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Interactive comment on "Regulators of coastal wetland methane production and responses to simulated global change" *by* Carmella Vizza et al.

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General comments:

In this manuscript, Vizza and colleagues were interested in investigating CH4 production in freshwater and brackish wetlands of the Copper River delta in Alaska, USA. They made measurements of CH4 production of sediment slurries and also measured a range of physicochemical parameters in order to assess what factors were most important in controlling CH4 production. The authors also conducted two separate experiments to look at how various changes expected due to climate change – namely, increased salinity and increased organic matter inputs to the sediment – would affect CH4 production. The authors concluded that CH4 production was higher in freshwater vs. brackish marshes, a finding that has been reported elsewhere, and that sulfate

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concentrations and the composition of the microbial community played important roles in those ecosystem-related differences in CH4 production. When salinity and sulfate levels were experimentally raised, there was (unexpectedly) no change in CH4 production rates, which was attributed to a slow response of the microbial community. After adding organic matter from four different plant species to sediment slurries, the authors reported that CH4 production rates increased for two of the species but did not change for the other two.

I was very interested in the topics covered by this manuscript and think that the measurements and experiments can provide some insight into questions about controls of CH4 production and how global changes will affect CH4 dynamics. However, I was a bit disappointed in the analysis and interpretation of the data, especially in how the authors tied their findings to existing knowledge. For example: One of the global changes studied in this manuscript was the movement of saline water into freshwater wetlands, as should occur due to sea level rise. Although the authors mentioned several studies that have examined CH4 emissions along existing estuarine salinity gradients, they did not include any papers from the (steadily-growing) body of literature that has used focused experiments in the field and lab to determine how carbon cycling (including rates of CH4 production) responds to saltwater intrusion. As a starting point, you should look at recent work (last ~5 years) by Lisa Chambers, Nathaniel Weston, John Marton, Gijs van Dijk, Ashley Helton, and myself. A 2015 wetland salinization review paper in Ecosphere (Ellen Herbert = primary author) is another good place to consult. These papers will help the authors place their findings in the context of what is already known about the effects of saltwater moving into freshwater wetlands. Similarly, I felt that the authors could have done a better job exploring the literature on how plants and organic matter inputs affect CH4 production.

Beyond those issues of interpretation, I had several comments and questions about the experimental design and the statistical analyses that were conducted. Those comments, plus some others, are listed below.

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Specific comments: 1) p. 1, line 13; p. 3, line 12; and throughout manuscript, “freshwater and intertidal wetlands”. As someone who studies tidal freshwater wetlands, I take issue with the way you are classifying wetlands as either freshwater *or* intertidal. Wetlands can be both! Indeed, there are over 19,000 ha of tidal *and* freshwater wetlands within the Copper River Delta (see Hall’s chapter in the 2009 “Tidal Freshwater Wetlands” book; Barendregt, Whigham, Baldwin (eds). Backhuys Publishers). A better way of characterizing your two groups of sites would be “brackish intertidal” and “non-tidal freshwater” (unless, of course, your freshwater sites were also intertidal).

2) p. 1, lines 15-16: Your data clearly show that rates of CH4 production and porewater sulfate were each higher in the brackish sites. But, how did you determine that the high sulfate was the cause of the lower CH4 production? You also reported differences in porewater nitrate and acetate (top of p. 8) between ecosystem types, and presumably there were differences in salinity as well. How did you conclude that sulfate was the driving factor? Sulfate wasn’t even the most important factor from your modeling exercise (p. 8, lines 11-12).

3) p. 1, line 19, “. . .increased organic matter generally enhanced CH4 production rates.” This statement is too strong for your data set. You used four different organic matter amendments (= 4 plant species). The CH4 production rates increased for only two of the species you tested (Fig. 5); the other two species had no effect. So, based on your data, I don’t see how you can justify saying that the amendments “generally enhanced CH4 production.” If something happening 50% of the time means that it is a "general" occurrence, you could also say that the amendments generally had no effect on CH4 production.

4) p. 2, line 4, “. . .21 times more effectively. . .” This value of the global warming potential for CH4 over a 100-year time period is quite old and has been updated in each of the two IPCC reports that have been published after the Whalen paper you cited. The bigger point of this comment is that the global warming potential may not be the best way to compare CO2 and CH4 when one is talking about ecosystem processes, where

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gases are emitted or sequestered year after year. I discussed this in a paper that was published last year (Neubauer and Megonigal. 2015. Moving beyond global warming potentials to quantify the climatic role of ecosystems. Ecosystems. 18:1000-1013).

