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Spatiotemporal dynamics of marine bacterial and archaeal communities in surface waters off the northern Antarctic Peninsula

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ABSTRACT

Seasonal changes in taxonomic and functional diversity of microbial communities in polar regions are commonly observed, requiring strategies of microbes to adapt to the corresponding changes in environmental conditions. These natural fluctuations form the backdrop for changes induced by anthropogenic impacts. The main goal of this study was to assess the seasonal and temporal changes in bacterial and archaeal diversity and community structure off the northern Antarctic Peninsula over several seasons (spring, summer, autumn) from 2013 to 2015. Ten monitoring stations were selected across the Gerlache and Bransfield Straits and nearby Elephant Island, and archaeal and bacterial communities examined by amplicon sequencing of 16S rRNA genes. Alpha-diversity indices were higher in spring and correlated significantly with temperature. Spring was characterized by the presence of SAR11, and microbial communities remaining from winter, including representatives of *Thaumarchaeota* (*Nitrosopumilus*), *Euryarchaeota*, members of *Oceanospirillales,* SAR324. Summer and autumn were characterized by a high prevalence of *Flavobacteria* (NS5 marine group and *Polaribacter*), *Alphaproteobacteria* (*Rhodobacterales* and SAR11 clade) and *Gammaproteobacteria* (*Oceanospirillales/Balneatrix* and *Cellvibrionales*), generally known to be associated with organic matter degradation. Relatively higher abundance of phytoplankton groups occurred in spring, mainly characterized by the presence of the haptophyte *Phaeocystis* and the diatom *Corethron*, influencing the succession of heterotrophic bacterial communities. Microbial diversity and community structure varied significantly over time, but not over space, i.e., were similar between monitoring stations for the same time. In addition, the observed interannual variability in microbial community structure might be related to an increase in sea surface temperature. Environmental conditions related to seasonal variation, including temperature and most likely phytoplankton derived organic matter, appear to have triggered the observed shifts in microbial communities in the waters off the northern Antarctic Peninsula.

1. Introduction

The Southern Ocean is characterized by strong seasonality in environmental conditions, such as ice cover, mixed layer depths, light levels, and temperature, which have direct implications on microbial diversity and community structure (Smetacek and Nicol, 2005; Doney et al., 2012; Grzymski et al., 2012; Fuhrman et al., 2015; Bunse and Pinhassi, 2017).

Studies on the structure of microbial communities in the Southern Ocean have suggested that sampling with seasonal frequency, i.e. monthly and yearly, is fundamental to understand the taxonomic and functional diversity of microorganisms and their strategies to adapt to changing environmental conditions (e.g. Manganelli et al., 2009; Ducklow et al., 2012; Ghiglione and Murray, 2012; Cavicchioli, 2015; Luria et al., 2016; Schofield et al., 2017). In spring, short-lived phytoplankton blooms occur in shallow surface layers following the ice retreat, supplying organic carbon and nutrients to the food web and providing varied ecological niches for heterotrophic bacteria and archaea (Rousseau et al., 2000; Ducklow, 2003; Croft et al., 2005; Sher et al., 2011; Mendes et al., 2012; Delmont et al., 2014; Luria et al., 2016; Mendes et al., this issue). Consequently, the spring and summer communities are mainly composed of eukaryotic phototrophs and prokaryotic photoheterotrophs, chemoheterotrophs and aerobic anoxygenic phototrophs, whereas the winter community harbors

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relatively higher proportion of archaeal and bacterial chemolitoautotrophs (Ghiglione and Murray, 2012; Grzymski et al., 2012; Luria et al., 2016; Bunse and Pinhassi, 2017).

Surveys using temporal and spatial approaches are of equal importance in assessing the variation of marine microbial communities (e.g. Gilbert et al., 2012, Jones et al., 2012), although the use of temporal approaches may offer unique ecological information on community stability and its response to disturbances that cannot be obtained any other way (Faust et al., 2015; Fuhrman et al., 2015). Understanding seasonal shifts of microbial communities, as well as the parameters that influence their distribution, is essential to reveal the microbial response to perturbations that are predicted due to climate change. It is expected that polar regions will be – and already are – affected rapidly by climate change. The region off the northwestern Antarctic Peninsula is characterized by decreasing sea-ice extent and increasing sea surface temperatures, particularly during the summer, leading to changing wind patterns and ocean circulation with impacts on the local, regional, and even global scale (Stammerjohn et al., 2008; Doney et al., 2012; Jones et al., 2016). These changes may also reflect the natural internal variability of the regional atmospheric circulation on the Antarctic Peninsula (Turner et al., 2016).

With the growing evidence of increasing sea surface temperatures in the northern and western Antarctic Peninsula (Vaughan et al., 2003; Meredith and King, 2005; Turner et al., 2005), it is urgent to understand the actual impact of physical changes on biological communities through temporal and spatial surveys to elucidate trends and relationships between environmental forcing and biological variables. It is expected that global warming will cause shifts in the cell size of plankton, spatial range and seasonal abundance of populations, as well as a stimulation of microbial activity and thus decreased food availability for organisms at higher trophic levels (Moline et al., 2004; Kirchman et al., 2009; Montes-Hugo et al., 2009; Schofield et al., 2010; Doney et al., 2012). However, predicting microbial responses to climate change represents a formidable challenge, as bacteria and archaea tend to be more resilient (e.g. faster response to environmental change) than larger organisms due to their fast growth rates, greater dispersal capability, high metabolic flexibility, and rapid evolution, not to mention their metabolic versatility and the fact that even very closely related taxa can differ in their function (Shade et al., 2012; Kashtan et al., 2014; Luria et al., 2014; Yawata et al., 2014; Martiny et al., 2017).

Seasonal sampling over multiple years has the potential to unveil the environmental conditions regulating taxonomic distribution, and how microbial interactions and metabolic capabilities help microbes to thrive in polar regions and respond to climate change (Bunse and Pinhassi, 2017). These natural fluctuations between seasons form the backdrop for changes induced by anthropogenic impacts. Thus, the ongoing time-series in the Bransfield and Gerlache Straits by the Brazilian Antarctic research initiative represents a unique opportunity to study the microbes in a changing ocean. Here, we used 16S rRNA gene based analysis to assess the seasonal and spatial changes in bacterial and archaeal diversity, and community structure in surface waters off the northern Antarctic Peninsula (NAP). Results from this study expand our current knowledge about the seasonal distribution of archaeal and bacterial communities in surface waters, reinforce the important role of phytoplankton structuring microbial communities, and emphasize the role of temperature in shaping the community structure in a rapidly changing NAP region.

