

Kiel, 31st of March 2020

Dear Tina Treude (editor) and reviewers,

please find below our revised manuscript (marked-up version).

At the end of the document we have added again our point-by-point response to the reviews (same as already uploaded to the discussion forum), including a listing of all relevant changes made in the manuscript.

Thank you for your consideration.

Be safe,

Kathrin Busch on behalf of the authors

On giant shoulders: How a seamount affects the microbial community composition of seawater and sponges

Kathrin Busch¹, Ulrike Hanz², Furu Mienis², Benjamin Müller³, Andre Franke⁴, Emyr Martyn Roberts⁵, Hans Tore Rapp^{5†}, Ute Hentschel^{1,6}

5 ¹GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

²NIOZ Royal Netherlands Institute for Sea Research and Utrecht University, 1790 AB Den Burg, Texel, The Netherlands

³University of Amsterdam, Science Park 904, P.O. Box 94248, Amsterdam, The Netherlands

⁴Institute of Clinical Molecular Biology (IKMB), Rosalind-Franklin-Straße 12, 24105 Kiel, Germany

10 ⁵University of Bergen, Department of Biological Sciences and K.G. Jebsen Centre for Deep-sea Research, P.O. Box 7803, 5020 Bergen, Norway

⁶Christian-Albrechts University of Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

[† deceased March 7, 2020](#)

Correspondence to: Ute Hentschel (uhentschel@geomar.de)

15 **Abstract.** Seamounts represent ideal systems to study the influence and interdependency of environmental gradients at a single geographic location. These topographic features represent a prominent habitat for various forms of life, including microbiota and macrobiota, spanning benthic as well as pelagic organisms. While it is known that seamounts are globally abundant structures, it still remains unclear how and to which ~~extend~~~~extent~~ the complexity of the seafloor is intertwined with the local oceanographic mosaic, biogeochemistry and microbiology of a seamount ecosystem. Along these

20 lines, the present study aimed to explore whether and to ~~which~~~~what~~ ~~extend~~~~extent~~ seamounts can have an imprint on the microbial community composition of seawater and of sessile benthic invertebrates, sponges. For our high-resolution sampling approach of microbial diversity (16S rRNA gene ~~Amplicon~~~~amplicon~~ sequencing) along with measurements of inorganic nutrients and other biogeochemical parameters, we focused on the Schulz Bank seamount ecosystem, a sponge ground ecosystem which is located on the Arctic Mid-Ocean Ridge. Seawater samples were collected at two sampling depths

25 (mid-water: MW, and near-bed water: BW) from a total of 19 sampling sites. With a clustering approach we defined microbial micro-habitats within the pelagic realm at Schulz Bank, which were mapped onto the seamount's topography, and related to various environmental parameters (such as suspended particulate matter (SPM), dissolved inorganic carbon (DIC), silicate (SiO₄⁻), phosphate (PO₄³⁻), ammonia (NH₄⁺), nitrate (NO₃²⁻), nitrite (NO₂⁻), depth, and dissolved oxygen (O₂)). The results of our study reveal a 'seamount effect' (sensu stricto) on the microbial mid-water pelagic community ~~up to~~

30 ~~approximately at least~~ 200 m above the seafloor. Further, we observed a strong spatial heterogeneity in the pelagic microbial landscape across the seamount, with planktonic microbial communities reflecting oscillatory and circulatory water movements, as well as processes of benthic-pelagic coupling. Depth, NO₃²⁻, SiO₄⁻, and O₂ concentrations differed significantly between the determined pelagic microbial clusters close to the seafloor (BW), suggesting that these parameters

were presumably linked to changes in microbial community structures. Secondly, we assessed the associated microbial community compositions of three sponge species along a depth gradient of the seamount. While sponge-associated microbial communities were found to be mainly species-specific, we also detected significant intra-specific differences between individuals, depending on the pelagic near-bed cluster they originated from. The variable microbial phyla (i.e. phyla which showed significant differences across varying depth, NO_3^{2-} , SiO_4^- , O_2 concentrations and different from local seawater communities) were distinct for every sponge-species when considering average abundances per species. Variable microbial phyla included representatives of both; those taxa traditionally counted to the variable community fraction, as well as taxa counted traditionally to the core community fraction. Microbial co-occurrence patterns for the three examined sponge species *Geodia hentscheli*, ~~(demosponge, HMA)~~, *Lissodendoryx complicata* ~~(demosponge, most likely LMA)~~, and *Schaudinna rosea* ~~(Hexactinellida, most likely LMA)~~ were distinct from each other. Over all, this study shows that topographic structures such as the Schulz Bank seamount can have an imprint ('seamount effect' sensu lato) on both; the microbial community composition of seawater and of sessile benthic invertebrates such as sponges by an interplay between the geology, physical oceanography, biogeochemistry and microbiology of seamounts.

1 Introduction

Seamounts and mid-ocean ridges are prominent geologic features that add to the complexity of the seafloor. In the traditional sense, seamounts are defined as isolated submarine volcanic features with a minimum height of 1.000 m from base to summit (Menard, 1964; Wessel et al., 2010). However, geologic features with a height of 50-100 m may also be considered as seamounts (Staudigel et al., 2010, Smith and Cann, 1992; Wessel et al., 2010). There may be up to 100.000 to > 25 million seamounts present in the oceans (IUCN, 2013), although the error rate associated with these estimations is ~~very~~ high (IUCN, 2013). Despite the lack of accurate numbers, there is no doubt that with an-estimated 10 million km² coverage, the area occupied by these habitats is globally significant. Elevated topographic features in the open ocean are often hotspots of biological diversity and productivity (IUCN, 2013; Morato et al., 2010). It appears that interaction of the topography with the hydrography creates a combination of amplified tidal flow, increased current speed, and the formation of internal waves, which strongly enhances vertical mixing around seamounts (Lavelle and Mohn, 2010; Van Haren et al., 2017; Roberts et al., 2018). Consequential upwelling of nutrient-rich deep waters stimulates primary productivity in this layer of enhanced mixing (IUCN, 2013). In addition to vertical mixing processes, also horizontal fluxes of organic matter may be affected by the presence of seamounts, as they may promote enclosed or semi-enclosed oceanographic circulation patterns, like Taylor caps or columns [(Chapman and Haidvogel, 1992; Roberts et al., 2018) and references therein], leading to a retention of organic and inorganic matter.

Above mentioned processes make seamounts important habitats for pelagic as well as benthic species (Morato et al., 2010; Rogers, 2018) due to beneficial prevailing conditions. Particularly areas with strong water flows (evoked by interactions of currents and tides with elevated topography), in combination with a steep and irregular hard substrate,

represent suitable habitats for benthic suspension feeders, which indeed densely populate most seamounts (Genin et al., 1986; IUCN, 2013). Sponges (Porifera (Grant, 1836)) often dominate these suspension feeder communities and are increasingly recognised as key components of shallow and deep marine ecosystems (DeGoeij et al., 2017; Maldonado et al., 2016). Due to their high filtering capacity and association with diverse microbial communities, sponges are considered to substantially influence the carbon, nitrogen, and silicate cycling in marine systems (Taylor et al., 2007; Maldonado et al., 2012; De Goeij et al., 2013; Rix et al., 2016a; Maldonado et al., 2019) and to contribute to benthopelagic coupling by actively removing particulate organic matter (POM) from the water column (Pile et al., 1997; Reiswig, 1971; Ribes et al., 1999). In addition to their influence on particulate organic matter pools, many sponges have been identified to primarily feed on dissolved organic matter (DOM) (De Goeij et al., 2008; Mueller et al., 2014; Hoer et al., 2018; Gantt et al., 2019). Energy and nutrients stored in this DOM are then transferred into particulate detritus, which fuels benthic food webs (De Goeij et al., 2013; Rix et al., 2016b).

Intimate sponge-microbe associations ~~were~~have been observed throughout diverse habitats, reaching from coastal shallow sites in tropical and temperate regions to the deep-sea and polar seas (Helber et al., 2019; Kennedy et al., 2014; Moitinho-Silva et al., 2014; Naim et al., 2014; Schmitt et al., 2012; Steinert et al., 2019; Thomas et al., 2016). According to their microbiome, sponges can be classified to either feature high microbial abundance (HMA) or low microbial abundance (LMA) (Hentschel et al., 2003; Moitinho-Silva et al., 2017; Weisz et al., 2008). The dichotomy between HMA and LMA sponges is considered a main driver of the microbial community structure associated with shallow water sponges (Moitinho-Silva et al., 2017). In comparison to shallow waters, comparably few studies have been conducted on the microbiology of deep-sea sponges (Borchert et al., 2017; Jackson et al., 2013; Kennedy et al., 2014; Reveillaud et al., 2014; Tian et al., 2016). However, for example for deep-sea sponges of the genus *Geodia* (*G. barretti*, *G. macandrewii*, *G. phlegraei*, *G. atlantica*), similar microbial phyla have been observed as in HMA shallow water sponges, such as Acidobacteria, Poribacteria and Chloroflexi (Luter et al., 2017; Radax et al., 2012; Schöttner et al., 2013). In addition to the HMA-LMA dichotomy, an important factor in structuring the microbiomes of shallow water sponges is host taxonomy, which is manifested in ubiquitous species-specific sponge microbiomes (Easson and Thacker, 2014; Steinert et al., 2017; Thomas et al., 2016). Systematic analyses of the influence of biogeochemical parameters (particularly dissolved inorganic substances) on sponge-associated microbial diversity and interactivity ~~is~~are still lacking, particularly in deep-sea sponges. Seamounts provide an ideal study system in this regard, as they offer the potential of examining steep environmental gradients over small spatial scales. Sponge ground ecosystems are areas harbouring high densities of structure-forming sponge individuals. The Arctic Schulz Bank seamount has been observed to host a rich and diverse community of sponges (Roberts et al., 2018; Meyer et al., 2019) and may be considered a sponge ground ecosystem harbouring a reservoir of yet unexamined microbial biodiversity.

The present study aimed to characterise the microbial community composition of seawater surrounding the Schulz Bank seamount ecosystem, located on the Arctic Mid-Ocean Ridge. Seawater samples were collected at two sampling depths from a total of 19 sampling sites and the corresponding microbiome data were mapped onto the topography of the Schulz

100 Bank seamount ecosystem. Secondly, we assessed the associated microbial community compositions of three sponge species along a depth gradient of the seamount. Diversity metrics, as well as changes in the abundance of individual microbial taxa were ~~cor~~related with a set of biogeochemical parameters. This study explores whether topographic structures such as the Schulz Bank seamount, can have an imprint on both the microbial community composition of seawater and of sessile benthic invertebrates, sponges.

105 2 Methods

2.1 Description of the Schulz Bank seamount

Schulz Bank is located on the Arctic Mid-Ocean Ridge (73.8 °N; 7.5 °E) between the Greenland and Norwegian Seas ([Supplementary Material S1](#)). It is exposed to three main water masses: (i) the Norwegian Deep Water (NwDW) that is present at the base and flanks of the seamount, (ii) the intermediate water mass (NwArIW), which is most likely Norwegian Arctic Intermediate Water and occurs at the summit and shallower areas, and (iii) the warmer surface water mass (NwAtW) which is Norwegian Atlantic Water. ~~Notably near-bed water masses at Schulz Bank's summit have unusually low temperatures of around 0 to -1 °C (Roberts et al., 2018). Notably near-bed water masses at Schulz Bank's summit have low temperatures of around 0 to -1 °C.~~ Estimations of the seamount's basal dimensions state ~~minimal-conservative~~ values of 10 x 4 km to 15 x 6 km (Roberts et al., 2018), ~~may however also be larger as Schulz Bank belongs to a ridge system.~~ The summit of the seamount is located at around 600 m below the water surface ~~and the base depth is at more than 2500 m below the water surface. In a two-dimensional view, Schulz Bank has a broadly elliptical shape (Roberts et al., 2018).~~ Bathymetry data presented in this study were derived from the Bathymetry Data Portal of the European Marine Observation and Data Network (EMODnet) and spatial analyses were performed in QGIS (version 3.4.4) as well as ArcGIS (version 10.6).

2.2 Sampling procedures

120 Three cruises were undertaken onboard RV *G.O. Sars* (campaign names 'GS2016109A', 'GS2017110', and 'GS2018108') during northern hemisphere's summer in the years 2016-2018. Seawater samples were collected with a rosette water sampler equipped with 12 x 10 L Niskin bottles combined with a CTD sensor system (SBE-9, Sea-Bird Electronics Inc., Washington, USA). In total, 19 CTD stations were covered, carried out along transects aligned with the seamounts' minor and major axes, and also with the ~~74-73.8~~ °N line of latitude. At each of the 19 stations, seawater samples for microbial analyses and 125 biogeochemical parameters were collected at two water depths during the CTD upcast ([Fig. 2C and PANGAEA for metadata](#)): (i) 400 m below the seawater surface (mid-water) and (ii) correspondingly, at 10 m above the seafloor (near-bed water). Naturally, the near-bed depths varied along with seamount topography, ~~which-and~~ ranged from 575 to 2966 m. ~~In particular, the near-bed water samples were collected at significantly different depths (ANOVA, p=0.01). Depth was lowest at the seamount's summit (average = 575 m), intermediate at the flanks (average ± SE = 919 ± 106 m and 922 ± 142 m, respectively), and greatest in the vicinity of the Schulz Bank seamount (average ± SE = 1836 ± 376 m).~~

—Sponges were sampled between 580 and 2184 m water depth, along the CTD transects, by a remotely operated vehicle (ROV Ægir 6000, University of Bergen). A total of 36 sponge individuals representing the most abundant species were ~~collected~~ randomly selected from a larger collection effort. This subset included, including 16 *Geodia hentscheli* (Cárdenas et al., 2010) (Demospongiae), 8 *Lissodendoryx complicata* (Hansen, 1885) (Demospongiae), and 12 *Schaudinna rosea* (Fristedt, 1887) (Hexactinellida). The sponges were taxonomically identified by visual inspection on-board the ship. In addition, whole specimens and additional sponge samples were fixed in 99 % EtOH for deposition in the collections of the University of Bergen.

