

Review #1

Fettrow et al. assessed C concentrations in relation to different important biogeochemical parameters at three hydrodynamically distinct sites in a coastal marsh system over the course of the course of four vegetation periods. They find that total C and DOC concentrations as well as DOC across the sites vary with biogeochemical regime across the hydrological gradient and with vegetation period. They argue that the variability in C concentrations across the sites and with depth should be taken into account when blue carbon assessments are considered.

Main comment:

The authors provide a really nice biogeochemical dataset that builds on prior in-depth characterization of the same sites ((Seyfferth et al. 2020; Guimond et al. 2020). Particularly the comparison of tidal influence versus vegetation dynamics at the three different sites yields some interesting results. I think those results are novel and useful, and should be the core of the manuscript. While I agree that C stock assessments in these kinds of systems are tricky, the study was not set up to rigorously evaluate spatial variability across these kinds of systems.

Three distinct sites seem to be deliberately chosen based on their distinct hydrological regimes (see prior work cited above) and three replicate cores at each site are just not enough to truly assess spatial heterogeneity across the sites. My suggestion is to focus the revised and streamlined version of the manuscript on how tidal versus vegetation dynamics might affect total C/DOC concentration and DOC composition. There are some interesting results there that should make for a much lighter and interesting paper. In other words, focus on the spatiotemporal variability of C/DOC from a biogeochemical perspective. The fact that it makes for difficult C stock assessment is an interesting discussion point or implication, but perhaps shouldn't be the focus of this research article.

We thank the reviewer for their time in reviewing the manuscript and for the helpful comments. As the reviewer mentioned, we chose subsites based on previous research that had identified these three locations as hydrologically unique; therefore, this work extends our previous research to further investigate biogeochemical differences between the hydrologically unique subsites. We agree that we should “focus on the spatiotemporal variability of C/DOC from a biogeochemical perspective”, which is what we aimed to do. In the revised version, we can revise the text accordingly to make it clear that this was our intention. We also agree that we were not able to fully spatially resolve all the spatial heterogeneity that exists in the marsh ecosystem. Doing so would require many more soil cores than we were allowed by the permit in this protected Natural Preserve area. Thus, we were only able to choose the three distinct hydrologic and biogeochemical zones to illustrate the spatial variability with replicate cores. In the revised version, we will include additional text to illustrate the limitations of the study.

Other general comments:

- I noted below that ANOVA results are missing from Fig. 1-4. But then they were presented in Table 1-2. I suggest really shortening the results sections around Figs. 1-4 and incorporating the

ANOVA results there. It is otherwise redundant, and the information provided doesn't always seem directly relevant for the questions asked and hypotheses posed.

We thank the reviewer for their feedback and we can work to shorten the results section to remove any redundancy. It should be noted that Fig 1 is a map of soil core locations; therefore, there is no ANOVA results for the map. Also it should be noted that the information in Figures 2, 3, and 4 are not the same thing as those reported in the ANOVA tables in Tables 1 and 2.

Figure 2 reflects depth profiles of individual replicate soil C measurements at each depth at each phenophase and each subsite so that readers can visually understand the specific variability of soil C at each depth, phenophase, subsite and replicate core, all in one figure. In contrast, Table 1 shows the ANOVA results of total C in the entire depth profiles and phenophase but separated by subsite/ vegetation zonation as well as 9 other biogeochemical variables that are not represented in Figure 2.

The goal of Figure 3 is to show the reader ranges in soil C and S concentration by depth but separated by subsite, while Table 2 shows the ANOVA results of total C and S as well as 8 other biogeochemical variables by subsite where depth and phenophase are averaged. Similarly, Figure 4 shows variability of DOC as a heatmap across all replicate cores and phenophases, depths and subsites. As the author points out, this paper is about the variability of soil C and DOC so we want to show how variable it is at different scales by showing all replicate points presented in a visual, easy to identify way, just as we have done with soil C.

- The authors discuss C storage rates, but what you are measuring is C concentrations and what you are estimating seems to be stocks.

Yes, we use our C concentration values to calculate stocks, because we know the bulk density of these subsite locations from previous research, which was cited in the paper (Wilson and Smith 2015) and stated in the paper in lines 512-516. In the revised version, we will be sure to state them as such. We also use previously estimated soil accretion rates to calculate soil C storage rates (Line 512-513).

