Bacterial communities of the Arctic sea ice, drifting towards an age of uncertainty

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In
Microbiology and Biotechnology

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University of Alberta

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Abstract

The Arctic Ocean sea ice is shifting from a system dominated by thick perennial ice (multiyear ice –MYI) to one dominated by thinner, seasonal ice (first year ice – FYI). The effects of this shift on the bacterial constituents of the Arctic sea ice have been grossly under studied, although it is a vast habitat for a bacterial community. This community plays a key role in the biogeochemical cycles and energy flux of the Arctic Ocean via regulating and reseeding the spring and summer algal bloom. Furthermore bacterial secondary productivity supplies the water column with nutrients during the polar winter. MYI and FYI are similar in that both are cold, semi-solid habitats originating from the freezing of sea water; however, they differ in many of their chemical and physical attributes. MYI has lower bulk salinity and volume than FYI mainly due to seasonal flushing with melt water; the temperature gradient is steeper and there is less light attenuation in FYI than MYI because FYI is thinner; and MYI has features, such as freshwater surface melt ponds and annual growth layers, that are absent from FYI. To date, there has been almost no comparing and contrasting the structure and composition of microbial communities from both ice types. I contend that, given the importance of sea ice bacterial communities to the ecological functioning of the Arctic Ocean, such a comparison is crucial to our understanding of the repercussions of declining MYI cover to the Arctic Ocean environment. Therefore, I tested whether bacterial communities in Arctic MYI and FYI differ in composition and membership, as well as richness and diversity. Furthermore, I tested the response of these bacterial communities to environmental disturbance.

To test this, MYI and FYI cores were sampled from land-fast ice in the Lincoln Sea off the shore of Northern Ellesmere Island, NU, Canada, during the months of April and May in four consecutive field seasons (2010-2013). For depth-specific assessments, cores were sectioned into

30 cm sub-sections; for assessment of FYI vs. MYI bacterial structure, whole cores were assessed. To simulate environmental disturbance, cores from MYI and FYI sites where inserted inverted back to the borehole to disrupt the temperature and brine salinity gradients that occur in sea ice.. Assessment of microbial community structure was performed by analysis of 16S rRNA gene sequences.

Bacterial communities from MYI cluster to three statistically distinct communities representing the top, middle, and bottom of the ice. Bacteria from the classes Flavobacteria, Bacteroidetes, Actinobacteria and Betaproteobacteria dominated the top communities and decreased in relative abundance with depth, where as Gammaproteobacteria and Alphaproteobacteria increased in relative abundance with depth. Rather than associating with gradients in salinity or temperature, these changes in bacterial communities were associated with three distinct layers within the ice: melt pond ice, old (i.e. more than one year) ice, or new (i.e. formed over the winter season prior to sampling) ice. My results also show that in contrast to MYI samples, communities from FYI do not show distinct top to bottom clustering, supporting my second hypothesis. Though communities from both ice types were dominated by members of similar class level groups, FYI and MYI had distinct membership at the operational taxonomic unit (OTU) level. Bacterial composition was highly variable in FYI from different sites and field seasons; however, bacterial community composition in MYI samples was much more similar to each other. Treatments designed to simulate environmental disturbance had mixed effects. Core inversion transplantation resulted in changes to the membership and composition of the top communities from the MYI core making them similar to communities from the middle sections of the core. Results from similar disruption experiments on FYI cores were confounded by the highly variable community composition and lack of vertical structure in the communities.

Taken together, these results indicate that the shift from a MYI dominated Arctic to seasonal ice dominated Arctic will lead to a sea ice bacterial community with more variability and less predictability. This shift may indicate a transition to a community less able to rebound from environmental disturbance. I discuss potential implications of these findings for understanding and modeling responses of the Arctic Ocean ecosystem to the challenge of a changing climate and proposed future directions for this research.

Preface

This thesis includes previously published and submitted work (Chapters 2&3). The work concepts for all chapters were designed and conceived by IH and BDL. IH collected the samples, analyzed the data and wrote all chapters with assistance, guidance and editing from BDL and coauthors as listed in the citation for each chapter (see individual chapters).

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"So I started to walk into the water. I won't lie to you boys, I was terrified! But I pressed on and as I made my way passed the breakers a strange calm came over me. I don't know if it was divine intervention or the kinship of all living things but I tell you Jerry at that moment I was a marine biologist!" (George Costanza, 1994)

Acknowledgments

First and foremost I would like to extend many thanks and gratitude to my supervisor, Dr. Brian Lanoil, for his support, patience and guidance over the past five years. Brian thanks for everything, thanks for giving me the opportunity to do research in places where I doubt otherwise I would have had the chance to go. Thanks for introducing me to the field of microbial ecology, thanks for driving and pushing me while acknowledging my stubbornness. I know it couldn't have been easy.

I would also like to extend special thanks to our collaborator and member of my advisory committee Dr. Christian Haas for adopting me as the pet microbiologist of the CASIMBO crew and giving me the opportunity to conduct research in literally such cool (some would say cold) places. Christian, if it wasn't for your contagious enthusiasm for sea ice I probably wouldn't have made sea ice microbiology the focus of my thesis.

I would also like to thank other members of the CASIMBO entourage, Justin Backers, Alec Casey and especially Ben "Benjo" Lange for all the assistance, comradery and beer in the field. You guys made things so much easier.

To all past and present Lanoil Lab entourage, fellow students and friends who helped me along the way both scientifically and recreationally: Sina Kazemi, Ann Hammad, Elham Taghavimer, Anna Bramuchi, Leen Labeeuw, Paul "Klaus" Kirchberger, Joleen Khey, Jessica Kozllowski, Dimitry Kirm Kits, Mo Ghashghavi, Rhianna Charchuk, Annabella yee, Ryan Fyfe-Brown, Dr. Alex smith, Albert Remus Rosana and Cec Anders. I thank you all.

I would like to extend my thanks to members of my candidacy and advisory committees, Dr. Rolf Vinebrooke, Dr. Rebecca Case, Dr. Julia Foght and Dr. Duane Froese for all the input

they provided. I would also like to thank Dr. Connie Lovejoy and Dr. Jens Walter for the critical review and comments that helped improve this thesis.

Lastly I would like to thank my family and especially my partner Carmit for their love and support without which I don't know that I could have concurred the highs and lows of this PhD.

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1 Introduction

1.1 A prelude to the prelude

Arctic sea ice is the most prominent feature of the Arctic in general and the Arctic Ocean in particular. The Arctic Sea ice can be classified into two main types: A) seasonal ice, which forms during the freeze-up period and undergoes complete melt during the spring and summer, a.k.a. first year ice (FYI); and B) perennial or multi-year ice (MYI), which is ice that survives at least one melt season (Wadhams, 2000).

The Arctic Ocean was dominated by MYI cover however over the past three decades, there has been a radical reduction in the averaged minimal summer sea ice extent making seasonal sea ice the de-facto prominent ice cover in the Arctic (Maslanik *et al.*, 2011). Sea ice is a key regulator of Arctic climate and also provides habitat to many species of organisms, from polar bears to viruses. Thus, the decline in sea ice extent stands to severely impact many aspects of the Arctic ecosystem (Vincent, 2010; Post *et al.*, 2013). One of the key challenges in Arctic research is to understand what impact this change will have on Arctic Ocean energy flux and biogeochemical cycles (Wassmann & Reigstad, 2011). Sea ice bacteria are key regulators of such cycles; therefore, understanding if there are differences in the composition of bacterial constituents of MYI and FYI and linking those differences to potential differences in function will be a first step in this direction.

In my literature review, I will discuss the impacts and expected implications of the shift to a seasonal ice cover on the Arctic and the Arctic Ocean. I will then discuss the bacterial components of the Arctic sea ice, focusing on sea ice as a habitat, the adaptations required to survive in sea ice, and the role of sea ice bacteria in biogeochemical cycles. I will present why

MYI and FYI should be considered as different habitats for bacteria and why we should expect to find different bacterial communities residing within them. Lastly, I will give an overview of my thesis and my research objectives.

1.2 Arctic sea ice- a brief overview of its importance to the Arctic and the effects of global warming

1.2.1 The decline in Arctic sea ice minimal extant, a harbinger of global warming

Covering $15x10^6$ km^2 at its maximal extent, over 1.5 times the size of Canada, Arctic sea ice is one of the most prominent features of the Arctic. It is also very dynamic and undergoes large seasonal fluctuations the area it covers between minimal extent at the end of September and maximal extent at the end of March as can be viewed in Figure 1.1 (Stroeve *et al.*, 2007).

One of the most conspicuous consequences of recent global climate change has been the increased size of these fluctuations between minimal and maximal sea ice extent (Serreze *et al.*, 2007; Stroeve *et al.*, 2007). Until recently, MYI accounted for approximately 75% of the sea ice covering the Arctic Ocean, however, in the past three decades there has been a dramatic decrease in the proportion of Arctic MYI of the total sea ice cover (Serreze *et al.*, 2007). A series of measurements, taken between March of 1983 and March of 2011, have revealed that the proportion of MYI of the total sea ice covering the Arctic Ocean has decreased from 75% to just 45% of the ice cover (Maslanik *et al.*, 2011). These measurements also indicated that while old sea ice is being exported out of the Arctic Ocean, it's being replaced by younger MYI, due to diminishing replenishment rates. As thinner FYI replaces MYI and the MYI remaining tends to be younger and therefore thinner, Arctic perennial sea ice cover is not only shrinking in extent, it is also losing volume (Maslanik *et al.*, 2011).

