

RESEARCH ARTICLE

Spatially resolved assembly, connectivity and structure of particle-associated and free-living bacterial communities in a high Arctic fjord

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ABSTRACT

The assembly processes that underlie the composition and connectivity of free-living (FL) and particle-associated (PA) bacterial communities from surface to deep waters remain little understood. Here, using phylogenetic null modeling, we quantify the relative influence of selective and stochastic mechanisms that assemble FL and PA bacterial communities throughout the water column in a high Arctic fjord. We demonstrate that assembly processes acting on FL and PA bacterial communities are similar in surface waters, but become increasingly distinct in deep waters. As depth increases, the relative influence of homogeneous selection increases for FL but decreases for PA communities. In addition, dispersal limitation and variable selection increase with depth for PA, but not for FL communities, indicating increased residence time of taxa on particles and less frequent decolonization. As a consequence, beta diversity of PA communities is greater in bottom than in surface waters. The limited connectivity between these communities with increasing depth leads to highly distinct FL and PA bacterial communities in bottom waters. Finally, depth-related trends for FL and PA beta diversity and connectivity in this study are consistent with previous observations in the open ocean, suggesting that assembly processes for FL and PA bacterial communities may also be distinct in other aquatic environments.

Keywords: community assembly; bacterial communities; particle-associated; free-living; high Arctic; Kongsfjorden

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INTRODUCTION

Microbial communities in the water column occupy diverse niches, and their lifestyles can be broadly categorized as particle-associated (PA) or free-living (FL), defined through an operational size-fraction cutoff (Grossart 2010). Their taxonomic composition (DeLong et al. 2006; Simon, Smith and Herfort 2014; Bižić-Ionescu et al. 2015; Jain et al. 2019), genome sizes and metabolic capabilities (Lyons and Dobbs 2012; Simon, Smith and Herfort 2014; Liu et al. 2020), and response to environmental perturbations often differ between the PA and FL fractions. However, the distinction between PA and FL bacterial communities can be confounded due to particle colonization and decolonization (Pedros-Alio and Brock 1983), resulting in members of the same population being identified in the two size fractions (Hollibaugh, Wong and Murrell 2000; Jain and Krishnan 2017). Microbial transitions between the PA and FL lifestyles, and their movement between heterogeneous particles, suggest that microbial assembly mechanisms and interconnectivity between these size fractions may depend in part on environmental conditions—the most relevant of which is likely particle quality and concentration (Yawata et al. 2020). Hence, PA and FL bacterial assembly mechanisms should be highly variable from surface to deep waters, along considerable particle concentration gradients. While the composition and connectivity of PA and FL taxa have been investigated from surface to deep waters (Eloe et al. 2011; Thiele et al. 2015; Mestre et al. 2018; Fadeev et al. 2020), the assembly processes underlying observed community structural patterns remain little understood.

The assembly of microbial taxa on particles may be controlled by both deterministic and stochastic processes (Stegen et al. 2012, 2013). Environmental selection of microbes—out of a highly diverse regional species pool—that can sense, move toward (Stocker et al. 2008; Lambert et al. 2017), attach to and degrade particles (Enke et al. 2018, 2019) constitutes a deterministic process. Differential selection due to particle heterogeneity could lead to differences in initial colonization and resulting PA bacterial communities (Bižić-Ionescu et al. 2015; Bižić-Ionescu, Ionescu and Grossart 2018). However, some stochasticity in the initial colonization of particles may also lead to these taxonomic compositional differences across particles (Cordero and Datta 2016; Datta et al. 2016). Once settled on particles, microbial communities undergo a succession driven in part by substrate quantity and quality, which can result in detachment of taxa, as well as an attachment by new taxa that can exploit newly formed niches or bioavailable substrates (Cordero and Datta 2016; Enke et al. 2019). These processes, most explicitly studied using model particles and marine microbial communities, are reflected in nature through a substrate-controlled succession of microbial communities during phytoplankton blooms (Teeling et al. 2012, 2016; Sperling et al. 2017).

Quantifying the relative influence of assembly processes that act on PA versus FL bacteria provides insights into whether these communities are differentially structured, but this information is presently limited to a few systems. For instance, studies from two freshwater lakes (Zhao et al. 2017; Xu et al. 2020) and a river-to-bay gradient (Wang et al. 2020) have identified differences in assembly mechanisms among PA and FL bacterial communities. Homogeneous selection—a deterministic process that selects for taxonomically coherent communities due to similarities in environmental conditions (Stegen et al. 2012, 2013)—plays a predominant role in structuring FL bacterial communities (Wang et al. 2020). In contrast, variable selection, due to heterogeneity in environmental conditions, such as particle composition,

may play a more important role in the assembly of PA bacterial communities (Xu et al. 2020). However, assembly mechanisms of PA and FL communities can be dynamic, for example, in response to a phytoplankton bloom (Xu et al. 2020). As the immediate effect of environmental changes on community assembly can have lasting impacts on community composition, variability in assembly mechanisms must be identified and contextualized across temporal and spatial gradients.

Our understanding of assembly mechanisms among PA and FL bacterial communities is notably scarce along vertical gradients. This information is useful in interpreting the mechanisms leading to observed vertical succession (Thiele et al. 2015; Pelve, Fontanez and DeLong 2017), connectivity (Mestre et al. 2018; Rapp et al. 2018; Fadeev et al. 2020) and compositional differences among PA microbial communities at various depths in the marine water column (Salazar et al. 2016; Balmonte, Teske and Arnosti 2018; Boeuf et al. 2019; Baumas et al. 2021; Poff et al. 2021). Defining assembly mechanisms among PA and FL microbial communities may contribute to models when mapped along environmental gradients (Stegen et al. 2015), which can be used to predict how community composition and potentially their biogeochemical function may be altered following pulse or press disturbances. Thus, quantifying assembly mechanisms bears significant ecological relevance, especially when investigated in systems prone to or undergoing rapid changes.

Here, we investigated the extent to which assembly processes for PA and FL bacterial communities differed spatially due to the stratification of water masses and vertical physicochemical heterogeneity. We applied a previously established phylogenetic null modeling approach to compare differences in the relative importance of four assembly processes—e.g. homogeneous selection, variable selection, homogenizing dispersal and dispersal limitation (Stegen et al. 2012, 2013)—between PA and FL bacterial communities. We chose Kongsfjorden, a fjord in Svalbard, Arctic, as a model system to investigate vertical differences in assembly processes between FL and PA bacterial communities due to (i) the strong stratification of the water column during the summer, (ii) the distinct origins of the water masses and (iii) well-characterized composition of total bacterial communities (Han et al. 2021), but limited understanding of the taxa that comprise the PA and FL fractions (Jain and Krishnan 2017, 2021; Jain et al. 2019), especially deeper in the water column. Building on previous observations that assembly mechanisms of FL and PA bacterial communities vary in space (Wang et al. 2020) and time (Xu et al. 2020), we specifically tested two related hypotheses. First, assembly mechanisms acting upon PA and FL communities would differ in surface versus deep waters in part due to vertically heterogeneous physicochemical conditions. Second, with increasing depth, the relative influence of these processes would become increasingly distinct between FL and PA bacterial communities, likely due to the limited exchange of taxa (Yawata et al. 2020). While investigated in a high Arctic fjord with physicochemical changes over a relatively shallow depth range (down to ~300 m), these distinct assembly processes of PA and FL communities are likely applicable to other systems in which steep vertical gradients of particle concentrations are observed.

