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 between the coral framework represents a separate microbial habitat. Further, 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). 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

92 Samples were collected during cruises 64PE360 (October 2012) and 64PE377 (October 2013) aboard
93 the RV Pelagia (NIOZ) in the Logachev Mound Province on SE Rockall Bank (Fig. 1a). The focus site for
94 this study was Haas Mound, one of the largest and highest carbonate mounds in the Logachev
95 Mound Province (Mienis et al., 2006) (Fig. 1b). Two transects (Fig. 1c), from the base to the summit
96 of Haas Mound, were surveyed with a tethered HD video camera towed at 2 m above the bottom
97 (mab). Videos were annotated on board and box-corer locations were selected representing the
98 variation in coral cover and megafauna composition.
99 Microbial community samples (Table 1) were collected from a range of putative biotopes across Haas
100 Mound that were operationally defined using video information, hydrographic data collected during
101 the 2012–2013 cruises and earlier (e.g. Mienis et al., 2007), and literature on coral microbe
102 interactions (Carlos et al., 2013; Kellogg et al., 2009; Schöttner et al., 2012; Wild et al., 2008). These
103 biotopes were: (i) water well above the mound i.e. at 400 m water depth; (ii) water overlaying the
104 coral framework at 5 and 10 mab; (iii) near-bottom water; (iv) sediment; (v) uneroded (recently
105 deceased) and eroded *L. pertusa* skeleton; and (vi) *L. pertusa* mucus.
106 Box-core samples were taken with a 50 cm diameter, NIOZ designed box-corer. This box-corer is
107 equipped with a tightly-sealing top valve that prevents the leakage and/or exchange of sea water
108 overlaying the sample during ascent enabling sampling of the near-bottom water once the box-corer
109 was on board. A total of 9 box-cores were collected on the two transects (Table 2, Fig. 1D) and from
110 these, *L. pertusa* skeleton, mucus and near bottom water were taken when available. We
111 differentiated between eroded and uneroded skeleton based on its discoloration (”white” for
112 uneroded skeleton, without biofilm, and “brown” for eroded, older skeleton with biofilm). The water
113 column overlaying Haas Mound was sampled using a rosette sampler equipped with 24 Niskin bottles
114 of each 11 L, attached to a conductivity-temperature-depth (CTD) meter. For each CTD drop, water
115 was collected from three different depths: 400 m water depth and 5 and 10 mab (Table 3, Fig. 1C).
116 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 per box core (preferably from different colonies) and handled as described in 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 sorted according to the reverse MID tags into the 3 replicates. In both procedures a maximum of 2 mismatches in both primers and tags was 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).

OTU-tables were imported in PRIMERv6 (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 PRIMERv6 and plotted in R. For a total of 40 samples (pooled from 121 independent methodological replicates: 38 triplo's and 2 duplo's namely water of 400 m at station 36 and near-bottom water at station 72), the average number of reads per sample was 16220 (with standard error 1090). Rarefaction curves of OTUs plotted against reads per sample almost reached a plateau at 14000 reads per sample (S.I. Fig. 2).

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 at 0.75 mab (Fig. 1c). The duration of each deployment was approximately 48 h.

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. 2a). 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. 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 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. 2b), while salinity dropped to 35.2 (Fig. 2c). 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. 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 increased to 27.60 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 kg m^{-3}.

Video recordings along transects crossing Haas Mound showed large heterogeneity in coral framework distribution. The mound S-slope 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) was sampled by box-cores (st46), which revealed a thick layer of coral framework (Fig. 3). Extensive coral framework was also sampled higher up the S-slope near the edge of the summit between 500-600 m depth (Fig. 3a). Density of the coral framework in box-core samples taken beyond the edge towards the central part of the summit contained reduced amounts of 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 (Fig. 3c).

3.2 Microbial communities and diversity in Haas Mound samples

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 (1878), mucus (1761) and overlaying water (1193). Chao1 indices showed the same trend, decreasing from 3089 in near-bottom water to 1845 in overlaying water (S.I. Table 1). Initial MDS plot of the similarities in OTU composition of the samples immediately showed that the samples of the overlaying water taken at 5 and 10 mab did not differ. This was confirmed by ANOSIM ($p > 0.1$; 999 permutations). Hence, these samples were pooled in one category indicated hereafter as 5 and 10 mab. Subsequent MDS plots were made of the similarities in the sample set and these revealed a consistent pattern, i.e. five different clusters which correspond with the biotopes of the samples (Fig. 4, S.I. 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 in close contact with 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 and within 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 Deltaproteobacteria (11%) and Alphaproteobacteria (9%). Other biotopes shared these 4 groups, however with different relative abundances (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. 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. 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%), 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 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 the distinct signatures of the biotopes. Near-bottom water (Fig. 5b) was distinct from other biotopes by the relative dominance of *Nitrosopumilus* (3.2%), uncultured Xanthomonadales (1,6%), *Defluviicoccus* (1.3%), *Marinicella* (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

273 Flavobacteriaceae NS5 group each contributed between 0.5 and 1.1% to the microbial community of
274 the overlaying water. A comparison of the relative abundance of the class Thaumarchaeota MGI with
275 the abundance of the genus *Nitrosopumilus* indicates that the latter contributed ~2.5% to this class in
276 overlaying water (~17% in near-bottom water and sediment, and ~35% in skeleton and mucus),
277 meaning that other, unknown genera contributed 97% to the Thaumarchaeota class in overlaying
278 water. Sediment was relatively rich in uncult. Xanthomonadales (2.9%) and *Nitrosococcus* (1.5%) in
279 comparison to other biotopes where percentages were <1.7% and <0.8%, respectively. Skeleton
280 samples contained relatively high percentages (>1%) of *Nitrosomonas, Nitrospira, Entotheonella,*
281 *Granulosicoccus, Rhodobium, Blastopirellula and Pseudahrensia*, while proportions in other biotopes
282 were <0.5%. Mucus samples contained large amounts of Alteromonadaceae BD1-7 clade (22%, SE
283 9%) and *Acinetobacter* (9%, SE 9%), with high variability between the samples. *Aquabacterium*
284 (1.9%), *Endozoicomonas* (1.5%), *Polaribacter* (1.3%), and *Pseudomonas* (1.0%) were most apparent in
285 mucus. *Mycoplasma* was not found in mucus but this genus was present in low percentages in
286 skeleton (0.03%) and near-bottom water (0.01%).
287 Specific indicators, i.e. taxa that showed a significant non-random association to a specific
288 biotope, were found for all biotopes (S.I. Table 1). The number of strong indicators (i.e., given the
289 indicator is present, the probability that the sample belongs to a certain biotope > 0.85) was highest
290 in near-bottom water and mucus (8 and 12 strong indicators, respectively) and low in overlaying
291 water, sediment and skeleton (4, 0, and 0 strong indicators, respectively). Brocadiaceae W4, and
292 Dehalococcoidia were the most abundant strong indicators in near-bottom water whereas SAR11
293 clade Deep 1 and Oceanospirillales ZD0405 were typical for overlaying water. Mucus was
294 characterized by Alteromonadaceae BD1-7 and *Acinetobacter*.
295
296 **3.2.2 Variation within biotopes**
297 Within clusters belonging to two of the five main biotopes, patterns were present that could be
298 related to additional factors (Fig. 7 and 8). Within the overlaying water cluster, depth category (400

