

Microbe biogeography tracks water-masses in a dynamic oceanic frontal system

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Abstract

Dispersal limitation, not just environmental selection, plays an important role in microbial biogeography. The distance-decay relationship is thought to be weak in habitats where dispersal is high, such as in the pelagic environment, where ocean currents facilitate microbial dispersal. Most studies of microbial community composition to date have observed little geographical heterogeneity on a regional scale (100s km).

We present a study of microbial communities across a dynamic frontal zone in the South West Indian Ocean and investigate the spatial structure of the microbes with respect to the different water masses separated by these fronts.

We collected 153 samples of free-living microorganisms from five seamounts located along a gradient from subtropical to subantarctic waters and across three depth layers, 1) the sub-surface chlorophyll maximum (c. 40m), 2) the bottom of the euphotic zone (c. 200m), and 3) the benthic boundary layer (300-2000m). Diversity and abundance of microbial operational taxonomic units (OTUs) was assessed by amplification and sequencing of the 16S rRNA gene on an Illumina MiSeq platform.

Multivariate analyses showed that microbial communities were structured more strongly by depth than by latitude, with similar phyla occurring within each depth stratum across seamounts. The deep layer was homogeneous across the entire survey area, corresponding to the spread of Antarctic intermediate water. However, within both the sub-surface layer and the intermediate depth stratum there was evidence for OTU turnover across fronts. The microbiome of these layers appears to be divided into three distinct biological regimes corresponding to the subantarctic surface water, the convergence zone, and subtropical. We show that microbial biogeography across depth and latitudinal gradients is linked to the water-masses the microbes persist in, resulting in regional patterns of microbial biogeography, that correspond to the regional scale physical oceanography.

Keywords: Southwest Indian Ridge, Microbe biogeography, Dynamic Frontal Systems

1 Introduction

The world's oceans are teeming with an enormous pool of diverse microscopic life forms. Ecologically, microbes play a vital role in marine food chains and global nutrient cycling and are involved in virtually all geochemical reactions occurring in the oceans [1, 2]. A few studies have tried to tease apart depth and geographical distribution patterns of microbial taxa [3–7]. In the first global study of prokaryotic microbes by Pommier *et al.* [6] only two taxa, the Alphaproteobacterium, *Pelagibacter ubique*, and the photosynthetic cyanobacterial genus, *Synechococcus*, were found to be cosmopolitan. Furthermore, 69% of the identified operational taxonomic units (OTUs) were unique to their collection location. It has been demonstrated that species richness varies with season and peaks in high-latitude waters during winter [8–10]. Sul *et al.* [11] showed that most microbial OTUs did not exhibit a bipolar distribution and argued that their findings suggest that Bacteria follow biogeographic patterns more typical of macroscopic organisms, and that dispersal limitation, not just environmental selection, likely plays an important role. The exact nature of the latitudinal gradients in richness, abundance and diversity in Bacteria is still uncertain because of the substantial unexplained spatial and temporal variation of taxon occurrence, however, OTU richness has been shown to correlate with temperature, salinity, primary productivity, and depth [5, 12–15]. Changes in ocean currents and productivity may therefore be responsible for changes in observed Bacterial and archaeal diversity. In addition, microbial community turnover has been observed across oceanic fronts in surface water masses [16], but less is known across water masses for deeper strata. The deep ocean is often considered a relatively uniform environment with stable physical parameters [4], with different microbial communities persisting in deep ocean water masses between ocean basins on a global scale.

The biogeography of microorganisms is undoubtedly directed by the evolutionary and ecological interaction of selection, genetic drift, dispersal, and genetic mutation [17]. According to Hanson *et al.* (2012) the distance-decay relationship, which states that the similarity between two locations declines as geographical distance increases, should be relatively weak in habitats where dispersal is high, such as in

85 the pelagic environment, where ocean currents facilitate microbial dispersal. A dis-
 86 tance effect on microbial community composition is most often observed at small
 87 (0–1 km) [18] or very large spatial (>5,000 km) scales, i.e. between ocean basins
 88 [4, 5]. A small-scale distance effect may be the result of microbial aggregation [19],
 89 which can be caused by dispersal limitation. Investigating microbial communities at
 90 regional scales (100s km) and across depth strata is imperative, as this is the scale
 91 at which different ocean masses create contrasting physical conditions and thus con-
 92 trasting microbial communities [20]. Little is known about the depth distribution
 93 of microorganisms in many ocean basins, especially across mid-ocean ridges and the
 94 influence of those ridges on microbial dispersal. Open-ocean seamounts are con-
 95 sidered to be “hotspots” of marine life but their role in microbial dispersal is still
 96 under discussion [21]. [22]. They are often considered unique ecosystems in terms
 97 of their structure and sometimes high biomass of the benthic and pelagic biological
 98 communities [21, 23–25]. While the ecology of metazoans on seamounts has received
 99 considerable attention, studies focusing on lower trophic levels, and microbial pro-
 100 cesses on seamounts in particular are lacking, despite their potential strong influence
 101 on biogeochemistry [26–29]. Seamounts are very dynamic hydrological habitats and
 102 may in some instances create local enhancement of large autotrophic cells and pi-
 103 coplankton (i.e. near the summit or flanks of seamounts) [27]. Surveying seamounts
 104 on the Southwest Indian Ridge we present a comparative study of the three di-
 105 mensional microbial biogeography around seamounts from the sub-Antarctic to the
 106 subtropics in a dynamic frontal system.

107 This study focuses on differences in community composition on local (1–2 km) and
 108 regional (100s km) geographical scales, as well as along a 2 km depth range at each
 109 site. We aim to horizontally and vertically delineate the microbial communities of
 110 different water masses across one of the world’s most hydrologically dynamic regions,
 111 the Southwest Indian Ocean [30, 31].

112 2 Materials and methods

113 2.1 Sampling

114 Sampling was carried out during the *RRS James Cook* voyage JC66 from 4th Novem-
115 ber – 20th December 2011. Conductivity, temperature, and depth (CTD) profiles,
116 as well as all water samples, were collected with a SeaBird Electronics SBE +911
117 CTD and rosette fitted with Niskin bottles of 10 l volume. Samples were collected
118 along transects across the seamounts with six CTD deployments on Coral, Melville,
119 Middle of What, and Atlantis seamounts and a single CTD deployment on the sum-
120 mit of Sapmer seamount (Fig. 1). An in-situ fluorometer measured Chlorophyll *a*
121 fluorescence to a maximum depth of about 300 m [32] on all CTD deployments.

122 1 l of sea-water was filtered on a 0.22 μm filter from each CTD deployment at the
123 chlorophyll maximum (40 - 80 m, referred to as shallow stratum), at the boundary of
124 the euphotic zone (\sim 200 m depth, referred to as mid stratum), and 10-20 m ($>$ 500 m
125 depth) from the seafloor (referred to as deep stratum). Two samples were collected
126 from each depth layer from separate Niskin bottles. During the fieldwork, a total of
127 153 samples were collected for sequencing of microorganisms and 223 samples were
128 collected for quantification of microorganisms through flow cytometry (Table 1).

129 For every CTD deployment, water samples for nutrient analysis were collected
130 at the same locations as particulate organic carbon (POC) and flow cytometry
131 (FC) samples. Data from the flow cytometry and POC analysis were acquired
132 from Djurhuus *et al.* 2015 [29]. We collected 173 samples for macronutrients, ni-
133 trite, nitrite+nitrate, phosphate and silicate. They were analysed using a 5 channel
134 Technicon AAII segmented flow analyser [33]. Analyses were calibrated, quality
135 controlled, and checked against KANSO-certified nutrient reference materials. All
136 environmental data was used in an analysis of drivers of microbial community struc-
137 ture, see multivariate regression analysis below.

