

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.

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Rebuttal letter to accompany manuscript by Pop Ristova et al.

Response to reviewer#2

General comment1: In the results section the biogeochemistry is split into different sections but these parameters are not discreet. Sulfide, ammonium, etc. are related to each other and to the fluxes discussed. It would be easy to read and to follow the story if this was one section without subheadings. Maybe not all sub-sections

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need to be combined but some need to be. - Following reviewer's suggestion, some of the subheadings in the “3.2 Biogeochemistry of different habitats at REGAB” have been removed. The revised manuscript thus now contains only 3 biogeochemistry-related results subsections: “3.2.1 Porewater geochemistry” (pg12 L10), “3.2.2 Rates of methane and sulphate consumption” (pg13 L17) and “3.2.3 In situ fluxes of oxygen and methane” (pg14 L15).

General comment2: Figure 3 contains a lot of information that is difficult to read due to the small size. I also feel like the rate data and cell counts are redundant to table 2. Do you really need to present all of this data in both forms in the main paper? Some of this might be best in the supplemental material so that there is a better focus on the key results. - Figure 3 has been modified according to reviewer's suggestions. The rate data, cell counts and alkalinity profiles previously shown in Fig.3 were shifted to the supplement (Supplement Fig.2). Thus now, Fig.3 comprises only depth profiles of H₂S, SO₄ (Fig.3a) and NH₄ (Fig.3b). Please note that based on the reviewer#1 request, the depth layers from which H₂S fluxes presented in Table 2 were calculated, are now depicted on the Fig. 3a with red symbols.

Specific comment1: The habitat description sounds like results. - Following the suggestion from both reviewers the habitat description text has been now incorporated in the results section (see pg11 L9 – 28 and pg12 L1-9).

Specific comment2: Please be thorough in defining abbreviations. SR is not defined in the first results section or in the methods, e.g., pg. 8343, l. 10. - Text has been corrected according to reviewer's comment.

Specific comment3: Pg. 8345, l.19, correct the spelling of microelectrodes - Text has been corrected according to reviewer's comment.

Specific comment4: Heading of 3.1.7: please define TOU here - Heading has been renamed due to comment 2.

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Specific comment5: Pg. 8366, l. 15: please correct the spelling of determination - The spelling has been corrected according to reviewer's comment.

Specific comment6: Figure 3: please define the symbols displayed for H₂S and sulfate and define SR in the legend. - Thank you for noticing this omission. The symbols for H₂S and SO₄ are now defined both on the graphs, as well as in the legend. The SR acronym is explained in the legend of Supplement Fig. 2.

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Response to reviewer#1 Chapter: Introduction

Comment 1: L. 26 Sibuet and Olu 1998, better Levins review - Reference to the review by Levin (2005) has been added.

Comment 2: 8339 L.22 anaerobic - Text has been changed according to reviewer's suggestion.

Chapter: Material and Methods

Comment 3: 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 - A general map of the areal distribution of megafauna has already been published in Ondreas et al. 2005 and is now referred to. In our Fig 1 the focus is on the clusters of samples, and the distance between habitats. We have added a black line indicating the extension of carbonates with associated mytilids and siboglinids, and labels for the different patchy clam habitats sampled.

Comment 4: 8342 L. 22 "Within the mussel patch individuals of the siboglinid polychaetes ... " please check whether the taxonomy with regard to polychaetes is correct - The taxonomy of the siboglinid polychaetes is correct. For detailed information please see Andersen et al. (2005) and Olu-Le Roy et al. (2007).

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Comment 5: The habitat description sounds like results. - As stated above, based on the suggestion by both reviewers the habitat description has been now incorporated in the results section (see pg11 L9-28 and pg12 L1-9).

Comment 6: 8343 L. 20 English style - "analysis" was corrected as "analyses".

