Response to Anonymous Referee #1

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 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 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 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.
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 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.

Response to Anonymous Referee #2

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 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.
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 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.

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 âAˇTinˇ factâAˇT the first to report Archaea in association with L. pertusa growing on a carbonate ˇ 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.

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:

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.

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.

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 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.
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.

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 supporting evidence for the pattern you found between coral skeleton and mucus?
Response: now put in relation to variability

Response to Anonymous Referee #3

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.

This manuscript describes the microbial community in a cold water coral environment and links it to environmental parameters including water flow and topography. For the first time, Archaea are detected in coral mucus. Major point: Neulinger et al and Kellogg at all report on two potential symbionts of Lophelia and Hansson et al. on one of Madrepora. It is somewhat startling that the authors completely ignore that. It is imperative to address this question: do their data support that or not.

Response: We now give rightful credit to Emblem et al. (2012). To our knowledge it is still the first time that archaea were found in mucus. We revised the text. 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). We also address TM7 and give credit to the authors you mention.

Minor points: Where are the sequences deposed?

Response: Datafiles will be available via ENA once the paper is accepted.

The size of the collected corals is not mentioned. Is there information on the type of branches (old vs young)?

Response: Now described in the text: ~0.5 cm. young branches were white and uneroded without biofilm; old skeleton was eroded, brownish and with biofilm.

How long lasted the incubation for collecting mucus?

Response: We collected ~0.5 mL mucus according to Schottner et al. 2012 and incubated 2-3 minutes.

Microbial assemblages on a cold-water coral mound at the SE Rockall Bank (NE Atlantic): interactions with hydrography and topography

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Keywords: cold-water coral mound, microbial assemblages, Roche 454 sequencing, hydrodynamics, biotopes

Abstract

This study ~~shows~~characterizes the microbial community composition over Haas Mound, one of the most prominent cold-water coral mounds of the Logachev Mound Province (Rockall Bank, NE Atlantic~~), outlining~~). We outline patterns of distribution ~~patterns both~~ , vertically ~~--~~from the seafloor to the water column-- and laterally --across the mound-- and ~~coupling this~~couple these to mound topography and hydrography. Samples of water, sediment and *Lophelia pertusa* were collected in 2012 and 2013 from ~~biotopes~~locations that were ~~partially~~ chosen based on high definition video surveys ~~that were conducted prior to sampling and included overlaying water (400 m depth and 5+10 m above the bottom) collected with a CTD/Rosette system and near-bottom water, sediment, *Lophelia pertusa* mucus, and *L. pertusa* skeleton samples collected with a box-core. Furthermore, temperature~~. Temperature and current measurements were obtained at two sites at the summit and foot of Haas Mound to study near-bed hydrodynamic conditions. ~~Community composition was determined by next generation Roche 454 sequencing yielding high-resolution records of 16S rRNA genotypes, improving~~Overlaying water was collected from depths of 400 m as well as 5 and 10 m above the bottom using a CTD/Rosette system. Near-bottom water, sediment, and *L. pertusa* mucus and skeleton samples were obtained with a box-corer. Of all these biotopes, Roche GS-FLX amplicon sequencing targeting both Bacteria and Archaea was carried out, augmenting our understanding of deep- sea microbial consortia. ~~With the methods we employed we were able to report for the first time Archaea in association with *L. pertusa*.~~ The pattern of similarities between samples, visualized by multi-dimensional scaling (MDS), indicates a strong link between the distribution of microbes and the specific biotopes. ~~All biotopes share a number of taxa, but biotopes are distinct on basis of relative abundances and a small number of unique taxa. Similarity in microbes indicates that water~~ The microbial OTU diversity was highest in

near-bottom water, which was sampled in the coral framework. For the first time, Thaumarchaeota MGI were found in *L. pertusa* mucus; *Ectozoicomonas* was detected in skeleton, mucus and near-bottom water; whereas *Mycoplasma* was only detected in skeleton and near-bottom water, however not in mucus. ANOSIM indicates that overlaying water is well-mixed at 400 m depth~~,~~ but less so at 5~~+~~ and 10 m above the bottom, where the composition of microbial communities differed significantly between summit, slope and off ~~mound. Even more variability was observed in the near-bottom water samples, which group according to sampling station. Likely the coral framework prevents~~-mound. At all locations, the near-bottom water differed significantly from water at 5 m above the bottom, illustrating that the near-bottom water in between the ~~branches to be vigorously mixed with the water overlaying the reef. The microbial consortium on Haas Mound appears strongly linked with the surrounding environment, making cold-water coral~~ coral framework represents a separate microbial habitat. Further, the observed spatial heterogeneity in microbial communities ~~sensitive~~is discussed in relation to ~~outside~~ environmental ~~influences.~~

conditions.

1. Introduction

Numerous mounds composed of a mixture of sediment and cold-water coral debris line the Southeast slope of Rockall Bank, between 500 and 1100 m water depth (Kenyon et al., 2003; van Weering et al., 2003). This so called 'Logachev Mound Province' consists of mounds varying from tens to hundreds of m in height and several km in length and width (Kenyon et al., 2003). These mounds have been developing since the middle Miocene - early Pliocene, largely as the byproduct of interacting hydrodynamic regimes, coral growth and sedimentation Numerous mounds composed of mixed sediment and cold-water coral debris line the Southeast slope of Rockall Bank between 500-1100 m water depth (Kenyon et al., 2003; van Weering et al., 2003). This so-called "Logachev Mound Province" consists of mounds varying from tens to hundreds of m in height and several km in length and width (Kenyon et al., 2003). These mounds have been developing since the middle Miocene-early Pliocene, largely as the by-product of interacting hydrodynamic regimes, coral growth and sedimentation (De Haas et al., 2009; Mienis et al., 2007)(De Haas et al., 2009; Mienis et al., 2007).

Living coral colonies of mainly *Lophelia pertusa* and *Madrepora oculata* inhabit the mound summits and flanks, providing habitat for a wide range of invertebrates and fish (Costello et al., 2005; van Soest et al., 2008)(Costello et al., 2005; van Soest et al., 2008).

Deployments of current and temperature sensors at different sites in the Logachev Mound province have provided evidence of large regional differences with respect to current strength, temperature fluctuations and organic carbon supply (Mienis et al., 2007). Also on the scale of individual mounds significant heterogeneity in environmental conditions was found for instance between the summit and foot of mound structures (Duineveld et al., 2007). More recent studies on the near-bed hydrodynamic regime in the Logachev Mound Province revealed intense mixing on the mounds as a result of internal waves interacting with the

~~topography (Mohn et al., 2014; van Haren et al., 2014). This mixing not only provides a constant food supply, but also ensures the removal of CO_2 from the area and the constant refreshment of dissolved oxygen and nutrients (Findlay et al., 2014). The relevance of this variation for the growth of cold-water coral framework and mounds as a whole is a subject of current studies (Mienis et al. 2014, pers. comm.)~~

~~Microbes have been found crucial for the fitness of tropical corals (Knowlton and Rohwer, 2003; Krediet et al., 2013; Rosenberg et al., 2007). Shifts in the composition or metabolism of coral-associated microbial consortia can significantly reduce resilience of tropical corals, increasing stress, disease, and death~~ . Measurements of currents and temperature around the Logachev Mound Province have provided evidence of large regional differences with respect to current strength, temperature fluctuations, and organic carbon supply (Mienis et al., 2007). Significant heterogeneity in environmental conditions has also been found within individual mounds, such as between the summit and foot of mound structures (Duineveld et al., 2007). Recent studies on the near-bed hydrodynamic regime in the Logachev Mound Province revealed intense mixing on the mounds as a result of internal waves interacting with the topography (Mohn et al., 2014; van Haren et al., 2014). Such mixing provides a supply of food particles, i.e., phytodetritus, and constant refreshment of dissolved oxygen and nutrients (Findlay et al., 2014). The relevance of the hydrodynamic mixing regime for the growth of cold-water coral framework and mounds as a whole is a subject of current studies (F. Mienis, personal communication, 2014).