299 m versus 5 and 10 mab) and year (2012 versus 2013) were discriminating factors as illustrated in the
300 MDS plot (Fig. 7) and determined by ANOSIM ($p < 0.01$ and $p < 0.0001$, respectively, 9999
301 permutations). Within the group of samples taken at 5 and 10 mab, three clusters were recognized
302 according to their geographic position. Samples taken on Haas mound summit clearly differed ($p <$
303 0.001, 9999 permutations) from samples taken at deeper locations on Haas mound slope and from
304 samples taken off-mound. Deeper samples contained relatively more Thaumarchaeota Marine Group
305 I (Fig. 6a). Opposite trends (decreasing with depth) were detected in the classes
306 Gammaproteobacteria, Alphaproteobacteria and Acidimicrobiia (Fig. 6a) and in the genera
307 *Pseudospirillum*, *Nitrospina*, and *NS5 marine group* (Fig. 6b). A small but significant inter annual
308 effect was present in the water samples taken at 400 m and at 5 and 10 mab on Haas Mound,
309 however the year effect was not shown in off-mound samples taken at 5 and 10 mab (Fig. 7).
310 Distance based Redundancy Analyses indicated that depth correlated variables, i.e. temperature,
311 salinity and density, only explained 17% of the total variation in microbial community composition of
312 overlaying water at 5 and 10 mab. Turbidity of the water explained an additional 14% and was
313 correlated with year (r=-0.97).
314 Within the cluster of skeleton samples, uneroded dead coral skeleton hosted a distinct microbial
315 community from eroded dead skeleton (Fig. 8). Uneroded dead skeleton contained more of the
316 classes Gammaproteobacteria and Sphingobacteria (Fig. 6c) whereas eroded skeleton communities
317 contained relatively more Acidobacteria and Planctomycetia (Fig. 6c). On genus level, uneroded dead
318 skeleton contained more *Nitrosopumilus*, *uncult. Xanthomonadales*, *Blastopirellula* and
319 *Pseudahrensia among others,* whereas eroded skeleton contained more *Rhodopirellula*, Pir 4 lineage
320 and *Rhodobium* (Fig. 6d). No patterns were found within the clusters of near-bottom water,
321 sediment and *L. pertusa* mucus samples.
322
323 **4 Discussion**
324 **4.1 Microbial communities and hydrography**

325 The temperature measurements made during this study on Haas Mound support previous
326 observations and models, showing that the S-slope of Haas Mound is subject to intensified mixing
327 caused by internal waves (Mohn et al., 2014; van Haren et al., 2014). By contrast, conditions on the
328 summit of the mound are less dynamic because the internal wave height is less than the mound
329 height and the deep cold water does not reach the summit, but flushes around the slopes of the
330 mound (van Haren et al., 2014). The distribution of dense, live coral framework on the slope seems
331 to match with the degree of mixing, as framework was found to be less abundant on the summit
332 (Figs. 3). This pattern suggests that mixing is important, for supplying food particles, i.e.,
333 phytodetritus (Duineveld et al., 2007), to the living corals, as well as transporting dissolved nutrients,
334 organic carbon, CO_2 and O_2, as is observed near tropical shallow water reefs (Genin et al., 2002;
335 Reidenbach et al., 2006).
336 The distribution of microbial communities across Haas Mound, in some aspects, also reflects local
337 hydrodynamic patterns, though small inter annual effects are apparent. Microbial communities in
338 the overlaying water at 400 m depth within a given year were very similar to each other. This result is
339 explicable since this depth is well above the direct influence of the mound and absolute distances
340 between successive CTD samples were small (< 1 km). Samples on- and off mound showed similar
341 microbial compositions at 400 m. In contrast, samples at 5 and 10 mab differed between mound
342 summit, mound slope and (deeper) off-mound locations (Fig. 7). To explain this differentiation of the
343 microbial communities according to mound site we infer that a gradient in environmental conditions
344 exists on the mound. This hypothetical gradient is caused by internal waves coming from the deep
345 and causing cold water to slosh up the slope, exposing the lower part to more intense mixing, lower
346 temperatures and different water chemistry for longer periods than the upper slope while the
347 summit is not reached by the wave (van Haren et al., 2014).
348 Microbial OTU diversity was highest in near-bottom water and decreased subsequently in sediment,
349 skeleton, mucus and overlaying water. Possibly the enhanced microbial diversity of near-bottom
350 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 could be expected in the near-bottom water sample and 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, 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). 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.

377

378 **4.2 Microbial communities associated with *Lophelia pertusa* skeleton and mucus**

379 Distinct communities were identified on dead coral skeleton and in freshly produced mucus of living
380 coral. Skeleton and mucus contained a substantial amount of Thaumarchaeota Marine Group 1 (9%
381 and 11 %, respectively) of which the majority was unclassified, and the genus *Nitrosopumilus* made
382 up 3% in both sample types and *Cenarchaeum* 0.4% in skeleton and 0.1% in mucus. In addition, small
383 amounts of the Euryarchaeota class Halobacteria (0.1% in skeleton and 0.3% in mucus) and in mucus
384 also Thermoplasmata (0.2%) were found. It is for the first time that Archaea are detected in coral
385 mucus. Archaea had been reported already in samples of *L. pertusa* tissue with corallites crushed
386 (Emblem et al., 2012), and with Archaea affiliated to three species prominently present in the top-10
387 of prokaryotic species based on 454 read data: *Nitrosopumilus maritimus*, *Cenarchaeum symbiosum*
388 and *Candidatus Nitrosoarchaeum* sp..
389 Although not detected by Yakimov (2006), two bacterial genera were previously reported to be part
390 of the *L. pertusa* biome, *Mycoplasma* and *TM7* (Kellogg et al., 2009; Neulinger et al., 2009; Neulinger
391 et al., 2008). In this study, using 454 sequencing, we detected these genera in low relative amounts:
392 *Mycoplasma* was detected in skeleton (0.028%), near-bottom water (0.013%) and overlaying water
393 (0.001%), however not in mucus and sediment. Candidate division TM7 was found in all biotopes,
394 with highest relative amounts in skeleton (0.115%) and mucus (0.071%). With high densities of
395 microorganisms, these small relative percentages of *Mycoplasma* and TM7 may still translate in
396 significant numbers. Moreover, the percentages we report for TM7 may be severe underestimations
397 because the primers we used have a low coverage for Candidate divisions WS6, TM7 and OP11
398 (Klindworth et al., 2013). In our samples of freshly collected mucus, the genera Alteromonadaceae
399 BD1-7 clade (22%) and *Acinetobacter* (9%) were highly represented, and also *Endozoicomonas*,
400 *Polaribacter, Pseudomonas, Aquabacterium* and *Thalassospira* were outstanding in mucus.
401 Representatives of *Acinetobacter* have been reported from cold-water coral (Hansson et al., 2009)
402 and from both healthy and diseased tropical corals (Koren and Rosenberg, 2008; Luna et al., 2010;

