

Biogeosciences Discuss., author comment AC1  
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## Reply on RC1

Huazu Liu et al.

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Author comment on "Effect of vegetation distribution driven by hydrological fluctuation on sedimental stoichiometry regulating N<sub>2</sub>O emissions in freshwater wetland" by Huazu Liu et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-208-AC1>, 2022

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## Reply on RC1

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## RC1

Overall, the idea of the study is interesting, however the major limitation is that it is based on only few grab samples (both soil and gas). Samples were collected once during low water level event and once during high water level event. Making concluding about the ecosystem based on few samples is not sufficient. For example, Figure 1 shows regression analyses that is based on only few points and same goes with other figures as well. To understand the dynamics at an ecosystem level, a much larger amount of samples should be collected. First to see the seasonal dynamics and secondly to have a realizable amount of data for statistical analyses.

**Author's response:** We thank the anonymous referee#1 for the detailed reviews with relevant and constructive comments to improve the quality of the manuscript. The received recommendations were carefully considered and incorporated into the current version of the manuscript. We focused on the differences between nitrogen cycle during high and low water levels in ecosystem. Therefore, we monitored N<sub>2</sub>O emissions, vegetations and soil in the steady period after the change of water level. In addition, influenced by the Three Gorges Dam, the annual change of water level in the study area were very regular. As a result, the variations between years in the change of water level were very small. Although we set up three sampling sites at each vegetation zone in the study area, we collected as many samples as possible at each sampling site for statistical analysis. And we ensured that the distances between the sampling sites made less interference between the sites. We agreed the detailed review and comments which will be helpful in our future researches. A point-by-point response to comments was given below.

- Figure 1 - Photos have low quality. Location of the region would be nice to show.

**Author's response:**

Thanks for the correction; we have replaced a photo with high quality.

- Lines 115-120 - You inserted pedestal into the soil and then started to collect gas samples. How long was the stabilisation period because this could create relatively large disturbance to the soil? How many gas samples were used to calculate flux? How did you access the site during high flood to avoid soil disturbance? The size of the chambers?

**Author's response:**

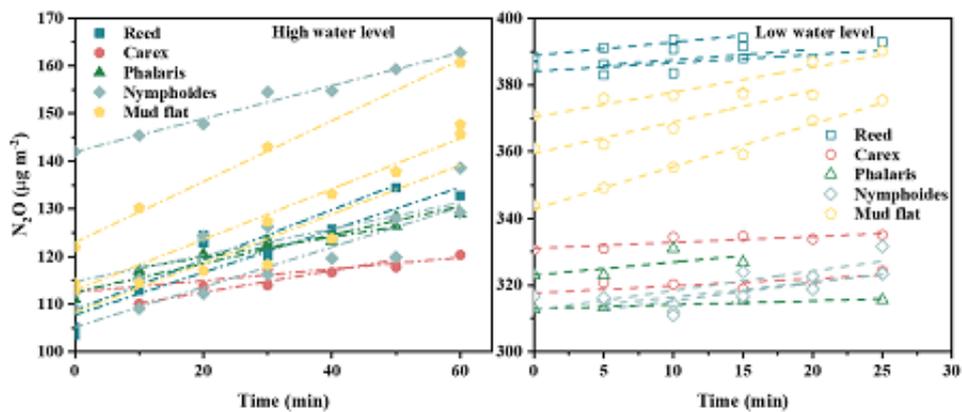
For the first sampling, we inserted the pedestal about 10cm into the soil. And we didn't take the pedestal to reduce the disturbance of subsequent samples. After the pedestal into the soil, we set a stabilization period of 30 minutes and a board to reduce soil disturbance from people (Wang, H. J., Wang, W. D., Yin, C. Q., Wang, Y. C., and Lu, J. W.: Littoral zones as the "hotspots" of nitrous oxide (N<sub>2</sub>O) emission in a hyper-eutrophic lake in China, Atmospheric Environment, 40, 5522-5527, 10.1016/j.atmosenv.2006.05.032, 2006.) (as shown in the figure below).



We shut down the ship's machinery in the study area and waited for an hour before sampling during high water level. Then, we let the chamber on the water for 30 minutes before collecting the gas. We used a 10m air pipe, which kept chamber as far away from the ship as possible to reduce disturbance (as shown in the figure below).



Seven gas samples were used to calculate flux in each sampling site. And three sampling sites were set in each vegetation zone.