Other studies have already shown that cold-water coral reefs are hotspots of carbon mineralization ~~(Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Gilbert et al., 2012; Rohwer and Kelley, 2004)~~(Rovelli et al., 2015; van Oevelen et al., 2009)~~.~~

~~Insight into the distribution and variability of microbial communities in cold-water coral ecosystems is progressing, and has revealed a distinction between cold-water coral-associated~~

~~and ambient microbial communities. Furthermore, substantial spatial variability of microbial consortia was found~~ and metazoan biodiversity and biomass ~~(Hansson et al., 2009; Neulinger et al., 2008; Penn et al., 2006; Schöttner et al., 2012; Yakimov et al., 2006)~~(Biber et al., 2014; Henry and Roberts, 2007)~~. Most of the studies thus far have been limited to estimates of the number of operational taxonomic units (OTU) present in cold-water coral associated microbial communities and have been comparing geographically separate sites with limited insight into the distribution of microbes throughout an individual cold-water coral habitat or consideration of local hydrographic conditions (Galkiewicz et al., 2011; Hansson et al., 2009; Jensen et al., 2012; Jensen et al., 2008; Kellogg et al., 2009; Neulinger et al., 2008; Schöttner et al., 2009). On the basis of samples taken at a variety of spatial scales in relatively shallow cold-water coral reefs off Norway, Schöttner et al. (2012) concluded that the composition of microbes associated with *L. pertusa* is a product of multiple factors at multiple scales. Especially towards the reef periphery Schöttner et al. (2012) found increased variability in microbial communities on local and regional scales, suggesting significant biogeographic (habitat) imprinting. Observed heterogeneity on cold-water coral mounds has led to the suggestion that the collection of a single sample from a site, or the sampling of a single location in a cold-water coral habitat cannot adequately offer insight into the microbial community as a whole (Findlay et al., 2014).~~

~~In the present study a more detailed analysis was made of the composition and distribution of microbial communities across a larger, individual deep cold-water coral mound, laterally from summit to base of the mound and vertically from sediment to the water column. Community composition involving both bacterial and archaeal domains in this case is determined by next-generation Roche GS-FLX sequencing yielding high-resolution records on the basis of 16S rRNA analysis. By exploring links between mound biotopes and the microbial community we made a first step towards a better insight into importance of cold-water coral habitats for~~

~~microbial diversity and function. Earlier studies have already shown that these habitats are hotspots of metazoan biodiversity and biomass, and of carbon mineralization~~ and as such deserve our attention and protection. Whether these reefs are also biodiversity hotspots for microbial communities was qualified "questionable" based on low bacterial OTU numbers in ARISA profiles (Schöttner et al., 2012). Microbes are crucial for the fitness of tropical corals (Knowlton and Rohwer, 2003; Krediet et al., 2013; Rosenberg et al., 2007). Shifts in the composition or metabolism of shallow-water coral-associated microbial consortia can significantly impair the health of tropical corals by increasing stress, the incidence and prevalence of disease, and causing mortality ~~(Biber et al., 2014; Henry and Roberts, 2007; van Oevelen et al., 2009)~~(Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Gilbert et al., 2012; Rohwer and Kelley, 2004).

~~2.~~In deep cold-water coral ecosystems insight into the distribution and variability of microbial communities is now also progressing. Research has begun to reveal patterns in the composition of microbial communities associated with cold-water corals (Emblem et al., 2012; Galkiewicz et al., 2011; Hansson et al., 2009; Kellogg et al., 2009; Neulinger et al., 2009; Neulinger et al., 2008; Penn et al., 2006; Schöttner et al., 2009; Schöttner et al., 2012; Yakimov et al., 2006) and the ambient environment (Jensen et al., 2012; Jensen et al., 2014; Jensen et al., 2008; Schöttner et al., 2012; Templer et al., 2011). Schöttner et al. (2012) concluded that bacteria in coastal CWC reefs are structured based on habitat (coral branch, mucus, water and sediment) and reef location (four reefs located off Norway). Jensen et al. (2014) found bacterial communities to be similar in water sampled proximal (~1 m) and distal (30 m) in one reef, whereas in another reef these communities clearly differed.
In the present study a detailed analysis was made of the composition and distribution of microbial communities across Haas mound, a deep cold-water coral mound in the NE

Atlantic. The main objective of this study is to provide insight into diversity of microbial communities (Bacteria and Archaea) within different biotopes at Haas Mound. Besides the water column these biotopes included the major surfaces that are in contact with the water, i.e., coral framework, coral mucus and sediment. Our hypotheses are: 1) microbial communities, including Bacteria and Archaea, will be structured based on above mentioned biotopes; 2) within the water column we expect a reef effect on the microbial community composition at close distance above the reef.

2 Materials and methods

2.1 Location and sample collection

Samples were collected during cruises 64PE360 (October 2012) and 64PE377 (October 2013) aboard the RV Pelagia (NIOZ) in the Logachev Mound Province on SE Rockall Bank ~~in October 2012 and 2013, respectively (Figure 1A).~~(Fig. 1a). The focus site ~~of~~for this study was Haas Mound ~~being,~~ one of the largest and highest ~~(360 m)~~ carbonate mounds in the Logachev Mound Province ~~(Mienis et al., 2006)~~(Mienis et al., 2006) (~~Figure 1B). Transects~~Fig. 1b). Two transects (Fig. 1c), from the base to the summit of Haas Mound, were ~~first~~ surveyed with a tethered HD video camera towed at ~~~~2 m above the bottom (mab). Videos were annotated on ~~the fly~~board and box-~~core~~corer locations were selected ~~based on annotations.~~ representing the variation in coral cover and megafauna composition. ~~Samples of the bacterial~~Microbial community samples (Table 1) were collected ~~in~~from a range of putative biotopes across Haas Mound that were operationally defined using video information, hydrographic data ~~from~~collected during the 2012~~–~~2013 cruises and earlier ~~(e.g. Mienis et al., 2007),~~(e.g. Mienis et al., 2007), and literature on coral microbe interactions ~~(Carlos et al., 2013; Kellogg et al., 2009; Schöttner et al., 2012; Wild et al., 2008)~~(Carlos et al., 2013; Kellogg et al., 2009; Schöttner et al., 2012; Wild et al., 2008). ~~The different~~ These

biotopes ~~that we sampled~~ were: (i) water well above the mound ~~(~~i.e. at 400 m water depth~~),~~ ~~iii~~; (ii) water overlaying the coral framework at 5 and 10 mab~~,~~; (iii) near-bottom water; (iv) ~~near-bottom water, v)~~ sediment~~, vi) *L.*~~ ; (v) uneroded (recently deceased) and eroded *L. pertusa* ~~dead~~ skeleton ~~(uneroded as well as eroded pieces) and vii) coral~~ ; and (vi) *L. pertusa* mucus.

Box-core samples were taken with a 50 cm diameter, NIOZ designed box-~~core.~~ ~~The~~corer. This box-~~core~~corer is equipped with a tightly-sealing top valve~~, which~~ that prevents ~~he~~the leakage and/or exchange of sea water overlaying the sample during ascent~~, allowing~~ enabling sampling of the near-bottom water once the box-corer was on board. A total of 9 ~~successful~~ box-cores were collected on the two transects~~, i.e. 5 were collected in 2012 and 4 were collected in 2013 (Figure 1C and 2). Of the 2012 box-cores,~~ (Table 2, Fig. 1D) and from these, *L. pertusa* skeleton, mucus and near- bottom water ~~samples~~ were ~~retrieved from 3~~taken when available. We differentiated between eroded and ~~both living and dead (~~uneroded ~~and eroded) *L. pertusa* samples were collected from 2 box-cores. Sediment samples were collected from box-cores taken during the 2013 cruise (Table 1).~~

~~———~~skeleton based on its discoloration (”white” for uneroded skeleton, without biofilm, and “brown” for eroded, older skeleton with biofilm). The water column overlaying Haas Mound was sampled using a rosette ~~of 11 L~~sampler equipped with 24 Niskin bottles of each 11 L, attached to a conductivity-temperature-depth (CTD) meter. For each CTD drop, water was collected from three different depths ~~were chosen to collect water: at~~: 400 m water depth and ~~at~~ 5 and 10 mab (Table ~~2). Samples from a total of 7 CTD stations were analyzed, 4 sampled in 2012 and 3 sampled in 2013 (Figure 1C and 2). In 2013~~3, Fig. 1C). Also, one off- mound station at 1200 m water depth, situated 10 km SE from Haas mound was sampled with the CTD ~~in the same way~~ to determine if water mass characteristics ~~above~~near the mound differ ~~much~~ from those off- mound and in deeper water.

Water sampled for microbial DNA analysis was filtered ~~through~~directly on 0.2 ~~µm~~µm polycarbonate filters (Whatman~~). At each~~) using mild under-pressure of 0.2 bar. From each water depth, 3 samples of 2 L were filtered~~.~~ from the same Niskin bottle. The near-bottom water collected from box-cores was sampled in a similar way (3 samples of 0,5 L were taken from the same ~~fashion.~~box-core). Between two casts, the box-corer was thoroughly cleaned and rinsed with seawater. All filters were immediately frozen in 6 ~~ml~~mL Pony vials at -80 ~~°C.~~ ~~Living and dead coral samples (*L. pertusa*) were collected from box-cores which contained framework. Living corals~~°C. Coral mucus as well as ~~uneroded skeletons~~skeleton were ~~collected~~sampled in at least three replicates per box core (preferably from different colonies) ~~from two box-core stations in 2012. The box-cores taken in 2013 contained no living coral, but extra samples of uneroded skeletons as well as eroded skeletons were collected from 2 stations.~~

~~Coral samples were briefly rinsed with demineralized water to wash off seawater and sediment. To sample mucus, coral branches were placed on clean aluminum foil in a 4 °C lab on board. As mucus accumulated around the contact points between the coral and aluminum foil it was collected with a sterile 5 ml syringe and needle. Some mucus could also be gathered directly from the surface of coral branches and polyps. Generally around 0.5 ml of mucus was retrieved. Skeleton samples were deeply scraped along the surface with scalpel blades. Mucus and skeleton scrapings were placed in individual 6 ml pony vials and immediately frozen at -80 °C. In 2013,~~and handled as described in Schöttner et al. (2009). Except for skeleton in 2013, when we replaced the scraping technique ~~was abandoned, due to the low amount of sample material retrieved, and 2-3~~described by Schöttner et al. (2009) by harvesting 0,5-1 cm of coral skeleton ~~was instead~~and directly ~~frozen~~freezing this at ~~-80°~~-80 °C on board. In the lab, these samples were ~~later~~ exposed to liquid nitrogen and homogenized

directly into the MoBio bead extraction tubes for DNA extractionwith sterile mortar and pestle.

