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ORIGINAL ARTICLE

An active bacterial community linked to high chl-*a* concentrations in Antarctic winter-pack ice and evidence for the development of an anaerobic sea-ice bacterial community

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Antarctic sea-ice bacterial community composition and dynamics in various developmental stages were investigated during the austral winter in 2013. Thick snow cover likely insulated the ice, leading to high (<4 µg l^{-1}) chlorophyll-*a* (chl-*a*) concentrations and consequent bacterial production. Typical sea-ice bacterial genera, for example, *Octadecabacter*, *Polaribacter* and *Glaciecola*, often abundant in spring and summer during the sea-ice algal bloom, predominated in the communities. The variability in bacterial community composition in the different ice types was mainly explained by the chl-*a* concentrations, suggesting that as in spring and summer sea ice, the sea-ice bacteria and algae may also be coupled during the Antarctic winter. Coupling between the bacterial community and sea-ice algae was further supported by significant correlations between bacterial abundance and production with chl-*a*. In addition, sulphate-reducing bacteria (for example, *Desulforhopalus*) together with odour of H_2S were observed in thick, apparently anoxic ice, suggesting that the development of the anaerobic bacterial community may occur in sea ice under suitable conditions. In all, the results show that bacterial community in Antarctic sea ice can stay active throughout the winter period and thus possible future warming of sea ice and consequent increase in bacterial production may lead to changes in bacteria-mediated processes in the Antarctic sea-ice zone.
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Introduction

Antarctic sea ice is an important component of the Southern Ocean ecosystem from both the geophysical and biogeochemical standpoints. It covers vast areas from 3×10^6 km^2 during the sea-ice minimum in February up to 18.3×10^6 km^2 in the September maximum (Comiso, 2010). In contrast to the diminishing sea ice extent in the Arctic (Perovich *et al.*, 2014), Antarctic sea ice is globally expanding (IPCC, 2013). However, this encompasses a large spatial heterogeneity meaning that there are indeed areas (for example, the Antarctic Peninsula) in which the surface ice temperatures are increasing and ice extent decreasing (Stammerjohn *et al.*, 2008; Comiso, 2010). Also, the inter-annual variation in sea ice is high: for example, in 2017 during February minimum sea ice extent was 2.35×10^6 km^2, which was 24.4%, below the 1981–2010 average (NOAA, 2017).

In the Southern Ocean, dynamic sea-ice growth through the pancake ice (PCI) cycle and deformed growth (for example, rafting) is common (Lange *et al.*, 1989; Haas, 2010; Petrich and Eicken, 2010). In addition, the thick snow cover and dynamic growth cause flooding, which introduces additional nutrients from seawater to the surface and internal ice horizons (Lange *et al.*, 1990; Eicken *et al.*, 1994; Fritsen *et al.*, 1994). These physical events induce a more distributed algal biomass than in the Arctic,

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that is, with chlorophyll-*a* (chl-*a*) peaks in the upper and middle horizons of the sea-ice cover, in addition to the more prevalent bottom-ice maxima (Meiners *et al.*, 2012; Arrigo, 2014). The highest chl-*a* concentrations in Antarctic sea ice occur during spring and summer. However, unlike in the Arctic, peaks in chl-*a* are also observed in autumn (Fritsen *et al.*, 1994; Delille *et al.*, 2002; Meiners *et al.*, 2012).

Sea-ice algae, together with sea-ice bacteria are the most productive and abundant organisms in ice. As in the water column, bacteria in sea ice drive an active microbial loop, in which dissolved organic matter is recycled back to the upper trophic levels as bacterial biomass (Kottmeier *et al.*, 1987; Smith and Clement, 1990; Laurion *et al.*, 1995; Kaartokallio, 2004; Cowie *et al.*, 2014). In addition, bacteria decompose particulate organic matter and remineralize nutrients (Sullivan and Palmisano, 1984; Deming, 2010). Sea-ice bacteria and their community structure are governed by abiotic and biotic factors such as temperature, salinity, light, substrate and nutrient availability, as well as grazing and viral lysis (Kaartokallio, 2004; Mock and Thomas, 2005; Kuosa and Kaartokallio, 2006; Riedel *et al.*, 2007; Collins *et al.*, 2008, 2010; Piiparinen and Kuosa, 2011; Collins, 2015). As the fluctuation of these factors is highly seasonal, they also induce the seasonal dynamics of bacterial abundance and community composition in sea ice.

Most sea-ice bacterial community studies have been conducted during spring and summer, when the sea-ice algal mass growth provides ample autochthonous substrate for bacteria. During the spring and summer seasons, copiotrophic (that is, bacteria adapted to growing best in nutrient-rich environments) *Gammaproteobacteria* (for example, genera *Glaciecola* and *Colwellia*) dominate the bacterial communities, together with copiotrophic *Flavobacteriia* (for example, genera *Polaribacter* and *Flavobacterium*) and *Alphaproteobacteria* (for example, genus *Octadecabacter*) both in first-year ice (FYI) and multiyear ice (Bowman *et al.*, 1997, 2012; Brown and Bowman, 2001; Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003; Kaartokallio *et al.*, 2008; Deming, 2010; Hatam *et al.*, 2014, 2016; Eronen-Rasimus *et al.*, 2015).