403 Rohwer et al., 2002). Members of this genus are well known for their resistance to numerous
404 antibiotics (Devi et al., 2011) and may play a role in the defensive-tactics of corals (Shnit-Orland and
405 Kushmaro, 2009). *Pseudomonas* strains are also known for their antibacterial activity (Ye and Karn,
406 2015) and this genus has been found before in *L. pertusa* (Emblem et al., 2012) and in soft corals
407 (Salasia and Lämmler, 2008).
408 *Endozoicomonas* contains aerobic and halophilic members reported to have associations with corals
409 (Alsheikh-Hussain, 2011; Bayer et al., 2013; Hansson et al., 2009; Kellogg et al., 2009; Pike et al.,
410 2013; Yang et al., 2010) and other marine invertebrates (Kurahashi and Yokota, 2007; Nishijima et al.,
411 2013). Recent results of Ainsworth et al. (2015) indicate that Endozoicimonaceae are likely localized
412 to either the outer coral surface mucus layer or the coral skeleton, as they were found exclusively in
413 the whole organism microbiome and not in isolated coral tissues. Our results confirm that both the
414 mucus (1.5%) and uneroded (recently deceased coral) skeleton (0.9%) are habitats for
415 *Endozoicomonas*. The *Endozoicomonas* found in near-bottom water (0.2%) is probably also related to
416 the presence of mucus. *L. pertusa* is able to produce large amounts of mucus that partly dissolve in
417 the water and stimulated oxygen consumption and microbial activity in near-bottom water up to 10x
418 that in overlaying water (Wild et al., 2008). In this sense *Endozoicomonas* may be an indicator for
419 reef or framework water; the genus was not found in sediment, nor in overlaying water at 5 and 10
420 mab.
421 Different microbial communities were associated with uneroded skeleton compared to eroded
422 skeleton. The microbial community apparently undergoes a major shift upon the death of the coral
423 host, and continues to change as the skeleton degrades over time. This is congruent with reports on
424 microbial succession in shallow-water tropical scleractinians that compare live tissue to recently
425 denuded coral skeleton (Le Campion-Alsumard et al., 1995). Schöttner et al. (2009) identified distinct
426 microbial communities on different areas along a single branch of *L. pertusa*, pointing to cold-water
427 coral framework forming a highly heterogeneous environment.

428 The variations between the different biotopes and within the biotopes that were sampled during this
429 study, emphasize that increasing insight in the role of microbes in cold-water coral ecosystems
430 requires both improved taxonomic resolution and actual knowledge of local biotopes, hydrography
431 and chemical oceanography. Although our study of this single carbonate mound is among few that
432 integrate information on hydrography with microbiology, it has for practical and logistic reasons by
433 no means been exhaustive, and numerous pathways of future research are still open. These include
434 further exploration of the diversity of microbial communities associated with living coral tissue, and
435 the potential reliance of cold-water corals on their microbial associates for chemically-produced
436 energy (Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Kellogg et al., 2009; Rohwer and Kelley,
437 2004). Also interactions with chemical oceanography (e.g. nutrients, oxygen gradients) need to be
438 explored similarly as with specific epifaunal organisms, especially sponges. Furthermore,
439 comparisons on somewhat larger scale between the prominent Haas Mound and nearby mounds of
440 smaller dimensions may shed light on the specific roles of microbes in mound development.
441
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448
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633 **Table 1**. Number of unique samples taken from different biotopes at Haas mound summit, slope and

634 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 (11) 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)

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

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	3984-86
							Skeleton-uneroded	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	3993-95
							Skeleton-uneroded	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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641

642 **Table 3**. List of sampling stations of the overlaying water column. See for abbreviation Fig. 4.

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
					895	w_10 mab	6.7	4040-42
					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
					553	w_10 mab	9	4049-51
					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
					573	w_10 mab	8.7	4058-60
					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
2013	Off mound	2	N 55° 25.95'	W 15° 43.83'	400	w_400 m	9.9	4069-71
					1192	w_10 mab	5.7	4072-74
					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
					522	w_10 mab	8.8	4081-83
					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
					709	w_10 mab	9.1	4090-92
					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
					550	w_10 mab	9	4099-101
					555	w_5 mab	8.9	4102-104

643

644 **Figure 1**. A. Location of Logachev Mound Province (yellow polygon). B. Multibeam map of Logachev
645 Mounds with Haas Mound encircled. C. Detail of Haas Mound with lander and CTD stations arranged
646 along two video transects (dotted lines). D. Detail of Haas Mound with box-corer stations indicated.
647 Note CTD02 is not on the map and lies 8 km SE of CTD10. Red and yellow symbols indicate stations
648 sampled in 2012 and 2013, respectively.

649

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

653

654 **Figure 3**. Photographs of box-cores taken at the S-slope (A, st25 and B, st46) and summit (C, st24 and
655 D, st72) of Haas mound. A clear difference in the amount and height of coral framework was
656 observed.

657

658 **Figure 4.** Microbial OTU composition of 40 samples shows clustering according to biotope: overlaying
659 water (w_400 m; w_5 and 10 mab), near-bottom water (w_bc), sediment, skeleton and mucus. The
660 MDS plot of all 121 samples analyzed, including replicates, shows a similar pattern (S.I. Fig. 1). The
661 same pattern is apparent for microbial classes and genera (not shown).

