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

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Abstract

This study 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). We outline patterns of distribution vertically--from the seafloor to the water column--and laterally--across the mound--and couple these to mound topography and hydrography. Samples of water, sediment and *Lophelia pertusa* were collected in 2012 and 2013 from locations that were chosen based on high definition video surveys. Temperature and current measurements were obtained at two sites at the summit and foot of Haas Mound to study near-bed hydrodynamic conditions. 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. 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. 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. At all locations, the near-bottom water differed significantly from water at 5 m above the bottom, illustrating that the near-bottom water in the framework represents a separate microbial habitat. The observed spatial heterogeneity in microbial communities is discussed in relation to environmental conditions.

1 Introduction

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). Living coral colonies of *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). 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 (Rovelli et al., 2015; van Oevelen et al., 2009) and metazoan biodiversity and biomass (Biber et al., 2014; Henry and Roberts, 2007) 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 (Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Gilbert et al., 2012; Rohwer and Kelley, 2004).

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). On the basis of samples taken at a variety of spatial scales in relatively shallow cold-water coral reefs, Schöttner et al. (2012) concluded that bacteria in these CWC reefs are structured based on habitat (coral branch, mucus, water and sediment) and reef location (four reefs located off Norway). Archaea were not included in this study and water was sampled near the reef only, and not higher up in the water column. Adding to this, recently, Jensen et al. (2014) found highly similar bacterial communities in water sampled proximal (~1 m) and distal (30 m) to a reef, whereas in another reef proximal and distal water 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 cold-water coral biotope; 2) the reef will have an effect on the microbial community composition of the associated water, and due to strong mixing, this effect will also be visible higher up in the water column, i.e., at 5 and 10 m above the bottom.

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 (Fig. 1a). The focus site for this study was Haas Mound, one of the largest and highest carbonate mounds in the Logachev Mound Province (Mienis et al., 2006) (Fig. 1b). Two transects (Fig. 1c and Fig. 2), from the base to the summit of Haas Mound, were surveyed with a tethered HD video camera towed at 2 m above the bottom (mab). Videos were annotated on board and box-corer locations were selected representing the variation in coral cover and megafauna composition.

Microbial community samples (Table 1) were collected from a range of putative biotopes across Haas Mound that were operationally defined using video information, hydrographic data collected during the 2012–2013 cruises and earlier 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). These biotopes were: (i) water well above the mound i.e. at 400 m water depth; (ii) water overlaying the coral framework at 5 and 10 mab; (iii) near-bottom water; (iv) sediment; (v) uneroded (recently deceased) and eroded *L. pertusa* skeleton; and (vi) *L. pertusa* mucus.

Box-core samples were taken with a 50 cm diameter, NIOZ designed box-corer. This box-corer is equipped with a tightly-sealing top valve that prevents the leakage and/or exchange of sea water overlaying the sample during ascent enabling sampling of the near-bottom water once the box-corer was on board. A total of 9 box-cores were collected on the two transects (Table 2, Fig. 1D and Fig. 2) and from these *L. pertusa* skeleton, mucus and near bottom water were taken when available. We differentiated between eroded and uneroded 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 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: 1) 400 m, 2) 5 mab, and 3) 10 mab (Table 3, Fig. 1C and Fig.2). Also, one off-mound station at 1200 m water depth, situated 10 km SE from Haas mound was sampled with the CTD to determine if water mass characteristics near the mound differ from those off-mound and in deeper water.

Water sampled for microbial DNA analysis was filtered directly on 0.2 µm polycarbonate filters (Whatman) 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 box-core). Between two casts, the box corer was thoroughly cleaned and rinsed with seawater. All filters were immediately frozen in 6 mL Pony vials at -80 °C. Coral mucus as well as skeleton were sampled in at least three replicates (preferably from different colonies) following Schöttner et al. (2009). Except for skeleton in 2013, when we replaced the scraping technique described by Schöttner et al. (2009) by harvesting 0,5-1 cm of coral skeleton and directly freezing this at -80 °C on board. In the lab, these samples were exposed to liquid nitrogen and homogenized with 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. The concentration of the DNA in the extracts was measured with a F-2500 Fluorescence Spectrofluorometer (Hitachi, Tokyo, Japan) using QUANT-iT™PicoGreen® dsDNA kit (Life Technologies, USA). The quality was checked incidentally on a 1% agarose gel. To amplify the V4 region of the 16 S rDNA, the universal prokaryotic primer set S-DArch-0519-a-S-15 (5-CAGCMGCCGCGGTAA-3) (Wang et al., 2007) and S-D-Bact-0785-b-A-18 (5-TACNVGGGTATCTAATCC-3) (Claesson et al., 2009) were used as recommended in 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. To avoid PCR bias, per DNA extract, two separate 50 µL 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 to aim for approximately 10 ng genomic DNA per reaction. The PCR was run on an iCycler™ Thermo Cycler (BioRad, USA). Cycle conditions were as follows: 30 s at 98 °C, then 30 cycles (10 s at 98 °C, 20 s at 53 °C, 30 s at 72 °C), followed by 7 min at 72 °C. PCR products were loaded entirely on a 2% (by weight) agarose gel pre-stained with SybrSafe and run at 80 V for 50 min. Blue-light excitation was used when excising the PCR products to avoid UV-damage. Duplo PCR-products were pooled and purified using the Qiaquick Gel Extraction kit. After fluorimetric quantification as described above, equal amounts (70 ng) of the purified PCR-products were pooled (18 samples with their unique forward-MID and reverse- MID combination per set). Using a MinElute kit (Qiagen),the volume was adjusted to 25 µL with a final concentration of > 50 ng μL^{-1} pooled PCR product per set. In total, 7 sets of samples were sent to Macrogen (Seoul, South Korea), each set sequenced using Roche GS-FLX instruments and Titanium chemistry on 1/8 region gasket.

2.3 Sequence processing, taxonomic assignment and diversity analyses

The sequence library of each sample set was filtered on length and quality, and sorted based on the forward MID using the Ribosomal Database Project (RDP) pipeline Initial process (Cole et al., 2014). Only sequences longer than 250 bases with average *Q*-score above 25 were kept. These sequences were reverse complemented and sorted according to the reverse MID tags into the 3 replicates. In both procedures only 2 mismatches in both primers and tags were accepted. At the end of the procedure, each of the seven 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 OTUs and > 93% classification similarity to closest relative in SILVA database 119).

169 OTU-tables were imported in PRIMERv6 (Clarke and Gorley, 2006). The number of reads per
170 taxonomic unit was normalized per sample to avoid biases caused by differences in sample size.
171 Methodological replicates (3 per unique sample) were pooled. Rarefaction curves and diversity
172 indices were calculated using QIIME (Caporaso et al., 2010) and plotted in R. For a total of 40 samples
173 (pooled from 121 independent methodological replicates: 38 triplo's and 2 duplo's namely water of
174 400 m at station 36 and near-bottom water at station 72), the average number of reads per sample
175 was 16678 (with standard error 1090). Rarefaction curves of OTUs plotted against reads per sample
176 almost reached a plateau at 14000 reads per sample (S.I. Fig. 2 in the Supplement).
177 Differences in the microbial OTU composition were identified in PRIMERv6 (Clarke and PRIMER,
178 2006; Clarke, 1993) by analysing Bray-Curtis distance for all pooled samples (n=40), and also for all
179 methodological replicates (n=121). Results were visualized with MDS plots. DBRDA was done in
180 PRIMERv6 on the samples taken at 5 and 10 mab with 7 variables (temperature, salinity,
181 transmission, fluorescence, oxygen, Par and Spar) to explain the variability in microbial community
182 composition within this sample group.
183 The OTU classification files were processed in Excel and class and genus data were selected for
184 representation to allow easy comparison with other CWC studies (references mentioned in text).
185 The fractions of reads that were assigned to specific taxonomic units were 99% to class, 58% to
186 family and 29% to genus level. Indicator OTUs, with significant non-random association ($p < 0.0001$,
187 9999 permutations) with one of the five biotopes, were identified with Indicator Species Analysis in R
188 using the indicspecies package 1.6.7. (De Caceres and Legendre, 2009) with display of both Indicator
189 Values "A" and "B" (Dufrene and Legendre, 1997).
190
191 **2.4 Near-bed temperature and current measurements**
192 During the 2012 cruise, temperature and currents were measured on the summit (st5 at 556 m) and
193 at the foot of Haas Mound (st41 at 861 m) with an FSI™ 3DACM acoustic current meter (Falmouth

instruments) with temperature probe, which was attached to a benthic lander at 0.75 mab (Fig. 1c). The duration of each deployment was approximately 48 h.

2.5 Data submission

SSU rRNA gene amplicon pyrosequences are available via the NCBI Sequence Read Archive (number to be assigned upon publication).