Comment 7: 8343 L. 24 please specify in more detail to what extend the method of Hall and Aller 1992 has been modified. Are you sure that this method is appropriate for high pore water ammonium concentrations? - The "modified" was deleted, as only minor aspects of the method were altered. In the paper by Hall and Aller (1992) it is explicitly stated that the method can be used for the determination of ammonium concentrations of at least 0.1 mM without any problems. The data presented in our manuscript (Fig. 3b) were within this range or slightly above (max. 0.13 mM), and obtained from the linear range of the calibration curve. Comment 8: 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? - To read more clearly, the above sentence (pg6 L26) was corrected as follows: "Values for sulphate reduction rates used in the model corresponded to the analytically results (see Table 2).

Comment 9: 8345 L. 1 ". . . was incubated in situ and changes monitored", please insert concentration before changes. - Upon reviewer's suggestion the sentence (pg7 L17-20) was modified as follows: "Briefly, 284 cm² of sediment with 10 – 15 cm overlying water was incubated in situ and changes in oxygen and methane concentrations were monitored over time by pre-programmed syringe sampling and optode measurements."

Comment 10: 8345 L. 4 please correct unit into mmol m⁻² d⁻¹. - Thank you for noticing the mistake; the unit was corrected accordingly.

Comment 11: 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 μm good enough that the DBL could be clearly

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resolved? - The DBL for most of the investigated sites was found to be on average 1 mm (0.2 – 1.6), thus the vertical resolution of the microprofiling was sufficient to capture the DBL. For the reader's convenience, all microsensor measurements were added to the supplement of this manuscript (Supplement Fig. 4).

Comment 12: 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? - Thank you for raising this issue. We agree with the reviewer that a short explanation on the various tests will facilitate the understanding of the statistics applied in this study. Accordingly, short explanations on the multivariate tests, the Hellinger and log-transformation, have been incorporated in the Material and Methods section (pg.9 L19-29 and pg10 L1-19). Comment 13: 8348 L.7 please introduce the abbreviation OTU if it was not already done earlier on - Text was corrected according to reviewer's suggestion.

Comment 14: 8348 L.9 why did you apply the log-transformation to the geochemical data? What are the consequences of that? - Please see response to comment 12. Short explanation on the application of log-transformation has been added to the Material and Methods section (pg11 L1-4).

Chapter: Results

Comment 15: 8348 L.25 could you please specify or indicate in Fig. 3 for which depth horizons the sulfide fluxes were calculated. - The depth layers used to calculate the sulfide fluxes are now marked with red symbols. Please see updated Fig.3a.

Comment 16: 8351 In situ CH₄ efflux: you mention that the CH₄ 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.

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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 sea bed 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. - Upon reviewer's request the data from all chamber incubations, showing oxygen concentration decline and methane concentration increase with time, is now shown as graphs in the supplement (Supplement Fig. 3). Careful choice of the measurement site, positioning and insertion of the chamber in the sediment was performed by visual inspection with the ROV. Moreover only soft sediment without carbonate crusts and shell debris were sampled. Finally, the initial water samples (taken within approximately the first 30 minutes of the incubations) always showed low methane concentrations, similar to the ambient bottom water CH₄ concentration. This assures us that no major fractures have been formed during the insertion of the chamber in the soft sediment, along which methane could escape.

Comment 17: 8351 In situ TOU measurements: same as for CH₄ 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 O₂ concentration over time. Did the chamber become anoxic at the end of the incubations? If yes how would this affect the methane efflux? - Although in some of the incubations the final oxygen concentrations were indeed very low (< 50 μM), please note that in most of the cases the TOU was calculated from the initial decrease in oxygen concentration during the incubation (see Supplement Fig. 3). Oxygen concentration decrease (raw data) during all chamber incubations is now shown in Supplement Figure 3. Information on the shape, size and total volume of the chamber has been incorporated in the Material and Methods part (pg.7, L15-16). The height range of the enclosed water column by the chamber had

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been already given in the text (please see pg7 L18), however please note that for each individual incubation the volume of the enclosed bottom water was different, as this directly depends on how deep was the chamber inserted in the sediment. In general, for all measurements the enclosed water column height ranged between 10 and 15 cm.

