Diversity and community composition of particle-associated and free-living bacteria in mesopelagic and bathypelagic Southern Ocean water masses: Evidence of dispersal limitation in the Bransfield Strait

Mathias Milici,1* Marius Vital,2 Jürgen Tomasch,1 Thomas H. Badewien,3 Helge-A. Giebel,3 Iris Plumeier,2 Hui Wang,1 Dietmar H. Pieper,2 Irene Wagner-Döbler,1 Meinhard Simon3

1Group Microbial Communication, Helmholtz Center for Infection Research, Braunschweig, Germany
2Group Microbial Interactions and Processes, Braunschweig, Germany
3Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany

Abstract

The Southern Ocean constitutes about 10% of the global oceans’ volume and is characterized by high primary production. Particulate organic matter (POM) is exported from the photic zone to the deep ocean and sustains life of particle associated (PA) and free-living (FL) bacterial communities in the dark realm. Little is known about the composition and diversity of PA and FL bacterial communities below the photic zone and how they differ among various regions of the Southern Ocean. Therefore, we investigated the composition of small (3–8 μm) and large (>8 μm) PA and FL (0.2–3 μm) bacterial communities between 500 m and 3600 m in the Bransfield Strait, Drake Passage, and the south Atlantic Ocean featuring also Southern Ocean water masses. PA bacterial communities had a higher OTU richness and evenness than FL ones. Taxonomic analysis revealed a different community composition between FL and PA bacteria. A large number of OTUs belonging to diverse phyla (Bacteroidetes, Planctomycetes, Betaproteobacteria, Deltaproteobacteria, and Verrucomicrobia) were significantly enriched on particles; in contrast very few bacterial lineages were FL specialists. Lifestyle (FL vs. PA) and region (Bransfield basin vs. other regions) strongly influenced bacterial communities. Depth explained only marginal fraction of the total variation (~12%), suggesting that selective processes driven by depth have a smaller effect in the Southern Ocean when compared to lifestyle (25%) and region (31%). Overall these data indicate a strong influence of isolated water masses such as the basin of the Bransfield Strait on the composition of bacterial communities in the dark ocean.

The Southern Ocean is an environmentally isolated biome but sustains a large diversity and biomass of phytoplankton and bacterioplankton communities (Griffiths 2010; De Monte et al. 2013; Wilkins et al. 2013c; Cavicchioli 2015). This oceanic region plays a major role in marine carbon cycling (Kirchman et al. 2009), in particular in the downward flux of particulate organic matter (POM), as large proportions of sinking POM and biogenic silica reach the sea floor (Falkowski et al. 1998). The low temperature strongly limits bacterial metabolism and growth (Simon et al. 1999; Bidle et al. 2002; Kirchman et al. 2009), thus shifting degradation of sinking POM to below the mixed layer and even to the sea floor (Treguer et al. 1995; Simon et al. 2004). The Bransfield Strait, between the Antarctic Peninsula and the South Shetland Islands, is one of the most productive regions in the Southern Ocean and exhibits a high export production (Kim et al. 2005; Manganelli et al. 2009; Teira et al. 2013).

Bacterial communities in the meso- and bathypelagic zone are usually linked to the epipelagic zone as the major inputs of energy and substrates originate from phytoplankton primary production and sinking POM (Herndl and Reinhhaler 2013; Worden et al. 2015). This is particularly true for productive oceanic regions with high amounts of sinking POM, such as large areas in the Southern Ocean (see above), but other sources of energy and substrates and even advection may contribute as well (Herndl and Reinhhaler 2013; Wilkins et al. 2013b; Frank et al. 2016). Organic aggregates and fecal pellets, the major form of sinking POM, are hot spots of microbial activity (Smith et al. 1992; Azam and Long 2001; Simon et al. 2002).
and undergo biogeochemical changes while sinking (Benner and Amon 2015). They are partly mineralized but also solubilized thus fueling the pool of dissolved organic matter (DOM) and leading eventually to disaggregation. The high microbial activities of POM-associated (PA) bacteria are reflected by their enhanced cell-specific rates of polymer hydrolysis and substrate uptake relative to the free-living (FL) bacteria in the surrounding water (Simon et al. 2002; Grossart et al. 2007). Both communities also exhibit a different composition. PA-bacterial communities were found to be relatively enriched in polymer-degrading bacteria such as Flavobacteria and Gammaproteobacteria and in Planctomycetes and Verrucomicrobia whereas the FL bacterial communities were enriched in Alphaproteobacteria (Mohit et al. 2014; Bizic-Ionescu et al. 2015; Milici et al. 2016a). The great majority of these studies have been carried out in the sun-lit near surface waters and only few reports are available from the dark ocean. In the Mediterranean Sea at 500 m depth the PA bacterial community was found to be relatively enriched in Bacteroidetes, Planctomycetes, Verrucomicrobia and Firmicutes, whereas the FL communities were enriched in Alphaproteobacteria (Mohit et al. 2014; Bizic-Ionescu et al. 2015; Milici et al. 2016a). The great majority of these studies have been carried out in the sun-lit near surface waters and only few reports are available from the dark ocean.

In the Southern Ocean, the great majority of the small number of studies on the composition of prokaryotic communities using state of the art molecular approaches deals with the near-surface waters down to a depth of 200 m (Manganelli et al. 2009; Ghiglione and Murray 2012; Cavicchioli 2015; Yu et al. 2015; Landa et al. 2016). Only very scarce information is available from mesopelagic and bathypelagic waters (Lopez-Garcia et al. 2001; Wilkins et al. 2013b). In order to expand our knowledge on this realm we investigated the composition of PA and FL bacterial communities in the Bransfield Strait between the Antarctic Peninsula and the South Shetland Islands and in the Drake Passage. As reference a station in the temperate south Atlantic Ocean east of the Patagonian shelf, featuring also Antarctic water masses, was included. Given the importance of the dark oceans’ bacterial communities for mineralizing sinking POM and DOM and the necessity to understand the mechanisms controlling particle export to the deep ocean in more detail, we asked how these communities are influenced by regional and water mass-specific environmental conditions.

