

Elucidating the effects of physicochemical variables on the structure, composition and functionality of microbiomes in the Prince Edward Islands

By

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Declaration

I, Mancha Mabaso, declare that the thesis/dissertation 'Elucidating the effects of physicochemical variables on the structure, composition and functionality of microbiomes in the Prince Edward Islands', which I hereby submit for the degree Magister Scientiae (M.Sc.): Genetics at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

A handwritten signature in black ink, consisting of a large, stylized 'M' and 'B' intertwined, with a horizontal line crossing through them. To the right of the signature is a small, empty square box.

SIGNATURE

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Date: 17th July 2020

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There are not enough pages in the world to thank all the people that had a hand in this but here goes.

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List of Abbreviations

Abbreviation	Definition
AABW	Antarctic Bottom water
AAIW	Antarctic Intermediate water
ACC	Antarctic circumpolar current
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
AP	Alkaline phosphatase
APF	Antarctic Polar Front
ASV	Amplicon sequence variants
BCP	Biological carbon pump
BG	β -Glucosidase
BX	β -Xylosidase

C	Carbon
Ca	Calcium
C_{ant}	Anthropogenic CO ₂
CDW	Circumpolar Deep Water
CO₂	Carbon dioxide
Cu	Copper
DOM	Dissolved organic matter
Fe	Iron
FL	Free-living
Fmax	Fluorescence Maximum
HMW	High molecular weight
ITS	Internal transcribed spacer

K	Potassium
LCDW	Lower Circumpolar Deep Water
N	Nitrogen
Na	Sodium
NADW	North Atlantic Deep Water
NAG	N-acetyl-glucosaminidase
NH₃	Ammonia
NH₄	Ammonium
NO₃	Nitrate
OM	Organic Matter
OMZ	Oxygen Minimum Zone
P	Phosphorus

PA	Particle-attached
PEI	Prince Edward Islands
PF	Polar Front
POM	Particulate organic matter
PSUW	Pacific Sub-antarctic Upper Water
PUL	Polysaccharide utilization loci
rRNA	Ribosomal RNA
SAF	Sub-Antarctic Front
SO	Southern Ocean
SO₄	Sulphate
SP	Solubility pump
STF	Subtropical Front

UCDW

Upper Circumpolar Deep Water

Zn

Zinc

Summary

The effects of climate change, due to increased levels of anthropogenic CO₂, have resulted in significant changes in both terrestrial and marine ecosystems. Contemporary studies have shown that microbial communities including bacteria, archaea, fungi and viruses play substantial roles in regulating productivity in these ecosystems. Yet, we lack a complete understanding of the precise role played by microbiomes in biogeochemical cycling. This knowledge deficit is particularly true for marine microbiomes, which play a central role in biogeochemical cycling in the global ocean. This is particularly true for the Southern Ocean, which is the largest carbon sink on earth.

This thesis evaluates the factors shaping microbial communities within the vicinity of the Prince Edward Islands (PEI). The phylogenetic diversity of PEI bacteria and fungi was evaluated using amplicon sequencing of the 16S rRNA gene and fungal ITS region, respectively. These findings were correlated to physicochemical parameters to assess the main drivers of community structure. Additionally, extracellular enzymatic activities were assessed to shed light on the potential functionality. Using several statistical correlation analyses, the links between abiotic factors, microbiome composition and metabolic activity were explored.

Results revealed that microbial communities were significantly influenced by a number of physicochemical factors. These included depth in the water column, water mass endemicity, temperature, oxygen, and phosphorus. Our analysis showed that particle-attached (PA) and free-living (FL) microorganisms were taxonomically distinct. Furthermore, we found depth-resolved differences between, as well as within, the PA

and FL sub-communities. Extracellular enzymatic activities exhibited patterns with depth and significant correlations to nutrients. Overall, this study showed that physicochemical variables shaped microbial composition and structure but was incongruent with extracellular enzymatic activities specific for carbon, nitrogen and phosphorus. The results of this study suggest that microbial functionality may be closely linked to substrate availability. The data also allude to the importance of niche partitioning in near-shore microbial communities. Together, we provide important insights regarding the interplay between biotic and abiotic factors as key determinants of near-shore microbial communities in the Southern Ocean.

Chapter 1

The Southern Ocean, Oceanic Processes and Marine
Microorganisms

1. Ocean ecosystem services and the Southern Ocean

The oceans constitute over 70% of the Earth's surface and are the largest ecosystem (Rahmstorf 2002; Costello *et al.*, 2010). The crucial role played by the oceans in recreational activities and in the transport of commercial goods and services is often recognized (Longo *et al.*, 2017; Aanesen *et al.*, 2018). However, marine ecosystem services including the transport of heat (Stewart 2008; Su *et al.*, 2018) and nutrients (Doughty *et al.*, 2016) are often unappreciated. The oceans are vital regulators of the Earth's climate due to their role in adjusting solar radiation (Perovich 2005; Frölicher *et al.*, 2015). Radiation warms the ocean surface, initiating the hydrological cycle and triggering evaporation. This evaporate is ultimately deposited to terrestrial environments as rainfall (Costanza 1999; Rahmstorf 2002).

The role played by the oceans, as the world's largest reservoir of carbon is arguably the most important ecosystem service (Sarmiento *et al.*, 2010; Khatiwala *et al.*, 2013; Mckinley *et al.*, 2016). The release of anthropogenic CO₂ (C_{ant}) is recognized as the leading contributor to global climate change (Menon *et al.*, 2007; Oreskes 2018; Cavicchioli *et al.*, 2019). The ocean plays a crucial role in mitigating the effects of climate change, sequestering roughly a third of the cumulative C_{ant} emissions from the atmosphere (Khatiwala *et al.*, 2009; Khatiwala *et al.*, 2013; Gruber *et al.*, 2019). While the short-term effects of climate change continue to be the subject of extensive research (Bett *et al.*, 2017; Henson *et al.*, 2017; Botté *et al.*, 2019; Carozza *et al.*, 2019; Giudice and Azzaro 2019; De Scally *et al.*, 2020; Rastelli *et al.*, 2020), we lack a comprehensive understanding on the long term consequences on marine biota

(Cavicchioli *et al.*, 2019). The Southern Ocean (SO) is central in CO₂ cycling and is responsible for up to 40% of oceanic C_{ant} uptake (Sabine *et al.*, 2004; Landschützer *et al.*, 2016; McKinley *et al.*, 2016; Le Quéré *et al.*, 2018). As the largest oceanic carbon sink on the planet, the capacity to sequester CO₂ in the SO exceeds that of terrestrial plants (Field *et al.*, 1998; Andrew *et al.*, 2019). The SO is located below 60° S and, as the southern-most ocean, surrounds the Antarctic continent (Figure 1). It connects the Indian, Pacific and Atlantic oceans (Rintoul 2018). The past five decades of research have confirmed the importance of the SO as the world's most biologically productive and important marine ecosystem (Mayewski *et al.*, 2009; Moore *et al.*, 2018).

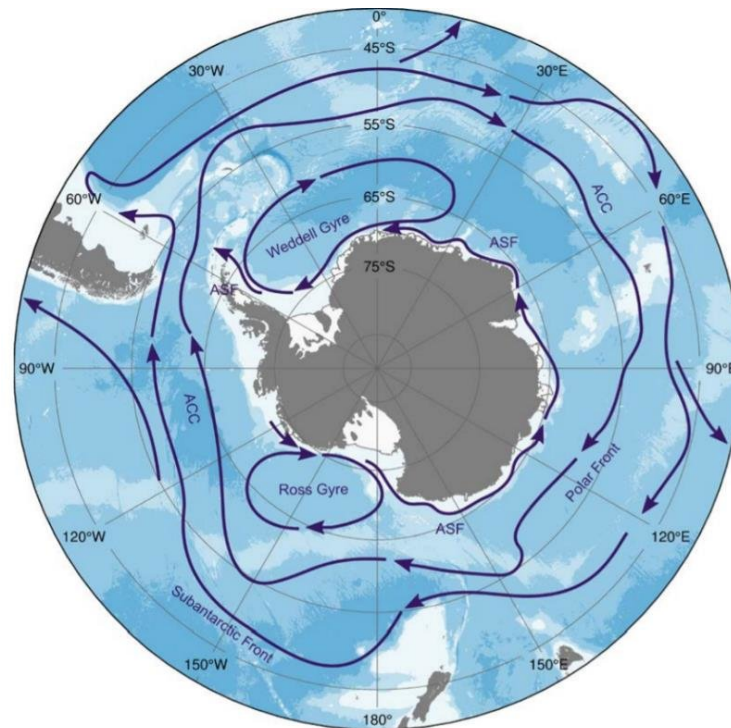


Figure 1: Southern Ocean circulation showing the Polar and sub-Antarctic Fronts of the Antarctic Circumpolar Current, sub-polar gyres and Antarctic Slope Front (ASF) (adapted from Rintoul *et al.*, (2001)). Background colours show bathymetry. Figure sourced from Moreau (2019).

1.1. Oceanographic features of the SO

The SO is characterized by strong westerly winds, which drive the Antarctic Circumpolar Current (ACC) (Rintoul *et al.*, 2001; Tamsitt *et al.*, 2016; Cheon and Kug 2020). The ACC is the world's strongest and predominant current system (Rintoul 2018). This current system is eastward-moving and extends from surface to the sea floor. Strong, katabatic winds as well as submarine topography are significant drivers of this current system (Ruan *et al.*, 2017; Rintoul 2018). This is the only current which connects the three major ocean basins (Carter *et al.*, 2008; Marshall and Speer 2012). This attribute makes it an important conduit for the cycling of heat, salt and gases throughout the ocean (Mayewski *et al.*, 2009; Turner and International Council for Science. Scientific Committee on Antarctic 2009; Frölicher *et al.*, 2015; Moore *et al.*, 2018).

The SO may be delineated into several zones and fronts marked by abrupt changes in water properties such as temperature, density and salinity (Deacon 1982; Whitworth *et al.*, 1985; Orsi *et al.*, 1995; Chapman *et al.*, 2020). A number of studies have used several approaches to define these zones and their precise geographic boundaries (Gordon *et al.*, 1978; Deacon 1982; Orsi *et al.*, 1995; Chapman *et al.*, 2020; Rosso *et al.*, 2020). To date, the northern-most boundary of sub-Antarctic waters is generally accepted as the Subtropical Front (STF), while the most southerly waters are recognized as the Antarctic Slope Front (ASF) (Graham and De Boer 2013). Frontal zones have been reported to change seasonally and inter-annually (Graham and De Boer 2013). As a result of the complexity of these frontal systems, the precise definition of these boundaries differs based on disparities between transects, satellites

and climatologies (Moore *et al.*, 1999; Carter *et al.*, 2008; Sokolov and Rintoul 2009; Graham and De Boer 2013; Chapman *et al.*, 2020).

The coldest, most dense water in the global ocean circulation is derived from SO water around Antarctica and sinks to the bottom of the ocean (Orsi *et al.*, 1999; Rintoul 2018; Abrahamsen *et al.*, 2019). These waters are transported north toward the Indian, Pacific and Atlantic oceans by deep western boundary currents (Carter *et al.*, 2008). At the onset of slow upwelling, deep cold waters rise and contribute to warm surface water circulation (Gruber *et al.*, 2019). These warmer and more saline waters are subsequently transported North, where the decrease in temperature causes these waters to sink forming the North Atlantic Deep Water (NADW) (Mccartney and Talley 1984; Mokeddem and Mcmanus 2017). Sandwiched between Antarctic Intermediate Water (AAIW) and Antarctic Bottom Water (AABW), the NADW migrates south, mixing with other water masses, and eventually surfacing at the Antarctic continental margin (Carter *et al.*, 2008; Dent., 2015; De Carvalho Campos *et al.*, 2017; Mokeddem and Mcmanus 2017) (Figure 2). SO circulation is the foremost connector of the physical oceanic environment and, by extension, of the associated marine ecosystem (Hunt *et al.*, 2016; Cavicchioli *et al.*, 2019).

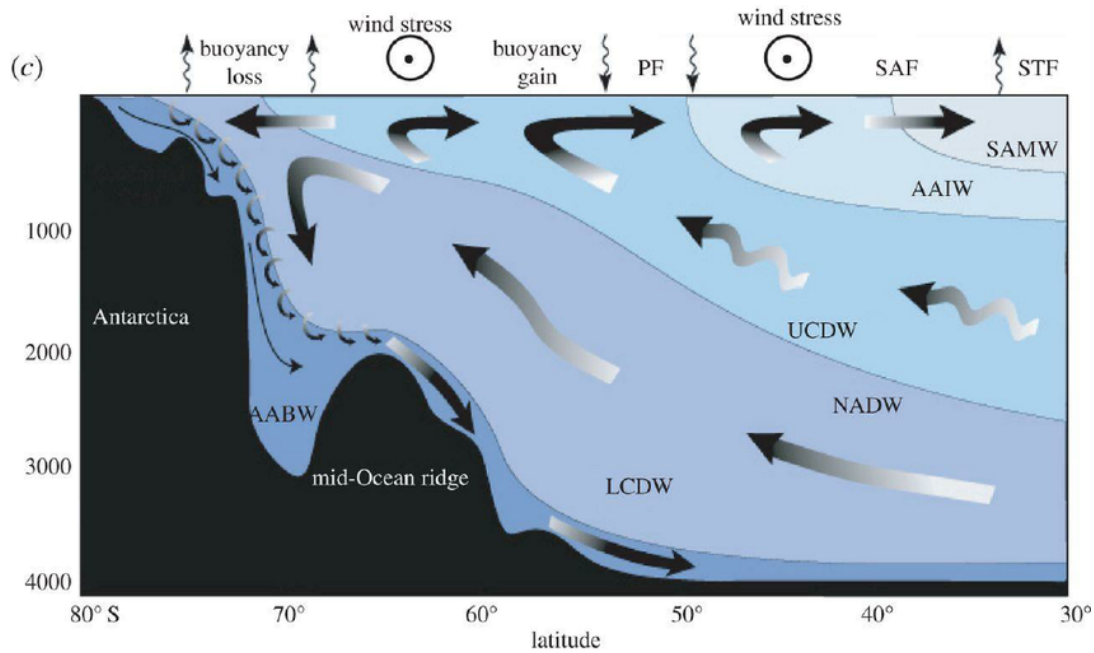


Figure 2: Schematic representation of Southern Ocean meridional overturning circulation, fronts and water masses, plus the atmospheric forces and fluxes The left hand side of the figure is indicative of the Antarctic continental shelf and the right side indicates North. Abbreviations are as follows: Lower Circumpolar Deep Water = LCDW, Upper Circumpolar Deep Water = UCDW Sub-Antarctic Front = SAF, Polar Front = PF, Subtropical Front = STF, Antarctic Bottom Water = AABW, North Atlantic Deep Water = NADW, Antarctic Intermediate Water = AAIW, Sub-Antarctic Mode Water = SAMW. Figure taken from Gent, (2015).

Terrestrial environments within the vicinity of these waters are marked by generally low temperatures (Convey *et al.*, 2014). Yet, several islands within the SO such as the Crozet and Kerguelen islands harbour a remarkable diversity of macro-organisms (Cherel *et al.*, 2017; Gubili *et al.*, 2017; Zbawicka *et al.*, 2019). The extent to which the prevailing environmental conditions shape the evolution of organisms has recently been the subject of several studies (Ertz *et al.*, 2017; Guillaumot *et al.*, 2018; Bottos

et al., 2020). These studies, based on SO islands such as the Prince Edward Islands, have revealed substantial insights regarding the interplay between biology and the environment (Leihy *et al.*, 2018; Venkatachalam *et al.*, 2019; Greve *et al.*, 2020).

1.2. ***The Prince Edward Islands***

The Prince Edward Islands (PEIs) are an archipelago within the Indian sector of the SO. This archipelago consists of Marion Island (46°54'S; 37°45'E) and smaller Prince Edward Island (46°38'S; 37°57'E). These islands are approximately 270km² and ~45km², respectively, and roughly 19 km apart (Lutjeharms 1985; Pakhomov and Froneman 1999). Both islands are within the path of the ACC between the sub-Antarctic front (SAF) and the Antarctic polar front (APF), which are highly complex in terms of circulation and variability (Duncombe Rae 1989). The proximity of the SAF and APF to the islands affects the temperature and speed at which water flows between and around the PEIs (Asdar 2019; Lamont *et al.*, 2019). As a result, changes in the location of the two fronts may affect the islands, the surrounding waters and, ultimately, the entire ecosystem (Lamont *et al.*, 2019).

The PEIs host a wide diversity of macro-organisms including seals (Bester *et al.*, 2011) penguins (Crawford *et al.*, 2009) and several species of sea birds (Schoombie *et al.*, 2017). These macro-organisms are top predators in the PEIs and substantially drive terrestrial and marine processes (Reisinger *et al.*, 2018). These organisms are critically dependent on island-associated ecosystem services including nutrients produced in the surrounding marine environment (Kaehler *et al.*, 2000). These

nutrients are imported to the island environment by top predators and cycled back to the near-shore marine environment, via run-off from the islands (Boelhouwers *et al.*, 2000). While the SO is a high-nutrient low-chlorophyll (HNLC) region (Blain *et al.*, 2001; Tagliabue *et al.*, 2017), terrigenous nutrient input and upwelling events result in increased productivity and phytoplankton blooms in the vicinity of SO islands like the PEIs (Doty and Oguri 1956; Boden 1988; Blain *et al.*, 2001). This phenomenon is termed the “island mass effect” and has been well studied in several marine islands (Blain *et al.*, 2001; Jena 2016; Messié *et al.*, 2020). In the SO, the island mass effect may elucidate several dynamics related to CO₂ sequestration and global biogeochemical cycling (Raapoto *et al.*, 2019; Messié *et al.*, 2020). Several studies have also shown that the island mass effect may explain species composition in the surrounding waters (Blain *et al.*, 2001; Caldeira *et al.*, 2002; Blain *et al.*, 2007; Gove *et al.*, 2016). These studies, based primarily on islands in the Pacific and Atlantic oceans, have demonstrated that discrete terrestrial patches profoundly impact nutrient availability and biodiversity in the surrounding waters (Gove *et al.*, 2016; Jena 2016). However, very few studies have investigated the island mass effect in the PEIs (Boden 1988; Hernández-León 1991; Gove *et al.*, 2016). Given the unique set of environmental conditions specific to this marine environment, the dearth in data for SO islands represents a clear knowledge deficit. Recent studies have suggested that microbial communities may be important drivers of the biological carbon pump in the SO (Cavan *et al.*, 2019). Yet, current research still lacks a mechanistic understanding regarding the role played by these microbiomes in SO nutrient sequestration.

2. Carbon cycling in the Southern Ocean

Marine CO₂ sequestration occurs by two major processes (Passow and Carlson 2012; Boyd *et al.*, 2019). The first is defined as the solubility pump (Stukel and Ducklow 2017). Where at high latitudes, CO₂ dissolves in cold surface water and sinks deeper as a result of temperature driven currents (Volk and Hoffert 1985; Sarmiento and Gruber 2006). The second process, termed the biological carbon pump (BCP), describes the fixation and export of carbon to the deep ocean through biologically mediated processes (Sigman *et al.*, 2010; Rafter *et al.*, 2017). These processes cumulatively explain the export of carbon to the deep ocean via passive flux of sinking particles, the active flux of particulate organic matter (POM) by zooplankton and the vertical transport of organic matter (OM) by physical processes (Jiao *et al.*, 2014). The BCP is responsible for the fixation of dissolved inorganic carbon (DIC), through phytoplankton and bacterial photosynthesis, to dissolved organic carbon (DOC) (Jiao *et al.*, 2010; Herndl and Reinthaler 2013; Boyd *et al.*, 2019; Le Moigne 2019). This DOC is consumed and recycled by other members of the marine ecosystem (Zhang *et al.*, 2018a). Subsequently, microorganisms assimilate organic matter below the surface and produce CO₂ through respiration (Chisholm 2000; Passow and Carlson 2012). The air-sea CO₂ exchange relies on a balance between the physical and biological processes that govern marine carbon cycle (Worden *et al.*, 2015; Hauck *et al.*, 2018). This cycling of CO₂ in the oceans has wide implications for regulation of the earth's climate (Doney *et al.*, 2011; Hülse *et al.*, 2017; Lønborg *et al.*, 2020).

The SO is the largest source of preformed nutrients in the ocean (Sarmiento *et al.*, 2004; Sigman *et al.*, 2010). Dissolved macronutrients and CO₂ are transported to surface waters by the wind-driven upwelling of deep water (Sarmiento and Gruber 2006). Surplus macronutrients are subsequently delivered to the low latitudes (Primeau *et al.*, 2013; Tamsitt *et al.*, 2017). In these oligotrophic regions, small scale mixing and upwelling support primary productivity (Sarmiento *et al.*, 2004). Given the SO's dominance in the global oceanic uptake of anthropogenic carbon, understanding SO carbon cycling may provide fundamental cues regarding nutrient sequestration in oligotrophic environments.

2.1. Marine food webs

Our understanding of the marine food webs has primarily focused on higher trophic levels (Pomeroy 1975; Hobson and Welch 1992; Brussaard *et al.*, 1996; Turner and Tester 1997; Ward *et al.*, 2012). In these studies, the role played by photosynthetic macroalgae as the production loci is often overstated (Pomeroy 1975; Williams 1981; Azam and Ammerman 1984; Azam and Malfatti 2007). Previous studies have shown that phytoplankton are fundamental for the export of carbon to the deep sea and form the base of oceanic food webs (Field *et al.*, 1998; Falkowski 2012; Worden *et al.*, 2015; Dutkiewicz *et al.*, 2019). These phytoplankton are the primary energy source for zooplankton and krill, which are consumed by organisms occupying higher trophic levels (Worden *et al.*, 2015). More importantly, primary production by phytoplankton is estimated at approximately 50 PgCyear⁻¹ and provides dissolved organic matter (DOM) directly or indirectly to all other marine organisms (Field *et al.*, 1998; Falkowski 2012; Higgins *et al.*, 2012; Smriga *et al.*, 2016). While phytoplankton play integral roles

in marine ecosystems, recent studies have shown that bacterioplankton, archaeoplankton and mycoplankton may be substantial contributors to primary production in the ocean.

2.2. Marine microbes and the carbon cycle

Marine bacteria were primarily recognized for their role in the decomposition of organic matter (Fukami *et al.*, 1985; Biddanda and Pomeroy 1988; Middelburg *et al.*, 1993). The discovery of several phototrophic bacteria, including ubiquitous marine Cyanobacteria *Synechococcus* and *Prochlorococcus*, have revealed the extent and importance of these taxa in marine ecosystems (Waterbury *et al.*, 1979; Chisholm *et al.*, 1988). These findings have also highlighted the importance of bacteria not only as degraders of OM, but as primary producers and important contributors to CO₂ fixation (Bunse and Pinhassi 2017; Gueneli *et al.*, 2018).

Autotrophic bacteria play a similar role to phytoplankton, in most marine environments, as the dominant primary producers through the sequestration of CO₂ to generate DOM as part of 'the microbial loop' (Chisholm 1992; Partensky *et al.*, 1999; Pomeroy *et al.*, 2007). The role played by microorganisms and the theory of the microbial loop was first described by Azam *et al.*, (1983) who showed that microbial communities were, in fact, essential active members of the biosphere. The microbial loop integrates microorganisms (that is, bacteria, archaea, fungi and viruses) as core constituents in marine food webs, highlighting their roles in the transformation and exchange of energy, carbon and nutrients (Azam *et al.*, 1983; Pomeroy *et al.*, 2007; Fenchel 2008; Steinberg and Landry 2017; Gladfelter *et al.*, 2019).

DOM is supplied to microbial communities via exudation by primary producers (Wetz and Wheeler 2007; Mühlenbruch *et al.*, 2018). The products of cell lysis, as a result of nutrient stress or viral attack, are also important sources of DOM (Azam and Smith 1991; Riemann and Middelboe 2002; Ma *et al.*, 2018). Following the production of DOM, heterotrophic microorganisms act as the primary consumers and remineralize both DOM and particulate organic matter (POM) (Cottrell and Kirchman 2000; Jiao *et al.*, 2010; Landa *et al.*, 2013). Microorganisms colonize OM throughout the water column and degrade it occasionally producing extracellular enzymes to act on high molecular weight (HMW) compounds (Hollibaugh and Azam 1983; Milici *et al.*, 2017). The by-products of remineralization are then available for use by other microorganisms and, ultimately, higher trophic levels (Sitta *et al.*, 2018). The direct consequence of these processes results in the drawdown of CO₂ to the deep ocean (Azam *et al.*, 1983; Fenchel 2008; Anderson and Tang 2010). The theory of the microbial loop proposes microorganisms at the core of marine productivity (Pomeroy *et al.*, 2007). Further, the theory extends their known roles from that of known consumers to potentially vital mediators of primary productivity (Andersson *et al.*, 2017). Studies have shown that 'the microbial loop' and the microorganisms it encompasses may be prominent in marine nutrient acquisition (Arrigo 2005; Armengol *et al.*, 2019). These studies support the proposal that microorganisms are active and functionally important constituents of marine food webs (Azam and Ammerman 1984; Falkowski *et al.*, 2008; Fenchel 2008; Jiao *et al.*, 2014; Worden *et al.*, 2015; Danovaro *et al.*, 2017a; Pang and Jones 2017; Salazar and Sunagawa 2017; Traving *et al.*, 2017; Sosa *et al.*, 2019; Steffan and Dharampal 2019).

3. Marine biogeochemical cycling mediated by microbial communities

The marine carbon cycle is closely coupled to the sequestration of other major nutrients including nitrogen and phosphorus (Hutchins *et al.*, 2009). The vast majority of biogeochemical cycles are mediated by marine microorganisms, which play substantial roles in the earth's functioning (Arrigo 2005). Expounding the processes that shape microbial communities spatially and temporally are important for predicting how biogeochemical cycles may change over time (Louca *et al.*, 2016).

Marine OM includes several compounds which contain carbon, phosphorus and nitrogen. The composition of OM further explains the association between marine biogeochemical cycles (Osterholz *et al.*, 2016; Ward 2017). Key nutrients in the upper ocean limit productivity in oligotrophic ocean regions (Moore *et al.*, 2013; Almahasheer *et al.*, 2016). Heterotrophic microbial guilds are involved in the uptake, assimilation and remineralization of inorganic phosphorus (Karl 2014; Karl and Björkman 2015). In order to assimilate phosphorus in marine systems, heterotrophic guilds employ a variety of metabolic mechanisms. These metabolisms include the capacity to upregulate enzyme-encoding genes under conditions of Pi limitation (Sebastián *et al.*, 2016). Examples of such systems include the C-P lyase pathway (Seweryn *et al.*, 2015; Sosa *et al.*, 2019) and the production of extracellular enzymes like alkaline phosphatase to aid in P acquisition (Browning *et al.*, 2017; Li *et al.*, 2019b).

Previous studies have shown that marine bacteria and archaea play vital roles in mediating steps in N-cycling (Daims *et al.*, 2016; Shah *et al.*, 2017). In the SO, recent studies have highlighted the broad range of genetic capacity for the sequestration of

several nutrients (Phoma *et al.*, 2018; Morales *et al.*, 2019). Several genes involved in nitrification, similar to those previously identified in members of the Betaproteobacteria and Thaumarchaeota were shown to occur widely in the water column (Phoma *et al.*, 2018). This capacity for nitrification was also mirrored in nitrogen assimilation and denitrification, which were also present from the ocean surface to the bottom. However, the extent to which these genes may encode functional enzymes has not been assessed. It may be argued that the bulk of functional processes in the oceans may be restricted to the surface microbiomes and that evidence of genes in the oxygen minimum and deep regions may be due purely to current transport of extant DNA. There is an urgent need to understand both the diversity and function of microbial communities in the SO.

4. Marine microbial diversity

Estimates suggest that marine biodiversity ranges between 10^3 to 10^9 distinct species (Pedrós-Alió 2006). Reports of novel lineages reported each year appear to confirm these estimates despite the high extinction rates (Parks *et al.*, 2017; Zaremba-Niedzwiedzka *et al.*, 2017; Zhang *et al.*, 2019b). Across the oceans, the high number of macro-organisms is accompanied by concomitantly high levels of micro unicellular and multicellular organisms (Moran 2015). There is also evidence that the marine biomass is dominated by microbial communities (~70%) made up of bacteria, archaea, fungi and viruses (Bar-On *et al.*, 2018).

4.1. Bacteria

Marine microbiomes are dominated by bacteria in terms of their abundances, diversity and metabolic activities (DeLong 2005; Pomeroy *et al.*, 2007; Salazar and Sunagawa 2017). The primary bacterial phyla include Bacteroidetes, Chloroflexi, Actinobacteria, Cyanobacteria and a diverse assortment of Proteobacteria (Pommier *et al.*, 2007; Wilkins *et al.*, 2013a; Sunagawa *et al.*, 2015). Proteobacteria are widely distributed (Pommier *et al.*, 2007; Ghiglione *et al.*, 2012; Delmont *et al.*, 2018) and typically the most abundant taxa globally and in the SO (Ghiglione *et al.*, 2012; Wilkins *et al.*, 2013a; Sunagawa *et al.*, 2015; Flaviani *et al.*, 2018), with members of Alpha- and Gammaproteobacteria being the most prevalent. Members of the Proteobacterial clade are mainly heralded for their contribution to heterotrophic activity in the ocean, aided by their remarkable capability of substrate utilization (Arístegui *et al.*, 2009; Landa *et al.*, 2013; Meier *et al.*, 2016). However, these bacteria are capable of a wide variety of metabolic functions and can serve a prime example of the functional diversity between and within many bacterial clades (Landa *et al.*, 2013; Zhou *et al.*, 2020). In addition to heterotrophy, Proteobacteria have been shown to contribute to primary production via sulphur and methane oxidation as well (Marshall and Morris 2013; Osborne and Haritos 2018; Menezes *et al.*, 2020). This metabolic flexibility made the Proteobacteria ideal for a study conducted by Zhou *et al.*, (2020) who evaluated the reasons for genome diversification in globally abundant bacteria. The authors suggest that bacterial lineages throughout the globe are not necessarily highly adapted to one specific environment but rather that widely distributed lineages such as the Proteobacteria have evolved strategies to adapt to the different environments in which they occur (Zhou *et al.*, 2020). One example of adaptability of bacteria is the phylum

Bacteroidetes. Members of this clade have been revealed to harbour unique degradation machinery in the form of polysaccharide utilization loci (PUL) (Bjursell *et al.*, 2006; Unfried *et al.*, 2018). This makes them exceptionally well suited for OM. They have evolved genes encoding gliding motility, specialised secretion systems and polymer degrading enzymes (Fernández-Gómez *et al.*, 2013; McBride and Zhu 2013; Krüger *et al.*, 2019). These features give them a competitive advantage in the scavenging, uptake and degradation of complex molecules in the surface waters they inhabit (Munoz *et al.*, 2020).

