Structure and function of high Arctic pelagic, particle-associated and benthic bacterial communities

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Summary

Arctic marine microbes are affected by environmental changes that may ultimately influence their functions in carbon cycling. Here, we investigated in concert the structure and enzymatic activities of pelagic, particle-associated and benthic bacterial communities in the central Arctic Ocean, and used these data to evaluate microbial structure–function relationships. Our findings showed influences of hydrographic conditions and particle association on community composition, and sharp pelagic-benthic contrasts. In addition to community compositional differences, regional and depth-related patterns in enzymatic activities were observed. Peptide hydrolysis rates were highest in surface waters, especially at ice-free and first year ice-covered regions, and decreased with depth. While the range of hydrolysed polysaccharides showed varying geographic patterns, particles often showed a wider spectrum of polysaccharide hydrolase activities. Summed benthic peptidase rates differed across stations but showed similar proportions of individual enzyme activities. Analysing for potential linkages between structure and function after subtracting the effect of environmental conditions revealed no direct link, indicating functional redundancy to carry out peptide hydrolysis among pelagic microbes. Thus, while community composition and activities are influenced by environmental conditions, bacterial functional redundancy suggests that compositional shifts – in response to the changing Arctic – may have complex and less predictable functional consequences than previously anticipated.

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Introduction

Microbial communities in the Arctic Ocean are diverse, distinct from lower latitude communities and structured by a range of features (e.g., Boetius *et al*., 2015). In Arctic marginal seas and the western Arctic Ocean, microbial communities are shaped by spatial variations in physicochemical regimes in the Arctic water column (Galand *et al*., 2010; Kirchman *et al*., 2010; Bowman *et al*., 2012; Fu *et al*., 2013; Winter *et al*., 2013; Han *et al*., 2015). Strong temporal fluctuations in environmental conditions result in seasonal changes in community composition in the upper water column (Bano and Hollibaugh, 2002; Alonso-Sáez *et al*., 2014; Han *et al.,* 2015). In addition to hydrography, particle association has emerged as a major factor affecting microbial community composition. While particle-associated microbial assemblages are mostly distinct from those of the water column and sediments (DeLong *et al*., 1993; Kellogg and Deming, 2009), recently observed compositional overlap between surface water microbial communities and deep water particle-associated assemblages highlights the role of particle colonization and vertical connectivity in shaping microbial communities (Thiele *et al*., 2015). Hydrographic regimes in overlying water (Hamdan *et al*., 2013; Buttigieg and Ramette, 2015) and particle association impact benthic microbial community structure, as shown for settling phytodetritus (Bienhold *et al*., 2012; Jacob *et al*., 2013), and sinking sea ice algae aggregates (Rapp *et al*., 2018).

Despite low temperatures and a narrow temporal window of primary production in the Arctic (Kirchman *et al*., 2009; Boetius *et al*., 2015), microbial communities in the Arctic Ocean carry out photosynthetic carbon assimilation and heterotrophic carbon degradation, and render the Arctic a net carbon sink (MacGilchrist *et al*., 2014). Microbial communities initiate organic matter degradation via the activities of their extracellular enzymes, which determine the quantity and quality of bioavailable substrates (Arnosti, 2011). The rates at which organic substrates are degraded, however, as well as the substrate specificities of microbially produced enzymes, are not well-constrained. Studies from the Arctic marginal seas (Kellogg and Deming 2009; Sala *et al*., 2010; Tamelander, 2013),

fjords (Steen and Arnosti, 2013, Teske *et al*., 2011; Arnosti, 2015), continental shelves (Bienhold *et al*., 2012; Boetius and Damm 1998) and the Canadian Archipelago (Kellogg and Deming 2014), suggest that microbial enzymatic activities are characterized by somewhat lower rates and considerably narrower substrate spectra than those in temperate and tropical latitudes (Arnosti *et al*., 2011). However, beyond pioneering initial surveys (Bano and Hollibaugh, 2002; Galand *et al*., 2010; Kirchman *et al*., 2010; Ghiglione *et al*., 2012), the composition and organic matter degrading capabilities of Arctic microbial communities remain poorly characterized, particularly in sea-ice covered regions and deep waters (Boetius *et al*., 2015).

Whether and to what extent these compositional and functional differences among microbial communities are linked to each other in a predictable manner is a question of considerable interest (Arnosti, 2011). For example, microbial communities and their carbon cycling capabilities may be affected by changes in the Arctic environment, including rising surface water temperatures and declining sea-ice cover (Wassmann, 2011). In coming years, these physical changes may lead to increases in the quantity of dissolved organic carbon routed through the microbial loop (Kirchman *et al*., 2009). Understanding variations in microbial community composition and activities along environmental and depth gradients is a critical step in determining the structure–function relationships of these communities. Consequently, the extent to which microbial communities are functionally dissimilar (i.e., changes in community composition have direct functional consequences) or functionally redundant (i.e., changes in environmental conditions, and not community composition, have proximate control over microbial functionality) in part determines whether shifts in microbial community composition may result in changes in ecosystem process rates (Reed and Martiny, 2007; Strickland *et al*., 2009).

To investigate this problem, we coupled community composition as determined on the basis of high throughput sequencing of 16S rRNA gene amplicons with empirical measurements of enzymatic activities to characterize the biogeographical distribution and organic matter degrading capabilities of pelagic, particle-associated and benthic bacterial communities in the central Arctic Ocean. Our aims were to identify the key environmental parameters that affect bacterial community composition and extracellular enzymatic activities along environmental and depth gradients. Using data on community composition and enzymatic activities in the water column, we investigated microbial structure–function relationships, and disentangled the relationships between environmental conditions, bacterial communities and peptidase activities. This integrated study illuminates surface-to-deep profiles of bacterial community structure and function at a time of rapid changes in the underexplored Arctic Ocean.

Results

Environmental context

With the exception of an open water station in the Laptev Sea, all stations were located in the Eurasian Basin (Fig. 1), and spanned a gradient of sea ice conditions ranging from open water at 79°N, to partially ice-covered (first year ice) and fully ice-covered conditions (multi-year ice) at 88°N (Table 1). Water masses targeted include the comparatively low salinity Polar Mixed Layer, the higher salinity Atlantic Layer and the Eurasian Basin Deep Water and Eurasian Basin Bottom Water (Supporting Information Fig. S1; Stein and Macdonald, 2004).

Bacterial community composition patterns

The dataset contains 1,088,000 sequences, with each sample rarefied to 34,000 sequences, and which represent 4055 unique OTUs at a 97% similarity cutoff. Nonmetric multidimensional scaling (NMDS) revealed that bacterial community composition differed by water mass and sample type (Fig. 2). Bacterial communities of the surface water and subsurface chlorophyll maximum (SCM) – bulk as well as particle-associated at SCM – clustered close to each other, signifying compositionally similar bacterial communities at these two depths. Bacterial communities from bulk water samples at 500 m and deep waters (≥2000 m) formed distinct groups that clustered closely together but separated from surface and SCM communities. Pairwise comparisons revealed that compositional differences among bulk bacterial communities in the surface and SCM are not significant (ANOSIM, $R = -0.076$, $p = 0.663$), while those between 500 m and deep waters are (ANOSIM, $R = 0.774$, $p = 0.001$). Particle-associated samples at the SCM formed a tighter cluster than those at 500 m. Sediment bacterial communities formed the tightest grouping (Fig. 2), indicating high compositional similarity across all sites.