2. Material and methods

2.1. The study area

The NAP region comprises the Bransfield and Gerlache Straits (Fig. 1). The circulation patterns in this area change seasonally, and the currents and eddies provide favorable physical conditions for plankton growth and dispersion. These straits are highly productive areas, all the way from phytoplankton and zooplankton to whales (Zhou et al., 2002), and exhibit a high export production of organic matter (Doval et al., 2002; Kim et al., 2005). The Bransfield Strait is a highly dynamic area, influenced both by the cold and salty waters from the Weddell Sea (called Transitional Zonal Water with Weddell Sea influence), and the relatively warm and fresh surface waters from the Bellingshausen Sea (called Transitional Zonal Water with Bellingshausen influence, TBW) (Tokarczyk, 1987; Garcia et al., 1994; Sangrà et al., 2011). Located south of Bransfield Strait, the Gerlache Strait is a relatively confined waterway that separates the Antarctic Peninsula from Brabant and Anvers Islands (see Kerr et al., this issue). The main surface circulation pattern is driven by the Gerlache Strait Current, which flows northeastward along the strait and carries Gerlache waters into the Bransfield Strait (Zhou et al., 2002, 2006).

2.2. Sampling strategy

The research cruises were conducted with the Brazilian Navy polar vessel *Almirante Maximiano* (H41) in spring (November 2013, 2014), late austral summer (February 2014, 2015) and autumn (March 2014, 2015). Ten oceanographic stations were sampled (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10) in the NAP region, comprising the Gerlache Strait, the Bransfield Strait and around Elephant Island (Fig. 1), to evaluate seasonal changes in the microbial community along an approximately 950 km long transect, which additionally allowed to assess the spatial variability of the microbial communities. Seawater and physical data (temperature and salinity) were collected at 5 m depth using a combined Sea-Bird CTD/Carrousel 911 system equipped with 24 5-l Niskin bottles. For microbial diversity, 3 l of seawater were filtered onto Sterivex filters (Millipore) with a pore size of 0.2 µm by using a peristaltic pump. Filters were immediately frozen onboard at −80 °C until further analyses in the laboratory.

2.3. DNA extraction, 16S rRNA gene amplification and sequencing

A protocol for low biomass samples developed by Boström et al. (2004) and Manganelli et al. (2009), with slight modifications (Signori et al., 2014) was used for DNA extraction. The microbial diversity was assessed by amplicon sequencing of the V4 region of the 16S rRNA gene with the Illumina platform and the universal primers 515F-806R, targeting both Archaea and Bacteria (Caporaso et al., 2011), with overall coverage of 85% and 86%, respectively (SILVA TestPrime 1.0, http://www.arb-silva.de/search/testprime/, tested in March 2017, allowing for 1 mismatch with no mismatches in the last 4 bases of the 3′end). A single-step 30 cycles PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used on 5 ng of DNA under the following conditions: 94°C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. Thereafter, all barcoded amplicon products from different samples were quantified using Qubit (Life Technologies), mixed in equal concentrations and purified using Ampure beads (Agencourt Bioscience Corporation, MA, USA). After these steps, samples were sequenced using Illumina MiSeq with 2 × 250 bp reads configuration, following the manufacturer's guidelines. The PCR and sequencing were carried out at Molecular Research Lab (Shallowater, Texas, USA). All sequence data have been deposited in the National Center for Biotechnology Information Sequence Read Archives (SRA) under BioProject ID PRJNA383940.

2.4. Sequencing data and statistical analyses

Raw sequencing reads were filtered for length (250–300 bp), quality score (mean, > 50), maximum number of ambiguous bases = 0, maximum homopolymer length = 6 and minimum expected error of 1.0, using Quantitative Insights Into Microbial Ecology (QIIME) 1.8.0 pipeline (Caporaso et al., 2010). Chimeras were checked using uchime2