3 Results

3.1 Haas mound physical environment and coral cover

The S-slope of Haas Mound is subject to strong daily variations in 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 as measured by the benthic lander. A much smaller temperature fluctuation i.e. less than 1 °C, was recorded on the summit (Fig. 3a). Temperature, salinity and oxygen profiles measured in 2012 and 2013 are shown for the water column at the off-mound (st2 and 11), mound S-slope (st33), and mound summit (st12) of Haas Mound (Fig. 3b-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 was 35.4 at 400 m depth and decreased slightly with depth. These temperature and salinity values are characteristic of Eastern North Atlantic Water. At the deeper off-mound st11 temperatures decreased to 6.6 °C at 1000 m water depth (Fig. 3b), while salinity dropped to 35.2 (Fig. 3c). Both values are indicative for the presence of Subarctic Intermediate Water (McGrath et al., 2012). The oxygen saturation was around 80% at 400 m depth. 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% (Fig. 3d). 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 increased to 27.60 where deep cold water was encountered. Bottom water

220 temperature at the far off-mound station (st2) was 5.3 °C, while salinity was 35.0 and density
221 27.7 kg m^{-3}.
222 Video recordings along transects crossing Haas Mound showed large heterogeneity in coral
223 framework distribution. The mound S-slope was characterized by dense framework while the mound
224 summit showed reduced framework alternating with mud patches. At parts of the summit coral
225 framework was replaced by a dense cover of large erect sponges (*Rosella nodastrella*). The foot of
226 the mound S-slope (~645 m depth) was sampled by box-cores (st46), which revealed a thick layer of
227 coral framework (Fig. 4B). Extensive coral framework was also sampled higher up the S-slope near
228 the edge of the summit between 500-600 m depth (Figs. 2 and 4A). Density of the coral framework in
229 box-core samples taken beyond the edge towards the central part of the summit contained reduced
230 amounts of coral framework, which was in line with video recording (Figs. 2 and 4C,D). One box-core
231 station (st24) yielded only mud and small fragments of coral skeleton (Fig. 4C).
232
233 **3.2 Microbial communities and diversity in Haas Mound samples**
234 The number of observed microbial OTUs (S.I. Table 1) was highest in near-bottom water (3858)
235 followed by sediment (3245), skeleton (2856), mucus (2663) and overlaying water (1712).
236 Corresponding Chao1 indices showed the same pattern, decreasing from 7876 in near-bottom water
237 to 2684 in overlaying water. Initial MDS plot of the similarities in OTU composition of the samples
238 immediately showed that the samples of the overlaying water taken at 5 and 10 mab did not differ.
239 This was confirmed by ANOSIM ($p > 0.1$; 999 permutations). Hence, these samples were pooled in
240 one category indicated hereafter as 5 and 10 mab. Subsequent MDS plots were made of the
241 similarities in the sample set and these revealed a consistent pattern, i.e. five different clusters which
242 correspond with the biotopes of the samples (Fig. 5, S.I. Fig. 1). The same clusters were apparent in
243 plots of microbial classes and genera. Overlaying water at 400 m grouped together with water at 5
244 and 10 mab and formed a tight cluster (Fig. 5). Near-bottom water, which is closely associated with
245 both reef and sediment, 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 clusters (biotopes) across the mound.

3.2.1 Variation between biotopes

In near-bottom water, Gammaproteobacteria (22%) and Thaumarchaeota marine group I (22%) were the most abundant classes followed by Deltaproteobacteria (11%) and Alphaproteobacteria (9%). Other biotopes shared these 4 groups, however with different relative abundances (Fig. 6A). 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. *L. pertusa* skeleton and mucus contained lower relative amounts of Thaumarchaeota MGI (9% and 11% respectively) than near-bottom water but still a substantial percentage of their total microbial communities.

Mucus was very rich in Gammaproteobacteria (49%) and also Flavobacteria (4.1%) whereas Betaproteobacteria (2.9%) were relatively high compared to other biotopes. Near-bottom water contained relatively high amounts of Halobacteria (1.2%) compared to other biotopes (<0.7%). Sediment contained a higher percentage of Acidobacteria (6.0%) compared to other biotopes (<4.2%) whereas skeleton was relatively rich in Acidimicrobiia (5.4%) and Planctomycetia (5.6%) compared to other biotopes (<2.9%, and <3.5% respectively). In overlaying water we found relatively high amounts of Deferribacteres (5.9%) and Thermoplasmata (6.1%) compared to other biotopes (<2%). Plotting the relatively most abundant genera (i.e., > 0.5% of all reads) confirmed a distinct signature of near-bottom water (Fig. 6B) with a top 6 of *Nitrosopumilus* (3.2%), uncultured Xanthomonadales (1,6%), *Defluviicoccus* (1.3%), *Marinicella* (1.2%), *Nitrosococcus* (0.8%) and the Brocadiaceae W4 lineage (1.1%) and a higher relative amount of *Colwellia* (0.6%) compared to the other biotopes (< 0.1%).

Overlaying water was relatively rich in Salinisphaeraceae ZD0417 marine group (1.9%) and Rhodospirillaceae AEGEAN-169 marine group (2,0%) compared to other biotopes (<0,4% and <0.3% respectively). *Pseudospirillum*, *Nitrosopumilus*, *Nitrospina* and the Flavobacteriaceae NS5 group 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* (1,5%) compared to other biotopes (< 1.7% and >0.8% respectively), whereas skeleton samples contained relatively high percentages (>1%) of *Nitrosomonas, Nitrospira, Entheonella, Granulosicoccus, Rhodobium, Blastopirellula and Pseudahrensia*, compared to other biotopes (<0,5%). Mucus samples contained large amounts of Alteromonadaceae BD1-7 clade (22%, SE 9%) and *Acinetobacter* (9%, SE 9%), with high variability between the samples. *Endozoicomonas* (1.5%), *Polaribacter* (1.3%), *Pseudomonas* (1.0%) and *Aquabacterium* (1.9%) were also outstanding in mucus compared to other biotopes. *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 in the Supplement). 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 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 (Fig. 8 and 9). Within the overlaying water cluster, depth category (400 versus 5 and 10 mab) and year (2012 versus 2013) were discriminating factors as illustrated in the MDS plot (Fig. 8) and determined by ANOSIM ($p < 0.01$ and $p < 0.0001$, respectively, 9999 permutations). Samples taken at 400 m differed significantly from samples taken at 5 and 10 mab. Within this latter group, three clusters were recognized according to their geographic position. Samples taken on Haas Mound summit (st12, 36, 10 and 15) clearly differed ($p < 0.001$, 9999 permutations) from samples taken at deeper locations on Haas Mound slope (st33 and 13) and from samples taken off Haas Mound (st2 and 11). Deeper samples contained relatively more Thaumarchaeota Marine Group I (Fig. 7A). Opposite trends (decreasing with depth) were detected in the classes Gammaproteobacteria, Alphaproteobacteria and Acidimicrobiia (Fig. 7A) and in the genera *Pseudospirillum*, *Nitrospina*, and *NS5 marine group* (Fig. 7B). A small but significant inter annual effect was present in the water samples taken at 400 m and at 5 and 10 mab on Haas Mound but not in samples taken off mound at 5 and 10 mab (Fig. 8). 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 (Fig. 9). Uneroded dead skeleton contained more of the classes Gammaproteobacteria and Sphingobacteria (Fig. 7C) whereas eroded skeleton communities contained relatively more Acidobacteria and Planctomycetia (Fig. 7C). On genus level, uneroded dead skeleton contained more *Nitrosopumilus*, *uncult. Xanthomonadales*, *Blastopirellula* and *Pseudahrensia among others,* whereas eroded skeleton contained more *Rhodopirellula*, Pir 4 lineage and *Rhodobium* (Fig. 7D). No patterns were found within the clusters of near-bottom water, sediment and *L. pertusa* mucus samples.

4 Discussion

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. 2 and 4). 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, CO2 and O2, as is observed near tropical shallow water reefs (Genin et al., 2002; Reidenbach et al., 2006).

The distribution of microbial communities across Haas Mound, in some aspects, also reflects local hydrodynamic patterns, though small inter annual effects are apparent. Microbial communities in the overlaying water at 400 m depth within a given year were very 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 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 (Fig. 8). To explain this differentiation of the microbial communities according to mound location 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. Likewise (Schöttner et al., 2009) found highest microbial OTU diversity in sediments followed by overlaying seawater, mucus and skeleton in a Norwegian cold water coral reef. 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).