### Materials and methods

**Sampling and bacterial abundance**

Samples were collected during cruises ANT-28/4 and ANT 28/5 between 16 March 2012 and 13 April 2012, with RV
Polarstern at three stations in the Southern Ocean and one in the South Atlantic Ocean, east of the Patagonian Shelf, between 61° and 45°S (Table 1; Fig. 1). Water masses were identified based on their hydrographic properties, i.e., temperature and salinity, according to Emery (2003). At all stations, samples were collected from the mesopelagic (500–1000 m) and from the bathypelagic zone (1000–3600 m, Table 1). Sampling was carried out with 12 L Niskin bottles mounted on a CTD rosette (Sea-Bird Electronics Inc. SBE 911 plus probe) equipped with double temperature and conductivity sensors, a pressure sensor, altimeter, chlorophyll fluorometer, and transmissometer. CTD data were validated during the cruise through regular reference measurements of water samples applying standard methods. Immediately after retrieval on deck, 10 mL subsamples were withdrawn from the bottles to determine bacterial abundance by flow cytometry (within the next 2 d) as described in Osterholz et al. (2015). For assessing the bacterial community composition, 12 L of water from one bottle was transferred to 20 L wide-mouth barrels, and filtered with three peristaltic pumps (Ismatec, IDEX Health & Science GmbH, Wertheim, Germany) through three successive stainless steel filtration devices (Druckfiltrationsgerät Edelstahl Typ 1627, Omnillab Laborzentrum, Braunschweig, Germany) equipped with the following membrane filters (diameter 142 mm): 8 μm (mixed cellulose ester SCWP14250, Millipore, Darmstadt, Germany), 3 μm (mixed cellulose ester SSWP14250, Millipore, Darmstadt, Germany), and 0.22 μm (polyethersulfone GPWP14250, Millipore, Darmstadt, Germany). After filtration, membranes were immediately stored at −80°C until DNA isolation. In total 39 samples were collected (Supporting Information Table S1).

**DNA isolation and bioinformatic analyses**

Total DNA was isolated with a combined chemical and mechanical procedure using the UltraClean Soil DNA Isolation KIT (MO BIO Laboratories, Carlsbad, California, U.S.A.) with modifications as described previously (Milici et al. 2016b,c). Library preparation was performed as described (Camarinha-Silva et al. 2014; Milici et al. 2016b,c) and 16S rDNA amplicons covering the V5-6 hypervariable region were subjected to 250 bp paired-end sequencing on the Illumina MiSeq platform. Bioinformatic analyses of raw reads were performed as previously described (Camarinha-Silva et al. 2014) with some modifications. Briefly, forward and reverse raw reads were merged (Cole et al. 2014) and subsequently aligned using MOTHUR (gotoh algorithm using the SILVA reference database version 123 NR) prior to pre-clustering (difs = 2). Obtained phylotypes that exhibited an abundance of ≥3 counts were considered for follow-up analysis. Raw sequences are available at the European Nucleotide Archive (ENA) under the accession number PRJEB14709.

**Data analysis**

Taxonomic classification was assigned using the SINA aligner (Pruesse et al. 2012) (version 1.2.11) employing the reference database SILVA (Pruesse et al. 2007) (version 123 NR). The OTUs were aligned and classified against a maximum of 100 sequences that had a minimum of 97% similarity with the query sequence, using the lowest common ancestor method (LCA). All OTUs that were not assigned to the domain bacteria were excluded from the analysis. Resampling, Kruskal–Wallis test and rarefaction analysis were performed in R (http://www.Rproject.org/, v. 3.0.1) with the library vegan: Community Ecology Package. Box plot analysis and calculation of alpha diversity indexes were performed in PRIMER (v.7.0.6, PRIMER-E, Plymouth Marine Laboratory, Plymouth, UK).

Fold change was calculated for each OTU using the factor filter size with R (http://www.Rproject.org/, v. 3.0.1) using the package edgeR (Empirical Analysis of Digital Gene Expression Data in R). Briefly, a generalized exact binomial test was performed after normalization using the weighted trimmed mean of M-values (Robinson and Oshlack 2010) on rarefied OTU counts. Only OTUs that had fold change values higher than log2 2 (absolute value) with a false discovery rate lower than 0.001 were included in the data representation.

PRIMER (v.7.0.6, PRIMER-E, Plymouth Marine Laboratory, Plymouth, UK), with the add-on PERMANOVA+ (v. 1.0.6 PRIMER-E, Plymouth Marine Laboratory, Plymouth, UK) was
used for beta diversity analysis (PERMANOVA, ANOSIM as well as PCO analysis).

Map representation was performed with Ocean Data View version 4.7.2 (Schlitzer, R., Ocean Data View, http://odv.awi.de, 2015).

Results

Stations and sequencing overview

All stations exhibited Atlantic Subarctic Upper Water (ASUW) at 500 m depth but different water masses below this depth (Table 1). Station 193, situated in the Bransfield Strait between the Antarctic Peninsula and the South Shetland Islands with an isolated basin of 2100 m depth, featured also ASUW at 1000 m but Antarctic Bottom Water (AABW) at 2000 m depth. Stations 287 and 179, in the Drake Passage south and north of the Polar Front, featured Arctic Intermediate Water (AIW) and Antarctic intermediate water (AAIW) at 1000 m, respectively. Station 298 in the south Atlantic was characterized by AIIW at 1000 and 1500 m and AABW at 2000 m depth. More detailed information on sampling location, day of sampling and sampling depth are reported in Table 1. Bacterial numbers at 500 m ranged between 9.29 × 10^4 and 14.10 × 10^4 mL^{-1} (Table 1). Below 500 m depth there was a strong decrease to less than 50% of the numbers at 500 m except at station 193 in the Bransfield Strait, where the cell numbers remained practically unchanged. The samples for the assessment of the bacterial community composition were serially filtered onto three membranes: 8 μm, 3 μm, and 0.22 μm. The fraction 0.22–3 μm was named free-living (FL), the fraction 3–8 μm small particle associated (SPA), and the fraction >8 μm large particle associated (LPA) bacterial community.

Overall more than 1.5 million raw reads were generated in our study, of which roughly 1.2 million were affiliated to the domain Bacteria (Supporting Information Table S1). An average of 32.681 ± 15.197 sequences per sample was obtained and a total of 4492 OTUs were identified. Rarefaction analysis showed that an adequate sequencing depth was reached (Fig. 2); all curves had overcome the exponential phase indicating that the vast majority of the phylotypes was detected.

Higher species richness and evenness on particles

Rarefaction analysis showed that samples belonging to the PA communities had a higher diversity in terms of OTUs recruited when compared to the FL fraction (Fig. 2C). We therefore investigated diversity patterns of the three size fractions. After resampling of the original data we calculated the species number (S) and Pielou index of evenness (J). For both indices we observed a gradual increase from the FL community towards the LPA community, with the SPA community in the middle (Fig. 2A,B). Differences between groups were tested with Kruskal–Wallis test (999 permutations) and were found to be highly significant (p < 0.001) for both alpha diversity indexes (S and J). When further comparing differences between each of the three size classes (pairwise tests) we found that for species richness (S) all pairwise tests were highly significant (p < 0.001), while for the Pielou (J) index of evenness only the pairwise test between FL and LPA was significant, and the other tests (FL vs. SPA and SPA vs. LPA) were not (p > 0.05).

The data show that most of the OTUs in the deep Southern Ocean and South Atlantic Ocean are adapted to live on particles, and furthermore that OTUs present on particles are more evenly distributed in terms of relative abundances compared to the free-living ones, where a small number of OTUs are dominating the community.

Analysis of the unique number of OTUs indicated a core community of the three bacterial size fractions of 43.6%, but roughly 25% of the OTUs were detected in only one of them. The LPA community contributed 17% unique OTUs, the FL community 5.6%, and the SPA community 3.1% (Fig. 2D). The SPA and LPA size fractions shared a larger proportion with each other than each of them with the FL fraction (Fig. 2D).

