Reply on RC2

Huazu Liu et al.

RC2

A:

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.

Author's response:

We would like to thank anonymous referee#2 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.

Hydrological conditions, such as flooding time, flooding depth, and flooding frequency, were the dominant factors driving vegetation distribution (Tan, Z. Q., Zhang, Q., Li, M. F., Li, Y. L., Xu, X. L., and Jiang, J. H.: A study of the relationship between wetland vegetation communities and water regimes using a combined remote sensing and hydraulic modeling approach, Hydrol. Res., 47, 278-292, 2016.; Toogood, S. E., Joyce, C. B., and Waite, S.: Response of floodplain grassland plant communities to altered water regimes, Plant Ecology, 197, 285-298, 2008.). Plant species had different amounts of carbon and nitrogen in their organisms (Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S. S., McCauley, E., Schulz, K. L., Siemann, E. H., and Sterner, R. W.: Nutritional constraints in terrestrial and freshwater food webs, Nature, 408, 578-580, 2000.; Yu, Q., Chen, Q., Elser, J. J., He, N., Wu, H., Zhang, G., Wu, J., Bai, Y., and Han, X.: Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability, Ecology Letters, 13, 1390-1399, 2010.). Therefore, we hypothesized that the biomass (carbon density and nitrogen density) of plants can be used as an index to distinguish plant species. And the change rate of water level (Figures 2a and 2b) can reflect the flooding time, frequency and depth. Thus, we explored the driving effect of hydrology on vegetation distribution by analyzing the relationship between the biomass (carbon density and nitrogen density) of plants and the change rate of water level. In fact, there were differences in the water level of all vegetation zones. Due to the large flooding depth, the difference in water level changes of these vegetation zones was not obvious in Figure 2a. In order to verify this, we scaled the image and showed part of the data during high water level (as shown in the figure below).



We thought that hydrology drove vegetation distribution, and the vegetation distribution effected the content of carbon and nitrogen in soil. The changes in the content of carbon and nitrogen (or stoichiometric proportion) varied the microbial processes and N₂O emission. As anonymous referee#2 said, there were many factors affecting N₂O emission, and we won't be able to conclude whether if it is the hydrology or the plant communities the ones driving the N₂O fluxes. However, our study attempted to reveal that the change of water level mediated by plant community drove the N₂O emission in wetlands. Additionally, we also discussed the influence of hydrology, plant communities and other factors on N₂O emission in the section of *Discussion*.

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.

Author's response:

We focused on the differences between nitrogen cycle during high and low water levels in ecosystem. Therefore, we monitored N₂O 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 ensured that the distances between the sampling sites made less interference between the sites. The approximate location of sampling sites was shown in the figure below, and the yellow box represents sampling sites.



We agreed with anonymous referee#2's reviews about the chambers. However, the water level in the study area varied greatly in different periods, which made it hard for us to use the same chamber to monitor N₂O. At present, using chambers on the ground and floating chambers was a common flux method (Wang, H. J., Wang, W. D., Yin, C. Q., Wang, Y. C., and Lu, J. W.: Littoral zones as the "hotspots" of nitrous oxide (N₂O) emission in a hyper-eutrophic lake in China, Atmospheric Environment, 40, 5522-

5527, 2006.; Cole, J.J., Bade, D.L., Bastviken, D., Pace, M.L., Van de Bogert, M.: Multiple approaches to estimating air-water gas exchange in small lakes, Limnol. Oceanogr. 8, 285–293, 2010.; Gålfalk, M., Bastviken, D., Fredriksson, S., Arneborg, L.: Determination of the piston velocity for water-air interfaces using flux chambers, acoustic Doppler velocimetry, and IR imaging of the water surface, J. Geophys. Res. 118, 770–782, 2013.).

We supplemented the description of the chambers in the revision as following:

L111-113. "The chamber technique on the ground was used to measure the in situ N_2O emission rate according to Wang (Wang et al., 2006). And we used a floating chamber to measure the in situ N_2O emission during high water level (Cole et al., 2010; Gålfalk et al., 2013)."

The initial concentration of N_2O was affected by weather, temperature and wind speed, which resulted to the focus of N_2O research on emission flux. And static chambers can effectively avoid the influence of these factors on N_2O emission flux. N_2O emission flux estimated by the static chamber method was calculated using Eq. below:

$$F = \frac{V}{A} \times \frac{\mathrm{d}C}{\mathrm{d}t}$$

where *F* is the N₂O emission flux ($\mu g m^{-2} min^{-1}$); *V* (m3) and *A* (m2) are the static chamber volume and surface area, respectively; and d*C*/d*t* is the time derivative of the N₂O concentration ($\mu g m^{-3} min^{-1}$).