Due to our method of collecting near-bottom water within the framework with a box-corer, a certain amount of suspended sediment could be expected in the near-bottom water sample and indeed in the MDS plot (Fig. 5) the cluster of near-bottom water is situated in between the clusters of overlaying water and sediment. However, from the inventory of microbial classes present in the biotopes it is apparent that near-bottom water supports a microbial community clearly different from a mixture of overlaying water and sediment. 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 2).

The 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 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) 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 a high diversity (this study). In contrast, Schöttner et al (2012) found low bacterial diversities in water sampled close to four Norwegian reefs. Also in contrast to our findings, Jensen et al. (2014) found very similar bacterial OTU compositions in water proximal (~1 m) and distal (30 m) to one reef. However, at another reef, these authors 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

375 in near-bottom water compared to overlaying water. We anticipate that samples taken at 1 m above
376 the reef not always (depending on the hydrodynamic conditions) reflect the typical microbial
377 community living in between the coral framework and that sampling water from between the
378 framework is preferred.
379
380 **4.2 Microbial communities associated with *Lophelia pertusa* skeleton and mucus**
381 Distinct communities were identified on dead coral skeleton and in freshly produced mucus of living
382 coral. Skeleton and mucus contained a substantial amount of Thaumarchaeota Marine Group 1 (9%
383 and 11 %, respectively) of which the majority was unclassified, and the genus *Nitrosopumilus* made
384 up 3% in both sample types and *Cenarchaeum* 0.4% in skeleton and 0.1% in mucus. In addition, we
385 found small amounts of the Euryarchaeota class Halobacteria (0.1% in skeleton and 0.3% in mucus)
386 and in mucus also Thermoplasmata (0.2%). It is for the first time that Archaea are detected in coral
387 mucus. Archaea had been reported already (Emblem et al., 2012) in samples of *L. pertusa* tissue with
388 corallites crushed, and with Archaea affiliated to three species prominently present in the top-10 of
389 prokaryotic species based on 454 read data: *Nitrosopumilus maritimus*, *Cenarchaeum symbiosum*
390 and *Candidatus Nitrosoarchaeum* sp..
391 Although not detected by Yakimov (2006), two bacterial genera were previously reported to be part
392 of the *L. pertusa* biome, *Mycoplasma* and *TM7* (Kellogg et al., 2009; Neulinger et al., 2009; Neulinger
393 et al., 2008). In this study, using 454 sequencing, we detected these genera in low relative amounts:
394 *Mycoplasma* was detected in skeleton (0.028%), near-bottom water (0.013%) and overlaying water
395 (0.001%), however not in mucus and sediment. Candidate division TM7 was found in all biotopes,
396 with highest relative amounts in skeleton (0.115%) and mucus (0.071%). With high densities of
397 microorganisms, these small relative percentages of *Mycoplasma* and TM7 may still translate in
398 significant numbers. Moreover, the percentages we found for TM7 may be severe underestimations
399 because the primers we used have a low coverage for Candidate divisions WS6, TM7 and OP11
400 (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). 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.

Different microbial communities were associated with uneroded skeleton compared to eroded skeleton. 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. 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 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; 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.

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635 **Table 1**. Number of unique samples taken from different biotopes at Haas mound summit, slope and

636 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 10 mab 5 mab	4 (23) 3 (9) 4 (12)	2 (6) 2 (6) 2 (6)	2 (6) 2 (6) 2 (6)	8 (23) 7 (21) 8 (24)
near-bottom water	w_bc	4 (11)		1 (3)	5 (14)
skeleton	uneroded eroded	2 (6) 1 (3)	2 (6) 1 (6)		4 (12) 2 (9)
mucus	mucus	1 (3)	1 (3)		2 (6)
sediment	sediment	2 (6)	2 (6)		4 (12)

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

Year	Site	Station nr	Latitude	Longitude	Depth (m)	Framework height (cm)	Biotope
2012	Mound slope	15	N 55° 29.45'	W 15° 48.41'	528	> 30	Mucus Skeleton-uneroded
	Summit	24	N 55° 29.77'	W 15° 48.05'	549	0-10	Near-bottom water
	Mound slope	25	N 55° 29.57'	W 15° 47.81'	568	>30	Mucus Skeleton-uneroded
	Mound slope	46	N 55° 29.45'	W 15° 47.64'	745	10-30	Near-bottom water
	Summit	72	N 55° 29.51'	W 15° 48.40'	562	0-10	Near-bottom water
2013	Mound slope	8	N 55° 29.45'	W 15° 47.64'	647	>30	Sediment
	Summit	9	N 55° 29.77'	W 15° 48.03'	547	0-10	Near-bottom water Sediment Skeleton-uneroded Skeleton-eroded
	Summit	11	N 55° 29.50'	W 15° 48.39'	564	10-30	Near-bottom water Sediment
	Mound slope	12	N 55° 29.26'	W 15° 48.45'	635	>30	Sediment Skeleton-uneroded Skeleton-eroded

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644 **Table 3**. List of sampling stations of the overlaying water column. See for abbreviation Fig. 5.

Year	Site	Station nr	Latitude	Longitude	Sample depth (m)	Sample type	Temperature (°C)
2012	Off mound	11	N 55° 28.92'	W 15° 48.33'	400	w_400m	9.7
					895	w_10mab	6.7
					907	w_5mab	6.6
	Mound summit	12	N 55° 29.50'	W 15° 48.50'	400	w_400m	9.6
					553	w_10mab	9
					562	w_5mab	8.9
	Mound slope	33	N 55° 29.57'	W 15° 47.83'	390	w_400m	10
					573	w_10mab	8.7
					578	w_5mab	8.6
	Mound slope	36	N 55° 29.94'	W 15° 48.29'	400	w_400m	10
					596	w_5mab	8.7
2013	Off mound	2	N 55° 25.95'	W 15° 43.83'	400	w_400m	9.9
					1192	w_10mab	5.7
					1200	w_5mab	5.4
	Mound summit	10	N 55° 29.76'	W 15° 48.04'	400	w_400m	9.8
					522	w_10mab	8.8
					530	w_5mab	8.5
	Mound slope	13	N 55° 29.25'	W 15° 48.44'	400	w_400m	9.7
					709	w_10mab	9.1
					718	w_5mab	9.2
	Mound summit	15	N 55° 29.50'	W 15° 48.39'	400	w_400m	9.8
					550	w_10mab	9
					555	w_5mab	8.9

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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 and CTD stations arranged along two video transects (dotted lines). D. Detail of 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.

