Dear Helga,

Please accept the revised manuscript and response to reviewer's comments for publication in Biogeosciences. We found the reviews to be very fair and also point out some areas for improvement, so we greatly appreciate that. We have tried to address every concern in detail and hope we have done a satisfactory job. Please let us know if other issues/concern arise during the re-review.

I apologize for the challenge in reading the earlier discussion post, it is my first time doing an interactive discussion in the review process. The Response to Reviewer's contain the same answers and I will make sure to have that uploaded in a readable fashion. We have addressed, in the methods, the mixing of microbial communities but did not see any reviewer comments directly on the statistical analysis. We did address in more detail the plot design and why we sampled from only a subset of the plots.

We thank you and the reviewer's again for taking the time to review this manuscript.

Kevan

Anonymous Referee #1

Received and published: 21 June 2019

General Comments The authors recognize the threat of saltwater intrusion caused by sea-level rise on non-tidal coastal forests and, using laboratory incubations, test whether additions of salt and coarse woody debris (CWD) change biogeochemical and microbial outputs. They find, among other factors, that salt water reduces total and soil organic carbon and microbial biomass, increases general seawater ions (SO4, Na, Cl, NH4, NO3, PO4, Ca, Mg, K), and over time, and stabilizes pH and Eh more quickly in the presence of CWD. Some enzymatic activity shifts, especially with coarse woody addition, d13C effects are largely unchanged with CWD but significant effects in absence of CWD. Cumulative CO2 and CH4 emissions are reduced with salt, but CWD with FW addition only stimulates CH4 production.

As noted, there is not a large literature on seawater intrusion into these non-tidal systems (I suspect because tidal systems will experience salt intrusion first, thus are the more timely systems of concern), but the postulated scenarios are reasonable, thus providing relative insights into responses of these systems. I appreciate the synthetic discussion and request a few details in my comments to help the reader advance from point to point in the same way the authors have.

1. Does the paper address relevant scientific questions within the scope of BG? yes
2. Does the paper present novel concepts, ideas, tools, or data? I'm not sure about novelty
3. Are substantial conclusions reached?
4. Are the scientific methods and assumptions valid and clearly outlined? I'd like to see hypotheses clearly stated
5. Are the results sufficient to support the interpretations and conclusions? Yes, with some specific clarifications requested

6. Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? yes
7. Do the authors give proper credit to related work and clearly indicate their own new/original contribution? adequate
8. Does the title clearly reflect the contents of the paper? I think so, but a comment included below seems to contradict the title and Figure 2
9. Does the abstract provide a concise and complete summary? yes
10. Is the overall presentation well structured and clear? yes
11. Is the language fluent and precise? Yes, with some subject-verb agreement errors and a few run-on sentences (L83) [There are many cases where subject-verb agreement is not in alignment. e.g.

L280 activity. . .were should be activity. . .was;

This has been corrected

L299 “enzyme . . . were” should be enzyme . . . was]

This has been corrected

L317 should be “a” one-way ANOVA, no?

An “a” has been added

12. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? yes

13. Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? Might include some of the data driving equations in supplemental sections

These are very common equations and are not necessary to include.

14. Are the number and quality of references appropriate? perhaps
15. Is the amount and quality of supplementary material appropriate? No supplemental received

Introduction:

I would have preferred to see clear hypotheses outlined in the last paragraph of the Introduction. The next to last paragraph reads more like Methods to me

We have added objectives to this section and changed the wording to sound less like methods.

Methods:

I cannot speak in depth to the methods used for isotopic analyses or microbial enzymatic processes. The authors do not disclose the methods used by the NCSU laboratory for the samples they sent to that unit for analysis; I would prefer they do (presumably ion chromatography, and NDIR?).

We have added this information.

Have the authors any general physicochemical descriptions of the field soils from where the incubation matrix was collected to help contextualize the work? It seems that other terminal electron acceptors (specifically nitrate) would be useful covariates across the plots that might affect whether a system reaches sulfate reduction, perhaps.

We measured soil ions in a study from 2013, using ion exchange probes (PRS probes) (Minick et al. 2019). These probes collected anions and cations over one six week period from July to August 2013 in the same plots used for this study. NO3- concentrations were very low and likely contributed little contributed little to the potential pool of electron acceptors. Alternatively S and Fe availability were much higher than NO3- in the hummocks, as measured using the same PRS probes. Given that the soils were completely saturated (e.g. flooded) with either fresh or salt water, and numerous ions were measured (regrettably not Fe though), we feel this represents an acceptable

Minick, K. J., Kelley, A. M., Miao, G., Li, X., Noormets, A., Mitra, B., and King, J. S.: Microtopography alters hydrology, phenol oxidase activity and nutrient availability in organic soils of a coastal freshwater forested wetland, Wetlands 39, 263-273, https://doi.org/10.1007/s13157-018-1107-5, 2019a.

It isn't essential that this be provided, but I suggest an interesting consideration if the data are available... The temperature and precipitation data provided are useful (L152), but I'd also like to see the range of these values since over such a long timespan.

We have kept this section as is.

Might the authors comment on the saltwater treatment levels they selected? These are rather high for a non-tidal system, and the high treatment would be oligohaline in a tidal system. Have levels this high been seen in some nearby areas?

Yes, the saltwater in the sound to the east (only a mile or so) ranges from approximately 1-5 percent saltwater, another couple miles into the sound and towards the ocean the water is up to 10-20 percent. So these values are reasonable. The 2.5 percent is a more likely, or relatively short term scenario, while the 5 percent represents a more extreme or long term scenario.

L210: Please allay any concerns of positive pressure effects in the chambers during the ~2week intervals between sampling toward the end of the incubation.

The lids were left loose between sampling periods. This is stated in the following lines (L211-212).

Results:

L339-341: The authors fall into a common trap suggesting that even though a mean is of a different magnitude, that the results vary. They do not. The statistics do not support that wood-amended soils were depleted – the statistics suggest equivalency if all of them are denoted with an "a". (and discussion)

The end of the sentence has been removed.

Figure 2. I'd like to see something in the discussion related to the pattern of CO2:CH4 reported in the Results. The trend in wood free is parabolic but linear upward in wood-amended. Is that useful? Does this suggest that there an optimal ratio of CWD and salinity that might be targeted to minimize GHG emissions as sea-level rises?

Our experiment was not intended to determine different levels of CWD inputs with all incubations receiving the proportionally same amount of wood additions and so we cannot test the combination of varying effects of wood and salinity. With that said, we think the reviewers observation is a good one and worth noting. We have added discussion on the CO2:CH4 trend in the discussion to this specifically

L396+: I believe this interpretation follows the same trap noted in L339-341. It is accurate to say MBC was lowest in the dry treatment across un-amended treatments and lowest in the 5ppt amended treatments.

We have made these changes

Discussion:

L424-425: what C cycling processes are the authors suggesting balance out the reductions in CO2 & CH4?

We have clarified this sentence

L426 & Figure 2L: I must be missing something, so I suspect other readers will as well. Panels B & E show that the wood-amended plots drop CO2 and CH4 with salt water addition (+2.5 & +5.0 ppt), but the text says it enhances CH4 under saltwater additions. Can you provide clarity? If this is actually referring to the difference (panels C & mostly F), then it seems that the CH4 emissions with CWD are essentially on balance (at the 0 line), no? I've interpreted that saltwater is different than freshwater amendment (A vs B), but the saltwater additions seem to cross the 0 line with the variance.

We have added clarification in the text to address this potential confusion. Panels C and F show the difference between wood free and wood amended soils which gives the wood-

associated CO2 and CH4 production. So within the wood free or wood amended treatments, salt water generally reduced gas production. But when comparing wood free and wood amended gas fluxes for each specific gas, we actually see that wood additions reduced CH4 from freshwater but enhanced it in salt water incubations. This is just another way of looking at the results in order to derive some interpretation of how wood versus non wood treatments influence gas production when incubated with fresh or salt water.

L432: the sentence is almost verbatim earlier in the manuscript (L154). Please revise so each occurrence is unique and not redundant Minor quibble: the hydroperiod operates constantly. I suggest these systems RESPOND over short time scales, but to state they operate on short time scales seems a bit misleading. Even no water is reflected in the hydroperiod in some way, isn't it? Technical corrections (in addition to a few pointed out previously)

We have made changes to the sentence in the discussion and changed operates to responds. We agree with the reviewers assessment

L126: The sentence beginning on L126 ("Although many studies. . .") is unnecessary. That statement was clearly outlined previously in the introduction and does not narrow their research into what they will test and what they expect to find (via the recommended hypotheses addition).

We have made some changes to this paragraph but have kept this sentence because we think it helps guide the reader in this summary introduction paragraph.

L142: why note 13 plots if you only used 4?

We have mentioned the thirteen plots because it is part of the description of the site. We feel it is important to note that this site is part of the Ameriflux network, which follows certain experimental design protocols. Of the thirteen plots, four of these are more intensively monitored for plant and soil processes. We have added information to this sentence to highlight why we chose four plots, to hopefully clarify why we chose to mention this.

L199: what year were the trees harvested?

2010, we added that it was harvested then.

L202 & L204: are the 6 rings mentioned in 204 the mean of the 5-7 rings in 202?

We have revised this section. It was six tree rings. We reduced mentioning it to only once.

L248: add (MBC) after spelling out microbial biomass C

This correction has been made

L286: enzyme XYL is not defined in the 5 above

This information has been added

L385: please be more precise than “the last couple”

We just removed that part of the sentence, due to it being somewhat subjective and not adding much to the overall results or interpretation

L421: recommend authors use the defined abbreviation “SLR” instead of sea-level rise (else, why define it earlier?)

This has been changed

L466: over time (add space)

This has been changed

Table 1: please provide units of the ions

This information has been added

Figure 2: Please confirm that the labels for panels B & E follow those of C & F (and not A & D). Would you consider a different title for panels C & F? It took me a while to understand that you were reporting the DIFFERENCE between the two, and it wasn’t some sort of range (the hyphen notation threw me off). Perhaps “Difference between wood-amended and wood-free”?

We have added a sentence to the figure caption to show this.

Referee #2

Friederike Gründger (Referee) friederike.gruendger@bios.au.dk

Received and published: 2 July 2019

My comments refer to the version of the manuscript that was uploaded by Kevan Minick at 10 May 2019.

The authors present a study that shows the influences of saltwater on CO2 production and CH4 formation processes in non-tidal freshwater-forested wetlands. Soil samples were collected from seven sites located in the Alligator River National Wildlife Refuge (ARNWR) in Dare County, North Carolina, a non-tidal pocosin wetland area that will be most likely effected by sea level rise and saltwater intrusion in the future. The study is based on laboratory incubation experiments testing the effects of freshwater, saltwater and added wood on soil microbial processes in freshwater forested wetland soils. Basic geochemistry, CO2 and CH4 concentrations in incubations, isotopic signatures, microbial biomass carbon measurements, and extracellular

enzyme analysis were carried out. The authors confirm that saltwater intrusion can result in reductions in CO2 and CH4 fluxes. Further, they found that coarse woody debris input to soils might reduce CH4 emissions under freshwater conditions, but enhance CO2 production and CH4 emissions under saltwater conditions. The authors also discuss shifts between hydrogenotrophic and acetoclastic methanogenesis dependent on certain incubation conditions. Please note, that I cannot comment on the validity and applicability of the methods used for the analysis of microbial biomass carbon and extracellular enzymes, because I am not an expert in that field.

General comments:

1. I wonder why and how soil samples were stored for such a long time (7 weeks) before initiating the incubation experiments. What were the conditions of storage – light, moisture level, oxygen availability? I'd assume that surface soil from hummocks is oxygenated, isn't it? Were the samples kept oxygenated during storage and, if yes, how? What was the temperature at the time of sampling? Only the mean annual temp. is given here. What were the incubation conditions –e.g. temperature, oxygen, volume of incubation? The incubation setup should definitely be more detailed.

> Samples were stored as other parts of the experiment were being initiated. Samples were stored at 4C, in a fridge, in the dark. The samples were stored based on their initial soil moisture levels, which were approximately 90% moisture. The hummocks are somewhat oxygenated but that depends on the water table depth. The hummocks are frequently inundated throughout the year when precip is high.
>
> We have added more detail on the incubation setup in the section 2.3.

2. How far/close were the sampling sites from each other? Would it be useful to add a map that shows soil, freshwater and saltwater sampling sites or pictures of the sampling site and the sampling procedure? I can't imagine the procedure of removing seven 10x10 cm-2 monoliths from hummocks to the depth of the root mat.

> We have added a new figure 1, a map with soil and water sampling locations and surrounding water bodies. The soils were sampled within a quarter mile of freshwater. The saltwater was sampled approximately 20 miles east of soil and water samples.
>
> Soils were removed using a saw and cutting in a 10 x 10 cm-2, using a pvc square as guidance. This is in the methods.