It is worth noting that the rate at which MYI cover is lost appears to be accelerating and that in the past twelve years, the Arctic has experienced nine record low years in MYI extent, with the lowest being 2012 (Parkinson & Comiso, 2013). It is forecasted that the accelerated loss of MYI cover and volume is expected to continue and conservative models predict that the Arctic Ocean will be largely ice-free during summer by the end of the 21st century (Boé *et al.*, 2009).

Since sea ice is such a prominent feature of the Arctic, it is tied and linked to many aspects of the Arctic as a whole. In the following sections, I will detail the implications that are expected for the climate, Arctic biology, and sea ice microbiology as consequence of the reduction in MYI.

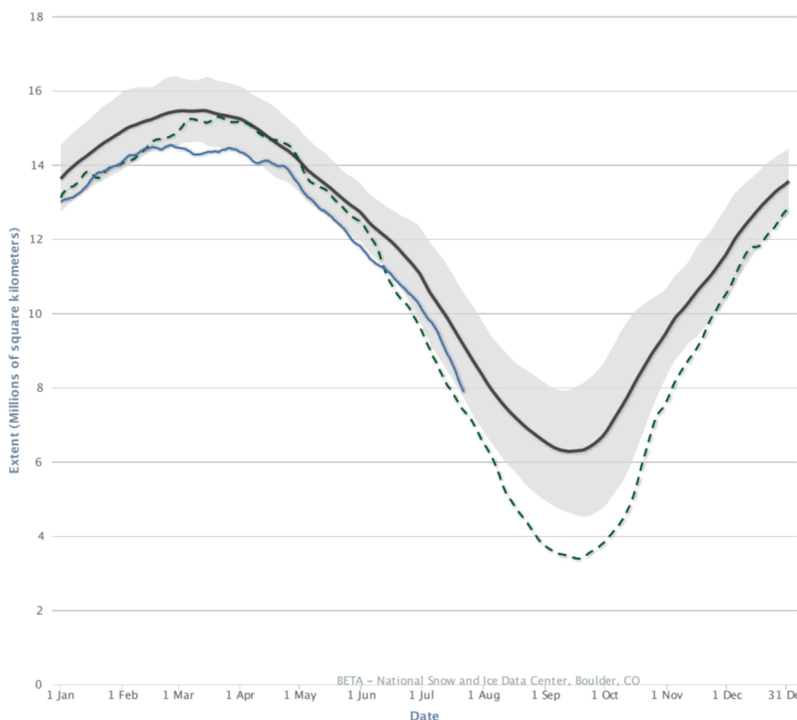

Figure 1.1 Annual fluctuation in Arctic sea ice extent: The black line represents 1981-2010 average extent and the gray shading represents ± 2 SD. The dashed black line represents 2012 record low MYI extent. The blue line represents the extent for 2015 i.e. this year

1.2.1.1 Implications of MYI loss to Arctic heat budget and climate

Sea ice plays an important role in the heat budget of the Arctic due to insulation of the Arctic Ocean, preventing heat transfer between the seawater and the atmosphere (Dieckmann & Hellmer, 2010). Furthermore, reflection of solar radiation during the spring and summer due to the high albedo of sea ice and the snow that accumulates on it also reduces the heat trapped by the Arctic Ocean (Hall, 2004; Perovich *et al.*, 2007). The diminishing summer extent of sea ice leads to larger open water areas with lower albedo, thus increasing the heat input to the Arctic Ocean both from exchange with the atmosphere and from solar radiation (Riihela *et al.*, 2013). The increased heat stored in the ocean in turn causes a delay in the onset of freezing and extending the period of open water in the Arctic Ocean (Dieckmann & Hellmer, 2010). This has a twofold effect; first the extended period of open water in the Arctic Ocean means that more heat can be radiated to the atmosphere from the Arctic Ocean thus raising the temperatures of the atmosphere and resulting in the accelerated warming of the Arctic as a whole (Overland *et al.*, 2012; Pithan & Mauritsen, 2014). Second, increased periods of open water where linked to changes atmospheric circulation over the Arctic region resulting in changes to climate patterns throughout the Arctic that accelerate the export of sea ice from the Arctic Ocean (Overland *et al.*, 2012). Accelerated export of sea ice in turn results in further increase the area of open waters which in turn radiate more heat to the atmosphere further increasing the rate in which the Arctic warms (Overland *et al.*, 2012). Thus it is clear that the reduction in MYI extant contributes significantly to the accelerated warming of both the Arctic Ocean and the Arctic in general.

The acceleration of warming in the Arctic due to the shrinking in MYI extent has had and expected to continue to have effects on habitats directly and indirectly tied to sea ice. Mainly this entails loss of these habitats or barriers between habitats due to warming. This stands to affect

many species dependant on these habitats thus the loss of MYI have severe consequences for their survival. Though this is an important topic, it is outside the scope of my thesis beyond the scope of this introduction to fully detail and is discusses in excellent reviews by Vincent and Post et al. (Vincent, 2010; Post *et al.*, 2013). Instead, since the focus of my thesis is sea ice bacterial communities of sea ice and since the Arctic sea ice is an integral part of the Arctic Ocean next I will discuss the expected effects of MYI loss on the Arctic Ocean food web and energy flux. Since sea ice bacteria are key components of both and the focal point of my thesis, I will then go deeper in their description starting from incorporation to sea ice, the ice as a habitat and their role in the environment.

1.2.1.2 Implications of MYI loss for Arctic Ocean energy flux and food web

The Arctic sea ice and its constituents are involved deeply in the regulation of energy flux to the Arctic Ocean. Due to ice attenuation of solar radiation, sea ice extent and thickness regulates light availability in the water column thus in conjunction with nutrient concentrations is also a regulator of phytoplankton blooms in the Arctic Ocean (Sakshaug & Skjoldal, 1989). It has been suggested that phytoplankton blooms in the Arctic Ocean only occur as the ice recedes and enough light can be reach the water column to trigger massive and yet short lived blooms: this geographical limitation is called the ice edge effect (Sakshaug & Skjoldal, 1989). This effect is also linked to the release of nutrients produced by sea ice microbes and this aspect will be detailed in upcoming paragraphs of this section (Sakshaug & Skjoldal, 1989; Niebauer *et al.*, 1995). However, it is becoming clear that the shift to a FYI-dominated Arctic is changing the paradigm linking phytoplankton bloom exclusively to sea ice retreat (Arrigo *et al.*, 2012; Lowry *et al.*, 2014).

Since FYI is much thinner than MYI, it allows higher levels of solar radiation to reach the water column. As the Arctic warms, the fraction of ice covered by melt ponds (i.e. fresh water ponds originating from snow melt) on these thin FYI sheets has also increased significantly over the past decade (Polashenski *et al.*, 2012). Water has a lower albedo than ice; as a result, melt-ponded ice transmits 3–10 times more light to the surface seawater than bare ice of the same thickness (Frey *et al.*, 2011). Thus, the combination of a thinner ice cover with larger proportion covered by melt ponds leads to a significant increase in light levels penetrating the ice covered Arctic Ocean (Perovich *et al.*, 2011). These new light regimes have enabled the frequent occurrence of massive under-ice phytoplankton blooms in the western Arctic Ocean over the past 15 years (Arrigo *et al.*, 2012; Lowry *et al.*, 2014). These blooms occur in continental shelves where there are no nutrient limitations due to the high amount of river discharge (Lowry *et al.*, 2014). However, more than half of the Arctic Ocean consists of shallow continental shelves with water rich in nutrients. Therefore, these under ice blooms will become more widespread as the proportion of thin heavily melt-ponded sea ice increases (Lowry *et al.*, 2014).

Changes in energy and biogeochemical cycles due to the decrease in MYI are not limited to areas of the Arctic Ocean where light is the limiting factor. Sea ice microbes, mainly diatom algae, provide much of the organic matter produced in the Arctic Ocean (Gosselin *et al.*, 1997). So long as sea ice is present, the organic matter enters the water column in slow servings of nutrients and feeds large quantities of sub-ice zooplankton grazing on the ice algae and bacteria. When the ice melts, stored organic matter within it is released in large quantity, which is another trigger for the ice edge effect (Sakshaug & Skjoldal, 1989; Niebauer *et al.*, 1995). The loss of MYI is expected to increase the area in which these short episodic blooms occur, at the expense of the longer, more stable ice algae blooms. This shift is expected to result in more net organic

matter production, but over a shorter time. Therefore, it is predicted that this “pulse” of organic matter and nutrients will be mainly available to the constituents of the microbial community of the Arctic Ocean, thus limiting the quantity and quality available for higher trophic levels (Wassmann & Reigstad, 2011).