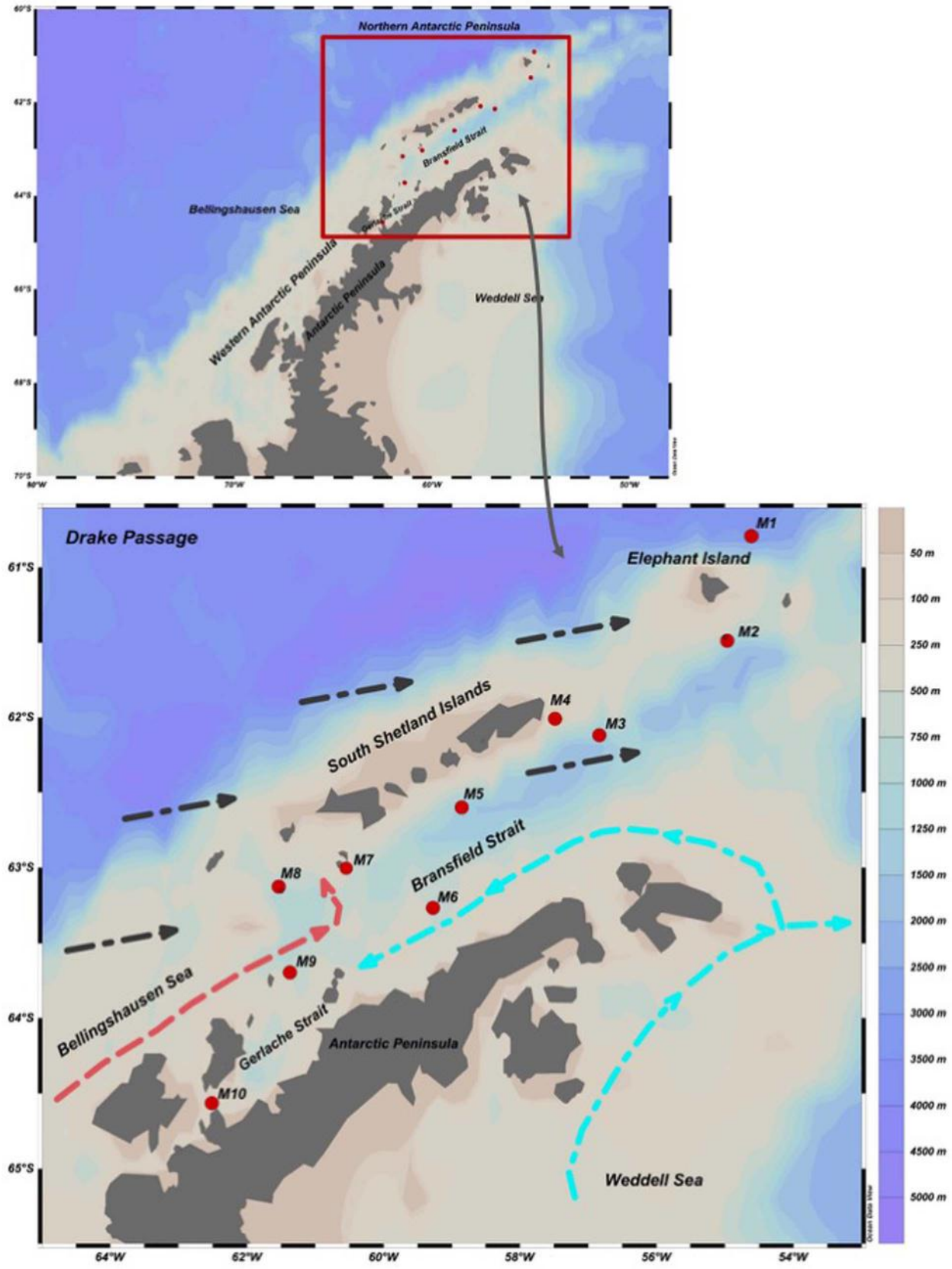


Fig. 1. Sampling map of ten selected monitoring stations (M1-M10, red dots) in the northern Antarctic Peninsula (NAP) to determine the spatiotemporal variation of surface microbial communities. The samples were collected in spring (November 2013, 2014), summer (February 2014, 2015) and autumn (March 2014, 2015) during the Brazilian Antarctic Expeditions. Basic scheme of surface circulation is represented: Black dashed arrows indicate the Antarctic Circumpolar Current, red dashed arrows indicate relatively warm and less saline water masses originating from the Bellingshausen Sea, blue dashed arrows indicate relatively cold and saline Weddell Sea water (modified from Bahk et al. 2003). The color scale bar on the right represents bathymetry. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

algorithm (Edgar, 2016). Sequences were clustered at 97% similarity using usearch and uclust pipelines (Edgar, 2010), and taxonomy was assigned to each Operational Taxonomic Unit (OTU) using SILVA database version 128 (Yilmaz et al., 2014). The OTU table was normalized with the cumulative sum scaling (CSS) method, which corrects the bias in the assessment of differential abundance introduced by total-sum normalization, using the metagenomeSeq Bioconductor package (Paulson et al., 2013). The CCS normalized reads were used for all downstream analyses. For each sample, alpha-diversity indices (number of observed OTUs, Chao1, Simpson and Shannon's diversity) were calculated using QIIME 1.8.0 (Caporaso et al., 2010), and differences in alpha-diversity estimates between groups of samples were tested using Student`s *t*-test in R (version 3.3.2). The number of shared OTUs between samples were visualized using ggplot2 package in R (Wickham, 2009). Beta-diversity between samples was visualized with non-metric multidimensional scaling (nMDS) based on weighted UniFrac distance (Lozupone and Knight, 2005), with fitting of the environmental gradients (temperature, salinity, season, year, longitude and latitude) applying the *envfit* function from the vegan R package (Oksanen et al., 2016). Differences in the relative abundances of taxa between groups of samples (spring *vs*. autumn, spring vs. summer, summer vs. autumn, spring vs. summer-autumn, within sampling periods, within monitoring stations and within sampling areas) were evaluated with a permutational multivariate analysis of variance (PERMANOVA, *adonis* function with 999 permutations, based on weighted UniFrac distance) using the vegan R package (Anderson, 2001; Oksanen et al., 2016). Analyses based on fixed-effects models (*lmer* function) using lme4 R package (Bates et al., 2015) were performed to test and decouple the relations between alpha-diversity indices and the values of temperature and salinity over years. Thereafter, only the significant relations between alpha-diversity indices and temperature were retained for calculating linear regressions. In addition, differences in temperature between groups of samples were tested using Student`s *t*-test in R (version 3.3.2).

3. Results

3.1. Thermohaline characteristics of Antarctic surface waters

Seawater temperatures varied from −1.54 to 1.91 °C, and salinities ranged from 33.23 to 34.86 in the study area (Fig. 2). Significant differences ($p < 0.05$) in thermohaline characteristics of surface waters were registered between austral spring, summer and autumn. Temperatures below zero were only found in spring, varying from −1.54 to −0.47 °C. In summer and autumn, temperatures were relatively higher and varied from 0.18 to 1.91 °C, and 0.35–0.95 °C, respectively. Salinities were more similar, ranging from 33.24 to 34.86 in spring, 33.62–34.38 in summer, and 33.23–33.89 in autumn.

3.2. Bacterial and archaeal community composition

After quality checking and data filtering, a total of 4,432,018 sequences (range of 5214–109,883 reads per sample) were obtained from 73 surface water samples, and clustering of these reads at 97% similarity threshold resulted in 894 OTUs. Removing chloroplasts and mitochondria-related sequences, we found 2,927,955 sequences (range of 3019–72,968 reads per sample) and 783 OTUs. Considering all sequences of bacteria and archaea retrieved in the present study (Fig. 3**a**), sequences belonging to *Bacteroidetes* (37.9%) and *Alphaproteobacteria* (29.9%) accounted for the largest fraction, followed by *Gammaproteobacteria* (26.1%). *Bacteroidetes* was almost exclusively represented by the order *Flavobacteriales* (37.6%) and the taxa *Polaribacter*, *Ulvibacter, Cryomorphaceae* and NS9. *Alphaproteobacteria* was mainly represented by the orders *Rhodobacterales* (17.4%), SAR11 clade (9.9%) and SAR116 clade (0.9%), whereas *Gammaproteobacteria* was represented by *Oceanospirillales* (19.1%), *Cellvibrionales* (5.1%) and *Alteromonadales* (1.3%). The classes *Betaproteobacteria* (1.3%) and *Deltaproteobacteria* (0.3%) were also identified, and mainly represented by the order *Methylophilales* and SAR324 clade, respectively. The archaeal phylum *Thaumarchaeota* represented 2.4% of all sequences, and *Euryarchaeota* was also identified, although relatively less abundant (0.5%). In general terms, *Thaumarchaeota/ Nitrosopumilus*, *Alphaproteobacteria/* SAR11 and *Gammaproteobacteria/ Oceanospirillales* represented a larger fraction of the microbial community in spring, with 6.2%, 14.5%, 26.3%, respectively (Fig. 3**b**). In summer and autumn, other taxa presented higher relative abundance: *Flavobacteria/ Flavobacteriales* (43.7% and 44.1%), *Alphaproteobacteria/ Rhodobacterales* (19.8% and 18.6%) and *Gammaproteobacteria/ Cellvibrionales* (5.9% and 6.3%) (Fig. 3**b**).

At various taxonomic levels for archaea and bacteria, samples from November 2013 and 2014, although with differences in relative abundance, were more enriched in the *Thaumarchaeota/ Nitrosopumilus* (6.7–5.7%), *Euryarchaeota* (1.6–0.8%), SAR11 clade (18.1–11.2%), *Rhodobacterales* (12.2–15.9%), *Oceanospirillales* (30.4–22.3%), *Cellvibrionales* (3.2–3.3%), *Flavobacteria/Flavobacteriales* (16.8–32.5%), and SAR324 clade (1.2–0.4%) (Fig. 4**a** and Supplementary Table S1). Samples from February 2014 and 2015 showed higher contribution of *Flavobacteria/ Flavobacteriales* (42.6–45.2%), *Rhodobacterales* (17.4–23%), SAR11 clade (7.8–6.7%), *Oceanospirillales* (18.6–16.3%) and *Cellvibrionales* (7.5–3.1%). Samples from March 2014 and 2015 presented higher relative contribution of *Flavobacteria/Flavobacteriales* (44.1–43.5%), followed by representatives of *Rhodobacterales* (19.8–16.1%) and SAR11 clade (8.2–7.3%), and gammaproteobacterial orders *Oceanospirillales* (13.6–11.8%), *Cellvibrionales* (6.3–6%) and *Alteromonadales* (0.4–5.4%).

