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Spatial variation in microbial communities associated with sea-ice algae in Commonwealth Bay, East Antarctica

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Abstract

Antarctic sea-ice forms a complex and dynamic system that drives many ecological processes in the Southern Ocean. Sea-ice microalgae and their associated microbial communities are understood to influence nutrient flow and allocation in marine polar environments. Sea-ice microalgae and their microbiota can have high seasonal and regional (>1000 km^2) compositional and abundance variation, driven by factors modulating their growth, symbiotic interactions and function. In contrast, our knowledge of small-scale variation in these communities is limited. Understanding variation across multiple scales and its potential drivers is critical for informing on how multiple stressors impact sea-ice communities and the functions they provide. Here, we characterized bacterial communities associated with sea-ice microalgae and the potential drivers that influence their variation across a range of spatial scales (metres to >10 kms) in a previously understudied area in Commonwealth Bay, East Antarctica where anomalous events have substantially and rapidly expanded local sea-ice coverage. We found a higher abundance and different composition of bacterial communities living in sea-ice microalgae closer to the shore compared to those further from the coast. Variation in community structure increased linearly with distance between samples. Ice thickness and depth to the seabed were found to be poor predictors of these communities. Further research on the small-scale environmental drivers influencing these communities is needed to fully understand how large-scale regional events can affect local function and ecosystem processes.

INTRODUCTION

The sea-ice surrounding Antarctica is a complex and dynamic system that varies significantly in space and time. The circumpolar extent of the sea-ice layer can cover from 5 to 20 million km^2 throughout the year [1], but forms to a greater extent during the austral winter. The rise of temperature in summer causes a reduction in the extent of the sea-ice around the continent and a change in the physical properties of the sea-ice, including its thickness and salinity gradients [2]. In addition, local icescape changes produced by glacier calving and relocation/grounding of icebergs to new areas may affect local hydrography, nutrient availability and sea-ice formation [3, 4]. Such seasonal and spatial shifts in the sea-ice can drastically influence biotic communities and are likely to alter large coastal areas in Antarctica with projected changes in extent under future warming [5].

Sea-ice communities are formed by diverse taxonomic groups that interact in complex ways and play a critical role in biogeochemical processes [2, 6–8]. Sea-ice microalgae communities are one of the most studied groups in Arctic regions [9], however less is known from Antarctic systems [10, 11]. These algal communities are generally concentrated under the surface of the ice in contact with seawater and up to ~20 cm within the ice matrix [12]. Sea-ice microalgae have a high biomass that varies seasonally and spatially, with diatoms being the most abundant group [13]. Multiple studies across the Antarctic continent and Arctic regions

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Abbreviations: ASV, amplicon sequence variant; CB, commonwealth bay; CD, cape denison; LMM, linear mixed model.

NCBI Sequence Read Archive (SRA), BioProject accession number: PRJNA765135.

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have shown that microalgae associated bacterial communities in the sea ice are dominated by some phyla and the recurring presence of specific genera (studies summarized in [14]). These include the phyla Proteobacteria (e.g. genera *Psychrobacter*, *Ruthia* and *Glaciecola*) and Bacteroidia (e.g. genera *Flavobacteriaceae* and *Rhodoferax*). In many cases, sea-ice microalgae are the main source of fixed carbon for other higher trophic levels in these systems, such as grazing heterotrophic protozoans (mainly ciliates and dinoflagellates [15], krill and larger metazoans (e.g. fish [16]. However, the coupling of sea-ice microalgae with associated bacterial communities can play an important role in the regulation of many ecologic processes [14].

Sea-ice bacterial communities play a significant role in nutrient turnover (e.g. N, P and Si) and organic matter degradation/remineralization from algal-derived photosynthetic products [17]. Specifically, sea-ice microalgae provide particulate and dissolved organic matter through cell degradation and extracellular substance release that bacterial communities can use as a source of nutrients [11]. At the same time, bacterial communities provide sea-ice microalgae with nutrients (P, trace metals and vitamins [14]), which support primary productivity and growth [18]. In addition to this microbial loop in nutrient turnover, the release of extracellular substances from both bacteria and sea-ice microalgae may also affect recrystallization of local ice and form refuges that increase survival of the sea-ice microalgae and bacteria [19]. This tight association between sea-ice microalgae and bacteria has been explored in recent years, but we do not yet have a clear understanding of the drivers regulating this important relationship [14], particularly at small spatial scales (i.e. areas ranging $<1000\,m^2$). Understanding the drivers of variation in diversity and structure of these communities at multiple spatial (and temporal) scales is critical to increase our predictive capacity of such communities and the functions they provide, particularly in the context of environmental change [20].

Multiple environmental and biotic factors are involved in the regulation of sea-ice microbial communities. Seasonality is one of the most important factors shaping sea-ice communities in Antarctica through direct effects driven by physical ice changes (e.g. annual ice formation cycles) that lead to effects on resource availability and allocation, and rates of productivity [10, 14]. Seasonal shifts can also change the sea-ice cover and thickness, which, in turn, can influence associated communities through changes in the amount of light penetrating the sea-ice [21], atmosphere-sea gas exchange [10] and sea-ice structure (frazil compared to pack ice). These changes alone can influence the rates of microbial growth and accumulation in sea-ice [22] and the formation of microbial habitats [14]. However, spatial variability in the physical properties of sea-ice can also contribute as a pivotal factor shaping sea-ice microbial communities. For instance, the structure, thickness and the presence of fractures in sea-ice vary at regional (distances at 1000's of kms of separation) and local scales (variability ranging from 100's to $1\,m^2$) around Antarctica [23]. Variation between locations or at smaller spatial scales can highly influence communities living within the sea-ice through microhabitat formation and resource allocation within different ice horizons [10, 24]. In addition, other site-specific variables are also involved in shaping sea-ice communities, including closeness to nutrient sources, date of ice formation, snow cover, site history or local geomorphology that determines closed or open sea-ice systems [10, 11, 25, 26]. These and other environmental factors are predicted to change at multiple scales in response to climate change [21, 27, 28].

