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# Diversity and community composition of active microbial communities in southern high latitude ecosystems


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*Dédicace*

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# Summary

Southern high latitudes ecosystems are highly sensitive to climate change, impacting physical, chemical and biological processes and ecosystem services, however, their prominent role in climate modulation and water masses circulation (at regional and global level), contrast with the relatively low number of studies on their functioning. Microorganisms are essential players in the marine ecosystem, where they influence its functioning through their distribution, abundance and activity. The study of bacterioplankton community structure over space and time allows to examine the effects of the different microbial processes that occur in the environment. The environment, in turn, influence the abundance and diversity of these communities with profound implications on ecosystem functioning. Relatively few studies on bacterioplankton community structure have been reported for southern Chilean Patagonia and for the Southern Ocean on a large scale, and none have targeted the active fraction of the bacterioplankton community. This study used 16S rRNA sequencing to analyze and describe the taxonomic composition of the active bacterioplankton communities in two southern polar zones: (i) the Subantarctic zone, by specifically targeting two fjords of the southern Patagonia and (ii) the Antarctic zone from New Zealand to South Shetland Island. The main objective of this thesis was to characterize the diversity and abundance of bacterioplankton communities along environmental and geographical gradients in southern high latitudes ecosystems. First, we investigated whether nearby fjords of the southern Chilean Patagonia, with similar climate and location but different freshwater inflows, had different communities (Chapter 1). Second, we investigated the interannual changes (2017-2018) experienced by the bacterioplankton community of the Yendegaia fjord (Chapter 1). Third, we examined the large-scale spatial structure of the bacterioplankton community along a transect across the Pacific sector of the Southern Ocean (Chapter 2).

In the first part of the thesis (Chapter 1) the study was conducted at small geographic scale in two adjacent fjords Pía and Yendegaia, within the Beagle Channel in the Magallanes region. Ours results shows that each fjord harbored distinct bacterioplanktonic community composition that vary according to the hydro-morphological characteristic of each fjord associated to the presence of marine-terminating glacier (Pía) or land-terminating glacier (Yendegaia). Differences in sediment load, light penetration, water column stratification, nutrient supply and the

presence/absence of sills drove the major taxonomic changes between bacterioplankton communities at both fjords. In addition, interannual changes in the bacterioplankton community composition of the Yendegaia fjord were observed between July 2017 and July 2018. The significant differences were mostly attributed to changes in nutrient concentration (nitrate and ammonium), chlorophyll-a, water column stratification and photosynthetic active radiation (PAR) between the two years. Our results illustrate that a shift in freshwater input from marine-terminating glacier towards land-terminating glacier due to accelerated climate change in the area would promote a modification in bacterioplankton community structure, which would subsequently affect biogeochemical processes in the subantarctic fjords.

In the second part of the thesis (Chapter 2), the study was conducted at a larger spatial scale (~ 6.500 km) through the Southern Ocean with focus on the distribution patterns of bacterioplankton communities over environmental and geographical distance gradients. We found marked biogeographic patterns in the active bacterioplankton community across the Southern Ocean transect. The compositional changes of bacterioplankton communities were significantly correlated to key environmental parameters such as temperature, salinity, silicic acid, PON and POC, which were characteristic of each sampled zone. Furthermore, spatial changes in the prokaryotic community composition were related to geographical distance. We suggest that spatial changes in community composition were enhanced by the presence of different oceanic fronts belonging to the Antarctic Circumpolar Current (ACC) that acted as biogeographic barriers limiting the dispersion of bacterioplankton communities. This study contributes to expand the scarce biogeographical knowledge of bacterioplanktonic communities in the Southern Ocean by demonstrating that contemporary environmental processes and oceanographic discontinuities (e.g., ocean fronts) influence the structure of bacterioplanktonic communities.

In summary, in this thesis we were able to demonstrate that southern polar bacterioplanktonic communities are structured according to physical, chemical, and biological parameters characteristic of the area. In addition, we also showed that changes in environmental, spatial, and temporal parameters also affect the abundance, diversity, and distribution of bacterioplanktonic communities and therefore their potential biogeochemical role, thus highlighting the importance of microbial ecology studies in areas sensitive to global climate change such as southern high latitude ecosystems.

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**GENERAL DISCUSSION**

# GENERAL INTRODUCTION

## 1. Marine Microbial Ecology

Until the mid-20th century, the most common methods to study microorganisms were based on plate counts, serial dilutions, or phase-contrast microscopy. These traditional methods led researchers to notice large discrepancies between what was growing on the agar plates and what was observed on the microscope for the same sample (Staley & Konopka, 1985). This phenom was later called as “the great plate count anomaly” by Staley & Konopka in 1985 (Staley & Konopka, 1985). Because of the lack of appropriate techniques for the study of environmental samples several ecosystems could not be deeply studied at the time. As a consequence, the role of bacteria in the water column was long underestimated and largely ignored in models of planktonic food chains and carbon flow under the evidence provided by plate counts (Steele, 1976).

One of the central goals of microbial ecology is to understand which factor drives the structure of the microbial communities through space and time which is of pivotal importance, as the structure of microbial communities inevitably impacts key functions of the ocean such as primary production and carbon cycling. Today, advances in sampling techniques and molecular methodologies are allowing a better characterization of microbial community structure, functional potential, and activity from a wide range of environments.

Molecular phylogeny has been the major breakthrough in the assessment of marine microbial diversity that has been possible through the use of nucleotide-sequence analysis of the small subunit ribosomal rRNA gene (Pace, 1997). This culture independent methodology allows to profile prokaryotic communities directly from their natural environments thus helping to report the true extent of phylogenetic diversity of a given habitat (Giovannoni et al., 1990; Hewson et al., 2006; Huber et al., 2002; Pace, 1985; Stahl et al., 1985).Furthermore molecular techniques like Denaturing Gradient Gel (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and automated ribosomal intergenic space analysis (ARISA) allows to compare microbial community patterns from a general perspective based on the characteristics migration distance of

PCR amplified DNA fragments in an electrophoretic device (Hugerth & Andersson, 2017). Then, the development of high-throughput sequencing (HTS) technologies allowed the processing of a large number of samples, and analyses at a deeper level of resolution, providing an in-depth description of the *in situ* microbial diversity (Caporaso et al., 2011; Sogin et al., 2006; Sunagawa et al., 2015) Marine microbial ecologist were among the first to apply HTS for describing microbial communities (Sogin et al., 2006). High amount of information and high coverage from environmental samples have been achieved through the use of HTS enabling deeper description of environmental communities and their associated potential metabolic pathways.

Great sampling effort combined with HTS techniques have provided visualization of the microbial marine diversity in large spatial scales. Ocean expeditions such as Sorcerer II Global Ocean Sampling (GOS) (Rusch et al., 2007), the Malaspina Expedition (Duarte, 2015) and the multiples surveys of TARA Oceans (Bork et al., 2015) have significantly contributed significantly to global knowledge of microbial diversity both in surface waters as well in deep oceanic waters. Through the mentioned expeditions, as well as others, not only biological data was obtained but also physical, chemical, and biogeochemical characteristics of the marine environment were registered, allowing to integrate environmental parameters with molecular diversity to better understand ecological processes within microbial community assembly.

## 1.1 Role of bacterioplankton in the ocean.

It is now known that unlike the terrestrial environment, the biomass of the ocean is dominated by microorganisms from all three domains of life (Azam & Fuhrman, 1984; Karl, 2007). Bacterioplankton (including bacteria and archaea) constitutes between 20-30% of planktonic biomass, it is responsible for up to 50% of the total water column respiration (Azam & Fuhrman, 1984; del Giorgio & Duarte, 2002; Fenchel, 2012; Whitman et al., 1998), and is estimated to contribute between 15-30% of the total primary production of the oceans (del Giorgio & Duarte, 2002; Ducklow, 2000). The notion that bacterioplankton affect the biogeochemistry of the environment in which they are found has been gradually developed during the 20th century, and has determined the importance of bacterioplankton within the flow of matter and energy, concept known as "microbial loop" (Azam et al., 1983; Pomeroy et al., 2007).

The microbial loop describes the role of bacterioplankton in the planktonic trophic web considering two fundamental roles: (i) its mineralizing activity, which is part of the self-purification capacity of the marine ecosystem, making nutrients available to primary producers, and (ii) the production of bacterial biomass that is effectively consumed by eukaryotic predators (Azam et al., 1983). Although bacterial biomass may be less when compared to larger organisms, its impact, in terms of organic matter transformation and energy flow, is much greater, due mainly to the high metabolic and growth rates of bacterioplankton. One of the main reasons for the important role of bacteria in marine ecosystems is their metabolic diversity, which can sometimes be very specific and sometimes very broad (Madigan et al., 1999). Within this physiological diversity, heterotrophic bacteria are the most studied because of their great importance as organic matter decomposer and because they are involved in a wide variety of fundamental processes in the marine ecosystem, ranging from nutrient recycling to sediment geochemistry (Fenchel, 2012; Madigan et al., 1999).

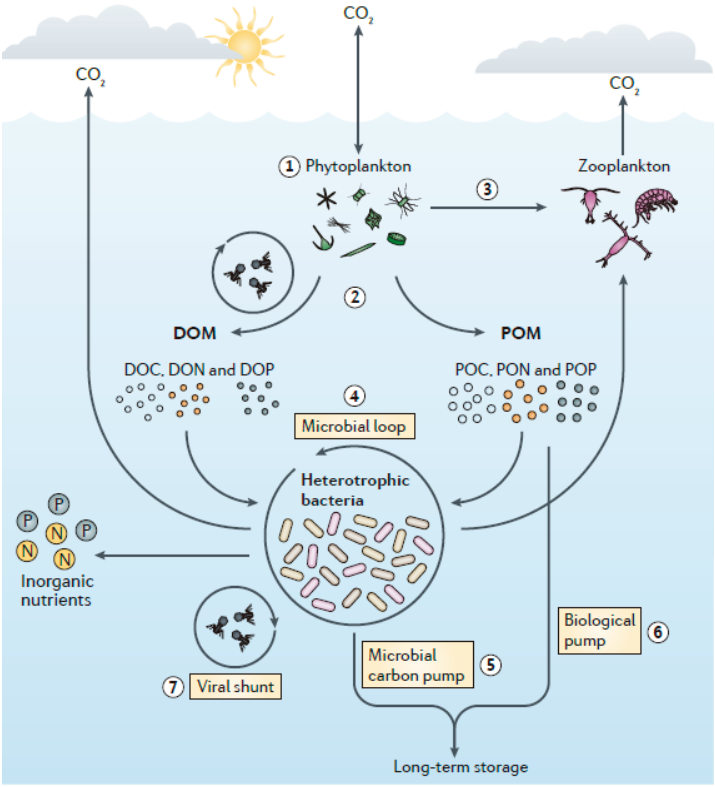



Figure 1: Microbial Loop (3) mineralization and recycling of organic matter by diverse heteroptrophic bacteria is consumed by zooplankton and the carbon is further transferred up to the food web. Heterotrophic bacteria also contribute the remineralization of organic nutrients, including DON and DOP, to inorganic forms which are then available for use by phytoplankton. (Figure from Buchan et al., 2014)

## 1.2 Microbial community structure.

Understanding the process and patterns that structure communities are the central goals of ecology. The community structure of a prokaryotic community can be described in terms of its taxonomic composition and relative abundance (Konopka, 2009; Nemergut et al., 2013). Biological diversity can be defined as "the variety and abundance of species in a defined unit of study" (Magurran, 2004). By studying biodiversity distribution over space and time, the processes that generate and maintain diversity can be elucidated (Martiny et al., 2006). Furthermore, by

quantifying and comparing biodiversity across ecosystems we can examine the effects that different ecological processes have on community structure (Nemergut et al., 2013). Whittaker (1972) proposed that species diversity can be described at three levels: within localities (alpha diversity), between localities (beta diversity) and over an entire geographical location (gamma diversity).

Alpha diversity can be expressed in terms of the number of species/OTUs (richness), or by computed metrics, per unit area or per sample and according to the even distribution of the species content by area (evenness) (Thukral, 2017). There are several alpha diversity metrics that describe biodiversity by considering the number of taxa present in each sample (e.g., Chao index) while others that also include information on evenness in terms of relative abundance (e.g., Shannon index) (Chao, 1987; Shannon & Weaver, 1962). To further understand how microbial communities can structure over space and time the study of beta diversity must be incorporated into the study of microbial community structure (Vellend, 2010). Among beta diversity two types of diversity can be described: turnover and nestedness (Anderson et al., 2011; Baselga, 2010). The turnover refers to changes in community structure (identity, relative abundance, biomass and cover of individual species) that occur from one place to another over a spatial, temporal or environmental gradient (Anderson et al., 2011). Nestedness refers to a variation in the composition of the community structure that can occur when the biota of a site with a lower number of species are subset of the biota of a richer site (Baselga, 2010). Since many factors can affect the number of species and alter species dominance over space and time, understanding what generates changes in the alpha or beta diversity of a community is important when considering the process that shape community structure.

Marine prokaryotic communities are extremely diverse with a wide range of diversity, morphology, physiology and behavior (Sunagawa et al., 2015). Due to the high heterogeneity of the microbial habitats and the distinct scales where microorganisms play an important role, the study and description of microorganisms diversity is extremely challenging (Nemergut et al., 2013). For instance, the detection of abundant microorganisms requires minimal sampling effort, whereas the recovery of genome sequences of low frequent microorganism requires a deep sampling effort (Sogin et al., 2006). Improvements in sequencing depth (high-throughput sequencing) has allowed the description of microbial communities to go from hundreds to thousands of sequences read (Galand & Logares, 2019). This has made it possible that a greater

diversity could be explored by including those taxa with a low abundance (Sogin et al., 2006). The discovery of the "rare biosphere", term coined by Sogin et al. (2006), revealed that low abundance taxa were important contributors to α-diversity and β-diversity assessments (Lynch & Neufeld, 2015).

Nowadays, it is increasingly recognized that microbial assemblages cannot be defined without reference to their surrounding environment on different spatial and temporal scales (Fuhrman et al., 2015). Marine bacterial communities participate in the biogeochemical cycles of important elements such as carbon, nitrogen, phosphorus, sulfur, and iron, contributing in this way to the marine ecosystem function (Fuhrman et al., 2015). Alpha diversity measurements at different locations and depths have revealed high diversity over a range of hundreds of OTUs in the marine ecosystem (Salazar & Sunagawa, 2017). Furthermore, recent evidence indicates that microbial community function correlates strongly with microbial community composition in the marine environment (Galand et al., 2018). Therefore, the study of community structure (diversity and abundance) acquires a fundamental role in understanding the role played by microbial communities in a specific ecosystem.

Marine ecosystems are dynamics and vary over space and time. Profound physical, chemical, and biological changes can occur in a transect from the coast to the open ocean, and from surface to the deep ocean (DeLong & Karl, 2005). Some factors are described to influence the distribution, abundance, and function of microbial communities. Physical, chemical and biological parameters of the marine environment such as temperature, salinity, depth and chlorophyl-a are the most well documented factors influencing microbial community structure in aquatic systems (Abell & Bowman, 2005; Balmonte et al., 2020; Bano et al., 2004; Campbell & Kirchman, 2013; Falcón et al., 2008a; Fortunato et al., 2013; Fortunato & Crump, 2011; Gutiérrez et al., 2018; Kim et al., 2020; Liu et al., 2020; Luria et al., 2016; Picazo et al., 2019; Sunagawa et al., 2015). Additionally, other physical and spatial variables such as latitude, water masses and oceanic fronts have also been described to influence the community structure of marine prokaryotes (Agogué et al., 2011; Brown et al., 2012; Friedline et al., 2012; Fuhrman et al., 2008; Galand et al., 2010; Ghiglione et al., 2012; Wilkins, et al., 2013a; b) thus highlighting the existence of biogeographical patterns in the structure of microbial communities. However, even though biogeographical patterns in free-living bacteria have been extensively addressed, certain

biogeographical mechanisms still need to be explored in poorly studied regions of the world such as the Southern Ocean.

The large number of publications showing that the diversity and abundance of marine bacteria differs according to locations, and that these differences may be correlated with environmental factors (Pommier et al., 2007), challenges the idea that "everything is everywhere" (Baas Becking, 1934). Microbial biogeography aims to reveal how the distribution and abundance of microorganisms are shaped by their physical context (Hanson et al., 2012). Microbial community diversity can be shaped across time and space due to a combination of fundamental ecological processes, selection, drift, dispersal and diversification (mutation) (Hanson et al., 2012; Nemergut et al., 2013). These four processes can be clearly observed in the most commonly biogeographic pattern described for microorganisms, the distance-decay relationship (Casteleyn et al., 2010; Cho & Tiedje, 2000; Green et al., 2004; Hewson et al., 2006; Horner-Devine et al., 2004). Selection that produces differences in microbial composition between locations will tend to produce a distance-decay relationship in which the compositional similarity between any two locations will decrease as the geographic distance between them increases (Hanson et al., 2012; Soininen et al., 2007). Drift differentiates microbial composition over space (Soininen et al., 2007), therefore, the distance-decay pattern associated to drift will be more important for microorganisms subject to restricted dispersal due to large spatial scales (e.g., between poles) or in less fluid environments (e.g., in oceanic fronts). Conversely to selection and drift, dispersion will act by decreasing the distance-decay pattern as its rate increases in homogenous environments (Hanson et al., 2012; Soininen et al., 2007). Finally, mutation will modify the distance-decay relationship by increasing local genetic diversity at all locations (Hanson et al., 2012). Cottenie (2005) examined 158 publishes data set from a diverse range of taxa, habitats, spatial scales, body sizes and dispersal mechanisms and found that 50% of the variation in community composition was explained by environmental and spatial variables. This highlights the need to incorporate local environmental parameters and geographic features in the analysis of community structure. However, despite the interest in distance-decay relationship the sum of the factors that can affect this interaction such as species-specific dispersal, specie-association interplay and seasonal patterns among others remain poorly tested. Furthermore, changes in the location of biogeographic barriers or in the environmental conditions due to global climate change could have a significant effect on the composition of microbial communities and a subsequent impact on ecosystem

functioning (Martiny et al., 2006). Thereby reinforcing the need to study in greater depth the processes that structure the biogeography of bacterioplankton communities in vulnerable environments.

In summary, the processes that structure microbial communities can be understood by describing the diversity and relative abundance of the organisms that compose them. However, it is also necessary to consider a detail study of the physical, chemical, and biological conditions that surround the community in order to suggest a role for the prokaryotic community in the functioning of the marine ecosystem.

## 2. Southern high latitude marine ecosystems

High latitudes marine ecosystems (HLME) refer to polar regions that include marine and freshwater regions. The southern HLME is an area that have experienced relatively low direct anthropogenic impacts, but face threats from global climate change because of vulnerabilities of their ecosystems and dependent human systems (Iriarte et al., 2019, 2018, 2010). The southern HLME can be divided into two main geographic regions, Subantarctic and Antarctic. The Antarctic and Subantarctic areas are separated by the Antarctic Circumpolar Current (ACC), which flows through Drake Passage acting as a natural barrier limiting the connection between these two southern areas (Lawver et al., 2013). However, it may been suggested that climate change may alter this barrier, generating an opportunity for connectivity between both geographical regions (Iriarte et al., 2019).

### 2.1 Subantarctic Region

*Hydrography and physico-chemical characteristics*

The Subantarctic region is one of the largest freshwater reservoir worldwide, and its numerous fjords and channels are regarded as a future key region for aquaculture and for hydroelectric power production (Iriarte, 2018). This region supports a unique ecosystem that is undergoing continuous change through evolutionary processes that have been operating since the

opening of the Drake Passage and the formation of the Antarctic Circumpolar Current (ACC) ca. 30 million years ago (Lawver et al., 2013). The Chilean Patagonia, located in the subantarctic region, is an extensive area composed of channels and fjords regulated by a mixture of continental and oceanic waters (González et al., 2013). Specifically, fresh and cold water from multiple sources such as river discharges, high rainfall and glacier melt, mixes along the channels and fjords with the warmer and saltier ocean water, generating an estuarine circulation (Iriarte et al., 2010; Sievers & Silva, 2008; Silva & Vargas, 2014). These freshwater inputs influence the biogeochemical and organic matter cycles in these ecosystems through the incorporation of nutrients such as silica, phosphate, nitrogen (Iriarte et al., 2018; Montero et al., 2017; Torres et al., 2014) and through the high content of particulate (POM) and dissolved (DOC) organic matter (González et al., 2013; Rojas & Silva, 2005).

Fjords are ecosystems highly sensitive to climate change that play a key role in the exchange of organic matter and carbon flow between terrestrial and marine environments (Bianchi et al., 2020; González et al., 2013; Iriarte et al., 2010). The microbial communities that integrate these ecosystems are largely structured by inputs of nutrients and organic matter (Montero et al., 2011). The productivity of the Patagonian fjords shows a latitudinal pattern of decrease towards the south mostly associated with changes in temperature, salinity, nutrients input (Si, N, P) and Photosynthetically Active Radiation (PAR) (Iriarte et al., 2018; Jacob et al., 2014; Torres et al., 2014). The low silicic acid (Si) and nitrogen (N) concentrations provided by glacier water runoff characteristics of the southern fjords are unable to support the high levels of primary productivity that is observed in more central and northern Patagonian fjords (Iriarte et al., 2018). Along with this, glacier runoff provides a plume of inorganic matter that attenuates light penetration limiting the depth of the euphotic layer (Montecino & Pizarro, 2008). The combination of low PAR irradiance coupled with a low supply of essential nutrients (N/Si) from glacier runoff would favors the growth of small autotrophic cells (< 20 µm) such as nano and picophytoplankton and cyanobacteria that are the main phototroph organisms sustaining sustaining primary production in southern fjords (Cuevas et al., 2019; Iriarte et al., 2018).

*Microbial communities*

The bacterioplanktonic community of high latitude fjords is mostly impacted by changes in the temperature/salinity of the water column (Gutiérrez et al., 2015, 2018) and by the amount

of organic/inorganic material transported through the proglacial river (Delpech et al., 2021; Montero et al., 2011). The input of organic matter from proglacial rivers is capable of sustaining a significant level of bacterioplanktonic secondary production even during autumn-winter months when primary production is low in the water column (Montero et al., 2011). Therefore, a large part of the organic material of allochthonous or local origin is processed by the bacterioplankton providing a permanent route for the transfer of organic matter to higher trophic levels (Montero et al., 2011). Furthermore, changes in DOC origin (snowmelt, spring bloom and permafrost thaw) and quantity, can generate temporal shifts in nearshore costal community structure of artic high latitude fjords (Delpech et al., 2021). DOC enrichment favor copiotrophs organisms such as *Sulfitobacter* and *Octadecabacter* (*Alphaproteobacteria*), meanwhile during low DOC concentrations and increased inflow of oceanic waters, the community structure change to an oligotroph dominance represented by *SAR11, SAR92, OM60* and *Verrucomicrobia* (Delpech et al., 2021). Because of the constant glacier freshwater input water temperature and salinity are probably the most important factor that drives the bacterioplankton community structure in artic high latitude fjords. In the northern section of Patagonia, changes in the composition of fjords bacterioplankton community have been related to variations in the temperature and salinity of the water column mediated by glacier input (Gutiérrez et al., 2015). During high meltwater discharge the surface community was dominated by microorganisms adapted to cold and freshwater such as *Cyanobacteria, Alphaproteobacteria, Betaproteobacteria,* and *Actinobacteria* (Gutiérrez et al., 2015). In contrast during winter months, predominance of marine prokaryotic community members such as *Thaumarchaeota, Gammaproteobacteria* and *Bacteroidetes* were found in higher proportions (Gutiérrez et al., 2015). Meanwhile in central Patagonian fjords the bacterioplankton community showed to be sensitive to the increase in water surface temperature evidenced by a decrease in richness and abundance of representative taxa such as *Flavobacteriaceae* (*Bacteroidetes*), *Rhodobacteraceae* (*Alphaproteobacteria*) and *Crenarchaeaceae* (*Thaumarchaeota*) (Gutiérrez et al., 2018).

## 2.2 Antarctic region.

*Hydrography and physical-chemical characteristics*

The Southern Ocean is composed of the southernmost waters of the world's oceans (Indian, Atlantic and Pacific) (Griffiths, 2010). It plays a critical role in global ocean circulation, biogeochemical cycles and in the global climate system through the connection of basins and the upper and lower limbs of the ocean driving circulation (Mayewski et al., 2009; Rintoul, 2011). Geographically it extends to 60° S, but the limit that comprise the Antarctic region is between the Antarctic convergence (Polar Front) and the coast of the Antarctic continent (Rintoul, 2011). The surface of the Southern Ocean is composed of several zones with sharp changes in temperature and salinity conditions which are separated by oceanic fronts (Orsi et al., 1995; Sokolov & Rintoul, 2002). The Antarctic Circumpolar Current (ACC) is a major component of the Southern Ocean that transport the water masses from west to east. It divides the Southern Ocean into three major zones: the Subantarctic Zone (SAZ), the Polar Frontal Zone (PFZ) and the Antarctic Zone (AAZ) that are separated from north to south by oceanics fronts: the Subtropical Front (STF), Subantarctic Front (SAF) and the Polar Front (PF) (Sokolov & Rintoul, 2002) (Fig. 2). Each zone and front possess different levels of productivity and are characterize by distinctive biological communities (Pollard et al., 2002).

The Southern Ocean is the most biologically productive ocean absorbing a large amount of $CO_2$ (Rintoul, 2011). Furthermore, it is the largest High Nutrient Low Chlorophyll region (HNLC) of the global ocean (Sarmiento et al., 1998) where the upwelling of deep waters returns nutrients to the surface ocean for phytoplankton utilization (Rintoul, 2011). The Antarctic zone, specifically the Antarctic Peninsula (AP) and Bransfield Strait (BS), is characterized as a biological hotspot that host a productive marine ecosystem strongly supported by phytoplankton blooms and oceanographic processes that sustain large biomasses of secondary producers (e.g. Antarctic krill *Euphausia superba*), benthic communities, and top predators (Perry et al., 2019; Santora & Veit, 2013; Prezelin et al., 2004; Schofield et al., 2010). However due to the severe environmental conditions of this region such as low temperature, long periods of darkness, changes in solar radiation and seasonal shifts in ice coverage microorganisms are the dominant form of life (Boetius et al., 2015; Cavicchioli, 2015). Eukaryotic phytoplankton is a major component of the Southern

Ocean microbial ecosystem which perform a large proportion of primary production in costal and continental shelf of the Antarctic zone (El-Sayed, 2005).

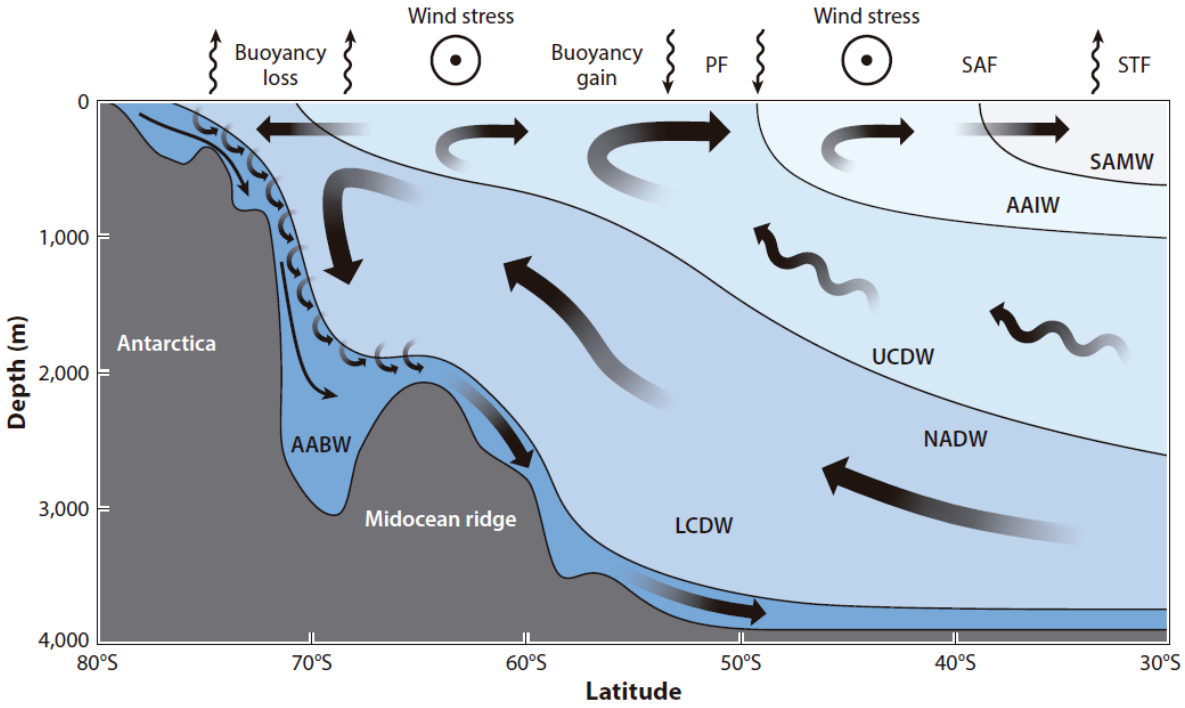



Figure 2: Schematic of the Southern Ocean meridional overturning circulation, the locations of the ocean fronts and water masses, and atmospheric forces and fluxes. Abbreviations: AABW, Antarctic Bottom Water; AAIW, Antarctic Intermediate Water; AAZ, Antarctic Zone; LCDW, Lower Circumpolar Deep Water; NADW, North Atlantic Deep Water; PF, Polar Front; PFZ, Polar Front Zone; STF, Subtropical Front; UCDW, Upper Circumpolar Deep Water. (figure from Gent, 2016).

*Microbial communities*

Many areas of the Southern Ocean have been sampled for the purpose of characterizing microbial community diversity allowing to have a good description of the main Southern Ocean marine pelagic microorganisms, but active microorganisms remain to be characterized. The distribution and abundance of prokaryotes in Antarctic waters are influenced by a complex

interplay between physical, chemical, and biological variables of each zone of the Southern Ocean. The seasonal dynamics of some heterotrophic prokaryotic communities have, for instance, been described in association with phytoplanktonic blooms in nutrient-enriched areas of the Southern Ocean (Kim & Ducklow, 2016; Liu et al., 2019, 2020; Luria et al., 2016; Obernosterer et al., 2011). In turn, in open oceanic areas, physical parameters such as temperature, depth, ice melting, advection, oceanic fronts and water circulation have been described as the major drivers shaping prokaryotic community structure (Alcamán-Arias et al., 2021; Piquet et al., 2011; Wilkins et al., 2013 a;b). In some coastal areas of the Antarctic region such as bays and fjords, the combination of environmental parameters, including salinity, nutrients and dissolved/particulate organic/inorganic matter, are described as major structuring factors (Moreno-Pino et al., 2016; Kim et al., 2020; Picazo et al., 2019).

The most abundant groups of heterotrophic prokaryotes in the Southern Ocean are *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes* (Abell & Bowman, 2005; Alcamán-Arias et al., 2021; Hernández et al., 2015a; Kim et al., 2014;Kim et al., 2020; Liu et al., 2019, 2020; Luria et al., 2016; Moreno-Pino et al., 2016; Picazo et al., 2019; Signori et al., 2014; Wilkins, et al., 2013a;b). Among Alphaproteobacteria, SAR11 is often dominating in both coastal and open ocean areas (Ghiglione et al., 2012; Giebel et al., 2009; López-García et al., 2001; Murray & Grzymski, 2007; Piquet et al., 2011). SAR11 has a biogeographic distribution in the Southern Ocean that is mainly mediated by water temperature (Brown et al., 2012). The clade is more abundant in subantarctic and polar front areas where HNLC conditions prevail, given it a competitive advantage compared to Antarctic zone characteristic where phytoplankton blooms result in increased concentrations of high molecular weight (HMW-DOM) that has been found to decrease their abundance (Ghiglione & Murray, 2012; Giebel et al., 2009). Furthermore, SAR11 abundance has been inversely correlated with chlorophyll-a levels and consistently found far from phytoplankton blooms (West et al., 2008). Among other Alphaproteobacteria, *Sulfitobacter* is the most abundant member of the *Roseobacter* clade in the Southern Ocean (Grzymski et al., 2012). Known for its ability to use organic sulfur compounds such as dimethylsulfoniopropionate (DMSP), commonly released during phytoplankton bloom decay (Moran et al., 2007). As for its distribution, *Sulfitobacter* is much more abundant in the spring-summer season than in autumn-winter (Ghiglione & Murray, 2012) associated to phytoplankton blooms and melting sea ice (Grzymski et al., 2012).