Impact of life-style and oceanic region on bacterial community composition

In order to assess the impact of size fraction and region on the bacterial community composition (BCC), we carried out a Principal Coordinate Analysis (Fig. 3). Along the first principal component samples were distinguished according to the region, with all samples from station 193 in the Bransfield Strait separated from the other stations in the Drake Passage and south Atlantic Ocean. Along the second principal component, samples were separated according to the filter size, i.e., lifestyle. Overall these data suggest that bacterial community composition was distinguishable by filter size as well as region while little effect of depth was shown. PERMANOVA test confirmed these observations: A statistically significant effect (p < 0.001) of region and filter size was observed, with 24.4% and 31.5% of the total variation explained, respectively (Supporting Information Table S2). Surprisingly, we found that the effect of depth (p < 0.004) was secondary and explained only 11.6% of the total variation. These results were confirmed and consistent with the ANOSIM test (Analysis of similarity) which showed a weak effect of realm (R = 0.2, p < 0.008) compared to the much larger effect of filter size (R = 0.7, p < 0.001) and region (R = 0.9, p < 0.001).

Distinct particle-associated bacterial communities in the Bransfield Strait

A total of 30 phyla (Proteobacteria were divided at the class level) were identified, of which only 10 phyla had a total relative abundance above 1% and only five contributed more than 5% to the total number of sequences (Fig. 4A). Almost half of the sequences were affiliated to Gammaproteobacteria, underlining the primary role of this class of
Proteobacteria in the dark ocean. Alphaproteobacteria, Bacteroidetes, and Marinomicrobia all had relative abundances close to 10%.

Several phyla were strongly enriched or depleted in the FL relative to the LPA communities. Furthermore, BCC was different between the Bransfield Strait and the rest of the samples (Fig. 4A,B).

Marinomicrobia, almost entirely represented by the SAR406 clade, accounted for almost 20% of the total community in the FL bacterial communities of all samples, and were strongly reduced in their relative abundances on particles. A similar pattern was observed for the Chloroflexi and Gemmatimonadetes, with a sharp reduction in their relative abundance in the LPA community. Gammaproteobacteria showed a similar trend at station 193 and at the other stations at depths of 500 m and 1000 m. Below 1000 m they consistently accounted for 40–50% of the total sequences in all three size fractions. By contrast, other taxa were depleted in the FL community and almost entirely present in the LPA community. Clear examples of this pattern were the Planctomycetes, which accounted for more than 20% of the total sequences in the LPA bacterial community of the stations in

Fig. 2. Diversity and evenness of FL, SPA, and LPA bacterial communities. (A) OTU richness (S), (B) Pielous index of evenness (J'). (C) Rarefaction analysis, (D) Venn diagram of shared OTUs between the three size fractions.
the Bransfield Strait and only 1% in the FL bacterial community. This pattern was conserved also at the other stations where the Planctomycetes amounted for about 10% in the LPA size fraction and only 2% in the FL bacterial community (Fig. 4A,B). Similar trends were observed for other phyla, e.g., Bacteroidetes, Betaproteobacteria, Verrucomicrobia, Paracoccus, Firmicutes and Deltaproteobacteria, indicating that a wide range of phyla inhabiting the deep ocean have a preference for the particle attached lifestyle, although these patterns were also distinct for the region of sampling (Bransfield Strait vs. other regions).

Further investigations at the clade and genus level confirmed the profound differences between the two lifestyles (FL vs. PA) as well as the region of sampling (Bransfield Strait vs. other regions). Members of genome streamlined clades like SAR11, SAR86, and SAR406 were among the most abundant groups in the FL fraction at all stations (Fig. 4C). By contrast, lineages belonging to Betaproteobacteria (Delftia and Janthinobacterium), Gammaproteobacteria (Marinobacter and Alteromonas), and to some Alphaproteobacteria (Thalassospira, Brevundimonas, and Sphingobium) were more abundant on the particles in the Drake Passage and south Atlantic Ocean. Other genera were more abundant on the particles at the station in the Bransfield Strait, e.g., the Bacteroidetes (NS9 marine group, Reichenbachiella, Loktanella (Alphaproteobacteria/Roseobacter) and Planctomycetes (OM190). Some lineages were more abundant in the SPA communities, namely the genera Acinetobacter, Rhodococcus, Rhodopirellula, and the group Arctic97B-4, the latter in particular in the Bransfield Strait.

Evidence of dispersal limitation in the Bransfield Strait

Our taxonomic analysis revealed that samples collected in the Bransfield Strait were significantly dissimilar from the rest of the samples, although they belonged to the same water masses. In order to understand the possible effect of dispersal limitation we performed a taxonomy independent analysis (Fig. 5). Our investigation, based on those OTUs contributing more than 0.1% to the total sequences of the study, suggests that similar water masses harbor distinct communities within the Bransfield Strait and in the rest of the Southern Ocean. Several sets of OTUs were either enriched or depleted in the Bransfield Strait and not found anywhere else. To further explore these differences we performed a core analysis (including the rare biosphere) of the Bransfield Strait against the rest of the samples and surprisingly found that 39.7% of the OTUs retrieved in this study were endemic of the Bransfield Strait. By contrast, only 5.6% of the total OTUs were exclusively retrieved in the other samples of the Southern Ocean water masses. These data
Fig. 4. Community composition of the three bacterial size fractions. OTUs were grouped at the level of phylum (class for the Proteobacteria) in (A and B). Only those phyla or classes that contributed more than 0.1% of the total sequences are displayed. The remaining groups are reported in Supporting Information Table S1. In panel (A) community composition for each individual sample is shown. The ID of each sample consists of the sampling station name, followed by the depth of sampling (m) and the filter size (FL, SPA, and LPA). On the upper part of the bar plot the macro regions are identified: Bransfield Strait, Drake Passage south (sampled south of the polar front), Drake passage north (sampled north of the polar front), and South Atlantic. In panel (B) the average relative abundance for the three size classes of the plankton in the Bransfield strait and other samples is shown. Panel (C) shows the relative abundance of genera or clades that contributed at least 5% of the total sequences. Color key identifies bacterial taxa.
therefore suggest that the Bransfield Strait harbors a distinct bacterioplankton community with respect to all three size classes of the plankton analyzed here, due to a combined effect of dispersal limitation and selection (see above).

