

**Authors' response to referee's comments (RC1) on the manuscript, "Organic matter and sediment properties determine in-lake variability of sediment CO<sub>2</sub> and CH<sub>4</sub> production and emissions of a small and shallow lake" by L.S.E. Praetzel et al.**

Dear reviewer,

thank you very much for your comments and suggested improvements on our manuscript "Organic matter and sediment properties determine in-lake variability of sediment CO<sub>2</sub> and CH<sub>4</sub> production and emissions of a small and shallow lake". We are sure that consideration of your raised points and ideas will considerably improve the manuscript and therefore we will especially focus on revising the discussions part. Please find our responses to each of your comments below, they will be structured as follows:

(1) comments from referee, (2) author's response, (3) author's changes in manuscript.

We hope to be able to answer your open questions and to adequately include your suggestions.

Kind regards,

Leandra Praetzel & Co-Authors

**Major points:**

(1) 1.L.79-81: The Gibbs free energies given in the ms are either not found in the quoted literature (Whiticar 1999) or are different (Conrad 1999). I assume the reason is that they were calculated using energies of formation for gases in dissolved rather than in gaseous state. This would be consistent with the Nernst equations mentioned later (L.250) also probably using gas concentrations rather than partial pressures. However, the authors should clarify the procedures.

(2) Gibbs free energies were calculated using formation energies  $G_f^0$  for the elements involved in the reactions in aqueous state. Used  $G_f^0$  values are reported by Stumm & Morgan 1996 and Nordstrom & Munoz 1994. In the Nernst equation, dissolved and gaseous concentrations in incubation vials were used for calculations. This will be mentioned in the revised manuscript.

We will include the following explanation on calculation of  $\Delta G_r^0$  in the revised manuscript:

(3) *"Values for  $\Delta G_r^0$  are calculated from standard formation energies  $\Delta G_f^0$  at 25°C in aqueous state listed in Stumm and Morgan (1995) and Nordstrom and Munoz (1994)."*

(1) 2.L.187-200: There are no isotopic data reported, therefore the description of IRMS methodology is not necessary.

(2)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are reported in Table 2 and will be discussed in more detail in the results section of the revised manuscript. IRMS was also used to determine mass contents of C and N. The authors therefore prefer to leave the methodology description in the manuscript.

(3) *"C and N isotopic values did not vary much between sites and were on average -27.6 ‰ and -0.9 ‰ respectively with only one outlier for  $\delta^{15}\text{N}$  at site 3.50 (-6.3 ‰)."*

*"Neither CO<sub>2</sub> nor CH<sub>4</sub> production rates were exhibited significant correlation with C content or, C/N ratio,  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ,..."*

(1) 3. Table 2: L.411-412 mentions strong FTIR absorption features of polysaccharides. However, this compound class is not listed in the Table.

(2) Absorption maxima for polysaccharides will be listed in Table 2 of the revised manuscript.

(1) 4.L.422-425. This is an overview of measured rates. However, the numbers seem to be slightly different from those shown in Fig.2. Although there is probably a reasonable explanation for these differences, I found it confusing. In fact I would be happy just looking at the data in the figure without reading the text. However, one could mention that the rates decreased from the shore to the centre, since this point is later relevant in the Discussion.

(2) We double-checked the accordance of production rates stated in the text and displayed in Figure 2. We confirm that values in the text are the same as in the figure. We note that production rates in the text are mean values, whereas bold lines of boxplots are median values. We suppose that this fact might have caused the perceived discrepancy.

Moreover, we will simplify the figure by removing data from 5-10 cm depth and instead include this data in the supporting information.

We will further add a sentence to emphasize that production rates increased from the center to the shore as follows:

(3) *“Overall, production rates decreased from the shore to the center of the lake.”*

(1) 5.L.477-487: Here applies the same as in point 4. The data in the text seem to slightly different from those seen in Fig. 7.

(2) See also answer to point 4. We double-checked the data and confirm that the values in text and figure are the same. Please note that the stated values in the text are mean values +/- standard deviations of triplicate measurements at the respective sites where minima and maxima were measured whereas boxplots in the figure represent the whole dataset at each sampling date.

