Response to comments on "Spatial gradients in soil-carbon character of a coastal forested floodplain are associated with abiotic features, but not microbial communities" by Aditi Sengupta et al.

Dear Dr. Ji-Hyung Park,

We greatly appreciate your and reviewers' thoughtful assessment of our manuscript "Spatial gradients in soil-carbon character of a coastal forested floodplain are associated with abiotic features, but not microbial communities" [Paper bg-2019-193]. Please see our response document for detailed responses to all your and reviewers' comments, with references to line numbers in the track-changes manuscript where changes can be found.

As a result of these changes we believe this work is significantly strengthened and hope you and the reviewers agree.

Sincerely, for the authors,

Dr. Aditi Sengupta

Response to Associate Editor comments are in blue.

The two reviewers recognized the scientific value and novelty of your manuscript. Agreeing with the two reviewers, I am pleased to tell you that you can now submit your revised manuscript to be considered for publication in Biogeosciences. However, given the large number of comments and some important issues raised by reviewers, I have to recommend 'reconsider after major revisiosn.'

Thank you for considering our manuscript for a revise and resubmit following major revisions. We have carefully considered your and reviewers' comments and have revised the manuscript. We have also responded point-by-point to your and reviewers' comments. Our responses are provided in italicized blue font. The line numbers of the revisions are of the marked-up copy with track changes. Additionally, we have also provided a new conceptual diagram line 682, 1199-1208 that summarizes the linkages revealed in our study among salinity, organic C thermodynamic favorability, and inferred levels of microbial activity.

1. I found that your indices of "biochemical transformations" based on FTICR-MS analysis might represent a key methodological approach. Although FTICR-MS has recently become a popular and common approach in various biogeochemical research fields, more detailed descriptions of the analytical procedures and accuracy, and its methodological limitations would help readers understand the unique approach employed in your study.

   Thank you for the suggestions. We have now provided detailed descriptions and methodological limitations as requested in lines 132-134, 266-269, 284-301, and 338-343. We have also added a new figure (Figure 2) lines 338-340, 1164-1169 to help illustrate the mass difference and transformation concept. The following have been added:

"An important caveat is that factors such as redox state, physical protection, mineral associations, and microbial community composition can alter this pure chemistry-based expectation (Schmidt et al., 2011)."

"Soil organic compounds were extracted using a sequential extraction protocol with polar {water (H_2O)} and non-polar {chloroform ($CHCl_3$) (representing mineral-bound fraction)} solvents per standardized protocols (Graham et al., 2017a; Tfaily et al., 2015, 2017), which extract about 2-15% of total organic carbon and represent both polar and non-polar soil organic carbon fractions. Importantly, our analyses do not depend on extracting a large portion of the C found within a given soil sample. Instead, we assume that the extracted fraction is a representative sub-sample."

"Briefly, samples were acidified to pH 2 with 85% phosphoric acid. The samples were passed through Bond Elut PPL cartridges (©Agilent Technologies) that were preactivated with CH_3OH. The cartridges were washed 5x with 10mM HCl followed by nitrogen-gas drying. Next, 1.5 ml CH3OH, a solvent that is compatible with direct analysis on the FTICR-MS, was used to elute the samples from the cartridge thus avoiding an additional evaporation step that might reduce the chance of losing volatile organic compounds. While SPE by PPL has shown not to be very effective in extracting several major classes of DOM compounds that have high ESI efficiencies, such as carboxylic acids and organo-sulfur compounds, and that out-competed other less functionalized compounds (e.g., carbohydrates) for charge in the ESI source (Tfaily et al., 2012), it is critical for marine and estuary DOM samples as it provides complete desalination of the sample. Loss of small molecules such as simple sugars is known to happen during SPE however this is not a concern for the current study as FTICR-MS is sensitive to compounds above ~200 Da. In this study, SPE by PPL isolated a major DOM fraction, that is salt-free, allowing for DOM characterization by FTICR-MS(Dittmar et al., 2008b). While we didn't measure SPE extraction efficiency for this study, it usually ranges between 40 and 62 % depending on the sample (Dittmar et al. 2008). Samples that are collected from the same ecosystem have shown to have similar extraction efficiency. For the purpose of this study, the WSOC (representing the water soluble fraction) and $CHCl_3$ (representing the mineral-bound fraction) fractions were used."

"All possible pairwise mass differences were calculated within each extraction type for each sample. As an example, Figure 2 shows the comparison of two peaks with a mass difference of 2.01586, which indicates a putative hydrogenation reaction between the two organic molecules represented by the associated peaks. It is important to note that direct injection electrospray ionization FTICR-MS cannot distinguish between isomers such as in the case of a mass difference corresponding to a loss of gain of glucose, fructose, or galactose."

2. First, please provide more details on the procedures of 'sequential phase extraction protocol to remove salts as per Dittmar et al., 2008' (e.g., SPE sorbent, eluent, recovery, etc.) in addition to the recovery of WSOC commented by the first reviewer. Please refer to the papers that have warned the technical limitation of SPE (e.g., Anal Bioanal Chem (2016) 408: 4809-4819)

Thank you for the suggestions. We have now provided a brief description of the SPE protocol, responded to the comment about recovery of WSOC by reviewer 1 and have also called out the limitation of SPE. The added text and line numbers are provided in response to the previous comment.

3. Considering the limitation of FTICR-MS as a tool for quantifying molecular peaks, you might also need to discuss the limitation of your approach of comparing mass differences in peaks. For instance, it has been criticized that FTICR-MS results cannot accurately quantify changes in peak intensity between samples from some incubation experiments.

Direct injection (DI) FTICR MS is known to be a qualitative or semi-quantitative approach and this is mainly due to the use of electrospray ionization. In general, a molecule's relative ionization efficiency is determined by the relative abilities of different functional groups to stabilize a negative charge. In negative ion mode, ESI preferentially ionizes molecules that can carry a negative charge as a result of deprotonation. It is possible to use DI FTICR MS in three ways: 1) considering the presence or absence of a peak in the mass spectrum and 2) MS peak intensities which are a function of the abundance of compound in the extract, the ionization efficiency, and their ability to compete for ionization with other compounds in the extract, and 3) accurate mass differences between compounds which doesn't take into consideration the intensity of the peak. In addition to offering putative identification of formula, FTICR MS, due to its ultra-high resolution, has the potential to identify the connectivity between related metabolites since chemically transformed species will be related by measurable and clearly defined mass differences regardless of their intensity.

In this study, we used approaches 1 and 3 and did not use peak intensities since the intensity-dependent approach is known to have issues with charge competition. That is, we used the presence or absence of a peak in the mass spectrum as well as the accurate masses to compare mass differences in peaks. In this approach metabolites whose mass differed by the expected amount (within 1 ppm) were considered to be putatively related by the corresponding metabolic transformation. One potential bias of this approach is that it doesn't take into consideration isomers. For example, a mass difference of $C_6H_{12}O_6$ could be either glucose or fructose or galactose. Using FTICR MS alone we can't differentiate between these three sugars. However, this approach was only used to classify sugar versus non-sugar transformations and therefore distinguishing between the simple sugars was not a goal of this study. It is important to note that approach 3 uses the masses of the peaks from the mass spectrum regardless of whether they are assigned a molecular formula or not.

As suggested, we added a sentence to the materials and methods that discusses briefly one of the main limitations of this approach. "It is important to note that the direct injection electrospray ionization FTICR-MS approach cannot distinguish between isomers such as in the case of a mass difference corresponding to a loss of gain of glucose, fructose, or galactose" in lines 340-343.

4. Second, please think about whether presenting some representative van Krevelen diagrams (as shown in Fig S1) would help readers better figure out your approach of mass difference. You have only four figures in the main manuscript, so one additional figure would not add too much load, I think.

We have now added a new figure (Figure 2, provided below), lines 338-340, 1164-1169to help illustrate the mass difference and transformation concept. to illustrate the mass difference approach. We consider this illustration more informative than van Krevelen and presents what is meant by mass difference of peaks affiliated with a biochemical transformation.

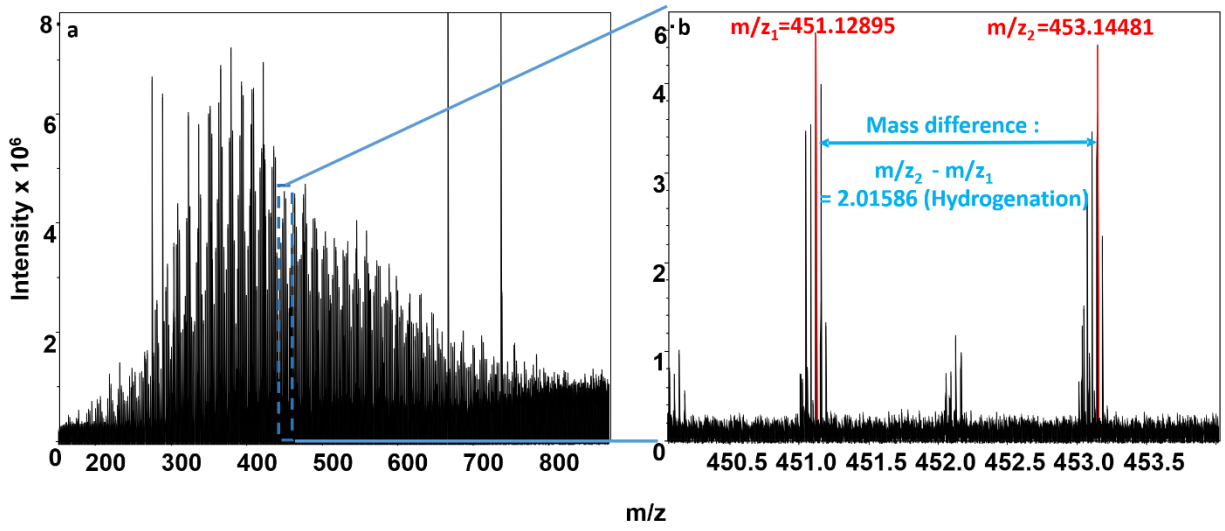

Figure 2. a) Negative mode FTICR-MS (full spectrum); b) zoom in at ~ 450 m/z showing an example of our FTICR-MS spectra overlain with peak mass assignments (red), and a biochemical transformation (mass difference between peaks, denoted in blue). Y axis denotes peak intensities, X-axis denotes mass-to-charge ratio.

5. I would like to ask you to make all the changes easily identifiable in a marked-up manuscript and a point-by-point reply to all the comments offered by the two reviewers and myself. I would also suggest that you specify the line numbers of the revised parts in your responses to the reviewers' and my own comments.

We have provided point-by-point response to your and reviewers' comments and have also provided edits in a track-change document. The line numbers provided in this response document correspond to the track-changed manuscript.

Responses to Referee #1 comments are in blue

Anonymous Referee #1 Received and published: 20 June 2019

1. This paper attempts to identify associations between soil carbon chemistry (molecular composition of SOC fractions revealed by FT-ICR MS analysis) and microbial communities (analyzed by 16S rRNA) at the coastal terrestrial-aquatic interfaces (TAIs) influenced by salinity gradients along a small first order stream in the Washington Coast. These two high-resolution techniques generate tons of information on organic matter chemistry and microbial community composition, which allows detailed examination of their linkages. The introduction part nicely lays out the rationale and hypothesis of this study and the paper is overall well written. However, there are a few issues that need to be addressed.

We appreciate that the reviewer recognizes the value in the data we report. We have carefully considered all the review comments and have provided responses.

2. First of all, the extracted fractions and analyzed molecules are only a small part of the SOC, which may (very likely) not reflect the overall chemistry of total soil organic matter. In this regard, the title and related descriptions should be clarified it is "chemical characteristics of soil carbon fractions" instead of "soil-carbon character".

*We have edited the title in the revised version to indicate this change and clarify in the text that we use "soil carbon character" in our text to indicate chemical characteristics of soil carbon fractions. For example, in line 558 we state that "we observed salinity-associated gradients in soil organic carbon fractions that were not associated with microbial community assembly processes", line 579-580 and 594-595 "*in chemical characteristics of soil carbon fractions",

3. It should also be mentioned in the Methods how much SOC was extracted by the employed method.

The following has been added in lines 266-271: "..which extract about 2-15% of total organic carbon and represent both polar and non-polar soil organic carbon fractions. Importantly, our analyses do not depend on extracting a large portion of the C found within a given soil sample. Instead, we assume that the extracted fraction is a representative sub-sample. This is a standard approach and assumption made in any study examining metabolites or other types of organic molecules in soil."

4. Given the lability of WSOC, it is hence more likely to be influenced by microbial decomposition compared to bulk SOC, but it is also strongly influenced by direct inputs of low-molecular compounds from root exudates, etc. This brings my second point. Despite the nicely formulated hypotheses for this paper, the authors seem to largely ignore (or underestimate) the influence of input processes on the molecular composition of extractable OC. Water- and solvent-extractable OC may derive from direct plant and algal inputs other than depolymerization of soil macromolecules by microbial-mediated enzyme attack. How would root exudates contribute to the thermodynamically less favorable C, for instance? Do you have an estimate of NPP (hence soil inputs) along the study gradient? The observed changes in C chemistry may well be a combined result of decomposition and input processes. Similarly, how would photo-oxidation affect the signal?

