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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 shows the microbial community composition over Haas Mound, one of the most prominent cold-water coral mounds of the Logachev Mound Province (Rockall Bank, NE Atlantic), outlining distribution patterns both vertically from the seafloor to the water column and laterally across the mound and coupling this to mound topography and hydrography. Samples were collected in 2012 and 2013 from biotopes that were partially chosen based on high definition video surveys that were conducted prior to sampling and included overlaying water (400 m depth and 5 + 10 m above the bottom (m ab)) collected with a CTD/Rosette system and near-bottom water, sediment, *Lophelia pertusa* mucus, and *L. pertusa* skeleton samples collected with a box-core. Furthermore, temperature and current measurements were obtained at two sites at the summit and foot of Haas Mound to study near-bed hydrodynamic conditions. Community composition was determined by next generation Roche 454 sequencing yielding high-resolution records of 16 S rRNA genotypes, improving our understanding of deep-sea microbial consortia. With the methods we employed we were able to report for the first time Archaea in association with *L. pertusa*. The pattern of similarities between samples visualized by multi-dimensional scaling (MDS), indicates a strong link between the distribution of microbes and specific biotopes. All biotopes share a number of taxa, but biotopes are distinct on basis of relative abundances and a small number of unique taxa. Similarity in microbes indicates that water is well-mixed at 400 m depth, but less so at 5 + 10 m above the bottom, where microbial communities differed between summit, slope and off mound. Even more variability was observed in the near-bottom water samples, which group according to sampling station. Likely the coral framework prevents the near-bottom water in between the branches to be vigorously mixed with the water overlaying the reef. The microbial consortium on Haas Mound appears strongly linked with the surrounding environment, making cold-water coral communities sensitive to outside environmental influences.

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1 Introduction

Numerous mounds composed of a mixture of sediment and cold-water coral debris line the Southeast slope of Rockall Bank, between 500 and 1100 m water depth (Kenyon et al., 2003; van Weering et al., 2003). This so called "Logachev Mound Province" consists of mounds varying from tens to hundreds of m in height and several km in length and width (Kenyon et al., 2003). These mounds have been developing since the middle Miocene–early Pliocene, largely as the byproduct of interacting hydrodynamic regimes, coral growth and sedimentation (De Haas et al., 2009; Mienis et al., 2007). Living coral colonies of mainly *Lophelia pertusa* and *Madrepora oculata* inhabit the mound summits and flanks, providing habitat for a wide range of invertebrates and fish (Costello et al., 2005; van Soest et al., 2008).

Deployments of current and temperature sensors at different sites in the Logachev Mound province have provided evidence of large regional differences with respect to current strength, temperature fluctuations and organic carbon supply (Mienis et al., 2007). Also on the scale of individual mounds significant heterogeneity in environmental conditions was found for instance between the summit and foot of mound structures (Duineveld et al., 2007). More recent studies on the near-bed hydrodynamic regime in the Logachev Mound Province revealed intense mixing on the mounds as a result of internal waves interacting with the topography (Mohn et al., 2014; van Haren et al., 2014). This mixing not only provides a constant food supply, but also ensures the removal of CO_2 from the area and the constant refreshment of dissolved oxygen and nutrients (Findlay et al., 2014). The relevance of this variation for the growth of cold-water coral framework and mounds as a whole is a subject of current studies (F. Mienis, personal communication, 2014).

Microbes have been found crucial for the fitness of tropical corals (Knowlton and Rohwer, 2003; Krediet et al., 2013; Rosenberg et al., 2007). Shifts in the composition or metabolism of coral-associated microbial consortia can significantly reduce resilience

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of tropical corals, increasing stress, disease, and death (Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Gilbert et al., 2012; Rohwer and Kelley, 2004).

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

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

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better insight into importance of cold-water coral habitats for microbial diversity and function. Earlier studies have already shown that these habitats are hotspots of metazoan biodiversity and biomass, and of carbon mineralization (Biber et al., 2014; Henry and Roberts, 2007; van Oevelen et al., 2009).

2 Materials and methods

2.1 Location and sample collection

Samples were collected during cruises 64PE360 and 64PE377 aboard the RV Pelagia (NIOZ) in the Logachev Mound Province on SE Rockall Bank in October 2012 and 2013, respectively (Fig. 1a). The focus site of this study was Haas Mound being one of the largest and highest (360 m) carbonate mounds in the Logachev Mound Province (Mienis et al., 2006) (Fig. 1b). Transects from base to summit of Haas Mound were first surveyed with a tethered HD video camera towed at ~2 m above the bottom (m ab). Videos were annotated on the fly and box-core locations were selected based on annotations.

Samples of the bacterial community were collected in a range of putative biotopes across Haas Mound that were operationally defined using video information, hydrographic data from 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). The different biotopes that we sampled were: (i) water well above the mound (400 m water depth), (ii) water overlaying the coral framework at 5 and 10 m ab, (iii) near-bottom water, (iv) sediment, (v) *L. pertusa* dead skeleton (uneroded as well as eroded pieces) and (vi) coral mucus.