2.2 DNA Extraction and 16S rRNA amplicon sequencing

DNA was extracted with Power Soil DNA Extraction Kits (MoBio) according to manufacturer's protocol and extracts were kept frozen at -20 °C. °C. The concentration of the DNA in the extracts was measured with a F-2500 Fluorescence Spectrofluorometer (Hitachi, Tokyo, Ja) Japan) using QUANT-iT™iT™PicoGreen® dsDNA kit (Life Technologies, USA). The quality was checked incidentally on a 1 % (by weight)% agarose gel. To amplify the V4 region of the 16 S rDNA, the universal prokaryotic primer set S-DArch-

To amplify the V4 region of the 16S rDNA the universal prokaryotic primer set S-D-Arch 0519-a-S-15 (5'5-CAGCMGCCGCGGTAA-3'3) (Wang et al., 2007)(Wang et al., 2007) and S-D-Bact-0785-b-A-18 (5'5-TACNVGGGTATCTAATCC-3'3) (Claesson et al., 2009)(Claesson et al., 2009) were used as recommended in (Klindworth et al., 2013)Klindworth et al. (2013). The forward primer was extended with a ten base molecular identifier (MID) barcode to distinguish the samples. Additionally the reverse primer also included a ten base barcode to distinguish the triplicates. Per sampleTo avoid PCR bias, per DNA extract, two separate 50 µlµL PCR reactions were performed, using 1 unit Phusion Taq each (Thermo Scientific) in 1x High-Fidelity Phusion polymerase buffer. The volume of template material was adjusted according to the respective DNA concentration of the DNA to aim for equal amounts of starting material (approximately 10 ng genomic DNA per reaction). The PCR was run on an iCycler™iCycler™ Thermo Cycler (BioRad, USA). Cycle conditions were as follows: 30 secs at 98 °C,°C, then 30 cycles (10 secs at 98 °C°C, 20 secs at 53 °C°C, 30 secs at 72 °C) x 30 cycles, finally °C), followed by 7 min at 72 °C.

~~All~~°C. PCR products were loaded entirely on a 2% (by weight) agarose gel pre-stained with SybrSafe and run at ~~80V~~80 V for 50 min. ~~A blue~~Blue-light ~~converter~~excitation was used when excising the PCR products to avoid UV-damage. ~~Products of the same sample~~Duplo PCR-products were ~~combined~~pooled and purified using the Qiaquick Gel Extraction kit. After fluorimetric quantification as described above, equal ~~concentrations~~amounts (70 ng) of the ~~cleaned~~purified PCR~~ ~~-products were pooled (18 samples with their unique forward-MID and reverse- MID combination per ~~1/8 lane) and with~~ set). Using a MinElute kit (Qiagen~~)),~~ the volume was adjusted to 25 ~~µl~~µL with a final concentration of ~~200~~> 50 ng ~~µl~~µL^{-1} pooled PCR product per set. In total, 7 ~~pooled~~sets of samples were sent to Macrogen (Seoul, South Korea~~)~~ ~~all~~), each set sequenced ~~by a~~using Roche GS-FLX instruments and Titanium ~~sequencer~~chemistry on 1/8 ~~lane each.~~ region gasket.

2.3 Sequence processing, taxonomic assignment and diversity analyses

~~The~~ ~~first steps of~~ sequence library of each sample set was filtered on length and quality, and sorted based on the ~~bio-informatic analysis were done with the~~ forward MID using the Ribosomal Database Project (RDP) pipeline ~~(Cole et al., 2014). To split the library of each lane (pooled sample) the routine "RDP Pipeline~~ Initial process~~" was used.~~ (Cole et al., 2014). Only sequences longer than 250 bases with average *Q*-score above 25 ~~and longer than 250 bases were analyzed. Subsequently, the~~ were kept. These sequences were ~~reverse complemented and~~ sorted~~-~~ according to the reverse MID tags into the 3 replicates. In both procedures ~~only~~a maximum of 2 mismatches in both primers and tags ~~were~~was accepted. ~~Finally~~At the end of the procedure, each of the seven ~~lanes~~libraries were split into 18 samples, 6 unique samples each with 3 replicates. All reads had a similar length of 251 bp.

~~~~ Reads were aligned with PyNAST and checked for chimeras using ChimeraSlayer in Qiime. The read files were classified using the SILVAngs web interface (Yilmaz et al., 2014)
~~~~

with default settings (> 98% similarity of ~~OTU's~~OTUs and > 93% classification similarity to closest relative in SILVA database 119). ~~The classified results were processed in Excel and the taxon's class and genus were extracted and~~ OTU-tables were imported in PRIMERv6 ~~(Clarke, 1993; Clarke and Gorley, 2006). The data were standardized~~(Clarke and Gorley, 2006). The number of reads per taxonomic unit was normalized per sample to avoid biases caused by differences in sample size.

Methodological replicates were pooled. Rarefaction curves and diversity indices were calculated using ~~QIIME (Caporaso et al. 2010)~~PRIMERv6 and plotted in R. For a total of ~~125~~40 samples (~~41~~pooled from 121 independent methodological replicates: 38 triplo's and ~~1 duplo~~2 duplo's namely water of 400 m at station 36 and near-bottom water at station 72), the average ~~amount~~number of reads per sample was ~~5627~~16220 (with standard error ~~228~~1090). Rarefaction curves of OTUs plotted against reads per sample almost reached a plateau at ~~3573 reads per sample (S.I. Fig. 1). The fractions of reads that were assigned to specific taxa were 99% to class, 58% to family and 29% to genus level.). A resemblance matrix was made on class and genus taxa based on a Bray-Curtis similarity coefficient. These resemblance matrices were visualized with MDS plots.~~14000 reads per sample (S.I. Fig. 2).

~~Genera with significant, nonrandom association ($p<0.0001$, 9999 permutations) with one of the five biotopes were identified with Indicator Species Analysis in R using the indicspecies package 1.6.7. (De Caceres and Legendre, 2009) with display of both Indicator Values 'A' and 'B' (Dufrene and Legendre, 1997).~~

Differences in the microbial OTU composition were identified in PRIMERv6 (Clarke and PRIMER, 2006; Clarke, 1993) by analysing Bray-Curtis distance for all pooled samples (n=40), and also for all methodological replicates (n=121). Results were visualized with MDS plots. DBRDA was done in PRIMERv6 on the samples taken at 5 and 10 mab with 7

variables (temperature, salinity, transmission, fluorescence, oxygen, Par and Spar) to explain the variability in microbial community composition within this sample group.

The OTU classification files were processed in Excel and class and genus data were selected for representation to allow easy comparison with other CWC studies (references mentioned in text).

The fractions of reads that were assigned to specific taxonomic units were 99% to class, 58% to family and 29% to genus level. Indicator OTUs, with significant non-random association ($p < 0.0001$, 9999 permutations) with one of the five biotopes, were identified with Indicator Species Analysis in R using the indicspecies package 1.6.7. (De Caceres and Legendre, 2009) with display of both Indicator Values "A" and "B" (Dufrene and Legendre, 1997).

SSU rRNA gene amplicon pyrosequences are available from the European Nucleotide Archive (ENA) via http://www.ebi.ac.uk/ena/data/view/PRJEB9766. Sample accession numbers are listed in Tables 2 and 3.

2.4 Near-bed temperature and current measurements

During the 2012 cruise, temperature and currents were measured on the summit (st5 at 556 m, 55° 29.677 'N, 15° 48.222 'W) and at the foot of Haas Mound (st41 at 861 m, 55° 28.94 'N, 15° 48.28 'W) with an FSI™ 3DACM acoustic current meter (Falmouth instruments) with temperature probe, which was attached to a benthic lander ~~(Figure 1C).~~ at 0.75 mab (Fig. 1c). The duration of each deployment was approximately 48 ~~hr~~h.

3~~.~~ Results

3.1 Haas mound physical environment and coral cover

The ~~SE~~ S-slope of Haas Mound is subject to strong daily variations in ~~watermass~~water mass properties due to internal tidal wave action causing deep, cold water to move up and

down the slope (see details in van Haren et al., 2014). This results in a daily temperature fluctuation at the foot of the mound of ~2.5 °C°C as measured by the benthic lander. A much smaller temperature fluctuation i.e. less than 1 °C°C, was recorded on the summit (Figure 3A).