Clustering patterns in the ordination space reflect the different relative proportions of taxonomic groups with depth and habitat based on our sequence datasets, which are sensitive to the sequencing approach. Methods such as fluorescent *in situ* hybridization would provide a more rigorously quantitative assessment. In any case, in the sea ice (melt pond and algae aggregate samples) and Polar Mixed Layer water samples (surface, SCM and SCM particle-associated samples), members of the class *Alphaproteobacteria* (20%–54%), *Flavobacteria* (20%–76%) and *Gammaproteobacteria* (18%–55%) were well represented (Fig. 3). Bulk seawater from the Atlantic

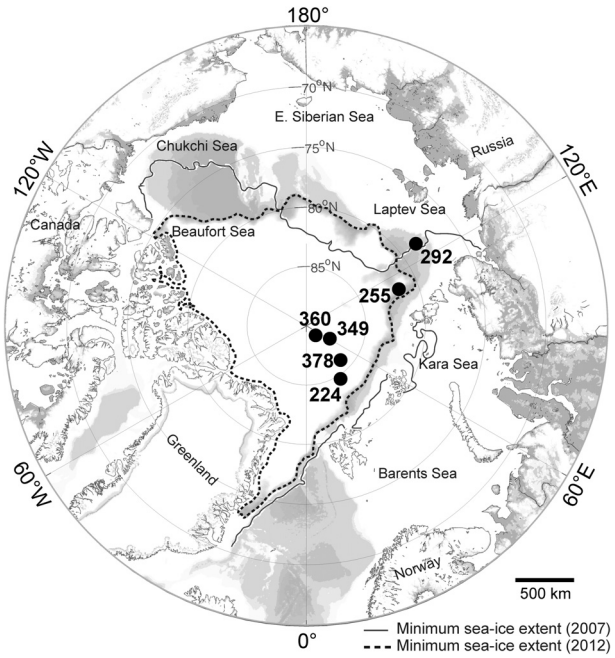

Fig. 1. A map of the central Arctic Ocean and marginal seas. The unbroken dark grey line shows the boundaries of the historic minimum in sea-ice extent in 2007, and the dashed line illustrates the historic minimum in sea-ice extent in 2012, around the time of sample collection. Black circles denote the sampling stations, with one in the open ocean (292), two in the ice-margin (224, 255) and three under full-ice cover (349, 360, 378). The map was modified from Marcel Nicolaus, Alfred Wegener Institute.

Layer and Eurasian Basin Deep Water and Bottom Water were comprised largely of bacterial taxa belonging to *Alphaproteobacteria* (30%–50%), *Gammaproteobacteria* (20%–48%), *Deltaproteobacteria* (7%–18%) and the AB16 clade within the phylum SAR406 (2%–9%). Sequences from particles at 500 m primarily correspond to members of the *Gammaproteobacteria* (62%–93%), *Alphaproteobacteria* (1%–32%) and *Flavobacteria* (1%–6%). Bulk water bacterial communities exhibited greater species richness and evenness with increasing depth (Supporting Information Fig. S2). Sediment bacterial communities yielded the highest diversity scores by any measure (Supporting Information Fig. S2); their most relatively abundant groups included *Gammaproteobacteria* (31%–46%), *Acidimicrobiia* (9%–15%), *Alphaproteobacteria*, (12%–17%), *Deltaproteobacteria* (6%–8%) and *Flavobacteria* (4%–8%). The most abundant OTU, most closely related to the alphaproteobacterium *Pelagibacter ubique* HTCC 1062, comprised 9.2% of the entire dataset. An OTU most closely related to the flavobacterium *Polaribacter irgensii* was the second most abundant OTU, comprising 5.4% of the dataset. Gammaproteobacterial OTUs related to *Colwellia piezophila*, an uncultured member of the family *Oceanospirillaceae* and an uncultured member of the OM60 clade represented the third, fourth and fifth most abundant OTUs in the entire dataset, respectively.

Influences of environmental conditions versus geographic distances

We sought to identify environmental factors that correlated with community dissimilarity. Temperature, salinity and oxygen concentrations contributed 29%, 24% and 21%, respectively, to the variation observed among surface-to-deep pelagic bacterial communities, all with statistical significance (ADONIS, $p = 0.001$); ca. 26% of variances were unaccounted for by the model. As a caveat, however, these correlations were scale-dependent. Taking into account only samples from the Polar Mixed Layer (surface and SCM), bacterial community dissimilarity was only significantly correlated with temperature (ADONIS, $R^2 = 0.42$, $p = 0.003$). Because environmental parameters can be spatially structured, we performed partial Mantel tests to measure the relative influence of environmental conditions on whole pelagic bacterial community dissimilarity, holding for the effect of geographic distances. We used temperature, salinity and oxygen to calculate an environmental condition dissimilarity matrix using Euclidean distances. Environmental parameters and bacterial community dissimilarity are strongly correlated (Mantel test, $r = 0.78$, $p = 0.001$); the relationship between geographic distances and community dissimilarity is not significant (Mantel Test, $r = -0.13$, $p = 0.99$).

Differentiation and depth-related trends of particle-associated communities

Particle association results in a community distinct from the bulk water samples both at the SCM (ANOSIM, $R = 0.74$, $p = 0.01$) and at 500 m (ANOSIM, $R = 1$, $p = 0.03$). Particle-associated bacterial communities also exhibited lower species richness and evenness compared with the bulk water community at the same depths (Supporting Information Fig. S2). SCM particle samples included substantial proportions of *Flavobacteria* and *Gammaproteobacteria,* with some contribution of *Alphaproteobacteria* (Fig. 2). Predominantly detected *Flavobacteria* on SCM particles include an OTU closely related to members of an uncultured cluster belonging to the family *Flavobacteraceae*, as well as OTUs closely related to *Polaribacter irgensii* 23-P (Supporting Information Fig. S3). Gammaproteobacteria on SCM particles are largely represented by members of the NOR5/OM60 clade (30%–65% of *Gammaproteobacteria*) (Supporting Information Fig. S4A), with some contribution of OTUs closely related to *Colwellia piezophila* and *Colwellia*

Table 1. List of samples, station ID, available data and physical parameters.

