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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 #1

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The manuscript (MS) addresses the distribution of microbial consortia associated with both cold-water corals and their abiotic environment along depth gradients on a sea mound in the NE Atlantic. The topic is clearly relevant and within the scope of BG. The paper presents novel data, as for the first time amplicon sequencing targeting both Bacteria and Archaea is carried out on cold-water coral samples. This has the potential to augment our understanding of deep sea microbiota. The authors conclude that variability in seawater microbiota at different heights above ground is a function of mixing efficiency, modulated by internal waves and coral framework.

While the MS clearly has the potential to convey interesting results, presentation leaves much to be desired. The MS misses a clear hypothesis, the only hint on the research

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that has been conducted being "By exploring links between mound biotopes and the microbial community [...]" (P 4, L 24-25). This is too vague. From table 1 it is hard to discover a rigorous sampling scheme for the box-core sampling. I strongly recommend to include a map with information on the sample types instead of the locations of CTDs, box cores, and landers. The uninformed reader should immediately comprehend what was collected when and where on the mound.

Sampling took place during the same month in two consecutive years, apparently trying to sample comparable locations but seemingly without trying to get complete sets of sample types (e.g., water from the box core was taken from station 46 in 2012, while from the comparable station 8 sediment was taken in 2013). Sadly, this weakens the impact of the MS, all the more since a time effect was discovered for the microbiota of the overlaying water column that cannot be seen in the box-core samples due to the incomplete sampling scheme.

Combination of multi-dimensional scaling and analysis of similarities (ANOSIM) is a standard approach in high-throughput sequencing analysis. Given that we are dealing with two factors in the water column data (Year and Biotope), the use of more sophisticated tools such as (distance-based) redundancy analysis [(db)-RDA] would be more appropriate: This method can control the effect of one factor when testing the other or test for an interaction of both effects. This analysis could be conducted with functions rda or capscale in the R package vegan.

Figure 5 shows an MDS plot based on taxonomic classification of microbial OTUs at the genus level. I would like to stress that taxonomy is an ever-changing and often rather arbitrary system. Unless there is a justified reason, analyses should be directly based on the OTU counts, since these provide the best resolution and do not depend on any external classification system.

Table 3 states different numbers of samples for the calculated indices with the same sample category (e.g., for w_bc, n = 14 for "reads/sample" and n = 9 for "observed

OTUs"). This is not comprehensible. Please base index calculation on the same number of samples.

Several studies mention Mycoplasma (Candidatus Mycoplasma corallicola) as one component of Lophelia pertusa-associated microbiota (Neulinger et al., 2008; Kellogg et al., 2009; Neulinger et al., 2009). This should also receive credit in the MS. Apparently, the authors did not detect Mycoplasma in their coral samples with the employed methodology. Probable causes of this should be discussed.

The authors state to have found Archaea on L. pertusa for the first time. However, an earlier study by Norwegian researchers has already shown Archaea to reside on this coral (Emblem et al., 2012). The authors should therefore revise their statement and give credit to the above-mentioned study.

The title clearly reflects the contents of the paper. The abstract provides a concise and complete summary of the MS. However, the authors should change "5+10m" to "5 and 10 m", as the plus sign is misleading here. I would also refrain from abbreviating "above the bottom" by "ab" in the abstract.

English language is used adequately. The number and quality of references appears appropriate, as does the supplemental material.

Minor points:

P 8, L 25: change "taxa" to "taxonomic units"

P 9, L 17: It is stated that hydrographic profiles are shown for the years 2012 and 1013 in Fig. 3b–d, but the respective figure only shows data for 2012. Please correct.

P 16, L 3: change "harbored" to "exhibited".

Table 1: For year 2013, there are three biotope samples listed between Station 9 and 11 (sediment, Skeleton uneroded, Skeleton eroded) for which no further description is given. Do they belong to Station 9 or was their station and description omitted

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accidentally? Please elaborate.

Table 3: There is one diversity index "PD_in_tree" that is neither explained nor referred to anywhere in the text. Please show only data that you are going to use. If you are going to discuss this index, please provide a definition for it.

Figure 8b: A grouping by station number is uninformative and forces the reader to look tediously for the properties of the stations. Please provide a more meaningful categorization (e.g., "Off/Slope/Summit").

Figure 8c: The first three categories in the legend (off w_400m, summit w_400m, slope w_400m) cannot be distinguished by their symbols/colors. Please improve.

References

Emblem, Å., Karlsen, B.O., Evertsen, J., Miller, D.J., Moum, T., and Johansen, S.D. (2012) Mitogenome polymorphism in a single branch sample revealed by SOLiD deep sequencing of the Lophelia pertusa coral genome. Gene 506: 344-349.

Kellogg, C.A., Lisle, J.T., and Galkiewicz, J.P. (2009) Culture-independent characterization of bacterial communities associated with the cold-water coral Lophelia pertusa in the Northeastern Gulf of Mexico. Appl Environ Microbiol 75: 2294-2303.

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Neulinger, S.C., Gärtner, A., Järnegren, J., Ludvigsen, M., Lochte, K., and Dullo, W.-C. (2009) Tissue-associated "Candidatus Mycoplasma corallicola" and filamentous bacteria on the cold-water coral Lophelia pertusa (Scleractinia). Appl Environ Microbiol 75: 1437-1444.

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