In Figure 3B-D, temperatureFig. 2a). Temperature, salinity and oxygen profiles measured in 2012 and 2013 are shown for the water column at the off-mound (st2 and 11), NW-mound S-slope (st87)st33), and mound summit (st12) of Haas Mound. (Fig. 2b-d). The temperature of the water column overlaying Haas Mound was around 10 °C at 400 m depth and decreased by 1 °C with every additional 156 m depth. Salinity iswas 35.4 at 400 m depth and decreasesdecreased slightly with depth. These temperature and salinity values are characteristic forof Eastern North Atlantic Water. At the foot of the deep SE slope the presence of cold water is clearly visible as an abrupt decrease in temperaturedeeper off-mound st11 temperatures decreased to 6.6 °C (Figure 3Bat 1000 m water depth (Fig. 2b), while salinity also drops sharplydropped to 35.2 (Figure 3CFig. 2c). Both values are indicative for the presence of Subarctic Intermediate Water (McGrath et al., 2012).(McGrath et al., 2012). The oxygen saturation was around 80% at 400 m depth and decreased with depth on the slope stations to about 75%.. In the cold water at the far off- mound station (st2) oxygen saturation decreased at 1000 m to less than 70% after which an increase was observed at 1200 m to around 80% (Figure 3DFig. 2d). Density of the water was ~27.30 kg m^{-3} at 400 m depth and gradually increased to 27.44 at 750 m, which is the depth of the slope of Haas Mound. Below 750 m, density abruptly increased to 27.660 where deep cold water was encountered. Bottom water temperature at the far off-mound station (st2) was 5.3 °C, while salinity was 35.0 and density 27.7 respectively. kg m^{-3}.

Video recordings along transects crossing Haas Mound showed large heterogeneity in coral framework distribution. The ~~lower parts of the SE~~ mound S-slope ~~at about~~ was characterized by dense framework while the mound summit showed reduced framework alternating with mud patches. At parts of the summit coral framework was replaced by a dense cover of large erect sponges (*Rosella nodastrella*). The foot of the mound S-slope (~645 m depth ~~were~~) was sampled by box-cores (~~st8, 46)~~st46), which revealed a thick layer of coral framework (~~Figure 2~~Fig. 3). Extensive coral framework was also sampled higher ~~on~~up the S-slope near the edge of the summit between 500 ~~and~~ 600 m depth (~~st12, 25, see Figure 2 and 4). The highest point sampled on Haas Mound was at 528 m depth on a ridge at the summit of the slope which had thick framework (st15). However the coral framework became rapidly reduced north of this ridge~~Fig. 3a). Density of the coral framework in box-core samples taken beyond the edge towards the ~~summit (st11,72, Figure 2 and 4). Box-cores taken at~~central part of the summit ~~plateau showed~~contained reduced amounts of ~~framework (st9) and one~~coral framework, which was in line with video recording (Fig. 3c,d). One box-core station (st24) yielded only mud and small fragments of coral skeleton (~~st24, Figure 4). Video footage of the summit showed that the ridges of denser framework alternated with valleys of reduced framework and mud. Overall the summit appeared much less densely covered by corals than the SE slope. At parts of the summit coral framework was replaced by a dense cover of large erect sponges (*Rosella nodastrella*~~Fig. 3c).

3.2 Microbial communities and diversity in Haas Mound samples

~~Microbial diversity based on~~ The number of observed microbial OTUs excluding overall singletons (S.I. Table 1) was highest in near-bottom water (2415) followed by sediment~~,~~ (2234), skeleton ~~and~~(1878), mucus~~. Lowest diversity was found in~~ (1761) and overlaying water (~~Table 3). Corresponding~~1193). Chao1 indices showed the same ~~pattern~~trend,

decreasing from ~~2829~~3089 in near-bottom water to ~~900~~1845 in overlaying water~~. A first~~ (S.I. Table 1). Initial MDS plot of the similarities in ~~microbial community~~OTU composition of the samples ~~based on of reads up to genera and up to classes~~ immediately showed that the samples of the overlaying water taken at 5 and 10 mab did not differ~~.~~. This was confirmed by ANOSIM (p~~>~~ > 0.1; ~~9999~~999 permutations). Hence, these samples were pooled in one category indicated hereafter as 5~~+~~ and 10 mab. ~~A subsequent~~Subsequent MDS ~~plot was~~plots were made of the similarities in the sample set and ~~this~~these revealed a consistent pattern, i.e. five different clusters which correspond with the biotopes of the samples (~~Figure 5~~Fig. 4, S.I. ~~Figure 2). Near~~Fig. 1). The same clusters were apparent in plots of microbial classes and genera. Overlaying water at 400 m grouped together with water at 5 and 10 mab and formed a tight cluster (Fig. 4). Unexpectedly, near-bottom water, which is ~~closely associated~~in close contact with both reef and sediment, ~~contained a microbial community that was clearly~~clustered distinct from overlaying water, sediment, *L. pertusa* skeleton and *L. pertusa* mucus. Following is an account of the composition of the bacterial communities encountered in the samples with emphasis on variation between ~~biotopes~~ and within ~~specific~~ clusters (biotopes) across the mound.

3.2.1~~.~~ Variation between biotopes

When plotting the composition of the samples according to class (Fig. 5a), differences become apparent between the biotopes. In near-bottom water, Gammaproteobacteria (22%) and Thaumarchaeota marine group I (22%) were the most abundant classes followed by ~~Delta~~ Deltaproteobacteria (11%) and Alphaproteobacteria~~. Sediment and overlaying water~~ (9%). Other biotopes shared ~~this top~~these 4 groups, however with different relative abundances~~, i.e. containing more Thaumarchaeota and~~ (Fig. 5a). Near-bottom water contained relatively high amounts of Halobacteria (1.2%), while other biotopes contained

<0.7%. Sediment and overlaying water both contained relatively less Gammaproteobacteria (14% in sediment; 18% in overlaying water) and more Thaumarchaeota MGI (24% in sediment; 31% in overlaying water) than near-bottom water (Figure . Sediment is characterised by a high percentage of Acidobacteria (6.0%) relative to <4.2% in other biotopes. In overlaying water we found relatively high amounts of Deferribacteres (5.9%) and Thermoplasmata (6.1%), while these were found <2% in the other biotopes. *L.*). Skeleton *pertusa* skeleton and mucus contained substantial lower relative amounts of Thaumarchaeota MGI (9-11%) but less than other biotopes. Skeleton exceeded in Alphaproteobacteria, Acidimicrobia, Acidobacteria and Planktomycetacia whereas mucus exceeded in % and 11% respectively) than near-bottom water but still a substantial percentage of their total microbial communities. Mucus was very rich in Gammaproteobacteria (Figure 6).49%) and also Flavobacteria (4.1%), and Betaproteobacteria (2.9%) were relatively high compared to other biotopes. Skeleton was relatively rich in Acidimicrobiia (5.4%) and Planctomycetia (5.6%) compared to other biotopes where these bars were below 2.9% and 3.5%, respectively.

Plotting the most abundant taxa (>composition of the samples using a higher taxonomic resolution, i.e. genera, on the basis of their relative abundance (each > 0.5% of all reads) confirmed athe distinct signaturesignatures of near the biotopes. Near-bottom water (Figure 7) with a top 6Fig. 5b) was distinct from other biotopes by the relative dominance of *Nitrosopumilus*, uncult. (3.2%), uncultured Xanthomonadales, (1,6%), *Defluviicoccus*, (1.3%), *Marinicella*, *Nitrosococcus* and the (1.2%), Brocadiaceae W4 lineage. (1.1%), *Nitrosococcus* (0.8%), *Colwellia* (0.6%) and OM60 clade (0.6%). Overlaying water was relatively rich in Salinisphaeraceae ZD0417 marine group, (1.9%) and Rhodospirillaceae AEGEAN-169 marine group, (2.0%) compared to other biotopes where proportions were <0.4% and <0.3%, respectively. *Pseudospirillum*, *Nitrosopumilus*, *Nitrospina* and the Flavobacteriaceae NS5 group. Sediment was rich in *Nitrosopumilus*, uncult. each contributed

between 0.5 and 1.1% to the microbial community of the overlaying water. A comparison of the relative abundance of the class Thaumarchaeota MGI with the abundance of the genus *Nitrosopumilus* indicates that the latter contributed ~2.5% to this class in overlaying water (~17% in near-bottom water and sediment, and ~35% in skeleton and mucus), meaning that other, unknown genera contributed 97% to the Thaumarchaeota class in overlaying water. Sediment was relatively rich in uncult. Xanthomonadales~~,~~ (2.9%) and *Nitrosococcus*~~,~~ ~~*Defluviicoccus* and the Pir4 lineage. In skeleton samples the top 6 genera were *Nitrosopumilus*, *Rhodobium*, the Pir4 lineage, *Entheonella*~~ (1.5%) in comparison to other biotopes where percentages were <1.7% and <0.8%, respectively. Skeleton samples contained relatively high percentages (>1%) of *Nitrosomonas*, *Nitrospira*~~ and ~~, *Entotheonella*, *Granulosicoccus*~~ whereas mucus~~, *Rhodobium*, *Blastopirellula and Pseudahrensia*, while proportions in other biotopes were <0.5%. Mucus samples contained ~~high~~large amounts of ~~the~~ Alteromonadaceae BD1-7 clade~~,~~ (22%, SE 9%) and *Acinetobacter*~~, *Nitrosopumilus*,~~ (9%, SE 9%), with high variability between the samples. *Aquabacterium*~~,~~ (1.9%), *Endozoicomonas* ~~and~~(1.5%), *Polaribacter*~~,~~ (1.3%), and *Pseudomonas* (1.0%) were most apparent in mucus. *Mycoplasma* was not found in mucus but this genus was present in low percentages in skeleton (0.03%) and near-bottom water (0.01%).

