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Interactive comment on "Microbial assemblages on a cold-water coral mound at the SE Rockall Bank (NE Atlantic): interactions with hydrography and topography" *by* J. D. L. van Bleijswijk et al.

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We wish to thank the reviewer for the efforts and input provided. We carefully went through all the comments and suggestions. We have adjusted the manuscript according to the comments made. Below we provide a description of the adjustments made, addressing the reviewers remarks.

Thank you very much for the opportunity to review this manuscript. The authors have made a good first attempt at conveying a complex data set and identifying possible drivers of microbial community assemblages in a very unique and under-explored environment. However, the manuscript falls short because it never clearly states objec-

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Interactive Discussion

Discussion Paper

tives or lists any testable hypotheses; it does not convey a rigorous sampling scheme; and it does not enable the reader to easily decipher how the data was assembled for analysis. These characteristics, along with other minor issues, make the manuscript–in its present form–unfit for publication. I feel that the authors have done good work but, in my humble opinion, need to substantially revise the entire manuscript before publishing.

Response: We substantially revised the entire manuscript according to your suggestions.

Are substantial conclusions reached? The manuscript has potential to convey interesting, meaningful results but it fails to achieve this goal due–in part–to inherent inconsistencies and other shortcomings pertaining to sample tracking and reporting. As result, it is difficult for the reader to determine the impact of any conclusions this manuscript offers.

Response: Agree. We revised the manuscript and now present objectives and results more clearly.

Are the scientific methods and assumptions valid and clearly outlined? The methods and assumptions could be better described. Please see suggested comments and edits in the supplement pdf provided.

Response: Thank you very much for the detailed comments in the supplement. We tried to address them as detailed as possible and provide all responses below.

Are the results sufficient to support the interpretations and conclusions? I am concerned about the threat of pseudoreplication in the dataset. This stems from the apparent inclusion of all sequence reads generated from the products of triplicate PCRs performed on individual environmental samples. If one were to assume a sample size that corresponds to the number of PCR replicates (i.e., N = 146 samples; 6 unique samples amplified in triplicate = 18 samples x 7 lanes on the NGS platform), as has

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been done here, then the data would contain pseudoreplicated units. Performing multiple PCRs on a single sample should be a step when preparing for 454 sequencing. At the very least, conducting replicate PCRs provides evidence that DNA template is actually present and will amplify using the chosen primers. However, treating pseudoreplicated units as replicate units–as appears to have been done here–will violate key statistical assumption of independence of samples. I would suggest the authors conduct their analysis on only one replicate per sample, which would appear to reduce their N from 126 to 42.

Response: We pooled the methodological replicates for all biotopes, resulting in 40 samples.

Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? I do not think it would be possible for others to reproduce this work given the manuscript's present format. For example, there appears to be either a miscommunication or misunderstanding about the next generation sequencing (NGS) platform used. The author's cite a "Roche 454 GS-FLX Titanium sequencer." This instrument should be referred to as the "Roche GS-FLX Sequencer using Titanium Chemistry." Regardless, the authors describe sending 7 pooled samples to Macrogen for sequencing using the above NGS platform on "1/8 lane each." To the best of my knowledge, the GS-FLX instrument uses a picotitre plate. DNA capture beads containing sequence template–DNA amplified via emulsion PCR–are flowed over the plate and captured in nano-sized wells. Sequencing of the DNA template library, therefore, occurs within individual wells. There are millions of wells per plate allowing for multiplexing different tagged samples on a single plate. Illumina platforms, such as the HiSeq, use lanes. It would be helpful if the authors would rectify this apparent conflict.

Response: We agree that there were some sloppy and incorrect descriptions in the former version of the manuscript. We revised the methods section.

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Interactive Comment

Do the authors give proper credit to related work and clearly indicate their own new/original contribution? Archaea have previously been reported in association with L. pertusa by Emblem et al. (2012). It may behoove the authors to conduct a more thorough literature review before making claims of first-discovery. However, it could be that the authors are âĂĒŸTinĒĞ factâĂĒŸT the first to report Archaea in association with L. pertusa growing on a carbonate ĒĞ mound in the Logachev Mound Province.

Response: We now give deserved credit to Emblem et al. and changed our text. To our knowledge and this of another anonymous reviewer, it was the first time that Archaea were found in mucus of L. pertusa.

Is the overall presentation well-structured and clear? No. Please see comments and suggested edits in the supplemental pdf provided. Most of these suggestions are copy-edits and can easily be included if accepted. Doing so may strengthen the overall presentation and clarity of this manuscript. However, there are other potential issues that may require the authors to re-analyze the entire data set (i.e., pseudoreplication caused by the inclusion of triplicate PCRs in the sample set).