2.2 Illumina MiSeq sequencing and preparation

DNA extraction, PCR and sequencing were performed using a modified version of the protocol presented in Caporaso *et al.* [34], adapted for the Illumina MiSeq according to the Earth Microbiome Project (EMP) standards [35] (<http://www.earthmicrobiome.org/emp-standard-protocols>). In brief, the genomic DNA was extracted from sub-samples of the water filters using a Powersoil-htp 96 Well DNA isolation kit (MoBio) with a 10 min. (65°C) incubation step modification. The V4-V5 region of the 16S rRNA gene was amplified with 515F/806R primers with 12 base pair (bp) barcodes. Amplification primers were adapted from Caporaso *et al.* [35] to include nine extra bases in the adapter region of the forward amplification primer that support paired-end sequencing on the MiSeq. Amplifications were done in triplicate and followed the EMP PCR protocol. PCR products were pooled at equimolar concentrations and cleaned using the UltraClean® PCR Clean-Up Kit (MoBio). 16S rRNA amplicon sequencing was conducted at the IGSB Next Generation Sequencing Core at Argonne National Laboratory using 151 bp paired-end sequencing on an Illumina MiSeq instrument.

Quality filtering of reads was applied, as described previously [35]. Reads shorter than 75 bases and chimera's and reads whose barcode did not match an expected barcode were discarded.

2.3 Bioinformatics

All bioinformatics were conducted using QIIME [36]. Forward and reverse raw sequences were combined using PEAR [37]. Joined reads were demultiplexed and quality trimmed. An open-reference OTU picking strategy was used, where OTUs were clustered against the GreenGenes 13_8 reference sequences using uclust ref [38] and reads with no hit to the reference sequence collection were subsequently clustered de novo at the 97% similarity level using uclust [38]. Reads were assigned to OTUs based on their best hit to this database at $\geq 97\%$ sequence identity. PYNAST [34] was used to align OTU sequences and OTU taxonomy was assessed using the RDP classifier retrained towards the GreenGenes database (97% similarity) [39].

Median sequence counts per sample after OTU picking were 22,522 with a standard deviation of 8,321. To generate a final OTU table, sequences not aligning in the PYNAST step were removed, and a subsampled OTU table was created by random sampling to an even depth of 11,270 sequences per sample and all singletons were removed.

Taxonomy was assigned to each read by accepting the Greengenes taxonomy string of the best matching Greengenes sequence. Data is available through DRYAD at <http://dx.doi.org/10.5061/dryad.qh767>.

2.4 Data analysis

R v3.2 (R Core Team, 2016) was used for all statistical analyses. For ordination and richness analyses, the R package *vegan* was used [40].

For species richness estimates we used observed richness. Differences in bacterial abundances and richness between stations were compared using an ANOVA and *post hoc* Tukey HSD tests.

Variation of microbial community structure with depth was assessed using non-metric multidimensional scaling (NMDS) analysis on Bray-Curtis dissimilarities, with 10,000 random permutations.

Multivariate Regression Tree (MRT) analysis [41] was used to identify a hierarchy of environmental factors and their individual contribution to microbial community structure. This method performs hierarchical dichotomous clustering of community data by selecting environmental parameters that maximize the homogeneity within groups of samples. Accordingly, these clusters are characterized by both a homogeneous assemblage structure and similar covariate values. MRTs do not employ significance testing but use cross-validation (CV) to determine the optimal number of dichotomous splits and the importance of predictor variables [41]. We used the R package *mvpart* 1.6-0 [42] to perform the analyses on Bray-Curtis dissimilarities [41] with salinity, temperature, depth, latitude, oxygen, Particulate Organic Carbon, phosphate, silicate, nitrate, and nitrite from previously published data [29]. Additionally, Indicator Species Analysis (ISA) [43–45] was performed using the R

196 package indicpecies [43, 45] to identify microbial taxa associated with splits of the
 197 MRT. This method aims at detecting OTUs that represent distinct ecological set-
 198 tings and indicate location-specific community types. This index is maximum when
 199 all individuals of a species are found in a single group of sites and when the species
 200 occurs in all sites of that group; it is a symmetric indicator.

201 Data was read into R, manipulated and visulaized using the ggplot2 and phyloseq
 202 packages [46].

203 3 Results

204 3.1 Depth distribution of microbial communities

205 As shown in [29] the seamounts reflect the environmental setting their situated
 206 in. Coral had a relatively higher nutrient availability and lower temperature in the
 207 surface, reflecting the mesotrophic environment, while Atlantis had oligotrophic con-
 208 centrations of nutrients, with a higher temperature in the surface layer. There were
 209 large differences in cell counts from the shallow strata between seamounts (Fig. 7).
 210 Coral (the southernmost seamount with lowest temperature) had the highest aver-
 211 age abundance (1.16×10^6 cells/ml ± 0.285) and Atlantis (northern most seamount
 212 with highest temperature) the lowest (0.50×10^6 cells/ml ± 0.179) (Tukey HSD,
 213 $df=2$, adjusted $p < 0.001$), Melville and Middle of What had similar average abun-
 214 dances in the shallow layer (0.926 and 0.925×10^6 cells/ml ± 0.172 , respectively)
 215 that were significantly different to Coral (Melville-Coral, Tukey HSD, $df=2$, ad-
 216 justed $p = 0.008$, MoW-Coral, Tukey HSD, $df=2$, adjusted $p = 0.006$) and Atlantis
 217 seamounts (Melville-Atlantis, Tukey HSD, $df=2$, adjusted $p < 0.001$, MoW-Atlantis,
 218 Tukey HSD, $df=2$, adjusted $p < 0.001$).

219 On Coral, we observed a total of 27,544 OTUs, while Melville had 28,821 OTUs,
 220 Middle of What had 31,996 OTUs, and Atlantis had 21,988 OTUs similar to what
 221 was found in marine environments by Zinger *et al.* [47]. The observed OTU richness
 222 did not vary significantly between seamounts (ANOVA, $F_{3,49} = 1.03$, $p = 0.39$;
 223 Fig. 8).

224 However, the middle stratum showed higher richness than the shallow and deep
 225 stratum (ANOVA, $F_{3,92} = 2.123$, $p = 0.039$: Fig. 8, Table 2). The strata also
 226 exhibited differences in their microbial community composition, demonstrated by the
 227 NMDS (Fig. 2; MANOVA $p < 0.01$) with clear separation between the depth strata.
 228 Gammaproteobacteria dominated all depth layers, but the shallow layer had a higher
 229 abundance of the photoautotrophic class Synechococcophycideae compared to the
 230 middle and deep layers. In addition the classes Flavobacteriia and Acidimicrobiia
 231 were more abundant in the shallow layer. Thaumarchaeota and Deltaproteobacteria
 232 both increased from shallow to deep (Fig. 9).

233 Bacteria accounted for 86.5 % and Archaea for 13.5 % of all sequences in the
 234 entire study. The most abundant phylum was Proteobacteria (61.3 %) with classes
 235 Gammaproteobacteria (26.6 %), Alphaproteobacteria (16.8 %), and Deltaproteobac-
 236 teria (8.7 %) being the most abundant. Other abundant classes were the Thau-
 237 marchaeota (9.7%), Acidimicrobiia (5.7 %), and Synechococcaceae (3.5 %, Fig. 9).
 238 Thaumarchaeota accounted for 72% of all Archaea sequences. Two OTUs dominated
 239 in terms of abundance and accounted for approximately 10% of all sequences: One
 240 is classified in the family SAR324 (Deltaproteobacteria and the other is from the or-
 241 der Oceanospirillales (Gammaproteobacteria). Both Oceanospirillales and SAR324
 242 have a high abundance at all seamounts and all depths (Fig. 9).

