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Biogeosciences Discussions
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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.

Anonymous Referee #2

Received and published: 23 April 2015

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 objectives 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.

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Interactive Discussion
Discussion Paper

1. Does the paper address relevant scientific questions within the scope of BG?

This manuscript attempts to characterize and compare microbial communities associated with 5 pre-determined biotopes from a single carbonate mound in the Logachev Mound Province. Further, the authors explore the role(s) of various abiotic factors associated with the Haas Mound environment as potential drivers for the assemblage of these microbial communities. These questions are relevant to and fall within the scope of Biogeosciences.

2. Does the paper present novel concepts, ideas, tools, or data?

The authors propose having discovered, for the first time, Archaea associated with the cold-water coral Lophelia pertusa. The identification of Archaea in addition to Bacteria has the potential to provide great insight into the functional ecology of this framework-builder of cold-water habitats. Accordingly, the paper attempts to correlate the environmental attributes of Haas Mound with the structure of relevant microbial communities. Testing for correlations between environmental factors and microbial community structureâĂŤand hence microbial ecologyâĂŤis fundamental within the purview of the marine sciences. Given the unique attributes of Haas Mound, the authors have put forward novel concepts for assessing the diversity and, by consequence, ecology of microbial communities associated with cold-water coral habitats.

3. 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.

4. 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.

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5. 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 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.

6. 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

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would be helpful if the authors would rectify this apparent conflict.

7. 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 areâĂŤin factâĂŤthe first to report Archaea in association with L. pertusa growing on a carbonate mound in the Logachev Mound Province.

8. Does the title clearly reflect the contents of the paper?

Yes.

9. Does the abstract provide a concise and complete summary?

Yes.

10. 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).

11. 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 punctu-

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ation, grammar, verb tense, and paragraph cohesion. Some suggestions have been provided in the supplement pdf.

12. 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.

13. 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.

14. Are the number and quality of references appropriate?

Yes.

15. Is the amount and quality of supplementary material appropriate?

Yes.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C1613/2015/bgd-12-C1613-2015-

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supplement.pdf

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