RESPONSE TO REVIEWERS' COMMENTS

Regular font: original comment by the reviewer
Italized text: response by the authors
"Italicized quotes":   revised text segments

Response to Comments by Referee #3

Reviewer's Comment: I have revised manuscript 'Shifting Mineral and Redox Controls on Carbon Cycling in Seasonally Flooded Mineral Soils'. This work focuses on an estimation of various factors (Eh, extractable Fe and Al, and root biomass) on the CO_2 emission from seasonally flooded forest soils in comparison to never flooded, and was done for the small scale transects. Moreover, the quality composition of SOM was determined in three soil horizons for these transect soils by the spectroscopic method (NEXAFS) and composition of DOC by the Fourier-transform ion cyclotron resonance mass spectrometry. During my revision, I found out that the manuscript has many drawbacks, especially in the methodological part and following results interpretation.

My main concerns are: i) statement that upland soils are not affected by water fluctuations (actually, they are affected, which I can see well from the Eh values), ii) interpretation of the composition of SOM by the methods, which can only provide qualitative estimation, but not quantitative; iii) discussion of the data which are not statistically significant, iv) absence of any soil microbiological analyses, which can explain the differences for CO_2 emission in various seasons.

Authors' response:
We thank the reviewer for these constructive comments and have taken the following steps to address them.

i) We agree with the reviewer that the upland soil undergoes seasonal changes in moisture that are reflected in the redox data. We have revised the description of the water table dynamics at the three landscape positions accordingly.

Author's changes: (Page 7, Ln 5-10): "The upland position does not experience flooding in a typical rainfall year, i.e., the water table does not rise above the soil surface. The transition position is located on the edge of the wetland, which typically does not get flooded in an average rainfall year but is under the influence of significant water table rise. The lowland position is in the lowest point of the transect and is flooded for several months throughout the year."

ii) We revised the description of the results to reflect the semi-quantitative nature of the mass spectrometry and spectroscopy results. We would further like to note that the methods used here are semi-quantitative. They allow to assess shifts in the relative abundance of different functional groups (C NEXAFS) or compound classes (FTICRMS) as is done in all other studies relying on these techniques. This is also the case for almost all other chemical methods for the

characterization of SOM (13C NMR, py-GC/MS, FTIR, XPS, etc), where relative abundances are commonly reported.

Authors' changes: (Page 16, Ln 12-16): "The composition of water extractable OM was similar across the transect (p-value > 0.05), but some general trends were noticeable. Both the modified aromaticity index (AI_{mod}) and the average molecular weight of the detected compounds showed gradual increases across the upland-to-lowland transitions in the surface horizons (Fig. 6a, Table S6). Paralleling that change, the relative contributions of lignin increased (+7%) and that of lipids decreased (-11%) moving from the upland to the lowland position (Fig. 6b, Table S5). In the subsurface horizons, however, both AI_{mod} and average molecular weight showed little changes (Fig. 6a, Table S6), while the relative abundance of lignin increased (+9%) and that of lipids decreased (-11%)."

(iii) We appreciate the reviewer's concern. Throughout the manuscript, we took greatest care to only report and discuss significant differences. The only data where we made an exception is the FTICRMS results. We decided to discuss the FTICRMS results because there are clear trends across the transect that are consistent with other observations (e.g., C concentration and functional groups chemistry) and was thus deemed of greater interest to the general public. To address the reviewer's comment, we revised the results section to clearly communicate the limitations in the data in the most transparent fashion possible.

Authors' changes: (Page 16, Ln 16-24): "To assess changes in oxidation state and molecular weight of compounds more readily available for microbial respiration, water extracts of all samples were analyzed by FT-ICR-MS (Fig. 6a-b, Fig. S1-3, Table S5, Table S6). The composition of water extractable OM was statistically indistinguishable across the transect (p-value > 0.05), but some general trends were noticeable. Both the modified aromaticity index (AI_{mod}) and the average molecular weight of the detected compounds showed gradual increases across the upland-to-lowland transitions in the surface horizons (Fig. 6a, Table S6). Paralleling that change, the relative contributions of lignin increased (+7%) and that of lipids decreased (-11%) moving from the upland to the lowland position (Fig. 6b, Table S5). In the subsurface horizons, however, both AI_{mod} and average molecular weight showed little changes (Fig. 6a, Table S6), while the relative abundance of lignin increased (+9%) and that of lipids decreased (-11%)."