5) p. 2, lines 5-7, "Currently, wetlands at northern latitudes. . ." Other studies that have taken a longer temporal perspective have concluded that many northern wetlands have had a net cooling effect for the last 8,000-11,000 years (Frolking and Roulet 2007. Global Change Biology. 13:1079-1088). Because CH4 is broken down in the atmosphere – and therefore the warming due to CH4 emitted in any given year is transient – but the cooling due to C sequestration lasts "forever," a wetland that is old enough can have a lifetime net cooling effect, even if its radiative balance over a shorter period implies net warming. So, a single wetland could have a warming or cooling effect, depending on what time scale you consider.

6) p. 3, line 28: Why is the word "wetlands" in quotes? Are you suggesting that your sites aren't actually wetlands? Also, why are you comparing brackish intertidal marshes with (unvegetated?) freshwater ponds? My understanding is that you are trying to compare sites that differ in salinity and sulfate due to their effects on CH4 production. Why not compare vegetated brackish marsh with vegetated freshwater marsh? Or, brackish ponds with freshwater ponds?

7) p. 4, lines 10-11 and 17: I'm a bit confused by your sampling design. You collected a single sample from five sites along the salinity gradient. Elsewhere (e.g., p. 3, lines 16-17), you explained that you expect that the availability of sulfate will be an important driver of rates of CH4 production. Given that, why would you combine all the sites along the salinity gradient into a single "brackish intertidal" value?

8) You have quite a wide range of sulfate values (Table 1); was there a significant relationship between porewater sulfate and CH4 production? This would be another way of getting at your hypothesis about the effects of sulfate on methanogenesis.

9) p. 4, line 20: Does this sulfate concentration indicate the sulfate concentration

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in the water that was added when making the slurry or does it indicate the sulfate concentration in the slurry itself? Can you also report the salinity of the water added for the slurries (or the final salinity of the slurry, whatever is consistent with the sulfate concentration)?

10) p. 4, "Increased organic matter simulation" section: How much organic matter did you add to each bottle? Did you characterize the organic matter (e.g., C, N, P contents? lignin content?)? Did you use aboveground or belowground tissues? Were the tissues first cut to a standard size (e.g., passed through a grinder) before added to the bottles? Details such as those should be added to this section.

11) p. 4, line 30: The genus is Menyanthes, not Menanythes. The same genus name is misspelled in some of the figure legends.

12) p. 5, lines 5 and 9, and elsewhere in the manuscript. Generally, you include a space between a number and its units (e.g., "60 mL" on line 5) but other times you don't (e.g., "250mL" on line 5). I also remember seeing some places where you said that your experiment lasted for "14d" (instead of "14 d"). When editing, check throughout the manuscript to see that you include a space between a number and its units.

13) p. 5, line 12: It is a flame ionization detector, not a flame ionizing detector.

14) In the context of comparing between treatments within your study, it is fine to report your CH4 production rates as μmol per bottle per time. However, this really limits your ability to compare your results with those of others. Note, for example, that you would have gotten different rates (on a per bottle basis) if you had used a different volume of sediment, even if everything else was identical. At a minimum, you should tell the reader the weight of sediment in each bottle (e.g., "The 60 ml of added sediment was equivalent to 80-90 g of dry sediment."). It would be even better if you reported your rates as μmol CH4 per gram sediment per time.

15) p. 6, lines 1-2, "Porewater concentrations were scaled. . ." I don't understand what

this means. Are you saying that you multiplied the anion concentrations by the volume of porewater in order to determine the total amount of each anion in the bottle? Based on your nutrient/anion results (bottom of p. 7 to top of p. 8), I think this is what you did. But, why did you do this? As with the CH4 production rates (previous comment), reporting things on a per-bottle basis makes your numbers completely dependent on the amount of sediment (and its water content) that you ran through the centrifuge. It seems more straightforward to report your nutrients using molar units (e.g., mM) because those numbers are independent of the volume of sediment that was processed and can be easily compared with other studies.