For *Gammaproteobacteria*, the SAR86 clade (*Oceanospirillales*) has been detected in surface waters of the Southern Ocean (Abell & Bowman, 2005; Obernosterer et al., 2011; West et al., 2008). Genomic analysis of partial SAR86 genomes showed a metabolic streamlining genome that appeared specialized for utilizing lipids and carbohydrates (Dupont et al., 2012). Other abundant member of the *Gammaproteobacteria* class are *Alteromonadales*, which include heterotrophs with broad substrate preferences. Within this class, *Cowellia* is a common member of polar systems with adaptation to cold environment through the production of cold active extracellular enzymes (Methe et al., 2005). The OMG group contains physiologically diverse heterotrophs of the clades OM60, BD1-7, KI89A, OM182 and SAR92 (Cho & Giovannoni, 2004). These groups have been detected in several zones of the Southern Ocean such as Subantarctic zone, Antarctic Peninsula and Kerguelen Island (Indic Ocean) (Grzymski et al., 2012; Obernosterer et al., 2011; West et al., 2008; Williams et al., 2013a;b). They are thought to degrade organic carbon produced by phytoplankton blooms because higher abundances were found inside bloom in the Kerguelen Islands (Obernosterer et al., 2011) and during the summer season (Grzymski et al., 2012; Williams et al., 2013c).

Among *Bacteroidetes*, members of the class *Flavobacteria* are major components of the planktonic communities in the Southern Ocean, especially prevalent in particle attached communities and in association with phytoplankton blooms (Abell & Bowman, 2005). This class has a well-described ability to degrade organic matter, suggesting an important role in the remineralization of primary production products (Kirchman, 2002). The biogeographical patterns associated with *Flavobacteria* show a greater presence in waters rich in nutrients and phytoplankton such as those found south of the polar front in the Antarctic zone (Abell & Bowman, 2005). The genus *Polaribacter* dominates *Flavobacteriales* populations and activity in the Southern Ocean (Abell & Bowman, 2005; Ghiglione & Murray, 2012; Murray & Grzymski, 2007; West et al., 2008).

## 2.3 Climate change effects.

Anthropogenic climate change is having a significant effect on the high latitude marine ecosystems in particular in the Subantarctic and Antarctic regions (González et al., 2019). The

Southern Ocean has been warming faster than the global ocean average (Rintoul, 2011). Along with this, the loss of continental ice in the Antarctic has accelerated more than six times in the last four decades been more critical in the west Antarctica (Rignot et al., 2019). Nearly 80% of South America's glaciers are in Chilean territory, between Patagonia and Tierra del Fuego (Rivera et al., 2000). According to records from 1986 to 2016 the total glaciers area has experienced a loss of approximately 8.6% (2,182 ± 1,276 $km^2$) (Meier et al., 2018) from which the greatest losses of ice are observed in the southern Chilean Patagonia (Dussaillant et al., 2019).

The effects of freshwater input on near marine ecosystems are complex and have multiple implications. In the Antarctic region the continental ice loss is responsible for a 10% of global sea level rise (Rignot et al., 2019) while the constant melting of the southern and northern ice fields (Patagonia) is equivalent to an annual sea rise between 0.078 and 0.105 mm (Rignot et al., 2003). In the Antarctic and Subantarctic regions most of the freshwater inputs are channeled through fjords to the costal ocean (Iriarte et al., 2014). It has been shown that the impacts of glacier discharge at mesoscale include nutrient upwelling, seawater iron enrichment, modification of the carbonate system and enhanced stratification (Hopwood et al., 2020; Iriarte, 2018).

On the West Antarctic Peninsula, the atmospheric warming has reached a 7°C over the last 50 years (Ducklow et al., 2013). This warming has contributed to the melting of glaciers and the collapse of numerous ice shelves by warming the sea surface (González et al., 2019). This warming has caused changes in the structure of the water column causing an increase in the stratification of the water column and a greater input of micronutrients such as iron (Höfer et al., 2019; Hopwood et al., 2020; Meire et al., 2017). This stratification acts by reducing the vertical supply of nutrients to the surface ocean and the concentration of dissolved oxygen in the ocean interior (Hopwood et al., 2020; Meire et al., 2017).

In summary, the impact of climate change on the southern high latitude ecosystems will have global ecological consequences. The response of marine microbial communities to climate change effect will have profound implications in the productivity of this ecosystems. Thus, the high latitude marine and freshwater environment provides ideal habitats for studying changes in microbial community composition and function in response to global climate change.

## 4. How to study microbial community structure in the ocean?

In 1980 the suitability of the small subunit (SSU) of the ribosomal RNA (rRNA) gene for inferring phylogenetic relationship between prokaryotic organisms was stablished (Fox et al., 1980). Latter Pace and colleagues were among the first to estimate the composition of natural mixed microbial communities from environmental samples through the analysis of 16S rRNA gene sequences obtained by cloning and subsequent Sanger sequencing (Pace et al., 1986). This work eliminated the limitation imposed by the dependence on culture methods for the study of microbial diversity. For decades, amplifying, cloning, and sequencing the entire 16s rRNA by Sanger technology was a widely used standard protocol for taxonomic identification and classification, however, the extensive cloning process and the total cost of the analysis were its major drawbacks.

High-throughput amplicon sequencing (HTS) of microbial communities is based on the amplification of housekeeping genes fragments through Polymerase Chain Reaction (PCR) obtained from DNA of environmental samples. The small subunit (SSU) ribosomal RNA (rRNA) gene (prokaryotes: 16S rRNA gene, eukaryotes: 18S rRNA gene) has been adopted as a universal phylogenetic marker gene for community analysis due to (i) its presence in all cells as a structural component of the ribosome, (ii) its lack of horizontal gene transfer, (iii) its high conservation, (iv) its sufficiently length, and because (v) it exhibits both conserved and hypervariable regions providing reliable binding sites for universal PCR primers (Pace et al., 1986). However, at molecular level, one of the major disadvantages of the 16S rRNA gene is that the copy number of rRNA genes varies greatly, from 1 to 15 copies in some bacteria (Rastogi et al., 2009). As a result of this variation the relative abundance of rRNA genes in environmental samples can be attributed both to variation in the relative abundance of different organism, and to variation in genomic rRNA copy number among those organisms (Fogel et al., 1999; Kembel et al., 2012). Despite of this molecular disadvantage, the 16S rRNA gene continues to be the method of choice for multiple surveys. Taxonomic classification is made through cross-referencing with sequences from databases therefore the taxonomic classification is as good as the underlying database. Among the more complete and update databases the Ribosomal Database Project (RDP) (Cole et al., 2014) and SILVA project (Pruesse et al., 2007) offer quality controlled databases of aligned rRNA sequences from the three domains of life.

Genomic surveys using HTS have been an efficient way to census the microbial diversity of the oceans. Certainly, HTS has enabled that marine microbial ecologist can directly access the diversity and functional potential of microbial species in natural communities. Since the work published by Sogin et al. (2006), many researchers have use of HTS technologies to describe the structure of marine microbial communities. However, the structure of bacterioplanktonic communities as well as the processes that generate them remain poorly explored in certain areas such as the Southern Ocean and southern Chilean Patagonia. Additionally, descriptions of bacterioplanktonic community composition in southern high latitude environments have been based traditionally on the total community while none have explored the active component of the community.

In summary, information provided by the amplification of 16s rRNA fragments is still valuable and provides the necessary foundation for subsequent field and laboratory experiments. However, the selection of primers, library preparation protocols, and sequencing analysis protocols should be thoroughly designed to avoid or minimize bias on the resulting profile of the microbial communities surveyed.

## 5. Objectives of the thesis

The findings summarized above shed light on the importance of studying the diversity and community composition of bacterioplankton in the world oceans, and in particular in the under sampled, but globally important, Subantarctic and Antarctic ecosystems. The general aim of this thesis was to investigate the structure of microbial communities in relation to geographical, physical, chemical, and biological parameters in southern ecosystems.

This thesis represents a microbial ecology study at a regional scale (Chapter 1) and at a global scale (Chapter 2). In chapter 1, the active microbial communities were characterized in the Chilean southern Patagonia. The aim was to study the local variability of microbial communities in two subantarctic fjords. More specifically we tested (i) if fjords with similar climate and localization, but with distinct freshwater inputs, have different prokaryotic communities, and (ii) if the prokaryotic community of Yendegaia fjord present inter-annual changes. In addition, the study helped to understanding the effect of changing climate on microbial community structure and thus indirectly on planktonic ecosystem functioning. In Chapter 2, the spatial structure of microbial communities was studied in the Southern Ocean. The aim was to investigate at a large geographic scale the community structure of the bacterioplankton in the Southern Ocean in relation to a physical, chemical, and biological gradient.

# CHAPTER I: Different active microbial communities in two contrasted subantarctic fjords

## Abstract

Microorganisms play a crucial role in biogeochemical processes affecting the primary production and biogeochemical cycles of the oceans. In subpolar areas, the increment of the water temperature induced by climate change could lead to changes in the structure and activity of planktonic microbial communities. To understand how the structure of the microbial community in Chilean Patagonian fjords could be affected by climate change, we analyzed the composition of the prokaryotic community (bacteria-archaea) in two fjords (Pía and Yendegaia) with contrasting morphological and hydrological features. We targeted both the standing stock (16S rDNA) and the active fraction (16S rRNA) of the microbial communities during two consecutive austral winters. Our results showed that in both fjords, the active community had higher diversity and stronger biogeographic patterns when compared to the standing stock. Members of the *Alpha-*, *Gamma-* and *Delta-proteobacteria* followed by archaea from the Marine Group I (MGI, *Thaumarchaeota*) dominated the active communities in both fjords. However, in the Pía fjord, which has a marine-terminating glacier, the composition of the microbial community was directly influenced by the freshwater discharges from the adjacent glacier, and indirectly by possible upwelling phenomenon that could bring deep sea bacteria such as SAR202 to the surface. In turn, in the Yendegaia, which has a land-terminating glacier, microbial communities were more similar to the ones described in oceanic waters. Furthermore, in the Yendegaia fjord, inter-annual differences in the taxonomic composition and diversity of the microbial community were observed. The Yendegaia fjord, without glacier calving, represents a fjord type that will likely be more common under future climate scenarios. Our results showing that it had different communities, with for example more nitrogen-fixing (*Planctomycetes*) microorganisms, indicate that, as a result of climate change, the changing planktonic communities could significantly impact biogeochemical processes in subantarctic fjords.

## Introduction

The Chilean Patagonia comprises one of the main fjord areas in the world (Iriarte et al., 2010). The numerous fjords are important ecosystems stressed by climate change, where freshwater input, ocean acidification and water temperature directly impact the water column of these fragile environments (Torres et al., 2011; Iriarte et al., 2018; 2019). The functioning, hydrological structure, productivity levels and dominant species of these marine and coastal systems are changing together with climate (Iriarte et al., 2010; González et al., 2013).

Two broad types of Patagonian fjords can be distinguished. Fjords of the temperate type, in which the water column does not freeze, and fjords of the subpolar type, in which water freezes in winter, but with average summer temperatures exceeding 0°C (Domack & Mcclennen, 1996; Gilbert, 2000; Howe et al., 2010). Both types of fjords may (or not) be associated with a glacier. Some of the associated glaciers can be land-terminating glaciers that are retreating and forming proglacial rivers that bring freshwater and terrestrial sediments to the fjords (Chu, 2013; Giesecke et al., 2019). Other glaciers are marine-terminating glacier where freshwater discharges occur directly from the glacier to the fjord surface, but also below (from 10 to 100 m), where the released meltwater can produce upwelling of deep sediments and nutrients (Chu, 2013; Giesecke et al., 2019). These two different types of freshwater inputs could have a distinct effects on the fjords' ecology, including their productivity (Meire et al., 2017) and phytoplankton size-structure (Cuevas et al., 2019), which in turn could lead to modifications of the fjord trophic state (Montero et al., 2011).

Two marked layers characterize the water column of Patagonian fjords. One layer is superficial with colder and less saline water that usually has high concentrations of silicic acid and suspended sediment (Valdenegro & Silva, 2003; Sievers, 2008; Silva, 2008; González et al., 2010; Iriarte et al., 2014). The other layer is more profound and influenced by a constant influx of subantarctic oceanic waters (SAAW), which has higher salinity and nutrient concentrations (nitrate and phosphate) (Valdenegro & Silva, 2003; Sievers, 2008; Silva, 2008; González et al., 2013). In some cases, the exchange of oceanic and estuarine waters can be limited by the presence of a sill at the mouth of fjords (Howe et al., 2010).

The distinct water masses characterizing fjords should promote the presence of distinct communities of planktonic microorganisms. Differences in distribution at the genus level have

been observed between the surface and bottom fjord waters in Svalbard, Norway (Zeng et al., 2009; Teske et al., 2011). In Chilean fjords changes in the physical and chemical properties of the water column, as a consequence of freshwater discharge, can also lead to variations in the microbial community composition (Piquet et al., 2010; Piquet et al., 2011; Zeng et al., 2013; Gutierrez et al., 2015). Furthermore, high sediment concentrations induced by mixing water can limit light penetration into the water column, which in turn reduces the euphotic zone, leading to changes in the microbial community composition as shown in arctic fjords of Svalbard (Piquet et al., 2010). Studies on particulate matter and sediments in fjords have also evidenced that glacial discharge has the potential to transform the microbial community structure through the transport of particles as seen in the Arctic (Bourgeois et al., 2016; Jain et al., 2019). Variations in microbial diversity can affect the microbial activity and thereafter have an impact on biogeochemical cycles (Gutiérrez et al., 2018; Balmonte et al., 2019).

The impact of fjord hydrography on the microbial community structure is now better understood, but it remains to be studied more thoroughly in the southern hemisphere. Besides, little is known about possible annual differences in community composition or variations between fjords from the same region. One study from Svalbard showed distinct microbial communities inhabiting adjacent fjords (Piquet et al., 2010), but there is no information from Patagonian fjords as the number of researches on the marine prokaryotic community is limited (Vargas et al., 2008; Mackenzie et al., 2012; Ugalde et al., 2013; Gutierrez et al., 2015; Undabarrena et al., 2016; Gutiérrez et al., 2018) in comparison to the northern and Arctic fjords (Zeng et al., 2009; Piquet et al., 2010; Zaikova et al., 2010; Piquet et al., 2011; Teske et al., 2011; Piontek et al., 2013; Roy et al., 2013; Sperling et al., 2013; Winter et al., 2013; Cardman et al., 2014; Bourgeois et al., 2016; Sinha et al., 2017a;b; Balmonte et al., 2019). Bacterial communities in high latitudes fjords are usually dominated by *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* (Zeng et al., 2009; Piquet et al., 2010; Gutierrez et al., 2015). Among archaea, *Thaumarchaeta* (MGI) appears as the most common in deep (Galand et al., 2008; 2009; 2010) and surface high latitude waters (Gutiérrez et al., 2018). However, the majority of the studies on prokaryotic community structure in high latitudes fjords have been performed on the total community (DNA fraction) (Zeng et al., 2009; Piquet et al., 2010; Teske et al., 2011; Zeng et al., 2013; Gutierrez et al., 2015; 2018) and little is known about how the active community (RNA fraction) respond to possible hydrographic variations.

The general aim of this study was to test if two Patagonian fjords with different geomorphological features had different microbial communities. We studied two fjords located along the Beagle channel in the southern Chilean Patagonia: Pía fjord (PF) featuring a marine-terminating glacier, and Yendegaia fjord (YF) featuring a land-terminating glacier, which represents a fjord under future climate scenarios. More specifically we tested (i) if fjords with similar climate and localization, but with distinct freshwater inputs, have different prokaryotic communities, and (ii) if the prokaryotic community of YF present inter-annual changes (July 2017 and July 2018). We analyzed both the total and active fraction of the communities, based on 16S rDNA and 16S rRNA, in relation to the physical, chemical, and biological properties of the water collected along horizontal and vertical transects.

## Material and Methods

Study area and sampling

Seawater samples were collected at YF and PF, which are located in the southern Chilean Patagonia (Fig. 1). Field campaigns were conducted during two consecutive austral winters (July 2017 and 2018) onboard the vessel M/N Forrest. All samples were collected with a rosette system with Niskin bottles from 0, 5, 10, 25 and 50 m depth. A total of 3 stations per fjord were sampled along a horizontal gradient which included waters close to the glacier terminus (head) and waters outside the fjord (mouth). For microbial biodiversity analysis, 2 L of water was filtered sequentially onto 3.0 µm and 0.22 µm pore size polycarbonate membrane filters (MilliporeSigma, Massachusetts, USA) and stored in RNAlater (Thermo Fisher Scientific, Massachusetts, USA) at -80°C (liquid nitrogen) until analyzed. Hydrographic data including salinity, photosynthetically active radiation (PAR) and temperature were recorded using an SBE 25 plus and an SBE 43 CTD (Sea Bird Scientific, USA).

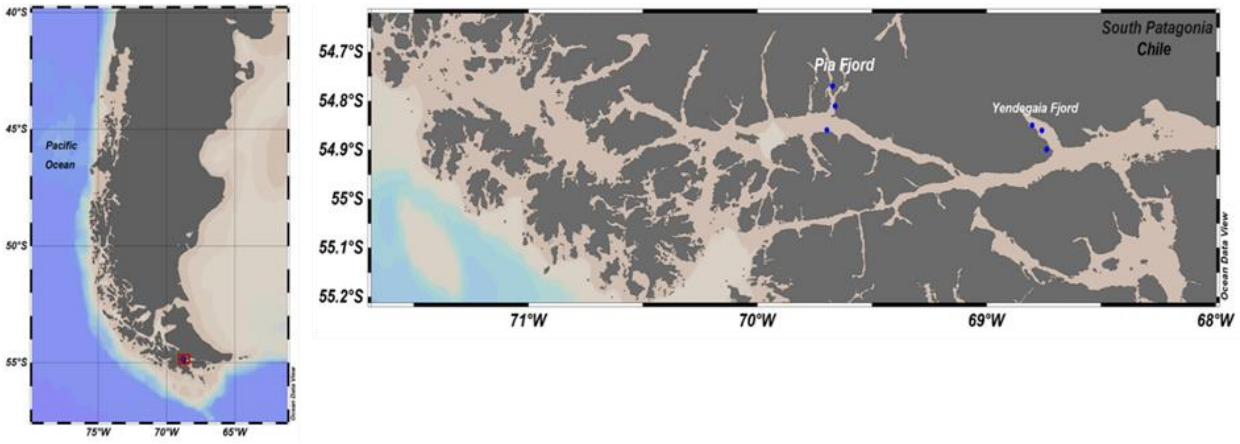



Figure 1: Study area and sampling sites in the Chilean southern Patagonia.

Samples for quantification of chlorophyll-a (Chl-a) and dissolved inorganic nutrients were collected from 1 L seawater filtered through 0.7 µm glass fiber filters (Whatman GF/F,

MilliporeSigma, Massachusetts, USA). For Chl-a determination, 200 ml of seawater was filtered (GF/F Whatman glass fiber filters, 0.7 µm nominal pore size) in triplicate and immediately frozen (−20°C) until later analysis via fluorometry (Turner Design TD-700), using acetone: water (90:10% v/v) for pigment extraction according to standard procedures (Parsons, 2013). Samples for nutrient analysis were filtered through GF/F filters and frozen at -20 °C before analysis by spectrophotometry at the Centro de Investigación en Ecosistemas de la Patagonia (CIEP) (Coyhaique, Chile) according to Strickland and Parsons method (Strickland and Parsons, 1968). To characterize Dissolved Organic Carbon (DOC), seawater samples were taken in duplicate and filtered through 0.22 µm pore size filters (Nucleopore) into pre-combusted (450°C) glass flask and acidified with hydrochloric acid at 37%. DOC samples were analyzed on an Ol Analytical Aurora Model 1030W TOC at the Hatch Stable Isotope Laboratory (University of Ottawa, Canada) according to earlier protocol (Lalonde et al., 2014). For microbial abundances, samples (1350 mL) were taken in 2 mL cryovials, fixed with glutaraldehyde (0.1% final concentration) and stored in darkness at 80°C until laboratory analysis at the Laboratory for Oceanographic Processes and Climate (Universidad de Concepción, Chile) by flow cytometry method (Marie et al., 2000). Samples for ammonium concentration were collected in triplicate and analyzed by fluorescence following earlier protocol (Holmes et al., 1999) adapted for extreme temperature ecosystems (water samples at 4°C; C. Fernández, unpublished data).

DNA and RNA extraction and sequencing

For nucleic acid extraction, the 0.2 µm frozen filters were cut with sterilized scissors into small pieces and incubated for 45 min at 37° in 840 µL of lysis buffer (40 mmol $L^{-1}$ EDTA, 50mmol $L^{-1}$ Tris hydrochloride pH 8.3 and 0.75 mol $L^{-1}$ sucrose) with 50 µL of lysozyme solution (20 mg $mL^{-1}$). Additionally, a second incubation with 50 µL of 20% Sodium Dodecyl Sulfate (SDS) and 10 µL of proteinase K (20 mg $mL^{-1}$) was completed in order to achieve cell lysis. Extraction of DNA and RNA was then performed from the lysate using an AllPrep DNA/RNA kit (Qiagen Inc, Germantown, USA) following the manufacturer instructions. The quality and the quantity of the extracted DNA and RNA were measured by spectrophotometry (Thermo Scientific NanoDrop 2000). The RNA samples were reverse transcribed to cDNA with random primers using

the SuperScript VILO cDNA synthesis kit (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) following the manufacturer's protocol. For both the DNA and cDNA, the V4-V5 region of the bacterial 16s rRNA gene was amplified using universal primers 515FB-GTGYCAGCMGCCGCGGTAA and 926R- CCGYCAATTYMTTTRAGTTT (Parada et al., 2016). Amplification and sequencing on Illumina Miseq were conducted in the commercial laboratory Integrated Microbiome Resource (IMR, Halifax, Canada) according to the protocol published earlier (Comeau et al., 2017).

## Sequence analyses

All the reads that had a mismatch with the 16S rRNA primers contained ambiguous nucleotides (N) or were <300 bp long beyond the forward primer were removed. In addition, a stringent quality trimming criterion was applied to remove reads that had ≥10% of bases with Phred values <27. This procedure is recommended to ensure that when clustering at 97% or more, the influence of erroneous reads is minimized (Huse et al., 2010; Kunin et al., 2010). The sequences were then de-replicated and clustered at a 99% sequence similarity threshold using UCLUST (Edgar, 2010) for de novo OTU picking. Representative sequences were classified against the SILVA v.128 database (Quast et al., 2013). Sequence data analyses were conducted with Pyrotagger pipeline (Kunin & Hugenholtz, 2010). Sequences selected for further analysis were compared manually to the Genbank database by BLAST. Putative chimeric sequences were removed. They were identified as sequences having the best Blast alignment <90% of the trimmed read length to the reference database and >90% sequence identity to the best Blast match.

## Statistical Analyses

The OTU sequences abundance table was transformed with an Hellinger transformation (Legendre & Gallagher, 2001) with the Vegan package (Oksanen et al., 2019) in R 3.5.3 (Team, R. C. 2018). An MDS based on Bray-Curtis similarity was conducted to visualize similarities in community composition between samples with the "vegan" package. Significant differences in

community structure among the different variables were tested with PERMANOVA with the adonis function. Indicator species analysis was conducted using the multipatt function of the "indicspecies" package in R (De Cáceres et al., 2010). Finally, Pearson correlation coefficient was calculated to analyze associations between abundance of indicator species and environmental variables.

## Results

### Environmental Variability

Temperature profiles in Pía fjord (PF) in 2017 were characterized by a strong vertical and horizontal gradient associated with the glacier freshwater discharge (Fig. 2A). The lowest temperatures (4.5 to 5.0 °C) were registered at the stations close to the glacier within the top 10 m of the water column (Fig. 2A). Warmer waters (6.0°C) were observed at 50 m depth (Fig. 2A). During 2017, Yendegaia fjord (YF) presented a relatively more homogenous temperatures distribution with overall higher temperatures (6.5°C) than PF (Fig. 2B). In turn, YF in 2018 had lower temperatures associated with the glacier at the head station at 0 to 10 m depth, and a homogenous temperature through the water column toward the Beagle Channel (Fig. 2C).

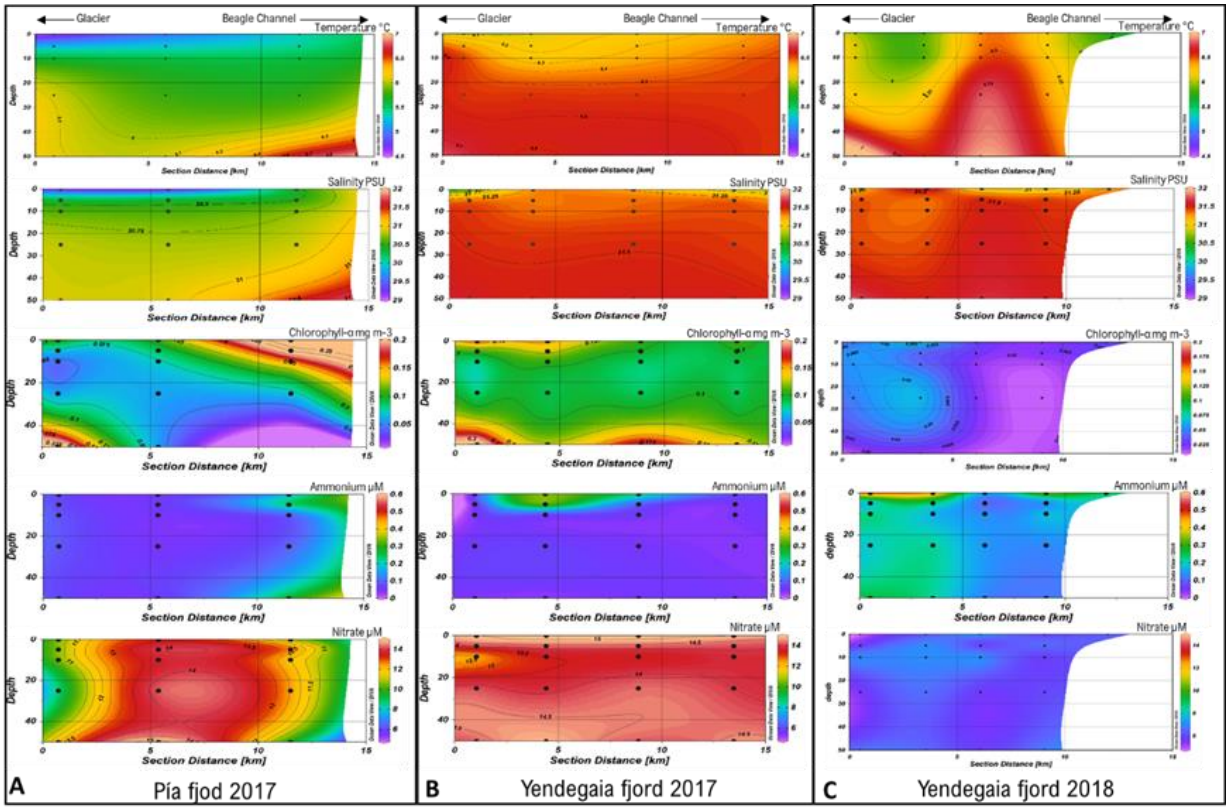



Figure 2: Distribution of environmental variables. Vertical distributions (from the top) of temperature (T°C), salinity (PSU), chlorophyll-a (mg m-3), ammonium (µM) and nitrate (µM) for Pía fjord (July 2017) and Yendegaia fjord (July 2017 and 2018).

Salinity values of PF showed an influence of the glacier freshwater inputs (28 - 29 PSU) within the top 5 m at the head and middle stations (Fig. 2A). Conversely, YF showed a homogenous salinity (around 31.5 PSU), during both years (2017 and 2018), along the horizontal and vertical transects (Fig. 2B-C), with an exception at the head station where salinity was 30.5 PSU at the surface in 2017 (Fig. 2B).

Chlorophyll a concentration varied from ~ 0.06 to 0.10 mg $mL^{-3}$ in the stations within the PF (Fig. 2A). For YF, there were marked differences between 2017 and 2018 (Fig. 2B-C). In 2017, the concentration of Chla ranged from 0.09 to 0.10 mg $mL^{-3}$ across the vertical and horizontal transects (Fig. 2B), whereas in 2018, concentrations were lower, varying from 0.01 to 0.07 mg $mL^{-3}$ across the water column (Fig. 2C).

Ammonium concentrations for PF and YF during 2017 were generally low (Fig. 2A-B), ranging from 0.04 to 0.3 µM with a maximum value (0.30 µM) in YF at the middle station within upper 5 m (Fig. 2B). In contrast, in 2018, YF had higher ammonium concentrations (0.1 to 0.5 µM) with maximal values (0.5 µM) observed at the head station in surface waters (0 m) (Fig.2C).

Nitrate concentrations in PF were lowest (8 to 10 µM) at the head station at all the depths compared to the middle station at 50 m depth (Fig. 2A). During 2017, YF had homogenous nitrate concentrations that ranged from 13 to 14 µM (Fig. 2B) across the water column. In contrast, in 2018, the nitrate concentrations decreased by half varying from 5 to 7 µM (Fig. 2C).

In terms of stratification of the water column, PF showed higher stratification than YF during 2017 with a surface layer (0 – 5 m) less saline and colder than the deeper layer (25 - 50 m) (Supplementary Fig. 1). Estuarine waters (EW) with salinity < 31 PSU (Sievers and Silva, 2008) occupied the upper 50 m of the water column. In comparison, YF 2017 had a low level of stratification with water less saline and warmer (Supplementary Fig. 1). For YF 2018, the presence of Modified Sub-Antarctic Water (MSAAW; Sievers and Silva, 2008) was observed in the whole water column across all the stations (Supplementary Fig. 2).

The maximum values of PAR were recorded in the surface layer of the water column (0-5 m) with highest values in YF 2017 across all stations (Supplementary Fig. 3). In July 2018, YF had lower PAR values compared to 2017 (Supplementary Fig. 3).

## Overall DNA and RNA community composition

We obtained a total of 2,722,567 16S rRNA/rDNA gene sequences of Bacteria and Archaea, from 78 samples taken during July 2017 and July 2018 in both fjords. A subset of the data was used to include only samples from which both DNA and RNA were successfully amplified (Supplementary Table 1). A total of 1,864,183 16S rRNA/rDNA gene sequences remained, divided between 44,006 different OTUs, of which 39,932 were assigned to Bacteria and 4,074 to Archaea.

We compared the microbial community composition among fjords and years based on both the active fraction of the community (16S rRNA) and the total community (16S rDNA) (Fig. 3). For both fjords, the RNA fraction was different from the DNA fraction on the non-metric

multidimensional scaling (NMDS) plot (PERMANOVA, p=0.001) (Fig.3). Furthermore, communities from the DNA fraction were more dispersed, as seen with the higher number of outlier samples, than communities from the RNA fraction (Supplementary Fig.4). The DNA communities also had lower community diversity than the RNA fraction (Supplementary Figure 5).

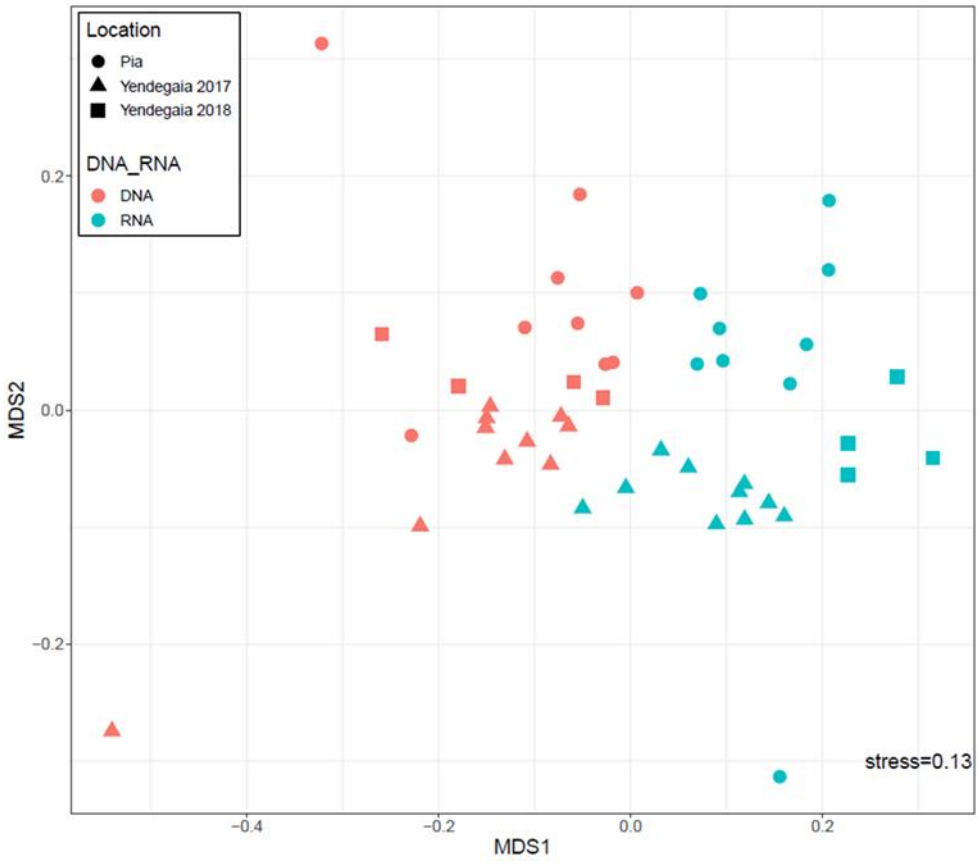



Figure 3: Non-Metric Multidimensional Scaling ordination (NMDS) based on Bray-Curtis similarity analysis of microbial communities identified in all the sampling sites. Colours represent DNA communities (red) and RNA communities (blue). Shapes represent the sampling site, Pía Fjord (circle), Yendegaia fjord year 2017 (triangle) and Yendegaia fjord year 2018 (square).

