, G. P., Bender, M. L., Luedke, N. A., Heath, G. R., Gullen, D., Dauphin, P., Hammond, D. and Hartman, B.: Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis, Geochim. Cosmochim. Acta, 43, 1075–1090, 1979.

Gao, H., Schreiber, F., Collins, G., Jensen, M. M., Kostka, J. E., Lavik, G., De Beer, D., Zhou, H. Y. and Kuypers, M. M. M.: Aerobic denitrification in permeable Wadden Sea sediments, ISME J., 4(3), 417–426, doi:10.1038/ismej.2009.127, 2010.

Garcia H.E. and Gordon L.I.: Oxygen solubility in seawater: Better fitting equations, Limnol. Oceanogr., 37(6), 1307-1312, 1992.

Glud, R. N.: Oxygen dynamics of marine sediments, Mar. Biol. Res., 4(4), 243–289, doi:10.1080/17451000801888726, 2008.

Gruber, N. and Galloway, J.N.: An Earth-system perspective of the global nitrogen cycle. Nature 451: 293–296. doi:10.1038/nature06592, 2008.

Hansen, H. P. and Koroleff, F.: Determination of nutrients, in Methods of Seawater Analysis 3rd ed.. Verlag Chemie, Weinheim, pp. 159–228, Wiley-VCH., 1999.

Hietanen, S. and Lukkari, K.: Effects of short-term anoxia on benthic denitrification, nutrient fluxes and phosphorus forms in coastal Baltic sediment, Aquat. Microb. Ecol., 49(3), 293–302, doi:10.3354/ame01146, 2007.

Jäntti, H. and Hietanen, S.: The effects of hypoxia on sediment nitrogen cycling in the Baltic Sea, Ambio, 41(2), 161–169, doi:10.1007/s13280-011-0233-6, 2012.

Jensen, M. M., Kuypers, M. M. M., Lavik, G. and Thamdrup, B.: Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea, Limnol. Oceanogr., 53(1), 23–36, doi:10.4319/lo.2008.53.1.0023, 2008.

Jensen, M. M., Petersen, J., Dalsgaard, T. and Thamdrup, B.: Pathways, rates, and regulation of N_2 production in the chemocline of an anoxic basin, Mariager Fjord, Denmark, Mar. Chem., 113(1–2), 102–113, doi:10.1016/j.marchem.2009.01.002, 2009.

Kalvelage, T., Jensen, M. M., Contreras, S., Revsbech, N. P., Lam, P., Günter, M., LaRoche, J., Lavik, G. and Kuypers, M. M. M.: Oxygen sensitivity of anammox and coupled N-cycle processes in oxygen minimum zones, PLoS One, 6(12), doi:10.1371/journal.pone.0029299, 2011.

Kamp, A., de Beer, D., Nitsch, J. L., Lavik, G. and Stief, P.: Diatoms respire nitrate to survive dark and anoxic conditions, PNAS, 108(14), 5649–54, doi:10.1073/pnas.1015744108, 2011.

Kaspar, H. F.: Denitrification, nitrate reduction to ammonium, and inorganic nitrogen pools in intertidal sediments, Mar. Biol., 74, 133–139, 1983.

Körner H. and Zumft, W. G.: Response to the dissolved oxygen level and respiratory substrate in continuous culture of *Pseudomonas stutzeri*, , 55(7), 1670–1676, 1989.

Kraft B., Tegetmeyer, H.E., Sharma, R., Klotz, M.G., Ferdelman, T.G., Hettich, R.L., Geelhoed, J.S., Strous, M.:The environmental controls that govern the end product of bacterial nitrate respiration, Sciences, 345(6197), 676-679, 2014.

Lazzaretti, M.A., and Hanselmann K.W., in collaboration with Brandl, H., Span, D., Bachofen, R.: The role of sediments in the phosphorous cycle in Lake Lugano. Seasonal and spatial variability of microbiological processes at the sediment-water surface. Aquat. Sci., 54, 285-299, doi:10.1007/BF00878141, 1992.

Lehmann, M. F., Bernasconi, S. M., McKenzie, J. A., Barbieri, A., Simona, M. and Veronesi, M.: Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling of eutrophic lake, Limnol. Oceanogr., 49(2), 415–429, 2004.

Lehmann, M. F., Barnett, B., Gélinas, Y., Gilbert, D., Maranger, R. J., Mucci, A., Sundby, B. and Thibodean, B.: Aerobic respiration and hypoxia in the Lower St . Lawrence Estuary : Stable isotope ratios of dissolved oxygen constrain oxygen sink partitioning, Limnol. Ocean., 54(6), 2157–2169, doi:10.4319/lo.2009.54.6.2157, 2009.