2. Fresh- and saltwater were mixed together to get the desired salt concentration for the saltwater treatments. That means, if the water samples weren't sterile-filtered, microbial communities from two different habitats were introduced to the soil microbiota in the incubations. The same applies to the addition of non-sterilized wood. In the manuscript, microbial interactions due to mixing of samples aren't discussed.

> Samples were filtered through Whatman #2 filters (8 µm) to remove particulates. This information has been added. This would not sterilize the water from microbial

populations by any mean. This mixing of microbial populations from the different water and soil sources were mixed together, although we would argue this represents what would occur during salt water inundation into these freshwater systems, either in short term pulses (such as storm surges) or longer term inundation periods with rising sea levels.

As I understand it, the incubations were held under oxic conditions (L213 "flushed at 20 psi for three minutes with CO2/CH4 free zero air). Would it be informative to explain how an aerobic incubation turns into an anoxic environment that promotes methanogenic processes? Also, the sequence of microbial processes that happen along the incubation time and the involvement of certain microbial groups in CO2/CH4 production could be emphasized more detailed.

We understand the reviewers concern but argue that our incubations were indeed anaerobic for the following reasons:

1) Although the incubations had oxic headspace (CO2 and CH4 free air, but containing O2), the soils were incubated at 100 % WHC which resulted in soils being completely flooded (either fresh- or salt-water) with water essentially covering the surface of the incubated soils, thereby allowing for the development of anaerobic conditions similar to that observed in the field and for subsequent production of CH_4 through the anaerobic process of methanogenesis. We have added that information at the beginning of section 2.3 of the methods. Further, O2 presence in the headspace would diffuse very slowly into the water (rates of O2 diffusion into water is approximately 5,800 to 9,500 times lower than that in water (Massman 1998)) and therefore would likely be of negligible effect on total Ch4 production.

2) We actually took measurements of redox potential throughout the experiment (see Figure 1C and 1D). This showed that incubations were indeed anaerobic, starting initially at +300 mV and dropping quickly to between approximately 100 and -400 throughout most of the incubation, with the wood additions dropping Eh much lower than non-wood treatments.

3) The rates of CH4 production are quite high, which in and of itself indicate that the incubations were anaerobic. We ran four blank incubations (jars with no soil) that were treated exactly the same (most importantly flushing with same air) and sampled on the same schedule as soil incubations. We have added a couple sentences about the blanks in the methods section. Further, when compared to anaerobic incubations (with N2 headspace) of soils from northern latitude wetlands, we see that our measurements are much much greater (see Treat et al. 2014; Walz et al. 2017 for instance).

Treat C, Wollheim WM, Varner R, Grandy AS, Talbot J, Frolking S (2014) Temperature and peat type control CO_2 and CH_4 production in Alaskan permafrost peats. Global Change Biology, **20,** 2674-2686.

Walz, J., C. Knoblauch, L. Böhme and E.-M. Pfeiffer (2017). "Regulation of soil organic matter decomposition in permafrost-affected Siberian tundra soils - Impact of oxygen

availability, freezing and thawing, temperature, and labile organic matter." Soil Biology and Biochemistry 110: 34-43.

4. Does the storage of the soil samples under 4◦C for 7 weeks cause a shift in microbial community composition and activity already, assuming that in situ temperature at the time of sampling were higher (quick online check for Feb 6 2018, Raleigh, North Carolina, shows 14◦C at noon)?

It is unlikely that storage temperature and time resulted in a significant shift in microbial communities and/or activity that would affect the results and inference from this experiment, given all samples were treated the same. Storing freshly collected soils at 4ºC (refrigerator temperature) is very common in soil microbial studies, and in fact many publications do not even state how long soils were stored before some kind of laboratory procedure! The reasoning is that at such a cold temperature forces the microbes and microbial processes to slow significantly, so that there is minimal decomposition/activity during storage and until incubation. There is also a fair amount of pre-incubation/processing that occurs before incubation of soils in these types of studies, making storage of soils in the most inert way possible necessary in order to complete those tasks before actually starting the incubation. Ideally, it would have occurred around 2-4 weeks post collection but in this case it was not possible.

5. Why were these five extracellular enzymes picked to be analyzed? A short description of what these enzymes are catalyzing and in what processes (with regards to your incubations) they are involved would help to understand the concept of the data acquisition (like in L299). Please, add measuring techniques for NAGase, AP, and AS!

We have added more information on what substrates/compounds these enzymes degrade. We have added NAGase, AP, and AS to the hydrolytic enzyme assay information.

6. Can you add a few thoughts about what it means to the environment and climate when CO2/CH4 production increases/decreases due to sea level rise in such areas? e.g. "Findings from this study indicate that substantial changes in the greenhouse gas flux" - how does it change - increase/decrease? What happens to the environment when dead trees provide a significant source of C to already C-rich peat soils? What do we have to expect after such a change? And why is it important to know what type of methanogenesis is dominant after saltwater intrusion? I am missing the wider picture of the impact of these processes e.g. (L439-442) what are the “important implications for above- and below-ground C cycling dynamics” in particular.

We have added a few sentences to the conclusion to expand somewhat on implications of this study, but hesitate to speculate too much about how well our lab experiment would represent ecosystem responses on a large scale (a common critique of studies like this in general). We have provided some detail on what to expect (e.g. C inputs to soils, ecosystem transition, etc), as well as suggestions for future directions. What this study does provide is insight into the ecosystem/soil response and provides mechanistic details on why we might find this response. For instance, this is why understanding the pathway of methane formation can be informative. The two different pathways appear to be

linked with very different magnitudes of fluxes, with hydrogenotrophic pathway having lower methane production than the acetoclastic pathway.

7. I find it a bit difficult to follow the discussion. You start nicely with an overview of your outcomes and the message is clear here. Then you discuss 'CO2 production' results, followed by 'CH4 production' results (L445-456) and the 'competition of the two methanogenic pathways' (L457-476). I suggest, at that point, continuing with the isotope section 'where different methanogenic pathways are discussed (from L505 on) and then bridge to the 'addition of wood part' (from L480 on). Further, it would help a lot to add a conceptual illustration as final figure showing the possible environmental changes at non-tidal freshwater-forested wetlands after a sea level rise scenario based on your results.

We have switched the two paragraphs as suggested.

Detailed comments:

L143 Why are only 4 plots used for that study? Isn't it redundant to mention that 13 plots were sampled, if only 4 were used for the study?

We have mentioned the thirteen plots because it is part of the description of the site. We feel it is important to note that this site is part of the Ameriflux network, which follows certain experimental design protocols. Of the thirteen plots, four of these are more intensively monitored for plant and soil processes. We have added information to this sentence to highlight why we chose four plots, to hopefully clarify why we chose to mention this.

L184 instead of: 4) soils incubated at 100% WHC with 5.0 ppt (5.0 ppt). correct to: 4) soils incubated at 100% WHC with saltwater (5.0 ppt).

"saltwater" was accidentally left out of the description and should have come after "with 5.0 ppt". We have added this information, which also keeps it consistent with treatment "3)" description.

L199 "dried at to a constant moisture level" – what does that mean? All cookies finally had similar moisture levels or were they dried until moisture per cookie didn't change any longer?

The latter, this means that the cookies were dried until no more change in moisture was measured.

L200 Are "control (non-fertilized) trees" different from the harvested trees that are mentioned before? Is it important to mention that they are non-fertilized? If this information isn't crucial, remove that sentence.

It is not important to mention. These are the same trees that were harvested. Some were from a fertilization treatment and some from a non fertilized treatment. We only used trees from the non fert trt. We have removed that sentence.

L221 How much soil exactly was removed from the incubation?

> Approximately 1.0 g dry soil weight was removed at each enzyme sampling date. We have added this information to this sentence.

L233 With "initial soil samples" you mean the soil that was stored at 4◦C for 4 week before the incubation experiment started or homogenized soil samples directly after sampling? Better define the term at some point in 2.3 incubation setup.

> The initial samples were removed from the homogenized bag prior to the start of the incubation. We have added this information

L240 "Soil pH was measured on fresh soil samples" – what is meant by fresh soil sample? Soil directly after sampling or after 7 weeks of storage? Instead of using the phrase "fresh", better find a term that clearly describes the condition of the sample (same for L250).

> This was measured the in soils after storage, the same day the incubations were started. We have added this information here.

L250 Avoid the term "fresh soil" when it was a soil subsample from an incubation. Fresh soil is anyway not a precise definition of a condition of a soil.

> Fresh has been removed from this sentence

L279 change into: enzymes were quantified on soil samples on days 0, 1, 8, 35, and 98 of the soil incubation.

> We have removed the "(day 0)" from the sentence to better reflect what was done. The measurements at "day 0" were done on soil samples before incubation. Therefore we have removed the "day 0" reference to avoid confusion that these were subjected to the different salt and fresh water treatments.

L285 Can't find enzyme XYL in the description of measured enzymes above.

> This information has been added

L383 "while the proportion of wood-derived CO2 remained steady for a good portion of the incubation but increased in the final couple measurements periods" – add something that indicates that you are referring to dry incubations. "for a good portion" and "final couple" isn't precise enough. Add proper terms for time scales.

> We have modified this sentence

L433 When parameters like the redox potential in an incubation were measured, they arent called "in situ" measurement. In situ would be, when the measurements were done at the ARNWR

sampling site. If the values shown are indeed in situ measurements, why aren't they mentioned in the result part? At least, I can't find them there.

> This is data collected from the field site. It is unpublished data and is not replicated but more observational from testing Eh during frequent field visits as a way to get an idea of what redox potentials we can maybe expect. More detailed studies of in situ redox potential are important and something we are very interested in, but cant provide that at this current time. We can state it is unpublished in parenthesis or leave as is. We feel it is important to mention though.

L458 "Numerous others studies have found that saltwater reduces CH4 fluxes compared to freswater, both within the field and laboratory." – add references. Correct typo in freshwater.

> This correction has been made

Saltwater reduces potential CO_2 and CH_4 production in ~~organic~~ peat soils from a coastal freshwater forested wetland

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Keywords: e*xtracellular enzyme activity, ~~peat~~sea level rise, methanogenesis, microbial biomass carbon, ^{13}~~C~~carbon isotopes*

Abstract A major concern for coastal freshwater wetland function and health are the effects of ~~is~~ saltwater intrusion ~~and the potential impacts~~ on greenhouse gas production from peat soils. Coastal freshwater wetlands are likely to experience increased hydroperiod with rising sea level, as well as saltwater intrusion. These potential changes to wetland hydrology may also alter forest structure and lead to a transition from forest to shrub/marsh wetland ecosystems. Loss of forested wetlands is already evident by dying trees and dead standing trees ("ghost" forests) along the Atlantic Coast of the US, which will result in significant alterations to plant carbon (C) inputs, particularly that of coarse woody debris, to soils. We investigated the effects of salinity and wood C inputs on soils collected from a coastal freshwater forested wetland in North Carolina, USA, and incubated in the laboratory with either freshwater or saltwater (2.5 or 5.0 ppt) and with or without the additions of wood. Saltwater additions at 2.5 ppt and 5.0 ppt reduced CO_2 production by 41 and 37 %, respectively, compared to freshwater. Methane production was reduced by 98 % (wood-free incubations) and by 75-87 % (wood-amended incubations) in saltwater treatments compared to the freshwater treatment. Additions of wood resulted in lower CH_4 production from the freshwater treatment and higher CH_4 production from saltwater treatments compared to wood-free incubations. The $\delta^{13}CH_4$-C isotopic signature indicated that in wood-free incubations, CH_4 produced from the freshwater treatment was from the acetoclastic pathway, while CH_4 produced from the saltwater treatments was more likely from the hydrogenotrophic pathway. These results suggest that saltwater intrusion into subtropical coastal freshwater forested wetlands will reduce CH_4 fluxes, but long-term changes in C dynamics will likely depend on how changes in wetland vegetation and microbial function influences C inputs to the soil.

1 Introduction

Sea-level rise (SLR) threatens coastal regions around the world. Significantly, the rate of SLR is not uniform around the globe, with the highest rate occurring along the Atlantic coast of North America between Cape Hatteras and Cape Cod, due to factors including local currents, tides and glacial isostatic rebound (Karegar et al., 2017; Sallenger et al., 2012). Along with economic and cultural impacts, health of coastal forested ecosystems are expected to be impacted by sea-level rise (Langston et al., 2017; Kirwan and Gedan 2019). For instance, salinization of coastal freshwater wetlands will likely impact vegetation community dynamics and regeneration in low lying (< 1m) wetlands (Langston et al., 2017). Understanding how coastal wetland ecosystems respond to extreme events, long-term climate change and a rapidly rising sea is essential to developing the tools needed for sustainable management of natural resources, and the building of resilient communities and strong economies. Because it has more than 5,180 km^2 of coastal ecosystems and urban areas below 1 m elevation, the state of North Carolina is highly vulnerable to climate change and SLR and therefore saltwater intrusion (Riggs and Ames, 2008, Titus and Richman, 2001).

