Sea ice dynamics drive benthic microbial communities in McMurdo Sound, Antarctica.

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Summary

Globally, Earth's warming climate is evidently driving sea ice variability in high latitude regions. In the Arctic, older and thicker multi-year sea ice is declining and transitioning into younger and more ephemeral first-year sea ice. Whereas in Antarctica, sea ice extent is increasing around the continent, masking strong regional reductions around the Western Antarctic Peninsula. Models predict that sea ice will continue to decline into the 21st Century globally. Reduced sea ice cover is expected to impact marine ecosystem functioning through increased primary productivity at the sea surface and increased flux of organic matter to the seafloor. Seafloor sediments are a critical component of the marine ecosystem as sites of organic matter remineralisation and nutrient regeneration, processes which are driven by microorganisms. Despite their importance, our understanding of sediment biogeochemical processes, their connectivity to the water column and to primary productivity at the surface is limited, particularly in polar regions. This impedes our ability to predict the impacts of climate change on marine ecosystem functioning. This study sampled marine sediments from two strategically chosen sites ~60 km apart in McMurdo Sound, Antarctica, where organic matter concentrations were high due to first-year ice conditions and productive open ocean source waters (Cape Evans), and low due to multi-year ice and oligotrophic source waters (New Harbour). Using high-throughput 16S rRNA gene amplicon sequencing and sediment geochemistry data (chlorophyll-α, phaeophytin, TOC, TN, δ13C, δ15N) this study compared the structure and composition of the sediment microbial communities at these two contrasting sites. The bacterial richness and evenness (alpha diversity) was comparatively greater in the low organic matter sediments underneath the multi-year ice cover than in the high organic matter sediments underneath first-year ice. Significant site-based compositional differences between the two study sites were found. Compositional differences in the high organic matter sediments were driven by known heterotrophic algal biopolymer degrading taxa *Flavobacteriales*, *Cytophagales*, and *Verrucomicrobiales*, and sulfate-reducing bacteria *Desulfobulbales*, potentially reflecting a sediment legacy of high algal-derived organic matter flux. Whereas the low organic matter sediment communities were driven by chemoautotrophic taxa *Nitrosopumilaeles, Nitrospirales*, and *Steroidobacterales* which are known to be involved in carbon fixation and nitrogen cycling, reflecting the legacy of oligotrophic conditions in these sediments. The marine sediments at New Harbour and Cape Evans were not influenced by wind-blown terrestrial surface sediments from the neighbouring Taylor Valley. Additionally, this study supported the hypothesis that subsurface brine channels from the Taylor Valley could be discharging into McMurdo Sound at New Harbour by detecting low abundances of taxa associated with high saline environments (e.g. *Thiohalorhabdales*). Overall, the findings from this study suggest that climate driven sea ice reductions and increased organic matter flux may shift sediment communities from autotrophy towards heterotrophy, thus impacting sediment biogeochemistry. This study contributes towards our understanding of marine sediment processes and marine ecosystem functioning. Additionally, this study provides a baseline of understanding of sediment microbial communities in McMurdo Sound which will support future research further examining community functional capabilities. Finally, this study contributes a first report of the direct impacts of climate driven sea ice change on sediment microbial communities in Antarctica.

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ASV	Amplicon sequence variant
bp	Base pair
CBB	Calvin–Benson–Bassham
CO_2	Carbon dioxide
CH_4	Methane
DNA	Deoxyribonucleic acid
N_2O	Nitrous oxide
M	Molar concentration
MNTD	Mean nearest taxon distance
MPD	Mean pairwise distance
NRI	Nearest relative index
NSTI	Nearest sequenced taxon index
NTI	Nearest taxon index
OTU	Operational taxonomic unit
PCoA	Principal co-ordinates analysis
PCR	Polymerase chain reaction
RCF	Relative centrifugal force
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
rTCA	Reductive tricarboxylic acid
SCUBA	Self-contained underwater breathing apparatus
TCA	Tricarboxylic acid
TOC	Total organic carbon
TN	Total nitrogen

Chapter 1

Literature Review

1.1 Climate change

Since the mid-20th century, Earth's climate has been warming at an accelerated rate, unprecedented over decades to millennia (Pachauri et al., 2014). The Fifth Assessment Report by the Intergovernmental Panel on Climate Change attributes recent global warming to anthropogenic activities driven by economic and population growth which have increased carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) concentrations in the atmosphere (Pachauri et al., 2014). The impacts of global warming are amplified at high-latitudes, particularly in the Arctic which has been warming at more than twice the global average over the past half century (Johannessen et al., 2004). Sea ice conditions are clearly changing which is altering global biogeochemical cycling and impacting marine ecosystem functioning (Dieckmann & Hellmer, 2003; Stammerjohn et al., 2012). There is a crucial need to understand the impacts of climate warming on polar marine ecosystems, not only for the ecological significance, but also for our economic, cultural, and social well-being.

1.2 Sea ice

1.2.1 Sea ice formation

The growth and decay of sea ice is controlled by thermodynamic properties between the ocean and atmosphere (Hibler, 1979). Sea ice begins forming during autumn when air temperatures drop below the freezing point of seawater and the exchange of heat from the ocean to the atmosphere drives the formation of ice crystals at the ocean surface. As sea ice grows, salt is rejected forming brine channels in the ice matrix. Sea ice continues to grow over winter typically reaching maximum thickness in spring before the onset of the summer melt season. Mean maximum sea ice thickness is 2-3 m in the Arctic (Kwok, 2018) and ~ 0.7 m in the Antarctic (Kurtz & Markus, 2012). Sea ice that completely melts and breaks out during summer leaving regions of open ocean is called first-year ice, whereas sea ice that survives past the summer melt season is called multi-year ice. Multi-year ice is considerably fresher and stronger than first-year ice due to the ongoing rejection of brine, often with steep ridges formed in the surface, and generally with a thicker layer of snow cover than first-year ice (Wadhams, 2000). Sea ice may grow as land-fast ice (fast ice) attached to coastal features such as the shoreline, glacier tongues, and ice shelves, or grounded over shoals or may grow as pack ice which is offshore ice that can be moved by wind and ocean currents. The area of sea ice that extends from the permanent ice zone to the winter maximum is called the seasonal ice zone.

1.2.2 Northern hemisphere sea ice

In the Northern hemisphere, sea ice persists in the Arctic Ocean which is a semi-enclosed area surrounded by continental landmasses. These landmasses restrict sea ice movement, allowing ice to survive throughout the summer melt season and grow to several meters thick (Laxon et al., 2003). Sea ice typically reaches a maximum extent of 15×10^6 km^2 in March and minimum of 6×10^6 km^2 in September (NSIDC, 2020a) (*Figure 1*). Passive microwave satellite records show annual sea ice extent has been steadily declining since 1979 (Meier et al., 2014), recently reaching a near 42-year record minimum extent in September 2020 (NSIDC, 2020b). The once dominant multi-year ice cover is rapidly declining and transitioning into more ephemeral first-year ice (Meier et al., 2014).

It has also been observed that sea ice retreat is occurring earlier in the season and freeze-up is occurring later, leading to a longer summer ice-free period (Stammerjohn et al., 2012; Stroeve et al., 2016). The loss of sea ice cover in the Arctic has been attributed to the effect of surface albedo feedback where shrinking snow and ice cover has increased absorption of solar radiation, thus driving further ice melt (Hall, 2004; Thackeray & Hall, 2019). Sea ice trends are expected to continue with climate models predicting that an ice-free summer in the Arctic could ensue by the end of the 21st century (Notz & Stroeve, 2018; Overland & Wang, 2013). It is also expected that precipitation in the future will be dominated by rain rather than snow (Bintanja & Andry, 2017), further impacting growth and decay rates of sea ice (Webster et al., 2014).

1.2.3 Southern hemisphere sea ice

In the Southern hemisphere, sea ice grows around the continental landmass of Antarctica and extends out into open ocean, with the northern extent mostly limited by the Antarctic Circumpolar Current (Martinson, 2012). During summer in the Antarctic, the sea ice retreats close to the continental coastline, therefore most of Antarctic sea ice exists as relatively thin (<1 m) first-year ice (Parkinson & Cavalieri, 2012). The majority of the multi-year ice that does exist in Antarctica resides in the Weddell Sea region (east of the Antarctic Peninsula), while the remainder persists in sheltered embayments around the continent (Parkinson & Cavalieri, 2012). Sea ice extent is more seasonally variable in Antarctica compared to the Arctic with extent typically reaching a maximum of 19×10^6 km^2 in September and minimum of 3×10^6 km^2 in February (NSIDC, 2020a) (*Figure 1*). In contrast to the Arctic, sea ice extent in Antarctica has been increasing overall during the past 4 decades, however this masks strong regional reductions in the West Antarctic Peninsula (Parkinson, 2019; Stammerjohn et al., 2012). Since the start of the record in 1978, annual Antarctic sea ice extent was highest in 2014 but was followed shortly after by major reductions until the record low was reached in 2017 (Parkinson, 2019). It is uncertain whether these reductions are part of normal inter-annual variability for Antarctica (Parkinson, 2019). Sea ice increases in Antarctica have been attributed to changes in atmospheric and oceanic circulation linked to stratospheric ozone hole depletion (Thompson & Solomon, 2002; Turner et al., 2009), variability of the Amundsen Sea Low (low pressure centre off west Antarctica) (Turner et al., 2015), variability of the El Niño–Southern Oscillation and Southern Annular Mode (Stammerjohn et al., 2008), and the accelerated basal melting of ice shelves (Bintanja et al., 2013). Climate models predict that substantial sea ice reductions will occur around Antarctica by the end of the 21st century with estimated reductions ranging from 16-67% in February and 8-30% in September (Collins et al., 2013).

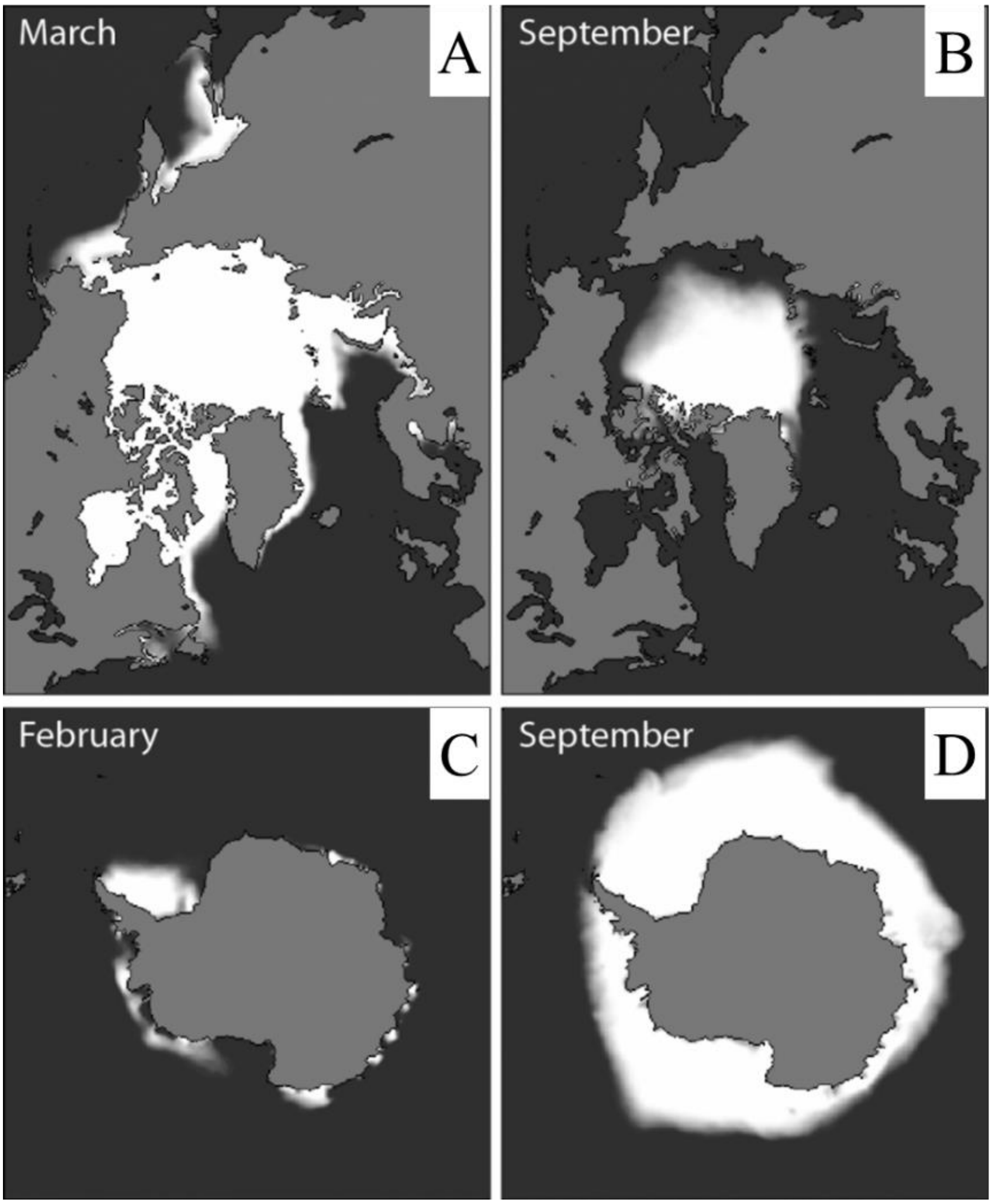

Figure 1. Sea ice extent depicted in white from 1981-2010 based on passive microwave satellite data. Arctic sea ice maximum extent in March (A) and minimum extent in September (B) and Antarctic minimum extent in February (C) and maximum in September (D). Image modified from the National Snow and Ice Data Centre, University of Colorado, Boulder (NSIDC, 2020b).

1.3 Polar ocean primary productivity

Sea ice within polar oceans plays a critical role in supporting ecosystem functioning and in regulating the global climate (Dieckmann & Hellmer, 2003). At the air-sea interface, anthropogenic CO_2 exchange is largely driven by biological uptake from autotrophic single-celled algae which convert CO_2 and inorganic nutrients into oxygen and organic matter (Feely et al., 2001). These microalgae are found in association with both the sea ice (sea ice algae) and the underlying water column (phytoplankton) where conditions are ideal for growth (Arrigo, 2014). The growing season of these primary producers is seasonally limited due to sunlight availability as 6 months of the year are spent in darkness, resulting in a large pulse of growth during spring and summer which stimulates a feeding response throughout the food web (Arrigo, 2014). Primary producers are fed on by zooplankton which are primarily fed on by fish in the Arctic and seabirds and marine mammals in the Antarctic (Murphy et al., 2016). While the contribution of sea ice algae to total annual productivity is often small compared to phytoplankton (1-10% (Arrigo, 2016)) its ecological importance is great as it serves as a sole food source for grazers throughout winter when phytoplankton stocks are low (Kohlbach et al., 2017). It has already been observed that climate-driven reductions in sea ice cover have altered sea ice algae and phytoplankton populations with cascading effects within the marine ecosystem (Post et al., 2013). For example, in the Arctic, sea ice reductions have altered distributions of Arctic cod, a key foraging species which is consumed by higher trophic levels such as ringed seals and beluga whales (Huserbråten et al., 2019). In the Southern hemisphere, the northern West Antarctic Peninsula has seen dramatic declines in sea ice extent which has resulted in a latitudinal shift of ice-dependant Antarctic krill and Adélie penguins poleward with a replacement by salps from the north which are fed on by Gentoo and Chinstrap penguins (Atkinson et al., 2004; Constable et al., 2014; Ducklow et al., 2007).

1.3.1 Seasonal bloom

Sea ice has a primary role in regulating the timing and magnitude of the sea ice algal and phytoplankton blooms (Arrigo, 2014). As sunlight and temperatures start to increase in spring, growth of the sea ice algae begins first. Sea ice algal communities are typically dominated by diatoms which are adapted to grow under low light conditions (Arrigo, 2014) as light can be reduced by more than 99% underneath 2.5 m of first-year ice cover (Schwarz et al., 2003). By late spring or early summer, sea ice algae typically reach peak bloom just prior to the melt and break out of sea ice. At this time, sea ice algae absorb most of the available sunlight, preventing light from reaching the upper water column (Arrigo et al., 1991). As the sea ice melts out during summer, the habitat is lost, and the algae are rapidly released as an intense seasonal pulse into the water column where they can be exported to the seafloor (Nadaï et al., 2020). After the sea ice algal biomass subsides, phytoplankton adapted to higher light levels typically bloom as more sunlight becomes available (Arrigo et al., 2012). Phytoplankton productivity is greatest at the edge of the sea ice where a layer of nutrient-rich melt water forms from the melting sea ice, providing a stable and well-lit environment (Smith & Nelson, 1986). The phytoplankton bloom is generally longer lived than the sea ice algal bloom (Wassmann & Reigstad, 2011).