MATERIALS AND METHODS

Sampling site and sample collection

Kongsfjorden is an open glacial fjord located on the northwest coast of the Svalbard archipelago in the Arctic Ocean (Fig. 1). Hydrography of Kongsfjorden is strongly influenced by

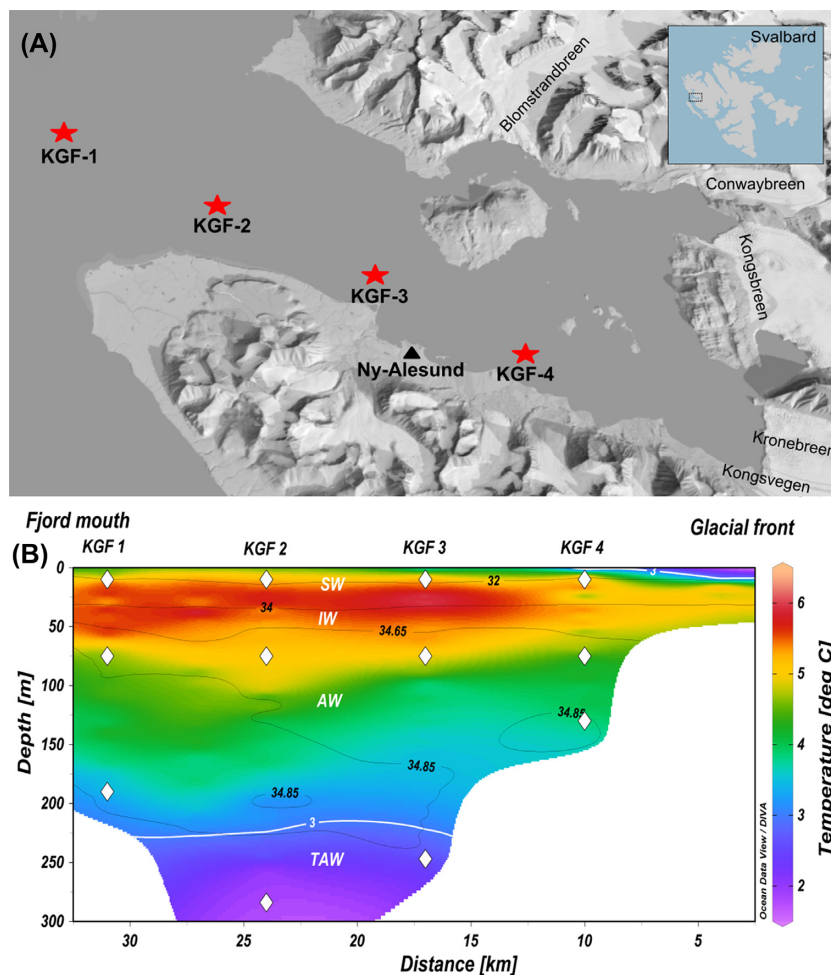


Figure 1. (A) Map of Kongsfjorden depicting the sampling locations (red stars); inset shows the location of Kongsfjorden in the Svalbard archipelago (right top corner). (B) Vertical distribution of temperature (colored area, °C) overlaid by salinity (black contours; top) in Kongsfjorden at the time of sampling. In the vertical distribution diagram (panel B), the x-axis denotes the distance of sampling/CTD stations from the Kronebreen glacier and y-axis denotes the depth; the water masses are demarcated by the 34 and 34.65 isohalines (black contours), as well as the 3.0°C isotherm (white contours), and the sampling depths are shown with filled diamonds (white). The water masses are represented as SW = surface water, IW = intermediate water, AW = Atlantic water and TAW = transformed Atlantic water.

the meltwater inputs from its tidewater glaciers, Kronebreen and Kongsvegen on the south coast, and Kongsbreen, Conwaybreen and Blomstrandbreen on the north coast (Fig. 1A) (Svendsen et al. 2002), and the subsurface intrusion of warm and saline Atlantic water (AW) from the Fram Strait (Cottier et al. 2005). Water samples were collected along the length of Kongsfjorden from four distinct locations at three different depths, i.e. surface, middle and bottom. The surface and middle depths were fixed at 10 and 75 m, respectively, whereas the bottom depths were variable (Fig. 1B). The sampling was conducted in September 2018 using workboat MS Teisten. Vertical variations in the seawater temperature and salinity were recorded using different sensors attached to the conductivity, temperature, depth (CTD) (SBE 19plus, Seabird Electronics, Bellevue, WA, USA). For bacterial community analyses, 2 L of water sample (in triplicates) was sequentially filtered through a 3- μm pore-size membrane (polycarbonate, Millipore, USA) followed by a 0.22- μm pore-size membrane (polycarbonate, Millipore, USA). After filtration, membranes were stored frozen at -80°C until further analysis. Microbial biomass retained on the 3- μm prefilter was considered as the PA fraction, whereas biomass within the size ranges of 3–0.22 μm as FL fraction (Jain et al. 2019). Several subsamples of seawater collected at each site were stored in high-density

polypropylene bottles at -20°C for measuring nutrients and total organic carbon (TOC). For analyzing various particulate parameters, 2 L of seawater sample (in several replicates) from each site was filtered onto the combusted (450°C , for 4 h) 0.7- μm pore-size GF/F filter (Whatman, USA) and stored at -20°C until analysis.

Nutrients and other biogeochemical parameters

The dissolved nutrients (nitrate, nitrite, silicate and phosphate) were measured following standard colorimetric methods (Grasshoff et al. 1985) using Seal AA3 analytical autoanalyzer. TOC in seawater samples was measured using potassium hydrogen phthalate as standard in the Shimadzu TOC-TN analyzer. Particulate organic carbon (POC) and total particulate nitrogen (TPN) were measured using an Elemental Analyzer (Isoprime, Vario Isotope Cube). Chlorophyll a was measured using Turner Fluorometer AU10 (Turner Inc., USA).

Bacterial community analyses

DNA was extracted from the 3- μm (PA fraction) and 0.22- μm (FL fraction) filters using the FastDNA spin kit for soil (MP Biomedicals, USA), as per the manufacturer's protocol. V3–V4 bacterial 16S rRNA gene was amplified from the DNA samples using

Pro341F/Pro805R primer (Takahashi et al. 2014), and amplicon sequencing were performed using Illumina MiSeq platform 2500 at the Agrigenome Pvt. Ltd (India). The sequencing data were processed in MOTHUR 1.44.3 following the standard operating procedure (http://www.mothur.org/wiki/MiSeq_SOP) (Kozich et al. 2013) using Pratyush High-Performance Computing facility at the Indian Institute of Tropical Meteorology (India) (for detailed method, please see Method S1, Supporting Information). Differentially abundant OTUs between FL and PA fractions, and between each sampling depth were identified using linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al. 2011) in MOTHUR 1.44.3, using the default parameters, except multiclass parameter was set to one-against-one. Operational taxonomic units (OTU) with P-value <0.05 and LDA score $[\log_{10}] \geq 4$ were considered as differentially abundant. Raw sequencing data were deposited in the NCBI-SRA under accession number PRJNA727972 (<https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA727972>).

Phylogenetic null modeling

To quantify the relative importance of different assembly mechanisms (e.g. variable selection, homogeneous selection, dispersal limitation, homogenizing dispersal, undominated), we applied a previously developed framework (Stegen et al. 2012, 2013, 2015). Briefly, β -nearest taxon index (β NTI) and Bray-Curtis-based Raup-Crick (RC_{bray}) scores were calculated from pairwise comparisons of phylogenetic turnover between all FL and PA communities, and the deviation of those values from null models (999 permutations). The β NTI was abundance-weighted, and the null model used was based on randomizing and shuffling taxa names and abundances across tips of the phylogenetic tree (Stegen et al. 2013). β NTI < -2 indicates significantly less phylogenetic turnover than expected by chance, indicating homogeneous selection. β NTI > +2 indicates significantly higher phylogenetic turnover than expected by chance, indicating variable selection. β NTI between -2 and +2 likely indicated stochastic processes at play, and RC_{bray} was calculated to separate different mechanisms. $RC_{\text{bray}} < -0.95$ suggests less phylogenetic turnover compared to the regional pool and is interpreted as homogenizing dispersal. In contrast, $RC_{\text{bray}} > +0.95$ suggests more phylogenetic turnover compared to the regional pool, indicating dispersal limitation. RC_{bray} between -0.95 and +0.95 indicates that phylogenetic turnover between microbial communities does not deviate significantly from comparisons to the regional pool. These values could be interpreted as ecological drift shaping communities (Stegen et al. 2012, 2013), or perhaps that the assembly of communities is 'undominated' by any single dispersal or selection mechanism (Stegen et al. 2015).

Microbial source tracking

SourceTracker2 was used under default conditions for running the microbial source tracking (MST) model (Knights et al. 2011). Our MST model was based on the assumption that the FL bacteria attach and colonize POM and are the source communities for the corresponding PA or sink communities (Fadeev et al. 2020). To test the predictive accuracy across various source communities (i.e. FL) from the different sampling depths we applied the leave-one-out approach. In this method, one of the source communities was hidden, in turn, from the model when it was trained. Our MST model accurately predicted the sampling depth of the surface and bottom FL communities and matched it with their original sampling depth (Fig. 3).