Box-core samples were taken with a 50 cm diameter NIOZ designed box-core. The box-core is equipped with a tightly-sealing top valve, which prevents he leakage and exchange of water overlaying the sample during ascent, allowing sampling of the near-bottom water on board. A total of 9 successful box-cores were collected on two tran-

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sects, i.e. 5 were collected in 2012 and 4 were collected in 2013 (Figs. 1c and 2). Of the 2012 box-cores, near-bottom water samples were retrieved from 3 and both living and dead (uneroded and eroded) *L. pertusa* samples were collected from 2 box-cores. Sediment samples were collected from box-cores taken during the 2013 cruise (Table 1).

The water column overlaying Haas Mound was sampled using a rosette of 11 L Niskin bottles attached to a conductivity-temperature-depth (CTD) meter. For each CTD drop, three depths were chosen to collect water: at 400 m water depth and at 5 and 10 m ab (Table 2). Samples from a total of 7 CTD stations were analyzed, 4 sampled in 2012 and 3 sampled in 2013 (Figs. 1c and 2). In 2013 one off mound station at 1200 m water depth situated 10 km SE from Haas mound was sampled with the CTD in the same way to determine if water mass characteristics above the mound differ much from those off mound and in deeper water.

Water sampled for microbial DNA analysis was filtered through 0.2 µm polycarbonate filters (Whatman). At each depth, 3 samples of 2 L were filtered. The near-bottom water collected from box-cores was sampled in the same fashion. All filters were immediately frozen in 6 mL Pony vials at −80 °C. Living and dead coral samples (*L. pertusa*) were collected from box-cores which contained framework. Living corals as well as uneroded skeletons were collected in three replicates (preferably from different colonies) from two box-core stations in 2012. The box-cores taken in 2013 contained no living coral, but extra samples of uneroded skeletons as well as eroded skeletons were collected from 2 stations.

Coral samples were briefly rinsed with demineralized water to wash off seawater and sediment. To sample mucus, coral branches were placed on clean aluminum foil in a 4 °C lab on board. As mucus accumulated around the contact points between the coral and aluminum foil it was collected with a sterile 5 mL syringe and needle. Some mucus could also be gathered directly from the surface of coral branches and polyps. Generally around 0.5 mL of mucus was retrieved. Skeleton samples were deeply scraped along the surface with scalpel blades. Mucus and skeleton scrapings were placed in

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individual 6 mL pony vials and immediately frozen at −80 °C. In 2013, the scraping technique was abandoned, due to the low amount of sample material retrieved, and 2–3 cm of coral skeleton was instead directly frozen at −80 °C on board. In the lab these samples were later exposed to liquid nitrogen and homogenized directly into the MoBio bead extraction tubes for DNA extraction.

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 % (by weight) agarose gel.

To amplify the V4 region of the 16 S rDNA the universal prokaryotic primer set S-D-Arch-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. Per sample, two separate 50 µL PCR reactions were performed using 1 unit Phusion Taq each (Thermo Scientific) in 1 × High-Fidelity Phusion polymerase buffer. The volume of template was adjusted to the concentration of the DNA to aim for equal amounts of starting material (approximately 10 ng genomic DNA per reaction). The PCR was run on an iCycler™ Thermo Cycler (BioRad, USA). Cycle conditions were as follows: 30 s at 98 °C, (10 s at 98 °C, 20 s at 53 °C, 30 s at 72 °C) × 30 cycles, finally 7 min at 72 °C.

All PCR products were loaded on a 2 % (by weight) agarose gel pre-stained with SybrSafe and run at 80 V for 50 min. A blue-light converter was used when excising the PCR products to avoid UV-damage. Products of the same sample were combined and purified using the Qiaquick Gel Extraction kit. After fluorimetric quantification, equal concentrations of the cleaned PCR products were pooled (18 samples with their unique

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forward-MID and reverse- MID combination per 1/8 lane) and with MinElute kit (Qiagen) the volume was adjusted to 25 µL with a final concentration of $< 25\ ng\ \mu L^{-1}$ pooled PCR product. In total, 7 pooled samples were sent to Macrogen (Seoul, South Korea) all sequenced by a Roche GS-FLX Titanium sequencer on 1/8 lane each.

2.3 Sequence processing, taxonomic assignment and diversity analyses

The first steps of the bio-informatic analysis were done with the Ribosomal Database Project (RDP) pipeline (Cole et al., 2014). To split the library of each lane (pooled sample) the routine "RDP Pipeline Initial process" was used. Only sequences with average *Q*-score above 25 and longer than 250 bases were analyzed. Subsequently, the 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. Finally, each of the seven lanes were split into 18 samples, 6 unique samples each with 3 replicates. All reads had a similar length of 251 bp.

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). The classified results were processed in Excel and the taxon's class and genus were extracted and imported in PRIMERv6 (Clarke, 1993; Clarke and Gorley, 2006). The data were standardized per sample to avoid biases caused by differences in sample size.