A research expedition to Cape Denison in Commonwealth Bay, east Antarctica, was conducted in December 2013 to survey marine benthic communities at several sites. Originally this location was characterized by an extensive coastal open water area (i.e. polynya) produced by strong katabatic winds that transported newly formed sea-ice offshore [29]. This coastal polynya provided large volumes of shelf water and salinity regulation to this region (Adelie-George V land [30]). However, the physical characteristics of sea-ice in the area drastically changed due to the grounding of an external iceberg (*B09B*,~$100\,km^2$ in size) in 2010 [29, 31]. The presence of *B09B* created a year-round sea-ice cover (~3 m ice thickness) that prevented the transport of sea-ice offshore and produced local changes in ocean circulation under a constant icescape (see Fig. 1 for a summary of the scenario created by B09B grounding). For example, changes in the icescape in this area had an effect on the properties and rate of formation of bottom water and high-salinity shelf water [29]. Local changes such as these have resulted in the decline of benthic algal communities and a shift to invertebrate-dominated benthic communities (e.g. ophiuroids and polychaetes) [25]. The newly formed sea-ice in Cape Denison provided an opportunity to describe the sea-ice microbial communities in an area not previously explored [14]. We used next-generation sequencing technologies (i.e. 16S rRNA-gene amplicon sequencing) to describe bacterial communities associated with sea-ice microalgae and determine spatial variability and potential environmental drivers involved in shaping these communities. Specifically, bacterial community structure was compared between sites near the shore and sites closest to the ice edge (~70 km apart). On-offshore spatial variation has been observed in other Antarctic communities (e.g. phytoplankton, bacterioplankton and archaea) and linked to hydrographic variability (i.e. higher water mixing nearshore and salinity shifts), presence of other communities (i.e. grazers) and nutrient availability (e.g. iron) [32] and [33]. Similarly, in this study, it was expected that sea-ice microalgae closest to the coast would hold a higher diversity of bacteria as ice here had an earlier date of formation and permanence (since 2010 with the arrival of iceberg B09B) and that variation in algal-associated bacterial assemblages would increase as the spatial distance between samples increased. Site-specific variables such as ice thickness and depth were also measured to determine their contribution to the variation in algal-associated bacterial communities. Results from the present work provide novel descriptions of the sea-ice microbial communities, which are poorly studied in east Antarctica. This study also provides a case study of the effects of a large-scale event that changed local icescapes in Antarctica. Such events are likely to become more common under current global-warming scenarios [34] and [35].

