Interactive comment 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.

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.

    We used FTICR-MS (Fourier Transform Ion Cyclotron Mass Spectrometry) and not FTIR (Fourier Transform Infrared Spectroscopy). FTICR-MS is a mass analysis that determines 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 profiling 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 ions.

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 review 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 will provide 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 279-289

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 infer these transformations.*

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 up the stage for our hypotheses in lines 167-173. However, for clarity, we will add sentences in the relevant Introduction paragraphs to link to the specific hypothesis listed lines 167-173.

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 in lines. **Salinity effects on soil processes as they relate to gas flux, dissolved organic carbon, and bulk carbon** *in TAIs are discussed in lines 76-95 and 105-108,* **gaps are discussed** *in lines 98-108, 115-116, 152-155 and how our* **study addresses those gaps** *by testing specific hypotheses in lines 123-150, 166-167. We will carefully review and attempt to clarify more examples in the revised version.*

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. However, we will rephrase certain sections of the abstract to convey a succinct message. To reiterate, Lines 30-32, and 40 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 Line 28 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.

   Added N-,S-,P- containing heteroatom. We have also explained what a heteroatom is in lines 135-136.

10. L34 please state salinity range here or previously

   Will add in revised version.

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.

   These biochemical transformations mean gain or loss of molecules based on gain or loss of mass in the spectra. These transformations can only be inferred (and not measured) from the FTICR-MS data by matching the mass differences in peaks to mass 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 (line 279-289). 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 will add the phrase "based on mass differences" in line 34 for clarity.

12. L35 which metrics of microbial activity were measured?

   We do not claim to measure microbial activity. We state that decreasing thermodynamic favorability, biochemical transformations, and heteroatom content imply less favorable organic carbon accumulation which in turn indicates 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, which suggests that communities are distinct from each other but their assembly is governed by stochastic processes (ecological drift arising through birth and death events that are randomly distributed across taxa). We will edit the revised manuscript to reflect this is simpler terms.

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

   Community assembly process is defined as the process governing composition of ecological communities (Stegen et al., 2012, 2013, 2015). Therefore, here it is the assembly of microbial communities. These are ecologically governed and driven by either deterministic processes (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, drift). We will edit the revised manuscript to reflect a succinct message.

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?

   There was no significant relationship between community assembly metrics (BNTI matrix which indicates phylogenetic relatedness of the samples, variable selection, homogeneous selection, dispersal limitation, and homogenizing dispersal) with C chemistry. The microbial community composition was determined using 16S rRNA amplicon sequencing approach, the microbial community assembly metrics were calculated using the Null modeling approach which used the 16S rRNA amplicon-sequence data derived composition information and phylogenetic tree.
   The community assembly metrics were compared to C chemistry. We will rephrase this sentence to better reflect the message.

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, and peak information (as written in methods section; lines 321-334).

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

   The community assembly process variables were disconnected from C chemistry. The community composition (βNTI) relationship with environmental variables were significant (p < 0.05 by Mantel test), but relatively weak (r=0.46 for pH and r=0.31 for specific conductivity), which was not true for Gibb's free energy which was strongly related to specific conductivity trends. We did not find any significant associations between community assembly process variables and C chemistry including Gibbs Free energy, transformation profiles and mass spectra peaks. These explanations are part of results section (Lines 435-460).

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 will edit the line in the revised manuscript.

   Microbial-driven carbon metabolism rates are decoupled from dissolved organic carbon concentrations.

   We are referring to both CO_2 and CH_4 flux trends and will restructure the sentence to reflect the same in the revised version.

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

    Yes.

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 when exposed to increasing salinity show increase in CO_2 flux while soils in saline environments exposed to increasing salinity show reduced CO_2 flux. This signifies that salinity exposure history is critical. We then go on to explain the implications of the salinity exposure history on 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 will rephrase Line 100 to read "Relatively consistent gas flux responses **with salinity exposure history….**"*

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

    We do not agree. Microbial activity drives carbon cycling.

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 will edit this paragraph and move the methods discussion to end of paragraph 4.

