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Biogeosciences Discussions

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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"The study offers a synthesis of the current knowledge on the microbial diversity at European deep-sea mud volcanoes based on studies that used traditional cloning/sequencing of (long) ribosomal RNA sequences. This may serve as baseline for future studies that target shorter sequences produced by NGS technologies for instance. The study identified the core phylotypes in those habitats, which are characterized by very distinct biogeochemical processes. Main comments -Table 1: please also indicate the total number of sequences in each study along with the number of unique OTUs. This may help understand whether large variation in coverage effort

may be expected. Indication on average sequence lengths in each case may be useful too."

Our first intention was to provide the total number of sequences for each study. But, we realized that the total number of sequences is not informative of the variation of the coverage effort. The reason is that researchers have different approaches regarding deposit of the sequences and providing OTU abundance data. So in some of the datasets retrieved the researchers have deposited all clones sequenced (so we clustered and used only the unique OTUs) while in other cases the researchers cluster, deposit only the unique and present (or not) the total number of clone sequenced and the relative abundance of each OTU in the corresponding paper. We faced similar problems with the sequence lengths. All the studies targeted the almost full length 16S rRNA gene (1300-1400nt). In a couple of cases researchers choose to deposit together with the full length sequences a few shorter ones (750-900nt). Although we personally don't agree with this approach, we choose not to discard those from the further analysis, in order to compare complete datasets, as deposited by the persons conducting the individual studies.

"- Line 183: Before comparing OTU richness between archaea and bacteria, it is important to ensure that the definition is about the same in both cases: Were the aligned rRNA gene sequences positioned at about the same nt position and covering about the same sequence length? If not, you may capture different levels of nt variability in the respective OTU definitions."

As we mentioned in the previous comment in all the studies almost full length sequences were targeted by the researchers. So for the majority of the comparisons a sequence length of 1300 nt was covered. We were also concerned about the different levels of nt variability and how the OTU definition might change for the shorter sequences, so we performed the comparisons (i) without manipulations [sequences were used as deposited] (ii) using the shorter sequence to trim all sequences in all databases. Using the 98% cut off criterion, the number of the common OTUs be-

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tween 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. Regarding the comparison between Archaea and Bacteria: of course we didn't do any direct sequence comparisons. We compared the total OTU richnesses. In all cases (all or only short sequences) the total bacterial OTU numbers were many times higher than archaeal. We also tested 97% sequence similarity as OTU definition and the trend remained the same.

"- Most of the analyses were done at the OTU level, but it may also be interesting to also provide a fuller picture of the similarity between MV communities at different taxonomic levels. This could then inform on the taxonomic levels at which endemism may be less pronounced and on the taxonomic resolution needed to either differentiate MV samples from each other or from typical deep sea sediments."

We choose to focus at the OTU level, in order to try to reveal specific key players. The dominant organisms present in those types of ecosystems belong to [regarding Archaea] Euryarchaeota (Methanosarcinales - ANME-2 and ANME-3, as well as ANME-1 group which is usually related to Methanosarcinales and Methanomicrobiales) and [regarding Beacteria] sulfate reducing (Desulfosarcina-Desulfococcus branch or Desulfobulbus). Other groups (e.g clades of epsilon and gamma Proteobacteria, Chloroflexi, candidate division JS1). These groups are quite widespread so we believe that it wouldn't be possible to reveal patterns related to marine mud volcanism.

Minor comments

-Line121-129: what is the final number of nt being retaind in the final aligment? Answer is given in the previous comment for line 183

-Line 122: "Taxonomic" not "Taxinomic" Corrected.

-Line 135: against which database? Added as suggested.

-Lines 159-162: A reference seems to lack here. Yes, it does. Added.

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Interactive Comment

-Line 178: the highest number [: : :] was found Added.

-Line 179: "deeper or fine-scaled analysis" it is not clear if you refer to spatial scale or to the resolution or diversity coverage of the techniques being used. The sentence should be clarified. In fact, when sequencing depth increases, more OTUs may be obtained, but also the chance that they differ in sequence may also increase (i.e. by going deeper in the rare biosphere). With a shallow sampling (reduced sequencing effort) you may find more shared types, because the latter would represent the dominant types. We mean that when we increase both the clone library coverage and analyse more samples from one site (e.g. see Pachiadaki et al. 2010) then we expect more sequences. We will rephrase this to clarify it.

-line 357: typo "environments" Corrected.

-Line 532: typo "datasets" Corrected.

-Figure 3. A legend with what the thickness and size of the points mean would be useful. We will include this information the revised legend.

-Line 373: "the" repeated. Corrected.

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