(iv) We agree with the reviewer that microbial processes are at the center of soil carbon cycling, and that differences in microbial mineralization rates are the cause for the differences in CO2 emissions across the transect. This study aimed to disentangle the biogeochemical processes that cause C accumulation and loss (through microbial mineralization) in seasonally flooded soils, and thus we focused on the coupled biogeochemical measurements rather than detailed microbial biology. While we did not include direct measures of microbial biomass, community composition or function, we did measure microbial activity (CO_2 production) and metabolic potential (redox potential). We fully agree that more detailed analyses of microbial community composition and function would be an interesting endeavor for the future.

General comments:

Reviewer's Comment: The article focuses on the C cycling in seasonally flooded mineral soils, however, had looked only on the CO2 emission, what about the methane? How strong can be the contribution of this gas to C flux out from the soil? What is known from the literature?

Authors' response: We addressed this concern briefly in the methods section (previously: Page 7, Ln 11-19). Briefly, CO_2 emissions are found to be orders of magnitude greater than CH4 emissions in comparable wetlands. We expanded this section as follows and moved it to the introduction section.

Authors' changes: (Page 6, Ln 9-14): "Based on other reports for comparable sites (Holgerson, 2015; Kifner et al. 2018), we expected methane production within these seasonal wetlands. However, CO_2 emissions were at least 15-times greater than methane production in those sites. While we acknowledge the disproportionate potency of methane as a climate-active greenhouse gas, this study aimed to determine the environmental and biogeochemical factors influencing C accrual or depletion in across the upland-lowland transition. We thus focused our monitoring efforts on quantitatively more important CO_2 emissions as the predominant C loss pathway."

Reviewer's Comment: Introduction in general: you have provided some information about the knowledge gaps, but did not explain any mechanisms for the processes going in the periodically flooded soils, and what is unclear. Please, provide more details.

Authors' response: We revised the following paragraph in the introduction to crystallize the knowledge gap. We further provided more detail on specific mechanisms as detailed in the specific comments below.

Authors' changes: (Page 5, Ln 15-21): "Water saturation thus likely governs C cycling in seasonally flooded soils through its combined impact on oxygen availability, root dynamics and mineral composition; but how the relative contribution of these biogeochemical controls vary across spatial and temporal gradients is still unknown. A recent study along hillslope transects in tropical forest soils representing an oxygen gradient (Hall and Silver, 2015), for example, found that a combination of Fe (II) (a proxy for reducing conditions), fine root biomass, and total Fe and Al concentrations explained the most variation of surface soil C content. How the relationships between C content and important biogeochemical controls differ in systems that are subject to seasonal flooding is still in question, especially with depth (Barcellos et al., 2018)."

Reviewer's Comment: Besides, you have a lack of information about microbial driven processes. From the results, I can see that you did not touch this topic, but actually, C fate in the soil is driven by microorganisms.

Authors' response:
(see IV above): As mentioned above, this study focuses on the environmental and biogeochemical controls on C in seasonal wetlands. Specifically, we examine how environmental and biogeochemical controls influence microbial OM decomposition and CO2 production. While we do not include measures of microbial biomass, diversity or function, we do assess microbial activity (as evidenced by CO2 efflux) and metabolic potential (as evidenced by redox potential,

and OM transformation (as evidenced by FT ICR MS and NEXAFS analyses). We do agree that more detailed microbial analyses of the linkage between seasonal flooding and microbial activity and function would be an interesting endeavor for the future. [TM-(1]

Reviewer's Comment: Moreover, you should point I the Introduction the reason why you focus only on the CO2, and not on methane estimations.

Authors' response: Please see our previous response and changes above.

Reviewer's Comment: No information is presented about the effect of temperature on the processes you are going to study.

Authors' response: We revised the introduction to add more information regarding the effects of soil temperature on carbon cycling in soil.

Authors' changes: (Page 4, Ln 1-4): " Temperature and soil moisture are principle controls on C cycling in soils (Lloyd and Taylor, 1994; Wang et al., 2014). Temperature regulates biological and chemical reaction rates and thus regulates the rate at which decomposition of soil organic matter can occur (Davidson and Janssens, 2006). However, water saturation is a critical driver of organic matter (OM) decomposition processes in seasonally flooded systems (Neckles and Niell, 1994)."

Reviewer's Comment: Hypothesis1 – it is not clear: 1) capacity of which minerals do you mean? 2) greater C accumulation for which period? 3) what does macromolecular and chemically-reduced OM mean?

Authors' response:
1) We specified the reactive minerals under consideration.

Authors' changes: (Page 6, Ln 7): "… lower capacity of Fe/Al (hydr)oxides to protect OM,…".

2) C accumulation here refers to the amount of C accumulated during the formation of lowland versus upland soils in this position.

