

## ***Interactive comment on “Efficiency and adaptability of the benthic methane filter at Quepos Slide cold seeps, offshore Costa Rica” by P. Steeb et al.***

**P. Steeb et al.**

ttreude@g.ucla.edu

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We could like to thank all four reviewers for their critical comments, which we think tremendously 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.

P. R. Dando pdando@mba.ac.uk Received and published: 13 January 2015

General comments This paper describes experiments, using 16 cm long sediment cores, to examine how the microbial community in sediments from a cold seep off Costa Rica respond to changes in seepage flow. In particular, how the rate of anaerobic

oxidation of methane changes over time. A synthetic, methane-rich, seepage solution was pumped into the core base and sulphate was allowed to diffuse into the core from the surface, to simulate natural conditions. Samples were withdrawn from the cores at approximately 1 cm depth intervals and at periods during the experiment. The experiment showed clear geochemical changes in the cores over time and demonstrated that the microbial rates adjusted, with time, to increased or decreased flows. A detailed critique of the experiment is given below. This paper would be enhanced by a section looking at the advantages and disadvantages of the technique and discussing alternative approaches.

Author Reply: A full discussion of the advantages and disadvantages of the SLOT system is provided in the original publication of this method (Steeb et al. 2014, *Limnology & Oceanography Methods*).

1. One problem with the experimental technique was that, even after 258 days, the system had not reached equilibrium, partly due to the long residence time of the pumped fluid.

Author Reply: We are aware of this issue. While the HFC reached quasi steady-state conditions, the LFC was still in the transition phase. You might argue if true steady state (or equilibrium) is ever reached in natural advective systems or if this is more of a theoretical concept. But that would be a different discussion. For our study, this is as good as it could get, since experimental runtimes of over 1 year are difficult to maintain. We already discussed the fact that steady state was probably not reached in the LFC (see 4.1) and added some more comments about it into section 4.2.

2. In addition, the method and frequency of sampling may have affected the rates. The least invasive technique to study changes in a flow through system would be to measure just the inflow and outflow.

Author Reply: Well...yes and no. If you just measure the in and outflow, which we fully agree would be the most non-invasive method, you have a black box and would learn

nothing about the evolution of geochemical gradients and the position of the SMTZ. Such systems have been created before (see, e.g., publications by Girguis or Wegener et al.) but they aimed at different research questions. For our purpose, this system was the best compromise. For a detailed comparison between the SLOT system and other systems, please refer to Steep et al. 2014.

The authors could discuss whether using short (syringe) cores, with sediment taken from the SMTZ and from above and below this zone, in a flow system might be more effective. Equilibrium should be reached more quickly and it would be simple to study how the microbial community at different depths responded.

Author Reply: We thank the reviewer for his comment, but will not discuss this aspect, because it is not the purpose of this paper to repeat the method discussion. Please refer to Steeb et al. 2014 for all the details. It would also not be advantageous to pick sediments from different depths and study them in short cores, as it would not be normal for deep sediments section to have such a short (diffusional) distance to the overlying, sulfate-rich water. This application would create even more artifacts.

A disadvantage of this is that it would not allow for the migration of bacteria in the seep fluid from deeper to shallower layers.

SLOT-System: 3. Subsampling the MUC core samples with the SLOT core tubes, if done by just pushing the latter into the former, as described in Steeb et al. (2014), given the core tube thickness, would cause sediment compression. There is no mention of the degree of compression caused, i.e. how much lower the sediment surface was in the SLOT tube compared with the MUC tube. The way to overcome this is to either use a piston, that is withdrawn at the same rate as the core tube is advanced, or by using a controlled vacuum applied to the top of the core to keep sediment levels equal as the tube is pushed deeper.

Author Reply: We tried sub-sampling with the SLOT liner (6 cm diameter) using a piston similar to what is done for the small rate liners (2.6 cm diameter), but given its

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much larger diameter, it was difficult to apply the right vacuum. In the end it worked pretty well by just slowly pushing the SLOT liner into the MUC core. The sediment level inside the SLOT liner remained on the same level as the MUC sediment level. No sediment compaction was obvious, although it might not have been completely avoided. Keep in mind that the piston method has its own flaws. If the applied vacuum is stronger compared to the speed at which the liners are pushed into the sediment, the sediment is sucked above the MUC core sediment level, which will also affect sediment properties.

