Title: Prokaryotic Niche Partitioning Between Suspended and Sinking Marine Particles

Running title: Suspended-/sinking-particle associated prokaryotes

Authors: Manon T Duret[1]*, Richard S Lampitt[2], Phyllis Lam[1]

[1]Ocean and Earth Science, University of Southampton

[2]National Oceanography Centre Southampton

PL, MTD and RSL designed the study. MTD collected and analysed samples, conducted data analysis and wrote the manuscript. PL assisted in the manuscript preparation. RSL provided feedback on the manuscript.

*Corresponding author:

Manon Duret,

Ocean and Earth Science, University of Southampton,

National Oceanography Centre Southampton

European Way, Southampton, SO14 3ZH,

United Kingdom

Tel: +44 2380 596478

Email: M.T.Duret@soton.ac.uk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1758-2229.12692

Originality & significance statement

This study is the first to distinguish the differences between prokaryotic microbial communities associated with sinking and suspended marine particles – as opposed to all previous work that has either treated the two particle types as one or focus only on sinking particles, such that the roles of suspended particles and their microbial inhabitants have been totally ignored. The two types of particles in this study were collected from the same water samples, but separated via the use of a marine snow catcher at four stations in the Scotia Sea (Southern Ocean). Using high-throughput amplicon sequencing of the 16S rRNA gene, our results revealed that suspended and sinking particles harbour different microbial communities, in terms of both composition and structure. Albeit present in both particle types, commonly known remineralising taxa were found to have preferences for one particle type or the other. This likely reflects different life strategies imposed by the nature of organic matter present within the two types of particle: *K*-strategists, specialised in the degradation of a narrower range of complex organic compounds, thrived on readily accessible yet semi-labile to refractory suspended particles; whereas the generalist fast-growing *r*-strategists preferred the sinking particles made of transient but more labile organic matter. This study provides new insights into the complexity of microbial communities in the pelagic ocean, and suggests different biogeochemical roles for suspended and sinking particle-associated prokaryotes in the marine carbon cycle. It also highlights the need to further assess the chemical composition and microbial activities in suspended and sinking particles in different oceanic regions, in order to gain better understanding of how the two types of particles may regulate the efficiency of the biological carbon pump and thus sequestration of atmospheric CO_2 by the oceans.

Summary

Suspended particles are major organic carbon substrates for heterotrophic microorganisms in the mesopelagic ocean (100–1000m). Nonetheless, communities associated with these particles have been overlooked compared to sinking particles, the latter generally considered as main carbon transporters to the deep ocean. This study is the first to differentiate prokaryotic communities associated with suspended and sinking particles, collected with a marine snow catcher at four environmentally distinct stations in the Scotia Sea. Amplicon sequencing of 16S rRNA gene revealed distinct prokaryotic communities associated with the two particle-types in the mixed-layer (0–100m) and upper-mesopelagic zone (mean dissimilarity 42.5 ± 15.2%). Although common remineralising taxa were present within both particle-types, gammaproteobacterial *Pseudomonadales* and *Vibrionales*, and alphaproteobacterial *Rhodobacterales* were found enriched in sinking particles up to 32-fold, while *Flavobacteriales* (*Bacteroidetes*) favoured suspended particles. We propose that this niche-partitioning may be driven by organic matter properties found within both particle-types: *K*-strategists, specialised in the degradation of complex organic compounds, thrived on semi-labile suspended particles, while generalists *r*-strategists were adapted to the transient labile organic contents of sinking particles. Differences between the two particle-associated communities were more pronounced in the mesopelagic than in the surface ocean, likely resulting from exchanges between particle-pools enabled by the stronger turbulence.

Introduction

The oceanic biological carbon pump annually removes about a third of anthropogenic atmospheric carbon dioxide (CO_2) (Sabine et al., 2004) via the export of organic carbon produced by phytoplankton in the sunlit surface ocean (euphotic zone) towards the deep ocean. Sinking particles are responsible for the rapid export of particulate organic matter (POM) into the mesopelagic (base of euphotic zone to ~1000 m) (Turner, 2015). The two major components of sinking particles are marine snow and faecal pellets (Ebersbach and Trull, 2008; Belcher et al., 2016). Marine snow particles are aggregates of ≥500 μm in size (Shanks and Trent, 1980), which include various materials such as microbial cells and phytodetrital materials (Alldredge and Silver, 1988; Simon et al., 2002) bound together by gel-like polymeric substances such as transparent exopolymer particles (TEP) (Alldredge et al., 1993). Faecal pellets can be produced by mesozooplankton (Stamieszkin et al., 2017) or microzooplankton (Gowing and Silver, 1985). They are a compacted form of biogenic material that is generally more resistant to remineralisation than marine snow (Ebersbach et al., 2011).

The depth reached by sinking particles is a key factor in oceanic carbon sequestration (Kwon et al., 2009) – the deeper POM reaches, the longer the carbon is removed from atmosphere-surface ocean reservoirs (Passow and Carlson, 2012). However, only 5 to 25 % of particles produced in the euphotic zone reach the mesopelagic zone (De La Rocha and Passow, 2007; Buesseler and Boyd, 2009) and even less reach the deep ocean (Francois et al., 2002). This is caused by POM remineralisation as particles sink, most of which takes place in the euphotic and upper-mesopelagic, leading to the release of CO_2 and dissolved inorganic nutrients. This remineralisation is responsible for the decrease in efficiency of the biological carbon pump (De La Rocha and Passow, 2007). It also results in the release of dissolve organic matter (DOM) and the disaggregation of sinking particles into smaller or suspended particles forming a trailing plume (Collins et al., 2015). Mesozooplankton, by their feeding behaviours (Lampitt et al., 1993), are responsible for most of sinking particle disaggregation (Giering et al., 2014) as well as the release of DOM (Strom et al., 1997). Complementary to this, final steps of remineralisation are mostly carried out by microbial communities (Cho and Azam, 1988; Smith et al.,

1992; Kiørboe and Jackson, 2001) that are either associated with particles or free-living in the water phase while feeding on the released DOM.

Prokaryotic communities associated with particles generally show higher concentrations, growth rates, enzymatic activities and metabolic diversity than their free-living counterparts (Grossart et al., 2007; Ganesh et al., 2014; Satinsky et al., 2014; Dang and Lovell, 2016). Both microbial communities are phylogenetically distinct. *Bacteroidetes*; *Vibrionales*, *Alteromonadales* and *Oceanospirillales* (*Gammaproteobacteria*); *Rhodobacterales* (*Alphaproteobacteria*); *Planctomycetes*; *Actinobacteria*; *Deltaproteobacteria*; *Campylobacterales* (*Epsilonproteobacteria*); and *Verrucomicrobia* are generally enriched in particle-associated communities (Delong et al., 1993; Crespo et al., 2013; Bižić-Ionescu et al., 2014; Lecleir et al., 2014; López-Pérez et al., 2016; Mestre et al., 2017). These taxa have adaptive capabilities for surface-attachment and enhanced abilities to assimilate and dissimilate single-carbon compounds and to degrade polysaccharides (Cottrell and Kirchman, 2000; Lyons and Dobbs, 2012; Fontanez et al., 2015). Particle-associated microbes are key actors in remineralisation processes as they are also typically detected during peaks of organic matter production such as phytoplankton blooms (Bauer et al., 2006; McCarren et al., 2010; Buchan et al., 2014) and oil spills (Mason et al., 2012). These observations, however, do not distinguish between sinking and suspended particle-associated microbial communities.

While the concentration of particulate organic carbon (POC) in sinking particles decreases exponentially with depth (Martin et al., 1987; Francois et al., 2002), the concomitant POC concentration in suspended particles remains largely constant and are ~1-2 orders of magnitude higher than that of sinking particles (Bishop et al., 1977; Bacon et al., 1985; Verdugo et al., 2004; Riley et al., 2012; Seitz et al., 2016). As current estimates of POC flux to the mesopelagic are not high enough to meet the carbon demand of heterotrophs (Boyd et al., 1999; Steinberg et al., 2008; Baltar et al., 2009), suspended particles have been considered a major organic carbon substrate for both microbes (Arístegui et al., 2009; Baltar et al., 2009, 2010) and metazoans (Gloeckler et al., 2017). In other words, while sinking particles as the main transporters of POM to depth are the starting point of remineralisation processes, suspended particles are a major sustaining POM substrate for mesopelagic

heterotrophs. This further suggests that sinking and suspended particles should have different ecological roles for microbes in the ocean, but no studies to date have investigated potential differences between microbial communities associated with these two particle types. Not only the species compositions of these associated communities remain unknown, but also the potentially different metabolic potentials and biogeochemical functions they confer. Any such differences would imply different modes of control on the efficiency of the biological carbon pump.

Methodologies traditionally used to sample particle-associated microbial communities are not suitable for collecting the two particle-types separately. They collect either solely sinking particles or bulk seston containing a mixture of sinking and suspended particles in unknown proportions. These techniques include:

(i) Direct particle-picking, biased towards larger-sized visible aggregates (Delong et al., 1993).

(ii) Sediment and gel traps for the collection of sinking particles (Lecleir et al., 2014; Fontanez et al., 2015).

(iii) *In situ* size-fractionated filtration with underwater submersible pumps collecting both suspended and sinking particles (McDonnell et al., 2015).

(iv) bulk seawater sampling with Niskin bottles followed by size-fractionated filtration collecting both suspended and sinking particles – main method used to date for microbial community analyses (Bižić-Ionescu et al., 2014).