As sea level changes, coastal plant communities move accordingly up and down the continental shelf. In recent geologic time, sea level has risen about 3 m over the past ~2,500 years from sea level reconstructions adjacent to our study site (Kemp et al., 2011). The rate of SLR has varied greatly over that time, with periods of stability and change, and a geologically unprecedented acceleration in recent decades. The current distribution of coastal forested wetlands reflects the hydrologic equilibrium of the recent past climate, but the widespread mortality of such forests suggests that the rate of SLR is in a time of rapid change at a rate

potentially faster than the forest's capacity to move upslope, resulting in widespread death of coastal freshwater forests (Kirwan and Gedan 2019). Furthermore, dying coastal forests will alter the quantity and quality of organic matter inputs to the soil as vegetation shifts occur, as well as introduce a large pulse of woody debris into soils. This has the potential to alter C cycling processes responsible for storage of C in the soil or loss of C as CO_2 and CH_4 (Winfrey and Zeikus, 1977).

Wetlands store more than 25% of global terrestrial soil C in deep soil organic matter deposits due to their unique hydrology and biogeochemistry (Batjes, 1996; Bridgham et al., 2006). Carbon storage capacity is especially high in forested wetlands characterized by abundant woody biomass, forest floors of *Spaghnum* spp., and deep organic soils. Across the US Southeast, soil organic C (SOC) in soils increases with proximity to the coast and is greatest in coastal wetlands (Johnson and Kern, 2003). Carbon densities are even higher in the formations of organic soils (Histosols) that occur across the region, typically ranging from 687 to 940 t ha^{-1}, but can be as high as 1,447 t ha^{-1} (Johnson and Kern, 2003). As noted, forested wetlands, which historically have contributed to terrestrial C sequestration, are in serious decline and processes leading to destabilization of accumulated soil C are not represented in broad-scale ecosystem and land-surface models. The extent of changes in soil C cycling processes attributable to altered hydroperiod, saltwater intrusion and structural changes in vegetation in these ecosystems remains unclear.

Saltwater intrusion, a direct result of SLR, into freshwater wetlands alters soil C cycling processes (Ardón et al., 2016; Ardón et al., 2018), particularly that of methanogenesis (Baldwin et al., 2006; Chambers et al., 2011; Dang et al., 2018; Marton et al., 2012), and microbial activity (e.g., extracellular enzyme activity, Morrissey et al., 2014; Neubauer et al., 2013). Saltwater

contains high concentrations of ions, particularly SO_4^{2-}, which support high rates of sulfate reduction compared to freshwater wetlands (Weston et al., 2011). Sulfate acts as a terminal electron acceptor in anaerobic respiration of soil organic C, and sulfate reducers will typically increase in abundance in response to saltwater intrusion and out-compete other anaerobic microorganisms particularly methanogens for C (Bridgham et al. 2013; Dang et al., 2019; Winfrey and Zeikus, 1977). The effect of SO_4^{2-} on soil C cycling and competitive interactions with other anaerobic microorganisms processes also appears dependent on the concentration of the ion (Chambers et al., 2011). Even within freshwater forested wetlands, hydrology and microtopography can interact to influence the amount of SO_4^{2-} within soils experiencing different levels of saturation and therefore rates of SO_4^{2-} reduction (Minick et al., 2019a). A majority of saltwater intrusion studies on soil C dynamics though have focused on tidal freshwater wetlands, whereas non-tidal freshwater wetlands have received relatively little attention, partially due to there being less dispersed geographically across the landscape. Nonetheless, they occupy critical zones within the coastal wetland ecosystem distribution and will be influenced by SLR differently than that of tidal wetlands. Tidal wetlands are likely to experience short-term pulses of saltwater with tidal movement of water, while ~~sea level rise~~SLR effects on saltwater intrusion into non-tidal freshwater wetlands may result in more long-term saltwater inundation. This difference in saltwater inundation period may influence rates of soil CO_2, CH_4 production, and microbial activity (Neubauer et al., 2013) and therefore should be considered in light of the hydrologic properties of ~~specific~~ non-tidal wetlands.

Saltwater intrusion into freshwater systems may also influence the CH_4 ~~producing~~ production pathways (Dang et al., 2019; Weston et al., 2011), as a result of saltwater-induced shifts in methanogenic microbial communities (Baldwin et al., 2006; Chambers et al., 2011;

Dang et al., 2019). Stable isotope analysis of CO_2 and CH_4 indicate that acetoclastic methanogenesis is the major CH_4 producing pathway in these freshwater wetlands (Angle et al., 2016~~, Minick et al., 2019b~~), but the influence of saltwater on the pathway of CH_4 formation in non-tidal freshwater forested wetlands has rarely been studied, particularly through the lens of CO_2 and CH_4 stable C isotope analysis. As ^{13}C isotopic analysis of CH_4 is non-destructive and is long-proven as a reliable indicator of the CH_4 production pathway (Whiticar et al., 1986), utilization of this analysis provides easily attainable information on the effects of freshwater compared to saltwater on CH_4 production dynamics in coastal wetland ecosystems experiencing SLR-induced changes in hydrology and vegetation.

~~We used a laboratory experiment~~Our goal in this study was to ~~investigate~~test whether ~~the effects of~~ saltwater ~~and wood~~ additions ~~on~~ alter the production of CO_2~~, production,~~ CH_4~~, production~~, and microbial activity from organic soils of ~~in~~ a non-tidal temperate freshwater forested wetland in coastal North Carolina, US, and whether effects differ in response to additions of wood. Although many studies have focused on salinity pulses in tidal freshwater wetlands, less attention has been given to the effects of sustained saltwater intrusion on soil C dynamics and we expect saltwater intrusion due to SLR will be more persistent in these non-tidal wetlands. Therefore, we ~~tested~~ investigated the effects of sustained saltwater ~~intrusion~~ inundation ~~over the course of a 98 day laboratory incubation~~using a laboratory microcosm experiment on ~~soil C cycling~~greenhouse gas production and microbial activity (e.g., microbial biomass C and extracellular enzyme activity). Wood additions to microcosms were utilized to mimic the potential large amount of wood inputs that will occur as forests dieback occurs along the aquatic-terrestrial fringes of the Atlantic Coast and these wetlands transition to shrub/marsh ecosystems (Kirwan and Gedan 2019), thereby providing a large and widespread pulse of coarse

woody debris to wetland soils and potentially altering soil C cycling. ~~Furthermore, we added wood to a subset of incubations in order to tease out effects of hydrology and wood inputs on C cycling.~~

2 Methods

2.1 Field Site Description

The field site was located in the Alligator River National Wildlife Refuge (ARNWR) in Dare County, North Carolina (35°47'N, 75°54'W) (Figure 1). The ARNWR was established in 1984 and is characterized by a diverse assemblage of non-tidal pocosin wetland types (Allen et al., 2011). ARNWR has a network of roads and canals, but in general contains vast expanses of minimally disturbed forested- and shrub-wetlands. Thirteen plots were established in a 4 km^2 area in the middle of a bottomland hardwood forest surrounding a 35-meter eddy covariance flux tower (US-NC4 in the AmeriFlux database; Minick et al., 2019a). Of the 13 plots (7 m radius), four central plots were utilized for this study which have been more intensively measured for plant and soil processes (Miao et al. 2013, Miao et al., 2017, Minick et al 2019a, 2019b, Mitra et al. 2019). Over-story plant species composition was predominantly composed of black gum (*Nyssa sylvatica*), swamp tupelo (*Nyssa biflora*), bald cypress (*Taxodium distichum*), with occasional red maple (*Acer rubrum*), sweet gum (*Liquidambar styraciflua*), white cedar (*Chamaecyparis thyoides*), and loblolly pine (*Pinus taeda*). The understory was predominantly fetterbush (*Lyonia lucida*), bitter gallberry (*Ilex albra*), red bay (*Persea borbonia*), and sweet bay (*Magnolia virginiana*). The mean annual temperature and precipitation from climate records of

an adjacent meteorological station (Manteo AP, NC, 35°55′N, 75°42′W, National Climatic Data Center) for the period 1981-2010 were 16.9 °C and 1270 mm, respectively. These wetlands are characterized by a hydroperiod that ~~operates~~ responds over short time scales and is driven primarily by variable precipitation patterns. Soils are classified as a Pungo series (very poorly managed dystic thermic typic Haplosaprist) with a deep, highly decomposed muck layer overlain by a shallow, less decomposed peat layer and underlain by highly reduced mineral sediments of Pleistocene origin (Riggs, 1996). Ground elevation is below < 1 m above sea level. Sea-level rise models of coastal NC show that ARNWR will experience almost complete inundation by 2100, with attendant shifts in ecosystem composition (DOD, 2010).

2.2 Sample Collection

Soil samples were collected on February 6, 2018, from surface organic soils by removing seven 10x10 cm^{-2} monoliths from hummocks to the depth of the root mat (approximately 6.3 cm) using a saw and a 10x10 cm^{-2} PVC square. The seven soil samples were composited by plot and stored on ice for transport back to the laboratory. In the laboratory, roots and large organic matter were removed by hand and gently homogenized. Soils samples were stored at in the dark at 4°C for seven weeks before initiating the laboratory incubation.

Freshwater and saltwater for the experiment was collected from water bodies surrounding the ARNWR on March 7, 2018 (Figure 1). Freshwater was collected from Milltail Creek, which runs Northwest from the center of ARNWR to Alligator River and is drainage for our forested wetland study site. Freshwater salt concentration was 0 ppt. Saltwater was collected from Roanoke Sound to the east of ARNWR and had a salt concentration of 19 ppt. Fresh- and salt-

water were mixed together to get the desired salt concentration for the saltwater treatments (2.5 and 5.0 ppt). Prior to mixing fresh- and salt-water was filtered through a Whatman #2 (8 µm). Neither salt- nor fresh-water were sterile filtered, therefore microbial communities from each water source were mixed together and added to the incubations. This could influence the response of soil microbes to the various treatments, but also represents what would occur under future projections of sea level rise in this region and the resulting mixing of fresh- and salt-water sources within the wetland. Four water samples of each fresh- and salt-water mixture were sent to the NCSU Environmental and Agricultural Testing Service laboratory for analysis of total organic C (TOC), ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^-), sulfate (SO_4^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), and chlorine (Cl^-). Analysis of TOC was made using a TOC analyzer (Schimadzu Scientific Instruments, Durham, NC). Analysis of NH_4^+, NO_3^-, and PO_4^-, was made using Latchat Quikchem 8500 flow injection analysis system (Lachat Insturments, Milwaukee, WI). For SO_4^{2-} and Cl^-, a Dionex ion chromatograph was used to measure concentration (Thermo Fisher Scientific, Waltham, MA). Finally, a Perkin Elmer 8000 inductively-coupled plasma-optical emission spectrometer (Perkin Elmer, Waltham, MA) was used to analyze water samples for Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^-.

2.3 Incubation Setup

Incubation water treatments included: 1) soils incubated at 65 % water holding capacity (WHC) (Dry); 2) soils incubated at 100% WHC with freshwater (0 ppt); 3) soils incubated at 100% WHC with 2.5 ppt saltwater (2.5 ppt); and 4) soils incubated at 100% WHC with 5.0 ppt saltwater (5.0 ppt). It is important to note that the 100% WHC moisture level resulted in soils

being completely flooded (either fresh- or salt-water) with water covering the surface of the incubated soils, thereby allowing for the development of methane producing conditions similar to that observed in the field. Soils were incubated in the dark in the laboratory for 98 d at 20 – 23 ºC in 1 L canning jars. After soil and water additions, the remaining headspace was estimated for each individual incubation vessel (approximately 750 mL) and used in the calculation of gas flux rates. A subsample of each soil was dried at 105°C to constant mass to determine gravimetric soil water content. Water holding capacity (WHC) was calculated by placing a subsample of fresh soil ~~(~~(approximately2 g fresh weight) in a funnel with a Whatman #1 filter and saturating with deionized H_2O (dH_2O). The saturated sample was allowed to drain into a conical flask for 2 h. After 2 h, the saturated soil was weighed, dried at 105°C to constant mass, and then weighed again to determine WHC.