Rarefaction curves and diversity indices were calculated using QIIME (Caporaso et al., 2010) and plotted in R. For a total of 125 samples (41 triplo's and 1 duplo), the average amount of reads per sample was 5627 (with standard error 228). Rarefaction curves of OTUs plotted against reads per sample almost reached a plateau at 3573 reads per sample (S.I. Fig. 1 in the Supplement). The fractions of reads that were assigned to specific taxa were 99 % to class, 58 % to family and 29 % to genus level. A resemblance matrix was made on class and genus taxa based on a Bray-Curtis similarity coefficient. These resemblance matrices were visualized with MDS plots.

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

2.4 Near-bed temperature and current measurements

During the 2012 cruise, temperature and currents were measured on the summit (st5 at 556 m) and 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 (Fig. 1c). The duration of each deployment was approximately 48 h.

3 Results

3.1 Haas mound physical environment and coral cover

The SE 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 less than 1 °C, was recorded on the summit (Fig. 3a).

In Fig. 3b–d, temperature, salinity and oxygen profiles measured in 2012 and 2013 are shown for the water column at the off mound (st2 and 11), NW slope (st87) and summit (st12) of Haas Mound. The temperature of the water column overlaying Haas Mound was around 10 °C at 400 m depth and decreased by 1 °C every 156 m depth. Salinity is 35.4 at 400 m depth and decreases slightly with depth. These temperature and salinity values are characteristic for Eastern North Atlantic Water. At the foot of the deep SE slope the presence of cold water is clearly visible as an abrupt decrease in temperature to 6.6 °C (Fig. 3b), while salinity also drops sharply to 35.2 (Fig. 3c). Both values are indicative for the presence of Subarctic Intermediate Water (McGrath

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et al., 2012). The oxygen saturation was around 80 % at 400 m depth and decreased with depth on the slope stations to about 75 %. In the cold water at the 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 ~ 27.3 at 400 m depth and gradually increased to 27.44 at 750 m, which is the depth of the slope of Haas Mound. Below 750 m, density abruptly increased to 27.6 where deep cold water was encountered. Bottom water temperature at the far off mound station (st2) was 5.3 °C, while salinity was 35.0 and density 27.7 respectively.

The lower parts of the SE slope at about 645 m depth were sampled by box-cores (st8, 46) which revealed a thick layer of coral framework (Fig. 2). Extensive framework was also sampled higher on the slope between 500 and 600 m depth (st12, 25, see Figs. 2 and 4). The highest point sampled on Haas Mound was at 528 m depth on a ridge at the summit of the slope which had thick framework (st15). However the coral framework became rapidly reduced north of this ridge towards the summit (st11,72, Figs. 2 and 4). Box-cores taken at the summit plateau showed reduced amounts of framework (st9) and one box-core station yielded only mud and small fragments of coral skeleton (st24, Fig. 4). Video footage of the summit showed that the ridges of denser framework alternated with valleys of reduced framework and mud. Overall the summit appeared much less densely covered by corals than the SE slope. At parts of the summit coral framework was replaced by a dense cover of large erect sponges (*Rosella nodastrella*).

3.2 Microbial communities and diversity in Haas Mound samples

Microbial diversity based on OTUs was highest in near-bottom water followed by sediment, skeleton and mucus. Lowest diversity was found in overlaying water (Table 3). Corresponding Chao1 indices showed the same pattern, decreasing from 2829 in near-bottom water to 900 in overlaying water. Initial MDS plot of the similarities in microbial community composition of the samples based on of reads classified up to genera and up to classes immediately showed that the samples of the overlaying water taken at

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5 and 10 m ab did not differ . This was confirmed by ANOSIM ($p > 0.1$; 9999 permutations). Hence, these samples were pooled in one category indicated hereafter as 5 + 10 m ab. 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. 5, S.I. Fig. 2). Near-bottom water, which is closely associated with both reef and sediment, contained a microbial community that was clearly distinct from overlaying water, sediment, *L. pertusa* skeleton and mucus. Following is an account of the composition of the bacterial communities encountered in the samples with emphasis on variation between biotopes and within specific biotopes across the mound.

3.2.1 Variation between biotopes

In near-bottom water, Gammaproteobacteria and Thaumarchaeota marine group I were the most abundant classes followed by Delta- and Alphaproteobacteria. Sediment and overlaying water shared this top 4, however with different relative abundances, i.e. containing more Thaumarchaeota and less Gammaproteobacteria than near-bottom water (Fig. 6). Skeleton and mucus contained substantial amounts of Thaumarchaeota MGI (9–11 %) but less than other biotopes. Skeleton exceeded in Alphaproteobacteria, Acidimicrobia, Acidobacteria and Planktomycetacia whereas mucus exceeded in Gammaproteobacteria (Fig. 6).