The main variable considered by these methods is particle-size, as sinking particles are generally considered larger than suspended particles (Verdugo et al., 2004). However, the sole use of particle-size as discriminant is inaccurate as other parameters, such as particle-shape (Lam and Marchal, 2015) and mineral content (Ploug et al., 2008) also influence particle sinking velocity. Sinking velocity is a more objective parameter to differentiate between sinking and suspended particles. The marine snow catcher (MSC) (Lampitt et al., 1993) is a 95 L gentle water-sampler using sinking velocity to separate collection of the two particle-types from the same water sample (Riley et al., 2012). After retrieval from the desired depth, the MSC is left undisturbed on the ship's deck for two hours to allow sinking

particles to settle at the bottom of the sampler while suspended particles remain in suspension in the upper part (Fig. 1).

This study compares the diversity and structure of prokaryotic communities associated with suspended and sinking particles collected with an MSC deployed in the mixed layer (10 m below the deep chlorophyll maximum, DCM) and in the upper-mesopelagic (110 m below the DCM). Amplicon sequencing of the V4 region of 16S rRNA encoding gene was used to characterise particle-associated communities sampled at four stations of contrasting nutrient and productivity regimes in the Scotia Sea (Southern Ocean) (Fig. 1 and S2) during the austral summer 2014. These stations included two low-productivity stations – ICE at the marginal ice edge and P2 in a high-nutrient-low-chlorophyll region – and two more productive stations – P3 in an iron-enriched region and UP in an upwelling region (Table 1). We hypothesise that suspended and sinking particles, due to the likely different organic matter compositions, harbour different prokaryotic communities, and such distinctions vary between surface mixed layer and mesopelagic depths.

Results

Amplicon Sequencing Data Overview

A total of 6,979,982 V4 16S rDNA paired-ends reads were recovered from particle-associated communities (≥ 10 µm), and 247,354 sequences from free-living communities (0.22 – 10 µm). Although free-living communities are not the focus of this study, they are presented here as a reference for the surrounding water-column prokaryotic community. After sequence trimming, pairing, merging and removal of chloroplast rDNA sequences, 1,638,059 sequences remained in particle-associated libraries with an average length of 460 bp (74,126 ± 118,652 sequences/library). Differences between the number of sequences per library was largely caused by unequal proportions of chloroplastic rDNA (median of 75 and 77 % for suspended and sinking particle-associated libraries respectively). Suspended particle-associated communities were sequenced to greater depths than sinking particle-associated communities (Fig. S3). In order to allow an unbiased comparison of communities associated with suspended and sinking particles of diameter ≥ 10 µm, libraries were rarefied to the smallest number of sequences per library.

Overall Community Structure in Various Particle-Fractions

A non-parametric multidimensional scaling (NMDS) analysis based on Bray Curtis dissimilarity distance of OTU (operational taxonomic unit) relative abundances (Fig. 2) revealed a clear distinction between (a) suspended particle-associated, (b) sinking particle-associated and (c) free-living communities. Suspended and sinking particle-associated communities and free-living communities formed distinct, despite loose, clusters in the mixed layer and the upper-mesopelagic. An unweighted pair group method with arithmetic means (UPGMA) tree at a 70% cut-off (Fig. 3) corroborated these observations showing similar clustering patterns at both sampling depths. The average dissimilarity between suspended and sinking particle-associated communities was higher in the upper-mesopelagic (48.1 ± 12.9 %) than in the mixed layer (36.8 ± 15.3 %), except at UP (Table S1). A permutational multivariate analysis of variance (PERMANOVA) (Table S2) revealed that particle-type (suspended or sinking particle-associated, or free-living) was a strong predictor of variability, explaining 27 % of OTU composition against 15 and 20 % attributed to the sample origin (station and depth, respectively) ($p < 0.05$).

Every station had >50 % dissimilarity compared to any other, and samples collected at ICE exhibited the highest dissimilarity (~70 % in the mixed layer and ~80 % in the upper-mesopelagic) (Table S1). Accordingly, communities sampled at ICE clustered individually from other stations (Fig. 2 and 3). Furthermore, a higher dissimilarity was observed between communities in the upper-mesopelagic (69.7 ± 12.8 %) than in the mixed layer (63.1 ± 14.9 %) at all stations. A PERMANOVA analysis of environmental parameters (Table S2) revealed that variation in temperature, oxygen and POC concentration (Table 1) altogether predicted 43 % of the OTU composition ($p < 0.05$).

A total of 8,156 OTUs were represented in this dataset with 139 OTU representing 75% of the overall sequences. The proportion of OTUs shared between sinking and suspended particle-associated communities was low in both the mixed layer (13.7 ± 4.6 %) and upper-mesopelagic (16.0 ± 2.2 %) (Fig. 4 and Table S3), although they represented a large proportion of the sequences in all samples. These sequences affiliated with OTUs shared between suspended (83.8 ± 14.5 %) and sinking particle-associated communities (74.0 ± 13.2 %) were proportionally higher in the mixed layer than in

the upper-mesopelagic (77.0 ± 11.3 % and 65.5 ± 21.1 % respectively). Sinking particle-associated communities had a higher proportion of unique OTUs (absent from their suspended particle counterpart and free-living communities) at both depths, comprising 25.0 ± 18.4 % of total OTU (representing 31.0 ± 9.6 % of sequences). Suspended particle-associated communities shared more sequences with free-living communities (85.0 ± 11.8 %) than did sinking particle communities in the mixed layer (57.3 ± 23.8 %) and in the upper-mesopelagic (75.5 ± 8.3 % versus 64.5 ± 14.2 % respectively). The alpha-diversity indices, Shannon's and Pielou's, as well as the unbiased number of OTUs, were higher for sinking particle-associated communities in the mixed layer (Fig. S4). In contrast, they were higher for suspended particle-associated communities in the upper-mesopelagic. Higher values indicate higher OTU richness and evenness.

Microbial Community Composition

Despite variability inherent to sampled stations, disparities between suspended and sinking particle-associated were apparent at both depths, even at lower taxonomic levels. *Proteobacteria* and *Bacteroidetes* were the two most abundant phyla, respectively representing 65.8 ± 13.4 % and 22.6 ± 12.2 % of the overall dataset (Fig. S5A). In the upper-mesopelagic the former was significantly enriched in sinking particle-associated communities, while the latter was enriched in suspended particle-associated communities (Kruskall-Wallis test, $p < 0.05$). Although not significantly, the phyla *Crenarchaeota* and *Planctomycetes* were more abundant in suspended particles while *Fibrobacteres* was more abundant in sinking particles at the same depth. In both mixed layer and upper-mesopelagic, the phyla *Firmicutes* and *Actinobacteria* were more abundant in sinking particles while archaeal phylum *Euryarchaeota* was more abundant in suspended particles.

With regards to the taxonomic class level (Fig. S5B), *Flavobacteria* and *Saprospirae* (*Bacteroidetes*), *Deltaproteobacteria*, *Thermoplasmata* (*Euryarchaeota*), *Thaumarchaeota* (*Crenarchaeota*), AB16 (SAR406), OM190 (*Planctomycetes*) and BS119 relatives, as well as *Acidobacteria* were in significantly higher proportion in suspended than in sinking particle-associated communities in the upper-mesopelagic (Kruskall-Wallis, $p < 0.05$) (Table 2). On the other hand, *Gammaproteobacteria* sequences were significantly more abundant in sinking particles (Kruskall-Wallis, $p < 0.05$). Other

taxonomic classes that appeared enriched within sinking particle-associated communities include *Alphaproteobacteria*, *Bacilli* (*Firmicutes*) and *Cytophagia* (*Bacteroidetes*), though the difference was not statistically significant. In the mixed layer, only *Bacilli* and *Betaproteobacteria* sequences were significantly enriched in sinking particle-associated communities (Kruskall-Wallis, $p < 0.05$). No other consistent and/or significant patterns was observed in the mixed layer across stations.

At lower taxonomic levels (order in Fig. 5 and family in Fig. 3), compositional differences between suspended and sinking particle-associated communities were also more evident in the upper-mesopelagic than in the mixed layer. A similarity percentage analysis (SIMPER) revealed that the family composition of suspended and sinking particle-associated communities was more dissimilar in the upper-mesopelagic than in the mixed layer, except at UP (Table S4). Furthermore, higher numbers of taxa explained the dissimilarity observed between suspended and sinking particle-associated communities in the upper-mesopelagic than in the mixed layer. Regarding the 50 most abundant taxonomic orders, 31 showed identical enrichment patterns across particle-associated communities sampled at every station in the upper-mesopelagic (19 within suspended and 12 within sinking particles), while only 10 showed similar enrichment patterns in the mixed layer (1 within suspended and 9 within sinking particles).

At the taxonomic order level (order in Fig. 5), suspended particle-associated communities were enriched in; *Bacteroidetes* (including *Saprospirales* and *Flavobacteriales*), *Planctomycetes* (*Planctomycetales*, *Pirellulales* and OM190 relatives), *Euryarchaeota* (*Cenarchaeales*) and *Crenarchaeota* (*Thermoplasmata*, E2), as well as in several orders from *Deltaproteobacteria* (*Spirobacillales*, *Myxococcales*, *Syntrophobacterales*, *Desulfobacterales*, and PB19, NB1-j and Sva0853 relatives), and from *Verrucomicrobia* (*Puneiceicoccales*, *Pedosphaerae*), the betaproteobacterial *Neisserales*, the actinomicrobial *Acidimicrobiales*, and the SAR406 relatives (ZA3648c and Arctic96B-7) in the upper-mesopelagic. On the other hand, sinking particle-associated communities were enriched in *Gammaproteobacteria* (including *Pseudomonadales*, *Vibrionales*, *Pasteurellales* and *Salinisphaerales*), *Alphaproteobacteria* (*Rhodobacterales* and *Rhizobiales*), *Firmicutes* (*Bacillales*, *Gemellales* and *Lactobacillales*) and *Actinobacteria* (*Actinomycetales*), as

well as the *Bacteroidetes* order *Cytophagales* and the *Fibrobacteres* order *Fibrobacterales* in the upper-mesopelagic.