243 Optimal tree size for the multivariate regression analysis, varied between 5 and
 244 10 (Fig. 10). An optimal tree size of 6 occurred most frequently (50% of all trees).
 245 Following Death *et al.* [41] we picked the tree of size 6 for further analyses, as it
 246 was within 1 standard error of the optimal tree, and also the most frequent optimal
 247 tree. It revealed that depth, and latitude together explained 66% of variation in
 248 community composition (Fig. 3). As indicated by the hierarchical order of splits
 249 and branch lengths of the MRT, depth was the main explanatory factor (split 1;
 250 Fig. 3), separating all seamount samples below 493 m (Cluster 3 in Fig. 3) from the
 251 rest. The cluster shallower than 493 m was again separated at 125 m, distinguishing
 252 the middle (Clusters 1, 4) and shallow layer (Cluster 2, 5, and 6). Coral seamount
 253 middle and shallow clusters are distinct from all other stations (Clusters 1, 6) and in

254 the shallow layer (at the chlorophyll maximum) Atlantis clustered separately from
255 all other stations (Cluster 2).

256 The indicator species analysis (ISA) revealed that there were 286 taxa associ-
257 ated with the different clusters (Table. 2). From those 286 the two taxa most
258 indicative of each cluster were as follows: chlorophyll maximum cluster of Coral
259 was distinguished with Marine group III and SAR202 and the middle strata with
260 the genera *Nitrosopumilus* (Cenarchaeaceae) and HTCC (Alteromonadales). The
261 surface clusters of Melville, Middle of What, and Sapmer were associated with the
262 genus *Prochlorococcus* while the middle layer was associated with *Synechococcus*.
263 The surface strata of Atlantis was associated with *Pseudomonas* and *Oceanospiril-*
264 *lales* and the middle was associated with *Synechococcus* and *Coralimargarita*. All
265 deep clusters were associated with *Candidatus "portiera"* and Halomonadaceae.

266 3.2 Regional biogeography of seamount microorganisms

267 As demonstrated by the multivariate regression tree, depth is the main predictor of
268 the microbial community structure across the survey area.

269 The relative abundance of microbes between the seamounts differs at the Class
270 (Fig. 9) and OTU levels (Fig. 4). Overall, the community structure, as captured
271 by the rank abundance of OTUs, changes substantially between Coral and Melville
272 and again between Middle of What and Atlantis. Melville and Middle of What are
273 very similar across all individual strata (Fig. 4). There are large changes in rank
274 abundances between the dominant OTUs from Coral to Atlantis. In the shallow layer
275 the ranks of Synecococcaceae (a) and Halomonadaceae decrease from dominating
276 on Coral (ranks 1 and 3, respectively) to uncommon on Atlantis (ranks 32 and 42,
277 respectively). The opposite latitudinal pattern, an increase in rank abundance, was
278 observed for OCS155 and *Synechococcus* (Prochl.) from ranks 21 and 50 at Coral,
279 respectively to ranks 2 and 1, respectively, at Atlantis. In the middle layer the
280 changes in rank abundance are similar to those in the shallow layer, with the most
281 abundant OTUs on Coral becoming the least abundant on Atlantis and vice versa. A
282 large cluster of OTUs in the middle layer decreased in relative abundance from Coral

283 to Melville (Fig. 4). These OTUs include Alteromonadales, HTCC2188 (OM182
 284 clade), Flavobacteriaceae, Pelagibacteraceae, Marine Group II, Crenarchaeacea, and
 285 Pseudoalteromonadaceae. The cluster occupies high ranks on Coral, but most of
 286 its OTUs are of lower rank on all other seamounts doing a taxa turn-over from
 287 Coral to Melville. The five most abundant OTUs in the deep layer are highly
 288 ranked throughout the seamounts. In general, the deep layer exhibits fewer rank
 289 changes of the dominant OTUs than the shallower strata, representing a more stable
 290 environment across the study area.

291 4 Discussion

292 4.1 Depth distribution of microbial communities

293 Our study reveals that the community composition of microorganisms along the
 294 SWIR is similar to open-ocean and deep-sea environments globally [5], with Al-
 295 phaproteobacteria and Gammaproteobacteria, being abundant throughout the wa-
 296 ter column. We found very similar relative abundances of all phyla to Sunagawa *et*
 297 *al.* [5], with the exception of much lower abundance of Alphaproteobacteria in our
 298 samples. In our study, the dominating primary producers are the Synechococcales,
 299 which have been found in similar abundances globally [29, 48, 49]. This is in contrast
 300 to Alves-Junior *et al.* [12] who found members from the order Prochlorales dominat-
 301 ing at the surface and chlorophyll maximum layer in the southwest Atlantic Ocean
 302 at a similar latitude. In the shallow layer we also found the actinobacterial clade,
 303 OCS155, which are heterotrophic and prolific producers of secondary compounds
 304 [50], likely relying on excretion of organic compounds from primary producers.

305 We found similar relative abundances of total Gammaproteobacteria in the deep
 306 layers to Alves-Junior *et al.* [12], although in our study it was dominated by the order
 307 Oceanospirillales, and not Alteromonadales. The MRT suggested distinct differences
 308 in community composition at depths >493 m indicated by characteristic deep-sea
 309 microorganisms SAR324 and Oceanospirillales. SAR324 has been implicated in
 310 sulphur oxidation, carbon fixation and heterotrophy. The versatile metabolisms

311 of SAR324 (lithotrophy, heterotrophy and alkane oxidation) all operate simultane-
 312 ously, and may explain SAR324's ubiquity in deep oceans [51]. Oceanospirillales
 313 is a psychropiezophilic microorganism, which explains its preference for the deep
 314 sea, and colder environments, such as the subantarctic Coral seamount rather than
 315 Atlantis, which is in subtropical waters [52]. Further, our samples collected at 200
 316 m, show higher abundance of Thaumarchaeota at depths just below the chlorophyll
 317 maximum, which is consistent with Sunagawa *et al.* [5].

318 Overall, we found that most of the abundant microorganisms in the deep layer
 319 were microorganisms with similar metabolic characteristics or environmental pref-
 320 erence, enabling widespread geographic distribution across the deep-layer. At the
 321 surface, we found a high abundance of microorganisms adapted to high light condi-
 322 tions, such as Synechococcaceae and the actinobacterial clade OCS155. The middle
 323 layer appears to be a mixture between the deep and shallow communities. It has
 324 a high abundance of several groups common in deep (e.g. Thaumarchaeota and
 325 Deltaproteobacteria) and shallow (Flavobacteriia, Alphaproteobacteria and Syne-
 326 chococcaceae). The layer below the chlorophyll maximum has been established
 327 as the area where most re-mineralisation takes place in the water column, which
 328 may explain the presence of high abundances of heterotrophic microorganisms such
 329 as Pelagibacteraceae capable of degrading dimethylsulfoniopropionate (DMSP) re-
 330 leased by decaying phytoplankton [53]. This might explain the pattern of higher
 331 species richness seen in the middle stratum (Fig. 8) with a habitat structure sim-
 332 ilar to that of the deep-sea but a stronger influence from the surface, creating an
 333 intermediate habitat where psychrophilic, piezophilic, and primary producing mi-
 334 croorganisms can co-exist: some of them thriving and some existing as transient
 335 members, sinking away from the surface. The higher richness of the middle layer is
 336 enhanced in the convergence zone (Melville and Middle of What), creating a mix-
 337 ture of the deep-sea and surface microorganisms of both subtropical and subantarctic
 338 watermasses.

339 It has been shown that primers used in this study can overestimate certain
 340 taxa, i.e. Thaumarchaeota and Gammaproteobacteria, and underestimate SAR11

341 in environmental samples [54, 55]. This probably influenced the results of this
 342 study, especially in terms of relative abundance of the different phyla, causing the
 343 Gammaproteobacteria to be dominating abundance and Alphaproteobacteria less so
 344 (Fig. 9, supplementary information). While we acknowledge the limitations of the
 345 primers used we do contend that any biases are consistent across the study, leaving
 346 the comparisons within the study unaffected. Since all samples were sequenced
 347 using the same primer, they would all be biased in a similar fashion and the main
 348 conclusions of the study are not affected. The purpose of this study was to delineate
 349 patterns of community structures between locations and get an inference of what
 350 drives the microbial communities. We would argue that our patterns still hold true
 351 given that all the samples are analysed in the same way. Independent of primer
 352 there will always be a bias towards or against certain taxa. It has been argued
 353 that reducing primer biases is especially important in the case of applications such
 354 as association networks or predicting functional processes [56], which is not the
 355 objective of this study.