Plotting the most abundant taxa (> 0.5 % of all reads) confirmed a distinct signature of near-bottom water (Fig. 7) with a top 6 of *Nitrosopumilus*, uncult. Xanthomonadales, *Defluviicoccus*, *Marinicella*, *Nitrosococcus* and the Brocadiaceae W4 lineage. Overlaying water was rich in Salinisphaeraceae ZD0417 marine group, Rhodospirillaceae AEGEAN-169 marine group, *Pseudospirillum*, *Nitrosopumilus*, *Nitrospina* and the Flavobacteriaceae NS5 group. Sediment was rich in *Nitrosopumilus*, uncult. Xanthomonadales, *Nitrosococcus*, *Defluviicoccus* and the Pir4 lineage. In skeleton samples the top 6 genera were *Nitrosopumilus*, *Rhodobium*, the Pir4 lineage, *Entheonella*, *Nitrospira* and *Granulosicoccus* whereas mucus samples contained high amounts of

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the Alteromonadaceae BD1-7 clade, *Acinetobacter*, *Nitrosopumilus*, *Aquabacterium*, *Endozoicomonas* and *Polaribacter*.

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 (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). Brocadiaceae W4, and Dehalococcoidia were the most abundant strong indicator species 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 the main biotopes, patterns were present that could be related to additional factors (Fig. 8a–c). Within the cluster of near-bottom water, microbial communities grouped according to station i.e. location on the Haas Mound (Figs. 2 and 8b). No relation was observed between the near-bottom water community and framework height (0–10 cm in stations 24, 72 and 9; 10–30 cm in stations 11 and 46).

Within the overlaying water samples, sample depth and year were discriminating factors as illustrated in the MDS plot (Fig. 8c) and determined by ANOSIM ($p<0.0001$ and $p<0.0001$, respectively, 9999 permutations). Samples taken at 400 m differed significantly from samples taken at 5 + 10 m ab. 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 ($p<0.0001$, 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 more Thaumarchaeota Marine Group I and more Oceanospirillales ZD0405. Opposite trends (decreasing with depth) were detected in the classes Gammaproteobacteria, Alphaproteobacteria and

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Acidimicrobia (Fig. 6c) and in the genera *Pseudospirillum*, *Nitrospina*, and NS5 marine group (Fig. 7c).

A year effect was present in the water samples taken at 400 m and 5 + 10 m ab on Haas Mound but not in 5 + 10 m ab taken off mound (Fig. 8c). Within the group of skeleton samples, uneroded dead coral skeleton hosted a distinct microbial community from eroded dead skeleton (Fig. 8a). Uneroded dead skeleton contained more of the classes Gammaproteobacteria, Flavobacteria and Sphingobacteria whereas eroded skeleton communities contained more Acidobacteria and Planctomycetia (Fig. 6b). On genus level, uneroded dead skeleton contained more *Nitrosopumilus*, *Endotheonella*, *Blastopirellula* and *Pseudahrensia* whereas eroded skeleton contained more *Rhodopirellula*, Pir 4 lineage and *Rhodobium* (Fig. 7b). Within the biotope clusters of sediment and *L. pertusa* mucus, additional patterns were not found.

4 Discussion

The temperature measurements made during this study on Haas Mound support previous observations and models, showing that the SE slope of Haas Mound is subject to intensified mixing caused by internal waves (Mohn et al., 2014; van Haren et al., 2014). By contrast, conditions on the summit of the mound are less dynamic because the wave height is less than the mound height and the deep cold water does not reach the summit, but flushes around the slopes of the mound (van Haren et al., 2014). The distribution of dense live coral framework on the slope seems to match with the degree of mixing, while much thinner framework was found on the summit (Figs. 2 and 4). Presumably mixing is important for the supply of particles towards the living corals and the exchange of dissolved nutrients, organic carbon, CO_2 and O_2, as is observed near tropical shallow water reefs (Genin et al., 2002; Reidenbach et al., 2006).

The distribution of microbial communities across Haas Mound is in some aspects also reflective of local hydrodynamic patterns, though small year effects are apparent. Microbial communities in the overlaying water at 400 m depth within a given year were

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very close. This result is explicable since this depth is well above the direct influence of the mound and absolute distances between successive CTD samples are small (< 1 km). Samples on and off mound showed similar microbial compositions at 400 m. In contrast, samples at 5 + 10 m ab differed between mound summit, mound slope and (deeper) off mound locations (Fig. 8c).

Due to our method of collecting near-bottom water within the framework with a box-corer, a certain amount of suspended sediment is expected in the water sample and hence similarity in microbes. However, sediment samples appear to support a community clearly distinct from the near-bottom water, indicating that influence of resuspended sediment on latter samples is small. Moreover, near-bottom water contained a number of strong indicator taxa that were highly specific (high A values in indicspecies analyses) for this biotope confirming its distinct signature (S.I. Table 1). The sharp difference between near-bottom water and overlaying water at 5 + 10 m ab 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, etc.) living in the framework and sediment, a biologically and chemically unique and sheltered environment is created for the development of a typical local microbial community with the highest diversity (this study) and probably also the highest activity: *Lophelia* produces large amounts of mucus that partly dissolve in the water and stimulated oxygen consumption and microbial activity in near-bottom water up to 10× that in overlaying water (Wild et al., 2008).