**Lineage-specific colonization of particles**

In order to further understand mechanisms of niche segregation between the three life-styles studied here we performed enrichment analysis at the OTU level. The fold change of abundance of each OTU was calculated between all combinations of categories, namely filter size (e.g., FL vs. LPA). Data were sorted for significance (false discovery rate FDR < 0.001) and log2 fold change ≥ ± 2. This enrichment analysis revealed that most of the differentially abundant OTUs were found when comparing LPA and FL (389 OTUs) while only a relatively small number was significantly enriched in the other two pair-wise comparisons: SPA-FL (37 OTUs) and LPA-SPA (28 OTUs). Substantial differences with respect to taxonomy could be observed between OTUs enriched in the FL community and those enriched in the LPA community (Fig. 6). Within the Bacteroidetes, all enriched OTUs on particles were assigned to the groups NS7, NS9, and NS10 as well as the genera Crocintomix, Reichenbachiella, and Owenweeksia, while the NS4 and NS5 groups were only enriched in the FL fraction. Of the Deltaproteobacteria, only a single OTU was not enriched in the LPA fraction (SAR324 clade), while the groups Sh76Sβ-Tzt-T29, P3OB-42 and GR-WP33-58 and the OM27 clade were represented by several OTUs which were significantly enriched in the LPA community. All OTUs belonging to the Planctomycetes were enriched in the LPA fraction, none was enriched in the FL fraction, and the groups OM 190, CL500-3, and FS140-16B-02 were represented by several OTUs. Similarly, verrucomicrobia were only enriched on particles, although with a lower number of OTUs compared to the Planctomycetes. Betaproteobacteria were also almost exclusively enriched on particles, with the only exception of a single OTU from clade OM43 which exhibited a positive fold change in the FL community. Interestingly, Janthinobacterium exhibited the highest negative fold change observed (log2 appr. –9) suggesting an obligate particle associated life-style.

Completely different taxa were enriched in the FL community. Chloroflexi, entirely represented by the SAR202 clade, were only enriched in the FL community. The SAR406 clade (Marinimicrobia) had a similar pattern, with most of the OTUs being present in the FL community; however we also detected enrichment of some OTUs in the LPA community, indicating the ability of some members of the clade to grow attached to particles. Members of Gammaproteobacteria and Alphaproteobacteria were enriched in both microenvironments, but with different OTUs. OTUs belonging to the clades SAR11, SAR86, AEGAN-169, and ZD0417 were only significantly enriched in the FL bacterial community and OTUs belonging to the Rhodobacteriaceae, like Loktanella and Pseudophaeobacter, were enriched in the LPA community, as well as several Gammaproteobacteria like members of the Alteromonadales and Cellvibrionales.

Comparison between the SPA and LPA, and SPA and FL communities, respectively. (Supporting Information Table S3; Fig. S1) showed that several members of Alphaproteobacteria and Actinobacteria were enriched on the small particles. For instance the genera Spingobium and Sphingomonas were always enriched in this fraction, suggesting a specialized life-style for those environments.

In conclusion, these results show clear niche segregation between the particle-associated and free-living life-style, which is particularly strong when comparing LPA and FL communities. The data suggest that most of the bacterial lineages inhabiting the deep ocean are adapted to a copiotrophic life-style on particles rather than a free-living one.

**Discussion**

**Bacterioplankton biogeography in the deep Southern Ocean is structured by organic matter input and water masses**

Bacterioplankton community composition (BCC) was marginally affected by depth but strongly influenced by the region of sampling, which explained 31% of variation. In particular, we found a sharp contrast between the station in the Bransfield Strait and the other three stations south and north of the Polar Front in the Drake Passage and in the South Atlantic Ocean. This contrast was also reflected in the bacterial cell numbers, which remained much higher below 500 m in the Bransfield Strait compared to further north where they decreased by 50% below this depth. The Bransfield Strait is a highly productive area of the Southern Ocean and much more productive than the Drake Passage, as documented by standing stocks of phytoplankton biomass (Manganelli et al. 2009; Teira et al. 2013). The eastern part, in which our station is located, appears to be the most productive area as documented by the highest sinking flux which occurs about a month after phytoplankton blooms (Kim et al. 2005). These features, and the strongly reduced microbial decomposition of phytoplankton-born organic matter in the mixed layer due to the low temperatures are typical for the Southern Ocean (Simon et al. 1999; Kirchman et al. 2009). They lead to an enhanced export of POM and a shift of its microbial decomposition to deeper layers (Bidle et al. 2002; Simon et al. 2004; Herndl and Reinthaler 2013), which can explain the higher bacterial numbers in the meso- and bathypelagic zones of the Bransfield Strait compared to the less productive regions further north. The deep basin of the Bransfield Strait is rather isolated from the Drake Passage despite partially similar water masses in the bathypelagic zone. These features may explain that BCC in the meso- and bathypelagic zone of the Bransfield Strait is mainly controlled by organic matter input and isolation/dispersal.
limitation, whereas in the deep Drake Passage and the deep South Atlantic Ocean it is mainly controlled by water masses circulation.

It is in fact remarkable that the samples from the deep Drake Passage and the South Atlantic Ocean exhibit similar BCC. These findings appear to reflect the oceanographic structure of the Southern Ocean and the south Atlantic Ocean which share several water masses (Carter et al. 2008) and suggest that BCC can be used to trace them, obviously due to the absence of dispersal limitation and by mixing processes (Hanson et al. 2012). A similar observation has been reported by Wilkins et al. (2013b) for various Southern

Fig. 5. The water masses of the Bransfield Strait harbor distinct bacterioplankton communities. In order to display the similarity between water masses between the Branfield strait and the rest of the samples Bray–Curtis similarity was calculated and averaged for each region and water mass independently from the filter size (particle associated and free-living grouped together). A hierarchal cluster was than calculated and is displayed on the upper part of the heat map. Different regions are identified with different symbols; the circle display the Bransfield strait, while the triangle the rest of the samples (Drake Passage sampled south of the polar front, Drake passage sampled north of the polar front and South Atlantic). Different colors instead distinguish between the different water masses (AAIW, ASUW, AABW, and AIW). On the y axis of the plot the Log10 relative abundance of the OTUs contributing more than 0.1% of the total sequences are displayed and clustered according to the index of association, which was calculated among OTUs (contributing more than 0.1%). Each line therefore displays the average Log10 relative abundance of an OTU which derived from any of the three size classes: FL, SPA, and LPA.
Fig. 6. Niche segregation of bacterial communities: OTU enrichment analysis was performed on standardized relative abundances between FL and LPA communities. The detailed results of the analysis can be found in Supporting Information Table S3. Only log2 fold changes higher than 2 were included in the dataset and the enriched OTUs were sorted for high statistical significance (FDR < 0.001). Negative fold change values indicate enrichment in the LPA community, while a positive fold change value indicates enrichment in the FL community. Color code identifies phyla (classes for Proteobacteria) and each bar represents the average fold change for genera and clades that were represented by more than one OTU. The other two pairwise comparisons FL-SPA and SPA-LPA are reported in Supporting Information Fig. S1.
Ocean water masses, but our data indicate that BCC can be used to trace Southern Ocean water masses beyond the Polar Front further north. In the deep North Atlantic and Arctic Ocean it has also been shown that BCC is shaped by water mass circulation (Galand et al. 2009; Agogue et al. 2011). Furthermore, our analysis suggests that besides processes of advection (Wilkins et al. 2013b) and water mass mixing (Hernando-Morales et al. in press) the Southern Ocean is also influenced by dispersal limitation. The Bransfield Strait exhibited a high proportion of endemic OTUs (~ 40%) when compared to the rest of the samples (~ 6%). We provide evidence that processes of dispersal limitation coupled with strong selective forces (higher amount of POM) can significantly alter the community structure of the deep Southern Ocean, as also proposed for the relationship between the Indo-Pacific Basin and the South Atlantic Ocean (Villar et al. 2015).