356 For marine macrofauna depth is a stronger predictor of metazoan community
 357 structure than geographical location [57]. Some studies have showed depth varia-
 358 tion in microorganisms, but most focus has been on coastal areas or the surface layer
 359 of the open-ocean [5, 12]. Here we emphasize that, like macrofauna, the microbial
 360 community is segregated by depth [5, 12, 58]. Temperature has been argued to be
 361 the most important driver of depth changes however, because temperature decreases
 362 with depth, its relative effects on microbial communities is difficult to disentangle
 363 [5]. Because temperatures decrease with depth, depth effect on microbial communi-
 364 ties might not be caused by temperature but by the fact that the general physical
 365 environment changes markedly with water mass through depth [29]. Temperature
 366 is also an indicator of water mass, and can thus be further confounded with loca-
 367 tion when investigating biogeography on a basin or global scale. Depth appeared
 368 to be a stronger predictor of microbial community structure than geographical loca-
 369 tion, although we did observe geographic differences in the microbial communities
 370 of the euphotic zone at the Northern and Southern extremes of the survey area, as

371 compared to the centre of the convergence zone.

372 Different seamount morphologies, as well as the variability of impinging currents,
373 result in a broad range of hydrodynamic patterns, the relative strength and persis-
374 tence of which may vary greatly in space and time [59]. Consequently, the effect
375 of seamounts on biological communities may be highly intermittent and difficult
376 to observe on the spatial and temporal scales accessible by vessel-based research.
377 Mendonca *et al.* [27] observed higher microbial biomass and abundance on the
378 summit of Seine and Sedlo seamounts in the North Atlantic Ocean, compared to a
379 reference background sample. We cannot provide insight into potential differences
380 between on-seamount and off-seamount samples, but we were able to investigate
381 within seamount differences. All seamounts were relatively homogeneous within
382 each depth layer and the MRT did not separate the summit or benthos of individual
383 seamounts from the remainder of the samples. Although significant heterogeneity of
384 microbial community composition has been described on local scales (1-10s km) [18],
385 the depth division of our samples is greater than the between sample differences at
386 the same depth at geographical distances in the order of (10-100s km). Metazoan
387 community differences between the pelagos and the benthic boundary layer are well
388 documented and have been observed on the SWIR [60], and elsewhere. However,
389 little is known about differences between demersal and pelagic microbial commu-
390 nities. We did not observe marked differences between the microbial communities
391 from samples with differing distances to seabed within a depth layer, although com-
392 parative samples are only available for Coral and Melville, given the deep summits
393 of the other seamounts. It has been shown previously that the particulate organic
394 carbon can be depleted on the summit of the same seamounts [29], however, this
395 was not reflected in differences in the community composition of samples from the
396 summit vs. the flanks. Given the sampling design of our study, distance to seabed is
397 confounded with depth and so the study cannot unravel potential differences of the
398 benthic boundary layer microbiome compared to open ocean microbial communities
399 at similar depth. There is no difference between samples of the same depth relative
400 to distance to seamount.

4.2 Regional biogeography of seamount microorganisms

The SWIR is located in an area where the Agulhas Return Current (ARC), the Sub-Tropical Front (STF) and the Sub-Antarctic Front (SAF), further to the south, create one of the most energetic and important hydrographic regions of the global ocean [61]. In the frontal zone (Melville, Middle of What, and Sapmer), peak chlorophyll concentration in excess of $1 \mu\text{g l}^{-1}$ has been recorded [61]. Outside this region, chlorophyll concentrations have been measured at $<0.9 \mu\text{g l}^{-1}$ [62]. Thus, seamounts along the SWIR are in contrasting productivity regimes and water masses depending on their proximity to the sub-tropical convergence zone and the sub-Antarctic front [29]. This trend is also reflected by the abundance of microorganisms, with higher abundances south of the sub-Antarctic front (Coral) and lowest abundances in the sub-tropical north (Atlantis) of the sub-tropical front (Melville and Middle of What).

Microbial communities of corresponding depth layers in the north (Atlantis), convergence zone (Melville and Middle of What) and south (Coral) have similar abundant microorganisms at the order and phyla level, indicating adaptation to habitat rather than location for similar types of organisms. However, at an OTU level, the microbial communities show quite large differences between each location based on rank abundance and the MRT (Figs. 3, 4). The MRT groups all deep seamount samples into one cluster, while separating out the surface and middle layer of Coral into two clusters, and the surface layer of Atlantis into another. As seen in the rank abundance, the surface layer on Coral is dominated by a *Synechococcus* OTU, which also dominates Melville and Middle of What, while Atlantis is dominated by *Prochlorococcus*. Both *Synechococcus* and *Prochlorococcus* have been shown in Djurhuus *et al.* [29] to be the dominant cyanobacteria at the surface of these seamounts. Interestingly *Prochlorococcus* and *Synechococcus* are indicator species of the surface and middle layer, respectively, of the convergence zone seamounts distinguishing the different strata with their niche adaptations to high light and low light conditions. Photosynthetically available radiation is a major driver of primary producers, which will influence the surface communities, due to

431 the strong environmental factor as seen on Coral, Melville, Middle of What, and
432 Sapmer (Fig. 5, 6) Sul *et al.* [11].

433 In the middle layer there is a group of OTUs that decreases from high rank
434 abundance on Coral to low rank abundance on all other seamounts. The middle
435 layer on Coral seamount was weakly stratified with maxima in temperature and
436 salinity at 250 m depth [63]. The deep layer formed a single cluster signifying
437 the stability of this environment with the deep Antarctic current dominating below
438 1000 m on all seamounts [64]. Atlantis was the seamount in the most strongly
439 stratified waters [63] creating a very stable environment. As depth is the strongest
440 predictor of microbial community structures, the stable stratified environment (i.e.
441 no mixing between depth strata) on Atlantis would explain the largest difference seen
442 in microbial community structure within the deep and shallow layers on Atlantis
443 (Table 2) between all samples, again indicating a depth effect that is stronger than
444 a geographical effect.

445 The rank abundance plots further demonstrate the change in the microbial com-
446 munities from south to north, through the convergence zone. The most abundant
447 taxa on Coral are rare on Melville, with a similar shift between Middle of What and
448 Atlantis, following the respective oceanic water masses and thus habitats (Fig. 6).
449 This provides further evidence that water masses influence prokaryotic community
450 composition can be considered barriers to microbial dispersal [65]. Similar to that
451 found by Agogue *et al.* (2011), where they suggest the deep-water masses act as
452 bio-oceanographic islands for bacterioplankton. In the deep sea, habitats have rel-
453 atively little environmental variation (e.g., temperature, salinity), which has led to
454 the evolution of species which have broad horizontal ranges [24]. However, because
455 abiotic and biotic factors vary greatly with depth, many species possess restricted
456 vertical ranges. Structuring of the microbial diversity is related to the physical,
457 chemical, and biological features of the water masses [65]; however, the definition
458 of water masses by physical properties can be enhanced by the microbial ecology
459 component as highlighted in this study (Figs. 5 and 6) [66–68].