4. Sediment would be further consolidated by the downwards pumping of water through the core during the 40 day "preparation phase". The pumping rate used during this phase is not stated. Thus the density of the sediment in the experimental cores may not reflect that of the sediment in situ. Both SLOT cores had a lower porosity than the MUC core (Figs. 8 & 9), suggesting sediment compression.

Author Reply: We added the pump rate (thanks for noticing), which was 20 ul/min, and added a comment about potential sediment compaction to the method (see 2.5).

5. The volume of pore water removed during the sampling periods appears to be excessive and may have had an effect on the experiment. Assuming that the core i.d is 6 cm (Steeb et al 2014), the core length is 16 cm and porosity is 0.85, then each core contains approximately 385 ml of pore water. With 16 sediment sampling points per core (Fig. 3) and 1.5-2ml/sample, then 24-32 ml of pore water is removed every sampling period, i.e 8 % of the pore water volume in the core. This would have to be replaced either by sediment compaction or by advection from the surface and I do not see how it can be stated "sulfate was transported into the sediment core solely via diffusion". Thus, for the low-flow experiment, advection from the surface to replace the pore water sampled, would be almost equivalent to the flow from the core base over the period to the 49 day sampling interval. This would have the effect of displacing the SMTZ downwards. In the high flow experiment the effect of removing pore water by sampling is reduced since the advective flow due to the pumped "seep water" would

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be much greater than the downward advection due to pore water removal.

Author Reply: The reviewer is absolutely right. The removal of porewater with rhizons is causing a smoothing of the geochemical profiles. This effect has been thoroughly discussed in the original publication of the method (Steeb et al. 2014). We added this information to the methods (see 2.5)

6. The bromine profiles in the low flow experiment are not adequately explained. It would help to visualize what happened by altering the scale of the bromide concentrations in Fig. 3. Why was Br elevated in the overlying water after 105 days but this disappeared subsequently? Could there be channels that opened and closed over time?

Author Reply: It is possible that a sudden channeling caused a spontaneous emission of advective water into the supernatant and then disappeared again. We can only speculate. We noted the increase of bromide in the supernatant in the results (see 3.2.1) and further highlighted channeling in the discussion (see 4.1).

7. The authors note that the "methane concentrations were lower than those potentially encountered under in situ conditions because the cores were not pressurized". Certainly some of the concentrations measured in core SO206-31 would lead to oversaturation at atmospheric pressure and bubble formation when the core was recovered. We are not told whether this occurred and gas channels could have formed in the SLOT cores, leading to channeled advection of the pumped "seep water".

Author Reply: Small bubbles formed after core retrieval (noticeable as "foamy" sediment) but no bubble release at the surface was visible. The bubbles disappeared after a while, probably through a combination of consumption, dissolution and diffusion. We can only speculate about channels formed through gas bubble formation. It is likely that the permeability of the sediment increased while foamy and decreased again after bubbles disappeared. Since the cores were not immediately set up in the SLOT system, but first stored on board and then transported to Germany (total ca. 170 days,

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information added to 2.5), there was plenty of time for the sediment to resettle.

It would also be interesting to know what effect the insertion of the line of rhizomes has on flow dynamics in the cores.

Author Reply: Of course the rhizons had some effect on the flow dynamics, but since the effect was the same for the high and the low flow core, we are confident that we can compare. We noticed that the sediment showed basically no resistance when the rhizons were introduced, which would suggest that the sediment was able to easily settle around them. But keep in mind that seep sediments are naturally heterogeneous. We like to compare it with crunchy peanut butter, due to the presence of carbonate pebbles. Plus sometimes clamshells are buried in the sediment. Seep sediments are complex and we can only try to mimic reality but will never fully reach it.

AOM rates cannot be accurately calculated in the LFC core by using differences in the methane inflow and outflow concentrations at 258 days. Firstly, equilibrium has not been reached at this time, only 186 ml of "seep water" had been delivered to the core at this time.

Author Reply: This is correct. We added a note to the results (see 3.2.1 and 3.2.2) and discussion (4.1 and 4.2) and also highlighted in the new Table 5 that AOM rates from the LFC/NHFC are most likely overestimated.