Microbial community composition of the active fraction

A Bray Curtis based dendrogram constructed from the RNA fraction revealed that microbial communities grouped according to their fjord of origin, and in the case of YF, grouping also followed the sampling year (Fig 4). The PERMANOVA test based on the Bray-Curtis dissimilarity distance matrix confirmed that the microbial community of each fjord differed following their sampling site and year ($p < 0.001$). The variable "location" better explained the differences in the microbial community composition for all the samples (Supplementary Table 2).

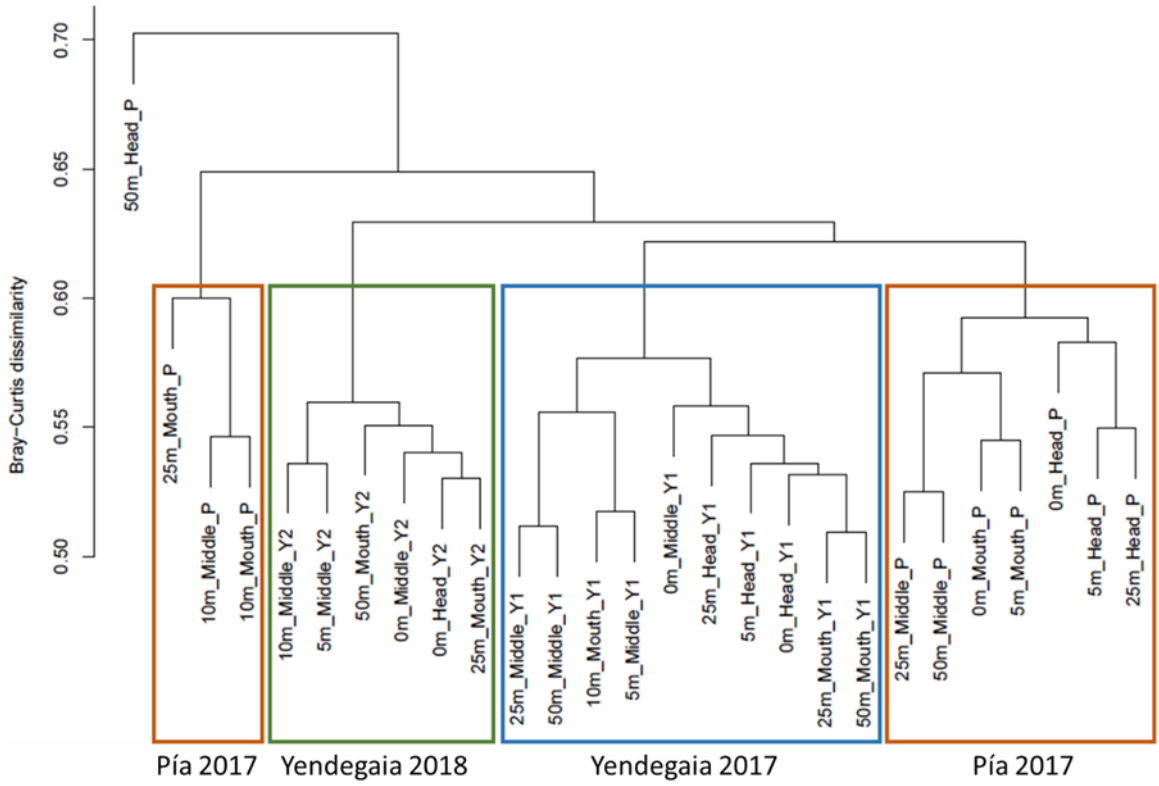



Figure 4: Hierarchical cluster dendrogram based on Bray-Curtis dissimilarity index showing the similarity between microbial community compositions for the RNA fraction in both fjords and years.

For YF 2017 and 2018, the samples did not group according to any of the spatial variables tested (horizontal and vertical). However, for PF the clustering dendrogram grouped the samples according to a horizontal gradient from head to mouth (Fig. 4), with a significant difference between the samples collected in the head of the fjord (closer to the glacier) versus the samples collected in the mouth of the fjord (PERMANOVA, p = 0.027; Table 1). No significant difference was noted for the vertical gradient (depth) in PF.

Table 1: Permutational Multivariate Analysis of Variance (PERMANOVA) examining the effects of the variables Depth (0-50 m) and Station (head, middle, mouth) in each of the locations. Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; R2, coefficient of determination; P, p-value. Significant results are bold

| Location | | Df | MS | F | R2 | P |
|---|---|---|---|---|---|---|
| Yendegaia 2017 | Depth | 1 | 0.17192 | 1.1286 | 0.12103 | 0.141 |
| | Station | 2 | 0.1673 | 1.0983 | 0.23556 | 0.178 |
| Yendegaia 2018 | Depth | 1 | 0.15031 | 0.95867 | 0.19743 | 0.7306 |
| | Station | 2 | 0.14872 | 0.94851 | 0.39068 | 0.8278 |
| Pía 2017 | Depth | 1 | 0.21996 | 1.1964 | 0.11185 | 0.135 |
| | Station | 2 | 0.27582 | 1.5002 | 0.14025 | ***0.027*** |

Across all the samples, *Proteobacteria* and *Thaumarchaeota* represented the dominant phyla, accounting for 56% and 20% of all OTUs, respectively (Supplementary Fig.3). Some less abundant phyla were also detected in most of the samples, including *Defirrebacteres* (3.7%), *Bacteroidetes* (3.4%), *Chloroflexi* (3%) and *Planctomycetes* (2.9%) (Supplementary Fig. 3). At the class level *Alphaproteobacteria* (24.8%), Marine Group I (Archaea) (19.7%), *Gammaproteobacteria* (17%) and *Deltaproteobacteria* (14%) were the most abundant in the water column among fjords and years (Fig. 5).

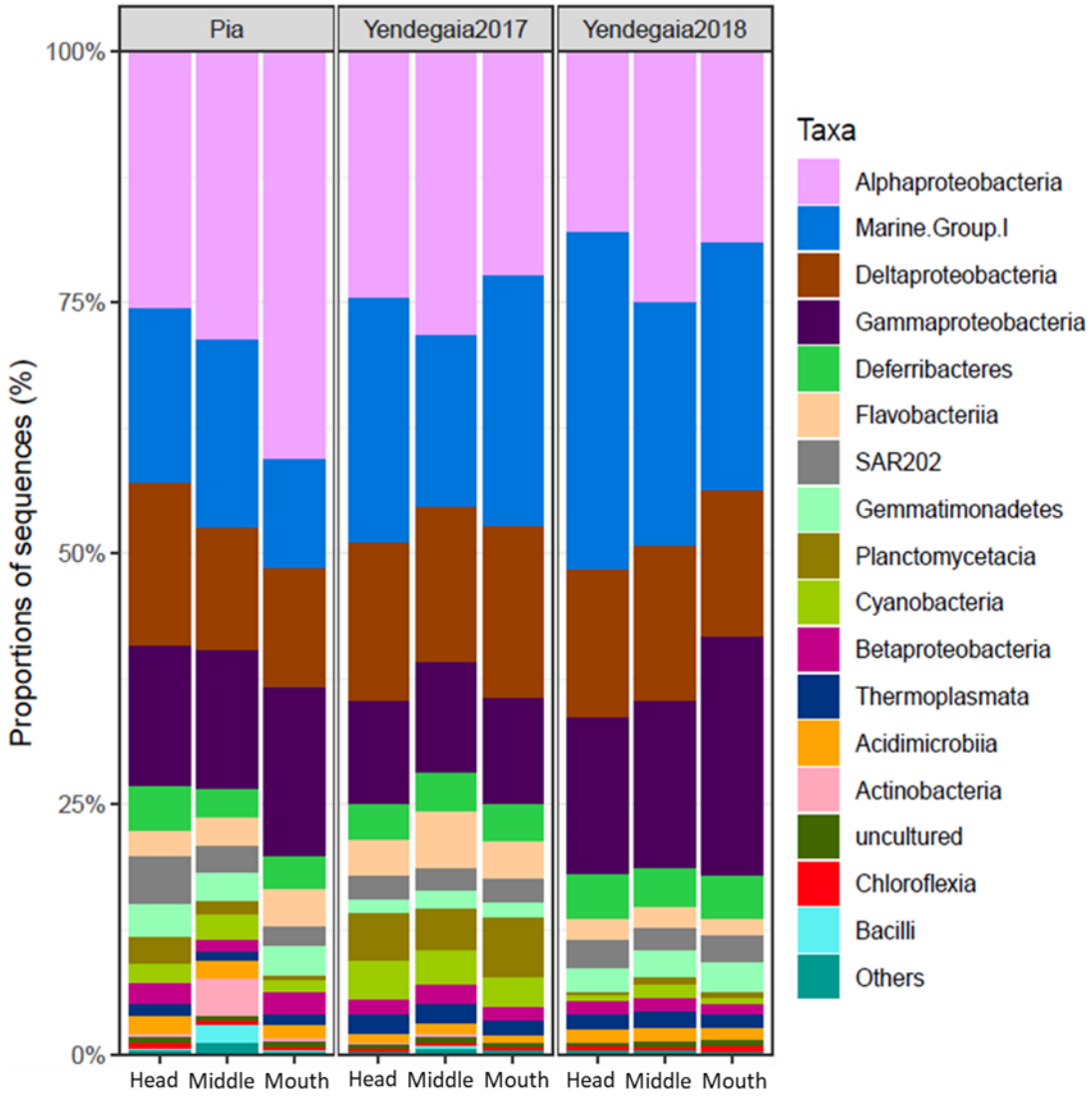



Figure 5: Relative proportion of microbial sequences at the Class level in the RNA fraction of each fjord.

The taxonomy of the microbial community at YF differed between years with a higher number of sequences of the archaea MGI *Thaumarchaeota* (26%) observed for the year 2018 in comparison to the year 2017 (21%) (Supplementary Fig. 6). Furthermore, the number of sequences for the phyla *Planctomycetes* and *Cyanobacteria* were more abundant in YF 2017 (5.4% and 3.3% respectively) in comparison to YF 2018 (0.9% and 0.9% respectively) (Supplementary Fig.6). Among *Proteobacteria*, the class *Alphaproteobacteria* were the most abundant for both years

(46.6% and 38%) (Supplementary Fig 6). The class *Deltaproteobacteria* was relatively more abundant in 2017 and the class *Gammaproteobacteria* in 2018 (Fig. 5)

Pía fjord was mainly characterized by members of the *Alpha-*, *Delta-* and *Gammaproteobacteria* with 60% of the total number of sequences, followed by *Thaumarchaeota* (14.24%) and *Bacteroidetes* (4.68%; Supplementary Fig.6). *Thaumarchaeota* were relatively more abundant at the head station compared to the inside fjord station. The classes *Actinobacteria* and *Bacilli* were only present in the PF and with more sequences at the middle station (Fig. 5), mostly in the deeper samples (25-50 m).

We identified the OTU that were responsible for the significant difference observed between fjords and years with the "Indicspecies" R package. A total of 374 indicators OTUs were significant ($p < 0.001$), indicating that they were specific for one of these environments (Fig. 6). The YF during 2018 hosted the highest number of indicator species (250 OTUs) and the greatest prokaryotic diversity (Supplementary Table 3). Members of the classes MGI, *Gemmatimonadetes* BD2-11, *Defirrebacteres*, *Thermoplasmata*, *Acidimicrobiia* and *Cyanobacteria* were indicator species present only in this fjord (Supplementary table 3). For PF, there were 15 OTU selected as indicator species, which included members of the classes *Acidobacteria* and *Chloroflexi*-SAR202 (Supplementary Table 3). In YF in 2017, 36 OTU were indicator species including members of the classes *Flavobacteriia*, *Planctomycetacia* and *Verrucomicrobiae* (Supplementary Table 3). The *Verrucomicrobiae* class was an indicator species found only in the YF during 2017. Lastly, the phylum *Proteobacteria* was selected as indicator species across all the locations with a high number of different OTU sequences (173) (Supplementary Table 3).

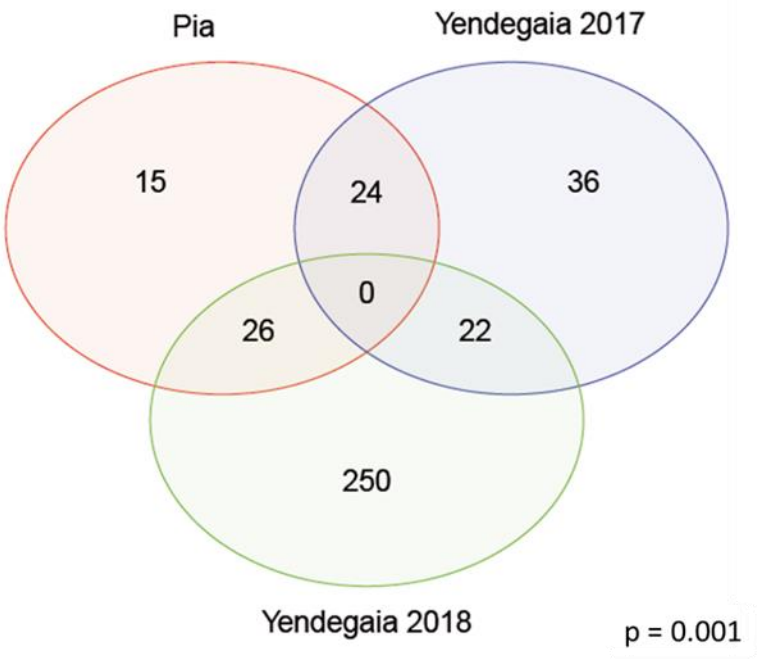



Figure 6: Venn diagram representing how many indicator microbial taxa are exclusive or share among the different sampling sites.

Correlations between indicator species and the environmental variables were determined for each fjord. In general, there were no significant correlations between indicator species and environmental variables in each fjord, except for the positive correlation between Nitrate and OTU105211_*Rhodospirillales* for the PF at the head station ($p = 0.05$) (Fig. 7), and the negative correlation for the OTU1238_*Alcanivorax* and temperature in YF during 2018 at the middle station ($p = 0.01$) (Fig. 9).

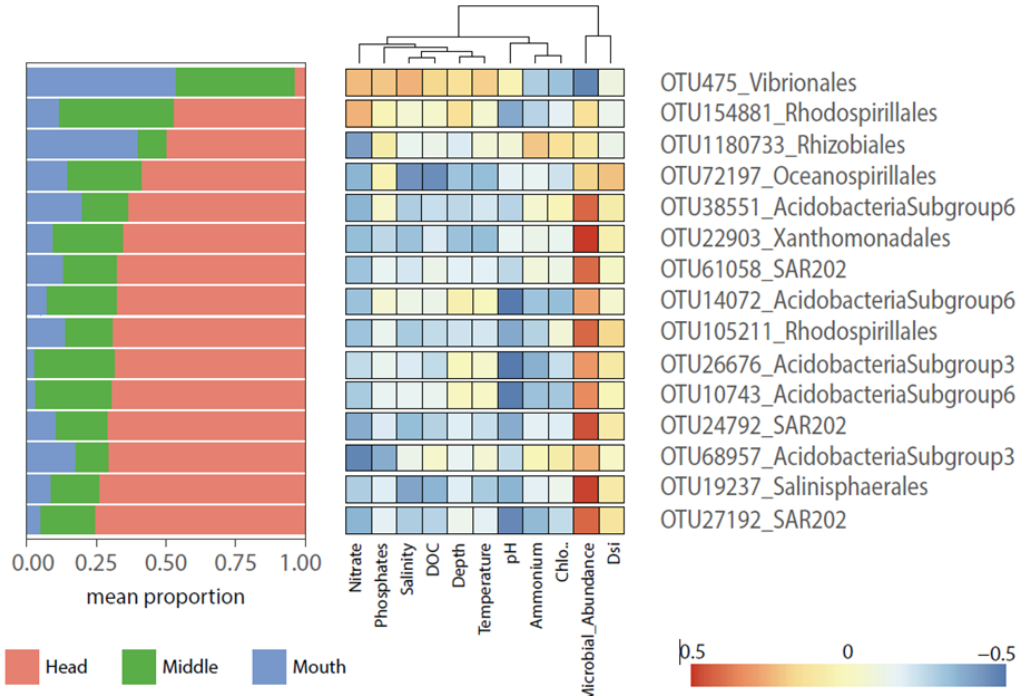



Figure 7: Heatmap displaying the indicator species (p = 0.001) for the Pía fjord. Left panel shows the mean relative contribution of the indicator species to microbial community detected by stations (Head, Middle and Mouth). Right panel shows heatmap of Pearson correlation between indicator species (OTU abundance) and environmental factors.

The major proportion of indicator species selected for PF were found at the head station except for the OTU475_*Vibrionales*, which was more present at the mouth station (Fig. 7). OTU475_*Vibrionales* show a pattern of correlation different to the rest of the OTU indicators, with a positive correlation with nitrate, phosphate and salinity and a negative correlation with microbial abundance (Fig. 7). In contrast, most of the other OTU indicators in PF (11 OTU) were negatively correlated with nitrate and positively with microbial abundance (Fig. 7). The OTUs *Acidobacteria* subgroup 6-3 (14072, 26676 and 10743) and the *Chloroflexi*-SAR202 (OTU 27192) were negatively correlated with pH, and the *Oceanospirillales* (OTU72197) was negatively correlated with phosphate and salinity.

For YF in 2017, 15 OTUs indicators were selected based on their abundance. In general, the OTUs showed to be more abundant at the middle station, except the OTU311408 (*Oceanospirillales*) and OTU165951 (*Rhodobacterales*) more abundant at the head station (Fig.

8). The environmental variables temperature, depth, and salinity were the variables that showed a better pattern of correlation with 5 OTUs (Fig. 8). A positive correlation was observed for the OTU43589/OTU60627 (*Bdellovibrionales*), OTU135042 (*Desulfuromonadales*) and OTU1033980 (*Verrucomicrobiales*), while the OTU45924 (*Rickettsiales*) and OTU45349 (*Plantomycetales*) correlated negatively (Fig. 8). Finally, strong positives correlations were observed for the OTU165951 (*Rhodobacterales*) and DOC and OTU44058 (*Rickettsiales*) with Chla and Dsi (Fig. 8).

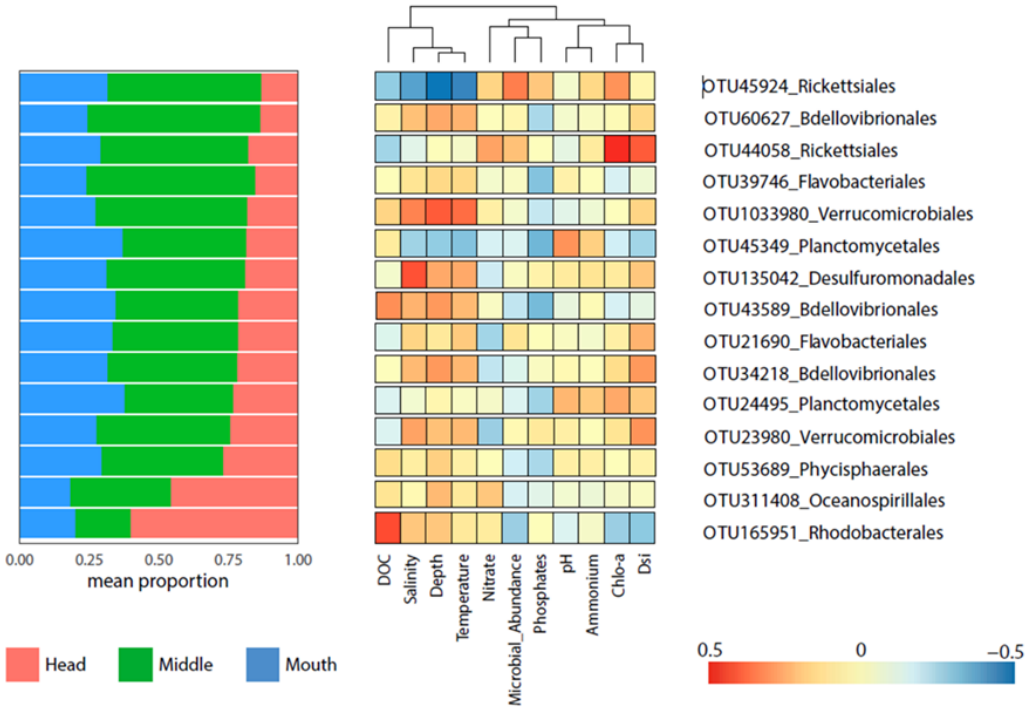



Figure 8: Heatmap displaying the indicator species (p = 0.001) for the Yendegaia 2017 fjord. Left panel shows the mean relative contribution of the indicator species to microbial community detected by stations (Head, Middle and Mouth). Right panel shows heatmap of Pearson correlation between indicator species (OTU abundance) and environmental factors.

For YF 2018, 15 OTUs indicators were selected with the same criteria as in YF 2017. The selected OTUs were mostly present at the stations Middle and Mouth (Fig. 9). The indicator species *Alteronomas* (OTU205/762), *Glaciecola* (OTU1468) and *Gemmatimonadetes*

(OTU86342), which were relatively more abundant at the mouth station, were positively correlated with salinity and temperature (Fig. 9). The OTU131586 (*Acidomicrobiales*) and OTU103964 (*Thermoplasmatales*), both relatively more abundant at the middle station, showed a similar pattern of correlation but in an inverse manner, with *Acidomicrobiales* positively correlated with salinity, nitrate and Dsi (Fig. 9). Furthermore, the OTU241565 (MGI) and OTU41001 (*Defirrebacterales*-SAR406) exhibited a positive correlation with temperature, phosphate, and depth, meanwhile both had a negative correlation with Chla, pH and ammonium. Conversely, the OTU1238 (*Alcanivorax*) correlated with the same set of variables previously described, but in the opposite way (Fig. 9). Finally, the OTU 27341 (*Pseudoalteromonadaceae*), with a similar proportion of sequences across the stations, presented positive correlation ammonium (Fig. 9).

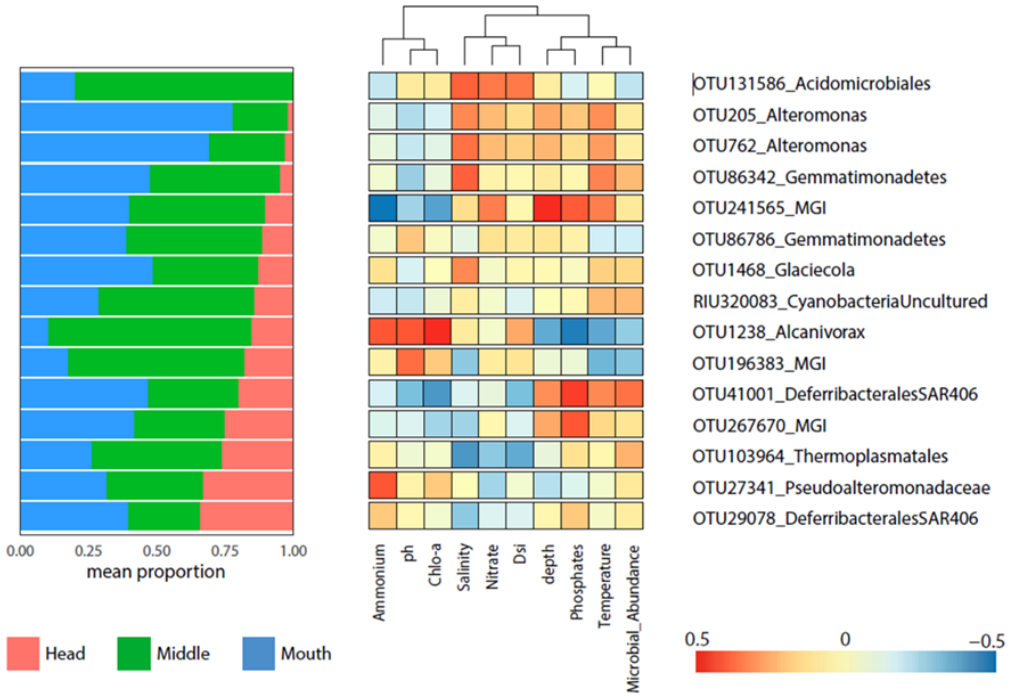



Figure 9: Heatmap displaying the indicator species (p = 0.001) for the Yendegaia 2018 fjord. Left panel shows the mean relative contribution of the indicator species to microbial community detected by stations (Head, Middle and Mouth). Right panel shows heatmap of Pearson correlation between indicator species (OTU abundance) and environmental factors.

## Discussion

### Differences between fjords

The characterization of the microbial communities of two contrasting Patagonian fjords revealed that each harbored a specific prokaryoric communities, highlighting the hybrid nature of these fjords between terrestrial and marine environments (González et al., 2013), and where prokaryotic communities from different origins may coexist pending on their phylogenetic and metabolic requirements (Fortunato & Crump, 2015). Most of the indicator species for Pia fjord (PF) were found at the head station toward the glacier terminus. They comprised OTUs belonging to *Acidobacteria*, typical members of freshwater systems, and *Alphaproteobacteria* abundant in fjord with mixed layers (Piquet et al., 2010; Fortunato et al., 2013). Members from the classes *Actinobacteria* and *Bacilli* (*Firmicutes*) were only detected in Pía fjord and with a more significant proportion in the station inside the fjord (Fig. 5). Studies across salinity gradients evidenced that phyla *Actinobacteria* and *Firmicutes* decrease with increasing salinity (Piquet et al., 2010; Fortunato & Crump, 2015), which is consistent with our observations. Interestingly, members of the SAR202 clade were also more present in PF compared to Yendegaia fjord (YF) 2017. This cluster is usually found in deep ocean waters below 200 m (Mehrshad et al., 2018), and described as sulfite-oxidizers with an essential role in the sulfur turnover in the dark ocean (Mehrshad et al., 2018). Its presence as one of the most abundant taxa and as an indicator species in our surface water data set provides new information on the distribution of this group and its potential role in sulfur biogeochemistry in Patagonian fjords where it has not been previously reported.

In YF during 2017, the number of sequences for the classes *Planctomycetia*, *Cyanobacteria* and *Thermoplasmata* were higher than in PF (Fig. 5). A recent study reported that members of the phylum *Planctomycetes* could fix nitrogen in surface waters of the Pacific and Atlantic oceans where the limitation of iron and nitrogen could favor these diazotrophs (Delmont et al., 2018). Our finding shows that these three classes doubled their sequence proportion in YF compare to PF, suggesting that Yendegaia environmental conditions are more suitable for the development of nitrogen fixation and photosynthetic microorganism. For *Archaea*, most of the sequences belonged to the Marine group II (MGII), which has earlier been shown to have contrasted abundance and distribution in polar waters (Galand et al., 2006; 2008). Church et al. (2003) reported that MGII

abundance was low along the entire water column and that it did not follow neither seasonal or horizontal patterns in the Southern Ocean. However, in the coastal Arctic Ocean, MGII was reported as the most common archaeal group in surface water samples (Galand et al., 2006; 2008). Furthermore, it was suggested that the presence of MGII along coastal waters could be linked to river waters containing high concentrations of particulate matter (Wells et al., 2006; Galand et al., 2008). This could explain the higher proportion of MGII observed in Yendegaia fjord, which receives waters from a proglacial river with a high input of particulate matter.

For YF 2017, the indicator species were dominated by members of the phyla *Planctomycetes*, *Bacteroidetes (Flavovacteriia*) and *Proteobacteria* (*Deltaproteobacteria*) (supplementary Table 3). *Deltaproteobacteria* is an important member of bacterial communities of marine sediments which has been related to sulfur cycle (Zeng et al., 2009), while *Planctomycetes* is associated to nitrogen fixation in environments with iron and nitrogen limitation (Delmont et al., 2018). *Flavobacteria* has been found to correlate positively with Chl-a in the Southern Ocean (Abell & Bowman, 2005). Finally, OTUs from the class *Verrucomicrobia* a common member of marine microbial communities with global distribution was also selected as an indicator species in YF.

The differences in community composition between the two fjords are possibly related to the morphological and hydrographical conditions of the fjords. Besides the salinity gradient, the PF is characterized by the presence of a pronounced sill at the outer part of the fjord (SHOA, Hydrographic and Oceanographic Service of the Chilean Navy), which limits the inflow of Subantarctic water (SAAW) into the fjord and increase the residence time of estuarine water. Moreover, in PF, water turbidity is high along the whole fjord and the entire water column, as opposed to YF where high turbidity associated to inorganic matter input and turbidity is only observed in the upper 20 m (Giesecke et al., 2019). The upwelling in PF produced by the discharge of freshwater from the marine-terminating glacier could favor the appearance of microorganisms from deep layers of water such as SAR202. Thus, differences in the morphological characteristic, sediment load and salinity/temperature could be responsible for the observed differences in microbial communities between both fjords.

Finally, there were also some common microbial features between the fjords. Consistent with previous studies in high latitude environments, members of the *Alpha- Gamma-* and *Delta-proteobacteria* were the most abundant classes in the entire water column accounting for 56% of

the sequences present in the active fraction for both fjords (Zeng et al., 2009; Teske et al., 2011; Gutierrez et al., 2015; 2018; Valdés et al., 2017). Concerning *Archaea*, members of the *Thaumarchaeota* (MGI) (20% of the total sequences across the whole set of samples) have been described as a major contributor to oceanic microbial diversity, but their presence varies according to depth and the environment (Santoro et al., 2019). In high latitude environments, higher proportions of MGI sequences have been found in surface water layers (Bano et al., 2004; Galand et al., 2008; Gutierrez et al., 2015; 2018) compared to subtropical ecosystems where MGI are found commonly in deep layers (Agogue et al., 2008; Church et al., 2010). Interestingly, no vertical community changes, on the total or active fraction of the microbial communities, were observed according to depth in the water column of both fjords and years. It probably reflects the fact that during winter, the water column of fjord tends to be vertically homogenous principally due to the low input of freshwater and the strong wind mixing (Bianchi et al., 2020). However, in PF, a clear horizontal community change was observed in the active fraction from the head to the mouth of the fjord (Table 1). Changes in the proportion of the sequences of phyla *Thaumarchaeota* and *Planctomycetacia* as well as *Gammaproteobacteria* and *Alphaproteobacteria* were recorded. The water column inside the PF was colder and less saline than at the mouth (Fig. 2A), which has been reported as possible drives for changes at the class level in estuarine and coastal saline gradients (Fortunato et al., 2012; Fortunato & Crump, 2015; Herlemann et al., 2016).

Differences between years.

In YF the prokaryotic community composition was compared between July 2017 and July 2018, and significant differences were observed. In 2018 there were more sequences belonging to MGI *Thaumarchaeota*, *Gemmantimonadetes* and *Gammaproteobacteria*, while in 2017, *Flavobacteria*, *Planctomycetacia* and *Cyanobacteria* were relatively more abundant. The differences are possibly explained by changes in the physicochemical characteristics of the water column and could be a result of climatological differences between years.