~~———~~Specific indicators, i.e. taxa that showed a significant non-random association to a specific biotope, were found for all biotopes (S.I. Table 1). The number of strong indicators (i.e., given the indicator is present, the probability that the sample belongs to a certain biotope > 0.85) was highest ~~-~~in near-bottom water and mucus (8 and 12 strong indicators, respectively) and low in overlaying water, sediment and skeleton (4, 0, and 0 strong indicators, respectively). Brocadiaceae W4, and Dehalococcoidia were the most abundant strong ~~indicator species~~indicators in near-bottom water whereas SAR11 clade Deep

1 and Oceanospirillales ZD0405 were typical for overlaying water. Mucus was characterized by Alteromonadaceae BD1-7 and *Acinetobacter*.

3.2.2 Variation within biotopes

Within clusters belonging to two of the five main biotopes, patterns were present that could be related to additional factors (Figure 8A-CFig. 7 and 8). Within the overlaying water cluster of near-bottom water, microbial communities grouped according to station i.e. location on the Haas Mound (Figure 2 and 8B). No relation was observed between the near-bottom water community and framework height (0-10 cm in stations 24, 72 and 9; 10-30 cm in stations 11 and 46).

Within the overlaying water samples, sample, depth category (400 m versus 5 and 10 mab) and year (2012 versus 2013) were discriminating factors as illustrated in the MDS plot (Figure 8CFig. 7) and determined by ANOSIM ($p <$ 0.0001001 and $p < 0.0001$, respectively, 9999 permutations). Samples taken at 400 m differed significantly fromWithin the group of samples taken at 5+ and 10 mab. Within this latter group, three clusters were recognized according to their geographic position. Samples taken on Haas Moundmound summit (st12, 36, 10 and 15) clearly differed ($p<$ < 0.0001001, 9999 permutations) from samples taken at deeper locations on Haas Moundmound slope (st33 and 13) and from samples taken off Haas Mound (st2 and 11).-mound. Deeper samples contained relatively more Thaumarchaeota Marine Group I and more Oceanospirillales ZD0405.(Fig. 6a). Opposite trends (decreasing with depth) were detected in the classes Gammaproteobacteria, Alphaproteobacteria and Acidimicrobia (Figure 6Acidimicrobiia (Fig. 6a) and in the genera *Pseudospirillum*, *Nitrospina*, and *NS5 marine group* (Figure 7).

Fig. 6b). A yearsmall but significant inter annual effect was present in the water samples taken at 400m400 m and at 5+10mab and 10 mab on Haas Mound but, however the year

effect was not shown in ~~5+10mab taken~~ off ~~mound (Figure 8C). Within the group of~~ -mound samples taken at 5 and 10 mab (Fig. 7). Distance based Redundancy Analyses indicated that depth correlated variables, i.e. temperature, salinity and density, only explained 17% of the total variation in microbial community composition of overlaying water at 5 and 10 mab. Turbidity of the water explained an additional 14% and was correlated with year (r=-0.97). Within the cluster of skeleton samples, uneroded dead coral skeleton hosted a distinct microbial community from eroded dead skeleton (~~Figure 8A~~Fig. 8). Uneroded dead skeleton contained more of the classes Gammaproteobacteria~~, Flavobacteria~~ and Sphingobacteria (Fig. 6c) whereas eroded skeleton communities contained relatively more Acidobacteria and Planctomycetia (~~Figure 6~~Fig. 6c). On genus level, uneroded dead skeleton contained more *Nitrosopumilus*, ~~*Endotheonella*~~*uncult. Xanthomonadales*, *Blastopirellula* and *Pseudahrensia* *among others*, whereas eroded skeleton contained more *Rhodopirellula*, Pir 4 lineage and *Rhodobium* (~~Figure 7). Within the biotope clusters of sediment and *L. pertusa* mucus, additional~~ Fig. 6d). No patterns were ~~not~~ found within the clusters of near-bottom water, sediment and *L. pertusa* mucus samples.

~~4.~~ Discussion

~~The temperature measurements made during this study on Haas Mound support previous observations and models, showing that the SE slope of Haas Mound is subject to intensified mixing caused by internal waves (Mohn et al., 2014; van Haren et al., 2014). By contrast, conditions on the summit of the mound are less dynamic because the wave height is less than the mound height and the deep cold water does not reach the summit, but flushes around the slopes of the mound (van Haren et al., 2014). The distribution of dense live coral framework on the slope seems to match with the degree of mixing, while much thinner framework was found on the summit (Figure 2 and 4). Presumably mixing is important for the~~

~~supply of particles towards the living corals and the exchange of dissolved nutrients, organic carbon, CO_2 and O_2, as is observed near tropical shallow water reefs (Genin et al., 2002; Reidenbach et al., 2006).~~

4.1 Microbial communities and hydrography

The temperature measurements made during this study on Haas Mound support previous observations and models, showing that the S-slope of Haas Mound is subject to intensified mixing caused by internal waves (Mohn et al., 2014; van Haren et al., 2014). By contrast, conditions on the summit of the mound are less dynamic because the internal wave height is less than the mound height and the deep cold water does not reach the summit, but flushes around the slopes of the mound (van Haren et al., 2014). The distribution of dense, live coral framework on the slope seems to match with the degree of mixing, as framework was found to be less abundant on the summit (Figs. 3). This pattern suggests that mixing is important, for supplying food particles, i.e., phytodetritus (Duineveld et al., 2007), to the living corals, as well as transporting dissolved nutrients, organic carbon, CO_2 and O_2, as is observed near tropical shallow water reefs (Genin et al., 2002; Reidenbach et al., 2006).

The distribution of microbial communities across Haas Mound ~~is~~, in some aspects, also ~~reflective of~~reflects local hydrodynamic patterns, though small ~~year~~inter annual effects are apparent. Microbial communities in the overlaying water at 400 m depth within a given year were very ~~close.~~similar to each other. This result is explicable since this depth is well above the direct influence of the mound and absolute distances between successive CTD samples ~~are~~were small (< 1 km). Samples on- and off mound showed similar microbial compositions at 400 m. In contrast, samples at 5~~+~~ and 10 mab differed between mound summit, mound slope and (deeper) off-~~-~~mound locations (~~Figure 8C).~~ Fig. 7). To explain this differentiation of the microbial communities according to mound site we infer that a gradient in environmental conditions exists on the mound. This hypothetical gradient is caused by internal waves

coming from the deep and causing cold water to slosh up the slope, exposing the lower part to more intense mixing, lower temperatures and different water chemistry for longer periods than the upper slope while the summit is not reached by the wave (van Haren et al., 2014). Microbial OTU diversity was highest in near-bottom water and decreased subsequently in sediment, skeleton, mucus and overlaying water. Possibly the enhanced microbial diversity of near-bottom water we encountered reflects the enhanced biodiversity of metazoans living on the coral framework (Bongiorni et al., 2010). Likewise (Schöttner et al., 2009) found highest microbial OTU diversity in sediments followed by overlaying seawater, and lower diversities in mucus and skeleton in a Norwegian cold water coral reef.

Due to our method of collecting near-bottom water within the coral framework with a box-corer,

a certain amount of suspended sediment ~~is~~could be expected in the near-bottom water sample and ~~hence similarity in microbes.~~indeed in the MDS plot (Fig. 4) the cluster of near-bottom water is situated in between the clusters of overlaying water and sediment. However, ~~sediment samples appear to support a~~ from the inventory of microbial classes present in the biotopes, it is apparent that near-bottom water supports a microbial community clearly ~~distinct~~different from ~~the near-bottom~~a mixture of overlaying water~~, indicating that influence of resuspended~~ and sediment ~~on latter samples is small~~. Moreover, near-bottom water contained a number of strong indicator taxa that were highly specific (high A values in indicspecies analyses) for this biotope confirming its distinct signature (S.I. Table ~~1).~~ 2).