Authors' changes: (Page 6, Ln 6-8): "We hypothesized that seasonally reduced conditions upon flooding in lowland positions will result in lower CO_2 efflux, greater C accumulation, lower capacity of Fe/Al (hydr)oxides to protect OM, and a selective preservation of macromolecular or chemically-reduced OM compared to the upland position."

3) We defined both terms in the introduction (Page 4, Ln 8). A macromolecule is "a molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass." (IUPAC Gold Book definition). Chemically-reduced compounds are in reference to organic carbon compounds with lower C oxidation states (Page 4, Ln 11-13).

<u>Reviewer's Comment</u>: 4) what are specific sites? Forest as well as grassland soils can be under periodic flood conditions in various climates and can be developed on different parent materials.

<u>Authors' response:</u> The specific sites are described in a detailed site description as part of the methods section (Page 6, Ln 17 – Page 7, Ln 8).

<u>Reviewer's Comment</u>: Moreover, you did not provide any information on how the quality of accumulated organic compounds in flooded condition is different from upland.

<u>Authors' response:</u> We are slightly confused by this comment. We present an in-depth molecular characterization of the organic matter that has accumulated in lowland and upland positions using two advanced analytical techniques, NEXAFS and FTICRMS.

<u>Reviewer's Comment</u>: There are many works done for the rice soils, for example, which are also under the pulse soil moisture conditions.

<u>Authors' response:</u> We agree that there is a growing body of literature on C cycling in rice paddies. While we discuss and cite relevant publications (see excerpt below), the specific objective of this study was to investigate the seasonal dynamics in natural, temperate, seasonally flooded wetlands of the northeastern US. They are distinctly different from rice paddies with respect to seasonal temperature, precipitation, climate and soil type.

Favre, F., Tessier, D., Abdelmoula, M., Génin, J. M., Gates, W. P. and Boivin, P.: Iron Reduction and Changes in Cation Exchange Capacity in Intermittently Waterlogged Soil, Eur. J. Soil Sci., 53(2), 175–183, doi:10.1046/j.1365-2389.2002.00423.x, 2002.

Hanke, A., Sauerwein, M., Kaiser, K. and Kalbitz, K.: Does Anoxic Processing of Dissolved Organic Matter Affect Organic–Mineral Interactions in Paddy Soils?, Geoderma, 228–229, 62–66, doi:<u>10.1016/j.geoderma.2013.12.006</u>, 2014.

Kögel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kölbl, A. and Schloter, M.: Biogeochemistry of Paddy Soils, Geoderma, 157(1–2), 1–14, doi:10.1016/j.geoderma.2010.03.009, 2010.

<u>Authors' changes:</u> (Page 18, Ln 10-17): "The diminished importance of Fe_o in C accumulation in our seasonally flooded lowland soils is consistent with the loss of reactive Fe phases observed in forest soils (Fiedler and Kalbitz, 2003; Zhao et al., 2017) and rice paddy soils (Favre et al., 2002; Kögel-Knabner et al., 2010; Hanke et al., 2014). High concentrations of organic C in rice paddy surface soils drives the reduction and dissolution of redox-active minerals, such as Fe(III) oxides, which is subsequently translocated vertically down the soil profile (Kögel-Knabner et al., 2010, Chen et al., 2017). In our sites, we found a noticeable, yet insignificant, increase in Fe_o contents in the lowland C_g-horizons (Table 3), which is likely a reflection of these vertical transport processes of soluble or colloidal Fe phases into the subsurface horizon, where they may reprecipitate during drained periods (Kögel-Knabner et al., 2010; Hanke et al., 2014)."

Reviewer's comment: Materials and method: not clear how far where the plots from each other; is it possible to know the approximate age of these relief form (I mean they were permanently here or formed only when the experimental station was established?); how many soil cores were collected and how from each horizon and from each site? How many samples were collected for root analysis from each site and each landscape position?

Authors' response: Fig. 1 depicts the distance between the individual positions, which ranged from 3 to 5 meters. The replicate transects are within approximately 100 meters of each other. The two sites (South Deerfield and Amherst) are approx. 9 mi apart. They are indistinguishable in terms of vegetation, climate and soil types. In fact, our analyses found no statistical differences between the two sites. We updated the site description to include this information.

Authors' changes: (Page 7, Ln 4-5): "Fig. 1 depicts the distance between the individual positions, which ranged from 3 to 5 meters. The replicate transects are within approximately 100 meters of each other."

We don't know the exact age of the relief form, but the wetlands are in natural depressions in areas unaffected by agricultural use. They can thus be considered historic seasonal wetlands, as evidenced in the development of horizons characteristic of seasonal wetlands. This pedogenic process will take 100s of years, reflecting the general age of the wetlands.