Alpha- and beta diversity and their correlates

Before calculating the alpha and beta diversity, sequencing depth was rarefied to 75945 reads per sample. Alpha diversity indices, including the observed number of OTUs or OTU richness, inverse Simpson index and Shannon diversity index, were calculated in MOTHUR 1.44.3. PRIMER v6 software package (Plymouth Marine Laboratory, UK) was used for calculating the beta diversity among samples. The square root transformed OTU abundance data were used to calculate the Bray-Curtis similarity index. The similarity matrix was used to perform cluster analysis using a group-average linking method and nonmetric multidimensional scaling (NMDS). Two-way permutational multivariate analysis of variance (PERMANOVA) was used for testing the effect of size fractionation, depths and their interaction on Bray-Curtis dissimilarity of the bacterial communities. The relationship between environmental variables, and distance from Kornebreen glacier, and the PA and FL bacterial community was evaluated using stepwise distLM and db-RDA in PRIMER V6. Before performing the distLM and dbRDA, a preliminary diagnostic was conducted using a draftsman plot to assess multicollinearity among the predictor variables. This analysis reveals that TPN and POC were highly correlated ($r = 0.9339$). In addition, nitrate and salinity were highly correlated to phosphate ($r = 0.767$ and $r = 0.899$, respectively). Therefore, to avoid redundancy, we did not include TPN and phosphate in the distLM and dbRDA. We have used detrended correspondence analysis (DCA) to analyze the species data and found the lengths of the gradients for both PA and FL species data to be <3. If the length of the gradient is <3.0, the result of RDA is better than that of CCA. Therefore, we chose RDA instead of CCA to evaluate the relationship between species and environmental factors.

RESULTS AND DISCUSSION

Water mass stratification in Kongsfjorden

Four distinct water masses in Kongsfjorden, during the sampling period (Fig. 1B), were identified as surface water (SW), intermediate water (IW), AW and transformed Atlantic water (TAW), as per the criterion reported earlier for Kongsfjorden (Cottier et al. 2005). We choose three different depths for sampling to collect water samples from at least three distinct water mass types (Fig. 1A). Since the bottom depth of Kongsfjorden is variable due to its topography, we decided to collect water samples from 5 m above the seafloor from each sampling location. Vertical profiles of the temperature and salinity (Fig. 1A) showed the SW (>3°C, <34 psu) lies atop the IW (>4°C, 34-34.65 psu), below which a cooler and saltier layer of AW was observed (>3°C, >34.65 psu). The warmer SW and IW observed during the study period could be attributed to the intrusion of AW at depth ranged between 50-190 m, resulting in a temperature inversion (David and Krishnan 2017). Kongsfjorden below 50 m was filled by the AW; however, a layer of TAW (<3°C, >34.65 psu) was found at the bottom in the central part of Kongsfjorden, i.e. between KGF-2 and KGF-3 (Fig. 1B). The intrusion of AW occurs throughout the summer and by the late summer period (August-September) reaches a quasi-steady state that can either be weakly or strongly dominated by AW or TAW (Cottier et al. 2005).

Nutrients and other biogeochemical characteristics

Nitrate and phosphate concentration increased with depth, reaching values ranging from 1.47 to 3.74 μM and 0.357 to 0.59

μM , respectively, in the bottom depths (Table 1). Both nitrate and phosphate showed a strong positive correlation with the salinity and depth (Table S1, Supporting Information), indicating that AW intrusion into Kongsfjorden represents a substantial source for these nutrients that can subsequently be transported to the surface via upwelling at the glacier front (Halbach et al. 2019). Chlorophyll (chl) *a* concentration in the surface waters ranged between 0.2 and 0.41 $\mu\text{g L}^{-1}$, and decreased with depth, as previously observed (Calleja et al. 2017). POC and TPN were higher in the surface water than middle and bottom waters, as previously reported (Calleja et al. 2017). C:N ratio ranged from 5.66 to 13.99, suggesting that POM is labile (Meyers 1994). TOC showed a strong negative correlation ($R = -0.976$; $P < 0.001$; Table S1, Supporting Information) with the distance from the glacier and could be attributed to the supply of dissolved organic carbon from the glacier (Hood et al. 2009). The high TOC concentration in the inner fjord location is also consistent with the previous observation on the elevated colored dissolved organic matter (CDOM) concentration in the near glacier location in Kongsfjorden (Sagan and Darecki 2018). These physicochemical parameters demonstrate substantial vertical heterogeneity, alongside observable—but less prominent—lateral variations.

Vertically resolved assembly and connectivity of FL and PA communities

The strong vertical stratification of Kongsfjorden and the potential development of distinct microbial communities afford the opportunity to investigate community assembly processes and resulting community connectivity and composition across well-defined depth layers. Using the previously developed phylogenetic null modeling framework (Stegen et al. 2012, 2013, 2015), we demonstrate similarities and divergences in community assembly mechanisms among FL and PA bacterial communities. Analysis of community phylogenetic turnover shows that the relative importance of ‘undominated’ assembly processes is consistently moderate to high among most of the communities (Fig. 2). This indicates that moderate to high fractions of community turnover in pairwise comparisons are not dominated strongly by either selection or dispersal, but that these processes may act simultaneously (Stegen et al. 2015). Earlier studies have attributed this to ecological drift (Stegen et al. 2013), or random birth and death of taxa; however, we instead adopt a more conservative definition to avoid definitive conclusions about a single assembly mechanism when the signal is weak.

From surface to bottom waters, distinct relative influences of assembly processes emerge between PA and FL communities. In surface waters and averaged across stations, PA and FL microbial communities are similarly structured by moderate levels of homogeneous selection (PA = 29.3%, FL = 27.2%) and dispersal limitation (PA = 19.6%, FL = 18.5%); minor levels of variable selection (PA = 6.5%, FL = 4.4%) can also be detected among these communities (Fig. 2A and B). With increasing depth in the water column, the relative influences of assembly processes become more distinct between FL and PA bacterial communities. Several specific features arise, including the following: (i) the decrease of homogeneous selection among PA bacterial communities (surface = 29.3%, middle = 18.5% and bottom = 10.8%), but moderate increase among their FL counterparts (surface = 27.2%, middle = 42.4% and bottom = 36.9%); (ii) increase of variable selection among PA communities (surface = 6.5%, bottom = 17.3%), but limited to no importance of this assembly process among FL communities in bottom waters (surface = 4.4%, bottom = 0%);

and (iii) increased influence of dispersal limitation among PA bacterial communities—often becoming the dominant assembly mechanism in bottom waters (surface = 19.6%, middle = 30.4% and bottom = 37.0%)—but its comparable relative importance among FL bacterial communities with increasing depth (surface = 18.5%, middle = 10.9% and bottom = 14.1%) (Fig. 2B).

That different assembly mechanisms show similar relative proportions among FL and PA bacterial communities in surface waters suggests greater exchange between the two size fractions. Additionally, the different relative influence of assembly processes in deeper waters likely indicates less interconnectivity between FL and PA communities. We hypothesized these to be the case because greater exchange would likely reduce or even eliminate size fraction-specific patterns, resulting in similar relative proportions of assembly processes across the two size fractions. When the opposite is true (i.e. low exchange), then size fraction-specific patterns likely arise, resulting in different relative influences of assembly processes. To evaluate these interpretations, we used MST, a Bayesian approach, treating the FL communities as sources of taxa that can colonize particles and comprise the PA fraction across depth layers. A similar approach has been applied to study the PA communities’ vertical connectivity in the Fram Strait, the gateway to the Arctic Ocean (Fadeev et al. 2020). Results from the MST model showed that the surface FL communities (as source taxa) contributed 80%, 20% and <10% to the PA communities from the surface, middle and bottom depths, respectively (Fig. 3). The high proportions of surface FL communities found in surface PA communities corroborate with our interpretation—based on similar assembly mechanisms in surface waters—that these communities are highly interconnected in the upper water column. High particle concentrations in surface waters could lessen the time spent by bacteria on particles as indicated by patch use theory (Yawata et al. 2020), facilitating frequent particle attachment/detachment that increases exchange between PA and FL communities (Simon, Smith and Herfort 2014; Thiele et al. 2015; Jain and Krishnan 2017).