Lehmann, M. F., Simona, M., Wyss, S., Blees, J., Frame, C. H., Niemann, H., Veronesi, M. and Zopfi, J.: Powering up the "biogeochemical engine": the impact of exceptional ventilation of a deep meromictic lake on the lacustrine redox, nutrient, and methane balances, Front. Earth Sci., 3, 1–13, doi:10.3389/feart.2015.00045, 2015.

Mohan, S. B. and Cole, J. A.: The dissimilatory reduction of nitrate to ammonia by anaerobic bacteria, in: Biology of the Nitrogen Cycle, edited by: Bothe H., Ferguson S., and Newton W. E., Elsevier B.V., pp. 93–106, 2007.

Moir, J. W. B. and Wood, N. J.: Nitrate and nitrite transport in bacteria, Cell. Mol. Life Sci., 58, 215–224, 2001.

Morley, N. and Baggs, E. M.: Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction, Soil Biol. Biochem., 42(10), 1864–1871, doi:10.1016/j.soilbio.2010.07.008, 2010.

Morley, N., Baggs, E. M., Peter, D. and Bakken, L.: Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations, FEMS Microbiol. Ecol., 65, 102–112, doi:10.1111/j.1574-6941.2008.00495.x, 2008.

Nielsen, L. P.: Denitrification in sediment determined from nitrogen isotope pairing, FEMS Microbiol. Ecol., 86, 357–362, doi:10.1016/0378-1097(92)90800-4, 1992.

Otte, S., Grobben, N. G., Robertson, L. A., Jetten, M. S. M. and Kuenen, J. G.: Nitrous oxide production by *Alcaligenes faecalis* under transient and dynamic aerobic and anaerobic conditions, Appl. Environ. Microbiol., 62(7), 2421–2426, 1996.

Papaspyrou, S., Smith, C. J., Dong, L. F., Whitby, C., Dumbrell, A. J. and Nedwell, D. B.: Nitrate reduction functional genes and nitrate reduction potentials persist in deeper estuarine sediments. Why ?, PLoS One, 9(4), e94111, doi:10.1371/journal.pone.0094111, 2014.

Patureau, D., Zumstein, E., Delgenes, J. P. and Moletta, R.: Aerobic denitrifiers isolated from diverse natural and managed ecosystems, Microb. Ecol., 39(2), 145–152, doi:10.1007/s002480000009, 2000.

Paulmier, A. and Ruiz-Pino, D.: Oxygen minimum zones (OMZs) in the modern ocean, Prog. Oceanogr., 80, 113–128, doi:10.1016/j.pocean.2008.08.001, 2009.

Payne, E. K., Burgin, A. J. and Hamilton, S. K.: Sediment nitrate manipulation using porewater equilibrators reveals potential for N and S coupling in freshwaters, Aquat. Microb. Ecol., 54(3), 233–241, doi:10.3354/ame01272, 2009.

Poock, S. R., Leach, E. R., Moir, J. W. B., Cole, J. A. and Richardson, D. J.: Respiratory detoxification of nitric oxide by the cytochrome c nitrite reductase of *Escherichia coli*, J. Biol. Chem., 277(26), 23664–23669, doi:10.1074/jbc.M200731200, 2002.

Prokopenko, M. G., Hirst, M. B., De Brabandere, L., Lawrence, D. J., Berelson, W. M., Granger, J., Chang, B. X., Dawson, S., Crane 3rd, E. J., Chong, L., Thamdrup, B., Townsend-Small, A. and Sigman, D. M.: Nitrogen losses in anoxic marine sediments driven by *Thioploca*-anammox bacterial consortia, Nature, 500(7461), 194–198, doi:10.1038/nature12365, 2013.

Rao, A. M. F., McCarthy, M. J., Gardner, W. S. and Jahnke, R. A.: Respiration and denitrification in permeable continental shelf deposits on the South Atlantic Bight: Rates of carbon and nitrogen cycling from sediment column experiments, Cont. Shelf Res., 27(13), 1801–1819, doi:10.1016/j.csr.2007.03.001, 2007.

Richardson, D.J., van Spanning R.J.M. and Ferguson S.J.: The prokaryotic nitrate reductases. In: Biology of the nitrogen cycle, edited by: Bothe H., Ferguson S.J., and Newton W.E., Elsevier B.V., 21-35, 2007.