Authors' changes: (Page 8, Ln 1-7): "Soil samples were collected using hand augers in each of the six replicate wetlands along the defined transects that included the three landscape positions (upland, transition, and lowland). In each landscape position, we collected soil cores from designated horizons, resulting in six replicate cores for each horizon and position. Horizons in the upland position were classified as A (0-25 cm), B (25-55 cm), and C (55-84+ cm) horizons; in the transition position as A (0-28 cm), C (28-48 cm), and C_g (48-69+); and in the lowland position as A (0-25 cm), C (25-35 cm), and C_g (35-68+ cm) (Soil Survey Staff, 1999) (Fig. 1a)."

Root biomass samples were collected in parallel with the selected representative transects as soil samples. We describe the method of collection in the revised manuscript (Page 8, Ln 14-18).

Authors' changes: (Page 8, Ln 15-16) "The cores were taken at 0-20, 20-40, and >40 cm and resulted in six cores per landscape position horizon (e.g., upland A-horizons n = 6)."

Reviewer's Comment: Fourier-transform ion cyclotron resonance mass spectrometry – method is not so used in soil science and can confuse readers, please provide additional information whether a liquid sample is introduced as a liquid for the measurement, or prepared additionally (dried or by any other way).

Authors' response: We have revised the section to include how the samples were prepared and introduced to the instrument. Briefly, soil samples were first extracted in ultrapure DI-H2O using one gram of soil and 10 mL of DI-H2O (1:10). After extraction, samples were filtered through 0.2-μm syringe filters. 100μl of the filtrate was then added to 200 μl of methanol (1:2) and injected directly (in liquid phase) onto the instrument.

Authors' changes: (Page 9, Ln 10-19) "*To determine the composition of bioavailable compounds that can potentially be used in microbial respiration (<600Da, Logue et al., 2016), water extracts of soil samples were collected on a 12 Tesla Bruker SolariX Fourier-transform ion cyclotron resonance mass spectrometer located at Environmental Molecular Sciences Laboratory (EMSL), a Department of Energy Biological and Environmental Research (DOE-BER) national user facility located in Richland, WA. Soil samples were extracted with ultrapure DI-H$_2$O using one gram of soil and 10 mL of DI-H$_2$O (1:10). The samples were sealed in 15 mL conical tip tubes and shaken for one hour. Samples were then centrifuged and filtered using syringe-filters. 100 µL of the filtrate was then added to 200 µL of methanol (1:2) and injected directly (in liquid phase) onto the instrument. A standard Bruker electrospray ionization (ESI) source was used to generate negatively charged molecular ions; samples were then introduced directly to the ESI source. The instrument was externally calibrated to a mass accuracy of <0.1 ppm weekly using a tuning solution from Agilent*".

Reviewer's Comment: For the results: there are many uncertainties in the method which you used to estimate the portion of various substances classes based on the FT-ICR-MS analysis, including ionization efficiencies, mobile phase composition, data acquisition parameters. Thus, I think it is better if you will re-do the figure 6, like it is originally advised in the article by Kim et al., 2003.

Authors' response: In response to the reviewer's comment, we added Van Krevelen Diagrams (VKD) for each sample in the SI (Fig. S1-3) and kept Figure 6 as is in the main manuscript. While FTICR MS is not a quantitative approach due to ionization competition during ESI process, all samples were run under the same exact conditions. As such comparison between samples is valid. One of the most common ways of visualizing FTICR MS data is through VKD (as suggested by the reviewer). This is a suitable approach when dealing with a handful of samples, but in this case we had roughly 70 samples, making it hard to visualize each sample through a VKD. Another way to use FTICR MS data is to group the peaks on the VKD into different compound classes based on their location on the VKD, for example lipid –like compounds are known to have low O/C and high H/C. After grouping all peaks based on their compound class, you can calculate relative abundance of each compound class. Since all samples were treated exactly the same, then this data can be used in a semi quantitative way to compare between different samples. This approach is highly used when dealing with a large number of samples. We followed the same approach in Figure 6 where soil samples were divided into three groups: upland, transition and lowland. Then each group was separated into three horizons. Only compounds/peaks that were unique to each horizon were then included in the calculation. We believe this approach works great for this data type especially to be able to see difference between samples, especially when it is hard to visualize 60 VKDs at the same time.

Reviewer's Comment: Another suggestion can be to determine at least some components by the true analytical methods in the bulk soil samples and not in the DOC (for example lignin and carbohydrates contents).