2.3 Biogeochemical analyses and measurements of environmental parameters

The following nine environmental parameters were analysed: depth, suspended particulate matter (SPM), dissolved inorganic carbon (DIC), silicate (Si), phosphate (PO_4^{3-}), ammonia (NH_4^+), nitrate (NO_3^{2-}), nitrite (NO_2^-), and dissolved oxygen (O_2). Depth and dissolved O_2 data were recorded during in situ water column profiling. Depth (pressure) was recorded with the CTD sensor system mentioned above. O_2 concentrations were derived from a dissolved oxygen sensor (SBE-43, Sea-Bird Electronics Inc., Washington, USA) that was attached to the rosette water sampler. For the analysis of suspended particulate matter (SPM), 2 x 10 L of water were filtered over pre-weighed combusted GFF filters, which were rinsed with demineralised water to remove salts (47 mm Whatman™ GF/F filters pre-combusted at 450 °C, stored at - 20 °C). Filters were freeze-dried and weighed before further analysis. For the analysis of inorganic nutrients (ammonia (NH_4^+), phosphate (PO_4^{3-}), nitrate (NO_3^{2-}), nitrite (NO_2^-), and silicate (Si)), seawater samples were filtered over 0.2 µm filters. Water samples for NH_4^+ , PO_4^{3-} , NO_x analysis were stored at - 20 °C and for Si analyses at 4 °C. Nutrients were measured with a QuAAtro Gas Segmented Continuous Flow Analyzer (Seal Analytical, Norderstedt, Germany). Measurements were made simultaneously on four channels for PO_4^{3-} (Murphy and Riley, 1962), NH_4^+ (Helder and de Vries, 1979) and NO_3^{2-} combined with NO_2^- (Grasshoff et al., 2009) and separately for Si (Strickland and Parsons, 1972). A freshly diluted mixed nutrient standard containing Si, PO_4^{3-} , and NO_3^{2-} was added to each run. The cocktail served as a guide to monitor the performance of the standards. All measurements were calibrated with standards diluted in low nutrient seawater (LNSW). For the analysis of dissolved inorganic carbon (DIC), seawater samples were transferred into a glass vial containing 15 µL HgCl_2 (mercury chloride) and analysed on a TechniconTraacs800 auto-analyzer (Technicon Instruments Corporation, Tarrytown, USA) following the methodology of Stoll et al. (2001). Analyses of variance (ANOVAs) were performed to test for statistical differences in the biogeochemical and physical parameters between the determined microbial near-bed water clusters (see below). As numbers of samples per mid-water cluster and per near-bed water cluster were unequal, we calculated Type III sums of squares for ANOVAs (unbalanced ANOVAs). We further calculated Spearman's rank correlations between depth and those biogeochemical parameters which turned out to differ significantly across the determined near-bed water clusters in the ANOVA analyses (see below).

2.4 Amplicon sequencing

Seawater samples were collected in triplicates from different Niskin bottles, yielding a total of 114 samples from all stations. Two litres of seawater sample were filtered onto polyvinylidene fluoride (PVDF) filter membranes (Merck Millipore) with a pore size of 0.22 μm and a diameter of 47 mm and stored at - 80 °C. ~~For sponge collection, Sponge samples were also collected at least in quadruplicates for each sampling region at Schulz Bank. Four~~ cubes of approximately 1 cm^3 were cut from the mesohyl with a scalpel, rinsed (sterile seawater), flash-frozen in liquid nitrogen and stored at - 80 °C. DNA was extracted from half a seawater filter or ~ 0.25 g of sponge tissue by using the DNeasy Power Soil Kit (Qiagen, Venlo, The Netherlands). The quality of the DNA extraction was assessed based on the 260/280 ratio using a NanoDrop spectrophotometer as well as by polymerase chain reaction with universal 16S primers and subsequent gel electrophoresis. The V3-V4 variable regions of the 16S rRNA gene were then amplified in a one-step PCR using the primer pair 341F-806R (dual-barcoding approach (Kozich et al., 2013); primer sequences: 5'-CCTACGGGAGGCAGCAG-3' & 5'-GGACTACHVGGGTWTCTAAT-3'). After verification of the presence of PCR-products by gel electrophoresis, normalisation (SequalPrep Normalisation Plate Kit; ThermoFisher Scientific, Waltham, USA) and equimolar pooling was performed. Sequencing was conducted on the MiSeq platform (MiSeqFGx; Illumina, San Diego, USA) with v3 chemistry. The settings for demultiplexing were 0 mismatches in the barcode sequences.

2.5 Bioinformatic analyses

For computation of microbial core-diversity metrics, sequences were processed within the QIIME2 environment (version 2018.11, (Bolyen et al., 2018)). Amplicon Sequence Variants (ASVs) were generated from forward reads (truncated to 270nt) with the DADA2 algorithm (Callahan et al., 2016). Phylogenetic trees were calculated based on resulting ASVs with the FastTree2 plugin. Representative ASVs were classified using the Silva 132 99 % OTUs 16S database (Quast et al., 2013) with the help of a primer-specific trained Naive Bayes taxonomic classifier. Alpha and beta diversity indices (e.g. Faith's Phylogenetic Diversity and weighted UniFrac distances, respectively) were calculated within QIIME2. To evaluate sample separation in ordination space, non-metric multidimensional scaling (NMDS) was performed on weighted UniFrac distances for seawater and sponge-associated microbiomes separately.

A machine learning approach was used to define microbial micro-habitats within the pelagic realm. Seawater microbiomes were clustered based on weighted UniFrac distances. The NbClust function was applied in R (version 3.0.2, (R Development Core Team, 2008)) to generate 30 indices to identify the best number of clusters based on the majority rule. A coordinate grid was set up as a basis for a georeferenced extrapolation of sampling points. Clustering regions were set up with the help of the k-Nearest-Neighbor-algorithm. The machine learning approach was fine-tuned in several ways: (i) the algorithm was trained in a way that in situ measured data points always belong to the cluster actually determined based on the sequencing data; (ii) a normalisation was applied with the help of a distance-weighted function meaning that closer data points have a higher weight; (iii) the probability of class membership was calculated and plotted as indication of confidence.

Permutational multivariate analyses of variance (PERMANOVAs) were performed with 999 permutations to determine whether microbiomes of selected clusters were statistically significantly different from each other. In detail, pair-wise tests across the determined clusters were conducted for the following samples separately: mid-water samples, near-bed water samples, *G. hentscheli*, *L. complicata*, and *S. rosea*. A significance level of $\alpha=0.05$ was applied for all statistical analyses in this study.

To evaluate co-occurrence patterns between microbial taxa across environmental gradients (i.e. determined near-bed water clusters), networks were constructed separately for every sponge species and seawater. Mean relative abundances of microbial phyla were calculated for all biological replicates of each sample type and for the corresponding near-bed water cluster. Microbial phyla, which showed significantly different enrichment between clusters, were determined and ranked using the Linear Discriminant Analysis Effect Size (LEfSe) algorithm (Segata et al., 2011). A correlation matrix was established for those taxa that differed significantly between clusters, to assess co-occurrences. In particular, the direction and strength of correlations were characterised for any significant phylum with all other significant taxa (as well as [the relations](#) with depth).

3 Results

3.1 Structure and composition of seawater microbial communities

A NMDS plot on weighted UniFrac distances separated the microbial communities of mid-water and near-bed water samples in ordination space with few exceptions (Fig. 1). Cluster analysis based on weighted UniFrac distances revealed two distinct clusters in the mid-water samples of which one (MW1) was located precisely above the summit of Schulz Bank, while the other (MW2) covered the wider seamount region and vicinity (Fig. 2A). Four distinct microbiome clusters were detected in the near-bed water samples (BW1-4). In terms of similarity, cluster BW1 was most distinct from all other clusters while clusters BW2 and BW3 were most similar to each other (Fig.2B). Moreover, BW1 cluster samples separated in ordination space in that they grouped with mid-water rather than near-bed water samples (i.e. consider the few black dots grouping together with the white dots in Fig. 1). Plotting the clusters on a spatial map revealed that near-bed water cluster BW1 was located near the summit of Schulz Bank seamount ([average depth = 575 m](#)), while clusters BW2 and BW3 covered its flanks ([average depth \$\pm\$ SE = 919 \$\pm\$ 106 m and 922 \$\pm\$ 142 m, respectively](#)), and cluster BW4 represented the vicinity close to the seamount ([average depth \$\pm\$ SE = 1836 \$\pm\$ 376 m](#)) (Fig.2 B). [Statistical testing of the individual depth data points contributing to a given cluster revealed a significant difference in the depth parameter between the clusters– \(ANOVA, \$p = 0.01\$, \$df = 3\$ \).](#)

Fig. 2C shows the bathymetry highlighting the contour lines of Schulz Bank seamount and its vicinity (reference West and East) as well as the 19 sampling stations. In addition to this representation, a 3D visualization of the microbiome clusters at and around Schulz Bank seamount was created (Fig. 3). Here, a digital elevation model of Schulz Bank seamount is depicted in combination with the overlaying water column structure and oceanographic context. Temperature profiles derived from whole water column sensing by CTD casts are plotted. Based on these profiles the vertical distributions of the surface water

(NwAtW), intermediate water (NwArIW), and Norwegian Deep Water (NwDW) were deduced in combination with the identified water masses as described in Roberts et al. (2018) (Fig. 3).

Microbial richness was overall slightly lower in the mid-water samples (mean Faith's Phylogenetic Diversity \pm standard error = 45.5 ± 0.8) than in the near-bed water samples (54.4 ± 0.9) (Supplementary Material S2A4). Near-bed water samples from the summit (BW1) ~~represented an exception to this pattern as they~~ displayed a slightly lower microbial richness than the other near-bed water samples (Supplementary Material S2B). The mid-water samples collected above Schulz summit showed also a slightly lower microbial richness than the other mid-water samples. Pairwise comparisons (PERMANOVA) revealed that the seawater microbial community clusters within the mid-water and near-bed water samples were significantly different from each other in terms of their microbial community composition (Supplementary Table S1). Furthermore, the pool of mid-water samples (MW1 - MW2) was significantly different from the pool of near-bed water samples (BW1 - BW4). Overall, the eight most dominant seawater microbial phyla, sorted in descending order of mean relative abundance, were: Proteobacteria (54 % of total community), Bacteroidetes (17 %), Verrucomicrobia (7 %), Marinimicrobia (SAR406 clade) (6 %), Actinobacteria (5 %), Chloroflexi (4 %), Acidobacteria (2 %), and Planctomycetes (1 %).

240 3.2 Seawater biogeochemistry at Schulz Bank seamount

When comparing the biogeochemical parameters of the mid-water samples clusters, only dissolved O₂ concentrations differed significantly (ANOVA, $p = 0.02$, $df = 1$) with slightly higher concentrations in MW1 (6.90 ± 0.04 mL L⁻¹) compared to MW2 (6.75 ± 0.03 mL L⁻¹) (Supplementary Table S2). All other tested biogeochemical parameters (SPM (2.22 ± 1.39 mg L⁻¹), DIC (2269.07 ± 26.63 μ mol L⁻¹), SiO₄⁻ (5.66 ± 0.06 μ mol L⁻¹), PO₄³⁻ (0.86 ± 0.01 μ mol L⁻¹), NH₄⁺ (0.11 ± 0.03 μ mol L⁻¹), NO₃⁻ (12.99 ± 0.08 μ mol L⁻¹), and NO₂⁻ (0.02 ± 0.01 μ mol L⁻¹) were not statistically different between MW1 and MW2. The values for mid-water samples are reported as average \pm standard error.

Of the eight biogeochemical parameters tested, the following three differed significantly between the near-bed water clusters. These were NO₃⁻ (ANOVA, $p = 0.0204$, $df = 3$), SiO₄⁻ (ANOVA, $p = 0.0203$, $df = 3$), and dissolved O₂ (ANOVA, $p = 0.01$, $df = 3$). Nitrate (range= 13.00-14.78 μ mol L⁻¹) and SiO₄⁻ (range = 6.00-10.65 μ mol L⁻¹) increased with depth, with lowest concentrations at the summit (BW1), intermediate concentrations at the flanks (BW2, BW3) and highest concentrations in the seamount vicinity (BW4) (Fig. 4). Dissolved oxygen (range= 6.48-6.99 mL L⁻¹) showed the reverse pattern in that its concentration was highest at the summit (BW1), intermediate at the flanks (BW2, BW3) and lowest in the seamount vicinity sites (BW4). Spearman's rank correlations calculated between depth and the three other significant parameters revealed indeed significant correlations in all cases (NO₃⁻ : $\rho = 0.77$, $p < 0.01$; SiO₄⁻ : $\rho = 0.85$, $p < 0.01$; dissolved O₂ : $\rho = -0.77$, $p < 0.01$) (Supplementary Material S3). The other biogeochemical parameters SPM (range= 0.49-1.87 mg L⁻¹), DIC (range= 2248.00-2265.67 μ mol L⁻¹), PO₄³⁻ (range= 0.90-0.97 μ mol L⁻¹), NH₄⁺ (range= 0.10-0.17 μ mol L⁻¹), and NO₂⁻ (range= 0-0.05 μ mol L⁻¹) were not significantly different between the near-bed water clusters. At the summit, no

pronounced differences in biogeochemical parameters were observed between the near-bed water (BW1) and mid-water samples (MW1) (Supplementary Table S2).

260 3.3 Structure and composition of sponge microbial communities

In order to analyze structure and microbial community composition of the sponges, we randomly selected at least four biological replicates per sponge species per BW cluster for statistical analysis. Overall, the three deep-sea sponge species *S. rosea*, *G. hentscheli*, and *L. complicata* showed host species-specific microbiomes, as indicated by a clear separation of their microbial communities in ordination space (Fig. 5). Sub-structuring based on near-bed water clusters in the non-metric multidimensional scaling plot as well as pairwise comparisons (PERMANOVA) revealed that the sponge microbial communities within each species differed significantly depending on the near-bed water clusters from which they were collected (Supplementary Table S1). The only exception was *S. rosea*, for which specimens from the flank (BW3) showed a microbial community composition that was intermediate between the summit (BW1) and the other flank cluster (BW2).

The dominant microbial phylum in *S. rosea* and *L. complicata* ~~were~~was Proteobacteria (Fig. 6A and Fig. 6B), whereas *G. hentscheli* microbiomes were dominated by Chloroflexi, Acidobacteria and Proteobacteria (Fig. 6C). Sponge microbiomes were more stable than seawater communities with less phyla exhibiting significant differences across the four near-bed water clusters or positively ~~cor~~relating with depth (Fig. 6). For the hexactinellid *S. rosea*, the relative abundances of five bacterial phyla (Acidobacteria, Chlamydiae, Kirimatiellaota, Planctomyces and Proteobacteria) were significantly different between individuals that were sampled from different near-bed water clusters. Out of these five phyla, the Acidobacteria, Chlamydiae, Kirimatiellaota, and Planctomyces were positively ~~cor~~related with depth while for the Proteobacteria neither a positive nor negative ~~cor~~relation with depth was discernable. Consequently, the Proteobacteria showed a negative ~~cor~~relation with the four other phyla in the network analysis. For the demosponge *L. complicata*, the relative abundances of the Bacteroidetes, Gemmatimonadetes, Nitrospinae, Planctomyces, Proteobacteria and Spirochaetes were significantly different between sponge individuals that were sampled from the different near-bed water clusters. For this sponge species, samples were only available from near-bed water clusters 1 and 2. Of the six phyla, the Planctomyces and Proteobacteria were positively ~~cor~~related with depth, while the other four were negatively ~~cor~~related with depth, which is also reflected in the network analysis. For the demosponge *G. hentscheli*, the relative abundances of eight phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Dadabacteria, Entotheonellota, PAUC34f and Schekmanbacteria) were significantly different between sponge individuals sampled from the near-bed water clusters BW1-BW4. Of those, Chloroflexi and Schekmanbacteria were positively ~~cor~~related with depth, while the others showed variable patterns over depth. The network analysis showed both positive and negative correlations between taxa for those increasing with depth as well as those displaying a variable response.