Risgaard-Petersen, N., Rysgaard, S. and Revsbech, N. P.: Combined microdiffusion-hypobromite oxidation method for determining nitrogen-15 isotope in ammonium., Soil Sci. Soc. Am., 59, 1077–1080, 1995.

Roberts, K. L., Eate, V. M., Eyre, B. D., Holland, D. P. and Cook, P. L. M.: Hypoxic events stimulate nitrogen recycling in a shallow salt-wedge estuary: The Yarra River estuary, Australia, Limnol. Oceanogr., 57(5), 1427–1442, doi:10.4319/lo.2012.57.5.1427, 2012.

Roberts, K. L., Kessler, A. J., Grace, M. R. and Cook, P. L. M.: Increased rates of dissimilatory nitrate reduction to ammonium (DNRA) under oxic conditions in a periodically hypoxic estuary, Geochim. Cosmochim. Acta, 133, 313–324, doi:10.1016/j.gca.2014.02.042, 2014.

Robertson, E. K., Roberts, K. L., Burdorf, L. D. W., Cook, P. and Thamdrup, B.: Dissimilatory nitrate reduction to ammonium coupled to Fe(II) oxidation in sediments of a periodically hypoxic estuary, Limnol. Oceanogr., 61(1), 365–381, doi:10.1002/lno.10220, 2016.

Robertson, L. A., Dalsgaard, T., Revsbech, N. and Kuenen, J. G.: Confirmation of 'aerobic denitrification' in batch cultures, using gas chromatography and ^{15}N mass spectrometry, FEMS Micriobiology Ecol., 18, 113–120, 1995.

Rysgaard, S., Risgaard-petersen, N. and Sloth, N. P.: Nitrification, denitrification, and nitrate ammonification in sediments of two coastal lagoons in Southern France, Hydrobiologia, 329, 133–141, 1996.

Seitzinger, S. P.: Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance, Limnol. Oceanogr., 33, 702–724, 1988.

Seitzinger, S., Harrison, J. A., Böhlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., Tobias, C. and van Drecht, G.: Denitrification across landscapes and waterscapes: A synthesis, Ecol. Appl., 16(6), 2064–2090, 2006.

Thamdrup, B. and Dalsgaard, T.: Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments, Appl. Environ. Microbiol., 68(3), 1312–1318, doi:10.1128/AEM.68.3.1312, 2002.

Smith E.J., Davison W. and Hamilton-Taylor J.: Methods for preparing synthetic freshwaters, Wat. Res., 36, 1286-1296, 2002.

Thauer, R. K., Jungermann, K. and Decker, K.: Energy conservation in chemotrophic anaerobic bacteria., Bacteriol. Rev., 41(1), 100–180, doi:10.1073/pnas.0803850105, 1977.

Tiedje, J.M.: Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Environmental microbiology of anaerobes, edited by: Zehnder A.J.B., John Wiley and Sons, New-York, 179-244, 1988.

Wenk, C. B., Zopfi, J., Gardner, W. S., McCarthy, M. J., Niemann, H., Veronesi, M. and Lehmann, M. F.: Partitioning between benthic and pelagic nitrate reduction in the Lake Lugano south basin, Limnol. Oceanogr., 59(4), 1421–1433, doi:10.4319/lo.2014.59.4.1421, 2014.

Zehr, J. P. and Ward, B. B.: Nitrogen Cycling in the Ocean : New perspectives on processes and paradigms, Appl. Environ. Microbiol., 68(3), 1015–1024, doi:10.1128/AEM.68.3.1015, 2002.

Zopfi, J., Kjær, T., Nielsen, L. P. and Jørgensen, B. B.: Ecology of *Thioploca* spp.: Nitrate and sulfur storage in relation to chemical microgradients and influence of *Thioploca* spp. on the sedimentary nitrogen cycle, Appl. Environ. Microbiol., 67(12), 5530–5537, doi:10.1128/AEM.67.12.5530-5537.2001, 2001.

Table 1: Overview of N transformation rates in O_2-controlled slurry incubation experiments. Negative and positive values correspond to net NO_3^- or NH_4^+ consumption and production rates over incubation time, respectively. Standard errors are indicated in bracket for *n* replicates. Average denitrification and DNRA rates (µmol N g^{-1} wet sed. d^{-1}) in anoxic control experiments were: 0.11 ± 0.01 and 0.12 ± 0.04, respectively, at Figino; 0.12 ± 0.01 and 0.11 ± 0.01, respectively, at Melide.