Drastic decline in the oxygen concentration in the chamber was only detected at the clam sites, where the methane efflux was very low (or hardly detectable). In contrast high methane effluxes were detected at the Mussel_S and Mussel_S_Env sites, where the oxygen concentration in the chamber remained above 200 μM even towards the end of the incubation (after app. 4h; please see Supplement Fig.3). Therefore we believe that the decline in oxygen concentration could not have an important impact on the measured methane effluxes.

Comment 18: 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. - To enrich the tabulated info on oxygen distribution and fluxes, as suggested by the reviewer we have added all measured microprofiles to the supplement of this manuscript (Supplement Fig. 4). Please note that due to the fragile nature of microsensors, all microprofiling was performed on soft sediment next to the living clam patches. The bottom water O₂ concentrations were the same for all sites (app. 250 μM) and all profiles had the typical diffusion-based shape (please see Supplement Fig. 4).

Comment 19: 8354 L.9 suggest removing the brackets and integrate this into the text. What is the difference between community structure and community composition? - The text was corrected according to the reviewer's remarks. Please note that the definition of β -diversity was shifted to the Material and Methods section (pg.8 L14-15). We have used the term "composition" according to Whittaker, 1972; to refer to the pres-

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ence/absence of species (or in our case OTUs), while "structure" encompasses both presence/absence as well as species (OTU) relative abundances.

Chapter: Discussion Comment 20: REGAB is an endmember of the Atlantic Equatorial Belt (AEB)" what do you mean with endmember? Endmember with regard to what? Please specify - In response to comment 22, this sentence has been removed.

Comment 21: 8355 L.19 "amphi-Atlantic Bathymodiolus" is this expression correct? - We have used the same terminology as in Olu-Le Roy et al. (2007), in which study the authors have identified the existence of two mussel species complexes that are widespread across the Atlantic Equatorial Belt. However, in response to comment 22, this sentence has been removed.

Comment 22: 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. - To provide a discussion more focused on the bacterial communities and the biogeochemistry, large part of the text dealing with megafauna has been removed (previous pg. 8355 L 11 – 24) and some sentences were slightly modified (pg. 18 L1-2).

Comment 23: 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. - While we think it is important to define that methane fluxes are proxy for the energy availability at cold seeps, to provide more precise discussion, as suggested by the reviewer, we have switched to the usage of methane fluxes throughout majority of the text.

Comment 24: 8357 L.3 which of these many references refer to the REGAB cold seep? I suggest using less references - The reason for more references was to guide the

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reader to other publications where data on microsensors measurements at other cold seep sites exist. This can help the reader to easily compare REGAB to other seep sites in terms of oxygen fluxes and distribution in the sediment. However, the sentence was slightly modified and the references split, so that it reads more clear which of the citations refer to only REGAB. The sentence (pg.18 L7-10) now reads as follows: "The REGAB pockmark (Menot et al., 2010; this study) comprises highly reduced, patchy habitats where due to the local upward transport of hydrocarbons oxygen is completely consumed within the first millimetres of seafloor, similar to what was found at other cold seeps (Beer et al., 2006; Girnth et al., 2010; Lichtschlag et al., 2010a; Grünke et al., 2011)".

Comment 25: 8357 L.8 suggest to use less references and only to mention the most important ones - Based on the reviewer's suggestion the list of references was reduced. The modified sentence (pg 18 L14) now reads as follows: "Previous investigations of deep water cold seeps have shown that free gas may escape from the seafloor within the gas hydrate stability zone, even at such high pressure and cold temperature as at REGAB (Suess et al., 1999; Fischer et al., 2011)."

Comment 26: 8357 L.10 "Methane concentrations in the bottom waters" – in which height above the sea floor were these measurements conducted? - The sentence (pg.18 L15–19) on the bottom water methane concentrations has been rewritten, to incorporate information on the location where the bottom water samples were taken from. The sentence now reads as follows: "Methane concentrations in the bottom waters (10 cm above seafloor) were highest in the vicinity of the Mussel_S_Env (3.6 μM), around 0.4 μM at Clam_S_Env to and decreased to 0.2 μM at the clam habitat (Clam_SW_Env) furthest away from the central gas vents. These values fall into the low range of values detected previously on top of the respective megafauna patches (Duperron et al., 2005; Olu-Le Roy et al., 2007a)".