During July 2017, the upper 50 m of the water column showed evidence of stratification in comparison to July 2018 (Supplementary Fig. 2). Moreover, the levels of Photosynthetically Active Radiation (PAR) recorded in the fjord were higher for July 2017 compared to July 2018

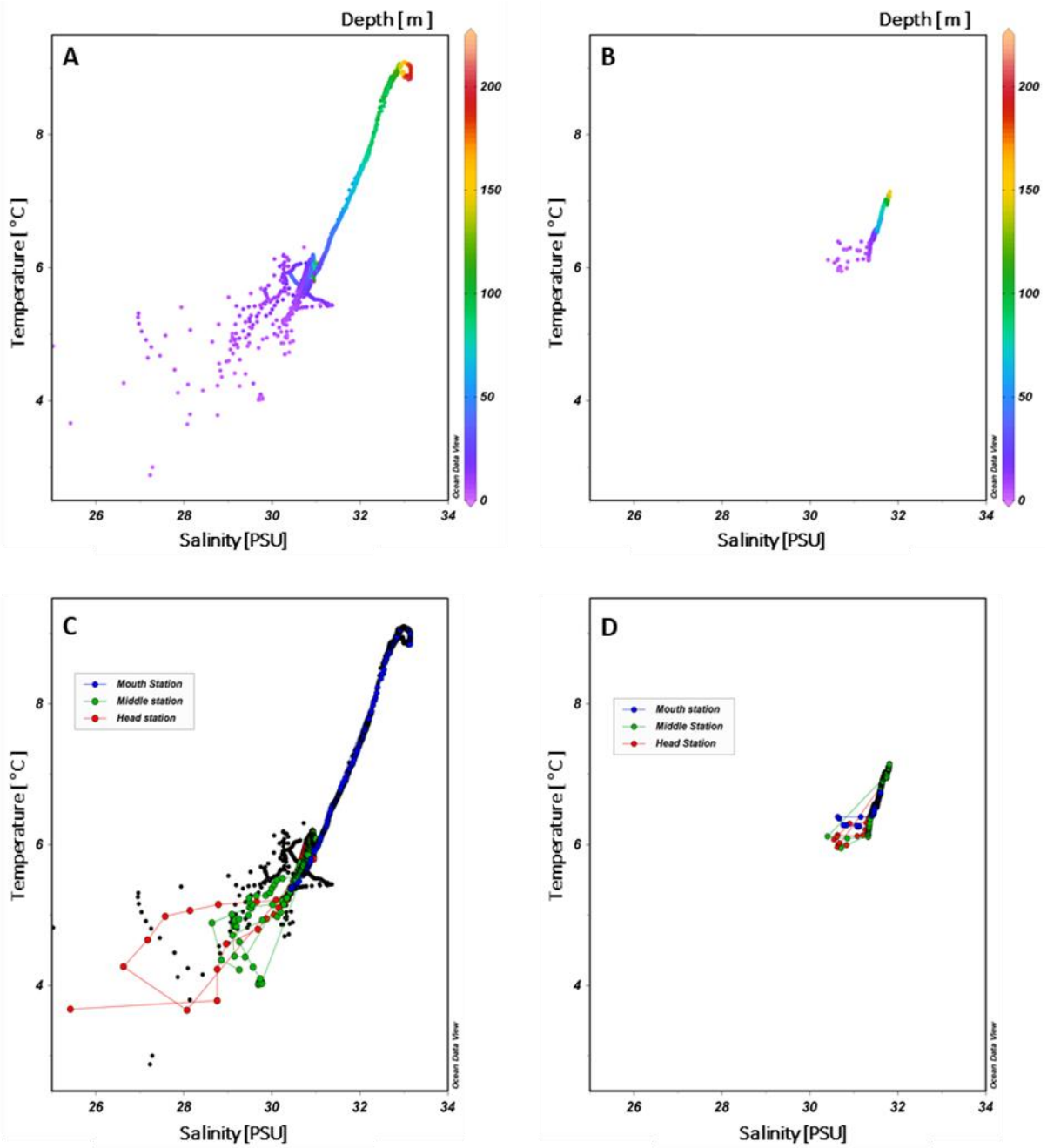
(Supplementary Fig. 3). The decrease in the PAR index during July 2018 is consistent with the decrease of Chl-a in the water column (Figure 2). Stratified water column and a high PAR index could favor low-size autotrophic cells such as *Cyanobacteria* (Cuevas et al., 2019). In the same way, the significant presence of *Flavobacteria* (phylum *Bacteroidetes*) during July 2017 could be related to a possible phytoplankton bloom related to the PAR levels and nitrate concentrations. *Flavobacteria* usually do not present a distribution pattern directly linked to physical or chemical environmental variables (Gomez-Pereira et al., 2010), but they seem associated to phytoplankton blooms because of their ability to degrade associated molecules (Kirchman, 2002). Conversely, the increase observed for MGI *Thaumarchaeota* in 2018 could be related to the decline in the PAR index and the increase of ammonium during July 2018. The presence of MGI in surface layers of high latitude environments has been related to low solar radiation intensity since MGI is sensitive to Reactive Oxygene Species (ROS) generated in the biological and photochemical process (Merbt et al., 2012; Luo et al., 2014; Pedneault et al., 2014; Tolar et al., 2016). Additionally, MGI is an ammonia oxidizer archaeon with high substrate affinity (Lehtovirta-Morley, 2018), which allows it to proliferate rapidly when ammonia concentrations increase. Furthermore, a high proportion of the OTUs indicator species for July 2018 was related to the non-photosynthetically activity such as OTU1238 (*Alcanivorax/Oceanospirillaceae*), OTU41001/29078 (SAR406), OTU27341 *(Pseudoaltemonadaceae*), MGI and *Thermoplasmatales* OTUs, among others. Some of these species, such as SAR406, *Pseudoaltermonomadaceae* and *Oceanospirillaceae*, are described to participate in sulfur and nitrogen biogeochemical cycles (Wright et al., 2014; Delmont et al., 2018).

Finally, in our dataset, the rDNA community did not reveal the clear differences seen with rRNA data (Supplementary Fig. 4). The fact that the RNA fraction presented greater diversity, as well as less dispersion and stronger biogeographical patterns, evidence that rRNA is a useful tool to detect the response of microorganisms to different environmental conditions. It must be mentioned, however, that the use of rRNA as an indicator of growing or active microorganism is controversial and it should be used as an indicator of potential activity rather than actual activity (Blazewicz et al., 2013).

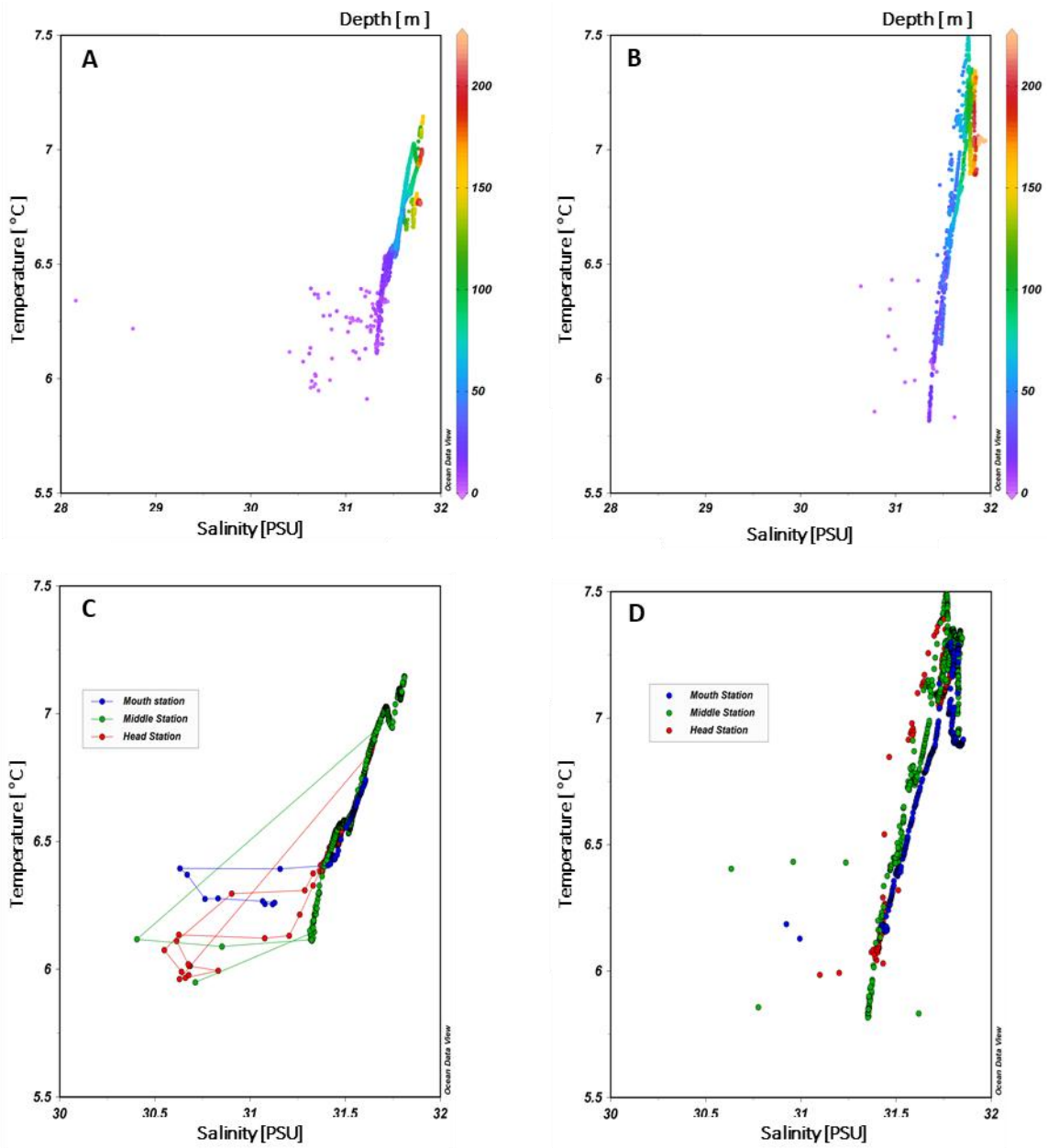
## Conclusion

In conclusion, our study shows that differences in the hydrographic properties of the water column, due to hydro-morphological characteristics of the fjords, can influence the structure of the prokaryotic community on the first 50 m of the water column. In PF, the prokaryotic community was influenced mainly from above (melt freshwater) and below (upwelled freshwater) due to the presence of the marine-terminating glacier. Meanwhile, in YF, which has a land-terminating glacier, the prokaryotic assemblage was similar to those generally described for oceanic waters. In addition, major inter-annual taxonomic changes in YF could be attributable to an increase of PAR that would promote the development of small size photosynthetic microorganisms leading to significant difference in prokaryotic community composition. Yendegaia could represent a typical fjord under future climate predictions, since Aquatic Critical Zones (ACZs) (Bianchi et al., 2020) reported that fjords are expected to experience substantial ice melting and glacial retreat (IPCC, 2019), which will modify the hydrographic properties, nutrients, light availability and climatologic conditions of this zone (Iriarte et al., 2018). The glacier associated to YF has indeed already retreated by 12 km from its origin. This retreat has impacted the physical, chemical, and biological properties of the fjord generating a state of low primary production (Meire et al., 2017; Giesecke et al., 2019). With this study we add data to the current knowledge on the impact of fjord glacier melting by showing that it favored microorganisms such as nitrogen fixing *Planctomycetes*. Alterations in the structure of the usual microbial community and subsequent changes in the trophic chain (Langenheder et al., 2003; Gutierrez et al., 2015) will affect the basic functionality of future fjords ecosystems.

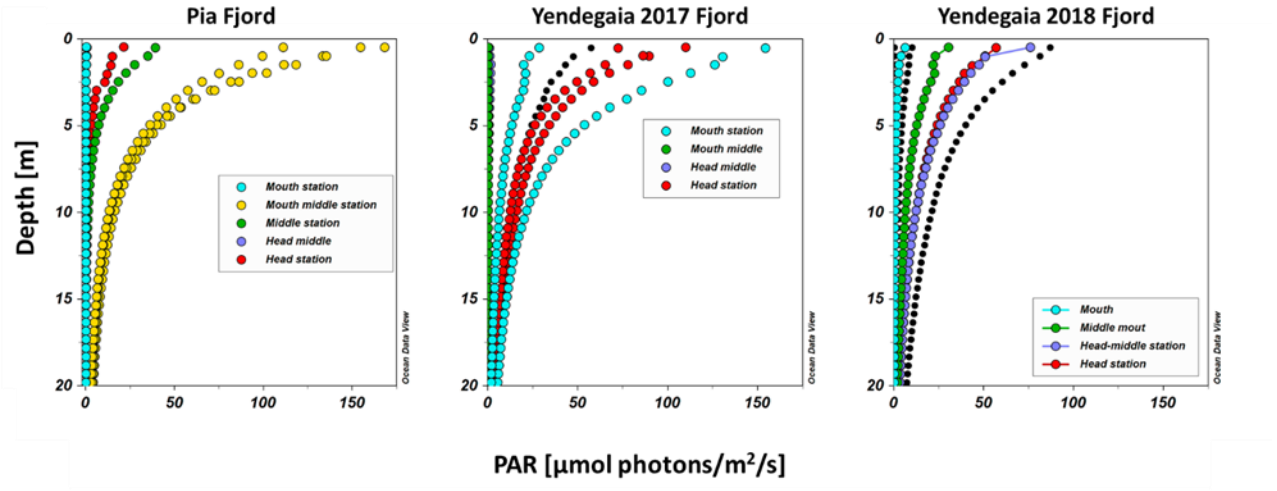
## Supplementary material



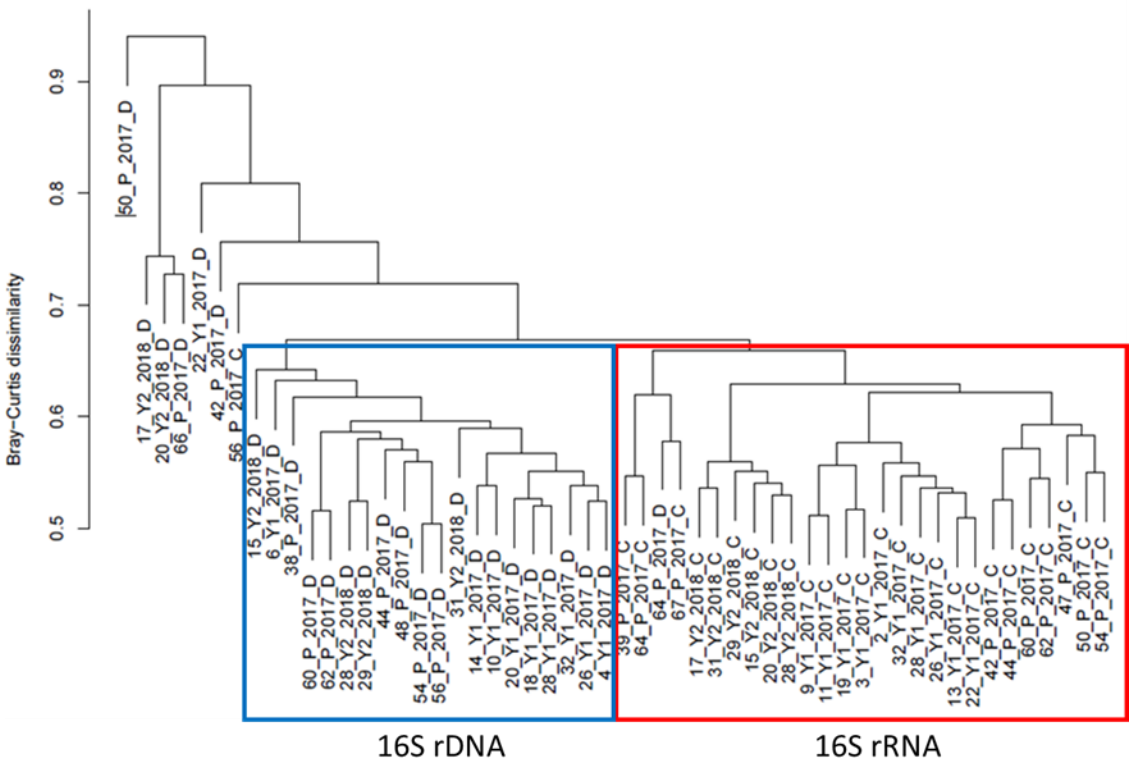

Supplementary Figure 1: Temperature/Salinity diagram. Left panels correspond to Pía Fjord. Right panels correspond to Yendegaia Fjord 2017.



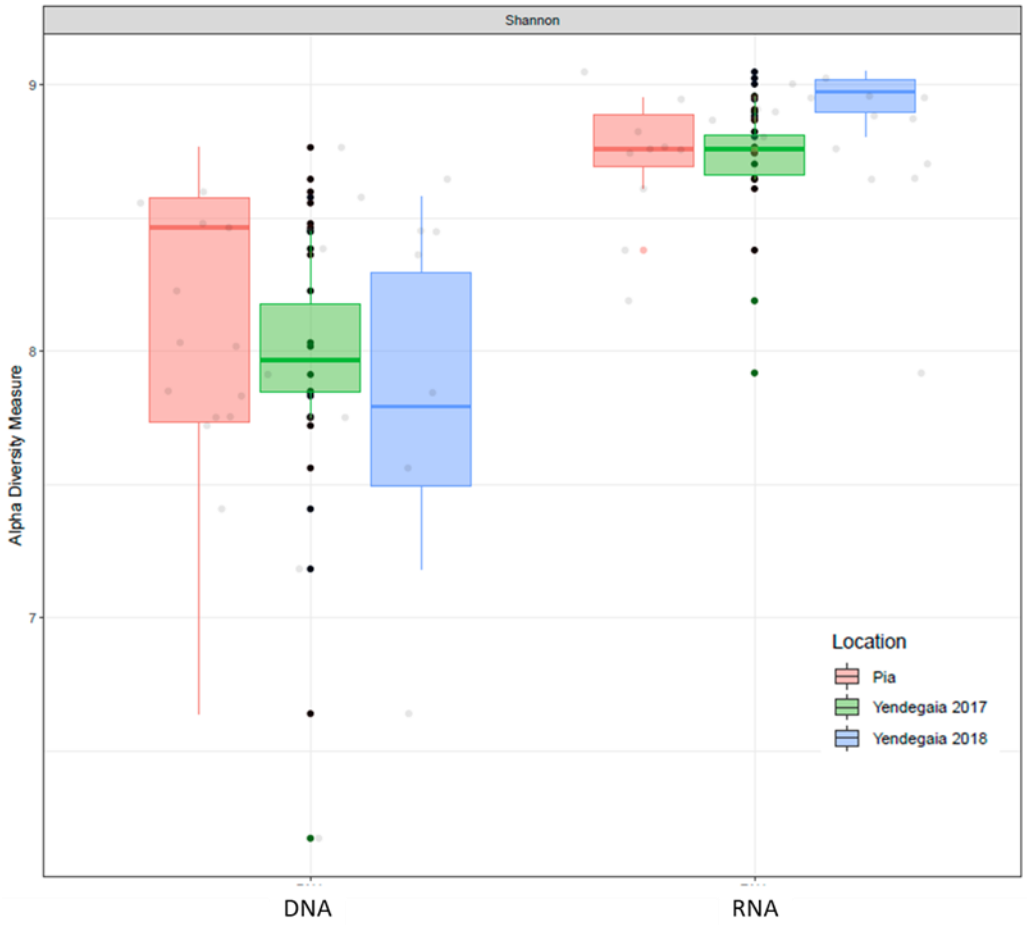

Supplementary Figure 2: Temperature/Salinity diagram. Left panels correspond to Yendegaia 2017 Fjord. Right panels correspond to Yendegaia Fjord 2018.



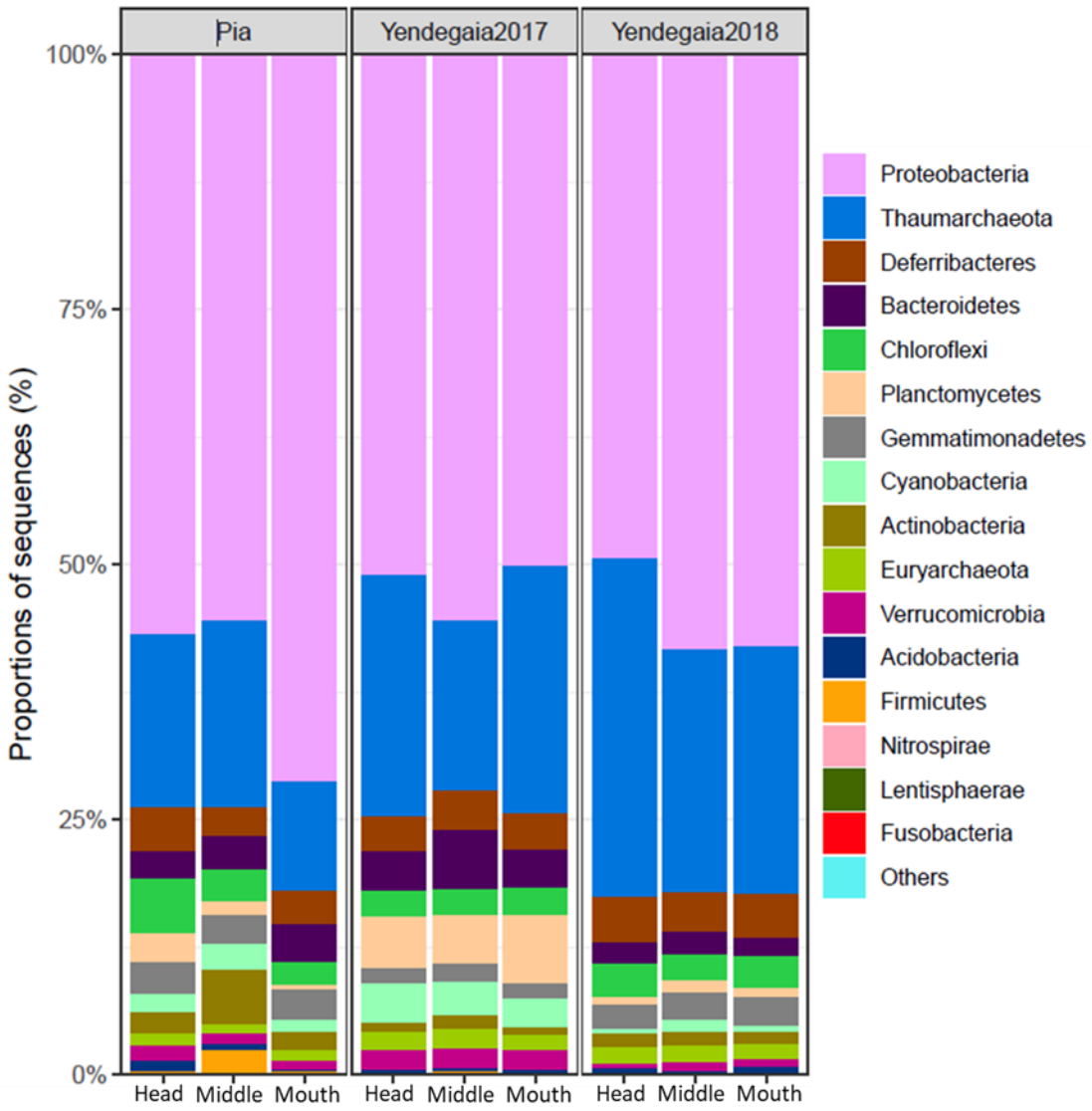

Supplementary Figure 3: Photosynthetically Active Radiation (PAR) from 0 to 20 m in each sampling location. Colours represent stations.




Supplementary Figure 4: Hierarchical cluster dendrogram based on Bray-Curtis dissimilarity index showing the similarity between microbial community compositions for the DNA and RNA fraction in both fjords and years.




Supplementary Figure 5: Boxplot based on Shannon diversity index, showing microbial community α-diversity for the DNA and RNA fraction in both fjords and years.




Supplementary Figure 6: Relative proportion of microbial sequences at the Phylum level in the RNA fraction of each fjord.

Supplementary Table 1: Number of sequences detected for prokaryotic assemblages in water samples from Pía (P), Yendegaia 2017 (Y1) and Yendegaia 2018 (Y2) fjords.

| Sample | Total Reads | DNA reads | RNA reads |
|---|---|---|---|
| 0m_Head_P | 68040 | 26120 | 41920 |
| 5m_Head_P | 60523 | 19455 | 41068 |
| 25m_Head_P | 74677 | 45615 | 29062 |
| 50m_Head_P | 79699 | 48293 | 31406 |
| 10m_Middle_P | 60160 | 15346 | 44814 |
| 25m_Middle_P | 89904 | 41320 | 48584 |
| 50m_Middle_P | 74049 | 24414 | 49635 |
| 0m_Mouth_P | 97325 | 48318 | 49007 |
| 5m_Mouth_P | 96352 | 50878 | 45474 |
| 10m_Mouth_P | 77071 | 32150 | 44921 |
| 25m_Mouth_P | 59150 | 31318 | 27832 |
| **Total Reads Pía** | **836950** | **383227** | **453723** |
| 0m_Head_Y1 | 56624 | 20665 | 35959 |
| 5m_Head_Y1 | 63363 | 28757 | 34606 |
| 25m_Head_Y1 | 59541 | 30225 | 29316 |
| 0m_Middle_Y1 | 48446 | 16479 | 31967 |
| 5m_Middle_Y1 | 50702 | 28310 | 22392 |
| 25m_Middle_Y1 | 64841 | 24701 | 40140 |
| 50m_Middle_Y1 | 83960 | 41670 | 42290 |
| 10m_Mouth_Y1 | 37592 | 19836 | 17756 |
| 25m_Mouth_Y1 | 48709 | 3949 | 44760 |
| 50m_Mouth_Y1 | 70693 | 24955 | 45738 |
| **Total Reads Y1** | **584471** | **239547** | **344924** |
| 0m_Head_Y2 | 76878 | 26231 | 50647 |
| 0m_Middle_Y2 | 57404 | 15269 | 42135 |
| 5m_Middle_Y2 | 63522 | 23056 | 40466 |
| 10m_Middle_Y2 | 96543 | 53161 | 43382 |

| | | | |
|---|---|---|---|
| 25m_Mouth_Y2 | 65242 | 31722 | 33520 |
| 50m_Mouth_Y2 | 83173 | 39231 | 43942 |
| **Total Reads Y2** | **442762** | **188670** | **254092** |

Supplementary Table 2: Permutational Multivariate Analysis of Variance (PERMANOVA) examining the effects of the factors "location" (Pía, Yendegaia2017, Yendegaia2018), Depth (0-50 m) and Station (head, middle, mouth) on the total of the samples for the RNA fraction. Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; R2, coefficient of determination; P, p-value. Statistically significant values are given in bold.

| | Df | MS | F | R2 | P |
|---|---|---|---|---|---|
| Depth | 1 | 0.1959 | 0.19592 | 0.03878 | 0.157 |
| Station | 2 | 0.3931 | 0.19653 | 0.0778 | 0.084 |
| Location | 2 | 0.9229 | 0.46144 | 0.18266 | ***0.001*** |

Supplementary Table 3: Distribution of Indicator microbial groups among the fjords (p = 0.001).

| Location | N° Taxa | N° Indicator taxa | N° OTU | Microbial taxa Class/Order Level | |
|---|---|---|---|---|---|
| Pía 2017 | 6600 | 15 | 1 | Gammaproteobacteria | Vibrionales |
| | | | 1 | Gammaproteobacteria | Salinisphaerales |
| | | | 1 | Gammaproteobacteria | Xanthomonadales |
| | | | 1 | Gammaproteobacteria | Oceanospirillales |
| | | | 3 | Acidobacteria | Subgroup 6 |
| | | | 2 | Acidobacteria | Subgroup 3 |
| | | | 2 | Alphaproteobacteria | Rhodospirillales |
| | | | 1 | Alphaproteobacteria | Rhizobiales |
| | | | 3 | Chloroflexi -SAR202 | |
| Yendegaia 2017 | 3499 | 36 | 3 | Alphaproteobacteria | Rickettsiales |
| | | | 1 | Alphaproteobacteria | Rhodobacterales |
| | | | 5 | Deltaproteobacteria | Bdellovibrionales |
| | | | 1 | Deltaproteobacteria | Desulfuromonadales |
| | | | 3 | Deltaproteobacteria | uncultured |
| | | | 8 | Flavobacteriia | Flavobacteriales |
| | | | 1 | Gammaproteobacteria | Oceanospirillales |
| | | | 1 | Gammaproteobacteria | uncultured |
| | | | 2 | Planctomycetes Uncultured | |
| | | | 2 | Phycisphaerae | Phycisphaerales |
| | | | 2 | Planctomicetacia-Pla3 | |
| | | | 3 | Planctomycetacia | Planctomycetales |
| | | | 4 | Verrucomicrobia-uncultured | |
| Yendegaia 2018 | 6508 | 250 | 6 | Acidimicrobiia | Acidimicrobiales |
| | | | 2 | Acidobacteria | Subgroup6 |
| | | | 2 | Alphaproteobacteria | Rhizobiales |
| | | | 11 | Alphaproteobacteria | Rhodobacterales |
| | | | 14 | Alphaproteobacteria | Rhodospirillales |

| | | |
|---|---|---|
| 4 | Alphaproteobacteria | Rickettsiales-SAR116 |
| 4 | Alphaproteobacteria | SAR11 |
| 1 | Betaproteobacteria | Nitrosomonadales |
| 1 | Betaproteobacteria | Methylophilales |
| 1 | Cytophagia | Cytophagales |
| 18 | Deferribacteres | Deferribacterales-406 |
| 15 | Deltaproteobacteria | Desulfobacterales |
| 15 | Deltaproteobacteria | SAR324 |
| 3 | Flavobacteriia | Flavobacteriales |
| 62 | Gammaproteobacteria | Alteromonadales |
| 17 | Gammaproteobacteria | Oceanospirillales |
| 1 | Gammaproteobacteria | Pseudomonadales |
| 1 | Gammaproteobacteria | uncultured |
| 1 | Gammaproteobacteria | Vibrionales |
| 1 | Gammaproteobacteria | Xanthomonadales |
| 18 | Gemmatimonadetes | BD2-11 |
| 2 | Chloroflexi-uncultured | |
| 5 | Chloroflexi-SAR202 | |
| 21 | Thaumarchaeota-MGI | |
| 2 | Cyanobacteria-uncultured | |
| 6 | Phycisphaerae | Phycisphaerales |
| 1 | Sphingobacteriia | Sphingobacteriales |
| 1 | Thermoleophilia | Gaiellales |
| 12 | Thermoplasmata | Thermoplasmatales-MGII |
| 1 | Proteobacteria-uncultured | |
| 1 | Verrucomicrobia uncultured | |

# CHAPTER II: Biogeography of Southern Ocean active prokaryotic communities over a large spatial scale.


## Abstract

Marine microorganisms are important ecosystem engineers due to their major participation in biogeochemical cycles. Their activity depends directly on community composition, yet little is known about the environmental and ecological processes that structure the composition and distribution of these microbial communities. The objective of this study was to test the effect of geographic distance and environmental parameters on prokaryotic community structure. We investigated the biogeography of bacterioplankton communities over a ~6.500 km longitudinal transect in the Southern Ocean by analyzing the total (16S rDNA) and the active fraction (16S rRNA) of the microbial communities. We found that the community composition of the total fraction was different from the active fraction among the oceanic zone investigated. In addition, higher α-diversity and stronger species turnover were displayed in the active community compared to the total community. Compositional changes in the bacterioplankton communities across the sampling zones were mainly explained by environmental parameters. *Alphaproteobacteria* and *Gammaproteobacteria* dominated overall the composition of the bacterioplankton communities, however, there were differences at lower taxonomical levels. Temperature, salinity, silicic acid, PON and POC impacted the composition of bacterioplankton communities by promoting taxonomic changes related to the specific conditions of each zone. Furthermore, changes in the bacterioplankton communities were also related to geographic distance. A strong distance-decay pattern between closer and distant bacterioplankton communities was observed. We hypothesize that it was related to the different oceanic fronts of the Antarctic Circumpolar Current (ACC) encountered through the sampling transect. Our findings contribute to a better understanding of the complex patterns that shape the structure of bacterioplankton communities in the Southern Ocean.

## Introduction

Although microbial marine communities are essential players of the marine ecosystem functioning, being the main drivers of the major marine biogeochemical cycles (Azam et al., 1983; Karl, 2002; Pomeroy et al., 2007), little is known about the ecological and oceanographic drivers that shape their distributions. Some known factors affecting the microbial community structure include geographical position from tropical to polar regions (Brown et al., 2012; Fuhrman et al., 2008; Ghiglione et al., 2012), and the physical-chemical parameters of marine environment such as temperature, salinity, depth and chlorophyll-a (Chl-a) concentration (Campbell & Kirchman, 2013; Crump et al., 2004; DeLong, 2006; Falcón et al., 2008; Fortunato et al., 2013; Herlemann et al., 2016; Sunagawa et al., 2015). Furthermore, oceanographic features like water masses, oceanic fronts and advection have also been described as physical barriers that can limit the dispersal of microorganisms and at the same time influence the structure of the communities at the ocean scale (Agogué et al., 2011; Galand et al., 2010; Wilkins et al., 2013a).