Sampling site	O_2 conc. in slurry	*n*	Inhibition compared to control (%)		NO_3^-	NH_4^+
	µmol L^{-1}		Denitrification	DNRA	*µmol N g^{-1} wet sed. d^{-1}*	
Figino	0 (control)	12			-1.4 (0.1)	0.7 (0.03)
	1.2	3	29	51	-0.8 (0.2)	0.8 (0.4)
	2	3	57	35	-0.4 (0.1)	0.8 (0.2)
	2.8	3	68	17	-0.4 (0.3)	0.7 (0.1)
	3.4	2	64	29	-0.6 (0.2)	0.6 (0.1)
	4.1	3	77	45	-0.9 (1.3)	0.5 (0.2)
	8.6	3	85	60	-1.1 (0.3)	0.4 (0.0)
	16	4	70	84	-0.2 (0.5)	0.1 (0.2)
	24.1	3	86	77	-0.2 (0.6)	0.2 (0.1)
	38	3	93	39	0.2 (2.1)	0.0 (0.2)
	61.3	3	94	64	-0.3 (0.3)	-0.2 (0.1)
	78.6	6	96	79	1.1 (1.4)	-0.1 (0.0)
Melide	0 (control)	9			-1.0 (0.4)	0.2 (0.0)
	0.8	2	43	37	-0.7 (0.2)	-0.1 (0.1)
	1.8	2	63	53	-0.6 (0.1)	0.2 (0.2)
	2.9	3	61	58	-0.5 (0.3)	-0.1 (0.2)
	3.8	4	58	65	-0.2 (0.1)	-0.1 (0.3)
	4.9	3	74	64	-0.3 (0.2)	-0.1 (0.3)
	9	7	73	69	0.0 (0.1)	-0.1 (0.2)
	13.1	2	69	37	-0.6 (0.1)	-0.1 (0.0)
	21.3	2	66	56	-0.4 (0.1)	-0.1 (0.1)
	44.4	2	67	34	-0.3 (0.2)	-0.1 (0.2)
	58.6	3	91	60	-0.1 (0.1)	-0.1 (0.2)
	73.2	4	93	75	0.2 (0.2)	-0.4 (0.1)

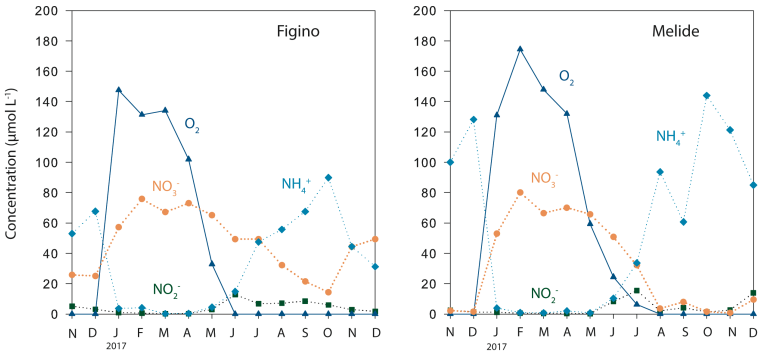


Figure 1: Concentrations of dissolved O_2 and reactive nitrogen in the bottom waters (2 m above the sediments) of the Lake Lugano South Basin in 2017.