Authors' response: The authors agree that analysis of bulk SOM composition is pertinent to this study, which is why we have conducted C (1s) NEXAFS analyses (Page 8, Ln 20-24). This type of analyses is optimal for measuring C functional group characteristics as it is performed on bulk

soil samples. Results of these analyses are reported in section 3.5 and discussed throughout the discussion section. Additionally, Fig. 5 shows the individual spectra and average NEXAFS spectra for the landscape positions and horizons. Peak deconvolution is then used to determine relative abundances of C functional groups within each bulk soil sample. Full relative abundance of these functional groups are reported in Table S4.

<u>Reviewer's Comment</u>: Try to put conclusions at the end of each resulting part, summarizing the findings.

<u>*Authors' response:*</u> *We have added short conclusions to sections 3.1 and 3.3 in the results section. However, we feel that adding further conclusion remarks to sections 3.2, 3.4 and 3.5 would be redundant.*

<u>*Authors' changes:*</u>
(Section 3.1, Page 13, Ln 13-15): "In sum, the rise in seasonal water table in lowland positions during the growing season was accompanied by decreased E_h and CO_2 fluxes compared to the upland positions. As the water table dropped during the growing season E_h and CO_2 fluxes increased markedly."
(Section 3.3, Page 14, Ln 3-5) "In sum, CO_2 emissions in the upland position were most strongly correlated to soil temperature, while water table depth and VMC correlated more strongly with CO_2 fluxes in the lowland position during the growing season."

<u>Reviewer's Comment</u>: Everywhere – when you speak about C in the soil, please use 'content' (g kg-1 soil) and 'concentration' when you speak about DOC.

<u>*Authors' response:*</u> *We have updated the terminology in the manuscript to reflect this suggestion.*

<u>Reviewer's Comment</u>: Table 1 – Actually shows no differences between CO2 effluxes, is it right?

<u>*Authors' response:*</u> *We appreciate the reviewer pointing out this discrepancy. We clarified that CO_2 effluxes were significantly different among the three landscape positions only when normalized to C content (revised Table 1).*

<u>Reviewer's Comment</u>: Fig. 1 and Fig. 2 – from the Fig 1 I can see, that actually all horizons C for all soils are actually all the time underwater. I have than a question: how did you measure the Eh? And how did you collect soils samples?

<u>*Authors' response:*</u>
We apologize for the confusion. The upland position, including the C horizon, are entirely drained during the dry season. We revised Fig. 1a to more accurately depict the water table dynamics in the upland landscape position. Moreover, we corrected Fig. 2b. In this figure, we had plotted the water table to be at 50 cm depth during the dry period, even though the water table had dropped below the measuring gauge. The measuring gauges were installed at 50 cm depth, thus when the water table was at or below 50 cm it was recorded at 50 cm and that was shown in Fig. 2b (October through December). This gave the false impression that the C-

horizons in the upland position are saturated during these months. To address this mistake, we removed the data points from Fig. 2b and noted that the water table dropped below this mark (Page 7, Ln 18-19).

<u>*Authors' changes*</u> *(Page 7, Ln 18-19): "Water table fluctuations were monitored using slotted PVC pipes installed to depths of 50 cm, therefore water table depths below 50 cm were undetectable and not reported (Fig.2b)."*

(Page 31, Ln 12-14): "Missing data points for the upland position in panel (b) are due to the water table dropping below the measuring gauge installed at a depth of 50 cm and is denoted with asterisks (*)."

As described in the methods section (Page 7, Ln 19-25), we measured Eh, we installed redox probes at each location to depths of 15, 30, and 45cm in triplicate in each landscape position of the six sites (Page 10, Ln 4-8). The redox probes were left in place for the entire year-long study and measured in parallel with CO_2, VMC and water table fluxes.

<u>Reviewer's Comment</u>: Moreover, Upland soil is also under the effect of water table rise (I can see it very well from the Eh values).

<u>*Authors' response:*</u> *We agree that the upland position does see a rise in water table depth during the fall recharge period, and that the subsurface horizons are affected by the fluctuations in moisture content. However, we do not make this claim in the manuscript, rather we are comparing flooded soils in the lowland positions which are submerged under water to their upland counterparts which are not submerged at any point throughout the year.*

(Page 7, Ln 5-8): "The upland position is in a forested landscape, approximately five meters away from the edge of the wetland, which does not undergo any flooding. The transition position is located on the edge of the wetland, which typically does not get flooded in an average rainfall year but is under the influence of water table rise. The lowland position is in the lowest point of the wetland and is flooded for several months throughout the year."

<u>Reviewer's Comment</u>: Fig 3 is not discussed in the result part.