When analysing host-associated microbiomes, ambient seawater microbiomes are valuable references for comparison. In this study, a total of 21 microbial clades were identified in ambient seawater, whose relative abundances varied significantly between the four near-bed water clusters. A total of nine taxa showed a positive ~~cor~~relation with depth,

one (Dadabacteria) showed a negative ~~cor~~relation with depth, and the remaining 11 taxa showed a variable response to depth. For seawater more phyla varied between near-bed water clusters than for the sponge samples. Overall, more microbial taxa showed significant positive ~~cor~~relations with depth, NO₃⁻, SiO₄⁻ and negative ~~cor~~relations with O₂ than vice versa. The microbial taxa showing a significant difference in relative abundance between the near-bed water clusters (as determined by LefSe) were different between sponges and seawater also between sponge species. Further, significant differences in relative abundances were observed for both abundant and less abundant sponge symbiont lineages. The microbial taxa showing a significant variability between near bed water clusters were different ones between sponges and seawater, and also between the sponge species. Further, the pattern applied to both abundant and less abundant bacterial lineages.

4 Discussion

300 Research records about seamount microbiology are sparse and comparably few studies have been conducted on deep-sea sponge microbiomes in general (Borchert et al., 2017; Jackson et al., 2013; Kennedy et al., 2014; Reveillaud et al., 2014). Our main aim was to assess whether and via which potential mechanisms a seamount can affect the community structure of pelagic and benthos (sponge)-associated microbial communities, using the Schulz Bank seamount as an exemplary field site. A total of 19 CTD sampling stations, each with two sampling depths, on and around Schulz Bank were analysed towards this
305 goal, and combined with sponge-associated microbial data gained during additional ROV dives.

4.1 A seamount imprint on seawater microbial communities

In this study we observed a pronounced similarity between the microbial community composition of the mid-water cluster located precisely above Schulz Bank's summit (MW1) and the microbial community composition of the near-bed water cluster at the summit (BW1). This is evident in Fig. 1, where few black dots representing the BW1 cluster group with mid-
310 water samples rather than near-bed water samples. In addition, the microbial community in the mid-water cluster above Schulz Bank's summit (MW1) was distinct from the community in the mid-water cluster covering the wider seamount region and vicinity (MW2), despite similar prevailing biogeochemical conditions in both mid-water clusters (MW1 vs MW2; exception = significant difference in O₂ concentrations between both clusters). From these two observations we conclude that the presence of a seamount can have an imprint on the microbial community structure in the overlying water column
315 ('seamount effect' sensu stricto). In particular we suspect that topography-induced vertical mixing processes occur at Schulz Bank seamount, which reallocate microbial communities within the water column and in turn influence the pelagic microbial diversity as far as approximately at least 200 m above the seamount's summit. In support of these interpretations, oscillating currents relating to the barotropic and baroclinic (internal) tide have been reported previously at the summit of the Schulz Bank seamount (Roberts et al., 2018) and other seamounts (Van Haren et al., 2017).

320 In addition to tide-induced vertical hydrodynamic processes, horizontal flow patterns can also help to explain the presence of seamount-specific microbial communities. Roberts et al. (2018) calculated that a Taylor cap or Taylor column

may be (temporarily) present at Schulz Bank. This oceanographic phenomenon describes an isolated anti-cyclonic flow circulation pattern over a seamount in the Northern hemisphere and hence may promote temporary spatial isolation of a seamount ecosystem from adjacent waters. Our conceptual schematic overview of ~~such events~~ hydrodynamics at Schulz Bank seamount is shown in Fig. 3 (dashed lines). This Figure recapitulates that similarities and dissimilarities in microbial signatures of seawater in this study (cluster dendrogram Fig.3) were ~~shown to be~~ consistent with the oscillatory water movements (i.e., due to internal tide-induced mixing) and possible circulatory flows (Taylor column) as predicted by Roberts et al. (2018).

Microbial richness was overall slightly lower at the summit of the seamount (BW1) than at the deeper locations (i.e. BW2-BW4). A similar trend was observed for the mid-water samples, where microbial richness was slightly lower for the microbial community above Schulz's summit in comparison to samples in its vicinity (MW1 vs MW2). On a macroscopic level, Morato et al. (2010) and others have described seamounts as hotspots of pelagic biodiversity, much less is however known about microbial diversity at seamounts. Our results of a lower microbial richness above the seamount summit might seem contradictory at first. However, Schulz Bank is a recognized sponge ground ecosystem, with a peak in sponge density and diversity at the seamount summit (Roberts et al., 2018; Meyer et al., 2019). Sponges are very efficient suspension feeders and are known for removing large amounts of particulate organic matter including prokaryotes and small eukaryotes from the water column (Leys et al., 2018). Benthic-pelagic coupling mediated by selective feeding of sponges on seawater microorganisms (McMurray et al., 2016; Van Oevelen et al., 2018) in combination with the discussed hydrodynamic patterns (vertical mixing) might explain the slightly reduced microbial richness of the water body residing directly above a sponge ground (BW1 and MW1). ~~S. The circumstance that~~ sponge density and community composition changes along the topography of Schulz Bank seamount in that density is highest at the summit (Meyer et al., 2019; Roberts et al., 2018). This natural variation, can further ~~aid to~~ explain the observed differences in microbial community composition between the other near-bed water samples (BW2-4). For these samples, we observed distinct pelagic microbial communities at a finer resolution than can be explained by the pure water masses distribution (consider depth of intermediate (NwArIW) and deep water (NwDW) layers in Fig. 3). Particularly the near-bed clusters BW2 and BW3, originated from a similar depth range and both were located at the seamount's flanks. Besides ecologically rooted explanations (i.e. ~~varying presence of dense ecosystems~~ variable presence of benthic organisms that influence biogeochemical cycles), also hydrodynamic processes (i.e. local flow direction linked to small scale topography and/or spatial orientation of the seamount's flanks) can explain the observation of distinct microbial community compositions within the near-bed water. We hence conclude that besides patterns related to water masses, we observe a much higher spatial heterogeneity of pelagic microbial communities than previously recognized. We call this kind of imprint on the pelagic microbial community composition, which is probably based on the topography combined with benthic-pelagic coupling processes and hydrodynamics, a 'seamount effect sensu lato'. These observations suggest that the presence of a seamount can have profound impacts on the distribution of microbial landscapes in the open ocean.

355 Seamounts are recognized as unique habitats in terms of ecosystem dynamics (Genin and Boehlert, 1985) and macroecology
(Morato et al., 2010). The present study reveals that seamounts also have a unique microbial signature that extends hundreds
of meters up into the overlying water column. In addition, we detected three distinct microbial clusters in seawater samples
taken near the seabed directly above Schulz Bank which were distinctly different from those of seawater collected in the
vicinity of the seamount (near-bed water clusters BW1-3 vs BW4). NO_3^- , SiO_4^- , and dissolved O_2 concentrations differed
360 significantly between the four near-bed water clusters. The observation that the seamount is intersecting with different
biogeochemical properties and microbial communities, is particularly interesting in regard to benthic organisms. In these
lines, the Schulz Bank seamount provides a platform for sponges and their associated microbial communities to respond to
topography-enabled environmental gradients (also a ‘seamount effect sensu lato’).

4.2 A seamount imprint on sponge-associated microbial communities

365 The investigated sponge species *S. rosea*, *G. hentscheli* and *L. complicata* were selected for this study as they represent key
taxa of the sponge community at Schulz Bank. Microbiomes of these three species clustered clearly apart from each other in
ordination space, indicating a dominant host species-effect on the associated microbial community structure. *S. rosea* and *L.*
complicata showed characteristic microbial signatures of LMA sponges (as defined in Moitinho-Silva et al., 2017) that are
being dominated by Proteobacteria. On the contrary, *G. hentscheli* displayed a microbial signature characteristic of HMA
370 sponges with dominant clades such as Chloroflexi and Acidobacteria, which is consistent with previous reports on sponges
of the genus *Geodia* (Luter et al., 2017; Radax et al., 2012; Schöttner et al., 2013).

When analysing each sponge species separately, sponge specimen microbiomes differed significantly between each
other and depended on the near-bed water clusters to which they belonged. This finding suggests that an environmental
signature is also detectable in sponge-associated microbial communities (seamount signature sensu lato). This observation is
375 striking, as sponge-associated microbial communities are considered as highly stable associations (Cárdenas et al., 2014;
Erwin et al., 2012, 2015; Pita et al., 2013; Steinert et al., 2016).

Previous studies have shown that abiotic factors (i.e. depth, geographical location) influence the microbial
community structure in shallow-water sponges, but stated that the core community is shaped by the intimate interaction with
the sponge host (Lurgi et al., 2019). Interestingly, in our study the major microbial players in terms of abundance, such as
380 Chloroflexi in *G. hentscheli*, show significant enrichment/depletion patterns across the four clusters. Traditionally (shallow
water) sponge-associated microbes have been classified into core, variable and species-specific communities (Schmitt et al.,
2012). The present study reveals that for the three investigated deep-sea sponges at Schulz Bank seamount the variable
community overlaps with the core-community when considering high taxonomy ranks. In addition, network analyses showed
both positive and negative correlations between taxa for those increasing with depth as well as those displaying a variable
385 response. We suspect that primary responders to environmental parameters have cascading effects on microbial lineages that
are not directly affected by water biogeochemistry.

In this study, silicate, oxygen and nitrate concentrations, as well as depth differed significantly between the four near-bed water clusters. While SiO_4^- and NO_3^- were positively correlated with depth, O_2 showed a negative relationship. An increase of nutrient concentrations with depth is consistent with our previous expectations [consider e.g. (Bristow et al., 2017)] and can be explained by remineralization processes of sinking marine snow within the deep water layers. Decreasing O_2 concentrations from the Intermediate Water (NwArIW) to the Deep Water (NwDW) are also consistent with our ~~prediction~~ expectation based on physical oceanography, as water layers more recently oxygenated at the ocean surface at their site of formation, typically carry more oxygen (Jeansson et al., 2017). In general, the absolute differences in the concentrations of all three significant environmental parameters were comparably small (especially NO_3^- and O_2). However, as microbial communities were significantly different between the clusters, we posit that the observed biogeochemical differences, albeit small, should be considered as drivers of sponge microbial community composition. In support of our hypothesis, the process of denitrification is for example known to be highly sensitive to nanomolar concentrations of O_2 concentrations (Dalsgaard et al., 2013). In addition, a previous study on the sponge *Xestospongia muta* demonstrated that changing NO_x concentrations over depth contribute to shaping the microbial community composition (Morrow et al., 2016). Furthermore, several studies have noted that depth is an important factor in structuring sponge-associated microbiomes (Indraningrat et al., 2019; Lesser et al., 2019; Steinert et al., 2016).

This is, to our knowledge, the first study that explores the impact of seawater biogeochemistry on deep-sea sponge microbiomes. We have used LEfSe analyses to identify sponge symbiont taxa whose relative abundance varies with depth, this being used as a proxy for selected biogeochemical parameters. We have further used co-occurrence networks to identify positively or negatively co-varying microbial clades. In the following we discuss one representative example, Chloroflexi in *G. hentscheli*. In this study, theThe relative abundance of Chloroflexi (among several other phyla) differed significantly between the four near-bed water clusters for *G. hentscheli* and seawater, showing a positive ~~cor~~relation with NO_3^- , SiO_4^- , and depth, and a negative ~~cor~~relation with O_2 . Members of the phylum Chloroflexi have been attributed to a relevant role in the degradation of organic matter, particularly in the deep ocean pelagic realm and within HMA-sponges (Bayer et al., 2018; Landry et al., 2017). High degradation rates of organic matter are often related with low O_2 and high nutrient concentrations, owing to biogeochemical feedbacks where nutrients enhance oxygen demand by increasing biological production and oxygen consumption during decomposition. Taken together, the differences in relative abundances of Chloroflexi in *G. hentscheli* and seawater could be driven by the NO_3^- , SiO_4^- , and O_2 concentrations in ambient seawater. While sponge microbiomes are generally considered as being highly stable in time and space, we provide a first evidence that small differences in water biogeochemistry may affect sponge microbiome composition. However, no uniform shifts in relative abundances of microbial taxa were observed for *G. hentscheli*, *L. complicata*, and *S. rosea*, but rather an individual response of each host species related to biogeochemical parameters. One explanation is that biological interactions between the sponge host and its microbiome, or between the microbes themselves might have masking effects.

Conclusions

420 We provide insights into the variability of pelagic and benthic (sponge-associated) microbiomes at the Arctic Schulz Bank
seamount [based on the microbiome analyses of 114 seawater and 36 sponge samples](#)~~with high-resolution sampling~~.
Interestingly, a ‘seamount signature’ is detected within the microbial community composition of samples originating as far
as 200 m above the seamount summit. We further show that the biogeochemistry of seawater which varies over depth (NO_3^- ,
 SiO_4^- , and O_2 concentrations) has a detectable, but variable influence on the composition of sponge-associated microbiomes.
425 This study provides new perspectives on the influence of seamounts on the microbial diversity in their vicinity. We conclude
that the geology, physical oceanography, biogeochemistry and microbiology of seamounts and similar structures are even
more closely linked than currently appreciated.

Data availability

Sample metadata and biogeochemical data were deposited in the ~~Pangaea~~ [PANGAEA](#) database:
430 <https://doi.pangaea.de/10.1594/PANGAEA.911304>. Raw sequences were archived in the NCBI Sequence Read Archive
under BioProject id: PRJNA600711.

Author contribution

KB, FM, BM, UHe designed the study. KB, UHa, FM, EMR, HTR participated in sampling. UHa, FM, KB focused on
biogeochemical parameters. HTR conducted the sponge taxonomic analysis. UHe ~~and~~ KB were responsible for the
435 microbial pipeline. AF was involved in sequencing. KB performed the data analysis (bioinformatics and visualisations). KB,
UHe wrote the manuscript. BM, FM, EMR, UHa, HTR reviewed and edited the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

440 [This work is dedicated to Hans Tore Rapp[†], sponge taxonomist, deep-sea explorer, colleague and friend. This study was
funded](#) [Funding was provided](#) by the European Union’s Horizon 2020 research and innovation program under Grant
Agreement No. 679849 (the SponGES project). This document reflects only the authors’ view and the Executive Agency for
Small and Medium-sized Enterprises (EASME) is not responsible for any use that may be made of the information it
contains.