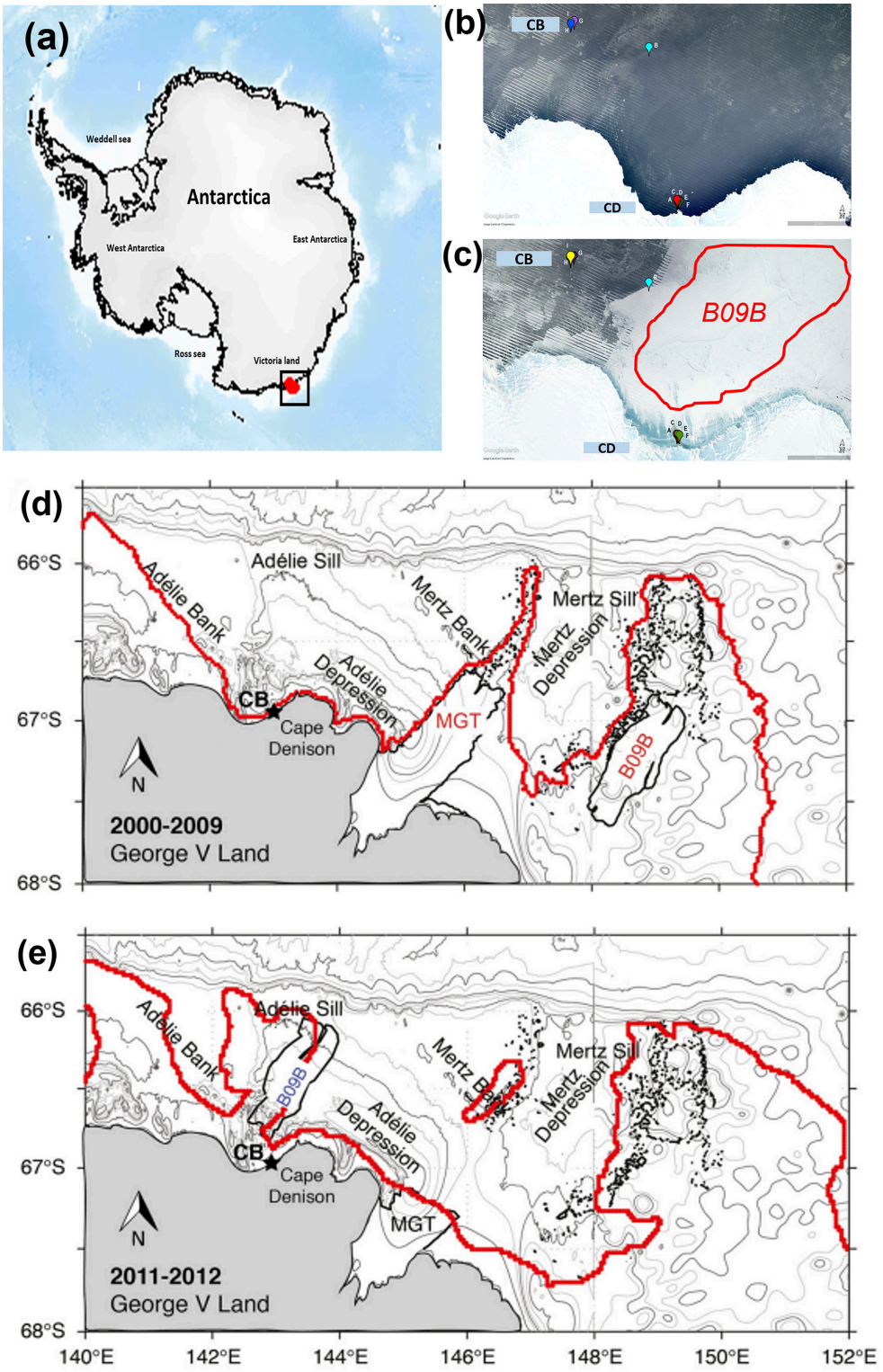

Fig. 1. Maps of the study area in east Antarctica showing the general location of the study site (a) based on maps from [66] and the extent of the sea-ice formation (red line) prior (b and d) and after (c and e) the grounding of B09B at Commonwealth Bay (CB). (b and c) show satellite imagery from CB prior to B098 (December 2010) and after grounding when the expedition for this study was conducted (December 2013) (Google earth pro v.7. 3.4.8248, acknowledged from Google, image Landsat/Copernicus). Sampled sites for this study are overlayed on the satellite imagery for both times. (d and e) are maps taken and modified from [25] and originally adapted from [30]. (d and e) also show the movement of B09B from the near Mertz Glacier Tongue area (MGT) in 2010 to the CB in 2010.

METHODS

Sampling algal-associated bacteria

Bacterial communities associated with sea-ice microalgae were sampled at two distinct areas in east Antarctica: along Cape Denison's shoreline (CD) and near the ice edge at Commonwealth Bay (CB; ~35 km to the shoreline and ~65 km of CD) on 19–20 December 2013 (Fig. 1 and refer to Fig. 1b in [25] for further detail on the site's location. Map in Fig.1a was made using he R package *ggmap* [36] based on data from the SouthernOcean maps [37]). At CD, sampling was undertaken at six sites ~200–900 m apart close to the shore (100–200 m from the shoreline). Within each site, three replicate cores ~5 m apart were obtained by drilling into the sea-ice using an ice corer (10 cm diameter), except for one site where only one core was obtained. Four replicate cores were sampled at a single CB site at ~400–900 m apart. A rope with a weight attached to the end was deployed through each drilled ice-hole to measure the depth from the surface of the ice to the seabed [25]. The length of each core was measured to estimate sea-ice thickness. Bacterial communities associated with ice-algae attached to the bottom of each core were sampled by swabbing the entire surface (~64 cm^2, avoiding a 1 cm edge) with sterile cotton swabs for 30 s [38–40]. Sampled bacterial communities in the sea-ice were considered to be associated with algae, however no direct interactions were evaluated to differentiate an actual symbiotic relationship (i.e. 'holobiont' [41], and co-occurrence (i.e. bacteria living exclusively in the sea-ice) of both biotic fractions. Swabs were placed in sterile cryo-tubes, which were stored in liquid N for ~12 h and then transferred to a −20 °C freezer in the vessel. Once back in Australia (January 2014) samples were transferred to a −80 °C freezer until they were processed.

DNA extractions, PCR and amplicon sequencing

DNA was extracted from swabs using the PowerSoil DNA extraction kit following the manufacturer's protocol (MoBio Laboratories). A polymerase chain reaction was done to amplify the hypervariable region V4 from 16S rRNA loci using targeted primers 515F (5′-GTGCCAGCMGCCGCGGTAA) and 806R (5′-GGACTACHVHHHTWTCTAAT) [42]. Amplified reads were then processed and sequenced at the Ramaciotti Centre for Genomics (UNSW, Australia) on a Miseq Illumina sequencer following the manufacturer's guidelines. All sequenced data was provided as pair-end demultiplexed FASTQ files by the sequencing centre and submitted to the NCBI Sequence Read Archive (SRA) database (BioProject accession number: PRJNA765135).