In the mixed layer, only the gammaproteobacterial *Oceanospirillales* was enriched within suspended particle-associated communities. Conversely, *Gammaproteobacteria* (*Pseudomonadales* and *Enterobacteriales*), *Betaproteobacteria* (*Burkholderiales* and *Neisseriales*), *Alphaproteobacteria* (*Sphingomonadales* and *Rhizobiales*) and *Firmicutes* (*Bacillales*, *Clostridiales* and *Gemellales*) orders, as well as the *Actinobacteria* order *Actinomycetales* was enriched within sinking particle-associated communities.

Discussion

This study is the first to compare microbial communities associated with suspended and sinking particles. Using next-generation amplicon sequencing of the V4 16S rRNA genes, our results reveal previously undiscovered differences between the two prokaryotic communities and clear differences between suspended and sinking particle-associated communities in terms of both structure and diversity. Taxa previously and collectively known as particle-associated were found to have their own specific and significant enrichment in one of the two particle-types. While significant at both depths, prokaryotic niche partitioning observed between the two particle-associated communities was more evident in the upper-mesopelagic than in the mixed layer.

Suspended and Sinking Particles Niche Partitioning

Regardless of site-to-site differences, particle-type was the factor explaining most of community composition variability, with suspended and sinking particle-associated communities displaying enrichments of specific taxa. Members of *Gammaproteobacteria*, along with alphaproteobacterial *Rhodobacterales* and *Bacteroidetes* are ubiquitous heterotrophic members of oceanic bacterial communities that often bloom in association with phytoplankton populations (Buchan et al., 2014). Although all are common particle-associated taxa (Delong et al., 1993; Crespo et al., 2013; Bižić-Ionescu et al., 2014; Lecleir et al., 2014; Fontanez et al., 2015; López-Pérez et al., 2016), they were significantly enriched in either suspended or sinking particle-associated communities.

In the upper-mesopelagic, sinking particle-associated communities were significantly enriched with *Gammaproteobacteria* (including *Pseudomonadales*, *Vibrionales* and *Pasteurellales*), *Alphaproteobacteria* (*Rhodobacterales* and *Rhizobiales*), *Actinobacteria* and *Firmicutes*. Gammaproteobacterial *Pseudomonadales* and *Vibrionales* (Pinhassi and Berman, 2003; Allers et al., 2007; Mason et al., 2012; Stewart et al., 2012; Logue et al., 2015; Liu et al., 2017), as well as members of Rhodobacterales, including Rhodobacteraceae (Buchan et al., 2005; Brinkhoff et al., 2008; Mayali et al., 2015), are generalist copiotrophs exhibiting surface-attachment capabilities (e.g., biofilm formation, pili, non-specific extracellular polymers and proteins). Their versatile metabolic capabilities allow them to utilise a wide spectrum of phytoplankton-derived organic matter substrates, ranging from monocyclic to aromatic compounds (labile to more refractory compounds) (Sperling et al., 2017). They respond rapidly to enrichments of organic matter, both in dissolved and particulate forms, in their environment facilitated by enhanced sensory chemotaxis and motility. They are commonly considered as primary colonisers of marine particles which initiate the process of remineralisation (Dang and Lovell, 2016). While microbial communities associated with sinking particles are thought to be relatively stable with depth (Mestre et al., 2018), recent reports further suggest that these communities at depth originate from the euphotic zone and have continuously been modified during particle sinking, as opposed to being the consequence of a succession of *in situ* colonisers at depth (Thiele et al., 2015).

Chemotaxis, motility, surface attachment capabilities, as well as acute competition strategies, would allow these taxa to rapidly sense, colonise and consume sinking particles. Subsequently, their ability to degrade low- to high-molecular-weight (LMW to HMW) organic compounds would allow them to degrade the transient composition of organic matter within sinking particles as they sink and become increasingly refractory. Furthermore, their aptitude to produce antimicrobial secondary metabolites and quorum sensing signalling molecules, coupled with biofilm forming strategies (Givskov et al., 1997; Whitehead et al., 2001; Gram et al., 2002; Buchan et al., 2005; Thomas et al., 2008; Dang and Lovell, 2016) could allow them to regulate specific traits (such as motility loss, exoenzyme production) to enhance their competitiveness to exploit the ecological niche within sinking particles.

On the other hand, the phylum *Bacteroidetes* (including *Flavobacteriales* and *Saprospirales*), *Planctomycetes* (*Planctomycetales* and *Pirellulales*), *Deltaproteobacteria* (*Desulfobacterales* and *Myxococcales*) and *Verrucomicrobia* were significantly enriched within suspended particle-associated communities in the upper-mesopelagic. *Planctomycetes* and *Verrucomicrobia* have been reported to have abilities for surface-attachment, such as on detrital aggregates (Delong et al., 1993; Crump et al., 1999), and for biopolymers degradation (García-Martínez et al., 2002; Woebken et al., 2007). *Flavobacteria* also have adaptations for surface-attachment (Gómez-Pereira et al., 2012). These taxa are typically associated with the decaying phase of phytoplankton blooms (Buchan et al., 2014) and are considered secondary particle colonisers (Dang and Lovell, 2016). Unlike their counterparts on sinking particles, *Flavobacteria* appears to be specialised in the degradation of complex and HMW organic compounds (Bauer et al., 2006; Williams et al., 2013; Kabisch et al., 2014). Accordingly, unlike *Roseobacter* whose genomes are abundant in ABC transporters (specialised in the uptake of LMW compounds) encoding genes, *Flavobacteria* genomes are abundant in genes encoding for TonB dependent-transporters that are active in the uptake of HMW molecules (Tang et al., 2012).

Reintjes et al. (2017) have shown that both *in vivo* and *in vitro Bacteroidetes* members are able to assimilate large polysaccharides in their periplasmic space, where subsequent hydrolysis to LMW and cell-uptake takes place. This "selfish" hydrolysis strategy leads to a reduction of diffusive loss to the cell surroundings. This uptake mechanism is more efficient than the one traditionally assumed to take place on sinking particles, from which hydrolytic exoenzymes released by the associated heterotrophic community leads to the formation of a plume enriched in dissolved organic matter consumed by free-living communities (Smith et al., 1992; Kiørboe and Jackson, 2001). If relevant to other members of the suspended particle-associated community, this restrictive strategy of organic matter consumption could be an ecological indicator of the differential nature of organic substrate present within suspended and sinking particles. In other words, suspended particle-associated communities may specialise in energy-efficient hydrolytic pathways to consume semi-labile organic matter with constant supply, while competitive colonisers in sinking particles are able to rapidly adapt to the transient nature of organic matter, ranging from labile to refractory as the particle sinks.

In addition to the observed niche partitioning, the structure and diversity of suspended and sinking particle-associated communities also varied among sampled stations, as driven by environmental factors like temperature, particulate organic carbon concentration and chlorophyll *a* concentration. UP and P3, for instance, are characterised by higher primary production levels than P2 and ICE owing to their influence by the South Georgia continental margin that upwells nutrients into surface waters (Atkinson et al., 2001). Samples from ICE, located in the marginal ice zone of the Antarctic continent, were on the other hand strong outliers. Melting ice provides nutrient-rich, cold and salty brines to the surface layer that are responsible for shaping prokaryotic communities. These communities are generally dominated by psychrophilic bacteria adapted to sub-zero temperatures and high-salinity conditions (Brown and Bowman, 2001), and are clearly distinct from those from other stations (Fig. 2 and 3).

Organic Matter as a Potential Driver for Life Strategy

Alteromonadales members (Lauro et al., 2009; McCarren et al., 2010) and *Rhodobacterales* (Dang and Lovell, 2016) are known primary particle colonisers. They grow rapidly to exploit competitively transient sources of organic matter and are usually the first to respond to organic pulses (e.g. Nelson and Wear, 2014; Pedler et al., 2014; Sheik et al., 2014) – and thus can be regarded as *r*-strategists. At the other end of the spectrum, *Bacteroidetes* are secondary colonisers highly specialised in the degradation of complex HMW organic compounds. Typically found in the decaying phase of a phytoplankton bloom (Bauer et al., 2006; Dang and Lovell, 2016), they are more akin to *K*-strategists. The differential metabolic capabilities of *r*- and *K*-strategists is enabled by their distinct genomic repertoires (Lauro et al., 2009). The two life strategies have previously been applied to explain the variability of free-living microbes in stable oligotrophic environments versus in transient nutrient-rich environments (Giovannoni et al., 2014). We here propose that a similar theory could be applied to particle-associated communities in order to explain the apparent enrichments of *r*-strategists on sinking particles and *K*-strategists on suspended particles. The niche differentiation between ubiquitous heterotrophic bacteria in suspended and sinking particles may be driven by the different nature of organic matter available within the two particle-pools in the Scotia Sea.