Minor contributions (<10–20%) of the surface FL communities to the middle and bottom water PA communities (Fig. 3) indicate weak vertical connectivity between the surface and the deeper waters of Kongsfjorden and this could be associated with the increased dispersal limitation among PA communities with increasing depth (Fig. 2). In addition, the proportions of unknown sources of taxa in PA communities increased with depth, quantified as 5%, 20% and 30% in the surface, middle and bottom depths, respectively (Fig. 3). This could be attributed in part to the increased proportion of variable selection, diversity, and numbers of rare and specialized PA OTUs with depth in Kongsfjorden. The bottom environment might harbor more diverse organic particles, providing more niches for the PA bacteria. Overall, MST-based results are consistent with the observation that the relative influences of community assembly processes change down the water column (Fig. 2). Concurrently, with increasing depth, the assembly mechanisms between FL and PA bacteria become increasingly divergent, suggesting less exchange between the two communities in deep waters. In deeper waters where particle concentrations are lower, bacterial residence times on particles increase to avoid longer search times for new particles (Yawata et al. 2020). Under this scenario, particles become increasingly disparate habitats due to the limited movement of taxa (Fig. 3), setting the stage for distinct trajectories of particle degradation and remineralization in deep waters (Balmonte et al. 2020). As particles undergo distinct degradation processes, increased compositional heterogeneity

Table 1. Details of the sampling locations, environmental variables, geographical distance and sampling depth. ND = Not detected.

Parameters	Surface					Middle					Bottom			
	KGF-1S	KGF-2S	KGF-3S	KGF-4S	KGF-1M	KGF-2M	KGF-3M	KGF-4M	KGF-1B	KGF2-B	KGF-3B	KGF-4B		
Lat (degrees.N)	79.0354	78.9931	78.9587	78.9228	79.0354	78.9931	78.9587	78.9228	79.0354	78.9931	78.9587	78.9228		
Long (degrees.E)	11.2836	11.5547	11.8224	12.0937	11.2836	11.5547	11.8224	12.0937	11.2836	11.5547	11.8224	12.0937		
Temp (deg C)	5.31	5.24	5.24	4.98	4.64	5.07	4.90	4.27	3.26	1.89	2.53	3.85		
Sal (psu)	32.02	31.83	31.75	32.20	34.83	34.77	34.75	34.68	34.85	34.81	34.84	34.86		
DO (mL L ⁻¹)	7.18	7.07	6.57	6.72	6.87	6.83	6.90	6.96	6.90	7.09	6.95	6.75		
TOC (µM C)	81.43	135.60	191.60	113.47	34.40	94.03	116.67	184.73	25.40	84.30	154.57	159.63		
POC (µM C)	22.67	33.13	18.33	20.05	11.67	11.57	11.58	13.07	11.46	10.18	13.32	14.08		
TPN (µM N)	4.01	4.31	2.09	2.39	1.06	1.42	1.45	1.73	0.82	1.41	1.75	1.62		
C/N	5.66	7.69	8.78	8.37	11.02	8.14	7.99	7.54	13.99	7.20	7.60	8.68		
Chl-a (µg L ⁻¹)	0.21	0.40	0.41	0.20	0.02	0.09	0.16	0.02	0.01	0.02	0.06	0.01		
Nitrate (µM)	ND	ND	ND	0.055	1.05	1.921	1.619	0.796	1.474	3.747	3.734	3.165		
Nitrite (µM)	0.053	0.06	0.096	0.042	0.093	0.241	0.247	0.125	0.1	0.095	0.067	0.163		
Phosphate (µM)	0.011	0.022	0.015	0.039	0.322	0.494	0.463	0.282	0.554	0.59	0.371	0.357		
Silicate (µM)	0.049	0.148	0.143	0.139	0.014	0.153	0.143	0.155	0.106	0.152	0.042	0.049		
Distance (km)	27	19	14	7	27	19	14	7	27	19	14	7		
Depth (m)	10	10	10	10	75	75	75	75	198	284	247	130		

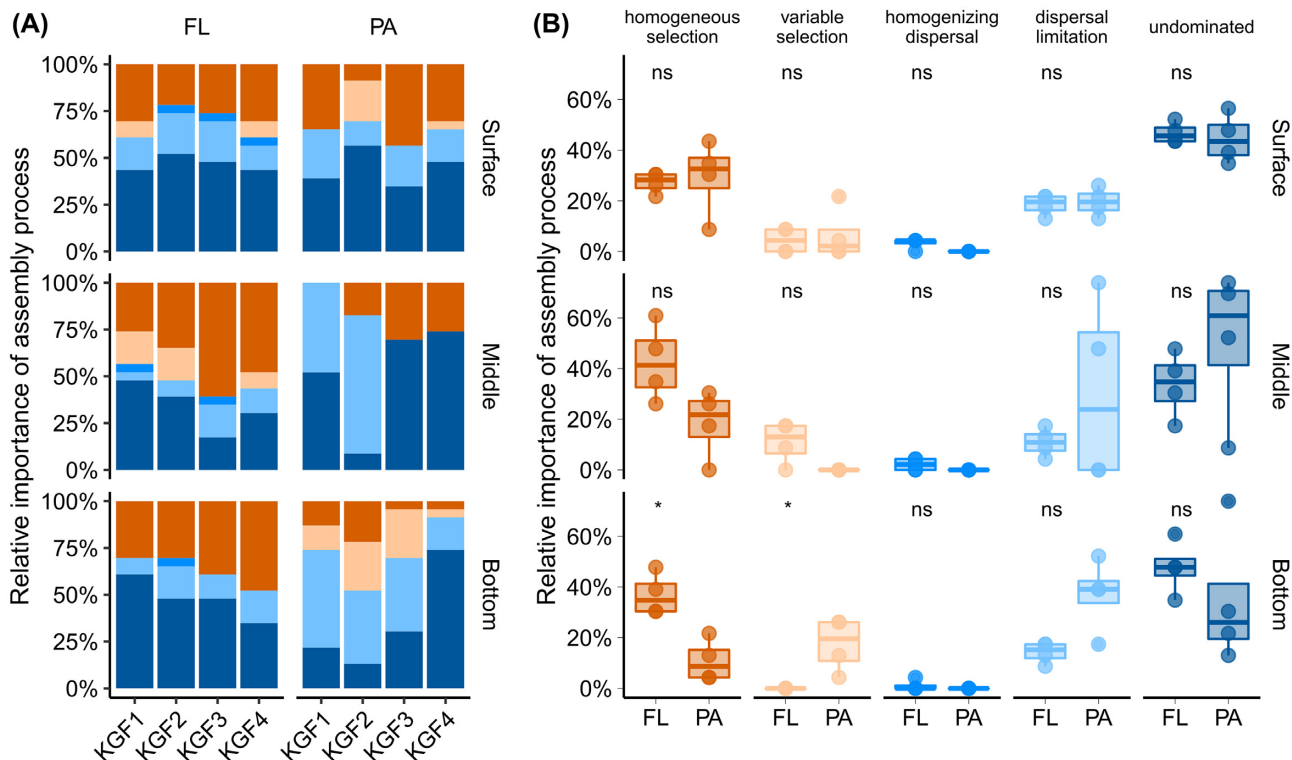


Figure 2. The relative importance of microbial community assembly processes (A) separated by depth and size fraction, and (B) separated by size fraction and assembly process in the surface, middle and bottom depths. Asterisks in panel (B) denote $P < 0.05$ using Wilcoxon test. NS = not statistically significantly different.