In contrast to spring and summer, bacterial communities during winter are poorly known. Members of the oligotrophic *Alphaproteobacteria* of the SAR11 clade dominate the sea-ice bacterial communities in the upper ice column during the Arctic winter and remain nearly unchanged until the algal mass accumulation in spring (Collins *et al.*, 2010). In newly formed Antarctic sea ice, bacterial activity is temporarily suppressed and later restored after consolidation of the sea ice (Grossmann and Dieckmann, 1994; Helmke and Weyland, 1995). With the consolidation of ice, psychrophilic (that is, bacteria that grow best at temperatures close to freezing point) predominante over psychrotolerant (that is, bacteria that tolerate cold temperatures) bacteria, which indicates change in community structure during winter (Helmke and Weyland, 1995). However, the Antarctic wintertime

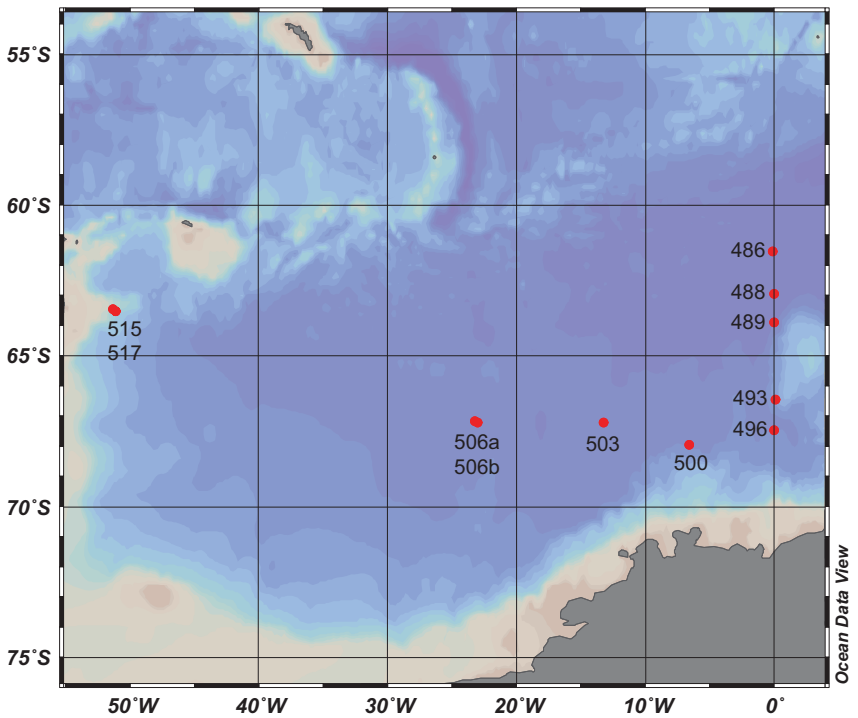

Figure 1 Map of the study area in the Weddell Sea, showing the sampling stations during the Antarctic Winter Ecosystem Climate Study (AWECS, leg ANT-XXIX/6) expedition. The map was generated with Ocean Data View (https://odv.awi.de; Schlitzer, 2016).

bacterial community structure has not been described to date, because the available information is limited to isolated bacterial strains (Brinkmeyer *et al*., 2003).

In this study, we describe the bacterial community dynamics in the different developmental stages of winter-pack ice in Antarctica. Understanding the community dynamics allows us to assess the biogeochemical role played by bacteria during periods of ice cover and provide insights on the potential consequences of climate change on the biogeochemical cycles and food webs of ice-covered seas.

Materials and methods

Study site, sampling and environmental variables

The samples were collected from 10 pack-ice stations along the Weddell and Lazarev Seas during the Antarctic Winter Ecosystem Climate Study (AWECS, leg ANT-XXIX/6)-expedition aboard the R/V Polarstern during the austral winter in June–July 2013 (Figure 1, Supplementary Table 1). The samples included different sea ice developmental stages: PCI, FYI and older fast ice/second-year ice (hereafter called SYI, Tison *et al*., 2014). Based on the preliminary interpretation of physical properties (Tison *et al*., 2014), in the Eastern Weddell Sea the ice was mostly frazil, deformed FYI with indication of rafting and signs of flooding (for example, station 500). At stations 515 and 517 close to the Antarctic Peninsula, the ice was predominantly columnar; however, 515 was SYI whereas 517 was FYI (Tison *et al*., 2014). All FYI and SYI stations were covered with a thick snow cover (mean 22.1 cm, range 14.5–35.6 cm), Tison *et al*., 2014) with generally increasing snow cover along the developmental stages of the ice (PCI < FYI < SYI), as previously described (Supplementary Table 1, Haas, 2010). At station 515, three sibling ice cores were collected two of which were SYI and one was 3 m long, from which only the middle horizons were sampled. The middle section had strong odour of H_2S, suggesting anoxic conditions (hereafter called ANOX).

The ice cores were drilled with a trace-metal-clean (electropolished steel) ice auger (Lannuzel *et al*., 2006, 2007), 14 cm in diameter. Two ice cores were collected and pooled at each FYI station for the microbiological analyses. We emphasised careful sampling and subsequent sample processing to avoid contamination. The ice cores collected were cut with an ethanol-wiped handsaw into two to seven pieces, depending on the ice thickness (each horizon 10–30 cm), crushed and placed in sterile plastic containers at 4 °C over night in darkness after which the rest of the ice was quickly melted in a water bath with constant stirring. The melted samples were immediately filtered after becoming fully melted. For the ANOX, only ice sections from 130 to 160 cm were sampled. Before melting, part of the ice core was taken for the bacterial production measurements. For the bacterial diversity analyses, direct melting (Helmke and Weyland, 1995; Kaartokallio, 2004) was used to avoid external DNA or nutrient contamination of the samples.