Agreed that extractable OC is influenced by inputs (plant and algal derived) and that the observed changes in C chemistry are a combined result of decomposition/input processes which we cannot separate out. We have added sentences in the Introduction (lines72-74) "While multiple processes impact TAI carbon pools (e.g., tidal-inputs, in situ root exudates and litter inputs, decomposition processes), there is some indication that microbial diversity and composition impact soil C storage and mineralization (Mau et al., 2015; Trivedi et al., 2016)." We also now discuss this in lines 566-568 as a caveat to indicate these multiple factors. "Future work should also use tools like Nuclear Magnetic Resonance and Gas Chromatograph-Mass Spectrometry to evaluate how low molecular weight OC (like those contributed by root exudates) varies with salinity."

While we agree that root exudates may impact the carbon signatures, this was not the focus of our study. A key reason is that FTICR-MS is not able to detect root exudates that have low molecular weight. This is a missing piece that can be filled in the future with NMR or GC-MS data. We thank the reviewer for the suggestion and it indeed will be an interesting new study to see how root exudate chemistry varies across the salinity gradient.

Unfortunately, we do not have a good estimate of NPP for the field site at this time. Using MODIS NPP products is also not a viable option because MODIS is 1 km pixel scale while the Beaver Creek site itself is only 3.8 km^2. However, we are in the process of collecting data to make such calculations for future studies focused on plant physiology at this same site. In the future we plan to examine changes in soil carbon chemistry as the floodplain soils become increasingly saline, and will include NPP information in our future efforts. Thank you for the recommendation.

We do not anticipate photo-oxidation at 10 cm and 19-30 cm soil depths.

5. Regarding the analysis and interpretation of the FT-ICR MS data, I am not convinced that the number of common/unique formulas is the best parameter to describe changes in OC chemistry.

We agree that this is not the best parameter/approach. This is a primary reason we focused much of our study on other features to describe changes in OC chemistry including Gibbs Free Energy, heteroatom content, and inferred biochemical transformations. The common/unique compound classes are a minor component of our analyses to show relative heterogeneity of compound classes between samples.

6. The relative abundance of these formulas should be considered.

We believe the reviewer is asking about the relative abundances of compound classes, as opposed to formulas. As such, we have provided relative peak abundances of compound classes in the water extracted organic carbon fraction (Table 1).

7. How representative are the unique formulas in the overall abundance of total MS peaks, for instance? How does the relative abundance of common formulas change with salinity gradient? Hemingway et al. 2017 GCA give a good example for such kind of analysis.

This is an interesting idea but it is beyond the scope of our study. The analysis being suggested would be adding additional concepts, questions, and hypotheses. We feel that our study is quite rich already with respect to concepts, questions, and hypotheses that are all linked together into a collective whole. The suggested analysis is intriguing, but doesn't clearly fit into our integrated vision for our study. We would therefore much prefer to explore this analysis in future work.

There are also some difficult issues that arise from the suggested analyses, as follows. First, we note that we did pairwise comparisons by grouping samples according to landscape position and depth (Lines 454-456), with common/unique features comparable between groups like Floodplain versus Inland, Floodplain versus Terrestrial, and Inland versus Terrestrial at two individual depths. However, comparing sample 1 to sample 2, and then sample 1 to sample 3, and so on to evaluate how common formulas change across the salinity gradient will lead to results that will be difficult to interpret. This is because the fraction of peaks that are common/unique is not a property inherent to a sample, but only emerges when comparing samples to each other. Therefore, we did not evaluate representativeness of unique formulas in the overall peaks because the unique/common feature are dependent on which groups are being compared. As such, we believe that additional methods development is needed to properly implement the suggested analyses.

8. Specific comments: Line 219: Why these two depths?

The two soil depths were chosen based on visual soil characteristics. The shallow depth was the organic-rich horizon, while the deeper depth was characterized by lighter colored, clay-rich soils. We did not go any deeper due to logistical constraints—during the time of sampling, the holes back-filled with water up to roughly the depth of the "deep" samples. The depth of distinct layers were consistent across all floodplain sites, though not as evident in the upland forest site.

9. Line 395: Relationship with what?

In line 481-482, the text now reads "No significant relationship between compound-class abundances and specific conductivity was observed (Table S5)."

Responses to Referee #2 comments are in blue

Anonymous Referee #2 Received and published: 20 June 2019

General Comments

1. This study investigated effects of salinity in coastal forested floodplains on soil carbon pools and microbial community structure. The authors use FTIR to characterize the chemical species within the soil C pool and molecular techniques to characterize and correlate microbial community structure to soil C chemistry, as well as compare all measurements between the different salinity sites.

   One important detail to note is that we used FTICR-MS (Fourier Transform Ion Cyclotron Mass Spectrometry) and not FTIR (Fourier Transform Infrared Spectroscopy). FTICR-MS quantifies mass-to-charge ratio of ions based on cyclotron frequency of ionized compounds in a fixed magnetic field, and therefore allows us to evaluate ultra-high-resolution profiles of

organic compounds from perspectives of thermodynamics, inferred biochemical transformations, and similarity to organic compound classes. FTIR measures infrared absorption and emission spectra and does not provide a mass-to-charge ratio of organic molecules.

2. The ecosystems studied are unique and interesting and at the fringe of TIAs which have clear importance as sea levels continue to rise and salt water intrusion into freshwater systems is likely to alter soil and ecosystem level C cycling dynamics within these fringe ecosystems. I think the study has value to be published and readers of BGC will be interested in the findings, although I have a few major suggestions, primarily in the writing style.

   We appreciate that the reviewer recognizes the value in the research and data we report. We have carefully considered all of the reviewer's comments and have provided detailed responses.

3. I find the writing to be good overall, but is too generalized in that there is not enough detail given for the use of specific terminology, particularly in the introduction but also throughout the manuscript.

   We thank Reviewer 2 for their constructive comments and feedback. We have provided definition of terminologies and/or refer readers to relevant citations that discuss the terminologies in detail in the revised version.

4. This is especially important to reach a broad enough audience and make this research have higher impact. For instance, microbial biochemical transformations, or biogeochemical transformations, were terms used a lot but it is not clear which transformations or processes the authors are referring too. See more comments on that below.

   The transformations refer to biochemical transformations that were potentially occurring within each sample. For which transformations we are referring to, please see lines 331-343 and (Breitling et al., 2006; Stegen et al., 2018) *which highlight the commonly observed biochemical transformations. The ultra-high mass accuracy of FTICR-MS allows us to putatively infer these transformations. We have additionally provided a new figure (Figure 2), line 338-340, 1164-1169to illustrare the mass difference based biochemical transformation approach.*

5. Further, I found that although the hypotheses were introduced in the introduction, the lack of specificity in the introduction regarding each hypotheses made it challenging to follow the authors' logic.

   Our introduction lays out the expectations based on literature review, setting the stage for our hypotheses in lines 192-204. For clarity, we have added sentences, for example, in (lines 125-129) "we derived a series of expectations by first recognizing that (1) our study system is a historically freshwater system, only recently being exposed to salt water due to removal of a culvert in 2014 (see Methods), and (2) microbial activity increases with increasing salinity in historically freshwater systems", lines 136-139 "however, we assume that OM

reactivity follows NOSC, thereby leading to our first expectation/hypothesis: the average ΔG^0_{Cox} of OM will increase with increasing salinity as organic compounds with greater thermodynamic favorability are preferentially depleted (LaRowe and Van Cappellen, 2011) due to microbial activity increasing with salinity", 173-176 "combined with evidence of increasing microbial activity with increasing salinity (discussed above) leads to a fifth hypothesis".

6. Overall, I think the authors should write the introduction with more specific examples from the literature they site, showing the gaps in knowledge on the subject (salinity effects on soil processes in TAIs) and how this study addresses those gaps by asking specific hypotheses.

   Relevant examples from literature are provided as follows. ***Salinity effects on soil processes as they relate to gas flux, dissolved organic carbon, and bulk carbon*** *in TAIs are discussed in lines 84-107,* ***gaps are discussed*** *in lines 103-107, 114-116, 157-160 and how our* ***study addresses those gaps*** *by testing specific hypotheses in lines 125-129 and 173-178, 192-204. We have carefully reviewed and attempted to clarify more examples in the revised version in lines 94, 98-100, 115-116.*

Abstract

7. Abstract is too vague, making it hard to follow what the authors studied, measured, and how to interpret these results.

   The abstract has been written for a general audience, capturing the essence of our analyses, results, and interpretation. We have rephrased certain sections of the abstract to convey a succinct message. To reiterate, Lines 30-32, and 40-42 demonstrate what we ***studied and measured*** *(salinity associated shift in organic C and associated microbial community assembly processes),* ***what we analyzed*** *(organic C thermodynamics, biochemical transformations, heteroatom content, relationship between microbial community assembly processes and C chemistry), and* ***the interpretation*** *(lines 42-48).*

8. L26 TAI doesn't really need an acronym here because it is never used in the abstract again.

   It is used in the next sentence and therefore the acronym is justified.

9. L31Heteroatom seems like a very specific term. It would be helpful to know the definition of a heteroatom or to use a more common term.

   We edited the Abstract text to indicate that heteroatoms are N,S, or P atoms contained within organic molecules. We have also explained what a heteroatom is in line 141.

10. L34 please state salinity range here or previously

    Added.

11. L34 what does inferred biochemical transformations mean? Are these the ones that were measured? It would be more direct to just state which biochemical transformations are being referred to.

    The biochemical transformations are putative gains or losses of molecules based on mass differences among peaks in the spectra. The transformations are not directly measured. They are inferred from the FTICR-MS data by matching the mass differences between pairs of peaks to molecular mass of known biochemical transformations. For example, a mass difference of 99.07 corresponds to gain or loss of the amino acid valine while a difference of 179.06 corresponds to gain or loss of a glucose molecule. We have provided this explanation in the methods (lines 338-343). There are 92 common biochemical transformations (based on commonly observed mass difference associated with biochemical transformations which we evaluated for in our data, and referenced from the following: (Bailey et al., 2017; Breitling et al., 2006; Graham et al., 2017a, 2018; Stegen et al., 2018). *We have also added a figure (Figure 2)(line 338-340, 1163-1168) to explain our approach to inferring putative biochemical transformations based on mass differences.*

12. L35 which metrics of microbial activity were measured?

    Sorry for any confusion. We did not measure microbial activity, and were careful to point that out (Line 36: "indicate lower microbial activity", and lines 100-103 "These observations suggest that microbial activity usually increases with salinity in soils that were not previously exposed to saline conditions, while simultaneously indicating reduced microbial activity with increasing salinity in soils that have a historical exposure to elevated salinity." We state in lines 597-60 that "decreasing biochemical transformations and heteroatom content (with increasing salinity) imply (but do not quantify) lower microbial activity at higher salinity".

13. L41 "Null modelling revealed strong influences on dispersal limitation" I am unclear what this means. So the microbial communities were spatially variable or distinct from each other depending on where the samples were taken?

    Strong influences of dispersal limitation influence microbial community composition by restricting the movement of organisms through space that, in turn, allows random demographic events (births and deaths) to cause unstructured divergence in community composition. This unstructured or stochastic divergence is known as ecological drift. In this case, divergence in community composition is not due to deterministic, selective forces systematically causing some taxa to have higher or lower fitness. We have edited the abstract to reflect this with more direct language.

14. L44 What is a community assembly process? Does this just mean C mineralization, or nitrification, or some other microbially driven process?

    Community assembly processes are those processes that govern the composition of ecological communities (Stegen et al., 2012, 2013, 2015). *Assembly processes are either deterministic (selection resulting from different organisms having different levels of fitness*

for a given set of environmental conditions including abiotic variables and biotic factors related to organismal interactions) or stochastic processes (random birth/death events leading to unstructured divergence in community composition). We have edited the manuscript to be more clearly define these terms and concepts in lines 161-169.

15. L44 "lack of an association" can the authors be more specific. How were microbial communities measured? PLFA? Molecular techniques? Which part of the microbial communities were compared to C chemistry?

    We have rephrased this sentence to be clearer and more direct. There was no significant relationship (based on regression) between C chemistry and the relative influences of different community assembly processes (as quantified by the bNTI metric, which is detailed in the Methods section). The microbial community composition was determined using 16S rRNA amplicon sequencing, and these data were used to run the null model underlying the bNTI metric (see Methods for our sequencing and null modeling approaches).

16. L44 "C chemistry" can the authors be more specific? Which C compounds?

    Associations were not evaluated with C compounds. They were evaluated with C chemistry information including Gibbs Free energy, transformation profiles, and heteroatom content, (as written in methods section; lines 316-329). We edited the sentence to give an example of what is meant by C chemistry.

17. L45 "disconnect btn community and C biogeochem" can you be more specific? What part of the community and biogeochemical processes were disconnected?

    The relative influences of different community assembly process were statistically uncorrelated with C chemistry (i.e., Gibbs Free energy, transformation profiles, and heteroatom content). The Abstract has been edited in line 47-52 for clarity.

Introduction

18. L100 change rates to processes. Rates are not microbially driven, processes are. Which rates/processes are decoupled? Which gas fluxes? CO2 and CH4?