445 Samples included in this study were collected in compliance with the Nagoya Protocol. We thank the crews and scientific parties of RV *G.O. Sars* cruises ‘GS2016109A’, ‘GS2017110’, and ‘GS2018108’ for great technical support while at sea. We are grateful for sponge sampling by Stig Vågenes and his ROV Ægir 6000-team (UiB), Christine Rooks (UiB) for sampling assistance on-board ship during ‘GS2016109A’, and Jasper de Goeij (UvA) for interesting discussions. We further acknowledge Ina Clefsen, Andrea Hethke, Ilona Urbach and Tonio Hauptmann (Kiel, Germany) for excellent laboratory support with the amplicon pipeline. Corinna Bang (IKMB, Kiel) provided valuable support with the sample sequencing and revised the manuscript before submission. We also thank two anonymous reviewers for their comments, which helped to improve this manuscript.

References

- 455 Bayer, K., Jahn, M. T., Slaby, B. M., Moitinho-Silva, L. and Hentschel, U.: Marine Sponges as *Chloroflexi* Hot Spots: Genomic Insights and High-Resolution Visualization of an Abundant and Diverse Symbiotic Clade, *mSystems*, 3(6), 1–19, doi:10.1128/msystems.00150-18, 2018.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Ghalith, G. A. Al, Alexander, H., Alm, E. J., Arumugam, M., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Colin, J., Brown, C. T., Callahan, B. J., Mauricio, A., Rodríguez, C., Chase, J., Cope, E., Silva, R. Da, Dorrestein, P. C., Douglas, G. M., Duvallet, C., Edwardson, C. F., Ernst, M., Fouquier, J., Gauglitz, J. M., Gibson, D. L., Gonzalez, A., Huttley, G. A., Janssen, S., Jarmusch, A. K., Kaehler, B. D., Kang, K. Bin, Keefe, C. R., Keim, P., Kelley, S. T., Ley, R., Loftfield, E., Marotz, C., Martin, B., Mcdonald, D., Mciver, L. J., Alexey, V., Metcalf, J. L., Morgan, S. C., Morton, J. T. and Naimey, A. T.: QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science, doi:10.7287/peerj.preprints.27295, 2018.
- 460 Borchert, E., Selvin, J., Kiran, S. G., Jackson, S. A., O’Gara, F. and Dobson, A. D. W.: A novel cold active esterase from a deep sea sponge *Stelletta normani* metagenomic library, *Front. Mar. Sci.*, 4, 1–13, doi:10.3389/fmars.2017.00287, 2017.
- Bristow, L. A., Mohr, W., Ahmerkamp, S. and Kuypers, M. M. M.: Nutrients that limit growth in the ocean, *Curr. Biol.*, 27(11), 474–478, doi:10.1016/j.cub.2017.03.030, 2017.
- 470 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. and Holmes, S. P.: DADA2: High-resolution sample inference from Illumina amplicon data, *Nat. Methods*, 13(7), 581–583, doi:10.1038/nmeth.3869, 2016.
- Cárdenas, C. A., Bell, J. J., Davy, S. K., Hoggard, M. and Taylor, M. W.: Influence of environmental variation on symbiotic bacterial communities of two temperate sponges, *FEMS Microbiol. Ecol.*, 88(3), 516–527, doi:10.1111/1574-6941.12317, 2014.
- 475 Cárdenas, P., Rapp, H. T., Schander, C. and Tendal, O. S.: Molecular Taxonomy and Phylogeny of the *Geodiidae* Gray, 1867 (*Porifera, Demospongiae, Astrophorida*) – combining Phylogenetic and Linnaean Classification, *Zool. Scr.*,

39, 89–106, 2010.

- Chapman, D. C. and Haidvogel, D. B.: Formation of Taylor caps over a tall isolated seamount in a stratified ocean, *Geophys. Astrophys. Fluid Dyn.*, 64(1–4), 31–65, doi:10.1080/03091929208228084, 1992.
- 480 Dalsgaard, T., De Brabandere, L. and Hall, P. O. J.: Denitrification in the water column of the central Baltic Sea, *Geochim. Cosmochim. Acta*, 106, 247–260, doi:10.1016/j.gca.2012.12.038, 2013.
- De Goeij, J. M., Van Den Berg, H., Van Oostveen, M. M., Epping, E. H. G. and Van Duyl, F. C.: Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges, *Mar. Ecol. Prog. Ser.*, 357, 139–151, doi:10.3354/meps07403, 2008.
- 485 De Goeij, J. M., Van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., De Goeij, A. F. P. M. and Admiraal, W.: Surviving in a marine desert: The sponge loop retains resources within coral reefs, *Science*, 342(6154), 108–110, doi:10.1126/science.1241981, 2013.
- DeGoeij, J. M., Lesser, M. P. and Pawlik, J. R.: Nutrient Fluxes and Ecological Functions of Coral Reef Sponges in a Changing Ocean, in *Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization*, edited by J. L. Carballo and J. J. Bell, pp. 373–410, Cham, Springer International Publishing, 2017.
- 490 Easson, C. G. and Thacker, R. W.: Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges, *Front. Microbiol.*, 5, 1–11, doi:10.3389/fmicb.2014.00532, 2014.
- Erwin, P. M., Pita, L., López-Legentil, S. and Turon, X.: Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance, *Appl. Environ. Microbiol.*, 78(20), 7358–7368, doi:10.1128/AEM.02035-12, 2012.
- 495 Erwin, P. M., Coma, R., López-Sendino, P., Serrano, E. and Ribes, M.: Stable symbionts across the HMA-LMA dichotomy: Low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts, *FEMS Microbiol. Ecol.*, 91(10), 1–11, doi:10.1093/femsec/fiv115, 2015.
- Fristedt, K.: Sponges from the Atlantic and Arctic Oceans and the Behring Sea, , 4, 401–471, plates 22-31, 1887.
- Gantt, S. E., McMurray, S. E., Stubler, A. D., Finelli, C. M., Pawlik, J. R. and Erwin, P. M.: Testing the relationship between
500 microbiome composition and flux of carbon and nutrients in Caribbean coral reef sponges, *Microbiome*, 7(1), 1–13, doi:10.1186/s40168-019-0739-x, 2019.
- Genin, A. and Boehlert, G. W.: Dynamics of temperature and chlorophyll structures above a seamount: an oceanic experiment., *J. Mar. Res.*, 43(4), 907–924, doi:10.1357/002224085788453868, 1985.
- Genin, A., Dayton, P. K., Lonsdale, P. F. and Spiess, F. N.: Corals on seamount peaks provide evidence of current
505 acceleration over deep-sea topography, *Nature*, 322(6074), 59–61, doi:10.1038/322059a0, 1986.
- Grant: Animal Kingdom, in *The Cyclopaedia of Anatomy and Physiology*, edited by R. B. Todd, pp. 107–118, Sherwood, Gilbert and Piper, London, UK., 1836.
- Grasshoff, K., Kremling, K. and Ehrhardt, M.: *Methods of Seawater Analysis*, 3rd Edition, WILEY-VCH Verlag GmbH, Weinheim, Germany., 2009.
- 510 Hansen, G. A.: Spongidae. The Norwegian North-Atlantic Expedition 1876-1878, *Zoology*, 13, 1–26, plates I–VII, 1885.

- Helber, S. B., Steinert, G., Wu, Y. C., Rohde, S., Hentschel, U., Muhando, C. A. and Schupp, P. J.: Sponges from Zanzibar host diverse prokaryotic communities with potential for natural product synthesis, *FEMS Microbiol. Ecol.*, 95(4), doi:10.1093/femsec/fiz026, 2019.
- 515 Helder, W. and de Vries, R. T. P.: An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters, *Netherlands J. Sea Res.*, 13(1), 154–160, 1979.
- Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J. and Horn, M.: Microbial diversity of marine sponges., in *Sponges (Porifera)*, edited by W. E. G. Mueller, pp. 59–88, Springer, Berlin, Heidelberg., 2003.
- Hoer, D. R., Gibson, P. J., Tommerdahl, J. P., Lindquist, N. L. and Martens, C. S.: Consumption of dissolved organic carbon by Caribbean reef sponges, *Limnol. Oceanogr.*, 63(1), 337–351, doi:10.1002/lno.10634, 2018.
- 520 Indraningrat, A. A. G., Micheller, S., Runderkamp, M., Sauerland, I., Becking, L. E., Smidt, H. and Sipkema, D.: Cultivation of Sponge-Associated Bacteria from *Agelas sventres* and *Xestospongia muta* Collected from Different Depths, *Mar. Drugs*, 17(10), 1–17, doi:10.3390/md17100578, 2019.
- IUCN: Seamounts Project: An Ecosystem Approach to Management of Seamounts in the Southern Indian Ocean, *Int. Union Conserv. Nat. Nat. Resour.*, 1, 1–60, 2013.
- 525 Jackson, S. A., Flemer, B., McCann, A., Kennedy, J., Morrissey, J. P., O’Gara, F. and Dobson, A. D. W.: Archaea appear to dominate the microbiome of *Inflatella pellicula* deep sea sponges, *PLoS One*, 8(12), 1–8, doi:10.1371/journal.pone.0084438, 2013.
- Jeansson, E., Olsen, A. and Jutterström, S.: Arctic Intermediate Water in the Nordic Seas, 1991–2009, *Deep. Res. Part I Oceanogr. Res. Pap.*, 128, 82–97, doi:10.1016/j.dsr.2017.08.013, 2017.
- 530 Kennedy, J., Flemer, B., Jackson, S. A., Morrissey, J. P., O’Gara, F. and Dobson, A. D. W.: Evidence of a putative deep sea specific microbiome in marine sponges, *PLoS One*, 9(3), 1–13, doi:10.1371/journal.pone.0091092, 2014.
- Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. and Schloss, P. D.: Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform, *Appl. Environ. Microbiol.*, 79(17), 5112–5120, doi:10.1128/AEM.01043-13, 2013.
- 535 Landry, Z., Swa, B. K., Herndl, G. J., Stepanauskas, R. and Giovannoni, S. J.: *SAR202* genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter, *MBio*, 8(2), 1–19, doi:10.1128/mBio.00413-17, 2017.
- Lavelle, J. W. and Mohn, C.: Motion, commotion, and biophysical connections at deep ocean seamounts, *Oceanography*, 23(1), 90–103, doi:10.5670/oceanog.2010.64, 2010.
- 540 Lesser, M. P., Mueller, B., Pankey, M. S., Macartney, K. J., Slattery, M. and Goeij, J. M. De: Depth-dependent detritus production in the sponge *Halisarca caerulea*, early view, 1–17, doi:10.1002/lno.11384, 2019.
- Leys, S. P., Kahn, A. S., Fang, J. K. H., Kutti, T. and Bannister, R. J.: Phagocytosis of microbial symbionts balances the carbon and nitrogen budget for the deep-water boreal sponge *Geodia barretti*, *Limnol. Oceanogr.*, 63(1), 187–202, doi:10.1002/lno.10623, 2018.

- 545 Lurgi, M., Thomas, T., Wemheuer, B., Webster, N. S. and Montoya, J. M.: Modularity and predicted functions of the global sponge-microbiome network, *Nat. Commun.*, 10, 1–12, doi:10.1038/s41467-019-08925-4, 2019.
- Luter, H. M., Bannister, R. J., Whalan, S., Kutti, T., Pineda, M.-C. and Webster, N. S.: Microbiome analysis of a disease affecting the deep-sea sponge *Geodia barretti*, *FEMS Microbiol. Ecol.*, 93(6), 1–6, doi:10.1093/femsec/fix074, 2017.
- 550 Maldonado, M., Ribes, M. and van Duyl, F. C.: Nutrient Fluxes Through Sponges. Biology, Budgets, and Ecological Implications, *Adv. Mar. Biol.*, 62, 113–182, doi:10.1016/B978-0-12-394283-8.00003-5, 2012.
- Maldonado, M., Aguilar, R., Bannister, R. J., Bell, J. J., Conway, K. W., Dayton, P. K., Díaz, C., Gutt, J., Kelly, M., Kenchington, E. L. R., Leys, S. P., Pomponi, S. A., Rapp, H. T., Rützler, K., Tendal, O. S., Vacelet, J. and Young, C. M.: Sponge Grounds as Key Marine Habitats: A Synthetic Review of Types, Structure, Functional Roles, and Conservation Concerns, in *Marine Animal Forests*, edited by S. Rossi, L. Bramanti, A. Gori, and C. Orejas, Springer International Publishing Switzerland, Switzerland., 2016.
- 555 Maldonado, M., López-Acosta, M., Sitjà, C., García-Puig, M., Galobart, C., Ercilla, G. and Leynaert, A.: Sponge skeletons as an important sink of silicon in the global oceans, *Nat. Geosci.*, 12(10), 815–822, doi:10.1038/s41561-019-0430-7, 2019.
- 560 McMurray, S. E., Johnson, Z. I., Hunt, D. E., Pawlik, J. R. and Finelli, C. M.: Selective feeding by the giant barrel sponge enhances foraging efficiency, *Limnol. Oceanogr.*, 61(4), 1271–1286, doi:10.1002/lno.10287, 2016.
- Menard, H.W.: *Marine Geology of the Pacific*, McGraw-Hill, New York, 271 pp., 1964.
- Meyer, H. K., Roberts, E. M., Rapp, H. T. and Davies, A. J.: Spatial patterns of arctic sponge ground fauna and demersal fish are detectable in autonomous underwater vehicle (AUV) imagery, *Deep. Res. Part I*, 153, article number 103137, doi:10.1016/j.dsr.2019.103137, 2019.
- 565 Moitinho-Silva, L., Bayer, K., Cannistraci, C. V., Giles, E. C., Ryu, T., Seridi, L., Ravasi, T. and Hentschel, U.: Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea, *Mol. Ecol.*, 23(6), 1348–1363, doi:10.1111/mec.12365, 2014.
- Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C. P., Wu, Y. C., McCormack, G. P., López-Legentil, S., Marchant, R., Webster, N., Thomas, T. and Hentschel, U.: Predicting the HMA-LMA status in marine sponges by machine learning, *Front. Microbiol.*, 8, 1–14, doi:10.3389/fmicb.2017.00752, 2017.
- 570 Morato, T., Hoyle, S. D., Allain, V. and Nicol, S. J.: Seamounts are hotspots of pelagic biodiversity in the open ocean, *Proc. Natl. Acad. Sci. U. S. A.*, 107(21), 9707–9711, doi:10.1073/pnas.0910290107, 2010.
- Morrow, K. M., Fiore, C. L. and Lesser, M. P.: Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient, *Environ. Microbiol.*, 18(6), 2025–2038, doi:10.1111/1462-2920.13226, 2016.
- 575 Mueller, B., De Goeij, J. M., Vermeij, M. J. A., Mulders, Y., Van Der Ent, E., Ribes, M. and Van Duyl, F. C.: Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC), *PLoS One*, 9(2), 1–7,

doi:10.1371/journal.pone.0090152, 2014.