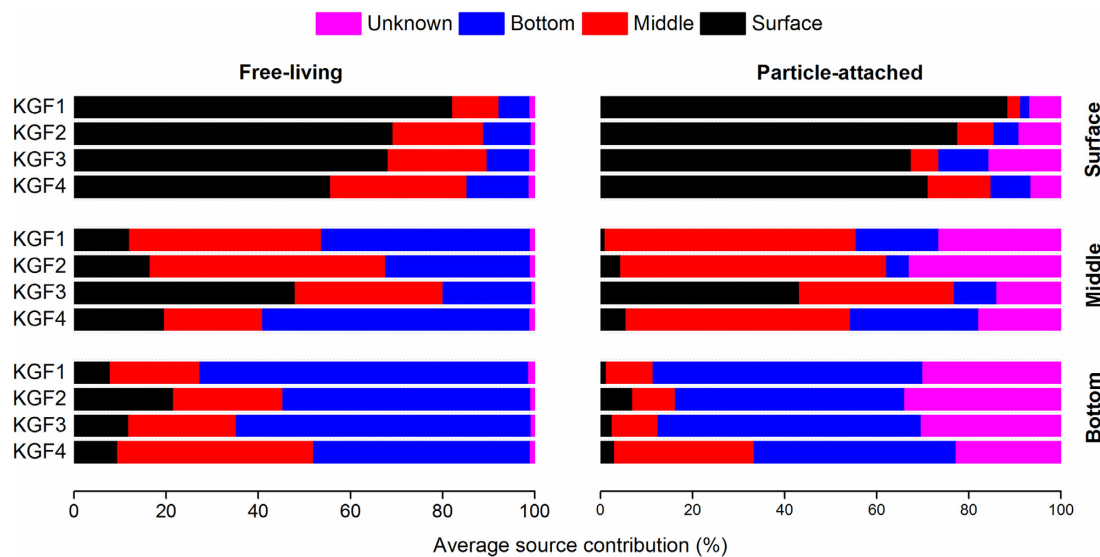


Figure 3. Percentage contribution of source (free-living) communities from the different depths (surface, middle and bottom) and unknown to the sink (particle-attached) communities, estimated using microbial source tracker model. A leave-one-out approach was used for estimating the percentage source contribution for the FL communities (right side).

within and across particles could lead to increased variable selection among PA communities in deep waters (Fig. 2).

Similarly, few other studies also show distinct assembly mechanisms acting upon FL and PA bacterial communities in a lake during a phytoplankton bloom (Xu et al. 2020), and in a river-bay system (Wang et al. 2020). Greater influence of variable selection among PA than FL bacterial communities (Xu et al. 2020),

and the greater importance of homogeneous selection among FL bacterial communities (Wang et al. 2020) are consistent with our results in deeper waters (Fig. 2). However, these patterns are temporally variable. For example, stochastic assembly mechanisms decrease in relative importance both for FL and PA bacterial communities during a phytoplankton bloom (Xu et al. 2020). Additionally, our results provide evidence that assembly pro-

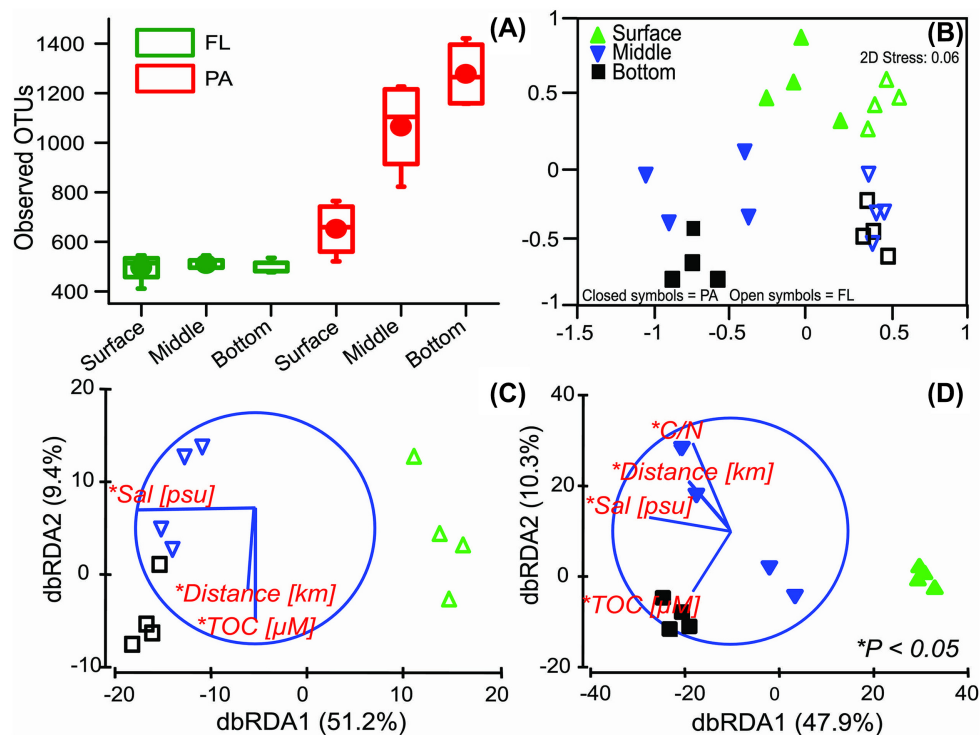


Figure 4. (A) Box plot comparison of bacterial OTU richness at different sampling depths (surface, middle and bottom) between FL and PA communities. In the box plot, the top and bottom boundaries of each box indicate 75th and 25th quartile values, respectively, and the line and a circle within each box represent the median and mean values, respectively. The ends of whiskers indicate the lowest and the highest values in each group. (B) NMDS 2D ordination of FL and PA bacterial communities. Distance-based linear modeling (distLM) and redundancy analysis (dbRDA) plots of (C) FL and (D) PA communities showing their relationship with the most significant ($*P < 0.05$) variables.

cesses can also be spatially variable. This spatial variability is most prominent when considering vertical distances—i.e. the surface to bottom waters (Fig. 2).

Quantifying the importance of assembly mechanisms, and distinguishing their influences between FL and PA communities, demonstrates simultaneous influences of selection and dispersal processes. A previous study that similarly investigated bacterial community assembly processes in the same fjord yielded contrasting results: only stochastic forces were detected among the examined communities (Han *et al.* 2021). However, our study distinguished assembly processes that act upon PA and FL taxa, as Han *et al.* (2021) analyzed bulk (non-size-fractionated) communities. Unless mass effects dominate microbial communities, purely stochastic assembly mechanisms are unlikely in nature, especially considering the observed phylogenetic conservation among FL and PA taxa that comprise the entire bacterial community (Schmidt *et al.* 2016), as well as the deterministic influence of salinity differences on microbial communities in fjords (Balmonde *et al.* 2020). Thus, a combination of environmental selection and stochastic forces simultaneously structure these distinct but often connected PA and FL communities, as observed here and in other studies (Wang *et al.* 2020; Xu *et al.* 2020).

Alpha diversity, beta diversity, and environmental sources of variation of PA and FL communities

A total of 3365592 V3–V4 bacterial 16S rRNA gene sequences were obtained from the 24 samples. After rarefying the sequencing depth of each sample to 75945 reads, a total of 4921 OTUs were obtained (Table S4, Supporting Information). All the samples showed a very high species coverage index (>99%) (Table

S2, Supporting Information) and the rarefaction curve showed asymptote in most samples (Fig. S1A–C, Supporting Information). This indicates that an adequate level of sequencing per sample has been performed. Species richness (observed number of OTUs; $T = 5.6$, $df = 22$, $P < 0.05$) (Fig. 4A) and the diversity (inverse Simpson; $T = 6.15$, $df = 22$, $P < 0.05$ and Shannon diversity index; $T = 7.99$, $df = 22$, $P < 0.05$) of the PA communities were significantly higher (two-tailed Student's *t*-test) than FL communities (Fig. S1D and E, Supporting Information). The higher diversity of the PA communities than FL concurs with previous reports from surface waters of Kongsfjorden (Jain *et al.* 2019), Canadian Arctic (Ortega-Retuerta *et al.* 2013) and the Baltic Sea (Rieck *et al.* 2015). With increasing depth, PA and FL alpha diversity exhibited distinct trends: whereas species richness increased with depth among PA communities, while those for the FL communities it remained relatively uniform (Fig. 4A). Advection of distinct PA communities via Atlantic waters might have contributed to the higher diversity among PA communities in the intermediate and bottom waters (Storesund *et al.* 2017). Overall, the compositional heterogeneity of particles and the microhabitats within particles likely promote the coexistence of a highly diverse bacterial community that occupies distinct niches (Simon, Smith and Herfort 2014; Schmidt *et al.* 2020).