To explain the differentiation of the near-bottom communities according to station we infer that a gradient in environmental conditions exists on the slope. This hypothetical gradient is caused by internal waves coming from the deep and causing cold water to slosh up the slope, exposing the lower part to more intense mixing and lower temperatures for longer periods than the upper slope while the summit is not reached by the wave (van Haren et al., 2014). The station effect could either be a direct response to

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such an environmental gradient (e.g. temperature, DOC, nutrients) or an indirect effect of different epifauna communities which in turn reflect the environmental gradient. As no detailed environmental measurements or epifauna samples are available from the slope, above remains speculative but signifies an avenue for further studies. The relevance of such studies lies in the fact that they could provide insight into mound build-up and its limits. For example defining the conditions for microbes associated with breaking down the skeleton may be vital to carbonate mound development and/or degradation in the deep-sea as cold-water coral mounds in the Logachev mound area are primarily composed of $CaCO_3$-based sediments, being the product of decomposed coral skeleton and associated species (Mienis et al., 2009).

Distinct communities were identified on coral skeleton and in mucus of living coral. For the first time Archaea were found to be associated with *L. pertusa*. Skeleton and mucus contained a substantial amount of Thaumarchaeota Marine Group 1 (9–11 %) and small amounts of the Euryarchaeota classes Halobacteria (0.1–0.3%) and Thermoplasmata (0–0.2 %). Earlier, Schöttner et al. (2009) identified similar distinct microbial communities on different areas along a single branch of *L. pertusa*, pointing to cold-water coral framework forming a highly heterogeneous microbial environment. Microbial diversity at the OTU level was largest in near-bottom water and decreased subsequently in sediment, skeleton, mucus and overlaying water, which is partly in agreement with previous studies on cold-water corals from the Logachev mound Province and elsewhere that however did not take into account archaea and extensive sequencing (Hansson et al., 2009; Schöttner et al., 2009). Our study is the first that found archaea to contribute significantly to the diversity and distinction between microbial communities associated with *L. pertusa* (cf. Yakimov et al., 2006; Kellog et al., 2009). Possibly the enhanced microbial diversity of near-bottom water also reflects the enhanced biodiversity of metazoans living on the coral framework (Bongiorni et al., 2010). The distinction between the microbial assemblages associated with *L. pertusa* and its surrounding environment suggest possible mediation by the coral host as has also been suggested in earlier studies (Schöttner et al., 2012).

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

The variations between the different biotopes and within the biotopes that were sampled during this study, emphasize that increasing insight in the role of microbes in cold-water coral ecosystems requires both improved taxonomic resolution and actual knowledge of local biotopes, hydrography and chemical oceanography. Although our study of this single carbonate mound is among few that integrate information on hydrography with microbiology, it has for practical and logistic reasons by no means been exhaustive, and numerous pathways of future research are still open. These include further exploration of the diversity of microbial communities associated with living coral tissue, and the potential reliance of cold-water corals on their microbial associates for chemically-produced energy (Ainsworth et al., 2010; Dinsdale and Rohwer, 2011; Rohwer and Kelley, 2004). 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

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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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Table 1. List of box-core sampling stations.

Year	Station	Latitude	Longitude	Depth (m)	Framework height (cm)	Biotope
2012	15	55°29.45′ N	15°48.41′ W	528	> 30	mucus Skeleton uneroded
	24	55°29.77′ N	15°48.05′ W	549	0–10	w_bc
	25	55°29.57′ N	15°47.81′ W	568	> 30	mucus Skeleton uneroded
	46	55°29.45′ N	15°47.64′ W	745	10–30	w_bc
	72	55°29.51′ N	15°48.40′ W	562	0–10	w_bc
2013	8	55°29.45′ N	15°47.64′ W	647	> 30	sediment
	9	55°29.77′ N	15°48.03′ W	547	0–10	w_bc
						sediment Skeleton uneroded Skeleton eroded
	11	55°29.50′ N	15°48.39′ W	564	10–30	w_bc sediment
	12	55°29.26′ N	15°48.45′ W	635	> 30	sediment Skeleton uneroded Skeleton eroded

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

Year	Station	Latitude	Longitude	Depth (m)	Biotope	Temperature (°C)
2012	11	55°28.92′ N	15°48.33′ W	400	400 m	9.7
				895	10 m ab	6.7
				907	5 m ab	6.6
	12	55°29.50′ N	15°48.50′ W	400	400 m	9.6
				553	10 m ab	9
				562	5 m ab	8.9
	33	55°29.57′ N	15°47.83′ W	390	400 m	10
				573	10 m ab	8.7
				578	5 m ab	8.6
	36	55°29.94′ N	15°48.29′ W	400	400 m	10
				596	5 m ab	8.7
2013	2	55°25.95′ N	15°43.83′ W	400	400 m	9.9
				1192	10 m ab	5.7
				1200	5 m ab	5.4
	10	55°29.76′ N	15°48.04′ W	400	400 m	9.8
				522	10 m ab	8.8
				530	5 m ab	8.5
	13	55°29.25′ N	15°48.44′ W	400	400 m	9.7
				709	10 m ab	9.1
				718	5 m ab	9.2
	15	55°29.50′ N	15°48.39′ W	400	400 m	9.8
				550	10 m ab	9
				555	5 m ab	8.9

