RESPONSE TO EDITORIAL COMMENTS

Dear Dr. Luo Yu,

Thank you for the opportunity to submit a revised manuscript to *Biogeosciences*. We have addressed the issues and concerns offered in in the two very thoughtful and constructive reviews as detailed in our point-by-point response below. A marked-up version of the revised manuscript follows our response below. Reviewer #1 had noted the discrepancy between the statistical results presented in the text and those reported in Table 3. That issue was simply due to the fact that we had mistakenly reported the results of statistical tests (ANOVA + Tukey's HSD) we had conducted on the entire dataset, rather than on a horizon-basis. We relied exclusively on this horizon-based comparison to assess our data for this manuscript. This problem was remedied by correcting the table to reflect the results of the horizon-based comparison and explaining the statistical approach in more detail. Reviewer #2 further requested substantial revisions of the abstract, which we rewrote almost completely. Reviewer #2 noted that the discussion section was repetitive and didn't sufficiently highlight the novelty of the results. In the revised manuscript, all discussion sections were significantly improved to sharpen the main points we would like to communicate. In addition, we completely rewrote the broader implications section to link our observations to potential climate change impacts on seasonally flooded mineral soils.

We hope that these changes adequately address your and the reviewers' concerns. Thank you for considering our revised manuscript for publication in *Biogeosciences*.

Sincerely,
Marco Keiluweit
Assistant Professor
University of Massachusetts Amherst

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 #1

Comment: Overall, I think this is a valuable dataset and a well-executed study. I support its eventual publication. The surface horizon data are well established, and I have just minor comments there as indicated below. However, the subsurface depth data are problematic, mostly because there are different overall soil depths in each of the sites and different horizon designations. This has led also I think to some statements that are not well supported by the statistics or that the statistics used are not well presented. For instance, reading the abstract while looking at Table 3 raises several questions if we interpret "significantly lower" to mean different statistical lowercase letter assignments, which most readers will. Much of my confusion occurs in section 3.3, where it appears in most cases the differences described are not statistically significant as shown on Table 3, but this is not pointed out in the text. At one point here the authors refer to a Tukey test for the topsoils (although I think they misplaced the word subsoil on pg. 12, ln 2) with a p value < 0.01 for the transition vs. lowland, but the those share a lowercase letter assignment in Table 3, which suggests they would not have a p value < 0.01. Correcting these presentation or interpretation issues is critical.

Authors' response: We thank Dr. Thompson for these extremely thoughtful and constructive comments. We took the following steps to assure that the statistical tests and the resulting estimates of significance are reported correctly.

First, we emphasize that the statistical analyses were done on square root transformed data, but the data provided in Table 3 is the non-transformed observational data. We have clarified this in the methods section on Page 10: "Statistical analyses were conducted on square root transformed data when assumptions of normal distribution were not met, although non-transformed observational values are reported within the text (Table 3)."

Second, we clarified our statements regarding the significant differences in C contents among surface and subsurface soils across the upland-to-lowland transect. We believe that the confusion arose from the fact that we made statements in the text (both abstract and main body) that were not reflected in the statistics reported in Table 3. We had initially conducted ANOVAs and Tukey's HSD tests on the whole dataset (i.e., across all landscape positions and horizons). But as we were primarily concerned with comparing C contents across individual horizons along the transect (e.g., comparing A horizons across upland, transition and lowland position), we conducted our final ANOVAs on a horizon-basis. Those tests indicated that both A and C horizons in upland and lowland positions are significantly different. We had presented the results from this analysis, including the p values indicating significant differences among the horizons, in the abstract (Ln: 20-22) and results section (Pg. 11 Ln:21-25). Unfortunately, the initial Table 3 mistakenly showed the results of the ANOVAs for the entire dataset, rather than the horizon-

based comparisons we used to test our hypotheses. The same mistake was made in Table S2 and S4. In the revised version, we updated the table letter designations, added detail on the statistical method, indicated which comparisons were made in the result section, and revised table footnotes to indicate that letter designations reflect the results from the horizon-basis analysis (Table 3, Table S2 and Table S4). We hope that this explanation and the measures taken will clarify this issue.

Assuming the stats letter values are correct, I think this could be resolved by looking at C stocks rather than C concentrations at depth and backing off on some of the subsurface interpretations that are not fully supported by the stats.

<u>*Authors' response:*</u> *We thank the reviewer for this suggestion. We believe the above explanation of the statistical approach taken to support our interpretations resolves the confusion about the stats letters.*

If the authors have data binned at finer depth intervals, that might help clarify things as well, but if not I suggest using C stock down to 68 cm, in which case one could compare equally across all the sites. One could examine surface C stock (0- 25 cm) and then a subsurface C stock value (25 – 68cm). Outside of this major issue, I think the paper has a lot of promise and the combination of field CO2 data and molecular-scale carbon chemistry is exciting.

<u>*Authors' response:*</u> *We thank the reviewer for this suggestion, but believe that the measures taken above to clarify our statistical approach sufficiently address this concern.*

Abstract

<u>Comment:</u> I read and reviewed the abstract without looking at any other parts of the MS to mimic a reader looking at the abstract on-line. Read alone, I am not clear on the findings and implications and thus the abstract needs to be clarified. I give a couple of specifics below in the line edits, but I encourage the authors to have someone unfamiliar with the study read the abstract alone after revision.

<u>*Authors' response:*</u> *We revised the abstract in response to the specific comments provided below, but we have also substantially revised the abstract in response to the general comments provided. Most importantly, added specific results that allow the reader to assess how we arrived at our conclusions.*

<u>Comment:</u> Ln 14: Is it really true that this is largely unknown? If this is just for seasonally flooded mineral soils (compared to wetlands in general), then this point escaped me on the first read. Perhaps it was the shift from "seasonally flooded soils" in the previous sentence to "seasonally flooded mineral soils" in this sentence. Use one term and stick with in, especially in the abstract where space is tight.

<u>*Authors' response:*</u> *We agree that the terminology should be more consistent and are using 'seasonally flooded mineral soils' here and throughout the revised manuscript.*

<u>Authors' changes:</u> Page 1, Ln12-14 "Redox conditions, plant root dynamics, and the abundance of protective mineral phases are well-established controls on soil C persistence, but their relative influence in seasonally flooded mineral soils is largely unknown."

<u>Comment:</u> Ln 16: Need to specify here that the lowlands are periodically flooded and the uplands are not if that is indeed the case. I am assuming that, but one could have uplands that are also periodically flooded due to high rainfall and perched water tables.

<u>Authors' response:</u> This point has been clarified in the abstract. The lowlands are in fact seasonally flooded whereas the transition and upland position are not.

<u>Authors' changes:</u> Page 1, Ln16-18 "Specifically, we contrasted mineral soils under temperate deciduous forests in lowland positions that undergo seasonal flooding with adjacent upland soils that do not, considering both surface (A) and subsurface (B/C) horizons."

<u>Comment:</u> Ln 17: This sentence is hard to follow. I read it twice and was still not sure what it was saying, where C was higher? I suggest "We found the lowlands had lower CO2 effluxes than the uplands. Lowland surface soils (0-20 cm...or whatever it is, also could give A or B or O classification) had higher C concentrations a higher abundance. . .than the uplands."

<u>Authors' response:</u> We revised the sentence as suggested.

<u>Authors' changes</u>: Page 1, Ln18-21"We found the lowland soils had lower total annual CO_2 efflux than the upland soils, with monthly CO_2 efflux most strongly correlated with redox potential (E_h). Lower CO_2 efflux as compared to the uplands corresponded to greater C concentration and abundance of lignin-rich, higher-molecular weight, chemically-reduced organic compounds in the lowland surface soils (A-horizons)."

<u>Comment:</u> Ln 20: Here I was confused again by subsoils slipping in there. I think you need to be much more upfront about this distinction as it is one of the main points of the abstract. At the end you also start to talk about C stocks (depth integrated concentrations), which would take into account bulk density. Consider discussing that here instead of concentration?

<u>Authors' response:</u> We revised the abstract to more clearly distinguish subsurface from surface horizon results as well as C stocks from C concentration.

<u>Authors' changes</u>: Page 1, Ln25-30 "Combined, our results suggest that low redox potentials are the primary cause for C accumulation in seasonally flooded surface soils, likely due to selective preservation of organic compounds under anaerobic conditions. In seasonally flooded subsurface soils, however, C accumulation is limited due to lower C inputs through root biomass and the removal of Fe phases under reducing conditions."

<u>Comment:</u> Ln23: It is not clear what non-reducible Al phases are being relied on for here? I assume mineral protection, but best not to have readers assuming in the abstract.

<u>Authors' response:</u> *The abstract was completely rewritten during revisions. We know specify that Fe_o and Al_o serve as proxies for protective metal phases. We further added a statement to clarify why we conclude that Al_o becomes more important in lowland soils.*

<u>Authors' changes</u>: *Page 1, Ln23-25 "Our linear mixed effects model showed that Fe_o served as the strongest measured predictor of C concentrations in upland soils, yet Fe_o had no predictive power in lowland soils. Instead, our model showed that E_h and Al_o became significantly stronger predictors in the lowland soils."*

<u>Comment:</u> Ln 24-25: The three reasons given for why you see more C in the topsoils than the subsoils are not supported in the abstract by any data. Either include this data upfront (i.e., lowland had low/zero O2, whereas uplands had O2 above X%; also data on roots and Fe presence/abundance) or you could simply state that these C findings correlated with O2, roots and Fe, implying the data is in the paper, but not fully presented. What you are asking the reader to do here is accept this statement without any sense that it is supported by data in this paper and that is not comfortable to many readers (and me I suppose).

<u>Authors' response:</u> *We recognize that the statements within the abstract are not presented with the data to support these claims. We have revised the abstract to reflect that there were correlations found between C and roots, Fe and redox potential and the supporting data is found in the main manuscript.*

<u>Authors' changes</u>: *Page 1, Ln20-25 "Lower CO_2 efflux as compared to the uplands corresponded to greater C concentration and abundance of lignin-rich, higher-molecular weight, chemically-reduced organic compounds in the lowland surface soils (A-horizons). In contrast, subsurface soils in the lowland position (C_g-horizons) showed lower C concentrations than the upland positions, coinciding with lower abundance of root biomass and oxalate-extractable Fe phases (Fe_o, a proxy for reactive Fe). Our linear mixed effects model showed that Fe_o served as the strongest measured predictor of C concentrations in upland soils, yet Fe_o had no predictive power in lowland soils. Instead, our model showed that E_h and Al_o became significantly stronger predictors in the lowland soils"*

<u>Comment:</u> Ln26: Again, without O2 data or mineral protection data, how could you conclude this. I assume it is in the rest of the paper. . .but I have not read that yet if I am most readers.

<u>Authors' response:</u> *We have addressed this issue in our revision of the abstract.*

Introduction

<u>Comment:</u> The introduction does a nice job setting the stage although I suggest line edits below.

<u>Authors' response:</u> *We appreciate this comment and the subsequent revision suggestions for clarity.*

Pg. 2

<u>Comment:</u> Ln 14-15: Revise for clarity.

<u>Authors' response:</u> The authors have revised the sentence as suggested.

<u>Authors' changes:</u> Page 3, Ln13-14 "Seasonal wetlands can be considered early warning ecosystems (Brooks, 2005); forecasting the impacts of climate change on permanently flooded mineral wetlands."

<u>Comment:</u> Ln 16: Maybe not "model ecosystems" but essential "endmembers".

<u>Authors' response:</u> We agree that 'endmembers' is a more suitable representation of these ecosystems.

<u>Authors' changes:</u> Page 3, Ln14-16 "*Thus, seasonally flooded wetlands represent essential endmembers to study the effects of climate change on larger permanently flooded wetland soils (Brooks, 2005).*"

<u>Comment:</u> Ln 19: This is an "endash" and you want an "emdash" here. A longer dash, that should not have spaces around it. On MS-Word you hit dash twice between words without adding spaces and word turns it into an emdash. Do this elsewhere in the text.

<u>Authors' response:</u> Thank you for noticing and correcting the endash/emdash inaccuracies. We have corrected all throughout the text.

<u>Comment:</u> Ln24: "seasonal wetlands" or seasonally flooded mineral wetlands, choose one term and stick with it through-out the MS.

<u>Authors' response:</u> We agree the terminology needs to be more consistent. We have revised the manuscript to be more consistent with the term 'seasonally flooded mineral soils'

<u>Authors' changes:</u> Page 3, Ln23-24 "Determining the controls on C cycling within seasonally flooded mineral soils thus requires specific consideration of the fluxes and dynamics across these terrestrial-aquatic transitions."

Pg. 3

<u>Comment:</u> Ln 5: "catalyze"

<u>Authors' response:</u> We revised the sentence, replacing "catalyzing.." to "..which catalyze..".

<u>Authors' changes: Page 4,</u> Ln4-6 "The resulting oxygen limitations inhibit the activity of oxidative enzymes which catalyze the depolymerization of higher-molecular weight OM into smaller, assimilable compounds (Megonigal et al. 2003)."

<u>Comment:</u> Ln 10: instead of chemically-reduced, "lower valance" would be more precise.

<u>*Authors' response:*</u> *The authors appreciation this suggestion. To be consistent with other publications on the subject, we would like to retain the 'chemically-reduced' terminology, but also reference "oxidation state" in the revised version.*

<u>*Authors' changes:*</u> *Page 4, Ln7-9 "Anaerobic conditions limit microbes to utilizing substrates that are chemically more oxidized, in turn preferentially preserving more chemically-reduced organic compounds (i.e., compounds with lower C oxidation states) in soils and sediments (Boye et al. 2017; Keiluweit et al., 2017)."*

<u>Comment:</u> Ln 17: ", but the impact of roots on soil C. . ."

<u>*Authors' response:*</u> *We thank the referee for this revision, which adds more clarity to the sentence.*

<u>*Authors' changes:*</u> *Page 4, Ln16-17 "Roots are the main contributors to C stocks in upland soils (Rasse et al., 2005), but the impacts of roots on soil C stocks in wetlands is less clear."*

<u>Comment:</u> Ln 18: "growth due to low DO (Day. . .)"

<u>*Authors' response:*</u> *We altered the sentence as suggested.*

<u>*Authors' changes:*</u> *Page 4, Ln17-18 "Water saturation directly inhibits root growth due to low dissolved oxygen concentrations (Day and Megonigal, 1993; Tokarz and Urban, 2015)."*

<u>Comment:</u> Ln 25: "distribution of high surface area minerals that are excellent sorbents for C in soils"

<u>*Authors' response:*</u> *We thank the reviewer for this constructive edit and revised the sentence as suggested.*

<u>*Authors' changes:*</u> *Page 4, Ln23-24 "In addition to restricting microbial metabolism and root growth, water saturation also influences the concentration and distribution of high surface area minerals that are excellent sorbents for C in soils …"*

Pg 4

<u>Comment:</u> I point out three of our recent papers that are highly relevant to this introduction/discussion, but which were not likely available when this was drafted.

<u>*Authors' response:*</u> *We thank the reviewer for pointing out these highly-relevant, recently published papers. We incorporated the following citations accordingly.*

<u>Comment:</u> Ln 10: See Chen et al 2018 ES&T and Chen and Thompson 2018 ES&T on these topics

<u>*Authors' response:*</u> *We incorporated these citations as requested.*

Authors' changes: *Page 5, Ln8-11 "Further, Al rather than Fe oxides are the predominate mineral phases contributing to OM retention in forested floodplain sediments because their solubility is controlled by pH rather than redox conditions (Darke and Walbridge, 2000), and may thus play a critical role in mineral protection in seasonally flooded soils (Chen et al., 2018; Chen and Thompson, 2018)."*

Comment: Ln 17: See Barcellos et al 2018 Soil Systems on this topic

Authors' response: *We incorporated these citations as requested.*

Authors' changes: Page 5, Ln15-16 *"How the relationships between C and important biogeochemical controls differ in systems that undergo longer, yet not permanent, periods of water saturation is still in question – especially with depth (Barcellos et al., 2018)."*

Comment: Ln 25: "measurements of soil. . ."

Authors' response: *We revised this sentence as suggested.*

Authors' changes: *Page 5, Ln21-23 "To accomplish our first objective, we related soil CO_2 efflux at three landscape positions (upland, transition, and lowland) spanning the transect over the course of a full drainage and flooding cycle to measurements of soil temperature, moisture, water table depth and redox potential."*

Methods

Comment: Well done, except that more description of the stats used are required potentially to clarify issues I raise above and below with regard to Table 3 lowercase lettering.

Authors' response: *As indicated above, we mistakenly had reported the ANOVA results for the whole dataset in Table 3, even though our results/discussion in the text were all based on horizon-based ANOVAs. We have updated the lettering and footnotes in Table 3. We further detail our horizon based approach in the revised methods section.*

Authors' changes: *Page 11, Ln8-15 "To test our hypotheses, differences among landscape positions were assessed individually for each set of horizons using analyses of variance (ANOVA) conducted in Rstudio (version 5.3.1) combined with Tukey's honesty significance difference (HSD) tests using R packages agricolae (de Mendiburu, 2017) and multcompView (Graves et al., 2015). Specifically, we compared values within surface (A), intermediate (B/C), and subsurface (C/C_g) horizons across the upland-to-lowland transect (Table 3). Alpha values of 0.05 were used for letter designations indicating significant differences among the landscape positions. Statistical analyses were conducted on square-root transformed data when assumptions of normal distribution were not met."*

Results

Comment: Main issue in this section is the depth that is considered ‘subsurface’. How does one determine this for soils with different depths or thicknesses? Normally, this doesn’t matter, but in this case the authors are making a key argument about the C and Fe interactions and chemistry “at depth”. Examining the C horizons, total C is actually higher in the lowland than in the upland and this would be true even if we examined C concentrations at 25 cm across the sites. If we go deeper, then the Cg of the transition and the lowland are equally low and the upland is higher, but not statistically higher based on the lower case letter assignments. The same is true in inverse for the lowest depth for Fe-o, it is highest at the lowland, but this is not significant from the other sites. This makes statements like “C concentrations were significantly lower in the lowland than in the upland subsoils”, which is in the abstract, incorrect based on the authors’ assignment of letters (see Table 3).