Heterotrophic bacteria differ in their ability to degrade organic matter, which is why modifications in phytoplankton composition lead to a succession in associated bacterial communities (Pinhassi et al., 2004; Teeling et al., 2012). Furthermore, as the phytoplankton bloom ages, the composition of organic matter produced changes; starting from labile LMW compounds at early stages (e.g., amino acids, carbohydrates), to HMW compounds at later stages (e.g., nucleic acids, polysaccharides) (Buchan et al., 2014). Accordingly, in both experimental and environmental settings of organic matter enrichments, alteromonads are the first detected, closely followed by *Rhodobacterales*, which is eventually outcompeted by *Flavobacteria*, as the organic matter lability decreases (environmental – e.g. Teeling et al., 2012, and experimental – e.g., Fuchs et al., 2000, Sheik et al., 2014, Yamada et al., 2016). This suggests that suspended particles in the upper-mesopelagic contained a higher proportion of semi-labile organic matter, which would be favourable for *K*-strategists. Meanwhile, the fast-growing *r*-strategists would colonise sinking particles in the euphotic zone, where organic matter is relatively fresh, and would subsequently modify their metabolism as the particle sinks in response to continuous changes in organic matter composition and lability. Therefore, organic matter quality is likely a major determining factor upon the selection of apparent *r-/K*-strategists in particle-associated communities, though other intrinsic capabilities may also add to the selection process, such as hydrolytic abilities, biofilm formation, quorum sensing mechanisms, chemotaxis sensitivity and motility.

Differential Particle Dynamics with Depth

More pronounced differences between suspended and sinking particle-associated communities were observed in the upper-mesopelagic than in the mixed layer (Fig. 4 and Table S3). Within the mixed layer, where most particulate organic matter is produced, a plethora of biotic and abiotic processes take place, including microbial remineralisation (leading to the release of dissolved organic matter – e.g., Carlson, 2002 – and reprocessed particulate organic matter – e.g., Turner, 2015), zooplankton feeding activities (Giering et al., 2014) and turbulent mixing. These processes lead to continuous aggregation and disaggregation of particles, altering their sizes and other physical and chemical properties (Lampitt et al., 1990; Burd and Jackson, 2009). Particle dynamic processes linking

suspended and sinking particle-pools together, as enabled by the stronger physical mixing and higher particle concentrations, are more intense in the mixed layer than in the upper-mesopelagic (Lam and Marchal, 2015). The higher proportion of taxa showing an enrichment in either particle-type in the upper-mesopelagic than in the mixed layer, corroborates the higher connectivity between the two particle-types in the mixed layer. Adding to the higher connectivity of particle pools, inherent physiological traits of heterotrophic bacteria could also reduce the differences between prokaryotic communities associated with either particle-type. For instance, motility of marine heterotrophic microbes is enhanced in highly productive environments (Dang and Lovell, 2016) such as within the mixed layer.

Conclusion

While typical ubiquitous particle-associated remineralising taxa were expectedly found in our samples, suspended and sinking particle-associated communities were not identical. In the upper-mesopelagic, the niche partitioning of heterotrophic taxa likely reflects a different life strategy imposed by the composition of organic matter available within the two particle-pools: *K*-strategists would exploit more refractory compounds bound to suspended particles, while *r*-strategists would benefit from their fast growth rates and broad hydrolytic capabilities to degrade the transient composition of organic compounds in sinking particles. In the mixed layer, continuous dynamic biotic and abiotic processes alter particles, which leads to a higher connectivity between the two particle-associated communities.

While sinking particles are primarily responsible for the rapid delivery of labile particulate organic carbon to the upper-mesopelagic, suspended particles constitute the majority of marine particulate organic carbon in the ocean and support most of the microbial carbon respiration in the mesopelagic (Baltar et al., 2009, 2010). This is consistent with extremely low respiration rates measured on sinking particles at the same stations (~ 5.19 ng C h^{-1} $particle^{-1}$) (Belcher et al., 2016). Consequently, suspended and sinking particle-associated communities are likely playing different roles in the oceanic biological carbon pump. As most suspended particles come from the disaggregation of sinking particles, their associated microbial communities coupled with free-living communities

undertake final remineralisation steps leading to most of the CO_2 production (Collins et al., 2015), thereby reducing the biological carbon pump efficiency. Further investigations are much needed to uncover active metabolic pathways in carbon remineralisation, such as with metatranscriptomic analyses, along with their rates of occurrence within the two particle-types, in order to gain better insights into the respective biogeochemical functions of the two particle-types, and hence more accurate understanding of how organic carbon is remineralised in the ocean.

Experimental procedures

Sampling stations and particle collection

Sampling took place during the austral summer 2014 (15 November – 17 December) on-board the RRS *James Clark Ross* (cruise JR304). Four stations of contrasting nutrient regimes and productivity in the Scotia Sea were sampled, including two low-productivity stations – ICE at the marginal ice edge and P2 in a high-nutrient-low-chlorophyll region – and two more productive stations – P3 in an iron-enriched region and UP in an upwelling region (Table 1). Surface chlorophyll *a* data represented on the map (Fig. 1) was constructed with the Ocean Data View software (https://odv.awi.de) using the mean values for December 2014 collected by the MODIS satellite (http://oceancolor.gsfc.nasa.gov/cgi/l3).

Particles were collected with a marine snow catcher (MSC) (Fig. S1) deployed in the mixed layer (10m below the deep chlorophyll maximum [DCM]) and the upper-mesopelagic (110m below the DCM) within ~ 30 minutes of each other, the former depth usually corresponding to a peak in particle abundance (Lampitt et al., 1993; Belcher et al., 2016). The DCM was defined by using fluorescence profiles from the conductivity-temperature-depth sensors (CTD Seabird 9Plus with SBE32 carousel) cast, at most, 4 hours prior to the MSC deployment (Fig. S2). Temperature, oxygen concentration and chlorophyll *a* concentration based on fluorescence measurements were obtained from CTD casts, and particulate organic carbon concentrations were measured from samples collected with the MSC as measured by (Belcher et al., 2016).

Suspended and sinking particles were collected from the upper part and the base of the MSC respectively. Sinking particles are here defined as the particles that have sunk to the base of the MSC

bottom after standing on deck for 2 hours (average sinking speed ≥ 12 m d^{-1}) and include both slow- and fast-sinking particles (Riley et al., 2012). Particles remaining in suspension in the upper part of the MSC are considered suspended. Microbial communities associated with suspended particles were sampled by sequentially filtering ~10 L of seawater collected from the upper part of the MSC through (i) a 100 µm pore-size nylon filter (47 mm diameter, Millipore), (ii) a 10 µm pore-size polycarbonate membrane filter (47 mm diameter, Millipore), and (iii) a 0.22 µm pore-size Sterivex cartridge filter (Millipore) driven by a peristaltic pump. Microbial communities associated with sinking particles were collected by gravity-filtering ~1.5 L of seawater from the bottom part of the MSC onto a 10 µm pore-size polycarbonate membrane filter. Both filtering steps were performed in under 1 hour, and filters were subsequently incubated with RNAlater (Ambion™, Thermo Fisher Scientific) for 12 hours at 4˚C, prior to being stored at -80˚C until further processing onshore. All equipment used for sampling procedures were cleaned in RNaseZap (Ambion™, Thermo Fisher Scientific) and rinsed with Milli-Q water prior to sampling.

DNA extraction, amplification and sequencing

Nucleic acids were recovered from the filters using a ToTALLY RNA kit (Ambion™, Thermo Fisher Scientific) followed by a DNA extraction step as described in Lam et al. (2011) – though only DNA extracts were considered in this study. Extracted DNA was further purified with a Wizard DNA clean-up system (Promega) following the manufacturer's recommendations.

Prior to amplification, the DNA extracted from 100 and 10 µm pore-size filters of suspended particles were pooled together to allow comparison with 10 µm pore-size filters collected from sinking particles, and hence consider particles ≥ 10 µm. DNA extracts from both filters were pooled in equal volumes in order to preserve the signal ratios and minimise distortion of the community structure on suspended particles ≥ 10 µm and were used as a template for PCR amplification.

Amplicon sequencing of prokaryotic 16S rDNA V4 region was performed according to Herbold et al. (2015). The primer set Pro341F (5'-CCTACGGGNBGCASCAG-3') and Pro805R (5'-GACTACNVGGGTATCTAATCC-3'; 464 bp) (Takahashi et al., 2014), and proofreading polymerase Kapa HiFi HotStart (Kapa Biosystems) were used at first for a 25-cycle PCR. Amplicons

were subsequently used as templates for a 10-cycle PCR to link them to specific Illumina adapters necessary for downstream sequencing. As one sample (UP station sinking particle in the mixed layer) had a total amount of extracted DNA less than 12.5 ng, a nested PCR approach was applied with an additional amplification with the universal 16S rDNA primers set, 27F (5'-AGAGTTTGGATCMTGGCTCAG-3') and 1492R (5'-ACCTTGTTACGACTT-3'; 1,465bp) prior to the two-step PCR described above. This procedure did not change the profile of the communities as evidenced by Fig. S6A and B, including the nested PCR sample from this study along with other samples collected during the same cruise which were amplified using the same method. After each PCR round, amplicons were purified with the Agencourt AMPure XP PCR clean-up kit (Beckman Coulter) following manufacturer's recommendations. The quality of purified amplicons was assessed with a DNA7500 Kit read on a 2100 BioAnalyser (Agilent Technologies), and the quantity measured with a Qubit dsDNA High-Sensitivity assay kit (Invitrogen™, Thermo Fisher Scientific).

Purified amplicons were pooled at equimolar concentrations (4 nM each) for the library preparation using a Nextera XT DNA kit (Illumina) following manufacturer's recommendations. Finally, the amplicons were sequenced with an Illumina MiSeq sequencing system (M02946, Illumina).