Sample	Type	Station ID	Latitude (°N)	Longitude (°E)	Depth (m)	BCC	EEA	Temp. (°C)	Salinity (PSU)	Oxygen (μM)
224A	Surface	PS 80/227–1	84.02	31.23	2	+	pep/poly	−1.5	33.4	389.7
224B	SCM	PS 80/231–1	84.02	31.21	50	+	pep/poly	−1.8	34.2	387.9
224G	SCM-PA	PS 80/231–1	84.02	31.21	50	+	pep/poly	−1.8	34.2	387.9
224C	500 m	PS 80/227–1	84.02	31.23	500	+	pep/poly	1.2	34.9	315.8
224H	500 m-PA	PS 80/227–1	84.02	31.23	500	*na*	pep/poly	1.2	34.9	315.8
224I	Deep	PS 80/227–1	84.02	31.23	2000	+	*na*	−0.7	34.9	303.6
224D	Deep	PS 80/227–1	84.02	31.23	3994	+	pep/poly	−0.7	34.9	303.6
224E	Sediment	PS 80/225–2	84.03	31.24	4014	*na*	poly	−0.7	34.9	303.6
255A	Surface	PS 80/254–1	82.71	109.14	1	*na*	pep/poly	−1.6	32.8	396.6
255B	SCM	PS 80/265–1	83.09	110.10	23	+	pep/poly	−1.7	33.4	395.3
255G	SCM-PA	PS 80/265–1	83.09	110.10	23	+	pep/poly	−1.7	33.4	395.3
255C	500 m	PS 80/254–1	82.71	109.14	500	*na*	pep/poly	1.1	34.9	316.3
255H	500 m-PA	PS 80/254–1	82.71	109.14	500	+	pep/poly	1.1	34.9	316.3
255D	Deep	PS 80/254–1	82.71	109.14	3548	*na*	pep/poly	−0.7	34.9	303.9
255E	Sediment	PS 80/260–2	83.95	109.81	3569	+	poly	−0.7	34.9	303.9
292A	Surface	PS 80/291–1	79.65	130.58	3	+	pep/poly	0.3	29.9	380.3
292B	SCM	PS 80/291–1	79.65	130.58	18	+	pep/poly	−1.3	31.2	384.3
292G	SCM-PA	PS 80/291–1	79.65	130.58	18	+	pep/poly	−1.3	31.2	384.3
292C	500 m	PS 80/291–1	79.65	130.58	500	+	pep/poly	0.7	34.9	318.9
292H	500 m-PA	PS 80/291–1	79.65	130.58	500	+	pep/poly	0.7	34.9	318.9
292D	Deep	PS 80/291–1	79.65	130.58	3365	+	pep/poly	−0.7	34.9	303.7
292E	Sediment	PS 80/292–2	79.65	130.60	3385	+	poly	−0.7	34.9	303.7
349I	Melt Pond	*na*	87.92	60.94	Ice	+	*na*	*na*	*na*	*na*
349K	Algae agg.	*na*	87.92	60.94	Ice	+	*na*	*na*	*na*	*na*
349A	Surface	PS 80/354–1	87.92	60.94	1	+	pep/poly	−1.8	33.1	405.6
349B	SCM	PS 80/354–1	87.92	60.94	14	+	pep/poly	−1.8	33.1	408.1
349C	500 m	PS 80/354–1	87.92	60.94	500	+	pep/poly	0.7	34.9	317.5
349D	Deep	PS 80/354–1	87.92	60.94	4300	+	pep/poly	−0.6	34.9	304.3
349E	Sediment	PS 80/350–2	87.93	61.04	4380	+	poly	−0.6	34.9	304.3
360A	Surface	PS 80/364–1	88.81	57.25	1	+	pep/poly	−1.8	33.0	413.4
360B	SCM	PS 80/364–1	88.81	57.25	20	+	pep/poly	−1.8	33.0	414.0
360G	SCM-PA	PS 80/364–1	88.81	57.25	20	*na*	pep/poly	−1.8	33.0	414.0
360C	500 m	PS 80/364–1	88.81	57.25	500	+	pep/poly	0.7	34.9	318.0
360H	500 m-PA	PS 80/364–1	88.81	57.25	500	+	pep/poly	0.7	34.9	318.0
360D	Deep	PS 80/364–1	88.81	57.25	4350	+	pep/poly	−0.6	34.9	304.7
360E	Sediment	PS 80/363–1	88.81	57.74	4378	+	pep/poly	−0.6	34.9	304.7
378A	Surface	PS 80/378–1	86.88	52.29	3	+	*na*	−1.8	33.1	400.3
378M	Deep	PS 80/378–1	86.88	52.29	4850	+	*na*	−0.6	34.9	304.2

BCC is an abbreviation for bacterial community composition; (+) indicates the presence of BCC data for the sample. EEA is an abbreviation for extracellular enzymatic activity; 'pep' indicates peptidase activity measurements, while 'poly' indicates polysaccharide hydrolase rate measurements. SCM-PA and 500 m-PA are the particle-associated samples for the SCM and 500 m depths. Temperature, salinity and oxygen values for SCM-PA, 500 m-PA and sediments were obtained from the SCM, 500 m and bottom water values respectively; bottom water samples are those with 'D' in the Sample column. Full environmental data for all station IDs are available on PANGEA (Rabe *et al*., 2013).

polaris. In contrast, sequences from the particle-associated samples at 500 m were, to a greater degree, dominated by members of *Gammaproteobacteria* (Supporting Information Fig. S4A), primarily consisting of OTUs closely related to *Colwellia piezophila* and *Colwellia polaris* (Supporting Information Fig. S5) – up to 57% and 41%, respectively, of the entire sequence dataset for some samples. Finally, members of the *Alphaproteobacteria* were detected in variable relative proportions on particles, and ranged from ca. 2% at Stn 255 to ca. 35% at Stn 360, both at 500 m (Fig. 2). The large contribution of *Alphaproteobacteria* on 500 m particles at Stn 360 was composed primarily of members of *Rhodobacteraceae* (Supporting Information Fig. S4B), in contrast to members of the *Pelagibacteraceae* (including the SAR 11 group) that predominated in water column samples.

Sediment bacterial communities

Bacterial communities in sediments differed significantly in composition compared with those from deep waters (ANOSIM, $R = 1$, $p = 0.006$). Members of the *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidimicrobiia*, *Deltaproteobacteria* and *Flavobacteria* were among the most highly represented bacterial classes in sequence datasets from sediments (Fig. 2). Moreover, across the entire dataset, benthic bacterial communities showed the highest α-diversity – based on OTU richness, evenness, and phylogenetic diversity (Supporting Information Fig. S2) – and lowest β-diversity, as indicated by the lowest group variance (Supporting Information Table S1). These results are consistent with patterns observed using Bray-Curtis-based community dissimilarities (Fig. 2).

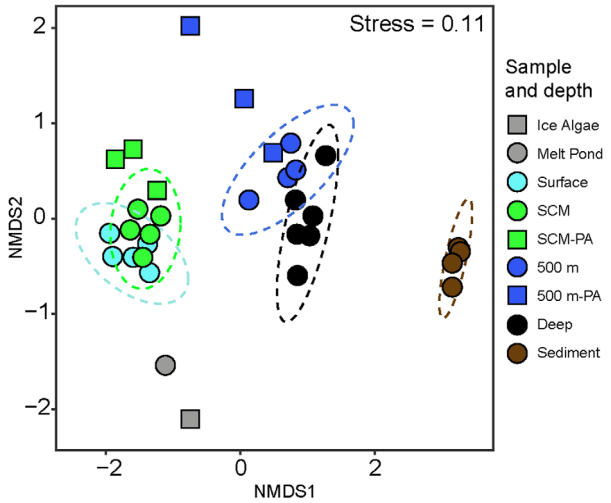

Fig. 2. Non-metric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity. Ordinations were conducted based on OTUs identified at 97% sequence similarity. Ellipses correspond to 95% confidence intervals for depth and sample types containing more than three samples. PA indicates particle-associated samples.