Authors’ response: We hope that additional explanation regarding the statistical tests and inferences provided above make it clear that there are statistical differences among A and C/Cg horizons, respectively. This statement was not made based on the ANOVA results for the full dataset we initially reported in Table 3, but for individual comparisons on a horizon-basis. We have updated Table 3 and other data tables to accurately reflect the horizon-based comparison.

Pg. 11

Comment: Ln 9: Assuming that Feb – June is the wet period, but you should tell readers that explicitly.

Authors’ response: The authors realize there had not been a clear definition of the growing and non-growing season designations within the methods section. We have added this definition to page 10 under statistical analyses. We added reminders in brackets on page 11.

Authors’ changes: Page 10, Ln24-25 “Regression analyses were conducted for the entire year-long dataset, and for the growing and non-growing seasons defined as May through September and October through March, respectively.”

Page 11:

Comment: Ln8-9: Assuming that Feb – June is the wet period, but you should tell readers that explicitly.

Authors’ response: We added a line to more clearly point out the flooded period of the lowland position in relation to the growing season parameter.

Authors’ changes: Page 11, Ln22-24 “The flooded period (February through June) of the lowland position extended into the first two months of the growing season.”

Comment: Ln 11: “significantly lower than in. . .”

Authors’ response: We have revised the sentence, but where the reviewer suggested ‘lower’ it should actually remain ‘greater’.

<u>*Authors' changes:*</u> *Page 11-12, Ln24-1"This general difference became even more pronounced when cumulative CO_2 emissions were normalized to C content, with the upland position showing significantly greater emissions than in the transition (p-value <0.001; Tukey's HSD) and lowland (p-value <0.001, Tukey's HSD) positions."*

<u>Comment:</u> Ln 13: "season lowland VMC. . ."

<u>*Authors' response:*</u> *We revised the sentence as suggested.*

<u>*Authors' changes:*</u> *Page 12, Ln11-13 "Soil moisture was consistently the greatest in the lowland position; during the growing season lowland VMC was 20% greater than the upland position (p-value < 0.05; Tukey's HSD), and 15% greater in the non-growing season (p-value < 0.05; Tukey's HSD) (Table S1)."*

<u>Comment:</u> Ln 21: Maybe it would be helpful to calculate the EH7 values here so that these could be compared with other studies and compared between the surface and subsurface horizons.

<u>*Authors' response:*</u> *The variations in pH values across our site is minimal and within the margin of error (4.98-5.43). Correcting the Eh values for pH would change Eh values by less than a decimal point. For example, an Eh value of 400 mV would correspond to Eh7 values of 399.91 mV at pH 5.43 and 399.88 mV at pH 4.98, respectively. These two pH values cover the range of values observed at our site, so we concluded that normalizing Eh to pH wouldn't change the numbers sufficiently to warrant inclusion.*

<u>Comment:</u> Ln 23: change "mineralogy" which is the study of minerals to "mineral composition". Do this elsewhere as well.

<u>*Authors' response:*</u> *We agree with this correction and have corrected it throughout the manuscript.*

Pg. 12

<u>Comment:</u> Ln 1: The data are more complex than this statement suggests. Please revise.

<u>*Authors' response:*</u> *We have revised the topic statement as suggested.*

<u>*Authors' changes:*</u> *Page 12, Ln7 "3.3 Relating carbon concentration to root biomass and mineral composition across upland-to-lowland transitions"*

<u>Comment:</u> Ln 2: Do you mean topsoil here???? Because actually it is over 8 times the subsoil, but according to the letters, the lowland and transition topsoil are equal within error.

<u>*Authors' response:*</u> *We're thankful the reviewer caught this misspelling. We revised accordingly.*

Authors' changes: *Page 12, Ln24-25 "C concentrations in the lowland position surface horizons were two and four times greater than the transition (p-value < 0.01; Tukey's HSD) and upland positions surface horizons (p-value < 0.001; Tukey's HSD), respectively."*

Comment: Ln 4: although this was not statistically significant, correct? I suggest adding that information.

Comment: Ln 7: Although again this was not statistically significant, right? Tell the reader that.

Authors' response: *In regards to the two comments above, we included a more detailed explanation of our statistical approaches as outlined in our response to the more general comments above. Again, these statements were supported by our horizon-based ANOVA results which are now shown in the revised Table 3. Conducting a horizon-based ANOVA test allowed us to compare only the comparable horizons and include the variances within those horizons when testing for differences. The Tukey's HSD results show a significant difference between the upland and lowland A-horizons (p-value = 0.03) and the upland C- and lowland Cg-horizons (p-value = 0.02). These findings support our statistical significance of the differences in C concentrations among the upland and lowland adjacent horizons. In addition to correcting Table 3, we revised Table S2 and S4 accordingly and made sure that the tables and results sections 3.3, 3.4 and 3.5 are consistent.*

Authors' changes: *Page 12, Ln22-24 "Along the upland to lowland transects, C concentrations in the surface horizons significantly increased (p-value < 0.05, Tukey's HSD) whereas concentrations in the subsurface horizons decreased along the transect (p-value < 0.05, Tukey's HSD) (Table 3)."*

Authors' changes: *Page13, Ln: 1-2 "In contrast, the subsurface soils in the upland positions had nearly double the C concentrations than in the transition and lowland positions (p-value < 0.05, Tukey's HSD) (Table 3)."*

Comment: Ln 9: True, except in the upland, right (Table 3 indicates it is not significant).

Authors' response: *Although we're not entirely sure, we believe the reviewer is referring to the silt and clay data. The statement has been adjusted in regards to significance.*

Authors' changes: *Page 13, Ln4-6 "Silt and clay content increased from the upland to the lowland positions, particularly in the subsoil, although shifts in silt and clay contents were not statistically significant (+33%, Table 3)."*

Comment: Ln 12: Change 'determine' to 'predict'

Authors' response: *We have made the suggested change.*

Authors' changes: *Page 13, Ln14 "To predict the relative influence of roots, mineral composition and* E_h *on C concentrations in each landscape position…"*

Comment: Ln 18: “concentrations decreased along...”

Authors’ response: We have made the suggested change.

Authors’ changes: Page 13, Ln19-21 “The model results show that as the importance of redox-active Fe_o *as a predictor for soil C concentrations decreased along upland-to-lowland transects, the importance of* Al_o *increased.”*

Comment: Ln 20: Change ‘identify’ to ‘predict’

Authors’ response: We have made the suggested change.

Authors’ changes: Page13, Ln22 “To predict the influence of the biogeochemical variables on soil C concentrations with soil depth…”

Comment: Ln 24: Maybe not Eh, but likely O2, right?

Authors’ response: We agree that it is oxygen availability, and not Eh, that is likely what causes higher C concentrations. We have adjusted the statement to reflect this detail.

Authors’ changes: Page 14, Ln1-2 “These results indicate that, among the tested biogeochemical variables, E_h*, a proxy for oxygen availability, has a predominant influence on C concentrations in the surface soils, while* Al_o *has the strongest influence on C concentrations at depth. “*

Comment: Ln 25: Change ‘effect’ to ‘influence’

Authors’ response: We have made the suggested change.

Authors’ changes: Page 13, Ln11-12 “while Al_o *has the strongest influence on C concentrations at depth.”*

Pg. 13

Comment: Ln 17: “across the upland to lowland transect. . .”

Authors’ response: We have made the suggested change.

Authors’ changes: Page14, Ln6 “increases across the upland to lowland transect”

Comment: Ln 18: “. . .(-11%) moving from upland to lowland.”

<u>*Authors' response:*</u> *We have made the suggested change.*

<u>*Authors' changes:*</u> *Page 14, Ln20-23 "Paralleling that change, the relative contributions of lignin increased (+7%) and that of lipids decreased (-11%) moving from upland to lowland position (Fig. 6b, Table S5)"*

<u>Comment:</u> Fig. 2: Symbols are hard to tell from one another. Consider using squares, triangles and circles. Cool could help too since other figures are in color.

<u>*Authors' response:*</u> *We thank the reviewer for this suggestion and have modified the figure to include color for a clearer designation between positions and depths.*

<u>Comment:</u> Fig. 2: Are the Eh values on these graphs corrected for pH? To allow comparisons between the depths/sites?

<u>*Authors' response:*</u> *We refer to our comment above about the minimal effect of pH on Eh values.*

Discussion

<u>*Authors' response: Please note that in response to Reviewer #1, we have substantially revised sections 4.1, 4.2 and 4.3.*</u>

Pg. 13

<u>Comment:</u> Ln 22: Change 'demonstrate' to 'suggest'

<u>*Authors' response:*</u> *We have made the suggested change.*

<u>*Authors' changes:*</u> *Page15, Ln4-5 "Our results suggest that the factors regulating CO_2 emissions and C accumulation shift…"*

<u>Comment:</u> Ln 23: "...transects, but exhibit potentially inverse trends in the subsurface."

<u>*Authors' response:*</u> *We have revised the statement leading into the discussion section..*

<u>*Authors' changes:*</u> *Page 15, Ln5 "*Our results suggest that the factors regulating CO_2 emissions and C accumulation shifted as predicted in surface soils along the upland-to-lowland transects, but exhibited inverse trends in the subsurface."

Pg. 14

Comment: Ln 1-2: delete sentence.

Authors' response: We deleted the suggested sentence.

Authors' changes: Page 15, deleted sentence "Our results show how seasonal flooding affects redox conditions, root biomass, and mineral composition as well as their impact on CO_2 efflux, C accumulation, and C chemistry across the upland to lowland transects."

Comment: Ln 4: "Our field data support our hypothesis that reducing. . ."

Authors' response: We have made the suggested change.

Author's changes: Page 15, Ln5-6 "Our field data support our hypothesis that reducing conditions under flooded conditions inhibit microbial respiration and thus reduce CO_2 emissions in the lowland position"

Comment: Ln 16-20: Clarify this section.

Authors' response: This section was comprehensively revised and significantly shortened for clarity.
Authors' changes: Page 15, Ln6-19 "Indeed, seasonal CO_2 emissions in the lowland positions were strongly correlated with VMC, water table depth, and E_h (Fig. 3). Conversely, in upland positions where oxygen limitations are not limiting, soil temperature was found to the best predictor variable for CO_2 emissions (Fig. 3a, Table 2). Our results further indicated that the impact of seasonal drainage of the lowland soils on CO_2 effluxes is limited by temperature effects (Fig. 2, Table 2). Oxygenation in other seasonally flooded soils usually results in increases in CO_2 effluxes due to enhanced aerobic microbial respiration (Laine et al., 1996; Krauss and Whitbeck, 2012). Although our lowland soils become oxygenated in the non-growing season due to the water table drop, we observed near equal CO_2 emissions from the three landscape positions (Table 1, Fig. 2a). A likely explanation for this convergence in CO_2 emissions is that oxygenation coincides with the low seasonal temperatures during the non-growing season (-1.7 to 10 degrees Celsius) which inhibit microbial activity (Lloyd and Taylor, 1994). In other words, even when seasonal drainage oxygenates the lowland soils, potentially allowing for aerobic microbial respiration to occur, CO_2 efflux in these seasonal wetlands still remains suppressed due to cold temperatures. It remains to be seen if shifts in the drainage period or higher temperatures during the non-growing season, as expected throughout the Northeastern US with continued global warming, disproportionally increase microbial respiration (and potentially C loss) from these soils."

Comment: Ln 24: Note that the figure shows topography that is not flat.

Authors' response: We appreciate this correction, and have updated the statement to reflect figure 1.

Authors' changes: Page 15, Ln23-25 "Given the proximity of our three positions and minor change in elevation, aboveground litter inputs can be considered equal across the transect."

Pg. 15

<u>Comment:</u> Ln 7: Consider using C stocks instead of concentration, which would help get around the depth issue.

<u>Authors' response:</u> The authors agree that using C stocks would be beneficial. Due to the lack of accurate bulk density data for this site, however, we have chosen to focus on concentrations.

Pg. 17

<u>Comment:</u> Ln 23: OK, but Cg in the lowland is 2nd highest across ALL sites/depths, so this statement doesn't ring fully true for me.

<u>Authors' response:</u> Despite the Cg horizon in the lowland positions being the 2nd highest horizon, it still had overall lower concentrations of both extractable Fe and Al compared to the other positions. Which has led us to conclude that even though new C inputs could occur belowground, there is still a limited mineral protection capacity within the lowland positions.

<u>Authors' changes:</u>

Page 16, Ln21-23 "A noticeable, yet insignificant, increase in Fe_o concentrations in the lowland Cg-horizons (Table 3) is likely a reflection of vertical transport of soluble or colloidal Fe phases into the subsurface horizon, where they may reprecipitate during drained periods."

Pg. 18, Ln 13-15: "As noted above, while the lowland horizons overall showed a decline in Fe_o and Al_o contents relative to the upland position (Table), a modest uptick in Fe_o content was observed in the lowland subsurface (Cg) horizon. One possibilities is thus that Fe precipitates in the seasonally flooded subsurface soil horizon trapped dissolved, partially-oxidized, lignin-derived OM leaching down the profile and so resulted in the accumulation of relatively oxidized OM"

Conclusions

<u>Comment:</u> Ln 10: change 'related to' to 'correlated with'

<u>Authors' response:</u> We have made the suggested change.

<u>Authors' changes:</u> Page 19, Ln16 "In the subsoil of seasonally flooded soils, anaerobic protection of C appears to be less important. C accumulation was low and primarily correlated with to Al_o,…"

<u>Comment:</u> Ln 12: But, again what about Fe-o in the lowland Cg?????

<u>Authors' response:</u> The reviewer points out here a trend in our dataset that we do not fully address, an apparent increase in Fe_o within the Cg horizon in the lowland position. While it is clear that Fe is lower

in the A and C horizons, this increase in Fe in the Cg horizon is likely due to vertical transport of soluble Fe. To address the slight increase in Feo in the lowland Cg we have added a comment in the 4.2 discussion section.

Authors' changes: Page 16, Ln25-26, "An observed, but insignificant, increase in Fe_o *concentrations in the lowland Cg-horizons are likely a reflection of vertical transportation of soluble Fe phases and reprecipitation at depth."*

Response to Comments by Referee #2

Comment: LaCroix et al. report findings on C storage and changes in the physical-geochemical composition of soils minerals and redox conditions under the seasonal flooding soils. Nevertheless, in different part of the manuscript several strongly weak points have been identified that must be addressed from the authors. Moreover, I do not find that results provided insight into the mechanism on C storage and the changes in the shifting minerals and other critical factors.

Authors' response: We thank the reviewer for taking the time to review out manuscript and believe we adequately addressed the specific concerns mentioned below.

Comment: 1. Synchrotron-based X-ray analyses and FT-ICR-MS analyses. There are plenty of literatures on these methods, as a reviewer also a reader, I suggest simplify these parts and move the detail descriptions into SI.

Authors' response: Biogeosciences allows for detailed methods sections. In the interest of providing sufficient information to less informed readers, we would like keep the level of detail in the methods section as is.

Comment: 2. Results. In figure 2, the symbols are too small and similar to be recognized, while and the resolution are lower.

Authors' response: We have updated figure 2 to include color to differentiate the different positions and depths, and to be more legible in general.

Comment: 3. In 3.3, compared with Feo and Alo, the authors haven't presented the detailed data of Fed and Ald, which are more sensitive to the changing of environmental factors. Meanwhile, the soil iron cycling is sensitive to the seasonal flooding, the recrystalliza- tion processes of iron oxides as well as aluminum oxides during the shifting of seasonal flooding soils are critical factors to the variation of the iron/aluminum species, which are further controlled the reactivity of iron/aluminum species in soil environment.

Authors' response: As discussed in the initial manuscript (Page 12 Ln13-14), the dithionite-extractable Fe and Al data is well correlated with the amount of oxalate-extractable Fe. In other words, the trends are the same. We thus believe that adding the dithionite extraction data to the main manuscript has no added values and left them in the SI.

Comment: 4. In 3.4, carboxylic/aromatic C ratios is a suitable indicator to present the different of oxidation degrees, however, from fig 5b, it's inaccurate to describe the increase trends of those values above in C horizons. It doesn't present significant different in fig 5b.

Authors' response: We changed the text to reflect that these differences are not significant at the $p < 0.05$ *values, but maintain that the trends are ecologically relevant and worthy of discussion. We would also* *like to note that this is one of the first manuscripts that reports variability in analyses such as C NEXAFS and FTICRMS. Due to the low throughput of these analyses, replicates are generally pooled before analysis and only analyzed as a composite sample in other publications, leaving the reader with no sense of the natural variability.*

<u>Authors' changes:</u> Page 14, Ln8-12 "Although the ratios were not significantly different among the landscape positions ($p > 0.05$, Tukey's HSD), noteworthy trends were found. In the surface horizons, the ratio gradually decreased across the upland-to-lowland transects in the surface horizons (Fig. 5b, Table S4). In the subsurface horizons, the opposite trend was observed, and the ratio steadily increased from the upland C horizons to the lowland Cg horizons (Fig. 5b, Table S4)."

<u>Comment:</u> 5. In discussion part, the authors just repeated the obtained results described in results part in another similar way, lacking of further discussion around the mechanism among C storage and the changes in the shifting minerals and other critical factors. As a reader, I find it's hard to get new information in this important parts.