Under future projections of thinning and reduced sea ice cover, it is expected that in regions where first-year ice replaces multi-year ice that there will be a general increase in primary productivity in response to increased light availability (Tedesco et al., 2019) (*Figure 2*). It is also likely that the earlier timing of the ice and snow melt season is expected to result in an earlier start to the under-ice algal bloom (Nadaï et al., 2020) and extension of the phytoplankton bloom (Post, 2017), however the impacts of associated nutrient limitation may restrict extended growth (Vancoppenolle et al., 2013) (*Figure 2*). Increased open water conditions are likely to favour water column species over sea ice dependant species (Tedesco et al., 2019).

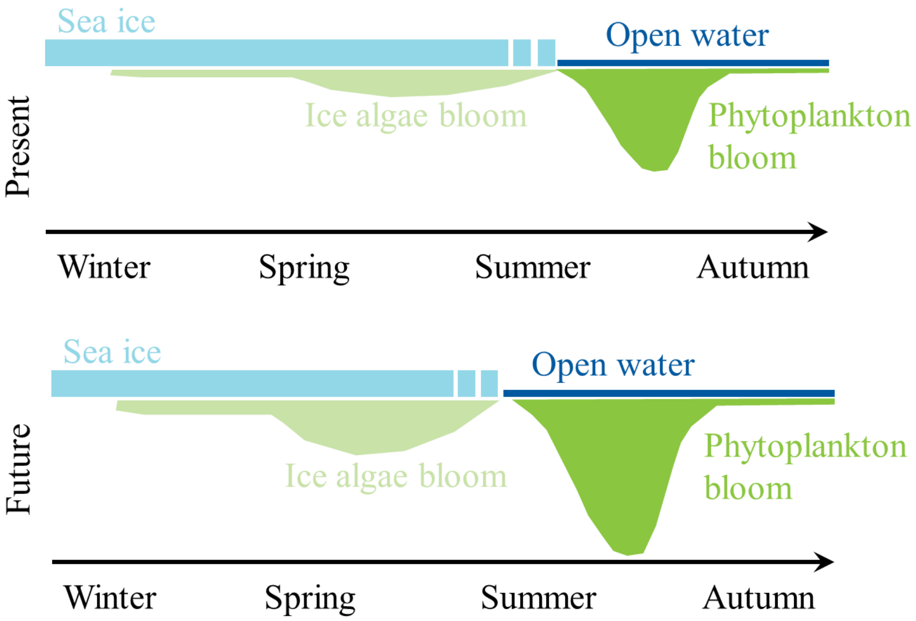

Figure 2. Timing and magnitude of sea ice algae and phytoplankton blooms in a seasonal ice zone region. Present day scenario (top) and predicted future scenario with climate warming (below). Illustration modified from (Wassmann & Reigstad, 2011).

1.3.2 Carbon export

Following the summer algal and phytoplankton blooms, consumer abundances increase in response to increased food supply (Leu et al., 2011) and the upper water column shifts from being autotrophic (organisms which synthesise their own food from inorganic nutrients) towards being more heterotrophic (organisms which consume organic material for nutrition) (Wassmann & Reigstad, 2011). Heterotrophic communities of zooplankton and bacteria with high demands for organic carbon rapidly consume and remineralise the majority of the particulate and dissolved organic matter within the upper water column (Harvey, 2006). Dissolved organic matter is primarily degraded by bacteria which use it for growth and respiration, the process of which regenerates nutrients such as nitrogen and phosphorus into the upper water column supporting primary production (Middelburg, 2019b). The bacterial biomass is also consumed by microzooplankton, thus supporting the heterotrophic food web by converting dissolved organic carbon to zooplankton (Middelburg, 2019b). Another important pathway for bacterial biomass involves viral lysis which causes the release of dissolved organic carbon into the water column (Suttle, 2005).

A small proportion of biological material that escapes consumption in the water column sinks to the seafloor, mostly in the form of particulate organic matter (Lønborg et al., 2020). For example, in the West Antarctic Peninsula it is estimated that ~10% of net primary production is exported to seafloor (benthic) sediments (Henley et al., 2017). Vertical export is largely determined by the dominance of the algal and phytoplankton communities and the grazing pressure of primary consumers (Wassmann et al., 2004). For example, sea ice algae are more rapidly exported to the seafloor than phytoplankton as they are able to accumulate into fast sinking aggregates or marine snow (Fernández-Méndez et al., 2014). It is generally assumed that rapid carbon export enhances the quality of carbon reaching the seafloor as it spends less time being degraded in the water column (Middleburg, 2019a). As consumer abundances increase later in the bloom season, high grazing impact and increased faecal pellet production can reduce the quality and quantity of carbon exported (Reigstad et al., 2008).

Under future climate warming scenarios, it is expected that reduced sea ice cover and increased surface productivity will increase the flux of organic matter to the seafloor (Boetius et al., 2013; Nadaï et al., 2020) and that an earlier timing of sea ice break out will drive earlier export of organic matter to the seafloor (Wassmann & Reigstad, 2011). Also, the intensity of the fluxes are expected to decrease and become steadier in the future (Wassmann & Reigstad, 2011). These alterations are likely to impact benthic sediment biogeochemical processes, however there is a current paucity of information on the connectivity of processes between benthic and water column (pelagic) systems and how such changes will impact marine ecosystem functioning.

1.4 Marine benthic sediments

Marine benthic sediments cover ~70% of the Earth's surface and are an accumulation of unconsolidated material originating from the erosion of continents, volcanic activity, biological productivity, hydrothermal vents, and cosmic debris (Seibold & Berger, 2017). Sediments are crucially important to marine ecosystem functioning as a habitat for diverse organisms of all domains of life which play a central role in organic matter degradation and remineralisation and biogeochemical cycling, thus impacting the global climate (Snelgrove et al., 2018). Sediment biogeochemical processes are largely driven by the deposition of organic matter and addition of oxygen from the water column (Snelgrove et al., 2018). Sediments are composites of particles and water and due to their porosity water is allowed to flow through them and nutrients can be exchanged with the water column, creating a close benthic-pelagic coupling (Huettel et al., 2014).

However, despite their importance our understanding of the processes which occur in marine sediments is limited and under-represented in marine research compared to what we know of the processes of the water column and beneath the sea ice, impeding our ability to predict the impacts of climate-driven sea ice change on the marine ecosystem.

1.4.1 Organic matter remineralisation

Organic matter decomposition is a central process within marine sediments (Aller, 1994). Organic matter is transformed primarily by diverse microorganisms (bacteria and archaea) within the sediments into essential inorganic nutrients that accumulate within the sediment porewater space which can then be diffused or advected back into the water column (Soetaert et al., 2000). Nutrient flux from the sediments to the water column is critically important for marine ecosystem functioning as it fuels primary productivity (Griffiths et al., 2017). The rate and direction of nutrient diffusion acts in response to nutrient concentration gradients between the porewater and water column, thus sediments may be a source or sink for nutrients (Huettel et al., 2014). Benthic macrofauna play a primary role in regulating porewater exchange rates as they burrow and rework the surface sediments during feeding, a process known as bioturbation (Huettel et al., 2014).

It is estimated that ~90% of the organic matter that reaches the seafloor is remineralised (Middelburg, 2019b). The quality and quantity of the organic matter within the sediments is an important factor controlling the rate at which remineralisation can occur (Mayor et al., 2012). For example, diatoms which are rich in labile compounds such as sugars, lipids, and amino acids are more rapidly mineralised than zooplankton faecal pellets which contain higher quantities of insoluble carbohydrates (Mayor et al., 2012). In general, organic matter lability decreases with sediment depth (Middelburg, 2019a).

1.4.2 Sediment biogeochemistry

In theory, during the process of remineralisation, organic matter proceeds along a degradation pathway based on the availability of terminal electron acceptors that are utilised in microbially-driven reduction-oxidation (redox) reactions to oxidise organic compounds (Aller, 1994). Redox reactions are thermodynamically driven and involve the transfer of electrons between chemical species to gain metabolic energy. Within marine sediments, a vertical redox profile forms due to preferential depletion of the highest energy-yielding electron acceptors associated with organic matter deposition (*Figure 3*). Oxygen is the most favourable electron acceptor and is depleted first due to aerobic respiration. In organic matter-rich sediments, such as in productive continental coast regions, high oxygen consumption typically results in a shallow oxic sediment layer only a few millimetres or centimetres deep (Middelburg, 2019b). Whereas in organic matter-poor sediments, such as beneath the deep sea South Pacific Gyre, oxygen can penetrate meters into the seafloor (Fischer et al., 2009). Once oxygen is depleted, organic compounds are oxidised by anaerobic bacteria using terminal electron acceptors such as nitrate, manganese (IV), iron (III), and sulfate, followed by CO_2, methanogenesis and/or fermentation (*Figure 3*). Sulfate reduction is the dominant anaerobic carbon metabolic process across many sediment environments due to the high sulfate abundance in seawater (~28 mM) (Capone & Kiene, 1988). Macrofauna bioturbation enhances microbial remineralisation processes by introducing higher quality organic matter and oxygen from the surface into deeper sediment layers (Lohrer et al., 2004).

Reduced compounds (metabolites) such as ammonium, manganese (II), iron (II), hydrogen sulphide, and methane contain a considerable amount of energy originally part of the organic matter and are efficiently utilised in re-oxidation reactions (Aller, 1994). Reduced metabolites can diffuse upwards through the sediment profile and react with an electron acceptor higher in the redox profile, often involving multiple reactions and intermediate compounds (Middelburg, 2019a). Chemolithoautotrophic metabolisms can use the energy released from re-oxidation reactions to support inorganic carbon fixation (Middelburg, 2011). Dark carbon fixation is an important carbon cycling process which sustains food webs by producing organic matter independently from photosynthesis (Vasquez-Cardenas et al., 2020).

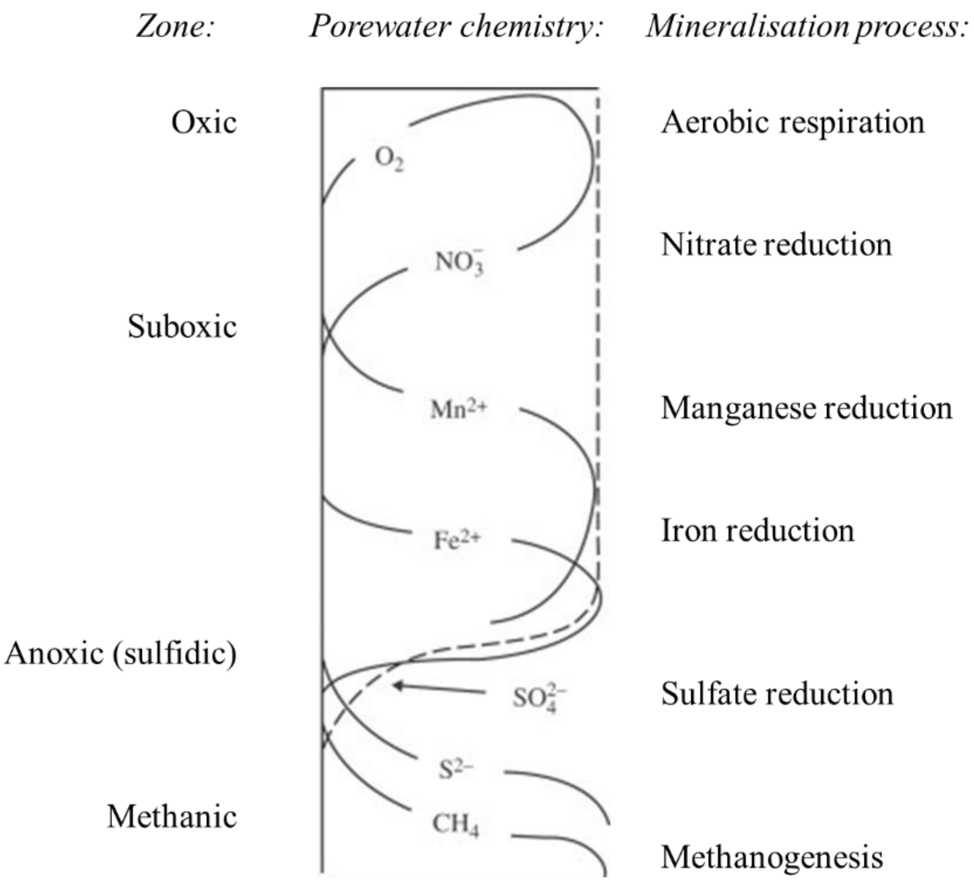

Figure 3. Schematic of simplified reduction-oxidation profile in marine sediments. The sediment-water interface is at the top of this schematic and is depicted by the oxic zone where oxygen is present. Each subsequent zone represents more reduced conditions as more favourable electron acceptors are depleted. To the right are the processes associated with available terminal electron acceptors during organic matter degradation. Illustration modified from (Megonigal et al., 2003).

1.4.3 Sediment microbial communities

Microorganisms are highly abundant within marine sediments, generally dominating the total benthic biomass (Kallmeyer et al., 2012) and the functions they perform are profoundly important for marine ecosystem functioning and global carbon cycling (Snelgrove et al., 2018). However, there is a paucity of information of sediment microbial communities, especially in polar regions. Microbial communities have been increasingly recognised as useful models to understand ecosystem functioning due to their central role in driving essential nutrient cycles (Falkowski et al., 2008) and ubiquitous distribution in marine sediments (Kallmeyer et al., 2012). Furthermore, their vast metabolic diversity and responsiveness to the environment identifies microorganisms as ideal sentinels for understanding the effects of climate change (Cavicchioli et al., 2019).

Studying the taxonomic diversity of microbial communities in relation to their environmental conditions is one approach to understanding and predicting how communities respond to environmental change. The two components that are used to measure community diversity are the taxon richness and the relative abundance of different taxa within the community (Konopka, 2009). Marine sediment communities can be directly and indirectly influenced by a variety of physical and chemical parameters including water depth (Li et al., 2020), oxygen availability (Zinger et al., 2011), temperature (Nguyen & Landfald, 2015), and biodiversity at the sediment-water interface (Austen et al., 2002). It has been shown in previous studies that organic carbon content is also a strong driver of microbial community diversity within marine sediments. For example, increased chlorophyll pigment concentrations (as a proxy for phytodetrital inputs to sediments) was correlated with increased bacterial richness in a transect study of the Siberian continental shelf in the Arctic (Bienhold et al., 2012). Another study comparing communities in sediments of high and low chlorophyll concentrations in the Crozet Islands, Antarctica, reported bacterial richness to be only marginally greater in the low chlorophyll sediments (Jamieson et al., 2013). In contrast to these studies, increased bacterial richness and evenness was correlated with decreased sediment organic carbon content in a transect study in west Antarctica, spanning the Ross Sea to the Antarctic Peninsula (Learman et al., 2016). More studies are needed to better understand the impact of sediment organic matter content on microbial community taxonomic diversity to predict change in ecosystem functions.

Key microbial taxa have been identified in previous studies of marine sediments that are distinctly reflective of the organic matter content and oxygen availability within the sediment profile. For example, in sediments with high organic matter concentrations a dominance of heterotrophic taxa such as *Gammaproteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* have been reported within communities, including in Northern Hemisphere sediments of the Helgoland Mud Area, North Sea (Oni et al., 2015) and in Southern Hemisphere sediments of the Antarctic Peninsula (Learman et al., 2016) and the Antarctic Polar Front (Ruff et al., 2014). Another well-known group often found in organic matter-rich sediments are *Deltaproteobacteria* which are associated with sulfate-reduction in anoxic sediment layers, such as in the Svalbard fjords of the Arctic (Jørgensen et al., 2020). In the organic matter-rich Adélie Basin sediments of Antarctica, methanogenic archaea such as *Methanosaeta* were found to be highly active members of the community (Carr et al., 2018).

In contrast, in sediments where organic matter content is low and oxygen penetrates deep through the sediment profile, such as in the South Pacific abyss, a dominance of chemolithoautotrophic ammonia-oxidising taxa such as *Thaumarchaeota* have been reported (Durbin & Teske, 2011). *Thaumarchaeota* were also found to be abundant in sediments beneath the Ross Ice Shelf in Antarctica where complete year-round darkness prevents *in situ* photosynthetic primary productivity (Carr et al., 2013). In sediments of the ultraoligotrophic South Pacific Gyre, *Alphaproteobacteria*, and nitrogen cyclers *Nitrospirae* and *Nitrospirae* were found to be dominant community members (Tully & Heidelberg, 2016).

1.5 McMurdo Sound

1.5.1 Regional setting

McMurdo Sound is a marine inlet ~60 km wide and 80 km long located in the southwestern corner of the Ross Sea, Antarctica. McMurdo Sound is bordered by three contrasting environments with the volcanic Ross Island to the east, the largest floating ice shelf on the planet ($\sim 4.7 \times 10^5$ km^2) (Stevens et al., 2020), the Ross Ice Shelf, to the south, and the Trans Antarctic Mountains and McMurdo Dry Valleys to the west (*Figure 4*). The bathymetry of McMurdo Sound is steep and rough with western shelf depths reaching ~200 m which gradually deepens to over 800 m nearer the east before rising to ~150 m at the eastern shelf (Barry, 1988).