3.3. Phytoplankton community composition based on 16S chloroplast sequences

The use of the utilized primer pair also resulted in the amplification of 16S rRNA genes from chloroplasts, allowing an assessment of phytoplankton diversity (Fig. 4**b**). Spring samples were characterized by relatively higher abundance of the haptophyte *Phaeocystis* (35.2–28.5%), the pennate diatom *Corethron* (29.9–12.2%), and the dinoflagellate *Dinophysis* (9.2–13.3%), totaling 74.23% and 53.93% of all chloroplast sequences in November 2013 and 2014, respectively (Fig. 4**b**). In summer, the phytoplankton community was dominated by the polar centric diatom *Thalassiossira* (39.5–27.7%), the haptophyte *Phaeocystis* (21.0–27.7%), and the pennate diatom *Fragilariopsis* (17.0–8.7%), making up 77.4% and 64.2% of all chloroplast sequences in February 2014 and 2015, respectively. In autumn, we identified higher relative abundance of *Fragilariopsis* (29.8–5.9%), *Thalassiossira* (22.5–34.5%) and *Phaeocystis* (17.6–20.8%), totaling 69.8% and 61.2% of all chloroplast sequences in March 2014 and 2015, respectively.

3.4. Alpha-diversity estimates for bacteria and archaea

The number of observed OTUs per sample varied from 150 to 395, both registered in November 2014 (Supplementary Table S2). Almost all the extreme values, maximum and minimum, for all indices were found in November 2014 and February 2015, respectively. Means of all alpha-diversity indices, except for Chao1, were significantly higher in samples originating from spring as compared to summer-autumn, with p-values equal to 0.01 (number of OTUs), 0.20 (Chao1), 0.0004 (Shannon), and 0.01 (Simpson).

After applying multiple-effects models to assess the relationships of alpha-diversity indices with both temperature and salinity over time, only temperature was found to be a significant parameter and was therefore chosen for linear regression analysis (Supplementary Table S3). Although with low r^2 values, the relations between temperature and different alpha-diversity indices were significant for the number of OTUs ($r^2 = 0.13$, $p = 0.005$), Shannon ($r^2 = 0.32$, $p < 0.001$), and Simpson ($r^2 = 0.19$, $p < 0.001$), suggesting higher richness and diversity in cooler compared to warmer surface waters (Fig. 5**a-d**).

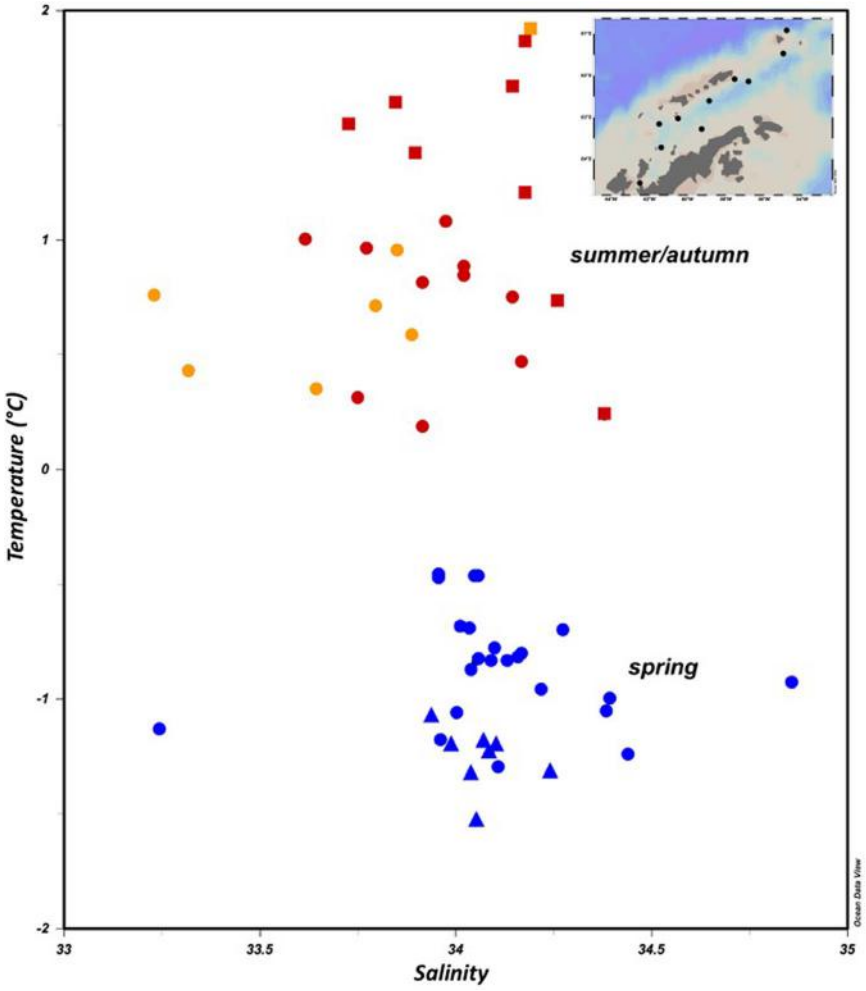


Fig. 2. Temperature and salinity distribution in surface waters in the northern Antarctic Peninsula (see little map on the top). Symbols in blue represent samples collected in spring, red symbols represent samples from summer, and yellow symbols, samples from autumn. Triangles represent samples from 2013, circles represent samples from 2014, and squares are samples from 2015. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Bacterial and archaeal community structure across space and time

Beta-diversity was determined after analysis of the relative abundance of the different taxa in relation to the sampling locations and the thermohaline characteristics of the surface seawater by using nMDS (Fig. 6). The results showed a clear segregation between samples collected in spring versus summer and autumn, which was supported by PERMANOVA tests with significant differences between spring and summer-autumn (r^2 = 0.34 and p = 0.001), spring and autumn (r^2 = 0.37 and p = 0.001), spring and summer (r^2 = 0.30 and p = 0.001), and within sampling periods (month/year) (r^2 = 0.51 and p = 0.001). Also, significant differences were found in microbial community structure between years, when we contrasted November 2013 *vs*. 2014 (r^2 = 0.31 and p = 0.001), February 2014 *vs*. 2015 (r^2 = 0.19 and p = 0.002), and March 2014 *vs*. 2015 (r^2 = 0.14 and p = 0.006). However, no significant differences were found among monitoring stations (r^2 = 0.08, p = 0.97), nor among sampling areas (r^2 = 0.02, p = 0.57) over all the expeditions (Supplementary Table S4). In addition, when plotting the environmental parameters, the influence of temperature (r^2 = 0.64 and p = 0.001) on the microbial community structure was found to be more important than salinity (r^2 = 0.07 and p = 0.15), and time of sampling (r^2 = 0.64 and p = 0.001 for all samples, r^2 = 0.54 and p = 0.001 for yearly comparisons, r^2 = 0.55 and p = 0.001 for seasonal comparisons) were more relevant than geographical location (r^2 = 0.10 and p = 0.08 for latitude, r^2 = 0.07 and p = 0.18 for longitude) (Supplementary Table S5).

