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Discussions

Interactive comment on "Microbial methanogenesis in the sulfate-reducing zone in sediments from Eckernförde Bay, SW Baltic Sea" *by* Johanna Maltby et al.

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We would like to thank the reviewer for her/his critical comments, which we think helped to improve the quality and clarity of this manuscript. We hope our responses and adaptations are adequate to accept this manuscript for publication in Biogeosciences. Please find our detailed responses below.

Anonymous Referee #1

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Shallow littoral sediments are a poorly constrained source of methane to marine and

brackish water columns. Normally, methane fluxes from marine sediments into the water column are restricted by the large fluxes of sulfate available to microbial sulfate reduction taking place in the sediments. This "microbial lid" on methane effluxes derives in part from the competitive advantage of organoclastic sulfate reducing bacteria versus methanogens for buried reactive organic carbon substrates, and also to the direct oxidation of upward diffusing methane by methanotrophic sulfate reducing prokaryotes. However, methane ebullition from deeper layers into the surface sediments, or the production of methane from non-competitive substrates (e.g. methyl amines, or methanol) may contribute significantly to the methane flux into bottom waters. It is the latter pro- cess that the authors of this study seek to address and quantify in Eckernförde Bay sediments. Their approach can be divided into two parts: 1) a seasonal study of sediment methane biogeochemistry, including rate measurements, and 2) an experimental enrichment to examine the effect of methanol as a potential non-competitive substratein Bognis Eck sediments. Maltby and co-authors present a detailed seasonal data set showing geochemical and experimental data collected over two years from the shallow, organic-rich sediments of Eckernförde Bay in the Baltic Sea. Although it has been known now for decades that minor amounts of methane forms in sulfate-reducing sediments from methanogenesis of non-competitive substrates, the role that this process plays in Eckernförde deep waters was not clear prior to this study. The data and outcome of the present study are consistent with previous studies of methanogenesis using non-competitive substrates and suggests that methane derived from non-competitive substrates may be a source of methane for the Eckernförde deep water. This study adds to the data and knowledge concerning sediment biogeochemical processes for Bognis Eck, which has been the site of a successful string of studies investigating the biogeochemistry of deep anoxic waters and the underlying sediments in Eckernförde Bay. The geochemical data is of high quality. The down core experimental tracer data is also of good quality, although I have reservations about interpretation of some of the experiments (see Major Issues below). Nevertheless, there are a number of points in the manuscript that the authors need to address.

Major issues: 1. Section 3.4.1 (and Methods – lines 235-244) Net methanogenesis: These rates do not necessarily represent methanogenesis in the presence of sulfate. Were the sulfate concentrations monitored during the incubations? There are no time course data of sulfate (nor methane) shown for these experiments. As the incubations were performed over four weeks, the chances that sulfate became depleted within several days at many of the depths is very likely given SR rates of up to 10000 nmol cmËĘ3 dayËĘ-1. Therefore, the direct comparison of the 14C labeled hydrogenotrophic rates with Net Methanogenesis rates are not at all valid. The Net MG rates are very likely a severe overestimation of actual in situ rates of methanogenesis.

Authors Reply: Thank you for initiating this discussion. We will add all methane development graphs to the supplementary material. Measurements of sulfate during the incubation was unfortunately not possible as we worked with closed headspace systems. We agree that sulfate likely declined during the incubations as we expected simultaneous sulfate reduction activity and because no sulfate was supplied from the overlying water. However, if sulfate would have been completely depleted over the course of the incubation, we would have expected a change in the steepness of the methane production, i.e. an increase in methane production after sulfate was exhausted, which was not the case. Secondly, a quick calculation using sulfate reduction rates from a past study (Bertics et al. 2013) and our sulfate concentrations tells us that sulfate was unlikely depleted. In the surface sediment, where sulfate reduction is usually highest ($\sim$200 nmol dm-3 d-1) together with sulfate ($\sim$18 μmol cm-3), sulfate would have theoretically been depleted to 12 μmol cm-3 within a 30 day incubation. In the deepest layers, where sulfate reduction is lowest ($\sim$10 nmol dm-3 d-1) together with sulfate ($\sim$2 μmol cm-3), sulfate would have theoretically been depleted to 1.7 μmol cm-3 within a 30 day incubation. As these calculations illustrate, we do not expect total exhaustion of sulfate and we also see no evidences for this in the methane production rates.

2. Likewise, the Manipulated Methanogenesis experiments are not described in enough detail to evaluate them properly. Were these experiments performed like the

Net Methanogenesis experiments? Or were they performed over shorter period of time using radiolabeled bicarbonate?