Response: we re-analysed the data and revised the figures and manuscript.

Is the language fluent and precise? Fluency has been demonstrated but there are numerous grammatical errors and a recurring theme of imprecision. The English language is inherently ambiguous. Sadly, this means great attention must be paid to word selection and grammar to ensure statements of objectives, methods, and conclusions cannot be misinterpreted or misunderstood. Though the authors demonstrate good command of the English language, it is recommended they revisit the entire text to ensure the appropriate use of punctuation, grammar, verb tense, and paragraph cohesion. Some suggestions have been provided in the supplement pdf.

Response: We revised the entire text and have accepted most suggestions provided in the supplement (see pdf).

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Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? There are numerous instances where units of measurement appear to be missing. These should be included when and wherever appropriate. Additional issues exist whereby the authors do not introduce abbreviations in the body of the text despite their use in Tables and Figures (e.g., near-bottom water = w_bc in Table 1; water column above the mound = w_CTD in Table 3). There are also some general inconsistencies throughout the text with regard to the use of abbreviations. For example, in the Abstract the term "5 + 10 m above bottom (ab)" is used. Later in the text this is written as "5 and 10 m ab." It is recommended that the "+" be replaced with "and" throughout the text and that the abbreviation "ab" not be used in the abstract. Generally, acronyms should not be used in the abstract unless the term is to be used frequently.

Response: We deleted acronyms from the abstract and used more consistent descriptions for sample categories.

Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? Figures 6 and 7 are very difficult to interpret due to their present size and quantity of information. It would be helpful to readers if these charts would be enlarged so each one occupies a single page.

Response: Figures 6 and 7 are enlarged and revised to make them well readable.

Detailed responses to comments from the supplement file

P1510:

Is it appropriate to propose a hypothesis in the abstract? Further, this does not seem to be one of the overall conclusions derived from your present work. You might consider removing this statement from the Abstract and relocating it to your Discussion/Conclusions.

Response: Rephrased

P1512:

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More detailed Than what?

Response: Removed more and rewrote the whole paragraph

This seems like your overall objective yet it is hidden within the text. I would suggest you re-write this last paragraph so that your objective is clearly conveyed in the first sentence.

Response: Rephrased and adjusted

P1513:

Consider adding Table 1

Response: New Table 1 made with clear overview of samples taken. See reference further down.

Please denote how many video transects were performed

Response: two transects, now added in text and indicated in Figure 1.

Explain “on board”

Response: video’s were analysed on board before sampling.

P1514:

Please clarify. Was the volume of each Niskin bottle 11 L? If so, how many individual Niskin bottles were in the rosette attached to the CTD?

Response: clarified: each bottle had 11 L volume

Did you conduct serial filtration using filters with different porosities? Were there any issues filtering 2L of seawater with only a 0.2-um filter? Please describe the filtration apparatus that you used.

Response: No issues with filtering encountered.

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Please clarify. At each of the 3 depths specified above (e.g., 400m, 5 m ab, and 10 m ab) how many Niskin bottles were fired?

Response: 1 bottle at each depth.

I am concerned that readers would interpret this statement to mean a single Niskin bottle with a volume of 11 L was fired at, for example, 400 m (N = 1 at 400 m). 2 L of water from this single Niskin bottle was then filtered through a 0.2 polycarbonate filter. This process was repeated two more times using water from the same Niskin bottle. This would be pseudoreplication.

Response: These were methodological replicates. To check for consistency of the whole process, from filtering up to the ngs sequencing. For comparisons of microbial communities we pooled the samples.

Did you clean and/or sterilize the components of the box core that contacted each specimen? If not, can you please justify why this was not done to prevent the possibility of cross-contamination?

Response: equipment was thoroughly cleaned with sea water.

P1515:

This needs a citation

Response: see next section describing the Mobio kit.

Please clarify. Did you PCR each DNA extract two times? Why?

Response: To avoid PCR bias each DNA extract was used in duplicate PCRs. The products of these PCRs were pooled later in the process. Text is rewritten to make this more clear.

How was this done? As described above?

Response: Yes. Changed text.

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P1516:

It would be helpful to describe what each of the 7 pooled samples consisted of?

Response: Text changed.

I believe this should be referred to as "Roche GS FLX Sequencer using Titanium chemistry."