Two sets of incubations were set up with the above mentioned water treatments. We added ^{13}C-depleted American sweetgum (*Liquidamber styraciflua*) wood to half the incubation vessels (0.22 g wood per g soil) (wood-amended), while the other half were incubated without wood (wood-free). Trees were grown at the Duke FACE site under elevated CO_2 concentrations (200 ppm CO_2 above ambient) using natural gas derived CO_2 with a depleted ^{13}C signature compared to that of the atmosphere (Feng et al., 2010; Schlesinger et al., 2006). The site was established in 1983 after clear cut and burn (Kim et al., ~~2015~~2016). Trees were grown under elevated CO_2 from 1994 to 2010 at which point they were harvested (Kim et al., ~~2015~~2016). Cookies were removed from harvested trees, dried ~~at~~ to a constant moisture level and stored at -20 °C until use. ~~For the current incubation study, wood from control (non-fertilized) trees grown in the elevated CO_2 were used.~~ The bark layer was removed and the outer ~~five to seven~~six tree rings of multiple cookies ~~was~~ were removed with a chisel. Wood was then finely ground in a

Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) and analyzed for C content and ^{13}C signature. Wood ~~removed from the outer six tree rings~~ had a C content of 45.6 ± 0.21 % and $\delta^{13}C$ value of -40.7 ± 0.06 ‰, which was within the range of -42 to -39 ‰ measured on fresh pine needles and fine roots (Schlesinger et al., 2006).

2.4 CO_2 and CH_4 Sample Collection and Analysis

Headspace gas samples were collected from incubation vessels 15 times over the course of the 98 d incubation (days 1, 4, 8, 11, 15, 19, 25, 29, 29, 47, 56, 63, 70, 84, 98). Incubation lids were loosened between measurements to allow for gas exchange with the ambient atmosphere. Four blank (no soil) incubations were set up and treated in the exact same manner as incubations containing soils. Blanks were used to measure soil-free CO_2 and CH_4 concentrations in incubations, which were always well below the detection limit of the gas analyzer (described below).~~-~~ Prior to each measurement, incubation vessels were removed from incubators, sealed tightly, and flushed at 20 psi for three minutes with CO_2/CH_4 free zero air (Airgas, Radnor, PA, USA). Following flushing, incubation vessels were immediately placed in the dark (2-6 h over the first 39 days and 12-18 h over the remainder of the incubation) before taking a gas sample for analysis. Approximately 300 mL of headspace gas was removed using a 50 mL gas-tight syringe and transferred to an evacuated 0.5 L Tedlar gas sampling bag (Restek, Bellefonte, PA, USA). Simultaneous analysis of CO_2 and CH_4 concentrations and $\delta^{13}C$ isotopic signature were conducted on a Picarro G2201-i Isotopic CO_2/CH_4 Analyzer (Picarro Inc., Sunnyvale, CA USA). Flux rates of CO_2-C and CH_4-C were calculated as well as daily cumulative CO_2-C and CH_4-C production summed over the course of the 98 d incubation. Small subsamples (approximately

1.0 g dry weight) of soil were removed periodically from each incubation vessel for extracellular enzyme analysis (see below). Removal of soil was accounted for in calculations of gas production rates. Incubation vessel water levels (mass basis) were checked and adjusted three times per week using either freshwater or saltwater.

The proportion and rate of wood-derived CO_2 at each sampling date was calculated using $^{13}CO_2$ data and using the ^{13}C of depleted wood (-40.07) in a two pool flux model, with the depleted wood signature as the one end-point and the $^{13}CO_2$ of wood-free incubations as the other endpoint. Total wood-derived CO_2 was calculated using cumulative CO_2 produced over the 98 d incubation and the average $^{13}CO_2$ across the whole incubation.

2.5 Soil Characteristics

Soil organic C concentration and $\delta^{13}C$ was analyzed on ~~initial~~ the four replicate soil samples prior to the start of the incubation (initial soil samples) and on soils from each of the thirty incubations following the 98 d incubation period. The initial C analysis was performed on samples removed prior to incubation. ~~Initial SOC properties were measured on the four plot replicates prior to incubation.~~ Soils were finely ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) prior to analysis on a Picarro G2201-i Isotopic CO_2/CH_4 Analyzer outfitted with a Costech combustion module for solid sample analysis (Picarro Inc., Sunnyvale, CA USA).

Soil pH and redox potential (Eh = mV) were measured in each incubation within one hour following sampling of headspace gas. Soil pH was measured on ~~fresh~~ the four replicate soil samples immediately prior to the start of the incubation with a glass electrode in a 1:2 mixture

277 (by mass) of soil and distilled water (dH_2O). Soil redox potential (Eh = mV) was measured
278 using a Martini ORP 57 ORP/ºC/ºF meter (Milwaukee Instruments, Inc., Rocky Mount, NC,
279 USA) .
280
281 **2.6 Microbial Biomass Carbon and $\delta^{13}C$ Isotopic Signature**
282
283 Microbial biomass C (MBC) was estimated on soils collected from incubations on day 1
284 (after 24 hour post-treatment incubation) and day 98 (following the end of the incubation). The
285 chloroform fumigation extraction (CFE) method was adapted from Vance et al. (1987) in order
286 to estimate MBC and $\delta^{13}C$. Briefly, one subsample of ~~fresh~~ soil (approximately ~~0.5~~1.0 g dry
287 weight each) was placed in a 50 mL beaker in a vacuum desiccator to be fumigated. Another
288 subsample was placed into an extraction bottle for immediate extraction in 0.5 M K_2SO_4 by
289 shaking for 1 hr and subsequently filtering through Whatman #2 filter paper to remove soil
290 particles. The samples in the desiccator were fumigated with ethanol-free chloroform ($CHCl_3$)
291 and incubated under vacuum for 3 d. After the 3 d fumigation, samples were extracted similar to
292 that of unfumigated samples. Filtered 0.5 M K_2SO_4 extracts were dried at 60 °C in a ventilated
293 drying oven and then ground to a fine powder with mortar and pestle before analysis of C
294 concentration and $\delta^{13}C$ on a Picarro G2201-i Isotopic CO_2/CH_4 Analyzer outfitted with a
295 Costech combustion module for solid sample analysis (Picarro Inc., Sunnyvale, CA USA).
296 Microbial C biomass was determined using the following equation:
297
298 $$MBC = EC / k_{EC}$$
299

where the chloroform-labile pool (EC) is the difference between C in the fumigated and non-fumigated extracts, and k_{EC} (extractable portion of MBC after fumigation) is soil-specific and estimated as 0.45 (Joergensen, 1996).

The $\delta^{13}C$ of MBC was estimated as the $\delta^{13}C$ of the C extracted from the fumigated soil sample in excess of that extracted from the non-fumigated soil sample using the following equation:

$$\delta^{13}C_{MBC}\ (‰) = (\delta^{13}C_f \times C_f - \delta^{13}C_{nf} \times C_{nf})/(C_f - C_{nf})$$

where C_f and C_{nf} is the concentration (mg kg^{-1} soil) of C extracted from the fumigated and non-fumigated soil samples, respectively, and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ is the ^{13}C natural abundance (‰) of the fumigated and non-fumigated soil samples, respectively.

2.5 Extracellular Enzyme Analysis

The potential activity of five extracellular enzymes ~~were~~ was quantified on ~~initial~~ soil samples ~~(day 0)~~ and on days 1, 8, 35, and 98 of the soil incubation. The enzymes chosen for this experiment represent a range of compounds they target, including fast and slow cycling C compounds, as well as ones that target nitrogen (N), phosphorus (P), and sulfate (S). The specific enzymes measured were: β-glucosidase (BG; EC: 3.2.1.21), xylosidase (XYL; EC 3.2.1.37), peroxidase (PER; EC: 1.11.1.7), β-glucosaminidase (NAGase; EC: 3.2.1.30), alkaline phosphatase (AP; EC: 3.1.3.1), and arylsulfatase (AS; EC: 3.1.6.1). Carbon-degrading enzymes BG, XYL, and PER degrade sugar, hemicellulose, and lignin, respectively, while the N-

degrading enzyme NAGase degrades chitin. Enzyme AP and AS degrade phosphorus and sulfate containing compounds, respectively. Substrates for all enzyme assays were dissolved in 50 mM, pH 5.0 acetate buffer solution for a final concentration of 5 mM substrate.

Hydrolytic enzymes (BG, ~~and~~ XYL, NAGase, AP, and AS) were measured using techniques outlined in Sinsabaugh et al. (1993). Approximately 0.~~5~~ 8 g dry weight of soil sample was suspended in 50 mL of a 50 mM, pH 5.0 acetate buffer solution and homogenized in a blender for 1 min. In a 2 mL centrifuge tube, 0.9 mL aliquot of the soil-buffer suspension was combined with 0.9 mL of the appropriate 5 mM p-nitrophenyl substrate solution for a total of three analytical replicates. Additionally, duplicate background controls consisted of 0.9 mL aliquot of soil-buffer suspension plus 0.9 mL of acetate buffer and four substrate controls were analyzed consisting of 0.9 mL substrate solution plus 0.9 mL buffer. The samples were agitated for 2-5 hr. Samples were then centrifuged at 8,160 g for 3 min. Supernatant (1.5 mL) was transferred to a 15 mL centrifuge tube containing 150 µL 1.0 M NaOH and 8.35 mL dH_2O. The resulting mixture was vortexed and a subsample transferred to a cuvette and the optical density at 410 nm was measured on a spectrophotometer (Beckman Coulter DU 800 Spectrophotometer, Brea, CA, USA).

The oxidative enzyme (PER) ~~were~~ was measured using techniques outlined in Sinsabaugh et al. (1992). PER is primarily involved in oxidation of phenol compounds and depolymerization of lignin. The same general procedure for hydrolytic enzymes was followed utilizing a 5 mM L-3,4-Dihydroxyphenylalanine (L-DOPA) (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) solution plus 0.2 mL of 0.3% H_2O_2 to all sample replicates and controls as the substrate. After set up of analytical replicates and substrate and background controls, the samples were agitated for 2-3 hr. Samples were then centrifuged at 8,160 g for 3 min. The

resulting supernatant turns an intense indigo color. Supernatant (1.4 mL) was transferred directly to a cuvette and the optical density at 460 nm was measured on a spectrophotometer.

For all enzymes, the mean absorbance of two background controls and four substrate controls was subtracted from that of three analytical replicates and divided by the molar efficiency (1.66/µmol), length of incubation (h), and soil dry weight. Enzyme activity was expressed as µmol substrate converted per g dry soil mass per hour (µmol g^{-1} h^{-1}).

2.6 Statistical Analysis

Water chemistry, cumulative CO_2 production, cumulative CH_4 production, cumulative enzyme activity, post-incubation SOC concentration and $\delta^{13}C$ SOC, and wood-derived and wood-associated SOC, CO_2, and MBC were analyzed using a one-way ANOVA (PROC GLM package). Microbial biomass C, MBC ^{13}C, pH, Eh, $\delta^{13}CO_2$, and $\delta^{13}CH_4$ were analyzed using repeated-measures ANOVA (PROC MIXED package) with time (Time) as the repeated measure and the incubation treatments as fixed effects. All data for wood-free and wood-amended soils were analyzed separately. Raw data were natural log-transformed where necessary to establish homogeneity of variance. If significant main effects or interactions were identified in the one-way ANOVA or repeated-measures ($P < 0.05$), then post-hoc comparison of least-squares means was performed. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA).

3 Results

3.1 Water and Soil Properties

Freshwater had higher concentrations of TOC compared to the saltwater treatments (Table 1). Concentration of SO_4^{2-}, Cl^-, Na^+, Ca^{2+}, Mg^{2+}, and K^+ were higher in saltwater treatments compared to freshwater and were approximately twice as high in the 5.0 ppt saltwater treatment compared to 2.5 ppt saltwater (Table 1).

Initial (pre-incubation) ~~hummock~~ SOC concentration was 490 ± 27 g kg^{-1} with a $\delta^{13}C$ value of -28.5 ± 0.32 ‰. After 98 d of incubation, SOC concentration in wood-free incubations was lower in the 5.0 ppt saltwater treatment, although no difference in soil $\delta^{13}C$ was found between treatments (Table 2). For wood-amended incubations, post-incubation SOC concentration was lower in the 5.0 ppt saltwater treatment compared to the dry and freshwater treatment (Table 2). The $\delta^{13}C$ of wood-free and wood-amended soils after 98 days of incubation was not different between treatments~~, but was depleted in ^{13}C compared to wood-free soils.~~ (Table 2).

Soil pH was significantly lower in the saltwater treatments in both wood-free and wood-amended soils compared to the dry and freshwater treatments (Table 3; Figure ~~1a~~2A-~~b~~B). After an initial drop of pH in saltwater treatments to between 3.2 and 3.4 pH, pH steadily climbed back up to between 4.0 and 4.2 p/H (Figure ~~1a~~2A-~~b~~B). In wood-free soils, differences in soil Eh between treatments was variable over time, with both the 5.0 ppt saltwater treatment and the freshwater treatment having the lowest redox potential at different time points throughout the incubation (Table 3; Figure ~~1c~~2C), but never got below -124 mV on average. In wood-amended soils, Eh dropped quickly to between -200 and -400 mV over the first 30 days for saltwater incubated soils (Table 3; Figure ~~1d~~2D), before rising to between -100 to 0 mV for the rest of the

incubation period. In freshwater incubated soils, Eh rose quickly back to between -50 to 0 mV by day 15 and remained at this level for the rest of the incubation period, while saltwater treatments had significantly lower Eh between days 8 and 25.