The ~~sharp~~large difference between near-bottom water and overlaying water at 5~~+~~ and 10 mab was not anticipated given the strong turbulent mixing in places. We hypothesize that this difference is due to the effect of the dense ~~3D~~3-D coral framework constraining the exchange between the near-bottom water in between the coral branches and the water overlaying the reef. As a consequence of prolonged residence time and close contact with the dense epifauna

(e.g. sponges, bivalves, foraminifera, crinoids~~, etc.~~)) living in the framework and sediment, a biologically and chemically unique and sheltered environment is created for the development of a typical local microbial community with ~~the highest diversity (this study) and probably also the highest activity: *Lophelia* produces~~a high diversity (this study). Jensen et al. (2014) found differences between proximal and distal water samples, comparable to the differences we found between near-bottom water and overlaying water at 5 and 10 mab: i.e. less Alphaproteobacteria and more Gammaproteobacteria and Planctomycetia in near-bottom water compared to overlaying water. However, in contrast to these findings, at a nearby reef, Jensen et al. (2014) found very similar bacterial OTU compositions in water collected proximal (~1 m) and distal (30 m) to the reef. We anticipate that samples taken at 1 m above the reef not always reflect the typical microbial community living in the coral framework depending on the hydrodynamic conditions.

4.2 Microbial communities associated with *Lophelia pertusa* skeleton and mucus

Distinct communities were identified on dead coral skeleton and in freshly produced mucus of living coral. Skeleton and mucus contained a substantial amount of Thaumarchaeota Marine Group 1 (9% and 11 %, respectively) of which the majority was unclassified, and the genus *Nitrosopumilus* made up 3% in both sample types and *Cenarchaeum* 0.4% in skeleton and 0.1% in mucus. In addition, small amounts of the Euryarchaeota class Halobacteria (0.1% in skeleton and 0.3% in mucus) and in mucus also Thermoplasmata (0.2%) were found. It is for the first time that Archaea are detected in coral mucus. Archaea had been reported already in samples of *L. pertusa* tissue with corallites crushed (Emblem et al., 2012), and with Archaea affiliated to three species prominently present in the top-10 of prokaryotic species based on 454 read data: *Nitrosopumilus maritimus*, *Cenarchaeum symbiosum* and *Candidatus Nitrosoarchaeum* sp..

Although not detected by Yakimov (2006), two bacterial genera were previously reported to be part of the *L. pertusa* biome, *Mycoplasma* and *TM7* (Kellogg et al., 2009; Neulinger et al., 2009; Neulinger et al., 2008). In this study, using 454 sequencing, we detected these genera in low relative amounts: *Mycoplasma* was detected in skeleton (0.028%), near-bottom water (0.013%) and overlaying water (0.001%), however not in mucus and sediment. Candidate division TM7 was found in all biotopes, with highest relative amounts in skeleton (0.115%) and mucus (0.071%). With high densities of microorganisms, these small relative percentages of *Mycoplasma* and TM7 may still translate in significant numbers. Moreover, the percentages we report for TM7 may be severe underestimations because the primers we used have a low coverage for Candidate divisions WS6, TM7 and OP11 (Klindworth et al., 2013). In our samples of freshly collected mucus, the genera Alteromonadaceae BD1-7 clade (22%) and *Acinetobacter* (9%) were highly represented, and also *Endozoicomonas*, *Polaribacter*, *Pseudomonas*, *Aquabacterium* and *Thalassospira* were outstanding in mucus. Representatives of *Acinetobacter* have been reported from cold-water coral (Hansson et al., 2009) and from both healthy and diseased tropical corals (Koren and Rosenberg, 2008; Luna et al., 2010; Rohwer et al., 2002). Members of this genus are well known for their resistance to numerous antibiotics (Devi et al., 2011) and may play a role in the defensive-tactics of corals (Shnit-Orland and Kushmaro, 2009). *Pseudomonas* strains are also known for their antibacterial activity (Ye and Karn, 2015) and this genus has been found before in *L. pertusa* (Emblem et al., 2012) and in soft corals (Salasia and Lämmler, 2008). *Endozoicomonas* contains aerobic and halophilic members reported to have associations with corals (Alsheikh-Hussain, 2011; Bayer et al., 2013; Hansson et al., 2009; Kellogg et al., 2009; Pike et al., 2013; Yang et al., 2010) and other marine invertebrates (Kurahashi and Yokota, 2007; Nishijima et al., 2013). Recent results of Ainsworth et al. (2015) indicate that Endozoicimonaceae are likely localized to either the outer coral surface mucus layer or the

coral skeleton, as they were found exclusively in the whole organism microbiome and not in isolated coral tissues. Our results confirm that both the mucus (1.5%) and uneroded (recently deceased coral) skeleton (0.9%) are habitats for *Endozoicomonas*. The *Endozoicomonas* found in near-bottom water (0.2%) is probably also related to the presence of mucus. *L. pertusa* is able to produce large amounts of mucus that partly dissolve in the water and stimulated oxygen consumption and microbial activity in near-bottom water up to 10x that in overlaying water ~~(Wild et al. 2008).~~(Wild et al., 2008). In this sense *Endozoicomonas* may be an indicator for reef or framework water; the genus was not found in sediment, nor in overlaying water at 5 and 10 mab.

~~To explain the differentiation of the near-bottom communities according to station we infer that a gradient in environmental conditions exists on the slope. This hypothetical gradient is caused by internal waves coming from the deep and causing cold water to slosh up the slope, exposing the lower part to more intense mixing and lower temperatures for longer periods than the upper slope while the summit is not reached by the wave (van Haren et al., 2014). The station effect could either be a direct response to such an environmental gradient (e.g. temperature, DOC, nutrients) or an indirect effect of different epifauna communities which in turn reflect the environmental gradient. As no detailed environmental measurements or epifauna samples are available from the slope, above remains speculative but signifies an avenue for further studies. The relevance of such studies lies in the fact that they could provide insight into mound build-up and its limits. For example defining the conditions for microbes associated with breaking down the skeleton may be vital to carbonate mound development and/or degradation in the deep-sea as cold-water coral mounds in the Logachev mound area are primarily composed of $CaCO_3$-based sediments, being the product of decomposed coral skeleton and associated species (Mienis et al., 2009).~~

~~Distinct communities were identified on coral skeleton and in mucus of living coral. For the first time Archaea were found to be associated with~~Different microbial communities were associated with uneroded skeleton compared to eroded skeleton. ~~*L.* *pertusa*. Skeleton and mucus contained a substantial amount of Thaumarchaeota Marine Group 1 (9-11%) and small amounts of the Euryarchaeota classes Halobacteria (0,1-0,3%) and Thermoplasmata (0-0,2%). Earlier, Schöttner et al. (2009) identified similar~~The microbial community apparently undergoes a major shift upon the death of the coral host, and continues to change as the skeleton degrades over time. ~~distinct microbial communities on different areas along a single branch of *L. pertusa*, pointing to cold-water coral framework forming a highly heterogeneous microbial environment. Microbial diversity at the OTU level was largest in near-bottom water and decreased subsequently in sediment, skeleton, mucus and overlaying water, which is partly in agreement with previous studies on cold-water corals from the Logachev mound Province and elsewhere that however did not take into account archaea and extensive sequencing (Hansson et al., 2009; Schöttner et al., 2009). Our study is the first that found archaea to contribute significantly to the diversity and distinction between microbial communities associated with *L. pertusa* (cf Yakimov et al., 2006; Kellog et al., 2009). Possibly the enhanced microbial diversity of near-bottom water also reflects the enhanced biodiversity of metazoans living on the coral framework (Bongiorni et al., 2010). The distinction between the microbial assemblages associated with *L. pertusa* and its surrounding environment suggest possible mediation by the coral host as has also been suggested in earlier studies (Schöttner et al., 2012).~~

This is congruent with reports on microbial succession in shallow-water tropical scleractinians that compare live tissue to recently denuded coral skeleton (Le Campion-Alsumard et al., 1995). Schöttner et al. (2009) identified distinct microbial communities on different areas

along a single branch of *L. pertusa*, pointing to cold-water coral framework forming a highly heterogeneous environment.

~~The microbial community apparently undergoes a major shift upon the death of the coral host, and continues to change as the skeleton degrades over time. Coral mucus collected from living *L. pertusa* and coral skeleton harbored the lowest similarity between samples within each biotope. The genus *Endozoicomonas* detected in uneroded skeleton and mucus contains aerobic and halophilic members reported to have associations with corals (Alsheikh-Hussain, 2011; Bayer et al., 2013; Pike et al., 2013; Yang et al., 2010) and other marine invertebrates (Kurahashi and Yokota, 2007; Nishijima et al., 2013). *Acinetobacter* was one of the most abundant genera in all mucus samples. Representatives of this genus have been reported from both healthy and diseased tropical corals (Koren and Rosenberg, 2008; Luna et al., 2010; Rohwer et al., 2002). Members of this genus are well known for their resistance to numerous antibiotics (Devi et al., 2011) and may play a role in the defensive tactics of corals (Shnit-Orland and Kushmaro, 2009). The chemoheterotrophic genus *Lutibacter* (Choi et al., 2013; Choi and Cho, 2006) was prevalent in uneroded skeleton and the de-nitrifying family Comamonadaceae (Khan et al., 2002) represented by the genus *Aquabacterium* was prevalent in mucus samples.~~

~~———~~The variations between the different biotopes and within the biotopes that were sampled during this study, emphasize that increasing insight in the role of microbes in cold-water coral ecosystems requires both improved taxonomic resolution and actual knowledge of local biotopes, hydrography and chemical oceanography. Although our study of this single carbonate mound is among few that integrate information on hydrography with microbiology, it has for practical and logistic reasons by no means been exhaustive, and numerous pathways of future research are still open. These include further exploration of the diversity of microbial communities associated with living coral tissue, and the potential reliance of cold-water corals

on their microbial associates for chemically-produced energy ~~(Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Rohwer and Kelley, 2004)~~(Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Kellogg et al., 2009; Rohwer and Kelley, 2004). Also interactions with chemical oceanography (e.g. nutrients, oxygen gradients) need to be explored similarly as with specific epifaunal organisms, especially sponges. Furthermore, comparisons on somewhat larger scale between the prominent Haas Mound and nearby mounds of smaller dimensions may shed light on the specific roles of microbes in mound development.