Bacterial abundance (flow cytometer)

The samples for bacterial abundance were fixed with paraformaldehyde (final concentration 1%). The cells were stained with SYBR Green I (Sigma-Aldrich Inc, St Louis, MO, USA) at a final dilution of 1:10 000 for 15 min in the dark and analysed with a CyFlow Cube8 flow cytometer (Partek GmbH, Münster, Germany) using a 488-nm laser (Gasol and Del Giorgio, 2000) within 30 min of staining.

Bacterial production

Bacterial production was measured as the incorporation of ^{3}H-labelled thymidine (Fuhrman and Azam, 1982) immediately after sampling, following the protocol described by Kaartokallio (2004). The ice sections were crushed, and from each section two aliquots and a formaldehyde-killed absorption blank (final conc. 1.85%) of approximately 7.5 g of crushed ice were taken. The samples were incubated in the dark at approximately –0.8 °C for 18 h with 2 ml of 0.2-µm-filtered and autoclaved seawater (salinity 33.9‰, measured on a unitless practical salinity scale; however, as it is essentially equal to ‰, it is used here; UNESCO, 1981), amended with [methyl-^{3}H]-thymidine (specific activity 20 Ci mmol^{-1}; PerkinElmer Inc, Waltham, MA, USA) at a final concentration of 20 nM. The incubations were stopped by adding formaldehyde (final conc. of 1.85%). The unincorporated ^{3}H-thymidine was removed with the standard cold-trichloroacetic acid extraction method (Fuhrman and Azam, 1980,1982). The samples were filtered onto 0.2-µm mixed cellulose ester filters (Advantec Mfs Inc, Dublin, CA, USA), the filters dissolved in Insta-Gel Plus scintillation cocktail (PerkinElmer) and the radioactivity measured with a Tri-Carb 2900TR scintillation counter (PerkinElmer) aboard the R/V Polarstern.

DNA extraction

For the DNA extractions, approximately 500 ml of the melted sea-ice were filtered onto sterile 0.22-µm membrane filters (Ø 47 mm; Whatman GE Healthcare, Little Chalfont, Kent, UK) and frozen in liquid nitrogen and later transferred to –80 °C.

The bacterial community DNA was extracted from the filters with a PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc, Carlsbad, CA, USA), as described by Eronen-Rasimus *et al.* (2014), 6 months after the cruise. In addition to the samples, negative controls without the sample were extracted.

Bacterial community analysis

For MiSeq sequencing, the 16S ribosomal RNA gene region from V1 to part of the V3 was amplified with a polymerase chain reaction, using the universal bacterial primers F8 (Chung *et al.*, 2004) and R492 (Edwards *et al.*, 1989). A two-step polymerase chain reaction and Illumina MiSeq (Illumina Inc, San Diego, CA, USA) paired-end multiplex sequencing were performed at the Institute of Biotechnology, University of Helsinki, Finland.

Bioinformatics for the MiSeq data

In all, approximately 6.2 million raw reads covering the V1–V3 region of the 16S ribosomal RNA gene were obtained with the Illumina MiSeq platform. Primer removal was done with Cutadapt (v. 1.7.1; Martin, 2011). The paired-end reads were merged with Paired-End reAd mergeR (Pear, v. 0.9.6; Zhang *et al.*, 2014). Quality filtering (> 400 bp, maximum expected error 2), chimera checking (UCHIME; Edgar *et al.*, 2011) and operational taxonomic unit clustering (Edgar, 2013) were done according to the UPARSE pipeline (Edgar, 2013). After quality filtering, a total of 1 298 455 sequences remained for further analyses. Taxonomic classification of the operational taxonomic units was done with Silva (v. 119, 60% confidence threshold; Quast *et al.*, 2013) in Mothur (v. 1.36.1; Schloss *et al.*, 2009). Before the statistical analyses, chloroplasts, mitochondria and singletons were removed based on the phylogenetic classification with Silva, and the libraries were normalised with metagenomeSeq (Paulson *et al.*, 2013) in R (v. 3.2.4) (R Development Core Team, 2011). In all, 1273 operational taxonomic units including 717 232 sequences were obtained. Raw reads were deposited into the Sequence Read Archive of National Center for Biotechnology Information under study accession number SRP094398.

Statistical analysis

All multivariate analyses were performed on the Bray–Curtis dissimilarity matrix derived from square-root-transformed, normalised values. Square-root transformation was applied to reduce the contribution of dominant species, because the Bray–Curtis resemblance measure does not scale individual sequences by the total values throughout all samples, and our samples showed large differences in abundance.

The bacterial community dynamics among all samples ($n = 50$) was visualised with non-metric multidimensional scaling (50 iterations). To determine whether the sea-ice bacterial communities differed significantly between different ice types (fixed factor; PCI = 7, FYI: $n = 29$, SYI: $n = 9$, ANOX: $n = 5$), a permutational multivariate analyses of variance (PERMANOVA) with pairwise comparisons (Anderson, 2001; McArdle and Anderson, 2001) was performed. As the data were unbalanced, all the tests were performed using type III sums of squares. A total of 9999 permutations, using unrestricted permutation of raw data (Manly, 1997) were performed, which is recommended for one-way designs (Anderson *et al.*, 2008). The homogeneity of dispersion was tested with permutational multivariate analysis of dispersion (Anderson, 2006), using the distance to the centroids. Homogeneity of dispersion (that is, homogeneity of variance) is an assumption in PERMANOVA and thus needed to discriminate whether the location, dispersion or both explains the variation in the bacterial communities.