(1) 6.L.507: Again the data in the text seem to slightly different from those seen in Fig. 8.

(2) We will adjust the mentioned values in the text according to the displayed flux rates in Fig. 8 as follows:

(3) *“CO<sub>2</sub> and CH<sub>4</sub> fluxes measured from intact sediment core incubations ranged from 10.8 ± 4.39 to 17.89 ± 2.04 and 0.02 ± 0.01 to 1.51 ± 2.63 mmol m<sup>-2</sup> d<sup>-1</sup> respectively.”*

(1) 7. The discussion is too wordy and should be focused to the really novel results. I also recommend a different structure for the Discussion. I think it is not ideal having individual chapters on spatial variability of OM quality, spatial variability of CO<sub>2</sub> and CH<sub>4</sub> production rates, and influence of OM quality on gas production, since such structure results in too much repetition and also is not very suitable for explaining gas production rates on the basis of OM quality.

(2) We will shorten the discussion and focus on novel results in the revised manuscript. The chapters 4.1.1, 4.1.2 and 4.1.3 will be summarized as one, entitled “4.1.1 Variability of CO<sub>2</sub> and CH<sub>4</sub>

production rates and influence of OM". Like this, we will considerably shorten the paragraph and thus avoid repetitions.

(1) 9. The discussion on temperature effects can be much shorter, since it is rather well reported in the literature.

(2) In the discussion on temperature effects, we emphasize the sensitivity of small lakes to temperature changes compared to large lakes. We will shorten that section in the revised manuscript but prefer to leave that statement in the discussion, as this strong and variable temperature effect on production rates has, to our knowledge, not been shown before for small and shallow lakes.

(3) *"In accordance with previous studies (den Heyer and Kalff, 1998; Sobek et al., 2009; Gudasz et al., 2015), we found that with a temperature increase of 10°C, production rates of CO<sub>2</sub> doubled and those of CH<sub>4</sub> were 2 to 11 times higher. Q<sub>10</sub>-values for CO<sub>2</sub> were thus within the range of earlier reported values by Liikanen et al. (2002) and Berström et al. (2010), whereas Q<sub>10</sub>-values for CH<sub>4</sub> production were slightly higher than values found by Duc et al. (2010). The large observed range of Q<sub>10</sub>-values, especially for CH<sub>4</sub>, implies that responses to temperature increases might not be homogeneously distributed within a lake. We point out that sediment CH<sub>4</sub> production is more sensitive to increasing temperatures compared to CO<sub>2</sub> production and that this leads to a stronger feedback on global warming when considering the higher global warming potential of CH<sub>4</sub> compared to CO<sub>2</sub> (Marotta et al., 2014). The observed negative correlation between Q<sub>10</sub>-values and FTIR peak ratios further suggests that sites with more labile OM are more susceptible to increasing temperatures in terms of CH<sub>4</sub> production, whereas at sites with more recalcitrant OM, this recalcitrant OM may limit the degradation processes. We therefore assume that sediment greenhouse gas production in small and shallow lakes might in the course of global warming increase to a larger extent than in deeper lakes, as shallow waters, compared to deeper lakes, do not get thermally stratified in summer and therefore shallow sediments warm much faster (Jankowski et al., 2006)."*

(1) 10. The discussion of methanogenic pathways (L.648-680) is not really relevant, since the data just show that both methanogenic pathways were exergonic and thus, could well operate. Everything else is speculation and not relevant. The magnitude of the Gibbs free energy does not allow to conclude whether the one pathway is more prevalent than the other. One could however discuss the correlation of the concentrations of H<sub>2</sub> and acetate, and the respective Delta G, with sediment OM quality, since correlations were reported in the Results.

(2) We will shorten the discussion of methanogenic pathways and increase the focus on the reported relationships between Gibb's free energies and OM quality in the revised manuscript.