The Southern Ocean (SO) plays an important role in global ocean circulation, biogeochemical cycles and climate (Broyer & Koubbi, 2014). The surface of the Southern Ocean is composed of several distinct zones defined by transitions in surface water temperatures and density (Orsi et al., 1995; Sokolov & Rintoul, 2002). The Antarctic Circumpolar Current (ACC) divide the Southern Ocean into three major zones: the Subantarctic Zone (SAZ), the Polar Frontal Zone (PFZ), and the Antarctic Zone (AAZ) (Pollard et al., 2002). These three major zones are separated from each other from north to south by the Subtropical Front (STF), the Subantarctic Front (SAF) and the Polar Front (PF) (Sokolov & Rintoul, 2002; Whitworth & Nowlin, 1987). Oceanic fronts are regions were environmentally distinct water masses meet, creating sharp physicochemical gradients over fine spatial scales (Belkin et al., 2009). Therefore, each zone and fronts possess characteristic temperature, salinity and productivity levels (Pollard et al., 2002; Sokolov & Rintoul, 2002), and tend to have distinctive biological communities (Sokolov & Rintoul, 2002). However as a consequence of climate change waters towards the pole side of the ACC waters have become warmer and more saline, while those to the north cooler and fresher (Haumann et al., 2016; Ning et al., 2008).

The west Antarctic Peninsula (WAP) and the Bransfield Strait (BS) in the AAZ is characterized by varied and complex coastal ecosystems, hosting a productive marine ecosystem

that is characterized by phytoplankton blooms and complex oceanographic processes (Prezelin et al., 2004; Schofield et al., 2010). This costal ecosystem is subjected to strong seasonal and interannual variability as a result of large scale climate change (Kim & Ducklow, 2016). Furthermore, the increasing air temperatures registered along the Antarctic Peninsula (AP) are expected to generate glacial melting causing glacier retreat and reduction in the ice-season period (Schofield et al., 2010). Along King George Island, many semi enclosed embayment such as Maxwell bay and it tributary Marian Cove, are exposed to freshwater input from glaciers surrounding the bay and oceanic water exchange with the Bransfield Strait (Llanillo et al., 2019). Evidence that this area is experiencing accelerated warming is the retreat of glaciers such as the glacier associated with Marian Cove, which has retreated 1.7 km from 1956 to 2012 (Ahn et al., 2016). As a consequence of the retreat of the Marian Cove glacier, the structure and function of the megabenthic epifauna has been affected and benthic diatom blooms have been recorded as unusual in this area (Ahn et al., 2016; Moon et al., 2015).

Several studies of diversity and community structure on bacterioplankton at regional scale have been carried out in the SO (Alcamán-Arias et al., 2021; Dinasquet et al., 2017; Hernández et al., 2015; Luria et al., 2016; Picazo et al., 2019; Piquet et al., 2011; Signori et al., 2014). However, only a few studies have been reported on a large scale (Luria et al., 2016; Wilkins et al., 2013a;b) and none has targeted the active fraction of the microbial communities. The composition of the bacterioplanktonic community in the SO show some variability across the different areas, however, members of the classes *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes* usually dominated the number of sequences (Abell & Bowman, 2005; Baltar et al., 2016; Ghiglione et al., 2012; Liu et al., 2019; Logares et al., 2020; Wilkins, et al., 2013b). Biogeographic patterns has been observed for the bacterioplanktonic communities in the SO mostly influenced by physical barriers such as the Polar Front and advection (Baltar et al., 2016; Wilkins et al., 2013a;b), and by biological association such as diatoms blooms (Liu et al., 2019). The predicted climate changes in the different areas that comprise the SO are expected to influence the composition of the biological communities demanding a better understanding of the key patterns of microbial ecology and biogeography as well as the specific ecosystem of the SO.

The objective of the present study was to explore the distribution, abundance and diversity of prokaryotic communities and to analyze its biogeographical patterns across different oceanographic areas in the SO. We analyzed both the total and active fraction of the communities,

based on 16S rDNA and 16S rRNA, over a large spatial distance (~ 6,000 km) comprising a global (ocean) and a local scale (fjord). We test how geographic distance and environmental parameters shape prokaryotic community composition.

## Material and Methods

### Study area and sampling

Field campaign was conducted from March to May 2018 on board the icebreaker ARAON as part of the expedition ANA08D of the Korean Polar Research Institute (KOPRI). Samples were taken along a transect from Christchurch New Zealand (52° 14.371 S/ 178° 4.22 W) to West Antarctic Peninsula (63° 23.711' S/ 61° 18.164' W) (Southern Ocean, Fig. 1A), and across the Bransfield Strait (62° 50.3990 S / 58° 12.0028 W) to the Marian Cove fjord (62° 12.8495 S/ 58° 46.3919 W) (Fig. 1B-C). For the Southern Ocean transect, a total of 31 stations were sampled (Fig.1A). The first 20 stations were sampled every 12 hours, while the rest of the stations (from 21 to 31) were sampled every 6 hours. The water was collected directly from the continuous ship water pipe system from a depth of 7 m. For the Bransfield Strait - Marian Cove transect, a total of 8 stations were sampled: 2 stations in the Bransfield Strait (Fig.1B), 3 stations in Maxwell Bay and 3 stations in Marian Cove Fjord (Fig.1C). Sea water from four different depths (0, 10, 25 and 50 m) was collected at each station from a rosette system with Niskin bottles. The 39 stations sampled on the Southern Ocean transect were grouped into 7 zones according to their geographic location and oceanographic parameters (water masses, major oceanographic fronts, costal vs oceanic influence). The first 5 stations were named as Subantarctic zone (SA) (52° 14.2651' S to 59° 28.0279' S) (Fig. 1A). The subsequent 5 stations were grouped as Polar Front (PF) zone (60° 57.023'S to 66° 12.1361'S), followed by 10 stations that were grouped as Amundsen Sea (AS) zone (67° 9.2437'S to 67° 26.722'S) (Fig. 1A). The next 11 stations were grouped as Antarctic Peninsula (AP) (67° 22.1291'S to 63° 24.9352'S) (Fig.1A) and the 2 stations sampled on the Bransfield Strait (BS) (62° 50.3983'S to 62° 28.8008'S) remain under the same name (Fig. 1B). Finally, the last 6 stations were divided into Maxwell Bay (MB) zone (62° 17.1021'S to 62° 13.8023'S) and Marian Cove (MC) zone (62° 13.0785'S to 62° 12.2998'S) (Fig.1C).

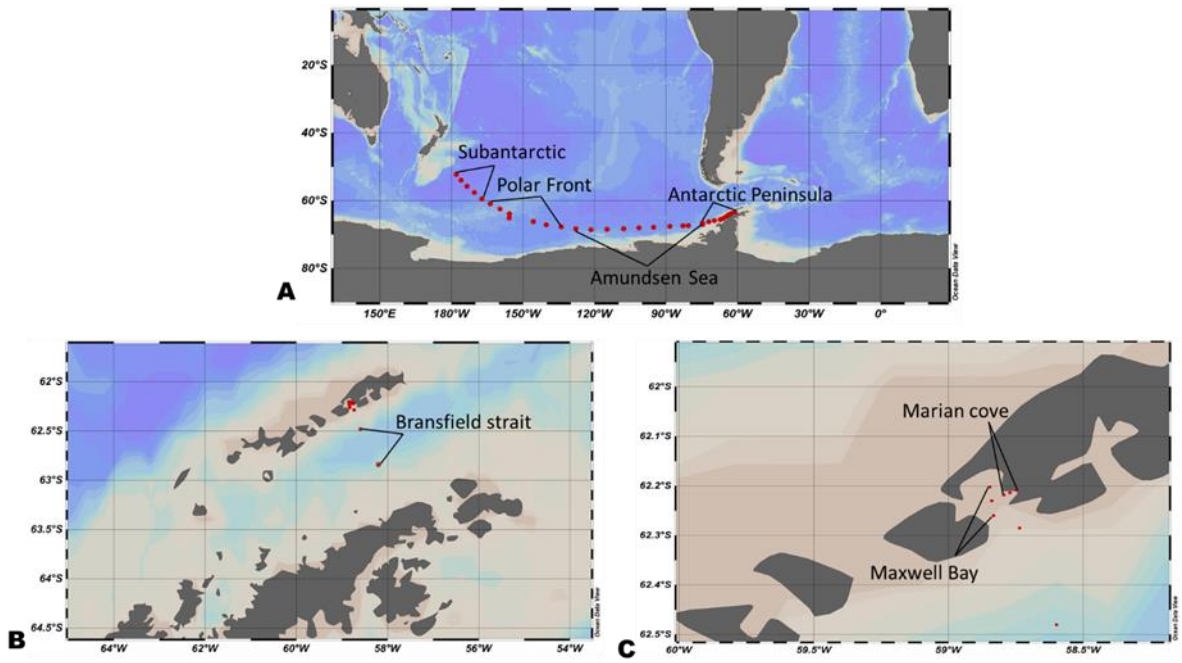



Figure 1: Study areas and sampling sites. A) Transect Christchurch, New Zealand, to the West Antarctic Peninsula. B) Bransfield Strait. C) Transect from Maxwell Bay to Marian cove fjord.

For microbial biodiversity analysis, 2 L of water was filtered sequentially onto 3.0 µm and 0.22 µm pore size polycarbonate membrane filters (MilliporeSigma, Massachusetts, USA) and stored in RNAlater (Thermo Fisher Scientific, Massachusetts, USA) at -80°C (liquid nitrogen) until analyzed. Hydrographic data including salinity, photosynthetically active radiation (PAR) and temperature were recorded using a Sea-Bird SBE 9 CTD (Sea Bird Scientific, USA).

Samples for quantification of chlorophyll a (Chla) and dissolved inorganic nutrients were collected from 500 mL seawater filtered through 0.7 µm glass fiber filters (Whatman GF/F, MilliporeSigma, Massachusetts, USA). For Chla determination, 400 ml of seawater was filtered (GF/F Whatman glass fiber filters, 0.7 µm nominal pore size) in triplicate and immediately frozen (−80°C) until later analysis via fluorometry (Turner Design TD-700), using acetone: water (90:10% v/v) for pigment extraction according to standard procedures (Parsons, 2013). For microbial abundances, samples (1350 mL) were taken in 2 mL cryovials, fixed with glutaraldehyde (0.1% final concentration) and stored in darkness at 80°C until laboratory analysis at the

Laboratory for Oceanographic Processes and Climate (Universidad de Concepción, Chile) by flow cytometry method (Marie et al., 2000). To characterize Dissolved Organic Carbon (DOC), seawater samples were taken in duplicate and filtered through 0.22 µm pore size filters (Nucleopore) into pre-combusted (450°C) glass flask and acidified with hydrochloric acid at 37%. DOC samples were analyzed with high-temperature oxidation (HTCO) method (Sugimura & Suzuki, 1988) using a Shimadzu TOC-V system. To maintain quality control of sample analyzed, 5-point calibration curve of seawater DOC reference standards was made. In addition, seawater DOC reference standards produced by the Hansell CRM program (http://www.rsmas.miami.edu/groups/organic-biogeochem/crm.html) were also analyzed each day. To maintain highest quality data control, samples were systematically checked against low-carbon water and deep and surface reference waters every sixth analysis (Hansell & Carlson, 1998). The between-day precision in the DOC measurement was 1–2 µM, or a CV of 2–3%. Dissolved nutrients samples (phosphate, silicate, nitrite + nitrate, and ammonium) were analyzed onboard ARAON vessel using a 4-channel continuous Auto-Analyzer (QuAAtro, SEAL Analytical, Southampton, UK) according to the manufacturer's instruction and standard colorimetric methods (Parsons et al., 1994). The channel configurations and reagents were prepared according to the 'QuAAtro Applications'. Standard curves were run with each batch of samples using freshly prepared standards that spanned the range of concentrations in the samples. Water samples for particulate organic carbon (POC) and nitrogen (PON) were filtered through 25 mm GF/F filters (precombusted 450 ºC, 4 hours). POC samples were stored frozen at –20°C until analysis. To remove the dissolved inorganic carbon, the filters were fumed with HCl overnight before analysis. Total amount of POC or PON was measured using an Elemental Analyzer at the stable isotope laboratory at the University of Hanyang, Korea.

DNA and RNA extraction and sequencing

For nucleic acid extraction, the 0.2 µm frozen filters were cut with sterilized scissors into small pieces and incubated for 45 min at 37° in 840 µL of lysis buffer (40 mmol $L^{-1}$ EDTA, 50mmol $L^{-1}$ Tris hydrochloride pH 8.3 and 0.75 mol $L^{-1}$ sucrose) with 50 µL of lysozyme solution (20 mg $mL^{-1}$), followed by a second incubation with 50 µL of 20% Sodium Dodecyl Sulfate (SDS) and 10 µL of proteinase K (20 mg $mL^{-1}$). Extraction of DNA and RNA was then performed from

the lysate using an AllPrep DNA/RNA kit (Qiagen Inc, Germantown, USA) following the manufacturer instructions. The quality and the quantity of the extracted DNA and RNA were measured by spectrophotometry (Thermo Scientific NanoDrop 2000). The RNA samples were reverse transcribed to cDNA with random primers using the SuperScript VILO cDNA synthesis kit (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) following the manufacturer's protocol. For both the DNA and cDNA, the V4-V5 region of the bacterial 16s rRNA gene was amplified using universal primers 515FB- GTGYCAGCMGCCGCGGTAA and 926R-CCGYCAATTYMTTTRAGTTT (Parada et al., 2016). Amplification and sequencing on Illumina Miseq were conducted in the commercial laboratory Integrated Microbiome Resource (IMR, Halifax, Canada) according to the protocol published earlier (Comeau et al., 2017).

Sequence analyses

All the reads that had a mismatch with the 16S rRNA primers contained ambiguous nucleotides (N) or were <300 bp long beyond the forward primer were removed. In addition, a stringent quality trimming criterion was applied to remove reads that had ≥10% of bases with Phred values <27. This procedure is recommended to ensure that when clustering at 97% or more, the influence of erroneous reads is minimized (Huse et al., 2010; Kunin et al., 2010). The sequences were then de-replicated and clustered at a 99% sequence similarity threshold using UCLUST (Edgar, 2010) for de novo OTU picking. Representative sequences were classified against the SILVA v.128 database (Quast et al., 2013). Sequence data analyses were conducted with Pyrotagger pipeline (Kunin & Hugenholtz, 2010). Sequences selected for further analysis were compared manually to the Genbank database by BLAST. Putative chimeric sequences were removed. They were identified as sequences having the best Blast alignment <90% of the trimmed read length to the reference database and >90% sequence identity to the best Blast match.

## Statistical Analyses

The OTU sequences abundance table was transformed with an Hellinger transformation (Legendre and Gallagher, 2001) with the Vegan package (Oksanen et al., 2019) in R 3.5.3 (Team, R. C. 2018). An MDS based on Bray-Curtis similarity was conducted to visualize similarities in community composition between samples with the vegan package. Significant differences in community structure among the different variables were tested with PERMANOVA with the adonis function. Indicator species analysis was conducted using the multipatt function of the indicspecies package in R (De Cáceres et al., 2012). In order to explore the impact of the environmental variables on the OTU data set and control the possible effect of geographic distance a partial Canonical Correspondence Analysis (CCA) was computed with the function cca from the vegan package. Distance-decay relationship between community assemblages were quantified using linear model (function lm), based on pairwise Bray-Curtis dissimilarity and the geographic distance separating each pair of communities. The geosphere package was used to calculate the geographic distance among communities with the function distHaversine.

## Results

### Environmental Parameters

Differences in environmental conditions were observed across the seven zones in the Southern Ocean transect. Temperature decreased from 12°C to -1.4°C from Subantarctic to Marian Cove (-1.4°C) (Fig. 2). Chl-a concentrations varied from 0.06 to 3 µg $L^{-1}$ with in general the lowest values measured at the first 3 zones of the transect (Subantarctic, Polar front and Amundsen Sea), and the highest values in Maxwell Bay and Marian Cove (Fig. 2). For Bransfield Strait zone Chl-a values ranged from 0.06 to 2.3 µg $L^{-1}$ (Fig. 2). Salinity did not show a high variability across the provinces with values ranging from 33.1 to 34.1 (PSU) (Fig. 2). Ammonium concentrations were generally low (<1µM) except for the Antarctic Peninsula zone were values fluctuated from 2.2 to 5.8 µM (Fig. 2). For inorganic nitrogen ($NO_3$+$NO_2$), an increment in the concentrations was observed across the transect from Subantarctic to Marian Cove zones (13.25 to 22 µM, respectively) with the highest average value recorded for the Maxwell Bay zone (23.5 µM) (Fig. 2). Phosphate concentrations did not show strong variations across the transect (ca. 1.5 µM, Fig. 2). A sharp increase in the concentrations of silicic acid was observed across the transect, with lowest values for Subantarctic zone (0.8 µM) and the highest values for Marian Cove zone (~70 µM) (Fig. 2). An opposite pattern in concentration was noticed for PON and POC with highest values of PON (~17 to 10 µM) associated to the main Southern Ocean transect (Subantarctic to Antarctic Peninsula zones), meanwhile the highest values of POC (~13 to 8 µM) were associated to the final part of the transect (Bransfield Strait to Marian Cove) (Fig. 2). DON concentration values ranged from ~24 to 33 µM across the transect with the highest values registered for the Polar Front zone (Fig. 2). Finally, DOC concentrations varied between 45 to 80 µM across the transect (Fig. 2).

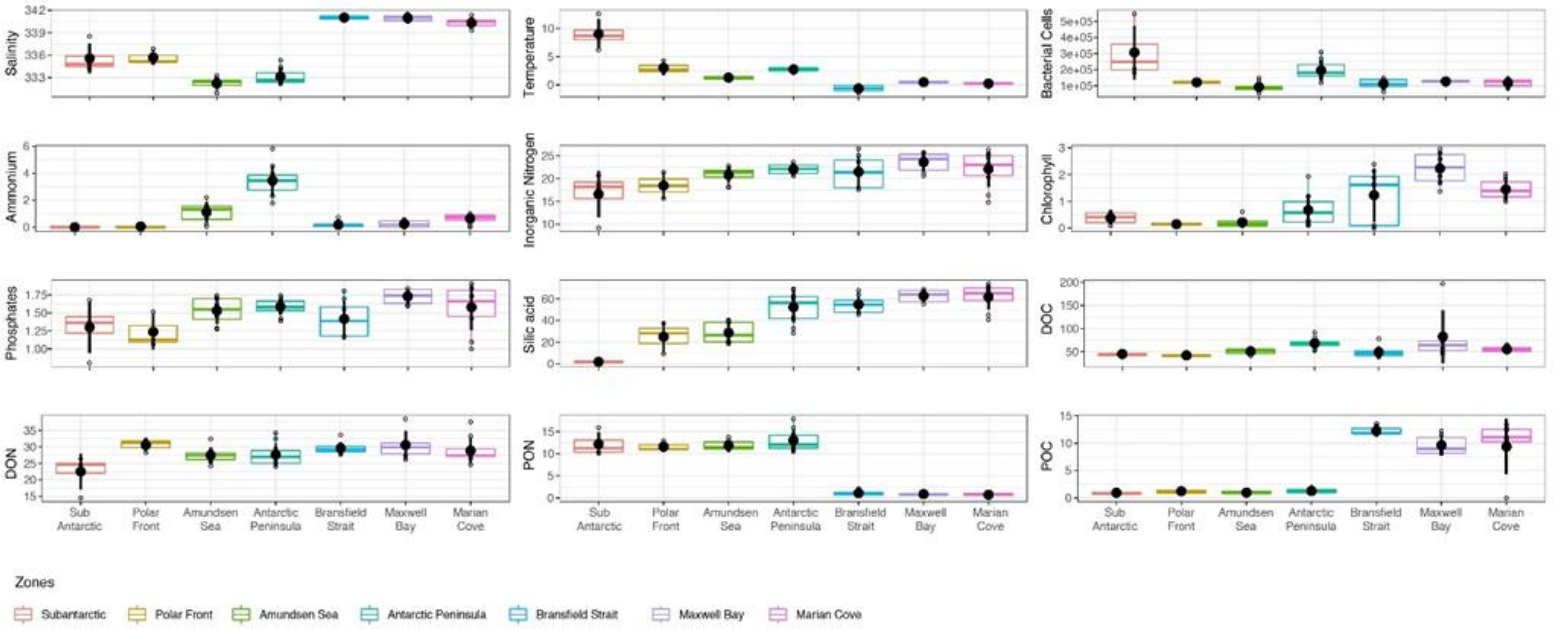



Figure 2: Environmental variables across the seven provinces. Variables are ordered from Subantarctic to Marian Cove fjord.

## Overall DNA and RNA community composition and diversity

We obtained a total of 3,185,567 16S rRNA/rDNA gene sequences of Bacteria and Archaea, from 63 samples taken from March to May 2018 in a Southern Ocean transect (Supplementary table 1). A total of 95,515 different OTUs were obtained, of which 91,860 were assigned to Bacteria and 665 to Archaea.

We compared the microbial community composition between zones based on both the active fraction of the community (16S rRNA) and the total fraction of the community (16S rDNA). The RNA and DNA fractions were separated on the non-metric multidimensional scaling (NMDS) ordination (PERMANOVA, p=0.001) (Fig. 3; Supplementary Figure 1; Supplementary Table 2). The dissimilarity between fractions was highest in the zones Bransfield Strait, Maxwell Bay and Marian Cove (Supplementary Figure 2). However, both fractions evidenced significant variation in the composition of the communities among zones (PERMANOVA, p=0.001) (Table 1; Supplementary Table 2).

Table 1: Permutational Multivariate Analysis of Variance (PERMANOVA) testing the effects of the variables Depth (0-50 m) and Station (Subantarctic, Polar Front, Amundsen Sea, Antarctic Peninsula, Bransfield Strait, Maxwell Bay and Marian Cove) in each of the fractions (DNA/RNA). Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; R2, coefficient of determination; P, p-value; BS, Bransfield Strait; MB, Maxwell Bay; MC, Marian Cove. Significant results are in bold.

PERMANOVA (adonis)

| Fraction | Source of variation | Df | MS | F | R2 | P |
|---|---|---|---|---|---|---|
| DNA (16S rDNA) | Zones (all zones) | 2 | 0.480 | 2.724 | 0.099 | ***0.001*** |
| | Depth (0,5,7,10,25,50) | 1 | 0.288 | 1.634 | 0.030 | ***0.030*** |
| RNA (16S rRNA) | Zones (all zones) | 3 | 0.643 | 2.985 | 0.132 | ***0.001*** |
| | Depth (0,5,7,10,25,50) | 1 | 0.307 | 1.424 | 0.021 | 0.053 |
| RNA (16S rRNA) | Zones (all zones) | 3 | 0.643 | 2.951 | 0.159 | **0.001** |
| | Depth (0,5,7,10) surface | 1 | 0.285 | 1.311 | 0.024 | 0.100 |
| RNA (16S rRNA) | Zones (BS, MB, MC) | 2 | 0.609 | 3.112 | 0.220 | **0.001** |
| | Depth (0,5,10,25,50) | 1 | 0.289 | 1.475 | 0.052 | 0.051 |

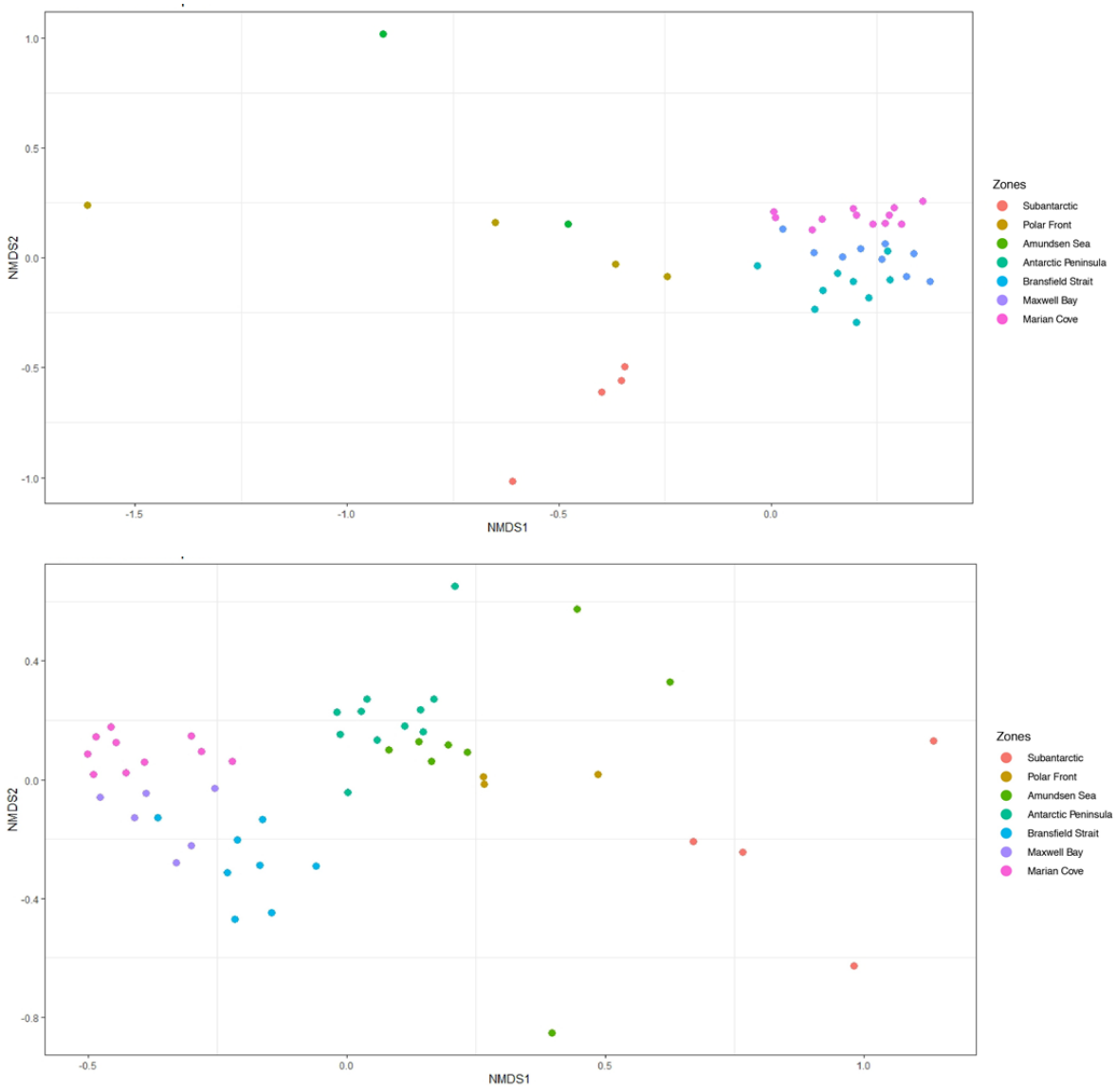



Figure 3: Non-Metric Multidimensional Scaling ordination (NMDS) based on Bray-Curtis similarity of microbial communities across the seven provinces. Colours represent provinces. Panel A correspond to DNA fraction and panel B to RNA fraction.

The Shannon diversity index showed a higher diversity for the RNA community compared to the DNA community in almost all the zones (Fig. 4). In general, the distribution of the α-diversity in the RNA community was less variable compared to the distribution of α-diversity observed for the DNA community (Fig.4). The zones Polar Front and Amundsen Sea in the DNA

community showed a high dispersion of the α-diversity values compare to the values of α-diversity exhibited in the RNA community (Fig.4). The Subantarctic zone showed the lowest values of α-diversity in the RNA community, however, these values were similar to those observed for the Subantarctic zone in the DNA community with a very similar median (Fig. 4). The highest values of α-diversity for the entire data set were obtained in the Marian Cove zone for the RNA community (Fig. 4).

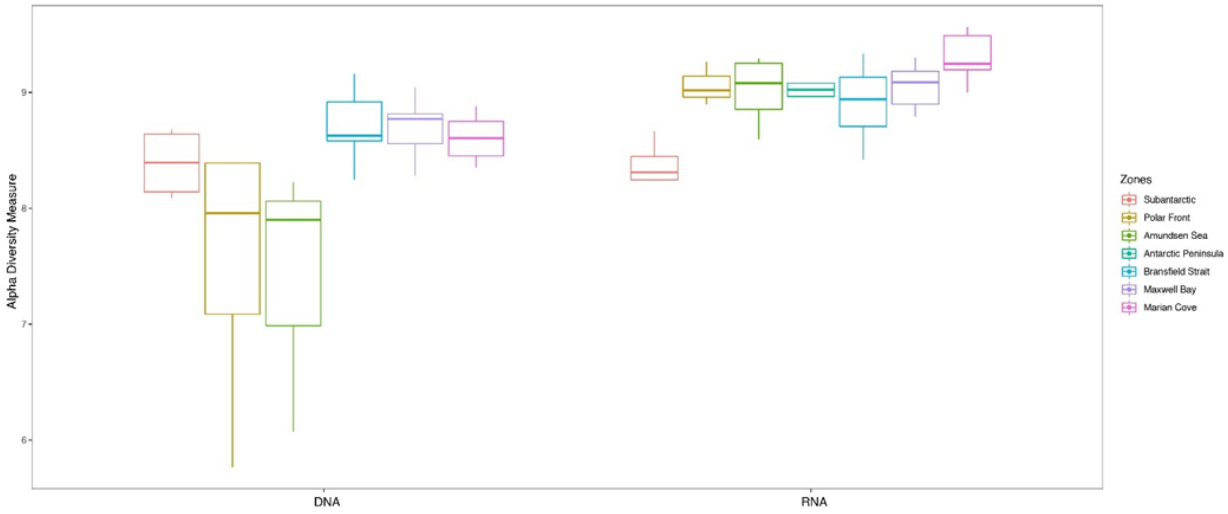



Figure 4: Shannon α-diversity index across seven provinces, comparing DNA and RNA fractions of the microbial community.

When comparing community composition across the transect, the variable depth was not significant in the RNA fraction when considering all depths (PERMANOVA, p=0.053) compared to the DNA fraction where variation in depths was significant (PERMANOVA, p=0.03) (Table 1). Additionally, in the RNA fraction no significant variation (PERMANOVA, P=0.1) in depth were appreciated when only surface samples (0,5,7, 10 m) were considered (Table 1)

We further focused our analysis on the RNA fraction because it included the complete data set containing data for the seven zones sampled, compared to the DNA fraction where the data for the Antarctic Peninsula zone was missing.

Environmental and distance effects on active community composition

The partial CCA between the community composition distance matrix and environmental variables controlling for the effect of geographical distance showed that 27% of the community variability was explained by environmental variables (salinity, temperature, ammonium, inorganic nitrogen, chlorophyll a, phosphate, silicic acid DOC, DON, POC, PON), while 10% corresponds to a geographic distance effect (Supplementary Table 3). The environmental variables structured the samples along the axis separating them into two groups, (i) main Southern Ocean transect (SA, PF, AS, AP), and (ii) BS-MB-MC. The first group one strongly correlated with Temperature (r = 0.79), PON (r = 0.72) whereas the communities from the second group correlated strongly with silicic acid (r = -0.71), salinity (r = -0.66) and POC (r =0.66) (Supplementary Figure 3; Supplementary Table 4).

The distance effect detected with the partial CCA was explored by comparing the geographical distance separating two samples against the similarity in community composition (Bray-Curtis) (Fig. 5). An overall comparison showed no significant linear relationship between geographical distance and community composition. However, when comparing specifically the different zones against each other, there was a significant correlation in 17 of the 21 comparisons (Fig. 5; Supplementary Table 5). The strongest relationships were for the more remote combinations such as Subantarctic vs Antarctic Peninsula (r=0.76), Bransfield Strait vs Subantarctic (r = 0.8), Maxwell Bay vs Subantarctic (r = 0.84), Maxwell Bay vs Amundsen Sea (r = 0.75), Marian Cove vs Subantarctic (r = 0.88) and Marian Cove vs Amundsen Sea (r = 0.7) (Supplementary table 5). However, some strong and significant distance-decay patterns were also detected in closer zones such as Subantarctic vs Polar front (r = 0.87), Amundsen Sea vs Antarctic Peninsula (r = 0.65) and Bransfield Strait vs Amundsen Sea (r = 0.62) (Supplementary table 5).