Similarly to Proteobacteria, many other bacterial lineages are not limited to their role as chief heterotrophs in marine ecosystems, but are capable of a wide variety of metabolic functions (Murray and Grzyski 2007). These include photoautotrophy which is (Falkowski 2012; Pinhassi *et al.*, 2016; Ivanovsky *et al.*, 2020), chemoautotrophy (Shah *et al.*, 2017; Ye *et al.*, 2020), methanogenesis (Wang *et al.*, 2019a; Yin *et al.*, 2019), sulphur oxidation (Hu *et al.*, 2018) and nitrification (Sakae *et al.*, 2019). Studies have suggested that some of these functions have been acquired by way of horizontal gene transfer (Sobecky and Hazen 2009; Terrapon *et al.*, 2014; Zhang *et al.*, 2019a). This is one mechanism that could explain the wide functional diversity amongst bacterial lineages. The high taxonomic and metabolic diversity of bacterial communities means they are able to colonize a large number of environments and perform a number of heterotrophic functions and offers insight into the ubiquity of bacteria in the SO (Wilkins *et al.*, 2013a; Salazar and Sunagawa 2017; Ruiz-González *et al.*, 2019).

4.2. Archaea

Archaea were regarded to be ecologically rare taxa and limited primarily to extreme environments (Danovaro *et al.*, 2017b; Santoro *et al.*, 2019). While Archaea are less abundant than bacteria (34% of prokaryotic biomass), earlier studies have demonstrated that they are ubiquitous (Delong 1992; Massana *et al.*, 1997; Karner *et al.*, 2001; Sunagawa *et al.*, 2015) in marine environments. Marine archaea are primarily dominated by Marine Group I (MGI) Thaumarchaeota (Delong 1992; Fuhrman *et al.*, 1992), Marine Group II (MGII), Euryarchaeota (Fuhrman and Davis 1997) as well as Marine Groups III (MGIII) and IV (MGIV) Euryarchaeota (López-García *et al.*, 2001) with other groups occurring at lower abundances (Delong 1992; Santoro *et al.*, 2019).

The ecological role played by archaea in the oceans varies considerably with depth (Hu *et al.*, 2011; Luo *et al.*, 2014; Junior *et al.*, 2015; Cheung *et al.*, 2018). A recent study on Thaumarchaeota from the depth of the ocean (the Mariana Trench) revealed several interesting insights regarding the metabolisms and potential adaptation mechanisms of these archaea (Zhong *et al.*, 2020). The authors reported that the capacity for organic compound import in some deep water Thaumarchaeota far exceeded those from shallow ocean regions and was above previous estimates (Zhong *et al.*, 2020). This study has interesting implications for our understanding of primary productivity in the aphotic zones of the ocean. However, there is a clear deficit in such data and the extent to which such findings hold for SO environments remains unknown.

Nevertheless, the contribution of archaea to the biological carbon pump is disproportionate to their abundances (Pachiadaki *et al.*, 2017; Meador *et al.*, 2020). Previous studies have demonstrated their capacity to breakdown DOM, fix atmospheric CO₂ and oxidise ammonia (NH₄) both aerobically and anaerobically (Herndl *et al.*, 2005; Yool *et al.*, 2007; Könneke *et al.*, 2014; Qin *et al.*, 2017). These metabolic roles have profound effects on marine biogeochemical cycles.

4.3. Fungi

Fungi are widely distributed in the ocean (Bochdansky *et al.*, 2017; Hassett *et al.*, 2019). Nevertheless, they remain the most understudied microbial group in the oceans (Rosa *et al.*, 2019). Recent studies suggest that the total diversity of marine fungi is vastly underestimated (Pang and Jones 2017). In contrast, fungi in terrestrial environments are relatively well known and several studies have provided insights regarding their distribution (Větrovský *et al.*, 2019) and biogeographic patterns (Tedersoo *et al.*, 2017; Gladfelter *et al.*, 2019). Fungi are vital to terrestrial and marine ecosystems and play key roles as decomposers of DOM and in the sequestration of nutrients (Raghukumar *et al.*, 2004). Current insights suggest that fungi from terrestrial environments vary considerably in terms of their ecological, morphological and phylogenetic diversity (Gladfelter *et al.*, 2019; Rosa *et al.*, 2019).

Although studies on marine fungi have provided important insights on their diversity and distribution, they have generally lacked sufficient spatial and temporal resolution (Gladfelter *et al.*, 2019). Specifically, these studies have shown that the dominant fungal taxonomic groups include Ascomycota and Basidiomycota, followed by Chytridiomycota and Zygomycota (Bass *et al.*, 2007; Richards *et al.*, 2015; Picard

2017). These taxa are dominant at global scales although their abundances differ markedly at local scales (Tisthammer *et al.*, 2016; Picard 2017). For instance, a study conducted in the Arctic showed that the distribution of Chytrids was closely correlated with sea ice melt and co-occurrence with diatoms (Kilias *et al.*, 2020). In contrast, in the South China Sea, the major factors shaping fungal community structure were dissolved oxygen and depth (Li *et al.*, 2018). This suggests that multiple factors may influence the structure of individual communities (Li *et al.*, 2019a; Hassett *et al.*, 2020). Other studies, conducted on a regional scale, suggest that fungi may not constitute substantial proportions of marine communities (Wang *et al.*, 2012; Orsi *et al.*, 2013; Richards *et al.*, 2015; Raghukumar 2017). Large scale datasets that are inclusive of fungi are limited to projects such as the Tara Oceans Expeditions and the Earth Microbiome Project (Gilbert *et al.*, 2010; Karsenti *et al.*, 2011; Tisthammer *et al.*, 2016; Gilbert *et al.*, 2018). These studies highlight the importance of environmental factors in shaping fungal communities and may explain the regional differences between communities.

4.4. Viruses

Viruses are the most numerically abundant group in marine microbiomes (Wommack and Colwell 2000). Studies estimate that viruses may comprise up to 10 million particles per milliliter of seawater (Suttle 2007; Arístegui *et al.*, 2009; Breitbart 2012; Gregory *et al.*, 2019). Contemporary studies are beginning to provide a clearer understanding of the role played by viruses in marine ecosystems. These studies have shown that viruses infect organisms on all trophic levels of the marine food web

(Coutinho *et al.*, 2017; Danovaro *et al.*, 2017b). Their hosts range all the way from large mammals (Bossart and Duignan 2018; Shin *et al.*, 2019) to microorganisms such like *Synechococcus* (Marston *et al.*, 2012; Flores-Urbe *et al.*, 2019; Kupczok 2019). Recent studies have shown that viral-host interactions may be important for metabolic control and ultimately influence marine ecosystem services (Nishimura *et al.*, 2017; Sotomayor-Garcia *et al.*, 2020).

Recent studies have shown that viruses have highly diverse taxonomic groups (Brum *et al.*, 2015; Roux *et al.*, 2016). In the SO, the viral communities are dominated by nucleocytoplasmic large DNA viruses like the chloroviruses and mimiviruses (Flaviani *et al.*, 2018). Others include, for example, double stranded DNA viruses such as vSAG 37-F6 (Martinez-Hernandez *et al.*, 2017) and *Pseudoalteromonas* phages as well as RNA viruses like *Picornavirales* (Vlok *et al.*, 2019). The lack of universal marker genes has limited the extent to which viruses may be characterized. This deficiency has also limited the efforts to provide biogeographic insights regarding viruses across the oceans (Sullivan 2015). With the help of genomics-based research, recent efforts have begun to expand our knowledge of the diversity and biogeography of marine viruses (Brum *et al.*, 2015; Coutinho *et al.*, 2017; Nishimura *et al.*, 2017). Studies have found that viral diversity and taxonomic composition are influenced by temperature, light, depth in the water column and host-cell densities (Hurwitz and Sullivan 2013; Luo *et al.*, 2017a).

Viruses play a role in controlling microbial populations through lysis (Riemann and Middelboe 2002; Danovaro *et al.*, 2017b). We now know that metabolic diversity through horizontal gene transfer events and host metabolic reprogramming by viral-

encoded auxiliary metabolic genes (Brum and Sullivan 2015; Hurwitz and U'ren 2016; Roux *et al.*, 2016; Coutinho *et al.*, 2019) is pivotal., Viruses may therefore be viewed as major constraints of biogeochemical cycling through their effects on microbial community composition, metabolism and evolution (Brum and Sullivan 2015; Duhaime *et al.*, 2017).

5. Factors shaping microbial communities

5.1. Physical and environmental drivers of microbiomes

Taxonomy and structure of microbial communities varies by geography (Flombaum *et al.*, 2013; Sul *et al.*, 2013). Bacterial and eukaryotic communities differ markedly when comparing the Arctic and Southern Oceans as well as between the two poles in comparison to tropical mid-latitudinal and equatorial regions (Milici *et al.*, 2016; Haggerty and Dinsdale 2017). In addition, the taxonomic distribution of fungi in oligotrophic regions differs markedly from nutrient rich areas such as the SO (Baldwin *et al.*, 2005; Ghiglione *et al.*, 2012). Studies suggest that these geographic differences are likely attributed to differences in various environmental variables (Ghiglione *et al.*, 2012; Sunagawa *et al.*, 2015; Morales *et al.*, 2019) such as sunlight (Hernández *et al.*, 2019), temperature (Baldwin *et al.*, 2005) and nutrient availability. Other studies have also confirmed the significance of environmental factors in shaping community structure and composition (Hanson *et al.*, 2012; Tisthammer *et al.*, 2016; Zhang *et al.*, 2018b)

5.2. Depth stratification

Environmental variables have been shown to vary with along the water column. Similarly, microbial community structure is greatly influenced by depth (Wilkins *et al.*, 2013a; Tisthammer *et al.*, 2016; Walsh *et al.*, 2016; Phoma *et al.*, 2018; Morales *et al.*, 2019). While prokaryotes are widely distributed, several studies have shown water column specific differences in their taxonomic composition. For example, Cyanobacteria are found throughout the water column but occur at higher abundances in surface waters (Gibberd *et al.*, 2013; Wilkins *et al.*, 2013a; Viljoen *et al.*, 2019). Archaea also display distinct patterns with increasing depth (Santoro *et al.*, 2019). For instance, Thaumarchaeota are more prevalent in the deep ocean while Euryarchaeota appear to dominate in the sunlight ocean (Massana *et al.*, 1997; Junior *et al.*, 2015). These differences are thought to be due to oceanographic and environmental conditions specific to different zones (Jing *et al.*, 2013; Bryant *et al.*, 2016). Surface ocean communities are subject to influence of fluctuations in temperature, sunlight and land-sea interactions making them more susceptible to short-term environmental selection (Bryant *et al.*, 2016). On the other hand, communities in deeper zones of the ocean appear to be more taxonomically even in distribution likely due to the connectivity of deep water masses (Agogu   *et al.*, 2011).

5.3. Water Mass Endemicity

In a number of studies, water masses have been identified as the most important variable explaining marine microbial diversity (Galand *et al.*, 2009; Agogu   *et al.*, 2011; Pernice *et al.*, 2016; Djurhuus *et al.*, 2017). Distinct water masses characterize the hydrodynamic conditions of the ocean (Tomczak and Godfrey 2003; Carter *et al.*,

2008; Agogu   *et al.*, 2011). Free-living microbes can be trapped within the boundaries of these waters resulting in water mass-specific community composition and functionality (Galand *et al.*, 2009; Hernando-Morales *et al.*, 2017). Thus, the community composition within a particular water mass is affected by the frequency of mixing between layers as well as the particular history of the water mass (Galand *et al.*, 2010; Hernando-Morales *et al.*, 2017).

While important drivers of microbial community structure have been identified, very little is known about how these are linked to one another. Further, minimal research has been done regarding the functional capacity of different marine microbes, the interactions between different taxa and whether or not these same factors influence the functional capacity of the microbiome and if it exhibits similar patterns.

The functional capabilities of many cultured microorganisms have been determined by culture-based methods. Thus, taxonomic profiling of the marine microbiome allows us to make inferences about its metabolic capabilities based on the community composition. However, one taxonomic group may be capable of multiple metabolic functions and multiple taxa are capable of executing similar functions. As a result, the relative importance of mechanisms influencing taxonomic diversity may not truly reflect on the functional capacity of the microbiome. Rather, the influence of factors such as water mass endemism, depth and resource availability on functional potential may be a better indicator of microbial community dynamics in marine systems (Louca *et al.*, 2016). Genome analyses may help to determine the adaptive mechanisms employed by microorganisms that allow them to persist under varying conditions and occupy distinct ecological niches (Grossart *et al.*, 2020).

6. Functional capacity and niche partitioning in marine microbial ecosystems

The capacity to degrade different forms of OM plays a role in the composition of the microbiome (Flint *et al.*, 2012; Gao *et al.*, 2017). It has been proposed that resource heterogeneity may be a significant driver of microbial diversity (Muscarella *et al.*, 2019). Deep sea microbial communities are highly dependent on the export of POM, in the form of 'marine snow', from the euphotic layer (Bochdansky *et al.*, 2017). Marine snow is produced by the aggregation of phytoplankton detritus and other organic matter (Kjørboe and Hansen 1993). This process mainly occurs in the surface ocean and, to a limited extent, the mesopelagic layer (Kriest and Evans 2000; Cavan *et al.*, 2019). The aggregation of particles is a major pathway for the formation and transformation of both organic and inorganic matter in marine systems and the export thereof is essential to supplying nutrients to bathypelagic life (Burd and Jackson 2009; Milici *et al.*, 2017).

Throughout the water column, heterotrophic microbes attach to sinking POM degrading HMW compounds and assimilate or release these into the water. These microorganisms regulate the availability of several nutrients by degrading HMW organic matter, producing smaller products that can be readily assimilated into the cell (Arnosti *et al.*, 2014; Baltar *et al.*, 2017). Heterotrophic microbes may include nanoflagellates, ciliates, bacteria, dinoflagellates, viruses and some small (< 200 µm) metazoans (Olson and Daly 2013). To consume organic carbon, many bacteria (e.g. Bacteroidetes and Verrucomicrobia) and microzooplankton colonize sinking particles

such as marine snow and dead phytoplankton that are rich in POM (Kiørboe *et al.*, 2003) often through exuding various enzymes.

Marine microbes may exist as either particle attached (PA) or free-living (FL) or alternating between the two lifestyles. This distinction depends on their interactions with POM in the water column (Simon *et al.*, 2014; Tang *et al.*, 2015). PA microorganisms remain primarily associated with a variety of particles as they have a higher capacity to degrade HMW OM than FL microbes which are less adept at degrading HMW OM and thus spend their entire life-cycle suspended in the water column (Grossart 2010; Bachmann *et al.*, 2018).

The abundance of PA bacterioplankton are estimated to be 100 - 1000 times higher than FL bacteria (Fenchel 2008; Herndl and Reinthaler 2013; Cavan *et al.*, 2019). PA and FL communities exhibit marked differences in their taxonomic composition (DeLong 1992; Zhang *et al.*, 2016). More importantly, these taxa perform different functions within the water column and are differentially influenced by environmental parameters (Lyons and Dobbs 2012; Zhang *et al.*, 2016; Gomez-Consarnau *et al.*, 2019). For example, Bachmann *et al.*, (2018) found that the biggest predictor of FL community structure was temperature while PA communities were best explained by both temperature and ammonium (Bachmann *et al.*, 2018). Bachmann *et al.*, (2018) also showed that PA and FL communities were most similar in surface waters than in the deep ocean. This is confirmed by another study (Liu *et al.*, 2018) which suggests an increasing relative importance of PA microorganisms with increasing depth given their higher capacity to hydrolyse less labile OM in the deep ocean. PA microbes also

have a higher propensity for producing and releasing extracellular enzymes (Baltar *et al.*, 2013), increasing their ability to the degrade HMW compounds.

Extracellular enzymes produced by heterotrophic microbes are major drivers of nutrient cycling in terrestrial, freshwater and marine environments (Hoppe 2003; Arnosti *et al.*, 2014; Liu *et al.*, 2018). The degradation of HMW compounds occurs through the release of extracellular enzymes into the immediate environment by secretion and (less frequently) by cell lysis (Arnosti 2011; Sinsabaugh *et al.*, 2014). Both bacteria and fungi are able to produce a range of enzymes which degrade different fractions of DOM and facilitate the acquisition of nutrients (Krause *et al.*, 2013; Yamada *et al.*, 2013; Arnosti *et al.*, 2014). The amount, distribution and functioning of extracellular enzymatic activities reflect differences in nutrient availability, the quantity of organic matter and microbial community composition as well as consumption (Arnosti *et al.*, 2014). Some examples of extracellular enzymatic activities include β -glucosidase (Luo *et al.*, 2017b) and β -xylosidase (Knob *et al.*, 2010), which are important for carbon acquisition through the degradation of sugar and hemicellulose, respectively, and N-acetyl glucosaminidase which degrades chitin for phosphorus acquisition (Arnosti *et al.*, 2014; Baltar *et al.*, 2017).

The majority of microbial extracellular enzymes are produced in surface waters (Davey *et al.*, 2001). Due to the higher availability of nutrients, the majority of DOM remineralization occurs in surface waters (Davey *et al.*, 2001). However, extracellular enzymatic activities have been detected throughout the water column (Davey *et al.*, 2001; Hoarfrost and Arnosti 2017b) and from coastal and deep-water sediments (Boetius *et al.*, 2000; Lloyd *et al.*, 2013; Luglia *et al.*, 2019). Several factors including

the substrate type and availability, pH and temperature have been identified as key influencers of enzyme expression in freshwater and terrestrial ecosystems (Smith *et al.*, 1992; Arnosti *et al.*, 2014). However, these relationships have not been demonstrated in marine environments where functional diversity is a better predictor of enzymatic activity (Arnosti *et al.*, 2014). In marine environments, the majority of studies have been performed on samples retrieved from surface or coastal waters (Baltar *et al.*, 2013; Luglia *et al.*, 2019). As a result, there is little information regarding enzymatic activities in the meso- and bathypelagic layers of the ocean (Pernice *et al.*, 2016). This is despite the fact that subsurface waters contain approximately 30% of the earth's microbial biomass (Kallmeyer *et al.*, 2012; Arnosti *et al.*, 2014). A better understanding of the enzymatic activity of communities in the deep ocean may reveal their contribution to the carbon pump (Hoarfrost and Arnosti 2017b). The enzymatic capacity of a microbial community determines the breadth of organic compounds, which may be recycled and can be a key indicator of its functional roles within an ecosystem (Hoppe 1991; Baltar 2018).

Taxonomic community profiling may reveal important insights regarding the variation between environments (Louca *et al.*, 2016). The interaction between environmental conditions and functioning is only partially reflected in overall community structure due to high functional redundancy in the marine microbial ecosystem (Martiny *et al.*, 2006; Louca *et al.*, 2016). Environmental conditions shape metabolic niches and selective processes and drives phylogenetically diverse groups to exhibit phenotypically similar traits (Stilianos *et al.*, 2017; Louca *et al.*, 2018). As a result, many metabolic phenotypes are dispersed irregularly across microbial clades (Louca *et al.*, 2018).

7. Molecular techniques for studying marine microbial communities

Previous studies aimed to enumerate microorganisms by counting the number of dividing cells per volume of seawater on solid growth media (Azam *et al.*, 1983; Richards *et al.*, 2012). Culture-based methods such as direct cell counts or plating on standard agar medium were later revealed as having largely underestimated the actual number of microbes ocean environments (Bernard *et al.*, 2001). This was largely due to the difficulties growing marine microbes in laboratories (Connon and Giovannoni 2002; Guo *et al.*, 2006). The inability to accurately replicate the precise growth conditions and the requirements of complex microbial systems may explain these underestimates (Guo *et al.*, 2006). In fact, Amann *et al.*, (1990) went on to estimate that less than 1% of prokaryotes could be cultivated in isolation. The precise numbers are still subject to debate. Martiny (2019) proposed that the 1% paradigm is weakened by the narrow definition of similarity between taxa. He argues that the presence of rare sequence variants may lead to the underestimation of observed cultivability and that appreciably more than 1% of taxa can, in fact, be cultured. Steen and colleagues (2019) argued that Martiny's theory could not be valid based on technical errors (Steen *et al.*, 2019). More specifically, they argued that the methods used by Martiny to calculate sequence similarity were flawed. Beyond this, they said that 'sequence similarity cannot infer culturability because physiology cannot be inferred from 16S sequences' (Steen *et al.*, 2019; Martiny 2020). Ultimately, the differences in the two arguments lie in criteria used to define taxa. The general

consensus remains that it is important to continue to develop cultivation-based model systems.

Nevertheless, present-day culture-dependent methods alone fail to provide accurate representations of microbial community composition (Richards *et al.*, 2012; Sunagawa *et al.*, 2015). The development of culture-independent methods served as a fitting answer to this problem (Hugenholtz *et al.*, 1998). Traditional fingerprinting techniques, such as amplified ribosomal intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) allow for more accurate profiling of the diversity of a microbial community (Muyzer and Smalla 1998; Osborn *et al.*, 2000; Ghosh *et al.*, 2015). These are, however, limited to detecting community members with abundances greater than 1% of the community (Galand *et al.*, 2009; Ramette 2009; Reich and Labes 2017; Reich *et al.*, 2017).

The advent of next-generation sequencing has revolutionized the field of microbial ecology allowing for the illumination of microbial diversity in the human gut (Mesnage *et al.*, 2018; Russell *et al.*, 2019), soil (Adriaenssens *et al.*, 2017; Bukar *et al.*, 2019) and marine habitats (Rusch *et al.*, 2007; De Corte *et al.*, 2018; Phoma *et al.*, 2018; Venkatachalam *et al.*, 2019; Sadaippan *et al.*, 2020). In other studies, amplicon sequencing has allowed for the identification of species in environmental samples (Kocher *et al.*, 2018). These approaches apply universal marker genes to reveal the diversity of bacteria, archaea and fungi, respectively (Richards *et al.*, 2012). With reference to the Southern Ocean, there have been a number of studies on the composition and structure of microbial communities (Murray and Grzyski 2007;

Wilkins *et al.*, 2013b). One of the possible criticisms with such studies is that they fail to accurately predict ecosystem functions by the identified taxonomic groups.

Shotgun sequencing presents a possible solution and applied to metagenomic studies, has provided insights regarding putative functions (Ranjan *et al.*, 2016). Shotgun metagenomics does not, however give an accurate description of the metabolic capacity of the microbiome (Rezasoltani *et al.*, 2020). Proteomics and transcriptomics allow for the detection of gene expression and thus are a better representation of the functioning of the community instead of mere taxonomic and phylogenetic diversity (Nunn and Timperman 2007; Kalita *et al.*, 2019; Rezasoltani *et al.*, 2020). The combination of these techniques and have become part of the standard in microbial ecology for community analyses (Pedrós-Alió 2006; Hiraoka *et al.*, 2019; Manck *et al.*, 2020; Singer *et al.*, 2020).

Next generation sequencing techniques are a good way to assign putative functionality to different taxonomic groups. Wu *et al.*, (2017) applied amplicon sequencing of bacterial and archaeal *amoA* genes in order to assess nitrification by Ammonia-oxidizing bacteria (AOB) and archaea (AOA) in acidic soils. The authors identified the functional AOA species and putatively assessed their ecological role in the acidic soil environment (Wu *et al.*, 2017). While these findings added some insight, they merely confirm the presence of the genes required for ammonia oxidation and thus are only an indication of the potential metabolic capacity of that community.

The detection of precursor genes, however, does not necessarily imply that the gene products are functional within that environment (Rocca *et al.*, 2015). It is therefore still essential to experimentally determine microbial community functionality. Methods such as stable isotope probing, fluorescence *in-situ* hybridization and extracellular enzyme activity assaying can also be used in conjunction with amplicon data to confirm the functions predicted using sequencing data (Moreira-Grez *et al.*, 2019; Hatzenpichler *et al.*, 2020). For instance, the determination of extracellular enzyme activities (discussed in the previous section) provides more accurate estimates of heterotrophic activity in different environments (Hoppe 1983; Hoarfrost and Arnosti 2017b). Fluorometric techniques provide a benefit over sequencing as they are able to measure the real-time degradation of substrates by exoenzymes (Hoppe 1983; Marx *et al.*, 2001; Arnosti *et al.*, 2014; Charkhabi 2018). The joint use of next generation sequencing and functional assays is important in order to add insight into the patterns and processes of marine microbial communities and the various factors that shape them.

8. Problem statement

Previous studies have established the taxonomic composition of microbial communities surrounding the Sub-Antarctic islands (Dinasquet *et al.*, 2019; Venkatachalam *et al.*, 2019). These studies revealed remarkable levels of phylogenetic diversity in marine waters proximal to these islands. However, we lack fundamental insights regarding the determinants of these microbiomes and their effects on functional processes. The 'island mass effect' may help to explain the ecological dynamics in these environments. More specifically, the theory may clarify

the role the environment plays and its influence on bacterial and archaeal communities.

Although these recent studies have provided some insights regarding the phylogenetic patterns of bacteria and archaea, there is a lack a basic understanding of fungal communities in this environment. All together, we lack a mechanistic understanding of the effects of hydrography and environmental variables on microbial community structure in this region of the SO.

A further deficit in our understanding of microbiomes around the PEIs is on the role played by depth as an evolutionary explanatory factor. We lack a clear understanding of depth-resolved differences throughout the water column and the effects of community-wide differences. The extent of niche partitioning among PA and FL microorganisms in this region is also unclear. To reduce this knowledge deficit it is important to study the taxonomy and the effects of physicochemical variables on functional dynamics in these environments.

Here, we elucidate the near-shore microbial communities of the PEIs. By exploring microbial community dynamics using 16S rRNA gene and ITS region sequencing, we shed light on the composition and structure of bacteria and fungi, respectively. We further investigate the differences between PA and FL microbiomes. A suite of nutrient analysis was used to determine the effects of physicochemical variables as drivers of these microbiomes. We further explore the extracellular enzymatic activities of these microbial communities. Together, our data provide comprehensive insights regarding phylogenetic diversity and the effects of physicochemical variables on functionality. Such data are vital for efforts to understand niche partitioning in the PEIs specifically

and the wider ocean microbiome. More generally, such studies are important for elucidating how climate change may affect the marine microbial ecosystem services.

9. Aim and Objectives

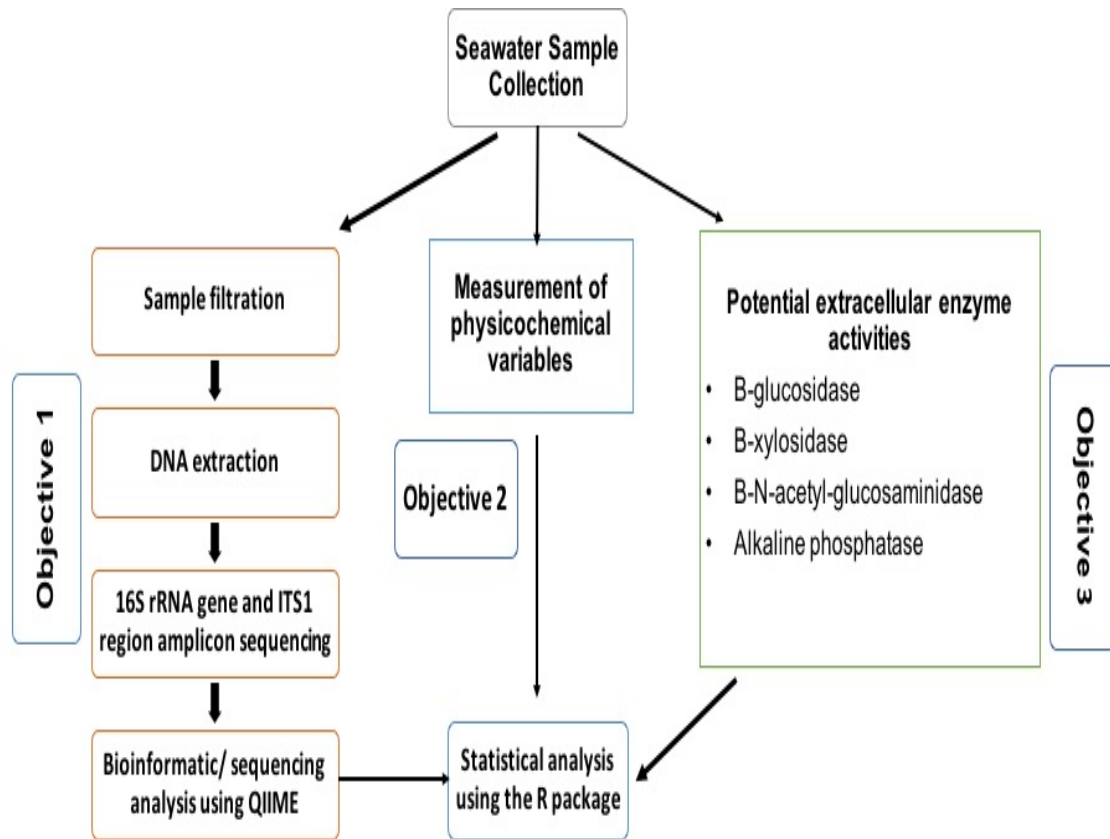
9.1. *Aim:*

The aim of this study was to elucidate the effects of physicochemical variables on the composition, structure and function of PEI microbial communities

9.2. *Objectives*

1. To determine the bacterioplankton and mycoplankton community structure and diversity using gene specific primers
2. To measure and assess physicochemical variables associated with the microbial community
3. To quantify microbial extracellular enzymatic activities linked to putative microbial heterotrophic taxa

9.3. Strategy flow diagram:



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Chapter 2

Community structure and functional determinants of
free-living and particle associated Bacterioplankton and
mycoplankton in sub-Antarctic islands

1. Abstract

Recent studies have shown that a range of biological and oceanographic features shape the composition, structure and function of Southern Ocean (SO) microbiomes. Within the vicinity of islands, the interactions between landmasses, the surrounding waters and biology result in complex tropic interactions. These interactions are expected to affect nutrient cycling and ecosystem services the bulk of which is driven by microorganisms. However, little is known regarding the microbial dynamics of near-shore regions of SO islands such as the Prince Edward Islands (PEIs). This knowledge deficit is particularly true for particle attached (PA) and free living (FL) bacterioplankton and fungal microbiota. Here, we provide insights regarding microbial community structure and composition of PEIs microbiomes. We assess the effects of physicochemical factors on PEIs microbial communities. Samples were collected from three depths, namely, the Fluorescence maximum (Fmax), the oxygen minimum zone (OMZ) and the bathypelagic zone (Deep) at 10 sites within the vicinity of the PEIs. Phylogenetic analysis of 16S and ITS rRNA gene regions and statistical analysis revealed a shift in bacterioplankton and fungal community structure and composition, respectively. This shift coincided with differences in depth and water mass specificity. The results also show that PA and FL PEI microbial communities were strongly influenced by oceanographic features and physicochemical variables. Extracellular enzymatic activities specific for carbon, nitrogen and phosphorus acquisition did not reveal any specific trends between community composition and potential functionality but exhibited a close linkage with substrate availability and depth. These results suggest that potential extracellular enzymatic functionality of the microbiome is driven by nutrient dynamics in this environment. This study provides valuable insights

regarding the influence of biotic and abiotic drivers in the dynamics of near-shore microbial communities in the SO.

