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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.***

**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.

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

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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 that has been conducted being "By exploring links between mound biotopes and the microbial community [...]" (P 4, L 24-25). This is too vague.

Response: We now clearly state our objectives and hypotheses (P3, L80-86)

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.

Response: A new Table (Table 1) has been added with an overview of all samples taken. Figures 1-2 and tables 2-3 with station information have been revised.

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

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could be conducted with functions `rda` or `capscale` in the R package `vegan`.

Response: The effect of year on the microbial community was significant for overlaying water, but very small compared to the differences due to biotope. Box core water (near-bottom water) samples were taken in both years but still clustered separately from other biotopes. We think that sampling over two years strengthens our manuscript because it shows that the patterns we find are consistent. For overlaying water we did additional dbRDA as you suggested and found influence of the variables Turbidity (correlating with year), and temperature, salinity and density (P. 14, L354-357).

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.

Response: We now show the MDS plots based on OTUs. (Fig. 5 and S.I. Fig. 2)

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.

Response: We first choose to calculate the index values on a fixed reads/sample value and because samples differed in total amount of reads, not all samples contributed to this value. We agree that this is confusing and recalculated the indexes

Several studies mention *Mycoplasma* (Candidatus *Mycoplasma coralicola*) 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.

**BGD**

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Response: Mycoplasma was reported for *L. pertusa* tissue. We did not sample tissue but fully agree that this aspect should get more attention in our manuscript. We found low amounts of Mycoplasma in uneroded (recently deceased) skeleton but not in mucus. (P13, L329-330; P18, L446-452).

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.

Response: We now give rightful credit to Emblem et al. To our knowledge it is the first time that archaea were found in mucus. We revised the text.

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.

Response: Agree and fixed.

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 C317 BGD 12, C315–C318, 2015 Interactive Comment Full Screen / Esc Printer-friendly Version Interactive Discussion Discussion Paper 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

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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.

Response: All of the above mentioned minor points were addressed in the revised manuscript.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C2678/2015/bgd-12-C2678-2015-supplement.pdf>

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