To determine the association between the chl-*a* and bacterial community composition, canonical analysis of principal coordinates (canonical correlation analysis; Anderson and Willis, 2003) based on the Bray–Curtis dissimilarity matrix derived from square-root-transformed, normalised values of the bacterial 16S ribosomal RNA gene sequences was performed (test statistics with 9999 permutations).

The correlation between chl-*a* and bacterial production (measured as thymidine incorporation) and abundance were calculated with two-way Spearman's rank-sum rho with a base package of R software (v. 3.2.4; R Development Core Team, 2011).

For the multivariate analyses, Plymouth Routines In Multivariate Ecological Research (PRIMER) v. 6 software (Clarke and Gorley, 2006) with the add-on package permutational ANOVA/MANOVA+ (PERMANOVA+) (Anderson *et al.*, 2008) were used.

Results and discussion

The bacterial communities in the different developmental stages of sea ice, that is, PCI, FYI, SYI and ANOX, were significantly different. Bacterial community dynamics was linked with the age of the ice and increasing chl-*a* concentrations, suggesting that as in spring and summer sea ice, the sea-ice bacteria and algae may be also coupled during the Antarctic winter. In addition, predominance of sulphate-reducing bacteria together with the odour of H_2S suggests that under suitable conditions reduction of sulphur compounds may occur in sea ice.

Based on the preliminary interpretation of physical properties (Tison *et al.*, 2014), all FYI and SYI stations were covered with a thick snow layer (mean 22.1 cm, range 14.5–35.6 cm), efficiently insulating the ice from cold air leading to warmer ice (Tison *et al.*, 2014). The ice was, in most cases, permeable (relative brine volume above 5%, calculated as a function of temperature and salinity—equation 2.6 of Petrich and Eicken (2010); Golden *et al.* (1998); Tison *et al.* (2014)). Ice permeability allows brine transport and therefore potentially favourable conditions for the sea-ice bacteria and algae.

The maximum chl-*a* concentrations, that is, a proxy for algal biomass, globally increased along