McMurdo Sound has been a valuable ecological research site for over the past 4 decades due to east-west environmental gradients (Dayton & Oliver, 1977), isolated conditions, and the close proximity to two major research bases, Scott

Base (New Zealand) and McMurdo Station (United States of America), which are located on the southern side of Ross Island (*Figure 4*).

1.5.2 Ross Sea productivity

The Ross Sea is the most biologically productive sector of the Southern Ocean due to the presence of the coastal Ross Sea Polynya, with an estimated annual productivity of ∼180 g C m^{-2} yr^{-1} (Arrigo et al., 2008). The Ross Sea Polynya is an ~25,000 km^2 area of open water that is maintained by large-scale atmospheric circulation and strong katabatic winds blowing from southern Marie Byrd Land and from glacial regions of the eastern Transantarctic Mountains (Bromwich et al., 1992). As the elevation of the sun increases in Antarctica during early spring, the open waters of the Ross Sea Polynya allow sunlight to reach primary producers earlier in the season than in ice-covered regions, thus greatly stimulating productivity (Smith et al., 2014). Macronutrients (nitrate, phosphate, and silicate) are rarely depleted in the Ross Sea, despite high rates of productivity, whereas iron is a limiting nutrient for growth (Smith et al., 2014). The McMurdo Sound marine system receives nutrient inputs from the highly productive Ross Sea via southbound ocean currents which enter the eastern side of McMurdo Sound as they follow the coastline of Ross Island (Barry, 1988) (*Figure 4*).

1.5.3 Oceanographic circulation in McMurdo Sound

Currents within McMurdo Sound follow a cyclonic pathway and have a net southward flow in the east and northward flow in the west (Barry & Dayton, 1988). Relatively warmer and nutrient-rich currents that enter McMurdo Sound from the Ross Sea continue to flow south down the coastline of Ross Island where most of the flow submerges into the cavity of the floating Ross Ice Shelf (Barry, 1988; Robinson et al., 2010) (*Figure 4*). The remainder of the flow is deflected westwards across the front of the ice shelf (Robinson et al., 2010). Underneath the Ross Ice Shelf there is an estimated cavity residence time of ~4-6 years (Holland et al., 2003; Smethie & Jacobs, 2005; Stevens et al., 2020) during which time advected phytoplankton settle through the water column (Vick-Majors et al., 2016). Currents emerge again from underneath the ice shelf in the west side of McMurdo Sound, relatively cooler and oligotrophic (Barry & Dayton, 1988), where they travel north following the Victoria Land coastline (*Figure 4*). Barry & Dayton (1988) reported that phytoplankton biomass in the water column was as low as 10% on the west side of McMurdo Sound compared to the east. Current velocities are typically weaker in the western McMurdo Sound than in the east (Dayton & Oliver, 1977), further reducing the advection of primary production in the west (Barry & Dayton, 1988).

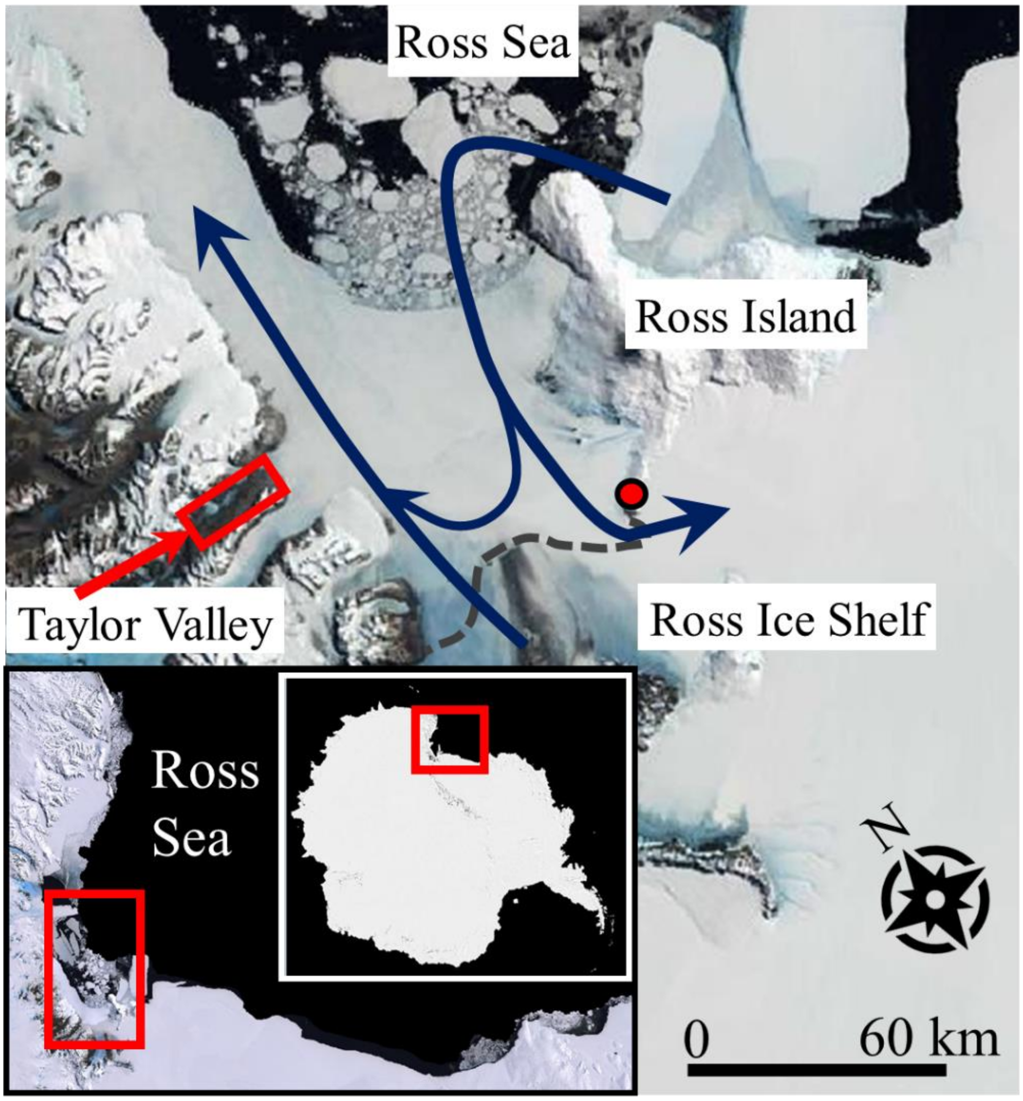

Figure 4. Landsat satellite image of McMurdo Sound in the Ross Sea, Antarctica. McMurdo Sound is bound by Ross Island to the east, the Ross Ice Shelf to the south (hashed line shows shelf edge), and continental mainland to the west where Taylor Valley is located. The research bases, Scott Base and McMurdo Station (red dot) are located on the south side of Ross Island. Dominant oceanographic current circulation patterns and directions (blue arrows) are overlaid on the map.

1.5.4 McMurdo Sound sea ice conditions

Fast ice is present in McMurdo Sound throughout the year with seasonal variations observed in the east and west sides of the Sound (Kim et al., 2018). In the east, first-year ice consistently persists until late spring and early summer when relatively warmer ocean swells from the Ross Sea break up the ice and ice floes are transported northwards out into the Ross Sea by wind (Kim et al., 2018). Under normal conditions, areas of the northern Sound are usually clear of first-year ice around October or November, by mid-December sea ice has broken out midway in the Sound, and by late February or early March the extent of the break-out nears the edge of the Ross Ice Shelf (Dayton & Oliver, 1977). Sea ice begins re-forming during late March around the southern margin from Ross Island and extends northwards (Dayton & Oliver, 1977). In the west along the Victoria Land coast, multi-year ice usually persists throughout the summer, remaining for multiple consecutive years (Kim et al., 2018). The infrequent and brief break up of sea ice in the western Sound would usually occur after February and may only last for several days, during which time ice may still remain in the area (Knox, 1990).

1.5.5 Surface primary productivity in McMurdo Sound

A major contribution to primary productivity in McMurdo Sound comes from sea ice algae and phytoplankton (Knox, 1990). The early bloom season is typically initiated in mid-November and is dominated by diatoms, followed by a bloom of water column associated *Phaeocystis* from mid-December to early January, and later another diatom bloom in late January-February (Knox, 1990). In the east McMurdo Sound, the presence of first-year ice that breaks out annually drives high surface primary productivity whereas in the west surface productivity is severely restricted by thicker multi-year ice cover (Knox, 1990). Diatom flux in the east Sound is an order of magnitude higher than in the west (Leventer & Dunbar, 1987).

1.5.6 Terrestrial influence on McMurdo Sound benthic sediments

1.5.6.1 Taylor Valley surface sediment transport

McMurdo Sound neighbours the McMurdo Dry Valleys located on the mainland which is the largest ice-free region in Antarctica (Levy, 2013). The Dry Valleys contain perennially ice-covered lakes, glaciers, transient streams, and extensive areas of soil and bedrock. The Taylor Valley is the southernmost of the McMurdo Dry Valleys and lies in an west-east direction ~30 km long with the valley terminus opening out into Explorers Cove of McMurdo Sound (*Figure 4*). Strong winds blow down the Taylor Valley during winter transporting sediment towards Explorers Cove (Nylen et al., 2004). Previous research has shown that the contribution of wind-blown surface sediment material from the Taylor Valley to benthic sedimentation in McMurdo Sound is minimal (Chewings et al., 2014). However, wind-blown sediment from the Taylor Valley does accumulate on the sea ice near the shoreline of Explorers Cove where ridges in the multi-year ice can reach up to 3 m high (Murray et al., 2013) (*Figure 5*). It has been reported that during summer as cracks form near pressure ridges in near-shore regions of the sea ice that small amounts of wind-blown sediment from the ice surface may be channelled through and deposited beneath the sea ice on the seafloor (Murray et al., 2013). Despite the high microbial diversity found in the Taylor Valley soils (Cary et al., 2010), microorganisms have not yet been used to investigate the connection between the terrestrial and benthic marine ecosystems in this region.

1.5.6.2 Subsurface brine aquifer discharge

Recent electromagnetic survey of the Taylor Valley has detected two deep subsurface brine aquifers lying beneath a layer of permafrost, reaching up to 300 m depth (Mikucki et al., 2015). These subsurface brine aquifers are hypothesised to discharge into McMurdo Sound at Explorers Cove and to be rich in soluble iron (Foley et al., 2019; Mikucki et al., 2015) (*Figure 5*). Brine inputs into the aquifer system are thought to originate from iron-rich subglacial water emerging from the Taylor Glacier at the head of the Taylor Valley which flow out through Blood Falls and into Lake Bonney (Mikucki et al., 2015) (*Figure 5*). Further down the valley, a second brine aquifer is hypothesised to connect Lake Hoare to Lake Fryxell before discharging along the coastal margin of McMurdo Sound (*Figure 5*). It has been estimated that 20 Gg yr^{-1} of soluble iron is discharged into McMurdo Sound via the subsurface aquifers (Foley et al., 2019). The findings from these studies supported earlier reports from the Dry Valley Drilling Project of the 1970's that boreholes drilled 183 m below sea level in the Taylor Valley near McMurdo Sound filled with liquid brine (Cartwright & Harris, 1981). Investigating the presence of the same bacterial taxa in sediments of New Harbour and in lake sediments of the Taylor Valley to validate the potential subsurface connectivity has not previously been done but is an approach commonly used to detect the origin of pathogens in water monitoring called microbial source tracking (Scott et al., 2002).

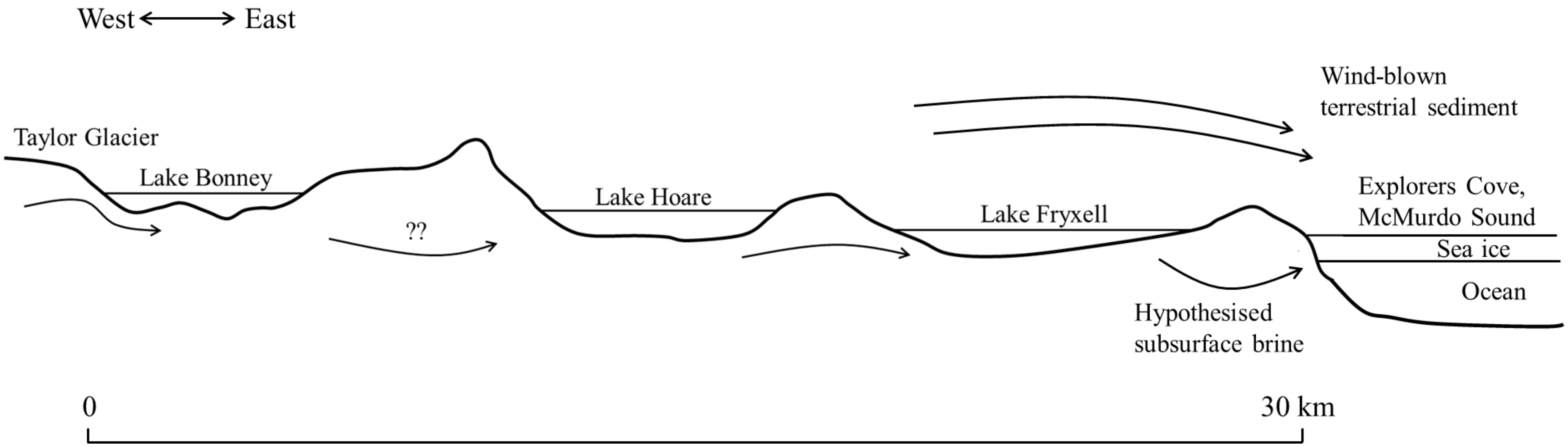

Figure 5. Schematic of the hypothesised terrestrial surface and sub-surface nutrient inputs from the Taylor Valley into the marine McMurdo Sound marine system at Explorers Cove. Based on proposed wind-blown sediment (Murray et al., 2013) and hypothesised brine aquifer discharge (Mikucki et al., 2015). Illustration modified from (Mikucki et al., 2015).

1.5.7 McMurdo Sound benthic ecosystem

Previous studies of the McMurdo Sound benthic ecosystem have been centred on describing the macrofauna communities and what drives their abundance and distribution. Considerable differences in macrofauna assemblages have been found between the west and east sides of McMurdo Sound and also, but to a lesser extent, across the north-south gradient and with depth (Dayton & Oliver, 1977; Thrush et al., 2006). Macrofauna densities in the east Sound are comparable to the most productive sites found anywhere in the world while those in the west are scarce and resemble those of the deep ocean (Dayton & Oliver, 1977). These east-west differences in benthic communities have been attributed to first-year ice persistence and considerably greater advected phytoplankton biomass from the Ross Sea Polynya in the east compared to the west where multi-year ice persists and oligotrophic waters flow from underneath the Ross Ice Shelf (Barry, 1988; Dayton & Oliver, 1977). A previous study in McMurdo Sound has shown that sediment organic matter content is higher underneath thinner first-year sea ice and lower under thicker multi-year sea ice (Lohrer et al., 2013).

More recently, research has been centred on better understanding the impacts of climate-driven sea ice change on the marine ecosystem in McMurdo Sound (Cummings et al., 2019; Dayton et al., 2019; Lohrer et al., 2013, 2020; Wing et al., 2018). Recent work using benthic incubation chambers to measure nutrient efflux across the sediment-water interface at two contrasting sites found that elevated quantities of algal detrital matter within sediments beneath thinner and less persistent sea ice corresponded to significantly greater benthic oxygen consumption and a tighter coupling of nitrogen and denitrification pathways (Lohrer et al., 2013). This study also reported that the role of benthic macrofauna in regenerating nutrients was enhanced at the site with more algal material in the sediments. In another recent study, the impact of increasing frequency of multi-year ice break out between the years of 2009-2017 in the western McMurdo Sound showed that in response to greater quantities of sediment algal material that benthic macrofauna abundances and benthic oxygen consumption increased (Lohrer et al., 2020). Together these studies have shown that increased algal flux to sediments enhances heterotrophic metabolic processes within the sediment environment. However, despite the central role that microorganisms play in organic matter remineralisation and biogeochemical cycling, and their use as sensitive sentinels for environmental change (Cavicchioli et al., 2019), the sediment microbial communities in McMurdo Sound have not been previously studied.

1.6 Methods to study microbial communities

1.6.1 Molecular approach

Cultivation-based techniques have only been able to achieve the isolation and characterisation of an estimated 1% of bacteria on Earth due to the difficulty of culturing bacteria in the laboratory (Schloss & Handelsman, 2004). The development of culture-independent molecular approaches based on sequencing nucleic acids (DNA) has significantly advanced the field of microbial ecology, unveiling deeper insight into the taxonomic and phylogenetic structure of microbial communities and their functions (Lasken & McLean, 2014). Two DNA sequencing based approaches that are commonly used to taxonomically profile a microbial community are gene amplicon sequencing and whole metagenome shotgun sequencing.