- 580 Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters, *Anal. Chim. Acta*, 27, 31–36, 1962.
- Naim, M. A., Morillo, J. A., Sørensen, S. J., Waleed, A. A. S., Smidt, H. and Siphema, D.: Host-specific microbial communities in three sympatric North Sea sponges, *FEMS Microbiol. Ecol.*, 90(2), 390–403, doi:10.1111/1574-6941.12400, 2014.
- 585 Van Oevelen, D., Mueller, C. E., Lundälv, T., Van Duyl, F. C., De Goeij, J. M. and Middelburg, J. J.: Niche overlap between a cold-water coral and an associated sponge for isotopically enriched particulate food sources, *PLoS One*, 13(3), 1–16, doi:10.1371/journal.pone.0194659, 2018.
- Pile, A. J., Patterson, M. R., Savarese, M., Chernykh, V. I. and Fialkov, V. A.: Trophic effects of sponge feeding within Lake Baikal's littoral zone. Sponge abundance, diet, feeding efficiency, and carbon flux, *Limnol. Oceanogr.*, 42, 178–184, 1997.
- 590 Pita, L., Turon, X., López-Legentil, S. and Erwin, P. M.: Host rules: Spatial stability of bacterial communities associated with marine sponges (*Ircinia* spp.) in the western Mediterranean sea, *FEMS Microbiol. Ecol.*, 86(2), 268–276, doi:10.1111/1574-6941.12159, 2013.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools, *Nucleic Acids Res.*, 41, 590–596, doi:10.1093/nar/gks1219, 2013.
- R Development Core Team: R: A language and environment for statistical computing., doi:<http://www.r-project.org>, 2008.
- Radax, R., Rattei, T., Lanzen, A., Bayer, C., Rapp, H. T., Urich, T. and Schleper, C.: Metatranscriptomics of the marine sponge *Geodia barretti*: Tackling phylogeny and function of its microbial community, *Environ. Microbiol.*, 14(5), 1308–1324, doi:10.1111/j.1462-2920.2012.02714.x, 2012.
- 600 Reiswig, H. M.: Particle feeding in natural populations of three marine demosponges, *Biol. Bull.*, 141(3), 568–591, 1971.
- Reveillaud, J., Maignien, L., Eren, M. A., Huber, J. A., Apprill, A., Sogin, M. L. and Vanreusel, A.: Host-specificity among abundant and rare taxa in the sponge microbiome, *ISME J.*, 8(6), 1198–1209, doi:10.1038/ismej.2013.227, 2014.
- Ribes, M., Coma, R. and Gili, J.: Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle, *Mar. Ecol. Prog. Ser.*, 176, 179–190, 1999.
- 605 Rix, L., De Goeij, J. M., Mueller, C. E., Struck, U., Middelburg, J. J., Van Duyl, F. C., Al-Horani, F. A., Wild, C., Naumann, M. S. and Van Oevelen, D.: Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems, *Sci. Rep.*, 6, 1–11, doi:10.1038/srep18715, 2016a.
- Rix, L., Goeij, J. M. De, Oevelen, D. Van, Struck, U., Al-horani, F. A., Wild, C. and Naumann, M. S.: Differential recycling of coral and algal dissolved organic matter via the sponge loop, *Funct. Ecol.*, 31(3), 1–12, doi:10.1111/1365-2435.12758, 2016b.
- 610

- Roberts, E. M., Mienis, F., Rapp, H. T., Hanz, U., Meyer, H. K. and Davies, A. J.: Oceanographic setting and short-timescale environmental variability at an Arctic seamount sponge ground, *Deep Sea Res. Part I Oceanogr. Res. Pap.*, 98–113, doi:10.1016/j.dsr.2018.06.007, 2018.
- 615 Rogers, A. D.: The Biology of Seamounts: 25 Years on, *Adv. Mar. Biol.*, 79, 137–224, doi:10.1016/bs.amb.2018.06.001, 2018.
- Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., Perez, T., Rodrigo, A., Schupp, P. J., Vacelet, J., Webster, N., Hentschel, U. and Taylor, M. W.: Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges, *ISME J*, 6(3), 564–576, doi:10.1038/ismej.2011.116, 2012.
- 620 Schöttner, S., Hoffmann, F., Cárdenas, P., Rapp, H. T., Boetius, A. and Ramette, A.: Relationships between Host Phylogeny, Host Type and Bacterial Community Diversity in Cold-Water Coral Reef Sponges, *PLoS One*, 8(2), 1–11, doi:10.1371/journal.pone.0055505, 2013.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S. and Huttenhower, C.: Metagenomic biomarker discovery and explanation, *Genome Biol.*, 12(6), R60, doi:10.1186/gb-2011-12-6-r60, 2011.
- 625 Smith, D.K. and Cann, J.R.: The role of seamount volcanism in crustal construction at the Mid-Atlantic Ridge (24°-30°N), *J Geophys Res Solid Earth*, 97, 1645–1658, 1992.
- Staudigel, H., Koppers, A.A.P., William Lavelle, J., Pitcher, T.J., and Shank, T.M.: Defining the word ‘seamount’, *Oceanography*, 23, 20–21, 2010.
- Steinert, G., Taylor, M. W., Deines, P., Simister, R. L., De Voogd, N. J., Hoggard, M. and Schupp, P. J.: In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity, *PeerJ*, 2016(4), 1–25, doi:10.7717/peerj.1936, 2016.
- 630 Steinert, G., Rohde, S., Janussen, D., Blaurock, C. and Schupp, P. J.: Host-specific assembly of sponge-associated prokaryotes at high taxonomic ranks, *Sci. Rep.*, 7, 1–9, doi:10.1038/s41598-017-02656-6, 2017.
- Steinert, G., Wemheuer, B., Janussen, D., Erpenbeck, D., Daniel, R., Simon, M., Brinkhoff, T. and Schupp, P. J.: Prokaryotic diversity and community patterns in antarctic continental shelf sponges, *Front. Mar. Sci.*, 6, 1–15, doi:10.3389/fmars.2019.00297, 2019.
- 635 Stoll, M. H. C., Bakker, K., Nobbe, G. H. and Haese, R. R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, *Anal. Chem.*, 73(17), 4111–4116, doi:10.1021/ac010303r, 2001.
- Strickland, J. and Parsons, T.: A practical handbook of seawater analysis, *Fish. Res. Board Canada*, 167, 65–70, 1972.
- 640 Taylor, M. W., Radax, R., Steger, D. and Wagner, M.: Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential, *Microbiol. Mol. Biol. Rev.*, 71(2), 295–347, doi:10.1128/MMBR.00040-06, 2007.
- Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., Olson, J. B., Erwin, P. M., López-Legentil, S., Luter, H., Chaves-Fonnegra, A., Costa, R., Schupp, P. J., Steindler, L., Erpenbeck, D., Gilbert, J., Knight, R., Ackermann, G., Victor Lopez, J., Taylor, M. W., Thacker, R. W., Montoya, J. M., Hentschel, U. and Webster, N. S.: Diversity, structure and convergent evolution of the global sponge microbiome, *Nat. Commun.*, 7,
- 645

1-12, doi:10.1038/ncomms11870, 2016.

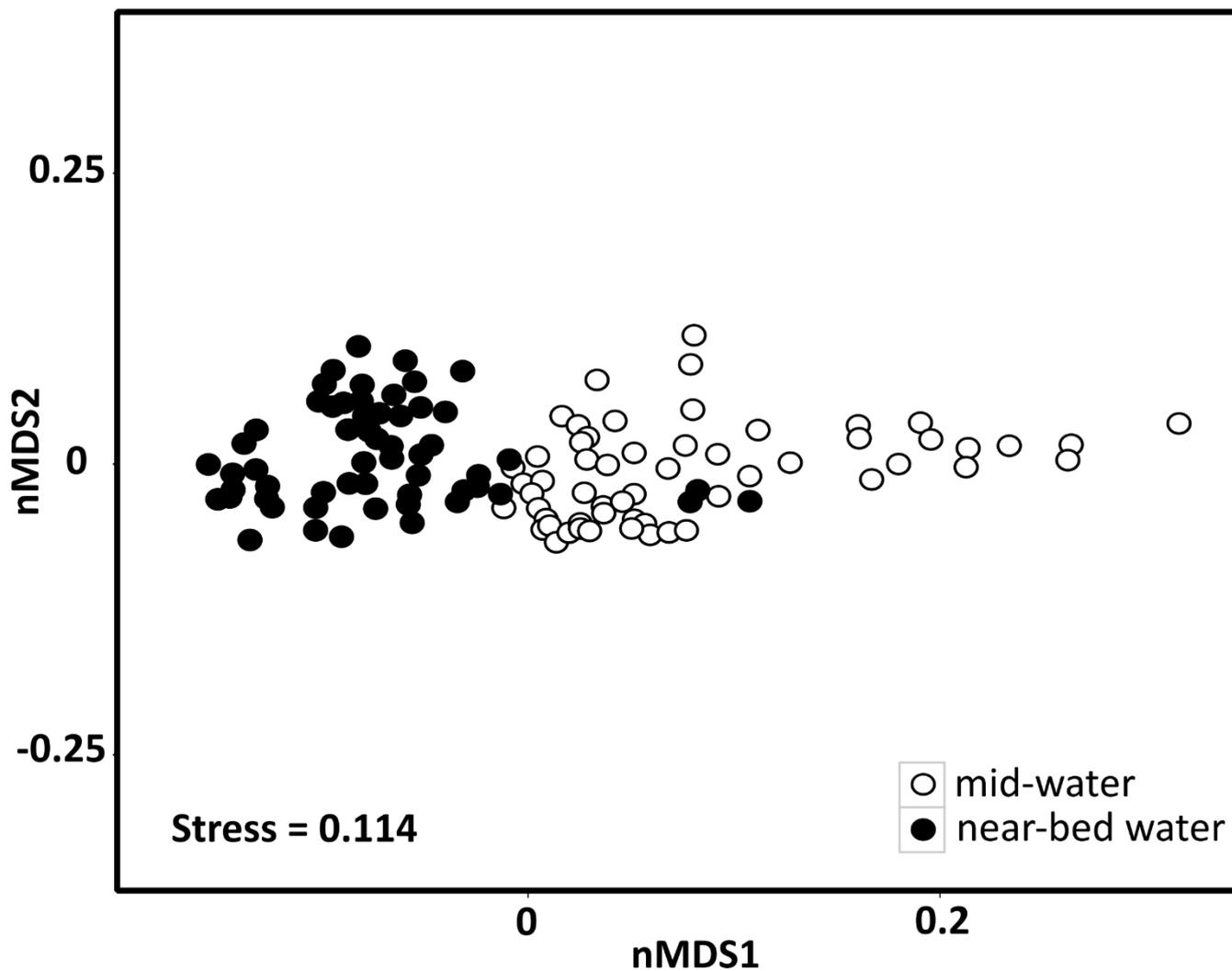
Tian, R. M., Sun, J., Cai, L., Zhang, W. P., Zhou, G. W., Qiu, J. W. and Qian, P. Y.: The deep-sea glass sponge *Lophophysema eversa* harbours potential symbionts responsible for the nutrient conversions of carbon, nitrogen and sulfur, *Environ. Microbiol.*, 18(8), 2481–2494, doi:10.1111/1462-2920.13161, 2016.

650 Van Haren, H., Hanz, U., de Stigter, H., Mienis, F. and Duineveld, G.: Internal wave turbulence at a biologically rich Mid-Atlantic seamount, *PLoS One*, 12(12), 1–16, doi:10.1371/journal.pone.0189720, 2017.

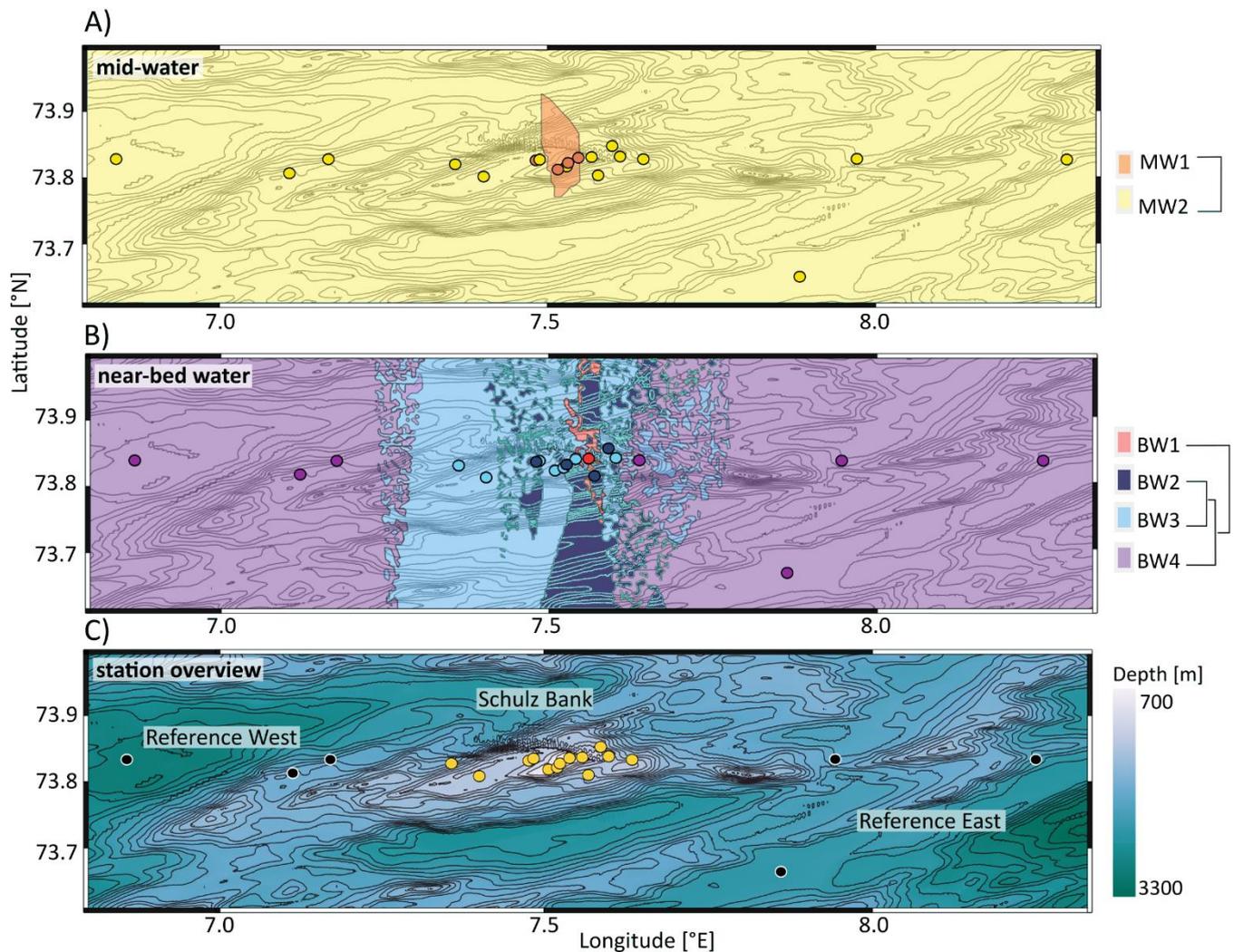
Weisz, J. B., Lindquist, N. and Martens, C. S.: Do associated microbial abundances impact marine demosponge pumping rates and tissue densities?, *Oecologia*, 155(2), 367–376, doi:10.1007/s00442-007-0910-0, 2008.