~~5.~~ *Acknowledgements*

. We would like to thank the captain and crew of the RV *Pelagia* and technicians of the NIOZ for their assistance during cruises 64PE360 (2012) and 64PE377 (2013), and Hans Malschaert for Linux support. This study was funded by the NIOZ Royal Netherlands Institute for Sea Research, Texel, ~~The~~the Netherlands and was partially funded by the Innovational Research Incentives Scheme of the Netherlands Organization for Scientific Research (NWO-VENI) awarded to FM.

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914 ~~**7. Figures and tables**~~

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Table 1. Number of unique samples taken from different biotopes at Haas mound summit, slope and off mound. Number between brackets is total number of samples analysed, including replicates.

Biotope	Sample type	summit	slope	off mound	total
overlaying water	400 m	4 (11)	2 (6)	2 (6)	8 (23)
	10 mab	3 (9)	2 (6)	2 (6)	7 (21)
	5 mab	4 (12)	2 (6)	2 (6)	8 (24)
near-bottom water	w_bc	4 (11)		1 (3)	5 (14)
skeleton	uneroded	2 (6)	2 (6)		4 (12)
	eroded	1 (3)	1 (6)		2 (9)
mucus	mucus	1 (3)	1 (3)		2 (6)
sediment	sediment	2 (6)	2 (6)		4 (12)

924 **Table 2.** List of box-core sampling stations.

925

Year	Site	Station nr	Latitude	Longitude	Depth (m)	Framework Height (cm)	Biotope	Accession nrs ERS78….
2012	Mound slope	15	N 55° 29.45'	W 15° 48.41'	528	> 30	Mucus Skeleton-uneroded	3984-86 3987-89
	Summit	24	N 55° 29.77'	W 15° 48.05'	549	0-10	Near-bottom water	3990-92
	Mound slope	25	N 55° 29.57'	W 15° 47.81'	568	>30	Mucus Skeleton-uneroded	3993-95 3996-98
	Mound slope	46	N 55° 29.45'	W 15° 47.64'	745	10-30	Near-bottom water	3999-4001
	Summit	72	N 55° 29.51'	W 15° 48.40'	562	0-10	Near-bottom water	4002-03
2013	Mound slope	8	N 55° 29.45'	W 15° 47.64'	647	>30	Sediment	4004-06
	Summit	9	N 55° 29.77'	W 15° 48.03'	547	0-10	Near-bottom water	4007-09

						Sediment	4010-12
						Skeleton-uneroded	4013-15
						Skeleton-eroded	4016-18
Summit	11	N 55° 29.50'	W 15° 48.39'	564	10-30	Near-bottom water	4019-21
						Sediment	4022-24
Mound slope	12	N 55° 29.26'	W 15° 48.45'	635	>30	Sediment	4025-27
						Skeleton-uneroded	4028-30
						Skeleton-eroded	4031-36

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928 **Table 3.** ~~**Table 2.**~~ List of sampling stations of the overlaying water column. See for

929 abbreviation Fig. 4.

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Year	Site	Station nr	Latitude	Longitude	Sample depth (m)	Sample type	Temperature (°C)	Accession nrs ERS78….
2012	Off mound	11	N 55° 28.92'	W 15° 48.33'	400	w_400 m	9.7	4037-39
						w_10		4040-42
					895	mab	6.7	
					907	w_5 mab	6.6	4043-45
	Mound summit	12	N 55° 29.50'	W 15° 48.50'	400	w_400 m	9.6	4046-48
						w_10		4049-51
					553	mab	9	
					562	w_5 mab	8.9	4052-54
	Mound slope	33	N 55° 29.57'	W 15° 47.83'	390	w_400 m	10	4055-57
						w_10		4058-60
					573	mab	8.7	
					578	w_5 mab	8.6	4061-63
	Mound slope	36	N 55° 29.94'	W 15° 48.29'	400	w_400 m	10	4064-65
					596	w_5 mab	8.7	4066-68

				N 55° 25.95'	W 15° 43.83'				4069-71
2013	Off mound	2				400	w_400 m	9.9	
							w_10		4072-74
						1192	mab	5.7	
						1200	w_5 mab	5.4	4075-77
	Mound summit	10		N 55° 29.76'	W 15° 48.04'	400	w_400 m	9.8	4078-80
							w_10		4081-83
						522	mab	8.8	
						530	w_5 mab	8.5	4084-86
	Mound slope	13		N 55° 29.25'	W 15° 48.44'	400	w_400 m	9.7	4087-89
							w_10		4090-92
						709	mab	9.1	
						718	w_5 mab	9.2	4093-95
	Mound summit	15		N 55° 29.50'	W 15° 48.39'	400	w_400 m	9.8	4096-98
							w_10		4099-101
						550	mab	9	
						555	w_5 mab	8.9	4102-104

~~**Table 3.** Sequence output and microbial diversity indices based on 3573 reads (average ± st. error, n=number of samples contributing) of five main categories of samples taken at Haas Mound.~~

Figure 1. A. Location of Logachev Mound Province (yellow polygon). B. Multibeam map of Logachev Mounds with Haas Mound encircled. C. Detail of Haas Mound with lander~~, box-core~~ and CTD stations arranged along two video transects (dotted ~~white~~ lines). ~~Light blue symbols represent the 2012 box-core samples and dark blue symbols those~~D. Detail of ~~2013. The position of the 2012 CTD casts is marked by black circles and those of 2013 by yellow circles.~~Haas Mound with box-corer stations indicated. Note CTD02 is not on the map and lies 8 km SE of CTD10. Red and yellow symbols indicate stations sampled in 2012 and 2013, respectively.

~~**Figure 2**. Bathymetric profiles of the two transects across the SE slope of Haas Mound (see Fig. 1). The position of the box-cores (squares) and some of the CTD casts (circles) is indicated. The yellow color filling of the squares represents the approximate percentage coral cover. Note that scales differ.~~

~~**Figure 3.**~~**Figure 2.** A. Temperature recorded *in situ* at the summit and foot of Haas Mound by a current meter on a benthic lander. B-D. Salinity, Temperature (°C), and Oxygen (% saturation), respectively, as recorded with the CTD on the slopes and summit of Haas Mound in October 2012 and 2013.

Figure 43. Photographs of box-cores taken at the ~~SE~~ S-slope (A, st25 and B, st46) and summit (C, st24 and D, st72) of Haas mound. A clear difference in the amount and height of coral framework was observed.

Figure ~~5~~4. Microbial ~~community on genus level~~OTU composition of ~~125~~40 samples shows clustering according to ~~sample category~~biotope: overlaying water (w_400 m; w_5 and ~~w_5+~~10 mab), near-bottom water (w_bc), sediment, ~~(*L. pertusa*)~~ skeleton and mucus. The MDS plot ~~on class level~~of all 121 samples analyzed, including replicates, shows a similar pattern (S.I. ~~Figure~~Fig. 1). The same pattern is apparent for microbial classes and genera (not shown).

Figure 5. Microbial community composition of five biotopes sampled at Haas mound. N= number of unique samples per biotope with a: total number of samples, including replicates. A. ~~**6.**~~ Most abundant (>1% of total ~~community~~reads) classes ~~of microbes in different categories of samples taken at Haas mound~~for water 5 and 10 mab (n=15, a45), near ~~. A. Near~~ bottom water ~~(~~w_bc~~)~~ ~~compared to~~ (n=5, a14), sediment ~~and water at 5+10mab. B. w_bc compared to *L. pertusa*~~(n=4, a12), skeleton (n=6, a21) and mucus~~. C. Overlaying water sampled at 400 m and 5+10 mab. The latter category shows differences related to sample location: on mound summit, mound slope or off-mound.~~

~~**Figure 7**~~ (n=2, a6). B. Most abundant (>0.5% of total reads) genera ~~of microbes in different categories of samples taken at Haas mound~~for water 5 and 10 mab, near ~~. A. Near~~ bottom water ~~(~~w_bc~~)~~ ~~compared to~~, sediment ~~and overlaying water at 5+10 mab. B. w_bc compared to *L. pertusa*~~, skeleton and mucus. ~~C. Overlaying water sampled at 400 m and 5+10 mab. The~~

~~latter category shows differences related to sample location: on mound summit, mound slope and off-mound~~Values are plotted as percentage, with standard error.