662

663 **Figure 5.** Microbial community composition of five biotopes sampled at Haas mound. N= number of
664 unique samples per biotope with a: total number of samples, including replicates. A. Most abundant
665 (>1% of total reads) classes for water 5 and 10 mab (n=15, a45), near bottom water w_bc (n=5, a14),
666 sediment (n=4, a12), skeleton (n=6, a21) and mucus (n=2, a6). B. Most abundant (>0.5% of total

667 reads) genera for water 5 and 10 mab, near bottom water w_bc, sediment, skeleton and mucus.
668 Values are plotted as percentage, with standard error.
669
670 **Figure 6.** Differences in microbial community composition within biotopes. N= number of unique
671 samples per biotope with a: total number of samples, including replicates. A. Microbial classes for
672 overlaying water at 400 m depth (n=8, a23), and at 5 and 10 mab on mound summit (n=7, a21),
673 mound slope (n=4, a12) and off-mound (n=4, a12). B. genera for overlaying water at 400 m depth,
674 and at 5 and 10 mab on mound summit, mound slope and off-mound. C. Microbial classes for
675 uneroded (n=2, a9) and eroded skeleton (n=4, a12). D. genera for uneroded and eroded skeleton.
676 Values plotted as percentage with standard error.
677
678 **Figure 7.** Zoom of microbial OTU composition of overlaying water (w_400 m and w_5 and 10 mab).
679 Roman capital I=2012, II=2013.
680
681 **Figure 8.** Zoom of microbial OTU composition of coral skeleton (eroded and uneroded). Roman
682 capital I= 2012, II=2013.

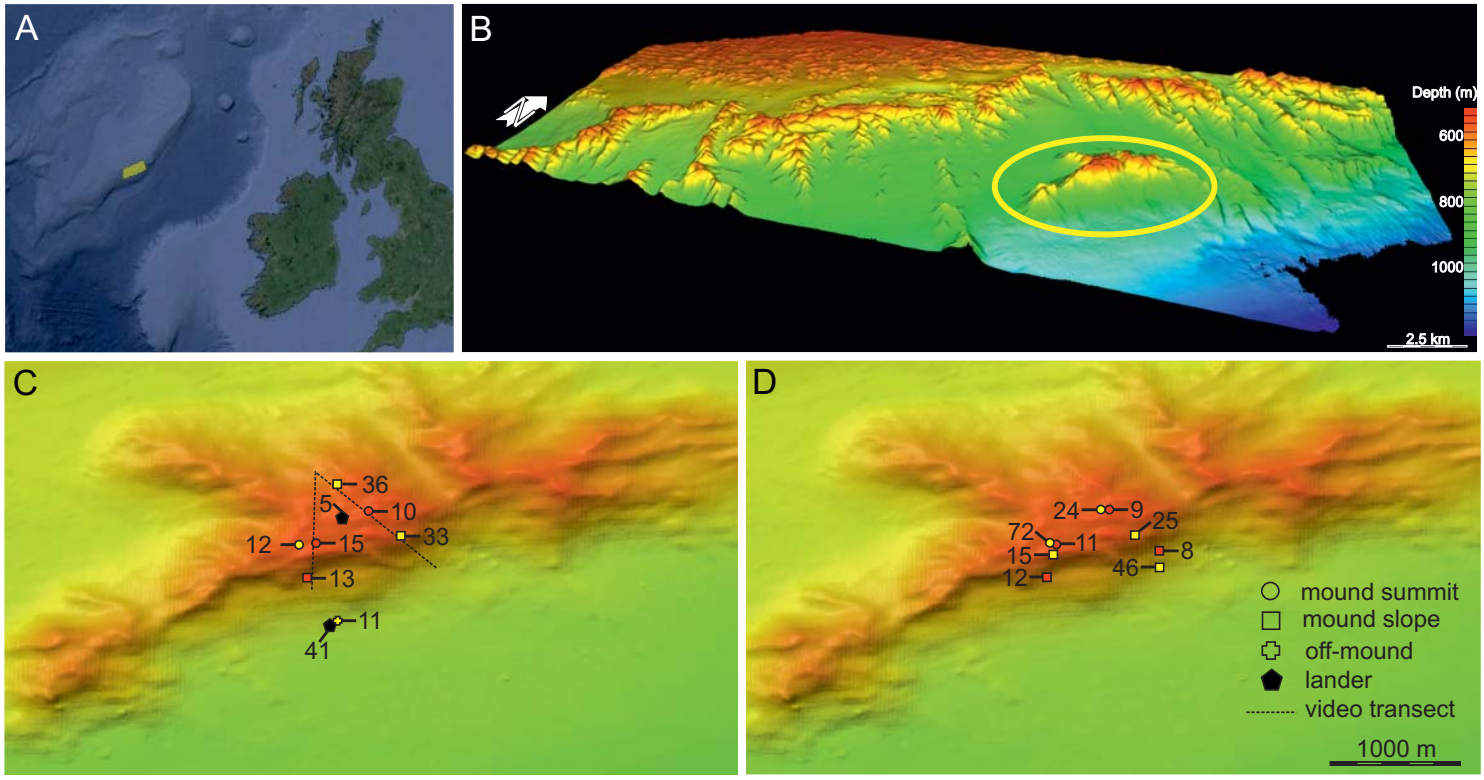
A
B
Depth (m)
600
800
1000
2.5 km
C
36
5
10
33
12
15
13
11
41
D
24
9
25
72
11
8
15
46
12
mound summit
mound slope
off-mound
lander
video transect
1000 m