Comment 27: 8357 L.18 "This Bathymodiolus type hosts sulphur- and methane-oxidizing endosymbionts and hence depends mostly on methane (Duperron et al.,

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2011).?" - according to their endosymbionts they also depend on the presence of sulfur. - Thank you for noticing the inconsistency in this sentence. In order to highlight the role of methane as main source of energy to the REGAB Bathymodiolus mussels, the sentence (pg 18 L23-25) has been corrected as follows: "This Bathymodiolus type hosts higher abundances of methane-oxidizing than sulphur-oxidizing endosymbionts in the gills, and hence appears to depend mostly on methane as the main source of energy (Duperron et al., 2011)."

Comment 28: 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? - To read more clear the sentence (pg. 20 L7) has been modified as follows: "The bottom dwelling clams can exploit subsurface sulphide, allowing them to populate cold seep of low geological activity, where methane and sulphide produced by AOM do not reach surface sediments (Fischer et al., 2012).

Comment 29: 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? - The sentence (pg20 L28) has been revised as follows: "The distribution patterns of the seep megafauna reflected methane fluxes and associated biogeochemical characteristics of the underlying seafloor. Thus megafauna assemblages are not only relevant indicators for the presence of seepage (Dando and Hovland, 1992), but also of local seepage activity i.e. of the magnitude of methane and oxygen fluxes, and the depth of sulphide production within the sediments". - With this sentence we would like to stress the fact that megafauna distribution

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is directly linked to the sediment geochemistry, and not only to specific bottom water conditions. Previous studies could show a general link between the distribution of megafauna and overall seepage activity by focusing on the bottom water rather than on spatial variations in seafloor geochemistry, using mainly *ex situ* approaches (Olu-Le Roy et al., 2007). In contrast, our study expands on these previous findings and furthermore reveals a direct spatial link between the distribution of megafauna and the *in situ* magnitude of methane and oxygen fluxes from the seafloor. Therefore we believe that these results represent an important finding and suggest to keep in the discussion part of the manuscript.

Comment 30: 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. - The sentence has been removed in the updated version of the manuscript.

Chapter: Discussion 4.2

Comment 31: 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. - Text (pg22 L7) has been modified as follows: “These results support the hypothesis (Cambon-Bonavita et al., 2009) that the bacterial community structure at cold seeps is influenced foremost by methane supply, as primary source of energy to anaerobic and aerobic methanotrophs”.

Comment 32: 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

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animals could be biologically influenced by sulphide as energy source or as toxin,” - Main aim of the sentence was to contrast the potential impacts of sulfide versus methane (as an energy source, or in the case of sulfide as a toxin) on the bacterial community structure. Hence, to clarify we have modified the sentence (pg22 L10-14) as follows: “One may assume that sulphide could biologically influence a higher proportion of bacteria and animals compared to methane. Many types of bacteria can use sulphide as an energy source (Campbell et al., 2006; Sievert et al., 2007), and it is a toxin to most animals (Bagarinao, 1992). However, the bacterial β -diversity was not significantly correlated to difference in sulphide fluxes among habitats. Rather, methane flux was the most important factor structuring the bacterial communities at REGAB.”.

Comment 33: 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. - The following short text focusing on the link between geochemistry and the bacterial community structure has been incorporated in the section 4.2 of the discussion (pg22 L18-28): “The substantial methane flux, as well as the relatively high rates of AOM coupled to SR (Table 2) detected at the mussel patch selected for distinct bacterial communities. In contrast, sites characterized by low to intermediate rates of AOM coupled to SR and hardly detectable methane fluxes, such as those measured at the clam patches and the bacterial mat, had highly similar bacterial community structure.” Chapter: Discussion 4.3

Comment 34: 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. - Upon reviewer’s suggestion the sentence has been removed, and moreover the following sentence has been slightly modified (pg23 L24): “A further aim of this study was to test if the distribution of the chemosynthetic megafauna,

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in addition to the sediment geochemistry, is also influencing bacterial community structure.”