    We have edited the line in the revised manuscript.

    Gas flux rates are decoupled from dissolved organic carbon concentrations.

    We are referring to both CO_2 and CH_4.
    The sentence has been revised.

19. L101 Size of C pool… is this referring to the concentration of DOC mentioned in L100? Clarify

    Yes and clarified in line 111-112.

20. L100-103 How does a decoupling between the C pool size and microbial activity in saline environments suggest it is due to salinity exposure history? Based on how this paragraph is written, it seems like the authors can only say it is due to elevated salinity. Clarify what is meant by salinity exposure history.

    We derive inference from our literature review in the previous paragraph that shows a general trend where soils from historically fresh environments show a positive relationship between CO_2 flux and experimentally manipulated salinity. In contrast, soils in historically saline environments show a negative relationship between CO_2 flux and experimentally manipulated salinity. This indicates that the history of salinity exposure strongly influences the effect of salinity on CO_2 fluxes. We further explain the implications of the salinity exposure history on the resource environment of microbes, bulk C signatures that cannot represent molecular-level changes, and often no observable shifts in bulk C even when a salinity exposure occurs in historically saline or fresh environment. We have rephrased the line to read "Relatively consistent gas flux responses ***to changes in salinity….****"Line 109.*

21. L107 Microbial-activity driven??? Needs to be reworded

    Changed to 'microbially driven'.

22. L98-120 this paragraph starts about discussion between relationships (or lack of) between gas fluxes, DOC, and microbes and ends in a discussion about methods for analyzing chemical constituents of SOC. This should be split up into two paragraphs or reworded to provide better flow. Maybe the first part can be incorporated into the previous paragraph.

    We have edited this paragraph and moved the section to line 180-191.

23. L135 please define heteroatom as it is not necessarily a common term when describing SOC

    We have defined it in the line (organic compounds containing N, S, P).

24. L137-138 What is it about increasing salinity that leads to greater heteroatom concentration? This point is unsupported by the first part of the sentence which seems to just be a general statement.

    It is expected that actively growing microbes increase heteroatom containing organic compounds (as indicated by the references cited). Since CO_2 fluxes increase with increasing salinity in freshwater systems, we hypothesized that as a historically freshwater system (that began changing to a saltwater system following removal of a culvert in 2014), Beaver Creek soils would also show increasing activity and therefore greater heteroatom content with increasing salinity. We edited this section for clarity, laying out expectations in lines 138-143.

25. L140 N mining…please be more specific…N uptake from soil? In the form of inorganic or organic N? Is it already available for uptake or do the microbes secrete enzymes to liberate organically bound N in order to take up inorganic N?

We edited the sentence to reflect a strategy that may require microbes to breakdown organic molecules to extract N (i.e. N mining).

26. L143 clarify that the flooding that results in marine derived OM is flooding from marine salt water terrestrial systems. I assume the terrestrial ecosystem is freshwater, but up to this point there has been no mention of whether the flooded environment is already saline or is freshwater.

We revised the line to clarify this as tidal flooding and that the upland site is freshwater.

27. L150-165 As a reader, I am having trouble following the logic of this paragraph mainly due to the lack of specificity in the use of terms such as community assembly processes, ecological assembly processes, biogeochemical processes, deterministic and stochastic assembly processes, and dispersal processes. Can the authors give examples of what processes they are specifically referring too? It is too general to build a hypothesis off of based on salinity changes in the environment. What is the difference between a community and ecological assembly process? And which can be grouped into deterministic and stochastic categories?

With respect to community assembly processes we point the reviewer to our responses and revised text discussed above under Reviewer2 comments 13 and 14.

We built our hypothesis based on combining two observations from the literature. First, Graham and Stegen (2017) showed that biogeochemical rates are higher when deterministic processes drive community assembly. Second, microbial activity and associated biogeochemical rates have been shown to increase with increasing salinity in historically freshwater systems. Putting these results together provides the hypothesis that the influence of deterministic assembly processes will increase with increasing salinity, due to our study system being historically freshwater (Lines 173-178).

28. L160 Why subsurface microbial ecology? Are the effects different in soil surface horizons?

Edited to include both surface and subsurface.

Methods

29. L184 provide lat and long coordinates at the end of the first sentence

Edited.

30. L186 Can any information be provided on the extent of inundation onto the landscape? Or the size of the floodplain?

    The Beaver Creek watershed is 3.8 km^2. The tidal floodplain makes up 0.5 km^2 of this total watershed area. Information added in the revised manuscript.

31. L189 define psu

    Edited.

32. L197-199 please provide common names for species as well

    Agrostis stolinifera (creeping bentgrass), Tsuga heterophylla (Western hemlock), Picea sitchensis (Sitka spruce). Edited.

33. L204-207 How long were the transects? At what distances along the transects were samples taken?

    Each transect is roughly 80-90 m. Samples were collected ~35 m apart (information provided in lines 244-245). Edited

34. L208-209 I prefer to see soil taxonomic information as well as soil series information. It gives readers a choice on what to interpret. I am not that familiar with Ocosta or Mopang soil series so it provides very little information to me about the soil characteristics without having to go look it up on the NRCS.

    Edited. These are Andisols.

35. L210 Any idea on water table depth? How deep is the water that pools on the surface?

    The water table depth in the floodplain is variable both seasonally and throughout tidal cycles/flood events. During floods, there can be almost 1 m of standing water depending on the tide height. During the summer we have observed the water table to be deeper than 60 cm below the ground surface, whereas in the winter it is higher (e.g. 20-30 cm). We have learned from a series of piezometer transects installed across the floodplain that the hydrology of this system is very complex. We are working on a 3-D hydrologic model to describe these dynamics along with salinity, but this effort is beyond the scope of the present manuscript, and unfortunately will not be published soon enough to be referenced here. We will provide the following info for context, while attempting to not lean too heavily on unpublished results in lines 236-239:

    "The transects experience periodic inundation episodes which result in surface pooling of tidal water, which can be up to ~1m deep. The water table varies seasonally and during tidal cycles and inundation events, ranging from 0 to ~1m below the ground surface (Ward, unpublished)."

36. L217-218 It would be nice to know the elevation of the floodplain, inland, and upland transects.

    Elevation provided in edited manuscript (lines 241-243)

37. L219 Are shallow samples 0-10 cm depth?

    Shallow samples were collected at 10 cm depth.

38. L224-229 There should be a little bit more detail here on each method, or maybe citations to the methods used at the very least. Provide make, model, company etc. for Lachat. How was pH measured, conductivity, GWC, BD, and porosity?!?! What about pre processing? Was large organic matter removed including roots and litter, or retained. Were samples air dried, sieved, etc..?

    We have added the relevant information in our revised manuscript in lines 256-261. We did not have any litter at the depths we collected the soils from. Sieving ensured removal of roots. All analyses were done on air-dried sieved soils.

39. L227-229 this doesn't need to be included here. It is in the following sections.

    Edited.

40. L243 followed by of….check wording

    Edited.

41. L294-295 It seems like more information should be provided on the microbial DNA procedures.

    All procedures were performed as per (Bottos et al., 2018) *that has been cited in the manuscript. We provide a brief overview (lines 350-361) of the associated methods and point the reader to Bottos et al. (2018) for additional details.*

Results

42. L352 Table S3 is almost unreadable in the small font size

    This is a large file. We can alternately have an excel table uploaded if allowed to do so or have this table be hosted with our data and provide a link. We would appreciate input from the editor on this point.

43. L392 missing comma after 14%

    Edited.

44. Why have the authors chosen to not include any taxonomical data on the microbial communities? It seems that this would be very useful information and I assume this information was obtainable from the methods used.

We agree that it can be useful to know the taxonomic composition but this information is not central to testing our hypotheses. As such, we have included a bar chart of major phyla and provided the OTU table with taxonomic assignments in our data package (See doi in the Data Availability section) so that others can more deeply evaluate taxonomic structure.

Discussion

45. L463-464 Here the authors have at least provided some examples of the biogeochem processes they are interested referring to.

Please note that we have indicated the same in lines 85-89.

46. L471 characteristics?

Edited.

47. L472 Authors mention spatially structure inputs. I assume this is in reference to land scape variation but it would be helpful to be more specific.

Yes. We edited this in the revised manuscript in lines 558-560 to read: "Our results are consistent with C chemistry being driven by a combination of spatially-structured inputs driven by landscape structure (i.e., terrestrial inputs further inland, marine inputs further downstream) and…"

48. L473 What metabolic responses of microbial communities were measured in this study?

We did not measure microbial metabolic responses. Instead, we find that microbial community composition is not related to SOC thermodynamic properties or indices that reflect microbial activity (i.e., number of biochemical transformations and heteroatom content). As such, any association between microbes and C chemistry must be mediated by changes in microbial metabolism. That is, our data are consistent with the interpretation that it doesn't matter 'who' is there, it matters what metabolisms they are expressing. Additional work is clearly needed that directly examines microbial metabolism, and we revised the manuscript to more directly indicate this need. We added the following text in lines 562-564: "An important caveat is that we did not measure microbial metabolism, but instead infer an influence of microbial metabolism due to microbial composition being independent of C chemistry."

49. L489 Suffering….awkward wording….Also this appears to be the first mention of forests/tress under stress. Can the authors elaborate on this or provide site level data confirming this?

Edited. We write about trees under stress in the methods section in lines 218-220 and 583-586. We edited the text for clarity and have provided additional reference from our recent vegetation survey publication at this site.

50. L494 The authors didn't measure mineral associated C. How then can comments be made about that fraction of the soil C pool? Maybe because these are generally silt and clay rich soils compared to the clearly much more organic surface soils?

    We edited the methods to indicate that we treated CHCl3-extracted organic carbon as proxy for mineral bound C (line 265) as per (Graham et al., 2017a).

51. L533 How did the authors determine dispersal limitation? Does this mean that the microbial communities were different between the sites? This would not be surprising but is hard to determine since microbial taxonomic structure was not provided.

    The influence of dispersal limitation (relative to other community assembly processes) was quantified using a previously established null modeling approach, as discussed in lines 366-388 (see Stegen et al. 2012, 2013, 2015). We edited the associated text for clarity. We chose to focus on ecological community assembly processes rather than look directly at taxonomic composition because of previously published simulation model-based predictions indicating a potential association between assembly processes and biogeochemical function (Graham and Stegen 2017). Null modeling is required because examining taxonomic composition directly does not provide information on community assembly processes.

52. L542 relatively fast dynamics…..unclear what this means….fast changes in the chemistry of the C? be specific.

    Edited.

53. L556-557 I find this statement to be highly speculative given the one sampling date and the lack of measurements of any actual microbial activity metrics. I would argue that there were no measures of biogeochemical functioning in this study, just measures of the outcome of biogeochemical processes (e.g. remaining C compounds, N compounds etc.).

    We recognize that our study captures one time point and we did not measure microbial activity or biogeochemical function. We are speculative within limits to suggest that community composition in our system is not associated with biogeochemical function. We are citing other papers that show poor association between composition and biogeochemical function. Our sentence structured is purposefully cautions, setup as 'combining our study with these previous…' We added additional language to make it clear that our results are 'consistent with' our inferences and previous literature, though we cannot make unequivocal statements. We also removed 'biogeochemical function' from this sentence. The text in lines 652-654 now reads: "Combining our study with these previous investigations provides evidence that is consistent with (but does not prove) that

soil microbial community composition can be independent of C chemistry, though this certainly varies across systems (e.g., Stegen et al. 2018).”

54. L159 is a more accurate statement…..microbial community (although I think the microbial community structure, abundance of different taxonomic groups, etc. should be shown) was compared to soil C chemistry.

    Line 559 response: we used the bNTI metric to show that microbial community composition and phylogenetic relatedness were not associated with C chemistry. We would also like to guide the reviewer to lines 640-651 that show that community composition may not change while function changes. We now also include a bar plot in in our data package to show the taxonomic groups and provide the OTU table as part of our data package.

55. L562-563 This is the first time, as far as I can tell, that the authors attempted to define dispersal limitation. This information needs to be given when this is first mentioned in the manuscript.

    We added a brief description in the Introduction with citations to guide readers to resources that explain community assembly processes in detail. In addition, we added explanatory text in the Methods section focused on Ecological Modeling. That text in lines 380-388 reads: “Pairwise community comparisons that do not deviate significantly from the null distribution (i.e., 2> βNTI>-2) indicate the dominance of stochastic processes (including homogenizing dispersal and dispersal limitation), or a scenario in which neither deterministic or stochastic processes dominate (referred to as undominated). Homogenizing dispersal occurs when rate of dispersal between two communities result in community composition becoming relatively similar between the two communities, and potentially overwhelming other assembly processes (e.g., variable selection). Dispersal limitation is the result of very low rates of organismal exchange between communities, which can result in the stochastic divergence of community composition through the accumulated outcomes of random birth/death events (i.e., ecological drift).”

56. L563-L566 How does restrictive movement of microbial communities in space lead to functional redundancy? It seems like this would actually reduce functional redundancy as spatially restricted microbial communities become more specialized over time especially in salinated and non salinated soils which likely has a marked effect on the microbial community structure.