A total of 96, 87 and 123 OTUs were present in all samples obtained in spring, summer and autumn, respectively (Fig. 7**a**). Spring and summer shared 70 OTUs, spring and autumn shared 80 OTUs, and summer and autumn shared 85 OTUs. The overall core microbiome of Antarctic surface waters consisted of 68 OTUs, belonging to *Alphaproteobacteria* (33.82%), *Gammaproteobacteria* (32.35%), *Bacteroidetes* (25%), *Betaproteobacteria* (5.88%), *Deltaproteobacteria* (1.47%), and *Thaumarchaeota* (1.47%) (Fig. 7**b,** Supplementary Table S6). Thus, even though the core microbiome of bacteria and archaea only consisted of 68 OTUs out of a total of 783, the main phylogenetic groups were still represented.

4. Discussion

4.1. Temporal dynamics

Bacterioplankton communities are known to greatly change at different time scales (Fuhrman et al., 2006, 2015), although few studies have investigated the temporal dynamics of microbial communities in polar regions (e.g. Ducklow et al., 2012; Ghiglione and Murray, 2012; Grzymski et al., 2012; Bowman et al., 2016; Luria et al., 2016; Schofield et al., 2017), largely due to the logistical challenges that are inherent in studying these systems. In the present study, the alpha-diversity indices that varied most significantly over time were the community diversity indices including evenness (Shannon, Simpson), suggesting that the relative abundance of different taxonomic groups is driving the difference between seasons and not necessarily species richness itself. This is also supported by the absence of a significant correlation with Chao1, and is also consistent with the fact that the core microbiome across all seasons contains representatives of all major phylogenetic groups. This

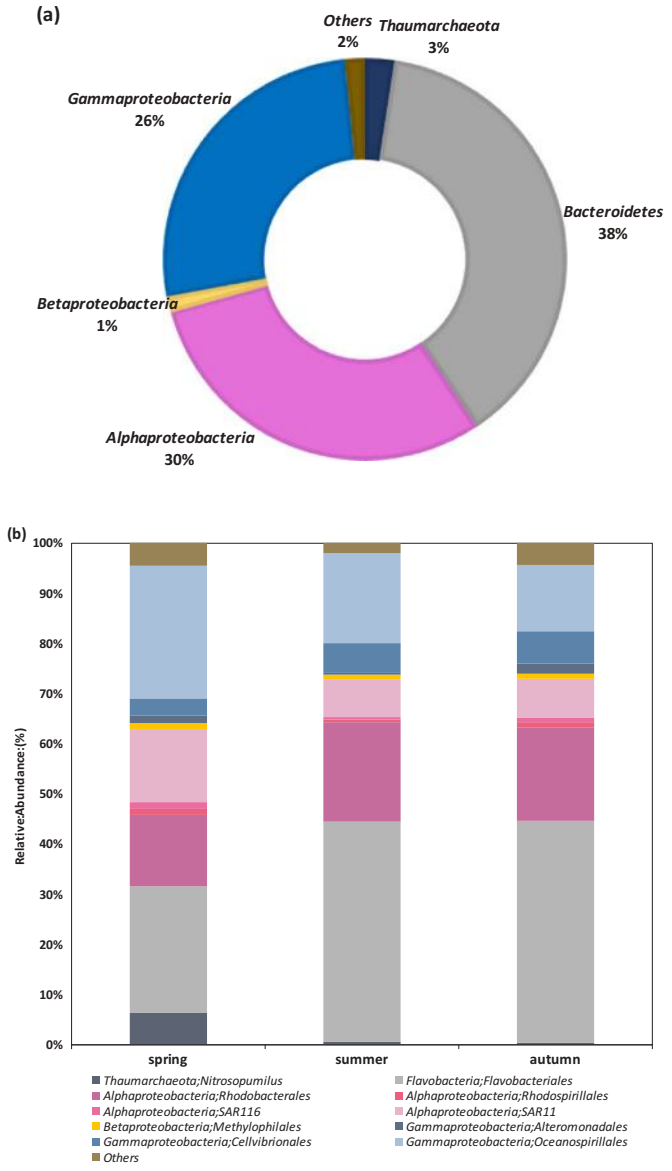


Fig. 3. Taxonomic composition of all sequences retrieved during this study (n = 4,432,018 sequences) in a total of 73 samples. The external pie chart shows the relative abundance (%) of the main archaeal and bacterial classes besides chloroplast sequences, and the internal pie chart represents the relative abundance of the main orders and genera identified for each group according to the color scales. The legend on the right corresponds to the internal pie chart, and the category 'Others' comprises taxa accounting for less than 1% of the total (a). Relative abundance (%) of microbial taxa identified in spring, summer and autumn (b). Color scales represent the main groups: pink shades for *Alphaproteobacteria*, blue shades for *Gammaproteobacteria*, grey for *Bacteroidetes*, green shades for chloroplast sequences, dark blue for *Thaumarchaeota*, brown for 'Others'. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is in agreement with other studies showing that estimators of alpha-diversity are higher during the Antarctic winter and spring, and that a significant decrease in richness and diversity occurs during summer (January and February), especially during phytoplankton blooms (e.g. Ghiglione and Murray, 2012; Grzymski et al., 2012; Hernández et al., 2014; Luria et al., 2016). However, our summer samples were likely taken after the summer phytoplankton bloom, that usually occurs in this area around January (Luria et al., 2016), explaining why the difference between spring and summer was not as pronounced in our case.