Thus, we thought that the chamber with different volume had less influence on N_2O monitoring. In order to reduce the disturbance, we inserted the pedestal about 10cm into the soil at the first sampling. 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. During high water level, we shut down the ship's machinery in the study area and waited for an hour before sampling. 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).



C:

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.

Author's response:

We described differences in N₂O emissions between vegetation zones in Results (L182-184: "The N₂O emissions flux showed significant differences between vegetation zones during each water level period (P < 0.05) (Fig. 4a)."). Meanwhile, we also discussed the differences of N₂O emission fluxes at reed zone, phalaris zone, and mud flat in "4.3 N₂O emission in hydrological-stressed wetlands". N₂O emissions were affected by multiple factors (Miya, R. K. and Firestone, M. K.: Phenanthrenedegrader community dynamics in rhizosphere soil from a common annual grass, Journal of Environmental Quality, 29, 584-592, 2000.; Stadmark, J. and Leonardson, L.: Greenhouse gas production in a pond sediment: Effects of temperature, itrate, acetate and season, Science of the Total Environment, 387, 194-205, 2007.; Wu, J., Zhang, J., Jia, W., Xie, H., Gu, R. R., Li, C., and Gao, B.: Impact of COD/N ratio on nitrous oxide emission from microcosm wetlands and their performance in removing nitrogen from wastewater, Bioresource Technology, 100, 2910-2917, 2009.). And the C:N ratio was considered the dominant factor of N₂O emission (Wei, X., Shao, M., Fu, X., Horton, R., Li, Y., and Zhang, X.: Distribution of soil organic C, N and P in three adjacent land use patterns in the northern Loess Plateau, China, Biogeochemistry, 96, 149-162, 2009.). We have updated Figure 4. and fixed the previous errors as shown below:



In Figure 4b., when the C:N < 25, the N₂O emission flux increased with the decrease of C:N ratio. This was consistent with many studies (Li, X., Hu, F., and Shi, W.: Plant material addition affects soil nitrous oxide production differently between aerobic and oxygen-limited conditions, Applied Soil Ecology, 64, 91-98, 2013.; Frimpong, K. A. and Baggs, E. M.: Do combined applications of crop residues and inorganic fertilizer lower emission of N₂O from soil?, Soil Use and Management, 26, 412-424, 2010.; Zhou, W., Jones, D. L., Hu, R., Clark, I. M., and Chadwick, D. R.: Crop residue carbon-to-nitrogen ratio regulates denitrifier N₂O production post flooding, Biology and Fertility of Soils 56, 825-838, 2020.). Although the C:N ratio from 30 to 45 didn't have any effect on the N₂O fluxes, we still found that when the C:N > 25, the N₂O emission flux gradually stabilized (0.23 μ g m⁻² min⁻¹). The data of N₂O emission fluxes and C:N ratio also accorded with normal distribution, which showed that the nonlinear fitting was reliable (as shown in the figure below).

N₂O Fluxes One-Sample Kolmogorov-Smirnov Test

		N2O
N		30
Normal Parameters ^{a,b}	Mean	0.4105
	Std. Deviation	0.25183
Most Extreme Differences	Absolute	0.147
	Positive	0.147
	Negative	-0.123
Test Statistic		0.147
Asymp. Sig. (2-tailed)		.095°
a. Test distribution is No	ormal.	

a. Test distribution is Non

b. Calculated from data.

c. Lilliefors Significance Correction.

C:N Ratio One-Sample Kolmogorov-Smirnov Test

		CNratio
N		30
Normal Parameters ^{a,b}	Mean	28.2203
	Std. Deviation	10.58221
Most Extreme Differences	Absolute	0.308
	Positive	0.308
	Negative	-0.216
Test Statistic		0.308
Asymp. Sig. (2-tailed)		.000 ^c

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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?

Author's response:

The microbial abundance can only reveal the structural changes in microbial community. Thus, we would like to further explore the change about *nirS* and *nirK* genes which were the N₂O producers in the microbial community. In the section of *Discussion*, we compared and explored N₂O emission fluxes with *nirS* and *nirK* genes to further verified the effect of water level mediated by vegetation on N₂O emission through microbial processes. In addition, anammox, as an important component of nitrogen cycle, was also affected by the change of water level in wetlands. And, there may be competition between the anammox and denitrification, which may indirectly affect the nitrogen cycle and N₂O emission. Thus, in order to more comprehensively study the dynamics of functional microorganisms involved in nitrogen cycling and reveal the N₂O emission process in wetlands, we also discussed the anammox.

E:

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.

Author's response:

We agreed the detailed review and comments which will be helpful in the revision. A

point-by-point response to comments was given below.

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.