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

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

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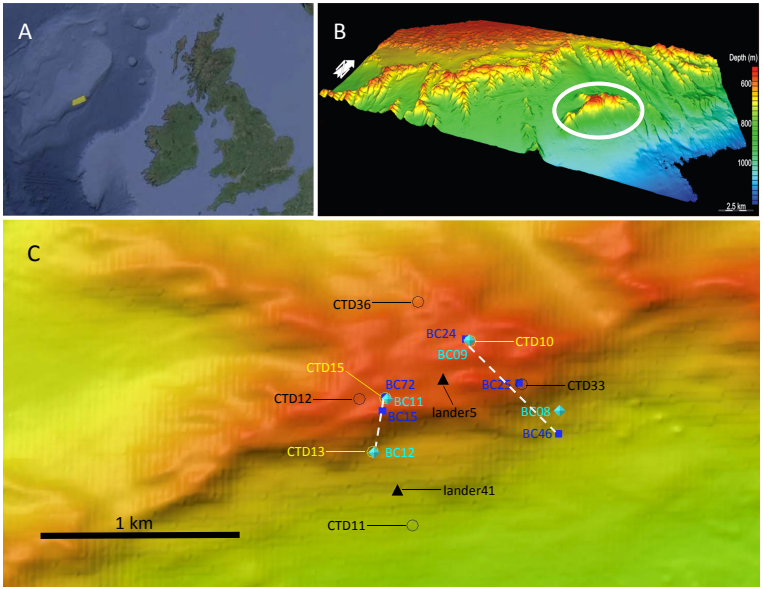

Figure 1. (a) Location of Logachev Mound Province (yellow polygon). **(b)** Multibeam map of Logachev Mounds with Haas Mound encircled. **(c)** Detail of Haas Mound with lander, box-core and CTD stations arranged along two transects (dotted white lines). Light blue symbols represent the 2012 box-core samples and dark blue symbols those of 2013. The position of the 2012 CTD casts is marked by black circles and those of 2013 by yellow circles. Note CTD02 is not on the map and lies 8 km SE of CTD10.

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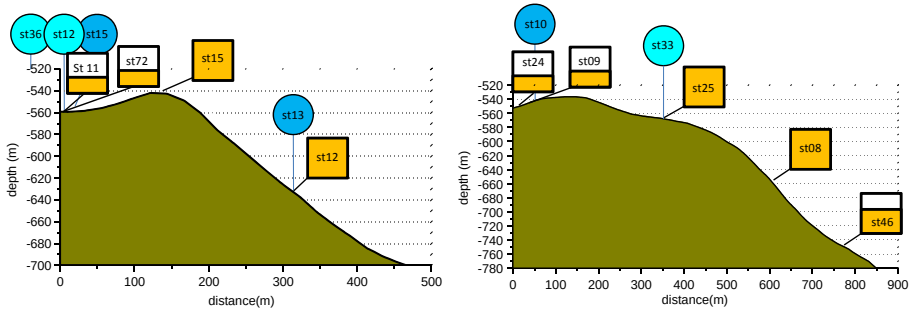

Figure 2. Bathymetric profiles of the two transects across the SE slope of Haas Mound (see Fig. 1). The position of the box-cores (squares) and some of the CTD casts (circles) is indicated. The yellow color filling of the squares represents the approximate percentage coral cover. Note that scales differ.

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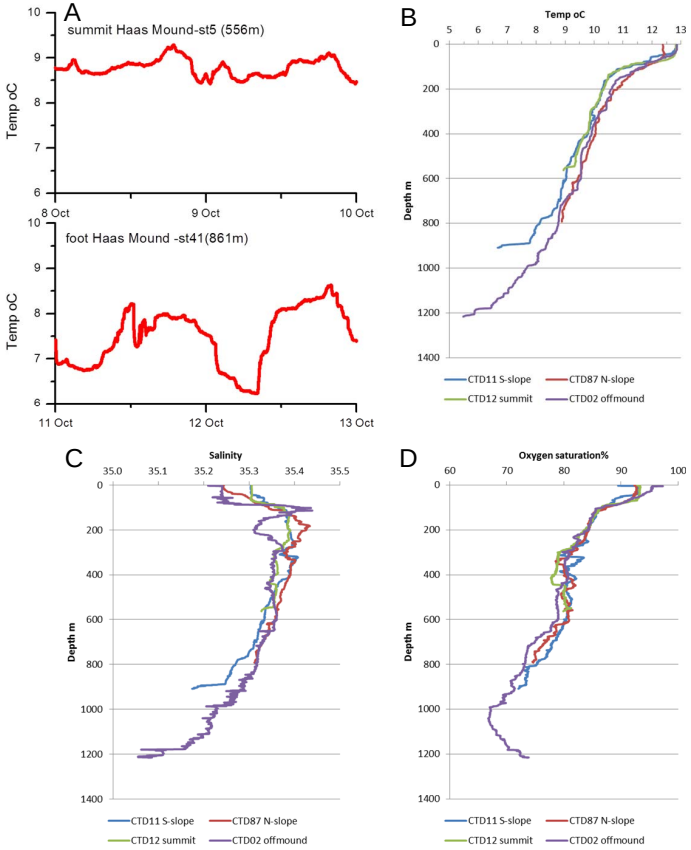