Bioinformatics

The sequence data has been submitted to the GenBank under accession number SUB2922837. Raw sequences were demultiplexed and their adapter sequences trimmed using the MiSeq Control software (v2.5.0.5, Illumina) directly after sequencing. The quality of demultiplexed raw read pairs was checked with FastQC (v 0.11.4; Babraham Bioinformatics). Forward and reverse reads with a maximum read length of 500 bp and Phred33 quality score of 0.8 were merged with the PANDAseq assembler software (v 2.8) (Masella et al., 2012). Open reference OTU clustering was subsequently performed under QIIME (MacQIIME v 1.9.1_20150604) (Caporaso et al., 2010), using the UCLUST algorithm with a minimum sequence identity of 97% against the 16S rRNA Silva database (v 128) (Quast et al., 2013). Singleton OTU and sequences affiliated with chloroplastic rDNA were removed from this dataset.

Data analyses

Statistical analyses were performed with the R statistics software (http://www.rstudio.com/) using the vegan package. All statistical analyses were performed on the rarefied dataset. The non-parametric multidimensional scaling (NMDS) analysis and the unweighted pair group method with arithmetic mean (UPGMA) tree were based on the Bray Curtis dissimilarity distance of OTU relative abundance compositions. The significance of factors influencing the communities OTU composition was investigated with permutational multivariate analyses of variance (PERMANOVA). Similarity percentage analysis (SIMPER) was used to investigate average differences between community compositions at the family level. The significance of relative abundance differences between particle-associated communities was assessed by a Kruskall-Wallis test with a p-value cut-off at 0.05.

Enrichment was calculated based on the $\log_2$(fold change) of taxonomic order relative abundance differences between sinking and suspended particle-associated communities in the rarefied dataset. It was calculated using the following equation:

$$Enrichment = \log_2\left(\frac{RA_{sinking}}{RA_{suspended}}\right) \quad \text{(Equation 1)}$$

with $RA_{sinking}$ as the relative abundance of the taxa in sinking particle-associated community at one depth and $RA_{suspended}$ as the relative abundance of the same taxa in suspended particle-associated community at the same depth. Unique and shared OTU between free-living, suspended and sinking particle-associated communities were also calculated on the rarefied dataset, while the phylum and class composition bar charts presented were based on the relative abundance of the non-rarefied dataset.

References

Alldredge, A.L., Passow, U., and Logan, B.E. (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. Deep. Res. Part I 40: 1131–1140.

Alldredge, A.L. and Silver, M.W. (1988) Characteristics, dynamics and significance of marine snow. Prog. Oceanogr. 20: 41–82.

Allers, E., Gómez-Consarnau, L., Pinhassi, J., Gasol, J.M., Šimek, K., and Pernthaler, J. (2007) Response of Alteromonadaceae and Rhodobacteriaceae to glucose and phosphorus manipulation in marine mesocosms. Environ. Microbiol. 9: 2417–2429.

Arístegui, J., Gasol, J.M., Duarte, C.M., and Herndl, G.J. (2009) Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54: 1501–1529.

Atkinson, A., Whitehouse, M.J., Priddle, J., Cripps, G.C., Ward, P., and Brandon, M.A. (2001) South Georgia, Antarctica: A productive, cold water, pelagic ecosystem. Mar. Ecol. Prog. Ser. 216: 279–308.

Bacon, M.P., Huh, C.A., Fleer, A.P., and Deuser, W.G. (1985) Seasonality in the flux of natural radionuclides and plutonium in the deep Sargasso Sea. Deep Sea Res. Part A, Oceanogr. Res. Pap. 32: 273–286.

Baltar, F., Arístegui, J., Gasol, J.M., Sintes, E., and Herndl, G.J. (2009) Evidence of prokaryotic metabolism on suspended particulate organic matter in the dark waters of the subtropical North Atlantic. Limnol. Oceanogr. 54: 182–193.

Baltar, F., Arístegui, J., Sintes, E., Gasol, J.M., Reinthaler, T., and Herndl, G.J. (2010) Significance of non-sinking particulate organic carbon and dark CO2 fixation to heterotrophic carbon demand in the mesopelagic northeast Atlantic. Geophys. Res. Lett. 37: 1–6.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., et al. (2006) Whole genome analysis of the marine Bacteroidetes Gramella forsetii reveals adaptations to degradation of polymeric organic matter. Environ. Microbiol. 8: 2201–2213.

Belcher, A., Iversen, M., Manno, C., Henson, S.A., Tarling, G.A., and Sanders, R. (2016) The role of particle associated microbes in remineralization of fecal pellets in the upper mesopelagic of the Scotia Sea, Antarctica. Limnol. Oceanogr. 61: 1049–1064.

Bishop, J.K.B., Edmond, J.M., Ketten, D.R., Bacon, M.P., and Silker, W.B. (1977) The chemistry, biology, and vertical flux of particulate matter from the upper 400 m of the equatorial Atlantic Ocean. Deep Sea Res. 24: 511–548.

Bižić-Ionescu, M., Zeder, M., Ionescu, D., Orlić, S., Fuchs, B.M., Grossart, H.-P., and Amann, R. (2014) Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization. Environ. Microbiol. 1–36.

Boyd, P.W., Sherry, N.D., Berges, J.A., Bishop, J.K.B., Calvert, S.E., Charette, M.A., et al. (1999) Transformations of biogenic particulates from the pelagic to the deep ocean realm. Deep. Res. Part II Top. Stud. Oceanogr. 46: 2761–2792.

Brinkhoff, T., Giebel, H.A., and Simon, M. (2008) Diversity, ecology, and genomics of the Roseobacter clade: A short overview. Arch. Microbiol. 189: 531–539.

Brown, M. V. and Bowman, J.P. (2001) A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol. Ecol. 35: 267–275.

Buchan, A., González, J.M., and Moran, M.A. (2005) Overview of the marine Roseobacter lineage. Appl. Environ. Microbiol. 71: 5665–5677.

Buchan, A., LeCleir, G.R., Gulvik, C. a, and González, J.M. (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12: 686–698.

Buesseler, K.O. and Boyd, P.W. (2009) Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean. Limnol. Oceanogr. 54: 1210–1232.

Burd, A.B. and Jackson, G.A. (2009) Particle aggregation. Ann. Rev. Mar. Sci. 1: 65–90.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. (2010) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108: 4516–4522.

Carlson, C. A. (2002) Production and Removal Processes. In, Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, pp. 91–151.

Cho, B.C. and Azam, F. (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332: 441–443.

Collins, J.R., Edwards, B.R., Thamatrakoln, K., Ossolinski, J.E., DiTullio, G.R., Bidle, K.D., et al. (2015) The multiple fates of sinking particles in the North Atlantic Ocean. Global Biogeochem. Cycles 29: 1471–1494.

Cottrell, M.T. and Kirchman, D.L. (2000) Natural Assemblages of Marine Proteobacteria and Members of the Cytophaga-Flavobacter Cluster Consuming Low- and High-Molecular-Weight Dissolved Organic Matter. Appl. Environ. Microbiol. 66: 1692–1697.

Crespo, B.G., Pommier, T., Fernández-Gómez, B., and Pedrós-Alió, C. (2013) Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. Microbiologyopen 2: 541–552.

Crump, B.C., Crump, B.C., Armbrust, E.V., Armbrust, E.V., Baross, J. a, and Baross, J. a (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.

Dang, H. and Lovell, C.R. (2016) Microbial Surface Colonization and Biofilm Development in Marine Environments. Microbiol. Mol. Biol. Rev. 80: 91–138.

Delong, E.F., Franks, D.G., and Alldredge, A.L. (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38: 924–934.

Ebersbach, F. and Trull, T.W. (2008) Sinking particle properties from polyacrylamide gels during KEOPS: controls on carbon export in an area of persistent natural iron inputs in the Southern Ocean. Limnol Ocean. 53: 212–224.

Ebersbach, F., Trull, T.W., Davies, D.M., and Bray, S.G. (2011) Controls on mesopelagic particle fluxes in the Sub-Antarctic and Polar Frontal Zones in the Southern Ocean south of Australia in summer-Perspectives from free-drifting sediment traps. Deep. Res. Part II Top. Stud. Oceanogr. 58: 2260–2276.

Fontanez, K.M., Eppley, J.M., Samo, T.J., Karl, D.M., and DeLong, E.F. (2015) Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre. Front. Microbiol. 6: 1–14.

Francois, R., Honjo, S., Krishfield, R., and Manganini, S. (2002) Factors controlling the flux of organic carbon to the bathypelagic zone of the ocean. Global Biogeochem. Cycles 16: 34-1-34–20.

Fuchs, B.M., Zubkov, M. V., Sahm, K., Burkill, P.H., and Amann, R. (2000) Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. Environ. Microbiol. 2: 191–201.

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

García-Martínez, J., Acinas, S.G., Massana, R., and Rodríguez-Valera, F. (2002) Prevalence and microdiversity of Alteromonas macleodii-like microorganisms in different oceanic regions. Environ. Microbiol. 4: 42–50.

Giering, S.L.C., Sanders, R., Lampitt, R.S., Anderson, T.R., Tamburini, C., Boutrif, M., et al. (2014) Reconciliation of the carbon budget in the ocean's twilight zone. Nature 507: 480–483.

Giovannoni, S.J., Cameron Thrash, J., and Temperton, B. (2014) Implications of streamlining theory for microbial ecology. ISME J. 8: 1–13.

Givskov, M., Eberl, L., and Molin, S. (1997) Control of exoenzyme production, motility and cell differentiation in Serratia liquefaciens. FEMS Microbiol. Lett. 148: 115–122.

Gloeckler, K., Choy, C.A., Hannides, C.C.S., Close, H.G., Goetze, E., Popp, B.N., and Drazen, J.C. (2017) Stable isotope analysis of micronekton around Hawaii reveals suspended particles are an important nutritional source in the lower mesopelagic and upper bathypelagic zones. Limnol. Oceanogr. 0:.