Peptidase activities

In order to quantify peptide and protein-cycling capabilities of Arctic microbial communities, we measured exo-acting (leucine aminopeptidase) and endo-acting peptidase activities (chymotrypsins, trypsins) of pelagic, particle-associated and benthic bacterial communities. Peptide hydrolysis was measurable at almost all stations and depths, with the exception of Stn 224 bottom water (Fig. 4A). Summed peptide hydrolysis rates generally decreased from surface to bottom waters, with rates in surface waters frequently an order of magnitude higher than at 500 m and in bottom waters (Fig. 4A). These trends are driven largely by the substantial contribution of leucine (Leu-MCA) hydrolysis at each station and depth. With the exception of Stn 360, summed peptide hydrolysis rates were higher in surface waters than at the SCM. Furthermore, summed peptidase rates in surface water were similar at the ice-free and first year ice-covered stations (Stns. 292, 255 and 224, respectively). These stations also showed the highest endopeptidase activities – particularly AAPF-chymotrypsin and/or FSR-trypsin. Summed rates in surface and SCM waters were notably lower at the multi-year ice-covered stations (Stns. 349 and 360), signifying comparatively lower potential for organic matter remineralization in the upper water column at the two stations with multi-year ice. However, the same stations showed slightly higher activities at depths of 500 m and in bottom water compared with other stations. Peptidase activities in bulk waters correlated weakly with temperature (ADONIS, R^2 = 0.088, p = 0.036), and strongly with salinity differences (ADONIS, R^2 = 0.653, p = 0.001).

A broader spectrum of peptidase activities was sometimes measured on particles than in bulk seawater, as evident at Stn 292 SCM and Stn 360 500 m (Fig. 4A and B). At many stations and depths, some endopeptidase activities, such as those measured by EGR-trypsin and AAF-chymotrypsin, were only detected on particles. Furthermore, summed particle-associated activities were higher at the SCM than at 500 m, with the exception of Stn 360, where summed particle-associated activities at 500 m were almost double those measured at the SCM (Fig. 4B). Overall, particle-associated peptidase activities accounted for ca. 4%–22% and 12%–100% of the bulk enzyme activities at the SCM and 500 m, respectively, demonstrating that a greater fraction of peptidase activities at 500 m were likely particle-associated.

Sediment peptidase activities were several orders of magnitude higher than activities in bulk seawater and particle incubations (Fig. 4C). All of the peptide substrates (except EGR-trypsin at Stn 255) were hydrolysed in sediments. The relative proportions of enzymatic activities in sediments varied little by station. The highest sediment peptidase rates were measured at Stn 292 and were due to the higher AAF-chym (ANOVA, $p < 0.01$) and AAPF-chym hydrolysis (ANOVA, $p < 0.001$) at this station compared with other stations. As in bulk seawater and on particles, L-MCA hydrolysis constituted the largest fraction (ca. 70%–80%) of summed peptidase activities.

Polysaccharide hydrolase activities

Of the six polysaccharide substrates, laminarin, pullulan, xylan and chondroitin were hydrolysed in bulk waters at multiple stations and depths, whereas arabinogalactan and fucoidan were not hydrolysed at any location (Fig. 5A). Laminarin was hydrolysed at all sites and depths, and chondroitin was hydrolysed from the surface to 500 m at Stns. 224, 349 and 360. Pullulan hydrolysis was measured at 500 m and in bottom waters, and xylan was hydrolysed only at a few depths and stations, with no evident spatial pattern. Summed polysaccharide hydrolysis rates varied with depth, although the highest summed rates were consistently measured at the SCM or at 500 m (Fig. 5A). Polysaccharide hydrolase activities only significantly correlated with differences in oxygen concentrations (ADONIS, R^2 = 0.258, p = 0.004).

On a volume-corrected basis, particle-associated polysaccharide hydrolase activities were lower than those for bulk seawater (Fig. 5B). As with bulk seawater, only four of the six substrates were hydrolysed in particle-associated incubations; arabinogalactan and fucoidan were not measurably hydrolysed. However, at most depths and locations, the spectrum of substrates hydrolysed in the particle-associated incubations was greater than for bulk seawater incubations – a trend that parallels some of the

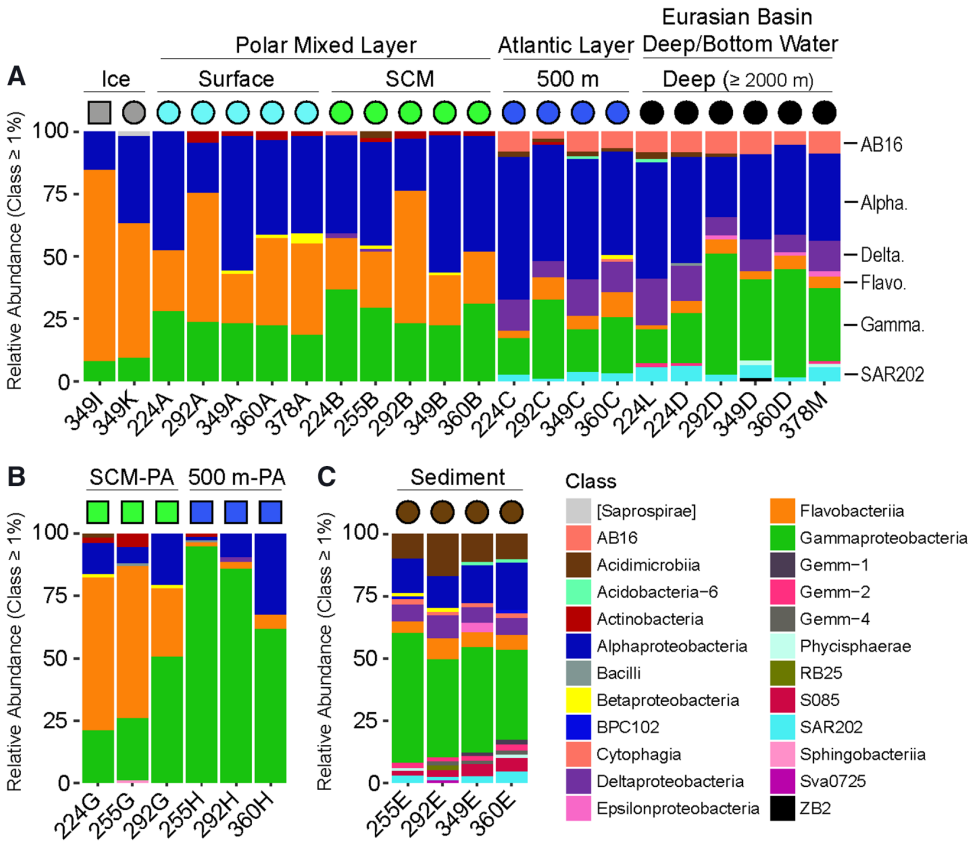

Fig. 3. Class-level breakdown of bulk pelagic (A), particle-associated (B) and benthic (C) bacterial communities in the Arctic Ocean.
The bar graph only includes classes with relative abundances of ≥1% of the entire data set. The sampling depth and sample type are indicated above the bar graphs, and the corresponding water masses for the samples are in bold. PA indicates particle-associated samples. Symbols above the bar graph correspond to those shown in Fig. 2.

observations with peptidase activities. In particular, xylan and pullulan hydrolysis were detected in the particle-associated incubations at depths and stations where they were not measured in the bulk water incubation (Fig. 5B).