460 The water mass separation based on temperature and salinity are in agreement

461 with our grouping based on microbial diversity [69]. Based on the differences in
 462 communities that run from the microbial level up, the north (Atlantis), convergence
 463 zone (Melville, Sapmer, Middle of What), and south (Coral) could be considered
 464 three biogeographical zones. This is consistent with findings for macrofauna and
 465 megafauna, chemical, and physical studies along the SWIR [29, 60, 69–71]. A mix-
 466 ture of environmental selection and dispersal limitation/facilitation likely plays roles
 467 in biogeographical patterns, although, such a clear water mass separation has not
 468 been found previously in microbial communities [12]. However, the ability to detect
 469 a biogeographic pattern may depend on taxonomic resolution. As demonstrated
 470 in this study, the distance–decay relationship is clear between the most abundant
 471 taxa. Accordingly, the latter half of Baas-Becking principle ‘the environment se-
 472 lects’ combined with distance-decay, might be appropriate in this example where
 473 currents might facilitate adequate dispersal between the studied locations to con-
 474 tinuously distribute microorganisms, but restriction of differing environments will
 475 compromise the success of the specific microbial taxa. However, the dispersal is
 476 not high enough to counteract the compositional differentiation imposed by the
 477 distance-decay relationship [17] and, contrary to ideas previously suggested by Han-
 478 son *et al.* [17], the distance-decay relationship is strong in the pelagic environment
 479 between seamounts in differing environmental setting (water-masses), indicating an
 480 environmental selection. This demonstrates a regional biogeographic structure in
 481 the dominant microbial taxa, with semi-restricted dispersal by currents and very
 482 limited community mixing across water masses.

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501

502 **Ethics**

503 Does not apply to this study.

504 **Data, code and materials**

505 All data is available through Dryad: <http://dx.doi.org/10.5061/dryad.qh767>

506 **Permission to carry out fieldwork**

507 Does not apply to this study.

508 **Competing interests**

509 The authors declare no competing interests.

510 **Author's contributions**

511 AD, ADR, and SOM conceived the study, participated in its design and coordination,
512 and helped draft the manuscript. AD collected all samples, conducted the laboratory
513 work and bioinformatics, and wrote the draft manuscript. AD and PBS analysed
514 the data and prepared figures and tables. All authors edited the manuscript.

Table 1: Overview of samples collected at the five seamount locations. FC is flow cytometry samples. DNA, FC and Nutr. (nutrients) are number of samples. WM: Water Mass. SA.: Subantarctic, ST.: Subtropical.

Station	Longitude	Latitude	Summit	WM	DNA	FC	Nutr
Coral	42°50'31"E	41°21'23"S	198 m	SA	43	79	86
Melville	46°45'74"E	38°31'56"S	120 m	CZ	32	64	73
MoW	50°22'16"E	37°56'76"S	1078 m	CZ	32	74	81
Sapmer	52°07'24"E	36°49'63"S	446 m	CZ	11	12	53
Atlantis	57°17'26" E	32°42'01"S	713 m	ST	35	66	91

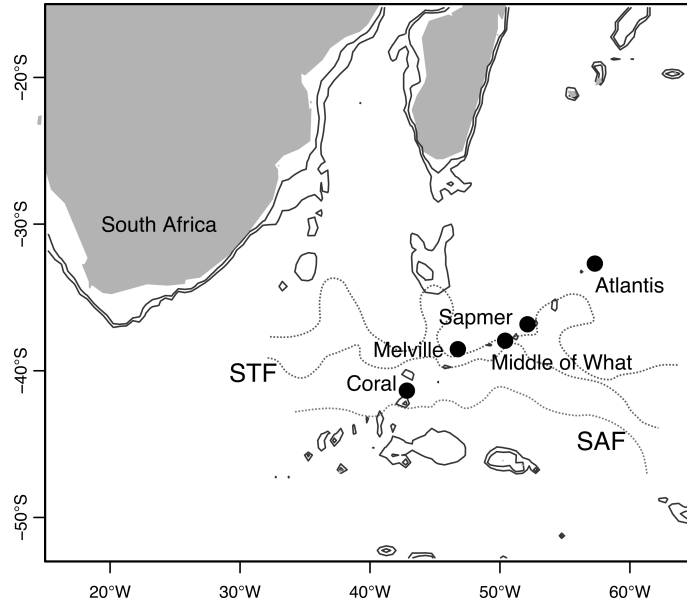


Figure 1: Locations of the five sampling stations on the South West Indian Ridge. The solid line represents the 1000 m contour. The dashed lines are the Agulhas return current, sub-tropical front (STF), and sub-Antarctic front (SAF), from North to South [63].

Table 2: OTU richness, cell abundances, and indicator species of the different strata of each seamount.

	Station	Observed richness	Abundance (10^6 cells/ml)	Indicator species	Most abundant taxa	Cluster (MRT)
Shallow	Coral	1410	1.16 (± 0.179)	Marine group III, SAR202	Synechococcaceae	6
	Melville	1486	0.926 (± 0.172)	<i>Prochlorococcus</i>	<i>Prochlorococcus</i>	5
	MoW	1530	0.925 (± 0.173)	<i>Prochlorococcus</i>	OCS155	5
	Atlantis	1421	0.50 (± 0.179)	Pseudomonadaceae, Oceanospirillaceae	OCS155	2
Mid	Coral	1134	0.297 ± 0.067	<i>Nitrosopumilus</i> , HTCC	Oceanospirillaceae	1
	Melville	1780	0.311 ± 0.095	<i>Synechococcus</i> , <i>Coralimargarita</i>	Cenarchaeacea	4
	MoW	1805	0.266 ± 0.118	<i>Synechococcus</i> , <i>Coralimargarita</i>	Cenarchaeacea	4
	Atlantis	1502	0.249 ± 0.0122	<i>Synechococcus</i> , <i>Coralimargarita</i>	Cenarchaeacea	4
Deep	Coral	1224	0.146 ± 0.101	Halomonadaceae, <i>Candidatus</i> "portiera"	Oceanospirillales	3
	Melville	1433	0.129 ± 0.105	Halomonadaceae, <i>Candidatus</i> "portiera"	SAR324	3
	MoW	1451	0.140 ± 0.074	Halomonadaceae, <i>Candidatus</i> "portiera"	SAR324	3
	Atlantis	994	0.081 ± 0.043	Halomonadaceae, <i>Candidatus</i> "portiera"	SAR324	3

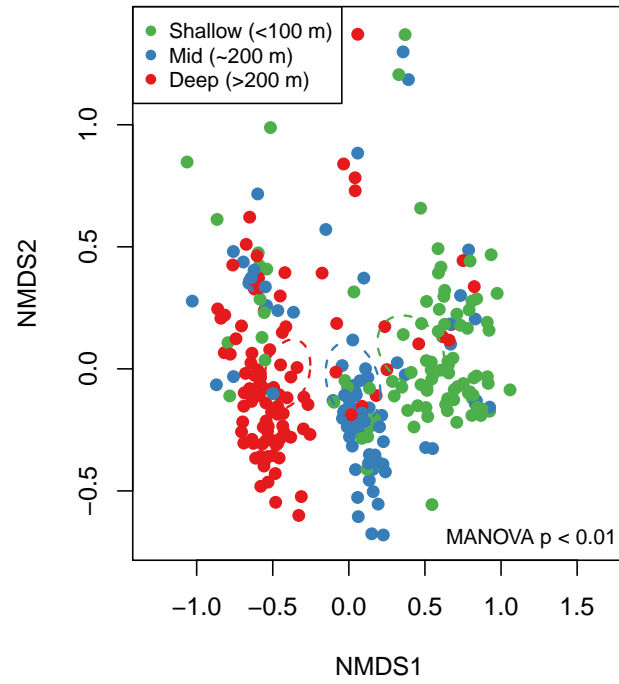


Figure 2: NMDS plot highlighting community differences between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). The ellipses represent the 99% confidence interval ellipses of the layer. MANOVA = Multivariate Analysis of Variance. Stress = 0.1225.