16) p. 7, line 4, "total sulfate and nitrate" Is this fourth factor the sum of sulfate and nitrate? If so, why did you add them together? I recognize that both sulfate and nitrate are electron acceptors. Adding them together makes the implicit assumption that one mole of nitrate is "the same" as one mole of sulfate. However, the thermodynamics (i.e., energy yield) and stoichiometry (i.e., moles of sulfate or nitrate reduced per mol of carbon oxidized) differ for sulfate reduction and nitrate reduction. So, in terms of competing with methanogens for electron donors, one mole of nitrate is not equivalent to one mole of sulfate.

17) p. 7, line 7: What is delta i?

18) GLMs: I am approaching this manuscript as someone who is interested in the questions you are addressing but is getting lost when trying to understand and interpret the GLMs. Admittedly, this is because I have not ever received formal (or informal) training in GLMs. I am not expecting the revised manuscript or your "Response to reviewers" document to provide a tutorial in how GLMs work, but I hope that you will be able to make some modifications to the manuscript text that will make it easier for someone who isn't familiar with GLMs (such as myself) to follow the analyses that you did. Here are some of the things that are causing me grief:

a. In a GLM, what is a parameter estimate (e.g., as mentioned on p. 7, line 7)? I

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am familiar with multiple linear regression analyses, where each explanatory variable has its own parameter (or, "slope"). But, multiple linear regressions use continuous explanatory variables. In contrast, you have some nominal variables (e.g., ecosystem type, time period, macrophyte species). I can't begin to translate from my experience with multiple linear regressions to guess how you would come up with a parameter estimate for a nominal variable, or what such a parameter would even mean.

b. How do you estimate the importance of different variables? And, what does "relative importance" mean? I first thought that relative importance would be where everything is expressed as a fraction of the importance of the most important variable (so, the relative importance of the most important variable would be 1 and everything else would have a lower relative importance). But, that must not be the case since none of the variables for the GLM from the organic matter addition experiment have a relative importance of 1 (p. 9, lines 10-11).

c. Given Figure 6, it is apparent that you can use GLMs to generate predictive equations. Would it be worthwhile to include your best predictive equations in the manuscript for the reader to see?

d. I have no idea how to interpret the "Akaike weights" numbers in Tables 2 and 3.

e. What is a null model?

19) p. 7, Statistical Analyses: What are you using as your level of statistical significance – 0.01? 0.05? 0.10?

20) p. 7-8, Water column and porewater chemistry results: A student of mine did an experiment where he measured porewater concentrations of 130 μM sulfate, 5 μM nitrate, and 4 μM acetate. How do those numbers compare with yours? I don't actually want you to make that comparison but I want to make the point (again) that it is impossible for the reader to make that kind of comparison. I only know that you processed "$\sim$50 ml" of sediment but I have no idea of the water content of that sediment. There-

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fore, I cannot convert your values of the stock of sulfate or nitrate or acetate in a ∼50 ml chunk of soil to a concentration.

21) p. 8, line 9 and Tables 2 & 3, "total sulfate/nitrate" Earlier, I thought that you calculated sulfate + nitrate. Did you actually use the ratio of these anions in your analyses? What is the rationale for doing that?

22) p. 9, lines 18-20 and p. 13, lines 4-5: I am unclear how you concluded that it is important to consider the interactions of multiple global change mechanisms. I totally agree with that idea, but do not see how your results demonstrate the importance of studying interactions. After all, you only looked at individual factors, not at interactions. If you had done an experiment where you manipulated salinity and organic matter availability individually and together and had found that the interacting factors gave results that were unexpected based on single factor experiments, then you would have support for the idea that it is important to consider the interactions of multiple global change mechanisms.

23) p. 9, line 22-26, "Many studies. . ." Weston et al. (2014; Biogeochemistry. 120:163-189) is an example of a study that measured CH4 fluxes along a salinity gradient *and* measured rates of methanogenesis. Neubauer et al. (2013; Biogeosciences. 10:8171-8183) reported CH4 fluxes and CH4 production for a wetland that experienced >3 years of experimental saltwater intrusion.

24) p. 9, line 27, "directly linked lower CH4 production to higher sulfate and nitrate concentrations" This is odd phrasing since you saw higher CH4 production where you had higher nitrate; your sentence suggests the opposite pattern. I think the confusion here is related to the way you did the GLMs with sulfate+nitrate (or perhaps sulfate/nitrate) as a model factor. You chemically analyzed these anions separately, but then combined them in some way for the statistical analyses. As noted in earlier comments, I do not understand how/why you combined these anions for statistical analysis.

