

## ***Interactive comment on “Interconnectivity vs. isolation of prokaryotic communities in European deep-sea mud volcanoes” by M. G. Pachiadaki and K. A. Kormas***

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“This is a nice study, which, for the first time, examines trends in microbial community composition across European deep-sea mud volcanoes, with aim to identify key community members of mud volcano sediment. Only a small number of OTUs is found across all mud volcanoes studied (please state which ones in the abstract).”

The GenBank numbers of these sequences will be added in the abstract.

“I have a few suggestions, which mainly pertain to the mode of presentation, which could be more clear and simple, and (I hope) not to the way the data was analyzed. (1) Why was a cut-off of 700 bp chosen for the analyses? Does this reduce the data set

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substantially? How does it affect the diversity that is actually studied?”

We will rewrite this part to clarify our approach. We decided to exclude studies containing DGGE sequences, which are relatively small so even low nt variation can have major impact on the % of sequence similarity. All the studies analysed here targeted the nearly full length of the 16S rRNA gene (1200-1400nt). In a couple of cases researchers choose to deposit together with the full length sequences a few shorter ones (700-950 nt). Although we personally don't agree with this approach, we chose not to discard those from the further analysis, in order to compare complete datasets, as deposited by the persons conducting the individual studies. So, there are some sequences in a couple of datasets that are "short", but longer than 700 nt (i.e. almost half of the whole gene's length). In order to test if the length of those sequences affects the % of sequence similarity (which would change the number of the shared OTUs), we performed the comparisons (i) without manipulations [sequences were used as deposited], (ii) using the shorter sequence to trim all sequences to this length in all databases. Using the 98% cut off criterion, the number of the common OTUs between the databases remained the same. It seems that the nt variability - that we all know it exists - didn't affect the percentage of sequence similarity.

“(2) The way the important data is presented in the text, i.e. the groups that are shared by different locations, is cumbersome to read. Please provide a table with an overview of this, e.g. sites as columns, OTUs that occur across two or more sites as rows, each OTU (e.g. representative accession number) with info on which group it belongs to (e.g. ANME-1, Marine Benthic Group D (MBG-D), etc.) in parentheses. Of this table, part (a) could be on Archaea, part (b) on Bacteria. Table 2 can provide an overview of it, if still useful, and otherwise removed.”

We will compile such a table as suggested, in order to help to reader to follow the most important OTUs in the text

“(3) Phylogenetic trees, displaying all OTUs, or if this is too crowded, representative

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OTUs for each group/cluster, would be extremely helpful. Please provide one for Bacteria and one for Archaea.”

We will construct and present phylogenetic of the shared phylotypes only, as the amount of all sequences analysed is too large to be included in an informative tree, plus, the published respective papers have the relevant trees.

“(4) As supplementary material, provide a table with clone and nucleotide accession numbers of all sequences that were included in this study. Within this table, please indicate the phylogenetic group each clone/nucleotide accession number has been assigned to (e.g. ANME-1), which site it is from, and which article it is from.”

We can compile such a table but we believe it is redundant as most of the requested clones are included in GenBank. Regarding the affiliation, some of it is really tentative and is not really informative if it is based only on base-by-base similarity (i.e. BLAST). Finally, this table will be a really long table (ca. 1500 sequences) that we are afraid it will be just overlooked. If the reviewer insists that this table will add to the value of the ms, we will compile this table.

“(5) Figs. 3 and 4 look nice, but I am not sure how useful they actually are in explaining the data. Either explain these better, ideally so the reader can get the key message from looking at them and reading the figure caption alone - or remove.”

The network visualization provides a better imaging than simple Venn diagrams as we can include more sites –a more than 3-circled Venn is rather intimidating to go through its information- and, additionally we can show on the same graph which sequences connect the different sites. We will comment a bit more detailed on these graphs so we can leave them in the text. Alternatively, we can move these in the supplementary material.

“(6) I think it would be important to explicitly state which of the groups that are shared between mud volcanoes have only been found in mud volcano sediments (or methane

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seeps). I know this is not true for any of the ANMEs, MBG-D, MBG-B, or MG-1 Archaea. At least for the ANMEs, this is not surprising, since anaerobic methanotrophy is a widespread process, simply because methane is produced and consumed in many places in marine sediments. But are there any groups that are truly unique to mud volcano (methane seep) sediments, based on existing data? If not, could it be that mud volcanoes are not unique/distinct microbial habitats, and thus there is no evolutionary benefit of specialization/endemism to this particular habitat?” “(7) Along the same lines as (6), are any of the OTUs, e.g. within the different ANME groups, shared between European mud volcanoes, but not found anywhere else? This would help address the question of interconnectivity vs. isolation, which is mentioned in the title, but then not really addressed in the manuscript.”

We will try to detect whether there are any sequences from the investigated datasets that are found solely on mud volcanoes, and if so we will cautiously say it in the revised ms. It is known that anaerobic methanotrophy is a widespread process. Marine sediments that are rich in methane -including mud volcanoes- hydrate bearing sediments, cold seeps and all subsurface sulfate-methane transition zones (SMTZ) seem to share common geochemical features which provide a suitable niche for the ANME Archaea. Similarly the sulfate reducing bacteria and the rest of the AOM related groups have been observed in a variety of environments. The SEEP SRB1a group has been identified as the ANME 2 partner in enrichment cultures from mud volcano sediments (Schreiber et al., 2010). It is exclusively associated with AOM in marine sediments and the majority of the published sequences are originated from mud volcanoes and cold seeps but it is also present in Black Sea mats. Whether any of these sequences is found only in MVs, can be inferred from the occurrence of similar sequences. It seems that the “connector” prokaryotes of the examined MVs are also found in other methane-related habitats.

Schreiber, L., Holler, T., Knittel, K., Meyerdierks, A., and Amann, R.: Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the

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ANME-2 clade, *Environmental Microbiology*, 12, 2327-2340.

“(8) In general, please be more specific about what you are looking for in the data, and then state whether or not the observed trends confirm your hypotheses/anticipated result. This will help add structure to the manuscript and make the implications more clear to the reader.”

We will provide at the end of the discussion a clear statement on the interconnectivity of the MVs considered in this paper.

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Interactive comment on *Biogeosciences Discuss.*, 9, 17377, 2012.