Here I discussed two possible ways by which the loss of MYI is expected to affect energy and nutrient cycles in the Arctic Ocean. The first was an increase in under ice phytoplankton blooms due to the thinning of sea ice. The second was an increase in areas where episodic ice edge phytoplankton blooms occur due to the larger areas of ice melts resulting in the release of organic matter produced by sea ice algae. The combined implications of both are not yet fully understood. However, it is clear that the second mechanism is heavily influenced by the work of sea ice microbes, since the organic matter is produced by ice algae and its availability to higher trophic levels is regulated by bacterial feeding on the organic matter the produce by the aforementioned algae. Therefore, here will be a good point to introduce the microbial constituents of the Arctic sea ice. Since my thesis deals with bacterial communities, I will mainly focus on members of this domain.

1.3 A deeper look into sea ice and sea ice bacteria: From sea ice formation and bacteria incorporation to biogeochemical cycles

1.3.1 The onset of sea ice formation and bacterial incorporation

Bacterial incorporation into sea ice begins with the early onset of sea ice formation during the fall, as the upper ocean Arctic Ocean is being cooled from above by the atmosphere. Due to the salinity values of the Arctic Ocean surface seawater (~27 ppts- 32 ppts), the freezing point is depressed compared fresh water (~-1.86° C). The surface water peak density coincides

with its freezing temperature rather than at 4° C, as is seen for freshwater (Eicken, 2008). Therefore, as the surface Arctic Ocean is cooled by the atmosphere to its freezing point, the densest water is found at the top (Wadhams, 2000). This generates mixing of the surface layer of the Arctic Ocean surface water layer in a process called thermo-haline convection, in which colder surface water sinks in-parallel to an upwell of warmer seawater from below (Wadhams, 2000). The thermo-haline convection continues until the entire upper water layer, stirred by wind and wave action as well, is at the freezing point at which point ice begins to form (Wadhams, 2000).

These early ice crystals are needle or platelet-shaped and are known as frazil ice. Frazil ice forms throughout the water column (Eicken, 2008). These needle-like ice crystals accumulate microbes as they travel up the water column and incorporate them in to the growing sea ice as they consolidate (Weissenberger & Grossmann, 1998). The incorporation of cells via harvesting or scavenging by frazil ice during the early stages of ice formation enriches bacterial cells in the sea ice by orders of magnitude relative to the surface water (Garrison *et al.*, 1983; Gradinger & Ikävalko, 1998).

Frazil ice crystals preferentially scavenge larger particles, thus, large algal cells (>10 μm) that secrete extracellular polymeric substances (EPS; see section 1.3.3.1 below for more details) are preferentially incorporated into the ice (Gradinger & Ikävalko, 1998). Marine algae with the ability to nucleate ice crystals are also preferentially scavenged by frazil ice as they are lifted towards the growing sea ice layer by the forming ice surrounding them (Knopf *et al.*, 2011). Although the surface seawater is the main inoculum source for sea ice, during turbulent conditions the formation of frazil ice can occur as deep as 25-30m below the water surface.

Therefore, in shallow shelves, frazil ice may harvest sediment particles and cells that associate with them directly from the benthos (Reimnitz *et al.*, 1992).

The scavenging of large particles from the water column and the benthos by frazil ice also indirectly facilitates the incorporation of bacteria into the forming ice. The indirect incorporation occurs through bacterial association with algal cells, sediment particles and aggregates of particulate EPS (pEPS) mainly of algal origin (Grossmann & Dieckmann, 1994; Weissenberger & Grossmann, 1998; Riedel *et al.*, 2007b). Based on artificial sea ice studies, bacteria producing EPS are also selectively and directly scavenged by frazil ice; however, field experiments designed to test for increased EPS production rates or capacity by sea ice bacteria have been inconclusive (Ewert & Deming, 2011).

Following further freezing, the frazil ice consolidates to form a surface slush layer, composed of frazil ice with granular texture. Subsequently, the microstructure and texture of the ice begins to change as new layers are added to the bottom (Eicken, 2008). Since wind and wave action are quieted by the formation of the slush layer, this phase of sea ice growth is termed quiescent growth. It is characterized by vertically elongated ice textures called columnar ice, which is a few cm in diameter and can grow to tens of cm in length (Eicken, 2008). At this point, bacteria are still incorporated into the growing ice via the sieving action of the columnar ice crystals as they grow (Weissenberger & Grossmann, 1998).

Due to its molecular structure, ice Ih (i.e. hexagonal ice crystal; the main form of crystalline ice found at surface temperatures and pressures) cannot incorporate impurities (e.g. ions, dissolved organic and inorganic molecules, microbial cells, etc.); therefore, as columnar ice grows and continues to consolidate, these impurities are rejected to the remaining liquid phase.

This liquid phase enriched with impurities becomes trapped as inclusions of highly saline and highly nutrient rich brine which is the source of the liquid phase found in sea ice (Weeks & Ackley, 1986; Weissenberger *et al.*, 1992; Light *et al.*, 2003). The columnar ice grows vertically; therefore, brine inclusions are mostly vertically oriented and tend to exhibit characteristic horizontal spacing (Wadhams, 2000). Trapped brine cells are not completely isolated; they form an interconnected network of fine channels and veins that results in the ice having a porous, sponge-like structure (Weissenberger *et al.*, 1992; Light *et al.*, 2003).

The brine network is where over 95% of bacterial cells are found, making brine the main habitat in sea ice (Junge *et al.*, 2001). In the following sections I will describe the conditions facing bacteria within this labyrinth.

1.3.2 Conditions in the brine network

The transformation from living in the water column to living within the brine introduces many challenges to the bacterial constituents of the sea ice. These challenges include higher salinity, lower temperature, higher nutrient concentrations and a reduction in habitable space compared to the water column (Cox & Weeks, 1983; Haas *et al.*, 1997; Krembs *et al.*, 2000). Due to the thickness of sea ice, the environment within the brine network varies and bacteria face different levels of extreme conditions depending on vertical location.

The foremost form of variability is the top-to-bottom temperature variations. During the autumn and winter, the temperature at the top layers of sea ice is influenced by air temperature, which can drop below -50° C. Sea ice temperatures do not normally get as cold as the air due to snow insulation, however it is not uncommon for bacteria residing in the upper portions of the Arctic sea ice to experience temperatures as low as -35° C during the winter, making it the

coldest marine environment on the planet (Haas *et al.*, 1997; Deming, 2007). On the other hand, bacteria found close to the ice-water interface experience much milder temperatures, as the bottom layer of the ice is in direct contact with seawater and thus is at the freezing temperature (-1.86° C). The difference in temperature between the relatively warm bottom and the cold top layers of the ice creates a linear temperature gradient that exists in sea ice throughout most of the year (Haas *et al.*, 1997). The steepness of this gradient is determined by the air temperature as the under ice water is constantly at near freezing. Thus, this gradient may vary throughout the year and is less significant during the short Arctic summer (Wadhams, 2000).

Brine salinity (i.e. the salinity that bacteria experience within the brine) is determined exclusively by temperature, thus the top-to-bottom temperature gradient also creates a linear gradient in brine salinity (Cox & Weeks, 1983). As ice temperatures drop, brine salinity increases due to the rejection of dissolved ions by the growing crystals which depresses the freezing point of the brine and maintains freezing equilibrium (Cox & Weeks, 1983). Thus, bacteria found in the top layer of the ice experience salinities as high as 220 ppts or more, greater than 7 times that of the Arctic Ocean surface seawater, throughout most of the year (Deming, 2007). It is worth noting that at temperatures lower than – 23° C, many ions dissolved in the brine reach their eutectic point. As the brine becomes saturated with these ions, they begin to precipitate. Thus, in some cases the brine salinity gradient will not be linear for the upper portion of the ice, as salinity will not change as temperature increases or decreases further (Thomas *et al.*, 2010). On the other hand, bacteria found near the ice-water interface experience salinities >50 ppts, only about 1.5 times that of the surface seawater (Deming, 2007). The high salinity concentrations in the surface brines reduce gas solubility, resulting in reduced concentration of biologically important gasses such as O_2 and CO_2 and a general trend of degasification of the sea

ice over time (Thomas *et al.*, 2010). This is especially pronounced at the top portions of the ice where the brine is supersaturated with soluble ions. Thus, bacteria residing in the colder portions of sea ice face in-ice oxygen minimum zones (Thomas *et al.*, 2010). However, pockets of low oxygen can occur throughout the ice due to the consumption of O_2 by biological processes.

Unlike brine salinity, which is determined solely by temperature, bulk salinity (salinity of the melted ice) is dependent on the salinity source water for the ice, but also the age of the ice. As the ice matures, brine drainage and flushing with melt-water generate a downwards movement of brine, concentrating it at the bottom of the ice sheet and the ice making over-all fresher (Wadhams, 2000). Gravity drainage occurs because brine slowly melts the ice, due to a micro scale top-to bottom temperature gradient within the vertical brine inclusions as well as downward movement of the dense brine (Cox & Weeks, 1975; Light *et al.*, 2003). However the majority of sea ice desalination occurs during the summer as the brine is flushed with fresh water originating from snow melt (Untersteiner, 1968).