Gómez-Pereira, P.R., Schüler, M., Fuchs, B.M., Bennke, C., Teeling, H., Waldmann, J., et al. (2012) Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. Environ. Microbiol. 14: 52–66.

Gowing, M.M. and Silver, M.W. (1985) Minipellets: A new and abundant size class of marine fecal pellets. J. Mar. Res. 43: 395–418.

Gram, L., Grossart, H., Schlingloff, A., and Kiørboe, T. (2002) Possible quorum sensing in marine snow bacteria: Production of acylated homoserine lactones by Roseobacter strains isolated from marine snow. Appl. Environ. Microbiol. 68: 4111–4116.

Grossart, H.P., Tang, K.W., Kiørboe, T., and Ploug, H. (2007) Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. FEMS Microbiol. Lett. 266: 194–200.

Herbold, C.W., Pelikan, C., Kuzyk, O., Hausmann, B., Angel, R., Berry, D., and Loy, A. (2015) A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. Front. Microbiol. 6: 731.

Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M., et al. (2014) Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes "Gramella forsetii" KT0803. ISME J. 8: 1492–1502.

Kiørboe, T. and Jackson, G. a. (2001) Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnol. Oceanogr. 46: 1309–1318.

Kwon, E.Y., Primeau, F., and Sarmiento, J.L. (2009) The impact of remineralization depth on the air–sea carbon balance. Nat. Geosci. 2: 630–635.

De La Rocha, C.L. and Passow, U. (2007) Factors influencing the sinking of POC and the efficiency of the biological carbon pump. Deep. Res. Part II Top. Stud. Oceanogr. 54: 639–658.

Lam, P., Jensen, M.M., Kock, A., Lettmann, K.A., Plancherel, Y., Lavik, G., et al. (2011) Origin and fate of the secondary nitrite maximum in the Arabian Sea. Biogeosciences 8: 1565–1577.

Lam, P.J. and Marchal, O. (2015) Insights into Particle Cycling from Thorium and Particle Data. Ann. Rev. Mar. Sci. 7: 159–184.

Lampitt, R.S., Noji, T., and von Bodungen, B. (1990) What happens to zooplankton faecal pellets? Implications for material flux. Mar. Biol. 104: 15–23.

Lampitt, R.S., Wishner, K.F., Turley, C.M., and Angel, M. V. (1993) Marine snow studies in the Northeast Atlantic Ocean: distribution, composition and role as a food source for migrating plankton. Mar. Biol. 116: 689–702.

Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S., et al. (2009) The genomic basis of trophic strategy in marine bacteria. Proc. Natl. Acad. Sci. U. S. A. 106: 15527–33.

Lecleir, G.R., Debruyn, J.M., Maas, E.W., Boyd, P.W., and Wilhelm, S.W. (2014) Temporal changes in particle-associated microbial communities after interception by nonlethal sediment traps. FEMS Microbiol. Ecol. 87: 153–163.

Liu, S., Wawrik, B., and Liu, Z. (2017) Different bacterial communities involved in peptide decomposition between normoxic and hypoxic coastal waters. Front. Microbiol. 8: 1–17.

Logue, J.B., Stedmon, C.A., Kellerman, A.M., Nielsen, N.J., Andersson, A.F., Laudon, H., et al. (2016) Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. ISME J. 10: 533–545.

López-Pérez, M., Kimes, N.E., Haro-Moreno, J.M., and Rodriguez-Valera, F. (2016) Not All Particles Are Equal: The Selective Enrichment of Particle-Associated Bacteria from the Mediterranean Sea. Front. Microbiol. 7:.

Lyons, M.M. and Dobbs, F.C. (2012) Differential utilization of carbon substrates by aggregate-associated and water-associated heterotrophic bacterial communities. Hydrobiologia 686: 181–193.

Martin, J.H., Knauer, G. a., Karl, D.M., and Broenkow, W.W. (1987) VERTEX: carbon cycling in the northeast Pacific. Deep Sea Res. Part A. Oceanogr. Res. Pap. 34: 267–285.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., and Neufeld, J.D. (2012) PANDAseq: PAired-eND Assembler for Illumina sequences. BMC Bioinformatics 13: 31.

Mason, O.U., Hazen, T.C., Borglin, S., Chain, P.S.G., Dubinsky, E.A., Fortney, J.L., et al. (2012) Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J. 6: 1715–1727.

Mayali, X., Stewart, B., Mabery, S., and Weber, P.K. (2015) Temporal succession in carbon incorporation from macromolecules by particle-attached bacteria in marine microcosms. Environ. Microbiol. Rep. 8: 68–75.

McCarren, J., Becker, J.W., Repeta, D.J., Shi, Y., Young, C.R., Malmstrom, R.R., et al. (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc. Natl. Acad. Sci. U. S. A. 107: 16420–7.

McDonnell, A.M.P., Lam, P.J., Lamborg, C.H., Buesseler, K.O., Sanders, R., Riley, J.S., et al. (2015) The oceanographic toolbox for the collection of sinking and suspended marine particles. Prog. Oceanogr. 133: 17–31.

Mestre, M., Borrull, E., Sala, Mm., and Gasol, J.M. (2017) Patterns of bacterial diversity in the marine planktonic particulate matter continuum. ISME J. 1–12.

Mestre, M., Ruiz-gonzález, C., Logares, R., Duarte, C.M., and Gasol, J.M. (2018) Sinking particles promote vertical connectivity in the ocean microbiome. 1–9.

Passow, U. and Carlson, C.A. (2012) The biological pump in a high CO2 world. Mar. Ecol. Prog. Ser. 470: 249–271.

Pedler, B.E., Aluwihare, L.I., and Azam, F. (2014) Single Bacterial Strain Capable of Significant Contribution to Carbon Cycling in the Surface Ocean. Proc. Natl. Acad. Sci. U. S. A. 111: 7202–7207.

Pinhassi, J. and Berman, T. (2003) Differential growth response of colony-forming alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. Appl. Environ. Microbiol. 69: 199–211.

Pinhassi, J., Havskum, H., Peters, F., and Malits, A. (2004) Changes in Bacterioplankton Composition under Different Phytoplankton Regimens. Appl. Environ. Microbiol. 70: 6753–6766.

Ploug, H., Iversen, M.H., and Fischer, G. (2008) Ballast, sinking velocity, and apparent diffusivity within marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria. Limnol. Oceanogr. 53: 1878–1886.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41: 590–596.

Reintjes, G., Arnosti, C., Fuchs, B.M., and Amann, R. (2017) An alternative polysaccharide uptake mechanism of marine bacteria. ISME J. 11: 1640–1650.

Riley, J.S., Sanders, R., Marsay, C., Le Moigne, F.A.C., Achterberg, E.P., and Poulton, A.J. (2012) The relative contribution of fast and slow sinking particles to ocean carbon export. Global Biogeochem. Cycles 26: 1–10.

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., et al. (2004) The oceanic sink for anthropogenic CO2. Science (80-.). 305: 367–371.

Satinsky, B.M., Crump, B.C., Smith, C.B., Sharma, S., Zielinski, B.L., Doherty, M., et al. (2014) Microspatial gene expression patterns in the Amazon River Plume. Proc. Natl. Acad. Sci. 111: 11085–90.

Seitz, K.W., Lazar, C.S., Hinrichs, K.-U., Teske, A.P., and Baker, B.J. (2016) Genomic reconstruction of a novel, deeply branched sediment archaeal phylum with pathways for acetogenesis and sulfur reduction. ISME J. 1–10.

Shanks, A.L. and Trent, J.D. (1980) Marine snow: Sinking rates and potential role in vertical flux. Deep. Res. Part a-Oceanographic Res. Pap. 27: 137–143.

Sheik, A.R., Brussaard, C.P.D., Lavik, G., Lam, P., Musat, N., Krupke, A., et al. (2014) Responses of the coastal bacterial community to viral infection of the algae Phaeocystis globosa. ISME J. 8: 212–25.

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

Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359: 139–142.

Sperling, M., Piontek, J., Engel, A., Wiltshire, K.H., Niggemann, J., Gerdts, G., and Wichels, A. (2017) Combined carbohydrates support rich communities of particle-associated marine bacterioplankton. Front. Microbiol. 8: 1–14.

Stamieszkin, K., Poulton, N., and Pershing, A. (2017) Zooplankton grazing and egestion shifts particle size distribution in natural communities. Mar. Ecol. Prog. Ser. 575: 43–56.

Steinberg, D.K., Van Mooy, B. a. S., Buesseler, K.O., Boyd, P.W., Kobari, T., and Karl, D.M. (2008) Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnol. Oceanogr. 53: 1327–1338.

Stewart, F.J., Dalsgaard, T., Young, C.R., Thamdrup, B., Revsbech, N.P., Ulloa, O., et al. (2012) Experimental Incubations Elicit Profound Changes in Community Transcription in OMZ Bacterioplankton. PLoS One 7: e37118.

Strom, S.L., Benner, R., Ziegler, S., and Dagg, M.J. (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. Linmol. Ocean. 42: 1364–1374.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., and Nishijima, M. (2014) Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS One 9: e105592.

Tang, K., Jiao, N., Liu, K., Zhang, Y., and Li, S. (2012) Distribution and functions of tonb-dependent transporters in marine bacteria and environments: Implications for dissolved organic matter utilization. PLoS One 7:.

Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., et al. (2012) Substrate-Controlled Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom. Science (80-.). 336: 608–611.

Thiele, S., Fuchs, B.M., Amann, R., and Iversen, M.H. (2015) Colonization in the Photic Zone and Subsequent Changes during Sinking Determine Bacterial Community Composition in Marine Snow. Appl. Environ. Microbiol. 81: 1463–1471.