Bioinformatic pipeline

Primers from raw FASTQ sequences were removed using the programme *cutadapt* v.3.4. ([43]; ran *as a conda* environment in *Python* v.3.9) with an initial deletion of ambiguous bases (Ns). Processed sequences were then quality trimmed using the function *FilterandTrim* from the *dada2* R package [44] and adjusting the trimming process to a maximum expected error of 2 and 6 (forward and reverse sequence, respectively). Maximum truncation lengths were decided for each paired read independently and based upon inspection of quality error plots where any base pairs found with a mean Q score <20 were dropped (final truncation at 240 bp for both forward and reverse reads). From the complete sequencing plate, maximum error rates were calculated independently on the forward and reverse reads and a model based on these values was constructed to be used in the denoising step. Complementary-base error plots were inspected to ensure a good fit of the error rate models to the observed data. Using the calculated error rates and the core denoising *DADA2* algorithm [44], sample inference was performed with a two-step 'pseudo-pooling' parameter to increase the detection of rare amplicon sequence variants (ASV) and chimeric sequences. After denoising, all paired reads were merged and an ASV abundance per sample table was constructed. Chimeric sequence removal was done on this ASV table through a consensus method (*removeBimeraDenovo*, [44]; i.e. samples in a sequence table are independently checked and a consensus decision on each ASV is made before removal). An average of 86±0.9% of reads per sample was kept after chimeric sequence removal. Taxonomic and species assignment on the constructed ASV table without chimeric sequences was done using SILVA v. 138.1 [45], a prokaryotic SSU taxonomic database trained and optimized for bacteria classification and formatted for DADA2. Depth of taxonomic assignment included seven taxonomic fields: kingdom, supergroup, division, class, order, family and genus. Unidentified phyla, differing kingdom level taxa from the targeted loci (i.e. Archaea and Eukaryotes) and sequences assigned to mitochondria and chloroplasts were removed. All steps of this pipeline were done using R v.3.6 and utilizing the *dada2* package [44].

Statistical analysis

The ASV abundance table produced from the bioinformatic pipeline was normalized to account for heterogeneous library sizes (sample size factor calculation, DESeq2, package *phyloseq* [46]. ASV with no counts and singletons produced by normalization were removed. Data were square root transformed for compositional analysis to account for bias generated through large variability in abundances of different ASV within each sample [47].

Alpha diversity indices were calculated for each sample, including bacterial richness (number of ASV), diversity (Shannon–Wiener index and Simpson index) and Evenness (Pielou index) (package *vegan* [48]). Linear mixed models (LMM, R package *lme4* [49]), were used to test for differences in alpha-diversity indices between the two areas: Cape Denison (CD; near the shoreline) and Commonwealth Bay (CB; ice-edge at ~70 km from the shoreline). Site (CD: 6 sites, n=3 replicates per site except one site with one sample; CB: 1 site, n=4 replicates) was included as a random factor nested within each area to account for correlations among

samples within each site and inter-site variability when testing for differences between CD and CB. All assumptions of linearity, normality, revision of influential outliers (Cook's distance) and homoscedasticity, which is an important assumption given the overall differences in sample size and the asymmetry of the design (i.e. multiple sites in CD vs one site in CB), were checked and met, validating the LMM with unequal sample size (package *performance* [50]). Fixed factor inferences were evaluated using the F-statistic with Satterthwaite approximation method for degrees of freedom [51] and effects of the random factor were inferred through a likelihood ratio test (alpha=0.05; function *anova* and *rand*, respectively; package *lmerTest* [4]. Individual Pearson correlations were also done to explore linear relationships between the alpha diversity measures and environmental data such as ice thickness (cm) and depth to the seabed (alpha=0.05).

Bacterial community compositional differences were also assessed between both areas (i.e. CD and CB), with ice thickness and depth included as covariates in the analysis. A nested permutational analysis of variance for a two-factor hierarchical model (nested PERMANOVA, function *nested.npmanova*, package *BiodiversityR* [52]) was used to determine compositional differences based on Bray–Curtis dissimilarities between each sample pair, including site as a random, nested factor within each area (alpha=0.05). To test for the assumption of equal variance between grouping factors [53], an analysis of multivariate homogeneity of group dispersions was done for area (CD and CB) and for site (function *betadsiper*, package *vegan* [48]). A principal coordinate analysis (PCoA) ordination was used to visualize bacterial compositional differences between areas (function *cmdscale*, package *vegan* [48]). Marginal tests and a distance-based redundancy analysis were conducted using the *dbrda* and *anova.cca* functions from the *vegan* package [48] to evaluate the influence of ice thickness and depth to the seabed on bacterial community composition. All environmental variables were log-transformed and scaled to be equally assessed in the marginal tests. In addition, Mantel tests were also done to determine the influence of spatial distances between sites on bacterial community composition. Bray–Curtis dissimilarities and Haversine distances (calculated as angular distances based on latitudinal and longitudinal data from each site; function *distm*, package *geosphere* [54], were used. An additional Pearson correlation was done to assess the strength of the linear relationship between the bacterial composition and spatial distance between sites. This was done by grouping contrasts obtained from Bray–Curtis dissimilarity and Haversine distance calculations into three groups: (1) CD and (2) CB for contrasts only between sites within these areas and (3) CD_CB for contrasts only between these two areas.

A multivariate generalized linear model was fitted to the normalized ASV abundance table using a negative-binomial distribution (*mvabund* R package, [55]); workflow based and documented in https://github.com/aliceyiwang/mvabund) to identify the ASVs that contributed to the structural differences between CD and CB. Differences in the abundance of individual ASV were evaluated through analyses of deviance (alpha=0.05, *P*-value adjustment for multiple testing using a step-down resampling algorithm, 1000 bootstraps, *mvabund* [55]).