The gene amplicon sequencing approach targets a highly conserved genetic marker common in all bacteria, the 16S ribosomal RNA (rRNA) gene. This ~1,500 base pair gene encodes the small subunit rRNA molecules of ribosomes and contains 10 highly conserved regions which are interleaved with 9 taxonomically informative variable and hypervariable regions (*Figure 6*). In this approach, following genomic DNA extraction from a sample, polymerase chain reaction (PCR) techniques are used to amplify a DNA region of interest by using a pair of primers which are designed to bind to the conserved regions and synthesise a complementary strand of DNA from the variable region (e.g. Parada et al., 2016). Millions of copies of the targeted DNA are produced during the PCR process which can then be effectively sequenced using high-throughput DNA sequencing technology. The raw sequencing data is processed in silico using bioinformatics to determine which taxa are present and their relative abundance. Targeted gene amplicon sequencing of environmental samples has been used extensively over the past 30 years to taxonomically profile and describe the diversity of bacterial and archaeal communities, revealing an unexpected huge diversity of microorganisms on Earth (Lozupone & Knight, 2007; Pace, 1997; Rappé & Giovannoni, 2003).

In the second approach, whole metagenome shotgun sequencing is used to investigate total DNA and generates information on taxonomy and functional genes. Following genomic DNA extraction from a sample, it achieves this by overlapping fragments of native DNA in parallel during high-throughput sequencing then assembles the fragments in silico into larger contiguous sequences (contigs) which then get reassembled into larger fragments (scaffolds) (Sharpton, 2014). Whole genomes of a community and can be used to taxonomically identify and functionally profile a microbial community. Whole metagenome shotgun sequencing provides a deeper resolution for functional identification of microbial community processes (Brumfield et al., 2020), but to taxonomically profile the community members gene amplicon sequencing of taxonomically conserved sequences are used.

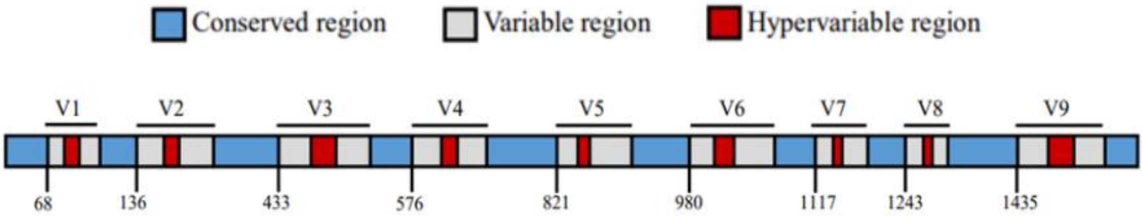

Figure 6. Diagram of the ~1500 base pair 16S rRNA gene and conserved, variable, and hypervariable regions. Blue = conserved region, grey = variable region, red= hypervariable region. Variable regions (V) V1-V9 are taxonomically informative regions interleaving conserved regions.

1.6.2 High-throughput DNA sequencing technology

Advances in high-throughput sequencing technologies have increased the speed and reduced the cost of DNA sequencing. A number of reliable platforms are now available including the Ion Torrent Personal Genome Machine (PGM), the Pacific Biosciences (PacBio) *RS* system, and the Illumina MiSeq. The performance of each of these platforms varies with differences in factors such as insert size (the length of DNA between the adapters to be sequenced), read length (the number of base pairs (bp) sequenced), depth (the number of times a base is represented within all sequencing reads), sequence accuracy, and cost (Kuczynski et al., 2011). A comparison of these 3 platforms by Kuczynski et al. (2011) shows Ion Torrent produces single reads of 200 bp with a low sequencing error rate, PacBio produces 1,110 bp reads but this comes at a higher sequencing error rate, and Illumina produces shorter 2 × 150 bp paired end reads but with greater depth and at lower cost. In another comparative study, Quail et al. (2012) found Ion Torrent was able to call a higher number of variants (single-nucleotide variants and insertion/deletion variants) compared to the PacBio and Illumina platforms. While Illumina is the current market leader in high throughput-sequencing, an advantage to using the Ion Torrent platform is that the reads are longer than those from Illumina and processing single reads is less computationally intensive than processing paired-end reads.

1.6.3 Taxonomic units

Analysis of PCR-amplified marker-gene sequencing reads has customarily been accomplished by clustering reads with a sequence similarity of typically > 97% and constructing molecular operational taxonomic units (OTUs) which can then act as a representative of a bacterial ‘species’ in downstream taxonomic characterisation applications (Konstantinidis & Tiedje, 2005). However, OTU-based approaches are limited by potential bias from the reference database as well as by the clustering approach which can combine different ‘species’ with a sequence similarity of > 97% into the same OTU (Callahan et al., 2017). Clustering to a higher threshold, for example 100% similarity, also has significant risk as sequencing errors can be falsely identified as a new OTU and create artificial inflation of diversity (Kunin et al., 2010).

A finer taxonomic resolution and higher confidence can be achieved by using amplicon sequence variants (ASVs) which are identified independently from a reference database and can distinguish sequence variants by as little as one nucleotide (Callahan et al., 2017). After determining the exact number of sequences generated an error model is used to compare similar reads which determines the probability of sequencing error. Sequences are then filtered by a confidence threshold value. The benefits of using ASVs rather than OTUs to analyse high-throughput marker-gene sequencing data are that ASVs can be validly compared across independent studies, independently processed datasets can be merged, and the accuracy of diversity measurements are improved (Callahan et al., 2017). A recent study by Prodan et al. (2020) compared 3 ASV bioinformatic pipelines for the analysis of amplicon sequence data, including USEARCH-UNOISE3 (Edgar, 2016), DADA2 (Callahan et al., 2016), and Qiime2-Deblur (Amir et al., 2017; Bolyen et al., 2019). They reported that comparatively DADA2 showed the greatest sensitivity in detecting all ASVs from the mock community dataset and was able to differentiate sequences at a single-base resolution. However, DADA2 was found to produce some spurious ASVs which were not produced by USEARCH-UNOISE3 and Qiime2-Deblur.

1.6.4 Community diversity metrics

Measuring and comparing microbial community structure is commonly achieved by using alpha and beta diversity metrics to analyse gene amplicon sequencing data. Alpha diversity aims to measure the taxonomic diversity within a single community by estimating bacterial richness (number of taxonomic units) and/or evenness (distribution of abundances of the groups) (Willis, 2019). Whereas beta diversity metrics aim to measure diversity (dis)similarity (variation) across two or more communities.

Estimating the number of taxa within a microbial community is inherently challenging due to the unique characteristics of microbial community datasets: high diversity, large numbers of rarely observed species, high counts of species observed only once (singletons), and a small number of very highly abundant taxa (Bunge et al., 2014). The nonparametric richness estimator Chao1 (Chao, 1984) is a commonly used metric to measure microbial community alpha diversity (e.g. Learman et al., 2016; Li et al., 2019). However, the validity of the Chao1 estimator has been argued as it depends on the assumption that the probability of observing every taxonomic unit is equal, which logically seems untrue for microbial communities. An approach reported by Willis & Bunge (2015) improves bacterial richness estimates within microbial community datasets by accounting for unobserved taxa and reducing the sensitivity to singleton counts.

The most commonly used metric to estimate richness with abundance is the Shannon–Wiener index (Hill et al., 2003; Shannon, 1948) which gives more weight to rare than common taxa. Similarly, dominance is most reliably estimated from the Simpson index (Hill et al., 2003; Simpson, 1949) which is determined from the probability that two randomly chosen taxa will be from the same taxonomic unit. Phylogenetic diversity is most often calculated using Faith’s phylogenetic diversity metric (Faith, 1992) which is determined from the sum of all branch lengths within the phylogenetic tree.

Species-based beta diversity measures are limited due to microbial “species” bring poorly defined and uncertain (Lozupone & Knight, 2008). For example, Jaccard distance is a species-based metric that qualitatively measures differences based only on presence or absence of taxa and is affected by sample size. Divergence-based diversity measures evaluate community similarity by considering phylogenetic distinctness of taxa. A quantitative weighted UniFrac approach takes into consideration the abundance and phylogenetic relatedness shared between taxonomic units by measuring the branch length of a phylogenetic tree (Lozupone & Knight, 2005). Using a weighted UniFrac metric for calculating beta diversity is more informative when comparing microbial communities at different sites.

1.7 Aim, hypothesis, and significance

The aim of this study was to characterise the structure and composition of sediment microbial communities at two contrasting sites in McMurdo Sound, Antarctica to understand how organic matter deposition drives sediment microbial communities. This study used 2 strategically chosen study sites, Cape Evans and New Harbour, based on their close proximity (~60 km distance) and contrasting differences in sediment organic matter content driven by different sea ice conditions and physical oceanography to test the hypothesis that sediment organic carbon content drives the resident microbial communities. Specifically, I applied 16S rRNA gene amplicon sequencing to address the following hypotheses:

1. The diversity of the microbial communities in the low organic matter New Harbour sediments would be greater than in the high organic matter Cape Evans sediments.
2. The composition of the microbial communities at Cape Evans and New Harbour would be distinctly different with heterotrophic taxa driving these differences at Cape Evans and chemoautotrophic taxa driving the differences at New Harbour.
3. The compositional differences between study sites would not be driven by inputs of wind-blown terrestrial surface material from the Taylor Valley.

In addition, to test the previously stated hypothesis of Mikucki et al. (2015) that subsurface Taylor Valley brine water discharges into McMurdo Sound via the sediments of New Harbour, I used 16S rRNA gene amplicon sequencing and microbial source tracking to test a secondary hypothesis:

4. New Harbour sediments will contain microbial taxa that have been previously reported from sediments of Taylor Valley lakes.

Primarily, this study will improve our understanding of how a climate-driven transition of multi-year ice to first-year ice will impact benthic biogeochemical processes and marine ecosystem functioning. Importantly, this study will contribute to our understanding of benthic sediment ecosystems and processes which are currently under-represented in marine research. In addition, this study will provide the first report of the direct impacts of climate-driven sea ice change on sediment microbial communities in Antarctica, supporting future predictions of the impacts of climate change. Lastly, the findings reported in this study will be useful in targeting future investigations into the functioning of the sediment microbial communities in McMurdo Sound.

Chapter 2

Sea Ice Dynamics Drive Benthic Microbial Communities in McMurdo Sound, Antarctica

2.1 Abstract

In polar marine ecosystems, thicker and older multi-year sea ice is transitioning into thinner and more ephemeral first-year sea ice due to a changing climate. This is expected to increase sea surface primary productivity and the vertical flux of organic matter to the seafloor, leading to compositional changes in sediment carbon that will likely alter microbially-mediated benthic biogeochemical processes. Despite this prospect for change, there is a current paucity of information on how organic carbon content influences sediment microbial community structure and composition in sea ice-influenced polar regions, thereby impeding our understanding of an essential component of the marine ecosystem. This study sampled organic matter-rich sediments driven by first-year ice and productive open ocean currents (Cape Evans) and low productivity sediments driven by multi-year ice and oligotrophic currents (New Harbour) within McMurdo Sound, Antarctica. Using high-throughput 16S rRNA gene amplicon sequencing and sediment geochemistry data (chlorophyll-α, phaeophytin, TOC, TN, δ13C, δ15N) this study compared the structure and composition of sediment microbial communities at these contrasting sites. Measures of bacterial richness and evenness (Shannon-Wiener index) revealed that community alpha-diversity was significantly greater in the oligotrophic multi-year ice sediments than the productive first-year ice sediments. This study identified significant site-specific compositional differences. The community

composition at Cape Evans supported known heterotrophic algal biopolymer degrading taxa *Flavobacteriales*, *Cytophagales,* and *Verrucomicrobiales,* and sulfate-reducing bacteria *Desulfobulbales*, reflecting the legacy of high TOC and chlorophyll-α conditions. Whereas at New Harbour, chemoautotrophic taxa *Nitrosopumilaeles*, *Nitrospirales,* and *Steroidobacterales* involved in carbon fixation and nitrogen cycling, were more abundant potentially reflecting the legacy of oligotrophic conditions at this site. The influence of wind-blown Taylor Valley terrestrial surface sediments on our sampled communities was negligible. The hypothesis that subsurface Taylor Valley brine channels could be discharging into McMurdo Sound was supported by our finding of low abundance taxa associated with high saline environments (e.g. *Thiohalorhabdales*) within New Harbour sediments, but not Cape Evans. Overall, these results suggest that sediment microbial communities will respond to climate driven sea ice reductions and increased organic matter flux by shifting the dependence on *in situ* chemoautotrophic carbon fixation and nitrogen cycling towards heterotrophic energy-yielding metabolisms, thus influencing benthic biogeochemical processes. This study contributes an important component to our understanding of benthic ecosystems in polar regions and is the first study to directly assess the impacts of climate driven sea ice change on benthic microbial communities in Antarctica.

2.2 Introduction

Globally, polar regions are experiencing sea ice variability in response to a warming climate (Stammerjohn et al., 2012). In the Arctic, thicker and older multi-year ice is declining and transitioning into thinner and more ephemeral first-year ice (Meier et al., 2014), with the lowest Arctic sea ice minimum extent recently measured (September 2020) on a 42-year satellite record (NSIDC, 2020b). Conversely, in the Antarctic, sea ice extent has been increasing overall around the continent, masking strong regional decreases in the West Antarctic Peninsula region that are comparable to the Arctic (Parkinson & Cavalieri, 2012; Stammerjohn et al., 2012). Multi-year ice was recently advected out of the Weddell Sea region where most of the Antarctic multi-year ice resides (Turner et al., 2020) in late 2016, a record year for rate of sea ice retreat in all sectors of Antarctica (Turner et al., 2017).

Polar oceans are a major sink for anthropogenic carbon dioxide (CO_2) and are critically important for global biogeochemical processes (Takahashi et al., 2009). Diverse communities of autotrophic microalgae, which assimilate CO_2 into biomass, live within and underneath the extensive sea ice (Arrigo, 2014), supported by oceanic upwelling of nutrient-rich subsurface waters, and regulating the export of particulate organic carbon to depths (Boetius et al., 2013). During spring, ice algal growth is stimulated as sunlight availability increases as ice and snow cover begins to melt (Arrigo, 2014), with concentrations of chlorophyll-α approaching 800 mg m^{-3} and 10,100 mg m^{-3} in the Arctic and Antarctic, respectively (Arrigo, 2016). Free-living phytoplankton in open water areas typically bloom later in the season once the ice algal biomass subsides and sunlight becomes available (Arrigo et al., 2012). Sea ice variability is altering the timing and magnitude of the blooms of ice-associated algae and free-living phytoplankton (Nadaï et al., 2020) which form the foundations of polar marine food webs (Arrigo, 2014), and as such, climate driven changes to sea ice dynamics are likely to have far reaching ecological impacts. For example, reductions in sea ice coverage in the northern West Antarctic Peninsula have already resulted in the displacement of sea ice-dependent species poleward, with replacement by ice-tolerant species (e.g. salps replaced krill and gentoo and chinstrap penguins replaced Adélie penguins) (Ducklow et al., 2007). As sea ice grows thinner, breaks out more frequently, and the ice-free period gets longer, the increased sunlight availability is expected to cause an increase in primary productivity (Lannuzel et al., 2020), increasing diatom and phytoplankton carbon fluxes to the seafloor (Anderson & Macdonald, 2015; Boetius et al., 2013; Nadaï et al., 2020), which may alter benthic (seafloor) ecological and biogeochemical processes.

Marine sediments play important roles in global biogeochemical cycles where the remineralisation of organic carbon is primarily regulated by diverse microorganisms which also play key roles in cycling other elements such as nitrogen and sulfur (Orsi, 2018). Organic matter remineralisation by the microbial communities residing within sediments is coupled with water column primary productivity processes as inorganic nutrients are released from the sediments back into the water column which sustains primary productivity at the surface (Nixon, 1981). Within the sediments, oxygen is consumed during heterotrophic respiration, and in organic matter-rich sediments it is rapidly depleted within the top few millimetres (Middelburg, 2019a), whereas in oligotrophic sediments with low sedimentation rates, oxygen is not fully consumed at the surface and can penetrate meters into the seafloor (D’Hondt et al., 2015). Organic carbon concentration and dissolved oxygen availability are significant factors driving microbial community composition within sediments (Hoshino et al., 2020; Learman et al., 2016; Orsi, 2018). Organic matter-rich sediments are often enriched with strictly anaerobic bacteria, such as sulfate-reducing bacteria and methanogens, whereas oligotrophic sediments are often selective of strictly aerobic and facultative anaerobic heterotrophs supported by carbon fixation and primary production from chemolithoautotrophic taxa (Orsi, 2018). Additionally, microbial activity within the sediments is enhanced by macrofauna bioturbation which introduces oxygen and fresh organic matter to depths and alters biogeochemical interactions (Lohrer et al., 2004).