3.2 CO_2, CH_4, $\delta^{13}CO_2$-C, and $\delta^{13}CH_4$-C

In wood-free incubations, cumulative CO_2 production was not different between the dry and freshwater treatments, but were higher than that produced from saltwater treatments (Table 4; Figure ~~2a~~3A). Cumulative CO_2 produced from wood-amended soils was highest in the dry treatment compared to all other treatments (Table 4; Figure ~~2b~~3B). Wood-derived CO_2 (calculated as the difference between cumulative CO_2 produced from wood-amended and wood-free incubations) was highest in the dry treatment (Table 4; Figure ~~2c~~3C). This finding was also confirmed by calculating cumulative wood-derived C using the ^{13}C two-pool mixing model, with the highest proportion found in the dry treatment (54 ± 4.6 %) compared to soils incubated with freshwater (42 ± 1.7 %), 2.5 ppt saltwater (37 ± 1.0 %), and 5.0 ppt saltwater (38 ± 1.5 %).

Cumulative CH_4 production was highest in the freshwater treatment compared to the saltwater treatments in both wood-free and wood-amended incubations (Table 4; Figure ~~2d~~3D-~~e~~E). The difference between cumulative CH_4 produced from wood-amended and wood-free incubations was lower (and exhibited a negative response to wood additions) in the freshwater treatment compared to both saltwater treatments (Table 3; Figure ~~2f~~3F), which both had a slight positive response to wood additions.

The CO_2:CH_4 ratio, in wood-free incubations, was calculated only for soils incubated under saturated conditions with freshwater or saltwater. The CO_2:CH_4 ratio, in wood-free

incubations, was highest in freshwater (6 ± 3.4), compared to the 2.5 ppt saltwater (136 ± 33.9) and 5.0 ppt saltwater (102 ± 30.3) ($F = 24.8$; $P = 0.0002$). The CO_2:CH_4 ratio, in wood-amended incubations, was highest in freshwater (9 ± 0.8), compared to the 2.5 ppt saltwater (53 ± 20.3) and 5.0 ppt saltwater (107 ± 37.7) ($F = 9.2$; $P = 0.007$).

The $\delta^{13}CO_2$-C and wood-derived CO_2 (estimated by ^{13}C two-pool mixing model) exhibited a time by treatment interaction for both wood-free and wood-amended incubations (Table 3; Figure ~~3a~~4A ~~b~~B). In general, $\delta^{13}CO_2$-C in wood-free and wood-amended incubations was depleted in the dry treatment (and remained steady throughout the incubation period) compared to all other treatments, especially after day 15. The proportion of wood-derived CO_2 was initially higher in saltwater treatments but gradually dropped over the course of the incubation, while the proportion of wood-derived CO_2 dropped quickly after the first sampling date (day 1) and remained steady (approximately ~~50~~ 40-60 %) for ~~a good portion of the~~the remainder of the- incubation period ~~but increased in the final couple measurements periods to a maximum of 75 %~~ (Figure ~~3c~~4C).

The $\delta^{13}CH_4$-C (Table 3; Figure ~~4~~5) exhibited a treatment and time effect (Table 3; Figure ~~4a b~~5A-B), but only for wood-free incubations. For wood-free incubations, average $^{13}CH_4$-C across the course of the incubation was most enriched in the freshwater treatment (-67.8 ± 2.4 ‰) compared to the 2.5 ppt (-80.1 ± 2.4 ‰) and 5.0 ppt (-82.3 ± 2.0 ‰) saltwater treatments (Figure ~~4C~~5C). No difference in the $\delta^{13}CH_4$-C was found in wood-amended incubations (Figure 4b, d), ranging from between -78 to -75 ‰ for all treatments.

3.3 Microbial Biomass Carbon and Extracellular Enzyme Activity

Initially, ~~in wood-free incubations,~~ MBC was ~~highest in the 2.5 ppt saltwater treatment~~lowest in the dry treatment of wood-free incubations and lowest in the 5 ppt treatment of wood-amended incubations ~~compared to the dry treatment~~ (Table 3; Table 5). Following the 98 day incubation, MBC ~~in wood-free incubations~~ was highest in the dry treatment of wood-free incubations, with no differences between the other treatments. In wood-amended ~~soils~~incubations, ~~no difference in MBC was found initially, but following the 98 day incubation~~final MBC was also highest in the dry treatment ~~followed by the freshwater treatment with the MBC of the~~and lowest in both saltwater treatments ~~being the lowest~~. Initial $\delta^{13}C$ of MBC did not differ between treatments in either the wood-free or wood amended soils (Table 3; Table 5). After the 98 day incubation, ^{13}C of MBC in the wood-free treatments was most depleted in the freshwater treatment and most enriched in the 5.0 ppt saltwater treatment. In wood-amended incubations, ^{13}C of MBC was most depleted in the dry treatment and most enriched in the freshwater and 5.0 ppt saltwater treatments. Furthermore, the proportion of wood-derived MBC (as estimated by ^{13}C mixing model calculations) was highest in the dry treatment (31 %) and the 2.5 ppt saltwater treatment (21%) compared to the freshwater treatment (4%) (Table 5).

In wood-free incubations, activity of BG, PER, and NAGase were higher in the dry treatment compared to the saltwater treatments (Table 4; Table 5). Activity of AS was higher in the dry and freshwater treatments compared to saltwater treatments, in both wood-free and wood-amended incubations. In wood-amended incubations, BG and NAGase were highest in the dry treatment compared to the saltwater treatments. In the freshwater treatment, wood addition reduced activity of BG and NAGase compared to wood-free incubations (Figure ~~5a-b~~6A-B), but

enhanced PER activity (Figure ~~5c~~6C). Wood addition also reduced AS and P activity across all treatments compared to wood-free incubations (Figure ~~5d-e~~6D-E).

4 Discussion

As forests within the lower coastal plain physiographic region of the southeastern US continue to experience increasing stresses from ~~sea level rise~~SLR on hydrology, changes in microbial C cycling processes should be expected. Our results, combined with other field and lab experiments, confirm that saltwater intrusion into coastal freshwater wetlands can result in reductions in CO_2 and CH_4 ~~fluxes~~ production (Ardón et al., 2016; Ardón et al.,2018) in the presence or absence of wood, but this ~~will~~ may be balanced by long- and short-term effects of saltwater intrusion on these C cycling processes (Weston et al., 2011) as well as changes in C inputs due to forest-to-marsh transition. Further, ~~increased coarse woody debris inputs~~wood additions to these wetland soils may reduce CH_4 ~~emissions~~ production under freshwater conditions compared to the absence wood additions (Figure 3C and 3F), but slightly enhance CH_4 ~~emissions~~ production under saltwater conditions. Our results also clearly demonstrate that substantial quantities of CH_4 can be produced from soils with redox potential between -100 to 100 mV, which may be related to the specific pathway of CH_4 production (acetoclastic versus hydrogenotrophic), and challenges the widespread assumption that methanogenesis only occurs at very low redox potentials. Changes in the water table depth at the ~~The~~ ARNWR ~~is characterized by a hydroperiod that operates over short time scales and is~~ driven primarily by ~~variable~~ precipitation patterns (~~Miao et al.~~Minick et al., ~~2013~~2019a), ~~which results~~resulting in the influx of oxygenated waters. Periodic *in situ* measurements of redox potential at the ARNWR

483 indicate that standing water is relatively aerated (Eh = 175 - 260 mV), while surface soils of
484 hummocks when not submerged are more aerated (Eh = 320 mV) than submerged hollow surface
485 soils (Eh = 100 - 150 mV) and deeper organic soils (20-40 cm depth; Eh = 50 - 90 mV).
486 Furthermore, our results indicate that additions of new C to soils as wood may result in short-
487 term reductions in redox potential as anaerobic processes are enhanced due to the added C
488 substrate and terminal electron acceptors are quickly reduced. As SLR continues to rise over the
489 next century, more persistent saltwater intrusion may occur as rising brackish waters mix with
490 non-tidal freshwater systems having important implications for both above- and below-ground C
491 cycling dynamics. Although our study only looked at these effects in a controlled laboratory
492 experiment, these data provide a baseline understanding of potential changes in C cycling
493 dynamics due to SLR.

494 Saltwater additions decreased CO_2 production compared to freshwater in the wood-free
495 soils, although MBC and extracellular enzyme activity were not different between these
496 treatments. This has been found in other pocosin wetland soils on the coast of North Carolina
497 (Ardón et al. 2018). Variable effects of salinity (and or sulfate additions) have been found on soil
498 respiration, with some studies showing an increase (Marton et al., 2012; Weston et al., 2011), a
499 decrease (Lozanovska et al. 2016; Servais et al. 2019), or no change (Baldwin et al., 2006).
500 Krauss et al. (2012) found that permanently flooded saltwater treatments (expected in non-tidal
501 wetlands) in a simulated coastal swamp mesocosm reduced soil respiration, whereas saltwater
502 pulses (expected in tidal wetlands) had a variable effect on soil respiration. Alternatively, CO_2
503 production was not reduced in the saltwater compared to freshwater treatments in wood-amended
504 soils, while MBC was lower in the saltwater compared to freshwater, which suggests a shift in
505 microbial carbon use efficiency.

Methane production was higher in the freshwater compared to saltwater treatments in both wood-amended and wood-free incubations. Numerous others studies have found that saltwater reduces CH_4 fluxes compared to freshwater, both within the field and laboratory. Reduced CH_4 production from saltwater treated soils primarily results from the availability of more energetically favorable terminal electron acceptors (primarily SO_4^{2-}), which leads to the competitive suppression of methanogenic microbial communities by sulfate reducing communities (Bridgham et al., 2013; Chambers et al., 2011; Winfrey and Zeikus, 1977), as methanogens and sulfate reducers compete for acetate and electrons (Le Mer and Roger, 2001). Dang et al. (2019) did find partial recovery over time of the methanogenic community following saltwater inundation to freshwater soil cores, but interestingly this community resembled that of microbes performing hydrogenotrophic methanogenesis and not acetoclastic methanogenesis. Activity of arylsulfatase was also lower in saltwater amended soils. This also indicates a functional change in the microbial community, as microbes in the saltwater treatment are utilizing the readily available SO_4^{2-} pool, while microbes in the freshwater and dry treatments are still actively producing SO_4^{2-}-liberating enzymes to support their metabolic activities. Findings by Baldwin et al. (2006) support the effects of saltwater on changing the microbial community structure as well, in which reductions in CH_4 production in NaCl treated freshwater sediments were accompanied by a reduction in archaeal (methanogens) microbial population, establishing a link between shifting microbial populations and changing CH_4 flux rates due to saltwater intrusion.

Changes in the CH_4 production due to saltwater additions appears to be related to the dominant CH_4 producing pathway. The $^{13}CH_4$ isotopic signature in wood-free freshwater incubated soils indicated that acetoclastic methanogenesis was the dominant CH_4 producing

pathway, while hydrogenotrophic methanogenesis dominated in the saltwater treatment. Acetoclastic methanogenesis produces isotopically enriched CH_4 compared to that of the hydrogenotrophic methanogenesis (Chasar et al., 2000; Conrad et al. 2010; Krohn et al. 2017; Sugimoto and Wada, 1993; Whiticar et al., 1986; Whiticar 1999), given that methanogens discriminate against heavier $^{13}CO_2$ during the hydrogenotrophic methanogenesis. The differences in C discrimination between the two pathways is greater for the hydrogenotrophic compared to the acetoclastic pathway which results in more depleted (-110 to -60 ‰) and more enriched (-60 ‰ to -50 ‰) $^{13}CH_4$, respectively. This has been confirmed in field and laboratory experiments (Conrad et al. 2010; Krohn et al. 2017; Krzycki et al., 1987; Sugimoto and Wada, 1993; Whiticar et al., 1986; Whiticar, 1999). Baldwin et al. (2006) also found that saltwater additions promoted the hydrogenotrophic methanogenic pathway. Further, recent studies have found that saltwater additions to soils result in a shift in the relative abundance of hydrogenotrophic methanogens (Chambers et al. 2011; Dang et al 2019), supporting the idea that saltwater may alter not only the flux of CH_4 but also the dominant pathway of methane production.