    Because ecological drift (enabled by dispersal limitation) can lead to the random loss of taxa within local communities, it can result in different communities containing different, but functionally redundant taxa. This has been added to the revised manuscript version in lines 663-665.

Tables and Graphs

57. Figure 1. It would be helpful to have a label for the waterway in the right hand side of the bottom panel. I think that is Beaver Creek but unsure. Maybe this tributary to Johns River does not have a name though?

Sorry for the confusion—the waterway in the bottom panel is in fact Beaver Creek. The confluence of Beaver Creek and Johns River is not shown in this panel. We have added a label accordingly.

58. Figure 2 and 3. I recommend color coding the points for each of the three sites so readers can see where they fall out on the regression line.

Edited. The salinity of the soil samples did not follow a clear spatial gradient.

59. Table S3 font size should be increased if possible

This is a large file. We can alternately have an excel table uploaded if allowed to do so or have this table be hosted with our data and provide a link. We would appreciate editorial guidance.

Spatial gradients in the characteristics of soil-carbon fractions are associated with abiotic features, but not microbial communities

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Abstract

Coastal terrestrial-aquatic interfaces (TAIs) are dynamic zones of biogeochemical cycling influenced by salinity gradients. However, there is significant heterogeneity in salinity influences on TAI soil biogeochemical function. This heterogeneity is perhaps related to unrecognized mechanisms associated with carbon (C) chemistry and microbial communities. To investigate this potential, we evaluated hypotheses associated with salinity-associated shifts in organic C thermodynamics, biochemical transformations, and nitrogen-, phosphorus-, and sulfur-containing heteroatom organic compounds in a first-order coastal watershed in the Olympic Peninsula of Washington state, USA. In contrast to our hypotheses, thermodynamic favorability of water soluble organic compounds in shallow soils decreased with increasing salinity (43-867 µS cm^{-1}), as did the number of inferred biochemical transformations and total heteroatom content. These patterns indicate lower microbial activity at higher salinity that is potentially constrained by accumulation of less favorable organic C. Furthermore, organic compounds appeared to be primarily marine/algal-derived in forested floodplain soils with more lipid-like and protein-like compounds, relative to upland soils that had more lignin-, tannin-, and carbohydrate-like compounds. Based on a recent simulation-based study, we further hypothesized a relationship between C chemistry and ecological assembly processes governing microbial community composition. Null modelling revealed that differences in microbial community composition—assayed using 16S rRNA gene sequencing—were primarily the result of limited exchange of organisms among communities (i.e., dispersal limitation). This results in unstructured demographic events that cause community composition to diverge stochastically, as opposed to divergence in community composition being due to deterministic selection-based processes associated with differences in environmental conditions. The strong influence of stochastic processes was further reflected in there being no statistical relationship between community

assembly processes (e.g., the relative influence of stochastic assembly processes) and C chemistry (e.g., heteroatom content). This suggests that microbial community composition does not have a mechanistic or causal linkage to C chemistry. The salinity-associated gradient in C chemistry was, therefore, likely influenced by a combination of spatially-structured inputs and salinity-associated metabolic responses of microbial communities that were independent of community composition. We propose that impacts of salinity on coastal soil biogeochemistry need to be understood in the context of C chemistry, hydrologic/depositional dynamics, and microbial physiology, while microbial composition may have less influence.

1. Introduction

The interface between terrestrial and aquatic ecosystems represent a dynamic and poorly understood component of the global carbon (C) cycle, particularly along the tidally-influenced reaches of coastal watersheds where terrestrial and marine biospheres intersect (Krauss et al., 2018; Neubauer et al., 2013a; Tank et al., 2018; Ward et al., 2017b). Moreover, the nutrient cycles occurring at these terrestrial-aquatic interfaces (TAIs) influence locally important ecosystem services like contaminant fate and transport and water quality (Conrads and Darby, 2017; Vidon et al., 2010). While coastal soil C stocks are being increasingly quantified (Hinson et al., 2017; Holmquist et al., 2018; Krauss et al., 2018), the impact of tidally-driven salinity gradients on molecular level features of the soil-C pool and the processes driving soil organic matter (OM) cycling are poorly studied (Barry et al., 2018; Hoitink et al., 2009; Sawakuchi et al., 2017; Ward et al., 2017b). This is particularly true in settings with low freshwater inputs that allows for significant seawater intrusion compared to large river systems (Hoitink and Jay, 2016).

While multiple processes impact TAI carbon pools (e.g., tidal-inputs, *in situ* root exudates and litter inputs, decomposition processes), there is some indication that microbial diversity and composition impact soil C storage and mineralization (Mau et al., 2015; Trivedi et al., 2016). This points to the intriguing possibility that processes governing microbial community assembly may be associated with OM chemistry, but evaluations of such associations are lacking. This lack of mechanistic knowledge combined with significant ecosystem heterogeneity in biogeochemical function across salinity gradients (more below), highlights a need to understand how molecular-level processes vary with seawater exposure along coastal TAIs. Doing so will help enhance predictive models of TAI biogeochemistry that can be potentially included in ecosystem models to more accurately represent the role of TAIs in the broader Earth system (U.S. DOE., 2017).

Modeling of coastal TAIs is currently impeded by poor knowledge of the mechanisms underlying salinity-driven variation in biogeochemical function of associated soils. Previous studies have evaluated function primarily as carbon dioxide (CO_2) and methane (CH_4) flux measurements from soil, and/or soil OM concentrations measured as bulk soil C, percent OM and porewater dissolved organic C (DOC) concentrations in large scale coastal plain river systems. Results from field-based natural salinity gradient studies, long-term field manipulations of salinity exposure, and lab-based incubation studies subjecting soils to varying levels of salinity broadly show the following trends: increases in CO_2 and decreases in CH_4 emissions in freshwater soils exposed to increasing salinity (Chambers et al., 2011, 2013, 2014; Liu et al., 2017; Marton et al., 2012; Neubauer et al., 2013a; Steinmuller and Chambers, 2018; Weston et al., 2006, 2011), and negative relationships between salinity and emissions of both CO_2 and CH_4 from soils with a history of exposure to high salinity (Chambers et al., 2013; Herbert et al., 2018;

Neubauer et al., 2005, 2013a; Weston et al., 2014) (also see Table S1). Exceptions have been observed where CO_2 emissions decreased in historically freshwater coastal wetland soils exposed to seawater (Ardón et al., 2018; Herbert et al., 2018). with Ardón et al. (2018) also reporting an increase in CH_4 flux with salinity and Steinmuller and Chambers (2018) reporting no change in CH_4 flux with increasing salinity. These observations suggest that microbial activity usually increases with salinity in soils that were not previously exposed to saline conditions, while simultaneously indicating reduced microbial activity with increasing salinity in soils that have a historical exposure to high salinity. In contrast to relatively consistent responses of gas fluxes to changes in salinity, there are strong inconsistencies in DOC responses, including no change (Weston et al., 2006, 2011, 2014), increased DOC (Chambers et al., 2014; Tzortziou et al., 2011), and decreased DOC (Ardón et al., 2016, 2018; Liu et al., 2017; Yang et al., 2018) with increasing salinity.

Relatively consistent gas flux responses to changes in salinity combined with inconsistent DOC responses to elevated salinity suggests decoupling between biogeochemical rates and the concentration of DOC. This apparent decoupling between the size of the C pool (in this case the concentration of DOC) and microbial activity suggests that C biogeochemistry is influenced by salinity-exposure history, which in turn influences nutrient resources available to soil microbial communities. Specifically, any systematic shifts in soil organic carbon (SOC) chemistry (along salinity gradients) that cannot be observed with bulk C measurements (e.g., changes in chemistry that reduce C bioavailability; Neubauer et al. 2013) may result in unpredictable carbon fluxes. Moreover, bulk C content can show no change across gradients of salinity (Neubauer et al., 2013a) and may fail to capture an integrated view of microbially driven C cycling dynamics at TAIs. In contrast, detailed molecular-level evaluation of SOC composition can provide a more

mechanistic view of OC transformations, relative to bulk measures of C content or gas flux measurements.

Despite its potential importance, a detailed understanding of the characteristics of soil organic compounds (Zark and Dittmar, 2018) and their association with microbial communities in coastal TAIs is currently not available. Nonetheless, we derived a series of expectations by first recognizing that (1) our study system is a historically freshwater system, only recently being exposed to salt water due to removal of a culvert in 2014 (see Methods), and (2) microbial activity increases with increasing salinity in historically freshwater systems (Nyman and Delaune, 1991; Smith et al., 1983; Tzortziou et al., 2011). In addition, it is generally expected that microbes preferentially degrade compounds with higher nominal oxidation states (NOSC) or lower Gibbs Free Energy (ΔG^0_{Cox}) due to greater thermodynamic favorability (Boye et al., 2017; Graham et al., 2017, 2018, Stegen et al., 2018, Ward et al., 2017a). An important caveat is that factors such as redox state, physical protection, mineral associations, and microbial community composition can alter this pure chemistry-based expectation (Schmidt et al., 2011). As a simple point of departure, however, we assume that OM reactivity follows NOSC, thereby leading to our first expectation/hypothesis: the average ΔG^0_{Cox} of OM will increase with increasing salinity as organic compounds with greater thermodynamic favorability are preferentially depleted (LaRowe and Van Cappellen, 2011) due to microbial activity increasing with salinity. To develop our second hypothesis we note that actively growing microbial communities are known to enhance biochemical transformations and generate heteroatom containing organic molecules [sulfur (S), nitrogen (N) and phosphorus (P)] (Guillemette et al., 2018; Koch et al., 2014; Ksionzek et al., 2016); therefore greater heteroatom content and more biochemical transformations are expected with increasing salinity. Our third hypothesis is based on

microorganisms adapting to saline conditions through the production or sequestration of osmolytes (Gouffi et al., 1999b, 1999a; Sleator and Hill, 2002), a strategy that may require microbes to break down organic molecules to extract N (i.e., N mining). We therefore hypothesize increases in N-containing biochemical transformation with increasing salinity. Our fourth hypothesis is based on the observation that soils in saturated environments like floodplains are expected to be less oxygenated and can also receive deposition of marine/algal derived OM and suspended sediments during tidal flooding. These factors can result in OM having lower oxygen to carbon (O/C) and higher hydrogen to carbon (H/C) ratios as compared to upland soils (Seidel et al., 2016; Tfaily et al., 2014; Ward et al., 2019b). We therefore hypothesize a greater relative abundance of lipid- and protein-like and less lignin- and tannin-like compounds in the floodplain soils, relative to upland (i.e., drained) soil.

While we expect systematic shifts in C chemistry across landscape scale salinity gradients, an open question is the degree to which C chemistry is associated with ecological assembly processes governing composition of microbial communities. Soil microorganisms transform soil C, but there is limited evidence of direct links between microbial community assembly processes and molecular-level soil C chemistry (Kubartová et al., 2015; Rocca et al., 2015; Trivedi et al., 2016; van der Wal et al., 2015). Assembly processes, broadly divided into deterministic (systematic impacts of selection) and stochastic (unstructured demographic events) factors, function over space and time to influence the composition of microbial communities, which in turn mediate biogeochemical cycles (Graham et al., 2016, 2017b; Nemergut et al., 2013a; Stegen et al., 2015). Deterministic processes lead to selection of microbial communities resulting from different organisms having different levels of fitness for a given set of environmental conditions including abiotic variables and biotic factors related to organismal interactions while stochastic

processes include random birth/death events and unstructured dispersal. The relative influences of stochastic and deterministic processes can be inferred from phylogenetic distances among microbial taxa using ecological null models. This approach has been widely employed to understand community assembly processes in surface and subsurface microbial ecology (Caruso et al., 2011; Dini-Andreote et al., 2015; Graham et al., 2017a, 2018; Sengupta et al., 2019; Stegen et al., 2012). Furthermore, a recent study used ecological simulation modeling to show that communities experiencing increased rates of dispersal are linked to reduced biogeochemical functioning (Graham and Stegen, 2017). This, combined with evidence of increasing microbial activity with increasing salinity (discussed above) leads to a fifth hypothesis that the influence of deterministic selection will progressively increase with salinity due to increased microbial activity.

Analyses of specific chemical biomarkers such as lignin phenols, amino acids, and lipids have been used in soils, sediments, and water to quantitatively evaluate the provenance of terrestrial-derived OM (Hedges et al., 1997), the reactivity of OM as it travels through a soil column (Shen et al., 2015), and microbial community composition (Langer and Rinklebe, 2009), respectively. While biomarkers provide quantitative details on OC cycling, they generally represent a small fraction of the total OM pool, thus, non-targeted approaches such as analysis of thousands of organic molecules via Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) have become increasingly widespread for determining molecular-level organic compound signatures (Rivas-Ubach et al., 2018) across a variety of terrestrial (Bailey et al., 2017; Simon et al., 2018), aquatic/marine (Lechtenfeld et al., 2015), and transitional settings such as hyporheic zones (Graham et al., 2017a, Stegen et al., 2016, 2018) and river-ocean gradients (Medeiros et al., 2015).