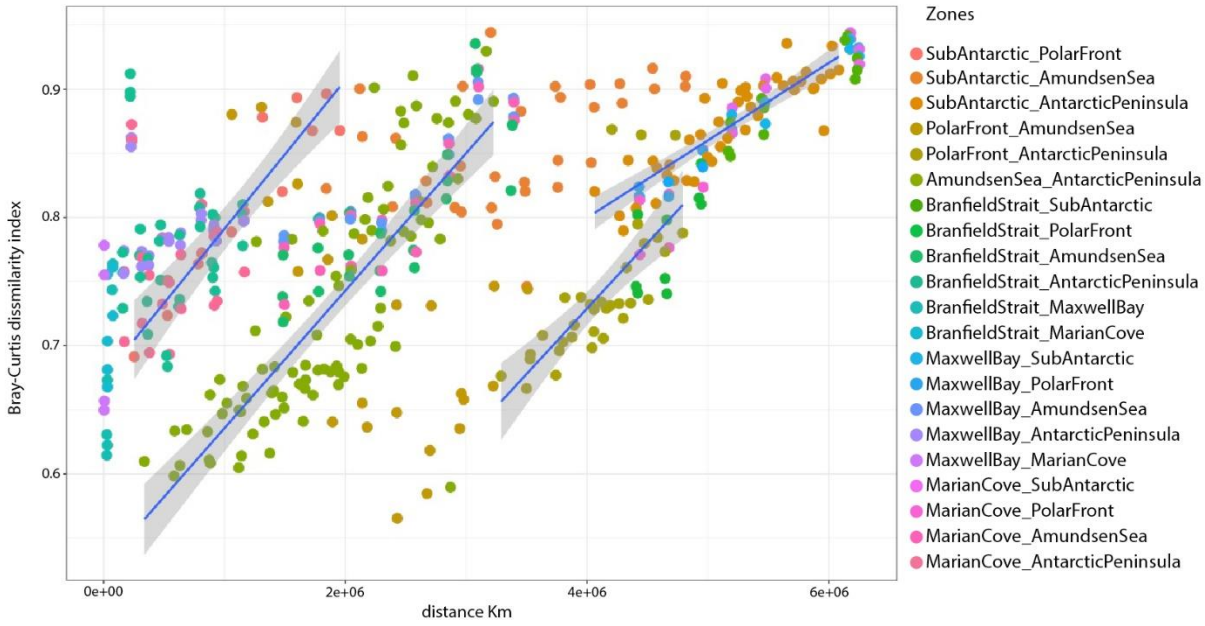



Figure 5 : Linear model. Geographic distance (Km) versus Abundance dissimilarity matrix (Bray-Curtis).

## Taxonomic composition of the active fraction

The taxonomy composition at order level showed that across the entire data set the *Oceanospirillales, Alteromonadales, Rhodobacterales and Flavobacteriales* were the dominant order, but at each zone a distinct composition was observed (Fig. 6). The ubiquitous *SAR11* was found as a major component of the community in all the zones, showing the larger percentage on the Antarctic Peninsula zone.

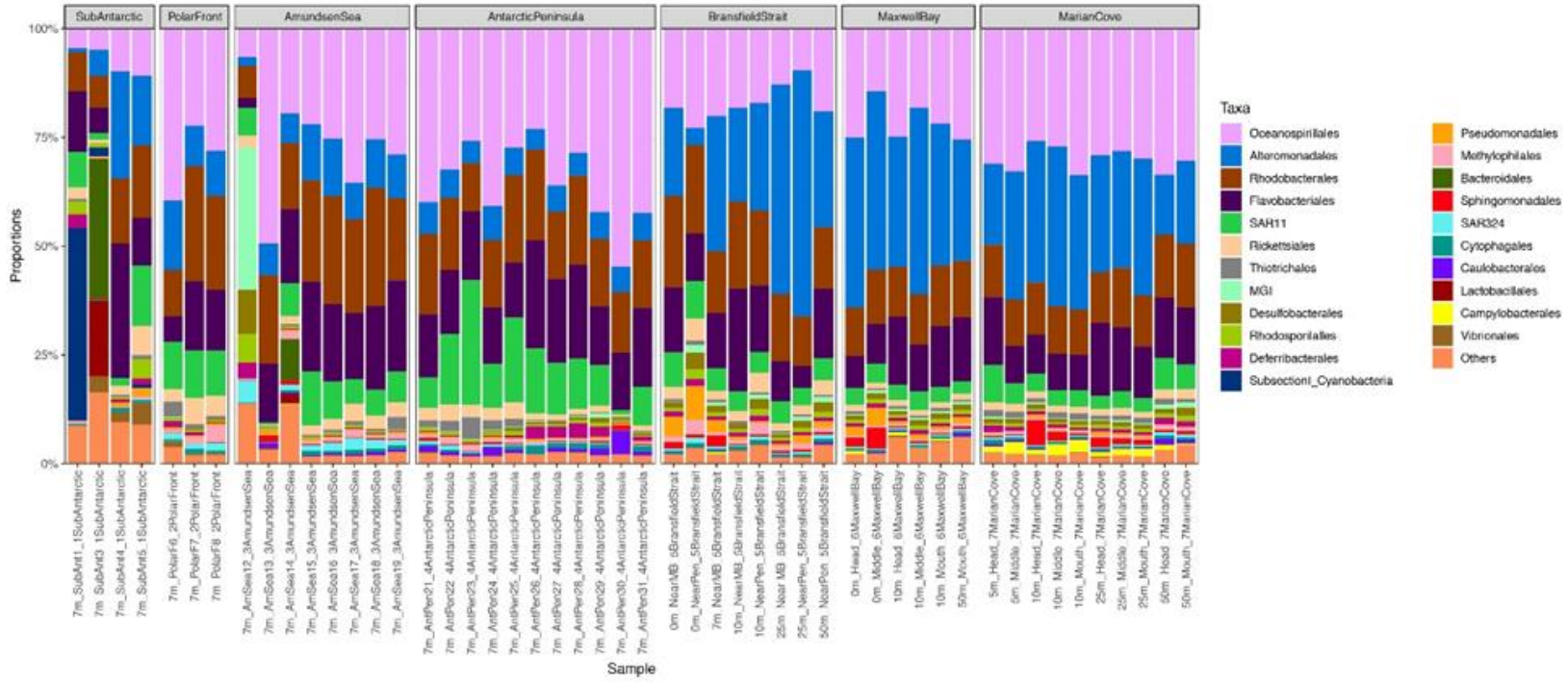



Figure 6: Relative abundance of taxonomical groups at the Order level in the RNA fraction of each province.

A distinct taxonomic composition was observed for the Subantarctic zone being dominated in its first two sampling points by *Cyanobacteria, Bacteroidales* and *Lactobacillales* (Fig. 6). The orders *Rickettsiales, Thiotrichales* and *Methylophilales* increased in relative abundance in the Polar Front zone (Fig. 6). *MGI* (*Thaumarchaeota*) dominated in one of the samples of Amundsen sea zone (Fig. 6). *Desulfobacterales*, *Rhodosporilalles*, and *SAR324* increased in relative abundance in the Amundsen Sea zone. The Antarctic peninsula station was dominated by *Oceanospirillales* meanwhile *Alteromonadales* exhibited the lowest values in the entire transect.

The last 3 zones, Bransfield Strait, Maxwell Bay and Marian Cove, showed a similar taxonomic composition dominated by *Alteromonadales, Oceanospirillales* and *Flavobacteriales* with a marked decrease of *SAR11* as dominant taxa (Fig. 6). Some differences were seen also for less abundant taxa in Bransfield Strait zone with an increase of *Pseudomonadales, Desulfobacterales* and *Methylophilales* (Fig. 6). In contrast with all the other zones, *Campylobacterales* had a higher relative abundance in all sampling points in Marian Cove zone (Fig. 6). Finally, an increase in the number of sequences was observed for the *Sphingomonadales* order at Maxwell Bay and Marian Cove zones (Fig.6).

We identified the OTUs that were responsible for the significant difference in community composition observed across the transect with the *Indicspecies* R package. A total of 695 indicators OTUs were specific for one of the seven zones ($p=0.001$; stat $>0.82$; IV $>0.8$) (Supplementary Table 6). The order *Cyanobacteria* characterized the indicator OTUs for the Subantarctic order, being the most abundant indicator OTU (56%) followed by *Rodhobacterales* (17%) and *Flavobacterales* OTU (9.8%) (Figure 7; Supplementary Table 6). However, OTUs of the order *Cyanobacteria* were not restricted to this zone. It was also found in the next zone, Polar Front, but with a very low percentage of abundance (0.23%) in relation to other indicator OTUs (Supplementary Table 6). Polar Front zone hosted the highest number of indicators OTUs (316) dominated by members of the *Oceanospirillales* and *Alteromonadales* orders (Fig. 7; Supplementary Table 6). *Rhodobacterales* was the third most dominant order in the Polar Front zone (Supplementary Table 6) and OTUs from the order *Pseudomonadales* were selected as exclusive indicators of this zone (Figure 7; Supplementary Table 6). Amundsen Sea zone, which has the lowest number of indicators OTUs (Supplementary Table 6), was dominated by *Oceanospirillales* (65%) followed by *Rhodobacterales* (34%) (Figure 7; Supplementary Table 6).

Indicator OTUs from the order *Caulobacterales* were selected as exclusive indicator OTU for the Antarctic Peninsula zone (Figure 7; Supplementary 6). The order *Rickettsiales* (*SAR116*) characterized the Bransfield Strait zone with a 77% of the indicator OTUs sequences (Fig. 7; Supplementary Table 6). *Puniceicoccales* was the second major OTUs indicators in Maxwell Bay zone (31%) being mostly restricted to this zone and the Subantarctic zone where it was found in less abundance among the indicators OTUs (0.67%) (Figure 7; Supplementary Table 6). Marian Cove was the second zone that presented a high number of indicators OTUs (281 OTUs). Furthermore, OTUs from the order Campylobacterales were selected as exclusive indicator OTUs (Figure 7; Supplementary Table 6).

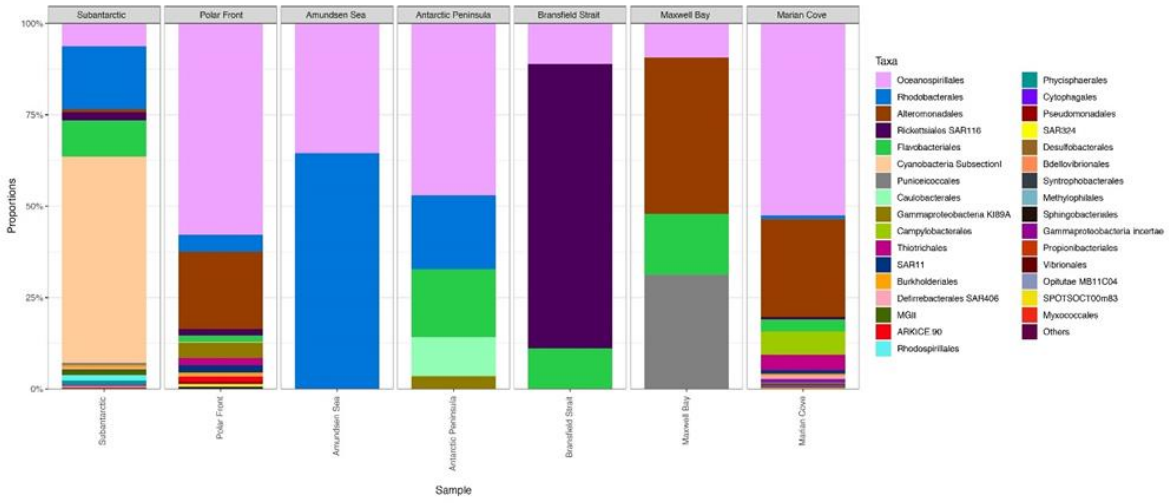



Figure 7: Relative abundance of most abundant indicator OTUs at the Order level in the RNA fraction of each province.

## Discussion

Our study reports the biogeography of marine microbes in a vast and sparsely studied area of the Southern Ocean. The patterns of community composition show that both the active fraction and the standing stock of bacterioplankton assemblages are structured by both key environmental parameters (temperature, salinity, POC PON and silicic acid) (27% of the variance) and by geographic distances (10% of the variance). The estimated amount of variance in our study were similar to the values reported in a review by Hanson et al. (2012). They point out that the average variance explained by environmental effect is 27% while for a distance effect it is 10%, highlighting that contemporary selection turns out to have a greater effect on microbial composition than historical processes on the biogeography of microorganisms.

Distance has been proposed as a barrier for dispersal in marine microorganisms. In most of the combinations tested in the present study, dissimilarity increased as the geographic distance between communities increased (Fig. 5). This strong distance-decay relationship can be associated to decreasing similarity in environmental parameters and hydrography with the increasing distance. However, the amount of variance that can be explained by distance (past events) versus environmental factors will depend on the sampling scale (Martiny et al., 2006). It is suggested that only when the distance is greater than 10,000 km the effects of historical separation may be greater than any effects that environmental factors may generate (Martiny et al., 2006). Our study covered a transect of ~ 6.500 km of distance with an average between sampling points of ~250 km in the first three zones (SA-PF-AS), an average of ~150 km between sampling points for the AP zone and an average of ~ 50 km between sampling points for the las three zones BS-MB-MC. It suggests that even though our sampling was extensive and detailed, the spatial resolution of this sampling survey might be not sufficient for distance alone to explain the observed biogeographic pattern.

According to the description made by Orsi et al. (1995) and Sokolov & Rintoul (2002), our sampling campaign crossed different oceanic fronts such as the southern branch of the Subantarctic Front (SAF), the southern branch of Polar Front (PF) and the Southern Fronts (SF) belonging to the Antarctic Circumpolar Current (ACC) divided into (i) the southern ACC front (sACCf) and (ii) the Southern Boundary of the ACC front (sbACC) (Fig. 8). Each of these fronts have distinct environmental characteristics that may promote distinct biological communities (Sokolov & Rintoul, 2002).

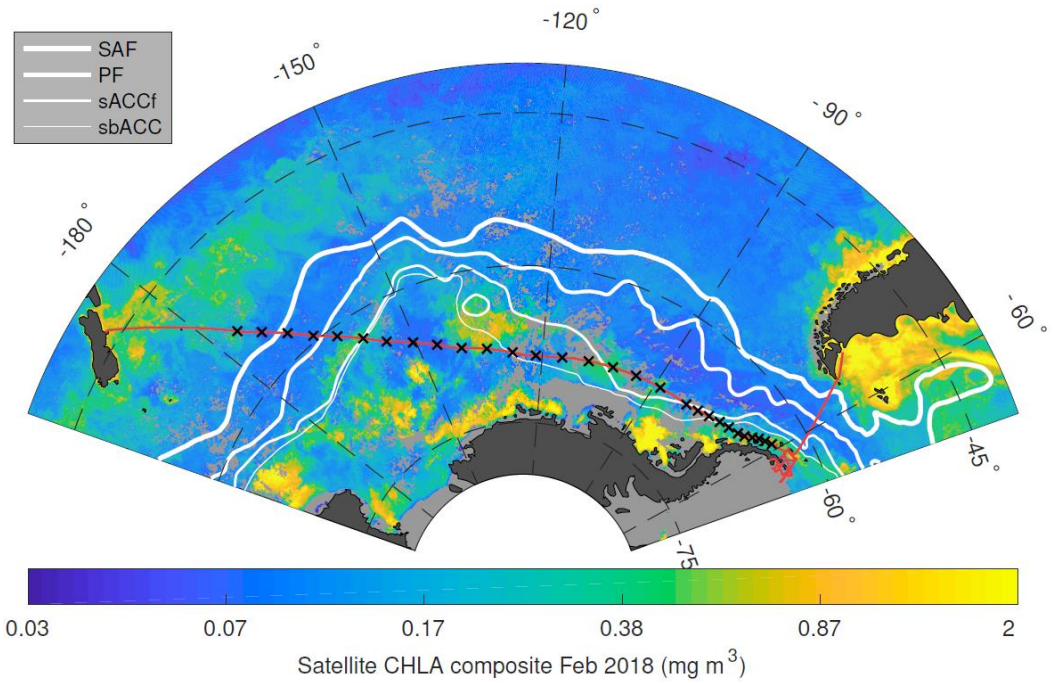



Figure 8: Oceanic fronts of Southern Ocean. SAF, Subantarctic Front; PF, Polar Front; sACCf, Southern Antarctic Circumpolar Current Front; sbACC, Southern Boundary Antarctic Circumpolar Current. The pink line shows the cruise transect and the X denote sampling sites.

Strong distance-decay patterns were also encountered in communities that were not geographically distant. It was the case for Subantarctic vs Polar Front zone, Amundsen Sea vs Antarctic Peninsula and Bransfield Strait vs Amundsen Sea, all of them separated by less than 250 km of distance. Several studies indicate that oceanographic properties of water masses and fronts play a significant role in the structure of bacterioplankton communities (Baltar et al., 2016; Djurhuus et al., 2017; Galand et al., 2010; Wilkins, et al., 2013a). The strong shift in community composition observed here within short distances could be due to the presence of different water masses. In particular, some samples obtained for Subantarctic zone were collected in the southern branch of SAF and PF, meanwhile the majority of the samples from Polar Front zone were obtained in the sACCf. Likewise, the samples collected for the Amundsen zone did not cross any front, unlike those collected in the Antarctic zone, which mostly established themselves in the sbACC front of the ACC. In the case of Bransfield Strait, the samples obtained were located within the sACCf. This result demonstrates that Southern Ocean fronts can act as significant biogeographic

boundaries generating significant changes in community composition of spatially near bacterioplankton communities.

The bacterioplankton communities were heterogeneously distributed along the Southern Ocean gradient being structured by the environmental parameters of each zone (Fig. 9). According to the environmental parameters the bacterioplankton communities of the seven zones were divided into two major groups: (i) Oceanic areas of the Southern Ocean transect composed of Subantarctic (SA), Polar Front (PF), Amundsen Sea (AS) and Antarctic Peninsula (AP) zones and (ii) coastal areas of the Southern Ocean composed of Bransfield Strait (BS), Maxwell Bay (MB) and Marian Cove (MC).

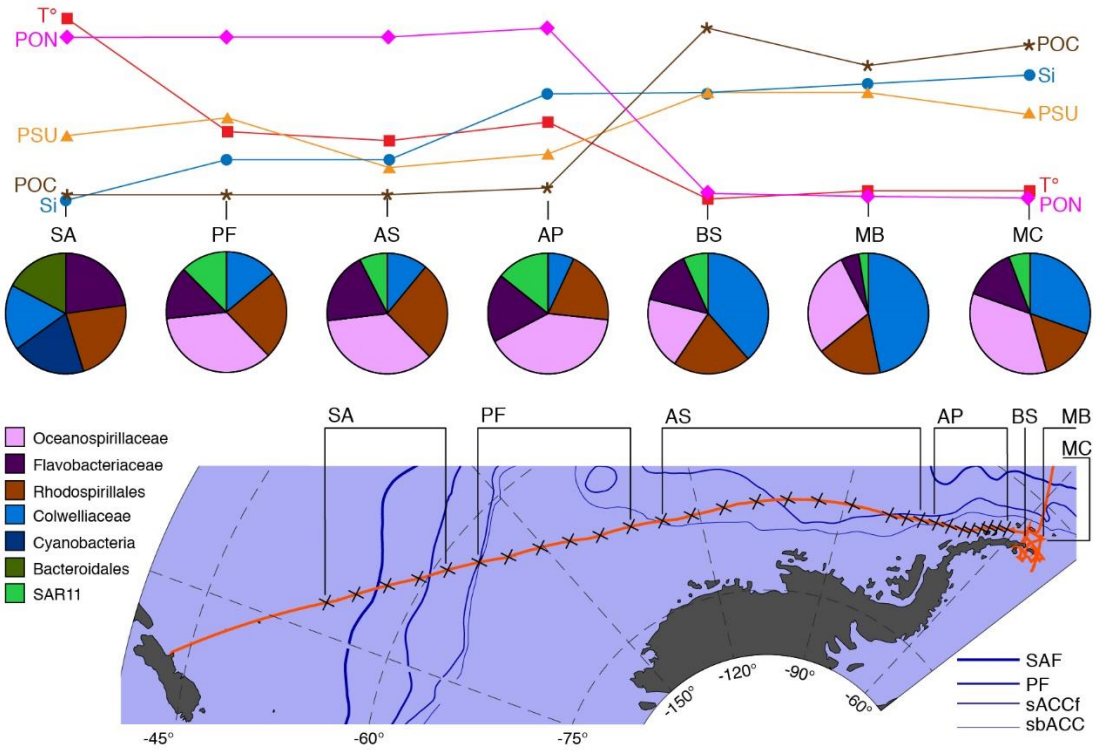



Figure 9: Conceptual description of major taxonomic changes in the microbial communities in relation to physical and chemical environmental parameter and oceanographic features that drive community changes along the spatial transect from Christchurch-New Zealand to South Shetland Island.

Temperature was the main environmental factor that structured the community composition among the zones possibly restricting the abundance of bacteria like *Alteromonadales* and SAR11 (Supplementary Table 4), while promoting the appearance of *Cyanobacteria* (Fig. 9). In agreement with these findings, it has been shown that the distribution of *SAR11,* (a globally distributed bacteria), can be significantly affected by temperature changes (Brown et al., 2012). The low temperatures recorded in BS, MB and MC could act as a growth factor for SAR11 for which high abundances are found at temperatures above 0°C (Brown et al., 2012). Conversely *Alteromonadales* are well cold-adapted heterotrophs that are normally found in cold waters of the Southern Ocean related to ice melt (Piquet et al., 2011). This may explain their high abundance as indicator taxa in the last part of the transect, a zone associated with ice formation and with the lowest temperatures registered (Fig. 9). Furthermore, one of the species that most contributed to the differentiation of the SA community from the rest of the zones was *Cyanobacteria*, which dominated in this zone while it was a minor taxonomic component (0.57%) of the entire dataset (Fig. 9). The first sample of the SA, where the number of sequences of *Cyanobacteria* was the most abundant, was collected in waters belonging to the southern branch of the SAF (-52.1400 °S) (Fig. 9). High abundances of *Cyanobacteria* have previously been reported further north of the STF where subtropical waters exhibit higher temperature and salinity (Liu et al., 2019; Wilkins et al., 2013a). Additionally, water surface temperature has been reported as a determining factor in the distribution of *Cyanobacteria* in the Southern Ocean (Marchant et al., 1987). The water temperature at which the highest abundance of *Cyanobacteria* was found corresponds to 12°C which represent the highest water temperature recorded in the entire transect, suggesting that the presence of *Cyanobacteria* is related to the water temperature in accordance with the characteristics described for the SAF.

The bacterioplankton communities of the PF, AS and AP zones were mainly structured by variations in the concentrations of silicic acid, POC, PON (Fig. 9) and secondly Chl-a, DON and ammonium concentrations. The most important nutrients for phytoplankton abundances are nitrogen and phosphorus and in particular silicate for diatoms (Sidabutar, 2016). However, ammonium has been reported to be an important nutrient able to alleviated the photoinhibition or the light-limitation at which phytoplankton is exposed in Antarctic waters (Agustí et al., 2009). Elevated ammonium concentrations (~ 3.3 µM) were recorded for the AP zone compare to the rest of the transect. Ammonium concentrations are seasonally variable in the West Antarctic Peninsula

with peak concentrations (>3.5 um) recorded in early autumn (March-May) (Henley et al., 2017). Previous studies have established successional patterns between phytoplankton blooms and some specific bacterioplankton members (Abell & Bowman, 2005; Choi, 2016; Kim et al., 2014; Liu et al., 2019; Luria et al., 2016; Obernosterer et al., 2011). In the PF, AS and AP zones, the generalist *Oceanospirillales*, *Rhodobacterales* and *Flavobacteriales* dominated de indicator taxa. *Flavobacteria*, *Rhodobacterales* and *Oceanospirillales* has been positively correlated with the abundance of large and small diatoms in the Southern Ocean (Kim et al., 2014; Liu et al., 2019; Luria et al., 2016). Considering the environmental parameters and the indicator OTUs selected, it is possible to indicate that the composition of the bacterioplankton community in the AS and AP are tightly related and structured by phytoplankton blooms.

In BS, MB and MC, the community structure of bacterioplankton were mainly driven by salinity, POC and silicic acid (Fig. 9; Supplementary Figure 3; Supplementary Table 4). The Bransfield Strait is characterized by a mixture of different bodies of water coming from different sectors of the Antarctic Peninsula. The surface layer during winter is characterized by temperatures lower than 1°C and salinity of 34.0 PSU which is define by Antarctic Surface Water (AASW) (Hofmann et al., 1996). Meanwhile Maxwell Bay and Marian Cove fjord present a typical estuarine circulation with superficial colder and less saline water (Llanillo et al., 2019). Furthermore, Marian Cove registered high concentrations of silicic acid and POC probably associated to meltwater streams with terrigenous particles that persist until late March (Yoo et al., 2015). The bacterioplankton community composition observed for BS, MB and MC zones is similar to the composition reported by others studies in the same area, relating the differences in the composition to the environmental gradients rather than spatial distance (Kim et al., 2020; Moreno-Pino et al., 2016; Zeng et al., 2014). In particular *Campylobacterales* (*Epsilonproteobacteria*) and *Puniceicoccales* (*Verrucomicrobiales*) were selected as indicator taxa of MC and MB respectively. *Campylobacterales* was detected in MC from head to mouth and from 0 to 25 m unlike that reported by Kim et al. (2020), who reports higher abundance of this order in areas near the mouth of the fjord influenced by water from MB. Furthermore, *Verrucomicrobiales* and *Campylobacterales* have been described as common members of particle-associated communities having the ability to degrade particulate organic carbon sources (Crespo et al., 2013; Duret et al., 2019; Fontanez et al., 2015). Differences in hydrographical properties of the water column like melt water input or mixed wind can lead to variation in the microbial

community composition of adjacent zones (Moreno-Pino et al., 2016; Zeng et al., 2014). Consequently, differences in the morphological characteristic, sediment load and meltwater discharge could be responsible for the observed differences in community composition between the zones BS, MB and MC.

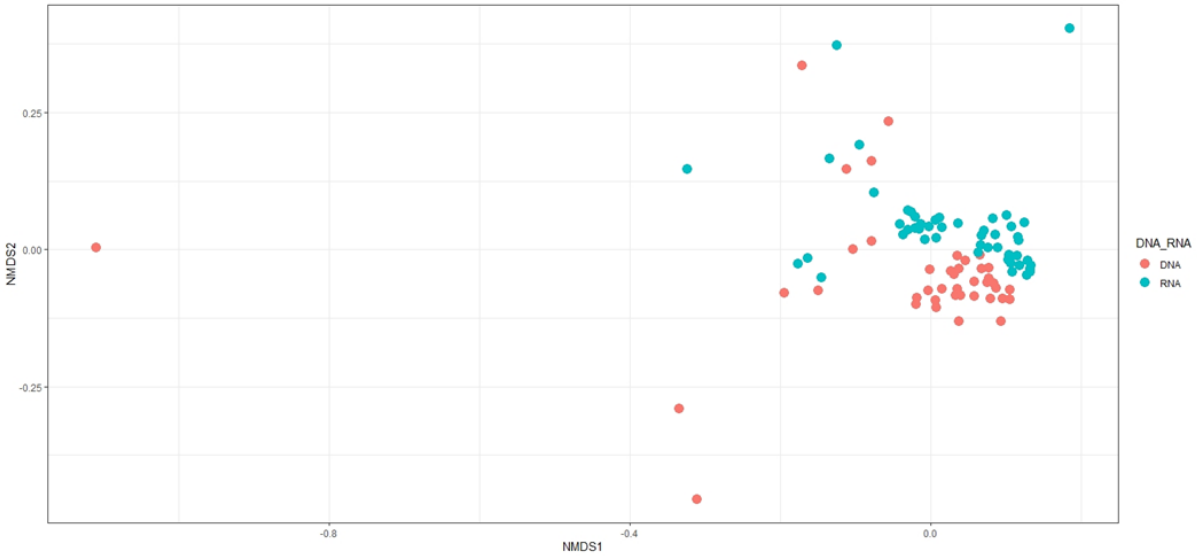
Therefore, the biogeographic patterns observed in our study are a combination of past events as a result of delimitation and geographic discontinuity produced by oceanic fronts, and current environmental conditions (temperature, salinity and nutrients). The main contribution of this study is to unveil the complex spatial pattern of the surface distribution of marine bacterioplankton concerning to an extended geographic area and increase knowledge about microbial ecology and biogeography in vulnerable ecosystems as the Southern Ocean.

## Conclusion

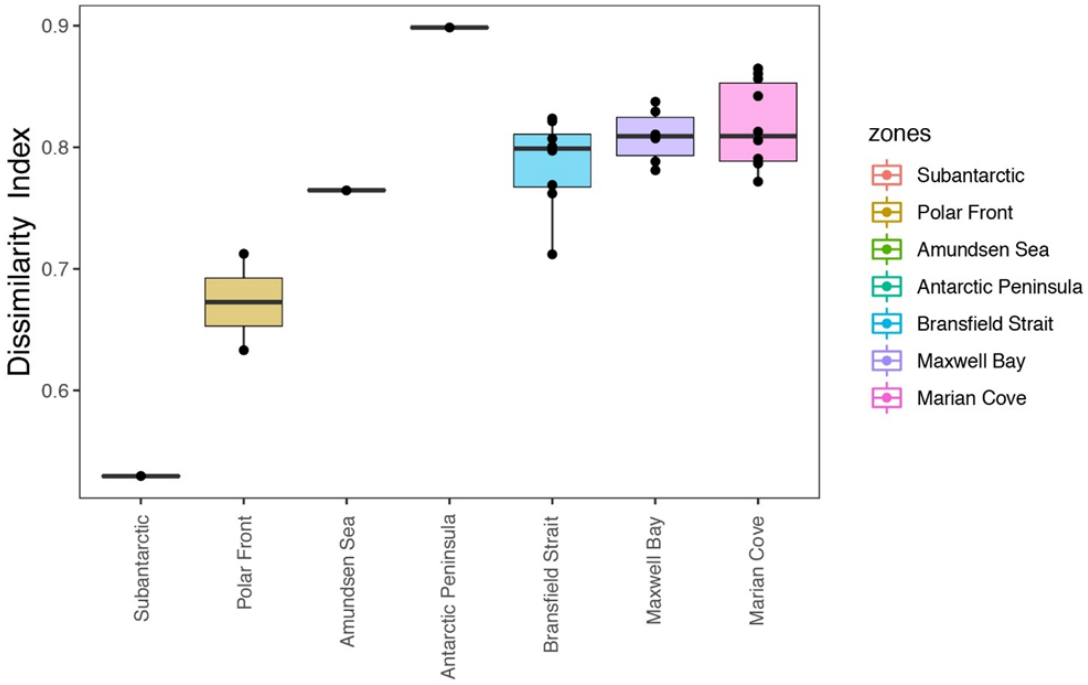
In conclusion, our results show that there are differences in the bacterioplankton community composition across the Southern Ocean transect from New Zealand to Antarctic Peninsula. The differences in taxonomic composition among the seven zones analyzed were evidenced at β-diversity level which strongly correlated with environmental parameters and with geographical distance. The turnover was mainly mediated by temperature, silicic acid, PON, salinity and POC. *Cyanobacteria*, *SAR11* and *Alteromonadales* were probably limited by water temperature, while the increase in *Oceanospirillales*, *Rhodobacterales* and *Flavobacteriales* was possibly driven by phytoplankton bloom. In the last part of the transect the increase in salinity favored a greater presence of *Gammaproteobacteria* meanwhile the appearance of *Campylobacterales* and *Puniceicoccales* might be associated to POC concentrations.

Ours results also indicate that ocean fronts shape microbial community composition by increasing the dissimilarity through the physicochemical and biological characteristics of the water column between geographically nearby and distant communities. However, the sampling resolution during the main SO transect did not allow us to characterize de different water masses encountered, and our analysis is based on previously published information that allow us to establish which oceanic fronts corresponding to the ACC were crossed during the sampling. Therefore, a rigorous confirmation of the effect of ocean fronts on the biogeographical patterns of bacterioplanktonic communities in the SO will require future work to measure physicochemical parameters as comprehensively as possible.