Response: corrected

To my knowledge, the GS FLX Sequencer does not use "lanes." Rather, it employs a picotiter plate on which all tagged, emPCR samples that are attached to DNA capture beads are contained. Sequencing then occurs within each hole on the picotitre plate containing a bead with template DNA. One fragment = One bead = One read. The use of "lanes" connotes Illumina. Please be sure you are not conflating NGS platforms and technologies.

Response: Corrected “lanes” to “region”

Did you use the RDP Pipeline Initial Process to first sort according to the forward-MID? It would be helpful if you could be more explicit.

Response: Yes, we did. See modified text.

It would be helpful if you could elaborate on what is meant by "lanes." To the best of my knowledge, Roche 454 sequencing does not employ "lanes." Rather, pooled samples are poured over a picotitre plate.

Response: text is changed to make this more clear.

Please include a small table summarize the 6 unique samples. If Table 3 summarizes these samples then why does it only include 5 main categories of sample?

Response: We processed 7 x 18 = 126 samples. These were all separate samples, separate filters and separate pieces of skeleton, processed independently with different

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DNA extractions and duplicate PCRs. However, since samples were taken from the same Niskin bottle, or box corer we now consider the samples as pseudoreplicates as was suggested.

Please be more explicit. Did you normalize according to relative abundances of sequence reads within a sample?

Response: Yes, text is rewritten to make this more clear.

What does "triplo's" and "duplo" mean? Triplicate PCRs of 41 samples? Duplicate PCRs of 1 sample? Why did you perform only duplicate PCRs on the one sample? Which sample is it?

Response: by default we took 3 samples of each biotope per station. In one case (. . ...) we lost 1 of the 3, remaining 2.

It is my understanding that a similarity matrix can be constructed using Bray-Curtis but that this is a dissimilarity metric. Please check this for accuracy and change your text if/where necessary.

Response: Yes, correct: Bray-Curtis calculates a distance matrix. Text is changed.

Why did you skip over family?

Response: This was the choice we made. Almost all reads were classified to class level. So this gives a good overview. The genera are informative in more detail and from genera the family can be deduced.

P1517:

Be sure that you have conjugated your verbs correctly. For the most part, they should all be past tense because you are talking about what you have measured.

Response: Text has been modified accordingly.

Fig. 3b-d describes data collected only in 2012, as per the Fig. legend.

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Response: Changed figure caption.

This is listed as N, not NW slope in the legend. Please be sure they are congruent.

Response: Text, figure captions and tables have been made congruent.

Please add a unit of measurement.

Response: Salinity is measured in psu.

It would be helpful to assign a depth to the foot of the deep SE slope.

Response: Depth is mentioned.

You might consider refraining from using subjective descriptors when describing the data. A decrease of 0.2 ppt may not be considered a "sharp" decrease by readers. Further, Fig. 3c does not reflect your description of the data.

Response: We agree, and changed text.

P1518:

It would be helpful to include the monikers you appear to use in Figure legends and tables for each environmental samples (e.g., near-bottom water = water_bc). I have not seen these introduced previously. It would help readers understand what is being conveyed in the tables and figures.

Response: Adjusted.

It would be helpful to briefly describe why you chose to report Chao1 and how this differs from the number of distinct OTUs associated with a sample type.

Response: described in text now.

You report PD_in_tree and Shannon in Table 3 yet do not discuss these metrics anywhere in the text. Why?

Response: PD value deleted since we do not discuss this in the text.

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Also, did you perform any tests of significance (e.g., t-tests) on the diversity indices associated with the different biotopes? If so, please report these and convey whether diversity associated with one biotope was, in fact, statistically significantly different from other biotopes.

Response: We report standard errors of the mean for the biotopes but did not do t-tests

This is a run-on sentence that needs to be re-written.

Response: Done

P1519:

Please report percentages for the relative abundances. It is not helpful to only report the names of the classes and list them as most abundant. For example, if Gammaprotebacteria was 10% relative abundance and all other classes were 1%, Gammaproteobacteria would be the most relatively abundant but perhaps not the most ecologically significant class of microbes.

Response: We report percentages now.

P1520:

This definition should be included in the methods section when "Specific indicators" was first introduced.

Response: We included the definition in the methods section.

P1521:

Particles of what?

Response: i.e. phytodetritus added in text.

P1524:

This seems a little out of place. Are you trying to cite Schottner et al. (2009) as

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supporting evidence for the pattern you found between coral skeleton and mucus?

Response: now put in relation to variability

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C2695/2015/bgd-12-C2695-2015-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 12, 1509, 2015.

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