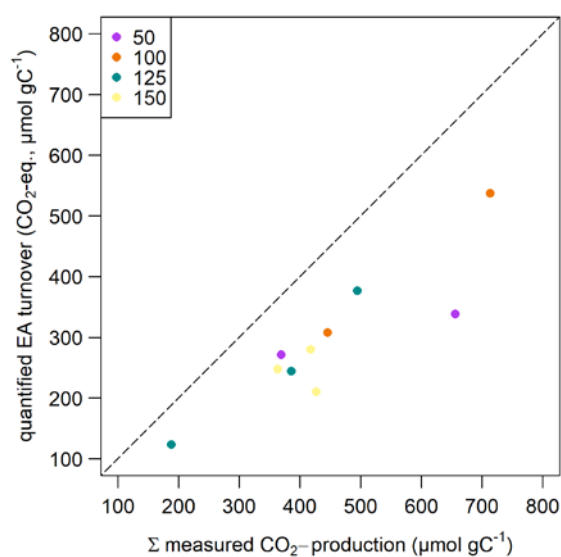
(3) *"[...] suggesting that both pathways could contribute to CH<sub>4</sub> production during the whole experiment. Still, this approach does not allow to evaluate which of the pathways predominates."*

*"[...] we would have expected a reverse pattern. Concomitantly, Gibb's free energy of hydrogenotrophic methanogenesis exhibited significant positive correlations with some FTIR peak ratios, although we expected that a high abundance of recalcitrant OM compounds would make hydrogenotrophic methanogenesis more feasible (Miyajima et al., 1997). Acetate and H<sub>2</sub> concentrations on the other hand, both exhibited significant negative correlations with some FTIR peak ratios. While this seemed reasonable for acetate concentrations (less acetate available in strongly decomposed OM), this result again proved to be against our expectations in terms of H<sub>2</sub> concentrations. One reason for these ambiguous findings might be that the system was not in a*

*steady state in terms of thermodynamic conditions. Similarly, long time scales of experiments still not reaching such steady state have also been observed earlier for peat columns (Bonaiuti et al., 2017)."*

(1) 11. The discussion of alternative electron acceptors (L.682-698) is rather short. The authors only discuss correlations. They miss the chance to discuss stoichiometric relations of reduced EAC with the amounts of CO<sub>2</sub> production. Although such mass comparisons apparently have recently been done by other members of the Knorr group (Gao et al. 2019), they would also be interesting for this particular lake. I have the impression that the magnitudes of reducible EACs might explain the CO<sub>2</sub> production in the beginning of the incubations, when rates of CO<sub>2</sub> production were larger than those of CH<sub>4</sub> production, while methanogenic decomposition of OM should result in equal rates. I wonder why this point is not addressed.

(2) In the revised manuscript, we will, besides observed correlations, also discuss the stoichiometric relationships between measured EAs and CO<sub>2</sub> production: We find that calculated CO<sub>2</sub> production from prevalent EAs is lower than the measured CO<sub>2</sub> production (see. Fig.). We propose this is due to unknown consumed EAs during the incubation that we did not capture; most likely iron in the solid phase. We indeed measured solid phase iron, but are not able to make statements about its speciation. But starting from the total Fe concentration in the solid phase (2-3%), we suppose that this is high enough to explain the missing EAs to reach a 1:1 ratio of measured CO<sub>2</sub> production and calculated EA turnover.



(1) 12. I noticed that lake sediments were anoxically preincubated for either one week (L:178) or 50 days (L.331). Please clarify! Anyway, the preincubation might have depleted most of the reducible iron and sulfur compounds. This may be the explanation for the low values of EAC<sub>inorg</sub> (Fig. 7), but is not discussed.

(2) The incubations were pre-incubated for one week, data from the sediment mesocosms was only used for analysis after 50 days of deployment in the climate chamber, in order to leave the cores adapt to laboratory conditions. We verified steady state conditions via the gas concentration in the sediment profile and observed constant conditions (i.e. no increase of concentrations) after 50 days. We will give a more detailed explanation on the procedure concerning sediment mesocosms in the revised manuscript:

(3) *“For statistical analyses and discussion, we only used measurements that were made >50 days after the deployment of the intact sediment core incubations in the climate chamber. This was done in order to ensure the system had adapted to experimental conditions and had reached a steady state. Steady state conditions were indicated by quasi-constant CO<sub>2</sub> and CH<sub>4</sub> concentrations in the sediment.”*

(2) We will additionally change the subtitle of the sections to clarify the difference between incubations and sediment mesocosms:

(3) *2.3 Intact sediment core incubations*

*2.3.1 CO<sub>2</sub> and CH<sub>4</sub> fluxes*

*2.3.2 Sediment gas stock change*

(2) Further, as suggested by the other reviewer, we will rename the sediment incubations to “slurry incubations” and the sediment mesocosms to “intact sediment core incubations”.