<u>*Authors' response:*</u> *We greatly appreciate the reviewer for noticing the missing section from our manuscript. This results section had been inadvertently deleted from the manuscript. This section has been added back into the manuscript.*

<u>*Authors' changes:*</u> *(Page 13, Ln 16 – Page 14, Ln 16):*
"3.2 Control on CO_2 fluxes
To determine which of the above environmental parameters best predict soil respiration across the hydrological gradient, we conducted a series of regression analyses (Fig. 3a-d). Regression analyses were carried out for subsets of the data representing the (i) full year, (ii) growing season or (iii) non-growing season (Table 2).
*   Soil temperature. The strength of the relationship between CO_2 flux and soil temperature, as expressed by how well the data can be described using the Arrhenius equation (Sierra, 2012),*

decreased along the upland-to-lowland transect (Fig. 3a). Soil temperature explained the most variance of CO_2 fluxes in the upland positions throughout the full year (r = 0.72, p < 0.001) and the growing season (r = 0.62, p < 0.001) (Table 2). Comparing the three landscape positions, soil temperature explained the least variation of CO_2 fluxes in all cases in the lowland position, especially in the growing season (r = 0.45, p < 0.001).

Soil moisture. As the relationship between CO_2 flux and soil temperature became weaker, that between CO_2 flux and water table depth gradually became stronger along upland-to-lowland transitions. CO_2 flux and water table depth (Fig. 2b) were significantly negatively correlated in the lowland positions in the full, growing season and non-growing season time periods (Table 2). The strongest correlation between water table depth and CO_2 flux occurred in the lowland position during the growing season (r = -0.55, p < 0.001), where it had a stronger relationship with CO_2 flux than soil temperature. Similarly, VMC and CO_2 fluxes were negatively correlated in the lowland position (r = -0.51, p < 0.001), with VMC showing a stronger relationship with CO_2 flux than soil temperature during the growing season (Fig. 2c) Soil redox potential, In keeping with a strong relationship between moisture and respiration at the transition and lowland positions, E_h was also most significantly correlated with CO_2 at the transition and lowland positions (Fig. 2d). E_h was a comparable predictor for CO_2 flux in both the lowland (r = 0.40, p-value <0.001) and transition (r = 0.41, p-value <0.001) positions during the growing season, but had no correlation with CO_2 flux in the upland position (Table 2). The strong correlations between E_h and CO_2 emissions were primarily limited to the lowland position.

In sum, CO_2 emissions in the upland position were most strongly correlated to soil temperature, while water table and VMC correlated more strongly with CO_2 fluxes in the lowland position during the growing season."

<u>Reviewer's Comment</u>: Discussion part (4.3) Discussion about fig. 5 does not support by statistical analyses (near all differences are not statistically significant). I suggest that you try to test differences between the horizons. Besides, data on figure 6 are qualitative and not quantitative (see comments above).

<u>*Authors' response:*</u> *The statistical analyses regarding the data in Fig. 5 are reported in Table S4. As the reviewer suggested, we did test differences based on a horizon basis.*

<u>Reviewer's Comment</u>: Part 4.4. This is basically a repetition of the discussion above, and suggest to include these references into the discussion part to make it deeper in sense.

<u>*Authors' response:*</u> *Section 4.4 explores how any moisture and temperature changes due to climate change may affect the dynamics within these vulnerable ecosystems. We feel these important implications are the core message of the paper and should be included.*

Specific comments

<u>Reviewer's Comment</u>: P3L11 '' seasonally flooded soils are metabolically more active'' – please be more precise here, what do you mean?

<u>*Authors' response:*</u> *We revised this sentence to address the reviewer's concern.*

Authors' changes: (Page 3, Ln 10-12): "This is surprising given that seasonally flooded soils are characterized by greater microbial activity than permanently flooded wetlands, resulting in significantly greater greenhouse gas emissions (Kifner et al. 2018)."

Reviewer's Comment: P4L5 Forgot oxidation state - SO4 2-

Authors' response: We have revised this sentence accordingly.

Authors' changes: (Page 4, Ln 21): "Further, once oxygen is depleted, microbes rely on alternative terminal electron acceptors (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-})…"

Reviewer's Comment: P3L25 – P4L12 Please be more precise here: the activity of which enzymes are inhibited;

Authors' response: We have revised the sentence for clarity of which enzymes we are referring to.

Authors' changes: (Page 4, Ln 5-8): "The resulting oxygen limitations inhibit the activity of oxidative enzymes, such as phenol oxidase or peroxidase (Freeman et al., 2001; Keiluweit et al., 2016), which catalyze the depolymerization of higher-molecular weight OM compounds (i.e., macromolecules) into smaller, assimilable compounds (Megonigal et al. 2003)."

Reviewer's Comment: which exactly levels of Eh are usually observed in the seasonally flooded soils? This is important to write because the various electron-donor system is active for various Eh values; how does soil C content effect on the Eh conditions?