The objective of the current study was to test the following hypotheses in a coastal forested floodplain and adjacent upland forest: (1) mean Gibbs Free Energy of organic compounds will increase with increasing salinity; (2 and 3) biochemical transformations, heteroatom content, and N-containing biochemical transformation will increase with increasing salinity; (4) lipid- and protein-like compound classes will be more prevalent in the floodplain soils compared to upland soils in which lignin- and tannin-like molecules will dominate; and (5) microbial community assembly processes will be increasingly deterministic as salinity increases. The chemical forms of C in these soils were characterized using FTICR-MS. We also employed ecological null model analysis to evaluate the relationship between C chemistry and the influences of assembly processes on microbial communities. Based on our results, we propose a conceptual model of organic C processing in a coastal forested floodplain in which landscape-scale gradients in C chemistry are driven by a combination of spatially-structured inputs and salinity-associated metabolic responses of microbial communities that are independent of community composition.

2. Materials and Methods:

2.1 Site Information and Soil Sampling

Soils along a coastal watershed draining a small first order stream, Beaver Creek, in the Washington coast were selected for this study (46.907, -123.976). Beaver Creek is a tributary of Johns River and experiences a high tidal range of up to 2.5 m that extends midway up the first-order stream's channel and inundates the landscape in its floodplains. The confluence of Beaver Creek and Johns River is roughly 2.5 km upstream of the Grays Harbor estuary and 14.5 km from the Pacific Ocean, and experiences variable exposure to saline waters at high tide (Fig. 1). Surface water salinity near Beaver Creek's confluence ranges from 0 practical salinity unit (psu) at low tide to 30 psu at high tide during dry periods (Ward, unpublished). The Beaver Creek

watershed is 3.8 km^2. The tidal floodplain makes up 0.5 km^2 of this total watershed area. Tidal exchange to Beaver Creek was restored after 2014 when a culvert near the creek's confluence with Johns River was removed (Washington Department of Fish and Wildlife, 2019). Due to the minimal past tidal exchange, the floodplain is dominated by gymnosperm trees (*Picea sitchensis*) that are rapidly dying since the culvert removal (Ward et al., 2019a). The headwaters (before the river channel forms) is a sparsely forested, perennially inundated freshwater wetland with tidal exchange blocked by a beaver dam, followed downstream by a densely forested setting along the river channel. Towards Beaver Creek's confluence salt tolerant grasses such as *Agrostis stolinifera* (Creeping bentgrass) become the most dominant land cover as forest cover becomes more sparse. The watershed's hillslope/uplands is dominated by *Tsuga heterophylla* (Western hemlock) trees, but *Picea sitchensis* (Sitka spruce) are also present.

Two sampling transects perpendicular to the river along the up/downstream salinity gradient were established and represent a high salt exposure site close to the culvert breach location and a moderate salt exposure site upstream of the high salt exposure site. These transects represent a coastal forested wetland with brackish (semi-salty) groundwater and consisted of three terrestrial sampling points at each transect extending from the riparian zone to the beginning of the steep upslope. An additional soil sampling point ~20m uphill from the moderate salt exposure site transect served as a purely terrestrial upland endmember. The soils are Andisols and floodplain transects represented hydric soils classified as Ocosta silty clay loam while the upland site was a well-drained Mopang silt loam. The transects experience periodic inundation episodes which result in surface pooling of tidal water, which can be up to ~1m deep. The water table varies seasonally and during tidal cycles and inundation events, ranging from about 0 to 1m below the ground surface (Ward, unpublished).

Soil samples were collected in triplicate at each of the seven locations (Fig. 1) [BC2 (2.94 m), BC3 (2.63 m), and BC4 (2.82 m) at the high-salt exposure transect, locations BC12 (2.82 m) , BC13 (2.67 m), BC14 (3.07 m) at the moderate salt exposure transect, and BC15 (13.45 m) as upland site]. The high-salt exposure transect was 230 m from the moderately saline transect (0.6 km from the confluence of Beaver Creek with Johns River), and each site at the transect was ~35 m apart from the next. Each transect is ~ 90 m. For data comparison's sake, we classify BC2, BC3, BC12, and BC13 as **floodplain** sites while BC4 and BC14 are further **inland** and ~75 m away from the creek at the base of the densely wooded hillslope. Soil samples for molecular characterization studies were collected at two depths—shallow (10 cm) and deep (19-30 cm). Samples were collected from the face of soil pits using custom mini-corers, placed into sterile amber glass vials, purged with N_2 to maintain anaerobic conditions, frozen in the field within an hour at -20 °C, and stored at -80 °C on return to the lab. Bulk samples were collected for soil physicochemical characterization including texture classification with hydrometer method after organic matter removal, dry combustion with direct measure of total C, nitrogen (N) and sulfur (S) by Elementar Macro Cube, plant-available N as ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) with 2M KCl quantified on Lachat QuikChem 8500 Series 2 (Hach, Loveland Colorado) as colorimetric reaction, pH measured as 1:2 soil:water slurry measured with Hanna benchtop meter, specific conductivity measured as 1:5 soil:water slurry measured on MP-6p portable specific conductivity meter, gravimetric water content measured after drying soils at 105 °C for 48 hours, and bulk density and porosity measured as per standard methods (Sumner,1999). Soil chemical characterization was performed on air-dried sieved soils.

2.2 FTICR-MS solvent extraction and data acquisition

Soil organic compounds were extracted using a sequential extraction protocol with polar {water (H_2O)} and non-polar {chloroform ($CHCl_3$) (representing mineral-bound fraction)} solvents per standardized protocols (Graham et al., 2017a; Tfaily et al., 2015, 2017), which extract about 2-15% of total organic carbon and represent both polar and non-polar soil organic carbon fractions. Importantly, our analyses do not depend on extracting a large portion of the C found within a given soil sample. Instead, we assume that the extracted fraction is a representative sub-sample. This is a standard approach and assumption made in any study examining metabolites or other types of organic molecules in soil. Briefly, extracts were prepared by adding 5 ml of MilliQ H_2O to 5 g of each of the replicate samples in sterile polypropylene centrifuge tubes (Genesee Scientific, San Diego, USA) suitable for organic solvent extractions and shaking for 2 h on a Thermo Scientific LP Vortex Mixer. Samples were removed from the shaker and centrifuged for 5 minutes at 6000 rpm, and the supernatant was removed into a fresh centrifuge tube. This step was repeated two more times, with the 15 ml supernatant pooled for each sample and stored at -80 °C until further processing. Next, Folch extraction with $CHCl_3$ and CH_3OH was performed for each soil pellet left over from the water extraction. Folch extraction entailed adding 2 ml CH_3OH, vortexing for 5 seconds, adding 4 ml $CHCl_3$, vortexing for 5 seconds, followed by 0.25 ml of MilliQ H_2O. The samples were shaken for 1 hr and another 1.25 ml MilliQ H_2O was added and left overnight at 4 °C to obtain bi-layer separation of upper (polar) layer and the lower (non-polar) layer. The extracts were stored in glass vials at -20 °C until ready to be used. The water soluble organic carbon (WSOC) fraction was further purified using a sequential phase extraction (SPE) protocol to remove salts as per Dittmar et al., 2008. Briefly, samples were acidified to pH 2 with 85% phosphoric acid. The samples were passed through Bond Elut PPL cartridges (©Agilent Technologies) that were preactivated with CH_3OH. The cartridges were washed 5x with 10mM HCl followed by nitrogen-gas drying. Next, 1.5 ml CH3OH, a solvent that is

compatible with direct analysis on the FTICR-MS, was used to elute the samples from the cartridge thus avoiding an additional evaporation step and reducing the chance of losing volatile organic compounds and saving time during sample preparation. While SPE by PPL have shown not to be very effective in extracting several major classes of DOM compounds that had high ESI efficiencies, such as carboxylic acids and organo-sulfur compounds, and that out-competed other less functionalized compounds (e.g., carbohydrates) for charge in the ESI source (Tfaily et al., 2012), it is highly efficient for marine and estuary DOM samples as it provides complete desalination of the sample. Loss of small molecules such as simple sugars is known to happen during SPE however this is not a concern for the current study as FTICR-MS is sensitive for compounds above 200 Da. In this study, SPE by PPL isolates a major DOM fraction, that is salt-free, allowing for DOM characterization by FTICR-MS(Dittmar et al., 2008b) . While we didn't measure SPE extraction efficiency for this study, it usually ranges between 40 and 62 % depending on the sample (Dittmar et al. 2008). Samples that are collected from the same ecosystem have shown to have similar extraction efficiency. The extracts were injected into a 12 Tesla Bruker SolariX FTICR-MS located at Environmental Molecular Sciences Laboratory (EMSL) in Richland, WA, USA. Detailed methods for instrument calibration, experimental conditions, and data acquisition are provided in Graham et al., 2017a and Tfaily et al., 2017.

2.3 FTICR-MS Data Processing

One hundred forty-four individual scans were averaged for each sample and internally calibrated using an organic matter 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 (100 - 900 m/z). Data Analysis software (Bruker Daltonik version 4.2) was used to convert raw spectra to a list of m/z values applying FTMS peak picker module with a signal-to-noise ratio

(S/N) threshold set to 7 and absolute intensity threshold to the default value of 100. Chemical formulae were then assigned using in-house software following the Compound Identification Algorithm, proposed by Kujawinski and Behn (2006), modified by Minor et al. (2012), and described in Tolić et al. (2017). Peaks below 200 and above 900 were dropped to select only for calibrated and assigned peaks. Chemical formulae were assigned based on the following criteria: S/N >7, and mass measurement error < 0.5 ppm, taking into consideration the presence of C, H, O, N, S, P, and excluding other elements. Detected peaks and associated molecular formula were uploaded to the in-house pipeline FTICR R Exploratory Data Analysis (FREDA) to obtain: (i) NOSC values from elemental composition of the organic compounds(Koch and Dittmar, 2006, 2016), (ii) thermodynamic favorability of the compounds calculated as Gibbs Free Energy for the oxidation half reactions of the organic compounds (ΔG^0_{Cox}) based on the equation ΔG^0_{Cox} = 60.3-28.5*NOSC (LaRowe and Van Cappellen, 2011), where a higher ΔG^0_{Cox} indicates a less thermodynamically favorable species than a lower value (LaRowe and Van Cappellen, 2011), (iii) abundance of compounds grouped into elemental groups (CHO, CHOS, CHOP, CHNOS, CHNO, CHNOP, CHOSP, and CHNOSP), and (iv) abundance of compound classes (carbohydrate-, lipid-, protein-, amino sugar-, lignin-, tannin-, condensed hydrocarbon-, and unsaturated hydrocarbon-like) based on molar H:C and O:C ratios of the compounds (Bailey et al., 2017).

Biochemical transformations potentially occurring in each sample were inferred from the FTICR-MS data by comparing mass differences in peaks within each sample to precise mass differences for commonly observed biochemical transformations (Breitling et al., 2006; Stegen et al., 2018b). The ultra-high mass accuracy of FTICR-MS allows precise mass differences to be

counted for the number of times each transformation was observed within each sample. Briefly, the mass difference between m/z peaks extracted from each spectrum were compared to commonly observed mass differences associated with 92 common biochemical transformations provided in previous publications (Graham et al., 2017a; Stegen et al., 2018c). All possible pairwise mass differences were calculated within each extraction type for each sample, as shown in Fig. 2 where a mass difference of 2.01586 indicates a hydrogenation reaction. It is important to note that direct injection electrospray ionization (ESI) FTICR-MS approach cannot distinguish between isomers such as in the case of a mass difference corresponding to a loss of grain of either glucose, fructose and galactose.

2.4 Ecological Modeling

Null modeling was used to estimate influences of ecological processes on microbial community composition from rarefied (10000) 16S rRNA amplicon data, providing estimates of microbial community composition and phylogenetic relatedness. The extraction, purification, and sequencing of soil microbial DNA were performed according to published protocol (Bottos et al., 2018). Briefly, microbial DNA was extracted from 0.25 g of each sample using the MoBio Power Soil DNA Isolation Kit and cleaned-up using Zymo ZR-96 Genomic DNA Clean and Concentrator-5 kit (Zymo Research Corporation, Irvine, CA) as per manufacturer instructions. The V4 region of the 16S rRNA gene was amplified by polymerase chain reaction and amplicons sequenced on Illumina MiSeq using the 500 Miseq Reagent Kit v2 (Illumina Inc., San Diego, CA) according to manufacturer's instructions. Sequence pre-processing, operational taxonomic unit (OTU) table construction and phylogenetic tree building were performed using an in-house pipeline, HUNDO (Brown et al., 2018). Briefly, sequence demultiplexing was done using EA-Utils (Aronesty, 2013), reads quality filtered with BBDuk2 (BBMap, 2014), and merged using

USEARCH (Edgar, 2010). Sequence de-replication and clustering was performed, taxonomy was assigned to operational taxonomic unit (OTU), and chimeras were removed using USEARCH. Raw sequences are archived at NCBI (BioProject PRJNA541992) at the following website:
https://dataview.ncbi.nlm.nih.gov/object/PRJNA541992?reviewer=b55qu29emsinvk3udb2rmuffqh.