2. Introduction

Understanding the effects of climate change on biodiversity remains a major ecological endeavour (Araújo and Rahbek 2006; Bellard *et al.*, 2012; Mantyka-Pringle *et al.*, 2012). This is especially true in ecosystems characterized by high levels of endemism, such as the SO, where losses in keystone taxa may ultimately affect ecosystem services (Chown *et al.*, 2015). Recent studies have provided quantitative evidence of species loss in the SO (Trathan *et al.*, 2007; Warnock and Scherer 2015). The impacts of such declines are evident across several ecosystems, including sub-Antarctic islands, which are major hubs for macro-organisms (Bester *et al.*, 2011; Labadie *et al.*, 2018). Efforts to reveal ecosystem dynamics across several habitats have highlighted the important role played by microorganisms in the cycling of carbon and organic matter (Yooseph *et al.*, 2007; Falkowski *et al.*, 2008; Passow and Carlson 2012; Pachiadaki *et al.*, 2017; Zhang *et al.*, 2018a). However, comparatively little is known regarding microbial communities associated with sub-Antarctic islands.

Studies of land-sea interactions have shown that the input of nutrients, as a result of island runoff, leads to increased primary productivity resulting in periodic phytoplankton blooms (Duncombe Rae 1989; Pakhomov and Froneman 1999; Treasure *et al.*, 2015; Von Der Meden *et al.*, 2017). These drifts, termed the 'island mass effect' (Doty and Oguri 1956; Boden 1988) are typically observed in the PEIs as well as other sub-Antarctic landmasses. Consequently, the PEIs harbour relatively elevated productivity levels in low productivity regions (Perissinotto and Rae 1990; Perissinotto *et al.*, 2000; Gurney *et al.*, 2014). A combination of climate, oceanographic

features and general land-sea interactions in the PEIs provide a valid model for understanding the effects of climate change on marine microbiomes.

Previous studies on the PEIs have primarily focused on terrestrial environments and macrofauna (Pistorius *et al.*, 2011; Greve *et al.*, 2017). Research on the surrounding marine environment has concentrated on the physical oceanography (Durgadoo *et al.*, 2010; Lamont *et al.*, 2019) and marine macrofauna (Haley *et al.*, 2017; Reisinger *et al.*, 2018). Few studies have provided phylogenetic and functional insights regarding microbial communities in the PEIs. Studies evaluating pelagic microbial assemblages and the factors that govern their composition and structure suggest higher than expected levels of diversity (Wilkins *et al.*, 2013a; Walsh *et al.*, 2016; Phoma *et al.*, 2018). These studies suggest that the microbiota, primarily bacteria, are shaped by several factors including the availability of light (Cory *et al.*, 2014; Bryant *et al.*, 2016), salinity levels (Crump *et al.*, 2004; Morrissey *et al.*, 2014; Mukhtar *et al.*, 2018), temperature (Sunagawa *et al.*, 2015; Morán *et al.*, 2017) as well as latitude and longitude (Wagner 2008; Ladau *et al.*, 2013; Haggerty and Dinsdale 2017).

Oceanic variability also has a major influence on the marine microbiome. For example, depth (Delong *et al.*, 2006; Walsh *et al.*, 2016; Phoma *et al.*, 2018) ocean fronts (Pinhassi *et al.*, 2003; Raes *et al.*, 2018) and water mass specificity (Agogue *et al.*, 2011; Wilkins *et al.*, 2013a; Hernando-Morales *et al.*, 2017) have been shown to substantially impact the structure and dynamics of marine microbiomes. It has previously been recorded that mesoscale eddies and shifts in ocean fronts may result in differences in community structure and composition in marine microbial communities (Venkatachalam *et al.*, 2019). However, these findings are insufficient to

conclude which of these factors best explains the differences observed between microbial communities. Additionally, very few studies have made these evaluations in the PEIs, where unique oceanographic conditions and physicochemical properties may affect microbial assemblages in alternative ways.

The manner and success with which individual microbes contribute to biogeochemical cycling in the marine environment is highly dependent on the availability of substrates (Meier *et al.*, 2016; Muscarella *et al.*, 2019). It also relies heavily on the microbes' ability to colonize and break down those substrates. Aquatic microorganisms can be classified according to their relationship with particulate matter (either truly free-living (FL), particle attached (PA) or alternating between the two lifestyles) (Grossart 2010; Tang *et al.*, 2015). PA microbes have a higher capacity to degrade high molecular weight (HMW) organic matter (OM) and thus remain primarily associated with a variety of particles (Lyons and Dobbs 2012; Liu *et al.*, 2018). FL bacteria are less adept at degrading HMW OM and thus spend their entire life-cycle suspended in the water column (Grossart 2010; Bachmann *et al.*, 2018).

Attachment to particles may increase the availability of OM for mineralization by microorganisms but there are circumstances under which the presence of a particular substance does not imply bioavailability (Milici *et al.*, 2017). Under these circumstances, the ability to convert OM into a form where it can be accessed is advantageous (Hoarfrost and Arnosti 2017a). The ability of some microorganisms to produce extracellular enzymes facilitates this process (Arnosti *et al.*, 2014; Baltar *et al.*, 2017). Several extracellular enzymes are produced by marine microbes that aid in the acquisition of essential nutrients like carbon, nitrogen and phosphorus (Benitez-

Nelson 2000; Sistla and Schimel 2013; Karl and Björkman 2015; Hoarfrost and Arnosti 2017a).

Relative to the increasing well of knowledge regarding marine microbial composition throughout the globe, it is still unclear to what extent the above-mentioned factors affect near-shore communities around the PEIs. Further it remains to be confirmed how the different factors are linked to one another and how they may affect the overall functioning of the community, if at all. There is a knowledge deficit with regards to biogeographical patterns of the PEI microbiome in general and more specifically with regard to how the physicochemical variables that determine community structure may be linked to the overall metabolic capabilities of the greater community. This study elucidates the effect of physicochemical variables on the community composition, structure and function of microbial communities in the SO.

3. Methods and Materials

3.1. Sampling

Samples were acquired aboard the RV SA Agulhas II from April to May 2018 during the Marion Island Relief Voyage in the austral spring. Seawater samples were collected from 10 oceanographic stations near the Prince Edward Islands (Figure 3) using a 24 x 20 litre Niskin bottles mounted on a CTD rosette multi-sampler (Sea-Bird SBE-911 plus V2 CTD system; Sea-Bird Electronics, Inc., USA). Samples were labelled H01, H03, H05, H07, H08, H11, H13, H17, H19 and H21, according to the sampling station. Samples were collected from three depths; the epipelagic (fluorescence maximum, F_{max}), the oxygen minimum zone (OMZ) and approximately

10 m above the seafloor (Deep). Physicochemical variables including temperature, salinity, conductivity and fluorescence were recorded at each sample site via sensors on the CTD rosette. Water was collected in 5 L seawater washed polyethylene bottles. This was immediately filtered through 0.45 μm and then 0.22 μm -pore cellulose acetate membrane filters (Merck Millipore Ltd., Germany) to capture the particle associated (PA) and free-living (FL) communities, respectively, prior to storage in cryovials at -20°C on board and then at the Centre for Microbial Ecology and Genomics (Pretoria, South Africa) until further analysis. Approximately 100 ml of unfiltered seawater was collected for nutrient analysis and extracellular enzymatic activity assays, respectively.

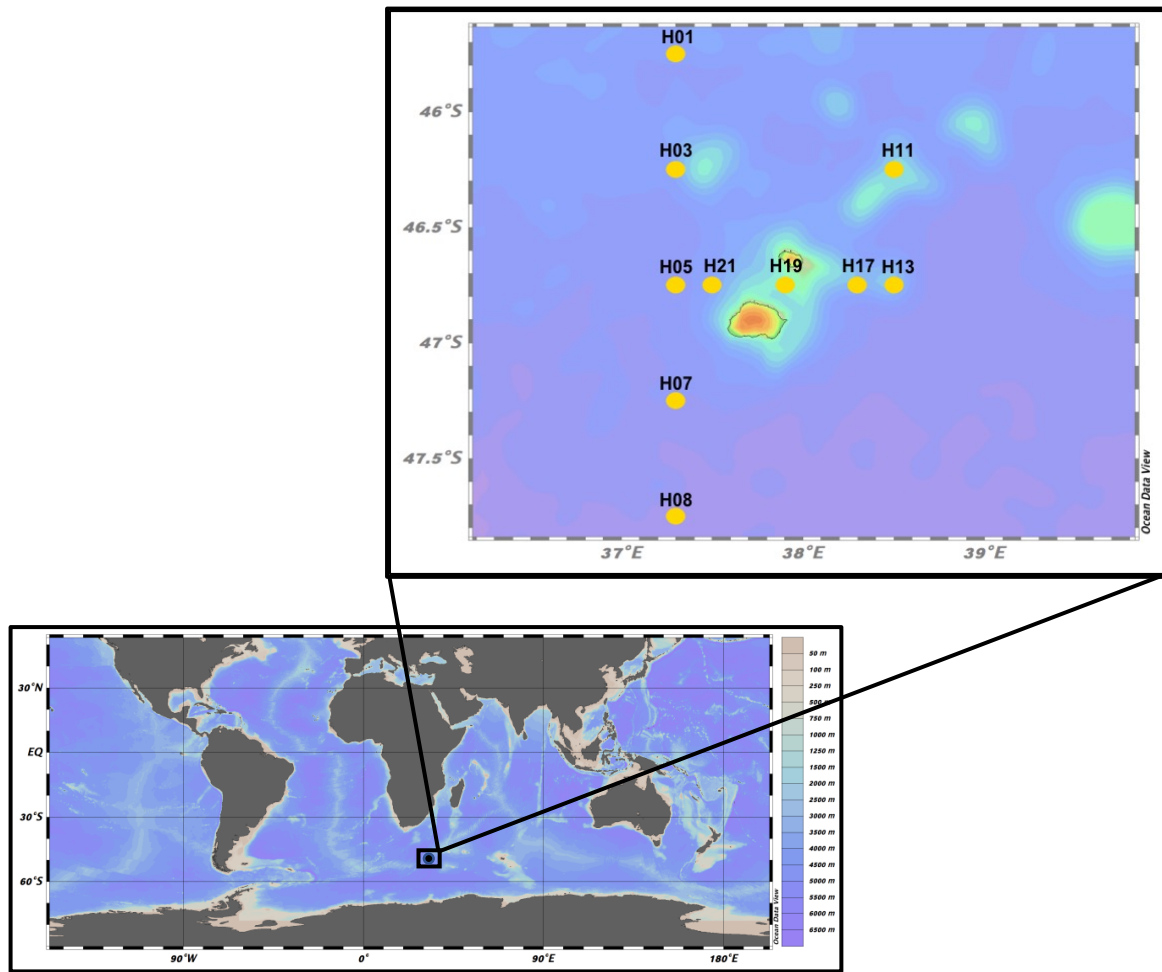


Figure 3: Map of the Prince Edward Islands showing the sampling locations and study area. Seawater samples were collected from 10 stations within the vicinity of the PEI's in the 'H-transect'. The bottom and top panels show the global positioning of the PEIs and the H-transect, respectively. Maps were created using Ocean Data View version 5.1.7 (<https://odv.awi.de>, 2019).

3.2. Nutrient analysis

Samples for nutrient analysis were sent to Bemlab (Somerset West, South Africa) where sodium (Na), potassium (K), calcium (Ca), iron (Fe), sulfate (SO₄), copper (Cu),

zinc (Zn), phosphorous (P), ammonium (NH₄), and nitrate (NO₃) concentrations were determined using a Varian Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent, Australia). For macro and trace element determination of Ca, K, SO₄, Cu, Zn and Fe, samples were filtered through a 0.45 µm syringe and transferred to an ICP tube for analysis on the ICP-OES as detailed previously (Quevauviller *et al.*, 1994). NH₄-N and NO₃-N samples were passed through a 0.45µm syringe and transferred to a test tube for analysis on the Gallery Discrete Analyzer (Thermofisher, USA). Details of these analyses were provided by Bemlab (Somerset West, South Africa).

3.3. Molecular Ecology Analysis

3.3.1. DNA extraction

Nucleic acids were extracted from a third of each membrane filter using the Power Water DNA isolation kit (MoBio, Carlsbad, USA), as detailed in the manufacturer's manual with minor modifications to maximize yield. Filters were cut into small pieces, using a sterile scalpel, and the initial lysis step was extended. The volume of elution buffer (Solution EB, Power Water DNA isolation kit) was reduced to 50µL. The DNA was quantified and quality checked using a NanoDrop Spectrophotometer 2000 (Thermo Scientific, USA) and agarose gel electrophoresis, respectively.

3.3.2. Polymerase Chain Reaction (PCR) and gel electrophoresis

Amplification was confirmed using PCR and gel electrophoresis. Each reaction consisted of OneTaq (0.025u/µL), 1X OneTaq buffer (New England Biolabs, Massachusetts, USA), universal bacterial/archaeal primers, 314F (5'-

CCTACXGGGAGGCAGCAG-3') (Hugenholtz *et al.*, 2001) and 908R (5'-CGTCAATTCMTTGTGAGTT-3') (Lane *et al.*, 1985), (0.2 mM), BSA (10 µg/µL), dNTPs (200 µM) and 1 µl of template DNA at a final reaction volume of 25µL. Amplification conditions consisted of 30 cycles of denaturation (at 95°C for 8 minutes), annealing (at 55°C for 30 seconds) and extension (at 72°C for 1 minute 30 seconds), followed by a final extension (72° for 10 minutes) in a BioRadT100™ Thermocycler (BioRad, Munich, Germany).

A 1% (w/v) agarose gel was prepared using 1 X TAE (10mM Tris pH 7.4, 1mM Na₂.EDTA₂H₂O pH 8) buffer (Sambrook *et al.*, 1989). DNA was mixed with 6X loading dye (Thermo Scientific, USA) then loaded onto the gel alongside the ThermoScientific Gene Ruler™ 1kb DNA ladder (0.5 µg/µL) (ThermoScientific, USA). Electrophoresis was done at 80 V for 40 minutes and the gel was visualised using a UV transilluminator (BioRad, Germany).

3.3.5. Sequencing

DNA extracts were sent to the University of Liverpool for high throughput 16S rRNA amplicon sequencing where all PCR amplification, product quality checking and purification as well as DNA library preparation was performed. PCR amplicon libraries of the V5–V6 region of 16S rRNA genes was prepared from genomic DNA. The marine specific primer pair 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926R (5'-CCGYCAATTYMTTTRAGTTT) (Parada *et al.*, 2016), targeting a region of approximately 411bp, was used to amplify hypervariable regions V4 and V5 of the bacterial and archaeal 16S rRNA gene. All 16S rRNA gene amplicons were

sequenced using the Illumina MiSeq Platform (2 x 250bp) NERC-funded Advanced Genomics Facility at the University of Liverpool (<http://www.liv.ac.uk/agf/>).

Fungal internal transcribed spacer (ITS) sequencing was performed at the Molecular Research DNA (MR DNA) sequencing facility (Texas, USA). All samples were quality checked prior to amplicon library preparation as detailed previously. Fungal-specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) were used as detailed previously (Kamutando *et al* 2019). Tagged amplicons were pooled in equimolar concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, USA) prior to paired-end sequencing (2 x 250bp) on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

A, more detailed description of the analyses performed by MR DNA is as follows. PCR amplification was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with cycle conditions: initial denaturation of 94°C for 3 minutes, 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 minute, with a final elongation step at 72°C for 5 minutes. PCR products were visualised on a 2% agarose gel to verify successful amplifications and observe band intensities. Thereafter, multiple PCR products were pooled equally according to DNA concentrations and molecular weight and then purified using calibrated Ampure XP beads. A DNA library was then prepared using the purified and pooled PCR products. Sequencing on the Illumina MiSeq Platform (2 x 300bp) was then performed according to the MR DNA analysis pipeline.

3.3.6. Bioinformatic analysis

The resultant sequence data were analysed using the Quantitative Insights Into Microbial Ecology (QIIME2) software package version 2018.8.0 (Bolyen *et al.*, 2018) as detailed previously (Kabwe *et al.*, 2020). We followed different methodologies the analysis of 16S rRNA genes and ITS region sequences, using specific databases for bacteria and fungi, respectively.

Raw bacterial reads were trimmed at both ends to remove barcodes (Phred quality score <64). Reads were dereplicated, denoised and filtered using DADA2, implemented in QIIME2, with the default parameters (Callahan *et al.*, 2016). These sequences were quality filtered prior to assigning amplicon sequence variants (ASVs) using the SILVA 99 database (Quast *et al.*, 2012). The resultant sequences were subsampled (rarefied) to a 14 300 reads per sample to ensure even sampling depth. Samples with sequences yielding less than 14 300 reads were omitted from subsequent analysis. The resultant bacterial ASV output file was exported in BIOM format.

Raw fungal reads were pre-processed by trimming 25 nucleotides from the start and 280 nucleotides from the end of each read. Reads were de-replicated and de-noised using DADA2 (Callahan *et al.*, 2016) in QIIME2 (Bolyen *et al.*, 2018). The detection of chimeric sequences was performed at 97% sequence similarity using USEARCH v11 (Edgar 2010). Assignment of ASVs was performed using the UNITE (release 7_99) database for fungi (Tedersoo *et al.*, 2018). Taxonomic assignments of ASVs was conducted based on 97% sequence similarity using USEARCH v11 (Edgar 2010).

Singletons were excluded from downstream processing. All samples were rarefied to the same number of sequences per sample (11705).

Diversity metrics were calculated from the 16S rRNA gene and ITS region feature tables generated in QIIME2. We calculated several diversity indices including alpha (Shannon's diversity, Observed OTUs, Faith's Phylogenetic Diversity and Pielou's Evenness) and beta (Jaccard distance dissimilarity, Bray-Curtis distance, unweighted UniFrac distance and weighted UniFrac distance) diversity indices.

3.3.7. Microbial diversity and Statistical Analyses

The majority of data analyses were performed using the R statistical package version 3.6.1 (R Core Team) and R Studio version 1.1.456 (Team 2019). Rarefaction curves were computed and visualized using *vegan*. Alpha diversity parameters were computed using *vegan* and visualized using *ggplot2* (Wickham 2011). Relative abundances were computed and visualised using the *phyloseq* (McMurdie and Holmes 2013) and *ggplot2* packages, respectively (Wickham 2011).

Pairwise similarities among samples were calculated using the Bray–Curtis index of dissimilarity from Hellinger-transformed ASV tables. The resultant data was represented in a two-dimensional non-metric multidimensional scaling (NMDS) ordination using the *ordplot* function in *vegan*. To assess differences between sample groupings in the NMDS ordinations, a permutational multivariate analysis of variance (PERMANOVA) based on 999 permutations of the data was performed. To infer the relative importance of factors shaping biogeographical patterns as well as environmental drivers, the environmental vectors were fitted to the ordination using

the `envfit` function (Oksanen *et al.*, 2007). Significant differences in community structure were determined using PERMANOVA in QIIME2 and the `betadisper` function in `vegan` and visualized using the `ggplot2` in R. Multivariate homogeneity of group dispersions, an assumption of permanova, was assessed through the `betadisper` function. Boxplots were used to visualise and to determine if these data conformed to requirements for statistically robust PERMANOVA.

Spearman's correlations between taxon abundances, environmental variables, diversity measures and enzymatic activities were computed using the `rcorr` function in the `Hmisc` package (Harrell Jr and Harrell Jr 2015). Correlations were recalculated in SparCC (Friedman and Alm 2012), which takes into account the proportion of reads contributed by each ASV. The correlation matrix was visualized in R using the `corrplot` package. Euclidean distances were used to perform hierarchical clustering. ASV data were $\log_{10}$ transformed, after which cluster analysis was performed. For these analysis, ASV data for the top 1% of the bacterial and archaeal taxa using the heatmap function were used. In order to assess the spatial correlation of multivariate data between the Bray-Curtis dissimilarity matrices of two sets of data, a Mantel statistic can be computed. Mantel correlations were computed for taxonomic relative abundances, environmental variables and extracellular enzymatic activities using the `mantel.test` function in `ape`.

3.4. Extracellular enzyme activity assays

Seawater samples collected from each site were assayed for the activity of 5 enzymes, β -glucosidase (BG), β -xylosidase (BX), β -N-acetyl-glucosaminidase (NAG), alkaline phosphatase (AP) and leucine aminopeptidase (LAP). Potential extracellular enzymatic activities for the selected enzymes were tested using fluorescence, where the substrate analogues used were linked either to 4-methylumbelliferone (MUB) (for BG, BX, NAG and AP) or 7-aminocoumarine (AMC) in the case of LAP (Table1). Potential enzyme activities were calculated as previously described by (Sinsabaugh 1994; Hoarfrost and Arnosti 2017b) using four replicate cells for each sample. Briefly, seawater (200 μ l) was added to black 96-well flat-bottom microplates (Thermoscientific, USA), to which between 100 mM to 500 mM of each enzyme substrate was added. Controls in each plate included a sample to which no substrate was added, a substrate control to which ddH₂O was added to the substrate analogue and a quench standard, which contained only the fluorescent molecule (MUB or AMC) and sample. Plates were incubated at 4°C in the dark for the time specified in Table 1. Fluorescence was measured from the black 96-well plates using a Spectramax® Paradigm Multimode Microplate reader (Molecular Devices, USA) with an emission wavelength of 450 nm and an excitation wavelength of 360nm. Enzyme activities were then calculated.

Table 1: Details regarding the specific substrates, concentrations and incubation times for extracellular enzymatic enzyme activity assays.

Enzyme	Substrate	Concentration	Incubation time
β -Glucosidase (BG)	4-Methylumbelliferyl β -D-glucopyranoside 6.77 mg/100 ml	500mM	3hrs
β -Xylosidase (BX)	β -D-xylopyranoside 6.17 mg/100 ml	200mM	4hrs
4-Methylumbelliferyl β -N-acetylglucosaminidase (NAG)	4-Methylumbelliferyl N-acetyl- β -D-glucosaminide 7.59 mg/100 ml	400mM	3hrs
Leucine aminopeptidase (LAP)	L-Leucine-7-amido-4-methylcoumarin hydrochloride 6.5 mg/100 ml (dissolve in 0.5 ml acetone first)	500mM	3hrs
Alkaline phosphatase (AP)	4-Methylumbelliferyl phosphate 5.12 mg/100 ml	100mM	11hrs

4. Results

4.1. Physicochemistry of the PEI

4.1.1. Physical characteristics

The CTD rosette measured physical and chemical properties including temperature, pH, salinity, conductivity, fluorescence and oxygen from various sensors at 3 depths for the 10 sampling locations (n=30). The fluorescence maximum ranged from 0,51 to 2,83 mg/m³ and steadily decreased along the water column to 0 mg/m³. Conductivity varied considerably demonstrating a general decrease with ocean depth and was lowest in samples collected in the bathypelagic layer. The highest recorded oxygen levels ranged from 5.30 ml/L (bathypelagic layer) to 6.38 ml/L (surface layer). Temperatures decreased steadily with an increase in depth. Salinity, increased with an increase in depth, up to a maximum of 34,8 PSU at approximately 2000 m. Thereafter there was a slight decrease in salinity to 35,65 PSU at around 4000 m.

CTD data (temperature, salinity and depth) was used to determine the water masses associated with each sample as detailed previously (Emery 2001). Four water masses were identified including the Pacific Sub-arctic Upper Water (PSUW), Antarctic Intermediate Water (AAIW), Antarctic Bottom Water (AABW) and North Atlantic Deep Water (NADW) (Table 2).

Table 2: Temperature-salinity and depth characteristics of PEI surrounding waters as used to define water masses according to previous publications (Emery 2001).

Sample ID	Depth (m)	Temperature(°C)	Salinity (PSU)	Water Mass
H01F	20.83	7.35	33.74	PSUW
H01M	1100.94	2.71	34.55	AAIW
H01D	2426.65	2.10	34.78	NADW
H03F	40.66	7.45	33.73	PSUW
H03M	1200.50	2.68	33.58	AAIW
H03D	2023.23	2.28	34.76	NADW
H05F	40.65	6.87	33.83	PSUW
H05M	1199.46	2.71	34.57	AAIW
H05D	3234.63	1.38	34.74	AABW

H07F	40.65	6.01	33.82	PSUW
H07M	1201.36	2.72	34.60	AAIW
H07D	3753.42	0.90	34.71	AABW
H08F	29.75	4.39	33.68	PSUW
H08M	950.76	2.47	34.58	AAIW
H08D	4154.25	0.17	34.67	AABW
H11F	75.36	6.67	33.79	PSUW
H11D	944.97	2.85	34.48	AAIW
H13F	45.61	7.17	33.75	PSUW
H13D	1000.21	2.82	34.44	AAIW
H17F	19.84	6.82	33.75	PSUW
H17D	927.16	2.91	34.42	AAIW

H19F	10.91	6.82	33.80	PSUW
H19D	200.25	4.04	34.12	PSUW
H21F	20.82	6.75	33.77	PSUW
H21M	1203.42	2.71	34.56	AAIW
H21D	2619.46	1.87	34.77	AABW

F=Fmax; M=OMZ; D= Deep

Permutational analysis combined with the nMDS ordination (Figure 4) showed that several environmental and physicochemical variables significantly affected bacterioplankton community structure. These parameters include depth ($p<0.001$), temperature ($p<0.001$), salinity ($p<0.001$), oxygen ($p<0.001$), fluorescence ($p<0.001$), phosphorus (P) ($p<0.05$) and the ratio of carbon, nitrogen and phosphorus (C:N:P) ($p<0.05$). The analysis further showed that communities were significantly influenced by depth (Fmax, OMZ or Deep) ($r^2=0.4504$, $p<0.001$) as well as water mass ($r^2=0.4511$, $p<0.001$).

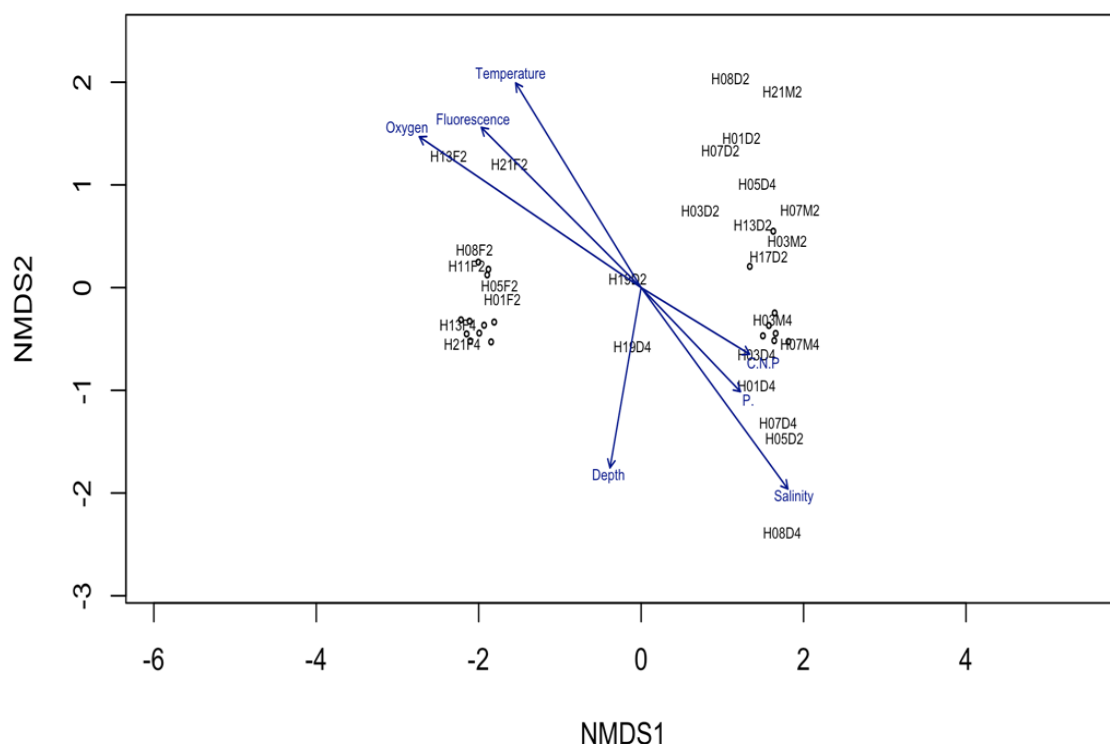


Figure 4: Non-metric multidimensional scaling (NMDS) ordination of marine bacterial and archaeal community in PEI waters. Vectors shown on the ordination plots were significant at * $p < 0.05$ and * $p < 0.001$.**

4.1.2. Chemical characteristics

Fe, Cu, Zn, NH_4 and NO_3 concentrations were consistently below the limit of detection at the majority of sample sites (Table S1) and were consequently excluded from all downstream analysis. Overall, nitrogen levels were very low, with the exception of samples from H3 and H7 (Figure 5). Mean concentrations of Ca, SO_4 , Na and K were significantly higher in H13, H17, H19 and H21 samples (Figure 5, Table S1). All other nutrients did not differ significantly (Kruskal-Wallis, $p > 0.05$) between stations (Figure

5). We also noted wide ranges of concentrations between individual samples (even at the same sampling site (Table S1)).