Author's response:

Our study found that the change of hydrological conditions effected by hydraulic regulation can induce the natural distribution of vegetation in Figure 2, and N₂O emissions varied with vegetation zones. For example, the N₂O flux of mud flat weas the highest, and the N₂O fluxes of reed and nymphoides zones were higher than those of carex and phalaris zones (Figure 4a). Therefore, we considered the mitigation of greenhouse gas by natural vegetation under hydraulic regulation as a highlight. However, we agreed that this sentence extended the results of the manuscript. In order to ensure that the abstract reflected the most important aspects of the manuscript, we deleted this sentence.

• *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

Author's response:

The ecological factors referred to the contents of carbon and nitrogen and the stoichiometric proportion in sediments. And we modified the description of the hypothesis based on reviews as following:

L82-83. "(2) the contents of carbon and nitrogen and the stoichiometric proportion of sediments changed with vegetations;"

• 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?

Author's response:

The upper left part was two images of the study area taken from different views (front view and top view). The lower panel was the schematic diagram of vegetation distribution and sampling in the study area. New caption was as following:

L86-87. "Figure 1. Location and distribution of sampling sites in Dongwa wetland of Dongting Lake, China (the upper left part was two images of the study area taken from front and top views; the lower left part was the schematic diagram of vegetation distribution and sampling in the study area; three sampling sites were used in each vegetation zone)"

• 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

Author's response:

During low water level, we used floating foam as a marker and connected sampling site with fishing line (about 6-7 m). We located every foam. Thus, we can accurately find the location of each sampling site during flooding.

• 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.

Author's response:

During high water level, we used a standard underwater plant picker $(0.25m \times 0.25m)$ to collect plants. The sample obtained by using this picker was the biomass of plant in 0.0625 m² (as shown in the figure below).



The collected plants were brought back to the laboratory for drying and weighing. The biomass (dry) of plants per unit area (g m⁻²) was calculated based on the area of the quadrat (or the area of the plant picker) and the dry weight. We pulverized and homogenized the dry plants, then analyzing the concentrations of carbon and nitrogen (g g⁻¹) in the plants. Finally, carbon and nitrogen densities per unit area (g m⁻²) were obtained according to the concentrations of carbon and nitrogen in plants (g g⁻¹) and biomass per unit area of plants (g m⁻²). In fact, both the biomass of plants and the concentrations of carbon and nitrogen in plants were changed.

• 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.

Author's response:

When comparing N_2O emission during different water levels, we only compared two sets of data at different water levels in the same vegetation zone. At this point, we only considered water level as a factor. Then we compared the N_2O emission between different vegetation zones at high and low water levels, respectively. We only considered vegetation as a factor at this moment. In the section of *Discussion*, we also discussed the effects of water level and vegetation on N_2O emission separately.

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.

Author's response:

Thank you for anonymous referee#2's reviews about the point in Figure 2b and 2c. We monitored all vegetation zones for a whole year using a self-calculating water level depth monitor. At the same time, the monitoring of plant biomass was rigorous. We set up multiple quadrats in each vegetation zone and averaged the biomass of each vegetation. And we measured the concentrations of carbon and nitrogen in each plant multiple times to reduce the error in the data. As mentioned in the previous reply, plant species had different amounts of carbon and nitrogen in their organisms (Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S. S., McCauley, E., Schulz, K. L., Siemann, E. H., and Sterner, R. W.: Nutritional constraints in terrestrial and freshwater food webs, Nature, 408, 578-580, 2000.; Yu, Q., Chen, Q., Elser, J. J., He, N., Wu, H., Zhang, G., Wu, J., Bai, Y., and Han, X.: Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability, Ecology Letters, 13, 1390-1399, 2010.). we hypothesized that the biomass (carbon density and nitrogen density) of plants can be used as an index to distinguish plant species. Many studies analyzed the effect of hydrological conditions on species composition and biomass of plants to reveal the interaction between hydrology and vegetations (Seabloom E. W., van der Valk A. G., Moloney K. A.,: The

role of water depth and soil temperature in determining initial composition of prairie wetland coenoclines, Plant Ecology, 138(2): 203–216, 1998.; Crosslé K., Brock M. A.,: How do water regime and clipping influence wetland plant establishment from seed banks and subsequent reproduction, Aquatic Botany, 74(1): 43–56, 2002.; Fraser L. H., Karnezis J. P.,: A comparative assessment of seedling survival and biomass accumulation for fourteen wetland plant species grown under minor water-depth differences, Wetlands, 25(3): 520–530, 2005.). Therefore, vegetation community was the key factor that mediated hydrodynamic process to affect N₂O emission.

• 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?