Null modeling was performed as described previously (Stegen et al., 2013, 2015) with a total of 35 samples to estimate relative influences of deterministic and stochastic selection processes. Briefly, samples that passed quality control and rarefaction threshold were evaluated for pairwise phylogenetic turnover between communities, calculated as the difference between the observed values of the β-mean-nearest-taxon-distance (βMNTD) and mean of the null βMNTD distribution, in units of standard deviation (see Stegen et al. 2012 for details). The difference between observed βMNTD and the null distribution is known as the β-nearest taxon index (βNTI). Deterministic assembly process are inferred to be dominant when βNTI > 2 or <-2. When βNTI is > 2 it indicates that deterministic processes have driven community composition apart, which is referred to as 'variable selection' (Dini-Andreote et al. 2015; Stegen et al. 2015). When βNTI is < -2 it indicates that deterministic processes have caused community composition to be similar between a given pair of communities, which is referred to as 'homogeneous selection' (Dini-Andreote et al. 2015; Stegen et al. 2015).

Pairwise community comparisons that do not deviate significantly from the null distribution (i.e., 2> βNTI>-2) indicate the dominance of stochastic processes (including homogenizing dispersal and dispersal limitation), or a scenario in which neither deterministic or stochastic processes

dominate (referred to as -undominated). Homogenizing dispersal occurs when rate of dispersal between two communities result in community composition becoming relatively similar between the two communities, and potentially overwhelming other assembly processes (e.g., variable selection). Dispersal limitation is the result of very low rates of organismal exchange between communities, which can result in the stochastic divergence of community composition through the accumulated outcomes of random birth/death events (i.e., ecological drift).

For pairwise comparisons that were not associated with deterministic processes (i.e., when 2> βNTI>-2), we use a second null model to test for influences of homogenizing dispersal or dispersal limitation. This second null model is referred to as RC_{bray}, and like βNTI accounts for variation in OTU relative abundances (Stegen et al., 2013, 2015). Homogenizing dispersal was inferred to be the dominant process for pairwise comparisons characterized by 2>βNTI>-2 and RC_{bray}<-0.95. Dispersal limitation was inferred to be the dominant process for pairwise comparisons characterized by 2>βNTI>-2 and RC_{bray}>0.95. The relative influences of variable selection, homogeneous selection, dispersal limitation, and homogenizing dispersal were quantified by the fraction of pairwise comparisons that were dominated by each ecological process (Stegen et al. 2013). The relative contribution of scenarios in which the system was undominated was estimated as the fraction of pairwise comparisons characterized by 2>βNTI >-2 and 0.95>RC_{bray}>-0.95 (Stegen et al. 2015).

2.5 Statistical Methods

Samples were separately analyzed for WSOC and $CHCl_3$ fractions. Within each solvent fraction, samples were grouped into shallow or deep depths. FTICR-MS dependent metrics including ΔG^0_{Cox}, and relative abundance of compound classes, total transformations, nitrogen-containing

transformations, and organic nitrogen containing compounds were regressed against specific conductivity. Regressions were considered significant if $R^2 \geq 0.50$ and $p \leq 0.05$. The transformation profiles were also regressed with the community assembly processes to determine the relationship between deterministic/stochastic processes and organic compound transformations. Mantel tests were used to evaluate similarity between BNTI matrix and Sorensen matrix of peak presence/absence. The Sorensen distance matrices of WSOC and $CHCl_3$ peaks were regressed against measured variables (soil physicochemical properties) and community assembly process-variables to determine correlations. Finally, a redundancy analysis –based stepwise model building with forward model choice was performed to determine variation in the Hellinger-transformed water-fraction peaks and $CHCl_3$ fraction peaks as explained by explanatory variables (which included measured soil variables, modeled community assembly variables, and categorical variables depth and location). All statistical analyses were performed in the statistical computing language R version 3.5.3 (R Development Core Team, 2019).

3. Results

3.1 Soil characterization. The percent of total soil C (%C) in the shallow soils (26.3 $\pm$ 8.3%) was higher than the deeper soils (4.0 $\pm$ 1.3%) for the lowland soils (i.e. "floodplain" and "inland" sites), while the upland site had an average %C of 7.4 $\pm$ 0.27% at 10 cm and 2.13 $\pm$ 0.06% at 30 cm (Table S2). No significant relation was observed between %C in the shallow inland and floodplain soils along the salinity gradient. The percent of total soil N (%N) of the shallow soils were higher (1.5 $\pm$ 0.40%) than the deeper soils (0.4 $\pm$ 0.08%) for the lowland soils and co-varied with %C (r^2=0.95). The pH of all soils were acidic (5.64 $\pm$ 0.70). The concentrations of NH_4-N and NO_3-N showed a consistent trend where NH_4-N was 1-2 orders of magnitude higher than

431 NO_3-N in all samples. The specific conductivity (used as a measurement of salinity in this study)
432 of the shallow soils ranged from 206-866 (±12) μS cm^{-1} in the lowland soils to 43±5 μS cm^{-1} in
433 the terrestrial end-member site. The deep soils exhibited specific conductivity ranging from to
434 148-524 (±11) μS cm^{-1} in the lowland soils to 29.2 ±8 μS cm^{-1} in the terrestrial end-member site.
435 Texture analysis revealed a broad range of sand (4.1 – 40 %), silt (21.4 – 57.9%), and clay (28.6
436 – 64.8%) fractions.
437
438 **3.2 Thermodynamics, compound classes, and elemental composition.** The calculated ΔG^0_{Cox}
439 WSOC (Table S3)in shallow soils was consistent with our hypothesis of decreasing
440 thermodynamic favorability with increasing conductivity. Average ΔG^0_{Cox} ranged from 53-71 kJ
441 mol C^{-1} (R^2= 0.78, p <0.00001), while no significant relationship between ΔG^0_{Cox} and specific
442 conductivity was observed for WSOC fraction in the deeper soils (averaging 51-54 kJ mol C^{-1})
443 for the floodplain and inland samples (Fig. 3). The upland site had significantly higher average
444 ΔG^0_{Cox} (67-70 kJ mol C^{-1}) than the soils near the lowland. The ΔG^0_{Cox} ($CHCl_3$) at both depths
445 (Table S4) was higher than the water fractions (ranging between 96-105 kJ mol C^{-1}) but did not
446 show significant relationship with respect to specific conductivity.
447
448 Peak profiles for each solvent extraction showed distinct compound classes in the van Krevelen
449 space, with peaks assigned to specific compound classes according to rules outlined in Kim et
450 al., 2003 and modified by Bailey et al., 2017. The WSOC fraction was dominated by compounds
451 classified as protein-, amino sugar-, lignin-, condensed hydrocarbon-, carbohydrate-, and tannin-
452 like compounds (Table 1), while the $CHCl_3$ fraction had relative high abundances (75% and
453 higher) of lipid-like compounds (data not shown). A modest percentage of peaks (11-17%) did
454 not have classes assigned. Unique and common peaks extracted in the WSOC fraction in samples

grouped according to their landscape position and depth [four sites in the floodplain (BC2, BC3, BC12, and BC13), two sites inland (BC4 and BC14), and one upland site (BC15)] are represented as H/C to O/C ratio of the compounds ($p = 0.05$) in Fig. S1.

The shallow WSOC in the floodplain had greater relative abundance of unique lipid (28%)- and protein (25%)-like compounds with relatively high H:C and low O:C ratios as compared to the upland site (BC15), which had an 31%, 30%, and 19% unique peaks representing lignin-, tannin-, and carbohydrate-like compounds respectively. About 23% of peaks were common in both groups, including lignin- and condensed hydrocarbon-like compounds (Fig. S1a). Between the floodplain and the inland samples, high H:C and low O:C ratios representing 47% lipid-, 38% protein-, and 22% amino sugar-like peaks were uniquely present in the floodplain samples (Fig. S1b). The inland shallow soils had 19% unique higher H:C peaks representing condensed hydrocarbon-like compounds compared to 1.2% in the upland soil , though most of the compound classes were observed at both locations (Fig. S1c). Linear regression with specific conductivity profiles showed significant positive correlation with amino sugar-, protein-, lipid-, and unsaturated hydrocarbon-like compounds, while condensed hydrocarbon-like compounds were significantly negatively correlated (Table S5)

For the deep soils, the upland site had 32% unique peaks with relatively high H:C ratios and low O:C ratios commonly associated with unsaturated hydrocarbon-like compounds, as compared to the 0.7% in the floodplain which had higher prevalence of unique peaks representing condensed hydrocarbon (36%)-, and tannin-like (35%) compounds (Table 1, Fig. S1d). The floodplain vs inland samples had thrice as many unique peaks with high H:C and low O:C ratios representing lipid-like compounds in the floodplain samples. Comparisons between inland and upland end-member samples revealed 43% and 37% unique peaks representing low H:C and high O: C ratio

hydrocarbon- and tannin-like compounds respectively in inland samples, while 32%, 14% 9%, and 12% of unique peaks were matched to unsaturated hydrocarbon-, lipid-, protein-, and amino sugar-like compounds respectively in the latter (Table 1, Fig. S1e, f). No significant relationship between compound-class abundances and specific conductivity was observed (Table S5). For the $CHCl_3$ fraction, peaks of lipid-like and unsaturated hydrocarbon-like compounds were observed to be common in all samples (data not shown) and regressions against specific conductivity were not significant for the compound classes.

Compositional differences of the organic compounds showed variable heteroatom abundances, with cumulative heteroatom abundance decreasing with increasing salinity (R^2=0.43, $p = 0.009$) for shallow fraction of the WSOC. For the WSOC fraction, heteroatom abundance of CHOP ($R^2 = 0.61$) and CHNOP ($R^2 = 0.50$) containing compounds was consistent with our hypothesis and significantly ($p < 0.01$) increased, while CHNOS ($R^2 = 0.66$), and CHNOSP ($R^2 = 0.62$) abundances were inconsistent with our hypothesis and significantly decreased with increasing specific conductivity. The elemental composition of the WSOC compounds for deep soils did not show any significant trend with respect to conductivity. For the $CHCl_3$ fraction, relative abundance of CHNOP in the shallow soils significantly decreased with specific conductivity ($R^2 = 0.57$, $p < 0.01$).

3.3 Transformation profiles. In contrast to our expectations, the number of transformations decreased with increasing salinity in the water fraction of shallow soils (R^2= 0.60, $p < 0.01$) (Fig. 4a, Table S3). We also evaluated N-containing transformations and the abundance of N-containing compounds in the system. Total nitrogen-containing transformations also decreased significantly with conductivity but the correlation was not as strong (R^2= 0.40, $p < 0.01$). Total N

503 containing compounds (Fig. 4b, Table S3) as well as their relative abundance decreased
504 significantly (R^2= 0.74, $p < 0.01$), with increasing conductivity in the shallow soils for water
505 fraction.
506
507 **3.4 Ecological processes impacting community composition**
508 Null modeling revealed that microbial community assembly processes were influenced by
509 variable selection (βNTI>2), homogenous selection (βNTI<-2), dispersal limitation (2>βNTI>-2
510 and RC_{bray}>0.95), homogenizing dispersal (2>βNTI>-2 and RC_{bray}<-0.95), and undominated
511 processes (2>βNTI>-2 and 0.95> RC_{bray}>-0.95) (Fig. 5). Dispersal limitation had the greatest
512 influence, responsible for 54% of the variation in community composition. The lowest signal
513 was for homogenizing dispersal (1%), and the signal for homogenous selection (23%) was higher
514 than variable selection (9%). Together, deterministic processes (variable selection plus
515 homogeneous selection) were responsible for 32% of the variation in community composition,
516 with 55% contributed by stochastic processes (dispersal limitation plus homogenizing dispersal).
517 Variation not accounted by dispersal or selection (i.e., influenced by a mixture of processes)
518 accounted for the remaining signal (23%). Consistent with influences from both stochastic and
519 deterministic processes, βNTI relationships with environmental variables were significant ($p <$
520 0.05 by Mantel test), but relatively weak (r=0.46 for pH and r=0.31 for specific conductivity)
521 (Fig. S2).
522
523 To evaluate associations between microbial community assembly processes and chemistry,
524 process estimates were regressed against features of the organic C profiles. Deterministic
525 processes decreased (Fig S3a) while community assembly processes influenced by non-
526 deterministic processes increased with increasing number of transformations of organic

compounds (Fig. S3b), although no strong relationships were observed between assembly processes and transformations ($p = 0.027$, $R^2 = 0.11$ for deterministic/non-deterministic processes, $p = 0.475$, $R^2 = 0.015$ for variable selection, $p = 0.054$, $R^2 = 0.10$ for homogenous selection, $p = 0.514$, $R^2 = 0.013$ for dispersal limitation, and $p = 0.627$, $R^2 = 0.007$ for homogenizing dispersal). No significant relationships were observed between assembly processes and the number of N-containing transformations. Sorensen dissimilarity values based on the detected FTICR peaks for the water fraction were correlated with distance matrices of measured environmental variables and estimates of community assembly processes. Weak positive correlations were observed with NH_4-N ($r = 0.28$), pH ($r = 0.27$), specific conductivity ($r = 0.41$), NO_3-N, silt, and clay ($r = 0.30$) while for the $CHCl_3$ fraction, weak positive correlations were observed with specific conductivity and NO_3-N ($r = 0.26$) (Fig. S4). A Mantel test of FTICR Sorensen dissimilarity vs βNTI values yielded a significant relationship ($r = 0.213$, $p = 0.003$) for water fraction but not for $CHCl_3$ fraction ($r=0.076$, $p = 0.152$). The stepwise model building yielded a combination of five variables that were weakly associated with the composition of water fraction peaks ($p=0.026$, adj. $R^2 = 0.217$), including sand, dispersal limitation, NH_4-N concentration, specific conductivity, and location. The model explaining variation in the composition of $CHCl_3$ fraction peaks was non-significant ($p = 0.1$, adj. $R^2 = 0.05$).

