

Interactive comment on “Saltwater reduces CO₂ and CH₄ production in organic soils from a coastal freshwater forested wetland” by Kevan J. Minick et al.

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We appreciate the reviewers detailed feedback and believe they have improved the manuscript greatly. We have provided responses to the reviewers comments. We hope our revisions are in line with what the reviewer wanted.

Referee #2

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My comments refer to the version of the manuscript that was uploaded by Kevan Minick

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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 claim 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

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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 cm² 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 cm², 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—Altered, 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 “ñCushed

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 CH₄ 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 CO₂ and CH₄ 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 4°C (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 (without altering soil biogeochemical conditions as much as 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 extracellular enzymes picked to be analyzed? A short de-

scription 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. And 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'

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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 [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 [Anally](#) 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

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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 aren't 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 can't 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 freshwater, both within the field and laboratory.” – add references. Correct typo in freshwater. This correction has been made

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