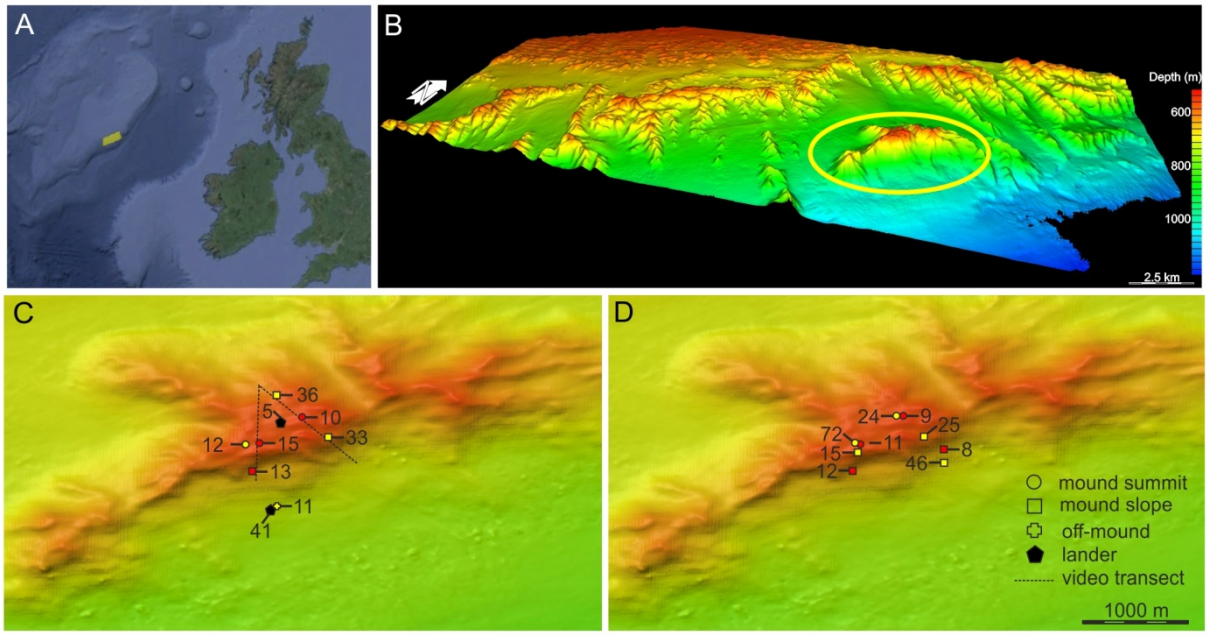

Figure 1

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

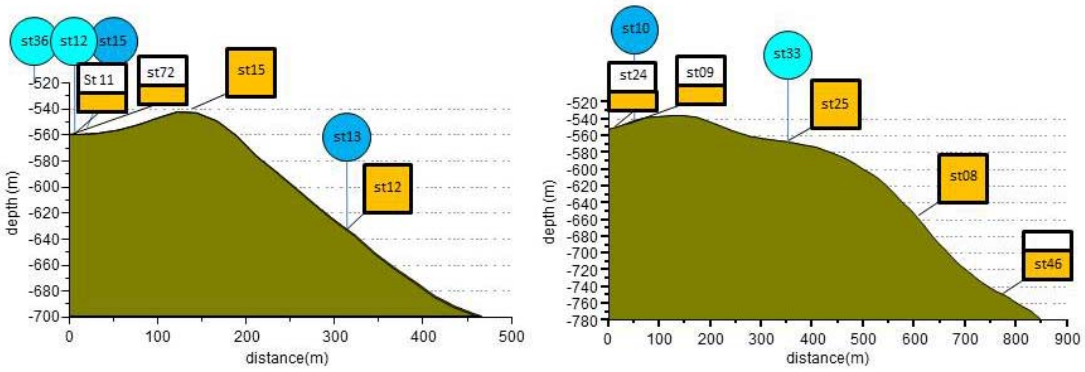

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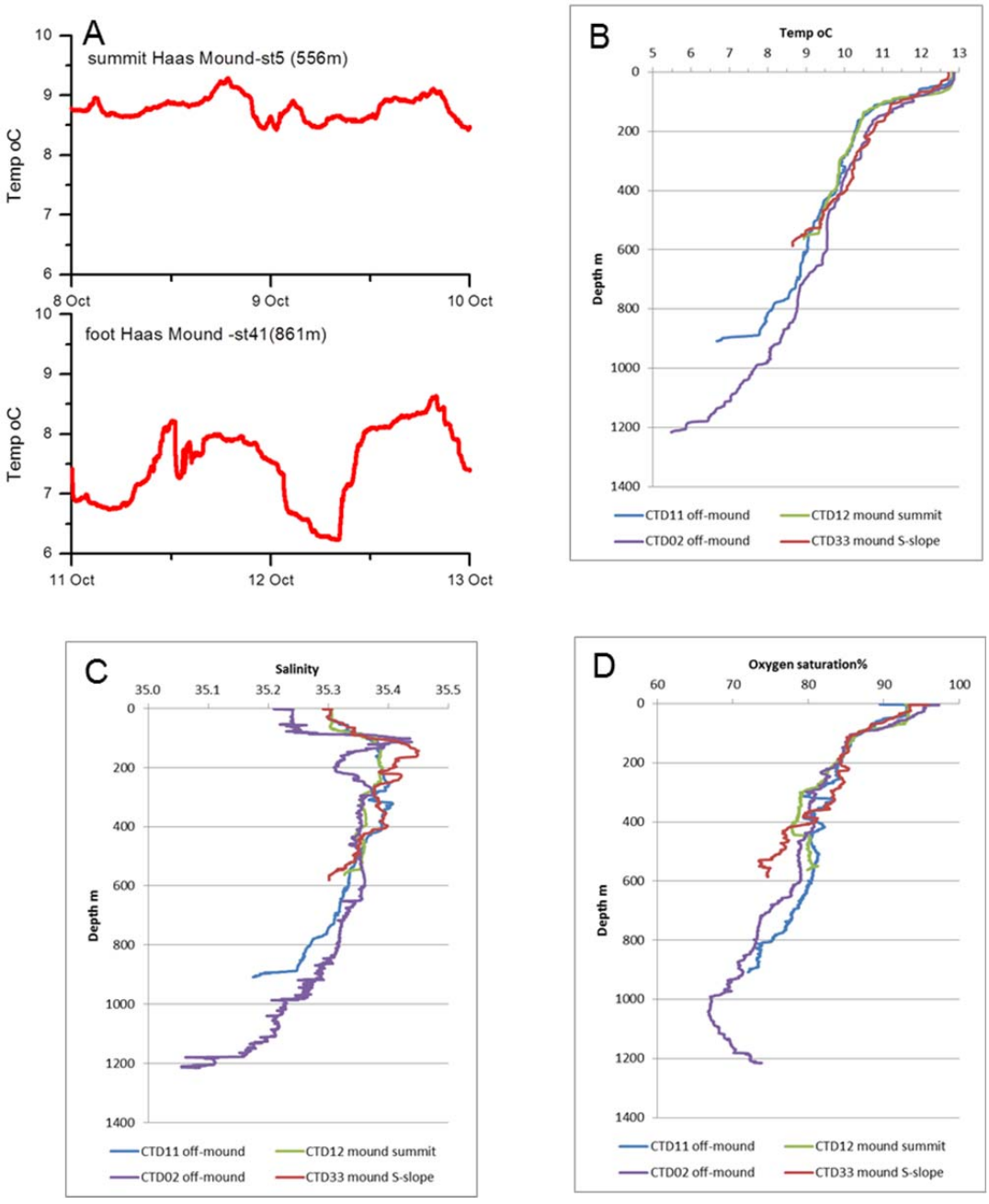
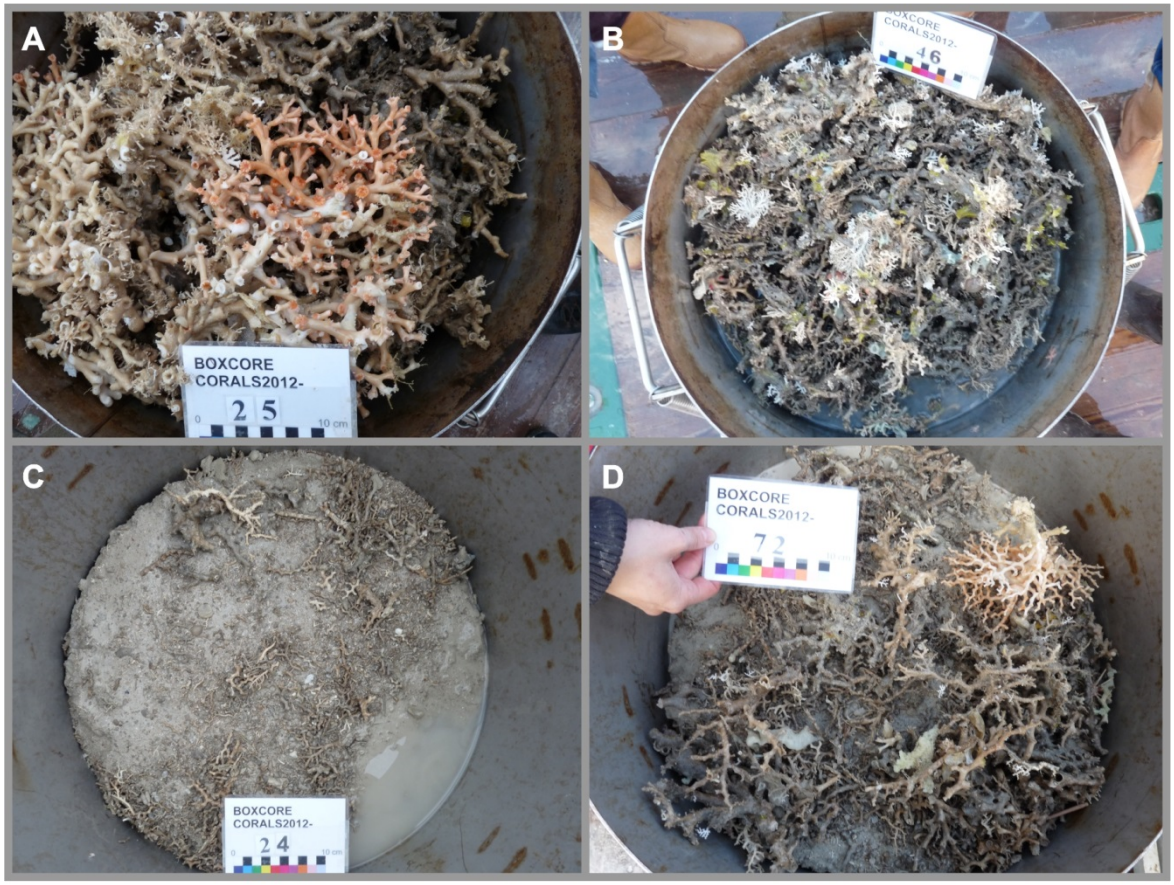
Figure 3. A. Temperature recorded *in situ* at the summit and foot of Haas Mound by a current meter on a benthic lander. B-D. Salinity, Temperature (˚C), and Oxygen (% saturation), respectively, as recorded with the CTD on the slopes and summit of Haas Mound in October 2012 and 2013.