Thomas, T., Evans, F.F., Schleheck, D., Mai-Prochnow, A., Burke, C., Penesyan, A., et al. (2008) Analysis of the Pseudoalteromonas tunicata genome reveals properties of a surface-associated life style in the marine environment. PLoS One 3: 1–11.

Turner, J.T. (2015) Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog. Oceanogr. 130: 205–248.

Verdugo, P., Alldredge, A.L., Azam, F., Kirchman, D.L., Passow, U., and Santschi, P.H. (2004) The oceanic gel phase: A bridge in the DOM-POM continuum. Mar. Chem. 92: 67–85.

Whitehead, N.A., Barnard, A.M.L., Slater, H., Simpson, N.J.L., and Salmond, G.P.C. (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol. Rev. 25: 365–404.

Williams, T.J., Wilkins, D., Long, E., Evans, F., Demaere, M.Z., Raftery, M.J., and Cavicchioli, R. (2013) The role of planktonic Flavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. Environ. Microbiol. 15: 1302–1317.

Woebken, D., Fuchs, B.M., Kuypers, M.M.M., and Amann, R. (2007) Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl. Environ. Microbiol. 73: 4648–4657.

Yamada, Y., Fukuda, H., Tada, Y., Kogure, K., and Nagata, T. (2016) Bacterial enhancement of gel particle coagulation in seawater. Aquat. Microb. Ecol. 77: 11–22.

Figure Legends

Figure 1. Location of sampling sites on a map of sea surface chlorophyll *a*. The map was constructed on Ocean Data View using data from NASA Ocean Color 9 km resolution level 3 browser. The chlorophyll *a* data was corrected with the OCx algorithm and the POC data was corrected by D. Stramski 2007 method (version 443/555) and correspond to a 32-day composition (17/11/2014-18/12/2014).

Figure 2. Non-metric multidimensional scaling plot of OTU composition. NMDS was calculated with Bray-Curtis distance of the rarefied dataset. The significance of environmental parameters (oxygen, fluorescence and POC concentrations and temperature) was tested using a PERMANOVA ($p < 0.05$). Significant environmental parameters are displayed and their respective arrow length is proportional to the community structure variability they explain. ML = mixed layer, UM = upper-mesopelagic.

Figure 3. Heatmap of taxonomic family composition and similarity clustering. The heatmap represents log (relative abundance) of the rarefied dataset. The similarity tree was calculated with an unweighted pair group method with arithmetic mean based on Bray-Curtis distance of the rarefied dataset.

Figure 4. Venn diagrams of average proportions of shared and unique OTU and affiliated sequences. The proportions of shared and unique OTU were calculated on the rarefied dataset and correspond to average values from all stations. Detailed numbers for each station are presented in Table S3. Numbers in regular black font are the average number of shared OTU normalised to the total number of OTU. Numbers in regular coloured font (red for suspended particles, blue for sinking particles and green for free-living) are the average number of unique OTU normalised to the total number of OTU. Numbers in bold coloured font are the relative abundance of sequences affiliated with shared or unique OTU.

Figure 5. Enrichment of taxonomic orders in suspended and sinking particle-associated communities. The $\log_2$ (fold change) was calculated for the 50 most abundant taxonomic orders and

is based on their relative abundance within sinking and suspended particle-associated communities (see Equation 1). Negative values (red) correspond to an enrichment in suspended particles and positive values (blue) in sinking particles. The legend values of 5/-5 correspond to Inf/-Inf, i.e., showing the absolute absence of an order in a sample compared to its counterpart.