RESULTS

Bacterial communities associated with sea-ice microalgae differed between CD, the area close the shoreline, and the ice edge area in CB. Alpha diversity indices calculated from sea-ice microalgae-associated bacteria showed higher ASV richness in CD compared to CB near the ice edge (P=0.03). However, no differences in bacterial diversity (Shannon–Wiener: P=0.83, Simpson: P=0.63) or evenness (Pielou: P=0.52) were found between CD and CB (Fig. 2, Table S1). The alpha diversity indices did not vary significantly among sites in each area (Table S1, available in the online version of this article, P>0.1). Homogeneous variances between the two areas for each alpha diversity index (Levene's test, P>0.05) provided additional evidence of the robustness of the models and their validity even with an unequal sample size. In addition, bacterial communities showed clear compositional differences between both areas (nested PERMANOVA: P=0.013; Fig. 3, Table S2) despite significant compositional variation among sites (PERMDISP: P=0.001, Fig. 3, Table S2).

Bacterial communities in both CD and CB were mainly dominated by four classes including Alphaproteobacteria, Gammaproteobacteria (phylum Proteobacteria), Bacteroidia (phylum Bacteroidota) and Verrucomicrobiae (phylum Verrucomicrobiota) (Fig. 4 and Fig. S1 for complete description of bacterial taxonomic levels). Nine genera were identified to differ in abundance between CD and CB including *Paraglaciecola*, *Polaribacter*, *Octadecabacter*, *Rubritalea*, *Bernardetia*, *Profundimonas*, *Lentimonas*, *Dasania* and *Owenweeksia* (Fig. 5, ASV that represent >1% of total abundance of the whole community). For all these cases, lower total abundances were found in sites in the CB area compared to CD with more than a 90% drop in total abundance in some cases (for example decrease in *Owenweeksia*: 99.9%; *Bernardetia* 99.2%; *Rubritalea* 98.8%; *Octadecabacter* 97.9%; and *Profundimonas* 97.1%). The genera *Polaribacter* and *Paraglaciecola* showed the highest total abundance in both areas compared to other genera however, they drastically decrease in abundance in the CB area (abundance drop of 97.4 and 78.3%, respectively).

Spatial variability in bacterial community composition was found with higher dissimilarity between sites closer to the shore (CD) compared to sites closest to the ice edge (CB). Through comparing the calculated Bray–Curtis dissimilarity and geospatial Haversine angular distances, a strong linear correlation was found (r=0.73, P<0.001) where dissimilarity increased at higher spatial distances (Fig. 6). These larger compositional dissimilarities were clear between the two sampled areas (CD vs CWB) compared to less dissimilar bacterial composition within each area (within CD or within CWB).

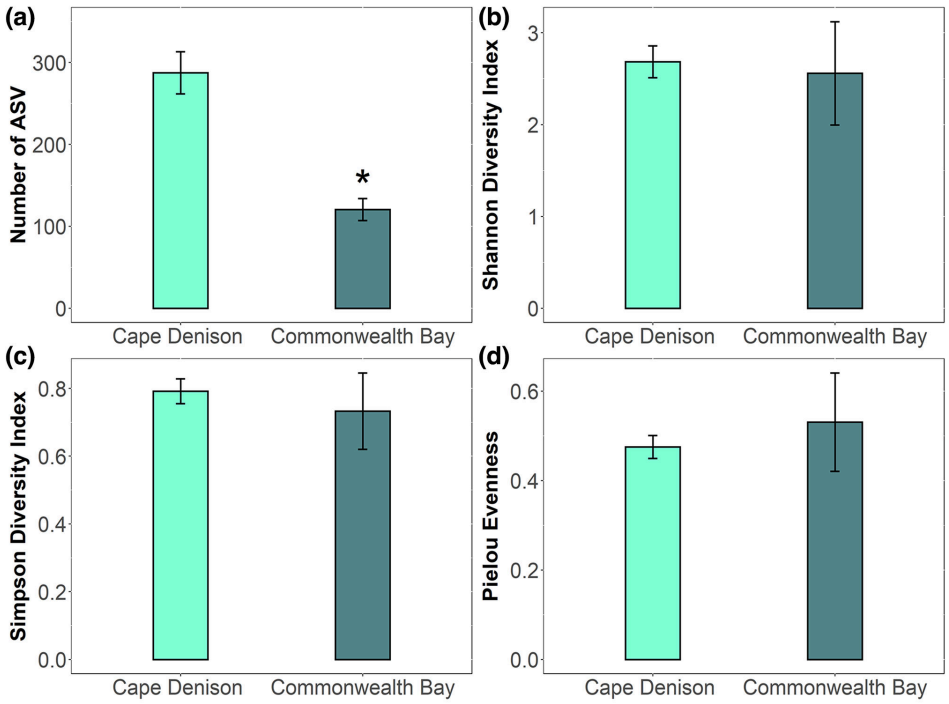

Fig. 2. Comparison of bacterial alpha diversity indices taken from sites close to shore (Cape Denison, *n*=16) and near the ice edge (Commonwealth Bay, *n*=4), including (a) richness (number of ASV), (b, c) diversity (Shannon Wiener and Simpson index) and evenness (Pielou index). Bar plots show mean±SE values for each alpha diversity index. Significant differences between areas are marked with an asterisk.