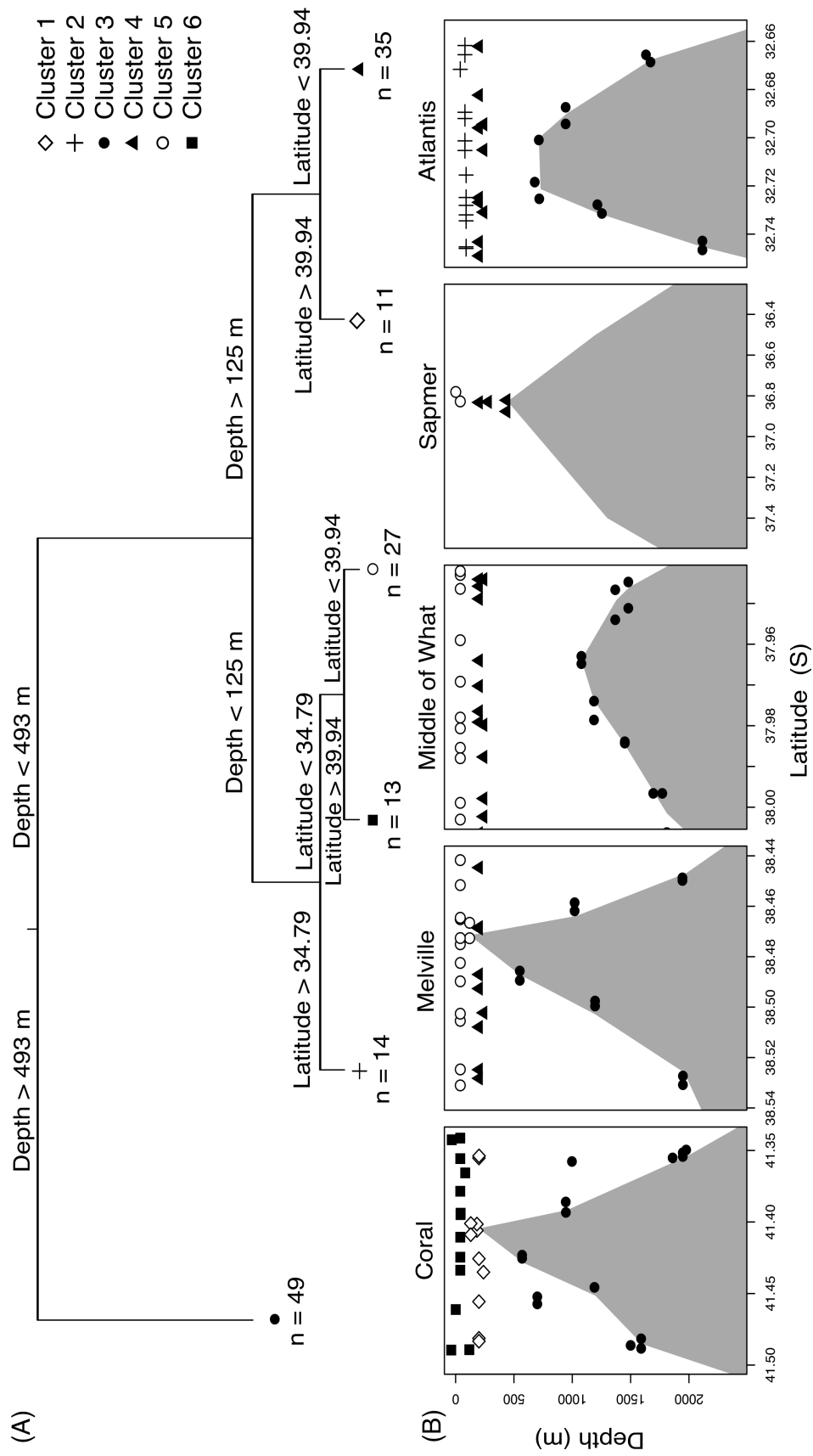


Figure 3: A) Multivariate Regression Tree (MRT) of microbial communities and their structuring by latitude and depth. B) Locations of MRT clusters across seamounts. Plotting symbols correspond to clusters in (A).

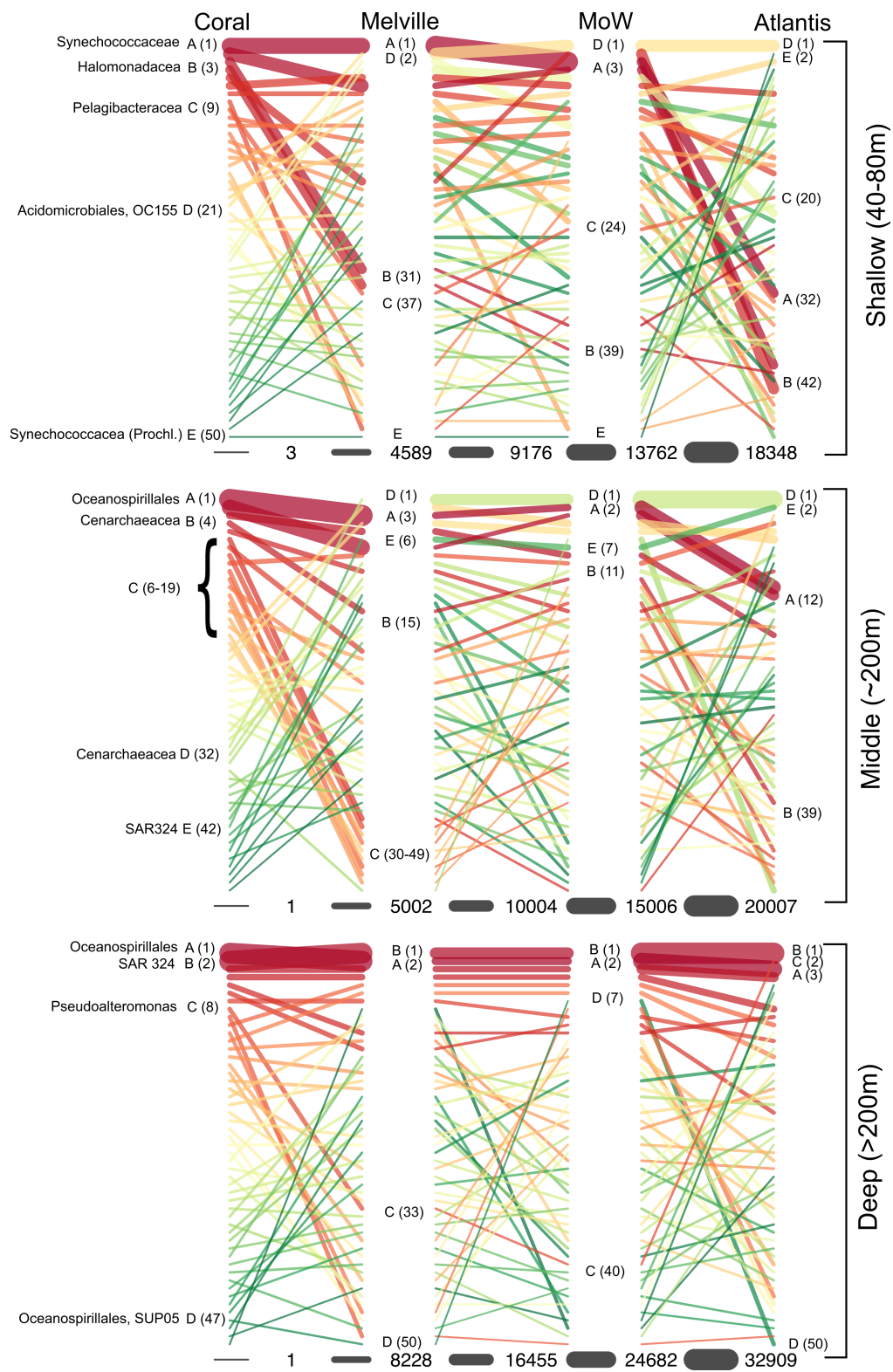


Figure 4: Rank abundance of the 50 most abundant OTUs on Coral, Melville, Middle of What and Atlantis. The rank abundance plots are divided between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). Each line signifies the changes in a particular OTU and the color of a specific line is to clarify the changes in rank across seamounts. Rank changes are indicated in parentheses on that specific seamount. Taxonomy of selected OTUs is specified on the left side of the figure. Line thickness represent relative abundance changes of a particular OTU as specified at the bottom of the figure.