Figure 6. Differences in microbial community composition within biotopes. N= number of unique samples per biotope with a: total number of samples, including replicates. A. ~~**Figure 8.** Zooms of microbial community composition on genus level. A. Skeleton of *L. pertusa*. B. Near-bottom water (w_bc). Numbers are station numbers. C. Overlaying water sampled at 400 m and at 5+10 mab along the slope of Haas Mound and at off mound stations.~~

~~**Table 1**. List of box-core sampling stations~~

Microbial classes for overlaying water at 400 m depth (n=8, a23), and at 5 and 10 mab on mound summit (n=7, a21), mound slope (n=4, a12) and off-mound (n=4, a12). B. genera for overlaying water at 400 m depth, and at 5 and 10 mab on mound summit, mound slope and off-mound. C. Microbial classes for uneroded (n=2, a9) and eroded skeleton (n=4, a12). D. genera for uneroded and eroded skeleton. Values plotted as percentage with standard error.

Figure 7. Zoom of microbial OTU composition of overlaying water (w_400 m and w_5 and 10 mab). Roman capital I=2012, II=2013.

Figure 8. Zoom of microbial OTU composition of coral skeleton (eroded and uneroded). Roman capital I= 2012, II=2013.

Supplementary information

S.I. Table 1. Sequence output and microbial diversity indices (average ± standard error) of five biotopes sampled at Haas Mound. Overall singletons were excluded in this analysis.

Yearbiotope	Stationreads/sample	Latitudeobserved OTUs	LongitudeChao1	Depth (m)Shannon
2012 15 N 55° 29.45' W 15° 48.41' 528 > 30 mucus Skeleton uneroded				
near-bottom water (n=5)	2410362 ± 823	N 55° 29.77'2415 ±176	W 15° 48.05'3089 ± 407	6.59 ±0-10 .09
25 N 55° 29.57' W 15° 47.81' 568 >30 mucus Skeleton uneroded				
46 N 55° 29.45' W 15° 47.64' 745 10-30 w_bc				
72 N 55° 29.51' W 15° 48.40' 562 0-10 w_bc				
sediment (n=4)	13372 ± 819	2234 ±201	2695 ± 256	6.19 ±0.16
skeleton (n=6)	917036 ±1789	N 55° 29.77'1878 ±144	W 15° 48.03'2374 ± 159	6.02 ±0-10 .07
mucus (n=2)	19896 ±2102	1761 ±653	2487 ±1121	4.86 ±0.85
overlaying water (n=23)	17420 ±1517	1193 ± 88	1845 ± 140	4.98 ±0.05
Skeleton eroded				

~~11~~	~~N 55° 29.50'~~	~~W 15° 48.39'~~	~~564~~	~~10-30~~	~~w_bc~~ ~~sediment~~
~~12~~	~~N 55° 29.26'~~	~~W 15° 48.45'~~	~~635~~	~~>30~~	~~sediment~~ ~~Skeleton uneroded~~ ~~Skeleton eroded~~

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S.I. Table 2. Indicator taxa given for five biotopes sampled at Haas Mound. Only those with the highest statistics values are listed. Numbers between brackets are number of strong indicators (A>0.85) over the total number of significant indicators (p<0.0001) found. w_CTD = water sampled at 400 m and 5 and 10 mab; Near-bottom water (w_bc). A = given the indicator is present, the probability that the sample belongs to the sample group. B = taking one sample from the group, the probability that it contains the indicator.

1012 **Table 2.** List of sampling stations of the overlaying water column.

Year Sample group (#strong indicators)	Station Indicator	Latitude A	Longitude B	Sample Depth (m) stat	Biotope p.value	Temperature (°C) Reads avg % in sample group
2012 w_CTD (4/38)	11 uncl. SAR11 clade Deep 1	N 55° 28.92' 0.8833	W 15° 48.33' 1.0000	400 0.940	400 m 0.0001	9.7 2.61
	Rhodospirillaceae AEGEAN-169 marine group	0.8796	1.0000	895 0.938	10 mab 0.0001	6.7 2.20
	uncl. Verrucomicrobia Arctic97B-4 marine group	0.8751	1.0000	907 0.935	5 mab 0.0001	6.6 0.45
	12 uncl. Thermoplasmatales Marine Group III	N 55° 29.50' 0.8721	W 15° 48.50' 1.0000	400 0.934	400 m 0.0001	9.6 1.00
	uncl. Oceanospirillales	0.8361	1.0000	553 0.914	10 mab 0.0001	9 2.85

	ZD0405					
w_bc (8/13)	uncl. Dehalococcoidia vadinBA26	0.9437	1.0000	5620.971	5 mab0.0001	8.90.36
	33uncultured Oceanospirillaceae	N 55° 29.57' 0.9460	W 15° 47.83' 0.8571	3900.900	400 m0.0001	100.05
	uncl. Dehalococcoidia GIF3	1.0000	0.7143	5730.845	10 mab0.0001	8.70.27
	uncl. BHI80-139	0.8931	0.7857	5780.838	5 mab0.0001	8.60.07
	36uncl. Dehalococcoidia Sh765B-AG-111	N 55° 29.94' 1.0000	W 15° 48.29' 0.6429	4000.802	400 m0.0001	100.09
	Sphingobacteriales KD1-131	0.8881	0.7143	5960.796	5 mab0.0001	8.70.09
	Thaumarchaeota Group C3	1.0000	0.5714	0.756	0.0001	0.03
	Brocadiaceae W4	0.9982	0.5000	0.706	0.0001	0.83

2013sediment (0/3)	2Phycisphaerae C86	N 55° 25.95' 0.6982	W 15° 43.83' 1.0000	4000.836	400 m0.0001	9.90.25
	uncl. Chloroflexi JG30-KF-CM66	0.5118	1.0000	11920.715	10 mab0.001	5.70.56
	uncl. Rhodospirillales AT-s3-44	0.3669	1.0000	12000.606	5 mab0.001	5.40.32
skeleton (0/12)	10uncl. Caldilineaceae	N 55° 29.76' 0.7979	W 15° 48.04' 1.0000	4000.893	400 m0.0001	9.80.71
	Granulosicoccus	0.7513	1.0000	5220.867	10 mab0.001	8.81.87
	Profundibacterium	0.7602	0.9524	5300.851	5 mab0.001	8.50.22
mucus (12/12)	13uncl. Oceanospirillales G02-CR02-full	N 55° 29.25' 0.9982	W 15° 48.44' 1.0000	4000.999	400 m0.0001	9.70.36
	Acinetobacter	0.9872	1.0000	7090.994	10 mab0.001	9.111

	uncult. Helicobacteraceae	0.9699	1.0000	7180.985	5 mab0.0001	9.20.48
	uncl. Oceanospirillales BPS-CK174	0.9651	1.0000	0.982	0.0001	0.29
	Alteromonadaceae BD1-7 clade	0.9636	1.0000	0.982	0.0001	22.00
	Corynebacterium	0.9259	1.0000	0.962	0.0001	0.11
	Staphylococcus	0.9169	1.0000	0.958	0.0001	0.06
	15*Sphingomonas*	N 55° 29.50' 0.9000	W 15° 48.39' 1.0000	4000.949	400 m0.0001	9.80.15
	Enhydrobacter	0.9963	0.8333	5500.911	10 mab0.0001	90.17
	Methylobacterium	0.9705	0.8333	5550.899	5 mab0.0001	8.90.24
	Tumebacillus	0.9106	0.8333	0.871	0.0001	0.13
	Micrococcus	0.9773	0.5000	0.699	0.0001	0.06

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Table 3. Sequence output and microbial diversity indices based on 3573 reads (average ± st. error, n=number of samples contributing) of five main categories of samples taken at Haas Mound.

	reads/sample	observed OTUs	Chao1	PD_in_tree	Shannon
w_bc	4295±285, n=14	1260±60, n= 9	2830±143, n= 9	126±5, n= 9	8,94±0,23, n= 9
sediment	5032±284, n=12	1001±48, n=10	1919±138, n=10	96±3, n=10	8,26±0,09, n=10
skeleton	6285±415, n=21	769±35, n=20	1421± 80, n=20	76±3, n=20	7,86±0,14, n=20
mucus	7034±561, n= 6	588±52, n= 6	919±116, n= 6	70±4, n= 6	6,08±0,17, n= 6
w_CTD	6212±357, n=68	488±42, n=54	900± 65, n=54	55±3, n=54	6,60±0,17, n=54

S.I. Figure 1. MDS plot of microbial community on OTU level of the individual samples showing clustering according to sample category: overlaying water (400 m and 5 and 10 mab), near-bottom water (w_bc), sediment, skeleton and mucus.

S.I. Figure 2. Rarefaction curves of OTU's plotted against reads per sample.