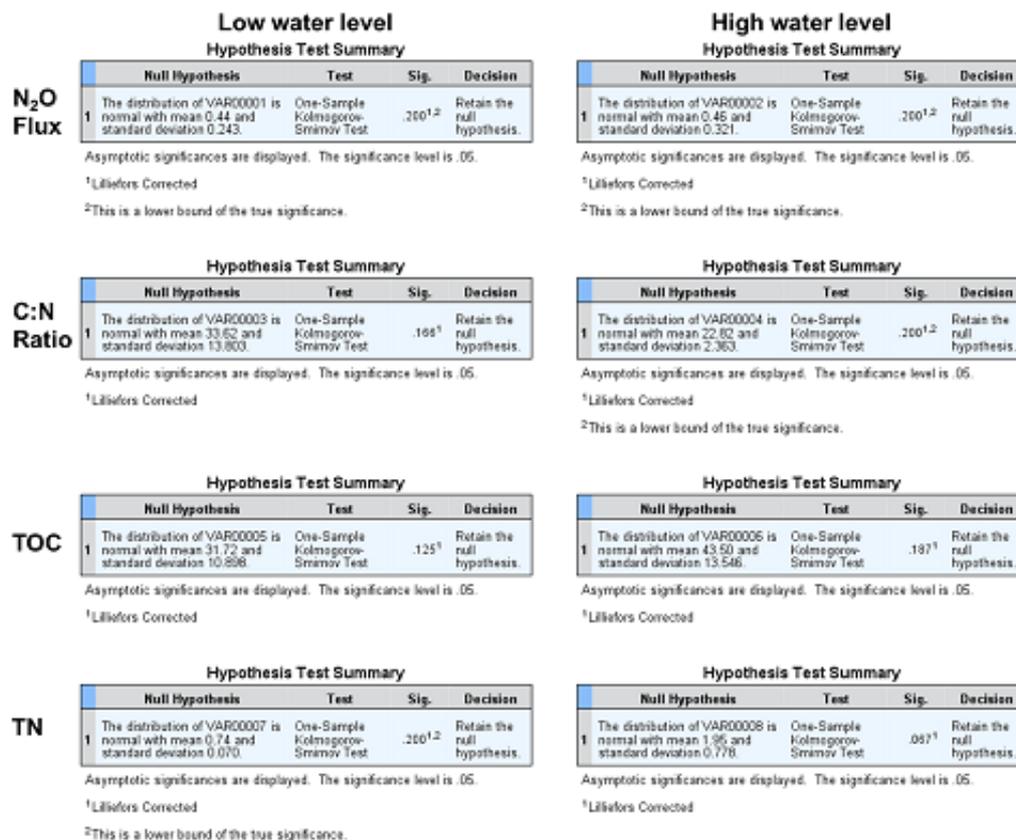
We described the size of the chambers in the manuscript as following:

L116-117. "The volume of the upper chambers used during low water level was 0.028 m<sup>3</sup> ( $h = 40\text{cm}$ ,  $\Phi = 30\text{cm}$ ), and the volume of the pedestal was 0.011 m<sup>3</sup> ( $h = 15\text{cm}$ ,  $\Phi = 30\text{cm}$ ). And the volume of the chambers used during high water level was 0.018 m<sup>3</sup> ( $l = 40\text{cm}$ ,  $w = 30\text{cm}$ ,  $h = 15\text{cm}$ )."

- Line 135 - Statistical analyses: was the data normally distributed? And what tests were used to control that?

**Author's response:**

We used KS-test to confirm that the data was normally distributed (as shown in the figure below).



- Figure 3 - caption is not referring to correct sub-plots. E.g. B is TOC not nitrogen density etc.

**Author’s response:**

New caption was as following:

L171-172. “Figure 3. Content of carbon and nitrogen in vegetation and sediments during different water levels. Carbon (a) and nitrogen (c) densities of vegetation in different zones. Concentration of TOC (b) and TN (d) in sediments in different vegetation zones”

- Figure 5 - text in the figure is so small that it is unreadable.

**Author’s response:**

We have resized the text in the figure as suggest.

- Line 350 - do you have data about N<sub>2</sub>O reducers: *nosZ* clade I and II genes? Currently the abundance of *nirS*, *nirK* and *hzsB* genes does not provide enough information about the entire N cycle.

**Author's response:**

In this study, we mainly focused on N<sub>2</sub>O emissions in the N cycle. Nitrite is converted to NO or N<sub>2</sub>O by nitrite reductase (NIR) in denitrification, the extensively used biomarkers for which are *nirK* (Cu-containing) and *nirS* (cytochrome cd 1) (Levy-Booth, D.J., Prescott, C.E., Grayston, S.J.: Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems, *Soil Biol. Biochem.*, 75, 11–25, 10.1016/j.soilbio.2014.03.021, 2014.). And the N<sub>2</sub>O emission varied with the abundance of *nirS* and *nirK* genes (Zhang, L., Jiang, M.H., Ding, K.R., Zhou, S.G., Iron oxides affect denitrifying bacterial communities with the *nirS* and *nirK* genes and potential N<sub>2</sub>O emission rates from paddy soil, *EUROPEAN JOURNAL OF SOIL BIOLOGY*, 93, 103903, 10.1016/j.ejsobi.2019.103093, 2019). Thus, the abundance of *nirS* and *nirK* genes became the main object of discussion. Meanwhile, in order to further explore the N cycle in the anaerobic environment such as reservoirs and lakes, we analyzed the functional gene (*hzsB* gene) of anammox to compare with the denitrification.

- Throughout the text: sometimes N<sub>2</sub>O has subscript (N<sub>2</sub>O) and sometimes not.

**Author's response:**

We double checked the subscripts and revised the incorrect subscripts.

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-208/bg-2021-208-AC1-supplement.pdf>