Within each sampled site in CD and CB, possible co-variates that were thought to modulate sea-ice bacterial communities were also evaluated including ice thickness and depth to the seabed. Ice thickness varied slightly among sampled sites (i.e. ranging from 170 to 230 cm), however, this variation was not found between areas (Fig. S2). These small variations in ice thickness had a low impact as a contributing factor shaping sea-ice microalgae-associated bacterial communities. There were some trends for linear relationships between ice thickness and diversity (Table S3a; Shannon Wiener: r=0.51, P=0.05 and Simpson: r=0.53, P=0.03) and evenness (Pielou: r=0.51, P=0.05). Marginal tests from the distance-based redundancy analysis did not support ice

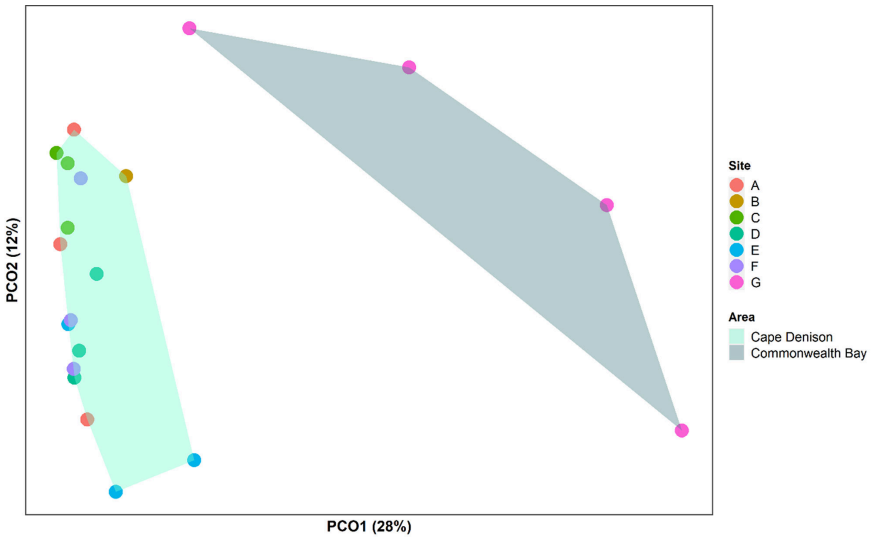

Fig. 3. Principal coordinate analysis to determine compositional differences between bacterial communities sampled in sites near the shore (CD) and close to the ice edge (CB).

Fig. 4. Relative abundance (%) of bacterial classes associated to sea-ice algae at sites close to shore (Cape Denison) and close to the ice edge (Commonwealth Bay). Only the most abundant taxa predominant at each taxonomic level are shown.

thickness as a relevant factor contributing to compositional dissimilarities (Table S3b; F=1.53, P=0.08; and Fig. S3). Depth to the seabed had a larger variability among sites (i.e. ranging between 1–14 m), however, no evidence was found of its influence on bacterial community diversity (Table S3a; P>0.4) or composition (Table S3b; P=0.06; Fig. S3). Although depth was not recorded in the CB area, it exceeded 100 m.

DISCUSSION

Sea-ice microalgae and their associated microbial communities play critical roles in the remineralization and turnover of nutrients (i.e. the 'microbial loop' [18]; in Antarctica [2] and [14]). Despite their importance in Antarctic ecosystems, we still lack a clear understanding of variation in these communities at multiple spatial scales and their potential drivers. A description of the bacterial communities associated with sea-ice microalgae was made in Commonwealth Bay, east Antarctica (Cape Denison), an area with no previous studies in sea-ice bacterial communities [14]. Bacterial communities associated with ice-algae varied strongly at spatial scales of 10's of km, with distance to the shoreline as a key potential driver, while ice thickness and distance to the seabed were not associated with the variation in the structure of these communities. Given the projected changes in sea-ice extent in response to contemporary and future environmental change [5], understanding how such changes at local scales influence sea-ice communities will be critical to predict impacts on the functioning of these systems.

Description of bacterial communities in Cape Denison, Antarctica

Sea-ice microalgae-associated bacterial communities have been described in many areas of Antarctica with a higher number of studies conducted in the Queen Maud land, Ross Sea in Victoria Land, and few in East Antarctica [14]. To our knowledge, no studies analysing sea-ice microalgae-associated bacterial communities have been previously conducted in Cape Denison at Commonwealth Bay [14] and the present work is the first to take this approach. In general, bacterial communities associated with sea-ice microalgae vary widely throughout the seasons as a response to resource availability from algal blooms [56], stress-related to shift in UV-B radiation [57] or changing salinity gradients brought by melting sea-ice [58]. The transition from late winter to early summer brings a shift in bacterial biomass and an increase in photosynthetic activity and respiration [59] compared to alternative energy sources [60]. Examples of dominant sea-ice bacteria throughout the year include mainly examples from the phyla Proteobacteria and Bacteroidota such as the genera *Psychrobacter*, *Ruthia*, *Glaciecola*, *Rhodoferax*, *Octadecabacter* and *Glaciecola* [14]. These have been seen to vary in response to algal biomass with shifts in abundance during summer [56]. In addition, clear 'zonation' within the sea-ice has been seen, with more psychrotolerant groups inhabiting the seawater-ice interface compared to more psychrophilic groups near the sea-ice centre [2]. In the current study, the sea-ice bacterial communities evaluated in the bottom of sea-ice cores in Cape Denison are overall dominated by psychrophilic heterotrophic Bacteroidota and Proteobacterial