Fig. 1

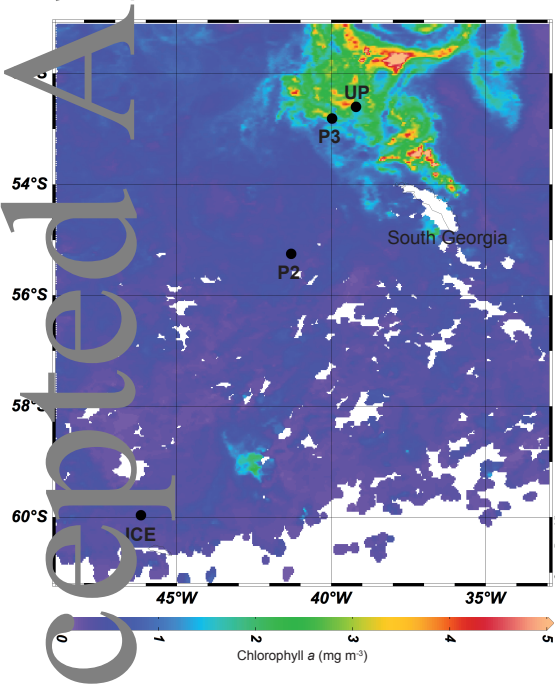

Fig. 2

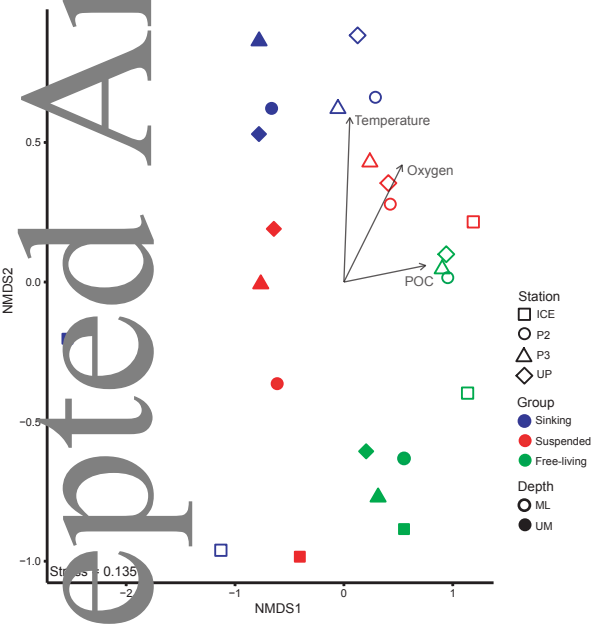

Fig. 3

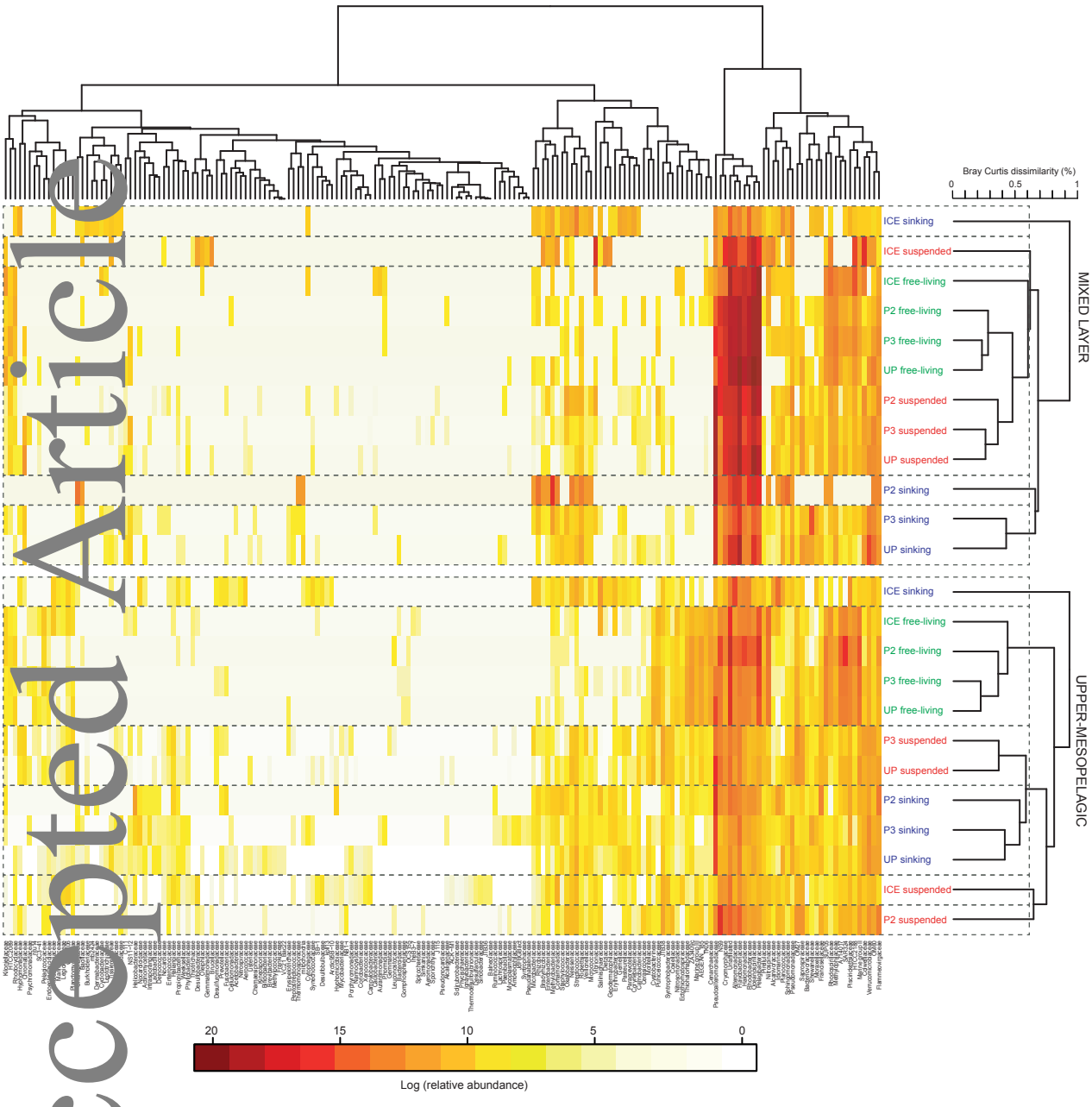

Fig. 4

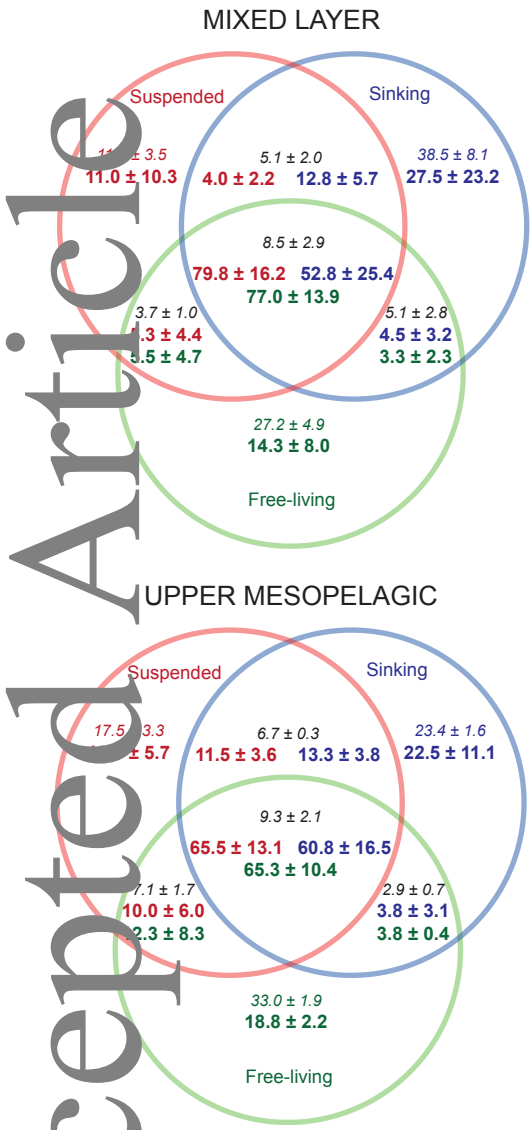

Fig. 5

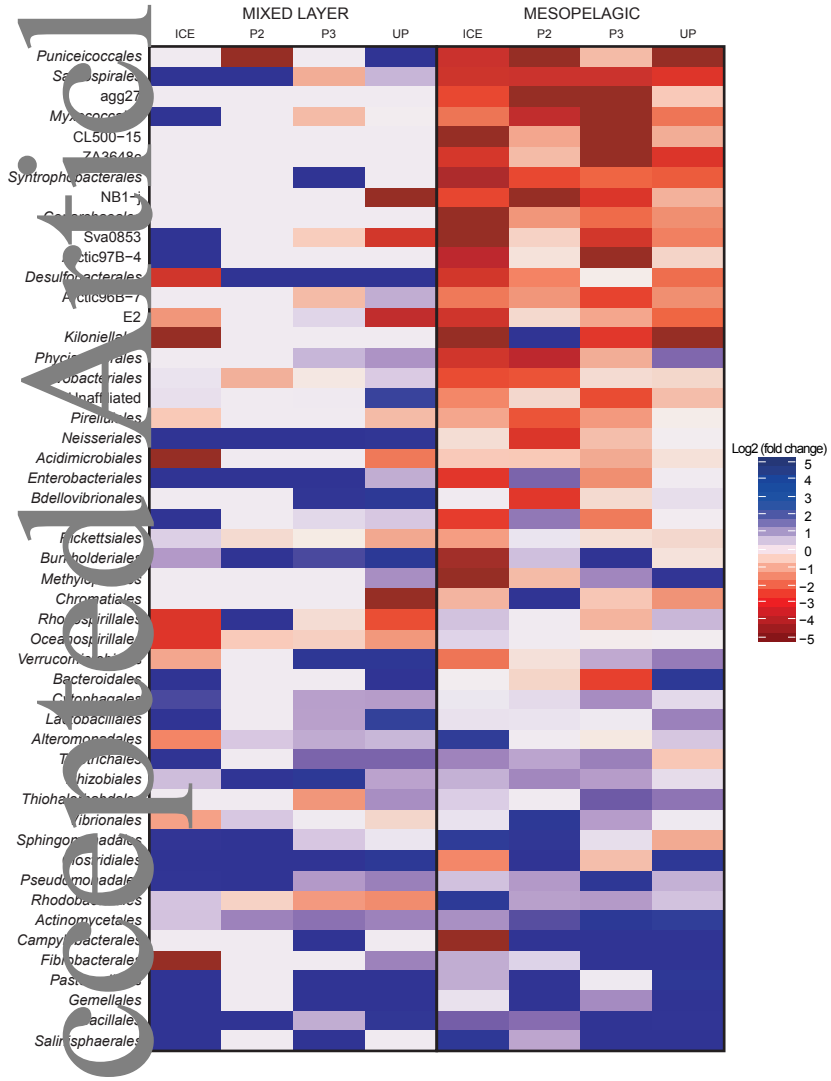

Table Legends

Table 1. Sampling stations description and associated hydrochemical variables. Mean chlorophyll a concentration corresponds to the average of values measured above the mixed layer depth as a proxy for productivity. PFZ = Polar Front Zone; AZZ = Antarctic zone.

Latitude (˚N)	Longitude (˚E)	Station	Station description	Mean chlorophyll *a* (µg L^{-1})	Depth (m)	Potential temperature (˚C)	Salinity	Oxygen (µM)	Particle-type	POC (µg L^{-1})
-59.9624	-46.1597	ICE	On the Antarctic continental ice edge.	0.40	60	-1.06	34.2	333.6	SS	75.7
									SK	2.5
					160	-0.76	34.4	253.6	SS	32.2
									SK	10.3
-55.2484	-41.264	P2	HNLC zone.	0.37	55	0.18	33.8	348.9	SS	124.6
									SK	14.1
					155	0.84	34.2	246.4	SS	38.6
									SK	2.7
-52.8121	-39.9724	P3	Iron fertilized zone, near South Georgia continental margin.	1.90	70	1.87	33.9	334.0	SS	124.2
									SK	5.6
					170	1.52	34.3	222.3	SS	31.4
									SK	3.9
-52.6018	-39.1994	UP	Upwelling station in proximity to P3, in frontal system PFZ and AAZ.	1.23	70	1.99	33.9	335.2	SS	92.2
									SK	22.5
					170	1.50	34.3	222.3	SS	45.8
									SK	9.1

Table 3.1

Table 2. Relative abundances of taxonomic classes in suspended and sinking particle-associated communities and comparison. The overall 30 most abundant taxonomical class classes are presented. Significant statistical differences between the two particle-types were tested with a Kruskall-Wallis test. Significant differences ($p < 0.05$) are highlighted with an asterisk (*) and the highest relative abundances with bold font.

	MIXED LAYER			**UPPER-MESOPELAGIC**		
	p-value	Sinking	Suspended	p-value	Sinking	Suspended
Gammaproteobacteria	0.69	44.4%	55.3%	0.03*	**59.5%**	29.8%
Flavobacteria	0.89	22.3%	23.5%	0.03*	13.1%	**34.3%**
Alphaproteobacteria	1	16.8%	16.4%	49.00%	7.5%	4.2%

Deltaproteobacteria	0.89	0.9%	0.5%	0.03*	1.7%	**10.3%**
Unaffiliated	0.2	4.1%	1.8%	11.00%	1.0%	1.8%
Bacilli	0.03*	**4.5%**	0.2%	11.00%	3.4%	1.2%
Thermoplasmata	0.69	0.1%	0.2%	0.03*	0.6%	**2.2%**
Verrucomicrobia	0.89	1.7%	0.3%	89.00%	3.8%	2.2%
Cytophagia	0.06	0.6%	0.3%	69.00%	3.1%	2.1%
Betaproteobacteria	0.03*	**1.5%**	0.3%	89.00%	1.1%	1.9%
AB16	0.46	0.0%	0.0%	0.03*	0.2%	**0.7%**
Saprospira	0.2	0.7%	0.2%	0.03*	0.3%	**2.8%**
Pedosphaera	1	0.0%	0.0%	0.03*	0.2%	**0.5%**
Actinobacteria	0.2	1.2%	0.6%	6.00%	1.3%	0.3%
Acidimicrobia	1	0.0%	0.0%	20.00%	0.3%	0.4%
Planctomycetia	0.77	0.1%	0.2%	20.00%	0.4%	0.9%
OM190	0.19	0.0%	0.0%	0.03*	0.1%	**1.3%**
Fibrobacteria	0.66	0.0%	0.0%	20.00%	0.6%	0.4%
Opituta	1	0.2%	0.1%	0.03*	0.0%	0.2%
SAR202	NA	0.0%	0.0%	89.00%	0.1%	0.0%
Thaumarchaeota	NA	0.0%	0.0%	0.03*	0.0%	**0.2%**
Clostridia	0.11	0.4%	0.0%	49.00%	0.3%	0.2%
Bacteroidia	0.41	0.2%	0.0%	69.00%	0.3%	0.2%
Phycisphaera	1	0.1%	0.0%	11.00%	0.1%	0.3%
Epsilonproteobacteria	0.87	0.0%	0.0%	36.00%	0.5%	0.1%
SHAB590	0.45	0.0%	0.0%	46.00%	0.1%	0.3%
BS119	0.19	0.0%	0.0%	0.03*	0.0%	**0.2%**
Sphingobacteria	1	0.0%	0.0%	20.00%	0.0%	0.1%
Halobacteria	0.45	0.0%	0.0%	100.00%	0.1%	0.1%

Table 3.2