Furthermore, a depth-specific trend was observed for individual nutrient levels. For instance, the mean Ca levels were significantly higher in majority of Fmax samples compared to those retrieved from OMZ and Deep locations (ANOVA, $F=3.583$, $p<0.05$) (Figure 6a). The Ca concentration reported for H3M, H8D, H13D and H21D were higher than the corresponding Fmax site sample concentration. A depth-specific trend was also observed when comparing P concentrations (ANOVA, $F= 13.23$, $p<0.001$) at the 3 designated depths (Figure 6f). Overall, nutrient levels did not vary significantly between the four water masses (Kruskal-Wallis, $p> 0.05$), with the exception of P where AABW had a higher mean concentration compared to the other three water masses (Kruskal-Wallis, $X^2 = 14.579$, $p<0.01$) (Figure S1).

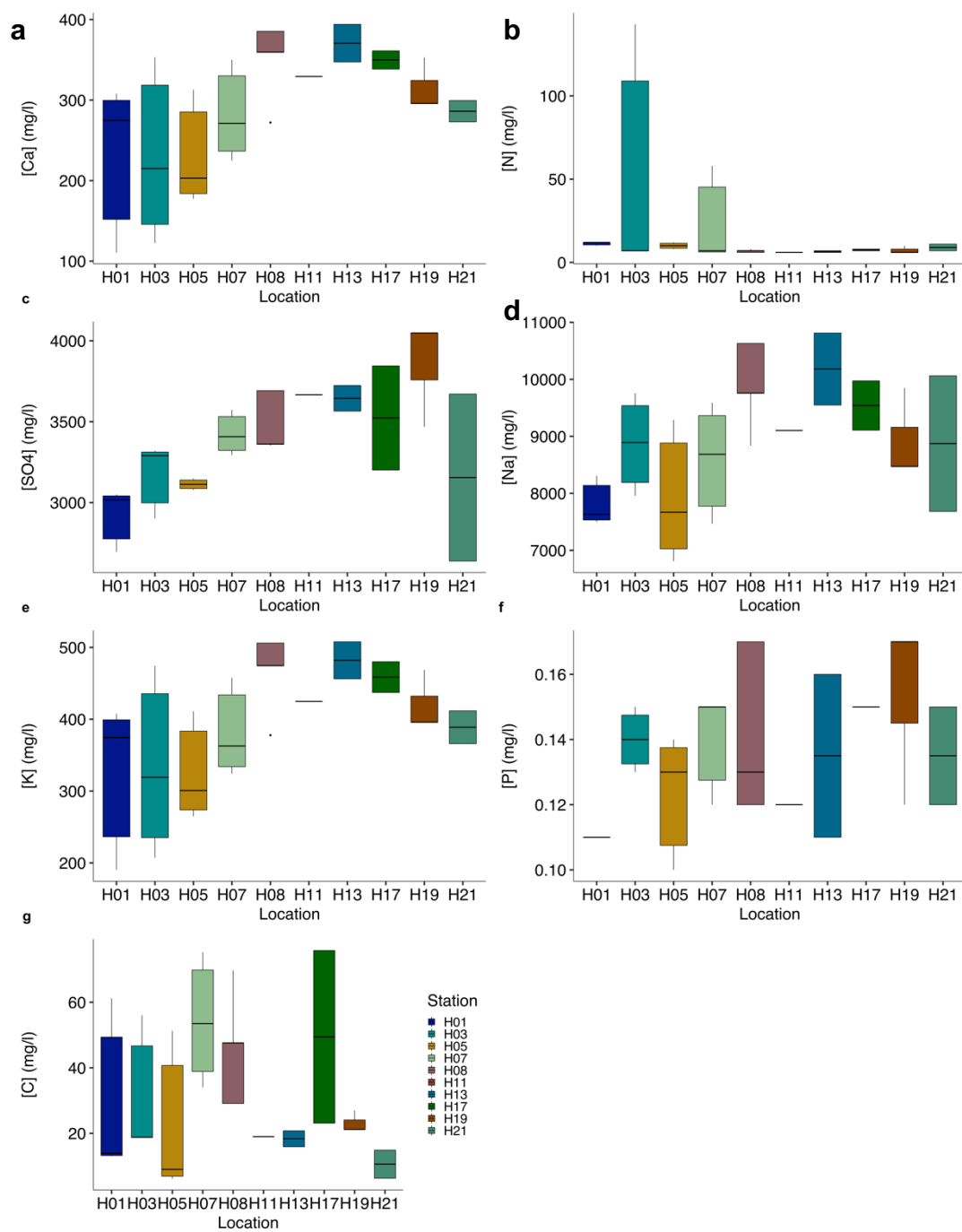


Figure 5: Boxplots showing mean nutrient concentrations at each location in the sample area indicated in mg/L for a) calcium, b) nitrogen, c) sulphate, d) sodium e) potassium, f) and g) carbon.

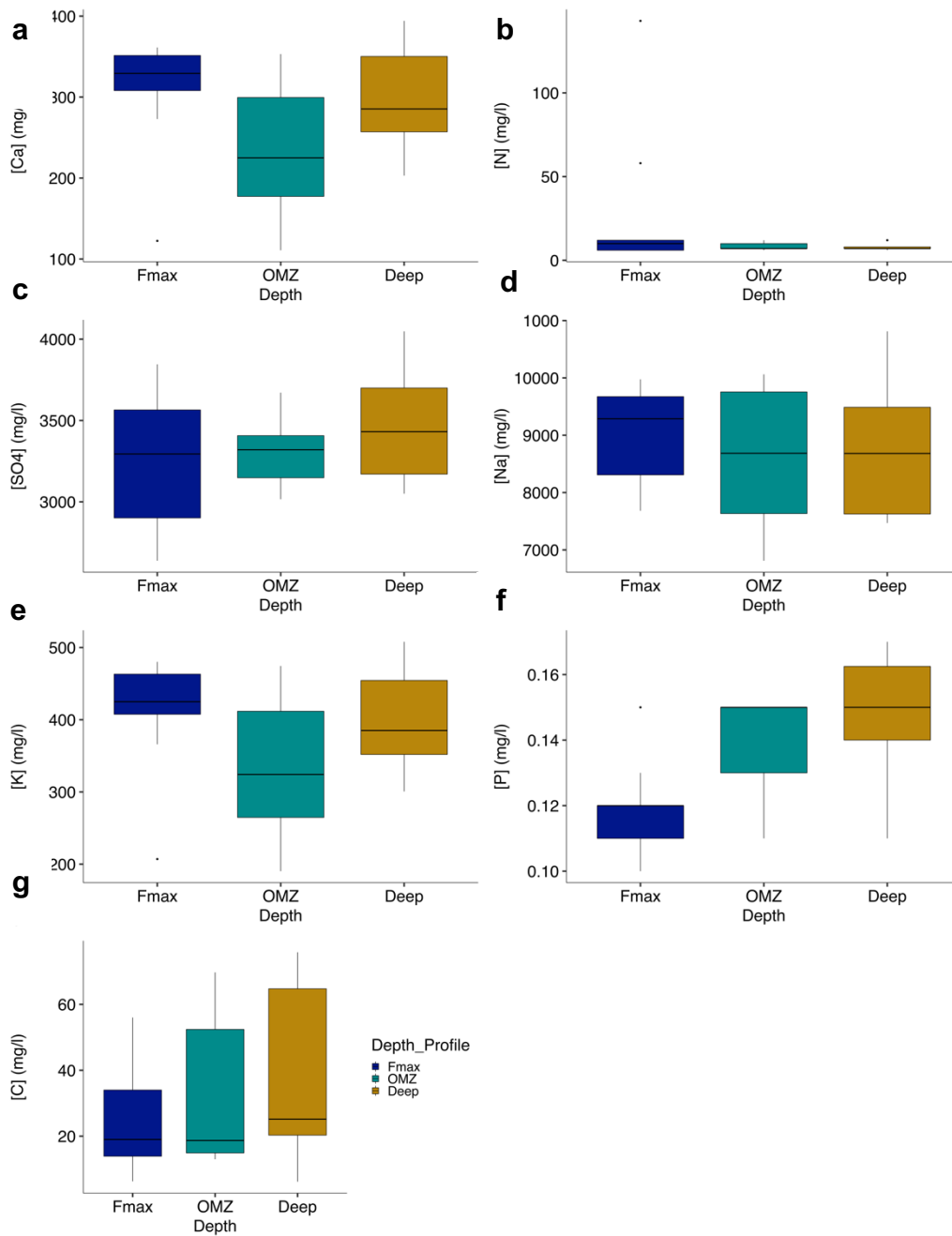


Figure 6: Boxplots showing the mean concentrations (mg/l) of a) calcium, b) sodium, c) potassium, d) carbon, e) nitrogen, f) phosphorus, g) sulphate at the Fmax (dark blue), OMZ (cyan) and Deep (gold) layers. Significant differences are indicated with an *.

4.2. Microbial Community analysis

4.2.1. Bacterial and archaeal composition

Amplicon sequencing of the 16S rRNA gene generated a total of 11 310 322 sequences (ranging from 54 628 to 397 686 reads per sample). After quality trimming, the number of sequences decreased to 10 080 857. Following chimera removal, the number of sequences was reduced to 5 657 529. Rarefaction to 14 230 sequences per sample resulted in the removal of 6 samples (H8M2, H11D2, H11D4, H19F, H21D2, H21D4), leaving a total number of 654 580 sequences in the remaining 46 samples. After quality filtering and rarefaction, based on a 99% taxonomic identity cut-off against the SILVA database, rarefied sequences clustered into 9 791 bacterial and archaeal ASVs. The number of bacterial and archaeal sequences per sample as a function of the number of ASVs is shown Figure 7a.

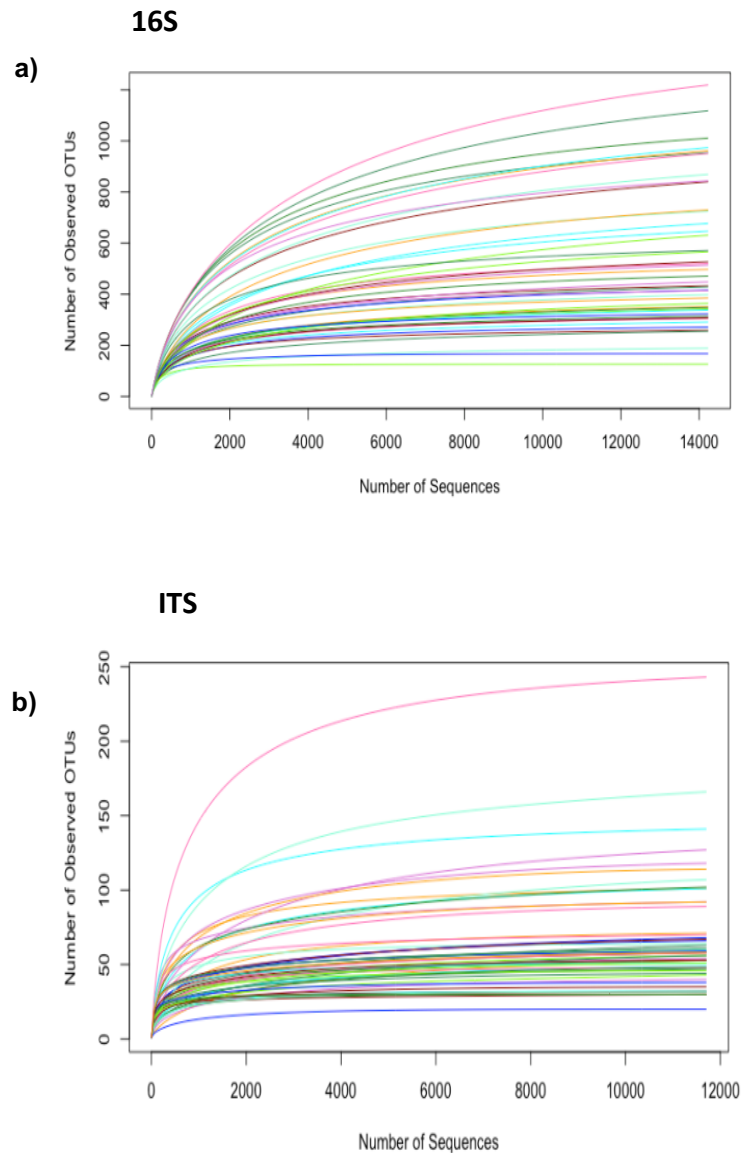


Figure 7: Rarefaction curve plotted as a function of observed OTUs (similarity threshold of 99% and 97% identity for 16S (a) and ITS (b) data, respectively).

The majority of bacterial and archaeal ASVs were taxonomically assigned with only 0.02% being unassigned sequences. Proteobacteria (39.94%) and Bacteroidetes (12.27%) were the dominant bacterial phyla identified across all samples (Figure 8a). Gammaproteobacteria (mostly order Thiomicrospirales), followed by Alphaproteobacteria (mostly SAR11 clade) were particularly common, being dominant

in samples recovered from most stations and depths. Archaea constituted 23.02% of 16S rRNA gene sequences with members of three archaeal phyla identified:

Thaumarchaeota (12.27%), Euryarchaeota (10.57%) and Nanoarchaeota (0.18%). Bacterioplankton community composition changed with depth profile. Phyla such as Cyanobacteria and Bacteroidetes were present at relatively higher abundances in the Fmax group, while Firmicutes and Thaumarchaeota increased in relative abundance down the water column. Taxa also differed in relative abundances depending on the specific water masses (Figure 8b) as well as between lifestyle (that is, either particle-associated or free-living bacteria). Verrucomicrobia and Cyanobacteria existed almost exclusively as particle-associated taxa (Figure 8) and were also more prevalent in PSUW in comparison to the other water masses.

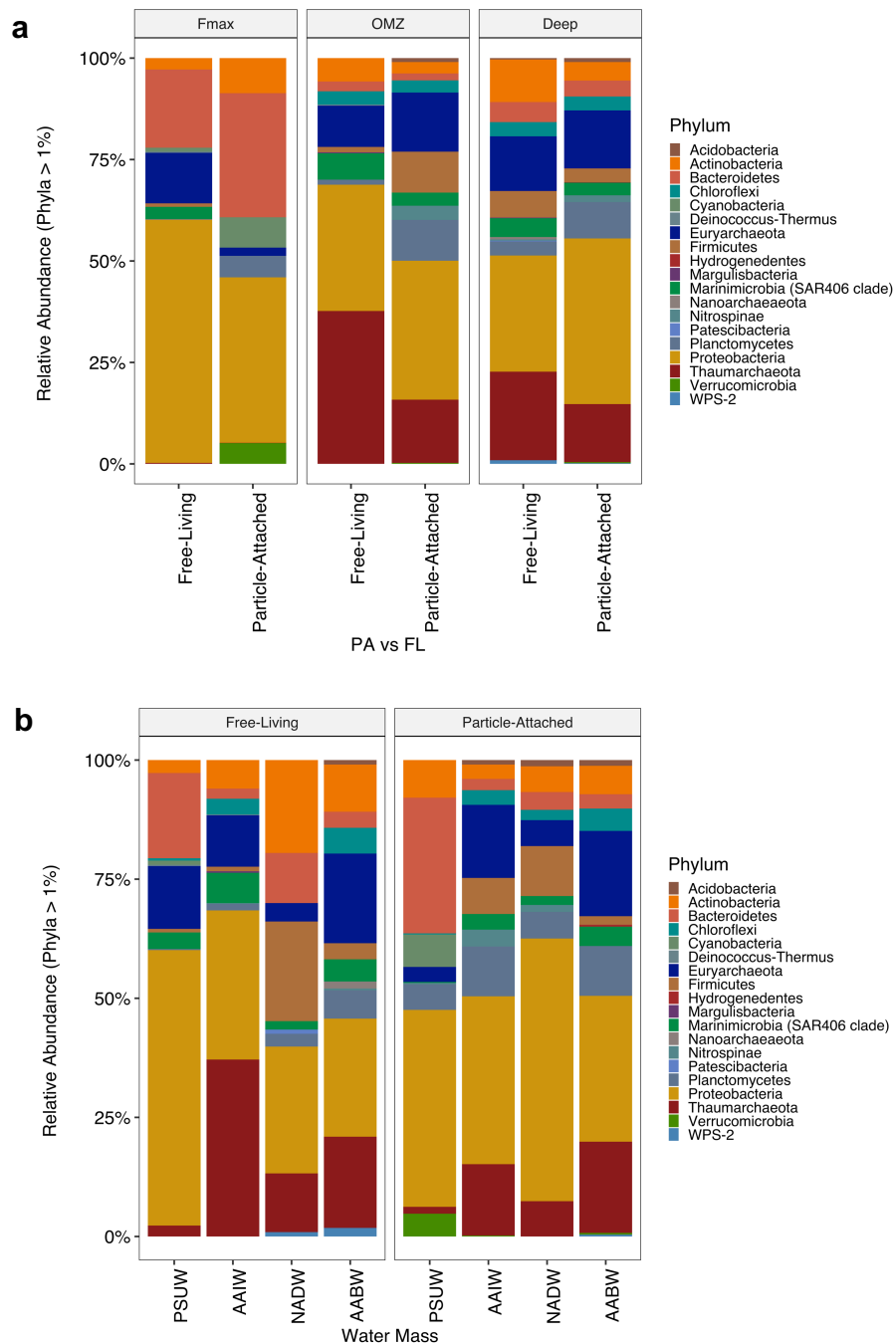


Figure 8: Bar graph showing the relative abundance of FL and PA bacterial and archaeal phyla across samples based on 16S rRNA amplicon sequencing between a) the three depths and b) the four different water masses as. This graph includes phyla that exist at a relative abundance of greater than 1%.

4.2.2. Bacterial and archaeal diversity

Overall, no disparity was observed between individual samples within the bacterioplankton community (Table S2). However, alpha diversity was significantly higher for bacterial communities in the Deep and OMZ depths compared to samples from the Fmax (Kruskal-Wallis, $p < 0.001$). When analysing the pairwise differences between bacterioplankton alpha diversity, significant differences were found between the Fmax and Deep samples (pairwise Kruskal-Wallis, $p < 0.001$) as well as between Fmax and OMZ samples (pairwise Kruskal-Wallis, $p < 0.001$), but not between Deep and OMZ samples (pairwise Kruskal-Wallis, $p = 0.7671703$) (Figure 9a). Particle attached (PA) bacterioplankton demonstrated a significantly higher alpha diversity than their free-living (FL) counterparts in the Deep (Wilcoxon, $p < 0.05$) and Fmax (Wilcoxon, $p < 0.05$) communities. However, no significant diversity differences were observed between PA and FL groups in the OMZ (Wilcoxon, $p = 0.082$) (Figure 9a). Water masses also revealed significantly different alpha diversity (Kruskal-Wallis, $p < 0.01$) between PA and FL, with the AABW and AAIW exhibiting higher diversity, compared to the other two water masses (Figure 9b).

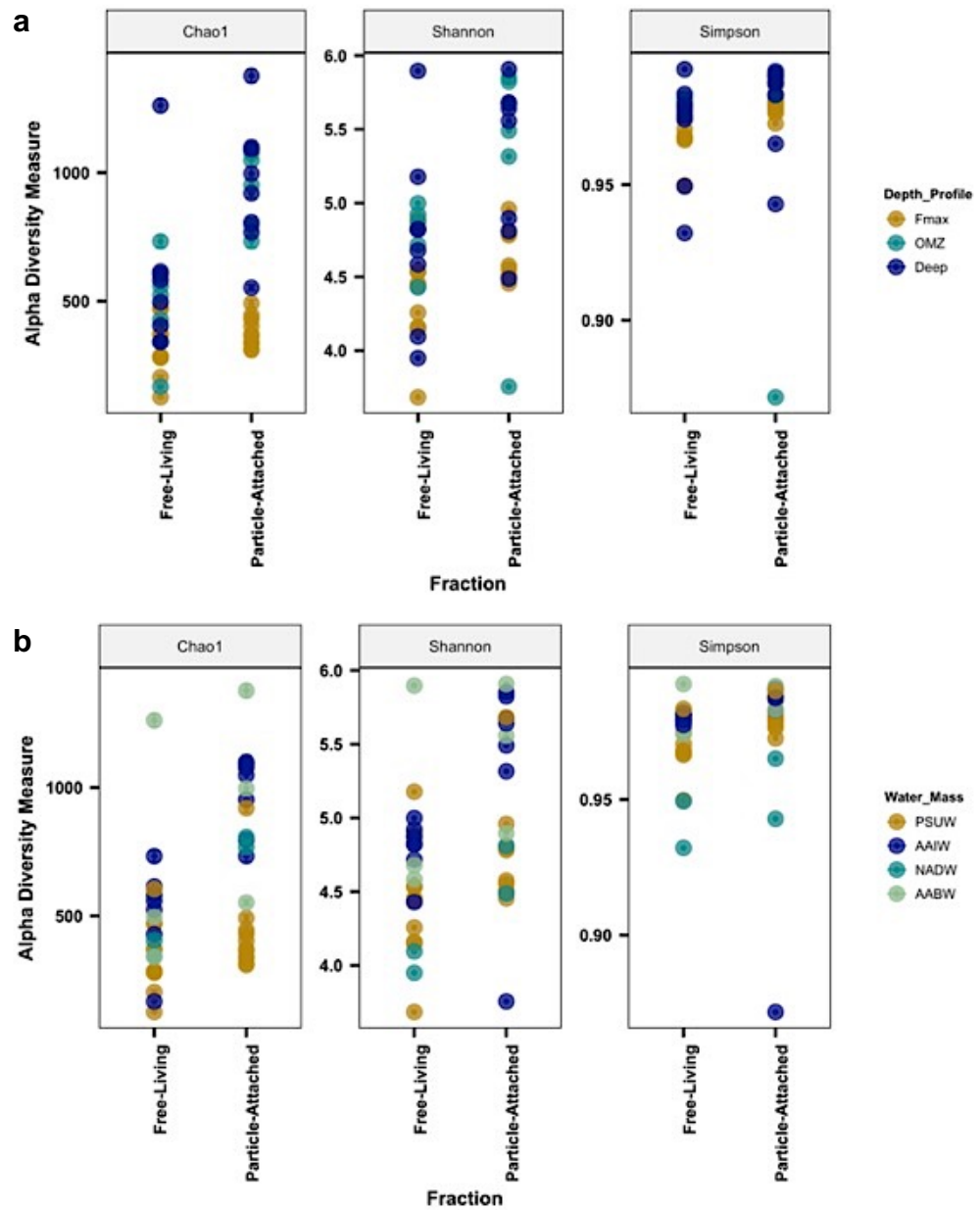


Figure 9: Alpha diversity measures for bacteria and archaea between PA and FL communities at (a) 3 depth profiles and (b) 4 water masses which are indicated by the colours in the legend. Individual points are representative of samples.

Deep water bacterioplankton communities had the highest number of unique ASVs, followed by OMZ and Fmax communities. The community structure was significantly different (PERMANOVA, p -value < 0.01) with a clear clustering of samples by depth (Figure 11a). The associated beta-dispersal was also statistically significant (beta disperse, p -value < 0.01), displaying a large degree of within-group variation (Figure S2). Furthermore, OMZ and Deep communities harboured more shared ASVs between the samples than found between Fmax and either of the other two sample depths (Figure 10a).

Water masses also harboured distinct bacterial and archaeal taxa. AAIW comprised a higher proportion of unique ASVs compared to samples from the other three water masses (Figure 10c). The lowest number of shared ASVs, between any two water masses, was observed between NADW and AABW. The highest proportion of shared ASVs was found between AAIW and NADW.

Bacterial and archaeal communities were also structured by water masses (PERMANOVA, p -value < 0.01; beta disperse, p -value > 0.05) (Figure 11a; Figure S3). The PA and FL bacteria shared less than 18% of the total number of ASVs (1 730 ASVs) (Figure 10e). Beta diversity also varied significantly between the FL and PA groups of prokaryotes (PERMANOVA, p -value < 0.05) (Figure 11; Figure S4). Additionally, nMDS revealed that in surface waters (Fmax and PSUW), PA and FL communities clustered separately. Further down the water column, PA and FL communities were indistinguishable from each other and within groups.

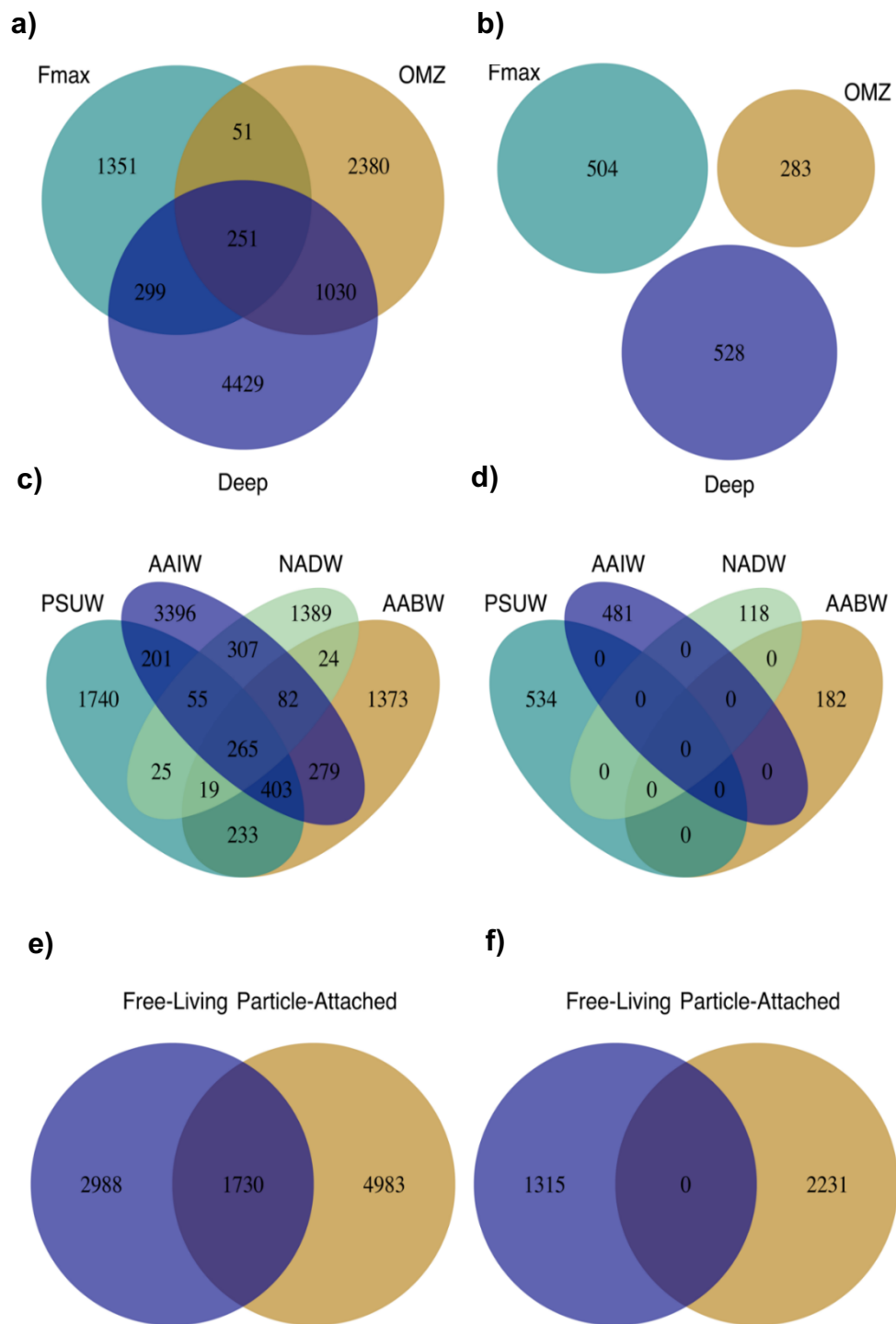


Figure 10: Venn diagrams showing the number of shared OTUs between depth profiles (a & b), water masses (c & d) and lifestyle (e & f) and for bacteria and archaea (a, c & e) and fungi (b, d & f).