Structure–function relationship

Variations in pelagic bacterial community composition weakly correlated with differences in peptidase activities (Mantel test, $r = 0.22$, $p = 0.014$). However, removing the influence of environmental conditions (temperature, salinity and oxygen) to evaluate a direct structure–function link indicates no statistically significant correlation (partial Mantel test, $r = 0.02$, $p = 0.4$). This analysis illustrates that the structure–function link does not persist independently of environmental variables for peptidase activities.

Discussion

Pelagic realm

In the central Arctic Ocean, differences in the extent of sea-ice coverage and sea-ice melting drive variations in temperature and salinity in surface waters. Seasonal variations in the upper water column, in tandem with the influence of North Atlantic waters on much of the Eurasian Basin, result in stratified water masses in the central Arctic (Stein and Macdonald, 2004). In our investigation, temperature and salinity correlated most strongly with bacterial community dissimilarity; these environmental parameters have also been correlated with patterns of community composition in global oceans (Zinger *et al*., 2011; Sunagawa *et al*., 2015) and across terrestrial and aquatic habitats (Lozupone and Knight, 2007). Thus, spatial environmental gradients and depth-related hydrographic features differentiate bacterial communities in the central Arctic (Supporting Information Fig. S6), consistent with previous observations in the greater Arctic (Galand *et al*., 2010; Han *et al*., 2015).

The upper water column of the seasonally-productive central Arctic Ocean was populated by organic matter degraders within the bacterial classes *Flavobacteria, Gammaproteobacteria* and *Alphaproteobacteria* (Dang *et al*., 2008; Teeling *et al*., 2012; Williams *et al*., 2013). The large relative contribution of these classes is

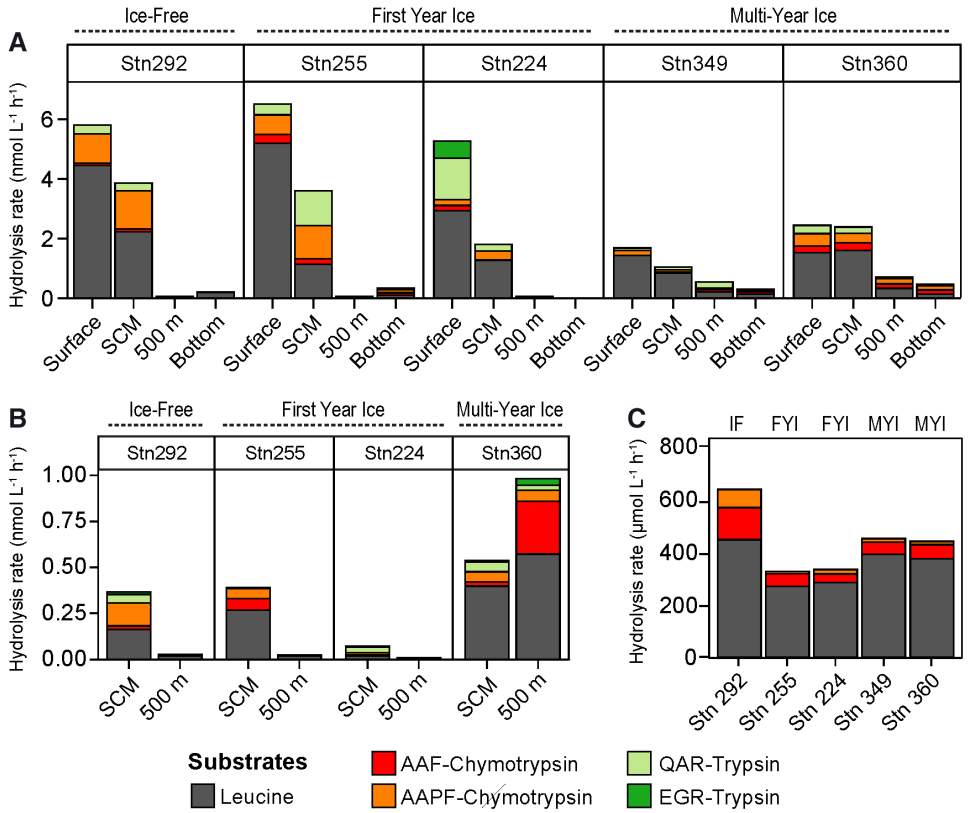

Fig. 4. Peptidase activities for (A) bulk seawater samples, (B) ≥3 µm particle-associated samples and (C) surficial sediment samples. For (A) and (B), each stacked bar represents a rate averaged from triplicate incubation across three timepoints; for (C), each stacked bar represents a triplicate-averaged rate only from the first time point (1 h incubation). Sediment activities are normalized by volume of sediment, not the sediment slurry. 'IF', 'FYI' and 'MYI' stand for Ice-Free, First Year ice and Multi-Year Ice respectively. Note the differences in scales and units. 'Bottom' water samples in this figure are classified as 'Deep' in Table 1 and Fig. 2.

consistent with trends previously observed in the Arctic (Bano and Hollibaugh, 2002; Boetius *et al*., 2015). Furthermore, variations in relative proportions of specific groups – the comparatively large contribution of *Flavobacteria* especially in the ice-free station – were in accordance with observations of increased flavobacterial proportions in the Canadian Arctic during the 2007 sea-ice minimum (Comeau *et al*., 2011) and in ice-free summer waters off Svalbard (Wilson *et al*., 2017). The absence of several flavobacterial genera in the melt pond and algal aggregate samples - but which were present in Stn 292 surface and SCM waters - indicates that the large contribution of *Flavobacteria* in these waters are linked to primary productivity rather than bacterial release from the melted sea-ice. Accordingly, net primary productivity was higher at stations with little to no sea-ice at the time of sampling (Fernández-Méndez *et al*., 2015).

Our analyses of bacterial communities extend into previously unexplored deep-water masses, including the Eurasian Basin Deep and Bottom Waters. At the mesopelagic (Galand *et al*., 2010) and extending down into the bathypelagic realm, a greater range of bacterial classes, most notably including the *Deltaproteobacteria*, SAR 202 (phylum *Chloroflexi*) and AB16 (within phylum *Marinimicrobia*), were detected. While many of the same bacterial groups – on a broad taxonomic level – were detected in the mesopelagic and bathypelagic, OTU-level analyses demonstrated significant community differences at 500 m and deep waters. The depth-related taxonomic differences and increase in phylogenetic diversity of pelagic bacterial communities observed in our study parallel observations from global oceans (Jing *et al*., 2013; Sunagawa *et al*., 2015; Wilson *et al*., 2017).