(2) The low EAC<sub>inorg</sub> values due to 1 week of preincubation will be discussed in the revised manuscript.

(3) *“Nevertheless, both absolute EAC<sub>inorg</sub> values as well as relative changes were very low, which might have been caused by the one-week preincubation, where most of the reducible inorganic compounds might have already been depleted.”*

#### **Minor:**

(1) 1.L.28: what means ‘sufficiently’ ?  $\rho=0.65$  is sufficient? Would  $\rho=0.6$  also be sufficient. Is there an objective criterion for sufficiency?

(2) The word sufficiently will be removed from the text.

(1) 2.L.30-32. I cannot follow the argument of this sentence. I suggest rephrasing.

(2) The sentence will be rephrased in the revised manuscript as follows:

(3) *“Our results show that within a small lake, CO<sub>2</sub> and CH<sub>4</sub> production show significant spatial variability, which is mainly driven by spatial differences in the degradability of the sediment OM.”*

(1) 3.L.67: cellulose is also a polysaccharide. I suggest rephrasing.

(2) Cellulose will be replaced by natural organic matter in the revised manuscript.

(1) 4.L.83: The Delta G-zero of hydrogenotrophic methanogenesis is more negative than of acetoclastic methanogenesis. Therefore the acetoclastic pathway is less (not more) energetically favorable.

(2) This will be corrected in the revised manuscript.

(1) 5.L.91: EAC has not yet been defined. Please check also for other abbreviations.

(2) This will be corrected in the revised manuscript. We will also check for other abbreviations and make sure that they will be defined when being used the first time and that afterwards, abbreviations will be used consistently throughout the whole manuscript.

(1) 6.L.107-109: The '4' in CH<sub>4</sub> as superscript

(2) This will be corrected in the revised manuscript.

(1) 7.L.168: 12 locations; please harmonize with the 13 sampling sites mentioned in the legend of Fig.1.

(2) Samples for incubations were taken from 12 sampling site, samples for sediment mesocosms were taken from 4 sites, whereas one of the mesocosms (S.150) represents the three sites of 150 cm depth from the incubations (1.150, 2.150 and 3.150) so that there are in total 13 sampling sites. This will be explained in more detail in the caption of Fig. 1 as follows:

(3) *"Location of the study area and 13 sampling sites within Lake Windsborn. Rhombus: Sampling sites for slurry incubations and intact sediment core incubations, circles: sites for slurry incubations only, asterisk: site for intact sediment core incubation only (reference for 1.150, 2.150 and 3.150)."*

(1) 8.L.207: 'relative abundance' compared to what?

(2) FTIR absorption peaks show relative abundances of the corresponding functional moieties in a single sample compared to another moiety. We therefore calculate peak ratios normalized to the polysaccharide peak. The sentence will be changed as follows in the revised manuscript:

(3) *"Distinct peaks at specific wavelengths were assigned to functional groups according to Artz et al. (2008) and normalized to the peak intensity at 1031 - 1035 cm<sup>-1</sup> (indicative of polysaccharides) in order to obtain inter-comparable peak-ratios of functional moieties in all samples as FTIR spectra only provide information about the relative abundance of certain functional moieties in one sample."*

(1) 9.L.268: EAC/EDC: I think you mean EAC & EDC rather than the ratio between both. I found similar possible confusions at many places in the text (e.g., L.293, L.369, 370, 383 and in the labels of Fig. 7. Please check carefully.

(2) Notations will be changed in the revised manuscript as suggested by the reviewer.