Authors' response: Seasonal variations in Eh are given in Fig. 2d and Table S2. Upland Eh ranges fall between approximately 390 and 500 mV during the growing season, and between 595 and 618 mV during the non-growing season. Transition Eh ranges fall between approximately 190 and 240 mV during the growing season, and between 480 and 580 mV during the non-growing season. Lowland Eh ranges fall between approximately 70 and 90 mV during the growing season, and between 415 and 450 mV during the non-growing season. There were no significant relationships found between C content and Eh in our systems.

Reviewer's Comment: P8L2 What does spline function mean?

Authors' response: An equal-area quadratic spline function is a mathematical function to estimate properties in soil, which fits curves piecewise throughout the entire soil profile based on given data points. The curves ensure there is equal areas to the left and right of the spline curve. This method enables us to use non-horizon based data points and estimate their values for given horizons. This method has been extensively used in soil science since first applied by Bishop et al. (1999), and later refined by Malone et al. (2009). The below references further explain how equal area spline functions can be used in soil science. We have revised this sentence with references.

Odgers, N. P., Libohova, Z. and Thompson, J. A.: Equal-area spline functions applied to a legacy soil database to create weighted-means maps of soil organic carbon at a continental scale, Geoderma, 189–190, 153–163, doi:10.1016/j.geoderma.2012.05.026, 2012.

Malone, B.P., McBratney, A.B., Minasny, B., and Laslett, G.M.: Mapping continuous depth functions of soil carbon storage and available water capacity, Geoderma, 154(1), 138-152, doi.org/10.1016/j.geoderma.2009.10.007, 2009.

Bishop, T.F.A., McBratney, A.B., and Laslett, G.M.: Modelling soil attribute depth functions with equal-area quadratic smoothing splines, Geoderma, 91(1), 27-45, doi.org/10.1016/S0016-7061(99)00003-8, 1999.

Authors' changes: (Page 8, Ln 17-19): "The initial values of root biomass were used to estimate biomass values for each soil horizon using an equal-area quadratic spline equation (Malone et al., 2009; Spline Tool v2.0, ASRIS). Mean E_h values for each soil horizon were also estimated using the equal-area quadratic spline equation (Malone et al., 2009)."

Reviewer's Comment: P8L23 Please, provide filter size for the DOC filtration.

Authors' response: Water extractable OM samples were filtered using 0.2-μm syringe filters.

Authors' changes: (Page 9, Ln 15): "Samples were then centrifuged and the supernatant filtered using 0.2-μm syringe filters."

Reviewer's Comment: P11L17-19 You can delete this sentence and start directly with results.

Authors' response: We have removed this sentence.

Reviewer's Comment: P11L20-P12L4 Please do not repeat data presented in the table, as well as the name of the stat. test you can remove, it is given in the table captions and in the mm section. Please, correct everywhere in the result section. Moreover, write only about significant results in the entire article, unless the goal of the paper is to show the absence of significance. Please, correct everywhere in the result section.

Authors' response: We thank to reviewer for these suggestions and have removed the names of the stats tests from the results section text. In regards to the discussion of non-significant results in the article, the only data we refer to that were not significant was the FT-ICR-MS data. We have previously explained the limitations of the statistical analyses (point iii in initial general comments) and have attempted to present these limitations in a transparent fashion to the reader: Page 16, Ln 11-13: "The composition of water extractable OM similar across the transect (p-value > 0.05), but some general trends were noticeable.".

Reviewer's Comment: P14L1-P14L16 from Table S4, I can see that only the second horizon was different from for the landscape positions, thus, please, delete description about insignificant

results. It is enough to write that no significant differences were found for other horizons or functional groups.

Authors' response: Table S4 shows significant differences for relative abundances of aromatic, aliphatic, carboxylic C functional groups between the three landscape positions on a horizon-basis in the surface horizons and second horizon groups. When insignificant trends were mentioned we addressed them as such.

Reviewer's Comment: Moreover, I do not see the reasons to present Fig 5 in the main result part, because it does not show any significant results. Please transfer to the supplementary materials.

Authors' response: As noted above, there are significant differences among the relative abundances of C functional groups shown in the NEXAFS spectra presented in Fig. 5. Therefore, we would respectfully keep this figure in the main part of the manuscript.

Reviewer's Comment: P15L1-2 Please, delete this sentence. Start with the discussion directly.

Authors' response: We have removed the suggested sentence.