Depth profiles of microbial activities, including the first empirical measurements of microbial enzymatic function in the Eurasian Basin Deep and Bottom waters, showed opposite trends for peptidases and polysaccharide hydrolase activities (Supporting Information Fig. S6). In the upper water column, summed peptidase activities were higher in the seasonally ice-free and partially ice-covered areas, and lower in the fully ice-covered stations, likely related to differences in primary production along a gradient of sea-ice regimes. Bulk peptidase activities substantially decreased below the SCM – a trend observed at all stations. In contrast, the highest summed rates and the broadest spectrum of polysaccharide hydrolase activities

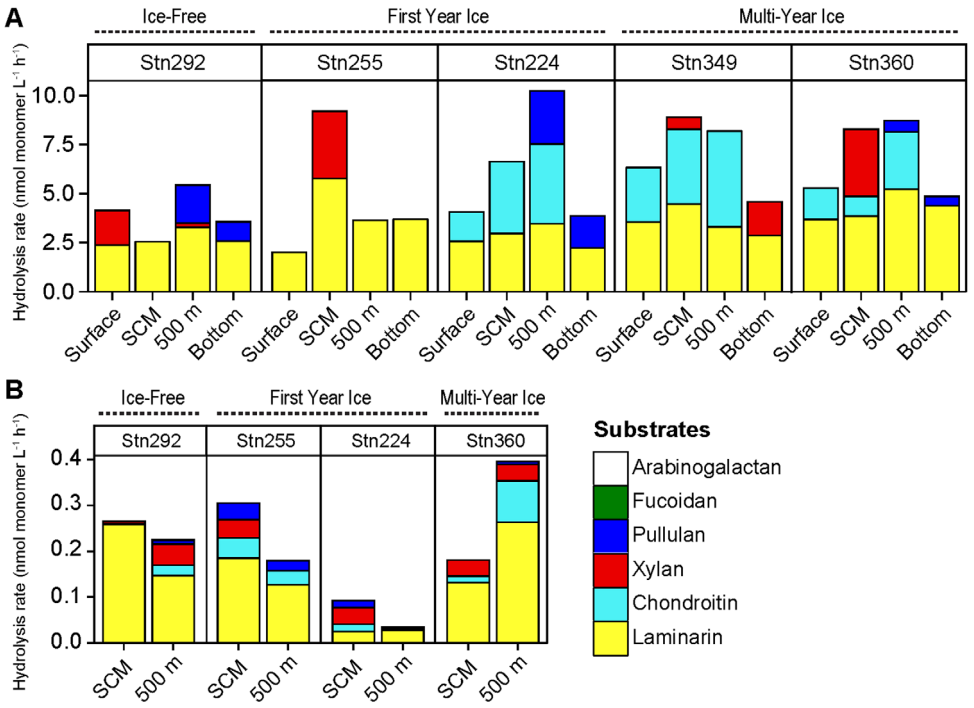

Fig. 5. Polysaccharide hydrolase activities for (A) bulk seawater samples, and (B) ≥3 μm particle-associated samples. No data for sediment samples are available. Reported rates are the maximum rates calculated at different timepoints throughout the incubation. The entire time-course data is available in the Supporting Information (Fig. S8). Note the differences in scale between (A) and (B). 'Bottom' water samples in this figure are classified as 'Deep' in Table 1 and Fig. 2.

were measured at either the SCM or at 500 m. These depth-related patterns are consistent with the hypothesis that organic nitrogen-containing substrates are preferentially removed from particles as they sink in the water column (Lee *et al*., 2000; Tamelander, 2013). Nevertheless, peptidase activities remain detectable in bottom waters (down to ca. 4350 m) at reduced rates, and with a narrower spectrum of enzyme activities. The relatively constant polysaccharide hydrolase activities throughout the water column at some stations (e.g., Stn 292, 349, 360) suggest that carbohydrates may fuel microbial metabolism in surface as well as deep waters, although substrate utilization patterns varied with depth.

While community composition and peptidase activities correlated with environmental conditions and therefore covaried, the lack of correlation between community structure and peptidase activities by themselves suggests that protein degradation is characterized by functional redundancy. Thus, environmental conditions, rather than community composition, ultimately control rates of specific ecosystem processes. We also note that community plasticity, the physiological or metabolic flexibility of community members under varying environmental conditions (Comte *et al*., 2013), may further confound efforts to identify structure–function links. These results suggest that, in the water column, the ability to utilize proteinaceous compounds are phylogenetically widespread, such that a strong structure–function link cannot be identified.

Particle-associated fraction

Particle association contributed significantly to community dissimilarity, although these effects were stronger in the mesopelagic than at the SCM. This observation may be indicative of population maintenance strategies. Particle-associated microbial taxa must maintain a free-living population to colonize newly formed particles in the upper water column, in order not to sink permanently from the photic zone as particles are exported to the seafloor (Pedrós-Alió and Brock, 1983). Under this scenario, particle-associated communities exchange with the free-living bacterial community. Alternatively, only a subset of the population attaches to the particles. In both scenarios, development of a distinct bacterial community on particles is limited. In the mesopelagic, where new particle production is less likely, continuous exchange with a free-living population is no longer needed; thus, particle-associated communities become more distinct from the whole community. Within-particle bacterial community changes during sinking, in addition to decolonization or detachment, may further enhance the development of a more distinct particle-associated community at depth

(Thiele *et al*., 2015). Both explanations are congruent with observations that taxa attached to deep-water particles are phylogenetically distinct from those of free-living assemblages (Eloe *et al*., 2011; Salazar *et al*., 2015).

The particle-associated niche was largely occupied by members of the class *Flavobacteria* (dominant on SCM particles), *Gammaproteobacteria* (dominant on 500 m particles) and *Alphaproteobacteria*, providing evidence for a distinct phylogenetic signal that became more prominent at depth. Taxa within these classes have been classified as particle-associated in other oceanic regions (Salazar *et al*., 2015; Thiele *et al*., 2015) and are among the most ubiquitous surface colonizers (Buchan *et al*., 2014; Dang and Lovell, 2016). Moreover, *Colwellia*-related OTUs – detected in moderate proportions on SCM particles – were proportionally dominant on particles at 500 m, highlighting the influence of epipelagic particle colonization to mesopelagic particle-associated communities (Tamelander, 2013; Thiele *et al*., 2015).

Irrespective of phylogenetically distinct particle-associated communities at the SCM and 500 m, however, polysaccharide hydrolase activities at the two depths overlapped. Such functional redundancy could be essential to initiate remineralization of structurally-diverse high molecular weight compounds within suspended and sinking particulate matter (D'Ambrosio *et al*., 2014). Functional redundancy among surface-associated microbes has also been observed between phylogenetically dissimilar algae-associated bacterial communities that share a core set of functional genes (Burke *et al*., 2011). However, the similarities in particle-associated enzymatic activities may also be attributed to the overlap of particle-associated taxa at the SCM and 500 m. In particular, members of the genus *Colwellia* have wide-ranging organic substrate utilization abilities (Bowman, 2014) and are detected in substantial proportions on particles at the SCM and 500 m.