663 **Figure 4**. Photographs of box-cores taken at the S-slope (A, st25 and B, st46) and summit (C, st24 and

664 D, st72) of Haas mound. A clear difference in the amount and height of coral framework was

665 observed.



666 Figure 4

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

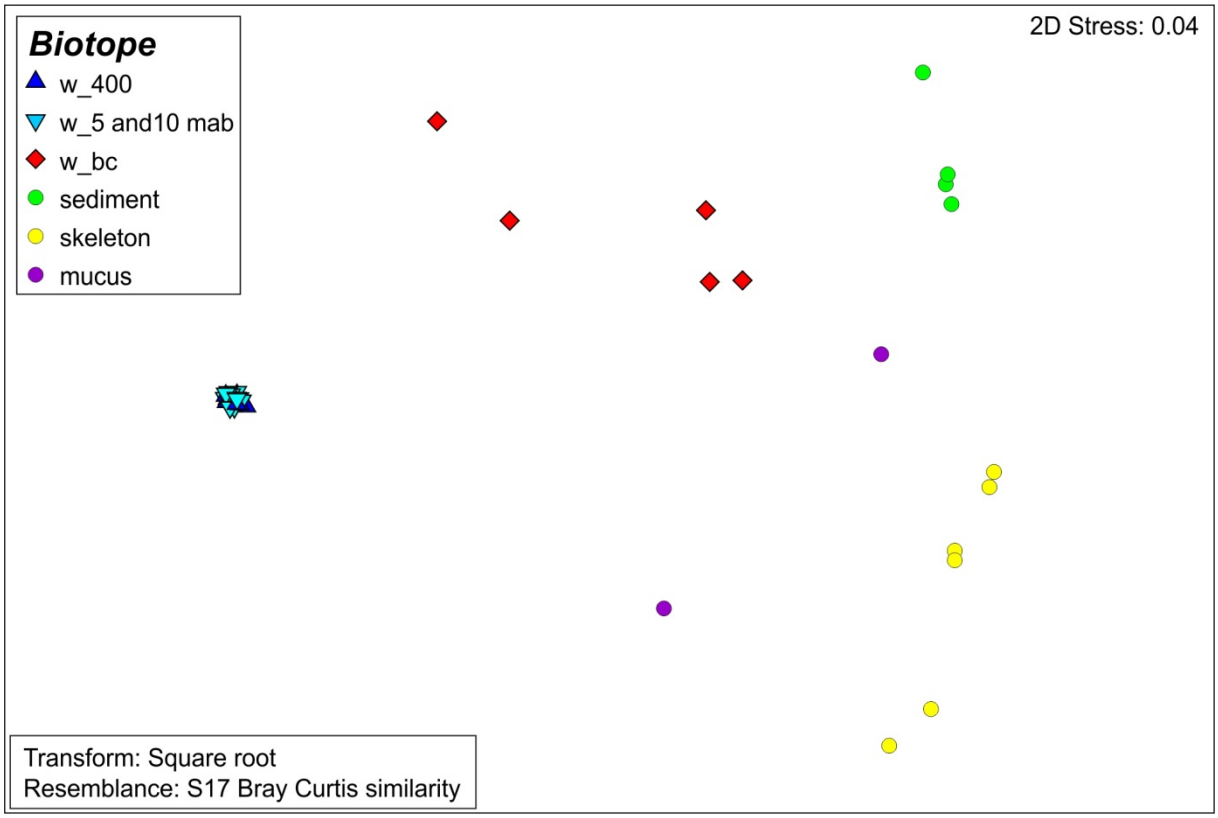
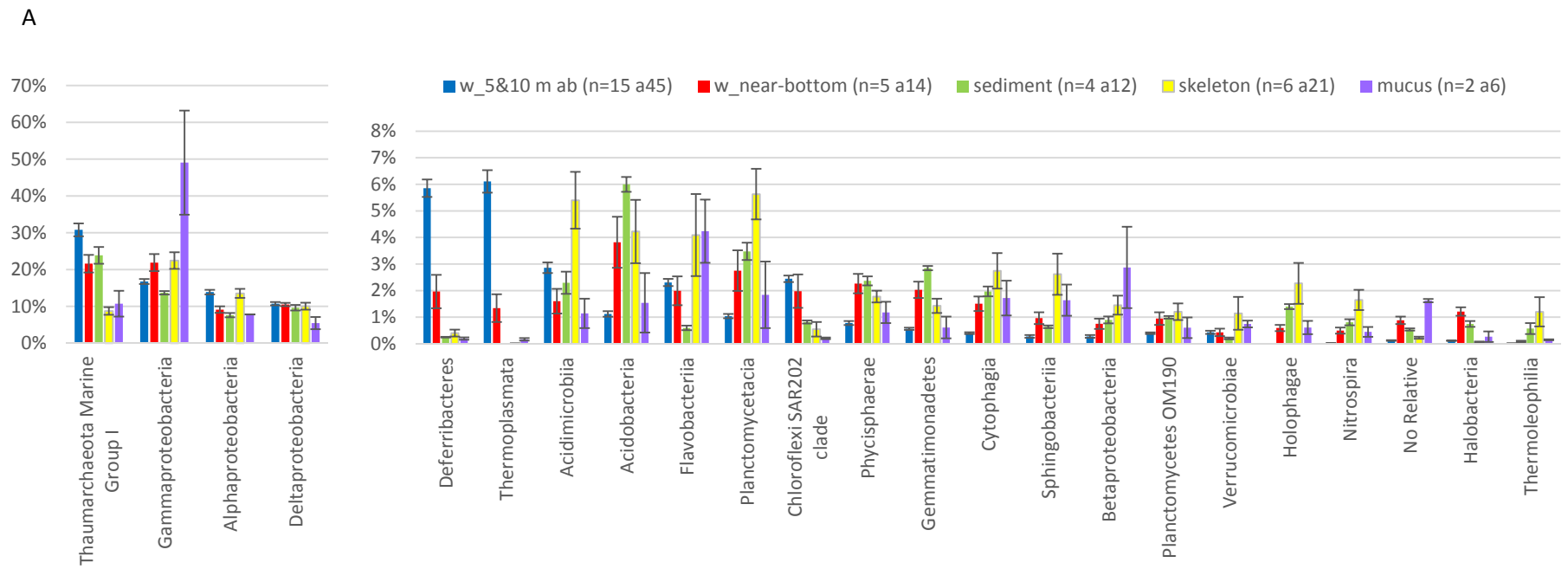


Figure 5

Figure 6. 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. Most abundant (>1% of total reads) classes. B. Most abundant (>0.5% of total reads) genera plotted as percentage, with standard error.



