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Interactive comment on "Saturated CO_2 inhibits microbial processes in CO_2-vented deep-sea sediments" *by* D. de Beer et al.

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We are grateful for the thoughtful review. We will cite the reviewer and then respond. We have improved the manuscript according to the comments of all 3 reviewers.

This study was focused on biogeochemical processes and microbial activity in sedimentsof a natural deep-sea CO2 seepage area of Yonaguni hydrothermal system.The aim was to assess the influence of the geochemical conditions occurring in acidic and CO2 / free carbonic acid saturated sediments on sulphate reduction (SR) and anaerobic methane oxidation (AOM). Without a doubt we are dealing with interesting manuscript dedicated to a very actual and important topic, i.e. CO2 leakage associated with CCS in the deep-sea floor and its possible influence on ecosystem functioning.

– Why traditional molecular techniques were not applied, such as the isolation of rRNA and mRNA, analysis of lipids? Simple analysis of pooled environmental RNA collected from different depths at venting site supported by simple cultivation/enrichment

Unfortunately, cultivations of environmentally relevant marine microorganisms are not that simple, and here we were dealing with deep-sea microrganisms adapted to 200 atm of CO2, i.e. conditions which are very difficult to be reproduced in the lab.

The use of rRNA methods to assess the distribution of cells at this and similar CO2 vents was done, and has been published separately (cited in the MS: Yanagawa K, Morono Y, de Beer D, Haeckel M, Sunamura M, Futagami T, Hoshino T, Terada T, Nakamura K, Urabe T, Rehder G, Boetius A and Inagaki F. (2012) Metabolically active microbial communities in marine sediment under high-CO2 and low-pH extremes. ISME doi: 10.1038/ismej.2012.124). However, the distribution of rRNA does not necessarily reflect the distribution and quantity/velocity of biogeochemical rates, which was the main focus of this study. rRNA studies only show the possible presence of cells of certain lineages and that might be active under given conditions. The presence of transcripts from functional genes does not necessarily mean that the protein in question is formed, nor that the enzyme is actually active. The use of microsensor and tracer methods to directly measure conversion rates was for the purpose of this study a straightforward approach to assess the effect of CO2 on geochemically relevant microbial activities, questions as to microbial phylogeny and abundance were answered earlier (Yanagawa et al. 2012).

– The energy yield of anaerobic methane oxidation with sulfate as electron acceptor is extremely low (-16.6 kJ). Based on thermodynamic considerations, AOM is not a process to be expected to occur at low pH and at high concentration of the end product(s). Thus, Impact of CO2 leakage on AOM would be easily explained by the end-product inhibition. The Authors are referring to presence of hydrogen at venting site, but did not indicate the H2 concentration. It could be very useful to have an idea about the in situ concentration. Regarding the very low free energy calculation and the elevated pres-

ence of one of the end products, AOM would be feasible only at one important caveat – at an extremely low ambient concentration of hydrogen, which means the presence of microorganisms, actively scavenging this important donor of electrons from the environment. So, to significantly improve the manuscript, the Authors should demonstrate these data.

The reviewer is correct with his criticism on the bioenergetic consequences of high CO2. Indeed CO2 will affect AOM by end-product inhibition. Carbonic acid will only dissipate the ΔpH and disrupt the cytoplasmic pH homeostasis, but will not dissipate the full PMF. The discussion is changed accordingly. As to the H2 concentration: The potential effect of H2 on AOM remains unclear – some studies have found no or low effect of H2 (e.g. Nauhaus, K., Boetius, A., Krüger, M., and F., W.: In vitro demonstration of anaerobic oxidation of methane coupled to sulfate reduction in sediment from a marine gas hydrate area, Env. Microbiol., 4, 296-305, 2002.), and alternative intermediates have been proposed, at least for some types of AOM consortia (e.g. Milucka, J., Ferdelman, T. G., Polerecky, L., Franzke, D., Wegener, G., Schmid, M., Lieberwirth, I., Wagner, M., Widdel, F., and Kuypers, M. M. M.: Zero-valent sulphur is a key intermediate in marine methane oxidation, Nature, 491, 541–546, 2012).

– More attention has to be dedicated to statistic analysis of present data. Where are the standard errors/deviations? These values should be mentioned at least in Methods.

We now included the standard deviations of the areal microbial rates. All local biogeochemical data are presented in the graphs.

specific comments:

1913, lines 8-9, lines 17-19. Much more references are needed for this statement. I assume, the transport of undissociated carbonic acid through the cell membranes is not such a simple process as stated and referring to the article of Terada (1990) only without providing other experimental evidences is not sufficient.

1) Indeed, the section is removed

1914. lines 1-15. As above, all these statements require much more deeply-grounded studies and experimental data. Observed inhibition of AOM and SR might also be affected both by (i) high ambient concentration of the end product(s) and/or by slightly acidic conditions (majority of AOM archaea and SRB are neutrophiles) (see comments above). What about to analyse the eventual presence of active either sulphuroxidizing chemolithoauto- or heterotrophic microaerophilic organisms and fermenting anaerobes? Lack of AOM and SR activities is not convincing enough to declare that at high concentration of free carbonic acid we are dealing with complete suppression of ALL microbial activities.

2) Indeed we measured only SR and AOM. The S-oxidizing and heterotrophic microaerophilic processes will only occur in the top mm of the sediments, and thus 5-7 cm away from the extreme CO2 levels.

1908, lines 3-5. Please, rephrase this sentence for clarity that oxygen is fuelling the respiration process, but not as an electron donor.

3) The sentence is split in two sentences to avoid the confusion.

lines 7-9. Please, rephrase this sentence: "Thus, the presence of liquid or supercritical CO2 in sediments will completely suppress microbial activity and conclusively change ecosystem function as observed in the Yonaguni subsurface sediments for anaerobic microbial respiration and microbial sulphide oxidation": : : As it stated by the Authors, as far as high CO2 / free carbonic acid concentration suppresses any metabolic activities, the ecosystem function can not be changed. It turns to do not be an ecosystem anymore.

4) Only a part of the ecosystem stops, namely the sediments adjacent to the liquid CO2. The sentence is changed.

Fig. 6B. Please, change the axis for MUC10 values to separate them more evidently

from the rest of data.

Fig. 6B: MUC10 did get a separate x-axis above the plot, because the values are almost one order of magnitude higher. This is now clarified in the subtitle of the figure.

Interactive comment on Biogeosciences Discuss., 10, 1899, 2013.