Beta diversity based on the Bray–Curtis dissimilarity index and ordinated using NMDS illustrates the compositional distinction between PA and FL communities (Fig. 4B; PERMANOVA, Table S3, Supporting Information), extending previous findings in surface waters of Kongsfjorden (Jain *et al.* 2019) to bottom waters. In addition, vertical trends of PA and FL beta diversity are consistent with results based on the phylogenetic null modeling and MST. First, the dissimilarity between PA and FL bacterial

communities increases with depth, evident in the increased distances between the two fractions in the NMDS (Fig. 4B). This prominent compositional separation between PA and FL in deep waters parallels the increasingly distinct assembly processes (Fig. 2) and connectivity, based on the MST analysis (Fig. 3). Second, higher beta diversity (greater dispersion) among deepwater PA bacterial communities (Fig. 4B), but lower beta diversity (tighter clustering) among their FL counterparts are consistent with the greater influence of variable selection and homogeneous selection on the PA and FL communities, respectively (Fig. 2). Large variations in the particle quality and chemical composition across sampling sites (Table 1) likely underlie the high beta diversity among PA bacterial communities (Jain et al. 2019). Moreover, these findings are consistent with observations that deepwater particles and the ambient water are occupied by the community with highly distinct niches and lifestyles (Thiele et al. 2015; Salazar et al. 2016).

Stepwise distLM and db-RDA were applied to find potential factors that impose both selection and stochasticity on FL and PA communities, including environmental variables, and geographic distance from the Kornebreen glacier, a proxy for terrestrial and freshwater influence. Salinity was the most significant ($P < 0.05$) variable that explained ~50% of the total variation in the FL and PA bacterial communities (Fig. 4C and D). Differences in salinity led to the vertical partitioning of the surface communities (both FL and PA) from the middle and bottom communities. Differences in salinity are due to the distinct origins and transformations of water masses in Kongsfjorden (Cottier et al. 2005). Physicochemical conditions are observed widely to shape bacterial biogeography locally in Kongsfjorden (Han et al. 2020), in the Atlantic Ocean (Agogue et al. 2012), in the Arctic Ocean (Galand et al. 2010; Balmonte, Teske and Arnosti 2018) and in global oceans (Sunagawa et al. 2015). Salinity, in particular, has been shown as an important selective force among bacterial communities in another high Arctic fjord (Balmonte et al. 2020), in other freshwater-to-marine systems (Simon, Smith and Herfort 2014; Fortunato and Crump, 2015; Satinsky et al. 2015); however, as salinity differences also in part distinguish water masses, they can also constitute a geographic barrier to dispersal (Galand et al. 2010), which can lead to stochastic community changes due to dispersal limitation.

Distance from the Kornebreen glacier seems to have a less prominent, but statistically significant ($P < 0.05$) influence on the horizontal variations of the FL and PA communities in Kongsfjorden (Fig. 4B). Lateral variations in the FL community structure, especially in the middle and bottom depths, are concurrent with the gradual increase in the homogeneous selection and dispersal limitation from the outer to the inner fjord (Fig. 2). On the other hand, the PA communities from the middle/intermediate layers were strongly influenced by the distance and C:N ratio, separating the communities into two groups (Fig. 4D). These depths (around ~50 m) at Kongsfjorden are highly influenced by the intrusion of AW, suggesting the influence of advective forcing on the FL and PA communities (Storesund et al. 2017). C:N ratio of POM at the intermediate depth showed a gradual decline with decreasing distance (Table 1), indicating qualitative differences of POM. In this fjord, these differences are likely due to distinct water mass origins (Jain et al. 2019), as observed for POM in another high Arctic fjord (Balmonte et al. 2020). Sustained lateral separation of different water masses especially in the intermediate layers in this fjord may also underlie the strong dispersal limitation observed among PA communities at the two outermost stations (KG1 and KG2; Fig. 2). Toward the glacier (e.g. KGF3 and KGF4), the strong influence of dispersal limitation among PA

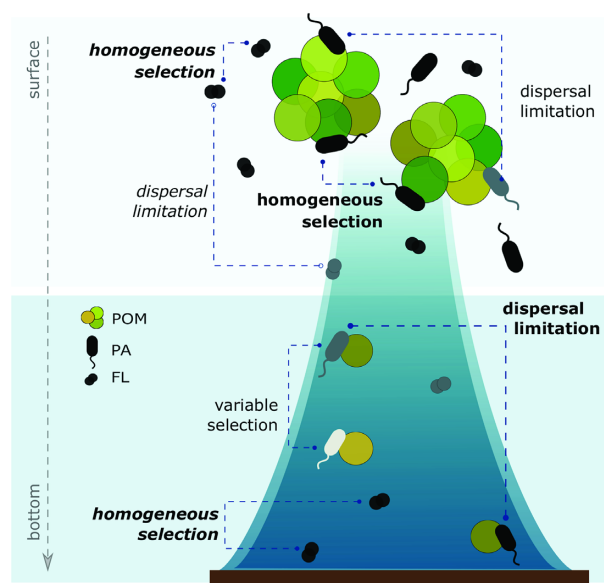


Figure 5. Schematic of the spatially distinct relative importance of assembly processes that influence varying composition and connectivity of microbial communities from surface to bottom waters. PA versus FL bacterial communities are depicted by different, individual symbols for simplicity. Assembly processes in bold font indicate the dominant selective (e.g. variable and homogeneous selection) versus stochastic (i.e. dispersal limitation) processes influencing PA versus FL bacterial communities in surface waters (top) versus middle and bottom depths (bottom). Assembly processes not shown in bold are those with minor influence. The relative importance of ‘undominated’ processes was substantial for all community, but it was not included to focus on mechanisms that differ in relative importance between PA and FL bacterial communities. POM = particulate organic matter.

communities disappears, indicating abrupt changes in assembly over a relatively short lateral distance. Overall, our results demonstrate strong vertical differences in FL and PA community assemblies, combined with subtle lateral changes in these processes that are linked to the intrusion of AW in the fjord.

Bacterial community structure and depth-specific indicator FL and PA taxa

Bacterial communities in the FL fractions, at all sampling depths, were dominated by the Alphaproteobacteria (phylum Proteobacteria), accounting for >50% of the total FL reads (Fig. S2, Supporting Information). The dominance of Alphaproteobacteria in the surface water of Kongsfjorden during the late summer season (August–September) has been reported earlier (Jain and Krishnan 2017; Jain et al. 2019, 2020; Thomas, Sinha and Krishnan 2019). Alphaproteobacteria, Gammaproteobacteria, Verrucomicrobiae and Bacteroidia were the most abundant taxa observed among the PA communities (Fig. S2, Supporting Information). Some of the bacterial taxa showed depth-specific distribution. For instance, members of the class Nitrospira (nitrite-oxidizing bacteria) and phylum Nitrospina were more abundant in the FL fractions at the bottom depths (Fig. S2, Supporting Information). However, in the PA fractions, Phycisphaerae and OM190 clade were more abundant in the middle and bottom depths, especially (Fig. S2, Supporting Information).

We performed differential abundance analysis using LEfSe to identify depth-specific indicator FL and PA taxa. This analysis showed five, one and three FL OTUs that were highly and differentially abundant in the surface, middle and bottom waters,

respectively. Among the PA communities, four, eight and five OTUs were differentially abundant in the surface, middle and bottom waters, respectively (Figs S3 and S4, Supporting Information). Identification of more differentially abundant PA OTUs suggests greater depth-specific differentiation of PA communities than observed among FL communities. FL and PA indicator taxa are likely metabolically diverse, based on the known ecological roles of closely related taxa. Among FL indicator taxa, OTUs belonging to Nitrospiraceae, Amylobacter, Sulfotobacter, Polaribacter, *Candidatus Actinomarina*, Nitrospinaceae, Nitrospira and Thioglobaceae suggest putative roles in nitrite oxidation (Füssel et al. 2012; Sun et al. 2019), sulfur oxidation (Rogge et al. 2017) and dark carbon fixation (Pachiadaki et al. 2017). PA indicator OTUs include members of *Roseibacillus*, *Luteolibacter*, *Fluviicola*, *Ulvibacter*, OM60, *Alteromonas* and Planctomycetota, among several others (Figs S3 and S4, Supporting Information). Taxa closely related to these OTUs have been identified as abundant on particles in Kongsfjorden (Jain and Krishnan 2017; Jain et al. 2019), as well as in deep marine waters (Salazar et al. 2015), with putative roles in the degradation of high molecular weight organic matter (Teeling et al. 2012) and utilization of oligopeptides and lipids (Fuchs et al. 2007; Jang et al. 2011). Identification of these putatively metabolically diverse FL and PA taxa further highlights the consequences of depth-specific assembly processes and resulting community structure on biogeochemical processes in different layers of the marine water column.