The presence of the archaeal phyla *Thaumarchaeota* (*Nitrosopumilus*) and *Euryarchaeota* in spring, and their very low abundance in summer-autumn are consistent with previous observations by Kalanetra et al. (2009), who found archaea to be almost completely absent during the summer season in an area where phytoplankton blooms occur. However, our seasonal patterns differ from Hérnandez et al. (2014), who found the presence of archaea throughout the entire year in an area not influenced by spring blooms. Possibly our results reflect the increase of phytoplankton occurrence in our study area in spring, followed by an increase in bacterial groups able to take advantage of the phytoplankton organic matter input (substrate availability) in summer and autumn, therefore, gaining importance in relation to archaeal groups. Other taxa present in spring, such as potentially chemoautotrophic groups SAR324 and *Oceanospirillales* (Sheik et al., 2014; Swan et al., 2011), have been previously registered in Antarctic deep waters in summer (Signori et al., 2014), and as part of the Antarctic surface winter community (Grzymski et al., 2012; Williams et al., 2012; Luria et al., 2014). Further, a combination of metagenomic and metaproteomic surveys showed that 18–37% of the bacterial and archaeal winter community were found to have the potential to fix CO_2 by performing chemolithoautotrophy (Grzymski et al., 2012; Williams et al., 2012), with an important contribution by ammonia-oxidizing *Thaumarchaeota* (Tolar et al., 2016). Apparently, three major taxonomic groups found in spring (i.e. *Nitrosopumilus*, SAR324, *Oceanospirillales*) remain from the previous winter. The identification of *Thaumarchaeota* and the SAR324 clade as part of the core microbiome provides evidence that these groups might contribute to chemosynthetic production not only in deep-waters and during the winter at the surface, but also in spring and even during summer, when any potentially present chemoautotrophs are overshadowed by the abundant heterotrophic bacteria that recycle organic matter during or after phytoplankton blooms.

For phytoplankton, the spring season represents the beginning of significant growth in response to increased irradiance and water column stabilization caused by favorable winds, warming, and/or freshening due to input of melt water from sea ice or glaciers (Prézelin et al., 2000; Ducklow et al., 2008; Mendes et al., 2012; Venables et al., 2013; Rozema et al., 2017a). In the present study, we observed the succession of different bacterial groups in response to the occurrence of different types of phytoplankton species. Our data based on chloroplast 16S rRNA gene sequences allowed a limited assessment of phytoplankton diversity, which was mainly characterized by haptophytes, diatoms and dinoflagellates. Previous studies have already shown high abundances of the haptophyte *Phaeocystis* and pennate diatoms (such as *Corethron*) in winter and early spring in the southern Antarctic Peninsula (Rozema et al., 2017a), which are more often associated with spring phytoplankton communities and sometimes with sea-ice melting (Annett et al., 2010; Rozema et al., 2017b). The presence of *Corethron* in spring followed by polar centric diatoms (such as *Thalassiossira*) from summer to autumn was also noticed by Rozema et al. (2017b), and the latter were previously reported as important components of the phytoplankton communities in coastal and open ocean regions of the Southern Ocean (Díez et al., 2004; Garibotti et al., 2005; Pike et al., 2009; Annett et al., 2010; Piquet et al., 2011; Ducklow et al., 2012). Similar results to the present study were found by Mendes et al. (2012); this issue), who used a different technique (HPLC analysis) to assess the spatiotemporal variability in the composition and biomass of phytoplankton in NAP. However, Mendes et al., (this issue) not only found *Phaeocystis antarctica* and small diatoms, but also cryptophytes as a major component of the phytoplankton community in the shallow mixed-layers (< 25 m). The lack to identify cryptophytes in our study is most likely due to the use of a primer set targeting Bacteria and Archaea instead of Eukarya, and possibly also the use of an DNA extraction procedure not tailored for phytoplankton.

Concomitant with a larger fraction of 16S chloroplast sequences in spring, indicative of a higher contribution of phytoplankton, SAR11 and *Oceanospirillales* exhibited their highest relative abundance, decreasing in summer and autumn. Sequences belonging to the SAR11 clade represent the most abundant bacterioplankton in the global ocean (Giovannoni, 2017), are characterized as aerobic and free-living

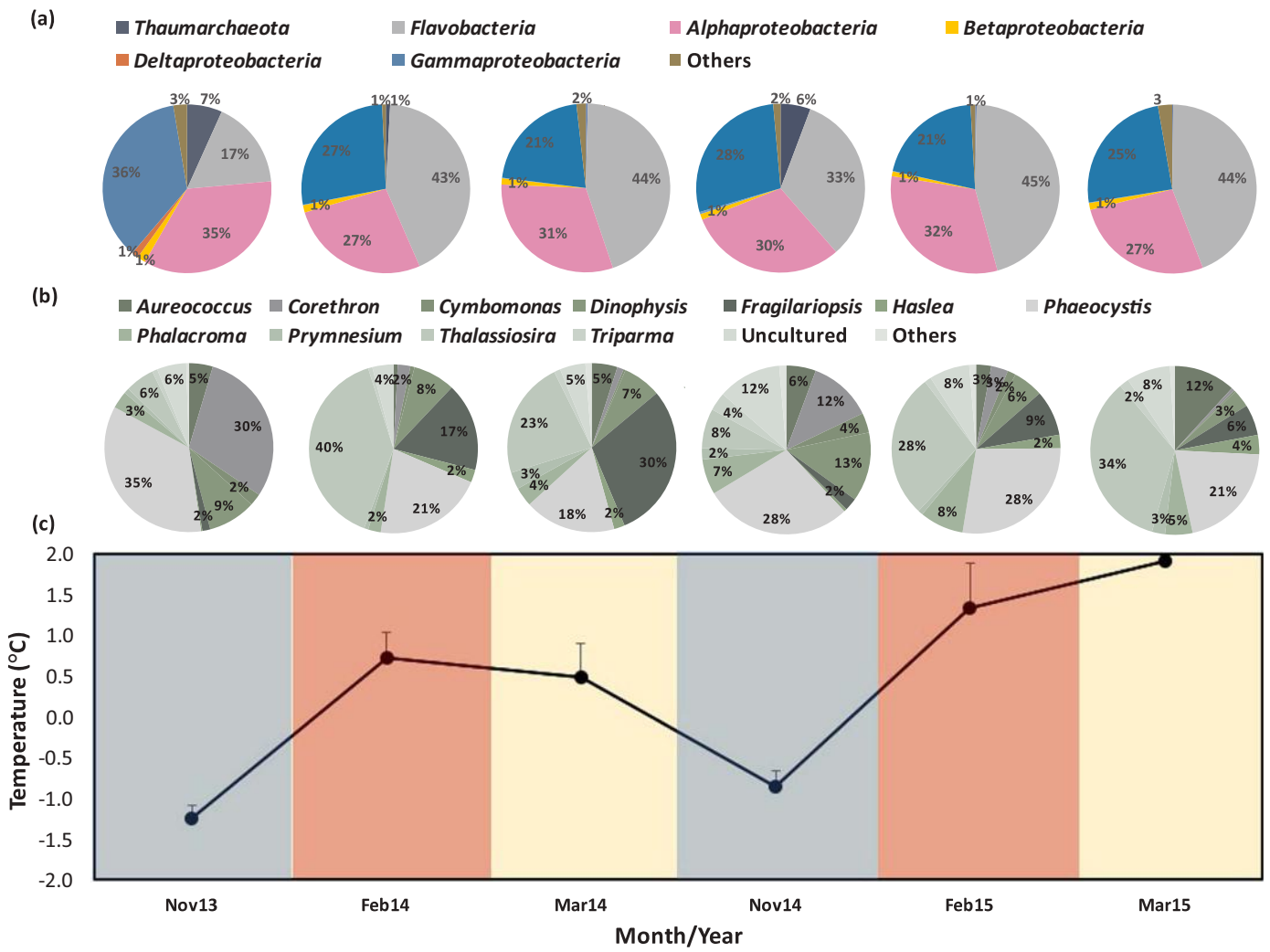


Fig. 4. Relative abundance (%) of archaeal phyla and bacterial classes (a), 16S eukaryotic genera (b), across temperature variation in Antarctic surface seawater between November 2013 and March 2015, with spring, summer and autumn represented by blue, red and yellow, respectively (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