Changes in fresh- and salt-water hydrology due to rising seas is leading to dramatic shifts in the dominant plant communities within the ARNWR and across the southeastern US (Connor et al., 1997; DOD, 2010; Langston et al., 2017; Kirwan and Gedan 2019). This has the potential to alter the soil C balance due to introduction of large amounts of coarse woody debris as trees die. In our laboratory experiment, additions of wood resulted in changes in both CO_2 and CH_4 production, but the direction of change depended on if soils were incubated with freshwater or saltwater. Wood additions increased CO_2 production except in the freshwater treatment. This was particularly evident in the dry treatment where wood additions increased CO_2 production by approximately 32 %. For the dry treatment, wood-amended soils had the highest MBC and

552 NAGase activity as microbes were likely immobilizing more N to support metabolic activities in
553 the presence of added C (Fisk et al., 2015; Minick et al., 2017). Higher respiration with wood
554 additions in the saltwater treatments likely resulted from enhanced metabolic activity of sulfate
555 reducing microbes in the presence of an added C source. On the other hand, wood additions
556 resulted in a decline in CH_4 production from the freshwater treatment, while slightly enhancing
557 CH_4 production from the saltwater treatments. Wood additions also resulted in much lower
558 redox potential, particularly in the saltwater treatments, and coupled with $^{13}CH_4$ stable isotope
559 composition may have driven the higher levels of CH_4 production (via hydrogenotrophic
560 methanogenesis) in the wood plus saltwater treatments. The suppression of CH_4 production by
561 wood additions in the freshwater treatment was somewhat surprising given the positive effects of
562 C additions on CH_4 production recently found in freshwater sediments (West et al. 2012), but
563 likely resulted from enhancement of other, more energetically favorable redox reactions with the
564 addition of a C source (e.g., wood). Furthermore, wood additions to freshwater incubations
565 resulted in a decrease in MBC and activity of BG and NAGase enzymes compared to wood-free
566 incubations, ~~but an~~and an increase in PER activity. This suggests that the microbial communities
567 have altered their functional capacity in response to wood-addition when exposed to freshwater.
568 The CO_2:CH_4 ratio further indicated that, in freshwater, CH_4 production was quite high in
569 relation to CO_2 production. This ratio was significantly higher though for saltwater treatments as
570 CH_4 production dropped drastically compared to freshwater. In wood-free incubations, the
571 CO_2:CH_4 trend between freshwater and saltwater treatments was parabolic but was linear upward
572 in wood-amended soils. This suggests that interactions between saltwater and coarse woody
573 debris (in the form of dead and dying trees; Kirwan and Gedan 2019) may be important to

understand in determining effects of salt water intrusion on greenhouse gas production in freshwater forested wetlands.

~~Changes in the CH_4 production due to saltwater additions appears to be related to the dominant CH_4 producing pathway. The $^{13}CH_4$ isotopic signature in wood-free freshwater incubated soils indicated that acetoclastic methanogenesis was the dominant CH_4 producing pathway, while hydrogenotrophic methanogenesis dominated in the saltwater treatment. Acetoclastic methanogenesis produces isotopically enriched CH_4 compared to that of the hydrogenotrophic methanogenesis (Chasar et al., 2000; Conrad et al. 2010; Krohn et al. 2017; Sugimoto and Wada, 1993; Whiticar et al., 1986; Whiticar 1999), given that methanogens discriminate against heavier $^{13}CO_2$ during the hydrogenotrophic methanogenesis. The differences in C discrimination between the two pathways is greater for the hydrogenotrophic compared to the acetoclastic pathway which results in more depleted (-110 to -60 ‰) and more enriched (-60 ‰ to -50 ‰) $^{13}CH_4$, respectively. This has been confirmed in field and laboratory experiments (Conrad et al. 2010; Krohn et al. 2017; Krzycki et al., 1987; Sugimoto and Wada, 1993; Whiticar et al., 1986; Whiticar, 1999). Baldwin et al. (2006) also found that saltwater additions promoted the hydrogenotrophic methanogenic pathway. Further, Dang et al (2019) showed that saltwater additions to soil cores resulted in a shift in the relative abundance of hydrogenotrophic methanogens, supporting the idea that saltwater may alter not only the flux of CH_4 but also the production pathway. Chambers et al. (2011) found a shift in the methanogenic microbial community under saltwater treatments as well, which could have implications for the dominant pathway of methane production. Previous work at our site showed that freshwater saturated soils from different microsites (hummocks, hollows, and subsurface Oa horizon soil) also had $\delta^{13}CH_4$~~

~~values more like that found from CH_4 produced via acetoclastic methanogenesis (Minick et al., 2019b).~~

Findings from this study indicate that substantial changes in the greenhouse gas flux and microbial activity are possible due to saltwater intrusion into freshwater wetland ecosystems but that the availability of C in the form of dead wood (as forests transition to marsh) may alter the magnitude of this effect. At ARNWR and similar coastal freshwater forested wetlands, salt water intrusion may reduce both CO_2 and CH_4 emissions from soils to the atmosphere. Sea level rise will likely lead to dramatic and visually striking changes in vegetation, particularly transitioning forested wetlands into shrub or marsh wetlands (Kirwan and Gedan 2019), which will reduce the primary productivity and the C uptake potential of these ecosystems as more productive forests transition to less productive marsh systems. As forested wetlands are lost, dead trees could provide a significant source of C to already C-rich peat soils, with the potential to also increase CO_2 emissions and slight increases in CH_4 production. The long-term effect of forest to marsh transition on ecosystem C storage will likely depend on the balance between dead wood inputs and effects of sea level rise and vegetation change on future C inputs and soil microbial C cycling processes. Future work should include investigation of these C cycling and microbial processes at the field-scale and expand to a wider range of non-tidal wetlands within the southeastern US region.

Author contribution

All authors contributed to the conception and design of the study. KM wrote the first draft of the manuscript. KM collected the samples from the field and performed laboratory analysis. All authors contributed to manuscript revision and approved the submitted version.

Competing Interest

The authors declare that they have no conflict of interest.

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Tables and Figures

Table 1**.** Total organic C (TOC) and ion concentrations (mg L^{-1}) in freshwater (0 ppt), 2.5 ppt saltwater, and 5.0 ppt saltwater. Standard errors of the mean are in parenthesis (n=4). Values with different superscript lowercase letters are significantly different ($P < 0.05$).

Treatment	TOC	SO_4^{2-}	Cl^-	Na^+	NH_4^+	NO_3^-	PO_4^{3-}	Ca^{2+}	Mg^{2+}	K^+
0 ppt	44 (0.3)^a	1 (0.1)^a	17 (0.2)^a	8 (0.1)^a	0.00 (0.000)^a	0.00 (0.000)^a	0.00 (0.000)^a	1 (0.0)^a	1 (0.0)^a	0.2 (0.0)^a
2.5 ppt	40 (0.7)^b	162 (1.3)^b	1391 (42.8)^b	538 (19.2)^b	0.06 (0.004)^b	0.06 (0.000)^a	0.01 (0.000)^a	23 (0.3)^b	64 (2.6)^b	19 (0.3)^b
5.0 ppt	38 (0.1)^b	319 (6.5)^c	2695 (22.6)^c	1039 (15.9)^c	0.07 (0.004)^b	0.07 (0.004)^a	0.01 (0.000)^b	44 (1.0)^c	125 (2.1)^c	36 (0.4)^c

Table 2. Post-incubation soil organic C (SOC) concentration (g kg^{-1}), SOC $\delta^{13}C$ (‰), and wood-derived SOC (%) (estimated from ^{13}C two pool mixing model) for soil samples collected from the field and incubated for 98 d in the laboratory under dry conditions (Dry) or fully saturated with freshwater (0 ppt) or saltwater (2.5 and 5.0 ppt) and with (+ Wood) or without addition of ^{13}C-depleted wood. Pre-incubation data was measured from the four replicates prior to incubation and therefore have the same for each treatment. Standard errors of the mean are in parenthesis (n=4). Data from wood-free and wood-amended soils were analyzed separately. Values followed by different superscript lowercase letters are significantly different between the four treatments of the non-wood or wood amended soils ($P < 0.05$).

Treatment	Post-SOC Concentration (g kg^{-1})	Post-SOC $\delta^{13}C$ (‰)	Wood-derived SOC (%)
Dry	495 (1.5)^{b}	-29.5 (0.20)^{a}	.
0 ppt	493 (3.3)^{b}	-29.5 (0.18)^{a}	.
2.5 ppt	488 (4.9)^{b}	-29.5 (0.20)^{a}	.
5.0 ppt	460 (8.6)^{a}	-29.5 (0.16)^{a}	.
Dry + Wood	491 (4.7)^{ab}	-30.4 (0.30)^{a}	8 (2.5)
0 ppt + Wood	502 (4.6)^{a}	-30.7 (0.22)^{a}	12 (0.4)
2.5 ppt + Wood	477 (4.9)^{bc}	-30.6 (0.35)^{a}	10 (1.4)
5.0 ppt + Wood	470 (4.6)^{c}	-30.4 (0.14)^{a}	10 (2.0)

Table 3. Results (F-values and significance) from the repeated measures ANOVA of pH, Eh, microbial biomass C (MBC), $\delta^{13}C$ isotopic signature of MBC, $\delta^{13}CO_2$ and $\delta^{13}CH_4$ measured in soils collected from a coastal freshwater forested wetland and incubated in the laboratory for 98 d under fully saturated with either freshwater or salt water (2.5 ppt and 5.0 ppt). Data from wood-free and wood-amended soils were analyzed separately.

Source	pH	Eh	MBC	MBC ^{13}C	$\delta^{13}CO_2$	$\delta^{13}CH_4$
Wood-Free						
Treatment	26.6***	4.5*	3.7*	3.2*	351.7***	60.5***
Time	4.4***	40.7***	40.9***	15.8**	24.2***	8.3***
Treatment x Treatment	1.22	3.7***	27.3***	3.3*	6.4***	1.1
Wood-Amended						
Treatment	29.0***	13.6***	39.9***	2.6	129.8***	0.3
Time	18.3***	30.1***	111.0***	3.7	34.8***	1.4
Treatment x Treatment	1.4	3.4***	24.2***	5.5**	8.3***	1.0

*P < 0.05, **P < 0.01, ***P < 0.0001

Table 4. Results (F-values and significance) from the one-way ANOVA of cumulative gas production and extracellular enzyme activity (BG: β-glucosidase; PER: peroxidase; NAGase: glucosaminidase; AP: alkaline phosphatase; and AS: arylsulfatase) from soils collected from a coastal freshwater forested wetland and incubated in the laboratory for 98 d under dry conditions or fully saturated with either freshwater or salt water (2.5 ppt and 5.0 ppt). Data from wood-free and wood-amended soils were analyzed separately.

Source	CO_2	CH_4	BG	PER	NAGase	AP	AS
Wood-Free							
Treatment	20.4***	15.6***	7.2**	11.9**	9.5**	0.9	15.8**
Wood-Amended							
Treatment	13.3**	36.7***	16.6**	2.5	32.0***	2.3	31.2***

*P < 0.05, **P < 0.01, ***P < 0.0001

Table 5. Initial (1 d) and final (98 d) microbial biomass C (MBC) concentration (mg kg^{-1}), MBC $\delta^{13}C$ (‰), wood-derived MBC (%) (estimated using ^{13}C two pool mixing model) and cumulative extracellular enzyme activity (μmol g^{-1}) (BG: β-glucosidase; PER: peroxidase; NAGase: glucosaminidase; AP: alkaline phosphatase; and AS: arylsulfatase) for soils incubated under dry conditions (Dry) or saturated with freshwater (0 ppt) or saltwater (2.5 and 5.0 ppt) and with (+ Wood) or without addition of ^{13}C-depleted wood. Standard errors of the mean are in parenthesis (n=4). Values followed by different superscript lowercase letters are significantly different between the four treatments for the wood-free or wood-amended soils ($P < 0.05$).