<u>**Response to Comments by Referee #4**</u>

Comment: This manuscript investigated the environmental and biogeochemical factors in controlling CO_2 efflux and organic matter composition along upland to lowland transition in seasonally flooded mineral soils. The experiment in this study is well designed, linked soil CO_2 emissions and related soil and plant properties in field condition. Some of results produced from this study are valuable, to certain extent, and provide additional data to fill the gap if considering the effects of seasonal flooding and mineral composition on C dynamics were rarely investigated in the field. I think the paper is publishable, but it requires minor revisions. Below, please find a list of comments that would be helpful to consider for revision of this paper.

<u>*Authors' response:*</u> *We thank the reviewer for the constructive comments have taken the suggested steps to improve the discussion section of our manuscript.*

General comments:

1) More recent literatures are needed in the discussion, to make clear what have been done. For example, Page 16 (Lines 15-20), Page 17 (Lines 5-10) the authors cited literatures about contribution of reactive Fe and Al on C accumulation.

<u>*Authors' response:*</u> *We thank the reviewer for suggesting more discussion around recent literature to ensure we are clearly identifying what is new and exciting about our study. We have included more discussion in sections 4.1, 4.2 and 4.3 in connection to prior literature in similar systems. However, there are limitations to comparing other study sites, such as wetlands in the Southeastern US and rice paddies, as they differ in climate, vegetation and parent material.*

<u>*Authors' changes:*</u>
(Section 4.1, Page 17, Ln15-17): "In contrast, CO_2 fluxes along a forested wetland gradient in the Southeastern US showed a lesser seasonal convergence (Krauss and Whitbeck, 2012), as the average annual air temperature of this study site is 7 degrees Celcius above the annual average in the Northeastern US."

(Section 4.2, Page 18, Ln 17-25): "The diminished importance of Fe_o in C accumulation in our seasonally flooded lowland soils is consistent with the loss of reactive Fe phases observed in forest soils (Fiedler and Kalbitz, 2003; Zhao et al., 2017) and rice paddy soils (Favre et al., 2002; Kögel-Knabner et al., 2010; Hanke et al., 2014). High concentrations of organic C in rice paddy surface soils drives the reduction and dissolution of redox-active minerals, such as Fe(III) oxides, which is subsequently translocated vertically down the soil profile (Kögel-Knabner et al., 2010, Chen et al., 2017). In our sites, we found a noticeable, yet insignificant, increase in Fe_o contents in the lowland C_g-horizons (Table 3), which is likely a reflection of these vertical transport processes of soluble or colloidal Fe phases into the subsurface horizon, where they may reprecipitate during drained periods (Kögel-Knabner et al., 2010; Hanke et al., 2014)."

(Section 4.3, Page 20, Ln 17-21): "A study of rice paddy soils showed that reductively dissolved Fe (hydr)oxide coatings on vermiculite clay surfaces were re-precipitated on mineral surfaces

upon re-oxygenation during water table drawdown (Favre et al. 2002). Therefore, it is likely that seasonally reduced Fe (hydr)oxides are transported down the soil profile and then re-precipitated in the subsurface soil horizon during water table drawdown (Kögel-Knabner et al., 2010)—trapping dissolved, partially-oxidized, lignin-derived OM also leaching down the profile and so resulted in the accumulation of relatively-oxidized OM."

2) I would like to see in depth discussion on the physiochemical mechanisms involved in the process of C dynamics with respected to Fe and Al phases in both upland and lowland filed should also be summarized here based on the published literatures. For example, page 16 (line 20-25) presented only reductive dissolution of Fe and Al phases.

Authors' response: We thank the reviewer for this suggestion and refer to our improved discussion of the role of Fe and Al phases in C retention in both reducing and oxidizing soils in the introduction (Page 5, Ln3-14) and discussion sections 4.2 (Page 18, Ln 17 – Page 19, Ln 16) and 4.3 (Page 20, Ln 16-21). Specifically, we point out how reducing conditions limit retention of C by Fe in the lowland sites compared to upland soils, due to reductive dissolution of Fe(III) oxides during anaerobic periods (Page 5, Ln 6-11 and Page 18, Ln 19-21). Conversely, we discuss how Al hydroxides are non-reducible and thus act as the primary sorbent for OM in flooded soils (Page 5, Ln 11-15 and Page 19, Ln 2-9).

3) Further experiment needed on abiotic component (MBC or community structure) and will add values on the obtained data and comprehensive understanding on processes. This would certainly help us better understand the role of wetting and drying cycles on GHG (CO_2, in this work).

Authors' response: The authors agree that microbiological approaches would enable us to answer more questions regarding the microbes and metabolic pathways mineralizing carbon along our transects. However, this aspect is outside the scope of this manuscript, which is focused on the influence of mineral composition and redox state on carbon accumulation and emissions. Future efforts at the site will surely look into furthering our understanding of seasonal shifts in microbial community composition and function within these ecosystems.