25) In your Statistical Analysis section (p. 7), you say that you used "general linear

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models." In the legends for Tables 2 and 3, you say that you used "general linearized models." A quick Google search for the exact phrase "general linearized models" did not reveal any statistics-related results in the first two pages of search results (I didn't look any deeper than that). A search for the same term (without the quotes) suggested Wikipedia pages for "general linear model" and "generalized linear model" as the top two search results. These are not the same thing. So, this comment is a long-winded request that you clarify whether you used "general linear models," "generalized linear models," or "general linearized models" (whatever they are) and to make sure that you use the correct terminology throughout your manuscript.

26) p. 10, lines 11-15: You cannot directly compare your CH4 production rates with those reported by Hines et al. (2008). Most importantly, Hines used 50 ml of slurry with 1 part soil to 3 parts total slurry volume; in each of your bottles, you used 120 ml of slurry that was 1 part wet sediment to 1 part water. All else being equal, we would expect higher CH4 production in your study simply because you had more sediment in your bottles. Expressing the rates per gram of soil/sediment, as I suggested earlier, would go a long way toward making your results comparable with those from other studies. [Helpfully, Hines et al. reported the typical weight of dry soil per milliliter of their slurry so you can get a rough idea of what their rates would be if expressed per gram of soil. Your data repository file lists sediment weights (wet or dry?) for each bottle.]

27) p. 10, last paragraph: Ultimately, this paragraph is unsatisfying. After seeing the huge June vs. August difference in rates of CH4 production, I was really hoping that you would be able to provide some strong insight into the cause(s) of that difference. I guess you are limited by data availability. Still, I wonder if others working in similar systems have reported order-of-magnitude changes in rates over such a short time period. I don't know anything about your system except what is in the manuscript but I'm wondering if the pattern could be related to the timing of soil thaw in the early growing season or perhaps the phenology of plant growth. Finally, I'll note that the measured

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concentration of acetate reflects the balance between rates of acetate production and acetate consumption. So, if higher acetate production in August was balanced by higher acetoclastic methanogenesis, you would see high rates of CH4 production without correspondingly high acetate concentrations.

28) p. 5, line 20 and p. 11, line 8: Change detectible to detectable.

29) p. 12, lines 5-8: The possible role of antimicrobial compounds is an interesting hypothesis and you presented some information to support it. However, I did not see where you tested for the effects of litter quality (e.g., C:N:P, percent lignin, lipid content) on CH4 production rates. Without having run those analyses (either the chemical analyses or the statistical analyses), why are you discounting the possible influence of those factors that have previously been shown to be important?

30) p. 12, line 13, "Fewer studies have examined. . ." There have been studies looking at CH4 emissions when wetland plants are grown in an elevated CO2 environment. Although there are important differences between CH4 emissions and CH4 production, the elevated CO2 studies generally find that CH4 emissions increase with elevated CO2, with this increase often being attributed to higher plant production (see, for example, Vann and Megonigal 2003. Biogeochemistry. 63:117-134).

31) Table 1: The legend says that you made 4-10 spot measurements per site. Given that, why aren't there any standard deviations or other estimates of error/variability for pond depth, temperature, pH, and salinity?

32) Data repository: I took a look at the data that you made available at the knd.informatics.org site and had a question about your CH4 production rates on the "All CH4 data" Excel worksheet. In column R, you reported "areal CH4 production (umol/m2/d)." How did you determine areal rates? What does an areal rate even mean in the context of a soil slurry in a bottle? Is the "area" the same as the cross-sectional area of the bottle? If so, that seems meaningless to me since a cylindrical bottle is going to have the same cross-sectional area whether the bottle is $\frac{1}{4}$ full, $\frac{1}{2}$ full, or $\frac{3}{4}$ full but,

presumably, would have different rates of CH4 production due to the different amounts of soil in the bottle. Given that you didn't report the areal rates in your manuscript, this whole things would be a question of curiosity. . .except that you used these areal rates to calculate the per-bottle rates (column S of the Excel file) that *are* reported in your manuscript. So, I need to know more about these areal rates before I can judge the validity of the per-bottle rates.

33) Data repository: I do not understand the formula that you used to go from areal rates (umol/m2/d) to per-bottle rates (umol/d): per-bottle rate = areal rate * 0.2 * sediment volume in liters. In order for the units to work out, the 0.2 factor must have units of m2/L. Those are odd units. Where does 0.2 m2/L come from and what does that conversion factor represent?

END OF REVIEW

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