Author's response:

L101-102. "The depth ratio was recorded by a self-calculating water level depth monitor (Onset HOBO U20-001-02) from October 2019 to October 2020". This sentence introduced the source of the data in Figure 2a. We used the ratio of flooding depth to flooding time as the change rate of water level in the x-axis of Figure 2b and 2c. The change rate of water level can reflect both the depth and time of flooding. And the photographs showed the plant in each vegetation zone.

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.

Author's response:

The result containing references were from other studies. We revised this issue as following:

L234-235. "Long-term fluctuations in water level affected the moisture of the soil in the wetland, which directly led to changes in the habitat for specific species (Boar, 2006)."

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.

Author's response:

In this study, vegetation decline mainly refers to the litter and death of plant caused by flooding. During the steady period of flooding, the litter and death of plants and the changes in the content of carbon and nitrogen in sediment have stabilized. In our experimental design, the biomass of plants and the content of carbon and nitrogen in sediments were analyzed and tested at each steady period of water level. And we compared and discussed the data obtained from the above process. Thus, the assimilation of plants and the dynamics of carbon and nitrogen in sediments can be accurately evaluated.

 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.

Author's response:

By comparing carbon (nitrogen) density of plant with the concentration of carbon (nitrogen) in sediment, we analyzed and discussed the carbon (nitrogen) cycling between plant and sediment with the change of water level. Due to the litter and death of plant caused by flooding, plants transported large amounts of carbon and nitrogen to sediments. This resulted in higher concentrations of carbon and nitrogen in sediments at high water level than that at low water level as shown in Figure 3b and 3d. This suggested that sediments acted as sinks of carbon and nitrogen during flooding. After the flooding, the sediment provided the nitrogen needed for the growth of plant. At this point, the sediment acted as a source of nitrogen. Thus, we used the

words like sink/source and assimilation/accumulation/decomposition. We will further consider and revise the use of these words in the revision.

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

Author's response:

The closed-chamber on the ground was divided into two parts: an upper chamber and a pedestal. There is a trough at the junction of the upper chamber and the pedestal to keep out the air. And we need to insert the pedestal before sampling. After putting the upper chamber on the pedestal injection of water into the trough, the inside of the chamber was isolated from the outside air. The inside of the floating chamber was also isolated from the outside air. Thus, this method was called closed-chamber technique (as shown in the figure below).





We supplemented the description of the chambers in the revision as following:

L111-113. "The chamber technique on the ground was used to measure the in situ N_2O emission rate according to Wang (Wang et al., 2006). And we used a floating chamber to measure the in situ N_2O emission during high water level (Cole et al., 2010; Gålfalk et al., 2013)."

• L123. Sediments were collected/sampled, I guess

Author's response:

We revised as following:

L123-124. "The microbial community structure in sediments was detected by using Illumina MiSeq sequencing of the 16S rRNA gene with the primers 515FmodF and 806RmodR"

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

Author's response:

The traits of vegetation referred to the carbon and nitrogen densities of vegetations and C:N ratio of plants.

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

Author's response:

We double checked and revised the data as following:

L142-144. "The water level in each vegetation zone reached its maximum on June 25, 2020 (mud flat: 6.493 m, nymphoides zone: 6.489 m, phalaris zone: 6.463 m, carex zone: 6.347 m, and reed zone: 5.405 m)."

 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"

Author's response:

L242-243. "Moreover, the niche of plants reflected the tolerance to disturbance and the ability to resist competition from species, to reveal the distribution of plants in habitats (Tilman, 1999)." According to this sentence, we further explained the cause of vegetation distribution based on niche theory. It meant that vegetation distribution was the response of plant niche to disturbance from hydrologic conditions.

• 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

Author's response:

According to Clymo et al.'s study, we inferred the possibility for the accumulation of organic matters as peat under anaerobic conditions. We revised as following:

L259-260. "Furthermore, compared to the aerobic state of the sediment without overlying water, anaerobic conditions in water-saturated layers significantly retard the decomposition of organic matters, possibly allowing it to accumulate as peat (Clymo et al., 1998)."

• 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 towater"), 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.

Author's response:

In this part, we discussed the influence of plants on N_2O emission. Before we start the discussion based on data, we would like to explore more ways about effects of plants on N_2O emission from other relevant studies. Therefore, at the beginning of this paragraph we addressed some relevant points. We repositioned the sentences and the references cited as following:

L290-294. "When aquatic plants grew, the oxygen released by photosynthesis ameliorated the conditions of the dissolved oxygen, redox potential, and pH, which affected the diffusion of N_2O from sediment to the overlying water (Zhang et al., 2010). The root system altered the rhizospheric environment and the attached microbes by releasing small molecule exudates, which also affected the emission of N_2O (Zhai et al., 2013)."