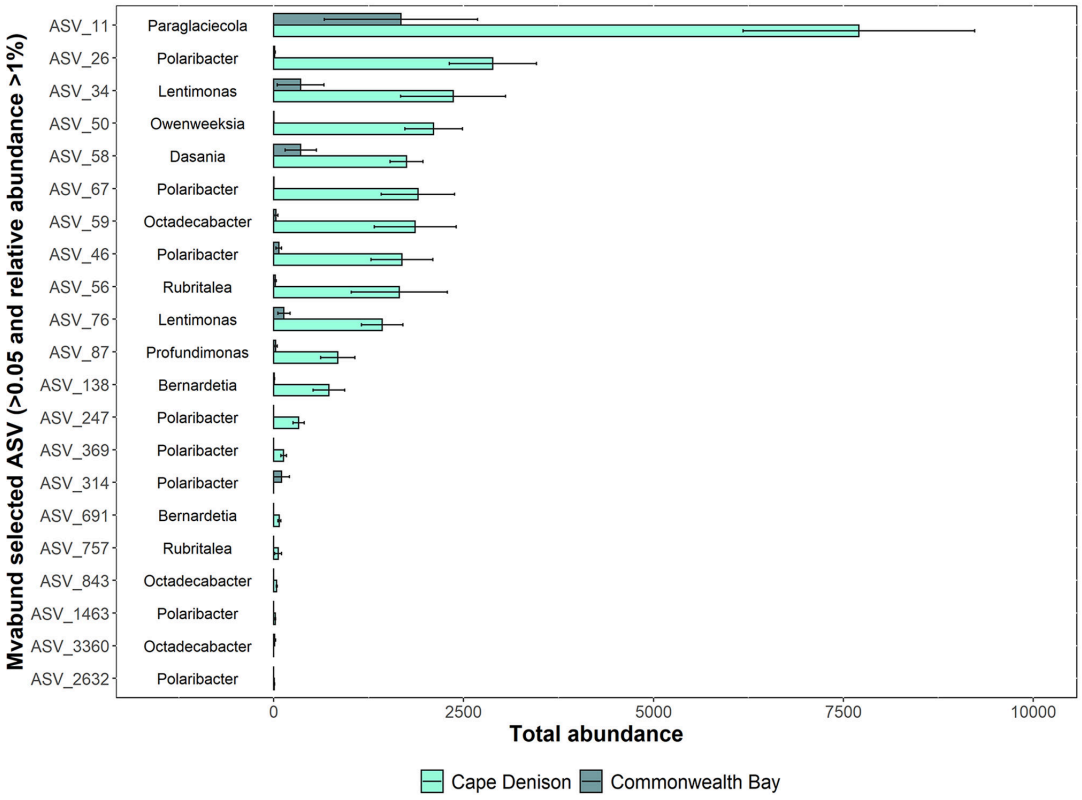

Fig. 5. Bacterial ASV identified to have significantly different abundance (total abundance as number of ASV counts; $P<0.05$) between sites near the shore (Cape Denison, $n=16$) and close to the ice edge (Commonwealth Bay, $n=4$). Labels with their corresponding genus are placed next to each ASV. Only ASVs with higher >1% relative abundance in the whole community were included. Values shown are mean±SE total abundance.

groups including the genera *Polaribacter* and *Paraglaciecola*. The dominance of these genera in the area is in concordance to other studies elsewhere in Antarctica, as these groups are known to have a strong association with sea-ice microalgae [61]. Other less abundant groups found include the genera *Lentimonas*, *Octadecabacter*, *Owenweeksia* and *Dasania*. Most of these groups have been seen in other regions of Antarctica or the Arctic and appear to be common heterotrophic bacteria in sea-ice and associated with algal communities [14, 56] and [62]. The overall presence of these groups in Cape Denison and other regions of Antarctica matches the general pattern of bacterial community composition of austral summer, however local spatial variation within Cape Denison brought by the area's history (i.e. the arrival of B09B iceberg), new ice formation in a previously free-ice area and spatial variability are given by the closeness to the shore, provide further evidence of the complexity of these communities in the sea-ice.

Small-scale spatial variability on sea-ice bacterial communities

A regional variation on sea-ice physical properties that may influence algae-associated bacterial communities has been described around Antarctica [10, 14, 23, 24]. Spatial variability in these communities at large scales has been attributed to multiple factors including changes in ice thickness, age of ice formation, type of ice (e.g. platlet or frazil ice), pre-existing conditions of the water column before winter freezing events (e.g. nutrient availability and dissolved organic matter content), snow cover (i.e. limiting light penetration to the sea-ice) and geomorphological characteristics [10, 11, 23–25]. However, similar effects of these factors on the bacterial communities associated with sea-ice microalgae have rarely been explored at local scales (some examples include [11, 26]). Results in the present study highlight the strong influence that local spatial variation and some physical attributes of the sea-ice at a smaller scale can have on shaping bacterial communities. Bacterial community abundance and composition change drastically from sites near the shoreline in CD compared to sites sampled closer to the ice edge in CB. A clear decrease in richness between both areas (58% decrease), compositional differentiation of the two communities (Figs. 3 and 6) and decrease of abundant taxa are clear evidence that spatial distance is a driver influencing these sea-ice communities and possibly closeness to shore may be an important factor involved. Nonetheless, it needs to be noted that even though these effects are clear, the diversity and evenness of these bacterial communities are unaffected by spatial distance and high variability in bacterial composition within the areas exist. Further, the constant dominance of psychrophilic bacteria such as the genera *Paraglaciecola* and *Polarbaicter* in both areas confirm that distance to the shoreline does not influence the presence of these groups, just their overall relative abundances.