Broader spatial and temporal implications

Our study demonstrates that the increasingly distinct PA and FL bacterial community assembly mechanisms with increasing depth in a stratified fjord water column coincide with decreasing connectivity and similarity between the two communities (Fig. 5). These patterns are brought upon by a combination of deterministic and stochastic processes that are intricately linked to environmental characteristics. Particle heterogeneity and decreasing concentration with depth (Table 1), and the presence of distinct water masses, impose variable selection and dispersal limitation, respectively, on PA communities (Fig. 5). These processes limit the exchange of taxa across particles, as well as between the PA and FL fractions that lead to increasingly distinct PA and FL communities in deep waters. Hence, while this study is carried out in a fjord model system, distinct mechanisms shaping PA and FL communities may also be observed where steep vertical gradients of particle concentrations are found, especially in coastal oceans. For example, our observation that PA and FL communities exhibit high and low beta diversity in deep waters, respectively, is in accordance with findings that PA—but not FL—communities show basin-specific groupings in bathypelagic waters (Salazar et al. 2016); these beta diversity trends are also consistent with organic matter-degrading capabilities of PA bacteria with increasing depth in the Pacific Ocean (Balmonte et al. 2021). Increasingly distinct assembly processes between these two communities, coupled with the limited exchange, might be among several mechanisms leading to prominent compositional separation between PA and FL communities in the mesopelagic (Baumas et al. 2021), bathypelagic (Salazar et al. 2015) and even in the hadopelagic systems (Eloe et al. 2011).

While likely relevant on a broader spatial scale, our findings additionally have importance in a temporal and more local context. These community assembly mechanisms and connectivity, identified in a well-studied, high Arctic fjord, are inherently tied

to water mass dynamics in this system. Alterations in the environmental context can conceivably result in immediate microbial community changes, taking into consideration the rapid responses of microorganisms to stimuli. Increasing freshwater flow from melting glaciers (Sejr et al. 2017) could alter present, active or surviving taxa in fjords, imposing selection on the basis of their tolerance to salinity changes (Balmonte et al. 2020), or genetic drift due to dispersal limitation should these waters increasingly stratify in the outer reaches of the fjord. In tandem, high discharge volumes could lead to homogenizing dispersal, or mass effects (Adams, Crump and Kling 2014), especially at—or closer to—points of discharge. Freshening and warming of surface waters intensify stratification, which throughout the polar regions are expected to coincide with stimulated microbial carbon processing and respiration (Kirchman, Moran and Ducklow 2009). Thus, in a system presently viewed as a surface area-normalized ‘hot spot’ of carbon burial (Smith et al. 2015), characterizing disturbance-related changes in microbial assembly mechanisms and their impacts on ecosystem functioning (Knelman and Nemergut 2014) is a critical priority in future studies.

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SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://www.femsec.org/) online.

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REFERENCES

- Adams HE, Crump BC, Kling GW. Metacommunity dynamics of bacteria in an Arctic lake: the impact of species sorting and mass effects on bacterial production and biogeography. *Front Microbiol* 2014;5:1–82.
- Agogué H, Lamy D, Neal PR et al. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. *Mol Ecol* 2012;20:258–74.
- Balmonte JP, Hasler-Sheetal H, Glud RN et al. Sharp contrasts between freshwater and marine microbial enzymatic capabilities, community composition, and DOM pools in a NE Greenland fjord. *Limnol Oceanogr* 2020;65:77–95.
- Balmonte JP, Simon M, Giebel HA et al. A sea change in microbial enzymes: heterogeneous latitudinal and depth-related gradients in bulk water and particle-associated enzymatic activities from 30°S to 59°N in the Pacific Ocean. *Limnol Oceanogr* 2021;66:3489–507.
- Balmonte JP, Teske A, Arnosti C. Structure and function of high Arctic pelagic, particle-associated, and benthic bacterial communities. *Environ Microbiol* 2018;20:2941–54.

- Baumas CMJ, Le Moigne FAC, Garel M et al. Mesopelagic microbial carbon production correlates with diversity across different marine particle fractions. *ISME J* 2021;15:1695–1708.
- Bižić-Ionescu M, Ionescu D, Grossart HP. Organic particles: heterogeneous hubs for microbial interactions in aquatic ecosystems. *Front Microbiol* 2018;9:2569.
- Bižić-Ionescu M, Zeder M, Ionescu D et al. Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization. *Environ Microbiol* 2015;17:3500–14.
- Boeuf D, Edwards BR, Eppley JM et al. Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. *Proc Natl Acad Sci USA* 2019;116:11824–32.
- Calleja ML, Kerhervé P, Bourgeois S et al. Effects of increase glacier discharge on phytoplankton bloom dynamics and pelagic geochemistry in a high Arctic fjord. *Prog Oceanogr* 2017;159:195–210.
- Cordero OX, Datta MS. Microbial interactions and community assembly at microscales. *Curr Opin Microbiol* 2016;31:227–34.
- Cottier F, Tverberg V, Inall M et al. Water mass modification in an Arctic fjord through cross-shelf exchange: the seasonal hydrography of Kongsfjorden, Svalbard. *J Geophys Res* 2005;110:1–18.
- Datta MS, Sliwerska E, Gore J et al. Magnetic alginate microparticles for purification of α -amylases. *Nat Commun* 2016;7:255–60.
- David TD, Krishnan KP. Recent variability in the Atlantic water intrusion and water masses in Kongsfjorden, an Arctic fjord. *Polar Sci* 2017;11:30–41.
- DeLong EF, Preston CM, Mincer T et al. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 2006;311:496–503.
- Eloe EA, Shulse CN, Fadrosch DW et al. Compositional differences in particle-associated and free-living microbial assemblages from an extreme deep-ocean environment. *Environ Microbiol Rep* 2011;3:449–58.
- Enke TN, Datta MS, Schwartzman J et al. Modular assembly of polysaccharide-degrading marine microbial communities. *Curr Biol* 2019;29:1528–35.
- Enke TN, Leventhal GE, Metzger M et al. Microscale ecology regulates particulate organic matter turnover in model marine microbial communities. *Nat Commun* 2018;9:2743.
- Fadeev E, Rogge A, Ramondenc S et al. Sea-ice retreat may decrease carbon export and vertical microbial connectivity in the Eurasian Arctic basins. *Nat Res* 2020. DOI: 10.21203/rs.3.rs-101878/v1.
- Fortunato CS, Crump BC. Microbial gene abundance and expression patterns across a river to ocean salinity gradient. *PLoS One* 2015;10:e0140578.
- Fuchs BM, Spring S, Teeling H et al. Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. *Proc Natl Acad Sci USA* 2007;104:2891–6.
- Füssel J, Lam P, Lavik G et al. Nitrite oxidation in the Namibian oxygen minimum zone. *ISME J* 2012;6:1200–9.
- Galand PE, Potvin M, Casamayor EO et al. Hydrography shapes bacterial biogeography of the deep Arctic Ocean. *ISME J* 2010;4:564–76.
- Grasshoff K, Ehrhardt M, Kremling K. *K.Grasshoff, M.Ehrhardt, K.Kremling (Editors):Methods of Seawater Analysis. Second, Revised and Extended Edition.–With 108 Figs, 26 Tab., 419 Pp. Weinheim/Deerfield Beach, Florida: Verlag Chemie 1983. ISBN 3–527-2599-8 (Weinheim) 0–89573-7 (Deerfield B. John Wiley & Sons, Ltd, 1985.*
- Grossart HP. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. *Environ Microbiol Rep* 2010;2:706–14.
- Halbach L, Vihtakari M, Duarte P et al. Tidewater glaciers and bedrock characteristics control the phytoplankton growth environment in a fjord in the Arctic. *Front Mar Sci* 2019;6:254.
- Han D, Richter-Heitmann T, Kim IN et al. Survey of bacterial phylogenetic diversity during the glacier melting season in an Arctic fjord. *Microb Ecol* 2021;81:1–13.
- Hollibaugh JT, Wong PS, Murrell MC. Similarity of particle-associated and free-living bacterial communities in northern San Francisco Bay, California. *Aquat Microb Ecol* 2000;21:103–14.
- Hood E, Fellman J, Spencer RGM et al. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* 2009;462:1044–7.
- Jain A, Krishnan KP, Begum N et al. Response of bacterial communities from Kongsfjorden (Svalbard, Arctic Ocean) to macroalgal polysaccharide amendments. *Mar Environ Res* 2020;155:104874.
- Jain A, Krishnan KP, Singh A et al. Biochemical composition of particles shape particle-associated bacterial community structure in a high Arctic fjord. *Ecol Indic* 2019;102:581–92.
- Jain A, Krishnan KP. Differences in free-living and particle-associated bacterial communities and their spatial variation in Kongsfjorden, Arctic. *J Basic Microbiol* 2017;57:827–38.
- Jain A, Krishnan KP. Marine Group-II archaea dominate particle-associated as well as free-living archaeal assemblages in the surface waters of Kongsfjorden, Svalbard, Arctic Ocean. *Antonie Van Leeuwenhoek* 2021;114:633–47.
- Jang Y, Oh HM, Kang I et al. Genome sequence of strain IMCC3088, a proteorhodopsin-containing marine bacterium belonging to the OM60/NOR5 clade. *J Bacteriol* 2011;193:3415–6.
- Kirchman DL, Moran XAG, Ducklow H. Microbial growth in the polar oceans – role of temperature and potential impact of climate change. *Nat Rev Microbiol* 2009;7:451–9.
- Knelman JE, Nemergut DR. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. *Front Microbiol* 2014. DOI: 10.3389/fmicb.2014.00424.
- Knights D, Kuczynski J, Charlson ES et al. Bayesian community-wide culture-independent microbial source tracking. *Nat Methods* 2011;8:761–5.
- Kozich JJ, Westcott SL, Baxter NT et al. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* 2013;79:5112–20.
- Lambert BS, Raina JB, Fernandez VI et al. A microfluidics-based in situ chemotaxis assay to study the behaviour of aquatic microbial communities. *Nat Microbiol* 2017;2:1344–9.
- Liu Y, Lin Q, Feng J et al. Differences in metabolic potential between particle-associated and free-living bacteria along Pearl River Estuary. *Sci Total Environ* 2020;728:138856.
- Lyons MM, Dobbs FC. Differential utilization of carbon substrates by aggregate-associated and water-associated heterotrophic bacterial communities. *Hydrobiologia* 2012;686:181–93.
- Mestre M, Ruiz-González C, Logares R et al. Sinking particles promote vertical connectivity in the ocean microbiome. *Proc Natl Acad Sci* 2018;115:E6799–807.
- Meyers PA. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem Geol* 1994;114:289–302.