Despite such strong evidence supported by the analysis of three size classes of the plankton, a limitation of our study is the small set of samples. Further studies are required to understand whether the Bransfield Strait is dispersal limited, i.e., separated from the main circulation of water masses in the Southern Ocean. Moreover, bacterioplankton communities are known to dramatically change also in a short time frame (Fuhrman et al. 2006, 2015), in particular in response to algal blooms (Needham and Fuhrman 2016; Teeling et al. 2012, 2016) and hence changes in POM and DOM composition and abundance. It is therefore possible that the observations made here are partially influenced by the season of sampling. Future studies should attempt to sample at a higher temporal resolution to determine whether the patterns observed here are depending on the season.

Higher bacterial diversity and evenness on particles

Particle-associated bacterial communities exhibited a higher diversity in terms of number of species than the FL ones. Moreover, they had a higher evenness, indicating a more homogeneous abundance distribution of the OTUs. These results are in accordance with studies of the Mediterranean Sea (Crespo et al. 2013) and the oxygen minimum zone (OMZ) of the Pacific off Peru (Ganesh et al. 2014). The study from the Mediterranean Sea, however, included only samples down to a depth of 500 m (Crespo et al. 2013) and the OMZ is a very special environment. Our results are in contrast to results of the Malaspina study of bathypelagic global ocean basins (Salazar et al. 2016). This study found a higher diversity in the FL relative to the PA bathypelagic bacterial community. A possible explanation for this discrepancy might be regional processes which are reflected in the latitudinal and thus temperature range sampled. The Malaspina cruise (http://scientific.expedicionmalaspina.es/; Salazar et al. 2015, 2016) covered only tropical and temperate oceanic regions (~ 30°S~30°N). We sampled much colder regions of the south Atlantic and the Southern Ocean between 45° and 61°S. This region is much more productive than the low latitude regions (Falkowski et al. 1998) and bacterial decomposition of sinking POM is strongly temperature limited, thus shifting microbial decomposition of sinking POM to deeper layers (see above). Therefore, the higher diversity of the PA bacterial community which we found in the meso- and bathypelagic waters presumably reflects the enhanced microbial activity on less degraded sinking POM, providing more ecological niches and requiring a higher functional diversity of the PA bacteria dwelling in these rich micro-patches.

Particle associated life-style: a conserved evolutionary trait in the deep ocean

Patterns in beta diversity in the deep Southern Ocean and South Atlantic Ocean were strongly dependent on filter size, which explained 24% of the total variation. Sample clustering was guided by filter size (FL, SPA, and LPA), demonstrating highly dissimilar communities. These results contribute to the growing body of evidence showing that bacterial communities from different planktonic size fractions are dramatically different not only in the epipelagic zone (Mohit et al. 2014; Bizic-Ionescu et al. 2015; Rieck et al. 2015; Milici et al. 2016a), but also in the mesopelagic and bathypelagic zones (Crespo et al. 2013; Ganesh et al. 2014; Salazar et al. 2015). Calculation of differential abundance at the OTU level demonstrated that many OTUs of the marine bacterioplankton are enriched (up to log2 of 9) on particles. This feature seems to be conserved at higher taxonomic rank (phylum and class). In particular, we observed that members of the Bacteroidetes, Planctomycetes, Verrucomicrobia, Firmicutes, Betaproteobacteria, and Deltaproteobacteria were almost exclusively represented on particles. These findings support the existence of an evolutionary adaptation to particle-associated environments for these lineages (Martinez-Garcia et al. 2012; Buchan et al. 2014; Lage and Bondoso 2014). Previous studies have reported an enrichment of these groups on particles from epi- to bathypelagic waters (DeLong et al. 1993; Acinas et al. 1999; Crump et al. 1999; Crespo et al. 2013; Mohit et al. 2014; Bizic-Ionescu et al. 2015; Rieck et al. 2015; Salazar et al. 2015; Milici et al. 2016a). However, the Southern Ocean is a poorly investigated biome in this respect (Griffiths 2010) and only very few studies have analyzed bacterioplankton communities of different size classes with high-throughput sequencing of the Southern Ocean (Wilkins et al. 2013a,b).

Among the Bacteroidetes, only the NS4 and NS5 groups were enriched in the FL community, while several other Flavobacteriaceae OTUs (lineages NS7, NS9, and NS10) were enriched on particles. Flavobacteriaceae are well known as colonizers and degraders of phytoplankton-derived POM and polymeric dissolved organic matter, in particular in temperate and polar environments, and occur as PA as well as FL bacteria (Simon et al. 1999; Abell and Bowman 2005;
Schattenhofer et al. 2009; Teeling et al. 2012; Buchan et al. 2014; Milici et al. 2016a; Teeling et al. 2016). Even though all flavobacteria appear to be able to hydrolyze polysaccharides, some lineages have evolved a PA life-style whereas others exhibit a FL life-style (Fernández-Gómez et al. 2013).

Several OTUs belonging to Deltaproteobacteria were enriched in the LPA community. They belong to the groups Sh765B-TzT-29, P3OB-42, GR-WP33-58, and OM27. Not much information is available about the biogeography of those groups. Salazar et al. (2015) reported the enrichment of the OM27 clade on particles, in accordance with our findings. Interestingly, only a single OTU from the Deltaproteobacteria (SAR 324 clade) was enriched in the FL community. SAR 324 was described as a PA lineage (Swan et al. 2011) and it is commonly retrieved in the Southern Ocean (Lopez-Garcia et al. 2001), but several studies of the epipelagic (Milici et al. 2016a), mesopelagic (Crespo et al. 2013), and bathypelagic zone (Salazar et al. 2015) showed an enrichment of this group in the FL community. Thus, the SAR324 clade might be able to thrive in both habitats, or different species not resolved by the amplicon analysis may exist, which are adapted to either ecological niche. However, we cannot rule out a bias due to serial filtration (Padilla et al. 2015) or predation (Hartmann et al. 2012, 2013).