B

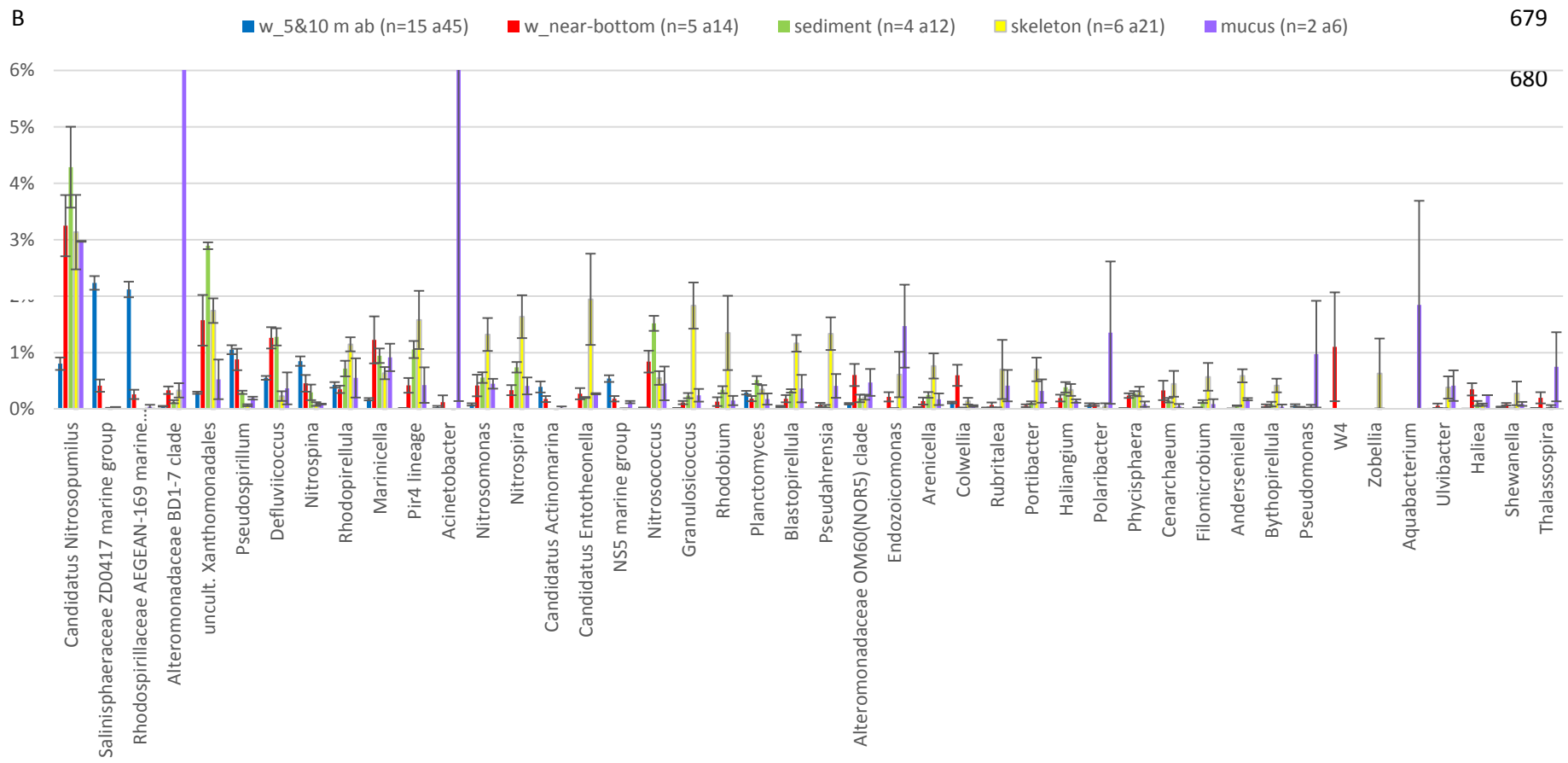

w_5&10 m ab (n=15 a45)
w_near-bottom (n=5 a14)
sediment (n=4 a12)
skeleton (n=6 a21)
mucus (n=2 a6)
6%
5%
4%
3%
1%
0%
Candidatus Nitrosopumilus
Salinisphaeraceae ZD0417 marine group
Rhodospirillaceae AEGEAN-169 marine...
Alteromonadaceae BD1-7 clade
uncult. Xanthomonadales
Pseudospirillum
Defluviicoccus
Nitrospina
Rhodopirellula
Marinicella
Pir4 lineage
Acinetobacter
Nitrosomonas
Nitrospira
Candidatus Actinomarina
Candidatus Entotheonella
NS5 marine group
Nitrosococcus
Granulosicoccus
Rhodobium
Planctomyces
Blastopirellula
Pseudahrensia
Alteromonadaceae OM60(NOR5) clade
Endozoicomonas
Arenicella
Colwellia
Rubritalea
Portibacter
Haliangium
Polaribacter
Phycisphaera
Cenarchaeum
Filomicrobium
Anderseniella
Bythopirellula
Pseudomonas
W4
Zobellia
Aquabacterium
Ulvibacter
Haliea
Shewanella
Thalassospira

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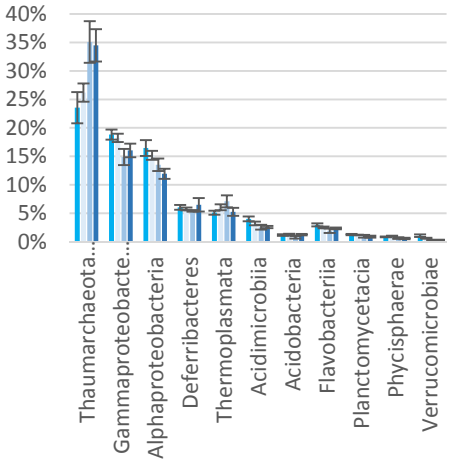
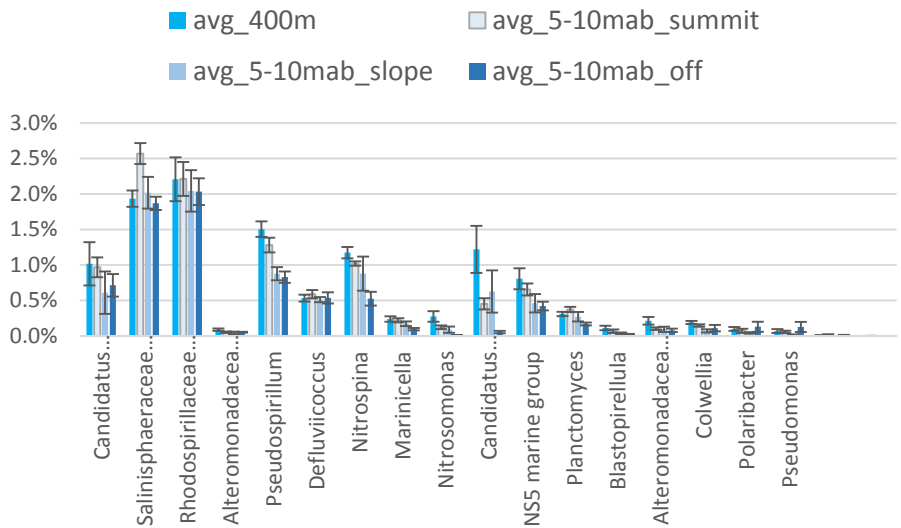
Figure 7. Differences in microbial community composition within biotopes. N= number of unique samples per biotope with a: total number of samples, including replicates. A. Microbial classes for overlaying water at 400 m depth, and at 5 and 10 mab on mound summit, slope and off-mound. B. genera for overlaying water at 400 m depth (n=8, a23), and at 5 and 10 mab on mound summit (n=7, a21), slope (n=4, a12) and off-mound (n=4, a12). C. Microbial classes for uneroded (recently deceased) and eroded skeleton. D. genera for uneroded (recently deceased) and eroded skeleton. Values plotted as percentage with standard error.

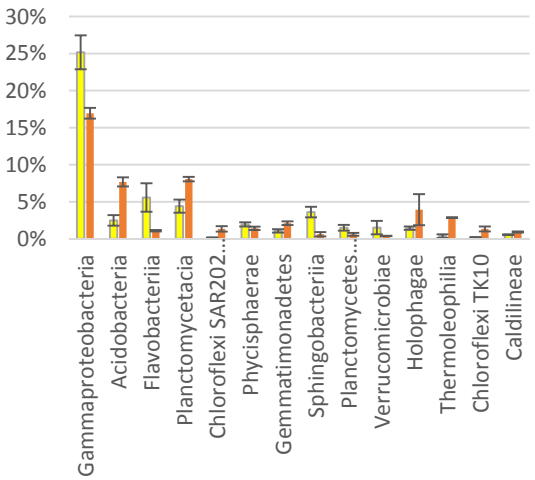
A



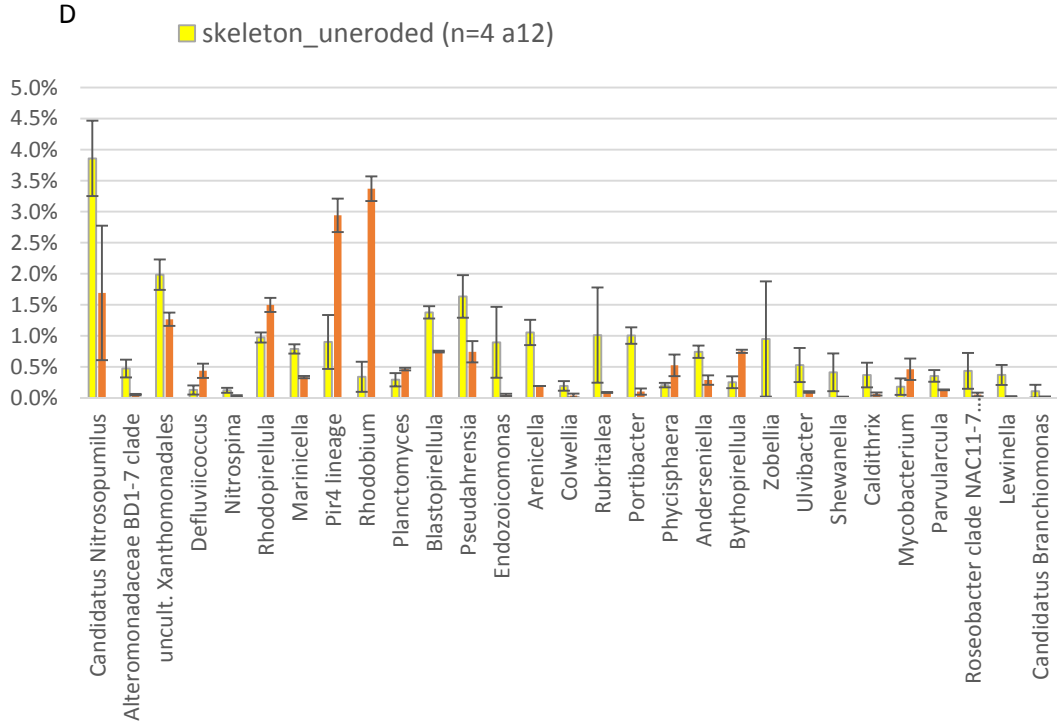
B



C


30%
25%
20%
15%
10%
5%
0%
Gammaproteobacteria
Acidobacteria
Flavobacteriia
Planctomycetacia
Chloroflexi SAR202...
Phycisphaerae
Gemmatimonadetes
Sphingobacteriia
Planctomycetes...
Verrucomicrobiae
Holophagae
Thermoleophilia
Chloroflexi TK10
Caldilineae

