

***Interactive comment on “Bacterial diversity and biogeochemistry of different chemosynthetic habitats of the REGAB cold seep (West African margin, 3160 m water depth)” by P. Pop Ristova et al.***

**Anonymous Referee #1**

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Review Biogeosciences Ristova et al. 2012-08-23

**General comments**

By combining pore water geochemistry, in situ flux measurements, and microbiological techniques in addition to advanced statistical methods that were applied to distinctively different seep habitats in the REGAB pockmark this MS represent an unique approach to understand the complexity cold seep ecosystems. Hence, this study bears a strong potential to provide novel insights and is of significance for the scientific community in

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this field.

However, I think that the MS has some substantial weaknesses that should be addressed before it can be considered for publication.

The discussion is not written well. It is lengthy and tedious to read. Often the statements are not very clear and sometimes they were superficial not providing novel insights (see details below). Especially, in section 4.1 but also in the other sections I had the impression that it often was not clear what to discuss and what it is aimed for. The discussion is often rather broad and touches many different topics without going into details. Overall, I suggest strongly shortening the discussion and to focus on the main story which I think is the difference of microbial communities and their link to the different geochemistry and megabenthic communities of the different habitats. As the distribution of megafauna is not the major focus of the paper, it might an idea that section 4.1 is strongly shortened or even left out. However, if the authors strive for the description of the whole cold seep ecosystem I suggest that the data (particularly the fluxes) should be explored in greater detail (see below). As the authors often talk about energy availability it would be interesting whether the authors could go into more detail and assess to what extend the methane from below is consumed during AOM, during aerobic methane oxidation or by the methanotroph endosymbionts in the Bathymodiolus mussels. It would be interesting – and I have the feeling that the data allow this – to see whether there major differences between the habitats in terms of usage of oxygen (e.g. sulfide oxidation vs. respiration) or with regard to CH<sub>4</sub> consumption during AOM vs. aerobic methane oxidation and how this relates to the distribution of microbial communities. Apart from this there are gaps with regard to the available literature in this field. It might be worthwhile to have a look on the work by Dando and colleagues. Boetius, Levin as well as Judd and Hovland provide nice reviews of cold seep ecosystems. Dale et al. conducted a nice study with regard to energy transfer at seep sites off New Zealand. However, its really important to carefully channel all this information and reshape the discussion section with a clear focus without becoming

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even more entangled in details that are not necessary within this context.

I am sure that the flux data are fine but nevertheless it would be great if some of the results (e.g. O<sub>2</sub> micro-profiles, raw data of chamber incubations) could be provided in the MS rather than just providing tabulated fluxes (for details see below). At least a selection could be provided in the MS, alternatively they could be provided in the supplements.

Lastly, I am not a native speaker, but I think the clarity of the MS would greatly benefit if a native speaker could edit the paper. To my feeling the sentences are often too long and – to my feeling – they do not really convey the intended message.

As the paper has a large potential deepening our understanding of cold seep ecosystems I recommend the paper for publication however only after substantial reworking of the discussion section.

Detailed comments

Introduction

L. 26 Sibuet and Olu 1998, better Levins review

8339 L.22 anaerobic

Methods

Fig.1 I think this figure could be improved, by annotating or highlighting the different habitats (siboglinids, bare sediments, carbonates etc.) by different shades or marks, it might be an idea to make an additional panel with a schematic sketch of the occurrence of the different megabenthic communities

8342 L. 22 “Within the mussel patch individuals of the siboglinid polychaetes ... “ please check whether the taxonomy with regard to polychaetes is correct

The habitat description sounds like results.

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8343 L. 20 English style

8343 L. 24 please specify in more detail to what extent the method of Hall and Aller 1992 has been modified. Are you sure that this method is appropriate for high pore water ammonium concentrations?

8344 L. 8344 “Values for sulphate reduction were adjusted to the integrated rates determined by the radiotracer injection method.” Please clarify what do you mean with adjusted?

8345 L. 1 “...was incubated in situ and changes monitored”, please insert concentration before changes.

8345 L. 4 please correct unit into mmol m<sup>-2</sup> d<sup>-1</sup>

8345 L.24 “Diffusive Oxygen Uptake (DOU) was calculated from the linear concentration gradient in the DBL (Diffusive Boundary Layer)” I am wondering a bit was the vertical resolution of 200  $\mu$ m good enough that the DBL could be clearly resolved?

8347 L.8347 As the paper addresses a wider community I suggest to spend a few more words to briefly describe the Mantel correlation test, e.g just by saying that it tests the correlation between two matrices. The same is true for the NMDS or ANOSIM. These tests are widely used in ecology, a geologist or chemist however might be less familiar with these types of statistics. What causes the Hellinger transformation to the data set?

8348 L.7 please introduce the abbreviation OTU if it was not already done earlier on

8348 L.9 why did you apply the log-transformation to the geochemical data? What are the consequences of that?

Results

8348 L.25 could you please specify or indicate in Fig. 3 for which depth horizons the sulfide fluxes were calculated.

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8351 In situ CH<sub>4</sub> efflux: you mention that the CH<sub>4</sub> emission was variable. Typically benthic chambers accumulate solutes over time hence variability mostly can be only discerned when the efflux becomes stronger with time as you described. The rates you measured belong to the highest seabed methane emission rate measured so far, hence it would be interesting to see the raw methane data over time (at least in the supplement). Could you please say some words on the quality of these measurements, especially in sediments with crusts of shell debris it might be difficult to measure seabed methane emission as during the insertion of the benthic chamber fractures within the sediment may occur along which methane might escape into the overlying bottom water.