4. DISCUSSION

Sea level rise is increasing the inland extent of tides and exacerbating storm surge, resulting in greater salinity intrusion and altered ecosystem behavior across coastal TAIs (Conrads and Darby, 2017; Ensign and Noe, 2018; Langston et al., 2017; McCarthy et al., 2018; Neubauer et al., 2013b). Site-driven variations in the responses of bulk soil biogeochemical processes (i.e.,

gas flux and DOC release) to elevated salinity suggests potentially important influences of underlying features such as C chemistry and microbial communities. To provide a foundation for understanding the role of C chemistry and microbial communities on biogeochemical cycling in coastal soils, we evaluated associations among a landscape-scale soil salinity gradient, molecular-level soil carbon chemistry, and microbial community assembly processes in order to ultimately inform future improvements for predictive models. In soils associated with a coastal first-order drainage basin, we observed salinity-associated gradients in soil organic carbon fractions that were not associated with microbial community assembly processes. Our results are consistent with C chemistry being driven by a combination of spatially-structured inputs driven by landscape structure (i.e., terrestrial inputs further inland, marine inputs further downstream) and salinity-associated metabolic responses of microbial communities that are independent of microbial community composition. An important caveat is that we did not measure microbial metabolism, but instead infer an influence of microbial metabolism due to microbial composition being independent of C chemistry. To more directly evaluate these inferences, additional work is needed that focuses on quantifying inputs (e.g., via stable isotopes) and measuring microbial metabolism (e.g., via metatranscriptomics). Future work should also use tools like Nuclear Magnetic Resonance and Gas Chromatograph-Mass Spectrometry to evaluate low molecular weight OC (like those contributed by root exudates) vary with salinity.

4.1 Molecular characterization reveals chemical gradients not seen in the bulk C pool

The systematic shifts observed in the molecular signatures compared to non-significant changes in bulk C chemistry shows that molecular-level investigations are particularly relevant to process-based resolution of C biogeochemistry. The absence of bulk C signals mimicking molecular C signals parallel studies indicating rapid change in molecular constituents of the soil

C pool with no change in gross C content (Graham et al., 2018; Reynolds et al., 2018). A faster turnover time of C has been observed in microbial biomass as compared to bulk soil organic matter (Kramer and Gleixner, 2008), which is likely to impact microbe-mediated biochemical C transformations and lead to chemically complex heterogeneous C signatures likely to be missed in bulk analysis (Tfaily et al., 2015). The systematic shifts in chemical characteristics of soil carbon fractions exhibited by samples at the shallow depth suggests that organic C compound pools in shallower soil depths are sensitive to salinity gradients while deeper depth signatures do not vary systematically across the landscape. The landscape gradient observed in the shallow soils is likely influenced by a combination of reduced litterfall due to trees suffering under recent increases in salinity, changing understory vegetation, and algae-rich particulate OM deposition during inundation events that presumably initiated after the recent culvert removal (Wang et. al, 2019). In contrast, the deeper soil depths were more similar to older organo-mineral complexed C in terrestrial soils across various ecosystems and land uses (Conant et al., 2011; Dungait et al., 2012; Jobbágy and Jackson, 2000; Kramer and Gleixner, 2006, 2008). The lack of any systematic gradients in the mineral-associated soil C provides further evidence in support of these interpretations, in addition to previous studies showing mineral-associated soil C to be less responsive to environmental forcings, relative to water soluble C (Reynolds et al., 2018).

4.2 Decreases in organic C thermodynamic favorability may restrict microbial activity

Consistent with our first hypothesis, systematic changes in chemical characteristics of soil carbon fractions were observed with thermodynamically less favorable C present at high salinity in shallow soils. This gradient was expected to emerge from increased microbial activity at higher salinity leaving behind less favorable organic C. However, decreases in the number of inferred biochemical transformations and heteroatom abundances with increasing salinity

suggests that microbial activity decreased with increasing salinity imply (but do not quantify) lower microbial activity at higher salinity. While difficult to infer direction of causality, these patterns suggest that less favorable C at higher salinities may constrain microbial activity, leading to fewer biochemical transformations of the organic C. Thermodynamic limitation of organic C transformation is likely due to anaerobic conditions (LaRowe and Van Cappellen, 2011), which are indicated by high-moisture content of soils, high NH_4-N, and low NO_3-N. Anaerobic conditions restrict oxidation of C compounds based on thermodynamic properties (i.e., NOSC and ΔG^0_{Cox}) (Boye et al., 2017), and our data suggest that this has the potential to lead to lower microbial activity in conditions with less favorable organic C.

4.3 Compound class landscape gradients suggest influences of spatially structured inputs

Similar to patterns in C thermodynamic favorability, C compound classes showed significant heterogeneity in shallow soils but had conserved characteristics in deeper soils. The lipid-like peaks observed in the shallow floodplain samples suggest marine-associated algal-derived lipid organic matter similar to results observed by Ward et al., 2019 in a coastal wetland setting. In contrast, lignin-like signatures in the upland site suggest terrestrially derived OM, as has been observed in other environments where terrestrially-derived organic molecules have a high abundance of vascular-plant derived material such as lignin (Hedges and Oades, 1997; Ward et al., 2013). These characteristics also align with reports of saturated soil environments (*e.g.*, floodplains) exhibiting greater abundance of less-oxygenated organic matter than aerobic environments (*e.g.*, upland soils) as reported by Tfaily et al., 2014 in organic matter transformation of a peat column. Our observed landscape gradients in compound class composition indicate spatially structured inputs of organic C such as particulate OM deposition (Langley et al., 2007). Combining this outcome with gradients observed in the total number of

biochemical transformations and the contribution of heteroatoms suggests that sources of C (marine vs terrestrial) and *in situ* processing combine to influence landscape-scale gradients molecular-level organic C chemistry.

4.4 Ecological assembly processes are weakly associated with organic C

Our results show that microbial community assembly is driven by a combination of dispersal limitation (a stochastic process) and deterministic selection most likely associated with pH, as is often observed in soils (Fierer, 2017; Fierer and Jackson, 2006; Garbeva et al., 2004). In contrast, variation in organic C character was associated primarily with specific conductivity. This suggests that the composition of microbial communities is not mechanistically related to C chemistry. Consistent with this inference, we found a very weak association between βNTI and organic C characteristics. Furthermore, and contrary to our hypothesis, we observed a weak negative association between the influence of deterministic processes and the number of organic C transformations.

Relatively fast changes of organic C chemistry compared to relatively slow changes in microbial composition may underlie the lack of association between assembly processes and C chemistry (Bramucci et al., 2013). Supporting this interpretation, a recent study evaluating microbial community composition and C biogeochemistry of soils in a mesohaline marsh following saltwater intrusion reported immediate changes in C mineralization rates with delayed shifts in microbial community composition (Dang et al., 2019). Similarly, a 17-year dryland soil transplant experiment showed large shifts in microbial activity with no change in community composition (Bond-Lamberty et al., 2016). Furthermore, studies across diverse systems show disconnect in function and composition. For example, C chemistry and not microbial community

structure or gene expression was found to significantly influence freshwater hyporheic zone organic matter processing (Graham et al., 2018); environmental conditions influenced the distribution of functional groups, but not taxonomic composition of marine bacterial and archaeal communities (Lima-Mendez et al., 2015; Louca et al., 2016); and dynamic community shifts did not impact functional stability of a methanogenic reactor (Fernández et al., 1999). Combining our study with these previous investigations provides evidence that is consistent with (but does not prove) that soil microbial community composition can be independent of C chemistry, though this certainly varies across systems (e.g., Stegen et al. 2018).

In our system, lack of an association between microbial composition and organic C chemistry is also likely due to a strong influence of stochastic community assembly. Our null modeling indicated that dispersal limitation was responsible for 54% of variation in community composition. Dispersal limitation influences composition by restricting the movement of organisms through space. Restricted movement enhances the influences of stochastic ecological drift, which arises through birth and death events that are randomly distributed across taxa (Green et al., 2004, 2008; Hubbell, 2001; Martiny et al., 2006; McClain et al., 2012; Stegen et al., 2015). Because ecological drift (enabled by dispersal limitation) can lead to the random loss of taxa within local communities, it can result in different communities containing different, but functionally redundant taxa (Loreau, 2004). Moreover, one can argue as per Louca et al., 2018 that in an open system with regular exposure to external inputs (e.g., via tides), functional redundancy is expected to occur and lead to a decoupling of microbial structure and function (Burke et al., 2011; Liebold and Chase, 2017; Nemergut et al., 2013b).

Conclusions

Our results have revealed landscape scale gradients in soil C chemistry in a coastal forested floodplain, but also show that such gradients are different across soil depths and OC fractions—occurring only in the shallow, water soluble C pool. In addition, we found little evidence of an association between C chemistry and microbial community assembly processes, likely due to a dominant influence of stochastic community assembly (as indicated by a strong influence of dispersal limitation). We propose that the disconnect between C chemistry and microbial communities is enhanced by differences in the time scales for which C chemistry and microbial community composition shift.

Our findings suggest that cross-system heterogeneity observed in coastal soil biogeochemical responses to salinity are likely associated with molecular-level C chemistry and microbial physiological responses that are contingent on historical conditions (Fig. 6) (Goldman et al., 2017; Hawkes and Keitt, 2015; Hawkes et al., 2017; Stegen et al., 2018a). We further suggest that microbial community composition may not strongly influence biogeochemical function in coastal soils. Processes associated with molecular-level C chemistry dynamics are therefore likely to be a critical component of ecosystem responses to changing salinity dynamics in coastal TAIs. A full elucidation of these processes will lay a foundation for the development of mechanistic models of coastal TAI biogeochemical dynamics, providing an opportunity for better representation of these ecosystems in local, regional, and Earth system models.

Code and data availability

Raw sequence data has been uploaded to the National Center for Biotechnology Information's (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA541992. All other data are available at DataHub (10.25584/data.2019-08.931/1558461, 10.25584/data.2019-

08.928/1558463, 10.25584/data.2019-08.929/1558462) upon manuscript acceptance. Original codes for community assembly metric calculation are available at Stegen_etal_ISME 2013 github repository https://github.com/stegen/Stegen_etal_ISME_2013.

Author contribution

AS designed the study, performed the experiments, conducted data analyses and interpretation, and wrote the original draft. JI and CG collected the samples and created site maps. MTF, RKC, and JT provided input on FTICR methodology, conducted the FTICR-MS instrument run, and handled quality filtering and pre-processing of FTICR scans. VLB and NDW contributed to funding acquisition, site selection, study design conceptualization, interpretation of results and editing. JCS contributed to funding acquisition, study design conceptualization, interpretation of results, reviewing and editing. All authors provided feedback on the manuscript.

Competing interests

The authors declare no conflict of interest.