- Ortega-Retuerta E, Joux F, Jeffrey WH et al. Spatial variability of particle-attached and free-living bacterial diversity in surface waters from the Mackenzie River to the Beaufort Sea (Canadian Arctic). *Biogeosciences* 2013;10:2747–59.
- Pedros-Alio C, Brock TD. The importance of attachment to particles for planktonic bacteria. *Arch für Hydrobiol* 1983;98:354–79.
- Pelvé EA, Fontanez KM, DeLong EF. Bacterial Succession on Sinking Particles in the Ocean's Interior. *Front Microbiol* 2017;8:2269.
- Poff KE, Leu AO, Eppley JM et al. Microbial dynamics of elevated carbon flux in the open ocean's abyss. *Proc Natl Acad Sci USA* 2021;118:e2018269118.
- Rapp JZ, Fernández-Méndez M, Bienhold C et al. Effects of ice-algal aggregate export on the connectivity of bacterial communities in the central Arctic Ocean. *Front Microbiol* 2018;9:1035.
- Rieck A, Herlemann DPR, Jürgens K et al. Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea. *Front Microbiol* 2015;6:1297.
- Rogge A, Vogts A, Voss M et al. Success of chemolithoautotrophic SUP05 and Sulfurimonas GD17 cells in pelagic Baltic Sea redox zones is facilitated by their lifestyles as K- and r-strategists. *Environ Microbiol* 2017;19:2495–506.
- Sagan S, Darecki M. Inherent optical properties and particulate matter distribution in summer season in waters of Hornsund and Kongsfjordenen, Spitsbergen. *Oceanologia* 2018;60:65–75.
- Salazar G, Cornejo-Castillo FM, Benítez-Barrios V et al. Global diversity and biogeography of deep-sea pelagic prokaryotes. *ISME J* 2016;10:596–608.
- Salazar G, Cornejo-Castillo FM, Borrull E et al. Particle-association lifestyle is a phylogenetically conserved trait in bathypelagic prokaryotes. *Mol Ecol* 2015;24:5692–706.
- Satinsky BM, Fortunato CS, Doherty M et al. Metagenomic and metatranscriptomic inventories of the lower Amazon River, May 2011. *Microbiome* 2015;3:39.
- Schmidt ML, Biddanda BA, Weinke AD et al. Microhabitats are associated with diversity-productivity relationships in freshwater bacterial communities. *FEMS Microbiol Ecol* 2020;96. DOI: 10.1093/femsec/fiaa029.
- Schmidt ML, White JD, Deneff VJ et al. Phylogenetic conservation of freshwater lake habitat preference varies between abundant bacterioplankton phyla. *Environ Microbiol* 2016;18:1212–26.
- Segata N, Izard J, Waldron L et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
- Sejr MK, Stedmon CA, Bendtsen J et al. Evidence of local and regional freshening of Northeast Greenland coastal waters. *Sci Rep* 2017;7:1–6.
- Simon HM, Smith MW, Herfort L. Metagenomic insights into particles and their associated microbiota in a coastal margin ecosystem. *Front Microbiol* 2014;5:466.
- Smith RW, Bianchi TS, Allison M et al. High rates of organic carbon burial in fjord sediments globally. *Nat Geosci* 2015;8:450–3.
- Sperling M, Piontek J, Engel A et al. Combined carbohydrates support rich communities of particle-associated marine bacterioplankton. *Front Microbiol* 2017;8:1–14.
- Stegen JC, Lin X, Fredrickson JK et al. Estimating and mapping ecological processes influencing microbial community assembly. *Front Microbiol* 2015;6:370.
- Stegen JC, Lin X, Fredrickson JK et al. Quantifying community assembly processes and identifying features that impose them. *ISME J* 2013;7:2069–79.
- Stegen JC, Lin X, Konopka AE et al. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J* 2012;6:1653–64.
- Stocker R, Seymour JR, Samadani A et al. Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. *Proc Natl Acad Sci USA* 2008;105:4209–14.
- Storesund JE, Sandaa RA, Thingstad TF et al. Linking bacterial community structure to advection and environmental impact along a coast-fjord gradient of the Sognefjord, western Norway. *Prog Oceanogr* 2017;159:13–30.
- Sun X, Kop LFM, Lau MCY et al. Uncultured Nitrospina-like species are major nitrite oxidizing bacteria in oxygen minimum zones. *ISME J* 2019;13:2391–402.
- Sunagawa S, Coelho LP, Chaffron S et al. Structure and function of the global ocean microbiome. *Science* 2015;348:1261359.
- Svendsen H, Beszczynska-Møller A, Hagen JO et al. The physical environment of Kongsfjorden–Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 2002;21:133–66.
- Takahashi S, Tomita J, Nishioka K et al. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. *PLoS One* 2014;9:e105592.
- Teeling H, Fuchs BM, Becher D et al. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 2012;336:608–11.
- Teeling H, Fuchs BM, Bennke CM et al. Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *eLife* 2016;5:e11888.
- Thiele S, Fuchs BM, Amann R et al. Colonization in the photic zone and subsequent changes during sinking determine bacterial community composition in marine snow. *Appl Environ Microbiol* 2015;81:1463–71.
- Thomas FA, Sinha RK, Krishnan KP. Bacterial community structure of a glacio-marine system in the Arctic (Ny-Ålesund, Svalbard). *Sci Total Environ* 2019:135264.
- Wang Y, Pan J, Yang J et al. Patterns and processes of free-living and particle-associated bacterioplankton and archaeoplankton communities in a subtropical river-bay system in South China. *Limnol Oceanogr* 2020;65:S161–79.
- Xu H, Zhao D, Zeng J et al. Distinct successional patterns and processes of free-living and particle-associated bacterial communities throughout a phytoplankton bloom. *Freshw Biol* 2020;65:1363–75.
- Yawata Y, Carrara F, Menolascina F et al. Constrained optimal foraging by marine bacterioplankton on particulate organic matter. *Proc Natl Acad Sci* 2020;117:25571–9.
- Zhao D, Xu H, Zeng J et al. Community composition and assembly processes of the free-living and particle-associated bacteria in Taihu Lake. *FEMS Microbiol Ecol* 2017;93:62.