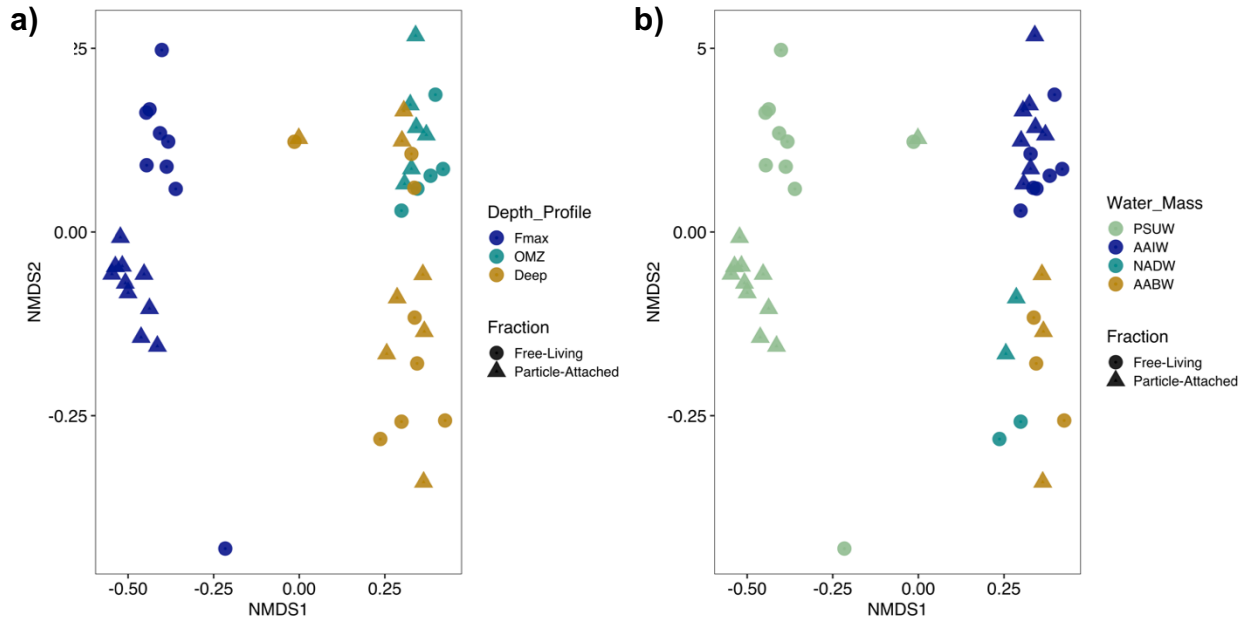


Figure 11: Non-metric multi-dimensional scaling (NMDS) ordination plots of bacterioplankton and mycoplankton communities constructed using Bray-Curtis dissimilarity. The colours in the legends represent (a) depth profiles and (b) water masses in the left and right panels respectively.

4.2.3. Fungal ITS composition

The ITS dataset had a total of 2 838 079 raw sequences (ranging from 14 323 to 123 199 reads per sample). Subsequent to quality filtering, the number of sequences was reduced to 2 324 716, and decreased to 2 286 677 after the removal of chimeras. These data were rarefied to 11 705, the lowest number of sequences per sample. The resulting number of ASVs detected, at a similarity threshold of 97% against the UNITE database, was 3 546. The number of fungal sequences per sample as a function of the number of ASVs is shown Figure 7b.

Approximately 20.84% of ASVs were identified as fungi after classification in QIIME against the UNITE database. However, the majority of sequences were accurately assigned kingdom level. Roughly the same proportion (22.46%) of reads were unassigned at PHYLUM level. More than half of the classified fungal ASVs belonged to the two most dominant phyla Basidiomycota (30.77%) and Ascomycota (25.93%) (Figure 12a). Mortierellomycota and Mucomuromycota were also detected at much lower relative abundances (<1%).

Ascomycota were the dominant fungal phyla in Deep and OMZ communities, while Basidiomycota were the most prevalent taxa in surface waters (Figure 12a). At the class level, there was a shift in the dominant taxa between depths. Exobasidiomycota was predominant at the surface while Dothideomycetes made up majority of the Basidiomycota phylum in the OMZ. There was a disproportionately large number of unassigned taxa in the OMZ, compared to the other two depths. The distribution of taxa also varied between free-living and particle associated fungi (Figure 12a) such that more unknown taxa were identified in the PA than in the FL. Some classes were differentially represented in different groups. For example, Tremellomycetes were found mostly in FL communities in the bathypelagic layer.

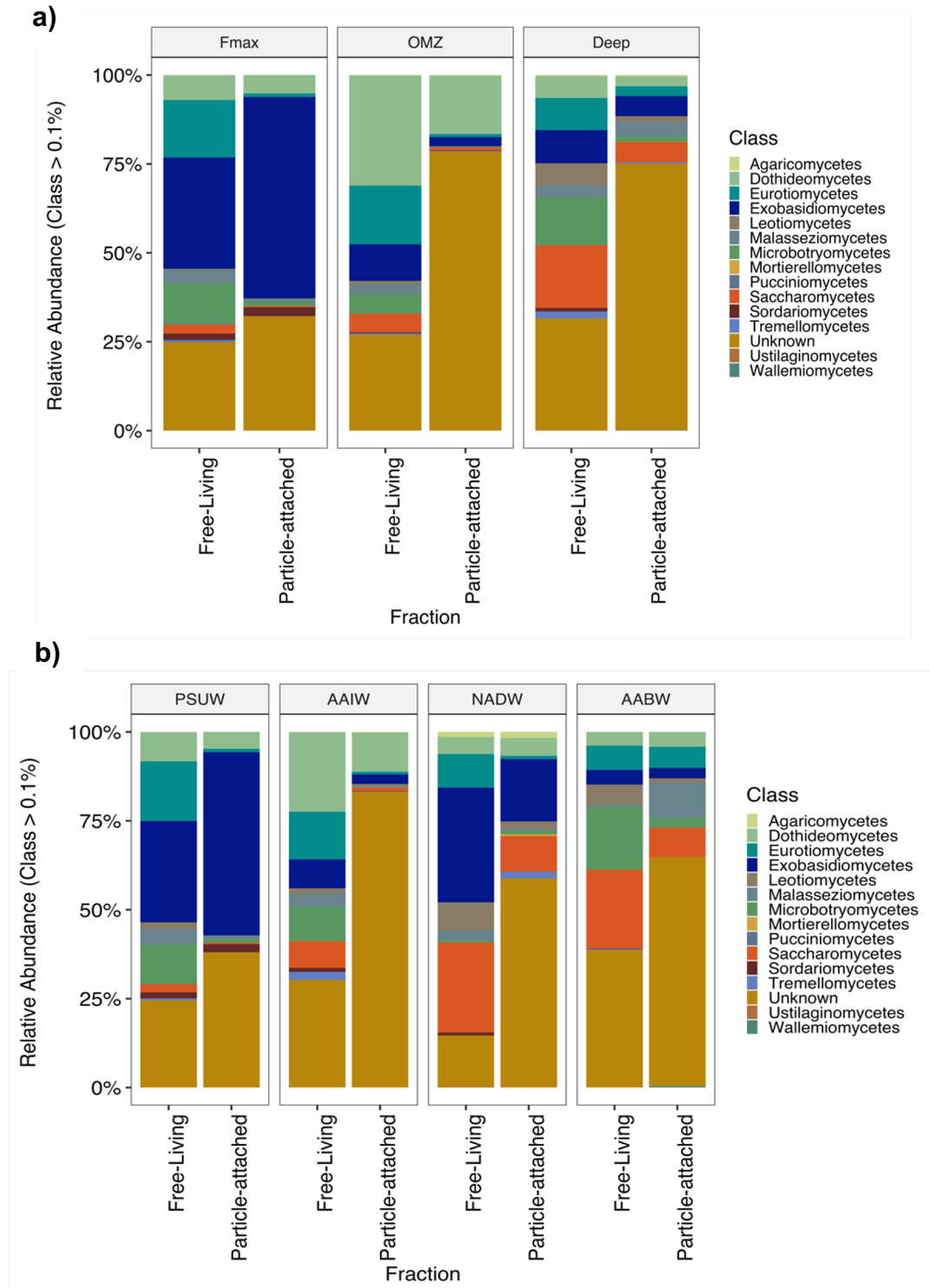


Figure 12: Bar graph showing the relative abundance of fungal classes across samples based on ITS 1-4 region amplicon sequencing between a) the three depth profiles and b) the four different water masses and between the PA and FL fungi in each group. This graph includes only classes that occur at a relative abundance of greater than 0.1%.

4.2.4. Fungal diversity

Fungal communities showed a similar trend to bacterial communities, with significant differences detected between the Deep and Fmax communities (Pairwise Kruskal-Wallis, $p < 0.001$) as well as the Deep and OMZ communities (Pairwise Kruskal-Wallis, $p < 0.05$) (Figure 13a). Additionally, PA fractions exhibited an overall higher diversity than FL fractions (Pairwise Kruskal-Wallis, $p < 0.05$) with deep communities showing a significantly higher alpha diversity than FL fungi in the deep (Wilcoxon, $p < 0.01$) and Fmax (Wilcoxon, $p < 0.01$) groups (Figure 13). In contrast, the OMZ fungal community diversity showed no significant difference between the two size fractions ($p = 0.31$). Significant differences in Shannon alpha diversity (Kruskal-Wallis, $p < 0.05$) were detected between the different water masses (Figure 13b). NADW and AABW exhibited higher diversity levels compared to the other water masses.

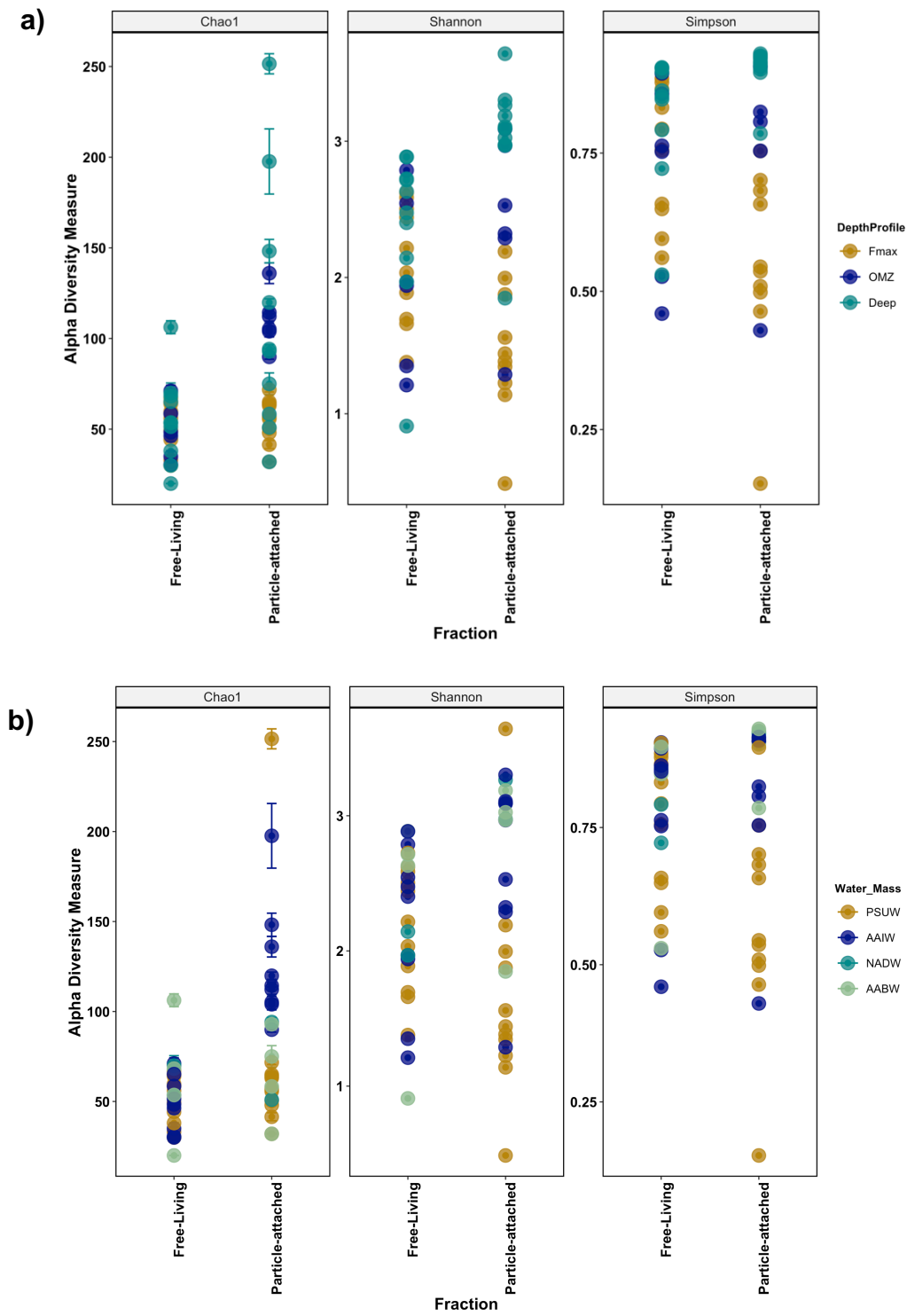


Figure 13: Alpha diversity measures for fungi across the (a) 3 depth profiles and (b) 4 water masses as well as between PA and FL communities. Individual points are representative of samples and the depths and water masses are indicated by the colours in the legend.

Fungal assemblages displayed unique communities in each discrete depth profile, with no common taxa prevalent (Figure 10b). A similar trend was observed between the four water masses and FL or PA fraction with no shared taxa were observed (Figure 10d). Non-metric dimensional scaling (nMDS) showed no significant difference in the structure of fungal communities based on the influence of FL and PA taxa (PERMANOVA, pseudo F= 1.0, p= 1.0), water mass (PERMANOVA, pseudo F= 1.0, p = 1.0) or depth (PERMANOVA, pseudo F= 1.0, p= 1.0) (Figure S5).

4.3. Enzymatic Activity Analysis

The extracellular enzymes β -glucosidase and β -xylosidase were detected in all samples. The AP activities of samples H08, H11 and H13 were higher compared to the other stations where the activity was negligible (Figure 14). The activity of NAG in samples H11, H13, H17, H19 and H21 was much higher compared to all other stations where values approached zero. Statistical analysis showed no significant difference in the activities of BG and BX between samples (Kruskal-Wallis, p-value> 0.05). This was also true between the three depths for AP, NAG and BG extracellular activities ((Kruskal-Wallis, p-value> 0.05) (Figure 15). The mean BX activity was significantly different between depths (Kruskal-Wallis, p-value< 0.05), with the highest levels recorded for surface waters (Fmax), followed by OMZ and then lowest activities in the deep samples. Water masses showed no significant influence (Kruskal-Wallis, p-value>0.05) on any of the extracellular enzyme activities evaluated (Figure.S6).

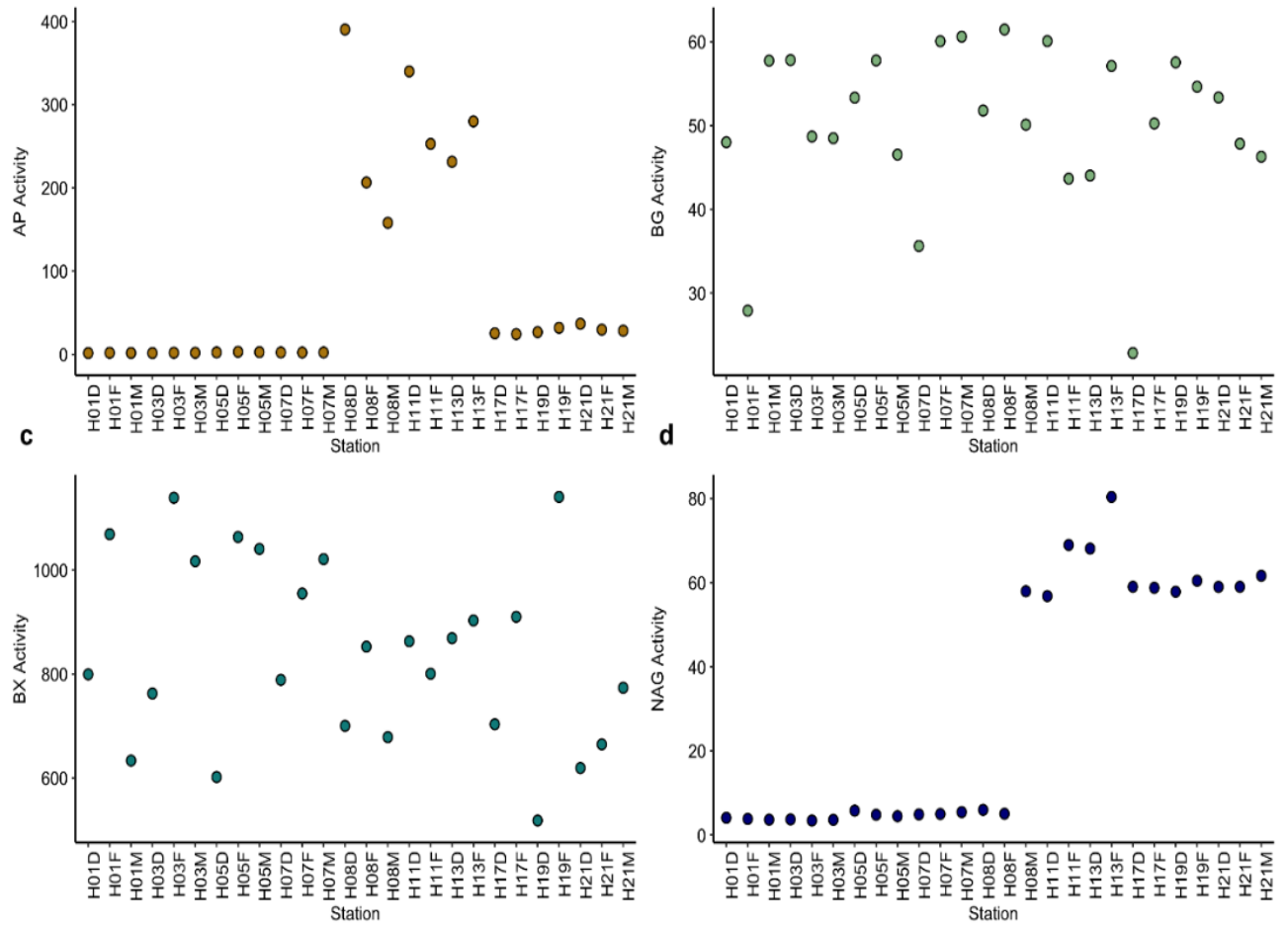


Figure 14: Extracellular activities for four enzymes involved in microbial nutrient acquisition for each sample (represented by individual dots).

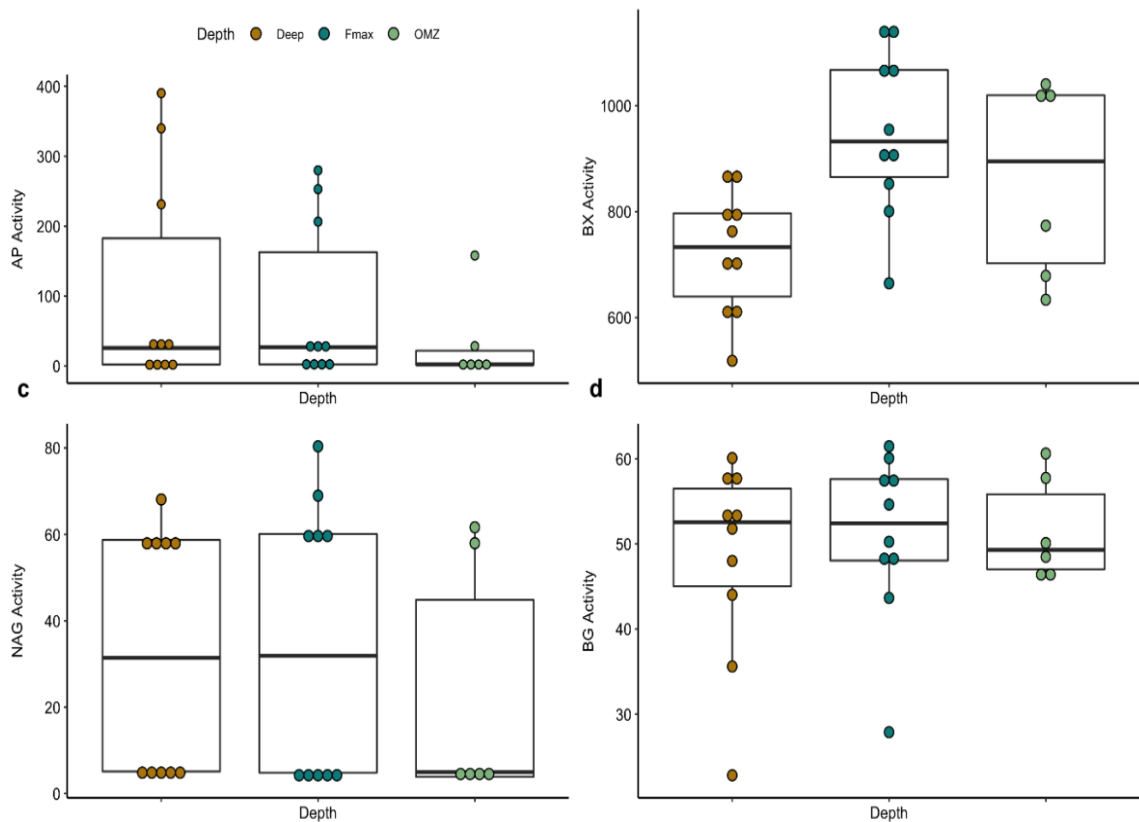


Figure 15: Extracellular activities for four enzymes involved in microbial nutrient acquisition for each sampling depth. Whiskers on each boxplot indicate the minimum and maximum values and solid black lines indicate the median value. Top and bottom vertical box limits show the 25th and 75th percentile, respectively.

4.4. Hierarchical Clustering Analyses

Samples clustered distinctly by depth (PERMANOVA, $P < 0.001$), where OMZ and Deep samples formed a single group separate from Fmax samples (cluster D). There was higher resolution between the different water masses (PERMANOVA, $p < 0.001$) with PSUW samples (including H19D2 and 4) all clustering together and samples from AABW and AAIW forming separate clusters. Samples from NADW waters were

scattered throughout cluster E (Figure 16). While a demarcation can be seen within cluster E, between the two size fractions (FL and PA), there was no significant clustering with regard to FL or PA communities (PERMANOVA, $p=0.15$).

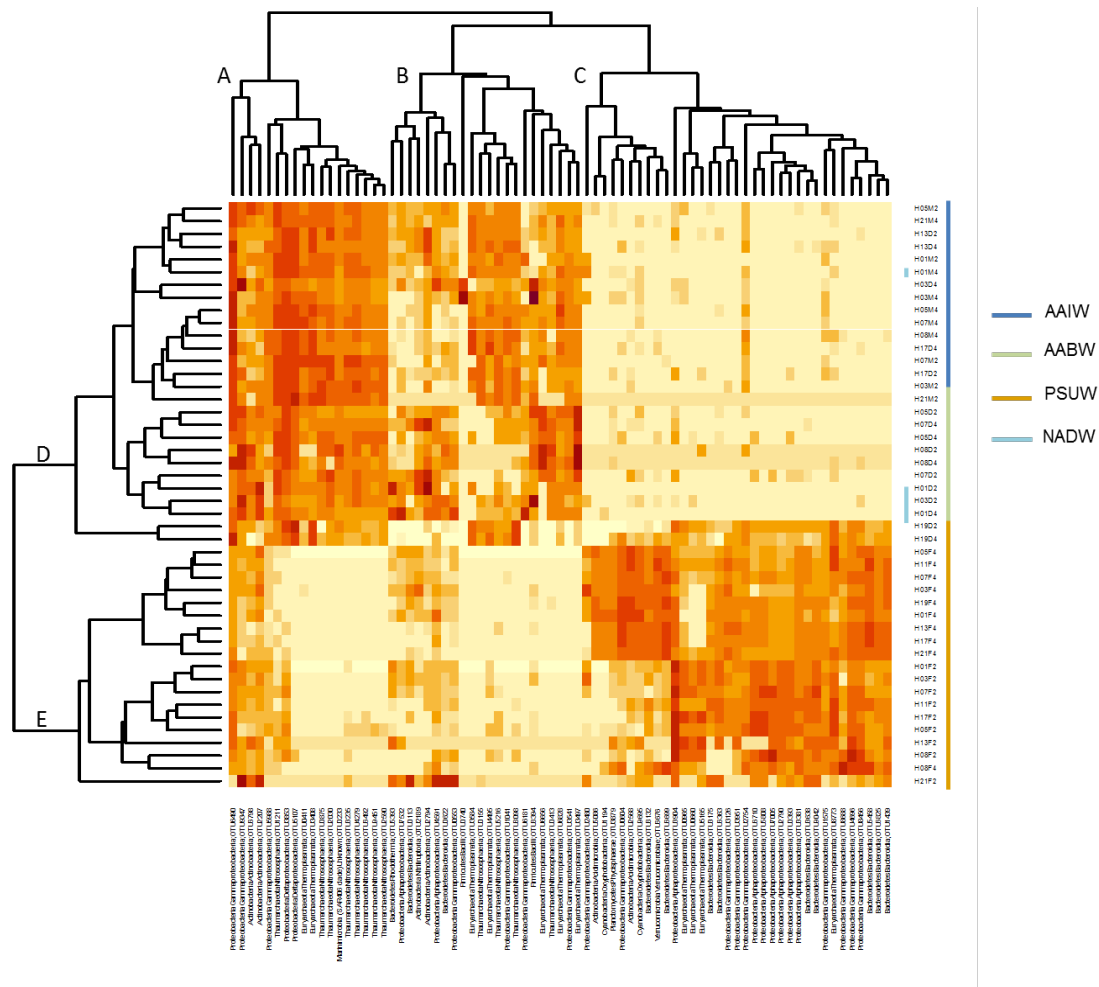


Figure 16: Heatmap with bidirectional clustering of bacterial OTUs and samples. Colours indicate clustering of samples collected from the four water masses.

5. Discussion

This study provides clear compositional, structural and functional trends of PEI microbiomes throughout the water column. Similar to previous studies, we show that depth, water mass endemicity and physicochemical variables are the main determinants of PEI community structure (Phoma and Makhalanyane, submitted). We highlight, for the first time, the importance of particle attachment and resource heterogeneity in these waters. Surprisingly, extracellular enzymatic activities for C, N and P acquisition did not exhibit any clear trends. These data suggest a disconnect between community composition and potential functionality.

5.1. Microbial communities and trends

At global scales, members of the phylum Proteobacteria (mostly affiliated to alpha-proteobacterial SAR11 clade) dominate marine ecosystems, followed by bacteria affiliated to Bacteroidetes and Cyanobacteria (Rusch *et al.*, 2007; Sunagawa *et al.*, 2015). The results of this regional study were broadly concordant with these studies revealing that Proteobacteria dominated bacterioplankton communities. In conflict with global-scale analyses, Gammaproteobacteria (20.31%) were overrepresented in this PEI dataset, compared to the Alphaproteobacteria (13.66%). However, this finding is similar to previous studies conducted in the Indian sector of the SO (Gupta *et al.*, 2015; Phoma *et al.*, 2018). Gammaproteobacteria appear to dominate in waters with higher levels of dissolved organic matter (DOM) (Landa *et al.*, 2013; Amaral *et al.*, 2016).

Members of the Gammaproteobacteria are also known to lead opportunistic lifestyles, allowing them to utilize a wide range of substrates and thus thrive in copiotrophic

environments such as the PEIs (Nikrad *et al.*, 2014; Amaral *et al.*, 2016; Liu and Liu 2020). Differences in PEI community composition are possibly due to physiological differences among taxa. These may exhibit clear differences in pathways related to the uptake of DOM, resulting in niche specificity due to differences in environmental conditions.

Compared to viruses, archaea and bacteria, a large proportion of fungal taxa comprise of unknown lineages (Picard 2017). The high proportion of unknown taxa is primarily due to database deficiencies characterized by a lack of representative fungal sequences (Ahrendt *et al.*, 2018). This deficit is even more pronounced for marine fungi, which despite recent developments in next generation sequencing approaches, remain understudied (Wang *et al.*, 2012; Grossart *et al.*, 2016; Hassett *et al.*, 2020). Previous global-scale analyses showed that Ascomycota and Basidiomycota dominate pelagic fungal communities (Tisthammer *et al.*, 2016; Morales *et al.*, 2019). These results are broadly consistent with our findings. However, Basidiomycota were present at higher relative abundances compared to Ascomycota in this study. In the South China Sea, 3 coastal sites were evaluated for fungal diversity and showed shifts in the dominance of either Basidiomycota or Ascomycota depending on their location (Wang *et al.*, 2019b). Basidiomycota in the PEIs were also shown to be positively correlated with BX extracellular enzyme activity. The dominance of Ascomycota and Basidiomycota coupled with putative enzymatic activity suggests that they may play an important role in OM cycling along coastal waters, similar to previous findings (Bochdansky *et al.*, 2017; Cunliffe *et al.*, 2017). These findings highlight that observations at global scales, resulting from pooling datasets, may differ starkly from localized sampling efforts and that fungal communities may be impacted by

environmental gradients globally and regionally (Tisthammer *et al.*, 2016; Lee *et al.*, 2019; Wang *et al.*, 2019b). Our study provides one of the first datasets on fungal communities and further studies are required to ascertain whether these findings are characteristic of this region of the SO.

While there are some studies on SO microbial communities, very few have focused on PEI waters. One study documented shifts in bacterioplankton community composition as a result of seasonal patterns, ocean variability and water mass endemism over several years concurrent with the present study (Lamont *et al.*, 2019; Venkatachalam *et al.*, 2019). Other findings (e.g. permutational analysis; Figure 4; and nMDS, Figure 11) of our study also highlight the importance of depth, lifestyle (PA or FL) and physicochemical variables (temperature, fluorescence, salinity, nutrients and oxygen) as key determinants of PEI community structure.

5.2. Extracellular enzymatic activities of PEI microbiomes

Overall, extracellular enzyme activities in the SO microbial community were highly variable among samples. This finding is similar to another study by Hoarfrost and Arnosti (2017a) who showed that potential enzymatic activity in the ocean varied according to spatial and depth-driven constraints on heterotrophic hydrolytic capacity.

In this study, one of these constraints could be lower substrate availability at some sampling locations which may result in attenuated enzyme activities (Pold *et al.*, 2017; Liu *et al.*, 2018; Noll *et al.*, 2019). Previous studies suggest that the dominant environmental conditions in this region may be energetically unfavourable due to low temperatures and highly variable substrate availability (Busto and Perez-Mateos 1995;

Cunha *et al.*, 2010). Several other factors known to inhibit enzyme- substrate associations may also explain the low activities. For example, complexation of substrates with colloidal organic matter and detritus may impede the association of enzymes with their substrates (Busto and Perez-Mateos 1995). Furthermore environmental factors such as low temperatures may constrain functional processes in the Sub-Antarctic bathypelagic layer resulting in generally reduced extracellular enzymatic activities (Pomeroy and Wiebe 2001). In addition, it has also been shown that in cold environments, enzymes are less likely to be degraded and thus can act on OM far away from the site of production (Baltar *et al.*, 2016).