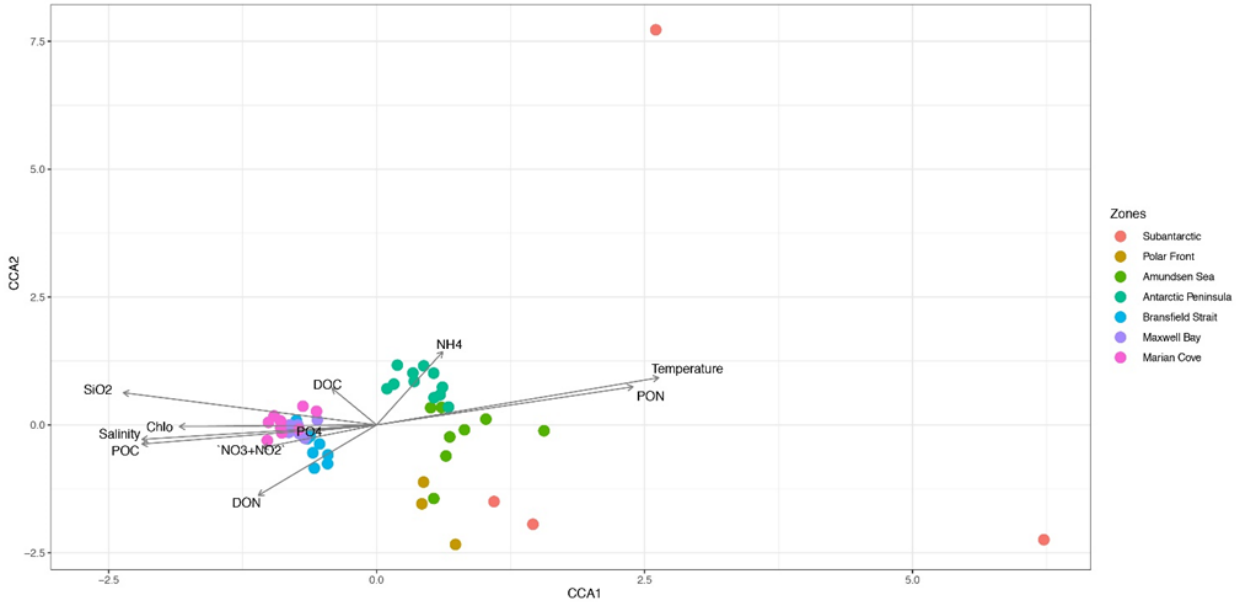
## Supplementary material




Supplementary Figure 1: Non-Metric Multidimensional Scaling ordination (NMDS) based on Bray-Curtis similarity of microbial communities identified across the seven provinces. Colours represent DNA communities (red) and RNA communities (blue). Stress 0.15.




Supplementary Figure 2: Bray-Curtis dissimilarity between the DNA and RNA fractions for microbial communities sampled. Only samples for which both the DNA and RNA fractions were successfully amplified were considered for this analysis. Colours represent provinces (Subantarctic, Polar Front, Amundsen Sea, Antarctic Peninsula, Bransfield




Supplementary Figure 3: Partial Canonical Correspondence Analysis (CCA) showing CCA1 and CCA2 plot of environmental and biological (OTU table) variables. Environmental variables correspond to temperature (°C), salinity (PSU), $NH_4$= ammonium, $NO_2$´+$NO_3$= inorganic nitrogen, Chla= total chlorophyll, $SiO_2$= silicic Acid, $PO_{4=}$ phosphate, DOC= dissolved organic carbon, POC= particulate organic carbon, DON= dissolved organic nitrogen, PON= particulate organic nitrogen.

Supplementary Table 1: Supplementary Table 1: Number of sequences detected for prokaryotic assemblages in water samples from Pía (P), Yendegaia 2017 (Y1) and Yendegaia 2018 (Y2) fjords.

| **Location** | **Total Reads** | **DNA reads** | **RNA reads** |
|---|---|---|---|
| Subantarctic | 192679 | 89818 | 102861 |
| Polar Front | 205831 | 126987 | 78844 |
| Amundsen Sea | 327900 | 303128 | 24772 |
| Antarctic Peninsula | 434881 | 434881 | NO DATA |
| Bransfield Strait | 624113 | 261736 | 362377 |
| Maxwell Bay | 510895 | 201339 | 309556 |
| Marian Cove | 889268 | 471733 | 417535 |
| **Total Reads** | 3185567 | 1889622 | 1089468 |

Supplementary Table 2: Permutational Multivariate Analysis of Variance (PERMANOVA) examining the effects of the variables Depth (0-50 m) and Station (Subantarctic, Polar Front, Amundsen Sea, Antarctic Peninsula, Bransfield Strait, Maxwell Bay and Marian Cove) and DNA/RNA in all the data set. Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; $R^2$, coefficient of determination; P, p-value. Significant results are in bold.

| Source of variation | Df | MS | F | R2 | P |
|---|---|---|---|---|---|
| Station | 3 | 0.682 | 2.517 | 0.070 | ***0.001*** |
| Depth | 1 | 0.264 | 0.974 | 0.009 | 0.498 |
| DNA_RNA | 1 | 1.028 | 3.790 | 0.035 | ***0.001*** |

Supplementary Table 3: Partial CCA

| CCA all samples | Inertia | Proportion | CCA 1 | % variation CCA1 | CCA 2 | % variation CCA2 |
|---|---|---|---|---|---|---|
| Total | 7.1388 | 100% | | | | |
| Geographic distance | 0.7028 | 10% | | | | |
| Environmental variables | 1.8948 | 27% | 0.49 | 7.6% | 0.262 | 4% |
| Unconstrained | 4.5412 | 63% | | | | |

Supplementary Table 4: Supplementary Table 4: partial CCA scores contrasting variables.

| Variable | CCA1 | CCA2 |
|---|---|---|
| Salinity | **-0.661** | -0.085 |
| Temperature | **0.795** | 0.279 |
| Ammonium | 0.187 | **0.432** |
| Inorganic Nitrogen | -0.321 | -0.134 |
| Chlorophyll a | **-0.555** | -0.010 |
| Silicic Acid | **-0.713** | 0.190 |
| Phosphate | -0.172 | -0.036 |
| Dissolved organic Carbon | -0.126 | 0.217 |
| Particulate Organic Carbon | **-0.661** | -0.113 |
| Dissolved Organic Nitrogen | -0.333 | **-0.416** |
| Particulate Organic Nitrogen | **0.722** | 0.225 |

Supplementary Table 5: Linear model geographic distance (Km) versus abundance dissimilarity matrix (Bray-Curtis).

| Zone combination | F-statistic | adjusted R-squared | p-value |
|---|---|---|---|
| Subantarctic vs Polar Front | 78.370 | **0.876** | **0.000** |
| Subantarctic vs Amundsen Sea | 6.803 | 0.158 | **0.014** |
| Subantarctic vs Antarctic Peninsula | **138.900** | **0.762** | **0.000** |
| Polar Front vs Amundsen Sea | 14.010 | 0.361 | **0.001** |
| Polar Front vs Antarctic Peninsula | 37.780 | 0.534 | **0.000** |
| Amundsen Sea vs Antarctic Peninsula | 163.400 | 0.651 | **0.000** |
| Bransfield Strait vs Subantarctic | **126.200** | **0.802** | **0.000** |
| Bransfield Strait vs Polar Front | 15.230 | 0.382 | **0.001** |
| Bransfield Strait vs Amundsen Sea | 107.100 | 0.628 | **0.000** |
| Bransfield Strait vs Antarctic Peninsula | 0.369 | -0.007 | 0.545 |
| Bransfield Strait vs Maxwell Bay | 14.750 | 0.226 | **0.000** |
| Bransfield Strait vs Marian Cove | 41.810 | 0.306 | **0.000** |
| Maxwell Bay vs Subantarctic | **121.800** | **0.840** | **0.000** |
| Maxwell Bay vs Polar Front | 18.180 | 0.503 | **0.001** |
| Maxwell Bay vs Amundsen Sea | **141.700** | **0.750** | **0.000** |
| Maxwell Bay vs Antarctic Peninsula | 0.047 | -0.015 | 0.830 |
| Maxwell Bay vs Marian Cove | 0.173 | -0.014 | 0.680 |
| Marian Cove vs Subantarctic | **292.200** | **0.882** | **0.000** |
| Marian Cove vs Polar Front | 18.850 | 0.381 | **0.000** |
| Marian Cove vs Amundsen Sea | **193.500** | **0.709** | **0.000** |
| Marian Cove vs Antarctic Peninsula | 0.174 | -0.007 | 0.678 |

Supplementary Table 6: Distribution of indicator microbial OTU among the seven provinces (IV >0.8 p =0.001; stat > 0.8).

| Province | N° Indicator microbial OTUs | N° OTUs per Class | Microbial Taxa Class/ Order Level | | Abundance % per Province |
|---|---|---|---|---|---|
| Subantarctic | 53 | 10 | Alphaproteobacteria | Rhodobacterales | 17.22 |
| | | 4 | Alphaproteobacteria | Rhodospirillales | 1.44 |
| | | | | Rickettsiales | |
| | | 4 | Alphaproteobacteria | SAR116 | 2.3 |
| | | 1 | Betaproteobacteria | Burkholderiales | 0.56 |
| | | 5 | Cyanobacteria | SubsectionI | 56.43 |
| | | 1 | Cytophagia | Cytophagales | 0.27 |
| | | | | Deferribacterales | |
| | | 1 | Deferribacteres | SAR406 | 0.56 |
| | | 1 | Deltaproteobacteria | Bdellovibrionales | 0.49 |
| | | 1 | Fibrobacteria | Fibrobacterales | 0 |
| | | 7 | Flavobacteriia | Flavobacteriales | 9.89 |
| | | 3 | Gammaproteobacteria | Alteromonadales | 0.75 |
| | | 6 | Gammaproteobacteria | Oceanospirillales | 6.25 |
| | | 2 | Gammaproteobacteria | Vibrionales | 0.15 |
| | | 1 | Opitutae | MB11C04 | 0.09 |
| | | 1 | Opitutae | Puniceicoccales | 0.67 |
| | | 2 | Phycisphaerae | Phycisphaerales | 1.22 |
| | | 1 | Planctomycetacia | Planctomycetales | 0.08 |
| | | 2 | Thermoplasmata | MGII | 1.56 |
| Polar Front | 316 | 29 | Alphaproteobacteria | Rhodobacterales | 4.53 |
| | | 6 | Alphaproteobacteria | Rhodospirillales | 1.66 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | Rickettsiales | |
| | | 13 | Alphaproteobacteria | SAR116 | 1.8 |
| | | 7 | Alphaproteobacteria | SAR11 | 1.98 |
| | | 3 | ARKICE-90 | uncultured | 1.24 |
| | | 2 | Betaproteobacteria | Burkholderiales | 0.87 |
| | | 1 | Betaproteobacteria | Methylophilales | 0.23 |
| | | 5 | Cyanobacteria | SubsectionI | 0.23 |
| | | 1 | Cytophagia | Cytophagales | 0 |
| | | | | Deferribacterales | |
| | | 2 | Deferribacteres | SAR406 | 0.23 |
| | | 1 | Deltaproteobacteria | Bdellovibrionales | 0 |
| | | 2 | Deltaproteobacteria | SAR324 | 0.5 |
| | | 1 | Fibrobacteria | Fibrobacterales | 0.09 |
| | | 14 | Flavobacteriia | Flavobacteriales | 1.71 |
| | | 48 | Gammaproteobacteria | Alteromonadales | 20.84 |
| | | 5 | Gammaproteobacteria | KI89A | 4.11 |
| | | 162 | Gammaproteobacteria | Oceanospirillales | 56.86 |
| | | 2 | Gammaproteobacteria | Pseudomonadales | 0.78 |
| | | 2 | Gammaproteobacteria | Thiotrichales | 1.84 |
| | | 2 | Gammaproteobacteria | Vibrionales | 0 |
| | | 1 | Opitutae | MB11C04 | 0.04 |
| | | 1 | Opitutae | Puniceicoccales | 0 |
| | | 2 | Phycisphaerae | Phycisphaerales | 0 |
| | | 1 | Planctomycetacia | Planctomycetales | 0 |
| | | 1 | Sphingobacteriia | Sphingobacteriales | 0.41 |
| | | 2 | Thermoplasmata | MGII | 0 |
| Amundsen | | | | | |
| Sea | 2 | 1 | Alphaproteobacteria | Rhodobacterales | 65.51 |
| | | 1 | Gammaproteobacteria | Oceanospirillales | 35.48 |

| | | | | | |
|---|---|---|---|---|---|
| Antarctic Peninsula | 23 | 1 | Alphaproteobacteria | Caulobacterales | 10.63 |
| | | 6 | Alphaproteobacteria | Rhodobacterales | 20.15 |
| | | 3 | Flavobacteriia | Flavobacteriales | 18.64 |
| | | 1 | Gammaproteobacteria | KI89A | 3.5 |
| | | 12 | Gammaproteobacteria | Oceanospirillales | 47.05 |
| Bransfield Strait | 3 | 1 | Alphaproteobacteria | Rickettsiales | 77.77 |
| | | 1 | Flavobacteriia | Flavobacteriales | 11.11 |
| | | 1 | Gammaproteobacteria | Oceanospirillales | 11.11 |
| Maxwell Bay | 17 | 3 | Flavobacteriia | Flavobacteriales | 16.66 |
| | | 8 | Gammaproteobacteria | Alteromonadales | 42.71 |
| | | 2 | Gammaproteobacteria | Oceanospirillales | 9.375 |
| | | 4 | Opitutae | Puniceicoccales | 31.25 |
| Marian Cove | 281 | 1 | Actinobacteria | Propionibacteriales | 0.15 |
| | | 7 | Alphaproteobacteria | Rhodobacterales Rickettsiales | 1.04 |
| | | 3 | Alphaproteobacteria | SAR116 | 0.67 |
| | | 3 | Alphaproteobacteria | SAR11 | 1.02 |
| | | 1 | ARKICE-90 | uncultured | 0.19 |
| | | 3 | Betaproteobacteria | Burkholderiales | 0.46 |
| | | 2 | Betaproteobacteria | Methylophilales | 0.19 |
| | | 2 | Cytophagia | Cytophagales | 0.65 |
| | | 4 | Deferribacteres | Deferribacterales 406 | 1.02 |
| | | 1 | Deltaproteobacteria | Desulfobacterales | 0.53 |
| | | 1 | Deltaproteobacteria | Myxococcales | 0.12 |
| | | 1 | Deltaproteobacteria | Syntrophobacterales | 0.48 |

| | | | |
|---|---|---|---|
| 25 | Epsilonproteobacteria | Campylobacterales | 6.39 |
| 10 | Flavobacteriia | Flavobacteriales | 3.3 |
| 74 | Gammaproteobacteria | Alteromonadales | 26.8 |
| 1 | Gammaproteobacteria | Incertae | 0.24 |
| 126 | Gammaproteobacteria | Oceanospirillales | 52.47 |
| 15 | Gammaproteobacteria | Thiotrichales | 4.13 |
| 1 | SPOTSOCT00m83 | | 0.13 |

# GENERAL DISCUSSION

This thesis aimed to investigate the influence of environmental parameters on bacterioplankton community composition and distribution in Southern Patagonia and Southern Ocean ecosystems. This discussion section will examinate and summarize the main findings of this thesis by highlighting our contribution to a better understanding of the structure of the bacterioplanktonic communities in southern high latitude environments.

## 1 Marine microbial community structure in southern high latitude fjords

Through this thesis we have studied two Subantarctic fjords, Pía and Yendegaia, and two Antarctic fjords Marian Cove and Maxwell Bay all presented in Chapter 1 and 2. In the following section I will synthetize and discuss the major findings including the fjord's characteristics, their bacterioplanktonic composition, and the future perspectives on southern high latitude fjords.

### 1.1 Characteristics of Subantarctic and Antarctic fjords.

Fjords are ecosystems where land-ocean environments overlap and where geological, chemical, biological and physical processes work together to maintain the functionality of the aquatic system (Bianchi et al., 2020). They are ecosystems highly sensitive to climate change that play a key role in the exchange of organic matter and carbon flow between terrestrial and marine environments (Bianchi et al., 2020; González et al., 2013; Iriarte et al., 2010). The studied fjords are located in the Subantarctic (southern Chilean Patagonia) and the Antarctic (King George Island) territory. These two zones are important ecosystems where great variations of climatic conditions have been taking place since the second half of the last century (Iriarte et al., 2019; Meredith & King, 2005; Schofield et al., 2010). In the Antarctic and Subantarctic ecosystems most of the freshwater input comes from glacier melt which is mostly channeled through fjords to the costal ocean (Iriarte et al., 2014). The result of this freshwater discharge in these ecosystems is complex and depends mostly on glacier-fjord interactions.

Among the subantarctic fjords studied, Yendegaia and Pía are located in the southern Patagonia of Chile, both fjords flow into the Beagle Channel and are separated by about 70 km of distance. The Yendegaia fjord has a land terminating glacier which has retreated approximately 12 km from the head of the fjord. Therefore, the water column of Yendegaia is impacted by freshwater discharges with high sediment content from the glacier transported by the 12 km river. In contrast, Pía Fjord has a marine terminating glacier which receives surface and deep freshwater discharge from the glacier.

The Antarctic fjords Marian Cove and Maxwell Bay are both located in the King George Island. Maxwell Bay is a deep fjord (~500 m) delimited on its western part by Nelson Island (Llanillo et al., 2019). Several tributary coves and bays border Maxwell Bay as well as large glaciers (Llanillo et al., 2019; Yoo et al., 2015). Marian cove is one of the tributary of Maxwell Bay, it is a narrow fjord approximately 4.5 km long and 1.5 km wide with a maximum depth of 120 m (Ahn et al., 2016; Yoo et al., 2015). Marian cove glacier ends in a vertical cliff (marine-terminating glacier) which has retreated approximately 1.7 km since 1956 (Moon et al., 2015). According to this description, the 4 fjords studied can be grouped into fjords with land-terminating glacier (Yendegaia) and with marine-terminating glacier (Pía, Marian Cove and Maxwell bay).

The impact of the freshwater discharge on the water column structure will depend greatly on the marine or land terminating connection presented between the fjord and the glacier. The proglacial river formed by retreating glaciers (land-terminating glacier) transport meltwater with suspended sediments which enter the fjord as a narrow buoyant plume (Chu, 2014). The most notorious implications of the sediment plume are the increased vertical stratification and limited light penetration due to increased surface water turbidity (Chu et al., 2009). Inversely, in the case of marine-terminating glacier, fresh water is discharged directly from the glacier to the surface of the fjord, but also from below the glacier down to approximately 100 m depth (Chu, 2014). The water discharged from depth can produce an upwelling of sediment and nutrient-rich water (Meire et al., 2017). The upwelling of nutrients mostly caused by water discharges in summer periods would produce a state of higher productivity in fjords with marine-terminating glacier than in those with land-terminating glacier (Giesecke et al., 2019; Meire et al., 2017).

As for water column hydrography, all the studied fjords presented a typical estuarine circulation with superficial colder and less saline water layer resulting from freshwater input, and a more profound layer with saltier and nutrient rich water as a result of oceanic waters inflow

(Llanillo et al., 2019; Sievers & Silva, 2008). This influx is called “estuarine circulation” where water column containing brackish water at the surface flow out of the fjord, and the more saline and nutrient rich coastal water beneath flow into the fjord through the mouth (Howe et al., 2010). However, the presence of entrance sills can limit the estuarine circulation and thus the oxygen renewal (Howe et al., 2010). Pía Fjord, Marian Cove and Maxwell Bay have a sill at the entrance of the fjord that significantly limits the inflow of water from the Beagle Channel in the case of Pía Fjord, the Maxwell Bay in the case of Marian Cove, and the water from Bransfield Strait in the case of Maxwell Bay (SHOA, Hydrographic and Oceanographic Service of the Chilean Navy; Llanillo et al., 2019; Yoo et al., 2015). This limitation of water circulation leads to an increase in the residence time of estuarine waters, which in turn can control the location and magnitude of phytoplankton blooms and the distribution of bacterial communities (Crump et al., 2004; Howe et al., 2010). The transport time scale calculated for residence time can vary widely between estuarine systems from 1-2 days to 7 months impacting the available time for the development of native bacterial populations (Crump et al., 2004).

In addition to their glacier regime, fjords can be classified according to the climatic regime in which they are found. According to this classification there are three fjord categories: (i) polar fjords which are permanently covered by sea ice or by an ice shelf, (ii) subpolar fjords located in places where the air temperature in summer exceeds 0°C, breaking up the ice formed during the winter, and (iii) temperate fjords in which the water column never freezes and in some cases there is no glacier present (Howe et al., 2010). Yendegaia fjord can be classified as a temperate fjord because the water column never freezes along the year. Meanwhile, Pía Fjord, Marian Cove and Maxwell Bay fall into the subpolar fjord classification because in the winter season a layer of ice forms on the surface of the water column, which melts as the temperature increases during early summer (Ahn et al., 2016; Llanillo et al., 2019; Yoo et al., 2015).

One of the major differences that we observed between the Subantarctic and Antarctic fjords sampled in this thesis is the concentration of nutrients. The sampling carried out in the Subantarctic region evidenced a state of oligotrophy for the fjords (Chapter 1 Fig. 2), while higher concentrations of nutrients such as phosphate, inorganic nitrogen and ammonium were recorded in the Antarctic fjords (Chapter 2, Fig. 2). In addition, differences in chlorophyll-α concentrations (~ 0.1 vs ~ 2.0 mg $m^3$) were recorded for both groups of fjords, with the highest concentrations occurring in the Antarctic fjords.

Differences in light availability, temperature, salinity, nutrients, chlorophyll, and water residence time could affect the microbial community function and composition of bacterioplankton communities producing communities that differ according to the characteristics of the sampled site.

## 1.2 Bacterioplanktonic community structure in the fjords

In order to have a full overview of the composition of the bacterioplanktonic community of the fjords studied and to understand how the characteristics of each fjord can influence the composition of the microbial community, a synthesis analysis of the data from Chapter 1 and 2 was done. Major differences were observed when comparing the composition of the bacterioplanktonic communities of the four fjords. The synthesis analysis shows that in the RNA fraction, the samples were divided in two major groups: Subantarctic fjords and Antarctic fjords. Furthermore, among these groups each fjord grouped according to their location, and in the case of Yendegaia, according to the year of sampling. In contrast, in the DNA fraction samples were more dispersed and with a higher number of outlier samples compared with samples of the RNA fraction (Fig.1). However, both fractions evidenced significant differences in the composition of the communities according to the sampling site (PERMANOVA adonis p=0.001) (Table 1)

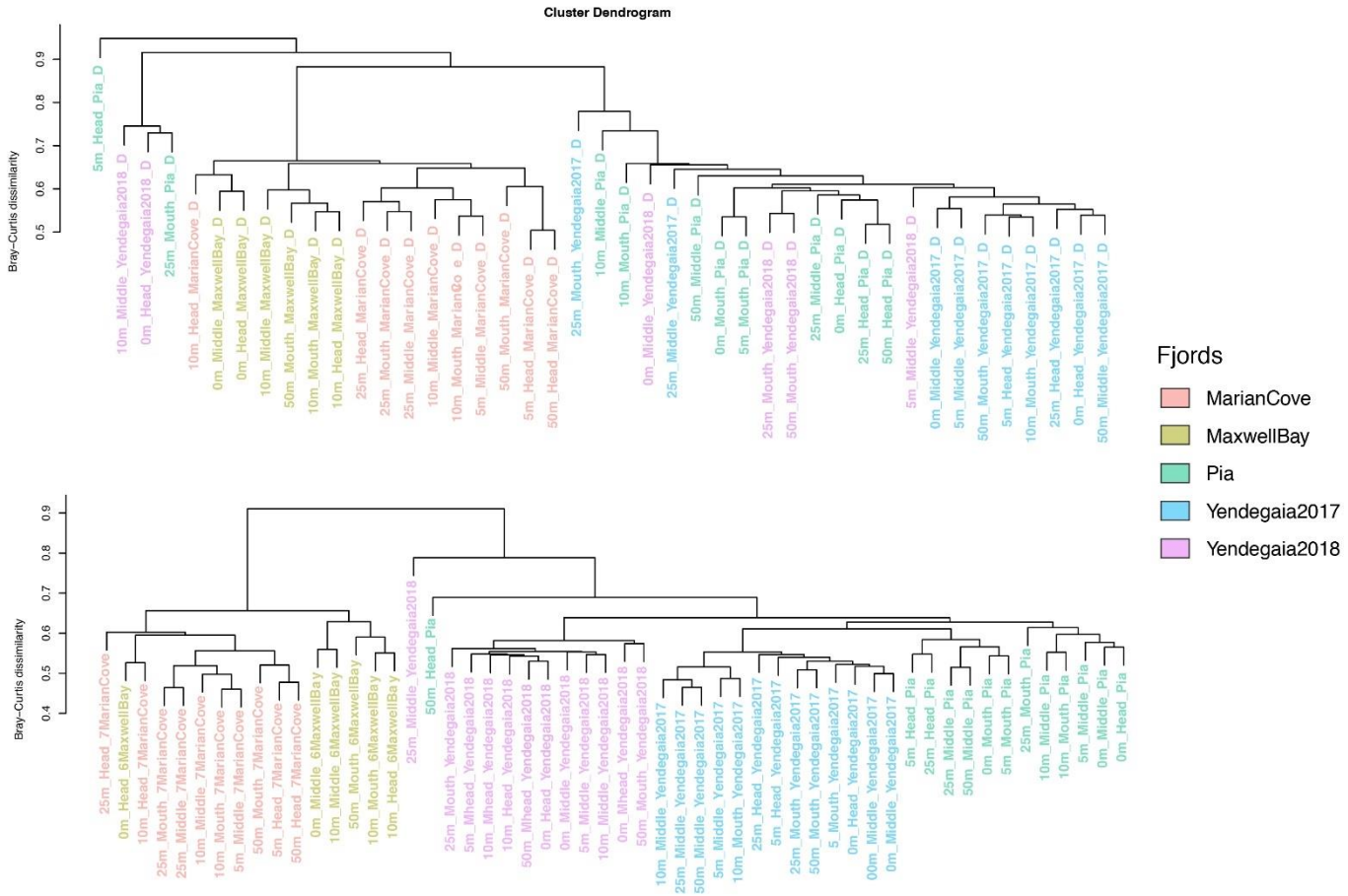



Figure 1: Hierarchical cluster dendrogram based on Bray-Curtis dissimilarity index showing the similarity between microbial community compositions for the rDNA(**A**) and rRNA (**B**) fractions in each fjords and years.

Table 1: Permutational Multivariate Analysis of Variance (PERMANOVA) examining the effects of the variables Depth (0-50 m) and Location (Maxwell Bay, Marian Cove, Pía fjord, Yendegaia 2017 fjord and Yendegaia 2018 fjord) and DNA/RNA in data set of four fjords. Key to abbreviations and column headings: MS, mean square; F, F ratio; $R^2$, coefficient of determination; P, p-value. Significant results are in bold.

**PERMANOVA (adonis)**

| Fraction | Source of variation | MS | F | R2 | P |
|---|---|---|---|---|---|
| DNA | Location | 1.176 | 5.110 | 0.350 | ***0.001*** |
| | Depth | 0.230 | 0.998 | 0.017 | 0.362 |
| RNA | Location | 1.719 | 10.161 | 0.449 | ***0.001*** |
| | Depth | 0.207 | 1.221 | 0.014 | 0.216 |
| Both Fractions | Location | 2.273 | 10.634 | 0.330 | ***0.001*** |
| | Depth | 0.253 | 1.185 | 0.009 | 0.197 |
| | DNA vs RNA | 1.212 | 5.669 | 0.044 | ***0.001*** |

The location explained 45% of the variation among the community composition in the RNA fraction (Table 1). Additionally, when testing the assumption of homogeneity of variance for both fractions a significant value was obtained by the RNA fraction (p=0.001) indicating variation between the composition of the communities (β-diversity) (Table 2).

Table 2: Permutation test for homogeneity of multivariate dispersions examining the effects of the variables Location (Maxwell Bay, Marian Cove, Pía fjord, Yendegaia 2017 fjord and Yendegaia 2018 fjord) and DNA/RNA in data set of four fjords. Key to abbreviations and column headings: MS, mean square; F, F ratio; N. Perm, Number of permutations; P, p-value. Significant results are in bold.

| Fraction | Source of variation | MS | F | N. Perm | P |
|---|---|---|---|---|---|
| DNA | Location | 0.017 | 1.955 | 999 | 0.103 |
| RNA | Location | 0.006 | 3.017 | 999 | ***0.017*** |
| Both Fractions | Location | 0.011 | 1.846 | 999 | 0.125 |
| | DNA vs RNA | 0.003 | 0.433 | 999 | 0.545 |

In general terms, the taxonomic composition of the bacterioplanktonic communities of the Subantarctic and Antarctic fjords was dominated by members of the phylum *Proteobacteria* (Fig. 2). However, differences in the proportion of these dominant groups were observed depending on the area of study. In the Antarctic zone *Gammaproteobacteria* was the most abundant class with a 58.8% of the total of sequences meanwhile in the subantarctic zone the *Alphaproteobacteria* dominated with a 24.8% of sequences (Fig. 2). Among the Orders that composed these classes *Oceanospirillales* and *Alteromonadales* (*Gammaproteobacteria*) were the most abundant in Antarctic fjords while *Rhodospirillales* and *Rhodobacterales* (*Alphaproteobacteria*) were the highly abundant in Subantarctic fjords (Fig. 2).

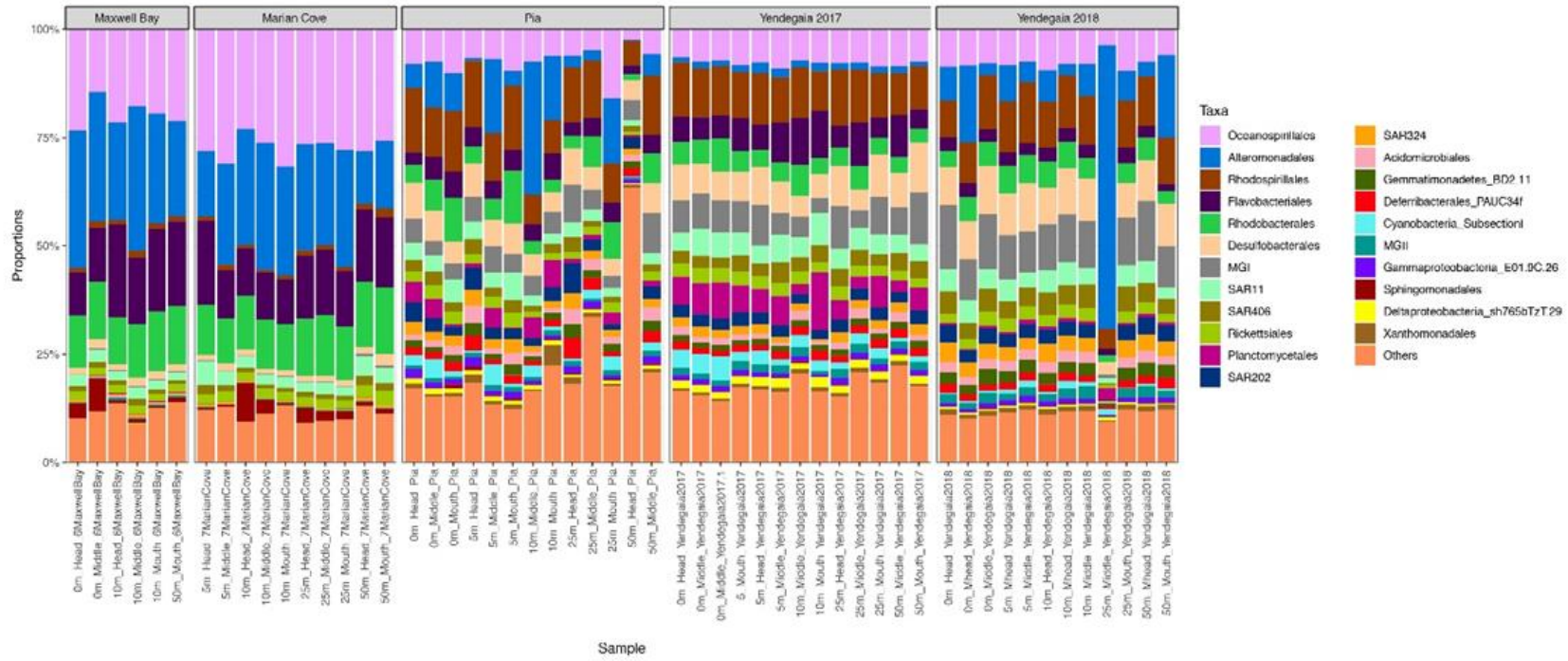



Figure 2: Relative abundance of taxonomical groups at the Order level in the RNA fraction of each fjord.