<u>Authors' response:</u> In response to this concern, we substantially revised the discussion sections to add information regarding the mechanisms under consideration. In particular, we substantially shortened section 4.1, comprehensively revised sections 4.2 and 4.3, and completely rewrote section 4.4.

<u>Comment:</u> 6. Further experiments should be designed and conducted to illustrate the mechanism on soil chemical-physical properties and C storage.

<u>Authors' response:</u> We believe that in-field measurements and analysis of field samples along environmental gradients is a suitable tool to examine the effects of different soil properties on soil carbon. While we acknowledge that this approach is based on correlations, and mechanisms can thus merely be inferred, it is one that is widely used in the biogeosciences community (Angst et al., 2018; Barcellos et al., 2018; Chen et al., 2017; Hall et al., 2016, Hall & Silver 2015; Olshansky et al., 2018; Torn et al. 1997). We believe this study uniquely combines environmental data with soil physical-chemical properties to infer variations in C storage mechanisms across upland-to-lowland transects.

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Shifting Mineral and Redox Controls on Carbon Cycling in Seasonally Flooded Mineral Soils

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Abstract. Although wetland soils represent a relatively small portion of the terrestrial landscape, they account for an estimated 20-30% of the ~~global~~global soil -carbon (C) reservoir. C stored in wetland soils ~~experiencing~~that experience seasonal flooding ~~are~~is likely the most vulnerable to increased severity and duration of droughts in response to climate change. Redox conditions, plant root dynamics, and the abundance of protective m~~ineral~~ineral -phases are well-established controls on soil C persistence ~~in soils~~, but their relative influence in seasonally flooded mineral soils is largely unknown. To address this knowledge gap, we assessed the relative importance of environmental (temperature, soil moisture, and redox potential) and biogeochemical (mineral composition and root biomass) factors in controlling CO_2 efflux, C quantity and organic matter composition along replicated upland-to-lowland transitions in seasonally flooded mineral soils. Specifically, we contrasted mineral soils under temperat~~ure~~e deciduous forests in lowland positions that undergo seasonal flooding with adjacent upland soils that do not, considering both surface ~~soil~~(A) and subsurface~~soil~~ (B/C) ~~soil~~ horizons. We found the lowland soils had lower total annual CO_2 efflux than the upland soils, with monthly CO_2 efflux in lowlands most strongly correlated with redox potential (E_h). Lower CO_2 efflux~~es~~ as compared to the uplands corresponded to greater C concentration~~s~~ and abundance of lignin-rich, higher-molecular weight, chemically-reduced organic compounds in the lowland surface soils~~s~~ (A-horizons). In contrast, subsurface soils~~soils~~ in the lowland position (C_g-horizons) showed lower C concentrations than the upland positions (C-horizons), coinciding with lower abundance of root biomass and oxalate-extractable Fe ~~phases~~ (Fe_o, a proxy for ~~reactive~~ protective Fe phases). Our linear mixed effects model showed that Fe_o served as the strongest measured predictor of C concentrations in upland soils, yet Fe_o had no predictive power in lowland soils. ~~In contrast~~Instead, our model ~~, our linear mixed effects model~~ showed that E_h and oxalate-extractable Al (Al_o, a proxy of protective Al phases) became significantly stronger predictors ~~across the upland-to-lowland transition~~in the lowland soils. Combined, our results suggest that ~~seasonal flooding and associated~~ low

redox potentials are the primary cause for C accumulation in seasonally flooded surface soils~~lsls~~, likely due to selective preservation of organic compounds under anaerobic conditions. In seasonally flooded ~~the~~ subsurface soils, however, ~~seasonal flooding limited~~ C accumulation is limited~~, which we attribute to~~ due to lower C inputs through root biomass and the removal of reactive Fe ~~and Al~~ phases under reducing conditions. Our findings demonstrate that C accrual in seasonally flooded mineral soil is primarily due to low redox potential in the surface soil, and that the lack of protective metal phases leaves these C stocks highly vulnerable to climate change.~~Soils contain three times the amount of carbon (C) than the atmosphere, with C turnover times ranging from centuries to millennia. Although wetland soils represent a relatively small portion of the terrestrial landscape, they account for an estimated 20-30% of the global C reservoir. Among wetlands, seasonally flooded soils are likely the most vulnerable to increased severity and duration of droughts in response to climate change. Yet, the relative influence of associated changes in oxygen limitations, root dynamics, and mineral protection on C cycling in seasonally flooded mineral soils is largely unknown. To address this knowledge gap, we combined seasonal monitoring of soil moisture, redox potential, and CO_2 efflux with a characterization of root biomass, mineralogy, C quantity and organic matter composition along upland-to-lowland transects of both top- and subsoils in temperate forested wetlands. We found that lower CO_2 effluxes in lowland than upland topsoils coincided with greater total C concentrations as well as a greater abundance of high molecular weight and chemically reduced organic compounds, indicating that selective preservation of organic compounds during anaerobic periods caused C accumulation in seasonally flooded surface soils. In subsoils, however, seasonal flooding and associated anaerobic conditions did not result in soil C accumulation. Instead, total C concentrations were significantly lower in lowland than in upland subsoils. Lower soil C accumulation in seasonally flooded subsoils coincided with lower abundance of root biomass and reducible Fe phases, and relied primarily on non-reducible Al phases rather than anaerobic conditions. Combined, our results demonstrate that seasonal flooding and associated anaerobic conditions accumulate C in topsoils, but limit C accumulation in subsoils by restricting root C inputs and removing of protective Fe phases through reductive dissolution. Our findings indicate that C accrual in seasonally flooded soil is due primarily to oxygen limitations in the surface soil, and that the overall lack of mineral protection leaves these C stocks highly vulnerable to climate change.~~

1 Introduction

Although wetland soils cover a relatively small portion of the Earth's land surface, they store an estimated 20-30% of the global soil C stocks (Mitsch et al., 2013). However, this C pool is under pressure from climate change, with increasing severity and frequency of droughts having substantial, yet largely unresolved consequences (Brooks et al., 2009, Fenner and Freeman, 2011). Increased droughts are expected to release previously stored C- in wetlands back into the atmosphere (Gorham et al., 1991). Prior studies focused on C cycling in wetland soils have been primarily aimed at organic wetlands, such as peats and bogs (Laine et al., 1996) or coastal wetlands (Kirwan and Blum, 2011). Although freshwater mineral wetlands are estimated to contain 46 Pg C globally (Bridgham et al., 2006), they have received comparatively little attention.

Previous studies on C cycling in mineral wetland soils are limited to permanently flooded, rather than seasonally flooded sites (Krauss and Whitbeck, 2012). This is surprising given that seasonally flooded soils are metabolically more active than permanently flooded wetlands, resulting in significantly greater greenhouse gas emissions (Kifner et al. 2018). Moreover, the consequences of climate change ~~is~~ are expected to be most immediately evident in seasonal wetlands due to their dependence upon precipitation and seasonal groundwater recharge (Tiner, 2003). Seasonal wetlands ~~are~~ can be considered as early warning ~~and detection~~ ecosystems (Brooks, 2005); forecasting the impacts of climate change on permanently flooded ~~mineral~~ wetlands. Thus, seasonally flooded wetlands represent essential endmembers ~~are ideal model ecosystems~~ to study the effects of climate change on ~~larger~~ permanently flooded wetland soils (Brooks, 2005).

Seasonal wetlands are geomorphic depressions in the landscape that have distinct hydrologic phases of flooding and draining (Brooks, 2005). These ephemeral wetlands are small (<1 hectare), but ubiquitous—~~—~~comprising nearly 70% of all temperate forest wetlands in the US (Tiner, 2003). Seasonal flooding and drainage not only create~~s~~ biogeochemical "hotspots" for soil C and nutrient cycling along upland-to-lowland transitions, but also "hot moments" as these transition zones move seasonally (Cohen et al., 2016). These transition zones are also relatively large, as the generally small size of seasonal wetlands results in a disproportionally large and dynamic terrestrial-aquatic interface relative to total wetland area (Cohen et al., 2016). Determining the controls on C cycling within seasonally flooded mineral soils thus requires specific consideration of the fluxes and dynamics across these terrestrial-aquatic transitions.

Though temperature and soil moisture are principle controls on C cycling in soils generally (Lloyd and Taylor, 1994; Wang et al., 2014), water saturation is a critically driver of ~~soil~~ organic matter (OM) decomposition processes in seasonally flooded systems (Neckles and Niell, 1994). Water saturation governs oxygen availability in soil pore spaces, as oxygen diffusion in water is 10,000 times slower than in air (Letey and Stolzy, 1964). The resulting oxygen limitations inhibit the activity of oxidative enzymes ~~catalyzing~~ which catalyze the depolymerization of higher-molecular weight OM into smaller, assimilable compounds (Megonigal et al. 2003). Further, once oxygen is depleted, microbes rely on alternative terminal electron acceptors (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2}) in heterotrophic respiration that yield less energy (Sutton-Grier et al., 2011). These thermodynamic constraints also dictate the types of organic substrate microbes are able to use in anaerobic heterotrophic respiration (LaRowe and Van Cappellen, 2011). Anaerobic conditions limit microbes to utilizing substrates that are chemically more oxidized, in turn preferentially preserving more chemically-reduced organic compounds (i.e., compounds with lower oxidation states) in soils and sediments (Boye et al. 2017; Keiluweit et al., 2017). While CO_2 emissions are often correlated with oxygen availability (or soil redox potential, E_h) (Koh et al., 2009), it is unclear to what extent such metabolic constraints result in the selective preservation of high-molecular weight, chemically-reduced OM in seasonally flooded systems where soils become aerated for prolonged periods.

Water saturation also impacts soil by controlling vegetation type and density—~~—~~thus acting as an indirect control on root growth and activity belowground. Plant roots contribute to soil C stocks through active rhizodeposition (exudates, secretions, dead border cells, and mucilage), dead root residues (Jones et al., 2009), and root-associated microbes (Bradford et al., 2013). Roots are the main contributors to C stocks in upland soils (Rasse et al., 2005), but the ~~root~~ impacts of roots on soil C stocks in wetlands is less clear. Water saturation directly inhibits root growth due to the associated low ~~redox potentials~~dissolved oxygen concentrations (Day and Megonigal, 1993; Tokarz and Urban, 2015). Indirectly, water saturation in soil selects for plant species that can tolerate water stress—~~—~~typically species that have developed advantageous traits to survive flooded conditions, such as shallow rooting systems (Tokarz and Urban, 2015). However, seasonally flooded soils select for an even smaller niche of plants, as they must be tolerant of both upland and lowland conditions (Brooks, 2005). How root inputs from facultative upland-to-lowland plant species contribute to soil C content and chemistry in seasonally flooded soils is still not clear.

In addition to restricting microbial metabolism and root growth, water saturation also influences the concentration and distribution of ~~reactive~~ high surface area minerals ~~that contribute to C accumulation~~that are ~~excellent~~ potent sorbents for C in soils (Chen et al., 2017; Torn et al., 1997; Wagai and Mayer, 2007). In upland soils, iron (Fe) or aluminum (Al) (hydr)oxides protect OM from microbial decomposition, thereby contributing to C storage for centuries to millennia (Torn et al., 1997; Wagai and Mayer, 2007). In flooded soils, however, the rapid depletion of oxygen upon flooding can result in the reductive dissolution of Fe(III) oxides (Chen et al., 2017), potentially causing the mobilization of previously Fe-bound OM (Zhao et al., 2017). During water table drawdown, Fe(II) may be leached from the profile or re-oxidized to Fe(III) oxides upon re-oxygenation of the soil (Wang et al., 2017). While redox-mediated transformations of Fe(III) oxides and export of Fe(II) is a well-known phenomenon (“gleying”) in seasonally flooded soils (Chen et al., 2017), their impact on mineral-associated OM has yet to be determined. Further, Al ~~oxides,~~ rather than Fe (hydr)oxides, are the predominate mineral phases contributing to OM retention in forested floodplain sediments because their solubility is controlled by pH rather than redox conditions (Borggaard et al., 1990; Darke and Walbridge, 2000), and may thus play a critical role in mineral protection in seasonally flooded soils (Chen et al., 2018; Chen and Thompson, 2018).

Water saturation thus likely governs C cycling in seasonally flooded soils through its combined impact on oxygen availability, root dynamics and ~~mineralogy~~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 contents. How the relationships between C and important biogeochemical controls differ in systems that undergo longer, yet not permanent, periods of water saturation is still in question, ~~—~~especially with depth (Barcellos et al., 2018).

In this study, we aimed to identify the predominant environmental and biogeochemical controls on CO_2 efflux, C content, and OM composition in seasonally flooded mineral soils. To accomplish this goal, we studied the impact of seasonal flooding on C cycling across complete soil profiles (0 to 1 m) in six replicated upland-to-lowland transects typical for the Northeastern US (Brooks, 2005). Our objectives were to (i) identify the environmental parameters that drive temporal dynamics of CO_2 efflux in seasonally flooded soils and (ii) examine the relative importance of biogeochemical controls on C concentration and ~~C~~ OM ~~chemistry~~composition with depth. To accomplish our first objective, we related soil CO_2 efflux at three landscape positions (upland, transition, and lowland) spanning the transect over the course of a full drainage and flooding cycle to measurements~~s~~ of soil temperature, moisture, water table depth and redox potential. To accomplish our second objective, we examined variations in C content and chemistry in both surface and subsurface horizons in relation to root distribution, ~~mineralogy~~mineral composition and redox potential. We hypothesized that seasonally reduced conditions upon flooding will result in lower CO_2 efflux, greater C accumulation, lower capacity of minerals to protect OM, and a selective preservation of macromolecular or chemically-reduced OM compared to the upland position. We anticipated that the transition position would represent an intermediate between upland and lowland positions.

2 Methods

2.1 Site description

Our study included six replicate forested wetlands in western Massachusetts that experience seasonal flooding through groundwater recharge; three sites are located at the UMass Experimental Farm Station in South Deerfield, MA, and three located within the Plum Brook Conservation area in South Amherst, MA. All sites consisted of soils that are glacially-derived sandy loams classified as mesic Typic Dystrudepts. Vegetation is dominated by red maple (*Acer rubrum*) and white oak (*Quercus alba*) stands with understory vegetation primarily composed of cinnamon fern (*Osmunda cinnamonea),* Canada mayflower (*Maianthemum canadense),* reed canary grass (*Phalaris arundinacea*), and jewelweed (*Impatiens capensis).* Mean annual air temperature is 9^{o}C and mean annual precipitation (rainfall and snowfall) is 120 cm (National Centers for Environmental Information (NCEI), National Oceanic and Atmospheric Administration (NOAA).

2.2 Field measurements

A transect in each seasonal wetland was delineated from an upland position to a lowland position (Fig. 1a-c). Three positions, termed “upland”, "transition”, and “lowland”, along each transect were established as monitoring stations and for soil sample collection. The upland position is in a forested landscape, approximately five meters away from the edge of the wetland, ~~and~~ 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~~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. 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). Each landscape position was monitored for CO_2 emissions, soil temperature, volumetric moisture content (VMC) at 0 to 10 cm, water table depth, and E_h. Field measurements were collected weekly at each designated landscape position in all six seasonal wetlands from May through August, then monthly from September through April. A field portable automated gas flux analyzer (LI-8100A, LI-COR Biotechnology, Lincoln, NE) was used to measure rates of CO_2 emissions, on permanently installed PVC collars, soil temperature and VMC. Three measurements of CO_2 fluxes were taken at each individual PVC collar using observation times of one minute, with 15 second dead band and pre- and post- purge times. The standard deviation of three observations was calculated in the field and a 15 % threshold was used for acceptable measurements. If the resulting standard deviation of the three measurements was greater than 15 % subsequent measurements were taken until the threshold was met. Based on other reports for comparable sites (Kifner et al. 2018), we expected methane production within these seasonal wetlands. However, in those sites methane production was 20-times lower than CO_2 production. While we fully acknowledge the disproportionate potency of methane as a climate-active greenhouse gas, our study aimed to determine the environmental and biogeochemical factors influencing C accrual or depletion in soils. We thus focused our monitoring efforts on quantitatively more important CO_2 emissions as the predominant C loss pathway. Water table fluctuations were monitored using slotted PVC pipes installed to depths of 100 cm. Platinum-tipped E_h probes were installed in triplicate at each depth of 15-, 30-, and 45-cm; each group (nine) of E_h probes were accompanied with a single salt bridge filled with saturated KCl in 3% agar for the reference electrode. In total, each landscape position had 18 redox probes installed at each depth. E_h was measured using a calomel electrode (Fisher Scientific, Pittsburg, PA) attached to a voltmeter and corrected to a standard hydrogen electrode by adding 244 mV to each reading (Fiedler et al., 2007).

2.3 Soil sampling and analysis

Soil samples were collected from all sites, positions and horizons using hand-augers. Coarse rocks and roots were removed from soil samples which were then sieved using standard 2 mm screens. Particle size distribution was determined using the pipette method outlined by Gee and Bauder (1986). Total C and N were determined with an elemental analyzer (Hedges and Stern, 1984). Extractable iron and aluminum concentrations were measured on each soil horizon from all three positions from the six pools (n=62) using ammonium-oxalate and citrate-bicarbonate-dithionite (CBD) extraction procedures (Loeppert and Inskeep, 1996). Ammonium-oxalate extractable Fe (Fe_o) and Al (Al_o) represent the poorly crystalline pool of Fe, while the CBD extractable Fe (Fe_d) and Al (Al_d) represent the total reducible Fe.