The intimate connection that microorganisms share with their environment identifies microorganisms as sensitive sentinels for climate change (Cavicchioli et al., 2019). Sea ice variability has the potential to alter sediment biogeochemical processes by increasing surface productivity and organic carbon flux to the sea floor. However, there is a current paucity of information on how polar sediment microbial communities will respond or if they will be impacted. Studies that address sediment microbial communities have occurred in polar sediments in the deep ocean (Li et al., 2020; Ruff et al., 2014) and along environmental gradients (Learman et al., 2016). However, no under-ice sediment studies have been conducted in direct relation to climate change in Antarctica to our knowledge, despite the critical importance of sedimentary processes to marine ecosystems.

McMurdo Sound is a marine inlet ~60 km wide located in the southwestern corner of the Ross Sea, adjacent to the Ross Ice Shelf in the south, Ross Island in the east, and the continental mainland in the west (*Figur*). Land-fast sea ice (ice fastened to the coastline) persists year round within McMurdo

Sound with marked differences in seasonal sea ice regimes in the eastern and western portions of the Sound (Kim et al., 2018). Comparatively thinner first-year ice persists in the eastern Sound which begins to break out during late spring, subsequently increasing sunlight and stimulating under-ice primary productivity. Whereas in the western Sound, thicker multi-year ice has historically persisted over many consecutive years with little or no breakout, thus severely limiting under-ice algal productivity. Fast-ice trends within McMurdo Sound have shown little change over the past 35 years, suggesting little impact from the atmospheric and oceanic climate drivers that influence Ross Sea ice pack (Kim et al., 2018; Parkinson, 2019).

The Ross Sea is one of the most biologically productive continental shelf regions in Antarctica (Smith et al., 2012), where intense phytoplankton blooms occur during periods of increased sunlight and reduced sea ice cover (Arrigo & van Dijken, 2004). A primary nutrient source for McMurdo Sound comes from the upwelling of nutrient-rich and relatively warm circumpolar waters onto the Ross Sea continental shelf (Smith et al., 2014) which enter McMurdo Sound via southward flowing currents near Ross Island (Robinson et al., 2010). These currents continue to flow south down the coastline towards the floating Ross Ice Shelf, the largest ice shelf on the planet ($\sim 4.7 \times 10^5$ km^2) (Stevens et al., 2020). Currents submerge into the cavity of the ice shelf where there is an estimated cavity residence time for oceanographic inputs of ~4 to 6 years (Holland et al., 2003; Smethie & Jacobs, 2005; Stevens et al., 2020). Beneath the ice shelf, advected phytoplankton settle through the water column (Vick-Majors et al., 2016) and perpetual darkness limits *in situ* primary productivity to bacterial chemosynthesis of reduced inorganic compounds (Horrigan, 1981). Depleted currents, which are considered oligotrophic

(Dayton & Oliver, 1977), emerge from underneath the Ross Ice Shelf and flow north along the western coastline of McMurdo Sound (Robinson et al., 2010).

Recent studies have considered two additional nutrient inputs (wind-blown terrestrial sediments and subsurface brine systems) into McMurdo Sound, potentially connecting the McMurdo marine system to the neighbouring terrestrial Taylor Valley, one of the ice-free valleys in the McMurdo Dry Valleys. Wind-blown terrestrial sediment from the Taylor Valley that gets channelled through cracks near pressure ridges in the sea ice has been suggested to make a small contribution to sedimentation in near-shore regions of New Harbour (Murray et al., 2013). Additionally, a subsurface brine system driven by hydraulic gradients between lakes in the Taylor Valley has recently been hypothesised to emerge as submarine groundwater discharge into McMurdo Sound at New Harbour, potentially providing a source of bioavailable nutrients such as iron (Foley et al., 2019; Mikucki et al., 2015). Despite diverse microbial communities being present in Taylor Valley soils (Cary et al., 2010) and lake sediments (Dillon et al., 2020; Tang et al., 2013), the potential connection between these terrestrial systems and the benthic marine system at New Harbour has not yet been investigated using microorganisms.

Previous work on benthic ecosystems in McMurdo Sound showed the quantity and quality of algal detrital matter was elevated in sediments underlying snow-free first-year ice compared to multi-year ice and that this correlated with increased benthic oxygen consumption and ammonium efflux from sediments into the water column, measured using benthic incubation chambers (Lohrer et al., 2013). Previous studies have also described the role of oceanographic circulation and productivity in supporting dense assemblages of benthic infauna in the east and scarce assemblages that resemble the deep ocean in the west (Dayton & Oliver, 1977). Another recent study showed that increased frequency of multi-year ice breakout between 2009 and 2017 in McMurdo Sound positively corresponded with sediment algal pigment concentration, infauna abundance, benthic oxygen demand, and nutrient regeneration rates (Lohrer et al., 2020). However, the association between sea ice and oceanographic conditions and the microbial communities in McMurdo Sound is unknown.

In this study, I applied microbial community structure, composition and taxonomically associated functional profiling of sediment microbial communities using 16S rRNA sequencing to understand how the transition of multi-year to first-year ice may impact benthic biogeochemical processes at two contrasting sites in McMurdo Sound. Two well-studied coastal sites, Cape Evans and New Harbour, were strategically chosen based on their close proximity (~60 km distance) and contrasting differences in sea ice conditions and physical oceanography which make these sites ideal to address the hypothesis that local sedimentary geochemistry and organic carbon loading would drive the resident microbial communities and that these differences would not be driven by Taylor Valley surface terrestrial inputs.

2.3 Materials and methods

2.3.1 Study sites, sampling, and sediment analysis

Marine benthic sediment was collected from the eastern Sound at Cape Evans (77°38.115′S, 166°24.410′E) from 14.2 m depth on 17/11/2017; from the western Sound at New Harbour (77°34.573′S, 163°32.608′E) at 18.5 m depth on 7/11/2017; and from an intertidal area in front of the New Harbour Jamesway hut (the “moat”, 77°34.372′S, 163°31.202′E) on 17/11/2017 (*Figure 7)*.

At Cape Evans and New Harbour, the subtidal sites were accessed through holes in snow-free sea ice (New Harbour 3.5 m thickness; Cape Evans 2.0 m thickness) and cores of surface sediment (2 cm internal diameter by 2 cm deep) for analysis of nucleic acids were collected by SCUBA at randomised positions along a 20 m long seafloor transect. After collection, each sediment sample was homogenised using a sterile spatula and a 5 ml subsample was transferred into a new 15 mL Falcon tube. Falcon tubes were centrifuged for 5 min at 1,000 RCF and the supernatant discarded. At least 5 ml of LifeGuard Soil DNA Preservation Solution (Qiagen, CA, USA) was added to each tube to preserve DNA. The tubes were capped, shaken by hand to mix, and stored at −20 °C until nucleic acid extraction.

Cores of sediment (2 cm internal diameter, 2 cm deep) for analysis of sediment algal pigments were collected from Cape Evans and New Harbour using previously published protocols (Cummings et al., 2006; Cummings et al., 2018; Lohrer et al., 2013; Norkko et al., 2007; S. F. Thrush & Cummings, 2011). Briefly, algal pigments (chlorophyll-α and phaeophytin (degradation product)) were extracted from freeze dried sediments in 90% ethanol and measured spectrophotometrically.

Sediment samples from Cape Evans and New Harbour were prepared for total organic carbon (TOC), total nitrogen (TN), and $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis following a previously published protocol (Kennedy et al., 2005). Briefly, 5 g of wet sediment per sample were dried in a 40 °C oven in aluminium pans then homogenised by grinding with mortar and pestle. Carbonate was removed using treatments of 1M HCl until samples stopped effervescing, with drying occurring in a 40 °C oven between each addition. An Isoprime 100 analyser (Elementar, Langenselbold, Germany) was used for TOC and TN measurements and a 20/20 isotope analyser (Sercon Ltd., Crewe, UK) for isotopic abundance at the Waikato Stable Isotope Unit (WSIU) (Hamilton, New Zealand).

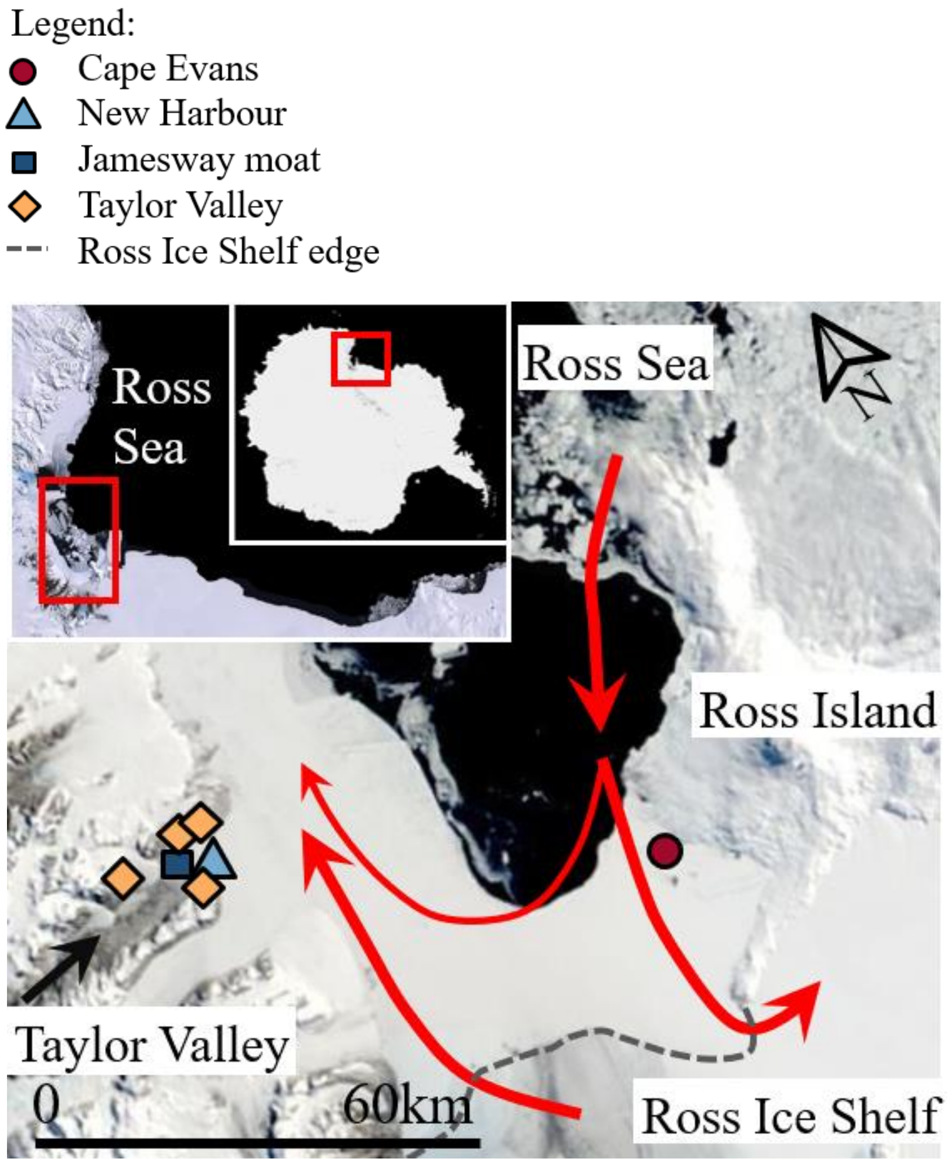

Figure 7. MODIS satellite image of McMurdo Sound and Ross Ice Shelf region during November 2017 with sample site locations (Cape Evans (14.2 m); New Harbour (18.5 m); Jamesway moat (0 m)); and Taylor Valley. Map overlaid with dominant oceanographic current circulation patterns (red arrows, dominance depicted by weight of arrow, based on (Robinson et al., 2010)). Land-fast sea ice breaks out annually at Cape Evans and persists for multiple consecutive years at New Harbour. Brackish water is present at the shallow intertidal Jamesway moat site during months of sunlight. The Taylor Valley sites are within 16 km of New Harbour. The Ross Ice Shelf has historically remained in place all year round and does not break out. Image source: NASA.

2.3.2 Amplicon sequencing

A modified DNA extraction protocol was developed using the Qiagen PowerSoil®DNA Isolation Kit (CA, USA). Briefly, for each sample 5 tubes each containing 200 mg of wet sediment were extracted and then pooled together onto a single elution column to concentrate the extracted material. The protocol was modified at the "prepare sample" step (C1 buffer heated to 60 ºC), "cell lysis" step (samples heated on a Thermomixer for 10 min at 60 ºC then shaken on a vortex mixer for 10 sec before bead beating), "inhibitor removal technology" step (incubation time increased to 10 min), "bind DNA" step (incubation time increased to 20 min, samples inverted every 3 min), "wash" step (5 representative extracts from each sample were pooled through the same spin column), and "elute" step (DNA eluted in 20 µL of 60 ºC nuclease free water and left on the spin column at room temperature for 2 min before centrifuging). DNA concentration was determined using a Qubit 2.0 Fluorometer (Life Technologies, CA, USA) and the quality confirmed by electrophoresis in 2% TAE agarose gel prior to downstream analysis.

The 16S hypervariable region V4-V5 was PCR amplified in triplicate reactions using the primer set 515F-Y and 926R (Parada et al., 2016; Quince et al., 2011) modified for Ion Torrent sequencing using a 1 step PCR strategy (IonCode Barcode Adapters, Thermo Fisher Scientific). Briefly, per 20 µL reaction were final concentrations of dNTPs (4.8 mM) (Invitrogen, CA, USA), 1× PCR buffer, $MgCl_2$ (120 mM) (Invitrogen), forward and reverse primer (4 mM each) (Integrated DNA Technologies, Inc, IA, USA), 1U Taq DNA polymerase (Invitrogen), and 3 ng total genomic DNA with the following conditions: 3 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 1 min at 50 °C, and 1.5 min at

72 °C, and final elongation for 10 min at 72 °C. Triplicate reactions were pooled and PCR amplicon products normalised using a SequalPrep Normalization Plate Kit (Invitrogen). Amplicons were sequenced using Ion Torrent Personal Genome Machine (PGM) DNA sequencer chemistry with an Ion 318v2 chip (Life Technologies) at the University of Waikato DNA sequencing facility (Hamilton, New Zealand).

2.3.3 Raw sequence data processing

Sequencing adapters, low quality reads and short reads (< 250 bp) were identified and removed with Mothur v1.40.5 (Schloss et al., 2009). Ion Torrent barcodes and sequencing primers were removed and new labels created using the Python script fastq_strip_barcode_relabel2.py in USEARCH v10 (Edgar, 2010). A total of 194,823 valid reads across 13 samples were processed using DADA2 v1.14.1 (Callahan et al., 2016) in R v3.6.3 (R Development Core Team, 2010) to generate amplicon sequence variants (ASVs). Briefly, reads < 250 bp, quality score < 2, and expected error > 2 were removed. After all quality steps, an ASV table was constructed containing 3,538 ASVs (average sequence length 225 base pairs). Representative ASV sequences were used to check for chimeras and 213 chimeric sequences were removed producing 3,325 unique ASVs for analysis. The lowest and highest read counts per sample ranged from 7,821 to 19,293 base pairs hence the dataset was not rarefied. Taxonomy was assigned to ASVs using the SILVA v138 database (Quast et al., 2012). Sequence alignment and phylogenetic UPGMA tree generation was completed using MUSCLE v3.8.31 (Edgar, 2004).

ASVs classified as eukaryotes, mitochondria, chloroplasts, or those sequences that were unclassified at the level of Kingdom, and Phylum were removed from the dataset. Before discarding chloroplast ASVs, these sequences were classified using Basic Local Alignment Search Tool (BLAST) against the nt database. Raw sequence data will be deposited into the Short Read Archive.

2.3.4 Sequencing data analysis

A rarefaction curve was generated to confirm completeness of sequencing. Analysis of ASVs was completed in R v3.6.3 (R Development Core Team, 2010). Plots were generated using ggplot2 v3.3.0 (Wickham, 2016). The taxonomic diversity within the bacterial communities (alpha diversity) at Cape Evans and New Harbour was calculated in Breakaway v4.6.10 (Willis, 2020) using a species-based metric of species richness (Willis & Bunge, 2015) and measure of richness and evenness (Shannon, 1948). The statistical differences in taxonomic diversity values between sample sites were assessed for each metric using Wilcoxon rank-sum tests. Phylogenetic diversity was measured for Cape Evans and New Harbour samples in Picante v1.8.1 (Kembel et al., 2010) using divergence-based measures of Mean Pairwise Distance (MPD) (relatedness of species deep in the tree) (Webb, 2000) and Mean Nearest Taxon distance (MNTD) (relatedness near branch tips) (Webb et al., 2002). MPD and MNTD were standardised to account for differences in species richness between sites, resulting in a Nearest Relative Index (NRI) and Nearest Taxon Index (NTI), respectively (Webb, 2000).