The differences in abundances of dominant groups could be explained by the distinct water masses characterizing each fjord. In the case of the Subantarctic zone, Pía fjords shows a clear influence of glacier discharge reflected in higher stratification at the head station and dominance of estuarine waters (21-31 PSU) at the top 50 m of the water column (Chapter 1, Supplementary Fig. 1). In comparison for Yendegaia fjord, interannual differences in the water column were observed. During July 2018 a clear presence of Modified Sub-Antarctic Waters (MSAAW) (31 -33 PSU) was detected across the entire fjord, meanwhile during July 2017, Yendegaia show a more stratified water column than 2018 and with a lower salinity (~ 30.5 – 31) (Chapter 1, Supplementary Fig. 2). In the case of Antarctic fjords, the water column of Marian Cove and Maxwell Bay were similar in salinity and temperature for the top 50 m. Marian cove showed signs of stratification at the head station with lower salinity and temperature than the rest of the stations, while in Maxwell Bay, a pycnocline below 50 m was registered. The Subantarctic fjords generally had lower salinity (30-32 PSU) than the salinity recorded in the Antarctic fjords (~34 PSU) (Chapter 1 Fig. 2; Chapter 2 Fig. 2). Studies of microbial community in salinity gradients have shown a higher abundance of *Oceanospirillales* (*Gammaproteobacteria*) associated with higher salinity concentration (Fortunato & Crump, 2015). Conversely, *Alphaproteobacteria* have been showed to be more abundant in areas with lower salinity concentration (Fortunato & Crump, 2015). Beside salinity, the bacterioplankton community composition of Antarctic fjords was also influenced by POC and silicic acid. *Campylobacterales* and *Puniceicoccales orders* were selected as indicator taxa for Marian Cove and Maxwell Bay, both taxa were previously reported as common members of particle-associated communities important in the remineralization process of particulate organic matter (Crespo et al., 2013; Fontanez et al., 2015).

In contrast in the Subantarctic fjord, none of the core environmental parameters (temperature, salinity, nutrients, organic matter) were shown to significantly influence the microbial community composition. However, the bacterioplankton composition of the subantarctic fjords could be influenced by the morphological characteristics of each fjord. Yendegaia fjord receives a large amount of suspended sediments transported to the long proglacial river (12 km) which enters the fjord as a floating plume affecting light penetration and water column stratification (Chu, 2014; Chu et al., 2009). While in the case of the Pía Fjord it receives surface and subsurface water discharges coming directly from the glacier which allow an upwelling of nutrients that in periods of high discharges promotes a state of high productivity for the fjord (Giesecke et al., 2019; Meire et al., 2017). *Thaumarchaeota*-MGI

contributed to 19% of the total sequences across the Subantarctic fjords, making it the second most abundant taxonomic group for this zone. While in the Antarctic fjords MGI was represented by a scarce 0.4% of the total sequences. The presence of MGI in surface waters of high latitude environments have been previously documented (Bano et al., 2004; Galand et al., 2008; Gutiérrez et al., 2015; 2018; Hernández et al., 2015; Santoro et al., 2019). However, differential patterns in abundance have been associated to season and the decrease in solar radiation intensity (Hernández et al., 2015; Luo et al., 2014; Pedneault et al., 2015; Tolar et al., 2016). Both zones were sampled during the autumn-winter season when solar radiation intensity is lower than in spring-summer. For which the highest abundance of this group found in Yendegaia fjord, are possibly explained by the limitation of light due to the surface plume of inorganic material from the land-terminating glacier. In addition during 2018 chlorophyll concentrations were even lower than in 2017 possibly leading to an increase in MGI abundance since a negative correlation has been described between MGI abundance and chlorophyll concentration (Murray et al., 1998). Another group of interest found in higher proportions in the Subantarctic fjords (2.9%) compare to Antarctic fjords (0.008%) was SAR202 which has not been previously described in surface waters of the Subantarctic zone. The higher abundances for this group were registered in Pía fjord (Subantarctic) and its occurrence could be associated with the subsurface glacier discharges (below 100 m) that would result in an upwelling of sediment and nutrients (Chu, 2014) favouring the appearance of species described in depth as SAR202 (Mehrshad et al., 2018).

In the Subantarctic fjords the values of nutrients, chlorophyll salinity and temperature remained almost constant across the stations possibly impeding a correlation of environmental parameters with the composition of the microbial community. It is therefore necessary to consider in more detail the sampling points within a fjord to ensure a correct environmental gradient that more accurately reflects fjord conditions. In the case of the Pía fjord, due to the dense ice cover encountered at the time of sampling, the head station corresponded to a location distant from the glacier discharge, which did not allow us to obtain a gradient that truly reflected the glacier water discharge. Likewise, in Yendegaia fjord the interannual sampling (2017 vs 2018) showed discrepancies in the number of points sampled within the fjord.

In summary, the four fjords studied presented environmental, hydrographic, and morphological characteristics that influence the composition of bacterioplanktonic communities by selecting a different community composition between the fjords. These differences are clearly displayed in the active fraction of the community rather than in the total

fraction. Our study indicate that analysis of the active fraction (16S rRNA) may result more informative than analysis of the total fraction (16S rDNA) in understanding how bacterial communities respond to variation in physical and other factors along fjords gradients. In section 3 a discussion related to this topic is provided.

## 1.3 Perspectives on the future of fjords ecosystems

Fjords are excellent natural laboratories to explore the structure of microbial communities because they are dynamics environments where temperature, salinity and nutrient gradients can be observed in a narrower scale than in the open ocean (Iriarte, 2018). Moreover, fjords are at the crossroad of terrestrial, cryosphere, oceanic and atmospheric interactions (Bianchi et al., 2020).

Fjords are also sensor ecosystems highly vulnerable to climate change due to their location in high latitude zones with warming rates higher than the global average temperature (Bianchi et al., 2020). The increase in atmospheric temperature will continue to promote the retreat of glaciers, so it is expected that marine-terminating glaciers typical of subpolar and polar areas will begin to disappear and that fjord-land-terminating glacier interface will prevail (Fig. 3B). This type of interface will result in changes in the inorganic supply and physical conditions of the fjord leading to a decrease in primary productivity and alterations in the carbon cycling (Meire et al., 2017) (Fig. 3). Effects of glacier retreat have evidenced a decrease in the abundance and functional diversity of epibenthic megafauna related to the high concentration of clay and silt (Moon et al., 2015) (Fig. 3B). Additionally, a reduction in phytoplankton abundance and consequent reduction in copepod biomass has been attributed to the characteristics of meltwater input from land-terminating glaciers (Giesecke et al., 2019) (Fig. 3B). The meltwater from land-terminating glaciers enters the fjords as a dense sediment plume that primarily limits light penetration and increases stratification of the water column (Chu et al., 2009) (Fig. 3B). The pronounced water column stratification prevents nutrient supplementation from deeper water while the high turbidity of the water column limits light penetration (Meire et al., 2017) (Fig. 3B). Both factors promote low chlorophyll concentration and low primary production (Meire et al., 2017).

Ours results adds to the current knowledge by showing that the active fraction of bacterioplankton communities is different between fjords with land terminating and marine

terminating glaciers. Ours results suggest that members of the phylum *Bacteroidetes,* mostly represented by *Flavobacteriales*, will markedly decrease in abundance in future fjords (Fig. 3B), which might be associated, as in Antarctic coastal areas, with the decrease in primary production prevented by the stratification of the water column (Piquet et al., 2011). It has been described that the combined effect of limited light penetration and low nutrient concentration (Si and N) in southern Patagonian fjords, will restrict the development of microphytoplankton (diatoms and dinoflagellates) resulting in a low primary production state (Iriarte et al., 2018). *Flavobacteriales* abundances is highly related to phytoplankton blooms due to their high remineralizing capacity of primary production products (Wilkins et al., 2013c) Similarly, *Alphaproteobacteria* members associated with phytoplankton blooms and algae abundance such as *Sulfitobacter*, *Planktomarina* and *Roseobacter* clade (*Rhodobacterales* order) will decrease in abundance contributing to a notorious decrease representation of the class (Fig. 3B). In contrast, the *Alphaproteobacteria* SAR11 will increase in abundance as phytoplankton bloom decay and chlorophyll concentration decrease (Fig. 3B) (Delpech et al., 2021; West et al., 2008; Wilkins et al., 2013c). According to our data the microbial community composition in marine terminating glaciers will be probably dominated by members of the class *Gammaproteobacteria* which are described as the dominant group in highly stratified water column (Fig. 3B) (Piquet et al., 2011). The dominance of *Gammaproteobacteria* will lead to a community with a high sulfur reduction and carbon fixation potential (Wilkins et al., 2013c). Furthermore, our data suggest that other less abundant groups such as *Desulfobacterales*, *Plantomycetes*, *Cyanobacteria* and MGI will take part in the community change increasing the potential for nitrogen fixation and sulfur reduction (Fig. 3B). In environments with low organic nitrogen and iron concentration *Planctomycetes* has been described as the main microorganism responsible for nitrogen fixation in oligotrophic surface waters (Delmont et al., 2018). *Desulfobacterales* (*Deltaproteobacteria*) are important members of the bacterial communities of permanent cold marine sediments (Teske et al., 2011). However, they have been detected in surface and bottom waters of polar environments (Ghiglione & Murray, 2012; Murray & Grzymski, 2007; Zeng et al., 2009). The suggested ecological role of this group in the deep waters of the Arctic fjords is related to the sulfur cycle. (Zeng et al., 2009). Limited light penetration and low nitrate and phosphate concentrations, which characterize fjords with land terminating glaciers (Yendegaia), would promote photosynthetic production through *Cyanobacteria* (Gutiérrez et al., 2015; Piquet et al., 2010). Finally, low chlorophyll concentrations and limited light penetration (Fig. 3B) will lead to an increase in the abundance

of MGI, described as sensitive to solar radiation and negatively correlated with chlorophyll concentrations (Murray et al., 1998; Pedneault et al., 2014; Tolar et al., 2016), which will further contribute to community carbon fixation via the 3-hydroxypropionate/4-hydroxybutyrate pathway (Alfreider et al., 2018; Berg et al., 2007).

Additionally changes in water temperature showed that warming enhances the community respiration rate of planktonic communities over net photosynthetic rates and favors the increase of picophytoplankton and the activity of heterotrophic bacteria (Cuevas et al., 2019; López-Urrutia et al., 2006; Morán et al., 2010; Regaudie-de-Gioux & Duarte, 2012). Furthermore, temperature controls the rate of metabolic process in bacteria limiting the thermal niche at which bacteria can efficiently interact with nutrients and compete for limiting resources (Davidson & Janssens, 2006; Hall et al., 2009). On the other hand, an increase in the supply of terrigenous organic carbon under warming scenario could result in an increased heterotrophic bacteria activity (Bianchi et al., 2020; Kirchman et al., 2009) with the potential for these to outcompete phytoplankton in nitrogen acquisition (Larsen et al., 2015).

In summary, our data suggest that alterations resulting from climate change such as describe above will have a cascading effect generating changes in the structure and abundance of the different resident populations. Which in turn will generating serious changes in the functioning of biogeochemical cycles and therefore ecosystem functioning. In this context is necessary to further study the implications of climate change in the microbial communities of southern high latitude fjords in order to better understand and possible predict the status of Patagonian fjords.

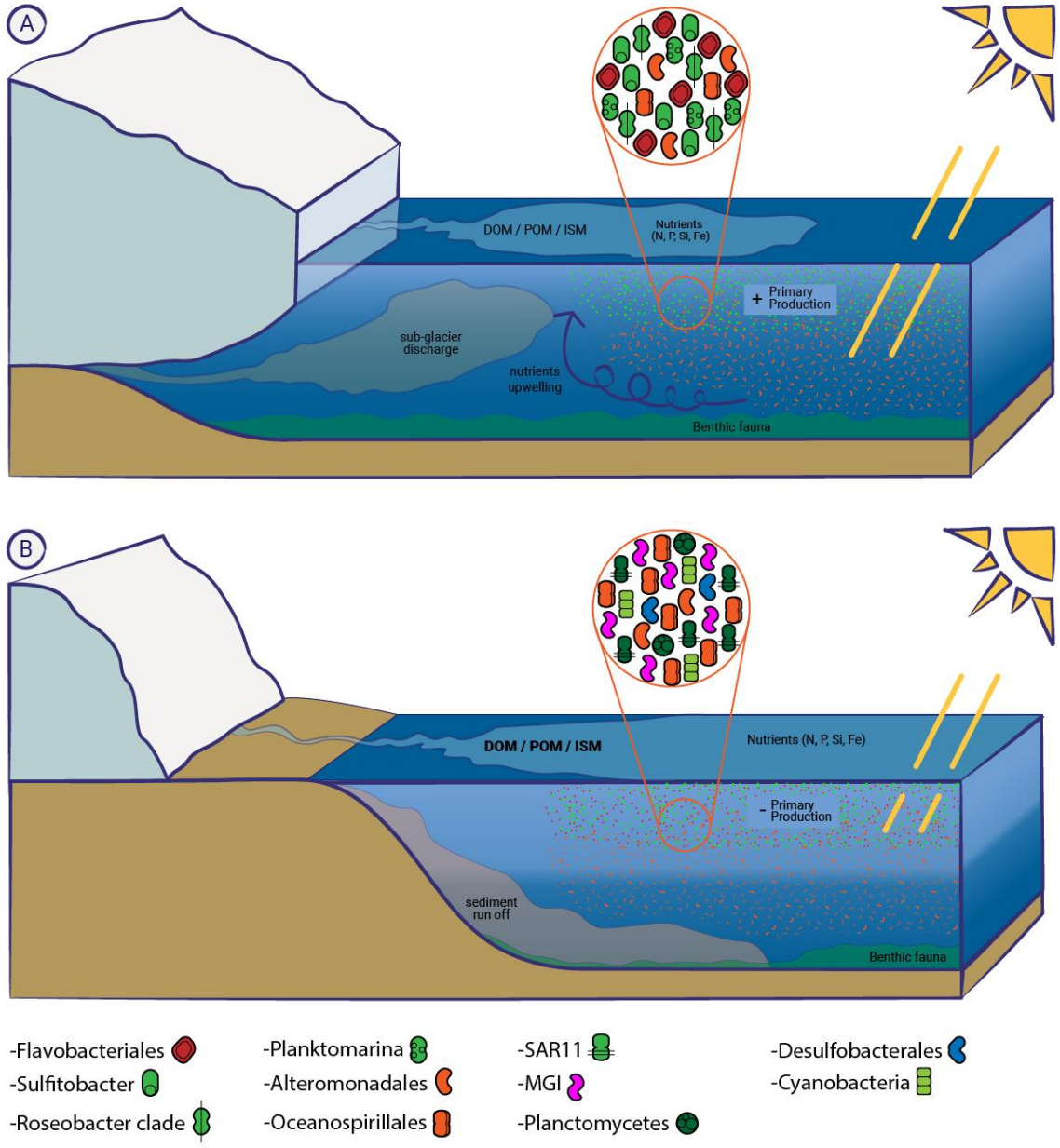



Figure 3: Conceptual model of fords hydrodynamic circulation and its impact on the biogeochemistry in a **A)** marine terminating glacier and **B)** land terminating glacier.

## 2 Factors structuring microbial communities at different spatial scales.

We conducted a large-scale study through the Southern Ocean and Antarctic Peninsula to detect possible biogeographic patterns in the bacterioplanktonic communities (Chapter 2). We showed that the bacterioplankton community composition was strongly driven by physical, chemical and biological parameters along environmental gradients. Additionally, we evidenced that sharp changes in community composition can occur over short (e.g. oceanic fronts) and large (e.g. open ocean) geographic distances.

In theory, geographically closer communities are expected to be more similar to each other than communities separated by a larger distance (Clark et al., 2021). It is thus expected that communities will show a decrease in community composition similarity associated with an increase in geographic distance. This assumption is known as the distance-decay relationship (Nekola & White, 1999). The relationship between community composition and geographic distance can be explained by at least three mechanism; first because of decreasing similarity of environmental characteristics, second because of the spatial configuration of the environment and third, due to the intrinsic capacity of organisms to limit their dispersal (Soininen et al., 2007). Several studies have observed distance-decay relationship in microorganisms in different types of habitats and at different taxonomic scales (Cho & Tiedje, 2000; Green et al., 2004; Horner-Devine et al., 2004; Casteleyn et al., 2010; Soininen et al., 2007; Hewson et al., 2006). Furthermore, robust patterns of biogeography in microbial communities have been detected in the marine environment (Falcón et al., 2008; Fuhrman et al., 2008; Galand et al., 2010; Ghiglione et al., 2012; Liu et al., 2019 ).

In the ocean, patterns of biogeography have been observed between microbial community composition and the physical, chemical and biological properties of water masses (Agogué et al., 2011; Galand et al., 2010; Ghiglione et al., 2012). This could challenge the usual distance decay theory. For instance, microbiological surveys of the oceans show that bacterial communities within a single body of water can be similar even over long spatial scales (e.g., 1000 km), while bacterial communities found in different water masses can differ in composition across small geographic scales (e.g., 100 m) (Agogué et al., 2011; Galand et al., 2010). Oceanic Fronts are areas where the water density changes abruptly accompanied by changes in current velocity and concentration of dissolved materials (Cromwell & Reid, 1956). Fronts have been previously reported to be important biogeographic barriers for microbial dispersal in Southern Ocean because they can limit physical migration of cells between regions

which is enhanced by the differences in environmental properties (Baltar et al., 2016; Pinhassi et al., 2003; Wilkins et al., 2013a;b). Another mechanism by which fronts can limit the spread of microorganisms is through the differences in physicochemical properties. In ocean fronts, biophysical factors such as temperature and chlorophyll concentration can vary abruptly, resulting in pronounced changes in the composition and activity of microbial communities (Baltar et al., 2016).

In the analysis of community composition made in the Antarctic (Chapter 2), strong distance-decay patterns were observed between the communities. We concluded that the pattern is first explained by a decrease in the similarity of environmental conditions as the geographical distance increases, and second by the spatial configuration of the studied territory associated mostly to oceanic fronts. During our sampling campaign, several fronts of the Antarctic Circumpolar Current (ACC) were crossed the polar front (PF), the southern boundary of the ACC (sbACC) and the southern ACC front (sACCf)). The presence of distance-decay pattern in our data set may indicate a low connectivity of the Antarctic areas investigated related to fronts characteristic (Barberán & Casamayor, 2010). This suggests that fronts can act as boundaries for bacterioplankton distribution by promoting a significant specie turnover between nearby and distant communities of the Southern Ocean.

## 3 Total community vs Active community.

In this study we analyzed the total and active fraction of bacterioplankton communities in Subantarctic and Antarctic zones. Total RNA and DNA were extracted in conjunction from the same filter with bacterioplankton samples to allow a proper comparison of total and active communities. In the case of total RNA reverse transcription to cDNA was performed with random primers. For both fractions (DNA and cDNA) the hypervariable regions V4-V5 of the gene 16S rRNA were amplified using the universal primers described by Parada et al. (2016).

Ours results clearly indicate differences between the total (rDNA) and active (rRNA) microbial community composition among the complete data set (Chapter 1, Fig. 3; Chapter 2, Supplementary Figure 1). In both studies (Chapter 1 and 2), the 16S rRNA (active fraction) the results appeared to be more resolutive for describing and explaining the bacterioplankton assemblages of each community compared to 16S rDNA gene (total fraction). In addition, in our study, higher alpha diversity values were observed for the active community compared to

the total community in Subantarctic and Antarctic zones (Chapter 1, Supplementary Fig. 2; Chapter 2, Fig. 4). Furthermore, better clustering level was observed for the RNA fraction in comparison to the DNA fraction, in which samples were more dispersed and with lower clustering level (Chapter 1, Supplementary Fig. 1; Chapter 2, Fig. 3; Fig. 1). Finally, a significant heterogeneous composition (β-diversity) between the communities analyzed in the Antarctic zone (Chapter 2) and between the Subantarctic and Antarctic fjords was evidenced in the active fraction as opposed to the total fraction where no changes in β-diversity were observed (Table 2). However, the degree of dissimilarity observed between the two fractions was not always equal throughout our data set (Chapter 2, Supplementary figure 2; Fig.4). In the Antarctic zone, Marian Cove and Maxwell Bay showed the greatest dissimilarity compared to the rest of the Antarctic locations (Chapter 2, Supplementary Figure 2). While among all the fjords sampled, Yendegaia 2018 showed the highest dissimilarity between the two fractions (Fig. 4).

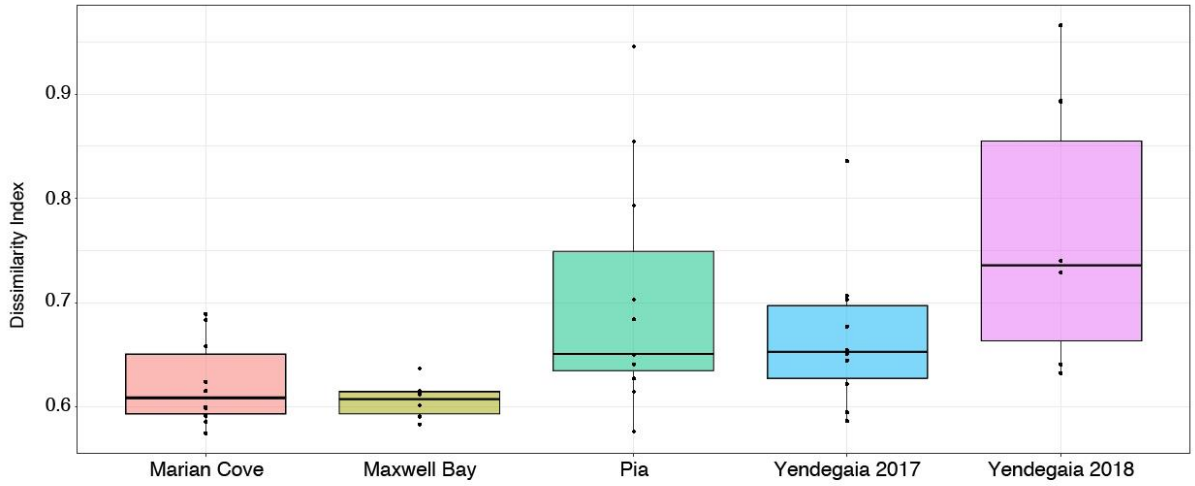



Figure 4: Bray-Curtis dissimilarity between the DNA and RNA fractions for microbial communities sampled. Only samples for which both the DNA and RNA fractions were successfully amplified were considered for this analysis.

The high compositional dissimilarity between rDNA and rRNA is due to differential changes in the relative abundance of certain OTUs. Traditionally, ribosomal RNA genes (rDNA) are used to identify microorganisms present in a specific environment regardless of

their metabolic state, while ribosomal RNA (rRNA) has been consistently used to characterize the growth state or activity of microorganisms in cultured and mixed microbial communities (Campbell et al., 2009; Campbell & Kirchman, 2013; Gaidos et al., 2011; Hunt et al., 2013). This is because a positive relationship between the response of bacteria to resource availability and the number of rRNA operons has been shown (Klappenbach et al., 2000), and ribosomal content has been found positively correlated with growth rate for many bacterial groups (DeLong et al., 1989; Schäfer et al., 2001; Troussellier et al., 2002). Therefore, the higher the number of 16S rRNA operons, the higher the number of ribosomes and the higher the expected growth rate, which will be reflected in a higher level of activity of the microorganism (Kirchman, 2016).

The number of 16S rRNA gene operons has been described as variable in bacteria ranging from 1 to 15 gene copies per species reflecting different ecological strategies of growth and activity (Moeseneder et al., 2005; Rastogi et al., 2009). However, the number of ribosomes can be even more abundant than rRNA operons depending on the growth rate of the bacterium (Kerkhof & Ward, 1993). Therefore, it could be assumed that in oligotrophic marine environments where bacteria tend to show a slow growth rate and a low number of rRNA operons, some marine bacteria might not be detected at the rDNA level but could be still detected at the rRNA level (Fegatella et al., 1998; Moeseneder et al., 2005). This could explain the higher alpha diversity observed in the active fraction compared to the total fraction. The Shannon index used in this study to explore α-diversity, measures the number of different species/OTUs (richness) and the proportional abundance of these species/OTUs (evenness) in a given site (Margalef, 1958).

In our data set, some changes in microbial community composition between locations (β-diversity) could only be observed at the rRNA level. Therefore, we can hypothesize that the changes associated with species turnover were related to the metabolic state of the community, which respond to specific environmental conditions, spatial restrictions, or historical events. Moreover, the highest levels of dissimilarity observed between the two fractions were recorded in Yendegaia 2018 fjord (Fig. 1) and for the Antarctic dataset in Marian Cove and Maxwell Bay fjords (Chapter 2, Supplementary Figure 2). Fjords are complex aquatic ecosystems where the interaction between fresh and oceanic waters generate changing gradients in salinity, temperature, nutrients, light availability and water circulation that will affect the development of native bacterioplankton communities (Campbell & Kirchman, 2013; Crump et al., 2004; González et al., 2013). Moreover, it has been described that in estuaries environmental variables

such as salinity and chlorophyll-a may affect differentially growth rate but not the abundance of individual taxa (Campbell & Kirchman, 2013). We can thus hypothesize that the bacterioplankton community fractions in these variable systems will show higher differences between the active and total community in comparison with communities living in more stable environmental gradients.

It should be noted that certain biases are related to the use of rRNA as an indicator of growth or activity. Blazewicz et al. (2013) provides a summary of these limitations indicating three main reasons. (i) The rRNA concentration and growth rate are not always easily correlated, studies conducted in *Vibrio fischeri* under non-static growth conditions showed that during periods of no growth the amount of rRNA continued to increase (Kerkhof & Kemp, 1999). (ii) The relationship between rRNA concentration and growth rate may differ significantly among taxa (Kemp et al., 1993) and (iii) the relationship between non-growth activities and rRNA concentration has not yet been investigated (Blazewicz et al., 2013). Therefore, the use of rRNA should be interpreted as the potential of a population to catalyse functions and this potential will reflect past, present and future events (Blazewicz et al., 2013). Consequently, the information provided by rRNA analysis will provide information of a potential activity and not of a current activity.

In addition to the biases mentioned above, some methodological aspects of the use of sequencing data should be addressed. Sequencing is a PCR-based technique for which nucleic acid extraction, amplification and sequencing errors has been mentioned extensively in the literature (Goodwin et al., 2016; Hugerth & Andersson, 2017; Kembel et al., 2012; Kennedy et al., 2014; Logares et al., 2012; Suzuki & Giovannoni, 1996). For example, it has been shown that samples kept frozen from sampling to the time of analysis show high levels of alpha diversity and differ less in their beta diversity than samples frozen at different time intervals. Our samples were properly frozen at -80°C in both expeditions and kept at that temperature until laboratory analysis.

In order to facilitate the comparison between both fractions, RNA and DNA analysis were performed from the same water filter. The PCR template content that has been reported as a source of variability in microbial community surveys (Chandler et al., 1997; Kennedy et al., 2014) was properly normalized (Comeau et al., 2017). Additionally, sequencing PCR relies in the correct selection of primers that will allow to improved accuracy in relative abundance, results comparation with prior studies, a good sequencing depth and a high coverage of taxonomic composition (Parada et al., 2016). In our study we use the primers 515F/926R which

allow a high coverage of bacteria and archaea with a more informative product of suitable length since it encompasses hypervariable regions V4-V5 (Parada et al., 2016).

In conclusion, rRNA-based analysis of the microbial community will provide taxonomic information on the potential active fraction of the community (Blazewicz et al., 2013) since it is able to isolate the active metabolic compound from the microbial community providing minimal distortion of metabolically inactive cells (Salazar & Sunagawa, 2017). Regarding the significant differences observed when comparing the composition of the total community versus the composition of the active community, we can conclude that variations in environmental parameters can affect the growth potential of certain taxonomic groups, producing notable differences between the DNA and RNA communities. Similar results to ours have been reported in estuarine, marine and Antarctic systems where strong gradients in the environmental parameters were observed (Campbell & Kirchman, 2013; Gentile et al., 2006; Moeseneder et al., 2005; Troussellier et al., 2002). Finally, RNA-based methods can provide more precise information on the effects of abiotic components on the composition of microbial communities. Moreover, the use of total RNA in oligotrophic environments (Subantarctic) and in environments subjected to constant climatic changes (Antarctic) proves to be a useful tool to explore the community composition of bacterioplankton providing a more comprehensive understanding of the complex dynamics that play microbial communities in challenged environments.

## 4 Future Work

The addition of unicellular eucaryotes to future studies on the description of active communities of southern high latitudes environments would be valuable. As we see in our data some of the major taxonomic components of southern high latitude marine ecosystems such as *Gammaproteobacteria* and *Bacteroidetes* are highly coupled to phytoplankton blooms, so the addition of this data to the analysis of the bacterioplankton community structure will apport valuable information. Furthermore, the analysis of gene expression patterns through metatranscriptomics of the microbial communities will deepen the understanding of the functional role of bacterioplankton in southern high latitude ecosystems.

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Claudia Maturana Martínez – Thèse de doctorat - 2021

Résumé :

Les écosystèmes marins des hautes latitudes méridionales (HLME) sont très sensibles au changement climatique, ayant un impact sur les processus physiques, chimiques et biologiques. Cependant, leur rôle important dans la modulation du climat et la circulation des masses d'eau, contraste avec le nombre relativement faible d'études sur leur fonctionnement. Relativement peu d'études sur la structure de la communauté bactérioplanctonique ont été rapportées pour le sud de la Patagonie chilienne et pour l'océan Austral (SO) à grande échelle, et aucune n'a ciblé la fraction active de la communauté bactérioplanctonique. Nous avons utilisé le séquençage de l'ARNr 16S pour analyser et décrire la structure communautaire des communautés bactérioplanctoniques actives dans le sud de l'HLME. L'objectif principal de cette thèse était de caractériser la diversité et l'abondance des communautés de bactérioplancton le long de gradients environnementaux et géographiques dans le sud de HLME. Tout d'abord, nous avons cherché à savoir si les fjords voisins du sud de la Patagonie chilienne, avec un climat et une localisation similaires mais des apports d'eau douce différents, présentaient des communautés différentes. Deuxièmement, nous avons étudié les changements interannuels subis par la communauté bactérioplanctonique du fjord de Yendegaia. Troisièmement, nous avons examiné la structure spatiale à grande échelle de la communauté bactérioplanctonique le long d'un transect traversant le secteur Pacifique du SO. Nos résultats montrent que les communautés bactérioplanctoniques du pôle sud sont structurées en fonction de paramètres physiques, chimiques et biologiques caractéristiques de la zone. De plus, nous avons également démontré que les changements des paramètres environnementaux, spatiaux et temporels affectent la structure des communautés bactérioplanctoniques. Ainsi, nous soulignons l'importance des études d'écologie microbienne dans les zones sensibles au changement climatique global comme le sud de l'HLME.

Mots clés : Écosystèmes marins des hautes latitudes australes, bactérioplancton, ARNr 16S, Patagonie Chilienne, Southern Ocean, fjords.

**Diversity and community composition of active microbial communities in southern high latitude ecosystems**

Abstract :

Southern high latitudes marine ecosystems (HLME) are highly sensitive to climate change, impacting physical, chemical, and biological processes, however, their prominent role in climate modulation and water masses circulation, contrast with the relatively low number of studies on their functioning. Relatively few studies on bacterioplankton community structure have been reported for southern Chilean Patagonia and for the Southern Ocean (SO) on a large scale, and none have targeted the active fraction of the bacterioplankton community. We used 16S rRNA sequencing to analyze and describe the community structure of the active bacterioplankton communities in southern HLME. The main objective of this thesis was to characterize de diversity and abundance of bacterioplankton communities along environmental and geographical gradients in southern HLME. First, we investigated whether nearby fjords of the southern Chilean Patagonia, with similar climate and location but different freshwater inflows, had different communities. Second, we investigated interannual changes experienced by the bacterioplankton community of the Yendegaia fjord. Third, we examined the large-scale spatial structure of the bacterioplankton community along a transect across the Pacific sector of the SO. Ours results show that southern polar bacterioplanktonic communities are structured according to physical, chemical, and biological parameters characteristic of the area. In addition, we also demonstrated that changes in environmental, spatial, and temporal parameters affect the structure of bacterioplanktonic communities. Thus, highlighting the importance of microbial ecology studies in areas sensitive to global climate change such as southern HLME.

Keywords: Southern high latitude marine ecosystems, Bacterioplankton, 16S rRNA, Chilean Patagonia, Southern Ocean, Fjords.