

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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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, saltwa-

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ter 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 can't 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.
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.
3. 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

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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. 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.

4. Does the storage of the soil samples under 4°C for 7 weeks caused 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)?

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!

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 aspect 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.

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'

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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 final figure showing the possible environmental changes at non-tidal freshwater-forested wetlands after a sea level rise scenario based on your results.

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?

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).

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?

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.

L221 How much soil exactly was removed from the incubation?

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.

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).

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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.

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

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

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.

L433 When parameters like the redox potential in an incubation were measured, then it isn’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.

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.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-174>, 2019.

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