Root biomass was determined by taking soil cores in all six wetlands at each position along the designated moisture transects. The cores were taken at 0-20 cm, 20-40 cm, and >40 cm. Root biomass was determined using a USDA NRCS hand sieving method (Soil Survey Staff, 1999). The initial values of root biomass were used to determine biomass values for each soil horizon using an equal-area quadratic spline function (Spline Tool v2.0, ASRIS). Mean E_h values for each soil horizon were also estimated using the spline function.

To determine the relative abundance of specific C functional groups and degree of oxidation, soil samples were analyzed using C (1s) near edge X-ray absorption fine structure (NEXAFS) spectroscopy at the Canadian Light Source (CLS) in Saskatoon, Canada. Soil samples from individual horizons were gently ground, slurried in DI-H_2O and pipetted onto clean In foils. After drying, C NEXAFS spectra were obtained using the spherical grating monochromator (SGM) beamline 11ID-1 (Regier, 2007). Step scan mode (0.25 eV steps from 270 to 320 eV) was used to minimize x-ray damage. A dwell time of 20 ms was used between scans. Individual spectra were collected at new locations on each sample for a total of 40 to 60 scans. The beamline exit slit was set at 25 mm, and the fluorescence yield data was collected using a two-stage microchannel plate detector. The resulting spectra were averaged for each sample and the averaged spectrum was then baseline normalized to zero and then normalized the beamline photon flux (I_o) from a separate Au reference foil. Each spectrum was calibrated to the carboxylic acid peak (288.5 eV) of a citric acid standard. Pre-edge (270-278 eV) and post-edge (310-320 eV) and an E_0 (290 eV) values were used to perform an edge step normalization. Peak deconvolution was conducted in Athena (Demeter version 0.9.25, 2006-2016); Ravel and Newville 2005) to determine the relative abundances of functional groups, with peak positions as described in Keiluweit et al. (2017). Gaussian peak positions, their full-width at half-maximum, and the arc tangent function were fixed. Peak height was set to vary freely during the fitting process. Parameters were adjusted until optimal fits for each spectrum were achieved and all spectra were fitted with these final parameters.

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_2O using one gram of soil and 10 mL of DI-H_2O (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 and the resulting filtrate solution was used for FT-ICR-MS analysis. 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, which contains the following compounds: $C_2F_3O_2$, $C_6HF_9N_3O$, $C_{12}HF_{21}N_3O$, $C_{20}H_{18}F_{27}N_3O_8P_3$, and $C_{26}H_{18}F_{39}N_3O_8P_3$ with an m/z ranging between 112 to 1333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid (SRFA)) standard. Blanks (HPLC grade MeOH) were also ran at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time (IAT) was varied to account for differences in C concentration between samples and varied between 0.1 and 0.3 s. Ninety-six individual scans were averaged for each sample and internally calibrated using OM homologous series separated by 14 Da (–CH_2 groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (i.e. 200 <m/z <1200). To further reduce cumulative errors, all sample peak lists for the entire dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would impact formula assignment. Putative chemical formulas were assigned using Formularity software (Tolić et al., 2017). Chemical formulas were assigned based on the following criteria: S/N >7, and mass measurement error <1 ppm, taking into consideration the presence of C, H, O, N, S and P and excluding other elements. Peaks with large mass ratios (m/z values >500 Da) often have multiple possible candidate formulas. These peaks were assigned formulas through propagation of CH_2, O, and H_2 homologous series. Additionally, to ensure consistent choice of molecular formula when multiple formula candidates are found the following rules were implemented: we consistently chose the formula with the lowest error with the lowest number of heteroatoms and the assignment of one phosphorus atom requires the presence of at least four oxygen atoms. Peaks that were present in the blanks were subtracted from the sample data sets. Additionally, all single peaks i.e. peaks that are present in only one sample were removed and are not included in the downstream analysis. To further identify only "unique" peaks, we compared samples with the same group against each other to keep the peaks in the sample set that occur at least half of the samples for that group; peaks that occurred in less than half the samples were discarded from the final data set.

To visualize differences in SOM composition, compounds were plotted on a V~~v~~an Krevelen diagram corresponding to their H/C (hydrogen to carbon) vs. O/C (oxygen to carbon) ratios (Kim et al., 2003). Van Krevelen diagrams provide a way to visualize and compare the average properties of OM and assign compounds to the major biochemical classes (i.e., lipid-, protein-, lignin-, carbohydrate-, - and condensed aromatic-like) (Kim et al., 2003). To identify the degree of oxidation of the SOM we calculated the nominal oxidation state of carbon (NOSC) (Keiluweit et al., (2017):

$$NOSC = -\left(\frac{-Z+4C+H-3N-2O+5P-2S}{C}\right) + 4 \qquad (1)$$

in which C, H, N, O, P, and S correspond to stoichiometry values measured by FT-ICR-MS, and Z is equal to the net charge of the organic compound (assumed to be zero). We utilized the calculated double bond equivalent (DBE) to determine the degree of saturation of the identified C compounds, using the equation set forth by Koch and Dittmar (2006):

$$DBE = 1 + \frac{1}{2}(2C - H + N + P) \qquad (2)$$

where C, H, N, O, P, and S correspond to stoichiometry values also measured by FT-ICR-MS. The DBE is a useful equation to determine the degree of unsaturation of organic carbon containing molecules, where higher DBE values indicates less H atoms and a greater density of C-C double bonds. We also analyzed aromaticity of water extractable organic matter using a modified aromaticity index (AImod) to determine the density of C-C double-bonds, using the amended equation by Koch and Dittmar (2016):

$$AI_{mod} = \frac{1+C-\frac{1}{2}O-S-\frac{1}{2}(N+P+H)}{C-\frac{1}{2}O-N-S-P} \qquad (3)$$

which takes into consideration the contributions of heteroatoms and π-bonds. To identify shifts in average molecular weights we calculated molecular weight using stoichiometry values measured by FT-ICR-MS:

$$MW = (C\ x\ 12.011) + (H\ x\ 1.008) + (O\ x\ 15.999) + (S\ x\ 32.06) + (P\ x\ 30.974) + (N\ x\ 14.007) \qquad (4)$$

where each element is multiplied by its molar mass.

2.4 Statistical analyses

All statistical analyses and plots were done using Rstudio (Version 1.0.136, R Core Team 2015). The lm() function in Rstudio was used to perform linear regressions with the seasonal data to determine how various environmental parameters (soil moisture, water table depth and redox potential) predicted CO_2 emissions in the three landscape positions. Arrhenius models were used to determine how soil temperature predicted CO_2 emissions in the three landscape positions using OriginPro (OriginLab) with the equation (Sierra et al. 2012):

$$k = Ae^{(-Ea/RT)} \qquad (5)$$

where Ea is the activation energy, A is the pre-exponential factor, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), and T is temperature in Kelvin (K). Relationships between total C and biogeochemical parameters were analyzed using linear mixed effects models with the lme4 package (Bates et al., 2015) in Rstudio. Regression analyses were conducted for the entire year-long dataset, and for the growing and non-growing seasons defined as May through September and October through March, respectively. Two sets of mixed effects models were conducted; the first to identify which biogeochemical variables (root biomass, Fe$_o$, Al$_o$, clay, and ~~redox~~E$_h$) predicted C content in the different landscape positions where wetland number (n=6) was a random effect and horizon (A, B/C, C/C$_g$) and one additional predictor variable were fixed effects. The second set of models aimed at identifying how the same variables predicted soil C at different soil depths, where wetland number was chosen as a random effect and landscape position (upland, transition, lowland) and one additional predictor variable as fixed effects. The mixed effects models were performed individually with one fixed effect parameter in addition to the blocking factor of either horizon or landscape position. To correct for multiple testing effects, we used the Bonferroni correction factor where $\alpha_{corrected}$ is equal to 0.01. To test our hypotheses, differences among landscape positions were assessed individually for each set of horizons using ~~Analyses were conducted on log transformed data when assumptions of normal distribution were not met. Analysis of variance (ANOVA) and Tukey's honestly significance difference tests were conducted in Rstudio.~~ A~~a~~nalyses of variance (ANOVA) conducted ~~were conducted~~ in Rstudio (version 5.3.1) ~~followed by~~combined with T~~t~~ukey's honesty significance difference (HSD) tests ~~conducted in R~~using R packages agricolae (de Mendiburu, 2017) and multcompView (Graves et al., 2015). Specifically, we compared values within surface (A), intermediate (B/C), and subsurface (C/C$_g$) horizons across the upland-to-lowland transect (Table 3). Alpha values of 0.05 were used for ~~different~~ letter designations indicating significant differences among the landscape positions. ~~Due to the conservation nature of using a Tukey's HSD on our entire dataset (36 comparisons), we tested differences among horizon groups (i.e., across A or C/C$_g$ horizons) rather than testing all nine horizons against each other. This method enables us to directly compare the horizons needed to test our hypotheses.~~Statistical ~~analysis~~ analyses were conducted on square-root transformed data when assumptions of normal distribution were not met.

3 Results

3.1 Seasonal dynamics

Although our positions along the upland-to-lowland transect (i.e., upland, transition, lowland positions) are only a few meters apart each, we found significant differences in the seasonal dynamics of soil respiration, water table depth, moisture content and redox conditions (Fig. 2).

Soil respiration. CO_2 fluxes in each landscape position began to rise in May and peaked in September. Thereafter, CO_2 efflux in all positions gradually declined to a baseline level until November~~. CO_2 fluxes~~, and remained ~~at that low baseline level~~low through April (Fig 2a). Cumulative CO_2 emissions during the growing season substantially decreased across the upland-to-lowland transect (Table 1). The flooded period (February through June) of the lowland position extended into the first two months of the growing season. Relative to the lowland position (24 mol CO_2 m^{-2} $year^{-1}$), cumulative CO_2 emissions were 38% greater in the transition position (33 mol CO_2 m^{-2} $year^{-1}$), and 58% greater in the upland position (38 mol CO_2 m^{-2}). This general difference became even more pronounced when cumulative CO_2 emissions were normalized to C content, with the upland position showing significantly greater emissions than ~~both~~ in the transition (p-value <0.001; Tukey's HSD) and lowland (p-value <0.001, Tukey's HSD) positions. In the non-growing season, the transition position registered the largest cumulative CO_2 ~~flux~~ emissions (20 mol~~e~~ CO_2 m^{-2}), but there were no noticeable differences between the upland and lowland positions (16 and 15 mol~~e~~ CO_2 m^{-2}, respectively) (Table 1).

Moisture dynamics. As typical in seasonal wetlands in the Northeastern US (Brooks, 2005), the water table in all three positions was highest from January to July and lowest from August through December (Fig. 2b). The lowland position had the greatest fluctuations in water table depth; the water table rose above the ground surface from February through June and dropped below the ground surface from July through January (-2 to -42 cm) (Table S1). The water table in the transition and upland positions showed similar seasonal dynamics, but the water table was significantly lower in the lowland position throughout the year. VMC generally followed water table fluctuations, although with less seasonal variation (Fig. 2c). Soil moisture was consistently the greatest in the lowland position; during the growing season lowland VMC was 20% greater than the upland position (p-value < 0.05; Tukey's HSD), and 15% greater in the non-growing season (p-value < 0.05; Tukey's HSD) (Table S1).

Redox dynamics. ~~Redox potential (~~E$_h$~~)~~ values typically mirrored the hydrologic conditions of each landscape position, with the lowest values generally occurring from May to July and the highest values between October and February (Fig. 2d). The lowland position had the largest seasonal amplitude, with values of less than 100 mV between May and July and above 500 mV from October to December. E$_h$ in the transition position only fell to values between 200 to 300 mV between May and July, and recovered to values near 600 mV by October. The E$_h$ values at the upland position remained above 450 mV throughout the entire year at 15 cm depth, but reached 400 mV or lower at 30 and 45 cm depths from May to July.

3.3 ~~Distribution~~ Relating~~of~~ carbon concentration to~~,~~ root biomass~~,~~ and ~~mineralogy~~mineral composition across upland-to-lowland transitions

To identify how roots and ~~mineralogy~~mineral composition affected ~~the~~ C distribution ~~of C~~ across the upland-to-lowland transect, we examined C concentrations in relation to root biomass, texture, extractable Fe and Al and E$_h$ (Table 3). Along the upland-to-lowland transects, C concentrations in the surface horizons increased (p-value < 0.05, Tukey's HSD) whereas concentrations in the subsurface horizons decreased along the transect (p-value < 0.05, Tukey's HSD) (Table 3). C concentrations in the lowland position ~~topsoil~~ surface horizons were ~~were two and~~ four times greater than the ~~transition (p-value < 0.01; Tukey's HSD) and~~ upland position~~s~~ ~~subsoils~~ surface horizons (p-value < 0.001; Tukey's HSD), respectively. In contrast, the ~~subsoils~~ subsurface soils in the upland positions had nearly double the C concentrations than ~~the subsoils subsurface soils of the~~in the transition and lowland positions (p-value < 0.05, Tukey's HSD) (Table 3). Root biomass significantly decreased from the upland to the lowland positions (p-value < 0.05, Tukey's HSD) (Table 3)~~(Table 3)~~, with a 10-fold decline in ~~.~~both ~~The upland position had nearly 10-times the amount of root biomass as the lowland position in the~~ surface and subsurface horizons.~~, however the differences observed in the subsurface horizons (C and C$_g$ horizons) were not statistically significant~~. Silt and clay content increased from the upland to the lowland positions, particularly in the subsurface soil~~oil~~ (+33%, Table 3), although shifts in silt and clay contents were also not statistically significant. Both Fe$_o$ and Al$_o$ ~~decreased by nearly 50 %~~significantly decreased ~~from~~ along the upland-~~-~~to-~~-~~lowland ~~positions~~ transects in the ~~topsoil~~surface horizons (p-value < 0.05, Tukey's HSD). However, in the ~~subsoil~~ subsurface horizons, Fe$_o$ ~~almost doubled~~showed a two-fold increase from the upland to the lowland positions~~s, yet this increase was not~~ (albeit not significantly~~ different~~), while ~~. The upland position had significantly more Al$_o$ than the transition and lowland positions in all horizons~~ the surface horizons ~~(p < 0.001, ANOVA), (p-value < 0.05, Tukey's HSD). In the subsurface horizon horizons of the upland position (C-horizon)~~Al$_o$

showed a four-fold decline ~~Al$_o$ was nearly four times that of the adjacent lowland position subsurface horizon (Cg horizons)~~ (p-value < 0.001, Tukey's HSD) (Table 3)~~and declined with depth in each landscape position~~. Fe$_d$ and Al$_d$ ~~strongly~~ followed the trends of Fe$_o$ and Al$_o$ (Table 3), thus we further limit our discussion to Fe$_o$ and Al$_o$.

3.4 Linear mixed effects models between total carbon and biogeochemical parameters

To ~~determine~~ predict the relative influence of roots, ~~mineralogy~~mineral composition and E$_h$ on C concentrations in each landscape position, we performed linear mixed effects models using total C as a response variable and root biomass, clay, Fe$_o$, Al$_o$ and mean E$_h$ in the growing season as predictor variables with horizon as a blocking factor (Fig. 4a). The relative importance of the predictor variables changed across the upland-to-lowland transects. In the upland positions, Fe$_o$ was the strongest predictor with the largest F-value (17.31, p-value < 0.001), followed by root biomass (13.31, p-value < 0.01). In the transition and lowland positions, however, only root biomass and particularly Al$_o$ were significantly correlated with C (p-value < 0.01; Table 4). The model results show that as the importance of redox-active Fe$_o$ as a predictor for soil C concentrations ~~became less important~~decreased along upland-to-lowland transects, the importance of Al$_o$ increased.

To ~~identify~~ predict the influence of the biogeochemical variables on soil C concentrations with soil depth, we performed linear mixed effects models on the different horizons, using landscape position as a blocking factor (Fig. 4b). In the A-horizon, E$_h$ had the highest F-value and strongest correlation to C (6.31, p-value < 0.05; Table S3). In the lowest horizons, Al$_o$ was the only significant predictor variable in the models (F-value = 16.10, p-value < 0.01, Table S3). These results indicate that, among the tested biogeochemical variables, E$_h$, a proxy for oxygen availability, has a predominant influence on C concentrations in the surface soils, while Al$_o$ has the strongest ~~effect~~ influence on C concentrations at depth.

3.5 Carbon chemistry across upland-to-lowland transitions

To examine variations in C chemistry ~~C~~ along upland-to-lowland transects, we analyzed solid-phase and water-extractable OM. C (1s) NEXAFS spectra showed a general ~~n overall~~ increase in abundance of chemically-reduced, solid-phase C across the upland-to-lowland transect~~s~~ in the ~~topsoil~~surface horizons, but an opposite trend in the ~~subsoil~~ subsurface horizons (Fig. 5a, Table ~~S3~~S4). Aliphatic~~, aromatic~~ and carboxylic C relative abundances were significantly different amongst the three landscape positions (p-value = < 0.05, ~~ANOVA~~Tukey's HSD). The relative abundance of chemically-reduced aliphatic ~~and aromatic~~ C increased from the upland to the lowland position in the surface horizons (p-value < 0.001 Tukey's HSD~~, Tukey's~~

~~HSD~~); though not statistically significant, ~~, but their~~ it~~’~~s contribution also decreased gradually along the same transect in the subsurface horizons (Fig. 5a, Table S4). Generally, chemically reduced aromatic C followed the same trend as aliphatic C~~, however these trends were not statistically significant~~. Chemically more oxidized carboxylic C decreased in the surface horizons from the upland to lowland positions (p-value < 0.01, Tukey’s HSD), yet increased slightly in the ~~subsoil~~ subsurface horizons along the same transect. As a measure of the degree of oxidation, we calculated carboxylic-to-aromatic C ratios (Fig. 5b), with higher ratios indicating a greater degree of oxidation. ~~Due to the high variance within the C (1s) NEXAFS spectra, the calculated~~ Although the ratios were not significantly different among the landscape positions (p-value > 0.05, Tukey’s HSD), ~~however it is worth noting the genera~~noteworthy~~l~~ trends were found. In the ~~topsoil~~surface horizons, the ratio gradually decreased across the upland-to-lowland transects in the ~~topsoils~~ surface horizons (Fig. 5b, Table S4). In the ~~subsoil~~subsurface horizons, the opposite trend was observed, and the ratio steadily increased from the upland C-~~-~~horizons to the lowland C$_g$-horizons (Fig. 5b, Table S4).