Comment 35: 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 sulfide 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 CH₄ is consumed by the mussels in contrast to the AOM or aerobic methane oxidation which might take place at the sediment surface? - Our data allows only rough and speculative estimates of the specific contribution of the bivalves to the total oxygen uptake and methane consumption, as we could not quantify their biomass. A brief discussion on potential bivalve consumption rates and comparison to the AOM has been incorporated in the manuscript (pg23 L29-30 and pg24 L-1-13): “Clam respiration accounted for a substantial local increase (25 – 30 times) in the total benthic oxygen uptake rates, as compared to the adjacent bare sediments (Table 2). At both Clam_S and Clam_SW sites roughly 96 – 97 % (calculated as the percentage of difference in TOU between the clam populated and bare sediment sites) of the total oxygen uptake was due to clam respiration. Difference in the TOU measured among the clam sites (590 mmol m⁻² d⁻¹ at the Clam_S and 294 mmol m⁻² d⁻¹ at the Clam_SW) can most probably be explained by the difference in clam density within the assemblages, rather than by variations in the individual respiration rates (Decker et al., 2012). In contrast, mussels contributed only 18% to the total oxygen uptake. In contrast to the clams, the Bathymodiolus mussels contain a high proportion of methanotrophic symbionts (Duperron et al., 2009, 2011; Petersen and Dubilier, 2009), causing a reduction of methane efflux within the mussel patch. If we assume that the difference in the methane effluxes between Mussel_S (81 mmol m⁻² d⁻¹) and Mussel_S_Env (334 mmol m⁻² d⁻¹) is due to the uptake by the mussels, they would consume approximately 12 times more methane (253 mmol m⁻² d⁻¹) than what is consumed via the process of anaerobic oxidation (AOM, 20 mmol m⁻² d⁻¹) (see Table 2). “.

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Comment 36: 8363 L.13 please shorten this sentence and rewrite it more concisely. - Upon reviewer’s suggestion the sentence (pg24 L14-19) has been modified as follows: “Similar to clam habitats at other cold seeps, sulphide was absent from the surface sediments at REGAB (Barry et al., 1997; Sahling et al., 2002; Levin et al., 2003; Fischer 15 et al., 2012). Bottom water sulphate penetrated till 6 cm depth at all clam patches. Such geochemical signature is usually ascribed to the dwelling activity of thiotrophic clams to access sulphide (Childress and Fisher, 1992), leading to a deeper sulphate penetration (Sahling et al., 2002; Cordes et al., 2005, 2010; Fischer et al., 2012)”.

Comment 37: 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. - One of the main aims of this study was to investigate whether clams are specifically associated to certain bacterial species in the underlying sediments. Hence, all sampling has been performed using pushcores of 8 cm diameter, which were afterwards immediately subsampled on board. Such strategy allowed capturing the direct contact clam-sediment zone, but not specifically the bacteria sitting on the clams or populating excrements or burrow linings. Accordingly, such direct associations were most likely not resolved. We clarified this accordingly in the MS: “This indicates that the abundant bacterial types in this cold seep ecosystem as detected by ARISA fingerprinting were directly affected by methane seepage and other geochemical processes, but only indirectly by the presence and absence of megafauna types. This finding may differ with high-resolution sampling targeting e.g. the surface of bivalves and their burrows, and with other types of molecular methods, which detect rare bacterial types.”

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Please also note the supplement to this comment:
<http://www.biogeosciences-discuss.net/9/C5440/2012/bgd-9-C5440-2012-supplement.pdf>

Interactive comment on Biogeosciences Discuss., 9, 8337, 2012.

