Biogeosciences Discussions

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Comment on bg-2021-208

Anonymous Referee #2

Referee comment on "Effect of vegetation distribution driven by hydrological fluctuation on sedimental stoichiometry regulating N_2O emissions in freshwater wetland" by Huazu Liu et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-208-RC2, 2021

The manuscript submitted by Liu and colleagues investigates relationships between plant species, hydrology and N2O fluxes. In their work, they evaluate four (or five?) vegetation zones in a Chinese wetland and analysed C and N contents in the vegetation and sediments, N2O fluxes, microbial communities and selected genes involved in the N cycle during high and low water levels. They conclude that "the distribution of plants under hydrological conditions modified the stoichiometric ratio of sediments, resulting in the variations of N2O emission fluxes and microbial communities in the vegetation zones".

While the topic is interesting and relevant for the journal, I have my serious doubts about the experimental design and the approach used. One of the main arguments of the manuscript is that the vegetation distribution is driven by hydrological changes; it is also argued that is the vegetation distribution the factor affecting the emission of N2O (Abstract, L3). Your first objective was indeed to examine the relationship between hydrology and species distribution. I was however not able to understand how your experimental set up was helpful to elucidate more about this matter, and which kind of data you use to support that this is indeed the case in your plots. You merely monitored the water level across the vegetation types and, actually, found that all vegetation types except reed were having exactly the same pattern (Figure 2a). And, even if you find a distinct pattern in the water dynamics across your vegetation zones, you won´t be able to conclude whether if it is the hydrology or the plant communities the ones driving the N2O fluxes.

Further, you focus on N2O emissions. We know that the temporal and spatial variability of N2O fluxes can be really high so I strongly suspect that measuring only twice a year over three (pseudo?) replicates is not enough to adequately catch the dynamics of the fluxes, especially with this highly contrasting environmental conditions. I also see that you measured on the soil surface (during low water level conditions) and on the water surface (high water level). This means your measurement conditions are totally different (different chamber setup, different diffusion coefficients, etc). I miss a clear explanation on how the different measurement conditions may have affected your results. For example, if I interpret Fig S2 correctly, I can see that the starting concentrations when setting the chamber (which should be the atmospheric N2O concentration) differ by a factor of three, which is hard for me to understand.

Further, I honestly think some results are misinterpreted, probably as a result of using flawed statistical methods. I found no mention to how the parameters measured were compared across vegetation zones, which in theory is your central point. For example, you highlight the role of the C:N ratio in the sediments and the N2O emissions (L190) but this is not supported by Figure 4b, probably because you ignored the effect of the different vegetation types in the approach. What I actually see in Figure 4b is how a change in the C:N ratio from 20-25 to 45-50 does not have any effect on the N2O fluxes. By the way, at least for the mud-flat, data from Fig 4a and 4b are different, so plese revise the consistence of your data.

I like to see that there is a part including microbial communities, but as it is now, this section is decoupled from the rest of the manuscript. How do these genes influence/are influenced by the rest of the parameters you are investigating? How do they fit into the big picture? As it is now, you provide a mere description of abundances (many times without any statistical analysis), but without a clear context. For example, why is annamox important here, if it doesn´t involve N2O turnover?

In summary, I strongly recommend to rethink the approach of the manuscript; you may merely compare different vegetation areas, considering that they are (or not) subjected to different hydrological regimes. Carefully think your hypotheses and reflect whether they can be tested with your experimental set up. Further, revise the methods, and provide a solid description of what was made in the statistical part. Then, you can explain the results which are relevant to these hypotheses, and discuss them accordingly.

Specific comments to the different sections (not exhaustive)

Abstract (L16-17): This is the closing sentence of your abstract, but it is actually coming out of the blue. I haven´t seen any reference to mitigation strategies at all in the rest of the manuscript. Please, be aware the abstract should reflect the most important aspects of the manuscript.

Objectives (L81-86): What are the "ecological factors" you refer to? As they are formulated now, the objectives are a mix between aims and hypotheses. For example, in 3) you are somehow assuming that C:N ratio of sediments will be the dominant factor for N2O fluxes. I suggest to clearly define your objectives and then, in order to achieve them, develop working hypotheses, that you will try to answer with your experimental design

Fig 1: The left-hand side part has no context at all. What are the photographs, why are there two sets? What is the lower panel?

I have a very practical question. According to Figure 2, the surface was covered by several meters of water when you sampled sediments and vegetation in June 2020. How did you sample? Did you differentiate between floating and submersed vegetation? When the water column is > 5 m, how can you differentiate between e.g. mud flat and nymphoides?

I miss the whole explanation on how the biomass was estimated. How is the 1 m x 1 m plot defined in the water? Was all the vegetation harvested and processed in the lab? There are huge changes in C and N densities (Figure 3). Are they coming only from changes in biomass, or did the concentrations in the plant change? Which sample did you take to determine C and N %? I can imagine this trait is not homogenous across the plant.

For the statistics, you mention one way ANOVA or t-test for comparind between high and low water levels, but I wonder how the vegetation factor was taken into account, and the interaction between water level and vegetation.

Results & Discussion:

L152: It seems that the main line of argumentation of the manuscript (and part of the title) originates from here "The correlation between water level variation and plant assimilation indicated that the long-term change of hydrological regime induced the stratification of vegetation". As explained before, I don´t get it; in case this correlation is true, it would tell you, at most (ie, assuming causation), about plant productivity, but not about vegetation types. And, in any case, I see a point cloud (including one with no vegetation) and the reed, clearly out of the region and likely highly responsible for your fitting.

Figure 2: I had troubles understanding this figure. First, there is no information (not in the caption, neither in the methods) on how the data from panel a were obtained. Second, what are the photographs? Third, what is the x-axis of panels b and c?

In the discussion, I was confused quite often because I was not sure if the results of this study or of others were discussed. This is quite often not clear (e.g. L234-235), probably

because of the past tense use. Please, revise this.

Some other comments:

L47, L83: You use the concept of "vegetation decline". This refers to vegetation dynamics, so it intrinsically involves a temporal component, which you are not covering with your approach.

Concepts of assimilation/accumulation/decomposition (e.g. L150, 280) and sink/source (e.g. L9) are usually misused. Only "densities" (in g m-2) are investigated, you are not looking at changes in stocks, neither you are looking at all components of the C or N cycle. I strongly suggest to revise these parts accordingly.

L112-114: specify what is the "closed-chamber technique". What is "a static chamber with an upper chamber"?

L123. Sediments were collected/sampled, I guess

L136: Which "traits of vegetation" do you mean?

L144: the maximum seems to be at about 6 meters, according to Fig 2. Please check your data.

L245: What does this sentence mean? "The discrepancies between the niches of plant species caused by hydrological conditions indicated the essence of stratification of vegetation zones"

L261: I think the mention to peat here is out of context here, unless you have evidence of peat being present in your study area

L292: You address interesting points here, but I don't see a relation to your data. Do you have any information about these parameters? I am not sure either if you claim that these

effets are having solely a physical effect ("affecting the diffusion of N2O from sediment to water"), or also biological effects on the production rates. As I mentioned before, this might be critical when comparing the two seasons you have conducted measurements.