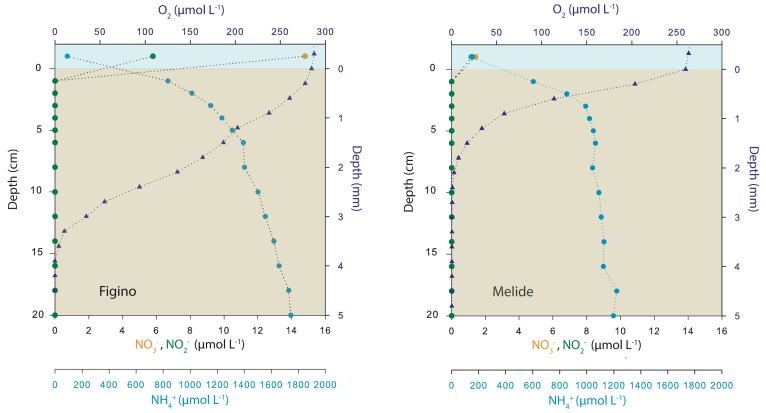
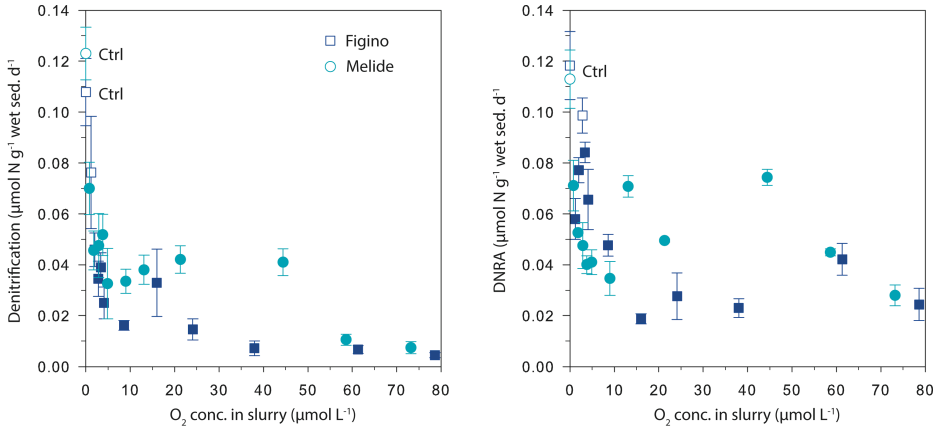


Figure 2: Ex situ sediment porewater profiles (O_2 and dissolved inorganic nitrogen) at the two sampling stations of the Lake Lugano South Basin in sediment cores collected in October 2017. Symbols correspond to the mean value of O_2 and dissolved N species concentrations measured in triplicate and duplicate cores, respectively. Oxygen concentration profiles (note different depth units) were determined in aerated cores, and thus are representative of the O_2 penetration during aerated conditions in the water column, as seen between January and April (see Fig. 1).



620

Figure 3: Denitrification and DNRA rates as a function of dissolved O_2 concentration in dilute sediment slurry from Figino and Melide. Statistically significant differences between denitrification/DNRA rates measured in the different O_2 treatments versus those in the respective control (Ctrl) experiments are shown by the filled symbols ($P < 0.05$, Supp. Table SI.2). Error bars represent the standard error of n replicate experiments and measurements (Table 1).

Commenté [MOU26]: Ref. 1, R17

Commenté [MOU27]: Ref. 2, R4

625

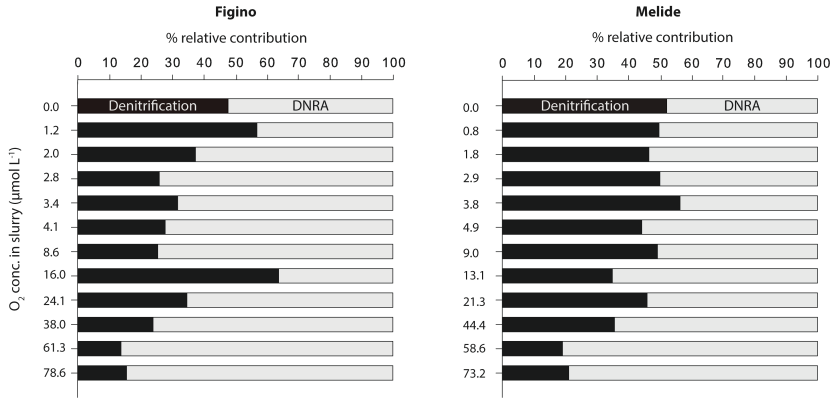


Figure 4: Relative contribution (%) of denitrification and DNRA to total nitrate reduction under variable O_2 conditions.

Supplementary Information

Direct O_2 control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments

Cojean, A.N.Y., *et al.*, Biogeosciences, 2019

Correspondence: adeline.cojean@unine.ch

Table SI.1: Average O_2 concentrations in replicate slurries (see Table 1) at different time points during the incubation period (~ 10 h). The first time point (T1) was always at 30 minutes after the beginning of the incubation while the other time points (T2, T3, …, T9) were different for each treatment (i.e. more frequent O_2 measurements at low O_2 levels).