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 trended to be increasing with increasing salinity in a freshwater system, we hypothesized that as a freshwater system (and changing to saltwater since 2014 after culvert removal), Beaver Creek would also see increasing activity and therefore greater heteroatom content with

increasing salinity. We will add a sentence in line 125 to indicate the salinity exposure history of our site and therefore our expectations of ecosystem behavior.

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 will edit 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 agree and will revise the line to clarify this as tidal flooding. Our site is unique in this sense that it used to be saline until a culver was built to divert water, blocking off tidal inundation. The site therefore turned fresh until 2014 when the culvert was removed and tidal activity resumed. We agree that we should mention that the system is freshwater in recent history and will rephrase line 100 to indicate this and therefore provide context to line 143. Further details are provided in Methods lines 190-194.

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?

   We will edit the text in the revised version to add clarity. Further, we direct the reviewer to (Graham et al., 2017a, 2017b, Stegen et al., 2012, 2013, 2015) references to gain detailed information about community assembly processes. As per our response to Reviewer 2 early in this response document: Community assembly process is defined as the process governing composition of ecological communities (Stegen et al., 2012, 2015). Therefore, here it is the assembly of microbial communities. These are ecologically governed and driven by either deterministic processes (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, drift).

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

   Edit will be made in the revised manuscript to indicate surface and sub-surface.

Methods

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

    Edit will be made in the revised manuscript.

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 km². The tidal floodplain makes up 0.5 km² of this total watershed area. Information will be added in the revised manuscript.

31. L189 define psu

    Edit will be made in the revised manuscript.

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

    Agrostis stolinifera (creeping bentgrass), Tsuga heterophylla (Western hemlock), Picea sitchensis (Sitka spruce). Edits will be made in the revised manuscript.

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

    Each transect was on roughly 80-90 m. Samples were collected ~35 m apart (information provided in line 216).

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.

    Edit will be made in the revised manuscript. 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:

"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)."

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

    We will provide the elevation in the revised manuscript.

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 will add the relevant information in our revised manuscript. We did not have any litter at the depths we collected the soils from. Sieving ensured removal of roots.

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

    Will edit in the revised manuscript.

40. L243 followed by of….check wording

    Will edit in the revised manuscript.

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. Without further specifics from the reviewer, we believe details in Bottos et al., 2018 are adequate.*

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.

43. L392 missing comma after 14%

Will correct in the revised version.

44. Why have the authors chosen to not include any taxonomical data on the microbial communities? Itseems 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 will include a bar chart as a supplemental figure, and also provide the OTU table with taxonomic assignments as a supplemental file so that others can more deeply evaluate taxonomic structure. For the response purpose, we have also included a phylum-level bar plot as an example of our supplemental addition to our revised manuscript.

Discussion

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

   Agreed. We have also indicated the same in lines 76-89.

46. L471 characteristics?

   We will edit in the revised version.

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 will edit this sentence in the revised manuscript to indicate landscape-variation and therefore differences in type (terrestrial versus aquatic) as well as terrestrial vegetation input differences.

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

   We did not measure any metabolic response but inferred thermodynamic favorability of organic compound metabolites. We will revise the manuscript to indicate that additional work is needed that focuses on quantifying inputs and measuring microbial metabolism.

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 line 193. We will provide 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 will edit the methods to indicate that we treated CHCl3-extracted organic carbon as proxy for mineral bound C 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 326-332 (see Stegen et al. 2012, 2013, 2015). We chose to focus on the 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. Null modeling is required because examining taxonomic composition directly does not provide information on the ecological processes governing community composition.

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

Yes, rapid changes in reactions. Will edit in revised manuscript to reflect the change.

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. We are speculative within limits to conclude that community composition in our system does not influence biogeochemical function. We are citing other papers that show poor association between composition and biogeochemical function. That's why the sentence is structured as 'combining our study with these previous…'

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 OC. We would also like to guide the reviewer to lines 544 to 555 that show that community composition may not change while function changes. We will also include a supplemental OTU table and bar plot in the revised manuscript to show the taxonomic groups.

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 will add brief description in the Introduction in the revised manuscript with detailed citations to guide readers to resources that explain community assembly processes in detail.

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. We will add this to the revised manuscript version.

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. We will edit this as requested in the revised version.

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.

We will make the change in the revised manuscript.

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.