Particle-associated communities additionally often demonstrated a broader range of polysaccharide hydrolase activities than the bulk water community. This trend could in part be due to differences in volumes sampled for our particle-associated (ca. 6–8 l) versus bulk seawater (45 ml) analyses. These results may nevertheless reflect contrasting abilities of particle-associated versus free-living taxa to utilize polysaccharides. Such differences have been documented, for example, among ecotypes of *Polaribacter irgensii*: the putatively algae-associated strain contained a wider range of polysaccharide utilization genes compared with the planktonic taxon (Xing *et al*., 2015). Furthermore, mechanisms such as quorum sensing on aggregates may lead to increased production of specific enzymes (Gram *et al*., 2002). Hydrolytic activity originating from particle-associated microbes can then be released as free enzymes to ambient water (Vetter *et al*., 1998; Ziervogel and Arnosti, 2008); however, due to low particle concentrations, the contribution of particle-associated microbes may be too dilute to be detected in bulk seawater measurements. Overall, our findings suggest that the degradation of particulate matter likely requires a broad spectrum of enzymatic 'tools' to access substrates. Consistent with this idea, particle-attached microbes possess a diverse genetic repertoire – compared with their free-living counterparts – necessary to internally transport and utilize substrates from particles (Teeling *et al*., 2012; Smith *et al*., 2013). The abundance of carbon sources on particles enables particle-associated microbes to maintain their copiotrophic lifestyle.

Sediments

Sediment bacterial communities exhibited considerably higher phylogenetic diversity than pelagic or particle-associated communities, in accordance with findings from a global-scale analysis of marine ecosystems (Zinger *et al*., 2011), and diverse terrestrial and aquatic habitats (Lozupone and Knight, 2007). In addition, β-diversity was lower among benthic bacterial communities compared with the bacterial communities of any depth from the water column or particles; nevertheless, subtle variations in community composition were detectable. Hydrographic conditions of overlying water masses may influence benthic bacterial communities (Hamdan *et al*., 2013); the similarity of benthic bacterial communities in the deep central Arctic might reflect in part the influence of relatively homogeneous and stable physical properties of the Eurasian Basin Deep Water and Eurasian Basin Bottom Water across the different stations. Additionally, low productivity regimes impose stronger selective forces than do high productivity ones, which may result in highly similar community composition across oligotrophic sites (Chase, 2010). Therefore, widely similar oligotrophic conditions in Arctic Basin sediments may select for phylogenetically similar bacterial communities across different sampling locations in this benthic habitat.

A specific feature of the peptidase activity patterns observed in sediments mirror those detected in the water column and on particles. Leucine aminopeptidase (exo-acting) activities contributed a large majority to the summed peptidase rates, while chymotrypsin and trypsin (endo-acting) activities contributed to a much lesser extent. Our findings of greater exo-acting versus endo-acting peptidase activities contrast with observations from Svalbard fjords (Steen and Arnosti, 2013; Arnosti, 2015) and coastal temperate waters off Japan (Obayashi and Suzuki, 2005; Obayashi and Suzuki, 2008). High leucine aminopeptidase activities have been proposed as microbial adaptations to carbon-poor conditions (Boetius and

Lochte, 1996; Polymenakou *et al*., 2008). Accordingly, a succession of proteases, from high endo-acting peptidase activities in unamended coastal temperate seawater to high exo-acting peptidase activity in the 112 day old 'aged' seawater (Bong *et al*., 2013), suggests an association between these hydrolytic enzymes and organic matter quantity and quality; however, the mechanistic basis for this linkage is unclear. Moreover, activities measured as leucine aminopeptidase may instead reflect the collective activity of several high-affinity, but low-specificity aminopeptidases (Steen *et al*., 2015). In any case, the expression of high substrate affinity exo-acting peptidases throughout the Arctic water column and sediments may be linked to the quality and quantity of organic matter (Bong *et al*., 2013).

Future outlook

Since previous observations (Comte *et al*., 2013) and our own findings indicate that the degree of functional redundancy and functional dissimilarity exhibited by microbial communities can be highly dependent on environmental conditions, it is imperative that structure–function relationships are evaluated in the context of the environment. We anticipate changes in bacterial community composition owing to physicochemical alterations in the Arctic (Kirchman *et al*., 2009). Projected shifts in carbon export to Arctic Ocean sediments (Wassmann, 2011) are already evident with widespread deposition of large sea-ice algae aggregates in the seafloor (Boetius *et al*., 2013), and can alter the composition of benthic microbial communities over time (Bienhold *et al*., 2012; Rapp *et al*., 2018). However, our observation that functional redundancy – within the context of peptidase activities – impacts the bacterial structure–function relationship in the water column may complicate efforts to predict the influence of Arctic environmental changes on specific microbially driven ecosystem processes. Moreover, the extent to which microbial communities are functionally redundant or functionally dissimilar for other relevant biogeochemical processes should be investigated in future studies.

Experimental procedures

Sites and sample collection

Samples were collected from the Eurasian Basin in the high Arctic between August and September 2012, during the ARK-27/3 cruise, and when a historic sea-ice extent minimum occurred (Fig. 1). Seawater from the surface (Polar Mixed Layer), from the subsurface chlorophyll maximum (SCM; found at different depths, and part of the Polar Mixed Layer), 500 m (Atlantic Layer), and bathypelagic zone (deep; ≥ 2000 m water; Eurasian Basin Deep Water and Eurasian Basin Bottom Water) were collected using Niskin bottles mounted on a CTD rosette (Supporting Information Fig. S1). At the SCM and 500 m, bulk seawater was also gravity-filtered through 3 μm pore size filters to separate bulk and ≥ 3 μm particle-associated microbial communities (Table 1). The upper 1 cm of surficial sediments – typically oxic, with the exception of patches of anoxic sediments beneath algal aggregate deposits at Stn. 349 and 360 (Boetius *et al*., 2013) – were collected at each station using a multicorer (MUC) and processed as detailed in the enzymatic activity measurements section. Melt pond and sea-ice aggregates were also collected using Duran bottles from Stn. 349. Environmental data for all ARK-27/3 stations are available in the PANGEA database (Rabe *et al*., 2013).

DNA sampling, extraction, sequencing and analysis

For whole community composition analyses, ca. 1 l seawater was filtered using a vacuum pump through a 0.2 μm pore size, 47 mm diameter Millipore membrane filter. Additional seawater from the SCM (0.8–3.2 l) and from a depth of 500 m (6.9–7.8 l) was gravity filtered through a 3 μm pore size, 47 mm Millipore membrane filter for large particle-associated community composition analyses (Supporting Information Table S2). Aggregates of the diatom *Melosira arctica* were also gravity-filtered. Samples were stored at –80 °C until DNA extraction. DNA was extracted using the PowerSoil DNA Isolation Kit (Qiagen), following the manufacturer's protocol. DNA samples were PCR-amplified and sequenced at the Genomic Sequencing and Analysis Facility at University of Texas at Austin. The V1-V2 hypervariable region of bacterial 16S rRNA genes was amplified with the Hyb8F_rRNA and Hyb338R_rRNA primer set (Supporting Information) and sequenced using Illumina MiSeq PE 2x300. Sequences from this primer may result in an underrepresentation of *Proteobacteria* and *Verrucomicrobia*, and an overrepresentation of *Bacteroidetes* (Campbell *et al*., 2012); however, bacterial proportions in this study match those from previously published and unpublished datasets produced using different primer pairs (Supporting Information Table S3). Sequences were demultiplexed, and adapters were trimmed before merging paired ends. Fastq-join on QIIME was used to merge paired ends (Caporaso *et al*., 2011), reducing the dataset to only the sequences with near-complete overlapping pairs. The quality of the merged ends was checked using FastQC, which showed that the mean Phred score for all sequences were above 34; thus, no merged pairs were further excluded from downstream analyses. OTUs were clustered *de novo* (97% sequence similarity), and the taxonomic affiliation of *de novo* OTUs were identified using

the GreenGenes database (DeSantis *et al*., 2006). Chimeric sequences were identified using ChimeraSlayer (Haas *et al*., 2011) and subsequently removed, along with singletons. Sequences were deposited into the NCBI Sequence Read Archive under the accession number SRP126633.