Wessel, P., Sandwell, D.T., and Kim, S.-S.: Seamount Census, *Oceanography*, 23, 24–33, 2010.

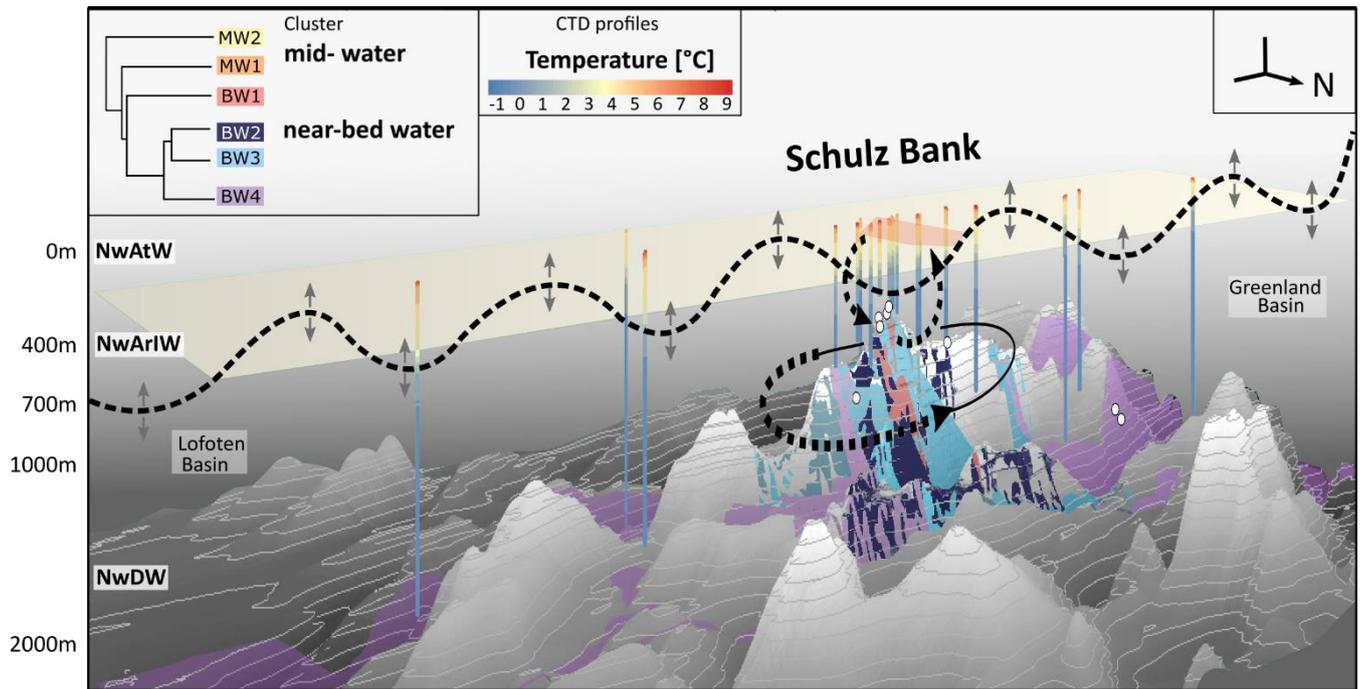
655



660 Figure 1: Seawater microbial community composition of mid-water and near-bed water samples visualised by a non-metric multidimensional scaling plot on weighted UniFrac distances. Each marker is one microbial community, with colors indicating the sample sub-type (i.e. mid-water or near-bed water).



665 Figure 2: Seawater microbial community structure across Schulz Bank. Contour lines in all three subplots represent the underlying topography. Colors in A) and B) represent clusters based on weighted UniFrac distances, where colored dots indicate stations with in situ sampling and filled areas represent extrapolations based on machine learning. **The further away predicted areas are from actual sample points, the higher is the associated uncertainty of these predictions.** A) includes all mid-water samples derived during the CTD transects. B) includes all near-bed water samples. Here, the degree of cluster similarity can be deduced from the dendrogram to the right of the plot(s). C) provides an overview of the sampling area, showing the locations of all 19 CTD stations. Stations directly located on the Schulz Bank are coloured yellow, while reference stations (west and east of Schulz Bank) are indicated by black colours. Colouring in sub-plot C) was done according to depth.

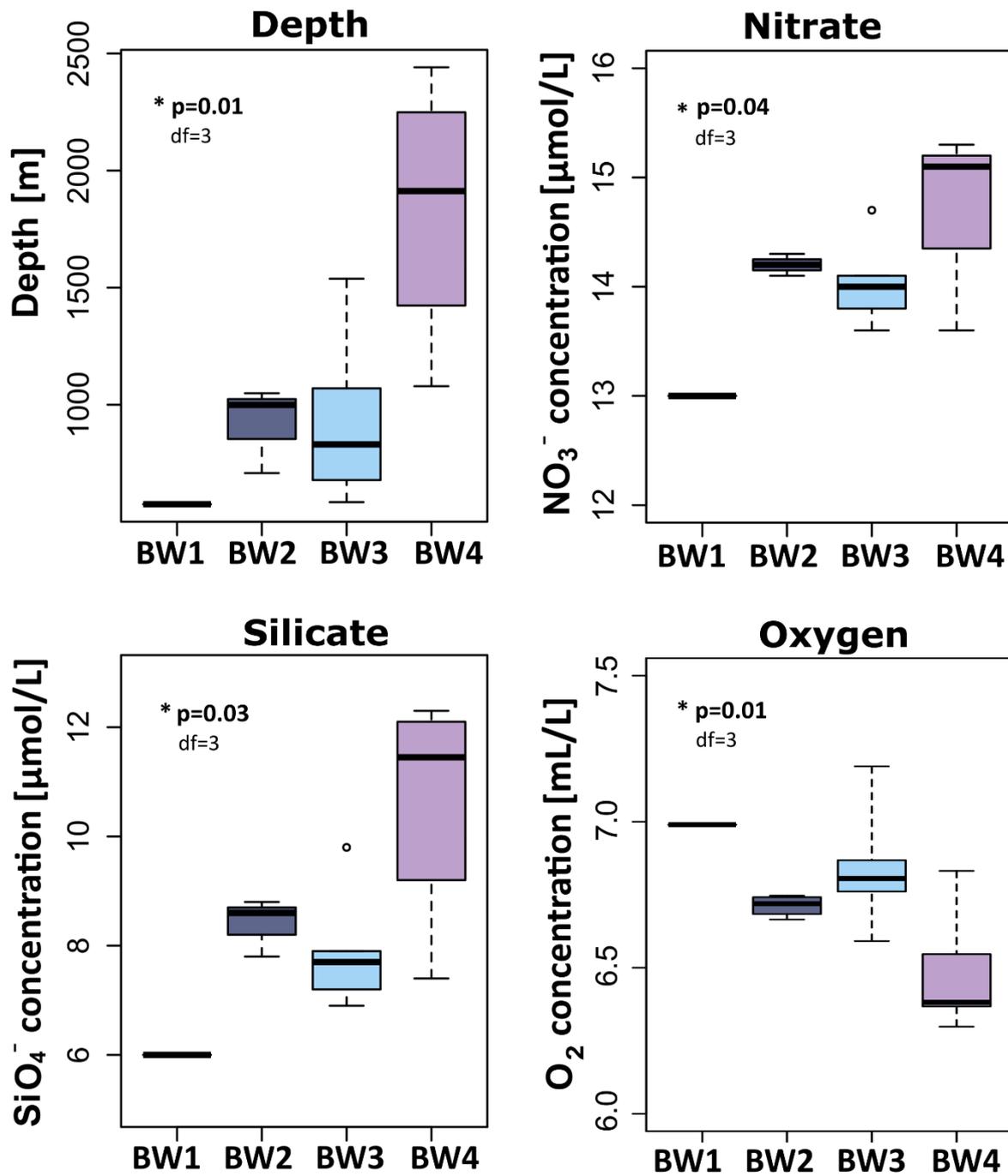


670

Figure 3: Conceptual overview and vertical 3D section showing spatial distribution of microbial clusters and oceanographic patterns on the Schulz Bank seamount. Extrapolated seawater microbial clusters are indicated by colored polygons: mid-water clusters are marked in orange (MW1) and yellow (MW2), while near-bed water clusters are marked in red (BW1), dark blue (BW2), light blue (BW3), and purple (BW4). The degree of cluster similarity can be deduced from the dendrogram in the left corner of the plot. Whole water column CTD profiles are indicated, showing the measured temperature values from surface to bottom at the respective sampling locations. Sponge sampling locations visible on this side of the seamount are indicated by white balls. Vertical positions of major watermasses: Norwegian Atlantic Water (NwAtW), Norwegian Arctic Intermediate Water (NwArIW), and Norwegian Deep Water (NwDW) are indicated. To give a broad orientation in space, a north arrow is depicted, as well as the major geologic features (Lofoten Basin and Greenland Basin). For Schulz Bank, water flows, such as a potential Taylor column circulating around the seamount, mixing between summit and pelagic realm, as well as tidally-driven internal motions (black horizontal line with bidirectional arrows) are indicated by dashed arrows and lines.

675

680



685 Figure 4: Concentrations and measurements of significant (ANOVA, $\alpha=0.05$) biogeochemical parameters for near-bed water samples, across the determined near-bed water clusters. p-values as well as degrees of freedom (df) for these parameters are written into the respective graphs. Colouring is the same as chosen for Fig. 2 and 3.

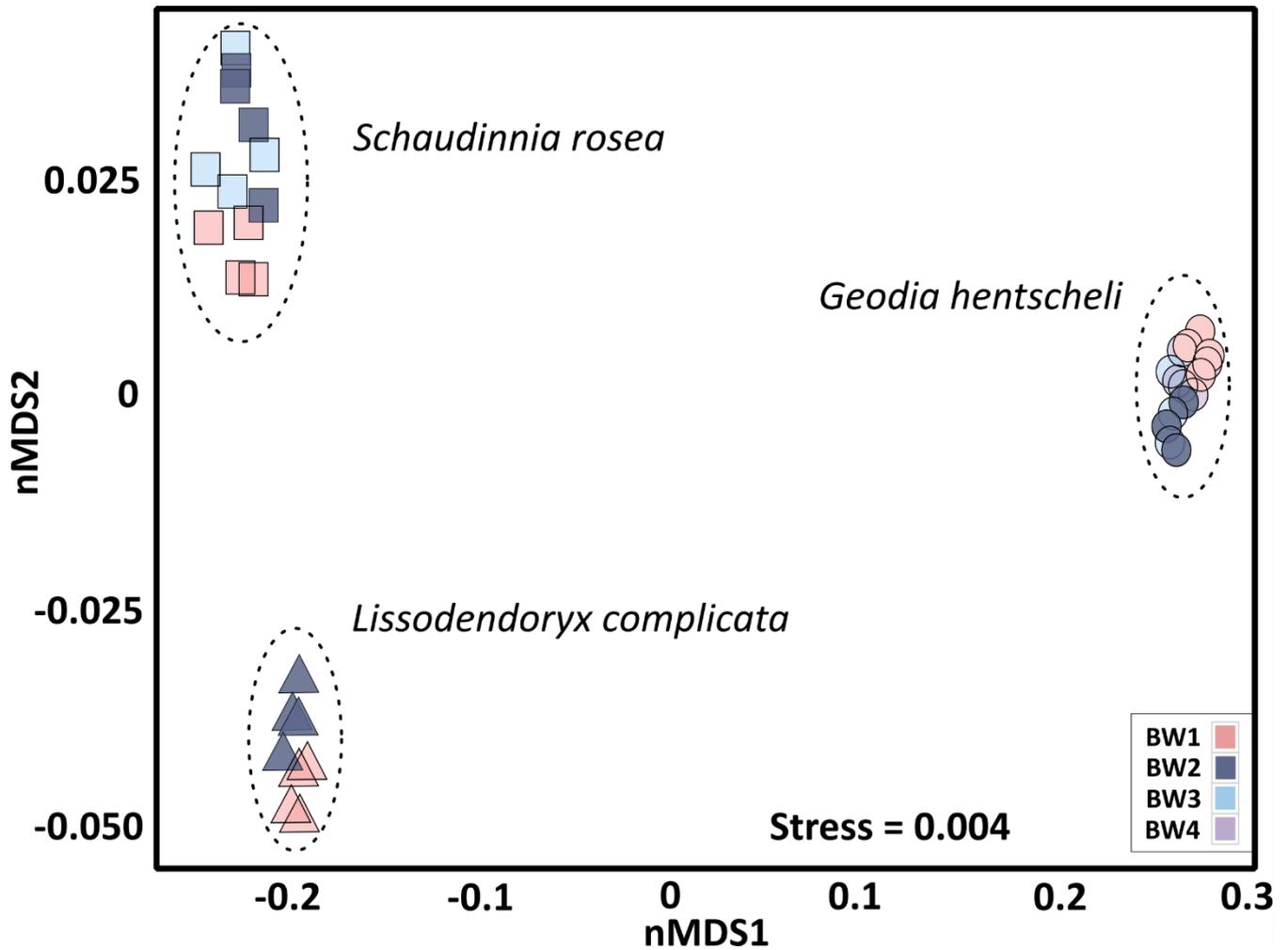
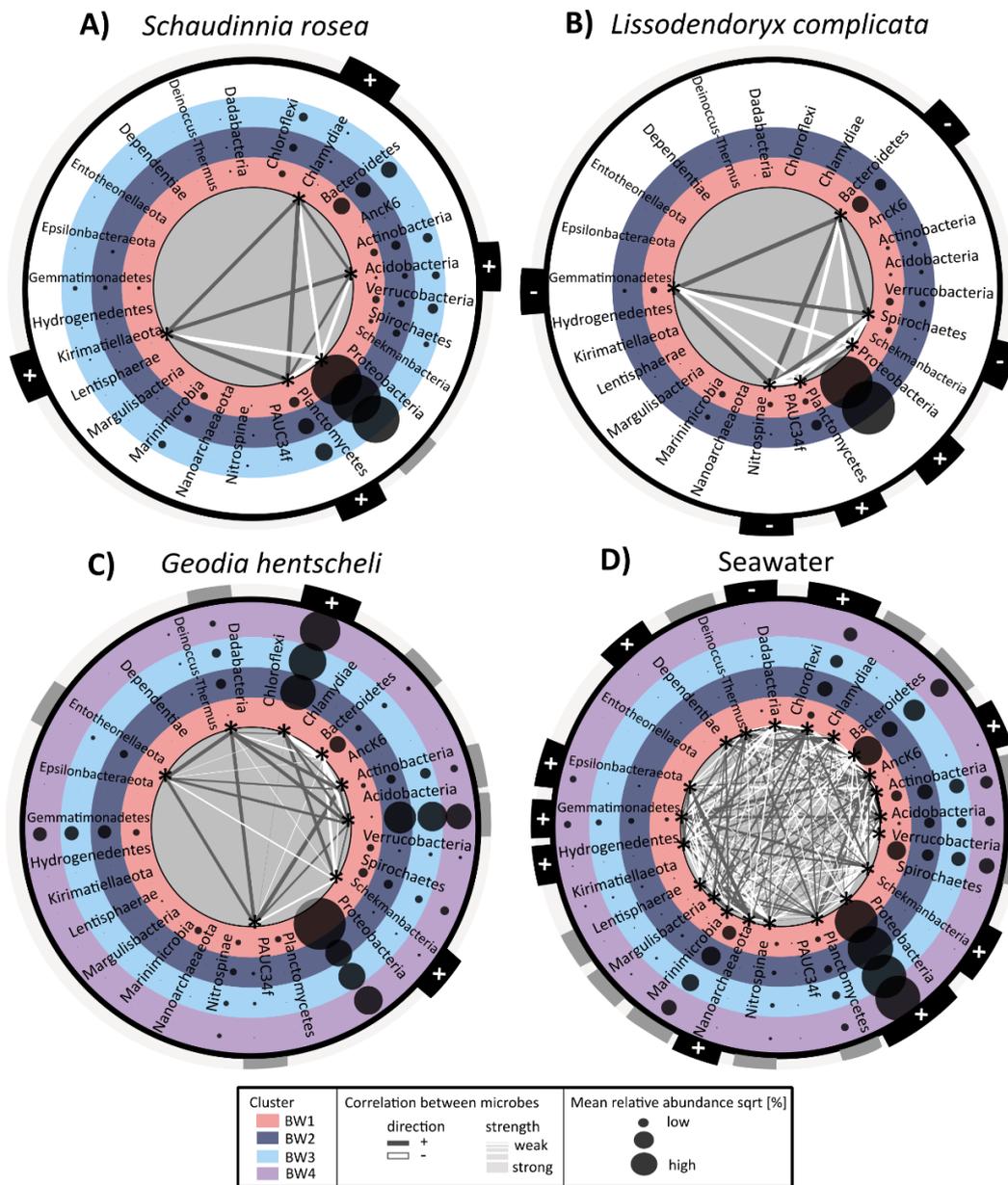