(3) *(EAC<sub>OM</sub>, and EDC<sub>OM</sub>)*

*EAC<sub>OM</sub> (EDC<sub>OM</sub>)*

$$\Delta CO_2 = ((c(CO_2)_{end} * V_{seg}) - (c(CO_2)_{start} * V_{seg})) / \Delta t \quad (11)$$

$$\Delta CH_4 = ((c(CH_4)_{end} * V_{seg}) - (c(CH_4)_{start} * V_{seg})) / \Delta t \quad (12)$$

*ΔCO<sub>2</sub> (ΔCH<sub>4</sub>)*

*total C and N*

*Fig. 7 label: EAC & EDC (μmol e<sup>-</sup> gC<sup>-1</sup>)*

(1) 10. L.299. The reference Tamura et al. (1974) only describes the analysis of Fe(II) (albeit in the presence of Fe(III)). How was Fe(III) analyzed?

(2) Fe (III) in the samples was reduced to Fe (II) with 10% ascorbic acid and determined likewise. The procedure will be explained in the revised manuscript as follows:

(3) *“Because 1,10-phenanthroline can only detect Fe<sup>2+</sup>, the Fe<sup>3+</sup> in the samples was reduced to Fe<sup>2+</sup> with 10% ascorbic acid. Then, the determined concentration of total Fe was used to calculate the concentration of Fe<sup>3+</sup> in the samples.”*

(1) 11. L.477-479: I cannot follow this sentence. Also compare major point 6 above. Please also note, that Fig. 7 is not mentioned in the text, and that Figure number should be exchanged with that of Fig. 6, since Fig.6 is reported later in the text than Fig. 7.

(2) The order of the figures will be changed and mentioning and numbering of figures will be adjusted in the revised manuscript. The sentence in ll. 447-449 will be changes as follows:

(3) *“EAC<sub>OM</sub> lay between 218.69 ± 97.15 and 545.71 ± 60.33 μmol e<sup>-</sup> gC<sup>-1</sup> at t0 and decreased on average by 44.85 μmol e<sup>-</sup> gC<sup>-1</sup> until t6. Highest values for EAC<sub>OM</sub> were found at site 3.125 corresponding to lowest measured CH<sub>4</sub> production rates at that site.”*

(1) 12. Table 3: Showing the time line as t0, t1, t2 etc. is awkward, since one has to consult the explanation in the methods section. I suggest listing the actual time points, i.e. 0, 1, 3 etc. days.

(2) The captions in Table 3 will be changed as suggested by the reviewer.

(1) 13. Table 4: The numbers in the table show too many decimal positions. Please report only those that are significant. In fact, at numerous places in the text numbers seem to show non-significant decimal positions. Please check and correct.

(2) The numbers in Table 4 as well as other number with non-significant decimal positions will be changed as suggested by the reviewer.

(3)

	CH <sub>4</sub> flux			CO <sub>2</sub> flux		
	rho	p	n	rho	p	n
Clay	0.648	< 0.05	12	0.605	< 0.05	12
Silt	0.497	<i>n.s.</i>	12	0.302	<i>n.s.</i>	12
Sand	-0.648	< 0.05	12	-0.605	< 0.05	12
Fats, waxes, lipids	-0.833	< 0.05	8	-0.333	<i>n.s.</i>	8
Phenols; humics	-0.833	< 0.05	8	-0.357	<i>n.s.</i>	8
Aromates	-0.595	<i>n.s.</i>	8	-0.524	<i>n.s.</i>	8
Lignin	-0.786	< 0.05	8	-0.381	<i>n.s.</i>	8
C/N	-0.881	< 0.01	8	-0.333	<i>n.s.</i>	8
C (%)	-0.714	<i>n.s.</i>	8	-0.190	<i>n.s.</i>	8
CH <sub>4</sub> sediment stock change	-0.222	<i>n.s.</i>	41	0.05	<i>n.s.</i>	35
CO <sub>2</sub> sediment stock change	-0.049	<i>n.s.</i>	41	-0.064	<i>n.s.</i>	35

(1) 14.L.535, 538: Should be Table 4 rather Table 5.

(2) The numbering will be changed in the revised manuscript.

(1) 15. References. Some of the references use capital letters for the titles.

(2) Capitals will be changed according to the reviewer's suggestion.