Non-metric multidimensional scaling (NMDS) ordination with Bray–Curtis dissimilarity index was performed to identify clustering patterns in the sequencing datasets of all sample types using the R package *phyloseq* (McMurdie and Holmes, 2013). To test for differences in groupings defined according to depth and sample type in the NMDS, we performed an Analysis of Similarity (ANOSIM) using both Bray-Curtis and UniFrac dissimilarities in the R package *vegan* (Oksanen *et al*., 2013). These analyses were also used for pairwise comparisons of groupings of bacterial communities from different depths, as well as bulk versus particle-associated communities. We used Analysis of Variance using Distance Matrices (ADONIS) with 999 permutations as implemented in *vegan* to investigate the correlation between measured environmental parameters (including temperature, salinity and oxygen) and bacterial community dissimilarity (Bray-Curtis), and to partition the variance that can be explained by each parameter. A partial Mantel test was used to determine the correlation between environmental conditions (temperature, salinity and oxygen) and community dissimilarity, holding for the effect of either geographic distances. We also tested for these correlations on community dissimilarity and geographic distances, holding for the effect of environmental conditions.

Enzymatic activity measurements

Exo- (terminal-unit cleaving) and endo-acting (mid-chain cleaving) peptidase activities were measured using the following substrate analogues, including their constituent single-letter amino acid codes: methylcoumarin (MCA)-labelled leucine (L-MCA), chymotrypsins (AAF-chymotrypsin, AAPF-chymotrypsin) and trypsins (QAR-trypsin, EGR-trypsin). Bulk seawater triplicate incubations were set up in 4 ml cuvettes, and substrates were added to a final concentration of 100 μM (Arnosti, 2015). Autoclaved ambient seawater with the same substrate concentration was used as killed control. Autoclaved and live seawater with no substrate addition were used as blanks. Subsamples were collected from the incubations at 0 h (upon addition of substrate), 24 h, 48 h and 72 h. Large-particle associated peptidase activities were measured using 1/12th sections of the 3 μm pore-size filters. For each particle-associated incubation, one filter piece, from the whole filter used also for particle-associated community composition analyses, was submerged in a 4 ml cuvette containing autoclaved ambient seawater; duplicate live incubations were set up, in addition to a killed control containing autoclaved ambient seawater and a sterile filter piece. These incubations were subsampled at 0 h (addition of substrate), 4 h, 8 h and 12 h. Peptidase activities were measured in sediments using 1:2 (vol:vol) sediment slurries made by adding cooled, autoclaved overlying bottom water to sediments. Live triplicates and one killed control per substrate were set up for the sediment incubations, and substrates were added to a final concentration of 100 μM. Sediment slurries were subsampled at 0, 1 h, 2 h and 4 h by taking 2 ml of the incubated slurries, centrifugation, filtration of the supernatant using SFCA-filters and addition of 1 ml filtered supernatant to 1 ml borate buffer. These procedures followed a previously established protocol (Arnosti, 2015). Fluorescence was converted to concentrations of hydrolysed substrate using a standard curve of fluorescence at different concentrations of the free fluorophore. All incubations were carried out at 0 °C in the dark.

The activities of specific endo-acting polysaccharide hydrolases that cleave their target polysaccharides mid-chain were measured using six fluoresceinamine-labelled (FLA) polysaccharides: pullulan, laminarin, xylan, fucoidan, arabinogalactan, chondroitin (Arnosti, 2003). Enzyme activities for these substrates have previously been measured in marine bacteria, and sequences corresponding to these hydrolytic enzymes have been identified in specific marine bacterial genomes (reviewed in Arnosti, 2011). Bulk seawater incubations were set up for each substrate in triplicates, plus one killed control. Each triplicate and killed control contained 15 ml of live and autoclaved seawater, respectively, to which one substrate per tube was added to a monomer concentration of 3.5 μM (Arnosti, 2003). All incubations were carried out at 0 °C and subsampled at 0 h, 120 h, 240 h, 360 h and 600 h. Additionally, large-particle-associated activities were measured on 1/12th sections of 3 μm pore-size filters. Each particle-containing filter piece was submerged in 15 ml of autoclaved ambient seawater amended with a polysaccharide substrate added to a final monomer concentration of 3.5 μM; killed controls were set up in a similar manner, but with 1/12th section of a sterile filter. These incubations were subsampled at the same time-points as bulk water incubations, and subsampling procedures followed an established protocol (D'Ambrosio *et al*., 2014). Subsamples were processed using gel permeation chromatography and fluorescence detection (Arnosti, 2003). Maximum potential rates, which were measured at different time points (Supporting Information Fig. S7A,B), are reported. We used ADONIS to test for correlations between enzymatic activities and the environmental parameters of temperature, salinity and oxygen concentration.

Some methodological limitations for the enzymatic assays have to be considered. Due to the comparatively long time-course of incubations, measurements with FLA-polysaccharides reflect long-term community potential. These measurements also incorporate a wider range of microbial responses (cellular growth, enzyme induction and community shifts) than the peptidase activities, which are measured over shorter time-courses and likely more closely reflect the microbial community at the time of sampling. However, due to the structural simplicity of the peptide analogues, the peptidase rates only weakly reflect the structural complexity of larger proteins.

Because of likely community shifts in long-term polysaccharide hydrolase enzyme assays, we chose peptidase rates to more directly assess whether differences in community composition are congruent with differences in enzymatic potentials. Thus, evaluation of the structure–function relationships does not include polysaccharide hydrolase activities. To evaluate the nature of this structure–function relationship, we quantified correlations between OTU-based community dissimilarity (Bray-Curtis) and peptidase activity dissimilarity (Euclidean distances), using Mantel test, as well as partial Mantel test to remove the effect of environmental conditions. A statistically significant, positive correlation indicates that increasing differences in community composition correlate with those in enzymatic potentials, which suggests functional consequences of community structural differences, or functional dissimilarity. Alternatively, differences in community composition that do not correlate with differences in peptidase potentials signify a functionally redundant or metabolically plastic community, where environmental factors, not community composition, shape functional differences between microbial communities (Reed and Martiny, 2007; Strickland *et al*., 2009).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information