

## RESPONSE TO REVIEWER COMMENTS

We thank both reviewers for their constructive comments and suggestions and we have revised the manuscript to address their concerns. Responses to specific comments are below, in blue.

### Response to Reviewer #1

This manuscript focuses on exploring the mechanisms for enhanced methane emissions under warming. The authors make use of a long-term project, SMARTX, at the Smithsonian's GCRew site. They measured methane fluxes, porewater methane concentrations, and porewater sulfate concentrations in two areas with differing plant communities, one dominated by *Schoenoplectus americanus*, C<sub>3</sub> grass, and the other dominated by *Spartina patens* and *Distichlis spicata*, C<sub>4</sub> grasses. Each area contained ambient plots, and plots with temperatures elevated 1.7 °C, 3.4 °C, and 5.1 °C above ambient. The authors found that methane flux increased with soil temperature and that mean annual CH<sub>4</sub> emissions were higher in the C<sub>4</sub> community than in the C<sub>3</sub> community with methane flux only increasing incrementally with temperature in the C<sub>4</sub> community and only increasing at the 5.1 °C treatment in the C<sub>3</sub> community. In the C<sub>3</sub> community, porewater dissolved methane also increased at the 5.1 °C treatment. However in the C<sub>4</sub> community, there was no difference in porewater methane across treatments in the surface sediment and methane concentration declined with increasing temperature in deeper sediment. Sulfate depletion also increased with increasing temperature. The authors used these data to explore the mechanisms behind methane emissions in these communities noting that these methane fluxes are likely a result of increased rates of methanogenesis, increased substrate availability, reduced competition with sulfate reducers, and indirect plant effects.

Overall this manuscript is well written and very well thought out. It is a significant contribution to the literature in that there are very few groups with enough funding to perform a long-term warming project such as this. I commend the authors on the hard work and dedication that went into this study. The introduction does a great job of setting up the rest of the paper and the discussion thoroughly explores the mechanisms responsible for these emissions. The authors provide a great comparison of their results to other studies.

Thank you for your thoughtful comments.

My main critique is the discussion around the pattern seen in CH<sub>4</sub> concentrations at depth in the C<sub>4</sub> community. The decline in methane concentrations with warming indicates a shift in where methanogenesis is occurring. With a shift from below the rhizosphere at lower temperatures, to within at higher temperatures. Why do you think this is occurring? Is there less labile C at depth at high temperatures? Is there a shift in the type of methanogenesis occurring to methylotrophic methanogenesis? There should be more discussion of this potential mechanism as methylotrophic methanogenesis has been found to be important in coastal sediments (Zhuang et al., 2016; Xiao et al., 2018).

This is an interested observation that was not discussed in the paper, so thank you for bringing it to our attention. We agree that warming caused a depth-dependent shift in porewater CH<sub>4</sub> in the

C<sub>4</sub> community, with a simultaneous increase at the surface (0-20 cm) and decrease at depth (40-120 cm). This is stated in the results (lines 249-252) and expanded on in the discussion (lines 429-438).

As with our primary observation that warming increased CH<sub>4</sub> emissions, there are multiple mechanisms or processes that could cause such a shift in porewater CH<sub>4</sub>. We have added the following text providing brief speculation on these potential mechanisms: “Of the four mechanisms outlined above, perhaps the simplest explanation is an increase in labile C at shallow depths and a decrease in deeper soil. This is consistent with DOC depth profiles from this the C<sub>4</sub> community in which porewater DOC increases with warming in shallow samples but decreases with warming in deep samples (Fig. S7). This shallowing of peak DOC concentrations could be due to a warming-induced increase in evapotranspiration, leading to slower downward hydrologic transport of DOC-rich surface porewater to lower depths, or a warming-induced shallowing of the root system, leading to a shift in the location of root exudates.” (lines 430-438).

As support for this explanation, we added a figure of porewater DOC data into the supplement. Unlike the broader set of porewater data, DOC was only measured in 2019 so not originally included in the manuscript. However, because it does provide some explanation of the dissolved CH<sub>4</sub> trends consistently observed over the years and because the reviewer pointed out that we did not sufficiently explain those trends, we have now included those data in the SI (Fig. S7).