Bulk salinity concentrations also influence the in-situ conditions experienced by bacteria residing in the sea ice. Ice phase equations dictate that, together with brine salinity and ice porosity, both controlled by temperature, bulk salinity controls the volume of the brine and the permeability of the ice (Cox & Weeks, 1983). Thus, for a given bulk salinity, temperature drops lead to decreased permeability of the ice (Cox & Weeks, 1983; Golden *et al.*, 1998). Channels and pockets of brine within the matrix are permeable (and thus can exchange brine) at a brine volume of 5% which normally occurs when the temperature is -5° C for a typical bulk salinity of the ice of 5 ppts (Cox & Weeks, 1983; Golden *et al.*, 1998). As temperature drops and sea ice reaches the point where it is no longer permeable, brine volume and pore size decrease and brine connectivity is lost. Thus, pockets of brine along with the bacteria residing within them become

isolated from the rest of the brine network (Golden *et al.*, 1998). While micrometer-scale connections remain possible, shrinking of brine channels drastically restricts gas and nutrient exchange with the water column and the rest of the brine network. In combination with biological activity, this isolation can lead to pockets of brine depleted of O_2 throughout the ice (Deming, 2007; Thomas *et al.*, 2010). Oxygen depletion was also reported to lead to the development of processes and possibly communities within the ice favored by anoxic conditions (Rysgaard & Glud, 2004).

Gradients within the ice are not limited to salinity and temperature, light levels and, as consequence, UV intensity experienced by sea ice bacteria are also dependent on vertical position within the ice. As light attenuates linearly with depth, bacteria residing at the top portions of the ice are exposed to higher intensities of UV irradiation and as consequence higher oxidative stress than those in deeper layers (King *et al.*, 2005). Higher light intensity at the top portions of the ice may also inhibit the growth of ice algae due to photo-inhibition.

Life within the brine imposes many challenges, with the main ones being cold temperatures and high salinities. In the next section, I will briefly describe the strategies bacteria employ to cope with such extreme conditions.

1.3.3 Bacterial adaptations to life in these conditions

Despite the harsh conditions in sea ice described in section 1.3.2 above, bacteria in the ice remain metabolically active and, in fact, maintain higher metabolic rates than that of pelagic bacteria found in the Arctic Ocean water column (Junge *et al.*, 2002; Riedel *et al.*, 2007a). Furthermore, they remain metabolically active even under conditions approximating sea ice during the polar winter (i.e. temperatures lower than –20 °C and brine salinity concentrations

higher than 200 ppts); thus, they remain metabolically active through the year (Junge *et al.*, 2001; Junge *et al.*, 2004; Junge *et al.*, 2006). There are many genotypic and phenotypic adaptations that enable bacteria to remain metabolically active in cold and saline conditions (Thomas & Dieckmann, 2002; Georlette *et al.*, 2004); here, I review some of these adaptations.

1.3.3.1 Adaptations to low temperatures

Psychrophilic microorganisms have an extensive array of adaptations aimed at coping with extreme low temperatures, including modifications of the membrane's lipid bilayer, the production of cryoprotectants, and the synthesis of enzymes with reduced structural rigidity (Georlette *et al.*, 2004).

The fluidity of the membrane is essential for its structural integrity and, thus, cellular functioning, since the cell membrane is a lipid bilayer, decreasing temperatures makes it a rigid gel that hinders the activity of membrane bound proteins (Gounot & Russell, 1999). The adaptation of membrane fluidity to extreme low temperatures revolves around the enrichment of the lipid bilayer with branched and unsaturated fatty acids. Unsaturated fatty acids contain double bonds that bend the carbon chain at the position of each bond making it less likely for neighboring lipids to align thus increasing the fluidity of the membrane. Sea ice bacteria have monounsaturated fatty acids (MUFA) that are regulated by temperature, depending on modification by a desaturase enzyme. Sea ice bacteria also express polyketide synthases that allow efficient production of MUFAs at low temperatures (Thomas & Dieckmann, 2002).

Another consequence of cellular freezing induces the formation of cytoplasmic ice crystals,

resulting in cellular damage and osmotic imbalance (Nichols *et al.*, 2000). Sea ice bacteria

produce cryoprotecting substances capable of changing the shape of growing ice crystals both within and out of the cell, thus protects them from lysis. These molecules also serve as solutes, depressing the freezing point of the cytoplasm thus maintaining liquid phase within the cell (Lizotte, 2003). One type of such cryoprotectant are ice binding proteins. Sea ice microbes were reported to produce antifreeze glycoproteins that are capable of binding ice crystals serving as cellular antifreeze (Nichols *et al.*, 2000; Thomas & Dieckmann, 2002). Interestingly, a similar adaptation utilizing ice nucleating proteins to control the growth of ice crystals within the cells, which is widespread in bacteria from other cold environments, is reported to be largely missing from sea ice bacteria (Junge & Swanson, 2008).

Perhaps one of the most studied array of cryoprotecting molecules are the extracellular polysaccharide substances (EPS). These are gelatinous high molecular weight carbohydrate polymers which sea ice bacteria and algae secrete in response to low temperatures (Nichols *et al.*, 2005). The EPS coats the cell, protecting it from damage caused by ice crystals forming outside the cell (Krembs & Engel, 2001). EPS also participates in cell adhesion to surfaces of the brine network and helps in the retention and protection of extracellular enzymes, required for bacterial activity, against cold denaturation (Nichols *et al.*, 2005). This ability to adhere to surfaces partly by using EPS may be the reason why sea ice bacteria are more active than pelagic bacteria (Junge *et al.*, 2004). The production of EPS by sea ice bacteria as well as by other microbes may alter the properties of the brine network; artificial sea ice containing EPS was demonstrated to have 15% more brine inclusions than EPS free sea ice (Krembs *et al.*, 2011). The presence of EPS also increased the size of these inclusions significantly and altered their geometrical characteristic making the shapes of the inclusions irregular (Krembs *et al.*, 2011). Thus, the production of EPS also helps in engineering the environment sea ice bacteria occupy,

making it more habitable by increasing permeability and the amount of space available for colonizing (Krembs *et al.*, 2011). ,

One of the greatest challenges to life in extreme cold is maintaining enzymatic function. There is an extensive array of identified modification to enzymes which are employed by bacteria to maintain active functionality in low temperatures, however they have rarely been studied systematically (Georlette *et al.*, 2004; D'Amico *et al.*, 2006). Nevertheless, though known modifications can be enzyme-specific, they generally favor increased structural flexibility. These are normally acquired through a lower content of proline and arginine, increased content of glycine, reduction in hydrophobic interactions, hydrogen bonds, and ionic interactions, and an increase in interaction with the solvent (i.e. the medium that enzymes are found in such as the cytoplasm or water) (D'Amico *et al.*, 2006). No cold-adapted proteins use all of these strategies; thus, it can be quite difficult to predict the temperature range of a protein based on coding sequence alone. Cold-adapted enzymes are biotechnologically useful and, as such, have been the subject of extensive study (D'Amico *et al.*, 2006).

1.3.3.2 Adaptations to High salinities

Bacteria of the sea ice need to adapt to rapidly changing salinity that can have a concentration as high as 220ppts during winter. This makes dehydration caused by the high brine salinities a major stressor for ice-trapped bacteruia. In many organisms, including sea ice bacteria, tolerance for low temperatures and dehydration follow similar mechanisms (Thomas & Dieckmann, 2002; Georlette *et al.*, 2004).

One adaptation to high salt concentrations is the synthesis of EPS. The cellular coating formed by the secretion of EPS helps retain water, reduces the cell surface exposure to the brine

and serves as hydrated gelatinous buffer (Nichols *et al.*, 2005). This prevents the cell from experiencing the full extent of salt concentration outside of its exopolymeric coating (Murray & Jumars, 2002). Another benefit of the buffering mechanism may be increased salinity in the remaining liquid outside the EPS matrix, thereby maintaining the volume of inhabitable pore space by further depressing the freezing point of the brine (Deming, 2007). Similar to the protection from cold denaturation, the association of EPS with extracellular enzymes allows these enzymes to remain active in the brine by stabilizing and buffering them from the hyper-saline condition (Huston *et al.*, 2000; Huston *et al.*, 2004).

Surviving the osmotic stress associated with high salinity also requires adjusting the cellular concentrations of osmolytes, in order to restore the osmotic balance between the environment and the cytoplasm. In response to the changing external osmotic pressure osmolytes, including inorganic ions and organic solutes (such as proline, mannitol, and glycine betaine), are accumulated or synthesized. Hyper-osmotic pressure in the in ice can change rapidly to hypo-osmotic pressure as the ice melts and flushed with fresh water during the summer, sea ice bacteria cope with these conditions by braking down or releasing osmolytes from the cell to the environment (Thomas & Dieckmann, 2002).

Now that I have discussed the adaptations of bacteria and other sea ice microbes to the harsh conditions within the brine it would be a good point to discuss what is it they do that makes them such a key component of the Arctic Ocean ecosystem.

1.3.4 Activity of sea ice bacteria

The Arctic Ocean in general and sea ice in particular are home to some impressive creatures such as whales, polar bears and many more. Due to their size and relatable looks they

draw a lot of the attention when discussing the effects of climate change in the Arctic. However the real movers and shaker in the Arctic Ocean and sea ice, like in any marine environment, are the unseen majority-the microbes. There are no higher plants in marine environments; thus, the majority of the primary production of organic matter comes from microbes, mainly unicellular algae (Azam & Malfatti, 2007). In the Arctic sea ice the situation is similar and the production of sea ice diatom algae is said to account for over 25% of the entire production in the Arctic region (Gosselin *et al.*, 1997). However much of the organic matter produced by microbes is then only utilized by other microbes, mainly bacteria, to understand why I will briefly describe the microbial loop.