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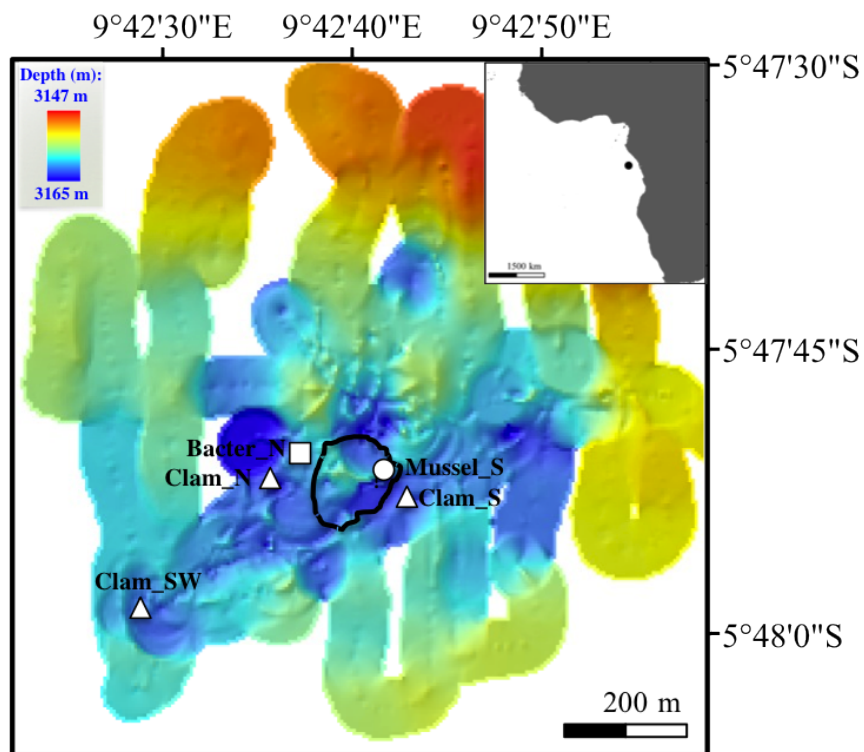


Fig. 1.

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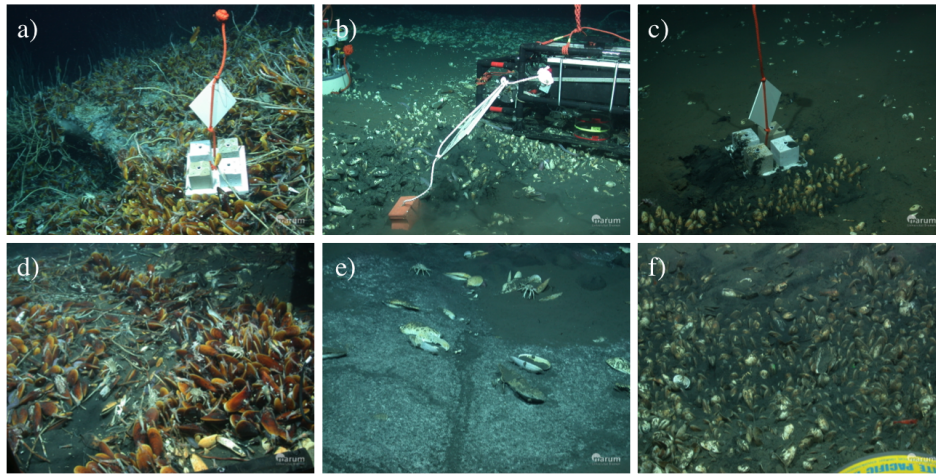


Fig. 2.