Figure 3. (a) Temperature recorded in situ at the summit and foot of Haas Mound by a current meter on a benthic lander. **(b–d)** Salinity, Temperature (°C), and Oxygen (% saturation), respectively, as recorded with the CTD on the slopes and summit of Haas Mound in 2012.

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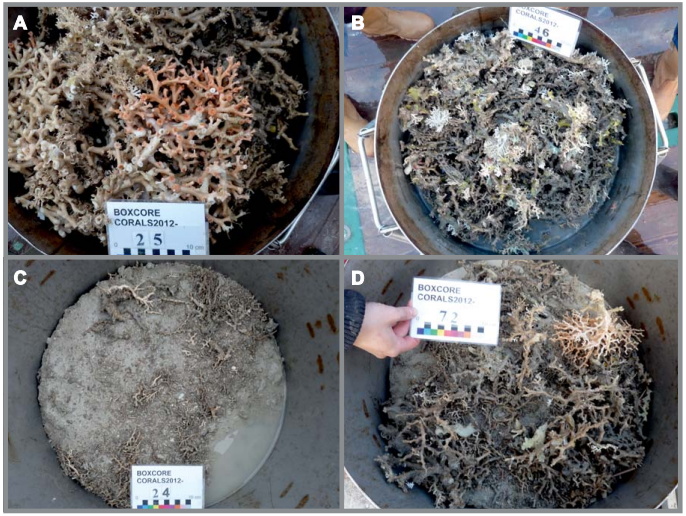

Figure 4. Photographs of box-cores taken at the SE slope **(a, b)** and summit **(c, d)** of Haas mound. A clear difference in the amount and height of coral framework was observed.

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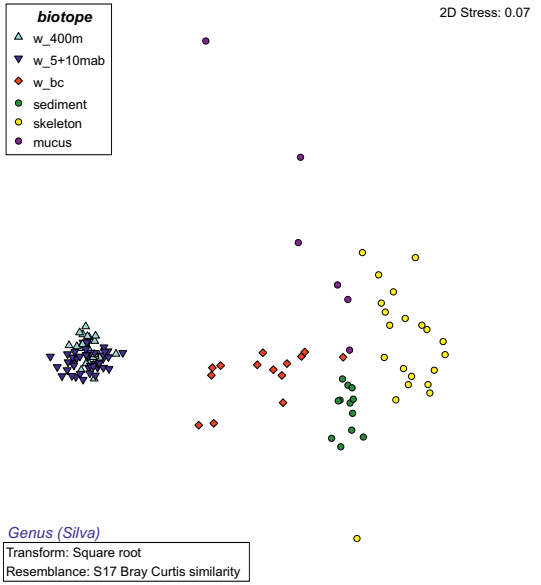

Figure 5. Microbial community on genus level of 125 samples shows clustering according to biotope: overlaying water (w_400 and w_5 + 10 m ab), near-bottom water (w_bc), sediment, (*L. pertusa*) skeleton and mucus. MDS plot on class level shows similar pattern (S.I. Fig. 1).

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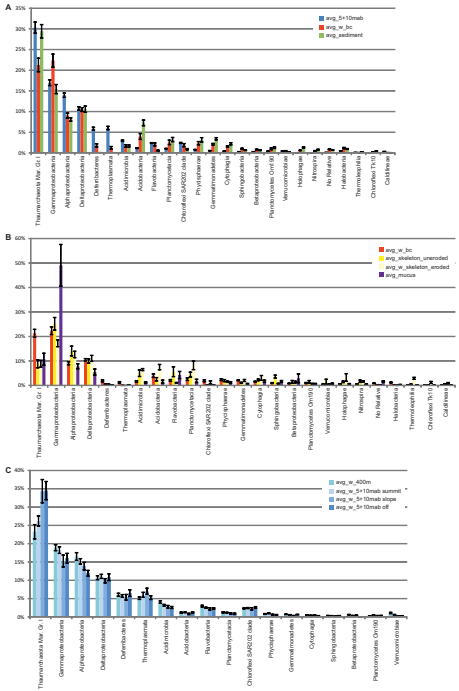

Figure 6. Most abundant (> 1% of total community) classes of microbes in different biotopes sampled at Haas mound. **(a)** Near-bottom water (w_bc) compared to sediment and water at 5+10 m ab. **(b)** w_bc compared to *L. pertusa* skeleton and mucus. **(c)** Overlaying water sampled at 400 m and 5 + 10 m ab. The latter category shows differences related to sample location: on mound summit, mound slope or off-mound.