chemoheterotrophs, and have previously been detected in high relative abundance during phytoplankton blooms (Landa et al., 2016). Among *Oceanospirillales* sequences, the genus *Balneatrix* was relatively dominant in our study, confirming previous observations of *Balneatrix* as an important member of coastal Antarctic bacterial communities (Nikrad et al., 2013; Moreno-Pino et al., 2016). These taxa might benefit from exudates released by actively growing phytoplankton. In summer and autumn, we observed a 1.5–2.5 times increase of *Flavobacteria* and *Cellvibrionales*, indicating degradation of phytoplankton biomass. Among *Flavobacteria/Flavobacteriales*, two taxa were the most abundant, the NS5 marine group and the genus *Polaribacter*, which have previously been identified in the Bransfield Strait as free-living bacteria (Milici et al., 2016) and frequently found in polar waters (Wilkins et al., 2013; Signori et al., 2014; Luria et al., 2016), respectively. Although relatively less abundant, *Cellvibrionales* were also present in late summer, particularly in February and March 2014. *Cellvibrionales* was recently proposed as a novel order within the *Gammaproteobacteria*, showing high seasonal abundances in coastal environments (Spring et al., 2015). Similarly, as *Flavobacteria*, members of this order have the capability to degrade complex carbohydrates and can occupy distinct nutrient ecological niches, like marine snow (Williams et al., 2013; Spring et al., 2015). In addition, some representatives of *Rhodobacterales* also increased in summer, which are known as dominant and primary colonizers of particulate organic matter (Dang et al., 2008), targeting common constituents of algal exudates, such as taurine, polyamines and glycolate, and forming commensal associations with phytoplankton (Buchan et al., 2005). Overall, the observed shifts in bacterial taxonomic groups may be intimately related to the differentiated use of organic matter that can shape the heterotrophic bacterial communities (Ducklow et al., 2012; Teeling et al., 2012; Kim and Ducklow, 2016; Landa et al., 2016; Luria et al., 2016).

Another important, but even less studied, component of the temporal variation in bacterial and archaeal communities in the Southern Ocean is the interannual variability. We found differences in microbial community composition between years, especially for spring (November 2013 and 2014) and summer months (February 2014 and 2015), that were corroborated by the statistics for beta-diversity (Supplementary Table S4). It is known from long-term studies on phytoplankton in the western Antarctic Peninsula that significant interannual variability of community composition and bloom magnitude are normally linked to winter sea ice cover and summer stratification strength, and changing temperatures (e.g. Rozema et al., 2017a; Schofield et al., 2017). A significant ($p < 0.05$) increase in in-situ temperature was observed in spring and summer between years, suggesting that the increase in temperature might play a role on the observed differences in microbial community structure.

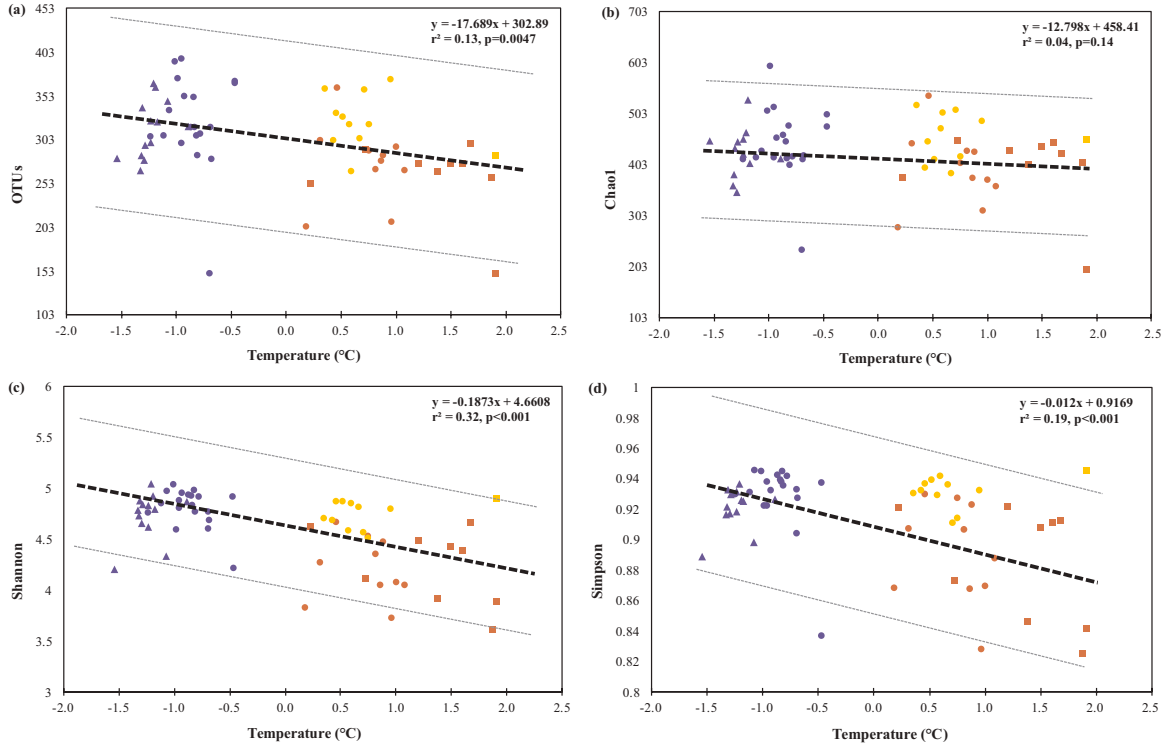


Fig. 5. Relationships between temperature (°C) and alpha-diversity indices: Number of OTUs (a), Chao 1 (b), Shannon (c) and Simpson (d), with the appropriate statistics information on the top right, and confidence intervals in light-colors lines. Symbols in blue represent samples collected in spring, red symbols represent samples from summer, and yellow symbols, samples from autumn. Triangles represent samples from 2013, circles represent samples from 2014, and squares are samples from 2015. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.2. Spatial variation

As part of our sampling design, we were also able to address the spatial variation of the microbial communities at a given time. Based on the literature, it is assumed that 2–20 km in horizontal extent corresponds to the size of a typical microbiologically coherent parcel of water, harboring a consistent microbial community composition (Hewson et al., 2006; Lie et al., 2013; Fuhrman et al., 2015). Surprisingly, in a transect of ca. 950 km length from the Gerlache and Bransfield Straits to the surrounding area of Elephant Island, we observed neither significant differences in microbial community structure among monitoring stations M1-M10, nor significant correlations between geographical distance and beta-diversity. This observation is consistent with Luria et al. (2014), who did not find a relationship with geographical distance, and observed that similar environmental conditions in summer lead to similar surface communities. Further, this reinforces previous findings of Signori et al. (2014), who found in a similar region that transitions between seasons (summer to autumn) could have led to differences in bacterial and archaeal communities. Based on our dataset, the geographical distance appears to be less relevant than the seasonal variation, as the microbial diversity and community structure in surface waters were very similar over space, while significant differences were found between seasons and years.