8351 In situ TOU measurements: same as for CH<sub>4</sub> efflux, it would be great if the raw data could be shown (at least in the supplement). These TOU's are very high and although I don't know the geometry of the chamber and the volume of the enclosed water column (which I suggest should also be mentioned somewhere) such a TOU requires a drastic decline of the O<sub>2</sub> concentration over time. Did the chamber become anoxic at the end of the incubations? If yes how would this affect the methane efflux?

Please do not misunderstand the past two comments as harsh criticism, I find these rates very interesting and there are indeed very little rate measurements from mussel or clam beds. Hence, I think it would be nice to provide the reader with more details on how these rates were measured.

8351 In situ oxygen microsensor measurements Same comment as above, I also suggest to show a selection of micro-profiles in the MS or in the supplement. As the DOU was determined using the DBL hydrodynamics in the bottom water is important which can be strongly affected on small spatial scales as for instance in close vicinity to clams protruding into the water column. Hence the reader should have the chance to have a closer look on the profiles.

8354 L.9 suggest removing the brackets and integrate this into the text. What is the

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difference between community structure and community composition?

Discussion

8355 L.12 "REGAB is an endmember of the Atlantic Equatorial Belt (AEB)" what do you mean with endmember? Endmember with regard to what? Please specify

8355 L.19 "amphi-Atlantic Bathymodiolus" is this expression correct?

The first part of the discussion including section 4.1 is a bit broad – I suggest focusing it by more concentrating on bacterial communities rather than on the megafauna.

8356 L.12 "energy availability" - why not simply referring to the fluxes of the different solutes or to their concentrations. I know I don't tell you something new but nevertheless I would be more careful with the term energy availability. The energy that becomes available using methane or the different electron acceptors mentioned is strongly dependent on the processes involved. High methane fluxes do not necessarily mean that a high amount of energy becomes immediately available.

8357 L.3 which of these many references refer to the REGAB cold seep? I suggest using less references

8357 L.8 suggest to use less references and only to mention the most important ones

8357 L.10 "Methane concentrations in the bottom waters" – in which height above the sea floor were these measurements conducted?

8357 L.18 "This Bathymodiolus type hosts sulphur- and methane-oxidizing endosymbionts and hence depends mostly on methane (Duperron et al., 2011)." ? - according to their endosymbionts they also depend on the presence of sulfur.

8359 L.9 "Apparently, the bottom dwelling activity of the clams enables them to populate cold seep habitats with low gas fluxes and hence low microbial activity, so that they dwell the subsurface sediments to exploit rather deep peaks in sulphide production via AOM (Fischer et al., 2012)." What do you mean with this sentence?

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8360 L.4 “Overall, the megafauna distribution reflects the underlying sediment characteristics, thus we propose that the megafauna assemblages can be used as reliable first visual indicator of the sediment geochemistry at cold seeps i.e. of the magnitude of methane and oxygen fluxes, and the depth of sulphide production within the sediments.” – I am sorry to say this but this is not a novel result and does not deserve to constitute a major conclusion. Is there anything else which we can learn by quantitatively comparing the different habitats?

8360 L.13 “The only other seep sites harbouring similar chemosynthetic habitats . . .” I suggest to be careful with such statements, as Hydrate Ridge harbour rich chemosynthetic communities, mud volcanoes in the Gulf of Cadiz harbour for example very diverse tube worm communities etc.

#### Discussion section 4.2

8361 L.20 “These results support the hypothesis that the bacterial community structure at cold seeps is influenced foremost by methane supply, as primary source of energy to anaerobic and aerobic methanotrophs (Cambon-Bonavita et al., 2009), and as a main indicator of the activity of geological processes such as gas overpressure, fluid flow and hydrate formation or dissociation.” I suggest deleting the latter part of the sentence as this statement is rather broad and not evidently supported by the data.

8361 L.25 what do you mean with this statement? Can you provide references for this? “Surprisingly, even though a much higher diversity of bacteria and animals could be biologically influenced by sulphide as energy source or as toxin,”

Although I find it very interesting, that at the different habitats different microbial communities prevail – I think that the discussion sometimes appears superficial. I miss a more detailed discussion of how changes of geochemistry or the occurrence of megabenthos affects bacterial diversity.

#### Discussion section 4.3

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8363 L.1: “Our data indicate that methane fluxes determine sediment geochemistry, which selects for different types of chemosynthetic megafauna at REGAB.” What do you mean with this sentence? As the statement is very general this sentence could be deleted.

8363 L.8: Could you provide actual figures (estimates) about the relative proportion of mussel and clam respiration from the TOU, what is the relative share of the sulphide oxidation? You mention that the mussels efficiently consume methane causing a reduction of the methane efflux. Rather than providing a reference could you please provide an estimate of how much CH<sub>4</sub> is consumed by the mussels in contrast to the AOM or aerobic methane oxidation which might take place at the sediment surface?

8363 L.13 please shorten this sentence and rewrite it more concisely.

8364 L.1 “Accordingly, no direct association of unique bacterial types with the different megafauna was detected. . . . This indicates that the abundant bacterial types in this cold seep ecosystem . . . were directly affected by methane seepage and other geochemical processes, but only indirectly by the presence and absence of megafauna types.” This is an interesting finding and difficult conceive. Could it be that this is due to the sampling strategy, it might be that specific bacteria colonize in micro-niches that were established by the megafauna e.g. during burrowing but were missed during sampling.

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