D


skeleton_uneroded (n=4 a12)
5.0%
4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
Candidatus Nitrosopumilus
Alteromonadaceae BD1-7 clade
uncult. Xanthomonadales
Defluviicoccus
Nitrospina
Rhodopirellula
Marinicella
Pir4 lineage
Rhodobium
Planctomyces
Blastopirellula
Pseudahrensia
Endozoicomonas
Arenicella
Colwellia
Rubritalea
Portibacter
Phycisphaera
Anderseniella
Bythopirellula
Zobellia
Ulvibacter
Shewanella
Caldithrix
Mycobacterium
Parvularcula
Roseobacter clade NAC11-7...
Lewinella
Candidatus Branchiomonas

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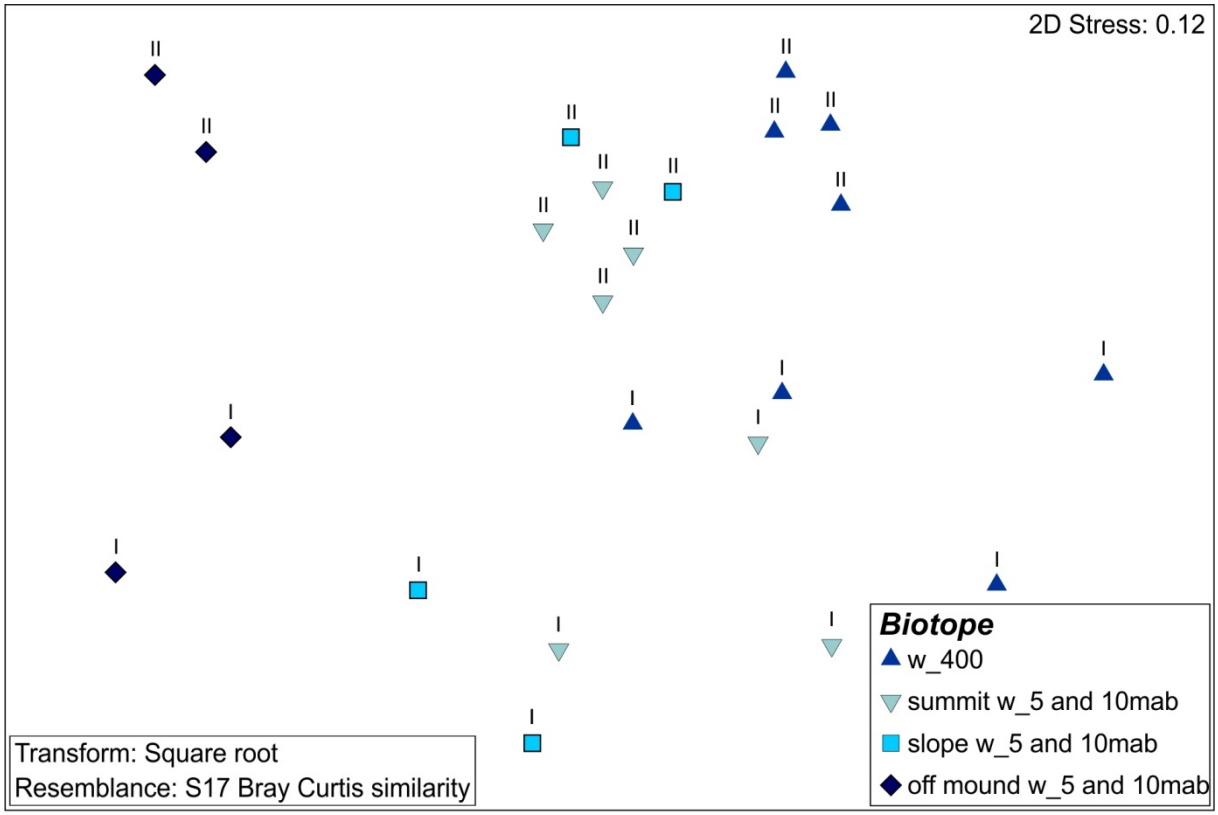
689 **Figure 8.** Zoom of microbial OTU composition of overlaying water (w_400 m and w_5 and 10 mab).

690 Roman capital I = 2012, II = 2013.

691



692 Figure 8

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694 **Figure 9.** Zoom of microbial OTU composition of coral skeleton (eroded and uneroded). Roman

695 capital I = 2012, II = 2013.

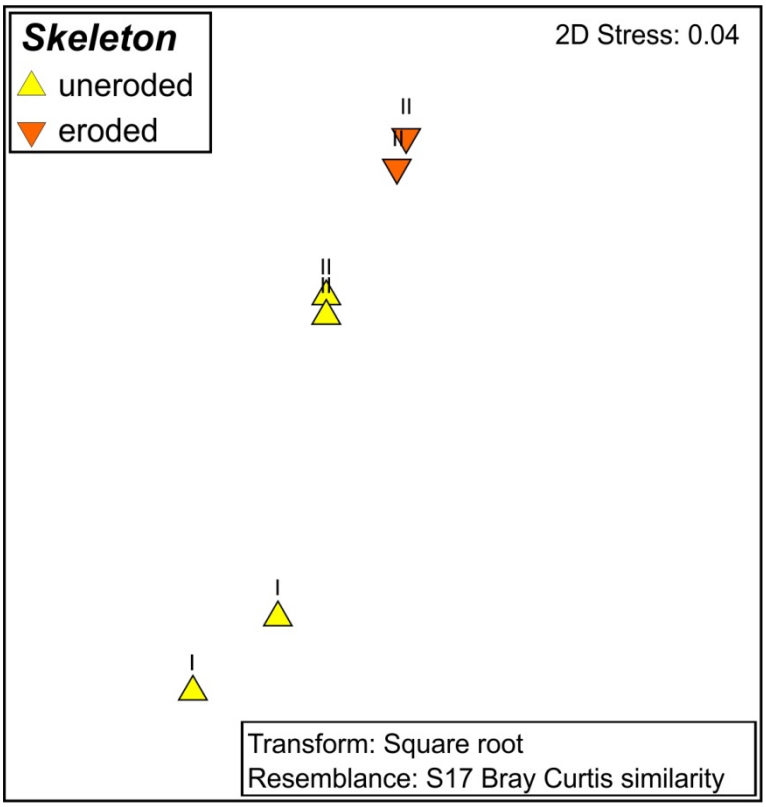

Figure 9

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699 **Supplementary information**

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701 **S.I. Table 1**. Sequence output and microbial diversity indices (average ± standard error) of five

702 biotopes sampled at Haas Mound. Singletons were not excluded in this analysis.

biotope	reads/sample	observed OTUs	Chao1	Shannon
near-bottom water (n=5)	11456 ±798	3858 ±567	7876 ±618	6.95 ±0.09
sediment (n=4)	14070 ±941	3245 ±104	5357 ±688	6.40 ±0.16
skeleton (n=6)	17713 ±1952	2856 ±300	4637 ± 709	6.19 ±0.07
mucus (n=2)	20140 ±2229	2663 ±665	2828 ±1123	4.93 ±0.87
overlaying water (n=23)	17651 ±1599	1712 ±119	2684 ± 306	5.04 ±0.06

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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.