1.3.4.1 The sea ice microbial loop

Originally, the transfer of organic matter and energy in marine food web was thought to flow from large phytoplankton such as diatoms and dinoflagellates through to higher trophic levels, the so called"classic food chain". However, Azam and colleagues argued that this depiction of the marine food web was incomplete and overly simplified (Azam *et al.*, 1983). Their main finding was that a significant amount of primary production that was thought to be lost to the environment as DOM was instead utilized by heterotrophic bacteria (Azam *et al.*, 1983). Thus, DOM produced by phototrophs is cycled through a "microbial loop" comprised of phototrophs, bacteria, viruses and phagotrophic protozoa. Heterotrophic bacteria initially consume the DOM and in turn are either consumed by Protozoa leading to carbon flow to higher trophic levels, or lysed by viruses thus recycling the DOM (Figure 1.2) (Azam *et al.*, 1983; Azam & Malfatti, 2007; Fenchel, 2008). This theory has not only ignited a complete reassessment of the ecological significance of microbes in marine environments and their role in

biogeochemical cycles but also fueled many studies increasing the understanding of microbial diversity and metabolic capabilities adding more levels to the loop (Fenchel, 2008).

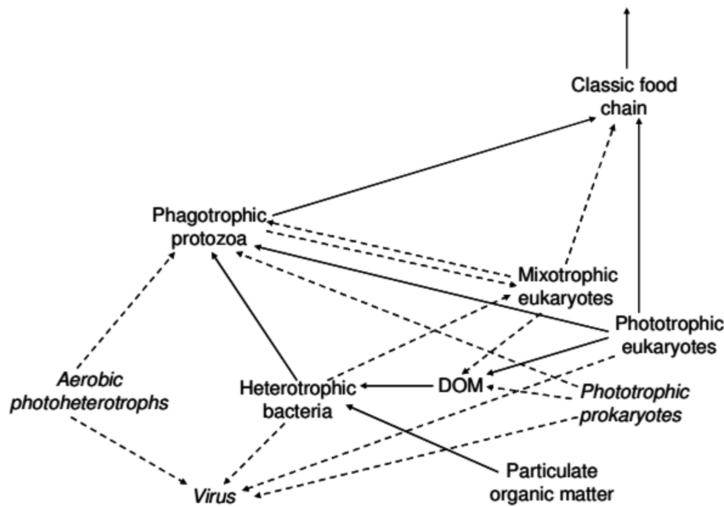

Figure 1.2 The microbial loop: The proposed, by Azam et al (1983) represented by full lines and updated with new participants of the loop in dashed line. This shows the central role bacteria play in DOM cycles in marine environments. Figure Adapted from Fenchel, 2008.

1.3.4.2 Sea ice microbial loop and its role in sea ice biogeochemical cycles.

Though not a lot is known regarding the microbial loop dynamics within sea ice, there is evidence of its function, as key components of it have been identified. I will briefly describe the central role the sea ice microbial loop and with it sea ice bacteria have on the Arctic Ocean biogeochemical cycles.

Much like in other marine systems, algae are the main contributors to the production of DOM as well as particulate organic matter (POM) to the ice environment during bloom period. This productivity is done directly via exuding photosynthate and EPS secretion and indirectly via cell lyses due to necrosis and disease. Combined with the release of dimethylsulfonioproprionate (DMSP, a compound use as an osmotic regulators) this supply the

microbial loop with organic carbon nitrogen and sulfur (Kirst & Wiencke, 1995; Staley & Gosink, 1999; Krembs *et al.*, 2002; Quillfeldt *et al.*, 2003; Riedel *et al.*, 2006).

The productivity of sea ice algae is episodic and occurs during the spring and summer (as long as the ice lasts). Bacteria in the ice, on the other hand, are active year round; thus, their involvement in the microbial loop is consistent of two components, spring/summer and autumn/winter. During algal blooms, bacteria heavily colonize aggregates of ice algae and their secreted EPS. These bacteria feed both on dead algal cells (cells that have undergone necrosis or lysis) and EPS. Much of the organic matter produced by algae cycles through bacteria first before traveling up the food web by the grazing of protists (Staley & Gosink, 1999; Riedel *et al.*, 2007a). In turn, the heterotrophic activity of bacteria is thought to facilitate continued spring/summer algal growth by providing re-mineralized inorganic nutrients such as phosphate, nitrate, and ammonia (McGrath Grossi *et al.*, 1984; Kottmeier & Sullivan, 1990). Thus, the activity of heterotrophic bacteria regulates the availability of algal produced organic matter to higher trophic levels in the ice. However since the ice and ocean are linked, algal production also sustains the underlying water column, as consequence the activity of sea ice bacteria also plays a role in regulating how much of the organic matter will be released to the Ocean (Deming, 2010).

During the winter, the secondary production of heterotrophic bacteria feeding on remnants of the algal spring/summer production makes the primary source of organic matter to higher trophic levels within the ice in lieu of algal production, as protozoa have only bacterial cells to feed on. Furthermore, aggregates of bacterial cells feeding on reservoirs of algal EPS also sink from the ice to the water column and the benthos, thus supplying the water column and benthos with a constant subsidy of organic matter (Renaud *et al.*, 2007; Wing *et al.*, 2012). As in

the spring and summer, the mineralization of nutrients by heterotrophic bacteria production is said to fertilize the ice in preparation for the spring diatom bloom (Deming, 2007).

This recycling of nutrients occurs also through the lysis of bacterial cells by viruses. Not much is known about viruses in sea ice and their dynamics with bacteria. However, the brine network hosts some of the highest concentrations of viruses in marine environments and possibly in the world. Virus-like particles can reach concentrations of up to 10^9 particles per milliliter of brine in “warm” summer/autumn ice, which exceeds the concentration of virus-like particles in underlying water by an order of magnitude (Maranger *et al.*, 1994; Wells & Deming, 2006). Though little is known about viral ecology in sea ice, active bacteriophages were isolated from the Arctic sea ice and microcosm experiments demonstrated that viruses from sea ice remain infective at high salinities and temperatures below -10° C (Borriss *et al.*, 2003; Wells & Deming, 2006). Due to the high concentrations of virus-like particles and the small volume of channels and pockets of the brine network, there are high contact rates between bacteria and phages which in turn lead to high infection and mortality rates of bacterial cells (Wells & Deming, 2006). This dynamic between viruses and bacteria regulate the availability of organic carbon and nutrients to higher trophic levels and during winter-time regenerate nutrients in preparation of the ice to the spring sea ice diatom bloom (Wells & Deming, 2006; Deming, 2007; Collins & Deming, 2011).

It is worth noting that bacteria are also involved in processes that reduce the amount of available inorganic nitrogen in the ice and contribute to loss of nitrogen from the ice. Probably due to the occurrence of anoxic zones within ice, both denitrification and anaerobic ammonia oxidation (anammox) take place in Arctic sea ice (Rysgaard & Glud, 2004; Rysgaard *et al.*, 2008). Denitrification occurs in higher intensities in MYI compared to FYI and annamox was not detected in FYI at all. This is likely due to a very slow growth of the bacteria involved in these

processes, thus the development of a community able to perform anammox and denitrification requires stable conditions and a long time to develop, conditions which MYI is able to supply better than FYI (Rysgaard & Glud, 2004; Rysgaard *et al.*, 2008). However, it is possible that since MYI has lower salinity than FYI, it contains more pockets of isolated brine inclusions with anaerobic conditions than FYI.

1.4 Changing sea ice and effect on sea ice bacterial communities.

Thus far, I have laid the grounds for understanding why sea ice is an important component of the Arctic and why the diminishing levels of MYI in the Arctic should be a cause for concern and a driver for Arctic research. I have also laid the grounds for understanding why the bacterial communities residing within sea ice are major players in biogeochemical cycles of the Arctic Ocean. One under-explored avenue of research is understanding whether FYI and MYI host bacterial communities made of different constituents which in turn would affect their function in the Arctic Ocean ecosystem, thus adding additional aspects to the implications of the demise of Arctic MYI. I will briefly cover the differences between MYI and FYI and will make the case for why they should be regarded as dissimilar habitats for microbes.

1.4.1 Isn't Ice just- ice? Should MYI and FYI represent different habitats for bacteria?

MYI is older than FYI. The lifespan of Arctic MYI can range up to 7 years, at which point water currents and atmospheric circulation governing the Arctic Ocean export it to the Atlantic Ocean, where it melts (Wadhams, 2000). Thus it can be argued that due to the fact the MYI survives longer than FYI, bacterial communities inhibiting it have longer time to develop and mature with respect to membership and composition (i.e. undergo succession processes).

Therefore, MYI bacterial communities will be made of different constituents than the younger FYI community.