Figure 1

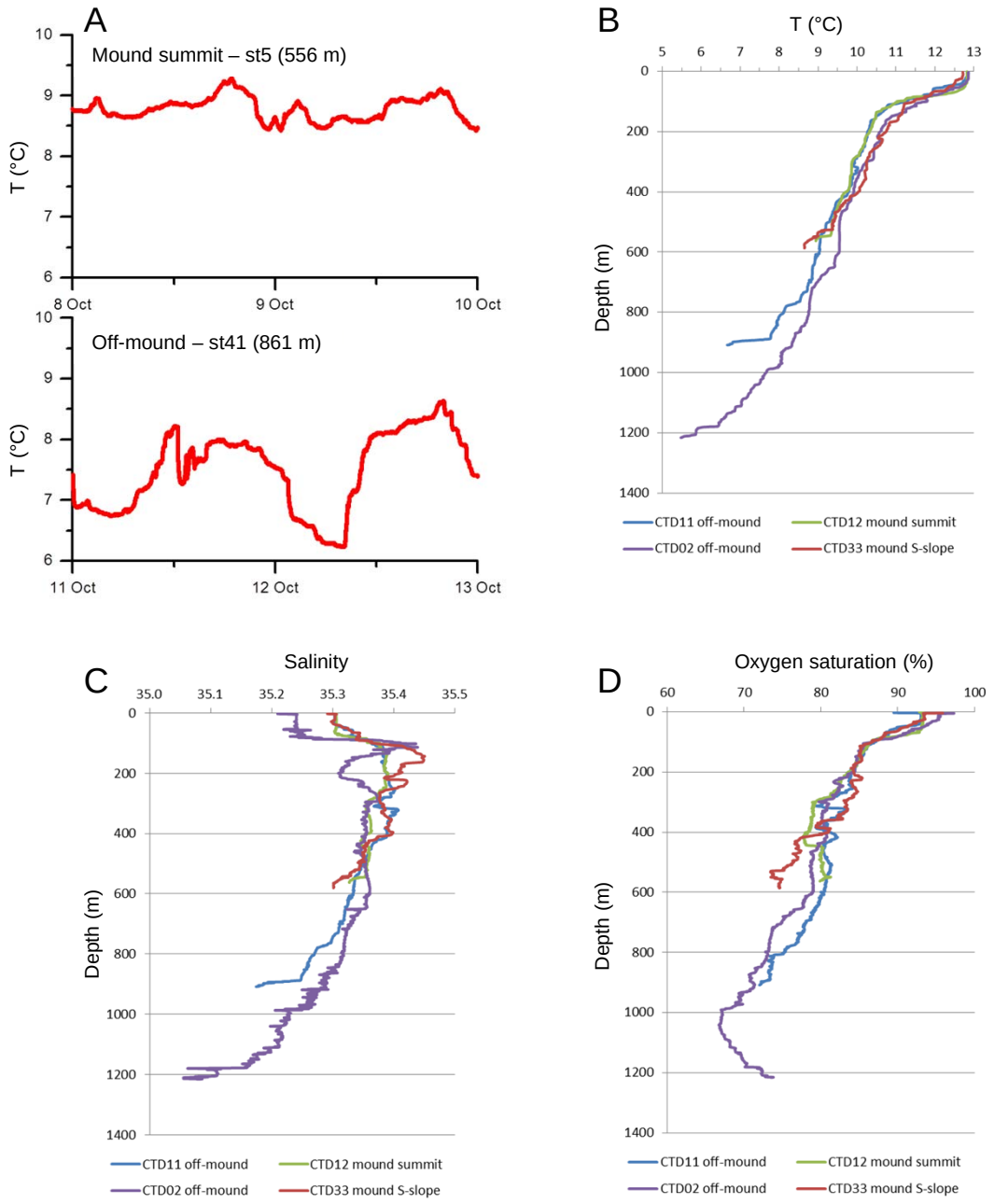
A
Mound summit – st5 (556 m)
T (°C)
8 Oct
9 Oct
10 Oct
Off-mound – st41 (861 m)
11 Oct
12 Oct
13 Oct
B
T (°C)
Depth (m)
CTD11 off-mound
CTD12 mound summit
CTD02 off-mound
CTD33 mound S-slope
C
Salinity
D
Oxygen saturation (%)

Figure 2

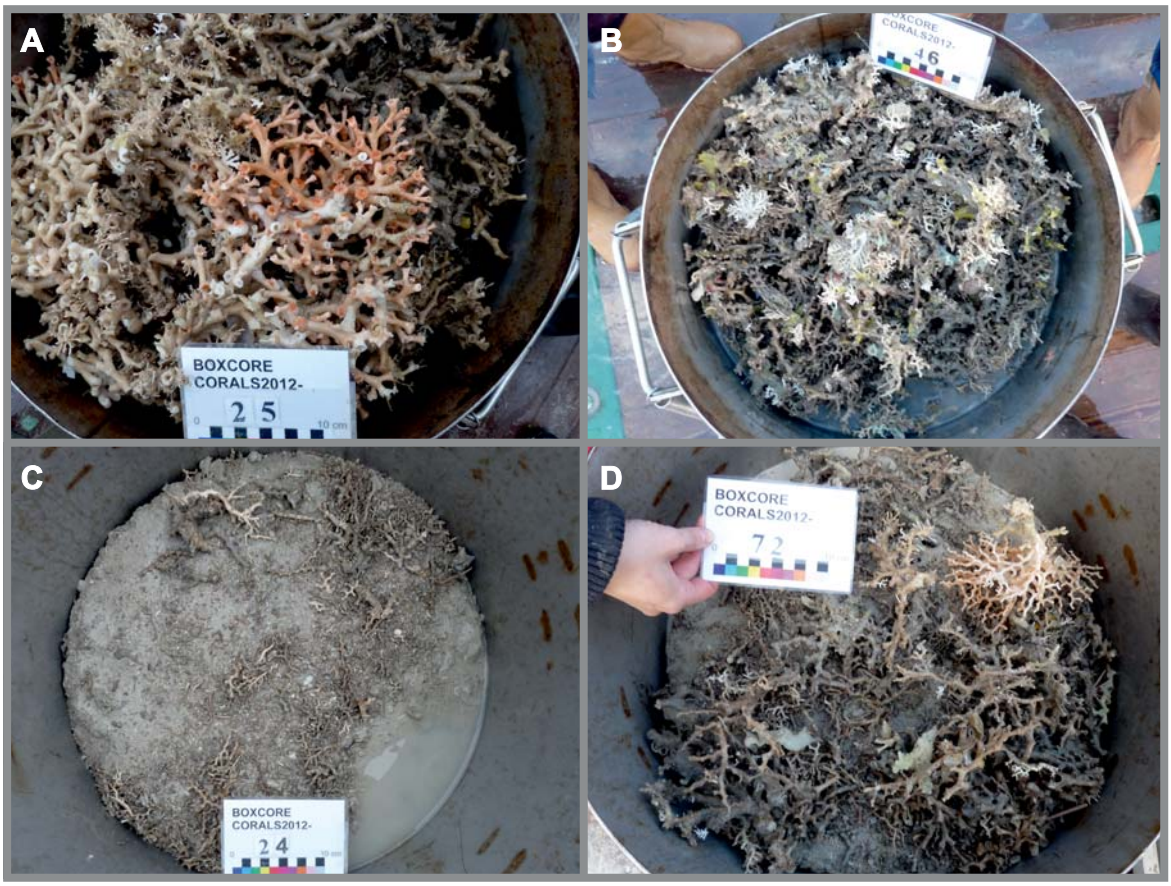
A
BOXCORE
CORALS2012-
25
B
BOXCORE
CORALS2012-
46
C
BOXCORE
CORALS2012-
24
D
BOXCORE
CORALS2012-
72