Figure 5: Sponge microbial community composition visualised by a non-metric multidimensional scaling plot on weighted UniFrac distances. Each marker is one microbial community, with symbols representing the sample sub-type (i.e. sponge species) and colors indicating the near-bed water cluster present at the respective sponge sampling location.



690

695

700

Figure 6: Co-occurrence network and differential abundance of microbial phyla across the four determined near-bed water clusters. Sub-plots A-C) show sponge data, with plot A) showing average *Schaudinnia rosea* data, B) presenting average *Lissodendoryx complicata* data and C) illustrating *Geodia hentscheli* data. Sub-plot D) contains seawater data. Near-bed water clusters are represented by differently colored rings. Each ring contains a list with microbial phyla which are alphabetically sorted. Average relative abundances of each of the respective phyla for the samples within a given cluster are indicated by bubble sizes. Those microbial phyla which are statistically significantly enriched or depleted across the four clusters (LEfSe/LefSE analysis), are marked with an asterisk inside the inner most ring. Only for those taxa where the difference is significant, correlation strength (indicated by size of connecting lines) and direction (represented by color of connecting lines: white = negative correlation, dark grey = positive correlation) with all other significant taxa are plotted. For all microbial phyla correlation with depth is indicated in the outer ring of each plot by + (meaning significant positive correlation) or - (meaning significant negative correlation).

Response to Reviewer Comments on Manuscript bg-2020-15:

On giant shoulders: How a seamount affects the microbial community composition of seawater and sponges

Kathrin Busch, Ulrike Hanz, Furu Mienis, Benjamin Müller, Andre Franke, Emyr Martyn Roberts, Hans Tore Rapp, Ute Hentschel

We thank the reviewers for the overall positive evaluation of our manuscript and for the time and effort taken. We have made considerable effort to address the points raised and also included three additional figures (Supplementary Material S1, Supplementary Material S2A, Supplementary Material S3), elaborated existing figures (Fig.3, Fig.4, Fig.6) and conducted further statistical analyses (i.e. TukeyHSD Posthoc tests, Spearman's rank correlation). Below please find our responses that are listed in the order raised. We show the referees' comments in black text, while our responses are formatted in red. The new line numbers refer to the revised manuscript (marked-up version). Thank you for your consideration.

Reviewer #1 (Comments for the authors):

Overall comments: This manuscript brings to bear physical oceanographic, biogeochemical, and microbiological data on the question of whether seamounts impact the microbial community composition of the water column and benthic organisms like sponges. I was skeptical of water column impacts, imagining that a given water mass would have a microbial signature irrespective of the seamount. However, the data in this paper convinced me of the unexpected findings that not only do seamounts exert an effect on the bacterial community composition up to 200 meters above their summit, but also a more subtle effect on the bacterial community composition of sponges growing at various depths on the seamount. The scientific methods are clearly described and appropriate for the work. CTD data were collected at stations both on and off the seamount, so there are appropriate mid-water and near-bottom water controls to assess the influence of the seamount. A sufficient number of sponge samples were collected for each species, which is not a small matter for deep-sea work. Moreover, the authors have put a tremendous amount of work into creating excellent visualizations of the data that clearly show how the results support their conclusions (particularly Figures 3 and 6). It takes real effort to combine such large amounts of information into figures and still have them clearly and cleanly illustrate the narrative points you are making in the discussion. The discussion is very concise and logically structured. The conclusions are well supported by the results. The references are appropriate in scope, including very recent findings (2018-2019) as well as citing classic papers from multiple decades (centuries). All of the references cited in the text are listed in the bibliography. I don't often have the pleasure of reviewing a paper that is so articulate and well organized and makes such surprising findings. This manuscript is excellent.

Typographic issues:

Abstract:

Line 17: 'extend' should be 'extent'

DONE

Line 19: 'which extend' should be 'what extent'

DONE

Line 21: amplicon does not need to be capitalized

DONE

Lines 38 and 42: remove the comma after 'both'

DONE

Lines 40-41: You do not define HMA or LMA prior to usage

The explanation of HMA or LMA as well as the taxonomic status has been removed here as it is explained later (LL79-81).

Introduction:

Lines 65-66: 'Rix, De Goeij et al. 2016' should be either Rix et al.2016a or Rix et al. 2016b

DONE

Line 71: 'Intimate sponge-microbe associations have been observed. . .'

DONE

Line 85: '. . .interactivity are still lacking'

DONE

Results:

Line 242: 'The dominant microbial phylum in *S. rosea* and *L. complicate* was Proteobacteria'

DONE

Throughout: There is inconsistency of the in-text citations throughout the manuscript. Some citations italicize 'et al.' while others do not. Please check the journal's preference and then make all of them the same.

DONE

References:

Line 463: Van Haren is listed under 'H' instead of 'V'

DONE

Lines 452-457: two De Goeij references are listed under 'G' instead of under 'D'

DONE

Line 569: Isme should be ISME

DONE

Response to Reviewer Comments on Manuscript bg-2020-15:

On giant shoulders: How a seamount affects the microbial community composition of seawater and sponges

Kathrin Busch, Ulrike Hanz, Furu Mienis, Benjamin Müller, Andre Franke, Emyr Martyn Roberts, Hans Tore Rapp, Ute Hentschel

We thank the reviewers for the overall positive evaluation of our manuscript and for the time and effort taken. We have made considerable effort to address the points raised and also included three additional figures (Supplementary Material S1, Supplementary Material S2A, Supplementary Material S3), elaborated existing figures (Fig.3, Fig.4, Fig.6) and conducted further statistical analyses (i.e. TukeyHSD Posthoc tests, Spearman's rank correlation). Below please find our responses that are listed in the order raised. We show the referees' comments in black text, while our responses are formatted in red. The new line numbers refer to the revised manuscript (marked-up version). Thank you for your consideration.

Reviewer #2 (Comments for the authors):

General evaluation: The paper describes pelagic microbial communities at a seamount and in the surrounding deep sea and compares these communities with sponge-associated microbial communities at the same locations. The authors conclude that biogeochemical properties of the water column and hydrodynamic effects induced by the seamount topography may shape these communities and explain differences between seamount summit, flanks and far field stations. These aspects have rarely, if ever, been investigated at seamounts, and the study is an important and interesting contribution to the knowledge of seamount ecology. Although the paper is generally well written, it lacks some important information regarding methodological aspects, the results are not always presented precisely, and the discussion is superficial in parts, with the results not being fully exploited. For example, the samples were taken in three consecutive years, which could have biased the results considerably, but it is nowhere mentioned which samples were taken in which year and how they differed, and interannual variability and its possible consequences are not discussed. The results of the hydrographic measurements are difficult to see; Fig. 3 is not helpful in this respect. The discussion of relative abundances focuses on only one phylum, but the interesting overall patterns are not considered. There are more issues throughout the text; details are given below. I think that a major revision can improve the paper considerably.

Detailed comments:

Abstract:

Line 28: I think it should read "at least 200 m", since only two depths were sampled

DONE

L.40/41: explain abbreviations HMA and LMA

The explanation of HMA and LMA has been removed from the abstract as it is given in the Introduction (LL79-81).

Introduction:

L46: although seamounts are widely recognised meanwhile, I would suggest to include a definition here
A definition was added (LL48-51).

L53: "stimulated primary productivity": insert citation here. This hypothesis has rarely been verified
A reference was added (L59).

Methods:

L100-108: a figure /map of the seamount location should be included here. Figure2C could be used as an inset to illustrate the bathymetry.

A map of the seamount location has been added (Supplementary Material S1).

Concerning Figure2C please see comment below where we explain that we prefer to keep Fig.2C together with Figs 2A and 2B.

More information is necessary of seamount features: base depth, shape
DONE (LL114-116).

L104: Why this isolated presentation of the near-sea bed temperature? The temperature distribution as derived from the CTD should be presented in the Results, including the allocation of water masses (see also below).

The near-sea bed temperature and the allocation of water masses are provided by Roberts et al (2018) and are not our own results. We have moved the sentence before the reference Roberts et al (2018) so that this becomes clearer (LL111-112).

L105: I do not quite understand what "minimal values" means in this context
This sentence has been clarified (L113).

L111: See also comment in the general evaluation. How were the samples distributed across the three years, which subset was taken in which year? This is essential information, since it is well known that temporal variability of water column properties is very high at different scales, and particularly at seamounts with their highly dynamic hydrographic regime.

All of the requested information along with an interactive map of the seamount and individual references on CTD and sponge samplings has been made publicly available on PANGAEA at the time of manuscript submission. Pls follow the link (L 430) to find this data depository.

L113: Again, a map of the sampling locations, including the sponge samples, should be included here, for example based on Fig. 2C.

See PANGAEA for the requested information. We think that this interactive visualization (PANGAEA) is more informative than a classical graph, as individual sampling points of seawater and sponges lay in very close vicinity to each other and overlap sometimes.

Looking at this figure, all stations were aligned, with some variation, along a W-E axis, with one exception, and I cannot see that any samples were taken along the 74°N-latitude.

The 74°N value has been corrected to 73.8°N (L124).

L117: replace "which" with "and".

DONE (L127).

L117-120: This statistic is strange here. First of all, it is not clear how an ANOVA can be applied to single measurements, but obviously some stations were pooled; the reason and which stations are not provided here (this becomes more clear only later). And even in this case, means and SEs (and hence an ANOVA) make no sense here, because the measurements of depth are no independent replicate measurements of a population, but are just taken at different locations. And, of course, it is trivial that the depth at the summit is shallower than at the flanks and at the base. . . That's how these regions are defined. Here, just the depth ranges should be indicated.

Thank you for raising this issue, this is indeed confusing. Once the MW and BW microbiome clusters were identified based on similarity of microbial communities irrespective of any metadata, we queried where they would fall into horizontal and vertical space. We found – based on ANOVA analyses of the individual depth data points underlying each cluster – that the clusters are indeed significantly different in depth. In fact, we consider depth as an independent replicate measurement at Schulz Bank. The sentences have therefore been moved from the methods into the results section (LL216-220).

L121: Were these sponges sampled at the same time as the water samples? The number of sponges sampled (i.e. four in each BW1-4 as presented later) indicates that a targeted sampling was done in the subareas defined by the microbial clusters of the near-seabed samples; i.e. probably much later. This information is important for the interpretation.

Sponges were sampled at the same time as the water samples. We had collected more sponges, of which a random subset was chosen so that the sampling design was balanced for statistical purposes. This is stated more clearly now (LL132-133).

L150: see also comment above. Without knowing the results, it is not clear what is meant here as "sampling region".

This is correct. The sentence was moved into the results after the clusters have been identified (LL261-262).

L186: the purpose of this correlation matrix is not clear. It is not dealt with in the discussion.

Please see our argumentation below.

Results:

L192ff: The extrapolations based on "machine learning" in the contour plots of Fig.2A and 2B appear very arbitrary, e.g. the N-S extension of MW1 in Fig. 2A, or, even worse, in Fig. 2B, where e.g. BW2 and BW3 extend far into a region which was not covered by samples and features different bathymetric (and most probably also hydrographic) conditions - this is highly unlikely. Even BW1, which was obviously found at only one station, appears to be present also in patches in the south and in the north. These extrapolations are confusing and also unnecessary for the interpretation of the results. I suggest to omit the extrapolations and show only the station dots with their respective colours indicating the allocations to the clusters.

We do not agree to remove this important figure which is key to our findings. While we have accrued a considerable amount of data on this remote location, machine learning is a very useful tool that allows us to expand our predictions into regions that could not be/may never be obtained by hands-on sampling. We are aware that these are "bioinformatic predictions" and have indicated that clearly in the legend. To make this predictive value even clearer, we have added the following sentence: "The further away predicted areas are from actual sample points, the higher is the associated uncertainty of these predictions."