The community compositional dissimilarity between samples sites (beta diversity) was determined using a principle co-ordinate analysis (PCoA) ordination of a divergence-based weighted UniFrac dissimilatory matrix using Phyloseq (v1.30.0) (McMurdie & Holmes, 2013). To determine if terrestrial surface soils from the Taylor Valley had an impact on the community composition of McMurdo Sound sediments I compared samples from the two marine sites to samples from the intertidal moat (Jamesway moat) at the shoreline of New Harbour as well as to 4 samples collected from the lower Taylor Valley as part of the New Zealand Terrestrial Biocomplexity Survey (NZTABS, 2017) (*Figure 7*). Analysis of Similarity (ANOSIM) (Clarke, 1993) was used to test the significance of the differences identified by the PCoA between sampling units.

Differentially abundant taxa of > 1% relative abundance within each of the microbial communities from Cape Evans and New Harbour were detected using Analysis of Composition of Microbiomes (ANCOM) (Mandal et al., 2015) with default settings from absolute abundance values based on compositional log-ratios. Differentially abundant taxa that were identified as significant ($p < 0.05$) by ANCOM were visualised in a heatmap at the taxonomic level of Order, generated using Phyloseq.

Functional gene abundances were predicted for ASVs in Cape Evans and New Harbour samples using PICRUSt2 v2.3.0 beta (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas et al., 2020) (using tools: EPA-NG (Barbera et al., 2019) and Gappa (Czech et al., 2020) for phylogenetic placement of reads, Castor (Louca & Doebeli, 2018) for hidden state prediction, and MinPath (Ye & Doak, 2009) for pathway inference). The Nearest Sequenced Taxon Index (NSTI) was used to estimate accuracy of PICRUSt2 predictions, ASVs with a NSTI value > 2.0 were removed. Averaged abundances of predicted pathways for each site derived by PICRUSt2 from the Metabolic Pathway Database (MetaCyc) (Caspi et al., 2020) were selected and a two-sided z-test statistically compared the predicted MetaCyc functions between sample sites in STAMP v2.1.3 (STatistical Analysis of Metagenomic Profiles) (Parks et al., 2014). The Newcombe-Wilson method was used to calculate the confidence interval (95%) and features with < 2 sequences and *p*-value > 0.01 were removed. The selected predicted metabolic functions of interest at Cape Evans and New Harbour were categorised at the parent level for broader interpretation and visualised in an extended error bar plot using STAMP.

2.4 Results

2.4.1 Sampling and sediment characteristics

A total of 13 surface sediment (0 – 2 cm) samples were collected from 2 marine locations, Cape Evans (n = 4) and New Harbour (n = 5), and 1 intertidal moat location, Jamesway moat (n = 4), in McMurdo Sound in the southwestern Ross Sea during November 2017.

The concentration of sediment chlorophyll-α (fresh algal material) and phaeophytin (degraded algal material) in Cape Evans (14.2 ± 0.8 µg g^{-1} and 7.2 ± 0.25 µg g^{-1}) was 14 × higher than that of New Harbour (1.0 ± 0.24 µg g^{-1} and 0.5 ± 0.14 µg g^{-1}). The ratio of fresh to degraded algal material (chlorophyll-α:phaeophytin) was 2:1 at both study sites.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values were determined from the homogenised sediment samples from Cape Evans and New Harbour. The $\delta^{13}C$ values were similar between Cape Evans and New Harbour sediments (–20.26‰ ± 0.01 and –21.90‰ ± 0.73 respectively (*Table 1*). The $\delta^{15}N$ values within the sediments at Cape Evans and New Harbour were 4.15‰ ± 0.23 and 4.31‰ ± 0.68, respectively (*Table 1*). Sediment TOC from Cape Evans was higher than New Harbour (0.33% ± 0.04 and 0.17% ± 0.01 respectively (*Table 1*)). Sediment TN from Cape Evans was higher than at New Harbour (0.05% ± 0.01 and 0.02% ± 0.00 respectively) (*Table 1*).

Table 1. Sea ice characteristics, sediment geochemistry, and solute fluxes* (*adapted from Lohrer et al. 2020 for Cape Evans 2013 and New Harbour 2017) for surface sediments collected from Cape Evans and New Harbour, McMurdo Sound, during November 2017, (mean ± standard deviation).

Sample site	**Cape Evans**	**New Harbour**
Sea ice characteristics:		
Thickness (m)	2.0	3.5
Snow cover	None	None
Persistence	First-year ice	Multi-year ice
Last fast-ice break out	Feb-Mar (2017)	Feb-Mar (2016)
Sediment geochemistry:		
Chlorophyll-α (μg g$^{–1}$ sediment)	14.2 ± 0.8	1.0 ± 0.24
Phaeophytin (μg g$^{–1}$ sediment)	7.2 ± 0.25	0.5 ± 0.14
δ13C (‰)	–20.26 ± 0.01	–21.90 ± 0.73
δ15N (‰)	4.15 ± 0.23	4.31 ± 0.68
% Total organic carbon (TOC)	0.33 ± 0.04	0.17 ± 0.01
% Total nitrogen (TN)	0.05 ± 0.01	0.02 ± 0.00
Solute fluxes:		
* Dissolved oxygen flux (μmol O_2 m^{-2} h^{-1})	222	54.4
* Ammonium efflux (μmol NH_4-N m^{-2} h^{-1})	13.2	0.6
* Nitrate+nitrite (μmol N m-^{2} h-^{1})	19.0	2.5
* Reactive phosphorus (μmol P m-^{2} h-^{1})	2.5	0.01
* Data adapted from Lohrer et al. (2020) for Cape Evans 2013 and New Harbour 2017		

2.4.2 Sequencing

Rarefaction curves confirmed sufficient completion of sequencing of samples from Cape Evans, New Harbour, and Jamesway moat (*Figure 8*).

ASVs classified as eukaryotes (115 ASVs), mitochondria (38 ASVs), chloroplasts (39 ASVs), or that were unclassified at the level of Kingdom (18 ASVs) and Phylum (144 ASVs) were removed from the dataset. Of the 39 ASVs identified as chloroplasts, BLAST analysis identified these sequences to be predominantly diatoms *Haslea nipkowii* (43.5%), *Skeletonema pseudocostatum* (22.7%), *Chaetoceros sp.* (9.0%), and *Asterionellopsis glacialis* (5.1%).

The filtered and quality checked 16S rRNA gene amplicon dataset included a total of 2,981 ASVs across all 13 samples. A total of 2,076 ASVs were identified from the 9 samples collected from Cape Evans and New Harbour. Of the total number of ASVs at these two sites, 1,308 ASVs (63%) were found at both sites. A total of 531 ASVs were unique to New Harbour samples representing 28.9% of the total relative abundance of ASVs at this site (1,839 ASVs). A total of 237 ASVs were unique to Cape Evans samples representing 15.3% of the total relative abundance of ASVs at this site (1,545 ASVs).

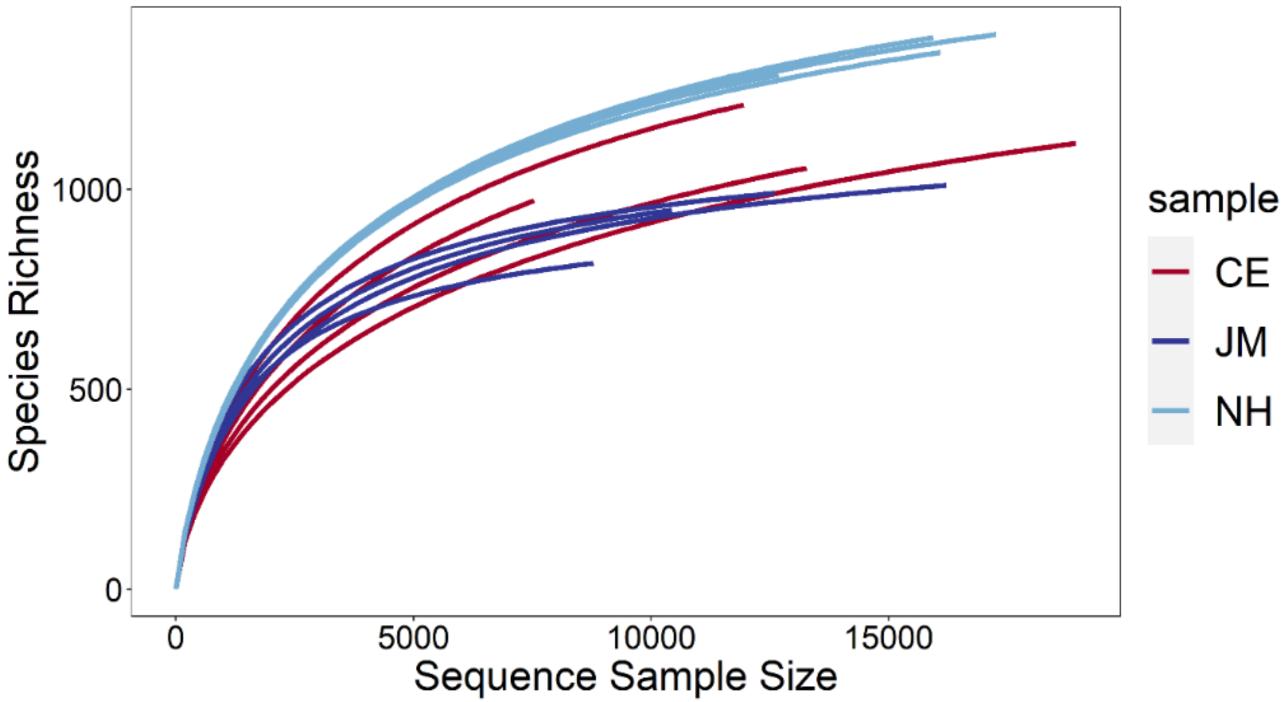

Figure 8. Rarefaction curve showing sequencing completion of the microbial communities in sediment samples from Cape Evans (CE), New Harbour (NH), and Jamesway moat (JM).

2.4.3 Taxonomic composition of microbial communities

Taxonomic classification of ASVs within the sediment samples across all 4 sites revealed that the dominant Phylum level taxa in Cape Evans, New Harbour and Jamesway moat samples were *Proteobacteria*, *Bacteroidota*, *Planctomycetota*, and *Verrucomicrobiota* whereas Taylor Valley samples were dominated by *Actinobacteriota, Acidobacteriota, Bacteroidota*, and *Deinococcota* (*Figure 9A, Table 2*). A large proportion of the phyla categorised as "Other" at Jamesway moat were represented by *Cyanobacteria*. Classification at the taxonomic level of Class revealed *Bacteroidia* were dominant across all 4 sites and that *Gammaproteobacteria* were dominant across all sites except Taylor Valley (*Figure 9B, Table 2*). *Planctomycetes* were dominant across Cape Evans and New Harbour sediments and *Alphaproteobacteria* were dominant across New Harbour and Jamesway moat sediments (*Figure 9B, Table 2*). *Verrucomicrobiae* were more dominant in Cape Evans sediments. *Thermoleophilia* and *Actinobacteria* were only dominant in Taylor Valley sediments (*Figure 9B, Table 2*).

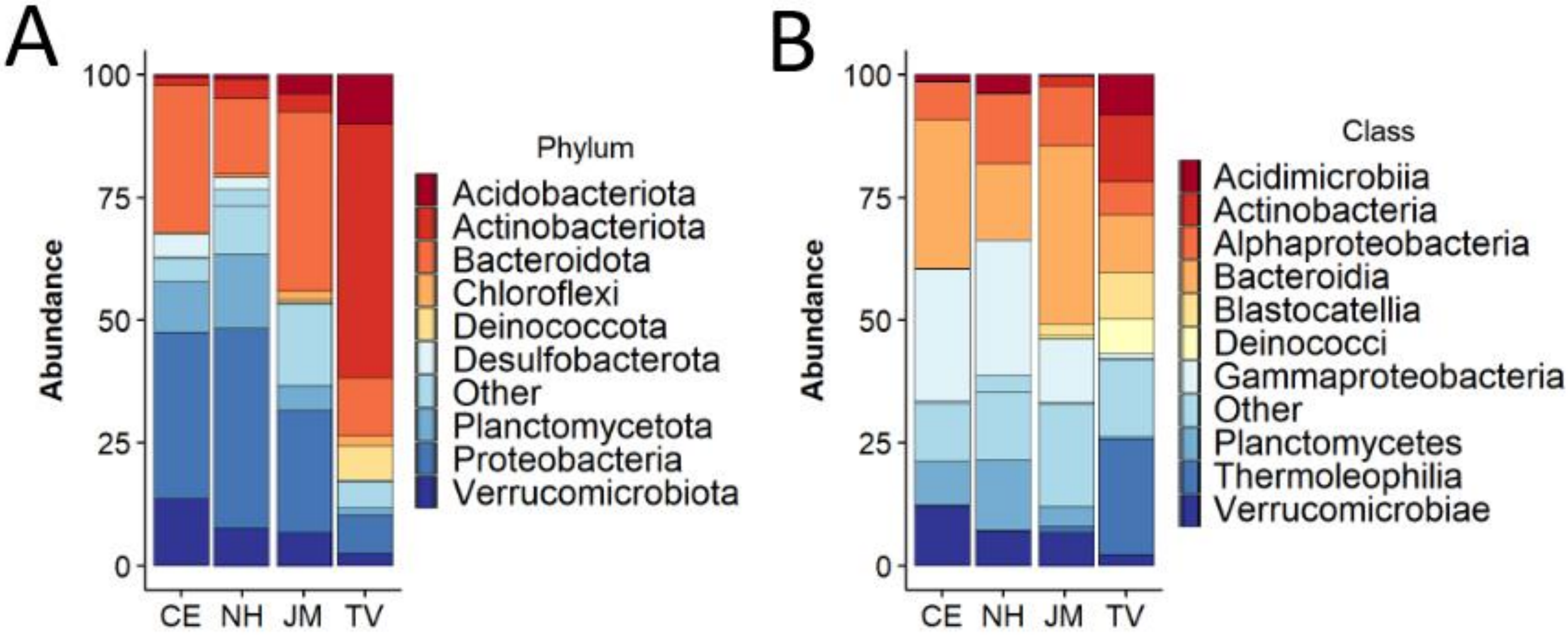

Figure 9. The 10 most abundant taxonomic groups of the microbial communities at the levels of Phylum (A) and Class (B) generated using 16S rRNA gene amplicon sequencing in surface sediment samples from Cape Evans (CE), New Harbour (NH), and Jamesway moat (JM), and Taylor Valley (TV) terrestrial soils.

Table 2. Relative abundance values of the 10 most abundant taxonomic groups of the microbial communities at the levels of Phylum and Class generated from 16S rRNA gene amplicon sequencing at Cape Evans (CE), New Harbour (NH), Jamesway moat (JM), and Taylor Valley (TV). The dominant Phyla or Class within each location is identified in bold.

		Relative abundance (%)			
		CE	**NH**	**JM**	**TV**
Phylum	*Acidobacteriota*	0.6	0.9	3.8	9.4
	Actinobacteriota	1.5	3.8	3.7	**52.8**
	Bacteroidota	29.6	15.1	**36**	11.7
	Chloroflexi	0.3	0.6	1.8	2.1
	Deinococcota	0.0	0.0	0.6	8.4
	Desulfobacterota	4.6	2.4	0.1	0.0
	Unassigned	6.5	15.4	17.7	5.6
	Planctomycetota	10	14.5	4.9	1.2
	Proteobacteria	**33.6**	**39.8**	24.7	6.8
	Verrucomicrobiota	13.3	7.5	6.7	2.0
Class	*Acidobacteriae*	0.0	0.0	0.4	0.1
	Actinobacteria	0.0	0.1	2.0	13.7
	Alphaproteobacteria	7.7	13.8	12.0	6.8
	Bacteroidia	**29.8**	14.8	**36.0**	11.8
	Blastocatellia	0.2	0.1	2.7	9.4
	Deinococci	0.0	0.0	0.6	6.9
	Gammaproteobacteria	26.3	**26.6**	12.8	1.1
	Unassigned	15.1	23.8	21.4	**23.9**
	Planctomycetes	8.8	13.7	4.1	0.6
	Thermoleophilia	0.0	0.1	1.2	23.3
	Verrucomicrobiae	12.0	6.8	6.7	2.4

2.4.4 Impact of terrestrial inputs on New Harbour sediments

Significant site based compositional differences were identified across all 4 sampling sites shown in the PCoA (*Figure 10*) where 56.8% of the variation was explained across the primary axis (ANOSIM R = 0.9, $p < 0.05$) (*Figure 10*). The most significant compositional differences occurred between the Taylor Valley and New Harbour samples across the primary axis. The composition of the Taylor Valley samples were more similar to the Jamesway moat samples than to any of the marine samples. A separation between Cape Evans and New Harbour samples was seen across the secondary axis of the PCoA where 26.5% of the variation was explained (*Figure 10*).