Authors Reply: We agree with the reviewer that some critical information concerning the methods for the manipulated experiment (e.g. incubation time) were missing. These experiments were performed like the net methanogenesis rates (besides the manipulation) and we have added this information to the methods section.

The handling of samples and incubations for the determination of hydrogenotrophic methanogenesis is described in chapter 2.7.1.2 “Hydrogenotrophic Methanogenesis”.The handling of samples with radiotracer was different from net methanogensis given the different nature of this rate determination method.

3. I am not sure how insightful the 13C-labeled methanol enrichments are for understanding the role of non-competitive substrates at this site. First of all, no in situ methanol concentrations are provided. Secondly, and more importantly, the authors added methanol up to 10 mM. These are enrichment concentrations that are not likely to reflect environmental conditions. Enrichment, or growthn methanol, is what they see in the experiments, as shown in Figures 6 and 7. The conclusion that these enriched organisms represent the in situ organisms and metabolisms is not tenable. This experiment does not even shed light on whether or not there was non-competitive methanogenesis occurring in the experiments slurries themselves. What happened to sulfate during this experiment? Was there still sulfate present after 10 days?

Authors Reply: Regarding sulfate concentration, please see our comment above. We are aware that methanol concentrations applied in our study were higher than usually observed under environmental conditions. The purpose of this experiment was to investigate the potential of the methanogenic community to use methanol as a non-competitive substrate. Other studies did similar stimulation experiments to demonstrate the activity of this metabolic group in the presence of sulfate reduction (see e.g. Oremland and Polcin 1982). We can of course not make any statement, how important this

process is under in situ conditions in relation to those that use other non-competitive substrates for methanogenesis, which we now discuss in more detail.

4. The Discussion needs to be made more concise. The authors should directly address the stated main point of the manuscript: Is there methanogenesis in the sulfate reducing zone, does it proceed via non-competitive substrates, and is it at all important for methane fluxes to the deep water? The discussion as written now is, to a large extent, a reiteration of the results with some commentary. It also tends to drift off into unwarranted speculation. Some parts that could be excised without detriment:

a. Lines 564 and following : "possible" additional sources of carbon and the production of hydrogen Authors Reply: We believe that this discussion is important to explain the observed higher rates in March; hence, we would like to keep it.

b. Lines 626 "Reaction of sulfide with methyl groups and organic matter. . .discussion is beside the point. Authors Reply: We agree that this part is too extensive and deleted it.

c. Lines 646 Discussion of dissolution of CO2 in water was already discussed earlier in Results. Authors Reply: We deleted this part as it is repetition.

d. Section 670 The discussion on temperature is speculative and I am not sure where it is leading. Authors Reply: We do think that the positive correlation between temperature and methanogenesis is an important point to mention, as temperature is a strong environmental factor in this temperate environment, which could explain some of the variations in methanogenesis. We clarified this.

e. Lines 783 and following: The discussion of deep methanogenesis (below the SMTZ) appears to be beyond the scope of the manuscript (i.e. methanogenesis in surface sediments)

Authors Reply: As the majority of methanogenesis occurs below the SMTZ, we believe it is very important to compare both surface and deep methanogenesis and their po-

tential to emit methane into the water column for assessing the relevance of surface methanogenesis.

One means of shortening the discussion might be to delete or severely scale-back to the discussion revolving around the PCA analysis. I do not see how the analysis and resulting discussion adds anything new to our understanding of the controls on methanogenesis in marine sediments. In considering such a discussion, it might be worth for the authors to revisit the seminal articles on this topic by Crill and Martens (L&O 1983 and GCA 1986).

Authors Reply: In our opinion, the PCA analysis is crucial, as it gives us statistical security about the potential environmental controls on surface methanogenesis. Without a statistical analysis, the discussion about environmental controls would be very speculative. Our study brings new insights into environmental controls, as we are one of the first ones studying environmental controls on surface methanogenesis within the sulfate-reducing zone. Therefore, we like to keep this part.

Specific Comments: Line 282. This sentence is confusing. "Fast oxygen consumption" does not correlate with "slowed microbial activity".

Authors Reply: What we meant was that due to quick exhaustion of oxygen in the core after retrieval, i.e. after capping the core from oxygen supply, organic matter degradation shifted to slower anaerobic processes. We clarified this in the manuscript.

Figures 1 and 2. The postage stamp size plots (at least in the BG Discussions version) are difficult to read. Perhaps taking he water column data out and combining it into a separate figure would help?

Authors Reply: Separating the water column data from the other profiles would make it harder for the reader to the connection between e.g. possible oxygen depletion in the water column and high surface methanogenesis rates. We therefore would like to keep it together. To make it easier to read, we increased the font size on the plots and also

left out some redundant axis titles.