The term carbon storage invokes that whatever carbon is there is persistent and stored. I would only use it where appropriate. Generally, the whole text would benefit from more clearly delineating when C accrual, storage, concentrations or stocks are discussed. Or when the authors talk about pools (stocks, concentrations,...) and rates.

We agree that terminology around the subject of soil C storage/ stocks can be confusing and often lacks a clear definition in each context. We agree that this terminology should be simplified in the text. In a revised version, we will only using the term "soil C accrual rate" when discussing ($\text{g C m}^{-2} \text{ yr}^{-1}$) and only using the term "soil C stocks" when discussing (kg C m^{-2}). Generally, this was our intention, but we agree there are a few areas where terminology can be better clarified.

ABSTRACT

Put more emphasis on results and less on hypotheses and approach.

We agree and will be sure to do this in the revised paper.

L41: maybe “plant phenology” instead of “ecological function”?

Yes, we agree that “ecological function” should be replaced with plant phenology on Line 41.

Maybe end on recommendation for sampling if one is interested in estimating/assessing C stocks?

We agree we should end the abstract on a recommendation, and we feel that is already at the end of the abstract. “It is, therefore, critical to consider spatial and temporal heterogeneity in soil C concentration when conducting blue C assessments to account for soil carbon variability and uncertainty in C stock estimates”. In a revised version we could also add further detail such as “we recommend that multiple locations and timepoints are sampled when conducting blue C studies to account for ecosystem-scale variability”.

INTRO

L86: exudates are by definition soluble, so perhaps omit “DOC”

We understand how this line might be confusing, but we want to introduce both “root exudates” and “DOC” into the story, and root exudates are just one source of DOC. We suggest rewording these sentences to “Belowground production of dissolved organic carbon (DOC) can come from root exudation (Luo et al. 2018)”.

Perhaps try to more clearly delineate the edaphic versus the plant controls on soil C stocks.

We thank the reviewer for this comment and agree we should add/ edit the intro to distinguish between plant vs. soil related effects more clearly on soil C stocks. In a revised version of the manuscript we will make those additions.

METHODS

157f: I assume the cores are extremely wet and take a long time to dry, especially given the high organic matter content. Wouldn't there be anaerobic metabolism in the glove bag, it's warm and wet in there, particularly in the presence of H₂?

We dried the soils in the glove bag using large trays of desiccant to rapidly remove the moisture from the drying soils. The glove bag itself would not be “wet” because the desiccant is constantly removing moisture from the air and we monitor the % humidity that is often <20%. We regenerate the desiccant daily to quickly remove water from the soils while also slowing down fast oxidative reactions.

So couldn't stem some of the seasonal variability in C content stem from differences in microbial activity at the time of sampling that then dictates how much C metabolism occurs in the glove bag? It seems like freeze-drying might be a better alternative.

That is possible, but all of the samples were treated in the same way. So, if H₂-fueled metabolism happened in the glovebag, it would have affected all cores equally and not be the main driver of the differences across sites or seasons. Also, the large size of the cores prevented us from being able to freeze-dry them with the equipment available. In the revised version, we can discuss this point as a possible limitation of the study.

180f: Is this a water extraction or really an extraction of the residual pore water in the cores? If it is the former, perhaps call it water extractable C. If it is the latter, isn't the extracted DOC concentration highly dependent on the moisture content at the time of sampling? And that moisture content will be a function of where in the tidal cycles it was sampled? Is it possible that the variability has more to do with that than site or season specific characteristics.

We extracted porewater by centrifuging the core, without any additives such as deionized water, so based on the reviewer's definition it would be "residual pore water" but we just refer to it as pore water.

The amount of porewater we obtain is a function of saturation and to be consistent across sampling timepoints, we core the locations at the same tidal inundation cycle each season. We will be sure to better articulate this in the text.

RESULTS

I don't quite understand why Fig. 2 and Fig 3 are necessary. I think the variability is nicely illustrated by Fig. 3. I would also add symbols indicating significant (where appropriate) in the latter.