MYI and FYI also differ in much of their chemical and physical properties. While FYI rarely reaches 2 m in depth, MYI sheets are usually thicker as new layers of ice are added to the bottom of MYI sheets during the autumn and winter. MYI typically reaches several meters thickness before the rate of summer melt catches up to the rate of winter growth (Wadhams, 2000). This is especially the case along the northern coast of Canada and Greenland where MYI is generally thicker than 3.5 m (Haas *et al.*, 2006; Haas *et al.*, 2010). Thus, many of the top-to-bottom gradients discussed earlier are steeper in FYI than in MYI.

MYI has a lower bulk solute content and therefore is fresher than FYI, due to the prolonged desalination (discussed in section 1.3.2 above). In particular, the brine network in MYI is flushed with summer melt multiple times during its lifespan (Untersteiner, 1968; Wadhams, 2000). The extensive desalination of MYI also results in a different bulk salinity profiles between the two types of ice. The bulk salinity profile of FYI resembles the shape of the letter C, since it has higher salinity values at the top and bottom of the ice and lower salinity in the middle (Cox & Weeks, 1975; Eicken, 2008). On the other hand, due to gravitational drainage, brine cell migration and mainly summer melt, the bulk salinity profile of MYI shows a general top-to-bottom increase in salinity concentrations where the upper parts are almost completely fresh and the lower portions approach sea water salinity (Untersteiner, 1968). The lower solute content reduces the volume of the brine network, making MYI less permeable than FYI with less habitable space (Golden *et al.*, 1998; Wadhams, 2000).

In addition to flushing the ice with fresh water, summer melt also accumulates as fresh water ponds at depression points on top of MYI sheets (Maykut, 1986). The water and surface of these melt ponds contain active mats of cyanobacteria along with other bacteria derived from snow and aeolian deposition, which serve as an inoculum source for MYI (Jungblut *et al.*, 2010; Harding *et al.*, 2011). During the winter, these fresh water ponds freeze, adding a layer of fresh water ice to the MYI sheet. Thus, MYI contain between two and three layers of ice: if a melt pond is present, there is fresh water ice at the top, newly formed ice forms a layer at the bottom, and older ice, which survived at least one summer melt season, forms a layer of old desalinated ice between them. None of these layers are present in FYI (Wadhams, 2000).

However, to date there have been only few studies done comparing the communities from MYI and FYI. Furthermore, despite the fact that MYI is more vertically complex than FYI little study was conducted thus far looking into the vertical distribution of bacterial communities from the two ice types.

1.5 Enter my thesis! Research objectives and thesis overview

To date there have been just a handful of studies looking at the composition of bacterial communities from Arctic sea ice. The majority of those efforts revolved around classic microbiological methods (i.e. isolation and identification of bacteria), or low resolution genomic techniques such as community fingerprinting and clone libraries using the 16S rRNA gene as marker for bacterial diversity and composition (Mock & Thomas, 2005).

Since seawater is the main source of inoculation for sea ice, it was assumed that the composition of sea ice bacterial communities would resemble that of bacterial communities from the Arctic Ocean. However, the composition of bacterial communities from sea ice is markedly

different than that of their source (Junge *et al.*, 2001; Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003b; Junge *et al.*, 2004). Phylogenetic analysis of bacteria cultured from Arctic sea ice show an exclusive relationship to known psychrophiles belonging to several genera from two phyla. These included bacteria classified as closely related to members of the genera *Alteromonas, Colwellia, Glaciecola, Octadecabacter, Pseudomonas* and *Shewanella* from the phylum *Proteobacteria* and bacteria classified as closely related to members of the genera *Cytophaga, Flavobacterium, Gelidibacter* and *Polaribacter* from the phylum *Bacteroidetes*. Bacteria closely related to member of the class *Bacilli* and the order Actinomycetales were also cultured (Junge *et al.*, 2002; Groudieva *et al.*, 2004). Community fingerprinting, in-situ hybridization and clone libraries based studies showed similar composition to cultivation-based approaches (Junge *et al.*, 2001; Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003b; Junge *et al.*, 2004).

Though these studies provide knowledge of the membership and composition of bacterial communities, the methodologies use in it are labor intensive and limited in the scope of the taxa they can identify due to their low coverage of the community members as well as the low resolution of fingerprinting techniques. Modern high throughput screening of 16S rRNA gene sequences was only introduced to sea ice research in 2012 (Bowman et al., 2012). Prior to the work described in Chapter 2 of this thesis, the study by Bowman et al. was the only study utilizing high throughput sequencing of 16S rRNA genes to describe bacterial community structure in sea ice (Bowman et al., 2012). This study confirmed the dominance of sequences closely related to members of the taxonomic groups seen in previous cultivation-based and clone library based studies (Bowman *et al.*, 2012). However, high throughput sequencing revealed the presence of sequences closely related to several taxonomic groups of bacteria that were not previously reported in sea ice. These include sequences classified as closely related to members

of the candidate phylum TM-7, the family *Micrococcineae* (which are normally found in soil environments), the genera *Methylmicrobium* and *Cycloclasticus* of the class *Gammaproteobacteria,* and sequences closely related to members of the phylum *Cyanobacteria* (Bowman *et al.*, 2012).

1.5.1 Research objectives

Of the previous studies of sea ice bacteria only one (Brinkmeyer *et al*. 2003b) compared FYI to MYI. Though the authors found similar groups, the limited scope of their methodology as was shown by Bowman and colleagues hints that there might be undiscovered differences in the composition of the two communities (Brinkmeyer *et al.*, 2003b; Bowman *et al.*, 2012). Of those studies only Junge *et al* (2001) looked and vertical changes in the composition of bacteria in sea ice (Junge *et al.*, 2001). This paper provides support for the idea that communities change in composition along the ice however it too due to methodological limitations only looked at a limited number of taxonomic groups.

Therefore to broaden the knowledge of how communities from MYI and FYI differ both with respect to each other and along the depth of the ice, the objectives of my thesis are as follow:

Objective one: Use high throughput sequencing of the 16S rRNA gene to examine if differences in the composition and membership of bacterial communities along the depth of MYI and FYI (Chapters 2 and 4).

Objective two: Use high throughput sequencing of the 16S rRNA gene to characterize and compare the overall community membership and composition from MYI and FYI (Chapter 3).

Objective three: Use in-situ disturbance followed by high throughput sequencing of the 16S rRNA gene to link any differences in the membership and composition (or structure) of bacterial communities from MYI and FYI to function (Chapter 4).

1.5.2 Thesis overview

In Chapter 2, I examined the vertical profile of the bacterial community composition in MYI. The *a priori* hypothesis was that the membership and composition of bacterial communities change with depth. I explored the possibilities that the community membership and composition (1) differ with ice depth due to strong physical and chemical gradients, (2) are relatively homogenous within the different layers that occur in MYI but differ between the layers, or (3) do not vary with ice depth.

In Chapter 3, I compared the membership and composition of MYI and FYI collected near each other at sites in the Lincoln Sea, north of Alert, Nunavut, Canada over three years of field sampling. The *a priori* hypothesis behind this study was that communities from similar ice types have greater similarities in membership and composition than between ice types, despite the year of collection or the collection site.

In Chapter 4, I examined the response of bacterial communities from FYI and MYI to *in-situ* environmental disturbance in the form of inversion transplantation, thus disrupting the inherent gradients within the ice. I predict that since communities in MYI are vertically distinct there will be noticeable changes to the communities of the top and bottom sections but not to those from the middle since these communities face the most significant change in in-situ conditions. Since this is the first research to do so, we provide a description of the vertical distribution of bacterial communities along the depth of FYI sheets. We predict that similar to

MYI response to the inversion will reflect in the attributes of the communities from the top and bottom sections.

Chapter 5 will include a synthesis of the findings presented in the previous chapters and future directions.

Throughout these chapters I will refer to the terms alpha and beta diversity, both were coined by R.H. Whittaker together with the term gamma diversity (Whittaker, 1972). As a plant ecologist Walker envisioned alpha diversity as species richness and diversity present in a habitat. Whittaker defined beta diversity as the change in the presence of species and their abundance between two habitats, and gamma diversity as a combination of alpha and beta diversity (Whittaker, 1972). These concepts where suggested originally for use in macro ecology however they are also used extensively in the field of microbial ecology though the term species is replaced by a more lenient definition of operational taxonomic unit, which represents a certain cut-off of similarity in the sequences of the 16S rRNA gene (Prosser *et al.*, 2007). Although Whittaker envisioned alpha diversity as a measure of diversity within a habitat, the concept has been broadened and many in the field of microbial ecology use alpha diversity measurements (i.e. species richness and diversity measurements) to compare different habitats (Prosser *et al.*, 2007). Thus the line between alpha and beta diversity has been somewhat blurred and throughout this thesis I will make use of this broader concept of alpha diversity and will also compare measurements of it between habitats as well as traditional beta diversity measurements.