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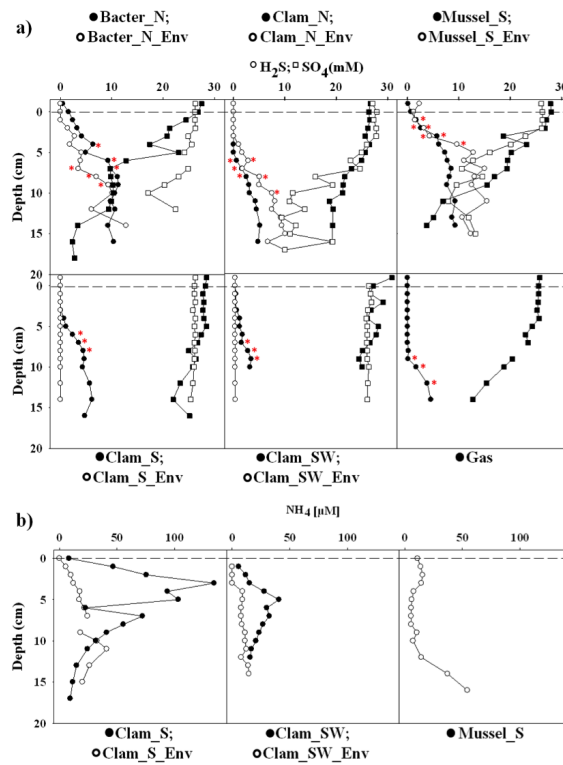


Fig. 3.

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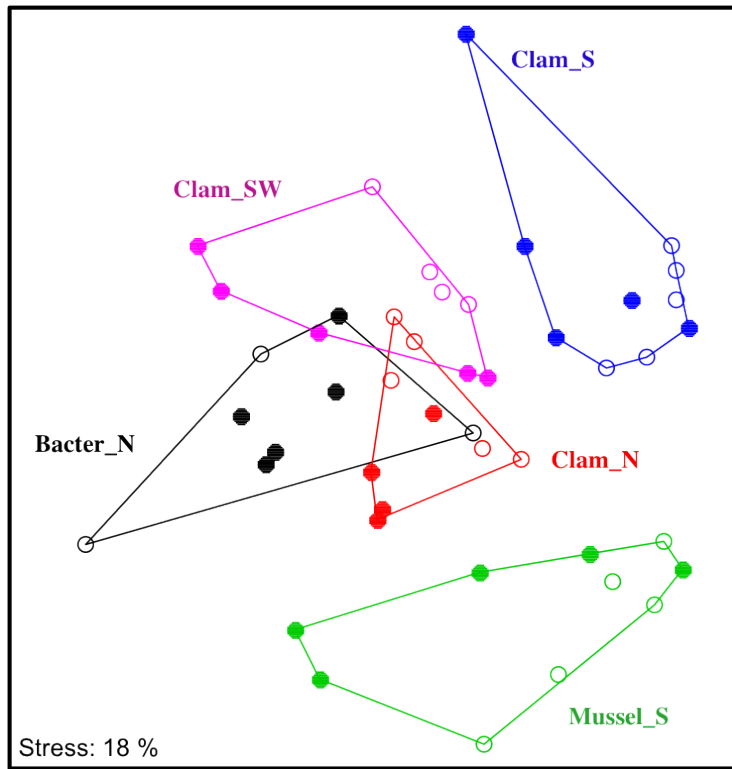


Fig. 4.

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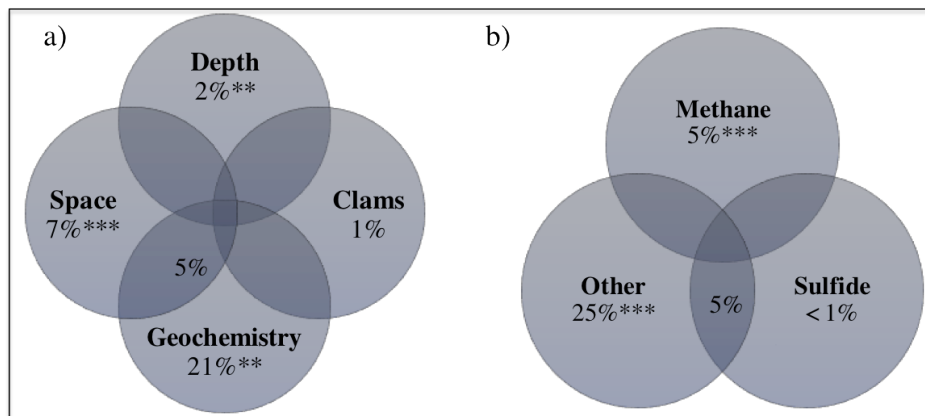


Fig. 5.

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