Within Betaproteobacteria, also many OTUs were enriched on particles, with the only exception of a single OTU from the OM43 clade, which was more abundant in the free-living community. This is in full accordance with its description as a typical oligotrophic bacterium with a streamlined genome (Giovannoni et al. 2008). However, many other Betaproteobacteria OTUs were enriched on particles. The genus *Janthinobacterium*, for instance, was represented by two OTUs which had the highest negative fold change in the whole study. These bacteria are usually rare members of the bacterioplankton, yet a recent study reported a bloom of a *Janthinobacterium* sp. population in the Arctic Ocean, where this genus reached up to 22% of the total number of bacteria in the epipelagic zone and was abundant down to the mesopelagic zone (~350 m; Alonso-Saez et al. 2014). A study conducted on the type strain *Janthinobacterium lividum* reported its ability to form biofilms, which is an important trait for particle associated life (Pantanella et al. 2007). The authors also found that *J. lividum* is able to shift from planktonic to biofilm growth during stress conditions caused by carbon source depletion and antibiotics. We found that *Janthinobacterium* affiliated OTUs were strongly over-represented on particles; this might be a response to the stressful conditions of the deep ocean compared to the epipelagic zone, which might represent its preferred ecological niche.

Only few lineages showed a typical FL life-style. Among them were SAR11, SAR86, and AEGAN-169 as well as SAR202 and SAR406 clades which are well known from other oceanic regions (Dupont et al. 2012; Swan et al. 2013; Giovannoni et al. 2014; Thrash et al. 2014). Interestingly, the recently described *Actinomarina minuta* (clade OM1; Ghai et al. 2013), which was shown to be numerically dominant in the FL community of the Atlantic Ocean (Milici et al. 2016a), was always present with low relative abundances, probably because of its phototrophic life-style (Ghai et al. 2013). The SAR406 clade is highly abundant in the deep ocean and was reported as FL in previous studies (Crespo et al. 2013; Salazar et al. 2015). In our study, however, we found that some OTUs belonging to this clade were significantly enriched on particles, suggesting that, as also proposed for SAR324, these bacteria might be able to switch between both micro-niches or the clade might contain several species or subspecies adapted to either FL or PA life-style, respectively.

Overall it seems that the PA life-style represents a typical adaptation of bacteria inhabiting the deep Southern Ocean, the FL life-style in fact being limited to a few lineages known to be oligotrophic and characterized by streamlined genomes (Grote et al. 2012; Giovannoni et al. 2014). This observation extends previous reports from the mesopelagic region of other oceans (DeLong et al. 2006; Herndl and Reinthaler 2013) to the Southern Ocean, the major origin of many bathypelagic water masses in the global oceans. Typical copiotrophic bacteria dominate the PA communities (Luo and Moran 2015), whose abundance is strongly reduced in the FL bacterial communities suggesting strong niche segregation in the dark ocean.

**Conclusion**

Our study provides new evidence for the remarkable taxonomic differences between PA and FL bacteria in the deep Southern Ocean water masses. Bacterial communities from the Bransfield Strait were strongly distinct from those in the Drake Passage and the south Atlantic Ocean. These differences are presumably due to the high primary production and high rates of carbon export in the Bransfield Strait and its isolation from the main water masses of the Southern Ocean, which consequently produces dispersal limitation. Overall, our data suggest that particles are a hotspot of bacterial diversity, and that bacterial lineages belonging to the Bacteroidetes, Planctomycetes, Deltaproteobacteria, Betaproteobacteria, and Verrucomicrobia represent an important link between POM and carbon cycle in the deep ocean. Our study did not include Archaea which are known to be important components of the prokaryotic communities in meso- and bathypelagic water masses of the Southern Ocean and other oceans (Murray et al. 1999; Gryzmski et al. 2012; Herndl and Reinthaler 2013). As most Archaea appear to be chemolithoautotrophic, they are presumably not directly involved in the decomposition of sinking organic matter like Bacteria, but rather indirectly, e.g., by oxidizing released ammonium. It would be most interesting to examine whether planktonic marine Archaea exhibit similar distribution...
patterns as Bacteria and whether similar factors control their dispersal in the Southern Ocean.

References

Abell, G. C. J., and J. P. Bowman. 2005. Ecological and biogeographic relationships of class Flavobacteria in the Southern Ocean. FEMS Microbiol Ecol. 51: 265–277. doi: 10.1016/j.femsec.2004.09.001

Acinas, S. G., J. Anton, and F. Rodriguez-Valera. 1999. Diversity of free-living and attached bacteria in offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl. Environ. Microbiol. 65: 514–522.

Agogue, H., D. Lamy, P. R. Neal, M. L. Sogin, and G. J. Herndl. 2011. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. Mol. Ecol. 20: 258–274. doi: 10.1111/j.1365-294X.2010.04932.x

Alonso-Saez, L., M. Zeder, T. Harding, J. Perntaler, C. Lovejoy, S. Bertilsson, and C. Pedros-Alio. 2014. Winter bloom of a rare betaproteobacterium in the Arctic Ocean. Front. Microbiol. 5: 425. doi: 10.3389/fmicb.2014.00425

Azam, F., and R. A. Long. 2001. Sea snow microcosms. Nature 414: 495, 497–495, 498. doi: 10.1038/35107174

Benner, R., and R. M. Amon. 2015. The size-reactivity continuum of major bioelements in the ocean. Ann. Rev. Mar. Sci. 7: 185–205. doi: 10.1146/annurev-marine-010213-135126

Bidle, K. D., M. Manganelli, and F. Azam. 2002. Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. Science 298: 1980–1984. doi: 10.1126/science.1076076

Bizic-Ionescu, M., M. Zeder, D. Ionescu, S. Orlic, B. M. Fuchs, H. P. Grossart, and R. Aumann. 2015. Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization. Environ. Microbiol. 17: 3500–3514. doi: 10.1111/1462-2920.12466

Buchan, A., G. R. LeCleir, C. A. Gulvik, and J. M. Gonzalez. 2016. Connectivity between surface and deep waters determines prokaryotic diversity in the North Atlantic Deep Water. Environ. Microbiol. 18: 2052–2063.

Carter, L., I. N. McCave, and M. J. M. Williams. 2008. Circulation and water masses of the Southern Ocean: A review, p. 85–114. In F. Florindo and M. Siegert [eds.], Developments in earth and environmental sciences, v. 8. Elsevier. doi:10.1016/S1571-9197(08)00004-9

Cavicchioli, R. 2015. Microbial ecology of Antarctic aquatic systems. Nat. Rev. Microbiol. 13: 691–706. doi: 10.1038/nrmicro3549

Cole, J. R., and others. 2014. Ribosomal Database Project: Data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42: D633–D642. doi: 10.1093/nar/gkt1244

Crespo, B. G., T. Pommier, B. Fernandez-Gomez, and C. Pedros-Alio. 2013. Taxonomic composition of the particle-attacted and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. Microbiol. Open 2: 541–552. doi: 10.1002/mbo3.92

Crump, B. C., E. V. Armbust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. 65: 3192–3204.