Line 200: see above; Fig.2C should be presented in 2.1

We prefer to keep Fig. 2C here because it serves as an important reference point for Figs 2A and 2B.

L203: replace "overlying" with "overlying".

DONE (L224).

L202-206: Fig. 3 is too complicated, and the additional results (oceanographic setting) cannot be adequately deduced from the figure. I suggest to provide either simple T/D-plots, or a 2-dimensional contour plot of temperature with a clear indication of water masses along the main sampling axis. The figure may be useful for interpretations, but then in the discussion section We feel strongly that this conceptual overview should remain in the manuscript. It presents a 3D visualisation of microbiome clusters as derived by machine learning in the context of real oceanographic data that have either been collected (CTDs) or

that have already been published (water masses) using the exact same T/S data of the 2016 cruise (Roberts et al. 2018). Simplification is not possible without the loss of data. We propose that it should remain here so that the connection to the 2D visualization (Fig. 2) using the same color scheme is maintained.

L209: this is not quite logical; the exception from the biodiversity in MW being lower than in BW would be a higher (or equal) biodiversity in MW, but not a difference between BW samples. Why are no data presented here like for the overall richness in BW and MW samples?

Correct. The sentence has been changed (L228-231). A plot showing the overall richness in BW and MW samples has been added (Supplementary Material S2A).

L212: does this apply only to the summit or to all regions? L213: the difference between this analysis and the one before is not clear. What is "pool" in this respect, and how did these differ?

The sentence (LL232-236) has been modified. The seawater microbial community clusters within the two groups (MW, BW) were significantly different from each other in terms of their microbial community composition. Moreover, the pool of mid-water samples (MW1 + MW2) was significantly different from the pool of near-bed water samples (BW1 - BW4). This has been stated more clearly.

L218: be precise: obviously not samples, but sample regions defined by microbial clustering were compared.

Correct. The term mid-water samples have been replaced with mid-water clusters (L241).

L224-234: it would be interesting to see which clusters differed from each other in their biogeochemical properties (e.g. pairwise comparisons). Acc. to Fig. 2B, cluster BW1 consists of only one sample; how was this considered in the ANOVAs?

We would like to clarify that cluster BW1 consists of three microbial samples that are overlaying in Fig. 2B. However, the reviewer is right that for BW1 only one biogeochemical sample is included in the ANOVA analyses. The sample numbers in the other BWs are consistently higher. As the ANOVA design was thus unbalanced, we calculated Type III sums of squares to account for this aspect while performing ANOVAs. We added an according remark for clarification into the manuscript (LL158-161).

Standard Posthoc tests (TukeyHSD, Bonferroni, etc) are generally sensitive against unequal sample sizes. However, following the reviewer's suggestion, we run TukeyHSD tests (based on linear model fits) for those parameters which turned out to be significant in ANOVA analyses (i.e. depth, oxygen, nitrate, silicate). When doing so, we only observed one significant difference of BW1 in comparison to the other BWs (for nitrate). The most significant difference was between BW3-BW4, which are also the clusters with the highest samples numbers. We think that these Posthoc test results are strongly biased by technical issues due to unequal sample sizes. We conclude that those tests are not helping in improving the manuscript content-wise. Trends to answer this question for the interested reader can however be deduced from the boxplots (Fig.4) included in the manuscript.

General remark: In respect to the limited number of biogeochemical samples for BW1, we agree that the design could have been improved with biogeochemical measurements at additional sites across Schulz Bank. However, as we are – to the best of our knowledge – the first to characterize the microbial seawater community of Schulz Bank (and also among the first seamount pelagic microbial studies globally) we could not know already during sampling if our (already higher) spatial resolution at the summit would be sufficient. As reviewer#1 states, we have compiled a considerable number of samples for deep-sea work. Due to expensive ship time also more sampling within one year would not have been possible, but we see and acknowledge (see Pangaea) the respective limitations that the reviewer brings up.

L226: "increased with depth"

DONE (L250).

L223: Here, only the summit stations were compared with respect to their biogeochemical parameters. What about the other locations?

We only had two clusters in the mid-water (LL241-246). The near-bed water clusters were compared in the following paragraph (LL247-257) and further details are given in Supplementary Table S2.

L248: Was this correlation with depth statistically tested? How?

We determined microbial taxa that differed significantly across the near-bed water clusters by LEfSe analyses. The identified microbial clusters of the near-bed water are obviously categorical explanatory variables (and not continuous variables). Therefore we did not perform a correlation between the determined microbial clusters and any microbial phylum. Depth turned out to be among those environmental parameters that differed significantly across the near-bed water clusters. In Fig.6 we use depth as a proxy for the microbial clusters and the other three significant biogeochemical parameters to minimize complexity for the reader and give an ecological context. We have included correlation analyses in the revised manuscript, which clarify that there is indeed a significant correlation between depth and those biogeochemical parameters.

Concerning the “correlation with depth” (e.g. L275) we are referring to the following: To assess “correlation with depth”, we plotted for all samples of every sponge species the relative abundances of each microbial phylum across all near-bed water clusters (boxplots with near-bed cluster on x-axis and relative abundance of the phylum on y-axis). By visual inspection we determined for those taxa turning out as significant in the LEfSe analysis, if they follow the same profile as depth (boxplot Fig.4).

We changed the term “correlation” to the term “relation” throughout the manuscript, where it was used in this context. Correlation now only refers to a statistically tested correlation *sensu stricto* (i.e. for example Spearman correlation).

L255: Interestingly, looking at Fig. 6, Protobacteria had a much higher relative abundance in BW1 than in the other clusters, whereas Gemmatimonadetes had much lower abundance in BW1, but in both phyla differences were not significant. Is there an explanation?

We are reporting here on those phyla that were statistically significantly different between the BW clusters. One explanation is that statistical significance will depend not solely on the abundance in one particular BW cluster but across all four BW clusters.

In this context, it would be very interesting which clusters differed from each other. For example, Fig. 6 suggests that the differences were mainly between BW1 and the other clusters, which showed only small differences. Could this be tested?

Please see our comment above about pairwise comparisons. In brief, we are limited in the number of biogeochemical samples particularly for BW1 as the reviewer has realized. We provide an argumentation above why we think our non-classical study design is leading to valuable, novel insights and prefer to put the focus of this study on overall trends across clusters, instead of pairwise considerations between clusters.

L258: see also comment in M&M. This information is not further used, and it is hardly or not at all discernible from Fig. 6.

We have added sentences evaluating on this result in the Discussion (LL383-386). Thanks for bringing this to our attention, as we think that this result is indeed very interesting. Based on this result we suspect that primary responders to environmental parameters have cascading effects on microbial lineages that are not directly affected by water biogeochemistry.

Concerning the suggested omission of the correlation matrix, we are the first to compute co-occurrence networks for deep-sea sponge microbiomes. It illustrates that different numbers and types of microbial taxa vary across the near-bed clusters in every sponge species and in seawater. This information is valuable for more mechanistic follow-up analyses.

L264: where is this analysis (correlation between biogeochemical parameters and relative abundances), and how was the statistics done (was this correlation independently tested?)? In Fig. 6, only some relationship between depth and relative abundances is indicated, with differences between depths always corresponding to differences between clusters

Please consider our detailed comment above. Depth is here taken as a proxy, because the detailed statistical analysis of relative abundances within each phylum in each sponge species against each biogeochemical parameter would be beyond the scope of this study. Here, we report on the general observation, which is novel and exciting as both reviewers acknowledge.

L266: it is not clear what "significant variability" means in this context, and how this variability was tested. L267: which pattern?

These two points have been clarified: The microbial taxa showing a significant difference in relative abundance between the near-bed water clusters (as determined by LEfSe) were different between sponges and seawater also between sponge species.

Further, significant differences in relative abundances were observed for both abundant and less abundant sponge symbiont lineages (LL293-296).

Discussion:

L276: But according to the results (L212ff) community clusters were significantly different between BW and MW samples. This contradiction has to be resolved.

With this statement, we are referring to the few black spots (representing BW1) that cluster with MW samples (Fig. 1). Even though these BW samples are more similar to the MW samples, they don't have to be identical. The wording has been revised accordingly (LL309-310).

L284: Since only one LW depth was sampled, this process could extend far higher than the 200 m, so it should better read "at least". But the process may not have necessarily been restricted to the summit, because due to the much greater distance between LW and BW samples at the other stations, a similar effect may just not have been detected.

The wording has been changed to "at least" (L317). While we agree that similar effects may apply to other depths, we prefer here to discuss the presented data.

L291: this applies also to the southern hemisphere!

To our knowledge, anti-cyclonic circulation patterns (as indicated by the counter-clock wise arrow in Fig. 3) are specific for the Northern hemisphere. We prefer to leave the sentence unchanged.

L293: this is far from clear and cannot be deduced from Fig. 3. Apart from the separated clusters at the summit, which may in fact be related to retention and vertical mixing by, e.g., a Taylor column, it is not shown how differences between stations could relate to oscillations of the water column.

The oscillations relate to tidal-induced variations in the water column structure, hence do not reflect differences between stations but rather similarities between mid-water and near-bed water samples at the summit. Please consider our interpretation in LL315-319. We have included a clustering dendrogram into the legend of Fig. 3 to clarify similarities and dissimilarities of microbial communities between the water clusters. Further we have added arrows for clarification of tidal-induced water movements.

L300: this comparison is hardly applicable here. The Morato et al paper deals with large pelagic predators, and their enhanced biodiversity at seamounts, which is not restricted to the summits, has underlying mechanisms completely different from microbial communities.

The sentence has been reworded (LL331-333).

L307: in which respect do they change? Some information would be helpful (without needing to consult the literature)

The sentence has been reworded (LL340-341).

L310: These are not discernible in Fig. 3. See also comment in the General Evaluation concerning Fig.3

Clarification has been added (LL343-345).

L312: it is not clear what is meant by "dense ecosystems"

The sentence has been reworded (LL346-349).

L316: include "probably" before "based" - there is no direct evidence

DONE (L351).

L350: "were positively correlated with depth. . ." No correlation analysis was done between biogeochemical parameters and depth, but discrete ANOVAs for each parameter which revealed differences between cluster regions. These appeared to covary with depth.

Thanks for this thoughtful suggestion. We calculated Spearman's rank correlation coefficients between depth and those biogeochemical parameters which turned out to differ significantly across the determined near-bed water clusters in the ANOVA analyses (LL159-161; LL253-255; S3).

L353: where does this prediction come from?

"Prediction" has been replaced with "expectation" (L392).

L354: this ("typically carry more oxygen. . .") is redundant to the statement before

The first statement refers to the process, the latter to concentration; this is an accurate sentence in our opinion.

L361: is it really depth (pressure?) that structures the communities, or depth-associated parameters?

We are here citing Indraningrat et al (2019) and prefer to be consistent with the authors' wording.

L363-370: Why are only Chloroflexi in *G. hentscheli* discussed? What about the other phyla and sponge species? Particularly with *G. hentscheli*, there are some interesting patterns which should be discussed in more detail. For example, whereas the relative abundances of most taxa are very similar in the BG2-BG4 clusters, the abundance of Chloroflexi and Acidobacteria are much lower in BW1, whereas Protobacteria are much higher - is there any explanation?

We have chosen this phylum as an example, because Chloroflexi are abundant and representative sponge symbionts, because the relative abundances are co-varying with depth, and because we show data for all BW clusters in *G. hentscheli*. We do not see the value to discuss this pattern with all clades because this would enormously inflate the discussion. An explanatory statement along these lines has been added (LL402-406).

L363: Acc.to Fig. 6, there is a big difference between BW 1 and the other clusters, which are very similar to each other. Has it been tested which clusters differ from each other?

The aim of our study is to explore changes in the sponge microbiome across the different near-bed water clusters. As explained above, depth serves as a proxy for the selected biogeochemical parameters that correlate with depth. We did not aim to relate changes in sponge microbiomes with each cluster separately. Please consider also our comments above.

L364: I cannot find any results about a positive correlation between these parameters and the clusters

Please see our comments above.

L367-369: this is not quite clear here. Usually, the oxygen demand is enhanced by the (microbial) degradation of OM, which on the other hand sets free nutrients such as NO₃⁻ and may enhance denitrification processes. It would be interesting in this respect to learn something about the metabolic pathways of Chloroflexi, e.g. whether they are involved in denitrification, which could explain a positive correlation with NO₃⁻.

We are not going into functions of individual phyla because the biological interactions between the sponge host and its microbiome, or between the microbes themselves might have masking effects. Pls see our statement (L417-418).

Conclusions:

L378:"high resolution sampling" is rather meaningless - distributing sampling over three years is rather not high resolution, and whether the spatial resolution is high is also questionable. I suggest to omit this; it is not necessary.

The sentence has been reworded (LL420-421).

L380: "has a detectable but variable influence. . ." I would be careful with this statement. There appeared to be some interrelation (a statistical correlation was not shown), but it could not be convincingly shown that a causal relationship with those parameters was highly likely, or which of the three was probably the key parameter. A possible mechanistic explanation would be interesting, for example with respect to metabolic functioning of the microbial phyla. What about interannual variability - the paper does not provide any information that would rule out a possible effect of the sampling dates.

“Interannual variability”: Please note that during the sampling process, we always sampled seawater references with a time lag of maximum a few hours to according sponge sampling. More details on the sampling dates have been made publicly available in the Pangaea database before submission.

“Variable influence”: We are referring to the observation that we observed no uniform patterns for all three analysed sponge species. Whether these patterns may be individual-specific or species-specific and which environmental parameter is the key parameter, exceeds the scope of this study.

We are reporting here correlation but not causation or mechanistic relations/functions between sponge microbiomes and seawater biogeochemistry. Without deep metagenomic data on functional gene inventories this is not the goal of the present study.

Figures:

Fig. 2: see comments in Results. Fig. 2C belongs into the Methods section

Please see comments above.

Fig. 3: This Figure should be placed into the Discussion and help interpreting the results. It is not suitable for the presentation of results, because, for example, the temperature profiles and water mass distribution are not readily identifiable in the 3D setting

The microbiome clusters presented in Fig. 3 come from our own analyses, the flow patterns and oscillations of the water column (dashed lines) were taken from Roberts et al (2018) who already provided the more detailed data and 2D-visualisations which the reviewer requests (above). The novelty in our study is the integration of this physical data with the newly generated microbial data. We have added a clustering dendrogram into Fig.3, which is an overarching result of our study. We prefer to leave this figure with the results.

Fig. 4: y-axis labelling is missing. Degrees of freedom of the ANOVAs should be included.

Thanks! We added y-axis labels, the degrees of freedom of the ANOVAs (into Fig.4), and adjusted the figure legend accordingly.

Fig. 6: the correlation matrix should be omitted - it is not used and is hardly (A and B) or not all discernible (C and D).

See comments above.

Panel C: *Geodia* in italics.

DONE.