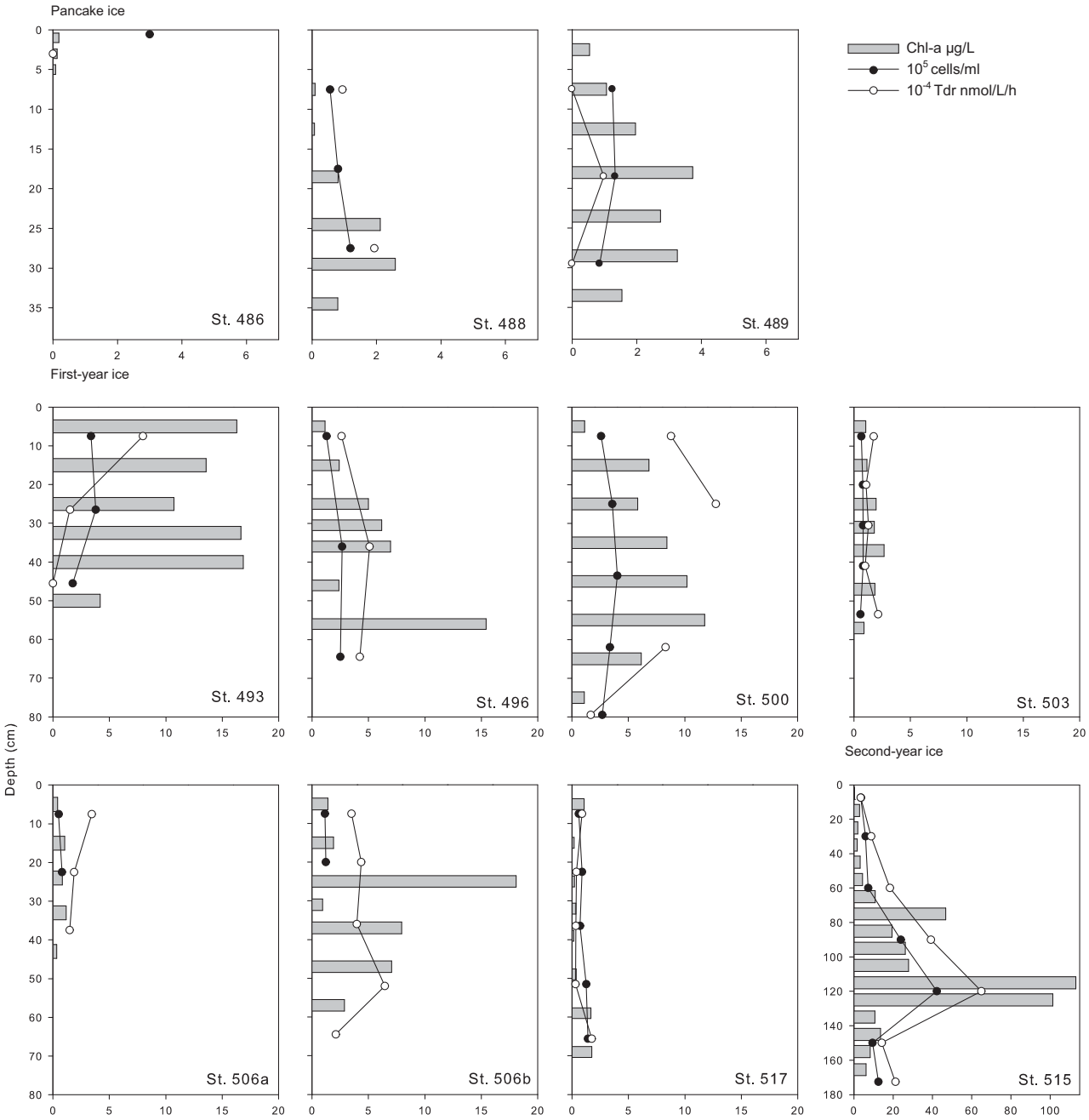

Figure 2 Bacterial production (measured as thymidine incorporation), bacterial abundance and chl-*a* concentrations in the sea-ice samples, Weddell Sea, Antarctica. Chl-*a* results are redrawn from Tison *et al.* (2014). Note the different scales.

with the age of ice from young PCI to thick SYI (Figure 2). The variation in chl-*a* concentrations in FYI was wide, ranging from stations with low ($<4\,\mu g\,l^{-1}$) chl-*a* concentrations to stations (493, 496, 498, 500 and 506b) that showed chl-*a* concentrations up to $18.1\,\mu g\,l^{-1}$ (Tison *et al.*, 2014). The highest chl-*a* ($113.15\,\mu g\,l^{-1}$) concentrations were observed in the middle of the SYI (110–120 cm), suggesting that it originated from the sea-ice-algal bottom 'bloom' from the previous summer. Unlike the typical bottom-ice-dominated chl-*a* peaks in the Weddell Sea (Meiners *et al.*, 2012), the chl-*a* peaks in our study were vertically distributed along the ice cores. This was the likely result of flooding, rafting and the predominance of frazil ice (preliminary ice-type observations from Tison *et al.*, 2014), which enhance scavenging of algae (Garrison *et al.*, 1983; Arrigo *et al.*, 2010). High chl-*a* concentrations in FYI were arguably preserved from the autumnal growth of sea-ice algae as the day length was very short and solar angle and light levels low. Low incident irradiation combined with thick snow cover likely prevented any noticeable photosynthetic activity. In addition, high cell-specific chl-*a* content because of

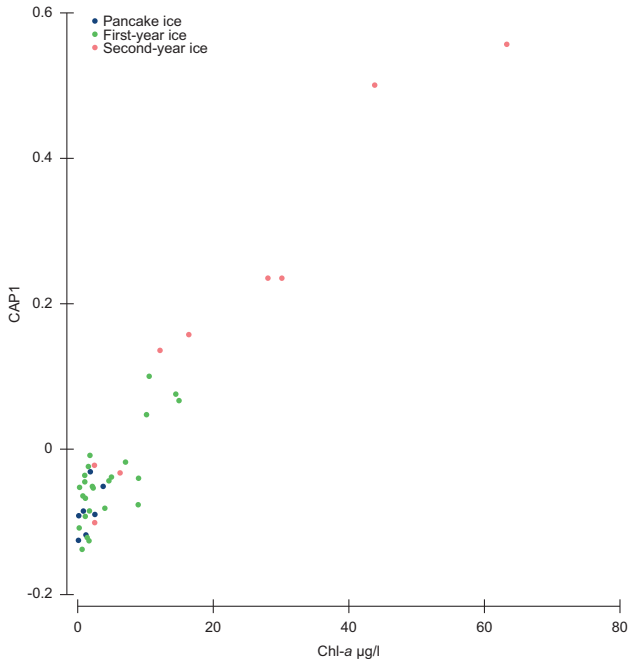

Figure 3 Canonical analysis of principal coordinates (CAP canonical correlation analysis; Anderson and Willis, 2003) based on the square-root transformed Bray–Curtis dissimilarity matrix of the bacterial 16S ribosomal RNA (rRNA) gene sequences. In total, CAP analysis explained 95% of the variation in the bacterial communities (choice of m = 19, $P = 0.0001$), with very high canonical correlation between the bacteria community composition and chl-*a* concentration (0.92, $P = 0.0001$).

the low-light adaptation may have contributed to the high chl-*a* concentrations. However, at the early-winter PCI stations with thin or no snow cover in the Eastern Weddell Sea, the growth of sea-ice algae could have been possible, as reported also earlier (Melnikov *et al*., 1998).