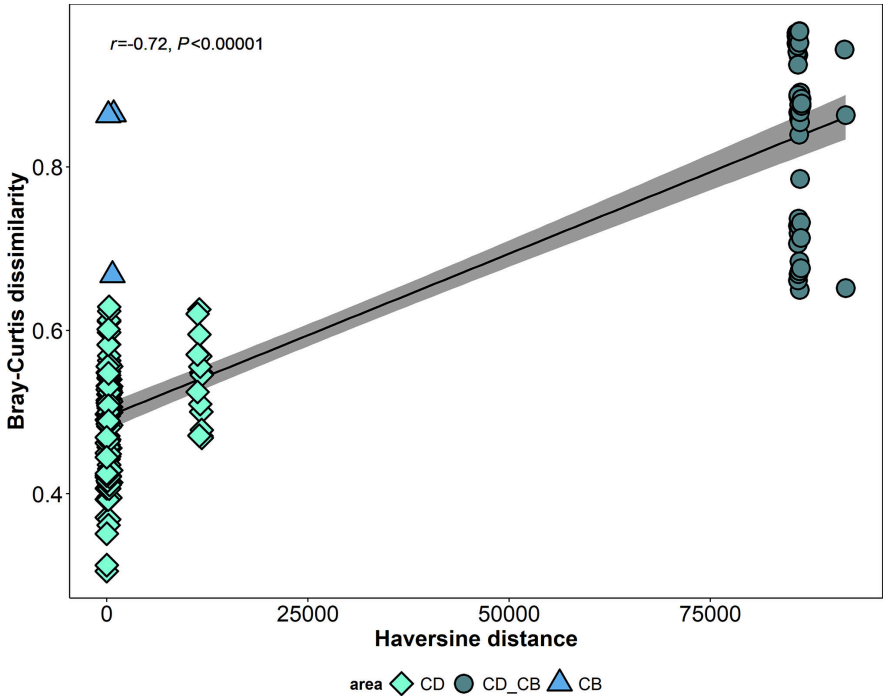

Fig. 6. Linear relationship of bacterial composition dissimilarity as distance increases between sites. Groups indicate contrasts between sites compared only within CD (closer to shore), sites compared only within CWB area (close to ice edge) and sites compared between both areas (CD_CWB). Compositional dissimilarities were calculated with Bray–Curtis distances and geographical distances were calculated as Haversine distances (angular distance between two sites) using latitudinal and longitudinal data.

Local effects of physical and environmental factors have been identified to be key regulators of algal biomass and patchiness [10, 58] and can subsequently affect associated bacterial communities. Specifically, such factors may change nutrient availability and quality sourcing from the host algae, and other algal resources such as light [58, 63]. At a local scale, these effects have been seen with distance to the shoreline as an important variable that delimits algal growth, presence of specific algal groups and the possible interactions between algae and bacteria (i.e. 'microbial loop'). Fiala *et al.* [11] obtained evidence of these local effects in the region of Dumont d'Urville station (Adélie Land, Antarctica), where in a transect from land to offshore, a decrease in algal biomass was registered. Another example was seen in Riaux-Gobin (2000, Adelie Land) [26] with a similar transect and showing how average chlorophyll-*a* content and other algae-bacterial compounds (nitric acid and silicic acid) decrease from nearshore to offshore sites. In both cases, changes in algal coverage would undoubtedly impact bacterial community development and provide evidence of high variability at short distances from the shoreline. For the present work, a possible decrement of algal coverage in sample cores far from the shoreline (CB) may explain the drastic drop in bacterial communities and some of the taxa present, but visual estimates suggested this is an unlikely explanation for the observed patterns. The underlying environmental factors explaining this pattern are not directly linked to ice physical properties such as thickness (as seen as drivers in other studies such as [64]) but instead to other properties such as the date of ice formation. Larger surface area for algal growth given by platelet ice compared to newly formed ice has been seen near floating ice shelves and land-fast ice [10, 11] and could explain these local differences. It is possible that the type of ice in CD and CB differ and is determined by the age of its formation and the advent of iceberg B09B (e.g. which brought year-round sea-ice cover nearshore [25]). This would provide CD with a better surface area for algal growth, better nutrient exchange, access to resources such as light, and the formation of new microbial microhabitats that would affect bacterial abundance. Additionally, specific algal taxa may also be present near the shore and provide different nutrients and resource quality to bacterial communities not present in the ice far from shore (for example, see [13] where *Fragilariopsis cylindrus* and *Fragilariopsis curta* were found exclusively in the nearshore). Further and more detailed studies of ice properties are needed to elucidate what factors determine variability at small spatial scales in bacterial communities but also how this can impact ecosystem functionality at local and regional scales.

This study highlights the importance of site-specific variability at small scales in bacterial communities associated with sea-ice microalgae in an area not previously explored in Antarctica. In addition, common physical factors of the ice that have been previously identified as drivers of these communities at larger scales (i.e. ice thickness or distance to the seabed), were found to have minimal influence at local scales. A clear limitation of this study was that we were unable to sample a complete distance gradient from the shore to the ice edge, or multiple sites at the ice edge or closer to shore that have either not been affected by

changes in ice cover (control sites) and/or that experienced such changes naturally (reference sites; see e.g. [65]), due to logistical constraints of the expedition. This prevented a thorough sampling and the construction of a balanced design for the analysis that would unambiguously decouple impacts of B09B on these microbial communities from natural spatio-temporal variation. However, new knowledge of the area and strong spatial effects on the bacterial communities generated from this work provide novel evidence of the localised response of Antarctic sea-ice communities to change at a higher resolution. Impacts of climate change and other environmental stressors in Antarctica are likely to vary at a range of spatial (and temporal) scales and studies at other Antarctic regions are necessary to examine the generality of these findings and potential implications. Further research is needed to understand the role of environmental and biotic factors in shaping local sea-ice communities and how this translates into effects on ecosystem function.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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