Manuscript: bg-2020-20

Title: Oxygen and light determine the pathways of nitrate reduction in sediments of a highly saline lake

RESPONSE TO REFEREE #3

We are grateful with the referee's comments, which were very helpful to improve the manuscript. In general, we have restructured the manuscript, rewritten some parts and revised the figures and tables included, moving some of them to supplementary material. In addition, Judith Prommer was added in the co-author list due to her significant contribution in explaining the role of nitrification in the discussion section. Our responses are shown below the reviewer's comments in blue.

Major concerns:

-Quality of the figures

I think that the authors have to remake the figures to illustrate their results better. Figures 1, 2, and 4 include the acclimation phase that, in my opinion, masks the "real" results. I suggest showing in these figures only the exact experimental time (i.e., since the addition of the 15N-nitrate, time 0). Most changes occur in the first 24h. I think these figures could be more illustrative showing only these 24h-36h that is the period until NO3 disappears. The complete figures could go to supplementary material.

We agree that results could be illustrated better. For this reason, we have deleted the stabilization phase from the main figures. Following your recommendations, Figure 1 now starts with time 0 (from the time of tracer addition), as well as the remaining figures of the main manuscript. The complete time incubation of N-species and the evolution of physico-chemical parameters (former Figure 4) have been moved to Supplementary Information (Figures S1 and S3, respectively).

About the end time of the figures included in the main manuscript, we decided to keep the whole incubation time. The reason for doing that is the valuable information we could miss by showing only this 24-36 h period. It's the case of the N_2O production in treatments OD and AD at the end of the experiments (Figure 1). Such concentrations (above 2 mmol/L) are presumably produced not only by denitrification but also by partial nitrification, fueled by OM mineralization (section 4.2, lines 368-395). This is a process triggered secondarily that, in our view, is important to discuss in the manuscript (especially as a source of N_2O) and hence our decision to keep the plots until 72 h of incubation.

Figure 3 should be cut at the same time that Figures 1,2 and 4. In this figure, I am also wondering if the specific times with more than 100% of recovery are errors. Please, consider deleting these times. For instance, time 3 in the treatment OD that reach almost 120%. I think there is an error mostly because time 2.5 and time 4 are pretty similar.

Figure 3 has been moved to Supplementary Information (Figure S3). About the information represented here, it refers to the mass balance (the recovery of ^{15}N) from our measurements. ^{15}N was added as NO_3^- but was measured in 4 different chemical compounds (NO_3^-, NH_4^+, N_2O,

N_2). Any measurement of concentration and ^{15}N enrichment comes with an error, implying a loss of ^{15}N (in gray) or an excess of it (as the case of time 3 in treatment OD). The accumulation of steps can inflate the total error on the final values. Therefore, the general recovery showing values at each time point so close to 100% actually demonstrates how precisely all the measurements were performed.

-The precision of some chemical analysis.

The concentration of ammonium shows tremendous values of standard deviation (Figure 1). I have concerns to assume the low replicability of this analysis. The concentration of DOC and its changes is high. I have some concerns here too. I did not see the complete protocol for DOC. In saline, endorheic lakes DOC is usually high (around 1-10 mM), but the values around 40 mM are extremely high even for this type of systems. Water from saline lakes needs a protocol for DOC with longer purge time and acid additions to make sure all the DIC has been removed. I was unable to see these details in the methods of acid addition and purge time. Changes in 20 mM are so extreme that I have concerns. Usually, phytoplankton blooms can change almost nothing DOC concentration in water.

Thank you for the comment. About DOC analysis, we followed the methodology provided by the manufacturer in the equipment manual, which is also briefly discussed in Stubbins and Dittmar (2012). So, samples were acidified to pH ≈ 2 with 2 M HCl. The optimal purging time for samples to remove all DIC after acidification was determined by measuring a series of identical samples in triplicates until results for DOC were achieved within a 95% confidence interval. The necessary time was determined to be 5 minutes, therefore this was the purging time used for all samples, even the ones with lower dissolved solids content. A sentence including this information has been included in the methodology.

Minor concerns

- Lines 42-48, I think this paragraph is not necessary since the paper is about nitrates removing and confuse the reader.

We agree with that, and therefore, these lines have been removed from the introduction.

- Lines 100-108, I found some incoherence here. Is Petrola lake submitted to nitrogen inputs or not?

Thank you for the comment, it is certainly something we did not express correctly. The last part of that paragraph has been rewritten (lines 105-111) as follows:

The lake has been classified as a heavily modified water body due to the inputs of agricultural pollutants as well as untreated wastewater directly spilled from Pétrola Village. Despite that the Pétrola endorheic basin was declared vulnerable to NO_3^- *pollution by the Regional Government of Castilla-La Mancha in 1998, it still receives a continuous supply of N mainly derived from inorganic synthetic fertilizers (Valiente et al., 2018). As a result, eutrophication of the water layer occurs leading to the dominance of phytoplankton, keeping out the light, and promoting bottom-water oxygen depletion because of bacterial decomposition.*

- Line 158, What is dissolved bound nitrogen?

Dissolved bound nitrogen (DNb) refers to the sum of dissolved N species (NO_3^-, NO_2^-, NH_4^+, organic N) excluding gaseous N forms (e.g. N_2 and N_2O). A short note has been added to Section 2.3 (line 175).

- Line 348, I think the reference of McCrackin and Elser is not about sediments

We checked this reference, where the authors “measured the rate of denitrification and nitrous oxide (N_2O) production during denitrification in sediments from 32 Norwegian lakes at the high and low ends of a gradient of atmospheric N deposition”. Therefore, we have decided to keep the reference in the manuscript.

References

McCrackin, M. L. and Elser, J. J.: Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes, Ecology, 91(2), 528–539, doi:10.1890/08-2210.1, 2010.

Stubbins, A. and Dittmar, T.: Low volume quantification of dissolved organic carbon and dissolved nitrogen, Limnology and Oceanography Methods, 10, doi:10.4319/lom.2012.10.347, 2012.

Valiente, N., Carrey, R., Otero, N., Soler, A., Sanz, D., Muñoz-Martín, A., Jirsa, F., Wanek, W. and Gómez-Alday, J. J.: A multi-isotopic approach to investigate the influence of land use on nitrate removal in a highly saline lake-aquifer system, Science of The Total Environment, 631–632, 649–659, doi:10.1016/j.scitotenv.2018.03.059, 2018.