De Monte, S., A. Soccolato, S. Alvain, and F. d’Ovidio. 2013. Can we detect oceanic biodiversity hotspots from space? ISME J. 7: 2054–2056. doi: 10.1038/ismej.2013.72

DeLong, E. F., D. G. Franks, and I. L. Aldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38: 924–934. doi: 10.4319/lo.1993.38.5.0924

DeLong, E. F., and others. 2006. Community genomics among stratified microbial assemblages in the ocean’s interior. Science 311: 496–503. doi: 10.1126/science.1120250

Dupont, C. L., and others. 2012. Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. ISME J. 6: 1186–1199. doi: 10.1038/ismej.2011.189

Eloe, E. A., and others. 2011. Going deeper: Metagenome of a hadopelagic microbial community. PLoS One 6: e20388. doi: 10.1371/journal.pone.0020388 doi: 10.1371/journal.pone.0020388

Emery, W. J. 2003. Water types and water masses, p. 1556–1567. In J. R. Holton, J. A. Curry and J. A. Pyle [eds.], Encyclopedia of atmospheric sciences, 2nd ed. Elsevier.

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. Biogeochemical controls and feedbacks on ocean primary production. Science 281: 200–207. doi: 10.1126/science.281.5374.200

Fernández-Gómez, B., M. Richter, M. Schüler, J. Pinhassi, S. Acinas, J. M. González, and C. Pedrós-Alló. 2013. Ecology of marine Bacteroidetes: A comparative genomics approach. ISME J. 7: 1026–1037. doi: 10.1038/ismej.2012.169

Frank, A. H., J. A. Garcia, G. J. Herndl, and T. Reinhalter. 2016. Connectivity between surface and deep waters determines prokaryotic diversity in the North Atlantic Deep Water. Environ. Microbiol. 18: 2052–2063.

Fuhrman, J. A., I. Hewson, M. S. Schwabach, J. A. Steele, M. V. Brown, and S. Naeem. 2006. Annually reoccurring bacterial communities in the North Atlantic determined by pyrosequencing of the 16S rRNA. Microbiol. Open 2: 541–552. doi: 10.1002/mbo3.92

Fuhrman, J. A., J. A. Cram, and D. M. Needham. 2015. Marine microbial community dynamics and their ecological interpretation. Nat. Rev. Microbiol. 13: 133–146. doi: 10.1038/nrmicro3417
Hartmann, M., M. V. Zubkov, D. J. Scanlan, and C. Lepere. 2009. Hydrography shapes bacterial biogeography of the deep Arctic Ocean. ISME J. 4: 564–576. doi:10.1038/ismej.2009.134

Ganesh, S., D. J. Parris, E. F. DeLong, and F. J. Stewart. 2014. Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone. ISME J. 8: 187–211. doi: 10.1038/ismej.2013.144

Ghai, R., C. M. Mizuno, A. Picazo, A. Camacho, and F. Rodriguez-Valera. 2013. Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Sci. Rep. 3: 2471. doi:10.1038/srep02471

Ghihlione, J. F., and A. E. Murray. 2012. Pronounced summer to winter differences and higher wintertime richness in coastal Antarctic marine bacterioplankton. Environ. Microbiol. 14: 617–629. doi:10.1111/j.1462-2920.2011.02601.x

Giovannoni, S. J., and others. 2008. The small genome of an abundant coastal ocean methylotroph. Environ. Microbiol. 10: 1771–1782. doi:10.1111/j.1462-2920.2008.01598.x

Giovannoni, S. J., T. J. Cameron, and B. Temperton. 2014. Implications of streamlining theory for microbial ecology. ISME J. 8: 1553–1565. doi:10.1038/ismej.2014.60

Griftiths, H. J. 2010. Antarctic marine biodiversity—what do we know about the distribution of life in the Southern Ocean? PLoS One 5: e11683. doi:10.1371/journal.pone.0011683

Grossart, H. P., K. W. Tang, T. Kiorboe, and H. Ploug. 2007. Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. FEMS Microbiol. Lett. 266: 194–200. doi:10.1111/j.1574-6968.2006.00520.x

Grote, J., J. C. Thrash, M. J. Landry, P. Carini, S. J. Giovannoni, and M. S. Rappe. 2012. Streamlining and core genome conservation among highly divergent members of the SAR11 clade. MBio. 3.

Grzymski, J. J., C. S. Riesenfeld, T. J. Williams, A. M. Dussa, H. Ducklow, M. Erickson, R. Cavicchioli, and A. E. Murray. 2012. A metagenomic assessment of winter and summer bacterioplankton from Antarctica Peninsula coastal surface waters. ISME J. 6: 1901–1915. doi:10.1038/ismej.2012.31

Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. Martiny. 2012. Beyond biomeographic patterns: Processes shaping the microbial landscape. Nat. Rev. Microbiol. 10: 497–506. doi:10.1038/nr micro2795

Hartmann, M., C. Grob, G. A. Tarran, A. P. Martin, P. H. Burkill, D. J. Scanlan, and M. V. Zubkov. 2012. Mixotrophic basis of Atlantic oligotrophic ecosystems. Proc. Natl. Acad. Sci. USA 109: 5756–5760. doi:10.1073/pnas.1118179109

Hartmann, M., M. V. Zubkov, D. J. Scanlan, and C. Lepere. 2013. In situ interactions between photosynthetic picocyanobacteria and bacterioplankton in the Atlantic Ocean: Evidence for mixotrophy. Environ. Microbiol. Rep. 5: 835–840. doi:10.1111/1758-2229.12084

Hernando-Morales, V., J. Ameneiro, and E. Teira. Water mass mixing shapes bacterial biogeography in a highly hydrodynamic region of the Southern Ocean. Environ. Microbiol. doi:10.1111/1462-2920.13538

Herndl, G. J., and T. Reintalker. 2013. Microbial control of the dark end of the biological pump. Nat. Geosci. 6: 718–724. doi:10.1038/geo201921

Kim, D. Y., J. S. Park, and Y. J. Kim. 2005. Interannual variation of particle fluxes in the eastern Bannsfield Strait, Antarctica: A response to the sea ice distribution. Deep-Sea Res. 1 52: 2140–2155. doi:10.1016/j.dsr.2005.06.008

Kirschman, D. L., X. A. Moran, and H. Ducklow. 2009. Microbial growth in the polar oceans - role of temperature and potential impact of climate change. Nat. Rev. Microbiol. 7: 451–459. doi:10.1038/nrmicro2115

Lage, O. M., and J. Bondoso. 2014. Planctomycetes and macroalgae, a striking association. Front. Microbiol. 5: 267. doi:10.3389/fmicb.2014.00267

Landa, M., S. Blain, U. Christaki, S. Monchy, and I. Obernosterer. 2016. Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. ISME J. 10: 39–50. doi:10.1038/is mej.2015.105

Lauro, F. M., and others. 2009. The genomic basis of trophic strategy in marine bacteria. Proc. Natl. Acad. Sci. USA 106: 15527–15533. doi:10.1073/pnas.0903507106

Lopez-Garcia, P., A. Lopez-Lopez, D. Moreira, and F. Rodriguez-Valera. 2001. Diversity of free-living prokaryotes from a deep-sea site at the Antarctic Polar Front. FEMS Microbiol. Ecol. 36: 193–202. doi:10.1016/S0168-6496(01)00133-7

Luo, H., and M. A. Moran. 2015. How do divergent ecological strategies emerge among marine bacterioplankton lineages? Trends Microbiol. 23: 577–584. doi:10.1016/j.tim.2015.05.004