Strong association between the bacterial community composition and chl-*a* was observed in canonical correlation analysis (canonical analysis of principal: 0.92, $P = 0.0001$; Anderson and Willis, 2003), indicating that the development of the bacterial community was associated with the availability of the algal-derived substrate (Figure 3). In addition, both the bacterial production and abundance correlated with the chl-*a* concentrations (Spearman's ρ: 0.63, P: 2.291×10^{-6}, ρ: 0.83, P: 1.51×10^{-11}), further supporting the coupling between the algal and bacterial communities. The coupling of the sea-ice bacterial community composition and chl-*a* has been also previously reported in early spring and summer sea ice (Cowie *et al*., 2014; Eronen-Rasimus *et al*., 2016). However, a previous mid-winter study showed no correlation between chl-*a* and bacterial biomass likely because of the low chl-*a* concentrations in the ice (Stewart and Fritsen, 2004). Our results suggest that warmer winter ice temperatures and associated high algal biomass in the ice may also sustain bacterial activity during the winter months, which may affect the bacteria-mediated biogeochemical processes in the ice. Mid-winter algal peaks and associated bacterial activity

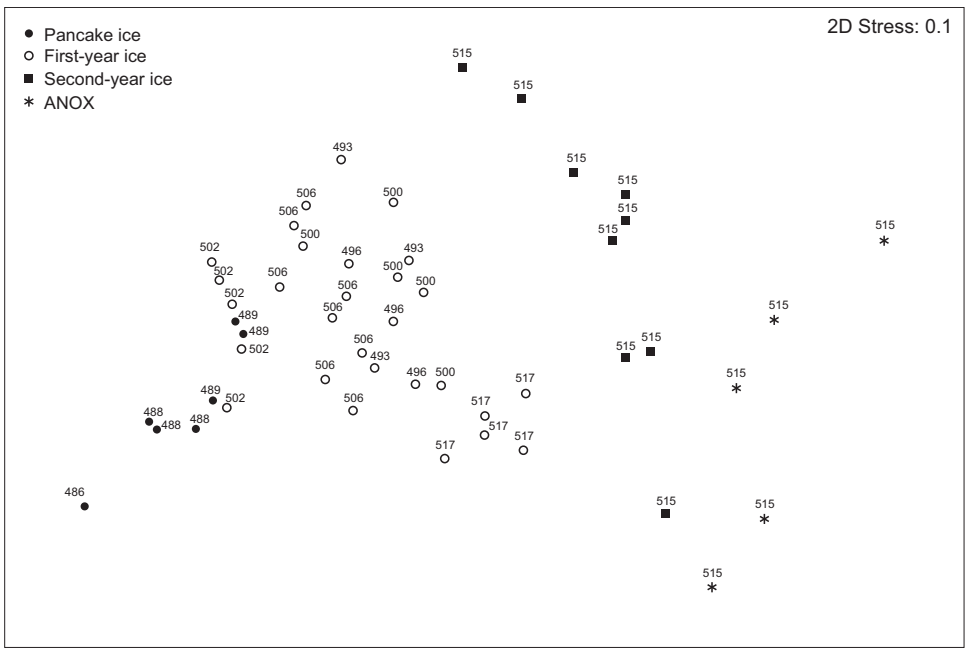

Figure 4 Non-metric multidimensional scaling (NMDS) plot showing changes in the sea-ice bacterial communities, based on 16S ribosomal RNA (rRNA) gene sequences, in the different developmental stages of the ice. The two-dimensional (2D) stress indicates how well the rank order relationships are described in two-dimensional space (0.1 = good ordination with no real prospect of a misleading interpretation; Clarke and Warwick, 2001).