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, Table S5, Table S6). The composition of water extractable OM was remarkable similar across the transect (~~While the nominal oxidation state of carbon (NOSC) did not change significantlyWhile there were few significant difference among the horizon groups in the three landscape positions~~p-value > 0.05, Tukey’s HSD), but s~~, we do note s~~ome ~~apparent~~ general trends were noticable. ~~across the upland-to-lowland transect.,~~ B~~b~~oth the modified aromaticity index (AI$_{mod}$) and the average molecular weight of the detected compounds showed ~~significant and~~ gradual increases across the upland-to-lowland transitions in the surface horizons (Fig. 6a, Table S6~~5~~). Paralleling that change, the relative contributions of lignin increased (+7%) and that of lipids decreased~~s~~ (-11%) moving from the upland ~~-~~to the ~~-~~lowland position ~~across the transect~~ (Fig. 6b, Table S5). In the ~~subsoils~~subsurface horizons, however, both AI$_{mod}$ and average molecular weight ~~did not change significantly~~ showed little changes (Fig. 6a, Table S6), while the relative abundance of lignin increased (+9%) and that of lipids decreased (-11%).

4 Discussion

~~Our results show how seasonal flooding affects redox conditions, root biomass, and mineralogy as well as their impact on CO$_2$ efflux, C accumulation, and C chemistry across the upland-to-lowland transects.~~ Our results ~~demonstrate~~ suggest that the factors regulating CO_2 emissions and C accumulation shifted as predicted in surface soils along the upland-to-lowland transects~~. However, in subsoils, the factors regulating C accumulation under seasonally flooded soils differed significantly from that in topsoils.~~, but exhibited ~~potentially~~ inverse trends in the subsurface.

4.1 Environmental parameters controlling CO_2 emissions

Our field data support our hypothesis that reducing conditions under flooded conditions inhibit microbial respiration and thus reduce CO_2 emissions ~~in seasonally flooded soil~~in the lowland position ~~is supported by our seasonal field data~~. Indeed, ~~We found strong~~Strong ~~correlations between~~ seasonal CO_2 emissions in the lowland positions were strongly correlated with~~and~~ VMC, water table depth, and E_h ~~in the seasonally flooded lowland positions of our study sites suggested that soil respiration in seasonally flooded mineral is largely a function of the redox regime~~ (Fig. 3). Conversely, in upland positions where oxygen limitations are not limiting, ~~The In the upland position position regressions between CO_2 effluxes and measured environmental variables further demonstrate that where oxygen availability is not limiting~~ soil temperature ~~, however, soil temperature explained~~~~is~~was found to be the best predictor variable for ~~the most variation in~~ CO_2 emissions (Fig. 3a, Table 2). ~~Our results indicate that CO_2 emissions are mainly controlled by soil temperature in upland soils, but in seasonally flooded mineral soils, water saturation and the associated low redox potentials become more important factors. While the effects of temperature (Lloyd and Taylor, 1994) on soil respiration and moisture (Neckles and Neill, 1994) on decomposition rates have been well-established, our results show that CO_2 emissions are differentially governed by these environmental parameters. The strength of the environmental parameter is largely a result of position within the landscape.~~

Our results further ~~showed~~ indicated that the impact of seasonal drainage of the lowland soils on CO_2 effluxes ~~CO_2 efflux is strongly regulated by water saturation and associated redox conditions, but only at~~is limited by temperature effects ~~temperatures sufficient for microbial activity~~ (Fig. 2, Table 2). Oxygenation in other seasonally flooded soils usually results in increases in CO_2 effluxes due to enhanced aerobic microbial respiration (Laine et al., 1996; Krauss and Whitbeck, 2012~~refs~~). ~~E_h in the lowland position were was typically less than 100 mV during the growing season, but greater than 400 mV during a majority of the non-growing season (October through January) (Fig. 2d, Table S2). The difference in E_h between the growing and non-growing season in the lowland position indicates that E_h is partly driven by the effects of temperature on microbial consumption of oxygen. We found significantly lower cumulative CO_2 emissions in the lowland position. The significant~~is ~~disparity of cumulative CO_2 emissions between the upland and lowland position was most pronounced during the growing~~

~~season (Table 1), where the lowlands showed 40 % lower CO$_2$ emissions than upland soils (Table 1). In the non-growing season, lowland and upland positions had near equal emissions (Table 1, Fig. 2a).~~ Although ~~the~~ our lowland soils bec~~o~~ame oxygenated in the non-growing season due to the water table drop,~~;~~ we observed near equal CO$_2$ emissions from the three landscape positions during that time period (Table 1, Fig. 2a). A ~~possible~~possible explanation for this convergence in CO$_2$ emissions ~~during the non-growing season could be the~~is that oxygenation coincides with the low seasonal temperatures during the non-growing season (-1.7 to 10 degrees Celsius) which inhibit microbial activity (Lloyd and Taylor, 1994). In other words, even when seasonal drainage oxygenates the lowland soils, ~~allows for aerobic metabolism in the lowland soils,~~allowing for aerobic microbial respiration to occur, ~~respiration rates~~CO$_2$ efflux in these seasonal wetlands still remains ~~limited~~suppressed due to ~~low~~cold temperatures. It remains to be seen if higher temperatures during the non-growing season, as expected throughout the Northeastern US with climate change (Karmalkar and Bradley, 2017), disproportionally increase microbial respiration (and potentially C loss) from these soils.~~These findings indicate that, although these seasonally flooded soils become oxygenated, the aerobic period occurs when low seasonal temperatures inhibit microbial activity. In other words, when these seasonally flooded soils experience drained periods with increased oxygen availability, aerobic respiration still remains limited due to low temperatures.~~

4.2 Contrasting impacts of roots, ~~mineralogy~~mineral composition and redox on C concentrations along the upland-to-lowland transect

C concentrations in the lowland ~~topsoil~~ surface soils were nearly four-times greater than in the upland ~~topsoil~~ surface soils (Table 3), which ~~are~~ was ~~more~~ most likely caused by lower microbial respiration rates (Fig. 2a) rather than ~~by~~ differences in C inputs. Given the proximity of our three landscape positions and ~~the flat topography~~minor changes in elevation, aboveground litter inputs can be considered equal across the transect. Moreover, if belowground C inputs were responsible for the greater C concentrations, we would expect root biomass to be higher in lowland than in upland positions. In fact, the opposite was the case (Table 3). ~~.~~Our linear mixed effects model further showed that C concentrations in the ~~topsoils~~ surface soils ~~was~~ were inversely related to E_h across the upland-to-lowland transect (Fig. 4b). In other words, low E_h values (i.e., oxygen availability) coincided with high C concentrations in the surface soil, an observation consistent with findings by Hall and Silver (2015) in tropical surface soils. Hence, greater C concentrations in lowland ~~topsoils~~ surface soil horizons are likely due to oxygen limitations rather than greater above or belowground C inputs.

Surprisingly, this relationship did not hold true in the ~~subsoils~~subsurface horizons, where our linear mixed effects model showed that E_h failed to predict C concentrations across the transect (Fig. 4b, Table S3). Lower C concentrations in lowland ~~subsoils~~ ~~subsurface horizons,~~ as compared to adjacent upland ~~subsoils~~ subsurface soils (Table 3)~~,~~ were likely a consequence of differences in root biomass~~. Root biomass in lowland subsoils was 10-times less than in upland subsoils (Table 3),~~; a difference that can be attributed to restricted root growth under oxygen limitations (Tokarz and Urban, 2015). With roots recognized as primary C inputs belowground, especially in the subsoil (Rasse et al., 2005), the lack of root-derived C may explain the low C ~~stocks~~ concentrations in deeper lowland horizons. With limited C inputs at depth, microbial oxygen consumption resulting from heterotrophic respiration may not be sufficient to cause prolonged oxygen limitations (Keiluweit et al. 2016). These results suggest that the effect of oxygen limitations on C accumulation in seasonally flooded mineral soils may be most pronounced in C-rich ~~topsoils~~surface soils, and less so in C-depleted ~~subsoils~~subsurface soils.

Contrasting trends between upland and lowland soils were also found for the relationship between C concentrations and the presence of reactive Fe and Al phases, which are known to contribute to C accumulation (Wagai and Mayer, 2007). The amount of Fe_o was significantly lower (Table 3) and had significantly less power to predict C concentrations (~~REFER TO LMM RESULTS FIGURE~~Fig. 4a-b, Table 4) in lowland soils than in the upland soils. ~~The amount of Fe_o in lowland soils was significantly lower than in upland soils (Table 3),~~ 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 flooded paddy soils (Hanke et al., 2012) and in gleyed forest soils (Fiedler and Kalbitz, 2003). Here, redox-active minerals such as Fe(III) oxides are frequently lost due to reductive dissolution under reducing conditions and subsequent translocation (Chen et al., 2017). ~~An observed~~A noticeable, yet insignificant~~, but insignificant~~, increase in Fe_o concentrations in the lowland C_g-horizons (Table 3) is~~are~~ likely a reflection of vertical transport~~ation~~ of soluble or colloidal Fe phases into the subsurface horizon, where they may reprecipitate during drained periods. ~~Despite this trend, there were overall lower concentrations of reactive metals in the lowland Cg horizon when taking into account both Fe and Al contents (Table 3).~~ ~~E_h values measured in lowland soil during the flooded period are sufficiently low for Fe(III) oxide reduction (Fig. 2d), likely causing the depletion in Fe_o observed here.~~ ~~The relatively low power of Fe_o to predict C concentrations in the lowland positions compared to the upland positions,~~ Our results therefore suggest~~s~~ that seasonal redox cycles, over pedogenic timescales, has progressively ~~reduced and depleted the soils of Fe oxides.~~lowered the capacity for C to accumulate with redox-active Fe oxides.

~~Even though the lowland soils also had an lower concentrations~~While the predictive power of- Fe$_o$ diminished across the upland-to-lowland transect, ~~of~~ Al$_o$ ~~, compared to the upland position~~became are stronger predictor of C concentrations (~~FIG/TABLE~~Fig. 4a-b, Table 4). ~~,~~ ~~Consequently, our linear mixed effects model showed that Fe$_o$ served as the strongest measured predictor variable for C in upland soils, yet Fe$_o$ had no predictive power in lowland soils (Fig. 4a). In contrast, our linear mixed effects models show that the strength of the relationship of C with Al$_o$ significantly increase~~ed~~s across upland-to-lowland transitions (Fig. 4a).~~. In contrast to Fe, Al hydroxides are not reducible to a more soluble lower oxidation state. Al hydroxides are thus more likely to accumulate in a dynamic redox environment such as our lowland soils. In fact, we found consistently higher Al$_o$ than Fe$_o$ contents in the lowland soils (Table 3). In similarly dynamic forested floodplain environments, C content was also found to be more strongly correlated with Al$_o$ than Fe$_o$, which was attributed to the formation of stable Al$_{3}{}^{+}$-OM complexes (Darke and Walbridge, 2000). ~~Stronger correlations between Al and C in seasonally flooded mineral soils could be attributed to the overall higher concentrations of Al$_o$ present in the soil, compared to Fe$_o$ (Table 3).~~ ~~Organic matter content in floodplain soils has also been reported to be more strongly correlated with Al$_o$ than Fe$_o$ (Darke and Walbridge, 2000).~~ ~~Stronger correlations between Al and C in seasonally flooded mineral soils could be attributed to the overall higher concentrations of Al$_o$ present in the soil, compared to Fe$_o$ (Table 3).~~ ~~Additionally, high soil OM contents,~~High OM contents, ~~typically found in wetland soils~~as found in our lowland soils, have also been found to stabilize Al$_{3}{}^{+}$-OM complexes by inhibiting crystallization of Al into more crystalline, and less reactive ~~can inhibit~~ Al oxides ~~crystallization if Al^{3+} if incorporated into Al-OM complexes~~ (Darke and Walbridge, 2000; Borggaard et al., 1990). ~~These Al-OM complexes result in more poorly crystalline, highly reactive, Al oxides.~~ Thus, ~~m~~~~Mineral protection of C~~C accumulation in our seasonally ~~reduced~~ flooded mineral wetland soils may partly ~~thus~~ depend on ~~non-reducible Al oxides~~non-reducible, poorly crystalline Al$_{3}{}^{+}$-OM complexes.

Together, ~~our~~ these results ~~indicate~~ clearly illustrate ~~the~~ that the relative importance of~~r~~ roots, mineral composition and redox conditions on ~~that~~ C storage shifts not only along the upland-to-lowland transect, but also with depth.- On the one hand, ~~in upland soils~~C accumulation in our upland soils relied ~~relies u~~upon both root inputs and the presence of both Fe and Al phases, as ~~previsouly~~previously documented ~~oxides, whereas C accumulation in lowland soils is more strongly linked to Al oxides and low oxygen availability.~~. .- On the other hand,

~~In sum, the seasonally flooded mineral soils in our study had sufficiently long periods of~~ reducing conditions (or oxygen limitations) in the seasonally flooded lowland soils are sufficient to cause C~~to~~ accumulation~~e~~ ~~C~~ in the ~~topsoil~~ surface horizon relative to upland soils~~horizons relative to the well-drained upland soils. C accumulated in the lowland surface soils is~~ , ~~most likely due to oxygen limitations and in spite of~~despite lower root C inputs and lower abundance of reactive Fe and Al phases. ~~However~~In contrast, ~~,~~ ~~subsoils~~ subsurface horizons~~soils~~ in seasonally flooded lowlands had much lower C concentrations than in the uplands; here C accumulation appears to be owed to non-~~redox active~~reducible Al~~phases~~$^{3+}$-OM complexes, but is limited by the lack of root C inputs belowground and the absence of ~~reactive~~ reactive Fe phases.

4.3 Divergent controls on ~~C~~ organic matter composition in seasonally flooded ~~top- and subsoils~~surface and subsurface soils

We hypothesized that anaerobic periods during seasonal ~~-~~flooding ~~of the lowland soils~~ limit the depolymerization of larger macromolecular compounds and/or the microbial respiration of chemically-reduced OM in the lowland soils. ~~(Keiluweit et al., 2016).~~ Conversely, we expected the upland positions to contain smaller and chemically more oxidized OM as a result of consistently largely aerobic conditions. While prior studies have primarily focused on total C in surface soils (Hall and Silver, 2015), subsurface soils (Olshansky et al., 2018), or DOM (Rouwane et al., 2018), this work represents the first examination of the depth-resolved chemical characteristics of C composition across upland-to-lowland transitions. Analysis of the composition of solid-phase and water-extractable C supported our predictions of a greater abundance of lignin-rich, higher-molecular weight, chemically-reduced OM in the lowland positions, but only in the surface horizons (Fig. 5, Fig. 6). ~~Analysis of the composition of solid-phase and water-extractable C supported our predictions of a greater abundance of higher-molecular weight, chemically-reduced OM in the lowland positions, but only in the topsoil surface horizons (Fig. 5, Fig. 6).~~ In the surface horizons, ~~S~~solid-phase OM across the upland-to-lowland transects ~~became relatively~~became more enriched in relatively reduced ~~reduced~~ aromatic and aliphatic C and ~~relatively~~ depleted in relatively oxidized ~~oxidized~~ carboxylic C, causing the ~~in the topsoil surface horizons~~average oxidation state to gradually decrease ~~-~~(Fig. 5a). Along the same transect, the average molecular weight, aromaticity and contribution from lignin compounds in ~~Water~~ water extractable OM increased ~~of the lowland topsoils surface soils showed greater average molecular weight, higher aromaticity and higher contributions from lignin compounds compared to the upland topsoils surface soils~~ (Fig. 6a~~-~~,b). ~~-~~The selective preservation of chemically-reduced, high-molecular weight OM in the lowland surface soils confirms our assertion above that comparatively low CO_2

fluxes and high C accumulation in the lowland surface horizons is controlled by ~~redox conditions~~oxygen limitations. ~~The fact that we observed only modest decreases in C oxidation state (Fig. 5b, Table S5) suggest thermodynamic limitations on microbial respiration (Keiluweit et al. 2017; Boye et al., 2017) play a limited role in topsoilssurface soils. Instead, the fact that lignin-rich, aromatic, higher-molecular weight OM preferentially accumulates indicates that limited oxidative depolymerization of plant-derived OM under anaerobic conditions is primarily responsible for C accumulation in seasonally flooded topsoilssurface soils.~~

Contrary to our expectation, ~~subsoils~~ subsurface soils showed the reverse trend. ~~and s~~Solid-phase C became ~~significantly~~ more oxidized along the upland-to-lowland transect (Fig. 5). Enhanced C oxidation in seasonally flooded soils is consistent with reports by Olshanksy et al. (2018), who showed that wet-dry cycles increased the interactions between more oxidized OM constituents (i.e. carboxylic C) and reactive soil minerals. It is also well known that subsurface soils in seasonally flooded mineral soils receive significantly ~~more~~greater amounts of dissolved OM leaching down from the ~~topsoil~~ surface horizons compared to upland soils (Fiedler and Kalbitz, 2003). In forest soils, dissolved OM leachates have been shown to consist of partially~~-~~-oxidized aromatic acids, presumably derived from lignin decomposition at the surface, that preferentially associate with reactive Fe phases in the subsurface (Kramer et al. 2012). As noted above, while lowland horizons showed an overall decline in Fe$_o$ and Al$_o$ contents relative to the upland position (Table), a modest uptick in Fe$_o$ content was observed in the lowland subsurface (C$_g$) horizon. One possibility~~ies~~ is thus that ~~GenerallyOverall, the lowland positions of our study showed a decline in Fe$_o$ and Al$_o$ compared to the upland positions.~~ ~~, but the lowland subsoil (Cg-horizon) showed an uptick in Fe$_o$ (Table 3). Such reactive~~ Fe precipitates in the seasonally flooded ~~subsoil~~subsurface soil horizon ~~phases could potentially~~ trapped~~trap~~ dissolved, partially-oxidized, ~~soluble compounds~~lignin-derived OM leaching down the profile ~~and so result~~and so resulted in the accumulation of relatively~~-~~-oxidized OM. ~~in seasonally flooded subsoils.~~

~~Additionally~~Additionally, changes in C oxidation state in the ~~subsoils~~ subsurface may be driven by variations in root C inputs along the upland-to-lowland transect (Table 3). Root C inputs are composed of chemically reduced aliphatic (e.g. suberin and cutin) and aromatic compounds (e.g. lignin and tannins) (Spielvogel et al., 2014). With root biomass in upland positions being noticeably higher, such root-derived inputs may have resulted in greater contributions of chemically~~-~~-reduced OM (Liang and Balser, 2008). In contrast, the lowland subsurface soils were nearly void of roots (Table 3). If OM in lowland subsurface soils predominantly stems from dissolved, partially-oxidized OM leaching down the profile, as discussed above, the lack of root-derived, reduced OM compounds may result in an average C oxidation state that is relatively more oxidized.