Commenté [MOU1]: Ref. 1, R8; Ref. 2, R2

	Average O_2 concentration during incubation (µmol L^{-1})	O_2 concentration in slurry at different time points (µmol L^{-1})								
		T1	T2	T3	T4	T5	T6	T7	T8	T9
Figino	1.2	1.4	1.2	1.4	1.2	1.2	1.1	1.0	1.1	1.2
	2.0	1.7	2.2	2.0	2.2	1.9	2.0	1.7	2.0	2.1
	2.8	2.2	2.6	2.9	3.1	2.9	2.9	2.9	2.8	3.0
	3.4	3.0	3.2	3.9	3.2	3.6	3.7			
	4.1	3.5	3.5	3.9	4.3	4.1	4.2	4.9		
	8.6	7.5	7.8	8.8	9.1	8.1	8.9	10.1		
	16.0	17.6	17.1	15.0	16.7	16.5	13.6	15.5	15.8	
	24.1	26.3	25.8	22.9	24.5	21.0	24.2			
	38.0	39.3	35.2	39.5	36.8	38.3				
	61.3	66.8	62.1	57.2	56.9	63.4				
	78.6	81.0	84.8	73.1	81.6	73.6	77.4			
Melide	0.8	0.8	0.8	0.6	0.8	0.8	0.7	1.0	1.1	
	1.8	1.2	2.0	1.7	2.0	1.8	1.7	1.9	2.1	
	2.9	2.7	3.0	2.9	3.1	2.8	3.0			
	3.8	3.2	3.5	3.3	3.8	3.7	4.1	3.5	4.3	4.4
	4.9	4.8	4.8	5.1	4.7	4.7	4.9	5.2		
	9.0	8.3	8.2	8.8	8.7	9.5	9.4	10.3	11.1	11.1
	13.1	11.3	11.3	13.3	12.7	13.8	13.8	15.1		
	21.3	20.6	19.0	21.4	21.4	24.2				
	44.4	40.3	36.4	47.8	46.7	50.6				
	58.6	44.6	53.3	56.3	52.0	57.8	66.6	68.9	69.0	
	73.2	39.3	70.8	73.8	69.7	76.8	81.2	86.4	87.3	

Table SI.2: Results of the t-test analysis of the difference between denitrification and DNRA rates in O_2 treatments and those in control experiments. *P* values with significant differences ($P < 0.05$) are highlighted in bold character.

Commenté [MOU2]: Ref. 2, R4

	O_2 concentration in slurry ($\mu mol\ L^{-1}$)	Denitrification	DNRA
Figino	1.2	0.3529	**0.0023**
	2.0	**0.0011**	**0.0127**
	2.8	**0.0003**	0.2137
	3.4	**0.0004**	**0.0305**
	4.1	**0.0001**	**0.0182**
	8.6	**< 0.0001**	**0.0003**
	16.0	**0.0056**	**< 0.0001**
	24.1	**< 0.0001**	**0.0093**
	38.0	**< 0.0001**	**< 0.0001**
	61.3	**< 0.0001**	**0.0002**
	78.6	**< 0.0001**	**< 0.0001**
Melide	0.8	**0.0099**	**0.0256**
	1.8	**0.0002**	**0.0008**
	2.9	**0.0055**	**0.0019**
	3.8	**0.0001**	**0.0002**
	4.9	**0.0045**	**0.0002**
	9.0	**< 0.0001**	**< 0.0001**
	13.1	**< 0.0001**	**0.0459**
	21.3	**< 0.0001**	**0.0005**
	44.4	**< 0.0001**	**0.0433**
	58.6	**< 0.0001**	**0.0062**
	73.2	**< 0.0001**	**< 0.0001**

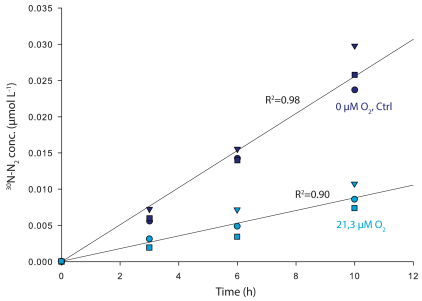


Figure SI.1: Exemplary times-series of $^{30}N_2$ concentration data during incubation experiments in two different O_2 treatments (triplicate) at Melide.

Commenté [A3]: Ref. 2, R3

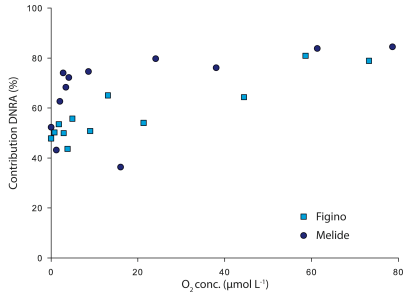


Figure SI.2: Relative contribution of DNRA (%) to total NO_3^- reduction at variable O_2 concentrations at Figino and Melide.