Figure 3

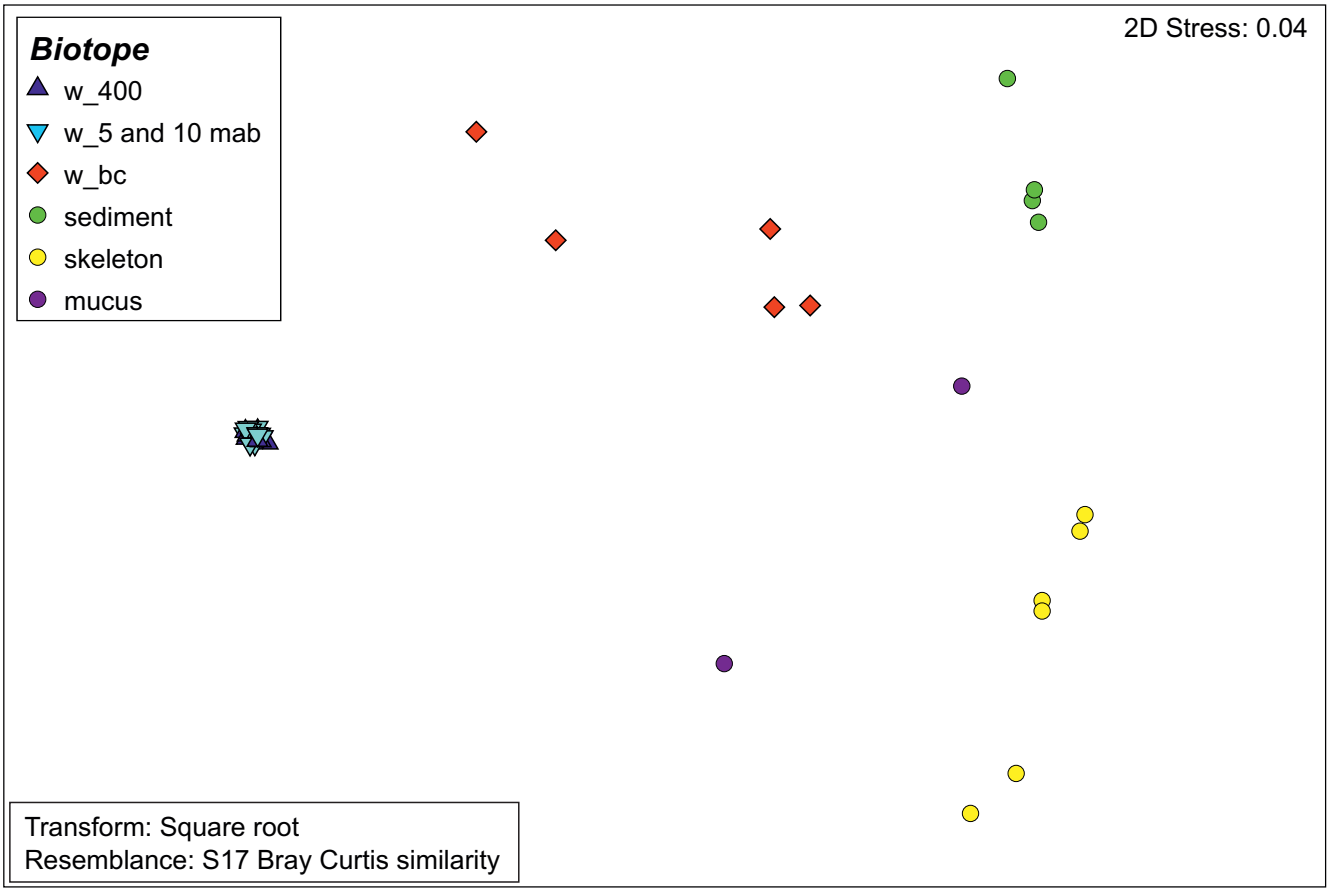
2D Stress: 0.04
Biotope
w_400
w_5 and 10 mab
w_bc
sediment
skeleton
mucus
Transform: Square root
Resemblance: S17 Bray Curtis similarity

Figure 4

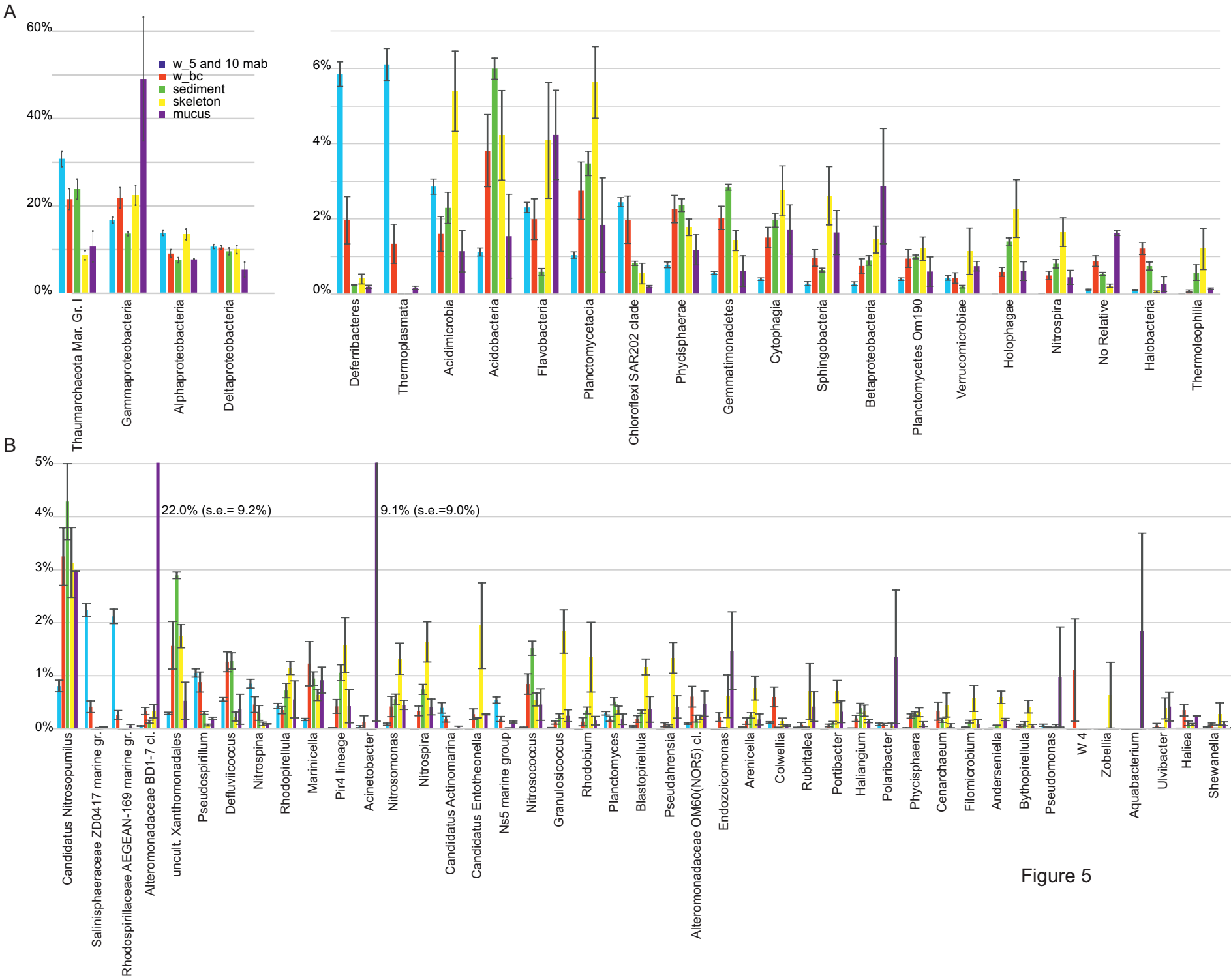
A
60%
40%
20%
0%
w_5 and 10 mab
w_bc
sediment
skeleton
mucus
Thaumarchaeota Mar. Gr. I
Gammaproteobacteria
Alphaproteobacteria
Deltaproteobacteria
6%
4%
2%
0%
Deferribacteres
Thermoplasmata
Acidimicrobia
Acidobacteria
Flavobacteria
Planctomycetacia
Chloroflexi SAR202 clade
Phycisphaerae
Gemmatimonadetes
Cytophagia
Sphingobacteria
Betaproteobacteria
Planctomycetes Om190
Verrucomicrobiae
Holophagae
Nitrospira
No Relative
Halobacteria
Thermoleophilia
B
5%
4%
3%
2%
1%
0%
22.0% (s.e.= 9.2%)
9.1% (s.e.=9.0%)
Candidatus Nitrosopumilus
Salinisphaeraceae ZD0417 marine gr.
Rhodospirillaceae AEGEAN-169 marine gr.
Alteromonadaceae BD1-7 cl.
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) cl.
Endozoicomonas
Arenicella
Colwellia
Rubritalea
Portibacter
Haliangium
Polaribacter
Phycisphaera
Cenarchaeum
Filomicrobium
Anderseniella
Bythopirellula
Pseudomonas
W 4
Zobellia
Aquabacterium
Ulvibacter
Haliea
Shewanella
Thalassospira