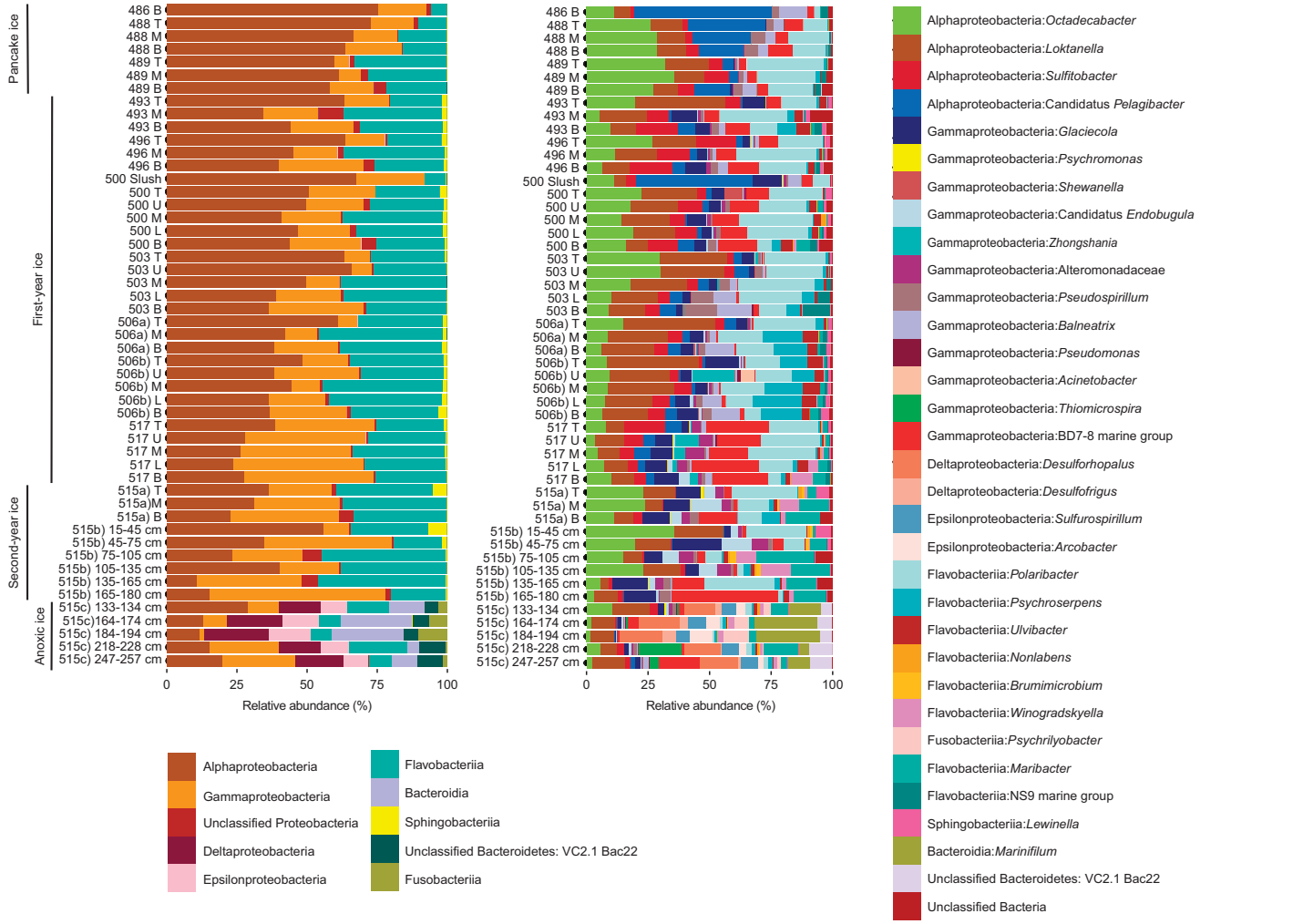

Figure 5 Class (on the left) and genus (on the right) level bacterial diversity of 16S ribosomal RNA (rRNA) gene sequences (~450 bp) representing >0.1% of all normalised operational taxonomic units (OTUs) derived from the Weddell Sea ice. T = top ice, U = upper intermediate ice, M = middle ice, L = lower intermediate ice and B = bottom ice. Plot created with ggplot2 (v. 2.1.0.; Wickham, 2009).

have also been described in warm and nutrient-rich Baltic Sea ice (Kaartokallio, 2004).

The bacterial communities differed significantly among the ice types (PERMANOVA: pseudo-F 12.124, $P = 0.0001$; pairwise tests: PCI vs FYI, PCI vs ANOX, FYI vs ANOX, FYI vs MYI and MYI vs ANOX P: 0.0001, PCI vs SYI 0.0002, Figure 4). As the differences in dispersion (that is, assumption of the homogeneity of variance is violated, permutational multivariate analysis of dispersion; $P < 0.05$) were detected in comparison with the PCI and other ice types, the differences were explained by both location (that is, ice type) and dispersion (that is, differences between group variances). The dispersion effect was visualised in the non-metric multidimensional scaling (Figure 4), because the PCI station formed a tighter group than did the other ice types. However, despite the dispersion effect, the development of the communities along with the age of the ice was evident according to non-metric multidimensional scaling (Figure 4), supporting the view that there were real differences in bacterial communities between the ice types.

Alphaproteobacteria, *Gammaproteobacteria* and *Flavobacteriia* dominated the bacterial communities in all ice types (PCI, FYI, SYI, ANOX; Figure 5). *Alphaproteobacteria* decreased along with the age of the ice (PCI: 48.8%; FYI: 40.0%; SYI: 31.2%) whereas *Gammaproteobacteria* and *Flavobacteriia* increased (PCI: 12.7 and 19.8%; FYI: 19.7 and 30.5%; SYI: 23.2 and 32.9%). The predominant bacterial groups in FYI and SYI were mostly common sea-ice bacteria such as *Rhodobacteraceae* (for example, genera *Octadecabacter* and *Loktanella*), *Alteromonadaceae* (for example, genera *Glaciecola* and Candidatus *Endobugula)* and the BD7-8 marine group (belonging to *Gammaproteobacteria*), as well as *Flavobacteriaceae* (for example, genus *Polaribacter*; Figure 5). However, the prevalence of the most abundant genera in PCI, FYI and SYI varied among the ice types, for example, showing a shift from predominance of common open-water SAR11