Lines 424-434. I would not put so much emphasis on the single bottom points of the gravity core.

Authors Reply: We think it is necessary to discuss the increase in sulfate at 350 cmbsf , as sulfate is a crucial factor for methanogenesis..

Line 469: The hydrogenotrophic methanogenic activity at 45 cm depth at the sulfatemethane transition zone may be in part due to tracer back flux associated with AOM (see Holler et al., PNAS 2011). Authors Reply: Thank you for this valid point. That peak in hydrogenotrophic methanogenesis is indeed situated at SMTZ, which is why tracer back flux from AOM is possible. We added this information to the discussion part under 4.1.1.

Figure 7: What is the difference between the methane concentration in this figure and in Figure 6? Why not combine Figures 6 & 7?

Authors Reply: Figure 6 and 7 (now 7 and 8) show the results of two different experiments even though both are from September 2014. Figure 7 shows sediment methane concentrations from the 0-1 cmbsf sediment interval over a more detailed sampling period (at least in the first 10 days) after the addition of non-labeled methanol. Figure 6 focuses on a different sediment interval (0-2 cmbsf) and the addition of 13C-labeled methanol with resulting headspace methane content and isotopic composition. We tried to clarify the figure captions.

Lines 525 and following: What are the criteria for calling something a “strong” or “weak” correlation.

Authors Reply: We decided to delete any characterization of “strong’ or “weak” correlations in the text, as it is hard to identify correlation strongness with PCA. We therefore focus on positive, negative or zero correlation.

Line 554 It might be good to briefly describe how BES works as an inhibitor, and why it

has no effect here.

Authors Reply: Thank you for this helpful comment. We added the function of BES and the possible explanation for BES insensitivity to this paragraph.

Line 566 How deep is bioturbation in Bognis Eck? And was the shell at 20 cm living or just debris?

Authors Reply: From previous studies we know that the bioturbation depth in Eckernfoerde Bay sediments is around 10 cm (e.g. (D’Andrea et al., 1996; Orsi et al., 1996; Bertics et al., 2013; Dale et al., 2013We added the bioturbation depth in line 588. The mollusk shells were empty, and we added this information to the text. It would not be correct in our opinion to call it shells debris, as we cannot be sure if the mollusk was still alive when we collected the core (and died during core storage).

Figure 8: Based on what criteria was 0-5 cm depth for integrated methanogenesis chosen, whereas, similar data, but from 0-25 cm is shown in Figure4?

Authors Reply: In Figure 8 we provide a closer look at methanogenesis directly at the sediment-water interface (0-5 cm), as this layer is likely to be most impacted by water column parameters. Figure 4 on the other hand provides an overview of the total integrated (0-25 cm) surface methanogenesis activity over the sampling period to investigate variations between months.

Line 614: Again, this looks like a growth curve.

Authors Reply: We agree with the reviewer and added this discussion.

Line 637: These organisms became dominant due to the highly enriched methanol concentrations employed. This does not say anything about their importance under in situ conditions.

Authors Reply: We thank the reviewer for this valid point. While we can make assumptions about the initial presence of methylotrophic methanogens under in-situ conditions,

we cannot make assumptions about their abundance. We adapted the interpretation accordingly.

Line 690 and following: Changing sulfate concentration-depth profiles as a response to changing salinity conditions indicates that this is a non-steady-state situation. Ergo, it is not possible to use this as an indication of microbial sulfate reduction.

Authors Reply: We are not sure if we understand the comment of the reviewer correctly. What we are trying to say (and which has been shown in other studies) is that due to the close coupling of sulfate to salinity, a decline in salinity would imply a decline in sulfate and hence a faster exhaustion of sulfate in the sediment leaving less organic matter to sulfate reduction. We clarified this part.

Line 841: How does the fueling of AOM above the SMTZ cause methanogenesis to play an “underestimated” role? I would expect that AOM would minimize the impact of methanogenesis on the water column methane budget.

Authors Reply: Thank you for this comment. Yes, AOM would minimize emissions and this is surely the case. But this close link between methanogenesis and AOM has been overlooked so far. We added a few comments on carbon cycling.

Technical comments: Line 138 “that” instead of “which”

Authors Reply: Done

Line 437 “Content” not “concentration” for POC wt%

Authors Reply: Done

Line 612: Sentence is confusing: “of” rather than “if”?

Authors Reply: Done

Also, the population changes to the new conditions; you do not have any evidence for adaption (and evolutionary concept).

Authors Reply: Formulation changed, deleted “adapted”.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-36/bg-2017-36-AC1-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-36, 2017.