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2.1 Abstract

Bacterial communities in Arctic sea ice play an important role in the regulation of nutrient and energy dynamics in the Arctic Ocean. Sea ice has vertical gradients in temperature, brine salinity and volume, and light and UV levels. Multiyear ice (MYI) has at least two distinct ice layers: old fresh ice with limited permeability, and new saline ice, and may also include a surface melt pond layer. Here, we determine whether bacterial communities (1) differ with ice depth due to strong physical and chemical gradients, (2) are relatively homogenous within a layer, but differ between layers, or (3) do not vary with ice depth. Cores of MYI off northern Ellesmere Island, NU, Canada, were subsectioned in 30-cm intervals, and the bacterial assemblage structure was characterized using 16S rRNA gene pyrotag sequencing. Assemblages clustered into three distinct groups: top (0–30 cm); middle (30–150 cm); and bottom (150–236 cm). These layers correspond to the occurrence of refrozen melt pond ice, at least 2-year-old ice, and newly grown first-year ice at the bottom of the ice sheet, respectively. Thus, MYI houses multiple distinct bacterial assemblages, and *in-situ* conditions appear to play a less important role in structuring microbial assemblages than the age or conditions of the ice at the time of formation.

2.2 Introduction

Sea ice is a habitat for a diverse and active microbial community (Junge *et al.*, 2004; Mock & Thomas, 2005; Junge *et al.*, 2006). The most easily recognizable feature of this

community is the algal bloom, usually dominated by diatoms, covering the bottom 10 cm of the ice during the spring and summer (Gosselin *et al.*, 1997; McMinn & Hegseth, 2007; McMinn *et al.*, 2007). Heterotrophic bacteria are key regulators of this bloom and of the subsequent energy and organic matter flux from the ice to underlying seawater via the microbial loop (Poltermann, 2001; Deming, 2007). Furthermore, during the polar winter, heterotrophic production and growth fertilizes the ice in preparation for the spring and summer bloom (Riedel *et al.*, 2008) and supplies the ice and underlying water column with a small but stable subsidy of organic matter through secondary production (Wing *et al.*, 2012).

Microorganisms that colonize sea-ice are exposed to a wide array of *in-situ* conditions. As the sea ice forms, salts within the water are excluded from the ice crystals, forming a highly saline brine matrix, with brine salinity and brine volume negatively and positively correlated with the temperature of the surrounding ice, respectively. These brines are interconnected within the ice; thus, sea ice could be considered a semi-solid brine matrix. Channels and pockets of brine within the matrix are crudely Considered permeable (and thus can exchange brine) at a brine volume of ~5% which normally occurs when the temperature is ~ -5°C for a typical bulk salinity of the ice of ~5‰, i.e. the Golden rule of fives (Golden *et al.*, 1998). Winter and early spring sea ice floes have a strong temperature gradient, with the top surface of the ice exposed to temperatures as cold as -40°C and the bottom of the ice at a constant -1.86°C (Eicken, 2008). Thus, brines near the top of the ice are as much as five-fold more saline than those near the ice-water interface (Collins *et al.*, 2010) and brine volume increases with depth (Wadhams, 2000). Due to scattering of incident light by the ice, both light intensity and ultraviolet radiation intensity decrease with ice depth. Higher intensity of UV irradiation at the upper portions of the ice leads to higher oxidative stress than in deeper samples (King *et al.*, 2005). The vast majority

of microbes within the ice reside within the brine matrix (Junge *et al.*, 2001); thus, sea ice microbes experience a less extreme environment, with higher temperatures, lower salinity, increased brine volume, lower UV irradiation, and lower oxygen radical concentration with increasing depth.

Sea ice is also a highly dynamic environment: the extent of sea ice in the Arctic region fluctuates by more than two-fold between minimal and maximal coverage in summer and winter respectively (Camiso, 2010). Thus, some Arctic sea ice is perennial, i.e. ice that has persisted through more than one summer (known as multiyear ice, or MYI), while other ice is seasonal (known as first year ice, or FYI). MYI proportions in the Arctic have dwindled rapidly over the past few decades (from >70% of total Arctic sea ice c. 1980 to approximately 45% today) and the Arctic Ocean is expected to be MYI free by end of the century (Boé *et al.*, 2009; Maslanik *et al.*, 2011). The implications of these shifts to Arctic Ocean ecosystem dynamics are not well understood.

MYI consists of at least two layers: an old layer that survived the previous season's melt and a new layer that is added to the bottom of the ice during the seasonal freeze period. The old ice layer undergoes a process of desalination due to gravitational brine drainage and fresh water flushing from snowmelt (Wadhams, 2000; Eicken, 2008). This flushing, along with the growth of new ice, leads to strong vertical stratification in the bulk concentrations (as opposed to brine concentrations) of solutes and particulate organic matter in MYI (Wadhams, 2000; Eicken, 2008). The old ice layer has lower bulk brine volumes than the new ice layer and is less permeable (Eicken, 2008). Melt water also tends to accumulate at low points on the uneven surface of MYI, creating fresh water melt ponds that become incorporated into the sea ice upon freezing (Wadhams, 2000; Jungblut *et al.*, 2010), potentially acting as a source of microbial

inoculum into the sea ice (Harding *et al.*, 2011). In these areas, the upper 15 to 30 cm of ice is comprised of fresh water (Wadhams, 2000), thereby adding another layer of complexity to the ice structure.

Despite these differences in the ice with depth, previous studies of Arctic sea ice bacterial assemblages were of vertically homogenized ice cores. In this study, we characterized the bacterial assemblage composition of different depths of a MYI core from the Lincoln Sea to determine whether the physical and chemical stratification in MYI leads to niche differentiation within the ice. We explore whether bacterial communities: 1) differ with ice depth due to strong physical and chemical gradients, 2) are relatively homogenous within a layer, but differ between layers, or 3) do not vary with ice depth.

2.3 Materials and Methods

2.3.1 Site description and sample collection

In collaboration with the Canadian Arctic Sea Ice Mass Balance Observatory (CASIMBO https://sites.google.com/a/ualberta.ca/casimbo/) expedition during May 2011, two landfast sea ice cores and underlying water samples (SW1, SW2) were sampled at one site off the shore of northern Ellesmere Island Nunavut, Canada (82.54905ºN, -62.37685ºW). The site was located in a depression in the ice, implying that a freshwater melt pond was present in summer and that the uppermost ice is, therefore, likely refrozen water from a freshwater pond. An accurate age of the sampled site was not determined; however, the ice was 2.4 m thick, which is typical of MYI and thicker than most FYI. Furthermore, the bulk salinity profile (as opposed to brine salinity) showed an increase in salinity with depth (Figure 2.1A) and texture analysis showed the

occurrence of a possible annual growth mark at approximately 125 cm from the surface (Figure 1B). Therefore, we conclude that the ice appears to have survived at least one melt season.

Three parallel cores were sampled using Kovacs Mark II 9cm corer (Kovacs Enterprise, Roseburg, Oregon) and a 36V electric hand drill. Prior to core extraction the core barrel was rinsed with sterile deionized water (Milli-Q Integral Water Purification System, EMD Millipore Corporation, Billerica, MA, USA). Two cores used for 16S rRNA gene pyrotag analysis were immediately sectioned on site to 30 cm intervals using a hand saw (rinsed in deionized water and wiped with an ethanol wipe) and placed in UV sterilized polypropylene bags. The third core was used for texture analysis and was placed in polypropylene bags with no processing in the field. For seawater sampling, duplicate 2 liter samples of water were pumped into polypropylene bags from the coring hole using a manual peristaltic pump (Cole Palmer, Montreal, Quebec, Canada). Both water and ice samples were covered, kept chilled, and processed within 4 hours of sampling. The temperature profile was estimated by measuring the temperature at the snow-ice interface and extrapolating a linear temperature gradient to the ice-water interface (which is at the freezing point of the water, i.e. -1.8ºC) (Figure 2.1S A).

2.3.2 Sample processing

Ice samples used for pyrotag analysis were thawed at room temperature in the dark. For each ice section and seawater sample, salinity was measured with an ExStikII® salinity meter (Extech Instruments, Nashua, NH, USA). A portion (45 mL) of each sample was preserved in formaldehyde (3.7% v/v final concentration; Fisher Scientific, Ottawa, ON, Canada) for later cell counts. The remaining ice melt and seawater samples were filtered individually through 0.22 µm pore size polyethersulfone membrane filters (Pall, Mississauga, ON, Canada). Direct melting

was used to avoid nonspecific addition of DNA and dilution of samples. Though previous papers reported some bacterial cell loss when using direct melting, it has been shown to be appropriate for sea ice bacterial samples (Helmke & Weyland, 1995; Kaartokallio *et al.*, 2005; Kaartokallio *et al.*, 2008; Deming, 2010; Ewert *et al.*, 2013).

All glassware was sterilized using 10% household bleach solution followed by thorough washing in sterile deionized water between samples to prevent cross contamination. Each filter was placed in a microfuge tube and submerged in RNA*later*® solution (Life Technologies, Burlington, ON Canada) and stored at -20°C for later DNA extraction.

The ice texture core was kept at -10°C and sectioned to 10 cm intervals using a band saw. Each subsection was then vertically trimmed with a band saw so that only the middle 3-5 mm were kept to provide a clean surface for imaging. Each vertical 10 cm core section was exposed to cross-polarized light and photographed using a Panasonic DMC-TZ3 for crystal structure analysis. Cell counts were performed on formaldehyde fixed cells stained with DAPI (Sigma-Aldrich Canada Co., Oakville, Ontario) using a Leica Microsystems DMRXA epiflorescent microscope (Leica, Concord, ON, Canada) as previously described (Hobbie et al. 1979; Porter and Feig 1980).