5.2.1. Carbon acquisition (BG and BX)

β -glucosidases and β -Xylosidases provide labile C and energy sources for microbial communities. These enzymes hydrolyze the beta bonds of glycol- and xylo-oligosaccharides, respectively, releasing monosaccharides (Galbe and Zacchi 2002; Luo *et al.*, 2017b; Thomas *et al.*, 2017; Shen *et al.*, 2019). These enzymes were active at all stations within this sampling area albeit inconsistently.

β -glucosidase (BG) activity was previously linked to heterotrophic microorganisms (Cunha *et al.*, 2010) and its activity has been related to both bacteria and fungi (Knight and Dick 2004; Gudmundsson *et al.*, 2016; Deng *et al.*, 2020). Heterotrophic bacteria (including Bacteroidetes and Proteobacteria) and fungi (Basidiomycota) have previously been recorded as producers of BG (Eichlerová *et al.*, 2015; Nicoletti and Andolfi 2018; Unfried *et al.*, 2018). In this PEI dataset, these taxa have been found throughout the water column at varying abundances, thus the presence of this enzyme at variable concentrations throughout the water column was expected. This signifies

that they may contribute to the production and thus activity of BG in Fmax, OMZ and Deep water.

In contrast, BX activity was significantly higher in surface waters demonstrating that the majority of xylan hydrolysis occurs in the epipelagic layer. BX activity was positively correlated with both Basidiomycota and Cyanobacteria, suggesting that fungi and bacteria cooperatively drive the production and consumption of BX. Filamentous fungi are potent producers of xylanolytic enzymes (Raghukumar *et al.*, 2004; Knob *et al.*, 2010; Huang 2019). The results from our study suggests that they may contribute considerably to the hydrolysis of xylan in surface waters. Further, these results suggest that xylan (derived principally from plant hemicellulases) levels in the upper region of the water column may be higher than previously suggested. Alternatively, these results suggest that BX activity may be energetically more favourable in surface marine microbiomes than BG.

BG and BX have diverse substrate ranges and function differentially based on their availability (Jordan *et al.*, 2013; Zhang *et al.*, 2017). It has been shown that complexity of some carbon source (such as lignocellulose) may require the action of complementary enzymes in a temporally and spatially dynamic manner (Hasunuma *et al.*, 2013; Cragg *et al.*, 2015; Cortes-Tolalpa *et al.*, 2017). Here, heterotrophic bacteria or fungi may be acting synergistically to break down organic matter throughout the water column (Jiménez- Fernández *et al.*, 2013). Additionally, it has been shown that the complexity of DOM can affect the way in which members of a community interact (Deng 2016) i.e. the more complex the substrate, the less antagonistic interactions occur (Deng and Wang 2017). Thus, dissimilarity in the type of available organic

matter is driving which enzymes and species are more metabolically active at any given time or sampling location. A previous study by Bauer *et al.*, (2006) used a whole genome shotgun sequencing approach to compare the gene family profiles of marine heterotrophic Bacteroidetes. The authors suggested that different members of marine bacterioplankton may consume specific substrates at different niches within an environment (Bauer *et al.*, 2006). We extend this idea and propose the inclusion of different members of the microbiome, where fungal and bacterioplankton groups may occupy distinct niches in the marine environment depending on the types of available substrates.

5.2.2. *Phosphorus acquisition*

Alkaline phosphatase (AP) is typically considered as a proxy for organic phosphorus mineralization potential and P availability (Krämer and Green 2000). This enzyme plays a vital role in hydrolysing phosphomonoesters and phosphodiester to release assimilable inorganic P for microorganisms (Luo *et al.*, 2017b). The activity of AP was higher at stations H08, H11 and H13, as well as in several deep samples. Extracellular phosphatases, such as AP, are upregulated in response to changes in external inorganic phosphorus concentrations and intracellular phosphorus demand (Hoppe 2003). However, the results of chemical analysis are not consistent with P limitation at these or other sampling locations. We also found no significant difference in activity between the three depths at the sampled sites. In fact, AP activities were high in deeper samples, where P levels were also elevated. Other studies support these observations (Hong *et al.*, 1998; Tamburini *et al.*, 2002; Baltar *et al.*, 2016) and it has been proposed that P-starvation may not be the only reason for higher AP activity.

This enhanced activity may be due to secondary roles of AP in the marine environment (Colman *et al.*, 2005; Karl and Björkman 2015).

Higher AP activity, at specific locations, may be linked to the increased availability of trace elements rather than the 'direct' effect of P limitation. Dominant marine bacteria routinely express APs, which hydrolyze phosphate under P stress conditions (Karl and Björkman 2015). PhoX, PhoA and PhoD constitute the dominant phosphate monoesterases in marine bacteria (Luo *et al.*, 2009), and their activities depend on the availability of obligate metal cofactors including Zn, Mg, Ca and Fe. Browning *et al.*, (2017) hypothesized that there would be a strong reliance on APs for phosphorus acquisition under Fe-enriched conditions. They further proposed that differences in co-factor requirements would provide some advantage for APases with alternative co-factor requirements. Here, AP was positively correlated with Ca (Spearman's, $p < 0.01$). Additionally, samples with higher AP activity also exhibit increased concentrations of Ca. This suggests that *phoX*, which relies on Ca as a metal co-factor, may be of some importance in this environment. However, *phoX* has mainly been shown to function in P-limited environments (Sebastian and Ammerman 2009; Sebastian and Ammerman 2011). As such, the observed differences in AP activity in the PEI may be explained by differences in the availability of these cofactors (found at very low concentrations in some samples) and the specific nutrient cofactor requirements of the microbial community at each location.

5.2.3. Nitrogen acquisition

N-acetyl glucosaminidase (NAG) is essential for nitrogen cycling due to its role in catalysing the terminal reaction in cellulose degradation of chitin and N-acetylglucosamine substrates (Shi *et al.*, 2012; Sistla and Schimel 2013). Thus NAG is used as the indicator for N acquisition (Luo *et al.*, 2017b). Fluxes of N in marine DOM are thought to be an important contributor to bacterial growth, carbon and nitrogen cycling (Riemann and Azam 2002). PEI locations, close to the coastline, exhibited higher NAG activity but low NH_3 and NH_4 concentrations. The trend observed near coastal sites is consistent with results from a previous study showing high NAG concentrations at near-shore localities (Li *et al.*, 2019b). However, possibly due to high riverine load and pollution, the N levels in the East China Sea were much higher compared to the pristine sub-Antarctic waters (Li *et al.*, 2019b). In fact, a decrease in NAG activity, under high N availability, has previously been recorded in wetland soils (Min *et al.*, 2011). The negative correlation of N to NAG activity may indicate enzyme activity is dependent on the availability of N in this environment. More specifically, in high N locations, there is a lower demand for N sources and thus it may be energetically unfeasible to expend energy via the production of an N-acquisition extracellular enzymes. In the case of the PEIs, our findings suggest that the higher activity of NAG may be driven by high demand for N source in an N-limited in the environment.

5.3. The influence of physicochemical variables

Environmental parameters are important factors which shape microbial community structure (Pomeroy and Wiebe 2001; Fuhrman *et al.*, 2015; Zhang *et al.*, 2018b). In the PEIs, we show that temperature, salinity, oxygen and fluorescence were major drivers of community structure. This is consistent with previous studies that have shown that the differences in community structure and composition are primarily due to temperature and other related abiotic features (Baldwin *et al.*, 2005; Agogu   *et al.*, 2011; Ngugi and Stingl 2012; Sunagawa *et al.*, 2015; Phoma *et al.*, 2018).

Concentrations of K, Ca, SO₄ and Na were significantly higher at stations closer to the two islands. PEI soils have higher nutrient concentrations compared to the surrounding oligotrophic waters (Duncombe Rae 1989; Perissinotto *et al.*, 2000). As such, the increased run-off from the land masses could result in the input of nutrients into these waters. This speculation is supported by previous studies which showed that nutrient levels were generally higher in near-shore PEI waters (Perissinotto *et al.*, 2000; Smith and Froneman 2008).

Mean Ca and P concentrations were significantly different among the three depths. Ca was highest at the surface and P was high in deep and Fmax samples relative to the OMZ. Studies have shown that Ca plays an important role as the primary environmental signal in biofilm formation (Chodur *et al.*, 2018), in flagella motility (Wheeler 2017), and is also released into the environment by surface microbes as a photophobic response (Verret *et al.*, 2010; Wheeler 2017). These functions are of particular importance in surface microbial communities and therefor may explain the elevated levels of Ca at the surface. Variable nutrient concentrations observed with

increasing depth may be due to upwelling events which draw cooler, nutrient-rich water to the euphotic zone (Pennington *et al.*, 2006; Mackenzie *et al.*, 2019). These upwelling events may explain the higher P concentrations in deep waters compared to Fmax samples (Figure 6f). However, the majority of P in the water column is likely derived from marine sediments rather than deposits from the islands or atmospheric deposition of P (Tyrrell 1999). These results are consistent with previous patterns observed, where P levels were higher in AABW compared to other water masses. AABW waters were likely to be associated with marine sediments (Blaeser and Ledbetter 1982), lending support to the idea that depth may be a main factor affecting nutrient availability.

5.4 Potential drivers of community structure in the PEI

5.4.1 Nutrient drivers of community structure

Phosphorus was the only significant nutrient driver of bacterial and archaeal community structure (Figure 4) suggesting its central role in SO microbial communities. P plays a fundamental role in cellular energy storage and transformations, membrane structure and genetic exchange (De Duve 1991; Karl and Björkman 2015). Microorganisms have evolved various strategies to cope with changing levels of P in marine environments, highlighting the importance of P availability in driving microbial evolution and niche adaptation (Dyhrman 2007). P deficiency limits microbial communities, as shown in studies conducted in regions such as the Arctic (Elser *et al.*, 2007; Browning *et al.*, 2017). This is unlikely to be the case in the SO, which is P-rich (Smith and Froneman 2008). Significant positive correlations were observed between P concentrations and Chloroflexi,

Thaumarchaeota and Firmicutes. In contrast, P was negatively correlated to Cyanobacteria, Bacteroidetes and Basidiomycota.

The effects of higher P concentrations are likely exacerbated by a lack of Fe availability between the islands. Fe is an important co-factor in the direct acquisition of P in marine environments and may limit P cycling (Wu *et al.*, 2000; Hutchins and Boyd 2016; Browning *et al.*, 2017). In an area subject to increased upwelling events and the deposition of micronutrients via run-off, it might be plausible to expect augmented levels of these required cofactors and thus increased P uptake. Contrary to this, concentrations of Fe and other trace elements in this region were very low (Boyd *et al.*, 2012; Boyd 2019). It can be assumed that the lack of a cofactor result in the inability of microbes effectively utilize any available P in the water, leading to its subsequent accumulation therein.

5.4.2. Depth stratification in the PEIs

Shared bacterial ASVs generally comprised of taxa found to be ubiquitous throughout the water column. These taxa likely represent bacteria with high metabolic plasticity, adapted to different environments. For bacterial communities, we found that the majority of ASVs were specific to designated depth profiles (Figure 10a), suggesting that depth may be a significant driver of community composition and structure. This was supported by the nMDS ordination (Figures 4 and 11) and beta dispersal results (supplementary figures) and was consistent with observations in previous studies (Wilkins *et al.*, 2013a; Sunagawa *et al.*, 2015; Walsh *et al.*, 2016; Phoma *et al.*, 2018).

On the phylum level, Bacteroidetes, Actinobacteria and Proteobacteria were ubiquitous throughout the sample set. However, there were differences in the dispersal of some groups at class level. Among the Actinobacteria, class Actinobacteria were found only in Deep and OMZ samples while Acidimicrobia were found exclusively in surface waters. Additionally, Gammaproteobacteria were persistent throughout the water column and in different water masses while Alphaproteobacteria were mostly restricted to surface (Fmax) samples and even more specifically to PSUW. Other taxa restricted to Fmax samples included Cyanobacteria, Verrucomicrobia and Planctomycetes. Of the Deep and OMZ samples, key taxa included Deltaproteobacteria, Firmicutes, Thaumarchaeota and Marinimicrobia (SAR406).

Surface waters are expected to mainly consist of photoheterotrophic bacteria (Flombaum *et al.*, 2013; Moran 2015). As an example, Cyanobacteria are recognized as the main photoautotrophs in marine environments (Flombaum *et al.*, 2013). Appropriately, Cyanobacteria were almost exclusively detected in Fmax samples (Figure 8a). Further, their presence was negatively correlated with depth. This change in the relative abundances of Cyanobacteria with depth is probably due to the change in light availability along the water column. Surprisingly, we did not find *Prochlorococcus* in our samples, even though they are known to be ubiquitous in marine surface waters (Partensky and Garczarek 2010). *Prochlorococcus* are known to demonstrate clear biogeographic patterns in response to temperature (Johnson *et al.*, 2006; Flombaum *et al.*, 2013) which may explain their low abundances in these sub Antarctic waters. Additionally, it has been demonstrated that the small cells of *Prochlorococcus* may be an advantage in low nutrient environments (Partensky and

Garczarek 2010). As the sampling area of this particular study is replete in nutrients, the combination of low temperature and high nutrient availability may explain the low relative abundances.

Bacteroidetes were also found at higher relative abundances in surface samples (Figure 8) and were negatively correlated to depth. This phylum have also been found to harbour proteorhodopsin, light sensing and CO₂ fixation genes that may be advantageous for survival in the photic marine environment (Fernández-Gómez *et al.*, 2013). Bacteroidetes includes groups of heterotrophic bacteria that are known degraders of high molecular weight compounds (both particulate and dissolved organic matter) (Bauer *et al.*, 2006; Kirchman 2010; Munoz *et al.*, 2020). Members of the Bacteroidetes are frequently enriched on organic matter particles (DeLong *et al.*, 1993; Milici *et al.*, 2017). Bacteroidetes also preferentially attach to the surfaces of aggregates of OM termed 'marine snow', which makes these taxa important for its colonization and breakdown in cold waters (Bauer *et al.*, 2006; Vojvoda *et al.*, 2014; Duret *et al.*, 2019). This knowledge, coupled with our findings, suggests that Bacteroidetes species may be largely responsible for the breakdown of organic matter at the surface. However, correlation analysis using extracellular enzymatic activities data do not support the centrality of these taxa in the PEIs.

Thaumarchaeota were also detected at higher relative abundance with increasing depth (Figure 8a). Thaumarchaeota are ubiquitous in marine environments and play important roles in the sequestration of carbon and nitrogen (Könneke *et al.*, 2005; Carini *et al.*, 2018; Dong *et al.*, 2019). However, marine Thaumarchaeota are generally known to be inhibited by light (Merbt *et al.*, 2012). This study shows a significant

positive correlation between the abundance of Thaumarchaeota and depth (Spearman's, $p < 0.001$) and highlights the importance of this taxa in surface water. This finding appears consistent with recent studies by Luo *et al.* (2014) showing that some Thaumarchaeota genera may persist in photic marine environments (Luo *et al.*, 2014). By comparing the genomes of Thaumarchaeota from epipelagic and mesopelagic zones the authors demonstrated major differences between distinct phenotypes. Key to these phenotypes was the ability of the surface Thaumarchaeota to circumvent the effects of ultraviolet-induced DNA damage. This allows them to persist in the photic zone although better adapted to life in the meso- and bathypelagic layers (Alonso-Sáez *et al.*, 2012; Carini *et al.*, 2018).

Depth stratification was also observed for fungal communities. In this study, fungal alpha diversity was higher in deeper samples. Additionally, the lack of shared fungal ASVs between the three depths indicated distinct fungal communities (Figure 10b). The highly variable conditions which characterize the different depths may selectively support distinct lineages (Christmas and Cunliffe 2019). This is supported by a change in the dominant phylum from Basidiomycota at the surface to Ascomycota in the OMZ and deep water. These findings are consistent with studies by Agogue *et al.*, (2011) and Morales *et al.*, (2019) which demonstrated that water column stratification results in dissimilarities in fungal diversity. This is probably due to distinct environmental conditions in the different pelagic layers (Agogue *et al.*, 2011; Morales *et al.*, 2019).

5.4.3. Water mass specificity

Four distinct water masses were identified in the PEI region based on previously established criteria (Emery 2001). At phylum level, clear similarities were observed in community composition between the deep-water masses (AABW and AAIW). This was confirmed by higher shared bacterial ASVs between AAIW and AABW and any other two water masses (Figure 10d). The mixing of water masses, which occurs between the layers during circulation, may explain these findings (Djurhuus *et al.*, 2017; Stukel and Ducklow 2017).

Overall, community composition and structure suggest that the different water masses supported diverse assemblages of bacteria and fungi. This is further supported by the low percentage of shared bacterial ASVs and more notably, the complete absence of shared fungal ASVs between the four water masses. Similar to the depth stratification of microbial communities, the most pronounced differences were observed between surface (i.e. PSUW) and deep-water masses.

It is reasonable to predict that these observed differences between water masses may be due to variation in depth rather than water mass specificity. However, permutational analysis suggests that both temperature and salinity may be significant determinants of community structure. As water mass encompasses depth, temperature and salinity (Emery 2001; Carter *et al.*, 2008), the assertion that the microbial community differences observed in the PEI are driven by water mass and not artefacts of the three individual parameters, seems viable. These findings provide further evidence that water masses play a significant role in microbial community composition and may be considered barriers to microbial dispersal (Acha *et al.*, 2015; Djurhuus *et al.*, 2017).

5.4.4. Particle-attached and free-living microorganisms differ in composition and ecological roles

Bathypelagic life is highly dependent on the export of particulate organic matter (POM), in the form of marine snow, from the euphotic layer (Bochdansky *et al.*, 2017). Marine microorganisms may be classified based on their lifestyles as either particle attached (PA) or free-living (FL). These lifestyles also determine microbial interactions with POM in the water column (Simon *et al.*, 2014; Tang *et al.*, 2015). We surveyed and compared size-fractionated samples in order to gain insights regarding the differences between the composition and structure of these lifestyles.

PA and FL microbiomes play significant yet distinct roles in marine ecosystems (Liu *et al.*, 2018). While FL microbes remain suspended in the water column, PA microorganisms are predominantly associated with various particles due to their capacity to degrade organic matter (OM) (Grossart 2010; Lyons and Dobbs 2012; Enke *et al.*, 2018). During sinking, PA microorganisms convert high molecular weight OM into smaller substrates that may be readily available to other microorganisms for cellular metabolism (Romaní *et al.*, 2006; Arnosti 2011; Milici *et al.*, 2017).

The findings in the PEI show that FL and PA communities were distinct from one another with regard to composition. This is confirmed by previous studies that found that PA communities were significantly different to FL communities in aquatic environments (DeLong *et al.*, 1993; Rieck *et al.*, 2015; Zhang *et al.*, 2016). The majority of PEI bacterioplankton were PA. Only 17,8% of Bacterial and Archaeal ASVs were shared between the two fractions. These shared taxa may correspond to bacterioplankton with a capacity to switch between the PA and FL lifestyles. This

capacity may be based on the availability of OM and bioavailable substrates (Lyons and Dobbs 2012; Yung *et al.*, 2016; Muscarella *et al.*, 2019). At phylum level, Basidiomycota were equally distributed between the PA and FL fractions. This group of fungi may play an important role in the degradation of high molecular weight compounds and mostly in association with OM in the form of marine snow in coastal (Taylor and Cunliffe 2016) and bathypelagic (Bochdansky *et al.*, 2017) waters. The ubiquitous presence in the free-living fraction suggests that this group is able to switch between the two lifestyles.

However, the higher proportion of ASVs unique to each fraction suggests that the two size fractions may be distinct and that those unique 'sub-communities' may harbour different sets of functional capabilities specialised for those microhabitats. Based on their ability to tolerate cold temperatures and high hydrostatic pressure, fungi have the potential to contribute substantially to OM degradation on bathypelagic marine snow (Bochdansky *et al.*, 2017). Our study confirms this, revealing the presence of fungal taxa both in the PA and FL fractions. Additionally, fungal community composition has been shown to differ significantly between those found on marine snow and in the ambient water (Bochdansky *et al.*, 2017; Hassett *et al.*, 2019). The absence of shared fungal ASVs between the PA and FL communities in this study suggests that fungal species may be largely restricted to either lifestyle. At the class level, Exobasidiomycetes were more prevalent in PA communities compared to the FL fraction. Ascomycota classes such as the Tremellomycetes and Microbotrymycetes occurred mainly as free-living fungi throughout the entire water column although they were observed in both FL and PA fractions.

The distribution within each sub-community also varied with depth. At the surface, Exobasidiomycetes were the dominant PA fungi and there were clear shifts in the composition of PA subcommunities with more Dothideomycetes in the OMZ and higher abundance of PA Saccharomycetes in the Deep layer. This is similar to a study by Bachmann *et al.*, (2018) that showed that PA and FL communities were most similar at the surface and displayed greater differences with increasing depth. A similar pattern was observed in the PEIs. Alpha diversities of PA and FL communities in Fmax samples were comparable to one another, whereas in Deep and OMZ, higher diversity was observed in the PA fraction as compared to FL bacteria and fungi.

Other PA microbes such as Eurotiomycetes and Dothidiomycetes, which were predominant in surface samples, were also present in the deep samples. This vertical connectivity is possibly due to higher sinking rates associated with PA subcommunities in contrast to FL microorganisms (Mestre *et al.*, 2018). Previous studies have suggested that particles originating from the surface may provide an inoculum of species to the bathypelagic layer (Mestre *et al.*, 2018; Zhao *et al.*, 2020). These species may be better able to thrive in the bathypelagic layer depending on the composition of that particulate matter as well as the physicochemical properties of the subsequent environment.

One of the ways in which PA bacterioplankton break down OM is via the release of extracellular enzymes. In fact, a study by Zhao *et al.*, (2020) showed that a number of PA bacteria (e.g. Gammaproteobacteria) tended to express genes encoding carbohydrate-active enzymes (CAZymes), peptidases and secretory mechanisms. Particle attachment sites are hot-spots for diversity and activity (Baltar *et al.*, 2010;

Bochdansky *et al.*, 2010; Bochdansky *et al.*, 2017) and Zhao *et al.*, (2020) suggest that enzymatic activity in the bathypelagic layer is more likely attributed to PA bacteria. Further, the products of this enzymatic activity may be released into the ambient water, providing substrates, enzymes and co-factors for FL communities (Murrell *et al.*, 1999; Romani *et al.*, 2006; Dang and Lovell 2016). This reveals possible interactions between PA and FL communities, particularly in the bathypelagic layer.

6. Synthesis

Taken together, the findings of this study highlight the various factors influencing the microbiome composition, structure and function in the PEI. More importantly, the results show that some of these factors may be inextricably linked. As a consequence, these combined factors cumulatively shape the structure and function of these microbiomes. Specifically, variables such as light availability, salinity, temperature, and nutrient availability are all depth-dependant variables that influence the structuring of marine microbiomes (Balmonte *et al.*, 2018; Easson and Lopez 2019).

As evidenced by the data, depth dependant variables are also linked to the functioning of extracellular enzymes. Factors such as temperature can directly affect the efficacy of enzymes already secreted into the environment (Cunha *et al.*, 2010; Baltar *et al.*, 2017). By influencing microbial composition, depth dependant variables also indirectly shape the suite of functional genes required for enzymatic activity (Zhao *et al.*, 2020). Enzymatic activities are also influenced by the quantity and form of available DOM and nutrients (Baltar *et al.*, 2017).

Marine snow plays a major role in connectivity of the epi-, meso-, and bathypelagic zones of the water column, acting as a dispersal vector for organic matter down the water column. There are lower intrinsic concentrations of DOM in the deep sea (Jiao *et al.*, 2010; Arrieta *et al.*, 2015) and the release of DOM from sinking particles using extracellular enzymes serves as a main source of DOM in the bathypelagic layer (Arístegui *et al.*, 2009). Additionally, the propensity of PA microorganisms to produce extracellular enzymes is key in the connection between lifestyle, the breakdown of organic matter and possible interactions between different groups with regard to the mineralization and acquisition of organic matter. All of these factors appear to be connected in many respects. It is essential that these connections be further scrutinised in order to improve our understanding of the functional roles of microbial communities in marine microbiomes.

The results of this study suggest niche occupancy by different taxonomic groups. Taxonomy is driven by regional and oceanographic differences. Many of the identified taxa have been shown to share some functional capabilities with regard to organic matter decomposition and DOM mineralization. This could be an indication of a high degree of functional redundancy in this environment. Correlations between nutrient availability and enzymatic activity lead to the suggestion that functional biogeography is driven by the pool of organic matter and not necessarily determined by the taxonomic composition of the community.

It must be stated that the caveat of amplicon sequencing is the lack of functional annotation, thus any postulation regarding function of any particular taxonomic group requires further scrutiny. In future, the use of shotgun sequencing,

metatranscriptomics or metaproteomics may help resolve some of the uncertainty pertaining to assigning function to taxonomic groups. Additionally, direct experimental approaches evaluating the relationships between each of the above-mentioned factors in the context of the microbiome are essential to sufficiently disentangle these factors from one another.

Our results provide valuable insight towards disentangling the influence of biotic and abiotic drivers in the dynamics of near-shore microbial communities in the SO. It confirms patterns of marine microbial community structuring and explores these in the context of fungal communities as well as PA and FL communities that have yet to be explored in the PEIs. This study will improve our understanding of near-shore marine microbiomes and potentially predict the ways in which future changes in climate or other perturbations may affect them in environments such as the PEIs.

7. References

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Supplementary Material

Table S1: Concentrations of nutrients for all sample sites at each depth.

Sample ID	Na mg/l	K mg/l	Ca mg/l	Fe mg/l	SO ₄ mg/l	Cu mg/l	Zn mg/l	P mg/l	NH ₄ -N mg/l	NO ₃ -N mg/l	N mg/l	TOC mg/l
H1F	8308,3	407,5	308	<0.04	2695	<0.02	<0.03	0,11	<0.28	<0.36	10,00	13,90
H1M	7633,1	190,3	110,8	<0.04	3016	<0.02	<0.03	0,11	<0.28	<0.36	12,00	13,00
H1D	7497,7	374,5	274,8	<0.04	3050	<0.02	<0.03	0,11	<0.28	<0.36	12,00	61,20
H3F	7956,1	207,1	122,4	<0.04	2901	<0.02	<0.03	0,13	<0.28	<0.36	143,00	56,00
H3M	9755,3	474,4	353,1	<0.04	3320	<0.02	<0.03	0,15	<0.28	<0.36	7,00	18,70
H3D	8891,5	319,2	215	<0.04	3290	<0.02	<0.03	0,14	<0.28	<0.36	7,00	18,80
H5F	9287,8	411,1	312,8	<0.04	3113	<0.02	<0.03	0,1	<0.28	<0.36	12,00	9,00
H5M	6810,8	264,7	177,3	<0.04	3148	<0.02	<0.03	0,13	<0.28	<0.36	10,00	51,30
H5D	7668,3	300,7	203	<0.04	3078	<0.02	<0.03	0,14	<0.28	<0.36	8,00	6,20
H7F	9589,3	457,6	349,8	<0.04	3294	<0.02	<0.03	0,12	<0.28	<0.36	58,00	34,00
H7M	8684,9	324,2	225	<0.04	3407	<0.02	<0.03	0,15	<0.28	<0.36	6,00	53,50

H7D	7469	362, 8	271, 1	<0.0 4	357 3	<0.0 2	<0.0 3	0,1 5	<0.2 8	<0.3 6	7,00	75,30
H8F	9757,7	474, 6	359, 5	<0.0 4	336 1	<0.0 2	<0.0 3	0,1 2	<0.2 8	<0.3 6	6,00	47,60
H8M	8837,2	377, 8	272, 1	<0.0 4	335 3	<0.0 2	<0.0 3	0,1 3	<0.2 8	<0.3 6	8,00	69,70
H8D	10630, 1	506, 1	385, 5	<0.0 4	369 2	<0.0 2	<0.0 3	0,1 7	0,3	<0.3 6	7,00	29,10
H11F	9102,6	424, 9	329, 3	<0.0 4	366 6	<0.0 2	<0.0 3	0,1 2	<0.2 8	<0.3 6	6,00	19,00
H11D	8337	303, 2	203, 7	<0.0 4	362 7	<0.0 2	<0.0 3	0,1 2	<0.2 8	<0.3 6	7,00	19,20
H13F	9548,1	456, 2	347, 3	<0.0 4	356 5	<0.0 2	<0.0 3	0,1 1	<0.2 8	<0.3 6	6,00	15,90
H13D	10812, 2	507, 9	394, 1	<0.0 4	372 4	<0.0 2	<0.0 3	0,1 6	<0.2 8	<0.3 6	7,00	20,80
H17F	9974,4	480, 1	361, 2	<0.0 4	384 5	<0.0 2	<0.0 3	0,1 5	<0.2 8	<0.3 6	7,00	23,10
H17D	9107,2	437, 2	338, 4	0,06	320 1	<0.0 2	<0.0 3	0,1 5	<0.2 8	<0.3 6	8,00	75,80
H19F	9848,1	468, 7	353	<0.0 4	346 8	<0.0 2	<0.0 3	0,1 2	<0.2 8	<0.3 6	10,00	27,00
H19D	8471,5	395, 8	295, 8	<0.0 4	404 8	<0.0 2	<0.0 3	0,1 7	<0.2 8	<0.3 6	6,00	21,20
H21F	7682,3	366	272, 9	<0.0 4	263 8	<0.0 2	<0.0 3	0,1 2	<0.2 8	<0.3 6	11,00	6,30

H21M	10061, 8	411, 8	299, 5	<0.0 4	367 1	<0.0 2	<0.0 3	0,1 5	<0.2 8	<0.3 6	7,00	14,90
H21D	11045, 1	521, 4	405, 9	<0.0 4	386 9	<0.0 2	<0.0 3	0,1 5	<0.2 8	<0.3 6	10,00	55,90

F=Fluorescence maximum (Fmax); M= Oxygen minimum zone (OMZ) ; D= Deep

Table S2: Raw alpha diversity values for each sample throughout the PEIs. Diversity is shown for bacteria and archaea