Figure 5

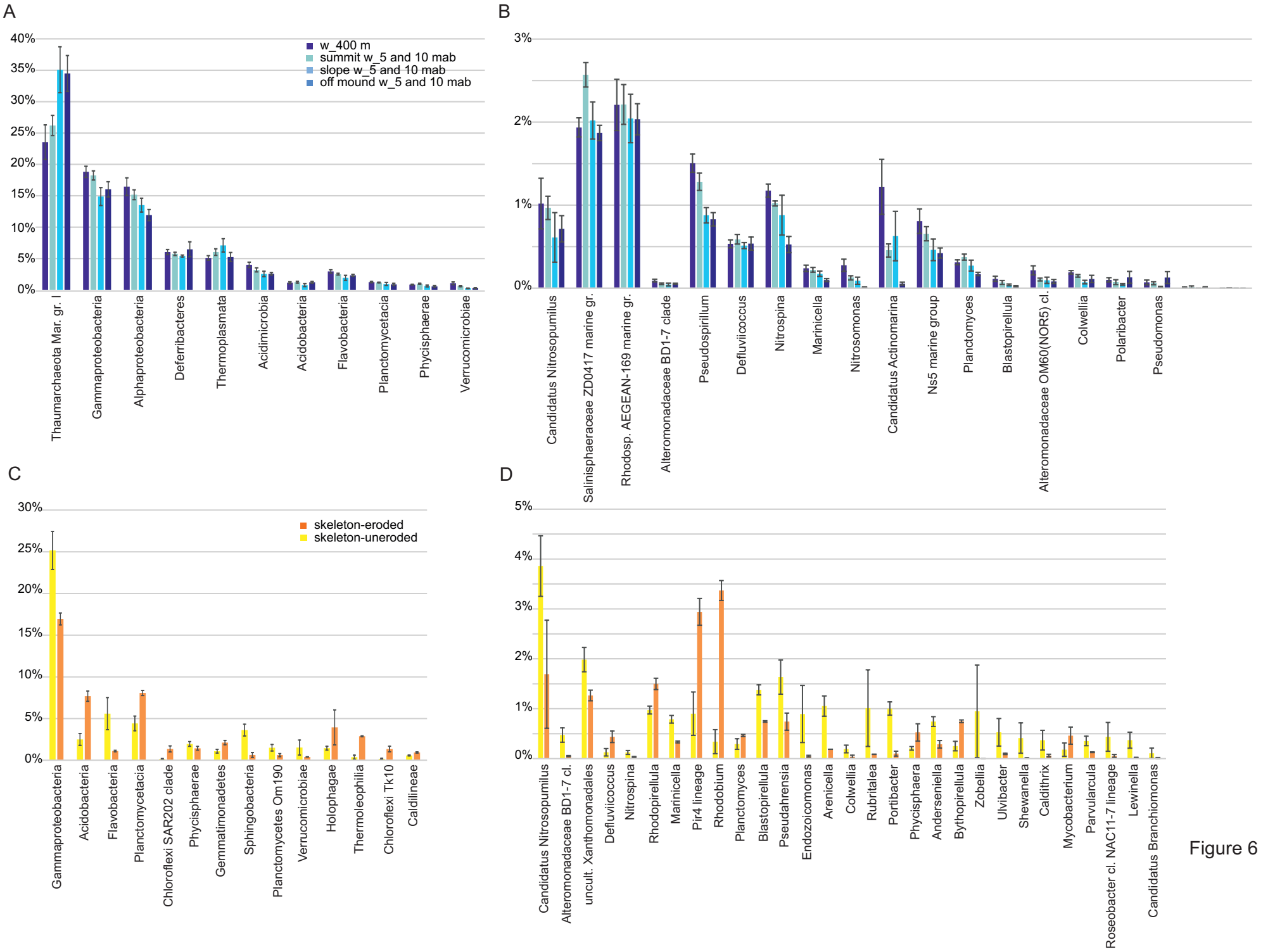
A
40%
35%
30%
25%
20%
15%
10%
5%
0%
w_400 m
summit w_5 and 10 mab
slope w_5 and 10 mab
off mound w_5 and 10 mab
Thaumarchaeota Mar. gr. I
Gammaproteobacteria
Alphaproteobacteria
Deferribacteres
Thermoplasmata
Acidimicrobia
Acidobacteria
Flavobacteria
Planctomycetacia
Phycisphaerae
Verrucomicrobiae
B
3%
2%
1%
0%
Candidatus Nitrosopumilus
Salinisphaeraceae ZD0417 marine gr.
Rhodosp. AEGEAN-169 marine gr.
Alteromonadaceae BD1-7 clade
Pseudospirillum
Defluviicoccus
Nitrospina
Marinicella
Nitrosomonas
Candidatus Actinomarina
Ns5 marine group
Planctomyces
Blastopirellula
Alteromonadaceae OM60(NOR5) cl.
Colwellia
Polaribacter
Pseudomonas
C
30%
25%
20%
15%
10%
5%
0%
skeleton-eroded
skeleton-uneroded
Gammaproteobacteria
Acidobacteria
Flavobacteria
Planctomycetacia
Chloroflexi SAR202 clade
Phycisphaerae
Gemmatimonadetes
Sphingobacteria
Planctomycetes Om190
Verrucomicrobiae
Holophagae
Thermoleophilia
Chloroflexi Tk10
Caldilineae
D
5%
4%
3%
2%
1%
0%
Candidatus Nitrosopumilus
Alteromonadaceae BD1-7 cl.
uncult. Xanthomonadales
Defluviicoccus
Nitrospina
Rhodopirellula
Marinicella
Pir4 lineage
Rhodobium
Planctomyces
Blastopirellula
Pseudahrensia
Endozoicomonas
Arenicella
Colwellia
Rubritalea
Portibacter
Phycisphaera
Anderseniella
Bythopirellula
Zobellia
Ulvibacter
Shewanella
Calditrix
Mycobacterium
Parvularcula
Roseobacter cl. NAC11-7 lineage
Lewinella
Candidatus Branchiomonas