Treatment	Initial MBC Concentration (mg kg^{-1})	Final MBC Concentration (mg kg^{-1})	Initial MBC $\delta^{13}C$ (‰)	Final MBC $\delta^{13}C$ (‰)	Wood-derived MBC (%)	BG	PER	NAGase	AP	AS
Dry	2238 (400)^{c}	4077 (387)^{a}	-27.0 (0.43)^{a}	-28.4 (0.28)^{ab}	.	547 (37)^{a}	176 (14)^{a}	240 (20)^{a}	7599 (1038)^{a}	47 (2)^{a}
0 ppt	3982 (196)^{ab}	2657 (344)^{b}	-27.3 (0.19)^{a}	-28.9 (0.16)^{a}	.	479 (18)^{ab}	197 (38)^{a}	194 (11)^{ab}	6308 (517)^{a}	47 (8)^{a}
2.5 ppt	7334 (1177)^{a}	2495 (195)^{b}	-27.8 (0.51)^{a}	-27.9 (0.03)^{ab}	.	389 (33)^{b}	412 (75)^{b}	159 (9)^{b}	6539 (183)^{a}	19 (3)^{b}
5.0 ppt	6483 (104)^{ab}	2114 (135)^{b}	-27.0 (0.30)^{a}	-27.4 (0.15)^{b}	.	379 (27)^{b}	490 (30)^{b}	154 (8)^{b}	6387 (529)^{a}	15 (2)^{b}
Dry + Wood	4444 (579)^{a}	5174 (249)^{a}	-29.3 (0.40)^{a}	-32.1 (0.44)^{a}	31 (4.9)^{a}	554 (37)^{a}	243 (22)^{a}	275 (17)^{a}	7247 (887)^{a}	40 (2)^{a}
0 ppt + Wood	5376 (330)^{a}	1832 (102)^{b}	-29.8 (0.37)^{a}	-29.4 (0.15^{b}	4 (1.1)^{b}	349 (24)^{b}	275 (44)^{a}	153 (11)^{b}	4965 (459)^{a}	36 (3)^{a}
2.5 ppt + Wood	5173 (405)^{a}	748 (124)^{c}	-30.1 (0.25)^{a}	-30.4 (0.95)^{ab}	21 (7.8)^{a}	368 (12)^{b}	365 (30)^{a}	150 (6)^{b}	5548 (653)^{a}	14 (3)^{b}
5.0 ppt + Wood	2123 (400)^{b}	790 (87)^{c}	-29.9 (0.43)^{a}	-29.7 (0.37)^{b}	18 (1.9)^{ab}	369 (13)^{b}	326 (38)^{a}	150 (6)^{b}	5893 (495)^{a}	13 (2)^{b}

Figure 1. Location of the Alligator River National Wildlife Refuge (ARNWR) in eastern North Carolina (NC) and the surrounding states water bodies. The enlarged map shows surrounding freshwater (Alligator River and Albermarle Sound) and saltwater (Pamlico Sound, Croatan Sound, and Roanoke Sound) bodies. The star represents the approximate location of soil and freshwater (from Milltail Creek) sampling locations within the freshwater forested wetlands of ARNWR. The black circle represents the approximate location of saltwater sampling (at the Melvin Daniels Bridge, Roanoke Sound) from the Roanoke Sound. The saltwater was sampled approximately 20 miles east of the soil and freshwater samples.

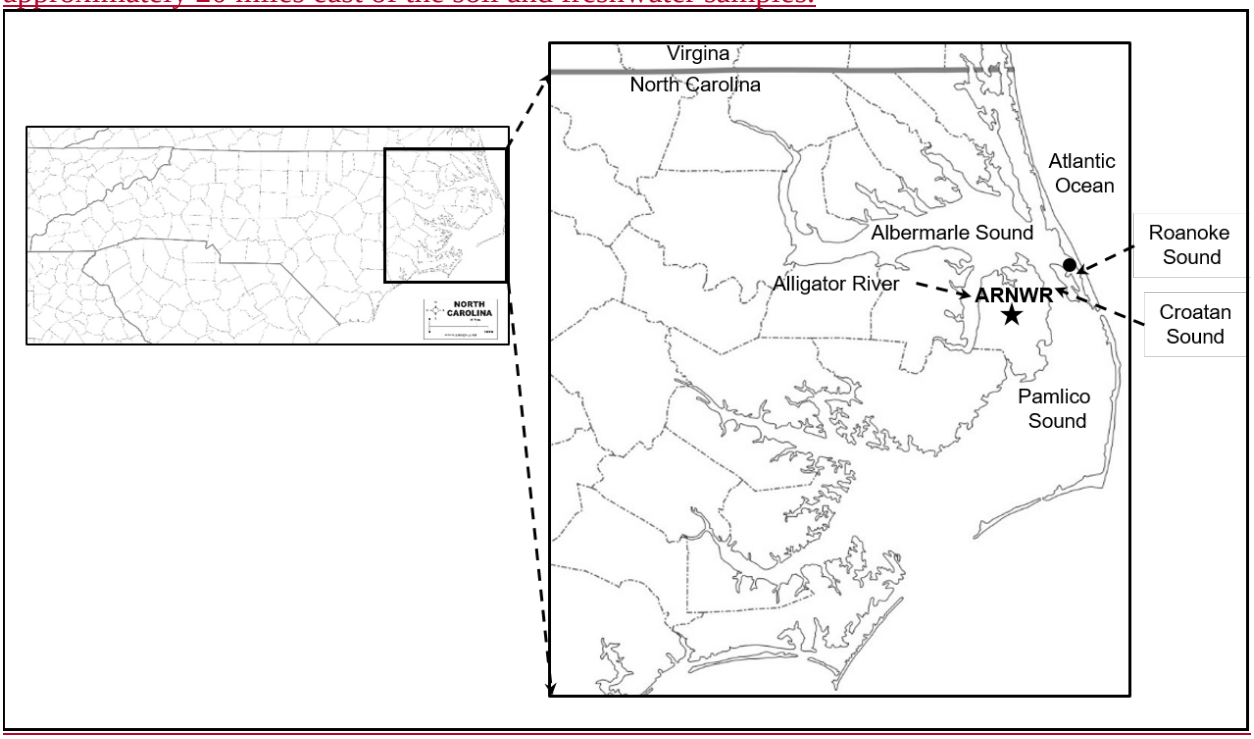


Figure ~~1~~2. pH for wood-free soils (A) and wood-amended soils (B) and redox potential for wood-free soils (C) and wood-amended soils (D) measured over the course of the 98 d laboratory incubation. Symbols represent mean with standard error (n=4). Treatment means with different lowercase letters are significantly different within a sampling time point ($P < 0.05$).

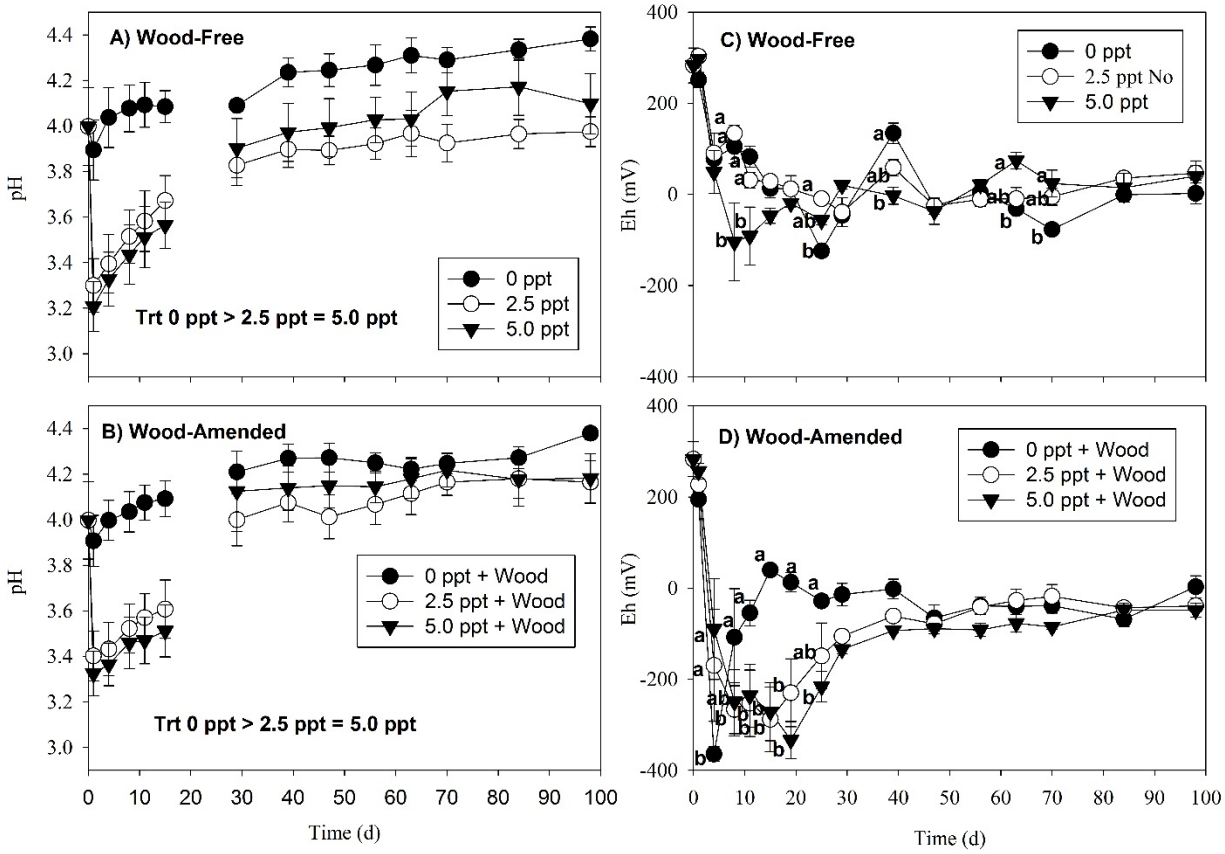


Figure ~~2~~3. Cumulative CO_2 production ~~for~~ from wood-free soils (A), wood-amended soils (B), and the wood-associated CO_2 production (C); and cumulative CH_4 production for wood free soils (D), wood amended soils (E), and the wood-associated CH_4 production (F). Panels C and F refer to the difference between wood-amended and wood-free soils. Bars represent mean with standard error (n=4). Bars with different uppercase letters are significantly different ($P < 0.05$).

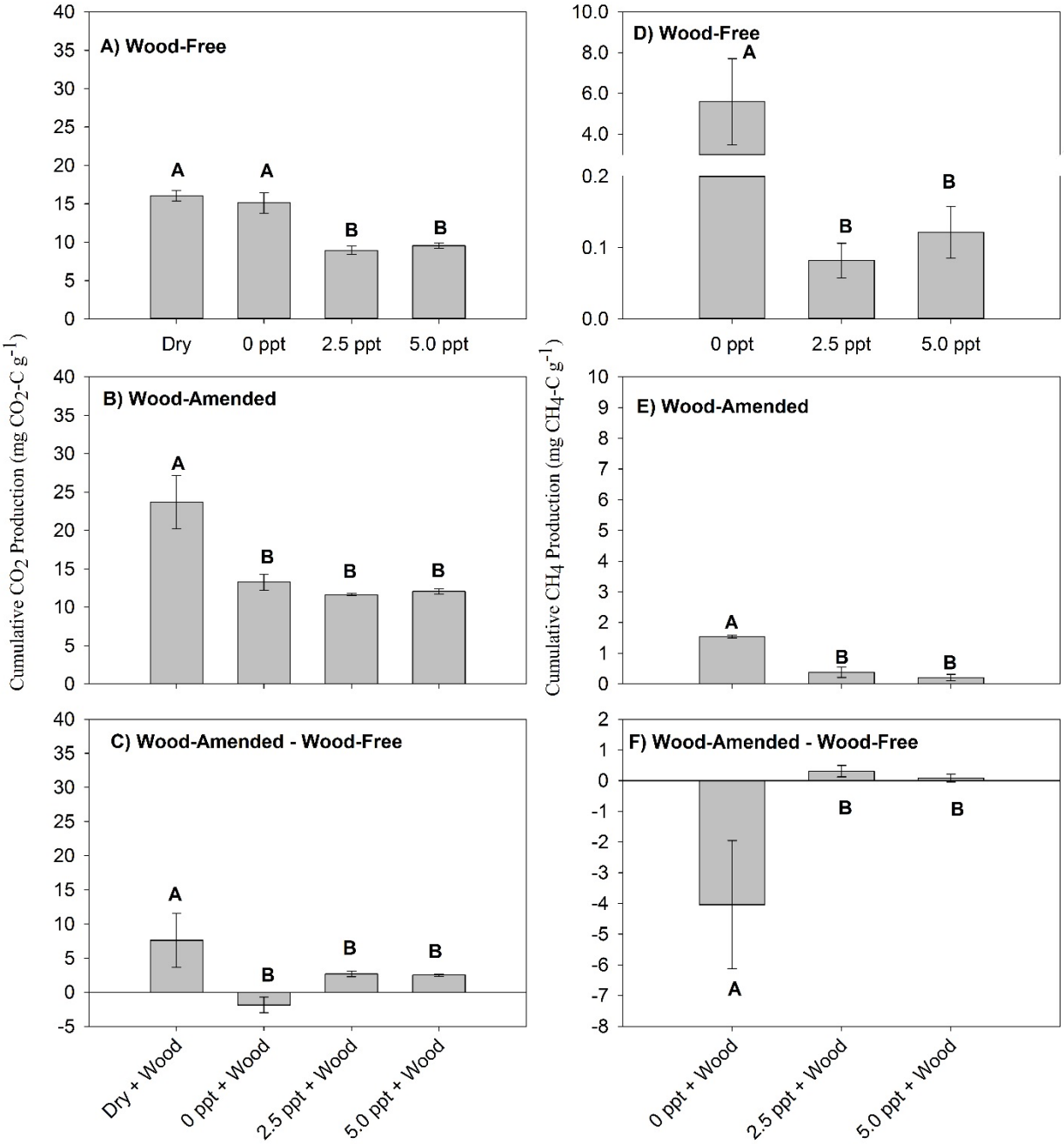


Figure ~~3~~4. The $\delta^{13}CO_2$ values measured over the course of the 98 d laboratory incubation for wood-free soils (A), wood-amended soils (B), and the proportion of wood-derived CO_2 (C). Bars represent mean with standard error (n=4). Treatment means with different lowercase letters are significantly different within a sampling time point ($P < 0.05$).