Sample group (#strong indicators)	Indicator	A	B	stat	p.value	Reads avg % in sample group
w_CTD (4/38)	uncl. SAR11 clade Deep 1	0.8833	1.0000	0.940	0.0001	2.61
	Rhodospirillaceae AEGEAN-169 marine group	0.8796	1.0000	0.938	0.0001	2.20
	uncl. Verrucomicrobia Arctic97B-4 marine group	0.8751	1.0000	0.935	0.0001	0.45
	uncl. Thermoplasmatales Marine Group III	0.8721	1.0000	0.934	0.0001	1.00
	uncl. Oceanospirillales ZD0405	0.8361	1.0000	0.914	0.0001	2.85
w_bc (8/13)	uncl. Dehalococcoidia vadinBA26	0.9437	1.0000	0.971	0.0001	0.36
	uncultured Oceanospirillaceae	0.9460	0.8571	0.900	0.0001	0.05
	uncl. Dehalococcoidia GIF3	1.0000	0.7143	0.845	0.0001	0.27
	uncl. BHI80-139	0.8931	0.7857	0.838	0.0001	0.07
	uncl. Dehalococcoidia Sh765B-AG-111	1.0000	0.6429	0.802	0.0001	0.09
	Sphingobacteriales KD1-131	0.8881	0.7143	0.796	0.0001	0.09
	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
sediment (0/3)	Phycisphaerae C86	0.6982	1.0000	0.836	0.0001	0.25
	uncl. Chloroflexi JG30-KF-CM66	0.5118	1.0000	0.715	0.0001	0.56
	uncl. Rhodospirillales AT-s3-44	0.3669	1.0000	0.606	0.0001	0.32
skeleton (0/12)	uncul. Caldilineaceae	0.7979	1.0000	0.893	0.0001	0.71
	Granulosicoccus	0.7513	1.0000	0.867	0.0001	1.87
	Profundibacterium	0.7602	0.9524	0.851	0.0001	0.22

mucus (12/12)	uncl. Oceanospirillales G02-CR02-full	0.9982	1.0000	0.999	0.0001	0.36
	Acinetobacter	0.9872	1.0000	0.994	0.0001	9.11
	uncult. Helicobacteraceae	0.9699	1.0000	0.985	0.0001	0.48
	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
	Sphingomonas	0.9000	1.0000	0.949	0.0001	0.15
	Enhydrobacter	0.9963	0.8333	0.911	0.0001	0.17
	Methylobacterium	0.9705	0.8333	0.899	0.0001	0.24
	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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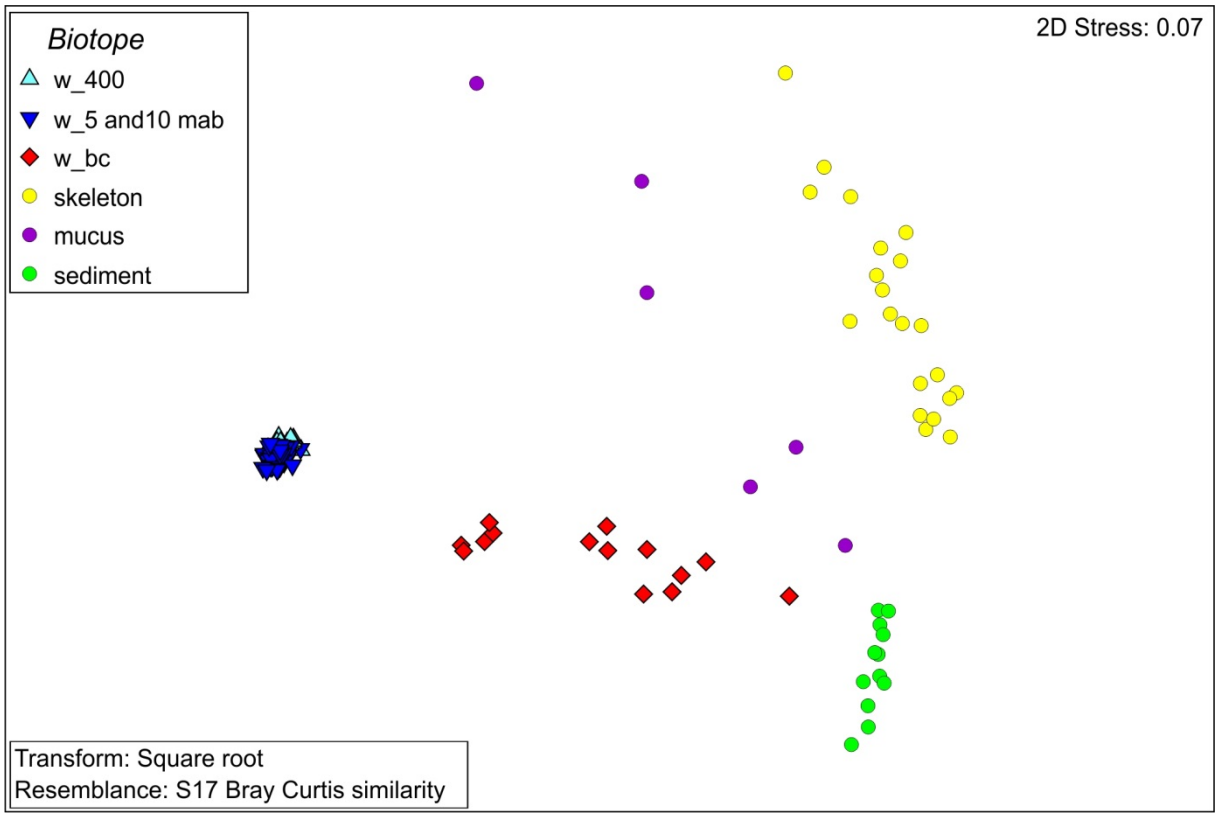
714 **S.I. Figure 1.** MDS plot of microbial community on OTU level of the individual samples showing
715 clustering according to sample category: overlaying water (400 m and 5 and 10 mab), near-bottom
716 water (w_bc), sediment, skeleton and mucus.
717



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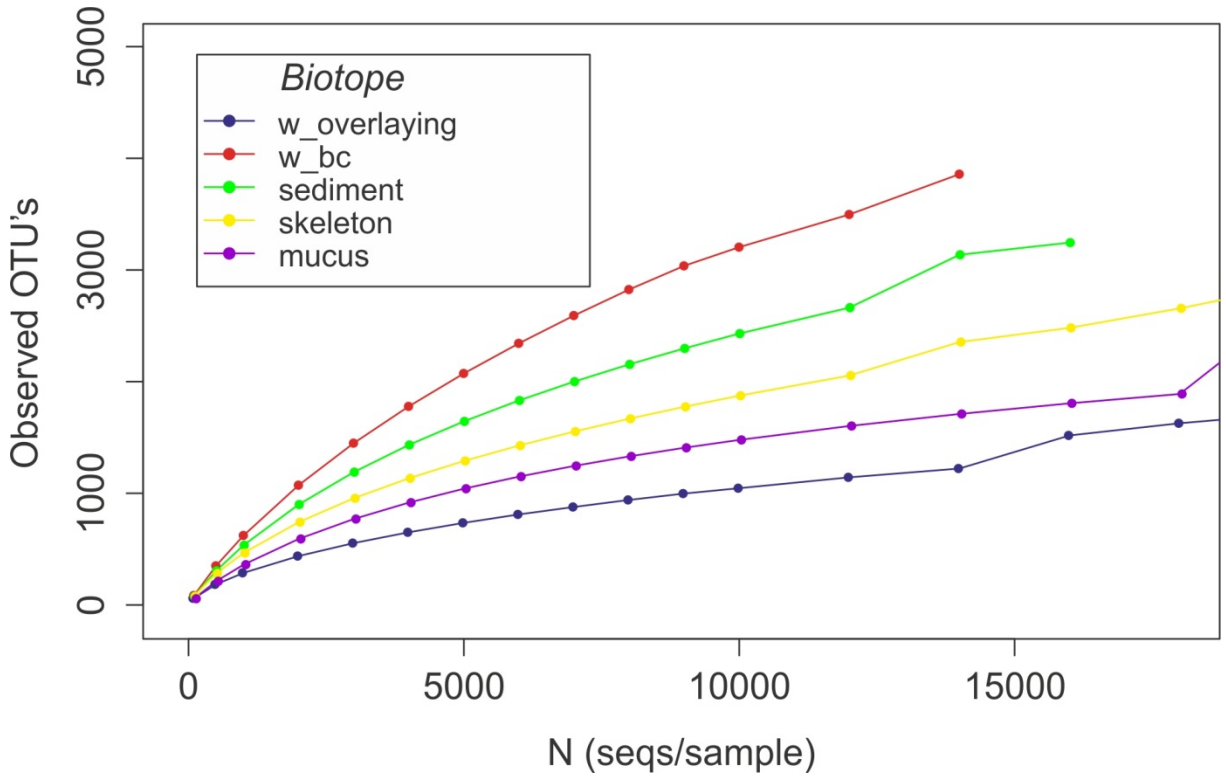
720 **S.I. Figure 2.** Rarefaction curves of OTU's plotted against reads per sample.



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