Figure 6

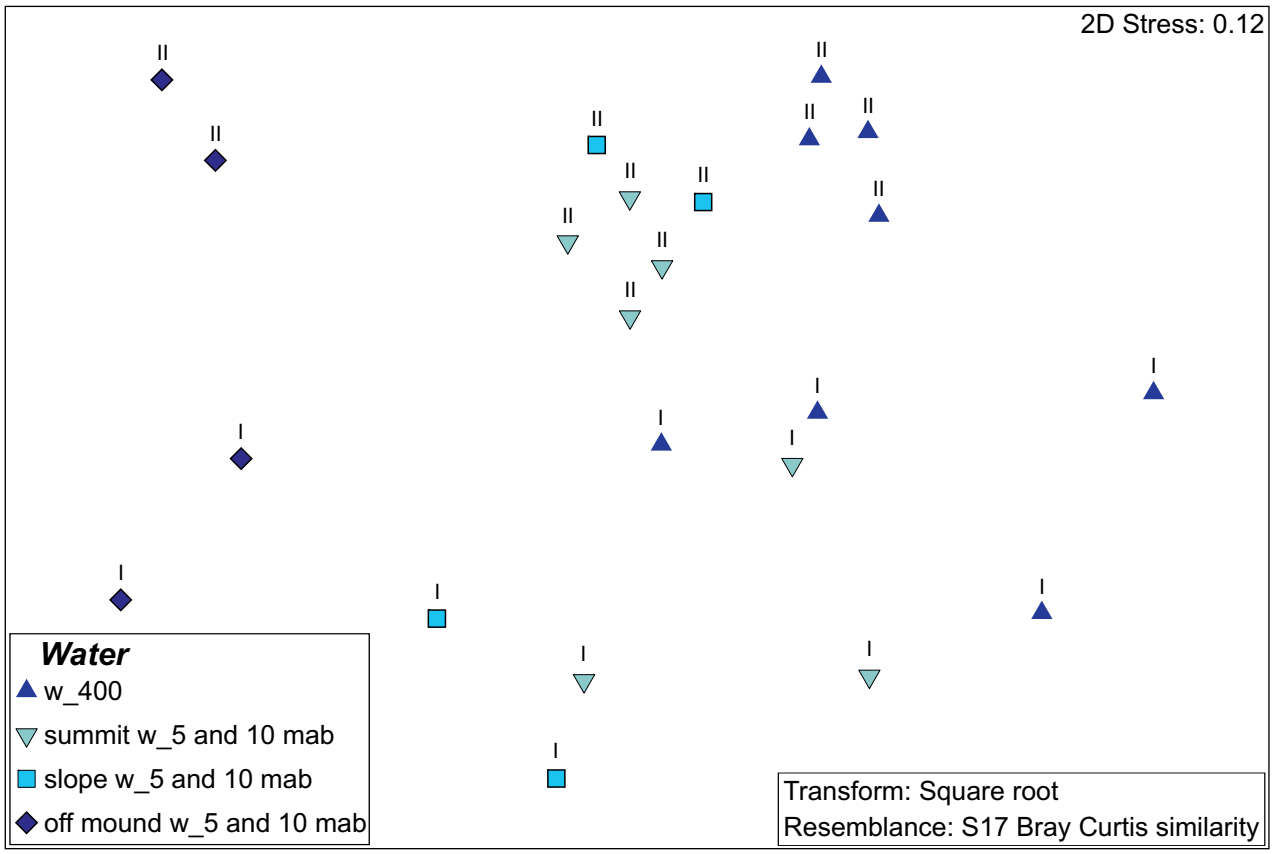
2D Stress: 0.12
Water
w_400
summit w_5 and 10 mab
slope w_5 and 10 mab
off mound w_5 and 10 mab
Transform: Square root
Resemblance: S17 Bray Curtis similarity

Figure 7

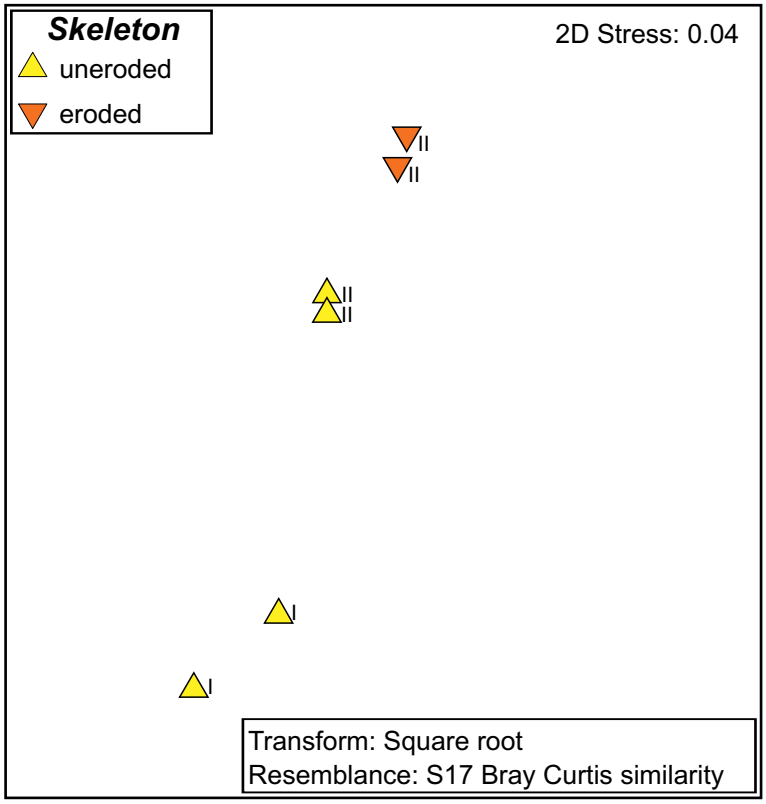
Skeleton
uneroded
eroded
2D Stress: 0.04
II
II
II
II
I
I
Transform: Square root
Resemblance: S17 Bray Curtis similarity

Figure 8