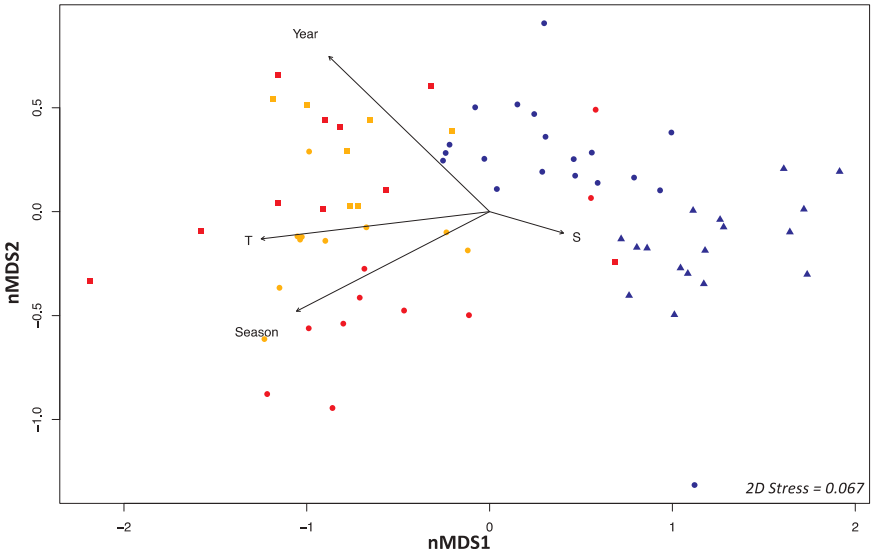
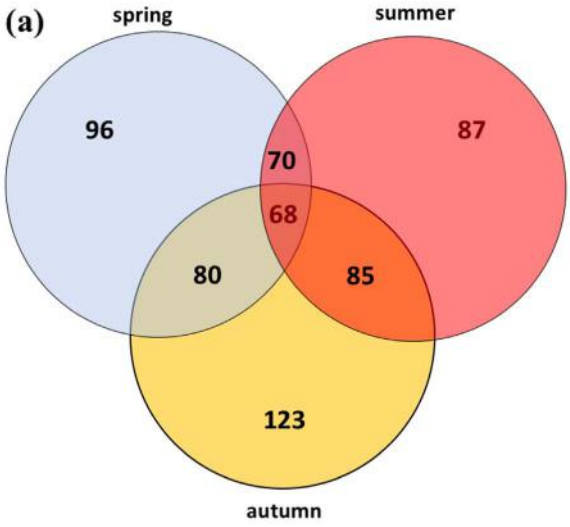


Fig. 6. Non-metric multidimensional scaling (nMDS) ordination based on weighted UniFrac distance. Symbols in blue represent samples collected in spring, red symbols represent samples from summer, and yellow symbols, samples from autumn. Triangles represent samples from 2013, circles represent samples from 2014, and squares are samples from 2015. Each arrow shows one gradient (Year, Season, T = temperature, S = salinity). The arrow points to the direction of the most rapid change in the environment (direction of the gradient) and its length is proportional to the correlation between ordination and environmental variable (strength of the gradient). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



(b)

Core Microbiome	
Alphaproteobacteria	33.82%
Betaproteobacteria	5.88%
Gammaproteobacteria	32.35%
Deltaproteobacteria	1.47%
Bacteroidetes	25.00%
Thaumarchaeota	1.47%

Fig. 7. Venn diagram of the core microbiome of Antarctic surface waters. Each circle (blue, red and yellow) contains numbers of OTUs present in 100% of samples within each group (spring, summer and autumn). Numbers of OTUs in the overlapping regions were shared by two or three groups (a). Relative abundance (%) of the taxonomic composition of 68 OTUs of Bacteria and Archaea shared between spring, summer and autumn (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. The influence of environmental factors

In a recent study, Moreno-Pino and collaborators (2016) determined that environmental factors control the spatial variation of bacterial communities in Fildes Bay, King George Island, Antarctica. Our results confirm this hypothesis on a larger scale, showing strong relationships between community composition and environmental conditions related to seasonal variation, but no spatial differences in microbial community structure. Our data suggest that an interplay of environmental conditions related to the seasonal variation in polar marine ecosystems is shaping the bacterial and archaeal communities, with temperature and organic matter being the most relevant regulatory factors (e.g. Kirchman et al., 2009; Ducklow et al., 2012; Signori et al., 2014; Torstensson et al., 2015; Bowman et al., 2016; Kim and Ducklow, 2016; Landa et al., 2016; Luria et al., 2016; Bunse and Pinhassi, 2017). Showing significant correlations with alpha- and beta-diversity, our study confirmed previous findings identifying seawater temperature as a key factor in determining the distribution of microorganisms in polar ecosystems (Fuhrman et al., 2006, 2008; Wilkins et al., 2013; Yung et al., 2015; Schofield et al., 2017), affecting the microbiome's diversity, activity and biogeochemical potential (Ward et al., 2017). In addition, climate-change induced sea-surface warming, together with other corresponding changes in oceanographic conditions, can further affect the structure and function of marine ecosystems, impacting the microbial composition, biomass and production, with possible cascading effects on higher trophic levels (e.g. Moline et al., 2004; Kirchman et al., 2009; Montes-Hugo et al., 2009; Doney et al., 2012; Ducklow et al., 2013; Mendes et al., this issue; Bowman et al., 2017; Schofield et al., 2017; Ward et al., 2017).

4.4. Final remarks

In conclusion, our study contributes to a better understanding of the shifts in marine microbial communities that occur every year between spring, summer and autumn in Antarctic surface waters. Interestingly, despite these shifts the main phylogenetic groups were part of the core microbiome, suggesting that the main functions of the community are retained throughout the year. Moreover, the relatively homogeneity of bacterial and archaeal communities found in Antarctic coastal waters across a 950 km transect suggests that a simple monitoring system of only a couple of stations may adequately cover the entire region. In addition, the observed tendency of interannual variability in community structure warrants further investigation. The observed changes in bacterial and archaeal community structure in response to environmental conditions related to seasonal variation and the lack of spatial variation provide critical knowledge to assess the ecosystem's response in the rapidly changing NAP region.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2017.12.017.

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