4.4 ~~Balance between~~Interplay among ~~mineral an~~redox controls, mineral protection and vegetation dynamics ~~d redox controls~~ will determine climate change response of C storage in seasonally flooded mineral soils ~~to climate change~~

Our results indicate that oxygen limitations ~~are~~ account for the significant C accumulation in surface horizons of seasonally flooded mineral soils.~~a significant control on C accumulation in seasonally flooded mineral soils.~~ The Northeastern US is the fastest warming region in the contiguous US, with winter temperatures rising at a higher rate than summer temperatures (K~~k~~armalkar and Bradley, 2017). Similarly, precipitation is expected to increase~~—, an increase~~an increase that is predicted to occur almost exclusively in the winter months (Karmalkar and Bradley, 2017). ~~Warmer temperatures and less rain in the summer months is predicted to shorten the~~ It is thus assumed that the duration and exten~~td~~ of ~~alter the timing of~~ flooding within similar wetland systems will change throughout the Northeastern US (Brooks, 2005). In the summer months, we would assume that increasing temperature will cause greater evapotranspiration if precipitation remains roughly the same. Consequently, summer drainage of seasonally flooded mineral soils will likely become more pronounced. The resulting ~~more o~~oxygenation of the surface soils may ~~ed,~~ lift~~ing~~ metabolic constraints on OM depolymerization and respiration~~, and~~, and is thus likely to cause ~~promoting~~ soil C loss and greater CO_2 emissions. ~~Additionally, seasonal flooding over pedologic time scales has resulted in a total overall loss of reactive minerals and metals and thus diminished the potential capacity of the soils to accumulate C through other means. Recent studies suggest that colonization by deep-rooting upland plants will offset some of the C loss upon drainage of former wetlands through additional C inputs (Gorham et al., 1991; Lal 2008). The overall lower concentrations of reactive metal phases observed in seasonally flooded soils investigated here suggests a low capacity for new C inputs to associate with reactive Fe or Al phases, and, consequently, a low potential to offset the losses of anaerobically protected C upon drainage.~~In the winter months, however, when both temperature and precipitation are expected to increase (Karmalkar and Bradley, 2017), seasonally flooded mineral soils around the Northeastern US will most likely remain flooded. Yet the increase in temperature may help overcome temperature limitations that we found to control emissions in winter months. To assess how the total C balance within seasonally flooded mineral wetlands may respond to climate change in the Northeastern US, it appears pertinent to explore how warmer winter temperatures affect anaerobic metabolic rates under fully ~~-~~saturated conditions over the winter months, and whether they have the potential to increase CO_2 or CH_4 emissions.

Additionally, recent studies suggest that colonization by deeper-rooting upland plants will offset some of the C loss upon drainage of former wetlands through additional C inputs (Laiho, 2006; Mueller et al., 2016). In our system, seasonal flooding over pedologic time scales has resulted in an overall loss of reactive metal phases. This result suggests a limited capacity for new C inputs to associate with reactive Fe or Al phases, and, consequently, a low potential to offset the losses of anaerobically protected C upon drainage in the short-term. The question whether increased root growth, and associated root-driven weathering of primary minerals, might also increase the abundance of reactive metal phases (Yu et al., 2017), and thus the potential for increased C storage in the long-term, warrants future research.~~Additionally, seasonal flooding over pedologic time scales has resulted in a loss of reactive minerals and metals and thus diminished the potential capacity of the soils to accumulate C through other means. Recent studies suggest that colonization by deep-rooting upland plants will offset some of the C loss upon drainage of former wetlands through additional C inputs (Gorham et al., 1991; Lal 2008). The lack of reactive metal phases observed in seasonally flooded soils investigated here suggests a low capacity for new C inputs to associate with reactive Fe or Al phases, and, consequently, a low potential to offset the losses of anaerobically protected C upon drainage.~~

5 Conclusions

Our examination of CO_2 emissions, C concentrations, and organic matter composition across six-replicated upland-to-lowland transects yielded important insights into the controls on C cycling in seasonally flooded mineral soils. Importantly, we see distinctly different mechanisms controlling C concentration and organic matter composition in surface versus subsurface soils, which sharply contrasts those governing the upland system. While Fe_o and Al_o predicted C concentrations at the upland sites, E_h and Al_o best explained the significantly larger C accumulation in lowland soils. In spite of seasonal re-oxygenation of the ~~topsoils~~surface horizons, periodic flooding (and the associated oxygen limitations) imposed sufficient metabolic constraints on depolymerization ~~and respiration~~ to cause the accumulation of plant-derived, aromatic, high-molecular weight OM in ~~topsoils~~surface soils. In the ~~subsoil~~ subsurface horizons of seasonally flooded soils, anaerobic protection of C appears to be less important. C accumulation was low and primarily ~~related to~~correlated with Al_o, and the OM preserved at depth was relatively oxidized. The fact that anaerobic periods during flooding restricted root growth and caused a relative depletion of Fe(III) oxides in the ~~subsoil~~ subsurface soil suggests that the lack of root C inputs and reactive ~~mineral surfaces~~metal phases are primarily responsible for the low subsurface C accumulation. Our findings suggest that anaerobically protected C in seasonally flooded surface soils may be particularly vulnerable to increased frequency of droughts. The extent to which associated C losses from surface soils may be compensated by upland plant encroachment and deeper root growth warrants further research.

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Figure Captions

Figure 1. Illustration of upland-to-lowland transects in forested seasonally flooded mineral soils ~~wetlands~~ used for this study. (a) Approximate distances and elevation change between landscape positions along the transects as well as the horizons sampled within each position. Approximate seasonal high and low water table depths are indicated by dashed lines. Example of (b) flooded and (c) drained seasonal wetland with marked upland (U), transition (T) and lowland (L) positions.

Figure 2. CO_2 efflux, water table, moisture and redox dynamics along upland-to-lowland transects. Mean monthly (a) soil CO_2 efflux, (b) water table depths, (c) volumetric moisture contents, and (d) depth-resolved redox potentials for the three landscape positions; upland, transition and lowland. Redox potentials are standardized from a calomel to a standard hydrogen electrode. Data are the means of measurements along upland-to-lowland transects in six replicate wetlands.

Figure 3. Pairwise ~~linear~~ regressions between soil CO_2 efflux and soil temperature, water table depth, moisture content and redox potential. Monthly averages for each environmental variable, recorded in six replicate upland-to-lowland transects over a full year, were combined for regression analyses. Regression analyses were conducted for both growing (red-scale markers) and non-growing season (blue-scale markers). (a) Relationship between soil temperature at 10 cm depth and soil respiration ~~modeled~~modelled using the Arrhenius equation. (b) Linear regressions of water table depths against CO_2 efflux. Water table depths less than zero are below soil surface; depths greater than zero are above soil surface. (c) Linear regression of volumetric moisture contents at 10 cm depth plotted against CO_2 efflux. (d) Linear regressions of soil redox potentials at 15 cm depth plotted against CO_2 efflux. Growing season (GS) and non-growing season (NGS) fits are shown for each regression.

Figure 4. Fixed effect parameters predicting total C in linear mixed effects models. (a) F-values of fixed effects for Al_o, Fe_o, clay, root biomass, and mean growing season E_h in each landscape position. (b) F-values of fixed effects for Al_o, Fe_o, clay, root biomass, and mean growing season E_h in the different horizons.

Figure 5. C (1s) NEXAFS analyses of solid-phase OM chemistry across upland-to-lowland transects. (a) NEXAFS spectra from six replicate wetlands (grey), plotted for each landscape position and depth, with the resulting mean spectra plotted (black). Peaks of particular interest are carboxylic C (285.35 eV), aliphatic C (287.20 eV), and aromatic C (285.03 eV) denoted by dotted vertical lines. (b) Average carboxyl-to-aromatic C (285.35 eV/285.03 eV) ratios plotted for each landscape position and depth; bars are standard error of the mean of the ~~six~~ replicates.

Figure 6. FT-ICR-MS analysis of water-extractable OM chemistry across upland-to-lowland transects. (a) Average relative abundances of compound classes as identified by O/C and H/C ratios in Van Krevelen plots. Grey-scale colors denote~~d~~ primarily plant-derived compound classes, while blue-scale compounds denote microbial-derived compound classes. (b)

Average AImod values, as an index for aromaticity, and molecular weights of all detected compounds. Averages represent the mean of replicate samples from six upland-to-wetland transects; bars are standard error of the mean.

Table 1 Average cumulative CO_2 emissions (n = 6 ± standard error) for each landscape position across upland-to-lowland transects

	Full year mol CO_2 m^{-2}	Growing season mol CO_2 m^{-2}	Non-growing season mol CO_2 m^{-2}
Upland position	$54^a \pm 1.1$	$38^a \pm 1.6$	$16^a \pm 0.8$
Transition position	$53^a \pm 0.9$	$33^a \pm 1.5$	$20^a \pm 0.9$
Lowland position	$39^a \pm 0.8$	$24^a \pm 1.3$	$15^a \pm 0.7$

Letter designations are Tukey's ~~honestly significance~~HSD test results. Different letter designations indicate a p-value of < 0.05.

Table 2 Regression analysis (r) results of potential environmental variables that predict CO_2 emissions along a moisture gradient

Environmental Variable	Season	Upland	Transition	Lowland
Soil temperature[#]	Full	0.72***	0.60***	0.53***
	GS	0.62***	0.56***	0.45***
	NGS	0.79***	0.81***	0.69***
Water Table Depth[S]	Full	-0.03	-0.05	-0.30**
	GS	-0.32**	-0.14	-0.55***
	NGS	-0.20	-0.17	-0.35**
Volumetric Moisture Content[S]	Full	0.20*	-0.44***	-0.32***
	GS	0.10	-0.72***	-0.51***
	NGS	-0.10	-0.37**	-0.37**
Soil Redox Potential[S]	Full	0.10	0.10	0.01
	GS	0.05	0.41***	0.40***
	NGS	0.06	0.08	0.27*

Full = entire year, GS = growing season, NGS = non-growing season.

[#] Arrhenius fit

[s] Linear fit

Significance codes: < 0.001 = '***', 0.01 = '**', 0.05 =' *'

Table 3 Average (n = 6 ± standard error) soil properties along the upland-to-lowland transect

Horizon	Total Carbon (%)	C:N	Root Biomass (mg g^{-1} soil)	pH	Silt + Clay (%)	Fe$_o$ (mg g^{-1} soil)	Al$_o$ (mg g^{-1} soil)
Upland							
A	2.3^a ± 0.5	11^a ± 2.5	61^a ± 27	4.98 ± 0.2	48^a ± 11	3.6^a ± 0.5	5.1^b ± 0.8
B	1.1^{ab} ± 0.3	13^a ± 3.4	14^a ± 3	5.22^a ± 0.2	39^a ± 11	2.4^a ± 0.7	5.7^a ± 1.9
C	0.64^a ± 0.1	13^a ± 5.3	6^a ± 3	5.29^a ± 0.1	37^a ± 12	1.7^a ± 0.4	3.6^a ± 0.8
Transition							
A	3.9^{ab} ± 1.5	14^a ± 1.6	48^{ab} ± 17	4.97^a ± 0.2	51^a ± 10	1.2^a ± 0.4	2.5^a ± 0.3
B/C	0.64^a ± 0.1	6.4^a ± 1.4	15^a ± 6	5.38^a ± 0.1	41^a ± 11	1.5^a ± 0.3	1.9^a ± 0.3
Cg	0.36^a ± 0.1	5.0^a ± 3.8	3^a ± 1	5.43^a ± 0.2	59^a ± 10	1.3^a ± 0.3	1.3^a ± 0.4
Lowland							
Ag	8.2^b ± 2.4	16^a ± 1.1	6^b ± 2	4.98^a ± 0.1	50^a ± 13	1.5^b ± 0.4	4.1^{ab} ± 1.1
C	1.9^b± 0.5	13^a ± 3.7	2^b ± 0.6	5.29^a ± 0.1	66^a ± 9	1.0^a ± 0.3	2.6^a ± 0.5
Cg	0.36^b ± 0.02	7.3^a ± 4.9	0.7^a ± 0.5	5.37^a ± 0.1	70^a ± 9	2.9^a ± 0.7	1.0^b ± 0.2

~~Table 3 Average (n = 6 ± standard error) soil properties along the upland-to-lowland transect~~

~~Horizon~~	~~Total Carbon (%)~~	~~C:N~~	~~Root Biomass (mg g^{-1} soil)~~	~~pH~~	~~Silt Clay (%)~~	~~Fe$_o$ (mg g^{-1} soil)~~	~~Al$_o$ (mg g^{-1} soil)~~
~~Upland~~							
~~A~~	~~2.3^{ab} ± 0.5~~	~~11^a ± 2.5~~	~~61^b ± 27~~	~~4.98 ± 0.2~~	~~48^a ± 11~~	~~3.6^b ± 0.5~~	~~5.1^c ± 0.8~~
~~B~~	~~1.1^{ab} ± 0.3~~	~~13^a ± 3.4~~	~~14^{ab} ± 3~~	~~5.22^a ± 0.2~~	~~39^a ± 11~~	~~2.4^{ab} ± 0.7~~	~~5.7^c ± 1.9~~
~~C~~	~~0.64^a ± 0.1~~	~~13^a ± 5.3~~	~~6^a ± 3~~	~~5.29^a ± 0.1~~	~~37^a ± 12~~	~~1.7^{ab} ± 0.4~~	~~3.6^{abc} ± 0.8~~
~~Transition~~							
~~A~~	~~3.9^{bc} ± 1.5~~	~~14^a ± 1.6~~	~~48^b ± 17~~	~~4.97^a ± 0.2~~	~~51^a ± 10~~	~~1.2^a ± 0.4~~	~~2.5^{abc} ± 0.3~~
~~B/C~~	~~0.64^a ± 0.1~~	~~6.4^a ± 1.4~~	~~15^{ab} ± 6~~	~~5.38^a ± 0.1~~	~~41^a ± 11~~	~~1.5^{ab} ± 0.3~~	~~1.9^{abc} ± 0.3~~
~~Cg~~	~~0.36^a ± 0.1~~	~~5.0^a ± 3.8~~	~~3^a ± 1~~	~~5.43^a ± 0.2~~	~~59^a ± 10~~	~~1.3^{ab} ± 0.3~~	~~1.3^{ab} ± 0.4~~
~~Lowland~~							
~~Ag~~	~~8.2^c ± 2.4~~	~~16^a ± 1.1~~	~~6^a ± 2~~	~~4.98^a ± 0.1~~	~~50^a ± 13~~	~~1.5^{ab} ± 0.4~~	~~4.1^{bc} ± 1.1~~
~~C~~	~~1.9^{ab} ± 0.5~~	~~13^a ± 3.7~~	~~2^a ± 0.6~~	~~5.29^a ± 0.1~~	~~66^a ± 9~~	~~1.0^a ± 0.3~~	~~2.6^{abc} ± 0.5~~
~~Cg~~	~~0.36^a ± 0.02~~	~~7.3^a ± 4.9~~	~~0.7^a ± 0.5~~	~~5.37^a ± 0.1~~	~~70^a ± 9~~	~~2.9^{ab} ± 0.7~~	~~1.0^a ± 0.2~~

Letter designations indicate significant differences among horizons of similar depth. Comparisons within surface (A horizons), intermediate (B/C horizons), and subsurface (C/Cg horizons) horizons were determined by ANOVA followed by ~~are~~ Tukey's HSD ~~results based on complimentary horizon basis~~. Different letter designations indicate a p-value of < 0.05.