We have also expanded the introduction to outline the three pathways of methanogenesis, including methylotrophic methanogenesis (lines 54-58). While we do not have sufficient data to speculate about changes in methanogenic pathways, we have added text to the discussion about the need to quantify these pathways to better understand the underlying mechanisms (lines 323-331).

Minor comments:

Lines 111 – 112: Can you change the 10-20% of high tides to a comparison as all tides like you did for the low elevation areas? This will make the comparison between the areas easier.

Both percentages are actually for high tides, but we realized our original phrasing was unclear. We revised this section to read “The marsh is typically saturated to within 5-15 cm of the soil surface, but inundation frequency varies across the site, from 10-20 % of high tides in high elevation areas to 30-60 % of high tides in low elevation areas.” (lines 123-125).

Lines: 147 – 149: Using 1/2 of the LOD is not a great way to deal with non-detects. Checkout Helsel (2006, Chemosphere). Here they outline why substituting value for non-detects is not a great idea and how to deal with these data. They are a pretty small percentage of your data, but it could be useful to use other methods in the future.

Thank you for drawing our attention to this. Because the non-detects are such a minimal part of the overall flux dataset, there is no substantial difference in the overall effects and conclusions when these values are removed from the analysis rather than set to 1/2 of the LOD. As a result, we plan to continue using 1/2 of the LOD for the non-detects in this dataset, but will consider other options for future analyses.

Figure 1: Can you add a legend describing the colors on your density plot?

We edited the figure caption to read “Density plot shows the range of soil temperatures in each treatment, delineated by color: ambient (blue), +1.7 °C (green), +3.4 °C (yellow), and +5.1 °C (red).

Lines 356 – 359: I really like your discussion of differing plant communities as net oxidizers or net reducers here.

Thank you!

Supplement Table S2: There is a typo in the figure legend. "2017, 2018, and 2018"

Fixed, thanks for catching that.

## Response to Reviewer #2

The manuscript “Biogeochemical and plant trait mechanisms drive enhanced methane emissions in response to whole-ecosystem warming” by Noyce and Megonigal is a very interesting study that explores mechanisms that drive enhanced CH<sub>4</sub> emissions in tidal wetlands under warming. The authors point out that in wetland CH<sub>4</sub> cycling research there is little information about the coupling of plant responses to the dynamics of electron donors, acceptors and rates of competing or opposing microbial processes. Within the scope of the long-term SMARTX experiment (The Salt Marsh Accretion Response to Temperature eXperiment; Chesapeake Bay, USA) the authors measured monthly CH<sub>4</sub> emissions from 2016 to 2019 along with porewater analysis and vegetation biomass and composition measurements. There were three replicate transects in a C<sub>3</sub>-dominated community (*Schoenoplectus americanus*) and three in a C<sub>4</sub>-dominated community (*Spartina patens* and *Distichlis spicata*). Each transect consists of unheated ambient plots and heated plots (1.7 °C, 3.4 °C, and 5.1 °C above ambient). Their results show that warming of 5.1 °C more than doubled CH<sub>4</sub> emissions in both plant communities via the complex biogeochemical interaction of several factors and that plant composition can modulate coastal wetland responses to climate change. I am not an English native speaker but the manuscript reads very well. It was a pleasure to read it! Overall, it is a very comprehensive, well designed and organized study. I have only few remarks.

Thank you for your thoughtful comments.

1) It would be nice to describe the statistical analysis of the data more in detail. Data were log-transformed. Were they all normally distributed after log-transformation? In my opinion you should use time series analysis because of your monthly measurements. You should consider the decrease of correlation between measurements with increasing time distance. With linear mixed models you can nicely separate growing seasons from other periods.

Thank you for drawing our attention to this; we now describe our analyses in more detail. The revised statistical section reads: “Pearson’s correlations were used to test the relationships between CH<sub>4</sub> flux and soil temperature, and CH<sub>4</sub> flux and plant biomass. Responses of CH<sub>4</sub> emissions to vegetation type and warming treatment were analyzed using linear mixed models with vegetation community and warming treatment as categorical variables, and plot and year as random effects. P-values were calculated using Satterthwaite’s method and Tukey’s post-hoc tests were used to compare individual means. Porewater data was averaged per year and then analyzed using one-way ANOVAs to determine the effects of warming treatment or plant community, applying Tukey’s HSD test for post-hoc analyses.” (lines 201-208).