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Table 1. Relative peak abundances (%) of compound classes in the water extracted organic carbon fraction averaged across replicates per site. Samples are ordered according to their depth profile (shallow and deep) and their relative position in the landscape: floodplain (Fp), inland (I), and upland (U). Abbreviations: Con HC (condensed hydrocarbon), UnsatHC (unsaturated hydrocarbon), Other (no classification assigned)

Site/Depth	Landscape position	Protein	Amino Sugar	Lipid	Lignin	Con HC	Tannin	Other	Carb	Unsat HC
BC2_Shallow	Fp	17.2	3.3	9.4	31.0	22.3	13.2	0.5	1.8	1.3
BC3_Shallow	Fp	21.6	3.8	11.5	27.3	23.0	9.8	0.4	1.5	1.2
BC4_Shallow	I	1.6	0.6	0.3	45.3	32.2	18.9	0.04	0.8	0.2
BC12_Shallow	Fp	7.6	1.8	4.0	38.1	31.2	15.3	0.1	1.2	0.7
BC13_Shallow	Fp	13.3	2.6	5.9	33.4	28.6	14.4	0.2	0.9	1.0
BC14_Shallow	I	6.1	1.7	1.6	37.0	35.8	16.	0.2	0.8	0.5
BC15_Shallow	U	3.7	1.5	1.3	51.8	18.5	21.0	0.2	1.5	0.5
BC2_Deep	Fp	2.3	0.5	1.5	41.2	27.2	25.7	0.2	1.1	0.3
BC3_Deep	Fp	3.2	0.3	3.1	34.1	33.4	24.4	0.3	0.9	0.2
BC4_Deep	I	2.8	0.8	0.6	50.4	27.7	16.5	0.2	0.7	0.2
BC12_Deep	Fp	2.29	0.40	1.43	43.3	27.9	22.9	0.2	1.2	0.3
BC13_Deep	Fp	3.47	0.62	2.00	39.8	33.6	19.2	0.2	0.8	0.3
BC14_Deep	I	1.71	0.76	0.57	43.7	32.5	19.34	0.2	1.0	0.2
BC15_Deep	U	9.51	2.55	4.70	63.8	5.1	9.93	0.7	1.0	2.6

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Field Code Changed

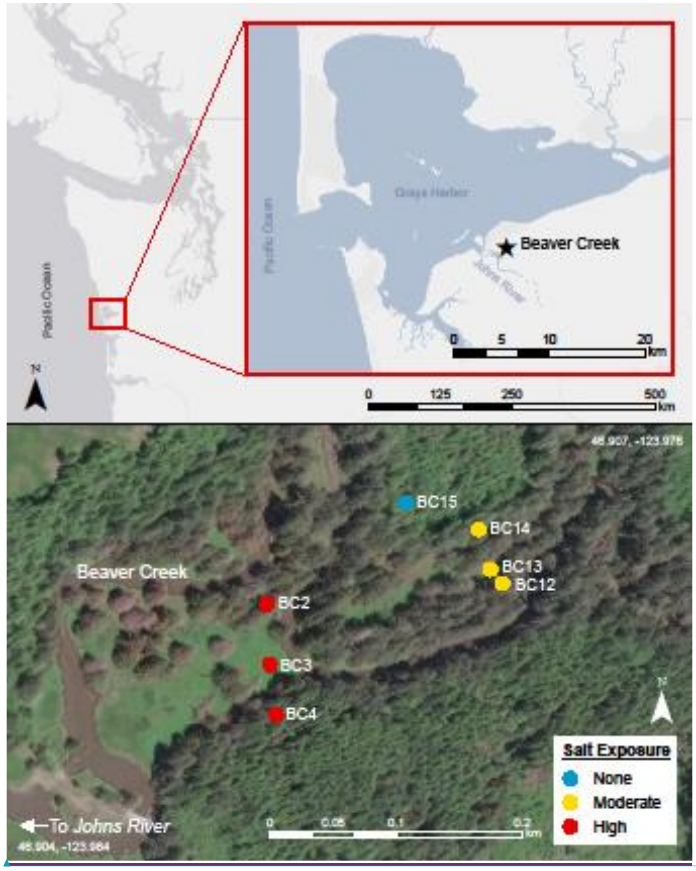

Figure 1. Study site Beaver Creek in the Olympic Peninsula in western Washington. The creek is a first order stream with tidal exchange restored in 2014. Top panel shows site location in western Washington with inset panel zoomed in to show site close to Johns River. Bottom panel shows soil sampling locations at the high salt exposure (BC2, BC3, BC4) transect, moderate salt exposure (BC12, BC13, BC14) transect, and terrestrial upland (BC15) site. The transects with six sampling sites experience periodic inundation episodes which result in surface pooling of tidal water. Map was created using ArcGIS 10.5 software (ESRI, 2017). Coordinate System: GCS WGS 1984.

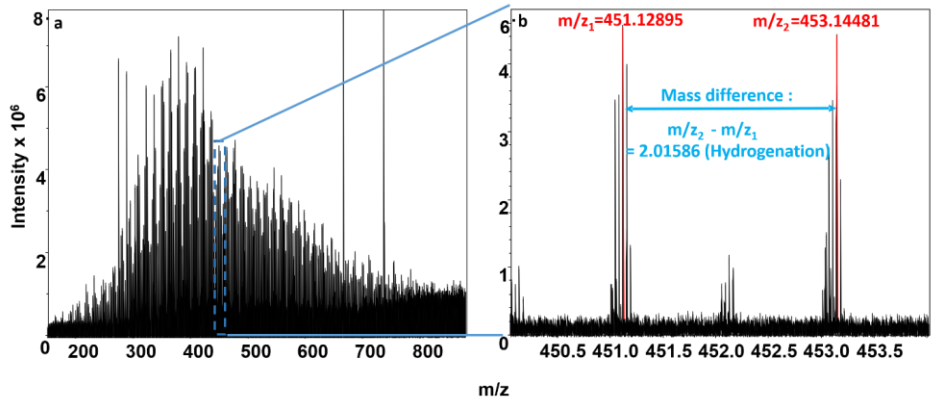

Figure 2. a) Negative mode FTICR-MS (full spectrum); b) zoom in at ~ 450 m/z showing an example of our FTICR-MS spectra overlain with peak mass assignments (red), and a biochemical transformation (mass difference between peaks, denoted in blue). Y axis denotes peak intensities, X-axis denotes mass-to-charge ratio.

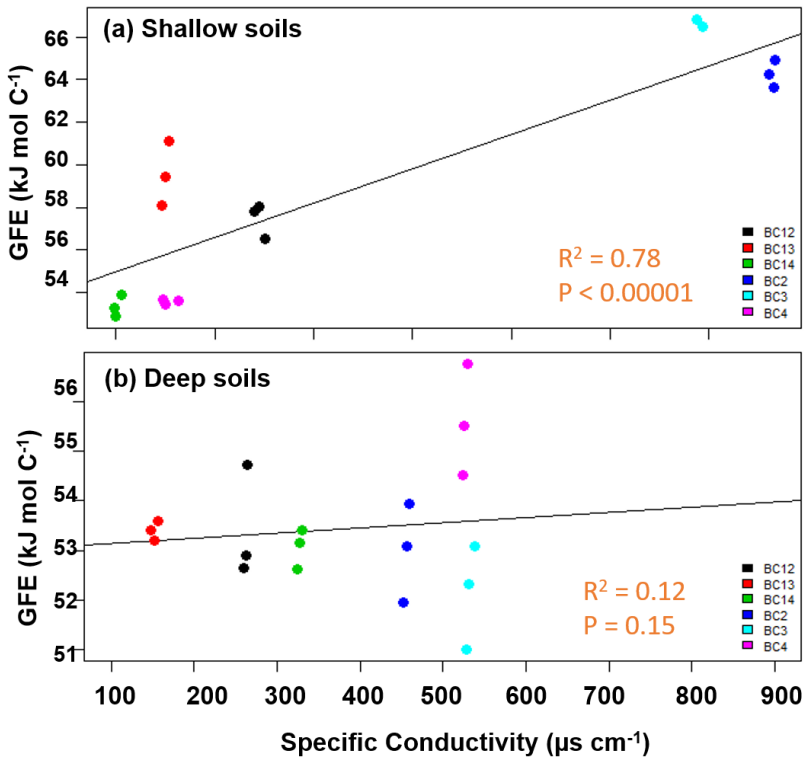

Figure 3. Average Gibbs Free Energy (GFE) of samples in the water fraction of shallow soils impacted by tidal inundation increased with increasing specific conductivity (a) while no change was observed in the deeper soils (b). The salinity of the soil samples did not follow a clear spatial gradient.

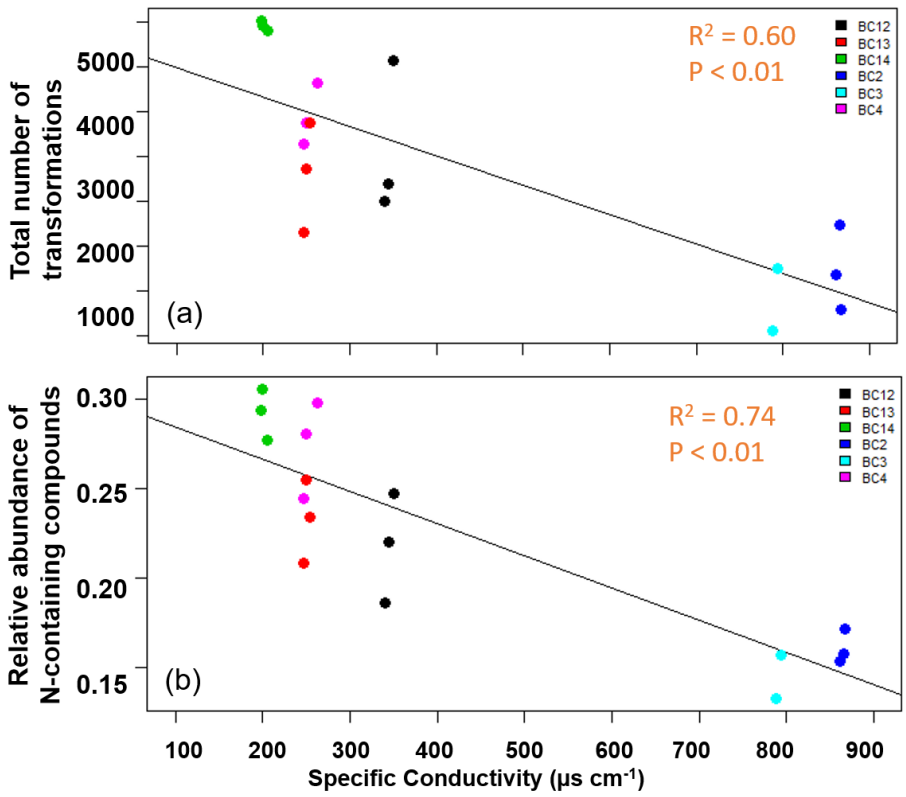

Figure 4. The total number of inferred transformations (a) and total abundance of N-containing compounds (b) in the water fraction of shallow soils impacted by tidal inundation show significant negative correlations with increasing specific conductivity. No significant relationships were observed for water fraction of deeper soils or for the $CHCl_3$ fraction in shallow or deeper soils. The salinity of the soil samples did not follow a clear spatial gradient.

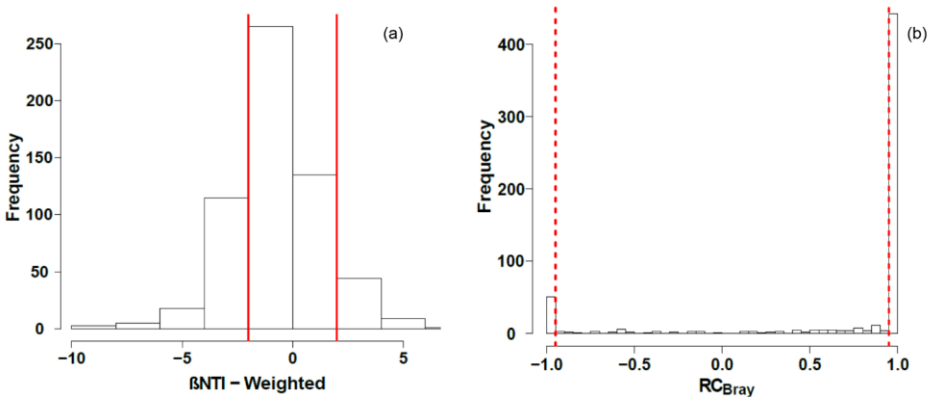

Figure 5. Histograms representing the observed distribution of comparisons based on (a) Beta-near taxon index (βNTI) and (b) Raup Crick metric (RC_{Bray}). Red lines represent the significance thresholds, whereby values outside their bounds are significantly different from the null distribution.

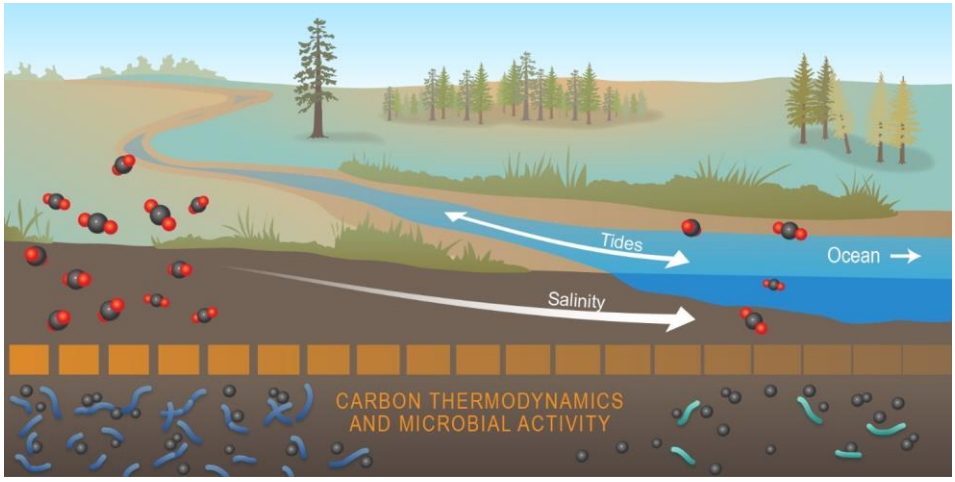

Figure 6. Conceptual model summarizing the key outcome of our study: Microbial activity at high salinity may be depressed by thermodynamically less favorable C. Collectively, our data revealed that organic C thermodynamic favorability, heteroatom content, and number of biochemical transformations all decreased with increasing salinity. This suggest that microbial activity was lower at higher salinity, and we hypothesize this was due to lower thermodynamic favorability of organic C. To evaluate generality, the salinity-associated gradients shown here need to be evaluated across coastal watersheds and mechanistically understood as they have implications for contemporary and future C cycling in coastal watersheds experiencing hydrologic disturbances (e.g., sea level rise and storm surge).