Secondly no rates of methanogenesis within the core were measured and since the outflow contained 0.9  $\mu\text{M}$  at this stage, in an anoxic core full of sulphate, it implies possible methane generation within the core or channeling.

Author Reply: We do not understand, why a core full of sulfate implies methanogenesis, as the presence of sulfate, i.e. sulfate reduction, usually excludes methanogenesis unless it is based on non-competitive substrates (e.g. not used by sulfate reducers). We cannot exclude non-competitive methanogenesis, but the methane production was probably minor compared to the methane supplied by the medium. We would assume

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that the established AOM community quickly removed the produced methane. We think that channeling is a more likely explanation for methane in the outflow of the LFC.

Without information on how much methane-free water was pumped through the core during the initial 40-day period it is not possible to assess how much methane might have been left in the core.

Author Reply: See information above. It is unlikely that any residual methane remained in the core during the ca. 170 days of storage and transport. The active AOM community most likely consumed all methane before the core was set up in the SLOT System.

It would have been helpful had the authors measured methane directly at each sampling period, since this can be done on sample volumes as small as 50  $\mu$ l, either by direct injection of pore water (as sometimes used for pore water bicarbonate) or by using small headspace vials.

Author Reply: We are not sure if we understand this suggestion. Does the reviewer suggest determining methane from rhizon samples? Rhizon sampling is not recommended for volatile substances, as vacuum is applied.

8. Hydrological residence time No units were given for this and I was unable to follow the calculation, "the average time of the seepage medium to flow through the sediment column and was calculated by dividing the pore water volume by the flow rate". For the low flow system, with an approximate pore volume of 390 ml and a flow of 0.5  $\mu$ l min<sup>-1</sup>, the HRT should be 541 days. How was the figure of 1080 derived?

9. Author Reply: The reviewer is right and wrong. It is correct that 1080 d is not the time the seepage medium needs to pass the core, but to pass from inflow to outflow (which was used to calculate areal rates, see Table 4). The residence of the medium to pass the core must take into account the volume below the core (between inflow and core) and the sediment porewater volume, which amounts to a residence time of 696 d for the LFC and 69 d for the NHFC. We corrected accordingly (see 2.5 and 4.1).

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#### Text queries and corrections

Abstract "Most of the dissolved methane reaching the seafloor at cold seeps is oxidized within the benthic microbial methane filter by anaerobic oxidation of methane (AOM)." Surely the methane reaching the seafloor has already passed the benthic AOM, since this is below the seafloor.

Author Reply: We rephrased.

Introduction "methane gas may be transported upwards in solution by molecular diffusion or by ascending fluids mobilized by - - (ii) formation of gas hydrates within the gas hydrates stability zone". How does hydrate formation aid upward transport of methane? Author Reply: We rephrased.

Methods The detector used for determining methane is not mentioned

Author Reply: FID, we added information to 2.2 for the field measurements. Analytical methods for the SLOT cores were identical to Steeb et al. 2014.

and it is not made clear how the concentrations are calculated, are they per liter of sediment or pore water?

Author Reply: We added information (for all parameters) to the figure captions. It was per sediment for the field data and per porewater for the SLOT experiment.

One would assume the latter but there is no mention of porosity measurements in the methods, other than for the SLOT cores. The use of mM, rather than mmol l<sup>-1</sup>, would have clarified this.

The incubation temperature for the SLOT cores is not given and we do not know how this differed from the in situ temperature (or how constant temperatures are in Slide sediments).

Author Reply: We added information to 2.5. The incubation temperature was 10°C, the in situ temperature was 8°C.

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Section 2.7 “At the end of the experiment, 1.5mL porewater from each depth was sampled for determinations of sulfide (0.5 mL), sulfate and bromide (1.0 mL) as well as total alkalinity (0.5 mL),” These individual volumes add to 2.0 ml, not 1.5.

Author Reply: Thanks for noticing. We actually took only 0.5 mL for sulfate and bromide (IC). We changed accordingly.

Results In section 3.2.3 it is stated that the experiment was terminated “after 350 day runtime” although runtime in Figures 3, 4 and elsewhere is calculated from the end of the 40 day initial period.

Author Reply: That is correct, all runtimes are given after the 40 days initial period. Hence, the total runtime is 350 days (not 390 days).

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Interactive comment on Biogeosciences Discuss., 11, 16033, 2014.