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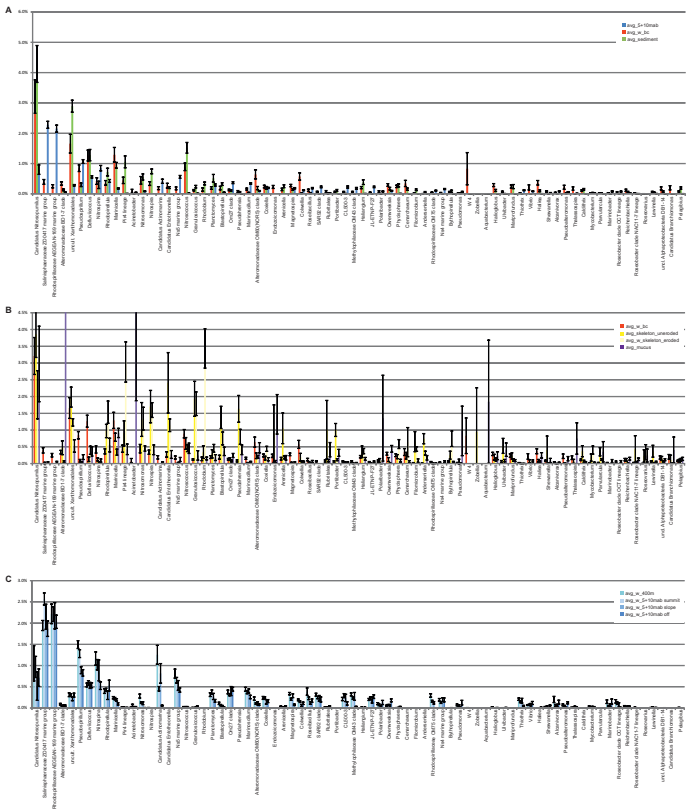

Figure 7. Most abundant genera of microbes in different biotopes sampled at Haas mound. **(a)** Near-bottom water (w_bc) compared to sediment and overlaying water at 5 + 10 m ab. **(b)** w_bc compared to *L. pertusa* skeleton and mucus. **(c)** Overlaying water sampled at 400 m and 5 + 10 m ab. The latter category shows differences related to sample location: on mound summit, mound slope and off-mound.

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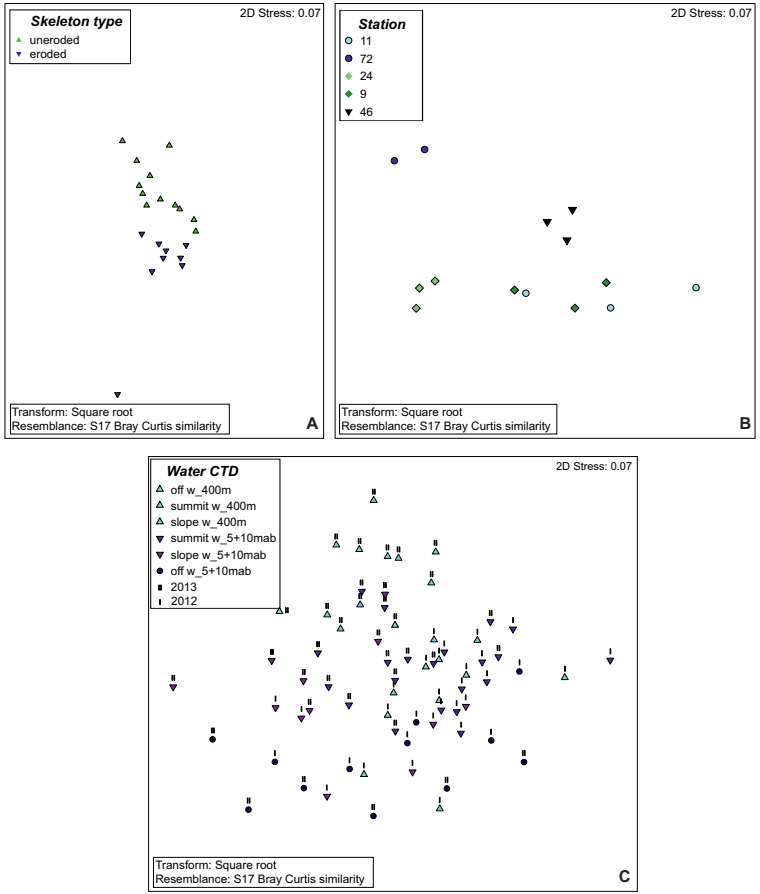

Figure 8. Zooms of microbial community composition on genus level. **(a)** Skeleton of *L. pertusa*. **(b)** Near-bottom water (w_bc). Numbers are station numbers. **(c)** Overlaying water sampled at 400 m and at 5 + 10 m ab along the slope of Haas Mound and at off mound stations.

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