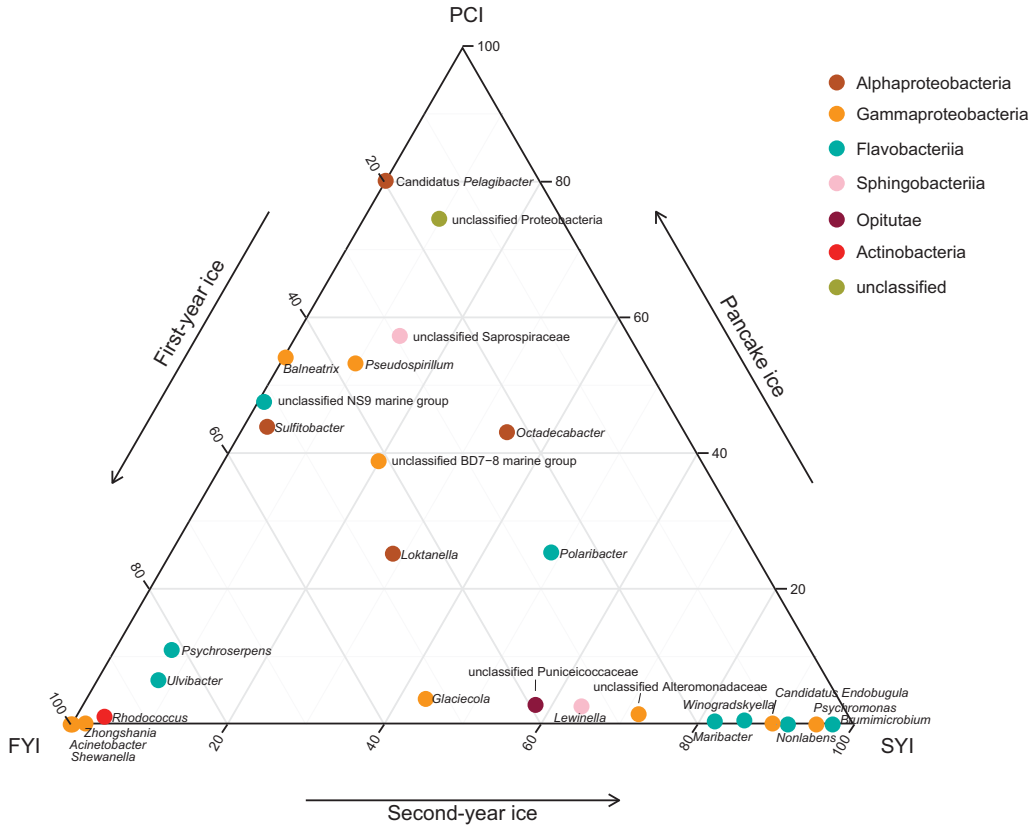

Figure 6 Ternary diagram (ggtern, v2.1.1; Hamilton, 2016) of 16S ribosomal RNA (rRNA) gene sequences (~450 bp) representing >0.1% of all normalised operational taxonomic units (OTUs) in each ice type (PCI, FYI and SYI) collected from the Weddell Sea, Antarctica.

clade bacteria (Morris *et al*., 2002) in PCI to the more typical sea-ice bacteria, such as *Polaribacter* (Figures 5 and 6). The predominance of *Gammaproteobacteria* and *Flavobacteriia* has been reported especially in spring/summer during the sea-ice algal 'bloom' (Brown and Bowman, 2001; Brinkmeyer *et al*., 2003; Bowman *et al*., 2012; Torstensson *et al*., 2015). However, they can also be predominant at other times of the year, for example, the Austral autumn, if algal-derived substrate is available (Brinkmeyer *et al*., 2003; Eronen-Rasimus *et al*., 2014). Thus, it is likely that these copiotrophic bacteria are present in Antarctic sea ice throughout the winter and able to maintain their activity, if the chl-*a* concentrations are high enough and physical conditions favourable. This is in line with an earlier culture-based study in the Weddell Sea ice (Helmke and Weyland, 1995), which showed the proportion of psychrophiles in bacterial community increasing with the age of the ice. The results also show that the wintertime bacterial community dynamics in Antarctic sea ice differ from those of the oligotrophic *Alphaproteobacteria* (SAR11 clade)-dominated winter Arctic sea ice (Collins *et al*., 2010).

In addition to these common sea-ice bacterial classes, the ANOX ice samples also showed a predominance of atypical sea-ice bacterial classes, such as *Deltaproteobacteria* (for example, genera *Desulforhopalus* and *Desulfofrigus*), *Epsilonproteobacteria* (for example, genera *Sulfurospirillum* and *Arcobacter*) and *Bacteroidia* (for example, genus *Marinifilum*; Figure 5). ANOX likely formed because of the entrapment of dense algal accumulations between rafted ice floes, where subsequent bacterial activity may have caused oxygen depletion and the establishment of anaerobic bacterial communities including potential sulphate-reducing bacteria. Based on the detected odour of H_2S, the bacteria had been actively growing and reducing sulphur compounds in the ice. There is also previous evidence of transient anoxic conditions and anaerobic reactions such, as denitrification, in the ice (Kaartokallio, 2001; Rysgaard and Glud, 2004; Rysgaard *et al*., 2008). Based on our data, we cannot conclude how commonly anaerobic bacteria occur in the Antarctic winter-pack ice or deduce the origin of the anaerobic bacterial community. However, the presented data suggest that, under specific

conditions, anaerobic bacteria may become locally predominant in sea ice.

Conclusions

Our study is the first of its kind to describe the wintertime bacterial community dynamics in Antarctic sea ice and show that under suitable conditions anaerobic bacteria may become predominant in the sea-ice bacterial community. The thick insulating snow cover warmed the ice sustaining high chl-*a* concentrations. In general, common sea-ice bacterial genera, for example, *Octadecabacter* (*Alphaproteobacteria*), *Polaribacter* (*Flavobacteriia*) and *Glaciecola* (*Gammaproteobacteria*), usually common in spring and summer sea ice, predominated in the communities. The bacterial community structure, abundance and activity were driven by chl-*a*, suggesting that permeable ice with associated high algal biomass sustain bacterial activity during the Antarctic winter. In addition, to the common sea-ice bacterial classes, predominance of sulphate-reducing bacteria (for example, *Desulforhopalus, Desulfofrigus* and *Sulfurospirillum*) was observed in the ANOX together with the odour of H_2S suggesting that under suitable conditions sulphur compounds may be reduced in sea ice. In all, the results suggest that sea-ice bacterial communities can remain dynamic throughout the winter if physical conditions are favourable and the chl-*a* concentrations high enough. Thus, the possible future warming of sea ice and consequent increase in bacterial production may induce changes in major bacteria-mediated biogeochemical processes in the Antarctic sea-ice zone.

Conflict of Interest

The authors declare no conflict of interest.

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