Table 4 Fixed effect parameters from the linear mixed models along the upland-to-lowland transects

Variable	Degrees of freedom	Regression Coefficient ± standard error	F - value	Landscape Prob > F	Horizon Prob >F
Upland					
Root Biomass	17	0.15 ± 0.09	13.31	<0.01	NS
Fe_o	17	0.37 ± 0.15	17.31	<0.001	NS
Al_o	17	0.31 ± 0.09	10.76	<0.01	<0.05
Clay	17	0.39 ± 0.23	8.56	<0.01	<0.05
E_h	17	0.24 ± 0.31	2.86	NS	<0.05
Transition					
Root Biomass	18	0.05 ± 0.07	21.81	<0.001	<0.05
Fe_o	18	-0.05 ± 0.08	2.86	NS	<0.0001
Al_o	18	0.14 ± 0.14	15.57	<0.001	<0.001
Clay	18	0.24 ± 0.07	1.68	NS	<0.0001
E_h	18	-0.08 ± 0.08	0.32	NS	<0.0001
Lowland					
Root Biomass	12	0.12 ± 0.38	11.22	<0.01	NS
Fe_o	12	-0.01 ± 0.17	0.45	NS	<0.01
Al_o	12	0.91 ± 0.15	137.36	<0.0001	NS
Clay	12	-0.08 ± 0.21	0.31	NS	<0.01
E_h	12	-0.47 ± 0.23	0.77	NS	<0.01

Model parameters with p-values > 0.05 are denoted as not-significant with the letters NS.

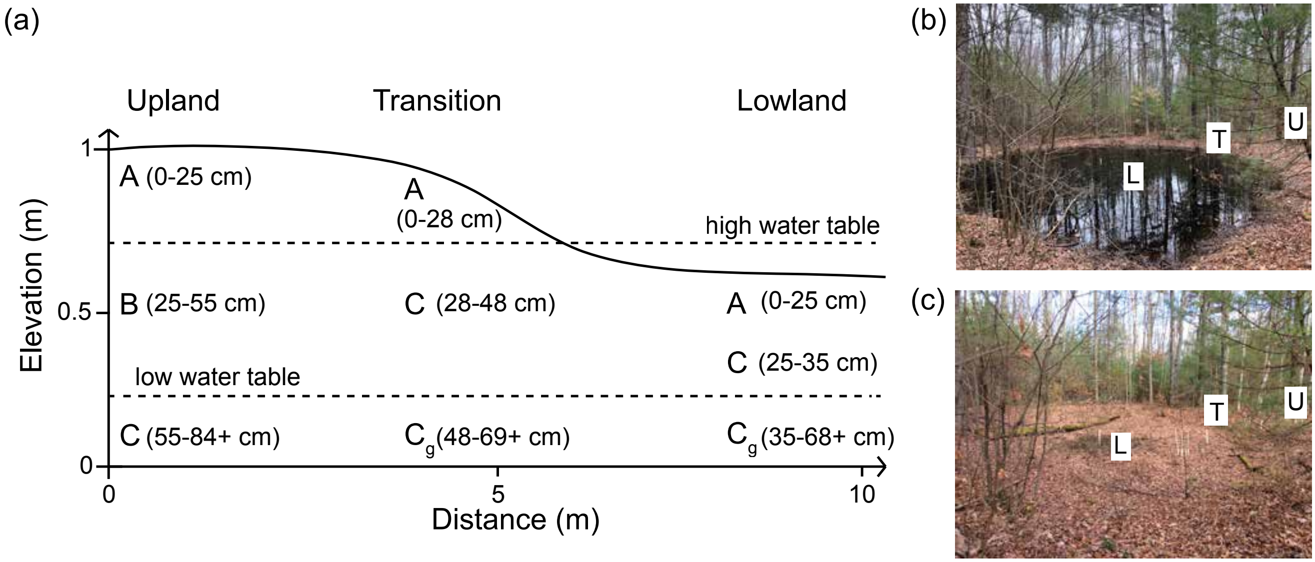
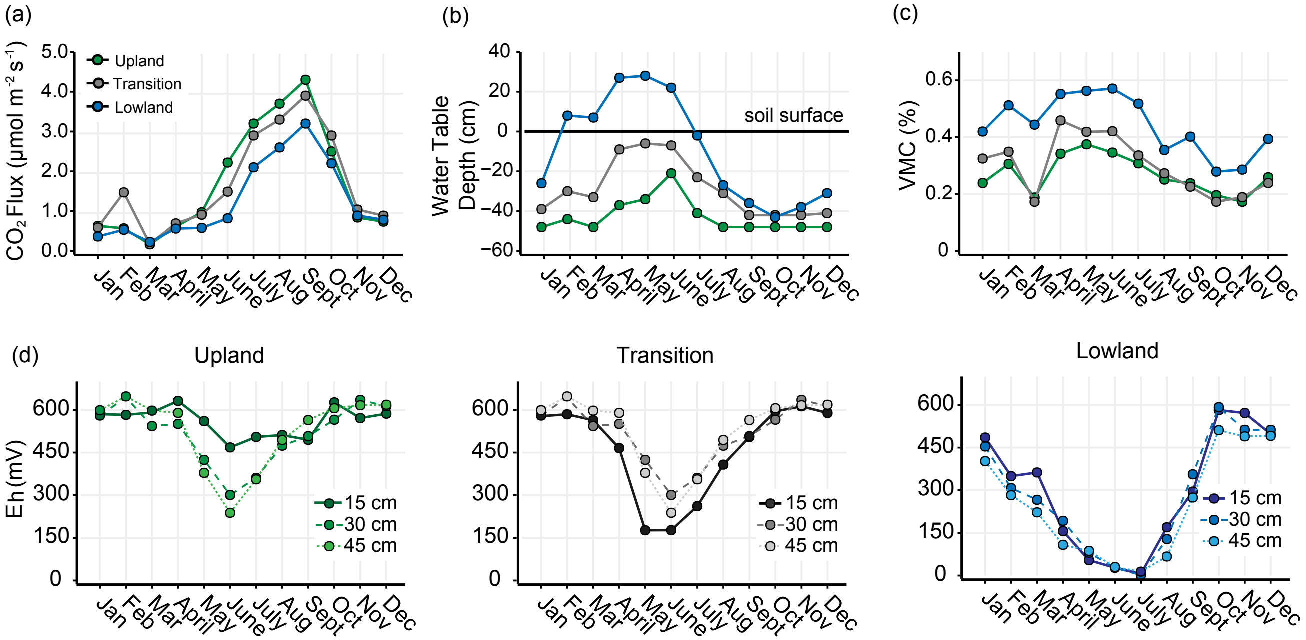

(a)
Upland
Transition
Lowland
Elevation (m)
1
0.5
0
A (0-25 cm)
A
(0-28 cm)
high water table
B (25-55 cm)
C (28-48 cm)
A (0-25 cm)
C (25-35 cm)
low water table
C (55-84+ cm)
C$_g$(48-69+ cm)
C$_g$(35-68+ cm)
0
5
10
Distance (m)
(b)
U
T
L
(c)
U
T
L

fig01



(a)
CO$_2$ Flux (μmol m^{-2} s^{-1})
5.0
4.0
3.0
2.0
1.0
0.0
Upland
Transition
Lowland
(b)
Water Table Depth (cm)
40
20
0
−20
−40
−60
soil surface
(c)
VMC (%)
0.6
0.4
0.2
0
Jan
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec
(d)
Upland
Transition
Lowland
Eh (mV)
600
450
300
150
0
15 cm
30 cm
45 cm

fig02

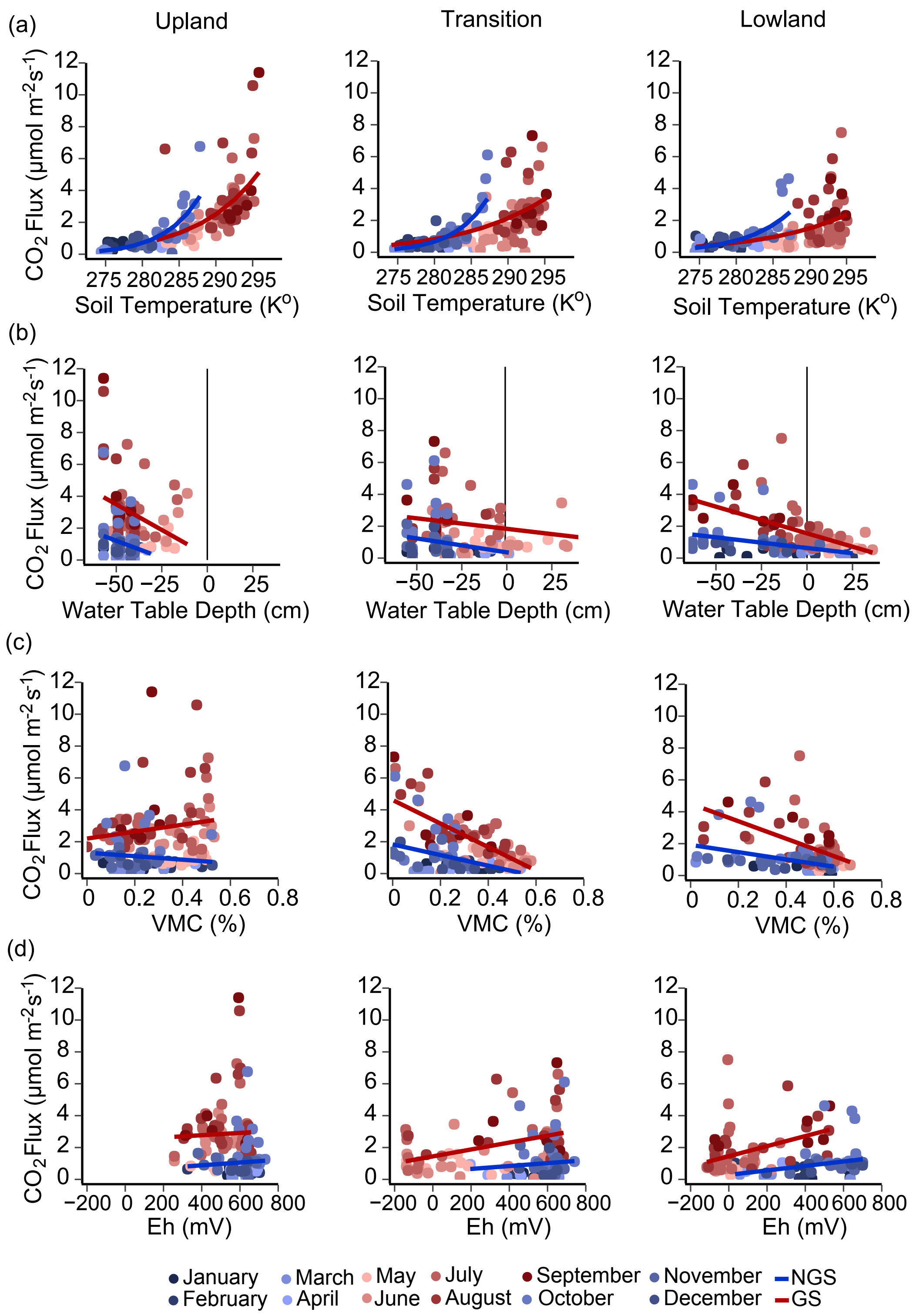

(a)
Upland
Transition
Lowland
CO_2 Flux (μmol $m^{-2}s^{-1}$)
0
2
4
6
8
10
12
275 280 285 290 295
Soil Temperature (K^o)
(b)
−50 −25 0 25
Water Table Depth (cm)
(c)
0 0.2 0.4 0.6 0.8
VMC (%)
(d)
−200 0 200 400 600 800
Eh (mV)
January
February
March
April
May
June
July
August
September
October
November
December
NGS
GS

fig03

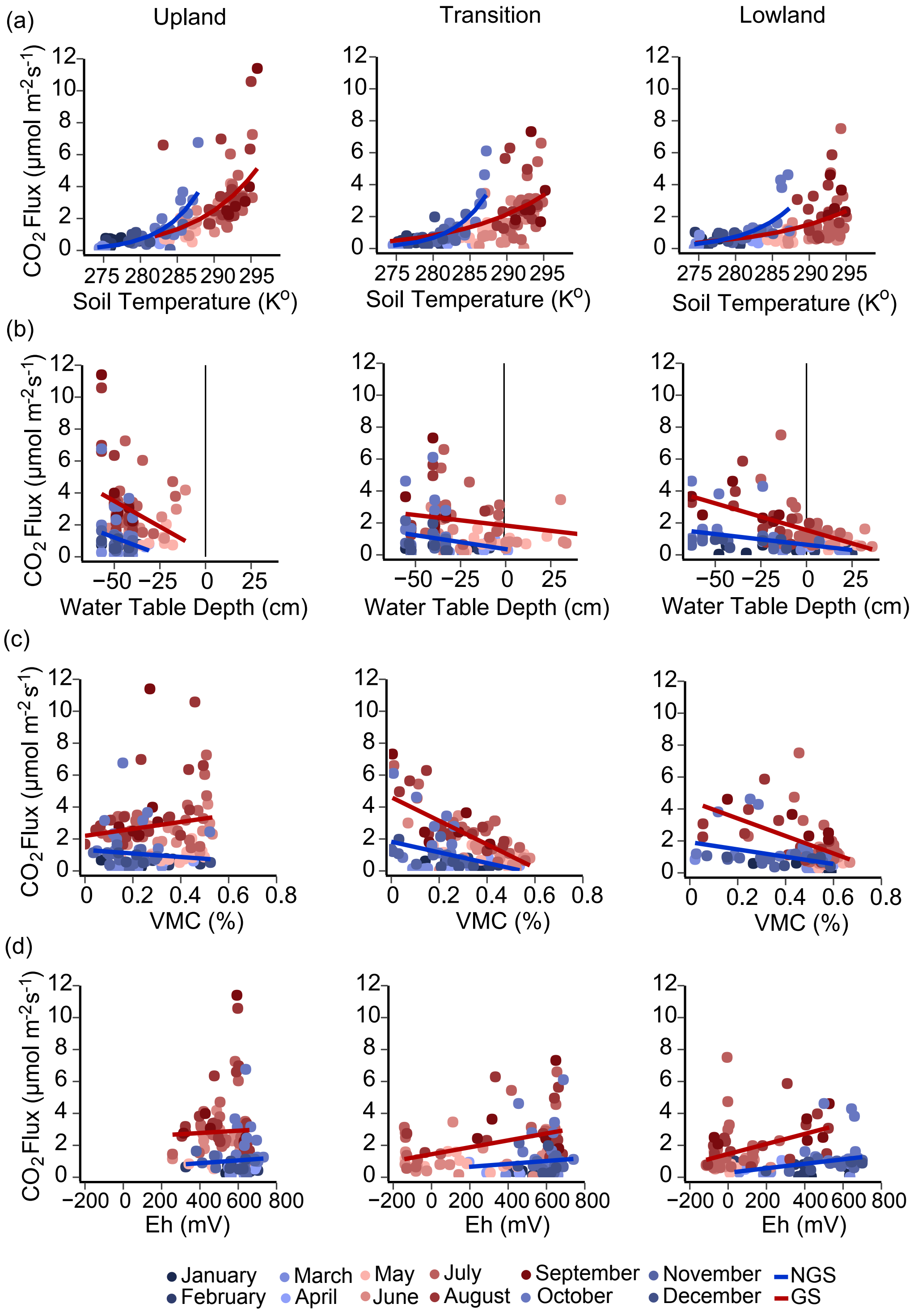

(a)
Upland
Transition
Lowland
CO_2 Flux (μmol m^{-2}s^{-1})
0
2
4
6
8
10
12
275 280 285 290 295
Soil Temperature (K^o)
(b)
−50 −25 0 25
Water Table Depth (cm)
(c)
0 0.2 0.4 0.6 0.8
VMC (%)
(d)
−200 0 200 400 600 800
Eh (mV)
January
February
March
April
May
June
July
August
September
October
November
December
NGS
GS

fig03

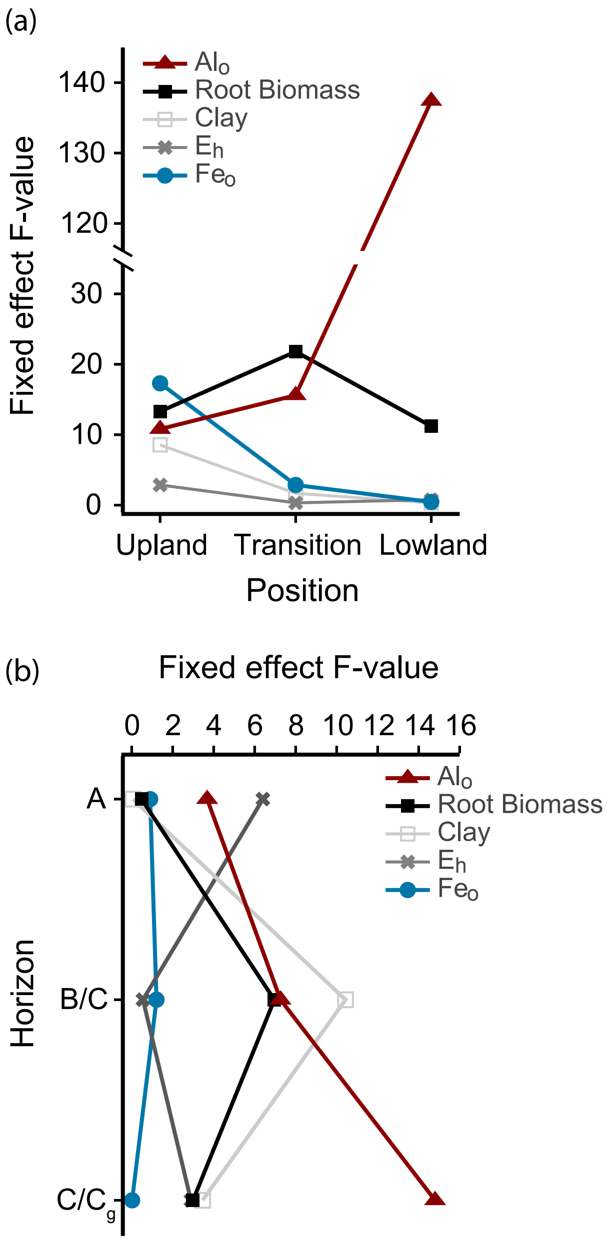

(a)
Fixed effect F-value
0
10
20
30
120
130
140
Upland
Transition
Lowland
Position
Al_o
Root Biomass
Clay
E_h
Fe_o
(b)
Fixed effect F-value
0 2 4 6 8 10 12 14 16
A
B/C
C/C_g
Horizon
Al_o
Root Biomass
Clay
E_h
Fe_o