The flux and porewater data were normally distributed after log-transformation. We state this in lines 199-200.

Our main research question was to understand the overall effects of temperature treatment and vegetation community on CH<sub>4</sub> emissions as summarized over the 4-year dataset. Our understanding of time series analysis is that it focuses on trends of one index over time, allowing for modeling and forecasting, but does not allow for comparisons between treatments. However, we acknowledge the reviewer’s concern that sampling the same plots each month could lead to autocorrelation in the data. To account for this, we have reanalyzed the flux data using linear mixed models with plot and year as random effects. For the porewater data, we calculated yearly means before applying ANOVAs. We describe this updated analysis in the methods section (lines 203-208) and have updated all the in-text statistical summaries accordingly (lines 225-226, 227, 231, 242-243, 245, 246, 250-251, 253, 254-255, 258-259). This slightly alters final P-values as the new analysis is more statistically conservative, but does not change the significance of any of the findings from the original manuscript.

2) Did bulk density and mineral N (and maybe other soil characteristics e. g. pH etc.) differ between treatments. I think the authors should present these results since they may be major drivers of methane cycling.

We know from previous data that bulk density is very uniform throughout the soil profile and is also unresponsive to treatments because of the fact that the soils are highly organic. When carbon is added to or removed from the soil profile, the result is a change in soil elevation, not a change in bulk density. In addition, the near lack of soil minerals means that mineral nitrogen effects are negligible. We recognize that we only briefly touched on the organic nature of these soils in the site description and have expanded that section in the revised version to state: “The very low mineral content (<20%) affects methane dynamics because negligible competition between methanogens and iron-reducing bacteria for electron donors is expected in the absence of a significant pool poorly crystalline iron oxides (Roden & Wetzel, 1996), as has been documented previously at this site (Weiss et al., 2004) (Weiss et al. 2004). Soil bulk density in the upper 60 cm averages 0.124 g cm<sup>3</sup> and ranges from 0.079 to 0.180 g cm<sup>3</sup>. The relatively uniform bulk density of the soil profile reflects the uniform soil organic matter content and the fact that bulk density becomes largely independent of organic matter and mineral content once organic matter content exceeds 50% (Holmquist et al., 2018).” (lines 116-123).

However, to thoroughly address the reviewer's comment, we also measured bulk density in 1-m soil cores collected from ambient temperature and +5.1 °C plots. After 4.5 years of warming, there was no difference in mean bulk density throughout the soil profile. We measure pH as part of the suite of porewater analyses, but there has also been no effect of warming on porewater pH over the past four years. We now state both of these results in lines 217-219 for increased transparency.

3) Why did the authors not measure acetate concentrations? It would have been nice to compare acetate concentrations between treatments to discuss potential changes in the ratio between hydrogenotrophically and acetoclastically produced CH<sub>4</sub>. That would have improved the discussion about changing CH<sub>4</sub> emissions very much. The authors mention the role of acetate throughout the manuscript but do not mention the methanogenic pathways and their potential role for changing ecosystem methane emissions.

We agree with the reviewer that the potential effect of shifting methanogenic pathways is clearly important to this topic and that concentration data of acetate and other organic compounds would support the rest of this dataset. Unfortunately, at the time these data were collected, we did not have the ability to measure acetate. Our future plans include measuring low-molecular-weight organic compounds (including acetate) and in the revised manuscript lists that as one of the key next steps (lines 327-329). We also acknowledge that phrasing in the original manuscript may have led readers to expect to see acetate data presented. To minimize that confusion, we have replaced 'acetate' with 'low-molecular-weight organic compounds' throughout the revised manuscript (e.g. lines 316, 332, 334).

We also agree that a discussion of methanogenic pathways is needed and thank the reviewer for pointing this out. We have expanded the introduction to outline the three pathways of methanogenesis (lines 54-58) and added text to the discussion about the need to quantify shifts in these methanogenic pathways to better infer mechanisms (lines 323-331). As outlined in our original manuscript, our working hypothesis (based on our data thus far) is that the availability of acetate and other low-molecular-weight organic C compounds is higher in the C<sub>4</sub>-dominated areas, so these areas are also likely to have a high ratio of CH<sub>4</sub> derived from acetoclastic methanogenesis. In the future, we plan to use stable isotopes to track methanogenic pathways and to look at the composition of the methanogen community, to support these initial findings.