The purpose of Figure 2 was to show how C concentrations in individual cores varied in space and time. Figure 3 is a summary of that variability in C and S with depth at all timepoints together. We could put Figure 2 in the supporting information, but we would worry that we are then not including the variability in C between cores that were intended to be replicates. Fig 2 represents the large amount of Soil C variability on different spatial (depth, replicate, subsite) and temporal (seasons) scales.

We save ANOVA significant results for a table when we assess overall summarized differences in all variables between subsite and phenology, but we agree that Fig 3 would benefit from having a significant difference letters report. We will be sure to add in sig differences to Fig 3 in a revised version of the manuscript.

212-214: if such a statement is made, it should be supported with adequate statistics 225-229: same as above

Note we did not say "significantly higher" in this sentence, we just note that its higher, but we can include modifier and say "appeared to be higher", which is later backed up statistically by the ANOVA results in Tables 1 and 2.

229-232: The regression approach is a very forgiving way to assess significant changes with depth. I think it would be more appropriate to run a ANOVA.

We thank the reviewer for their comments on our statistical approach. We could try a 2-way ANOVA with depth and subsite as factors for each of the phenophases. We could also attempt a 3-way ANOVA with depth, phenophase, and subsites as factors, and provide the results in a revised version of the manuscript

But, frankly, I don't really see how they are significantly different given the large variation among the three reps.

The purpose of the Figure 2 heatmaps is to show the variation in soil C with subsite, phenophase, and depth, and contrast those with Table 1's ANOVA results that are grouped by subsite and averaged across phenophase and depth. When grouped by subsite and averaged across phenophase and depth, there are significant differences across subsites (Table 1) but when grouped by phenophase and averaged across depth subsite, there are fewer significant differences (Table 2). The ANOVAs presented in Tables 1 and 2 clearly illustrate significant differences with the connecting letter report.

239-243: Again, it's ok to point out trends, but if it is claimed they are different, there should be statistical tests to support that claim.

In the revised version, we will be sure to clarify that these are trends. We can also run the ANOVA on these Figure 3 and include those as well.

Fig. 4: I would suggest plotting DOC concentrations analogous to Fig. 3, i.e., as a box plot and run the appropriate statistics. This data is really neat and I would like to see it highlighted like that.

We agree and will include that in the revised version.

276-277: why isn't this discussed? Wouldn't it make sense to highlight differences across the sites as well?

We saved the discussion for the discussion section and discussed differences across the subsites on lines 391-445.

Fig. 6: I don't love this figure. Could you make the lines a bit thinner so it's easier to see the individual traces?

We made the markers different shapes and colors so its easier to follow the line and keep track of sample site location with depth. We can try to make the lines thinner and plot markers slightly smaller, but we worry that when shrunked down to PDF size, they could be unreadable. The lines that overlap have similar values, therefore making the lines thinner would not necessarily resolve the overlapping issue.

Everything is also very compressed. For example, Eh varies quite a bit with season, but it's hard to see because the scale is so compressed.

This figure is a compromise between trying to show all of the data for each of the variables at all times and space versus showing the overall trends by season. What we hoped the reader would get out of this figure is that something like Eh varies substantially by season even more than by subsite. The Eh scale ranges from -400 to 600 so the scale is quite broad. This is so that we could fit all the variability across seasons and subsites onto one scale, rather than making a different range in scale for each figure. This way, the reader can more easily compare the broad differences in redox across sites, seasons and depth.

Table 1-3 header: Soil C % is not really a porewater biogeochemical variable. The table includes the solid phase.

We thank the reviewer for this comment. We agree that Soil C and S is not a porewater variable, and in a revised version we can edit the table header from “porewater biogeochemical variables” to “soil and porewater chemical variables”.

Also, wouldn't a two way ANOVA be more appropriate to assess the influence of both vegetation and season?

We can try a two-way ANOVA to look at the combined influence and interactions of both subsite and season. We will include those results in a revised version.

378f: it would help to better explain the step-wise linear regression approach. Which factors were included and which were eliminated in the process?

We thank the reviewer for this comment and agree the step wise regression approach could use more detail. We used all variables listed in Table 1 and 2 and the stepwise regression model was run to maximize the R^2 while using the least amount of variables to explain the variance. That is, the model was run to determine the most important (significant) biogeochemical variables we measured for predicting soil C concentration. In a revised version of this manuscript, we will add more detail about the variables used in the regression model, and how these final variables were chosen to represent the final model.