fig04

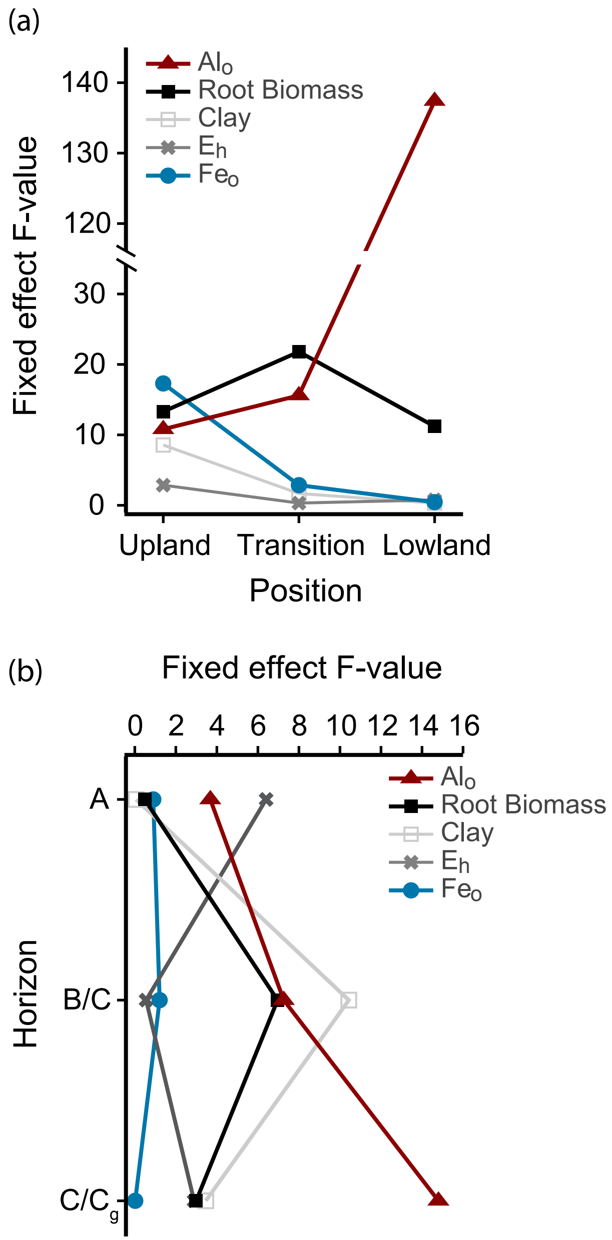

(a)
Fixed effect F-value
140
130
120
30
20
10
0
Al_o
Root Biomass
Clay
E_h
Fe_o
Upland
Transition
Lowland
Position
(b)
Fixed effect F-value
0
2
4
6
8
10
12
14
16
A
B/C
C/C_g
Horizon
Al_o
Root Biomass
Clay
E_h
Fe_o

fig04

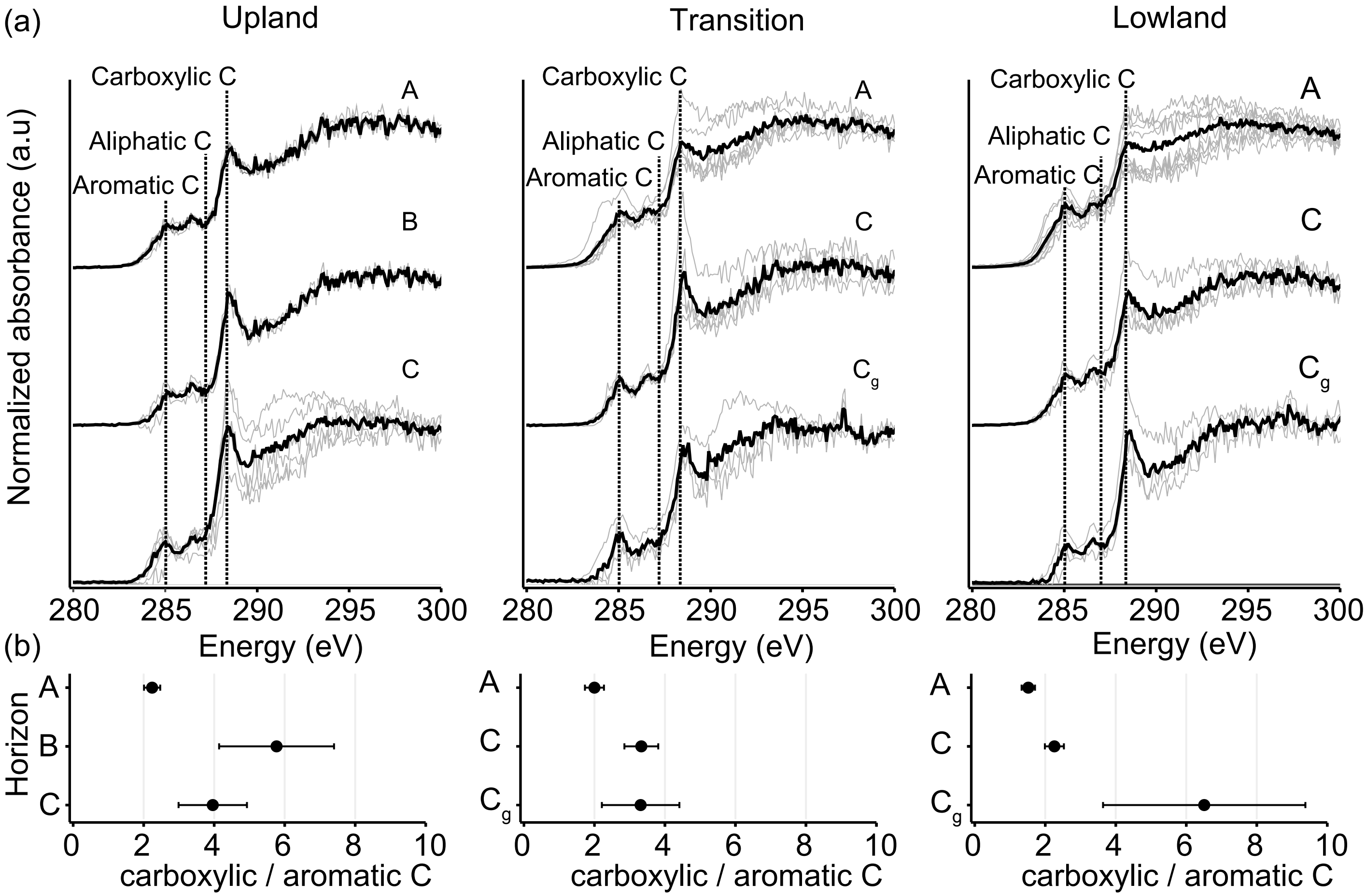

(a)
Upland
Transition
Lowland
Normalized absorbance (a.u)
Carboxylic C
Aliphatic C
Aromatic C
A
B
C
A
C
C_g
A
C
C_g
280
285
290
295
300
Energy (eV)
(b)
Horizon
A
B
C
A
C
C_g
A
C
C_g
0
2
4
6
8
10
carboxylic / aromatic C

fig05

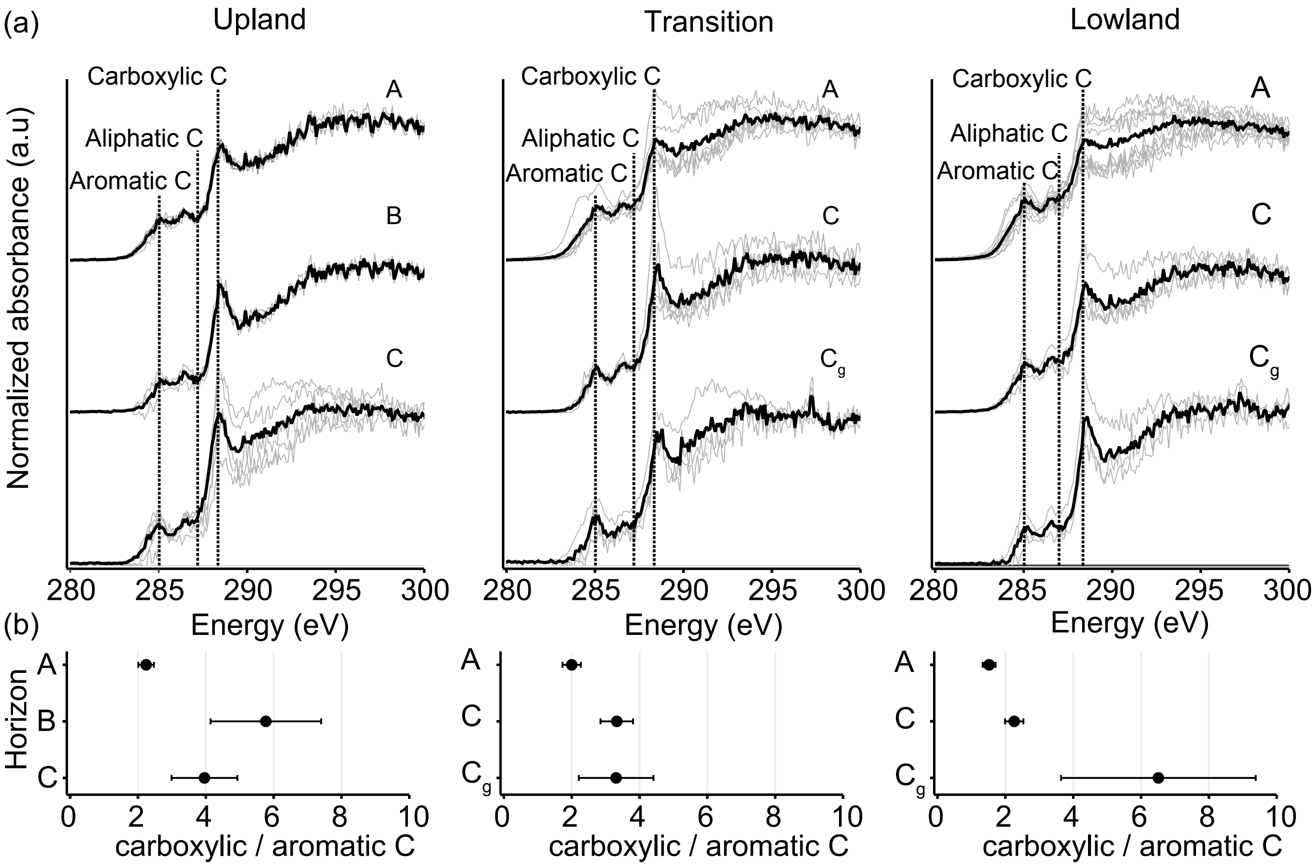

(a)
Upland
Transition
Lowland
Normalized absorbance (a.u)
Carboxylic C
Aliphatic C
Aromatic C
A
B
C
C_g
280
285
290
295
300
Energy (eV)
(b)
Horizon
carboxylic / aromatic C
0
2
4
6
8
10

fig05

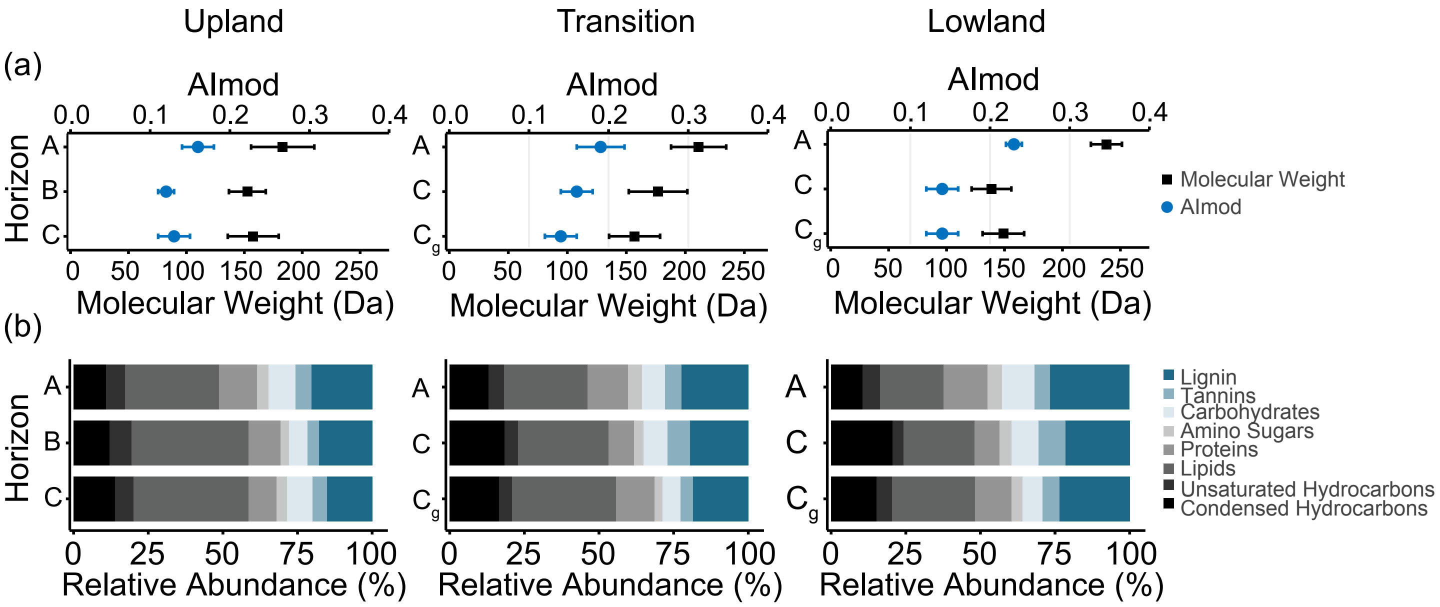
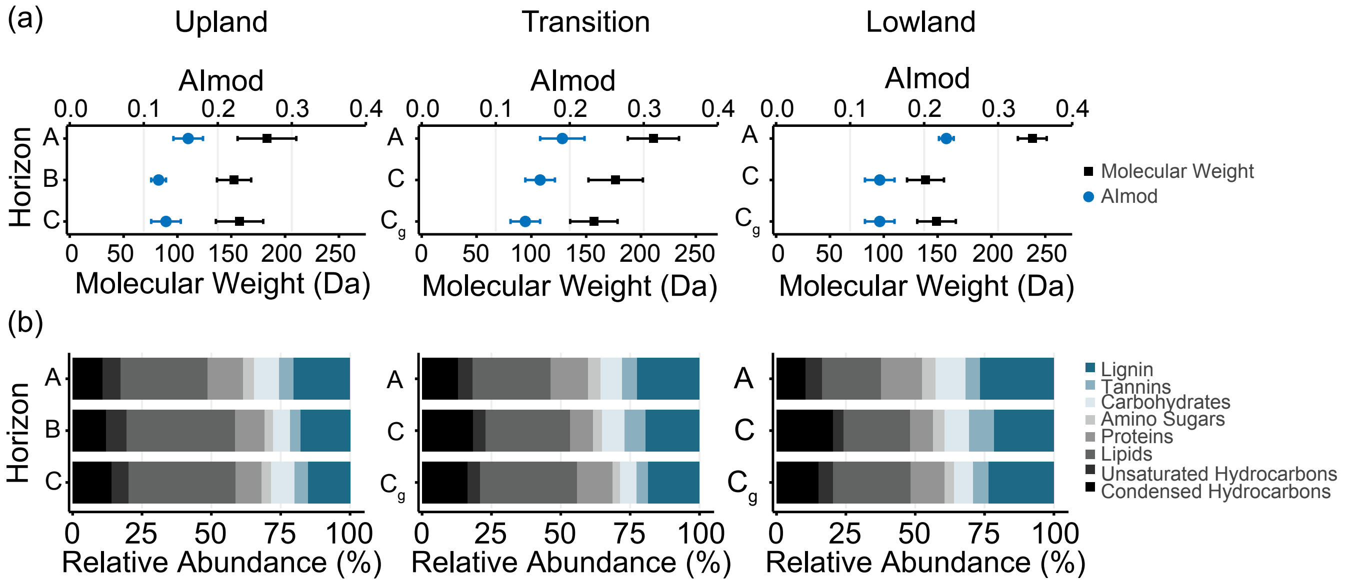

Upland
Transition
Lowland
(a)
AImod
0.0
0.1
0.2
0.3
0.4
Horizon
A
B
C
C
g
0
50
100
150
200
250
Molecular Weight (Da)
Molecular Weight
AImod
(b)
0
25
50
75
100
Relative Abundance (%)
Lignin
Tannins
Carbohydrates
Amino Sugars
Proteins
Lipids
Unsaturated Hydrocarbons
Condensed Hydrocarbons

fig06


(a)
Upland
Transition
Lowland
AImod
0.0
0.1
0.2
0.3
0.4
Horizon
A
B
C
C
g
0
50
100
150
200
250
Molecular Weight (Da)
Molecular Weight
AImod
(b)
0
25
50
75
100
Relative Abundance (%)
Lignin
Tannins
Carbohydrates
Amino Sugars
Proteins
Lipids
Unsaturated Hydrocarbons
Condensed Hydrocarbons

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