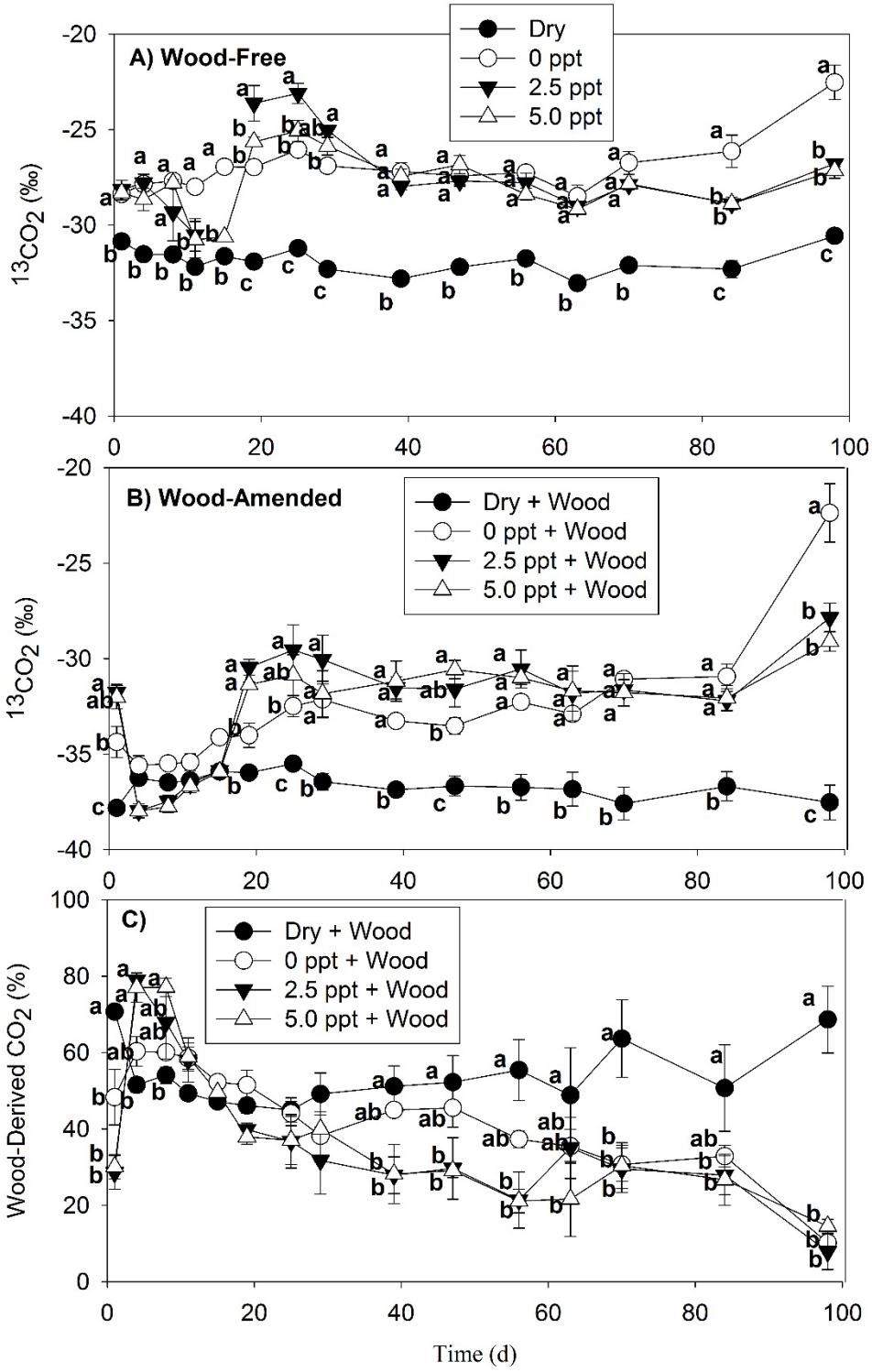


Figure 45. The $\delta^{13}CH_4$ values measured over the course of the 98 d laboratory incubation for wood-free soils (A) and wood-amended soils (B) and the average $\delta^{13}CH_4$ across the entire incubation for wood-free soils (C) and wood-amended soils (D). Symbols or bars represent mean with standard error (n=4). Treatment means with different lowercase letters are significantly different within a sampling time point ($P < 0.05$).

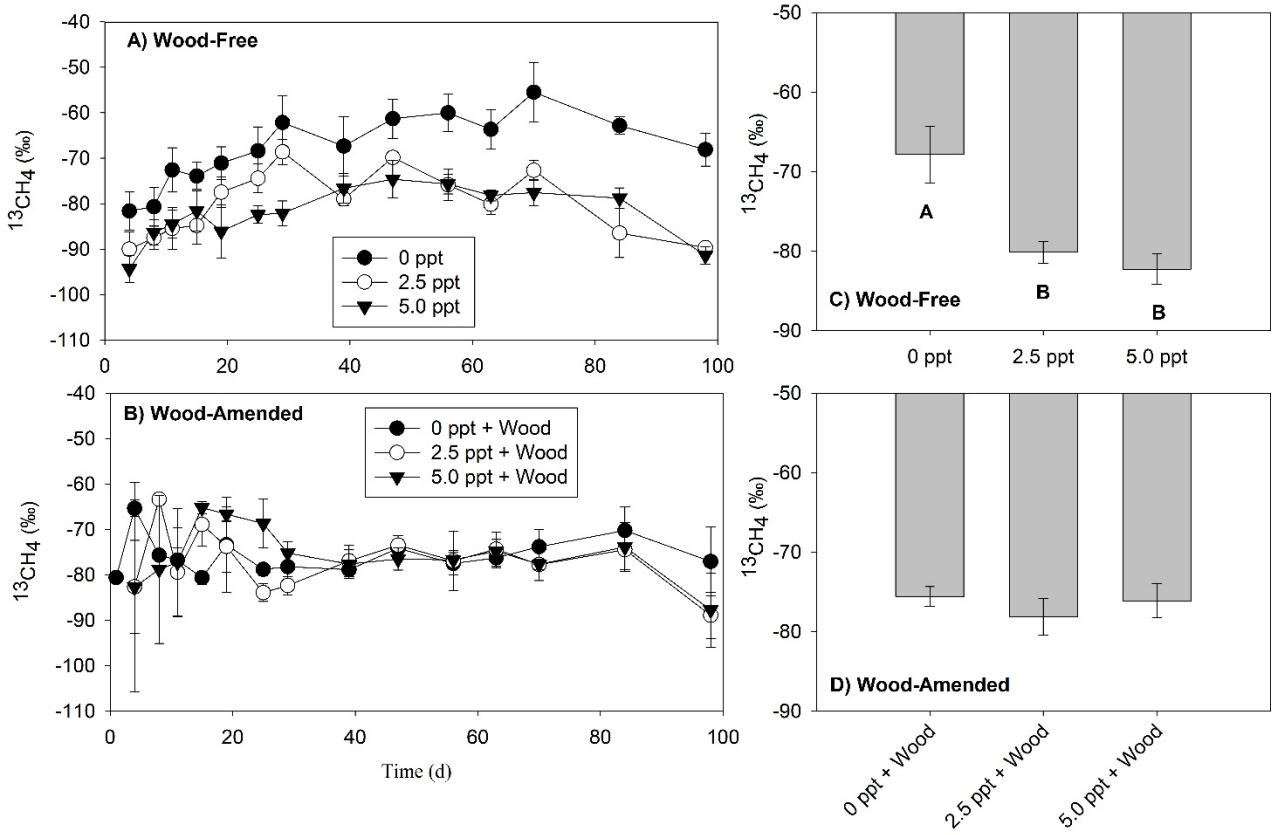
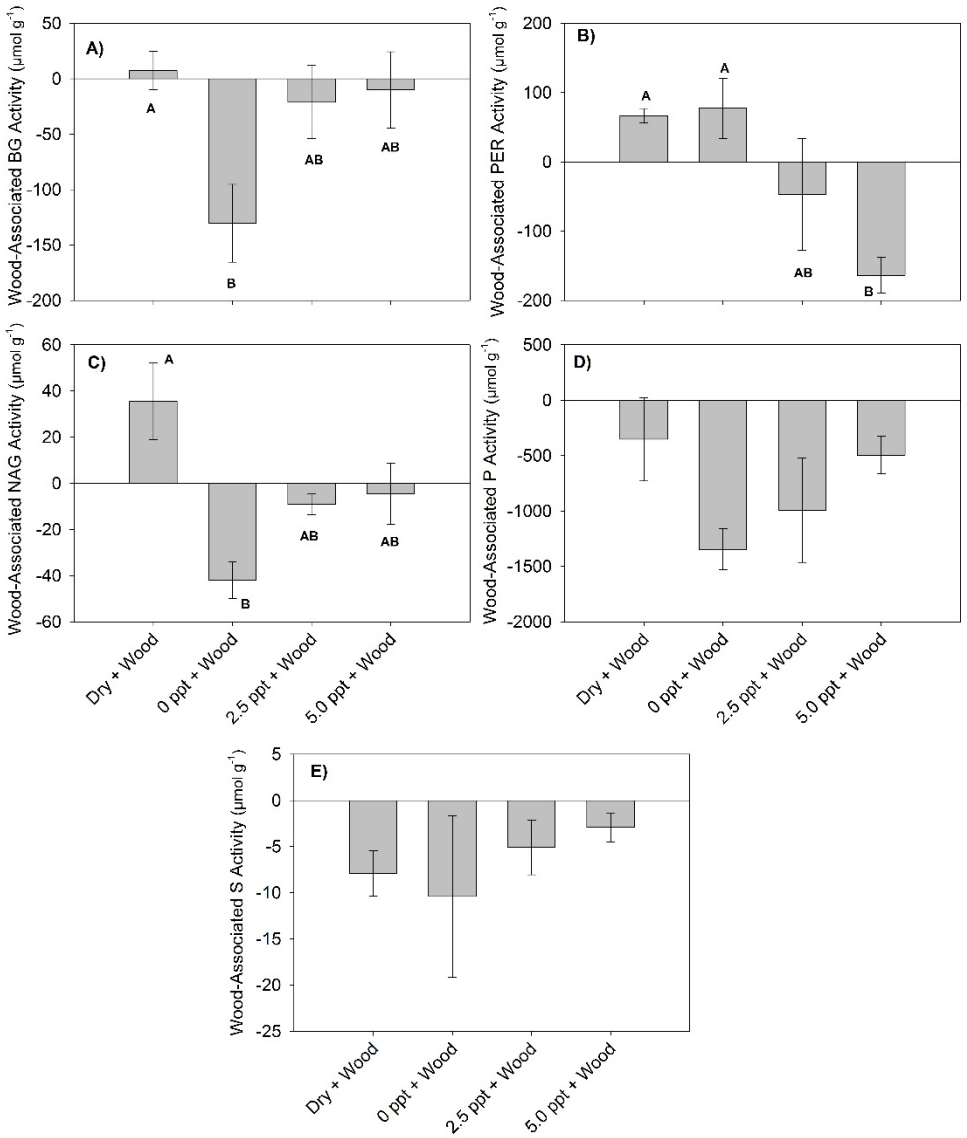


Figure 5~~6~~. Wood-associated (Wood-Amended – Wood Free) enzyme activity. Bars represent mean with standard error (n=4). Treatment means with different upper letters are significantly different ($P < 0.05$).


A)
Wood-Associated BG Activity (µmol g-1)
50
0
-50
-100
-150
-200
A
B
AB
AB
B)
Wood-Associated PER Activity (µmol g-1)
200
100
0
-100
-200
A
A
AB
B
C)
Wood-Associated NAG Activity (µmol g-1)
60
40
20
0
-20
-40
-60
A
B
AB
AB
D)
Wood-Associated P Activity (µmol g-1)
500
0
-500
-1000
-1500
-2000
E)
Wood-Associated S Activity (µmol g-1)
5
0
-5
-10
-15
-20
-25
Dry + Wood
0 ppt + Wood
2.5 ppt + Wood
5.0 ppt + Wood

1013