Manganelli, M., F. Malfatti, T. J. Samo, B. G. Mitchell, H. W. Wang, and F. Amaz. 2009. Major role of microbes in carbon fluxes during Austral winter in the Southern Drake Passage. PLoS One 4: e6941. doi:10.1371/journal.pone.0006941

Martinez-Garcia, M., and others. 2012. Capturing single cell genomes of active polysaccharide degraders: An unexpected contribution of Verrucomicrobia. PLoS One 7: e35314. doi:10.1371/journal.pone.0035314

Milici, M., and others. 2016a. Co-occurrence analysis of microbial taxa in the Atlantic Ocean reveals high connectivity in the free-living bacterioplankton. Front. Microbiol. 7: 649. doi:10.3389/fmicb.2016.00649

Milici, M., and others. 2016b. Bacterioplankton biogeography of the Atlantic Ocean: A case study of the distance-decay relationship. Front. Microbiol. 7: 590. doi:10.3389/fmicb.2016.00590

Milici, M., and others. 2016c. Low diversity of planktonic bacteria in the tropical ocean. Sci. Rep. 6: 19054. doi: 10.1038/srep19054

Mohit, V., P. Archambault, N. Toupoint, and C. Lovejoy. 2014. Phylogenetic differences in attached and free-living
bacterial communities in a temperate coastal lagoon during summer, revealed via high-throughput 16S rRNA gene sequencing. Appl. Environ. Microbiol. 80: 2071–2083. doi:10.1128/AEM.02916-13

Murray, A. E., K. Y. Wu, C. L. Moyer, D. M. Karl, and E. F. DeLong. 1999. Evidence for circumpolar distribution of planktonic Archaea in the Southern Ocean. Aquat. Microb. Ecol. 18: 263–273. doi:10.3354/ame018263

Needham, D. M., and J. A. Fuhrman. 2016. Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. Nat. Microbiol. 1: 16005. doi:10.1038/nmicrobiol.2016.5 doi:10.1038/nmicrobiol.2016.5

Osterholz, H., J. Niggemann, H. A. Giebel, M. Simon, and T. Dittmar. 2015. Inefficient microbial production of refractory dissolved organic matter in the ocean. Nat. Commun. 6: 7422. doi:10.1038/ncomms8422

Padilla, C. C., S. Ganesh, S. Gantt, A. Huhman, D. J. Parris, N. Sarode, and F. J. Stewart. 2015. Standard filtration practices may significantly distort planktonic microbial diversity estimates. Front. Microbiol. 6: 547. doi:10.3389/fmicb.2015.00547

Pantanella, F., F. Berlutti, C. Passariello, S. Sarli, C. Morea, and S. Schippa. 2007. Violacein and biofilm production in Janthinobacterium lividum. J. Appl. Microbiol. 102: 992–999. doi10.1111/j.1365-2672.2006.03155.x

Pruesse, E., C. Quast, K. Knittel, B. M. Fuchs, W. Ludwig, J. Peplies, and F. O. Glockner. 2007. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35: 7188–7196. doi10.1093/nar/gkm864

Pruesse, E., J. Peplies, and F. O. Glockner. 2012. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28: 1823–1829. doi:10.1093/bioinformatics/bts252

Rieck, A., D. P. Herlemann, K. Jurgens, and H. P. Grossart. 2015. Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea. Front. Microbiol. 6: 1297. doi10.3389/fmicb.2015.01297

Robinson, M. D., and A. Oshlack. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11: R25. doi:10.1186/gb-2010-11-3-r25 doi:10.1186/gb-2010-11-3-r25

Salazar, G., and others. 2015. Particle-association lifestyle is a phylogenetically conserved trait in bathypelagic prokaryotes. Mol. Ecol. 24: 5692–5706. doi10.1111/mec.13419

Salazar, G., F. M. Cornejo-Castillo, V. Benitez-Barrios, E. Fraile-Nuez, X. A. Alvarez-Salgado, C. M. Duarte, and S. G. Acinas. 2016. Global diversity and biogeography of deep-sea pelagic prokaryotes. ISME J. 10: 596–608. doi:10.1038/ismej.2015.137

Schattenhofer, M., B. M. Fuchs, R. Amann, M. V. Zubkov, G. A. Tarzan, and J. Pernthaler. 2009. Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. Environ. Microbiol. 11: 2078–2093. doi:10.1111/j.1462-2920.2009.01929.x

Simon, M., F. O. Glöckner, and R. Amann. 1999. Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. Aquat. Microb. Ecol. 18: 275–284. doi:10.3354/ame018275

Simon, M., H. P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28: 175–211. doi:10.3354/ame028175

Simon, M., B. Rosenstock, and W. Zwisler. 2004. Coupling of epipelagic and mesopelagic heterotrophic picoplankton production to phytoplankton biomass in the Antarctic Polar Frontal Region. Limnol. Oceanogr. 49: 1035–1043. doi10.4319/lo.2004.49.4.1035

Smith, D. C., M. Simon, A. L. Aldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359: 139–142. doi:10.1038/359139a0

Swan, B. K., and others. 2011. Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. Science 333: 1296–1300. doi:10.1126/science.1203690

Swan, B. K., and others. 2013. Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean. Proc. Natl. Acad. Sci. USA 110: 11463–11468. doi:10.1073/pnas.1304246110

Teeling, H., and others. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336: 608–611. doi:10.1126/science.1218344

Teeling, H., and others. 2016. Recurring patterns in bacterioplankton dynamics during coastal spring algal blooms. ELife: e11888. doi:10.7554/eLife.11888

Teira, E., D. Mourino-Carballido, S. Martinez-Garcia, C. Sobrino, J. Ameneiro, S. Henrnandez-Leon, and E. Vazquez. 2013. Primary production and bacterial carbon metabolism around South Shetland Islands in the Southern Ocean. Deep-Sea Res. 169: 70–81. doi:10.1016/j.dsr.2012.07.002

Thrash, J. C., B. Temperton, B. K. Swan, Z. C. Landry, T. Woyke, E. F. DeLong, R. Stepanauskas, and S. J. Giovannoni. 2014. Single-cell enabled comparative genomics of a deep ocean SAR11 bathytype. ISME J. 8: 1440–1451. doi:10.1038/ismej.2013.243

Treguer, P., D. M. Nelson, A. J. Van Bennekom, D. J. Demaster, A. Leynaert, and B. Queguiner. 1995. The silica balance in the world ocean: A reestimate. Science 268: 375–379. doi:10.1126/science.268.5209.375

Villar, E., and others. 2015. Ocean plankton. Environmental characteristics of Aguilas rings affect interocean plankton transport. Science 348: 1261447. doi:10.1126/science.1261447 doi:10.1126/science.1261447

Wilkins, D., and others. 2013a. Biogeographic partitioning of Southern Ocean microorganisms revealed by metagenomics.
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Conflict of Interest

None declared.