2.4.5 Impact of sea ice and oceanographic conditions on Cape Evans and New Harbour sediments

The bacterial diversity within the communities collected from Cape Evans and New Harbour were assessed using estimates of species richness which was higher (*NS* $p > 0.05$) in New Harbour sediments (mean 1,794 ± 24) than in sediments collected from Cape Evans (mean 1,702 ± 74) (*Figure 11A*). The Shannon-Wiener diversity index, used to evaluate bacterial richness and evenness, was higher in New Harbour samples (H' 6.2 ± 0.0) than in Cape Evans samples (H' 5.7 ± 0.2) ($p < 0.05$) (*Figure 11B*). The phylogenetic diversity within samples from Cape Evans and New Harbour measured by the NRI and NTI revealed negative values across both sites. NRI and NTI values were more negative in New Harbour samples (–12.8 ± 1.2 and –19.1 ± 1.0, respectively) than in Cape Evans samples (–11.5 ± 1.0 and –17.3 ± 1.0, respectively) (*Figure 11C, D*).

Differentially abundant taxa within samples from Cape Evans and New Harbour were identified by ANCOM which identified the relative abundances of

218 ASVs differed ($p < 0.05$) with 96 ASVs more abundant at Cape Evans and 122 ASVs more abundant at New Harbour. Of the 96 significant ASVs identified at Cape Evans the dominant phyla were *Proteobacteria* (28 ASVs; 10.4%), *Bacteroidota* (25 ASVs; 14.5%), *Verrucomicrobiota* (16 ASVs; 3.6%), and *Desulfobacterota* (8 ASVs; 3.3%) (*Figure 12*). Of the 122 significant ASVs identified as more abundant at New Harbour the dominant phyla were *Proteobacteria* (52 ASVs; 13.7%), *Bacteroidota* (25 ASVs; 5.1%), *Planctomycetota* (19 ASVs; 3.1%), and *Crenarchaeota* (5 ASVs; 2.0%) (*Figure 12*). Resolution of these ASVs at the taxonomic level of Order at Cape Evans revealed these sediments were represented by *Flavobacteriales* (12.2%), *Cytophagales* (2.1%), *Verrucomicrobiales* (3.1%), and *Desulfobulbales* (2.8%) (*Figure 12*). Whereas, New Harbour sediments were represented by *Steroidobacterales* (3.2%), *Rhodobacterales* (3.1%), *Nitrosopumilales* (2.0%), *Pirellulales* (2.6%), and *Nitrospirales* (1.4%) (*Figure 12*).

Taxonomically aligned metabolic associations were attributed for those sediment samples using PICRUSt2. A total of 29 ASVs were removed from the pipeline due to NSTI cut-off scores of > 2. Following filtering, a total of 211 statistically significant MetaCyc pathways were inferred by PICRUSt2 within the Cape Evans and New Harbour samples. Cape Evans sediments were represented by pathways associated with the tricarboxylic acid (TCA) cycle, carbohydrate degradation, fermentation, glyoxylate cycle, and sulfur and nitrogen compound metabolisms, whereas New Harbour sediments displayed greater abundances of pathways involved in C1 compound utilisation and assimilation and osmoregulation (*Figure 13*).

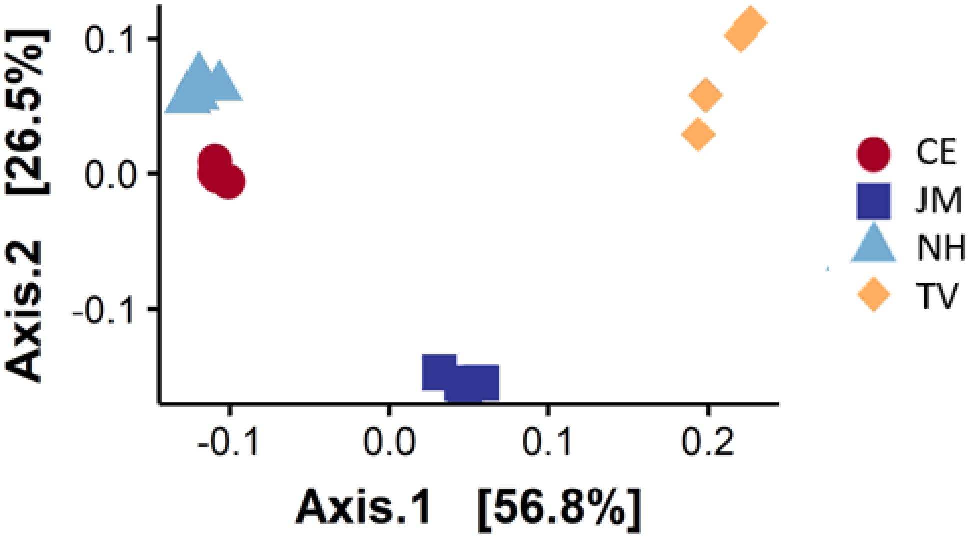

Figure 10. PCoA of a weighted UniFrac dissimilatory matrix of microbial communities in surface sediment samples from Cape Evans (CE), New Harbour (NH), Jamesway moat (JM), and Taylor Valley (TV) (ANOSIM R = 0.9, *p* < 0.01) (D). Site legend: Cape Evans = red circle; New Harbour = light blue triangle; Jamesway Moat = dark blue square; Taylor Valley = peach diamond.

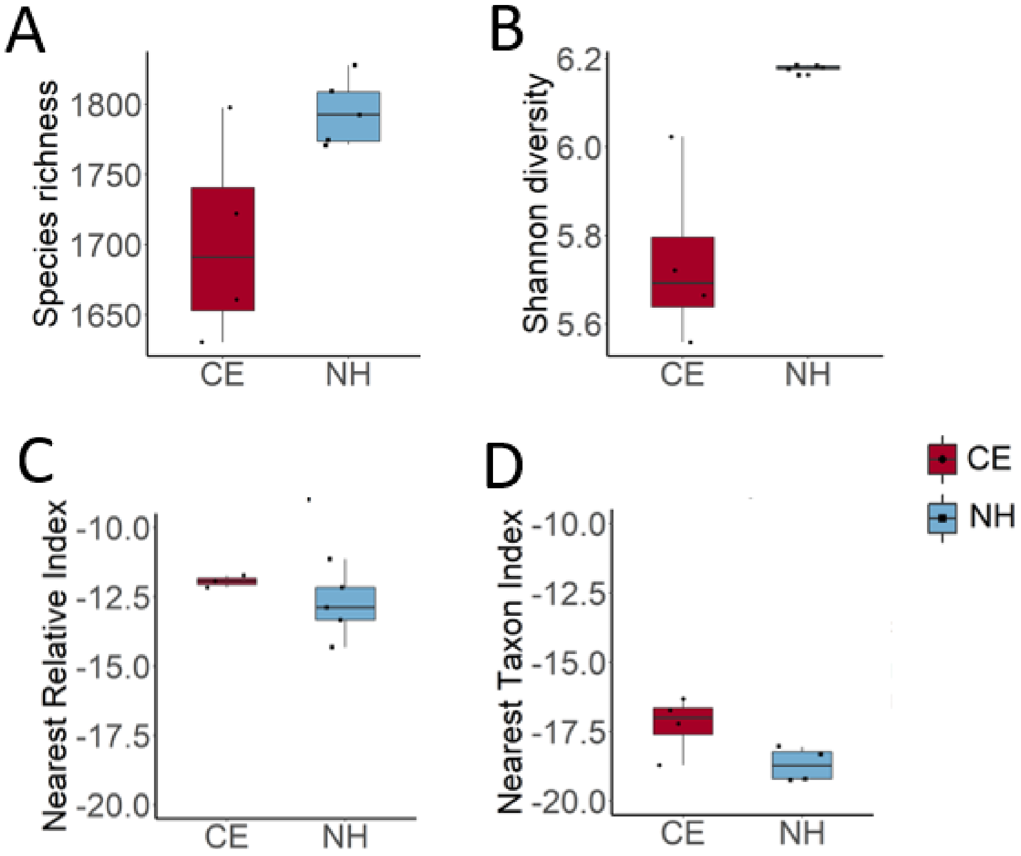

Figure 11. The taxonomic and phylogenetic alpha-diversity of the microbial communities in surface sediment samples per study site (Cape Evans, CE = red; New Harbour, NH = light blue) estimated using metrics of species richness (A), Shannon-Wiener diversity index (B), Nearest Taxon Index (C), and Nearest Relative Index (D).

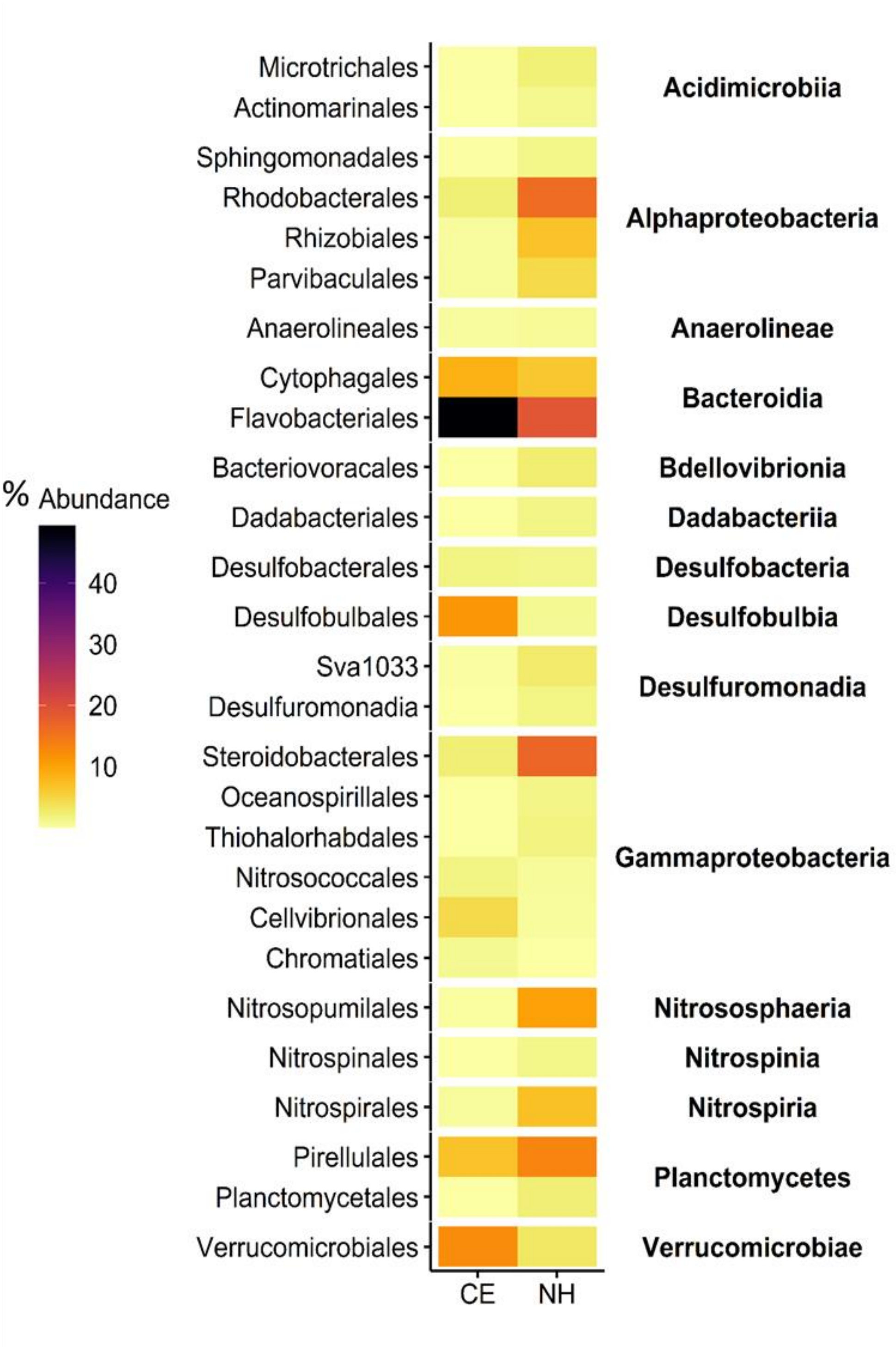

Figure 12. Differentially abundant taxa identified by ANCOM (Analysis of Composition of Microbiomes) in surface sediment samples from Cape Evans (CE) and New Harbour (NH), with *p* > 0.05 and relative abundance >1%, visualised in a heatmap at the taxonomic level of Order (left) and grouped by Class (right). Darker scale colours represent higher abundances.

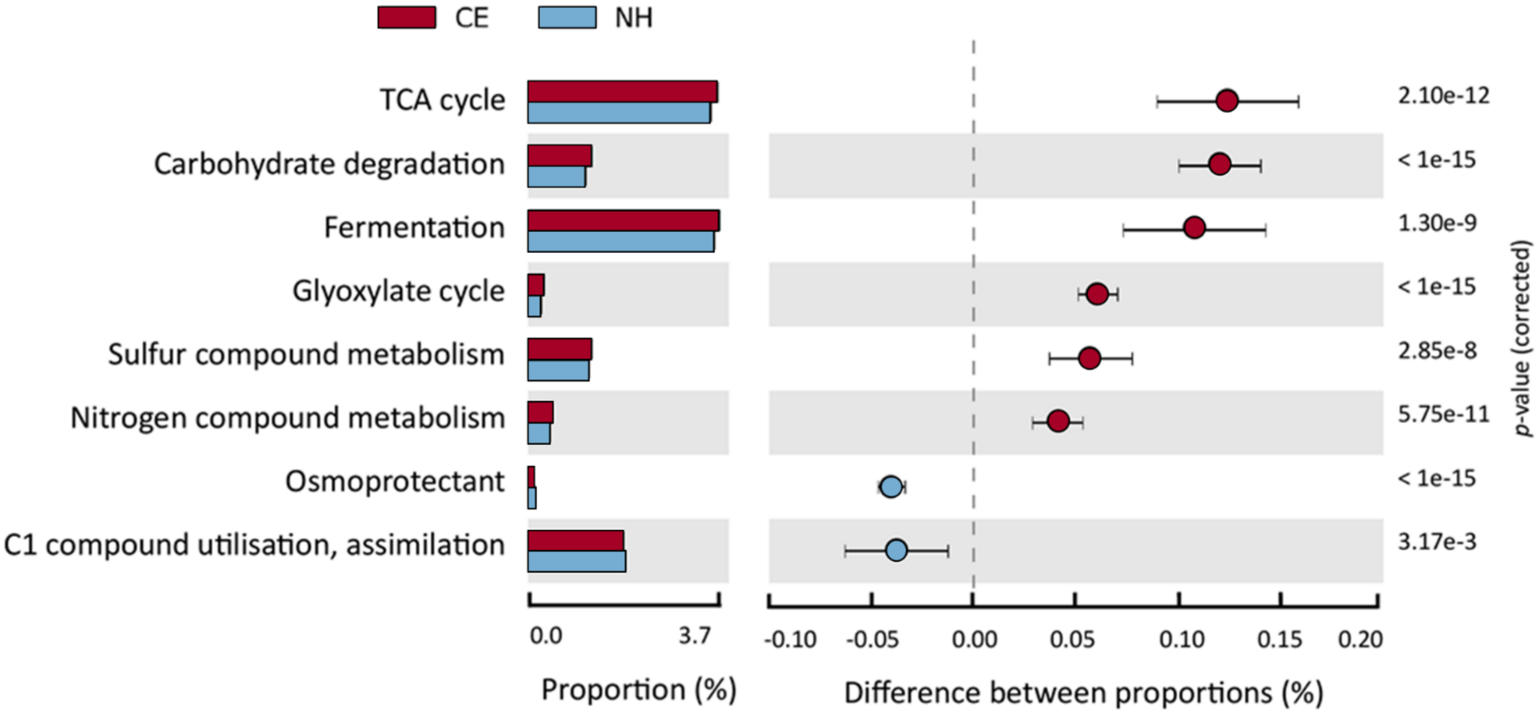

Figure 13. The relative abundances of selected PICRUSt2 predicted functional pathways based on the MetaCyc database in sediment samples from Cape Evans (CE) and New Harbour (NH), with $p > 0.05$ and minimum sequences = 2, grouped according to parent level categories. Selected MetaCyc pathways are shown that were representative of the microbial taxa of interest within this study at each site.

2.5 Discussion

This study investigated how a legacy of high and low sediment organic carbon loading drives benthic microbial community structure and composition beneath multi-year ice versus first-year ice in McMurdo Sound, Antarctica. The aim was to better understand the potential impacts of climate driven sea ice transitions on benthic biogeochemical processes. For this study, I hypothesised that local sedimentary geochemistry and organic carbon loading driven by sea ice and oceanographic conditions drives the taxonomic structure and composition of Cape Evans and New Harbour sediment microbial communities, and that differences would not be driven by surface terrestrial inputs from the Taylor Valley.