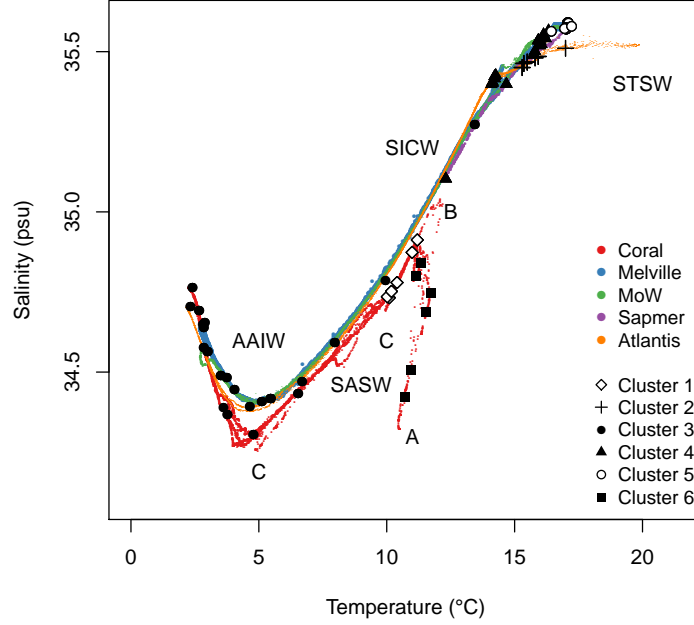


Figure 5: Temperature and salinity of water masses on SWIR seamounts. The points represent the clusters from the multivariate regression tree analysis. STSW is Subtropical surface water, SICW is South Indian Central Water, SASW is colder Subantarctic Surfaces Water, and AAIW is Antarctic Intermediated Water. The t/s properties showed a sub-surface salinity minimum (A). At the base of the fairly well mixed surface layer, strong stratification marked the transition to a salinity maximum of 34.7–34.8 associated with a temperature maximum (B). At about 250 m an inflexion in the t/s curve marked interleaving and small-scale minima and maxima in temperature and salinity (C). The segment C–B points to the end member of the STSW. C–D marks the area below the inflexion point and points to the AAIW.

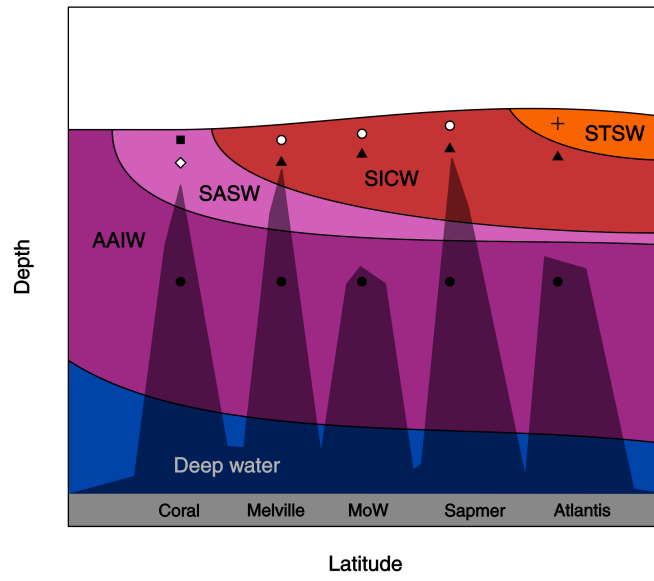


Figure 6: Schematic of watermasses of the Southwest Indian Ridge according to Emery *et al.* and Tomczak *et al.* [72, 73]. Multivariate regression tree clusters, representing different microbial community structure, are illustrated according to Figs. 5 and 3.

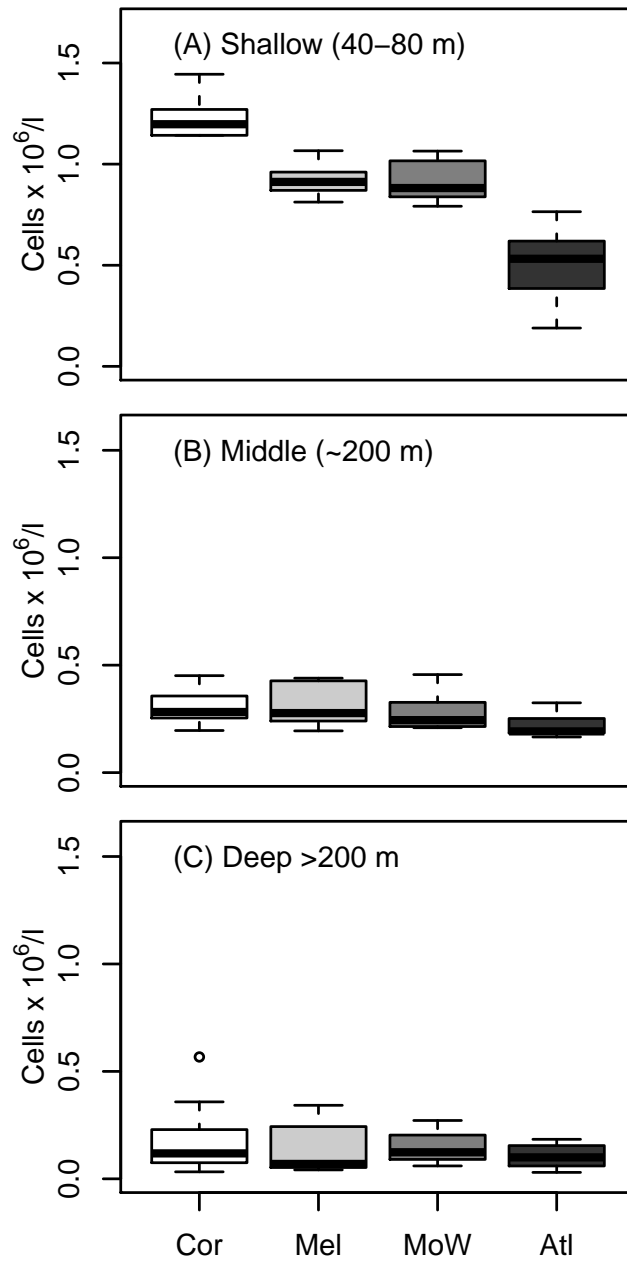


Figure 7: Abundance of microorganisms by depth stratum at the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW), and Atlantis (Atl). A: shallow (40-80 m). B: mid (200 m). C: deep (>200 m). Clearly visible abundance difference between Coral and the convergence zone (Melville and MoW) and between the convergence zone and Atlantis in the shallow.

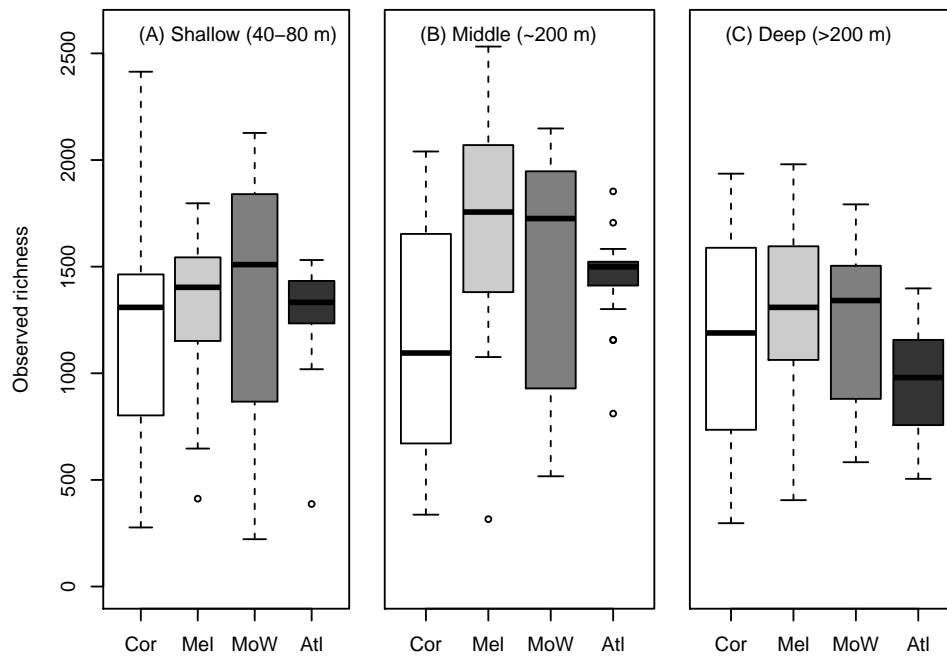


Figure 8: OTU richness across the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW) and Atlantis (Atl). The richness plot is separated into the shallow (A, 40–80 m), middle (B, 200 m) and deep (C, >200 m) stratum.