Sample ID	Observed	Chao1	ACE	Shannon	Simpson	InvSimpson	Fisher
H01F2	433,00	470,94	457,34	4,89	0,98	51,27	84,34
H01F4	351,00	371,50	373,36	4,45	0,97	36,37	65,10
H03F2	342,00	369,97	370,69	4,53	0,97	39,32	63,06
H03F4	338,00	362,41	366,38	4,51	0,98	44,23	62,16
H05F2	429,00	470,14	471,64	4,54	0,97	31,16	83,37
H05F4	448,00	491,81	495,40	4,78	0,98	52,05	87,98
H07F2	348,00	371,65	376,11	4,45	0,97	33,82	64,42
H07F4	398,00	430,34	431,52	4,81	0,98	58,43	75,98
H08F2	271,00	285,44	280,49	4,26	0,97	30,37	47,50
H08F4	305,00	314,85	317,90	4,50	0,98	42,33	54,83

H11F2	259,00	282,00	270,60	4,44	0,98	40,72	44,96
H11F4	385,00	403,50	405,72	4,80	0,98	56,78	72,94
H13F2	126,00	126,00	126,00	4,16	0,97	39,35	19,04
H13F4	291,00	309,21	313,00	4,49	0,98	47,23	51,78
H17F2	255,00	279,39	275,90	4,15	0,97	29,78	44,12
H17F4	310,00	338,64	333,39	4,55	0,98	48,20	55,93
H19F4	314,00	336,88	335,26	4,58	0,98	50,20	56,81
H21F2	189,00	203,62	200,69	3,68	0,95	19,89	30,79
H21F4	416,00	445,40	446,14	4,96	0,99	74,20	80,25
H01M2	520,00	558,89	547,92	4,92	0,98	53,20	105,96
H01M4	840,00	953,16	949,71	5,49	0,99	84,28	195,23
H03M2	497,00	524,05	528,63	4,87	0,98	49,44	100,13

H03M4	631,00	733,03	754,51	3,76	0,87	7,79	135,25
H05M2	647,00	732,88	718,81	5,00	0,98	55,73	139,62
H05M4	954,00	1 048,03	1 030,60	5,82	0,99	109,59	230,49
H07M2	414,00	428,59	428,14	4,72	0,98	47,24	79,78
H07M4	1 011,00	1 091,19	1 097,60	5,85	0,99	108,77	248,78
H08M4	725,00	795,31	792,21	5,32	0,98	58,87	161,47
H21M2	167,00	167,00	167,00	4,43	0,98	52,87	26,57
H21M4	951,00	1 077,46	1 084,87	5,68	0,99	103,19	229,54
H01D2	307,00	342,00	325,47	4,09	0,95	19,75	55,27
H01D4	730,00	807,41	815,76	4,81	0,97	28,62	162,90
H03D2	364,00	404,67	402,58	3,95	0,93	14,74	68,07
H03D4	677,00	767,89	771,27	4,49	0,94	17,49	147,92

H05D2	1 118,00	1 261,85	1 270,18	5,90	0,99	134,03	284,26
H05D4	514,00	552,33	556,08	4,90	0,98	59,35	104,43
H07D2	471,00	496,05	497,43	4,68	0,97	38,69	93,63
H07D4	869,00	996,89	981,30	5,56	0,99	100,05	204,04
H08D2	323,00	341,00	337,50	4,58	0,98	41,07	58,80
H08D4	1 219,00	1 377,35	1 400,29	5,91	0,99	122,24	319,14
H13D2	527,00	582,02	574,74	4,83	0,98	44,36	107,75
H13D4	962,00	1 099,69	1 080,22	5,67	0,99	80,59	233,03
H17D2	566,00	615,07	634,30	4,82	0,98	48,16	117,87
H17D4	974,00	1 092,13	1 106,09	5,64	0,99	79,54	236,86
H19D2	571,00	604,55	600,29	5,18	0,98	60,31	119,19
H19D4	844,00	919,79	916,44	5,68	0,99	99,99	196,44

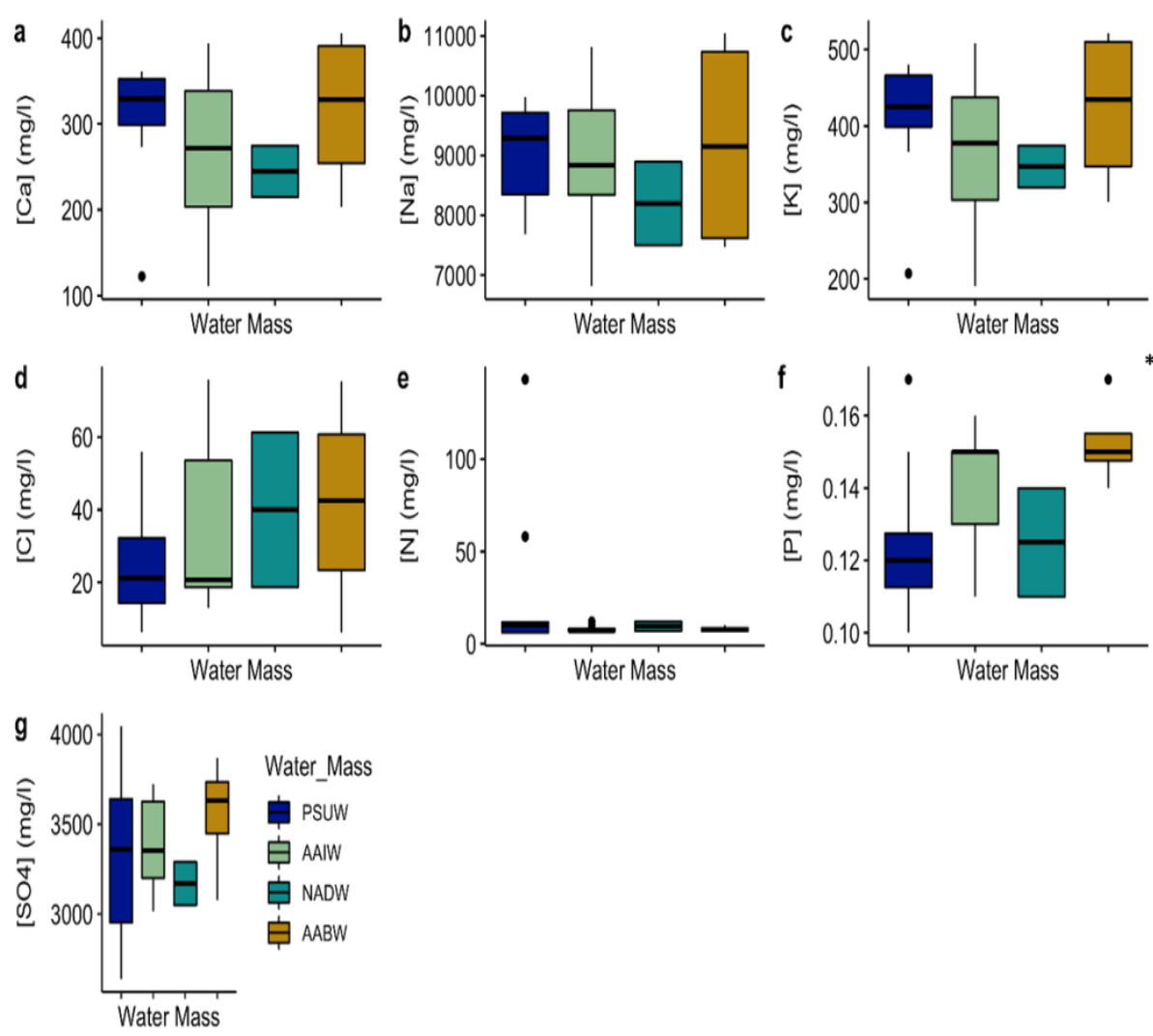


Figure S1: Boxplots showing differential concentrations (in mg/l) of a) calcium, b)sodium, c) potassium, d) total carbon, e) nitrogen, f) phosphorus, g) sulphate in the PSUW (dark blue), AAIW (green), NADW (cyan) and AABW (gold) layers. Significant differences are indicated with an *.

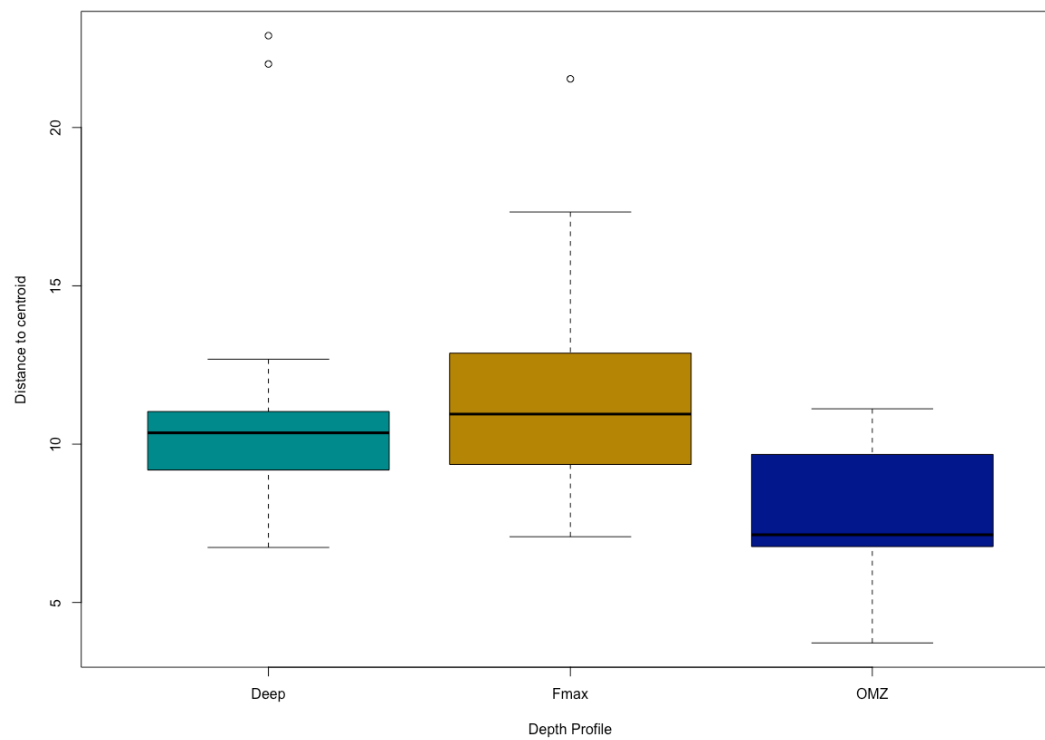


Figure S2: Betadisp plot. Colours indicate the three depths. Open circles above and beneath each box indicate outliers. Whiskers indicate minimum and maximum values and solid black lines within each boxplot represent the median value. Top and bottom vertical box limits show the 25th and 75th percentile, respectively.

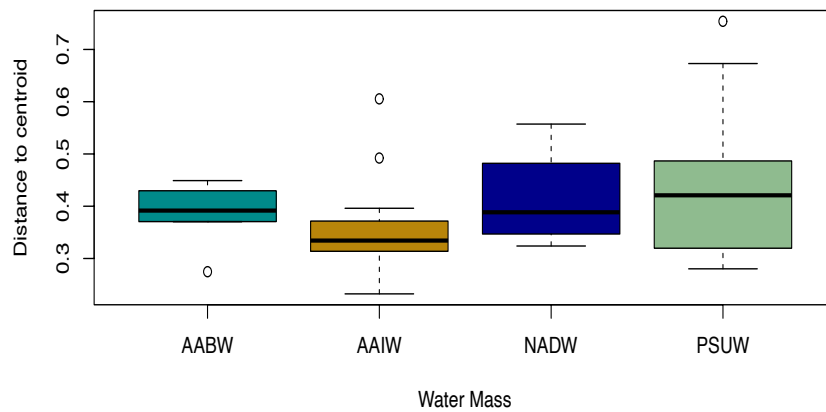


Figure S3: Betadisp plot. Colours indicate the three depths. Open circles above and beneath each box indicate outliers. Whiskers indicate minimum and maximum values and solid black lines within each boxplot represent the median value. Top and bottom vertical box limits show the 25th and 75th percentile, respectively.

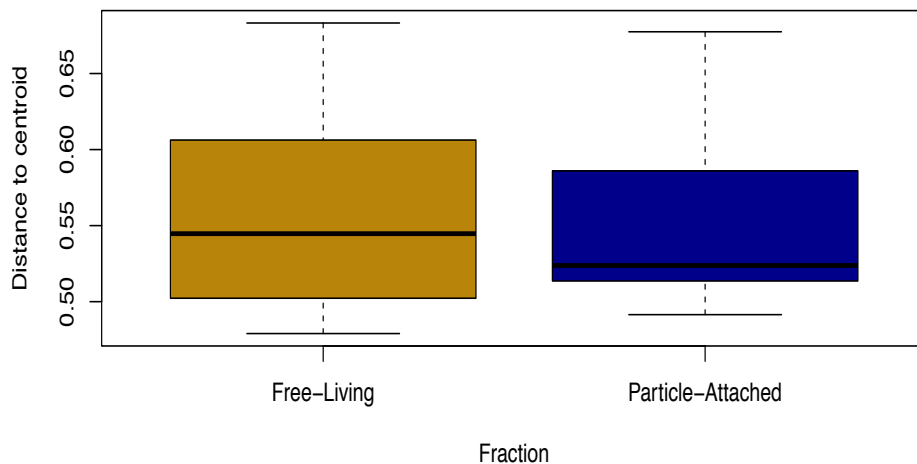


Figure S4: Betadisp plot. Colours indicate free-living (blue) or particle-attached (gold). Open circles above and beneath each box indicate outliers. Whiskers indicate minimum and maximum values and solid black lines within each boxplot represent the median value. Top and bottom vertical box limits show the 25th and 75th percentile, respectively.

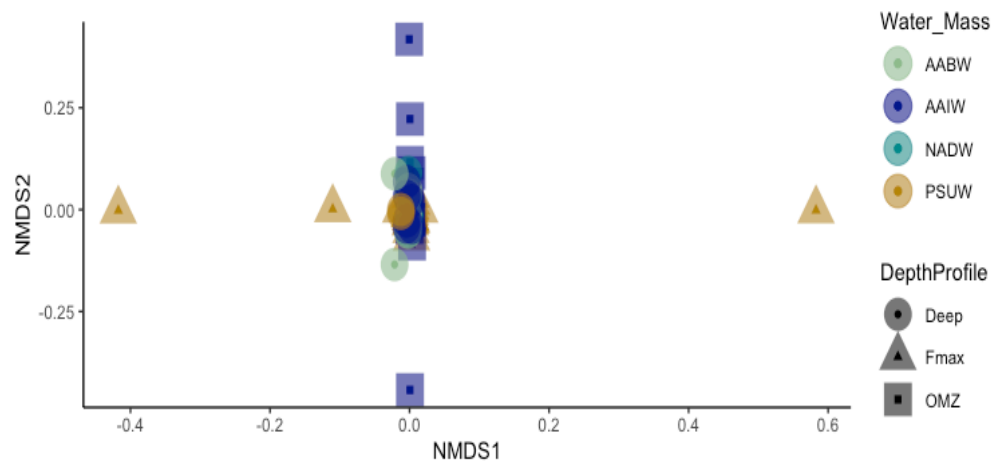


Figure S5: Non-metric multi-dimensional scaling (NMDS) ordination plots of mycoplankton communities constructed using Bray-Curtis dissimilarity. The colours in the legends represent (a) depth profiles and (b) water masses in the left and right panels respectively.

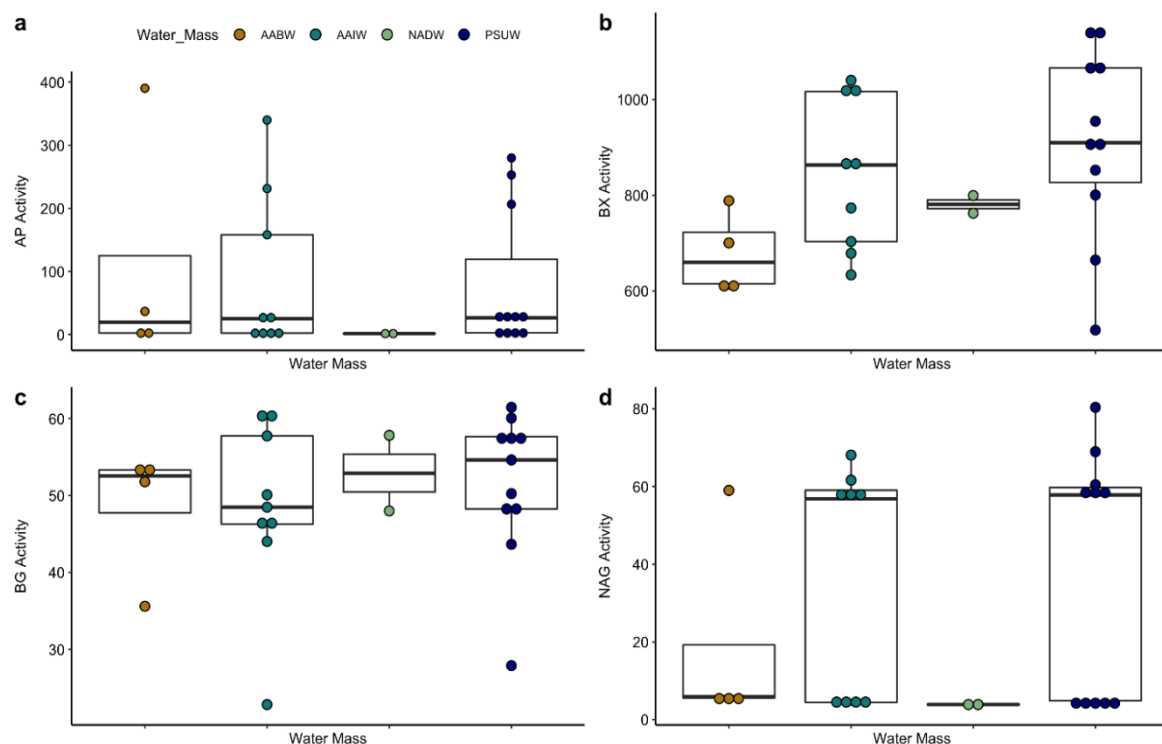


Figure S6: Extracellular activities for four enzymes involved in microbial nutrient acquisition for each of the identified water masses. Whiskers on each boxplot indicate the minimum and maximum values and solid black lines indicate the median value. Top and bottom vertical box limits show the 25th and 75th percentile, respectively.

Community structure and functional determinants of free-living and particle associated Bacterioplankton and mycoplankton in sub-Antarctic islands

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Microbiome; biogeochemical cycling; functional biogeography; particle attachment; extracellular enzyme activity

This PDF file includes:

Main Text
Figures 1 to 5
Tables 1 to 2

Abstract

Microbiomes breakdown particulate organic matter and, consequently, drive nutrient cycling in marine environments. In Southern Ocean islands such as the Prince Edward Islands (PEIs), the interactions between landmasses, the surrounding waters and macrofauna result in complex trophic interactions underpinned by microbial communities. However, we lack a clear understanding regarding particle attached (PA) and free living (FL) and their role as drivers of functional processes. To reduce this knowledge deficit, we provide insights regarding microbial community structure and composition of PA and FL microbiomes in the PEIs. Fluorescence maximum (F_{max}), the oxygen minimum zone (OMZ) and the bathypelagic zone (Deep) samples were collected from 10 sites. By measuring several physicochemical factors, we assess the extent to which these nutrients shape PEI microbial communities. Phylogenetic analysis of 16S and ITS rRNA gene regions together with statistical analysis revealed shifts in bacterioplankton and fungal community structure and composition, respectively. These shifts in PA and FL microbiomes coincided with differences in depth, oceanographic features and physicochemical variables. Extracellular enzymatic activities specific for carbon, nitrogen and phosphorus acquisition were incongruent with community composition but were closely linked to substrate availability and depth. These results suggest that microbial extracellular enzymatic activities were driven primarily by nutrient availability. Together, these data provide important insights regarding the influence the complex interplay between biotic and abiotic drivers as drivers of near-shore PA and FL microbial communities in the SO.

50 **Significance Statement**

51 Marine microbes play a significant role in global biogeochemical cycling. Thus, they are important
52 sentinels for climate change. However little is known about how the microbiome is related to abiotic
53 factors and how that corresponds to functionality. This study adds insight into the how future climate
54 change may affect marine microbiomes and the health of global ecosystems.

55

1 Introduction

Understanding the effects of climate change on biodiversity remains a major ecological endeavor (Araújo and Rahbek 2006; Bellard et al. 2012; Mantyka-Pringle et al. 2012). This is especially true in ecosystems characterized by high levels of endemism, such as the Southern Ocean, where losses in keystone taxa may ultimately affect ecosystem services (Chown et al. 2015). Recent studies in the Southern Ocean have provided quantitative evidence of species loss in several sub-Antarctic islands (Trathan et al. 2007; Warnock and Scherer 2015). These islands are major breeding hubs for macro-fauna, which profoundly affect terrestrial ecosystems (Bester et al. 2011; Labadie et al. 2018). Efforts to reveal ecosystem dynamics across several habitats have highlighted the important role played by microorganisms in the cycling of carbon and organic matter (Yooseph et al. 2007; Falkowski et al. 2008; Passow and Carlson 2012; Pachiadaki et al. 2017; Zhang et al. 2018). However, comparatively little is known regarding microbial communities associated with sub-Antarctic islands.

Studies of land-sea interactions have shown that nutrient input, as a result of island runoff, leads to increased primary productivity resulting in periodic phytoplankton blooms (Duncombe Rae 1989; Pakhomov and Froneman 1999; Treasure et al. 2015; Von Der Meden et al. 2017). These drifts, termed the 'island mass effect' (Doty and Oguri 1956; Boden 1988) are typically observed in the PEIs as well as other sub-Antarctic landmasses. Consequently, the PEIs harbour relatively elevated productivity levels in low productivity regions (Perissinotto and Rae 1990; Perissinotto et al. 2000; Gurney et al. 2014). A combination of climate, oceanographic features and general land-sea interactions in the PEIs provide a valid model for understanding the effects of climate change on marine microbiomes.

Few studies have provided phylogenetic and functional insights regarding microbial communities in the PEIs (Venkatachalam et al. 2019). Studies evaluating pelagic assemblages, and the factors that govern their composition and structure, suggest higher than expected levels of microbial diversity (Wilkins et al. 2013; Walsh et al. 2016; Phoma et al. 2018). These studies suggest that the microbiota, primarily bacteria, are shaped by several abiotic factors including the availability of

light (Cory et al. 2014; Bryant et al. 2016), temperature (Sunagawa et al. 2015; Morán et al. 2017) as well as latitude and longitude (Ladau *et al.* 2013; Haggerty and Dinsdale 2017) amongst others. It has previously been proposed that oceanic variability also has a major influence on the marine microbiome (Venkatachalam et al. 2019). Depth (DeLong et al. 2006; Walsh et al. 2016; Phoma et al. 2018), ocean fronts (Pinhassi et al. 2003; Raes et al. 2018) and water mass specificity (Agogue et al. 2011; Wilkins et al. 2013; Hernando-Morales et al. 2017) substantially impact the structure and dynamics of marine microbiomes. However, these data are insufficient to conclude which of these factors best explains the differences observed between microbial communities. Additionally, few studies have evaluated PEI waters (Venkatachalam et al. 2019). These studies show that a combination of unique oceanographic conditions and physicochemical properties affect microbial assemblages in various ways.

Aquatic microorganisms can be classified according to their relationship with particulate matter into three broad categories (either truly free-living (FL), particle attached (PA) or alternating between the two lifestyles) (Grossart 2010; Tang et al. 2015). PA microbes have a higher capacity to degrade high molecular weight (HMW) organic matter (OM) and thus remain primarily associated with a variety of particles (Lyons and Dobbs 2012; Liu et al. 2018). FL bacteria are less adept at degrading HMW OM and thus spend their entire life-cycle suspended in the water column (Grossart 2010; Bachmann et al. 2018). Particle associations may increase the availability of OM promoting mineralization by microorganisms (refs). However, under some conditions, the presence of particular substrates is not correlated to its bioavailability (Milici et al. 2017). Under such circumstances, the ability to convert OM to an accessible form is a clear advantage (Hoarfrost and Arnosti 2017b). The capacity to produce extracellular enzymes, by selected microorganisms, facilitates these processes (Arnosti et al. 2014; Baltar et al. 2017). Several extracellular enzymes are produced by marine microbes and recent studies have shown that these are important in the acquisition of essential nutrients including carbon, nitrogen and phosphorus (Benitez-Nelson 2000; Sistla and Schimel 2013; Karl and Björkman 2015; Hoarfrost and Arnosti 2017b).

To reduce the knowledge deficit regarding the PA and FL microbiomes in sub-Antarctic islands, we assess near-shore communities in the PEIs. We assess biogeographical patterns of bacterioplankton and mycoplankton in several sites providing insights on the effects of physicochemical variables. We assess the extracellular enzymatic activities of these community and examine their links to phylogeny.

Results

Physicochemical parameters

Depth-related trends of physicochemical variables are shown in (**S. Figure 1**). We identified four water masses including the Pacific Sub-arctic Upper Water (PSUW), Antarctic Intermediate Water (AAIW), Antarctic Bottom Water (AABW) and North Atlantic Deep Water (NADW) (**S. Table 1**). The concentrations of Ca, SO₄, Na, C, and K were significantly different between sites (ANOVA, $p < 0.05$) (Figure 1). We observed depth related trends in nutrient concentrations. Ca was significantly higher in Fmax than in OMZ and Deep samples (ANOVA, $p < 0.05$). P was significantly higher in Deep samples (ANOVA, $p < 0.001$) and was also significantly correlated with depth (Spearman, $p < 0.001$).

Bacterial, archaeal and fungal taxonomic composition

Proteobacteria and Bacteroidetes were the most dominant phyla in the entire water column, accounting for 39.94% and 12.27% of bacterial and archaeal ASVs, respectively. Archaea constituted 23.02% of 16S rRNA gene sequences with Thaumarchaeota (12.27 %) at the highest relative abundances. In contrast to bacteria and archaea, the vast proportion of fungal sequences were unassigned. This was irrespective of the water mass affiliation, sampling depth or location. Nevertheless, the assigned fungal ASVs were dominated by Basidiomycota (30.77%) and Ascomycota (25.93%) (**Figure 2**). At class level Exobasidiomycetes were present at high relative abundances followed by Dothidiomycetes and Saccharomycetes. When assessing taxonomic composition by size fraction (FL/PA) we found that xx dominated microbiota.

PA and FL microorganisms are taxonomically distinct

PA and FL communities differed significantly in abundance and diversity (Figure 2). PA were more abundant than FL microbes throughout the water column. PA and FL communities were also distinct in terms of taxonomic composition. Bacterial, archaeal and fungal species richness differed substantially between the two fractions, with generally higher alpha diversity in the PA fraction compared to the FL (Figure 2 a & c). Venn diagrams showed that bacterial and archaeal PA and FL communities shared less than 18% of the total number of ASVs. Remarkably, fungal PA and FL communities had no shared taxa at ASV level (Figure 3).

Depth stratification was a major driver in community composition. For bacteria and archaea, key taxa in Fmax samples include members of several phyla such as Cyanobacteria, Bacteroidetes and Verrucomicrobia (Figure 2a). OMZ and Deep samples were marked by higher relative abundances of Thaumarchaeota and Firmicutes. Fungal communities also exhibited clear taxonomic shifts with increasing depth. Basidiomycota dominated surface samples, while Ascomycota increased in relative abundance in the meso- and bathypelagic layer (Figure 2c). Additionally, species richness followed a depth-dependant trends in bacterial and archaeal as well as fungal alpha diversity. Species richness (for both groups) increased from Fmax to OMZ and Deep (Figure 2 b & d).

We also found that communities exhibited depth-resolved differences between microbial lifestyles (PA or FL), particularly in Fmax samples. nMDS showed that PA and FL communities clustered together in Fmax samples. FL *Saccharomyces* increased in relative abundance deeper in the water column and Exobasidiomycota were predominantly particle-associated in surface samples. Additionally, PA Dothidiomycetes were more abundant in the OMZ than the other depths.

Physicochemical variables shape microbial communities

Permutational analysis combined with the nMDS ordination (Figure 4) showed that several environmental and physicochemical variables significantly affected microbial community structure.

Several variables including temperature ($p<0.001$), salinity ($p<0.001$), oxygen ($p<0.001$), fluorescence ($p<0.001$), phosphorus (P) ($p<0.05$), the ratio of carbon, nitrogen and phosphorus (C:N:P) ($p<0.05$), water mass ($r^2=0.4511$, $p<0.001$) and depth ($p<0.001$).

Enzymatic activity analysis

The extracellular enzymes β -glucosidase and β -xylosidase were detected in all samples. The AP and NAG activities of some samples were higher compared to the other stations where the activity was negligible (Figure 5). The mean BX activity of was significantly different between depths (Kruskal-Wallis, $p\text{-value}< 0.05$), with the highest levels recorded for surface waters (Fmax), followed by OMZ and then lowest activities in the deep samples. The activities of extracellular enzymes were significantly correlated to environmental variables and nutrient concentrations (Table 1).

Discussion

We assessed the taxonomic composition of microbial communities in the PEIs. At global scales, members of the phylum Proteobacteria (mostly affiliated to alpha-proteobacterial SAR11 clade) dominate marine ecosystems and are followed by bacteria affiliated to Bacteroidetes and Cyanobacteria (Rusch *et al.* 2007; Sunagawa *et al.* 2015). The results of this regional study were broadly concordant with these findings. However, in contrast with global-scale analyses, Gammaproteobacteria were overrepresented in this PEI dataset and were found at higher relative abundances compared to the Alphaproteobacteria. The results are similar to previous studies conducted in the Indian sector of the SO (Gupta *et al.* 2015; Phoma *et al.* 2018). Previous global-scale analyses also showed that Ascomycota and Basidiomycota dominate pelagic fungal communities (Tisthammer *et al.* 2016; Morales *et al.* 2019). These results are broadly consistent with the biogeographic analysis of fungi in our sample set with the exception that Basidiomycota were present at higher relative abundances compared to Ascomycota in this study.