The $\delta^{13}C$ and $\delta^{15}N$ values of the Cape Evans and New Harbour sediment samples were comparable to sediments from the Antarctic Peninsula (Learman et al., 2016), supporting the role of diatom (Meyers, 1994) derived organic matter within this system. During this study, sea ice conditions were at near annual maximum thickness (Parkinson, 2019) and sediments were representative of an over-winter ecosystem which had not yet been highly impacted by new transient summer algal- and phytoplankton blooms (McMinn et al., 2010). As expected, sediment chlorophyll-α (an algal pigment), total organic carbon (TOC) and total nitrogen (TN) content were higher in Cape Evans sediments where thinner first-year ice conditions can support higher concentrations of under-ice algae (Cummings et al., 2019; Lohrer et al., 2013) and where phytoplankton-rich waters are advected into the area via southbound currents from the productive open ocean (Barry & Dayton, 1988). Whereas, the lower chlorophyll-α, TOC and TN content within the New Harbour samples were reflective of the thick multi-year ice cover which severely restricts under-ice algal-productivity and the inputs of oligotrophic source waters that emerge from underneath the Ross Ice Shelf (Barry & Dayton, 1988). These findings were supported by recent reports that inorganic solute fluxes are higher in sediments at Cape Evans than New Harbour (Lohrer et al., 2020) indicating that organic matter remineralisation is an important process at Cape Evans. Substantially higher abundances of benthic macrofauna at Cape Evans (Lohrer et al., 2020) could enhance microbial activity by burrowing and turning over the sediment which introduces fresh organic matter and oxygen to depths (Lohrer et al., 2004). The contrast in sediment geochemistry between the sites is indicative that the microbial communities will also differ, confirming that these are ideal sites to understand the impacts of sea ice variability on benthic ecosystem processes.

Measures of community alpha-diversity revealed the oligotrophic conditions underneath multi-year ice at New Harbour selected for significantly greater sediment bacterial diversity which were more phylogenetically over-dispersed than the organic matter-rich sediments underneath first-year ice at Cape Evans. A legacy of multi-year ice coverage that has largely persisted for the last 2,000 years (Emslie et al., 2003), together with consistent oligotrophic source water inputs from underneath the Ross Ice Shelf, has established a sediment environment at New Harbour that is likely to be more environmentally stable with persistent nutrient limitation. Long-term stable environmental conditions are thought to promote opportunities for metabolic specialisation and niche differentiation within microbial communities (Hibbing et al., 2010). Comparatively higher community diversification and the greater number of unique ASV identified within these New Harbour sediments support my hypothesis that New Harbour communities are reflective of these limited resources and stable environmental conditions. Whereas, the dynamic and more seasonally variable environment at Cape Evans could select for sediment taxa with broader environmental tolerances and metabolic flexibility (Chen et al., 2020). These findings are consistent with a study by Learman et al. (2016) who reported finding greater bacterial diversity in the lower organic carbon sediments of the Ross Sea compared to the high organic carbon sediments of the Antarctic Peninsula.

The sediments of Cape Evans and New Harbour were both represented by comparable Phylum level taxa, *Proteobacteria, Bacteroidota,* and *Planctomycetota*, which are ubiquitous and widely distributed in marine sediments, including beneath the Ross Ice Shelf (Carr et al., 2013). The high proportion of shared ASVs (63%) across our study sites which are ~60 km apart indicates the clockwise ocean current that circulates through McMurdo Sound and beneath the Ross Ice Shelf is an important oceanographic connection likely acting as a mechanism for microbial dispersal (Hamdan et al., 2013; Müller et al., 2014; Wilkins et al., 2013). The oceanic connectivity is likely working simultaneously with environmental selection, driven by differences in sediment organic matter content, nutrient inputs and primary productivity to impact structural differences between the sediment communities at Cape Evans and New Harbour.

Despite the Phylum level similarities identified within Cape Evans and New Harbour samples, significant site-based compositional differences were evident at the Order level. At Cape Evans, these differences are driven by key metabolic taxa that have previously been found to be abundant in other organic matter-rich coastal sediment environments. For example, *Flavobacteriales*, *Cytophagales*, and *Verrucomicrobiales* are known algal degraders and were found to be prevalent in the productive sediments of the Antarctic Peninsula (Learman et al., 2016) as well as in the Northern Hemisphere Helgoland Mud Area (Oni et al., 2015) and Svalbard fjord (Cardman et al., 2014). Sulfate-reducing bacteria *Desulfobulbales* are associated with degrading organic compounds in the anoxic sediment layer and may also disproportionate sulfur compounds (i.e. an 'inorganic fermentation'), providing metabolic versatility that would be advantageous under dynamic environmental conditions (Wasmund et al., 2017). These strict anaerobes are indicative that anoxic sediments (Middelburg, 2019a) lie within the top 2 cm of the sediment profile at Cape Evans. Together, these signature taxa are likely selected for by seasonally high algal flux driven by first-year ice conditions and the advection of productive and nutrient-rich waters from the open ocean. The pathways predicted by PICRUSt2 including carbohydrate and sulfur compound metabolism and fermentation supports the importance of organic matter deposition in structuring the sediment community composition (Jørgensen, 1977; Middelburg, 2019a) at Cape Evans.

In comparison, the microbial community within New Harbour sediments was represented by taxa that have previously been found to exist in other oligotrophic marine sediment environments. For example, *Alphaproteobacteria* were found to be dominant community members in the ultraoligotrophic sediments of the South Pacific Gyre (Morono et al., 2020; Tully & Heidelberg, 2016). Within New Harbour sediments, chemolithoautotrophic taxa *Nitrososphaeria, Woeseiaceae,* and *Nitrospira* were refelctive of a community dependance on inorganic nitrogen species (e.g. ammonium and nitrate) (Orsi, 2018), and *in situ* chemoautotrophic carbon fixation, providing heterotrophic taxa with a source of organic matter (Tully & Heidelberg, 2016). *Nitrososphaeria* are known thrive in nutrient limited environments (Könneke et al., 2014) and *Woeseiaceae* may be capable of performing light independent carbon fixation coupled to sulfur oxidaiton (Dyksma et al., 2016; Mußmann et al., 2017). These signature taxa are indicative of low sediment organic compound concentrations driven by low algal flux from multi-year ice cover and oligotrophic source waters from the Ross Ice Shelf and that oxygen penetration is deeper in these sediments than at Cape Evans (Orsi, 2018). In support of these findings, PICRUSt2 predicted higher abundances of genes involved in carbon fixation pathways within New Harbour samples which included the reductive tricarboxylic acid (rTCA) and Calvin–Benson–Bassham (CBB) cycles. Predicted genes for the CBB cycle were comparatively in lower abundance in New Harbour sediments than the rTCA cycle which may be due to the CBB cycle being a more energetically expensive carbon fixation pathway (Erb, 2011) and thus less favourable in a energy limited environment such as New Harbour. These stark compositional differences between New Harbour and Cape Evans suggests that a transition of multi-year to first-year ice will cause significant shifts in sediment microbial communtiy strucutre and composition, reflected by a shift from dominant chemolithoautotrophic to heterotrpohic metabolisms.

A recent electromagnetic survey of the Taylor Valley has suggested the existence of two subsurface brine aquifers, possibly rich in soluble iron, that flow down valley from the Taylor Glacier to Lake Bonney and from Lakes Hoare to Fryxell and into New Harbour (Foley et al., 2019; Mikucki et al., 2015). This microbial community analysis of the New Harbour sediments has identified a previously described extreme halophile, *Thiohalorhabdales*, which had been isolated from hypersaline lakes in the Mediterranean (Sorokin et al., 2008). While the abundance of *Thiohalorhabdales* within the community was low (1.3%), it was significantly higher than in the Cape Evans samples and provides support for the hypothesised presence of brine channels (Foley et al., 2019; Mikucki et al., 2015) potentially from subsurface Taylor Valley brine aquifer discharge. This finding was also supported by PICRUSt2 which predicted a significantly higher abundance of osmoregulation pathways within New Harbour sediments than Cape Evans which is reflective of organisms that grow in high-osmolarity environments. Within New Harbour sediments this study also found low abundances of iron (II) oxidising and iron (III) reducing bacteria that have previously been reported in Lake Fryxell and Blood Falls, including *Geopsychrobacter* (0.2%) (Li & Morgan-Kiss, 2019), *Cryobacterium* (< 0.1%) (Campen et al., 2019), and *Comamonadaceae* (< 0.1%) (Dillon et al., 2020). Blood Falls is estimated to deliver 20 Gg/year of soluble iron to McMurdo Sound via New Harbour (Campen et al., 2019) which may be a potential iron source for these taxa. These findings further demonstrate the value of microorganisms for identifying potential connections between ecosystems.

Understanding the taxonomic composition of microbial communties using 16S rRNA gene amplicon based approaches has greatly improved our understanding of the taxonomy and phylogeny of microbial communities Lozupone & Knight, 2007. Although, it is the most appropriate appraoach for studying taxonomy, it is limited in its ability to assign and identify functional attributes (e.g. PICRUSt2) of the microbial communities. Further analysis focued at addressing the functional potential of these sediment communites (e.g. metagenomics) could yield greater insight at a higher resolution into the structure, composition, and functioning of the microbial communities of these Cape Evans and New Harbour sediments.

Overall, this study shows that legacy sea ice conditions and consistent oceanography drive sediment microbial communtiy strucutre and composition. The two contrasting sites chosen have provided important detail on the microbial community structure and composition of sediments found under multi-year and first-year ice. Climate driven transitions of multi-year ice to frist-year ice are likely to drive supply changes in algal-derived organic carbon to the benthic ecosystem. Increased organic mater deposition is likely to drive changes in the benthic micorbial communities of New Harbour, which could be reflected by a transition from *in situ* chemoautotrophic carbon fixation and nitrogen cycling towards a dominance of heterotrophic metabolisms. These findings imply that these commnities could be used as sentinels for change in these difficult to study ecosystems. This first characterisation of the microbial communities at these two well-studied sites contributes a baseline of understanding to the benthic marine ecosystem in McMurdo Sound and as the first study to evaluate the possible functional implications of sediment microbial communities under projected climate change conditions in Antarctica, contributing a critical component to our understanding of this ecologically significant marine ecosystem.

Chapter 3

General Conclusions

Globally, some of the most evident impacts of anthropogenic global climate warming are demonstrated in the Arctic where reductions in sea ice thickness, persistence and snow cover and earlier timing of sea ice breakout are altering the timing and magnitude of seasonal algal and phytoplankton blooms (Boetius et al., 2013; Lannuzel et al., 2020; Nadaï et al., 2020), which directly impact the whole ecosystem (Macias-Fauria & Post, 2018). In Antarctica, dramatic reductions in sea ice extent are occurring in the northern West Antarctic Peninsula (Parkinson, 2019) which has decreased primary productivity (Montes-Hugo et al., 2009; Schofield et al., 2018) resulting in cascading effects within the marine trophic food web with observable latitudinal shifts in biota (Massom & Stammerjohn, 2010; Montes-Hugo et al., 2009). A striking example is the poleward shift of the ice-dependent Antarctic krill and Adélie penguins, with a replacement by salps as well as Gentoo and Chinstrap penguins coming from the north (Atkinson et al., 2004; Constable et al., 2014; Ducklow et al., 2007). The sea ice and productivity changes in the West Antarctic Peninsula (Montes-Hugo et al., 2009) and Arctic (Lannuzel et al., 2020) are now well documented, whereas regions significantly further south such as McMurdo Sound in the Ross Sea have yet to experience any significant climate-driven changes (Kim et al., 2018). Climate models are projecting that substantial losses of Antarctic sea ice will occur in the region over the 21st century (Solomon et al., 2007). Despite this, we are only now beginning to understand how climate induced shifts in sea ice primary productivity are impacting each level of these fragile polar food webs, from primary consumers to benthic microbial processes. This paucity of information on the interconnectedness between these habitats impedes our understanding of ecosystem level responses to the impacts of climate driven change.

Within polar studies of primary productivity, the sediment ecosystem is an under-represented habitat. This is especially true within Antarctica with this study providing the first report of the potential impacts of climate change on under-ice sediment microbial communities in Antarctica. The aim of this study was to characterise and compare the structure and taxonomic composition of surface sediment microbial communities underneath contrasting sea ice and oceanographic conditions in McMurdo Sound. This study provides insights into this difficult to study ecosystem and provides a better understanding of how altered fluxes of algal-derived organic matter resulting from reduced sea ice cover may impact benthic biogeochemical process. The findings from this study revealed that sediments beneath the thinner first-year ice were higher in chlorophyll-α concentration and TOC and TN content than the those beneath the thicker multi-year ice, thus reflecting higher primary productivity at the surface, advection of productive open ocean waters, and increased organic matter flux to the seafloor. Within the organic matter-rich sediments, lower microbial diversity was indicative of reduced bacterial competition for limited carbon resources (Hibbing et al., 2010) which might otherwise drive diversification and niche specialisation within a community (Schluter, 1996). Taxonomic signatures of sediment organic matter content were identified with heterotrophic algal biopolymer degrading taxa and sulfate reducing bacteria reflecting high organic matter flux from first-year ice and advected open ocean currents (Cardman et al., 2014; Jørgensen et al., 2019). Comparatively higher benthic oxygen consumption and solute flux from the organic matter-rich sediments was indicative of enhanced heterotrophic organic remineralisation processes, likely resulting in a shallower oxygen penetration depth and enriching facultative and strictly anaerobic bacteria (Middelburg, 2019a). In comparison, a selection for nitrogen cycling taxa and chemolithoautotrophic carbon fixing bacteria and archaea in organic matter-poor sediments was reflective of the low organic matter flux (Parro et al., 2019) from multi-year ice and oligotrophic source waters from underneath the Ross Ice Shelf. By predicting community functional pathways from the taxonomic dataset this study provides support that compositional differences could be reflective of functional differences (Douglas et al., 2020) within these sediments. This suggested that organic matter-rich sediments were distinguished by pathways involved in the aerobic and anaerobic oxidation of organic compounds whereas the organic matter-poor sediments were more represented by chemoautotrophic carbon fixation pathways.

The benthic ecosystem of McMurdo Sound has been used as an ecological research site for over the last 4 decades due to the ‘natural laboratory-like’ conditions, strong east-west gradient (Dayton & Oliver, 1977), and its proximity to two major research bases. As this is the first report describing the sediment microbial communities in McMurdo Sound, the findings from this study contribute a critical component to our understanding of the McMurdo Sound benthic ecosystem and trophic processes. This dataset could be used within a long-term environmental monitoring programme to capture shifts in sediment biological processes over time to support our understanding of the impacts of climate change. For example, microorganisms have been used in monitoring change in coral reef systems which are under pressure from both regional and global changes (Glasl et al., 2019). Due to the oceanographic connectivity of McMurdo Sound and the Ross Ice Shelf, these sites could also be utilised as external oceanographic control points (inflow and outflow) for any research examining the unexplored sediment microbial communities deep under the Ross Ice Shelf.

The bacterial 16S ribosomal RNA (rRNA) gene is the most commonly used marker gene to taxonomically identify bacteria and archaea with high-throughput sequencing (Janda & Abbott, 2007). Amplicon based sequencing approaches have greatly improved our understanding of bacterial phylogeny and taxonomy, however our ability to understand microbial community functional attributes lies in the capabilities of predictive tools such as PICRUSt2 (Douglas et al., 2020) which utilises a limited genome database and is limited as it infers an associated function using taxonomic maker genes. Other molecular approaches such as metagenomics could now be applied to the same Cape Evans and New Harbour samples examined in this study to provide further insight beyond that offered by 16S rRNA gene amplicon sequencing. This additional approach would provide deeper taxonomic resolution but more importantly would generate an assessment of microbial community functional capacity at each site which was not possible with the taxonomic approach used in this study or by PICRUSt2. By using a metagenomics approach, the presence, absence and relative abundance of functional genes of interest could be determined, which will allow questions around ecosystem stability and resilience in response to climate driven sea ice change to be answered. In potential future studies even deeper insight into community functioning could be gained by sampling for metatranscriptomics and complementary metabolomics approaches. These methods provide an assessment of which taxa are active and what they are doing by profiling expressed transcripts (messenger RNA) and the intermediates or end products of cellular metabolism (metabolites).

The changes that have occurred in the West Antarctic Peninsula provide a valuable example of how changing sea ice and primary productivity dynamics will have cascading effects on polar marine ecosystems (Massom & Stammerjohn, 2010; Montes-Hugo et al., 2009). This baseline study makes an important contribution towards building our understanding of the benthic microbial communities in Antarctica. This study has shown that sea ice thickness and persistence and oceanographic conditions drives sediment microbial community structure and taxonomic composition and their predicted associated functions, which further demonstrates the use of microorganisms as sensitive sentinels for environmental change (Cavicchioli et al., 2019). With projections of sea ice reductions to occur in Antarctica this century (Solomon et al., 2007), sediment biogeochemical processes are likely to be impacted as the composition of the sediment microbial communities shift in response to changes in surface primary productivity and organic matter fluxes. Potential implications for nutrient cycling and marine ecosystem functioning via the connectivity of the food web incites the need for more co-ordinated studies that link sediment microbial processes to water column and surface primary productivity processes. This will enable a better understanding of the impacts of sea ice variability on high-latitude ecosystems and prediction of future changes under current climate change scenarios.

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