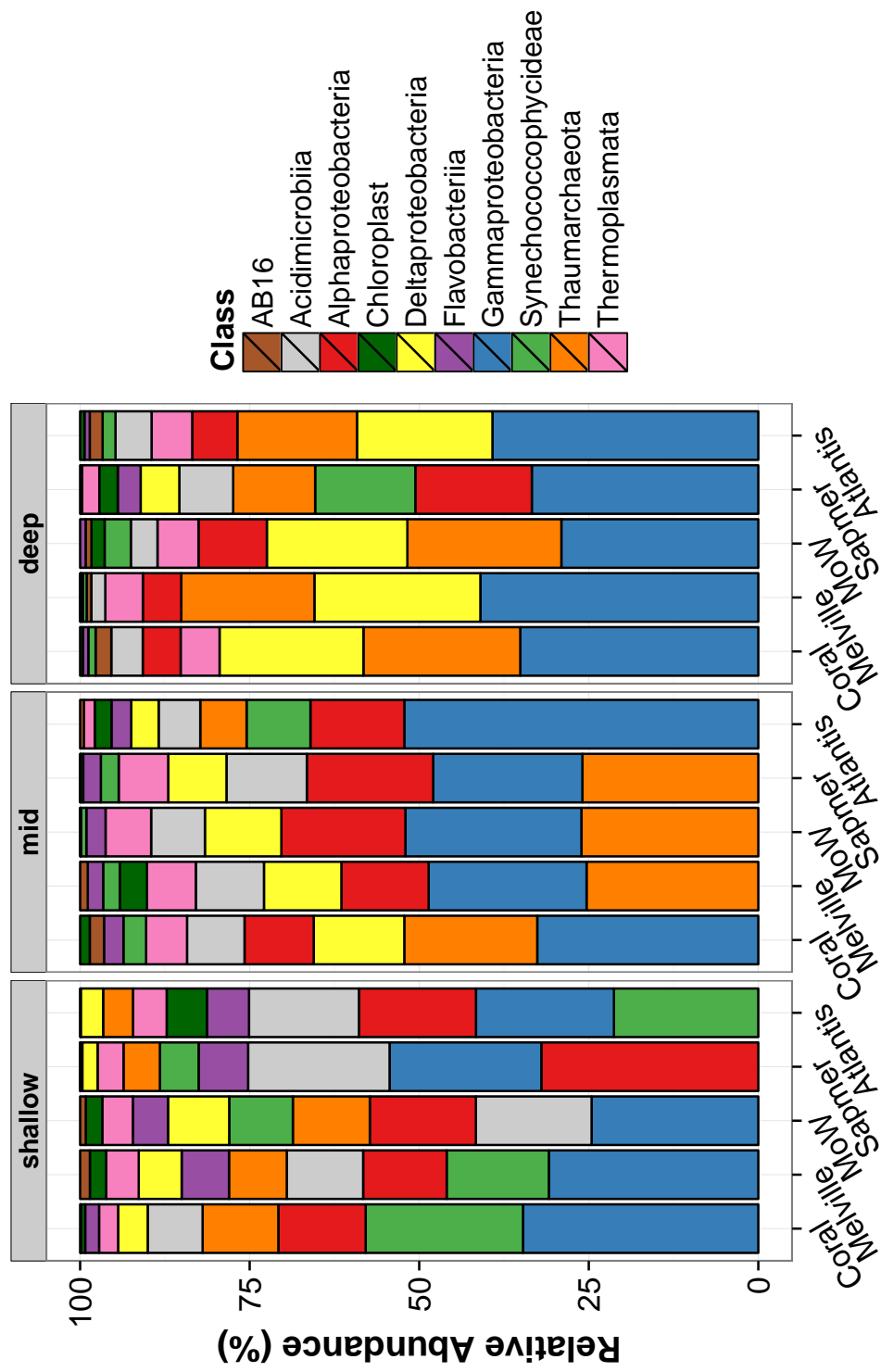


Figure 9: Barplot of the relatively most abundant microbial classes between depths (shallow=40-80 m, middle= 200 m, deep=>200 m) on all seamounts. MoW is Middle of What.

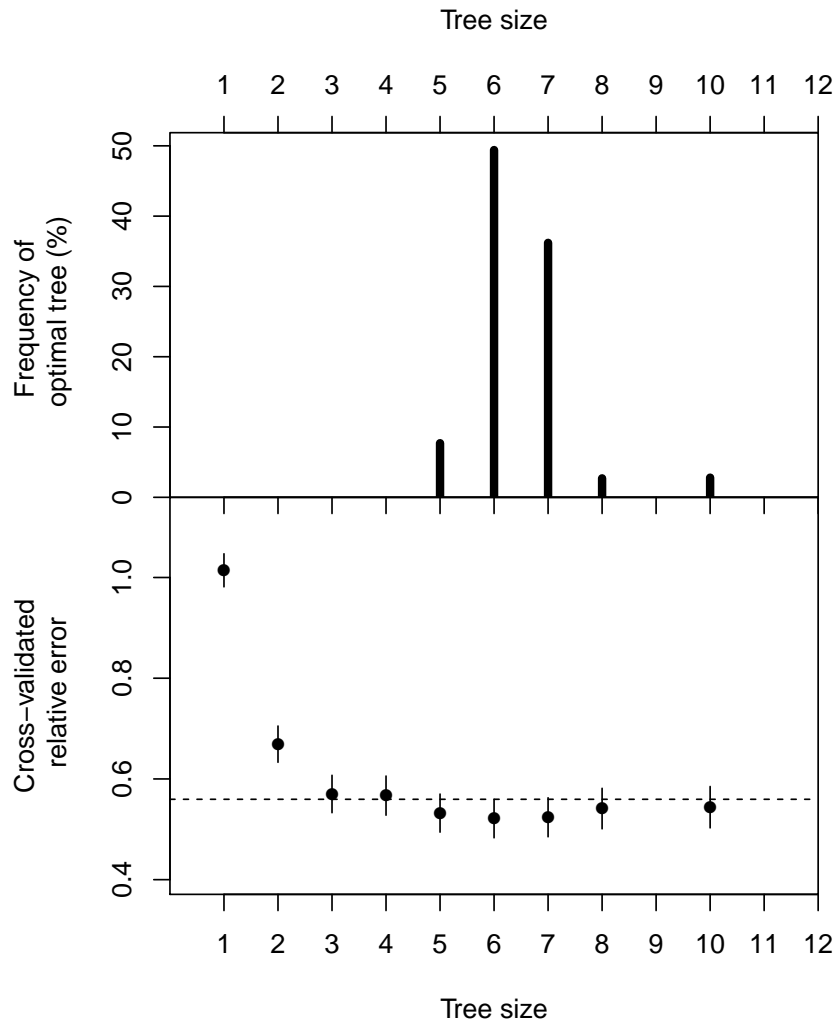


Figure 10: Multivariate Regression tree error output. The top plot is a histogram of how often the optimal tree is selected. The bottom graph displays the cross-validated error between the tree sizes. Optimal tree sizes were between 5-11. The most parsimonious were tree sizes 5 and 9.

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