187

188 Our findings show that FL and PA communities were distinct in terms of taxonomic composition.
189 Our analysis revealed that a high proportion of bacterial ASVs were unique to each lifestyle.
190 Comparison of PA and FL fungi revealed no shared ASVs. These findings suggest that the two size
191 fractions are taxonomically distinct in line with previous studies showing that PA taxa were
192 significantly different to FL communities in aquatic environments (DeLong *et al.* 1993; Rieck *et al.*
193 2015; Zhang *et al.* 2016).

194

195 We also observed several depth-dependent variations in these sub-communities with clear
196 distributional patterns. At the surface, Exobasidiomycetes were found in higher relative
197 abundances in PA fungi. There were clear shifts in the composition of PA subcommunities with
198 more Dothideomycetes in the OMZ and higher relative abundances of PA Saccharomycetes in the
199 Deep layer. We also noted that species richness in PA and FL sub-communities was comparable
200 in Fmax samples but increased significantly in PA microbes with increasing depth. This is similar
201 to a study by Bachmann *et al.* (2018) that showed that PA and FL communities were most similar
202 at the surface and displayed greater differences with increasing depth.

203

204 Species richness was generally higher in PA communities than in FL groups. This is concordant
205 with other studies of SO microbiomes that showed higher alpha diversity in PA bacteria than FL
206 (Milici *et al.* 2017). Studies have suggested that PA and FL microbes may have distinct ecological
207 roles in marine environments (Liu *et al.* 2018). FL microorganisms adopt to oligotrophic lifestyles
208 while PA microbes are generally copiotrophic preferring nutrient enriched environments (Herndl
209 and Reinthaler 2013; Giovannoni *et al.* 2014).

210

211 The SO is a HNLC environment (Blain *et al.* 2001; Tagliabue *et al.* 2017). It is therefore unsurprising
212 that PA microorganisms predominate in this environment. Further, the proximity to the islands,
213 suggests that there may be additional nutrient input from the terrestrial environment (Treasure *et*
214 *al.* 2015). This may result in greater heterogeneity of the OM resource pool and thus higher
215 abundance and diversity of PA microorganisms (Muscarella *et al.* 2019). The slow sinking of POM
216 in the SO and heterogeneity of the resource pool makes particulate matter provides a wide range
217 of ecological niches and may result in higher functional diversity.

218 The ability of PA to attach to particles is advantageous and may increase the availability of OM for
219 mineralization by microorganisms (Hoarfrost and Arnosti 2017b; Milici *et al.* 2017). Some
220 microorganisms produce extracellular enzymes to facilitate the process of OM degradation (Arnosti
221 *et al.* 2014; Baltar *et al.* 2017). Several extracellular enzymes are produced by marine microbes
222 that aid in the acquisition of essential nutrients like carbon, nitrogen and phosphorus (Benitez-
223 Nelson 2000; Sistla and Schimel 2013; Karl and Björkman 2015; Hoarfrost and Arnosti 2017b).
224 Enzymatic activity is higher in PA than in FL microorganisms (Baltar *et al.* 2013). We observed
225 some of these extracellular enzyme producing bacteria and fungi (e.g. Actinobacteria,
226 Gammaproteobacteria and Basidiomycota) within the PEI microbial community. These taxa may
227 be responsible for the enzymatic activity detected.

228 We found that enzymatic activities were mostly linked to nutrient availability. For instance, AP is
229 typically considered as a proxy for organic phosphorus mineralization potential and P availability
230 (Krämer and Green 2000). AP plays a vital role in hydrolysing phosphomonoesters and
231 phosphodiester to release assimilable inorganic P for microorganisms (Luo *et al.* 2017).
232 Extracellular phosphatases, such as AP, are upregulated in response to changes in external
233 inorganic phosphorus concentrations and intracellular phosphorus demand (Hoppe 2003).
234 However, the results of chemical analysis are not consistent with P limitation at these or other
235 sampling locations. In fact, AP activities were high in samples where P levels were also elevated.
236 Other studies support these observations (Hong *et al.* 1998; Tamburini *et al.* 2002; Baltar *et al.*

2016) and it has been proposed that P-starvation may not be the only reason for higher AP activity. This enhanced activity may be due to secondary roles of AP in the marine environment (Colman *et al.* 2005; Karl and Björkman 2015).

Higher AP activity, at specific locations, may be linked to the increased availability of trace elements rather than the 'direct' effect of P limitation. As such, the observed differences in enzymatic activity in the PEI may be explained by differences in the availability of nutrients and cofactors (found at very low concentrations in some samples) and the specific nutrient cofactor requirements of the microbial community at each location.

Particle attachment sites are hot-spots for diversity and activity (Baltar *et al.* 2010; Bochdansky *et al.* 2010; Bochdansky *et al.* 2017) and Zhao *et al.* (2020) suggest that enzymatic activity in the bathypelagic layer is more likely attributed to PA bacteria. Further, the products of this enzymatic activity may be released into the ambient water, providing substrates, enzymes and co-factors for FL communities (Murrell *et al.* 1999; Romaní *et al.* 2006; Dang and Lovell 2016). This reveals possible interactions between PA and FL communities, particularly in the bathypelagic layer.

The results of this study suggest niche occupancy by different taxonomic groups. Taxonomy is driven by regional and oceanographic differences. Many of the identified taxa have been shown to share some functional capabilities with regard to organic matter decomposition and DOM mineralization. This could be an indication of a high degree of functional redundancy in this environment. Correlations between nutrient availability and enzymatic activity lead to the suggestion that functional biogeography is driven by the pool of organic matter and not necessarily determined by the taxonomic composition of the community.

Materials and Methods

263

264 *Sampling*

265 Samples were acquired aboard the RV SA Agulhas II from April to May 2018 during the Marion
266 Island Relief Voyage. Seawater was collected from 10 oceanographic stations near the Prince
267 Edward Islands (Figure 1) using a 24 x 20 litre Niskin bottle CTD rosette multi-sampler (Sea-Bird
268 SBE-911 plus V2 CTD system; Sea-Bird Electronics, Inc., USA). Samples were collected from three
269 depths; the epipelagic (fluorescence maximum, Fmax), the oxygen minimum zone (OMZ) and
270 approximately 10m above the seafloor (Deep). Temperature, salinity, conductivity, oxygen and in
271 situ fluorescence were recorded at each site via sensors on the CTD rosette. Water was collected
272 in 5 L seawater washed polyethylene bottles. This was immediately filtered through 0.45µm and
273 then 0.22µm-pore cellulose acetate membrane filters to capture the particle associated (PA) and
274 free-living (FL) communities, respectively, prior to storage in cryo-vials at -20°C until further
275 analysis. Approximately 100ml of unfiltered water was collected for nutrient analysis and
276 extracellular enzymatic activity assays, respectively.

277 *Nutrient analysis*

278 Samples for nutrient analysis were sent to Bemlab (Somerset West, South Africa). Sodium (Na),
279 potassium (K), calcium (Ca), iron (Fe), sulphate (SO₄), copper (Cu), zinc (Zn), phosphorous (P),
280 ammonium (NH₄), and nitrate (NO₃) concentrations were determined using a Varian Inductively
281 Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent, Australia). For macro and
282 trace element determination of Ca, K, SO₄, Cu, Zn and Fe, samples were filtered through a 0.45
283 µm syringe and transferred to an ICP tube for analysis on the ICP-OES as detailed previously
284 (Quevauviller et al. 1994). NH₄-N and NO₃-N samples were passed through a 0.45µm syringe and
285 transferred to a test tube for analysis on the Gallery Discrete Analyser (Thermofisher, USA).

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Bacterial, archaeal and fungal amplicon sequencing

Nucleic acids were extracted from a third of each membrane filter using the Power Water DNA isolation kit (MoBio, Carlsbad, USA), as detailed in the manufacturer's manual with minor modifications to maximize yield. DNA extracts were sent to the University of Liverpool for high throughput 16S rRNA amplicon sequencing where all PCR amplification, product quality checking and purification as well as DNA library preparation was performed. The marine specific primer pair 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926R (5'- CCGYCAATTYMTTTRAGTTT) (Parada et al. 2016) was used to amplify hypervariable regions V4 and V5 of the bacterial and archaeal 16S rRNA gene. All 16S rRNA gene amplicons were sequenced using the Illumina MiSeq Platform (2 x 250bp) at the NERC-funded Advanced Genomics Facility at the University of Liverpool (<http://www.liv.ac.uk/agf/>).

Fungal internal transcribed spacer (ITS) sequencing was performed by the Molecular Research DNA (MR DNA) sequencing facility (Texas, USA). Fungal-specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) were used as detailed previously (Kamutando et al 2019). Tagged amplicons underwent paired-end sequencing (2 x 250bp) on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Bioinformatic analysis

The resultant sequence data were analysed using the Quantitative Insights Into Microbial Ecology (QIIME2) software package version 2018.8.0 (Bolyen et al. 2018). We followed different methodologies the analysis of 16S rRNA genes and ITS region sequences, using specific databases for bacteria and fungi, respectively.

Raw bacterial reads were trimmed at both ends to remove barcodes (Phred quality score <64). Reads were dereplicated, denoised and filtered using DADA2, implemented in QIIME2, with the default parameters (Callahan et al. 2016). These sequences were quality filtered prior to assigning amplicon sequence variants (ASVs) using the SILVA 99 database (Quast et al. 2012). The resultant

sequences were subsampled (rarefied) to a 14 300 reads per sample to ensure even sampling depth. Samples with sequences yielding less than 14 300 reads were omitted from subsequent analysis. The resultant bacterial ASV output file was exported in BIOM format.

Raw fungal reads were trimmed (25 nucleotides from the start and 280 nucleotides from the end of each read). Reads were de-replicated and de-noised using DADA2 (Callahan et al. 2016) in QIIME2 (Bolyen et al. 2018). The detection of chimeric sequences was performed at 97% sequence similarity using USEARCH v11 (Edgar 2010). Assignment of ASVs was performed using the UNITE (release 7_99) database for fungi (Tedersoo et al. 2018). Taxonomic assignments of ASVs was conducted based on 97% sequence similarity using USEARCH v11 (Edgar 2010). Singletons were excluded from downstream processing. All samples were rarefied to the same number of sequences per sample (11705).

Microbial diversity and Statistical Analyses

The majority of data analyses were performed using the R statistical package version 3.6.1 (R Core Team) and R Studio version 1.1.456 (Team 2019). Rarefaction curves were computed and visualized using vegan. Alpha diversity parameters were computed using vegan and visualized using ggplot2 (Wickham 2011). Relative abundances were computed and visualised using the phyloseq (McMurdie and Holmes 2013) and ggplot2 packages, respectively (Wickham 2011).

Pairwise similarities among samples were calculated using the Bray–Curtis index of dissimilarity from Hellinger-transformed ASV tables. The resultant data was represented in a two-dimensional non-metric multidimensional scaling (NMDS) ordination using the plot_ordination function in phyloseq. To assess differences between sample groupings in the NMDS ordinations, a permutational multivariate analysis of variance (PERMANOVA) based on 999 permutations of the data was performed. To infer the relative importance of factors shaping biogeographical patterns as well as environmental drivers, the environmental vectors were fitted to the ordination using the envfit function (Oksanen et al. 2007). Significant differences in community structure were determined using the adonis function in vegan and visualized using ggplot2 in R. Multivariate

homogeneity of group dispersions, an assumption of permanova, was assessed through the betadisper function in vegan. Boxplots were used to visualise and to determine if these data conformed to requirements for statistically robust PERMANOVA.

Spearman's correlations between taxon abundances, environmental variables, diversity measures and enzymatic activities were computed using the rcorr function in the Hmisc package (Harrell Jr and Harrell Jr 2015). Correlations were recalculated in SparCC (Friedman and Alm 2012), which takes into account the proportion of reads contributed by each ASV. The correlation matrix was visualized in R using the corrplot package. Euclidean distances were used to perform hierarchical clustering. ASV data were log₁₀ transformed, after which cluster analysis was performed. For these analysis, ASV data for the top 1% of the bacterial and archaeal taxa using the heatmap function were used.

Extracellular enzyme activity assays

Seawater samples collected from each site were assayed for the activity of 5 enzymes, β -glucosidase (BG), β -xylosidase (BX), β -N-acetyl-glucosaminidase (NAG), alkaline phosphatase (AP) and leucine aminopeptidase (LAP). Potential extracellular enzymatic activities for the selected enzymes were tested using fluorescence, where the substrate analogues used were linked either to 4-methylumbelliferone (MUB) (for BG, BX, NAG and AP) or 7-aminocoumarine (AMC) in the case of LAP (Table1). Potential enzyme activities were calculated as previously described by (Sinsabaugh 1994; Hoarfrost and Arnosti 2017a) using four replicate cells for each sample. Briefly, seawater (200 μ l) was added to black 96-well flat-bottom microplates (Thermoscientific, USA), to which between 100 mM to 500 mM of each enzyme substrate was added. Controls in each plate included a sample to which no substrate was added, a substrate control to which ddH₂O was added to the substrate analogue and a quench standard, which contained only the fluorescent molecule (MUB or AMC) and sample. Plates were incubated at 4°C in the dark for the time specified in Table 2. Fluorescence was measured from the black 96-well plates using a Spectramax® Paradigm

364 Multimide Microplate reader (Molecular Devices, USA) with an emission wavelength of 450 nm and
365 an excitation wavelength of 360nm. Enzyme activities were then calculated.

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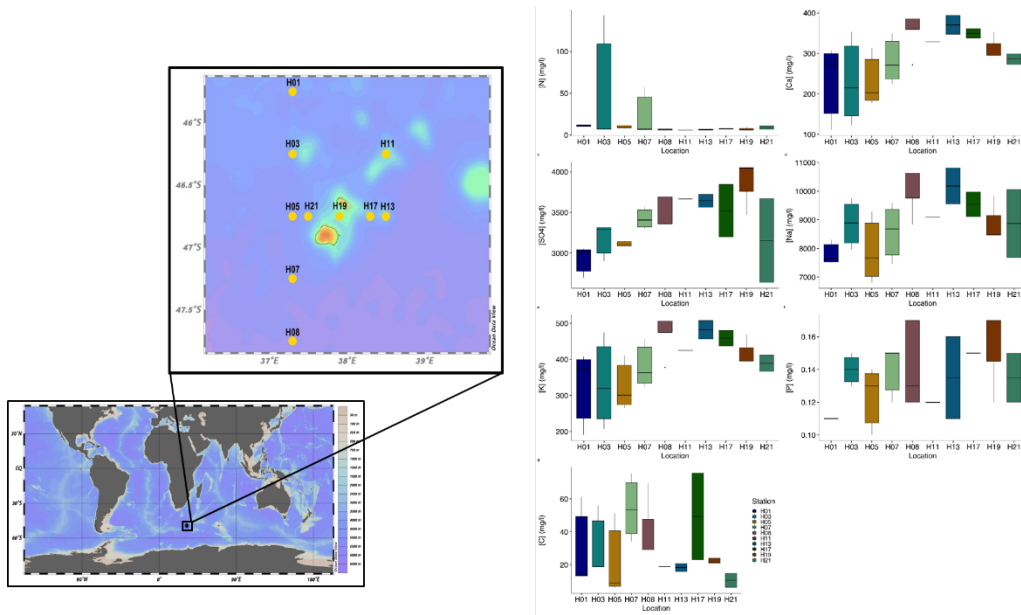


Figure 1: Panel 1) Map of the Prince Edward Islands showing the sampling locations and study area. Water was collected from 10 stations within the vicinity of the PEI's in the 'H-transect'. The sampling distances between South Africa and Antarctica as and the transect are indicated in the bottom and top panels, respectively. Maps were created using Ocean Data View version 5.1.7 (<https://odv.awi.de>, 2019). **Panel 2)** Boxplots showing nutrient concentrations at each location in the sample area indicated in mg/L for a) potassium, b) calcium, c) sulphate, d)carbon, e)phosphorus f)sodium and g) nitrogen.

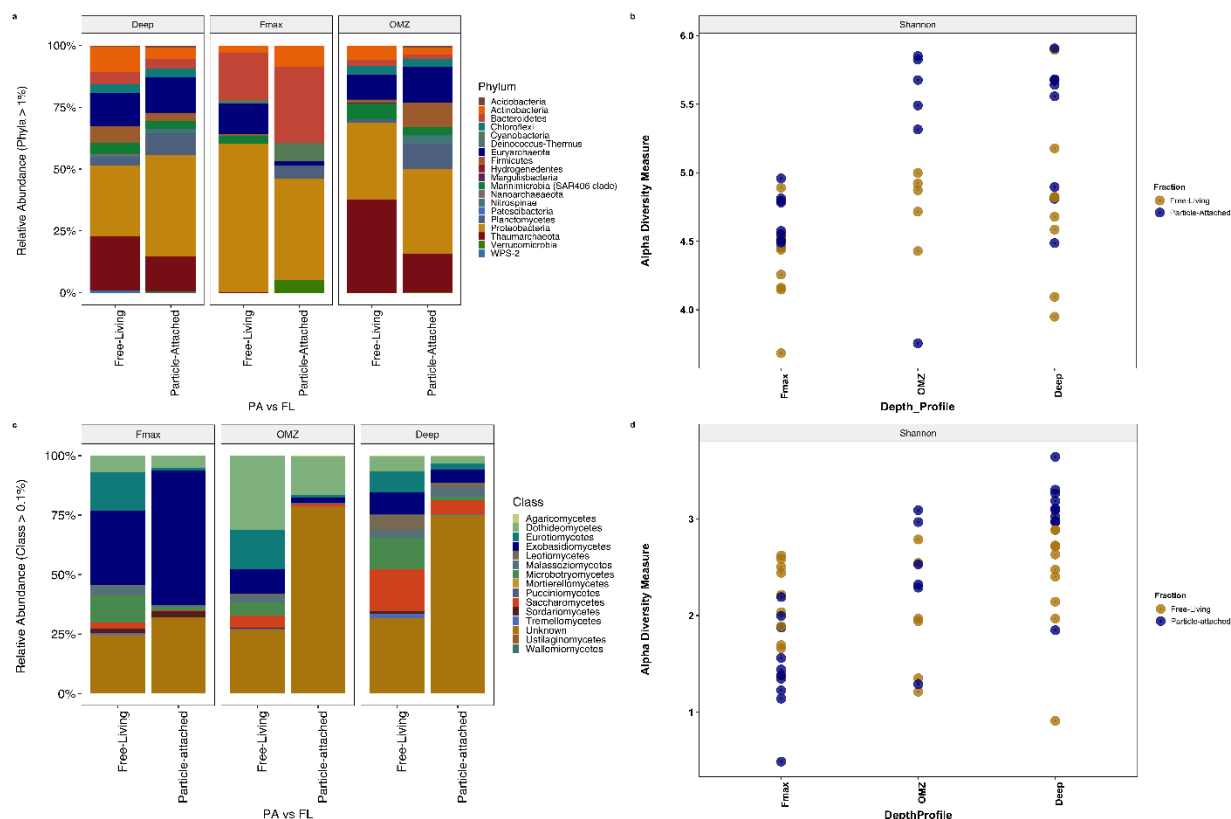


Figure 2. (a) Bar graph showing the relative abundance of FL and PA bacterial and archaeal phyla across samples based on 16S rRNA amplicon sequencing between the three depths. This graph includes phyla that exist at a relative abundance of greater than 1%. (b) Alpha diversity measures for bacteria and archaea across the 3 depth profiles and between PA and FL communities. Individual points are representative of samples and PA and FL samples are indicated by the colours in the legend. (c) Bar graph showing the relative abundance of fungal classes across samples based on ITS 1-4 region amplicon sequencing between the three depth profiles and between the PA and FL fungi in each group. This graph includes only classes that occur at a relative abundance of greater than 0.1%. (d) Alpha diversity measures for fungi across the 3 depth profiles and between PA and FL communities. Individual points are representative of samples and PA and FL samples are indicated by the colours in the legend.

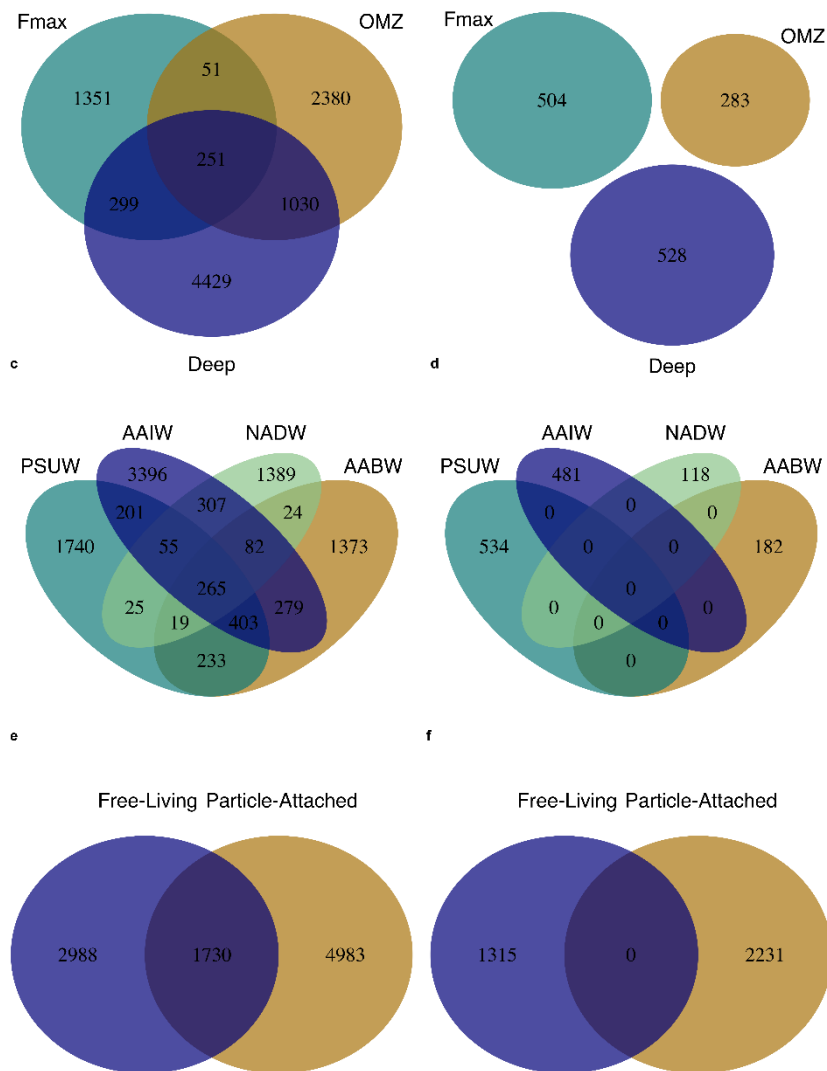


Figure 3: Venn diagrams showing the number of shared OTUs between depth profiles (a & b), water masses (c & d) and lifestyle (e & f) and for bacteria and archaea (a, c & e) and fungi (b, d & f).

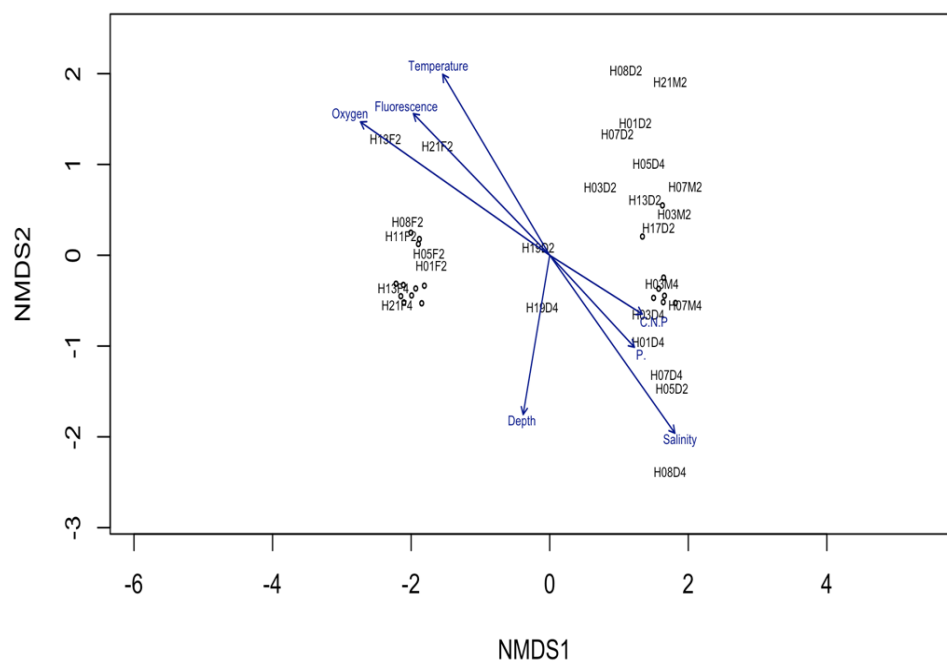


Figure 4: Non-metric multidimensional scaling (NMDS) ordination of marine bacterial and archaeal community in PEI waters. Vectors shown on the ordination plots were significant at $*p < 0.05$ and $***p < 0.001$.

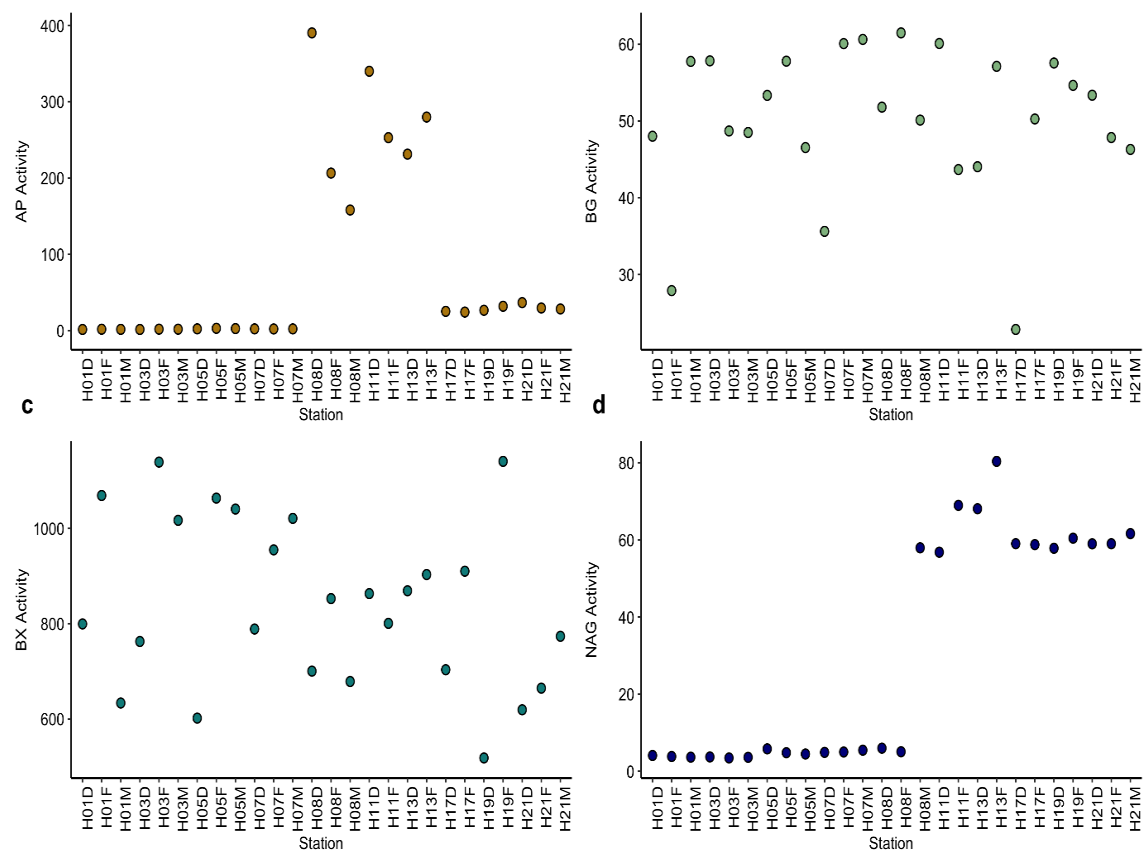


Figure 5. Extracellular activities for four enzymes involved in microbial nutrient acquisition for each sample (represented by individual dots).

683 **Table 1.** Table showing enzymatic activities and significant correlations to environmental
684 parameters

Enzyme	Environmental Parameter	Correlation (r)	p-value	685 686
AP Activity	Na	0.49	0.0010	
AP Activity	K	0.48	0.0013	
AP Activity	Ca	0.46	0.0025	
AP Activity	N	-0.46	0.0025	
AP Activity	Region	0.39	0.012	
AP Activity	NP	-0.32	0.055	
BX Activity	P	-0.32	0.050	
BX Activity	Surface Salinity	0.30	0.076	
BX Activity	Air Temp	0.29	0.082	
NAG Activity	Na	0.49	0.0010	
NAG Activity	K	0.49	0.0010	
NAG Activity	Ca	0.46	0.0022	
NAG Activity	N	-0.42	0.0064	
NAG Activity	NP	-0.34	0.039	

Table 2: Details regarding the specific substrates, concentrations and incubation times for extracellular enzymatic enzyme activity assays.

Enzyme	Substrate	Concentration	Incubation time
β-Glucosidase (BG)	4-Methylumbelliferyl β-D-glucopyranoside 6.77 mg/100 ml	500mM	3hrs
β-Xylosidase (BX)	β-D-xylopyranoside 6.17 mg/100 ml	200mM	4hrs
4-Methylumbelliferyl β-N-acetylglucosaminidase (NAG)	4-Methylumbelliferyl N-acetyl-β-D-glucosaminide 7.59 mg/100 ml	400mM	3hrs
Leucine aminopeptidase (LAP)	L-Leucine-7-amido-4-methylcoumarin hydrochloride 6.5 mg/100 ml (dissolve in 0.5 ml acetone first)	500mM	3hrs
Alkaline phosphatase (AP)	4-Methylumbelliferyl phosphate 5.12 mg/100 ml	100mM	11hrs