2.3.3 DNA extraction amplification and sequencing

DNA was extracted from preserved filters by bead beating using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) as instructed by the manufacturer. Filters from equivalent 30 cm sections of duplicate ice cores were combined prior to DNA extraction in order to ensure sufficient DNA yield. Seawater duplicate samples were processed individually.

Molecular Research LP (Shallowater, Texas, USA) performed pyrosequencing of the V1-V3 regions of the bacterial 16S rRNA gene as previously described (Dowd *et al.*, 2008). Amplification was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: 94ºC for 3 minutes, followed by 28 cycles of 94ºC for 30 seconds; 53ºC for 40 seconds and 72ºC for 1 minute with a final elongation step at 72ºC for 5 minutes. Gene-specific forward PCR primer sequences were tagged with the sequencing adapters for GS FLX Titanium chemistry, an 8 base barcode, and a linker sequence. All PCR reactions were performed in triplicate, mixed in equal concentrations, and purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, MA, USA). FLX-Titanium amplicon pyrosequencing was performed using the Genome Sequencer FLX System (Roche, Branford, CT). A list of all primers and barcodes is provided in Table 2.1S.

2.3.4 Pre-processing and quality control of raw sequences

All pre-processing and sequence quality control steps were performed using Mothur v. 1.33.3 following the Mother 454 SOP (http://www.mothur.org/wiki/454_SOP) (Schloss *et al.*, 2009; Schloss *et al.*, 2011). In brief, row flowgram data were used to discard sequences not in the range of 360-720 flows, as recommended (Quince *et al.*, 2011). Noise reduction was performed using the Mothur implementation of the PyroNoise algorithm using the shhh.flows command, which corrects PCR- and pyrosequencing- generated errors (Quince *et al.*, 2009; Schloss *et al.*, 2011). Sequences shorter than 200 bp or those containing primer/barcode mismatches or homopolymers longer than 8 bp were discarded. Remaining sequences were aligned against the SILVA reference data set, and then pre-clustered for further noise reduction as recommended (Pruesse *et al.*, 2007; Huse *et al.*, 2010; Schloss, 2010; Schloss *et al.*, 2011). Chimeras were detected and removed using the Mothur implementation of UCHIME (Edgar *et*

al., 2011; Schloss *et al.*, 2011). OTUs were assigned using the average-neighbor clustering algorithm for an OTU definition of 97% similarity. The assigned OTUs were classified to phylum, class, and/or genus level using the Ribosomal Database Project classifier (train set 9) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). Based on this classification, sequences of mitochondrial origin were removed from the data set. The sequences were subsampled to the smallest library size (n=1800) to allow identical sequencing depth for each sample before further alpha and beta diversity analyses (Gihring *et al.*, 2012) . Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under accession number SAMN02768838- SAMN02768847.

2.3.5 Assemblage analysis

Mothur v. 1.33.3 was used for all assemblage analysis and statistics (Schloss *et al.*, 2009). Alpha diversity was estimated using the Chao1 and ACE nonparametric richness estimators, reciprocal Simpson's diversity index (invsimpson), and Shannon diversity index (Shannon, 1947; Simpson, 1949; Chao, 1984; Chao & Shen, 2003). Yue & Clayton's theta measure of dissimilarity (θyc) was used to compare the assemblage structure of all samples in an OTU-based, phylogeny-independent manner (Yue & Clayton, 2005). A UPGMA dendrogram was generated based on the distance matrix resulting from the θyc analysis. The statistical support for clusters in the dendrogram was tested with a Mothur-modified implementation of weighted UniFrac (Lozupone *et al.*, 2006; Schloss *et al.*, 2009). Phylogenetic tree was generated with Mothur implementation of Clearcut using the relaxed neighbor joining algorithm (Evans *et al.*, 2006; Schloss *et al.*, 2011). Phylogeny-based analysis of assemblage similarity was performed using the Mothur implementation of un-weighted UniFrac (Lozupone *et al.*, 2006; Schloss *et al.*, 2009).

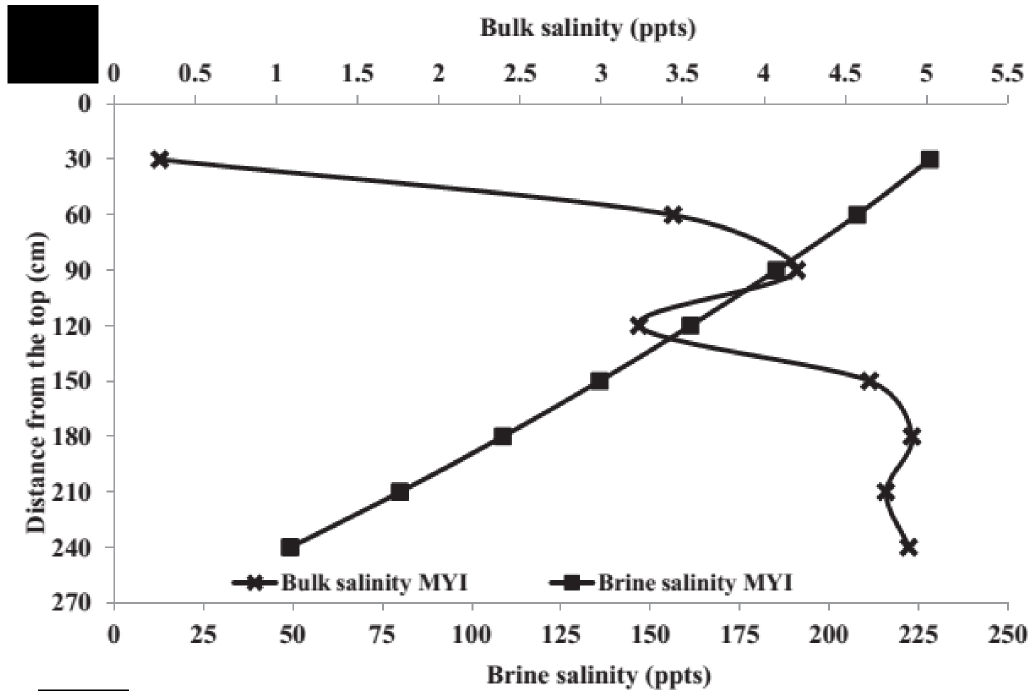
2.4 Results

2.4.1 *In-situ* conditions

The core length (236 cm) and bulk salinity profile (ranging from 0.28 ppts for the top 30 cm to 4.89 ppts for the bottom 30 cm) (Figure 2.1 A) were typical of MYI (Eicken *et al.*, 1995; Haas, 2004). Based on ice crystal texture analysis, the core had a possible annual growth mark at approximately 125 cm from the surface of the ice (Figure 2.1 B), indicating the presence of at least two annual layers. Furthermore, bulk salinity for the topmost layer was extremely low (Figure 1A), indicating a melt water pond origin. Thus, the ice has at least three layers: melt pond (0-30 cm), two-year old ice (subsequently referred to as old ice, represented by ice sections from 30-150 cm), and first year ice (subsequently referred to as new ice, represented by ice sections from 150-236 cm).

There were strong gradients of temperature and salinity within the ice. The average estimated temperature for the 30 cm intervals ranged from -16.08°C at the top to -2.81°C for the bottom (Figure 2.1S A). Brine salinity was calculated as described by Cox and Weeks (Cox & Weeks, 1983) and ranged from 228.21 ppts at the top to 49.2 ppts at the bottom, reversing the trend observed for the bulk salinity (as expected because the brine salinity is determined solely by temperature) (Figure 2.1A). Based on these salinity and temperature profiles, the ice core was permeable to the underlying seawater for only the bottom 90 cm (Golden *et al.*, 1998). Prokaryotic cell abundance showed no clear distribution trend (Figure 2.1S B). Cell counts ranged from high 10^4 to 10^5 cells ml^{-1} of ice melt: these values are on the low end of expected values for sea ice bacterial cell concentration (Junge *et al.*, 2002), furthermore cell concentrations seem to follow the trend of bulk salinity concentrations. Seawater samples had

salinities typical of the Arctic Ocean surface waters (Wadhams, 2000): 31.01 ppts and 32.25 ppts for SW1 and SW2 respectively.



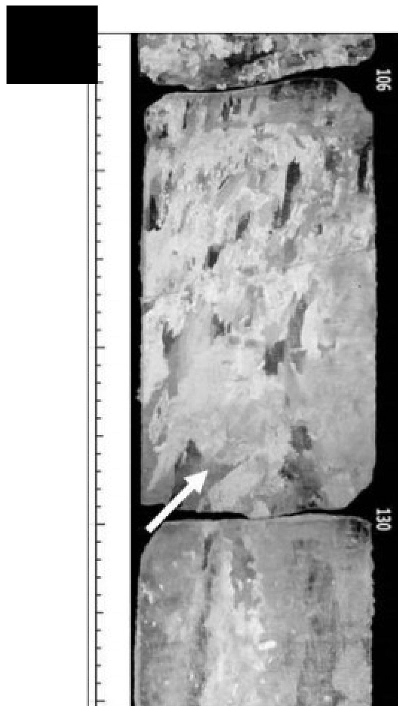


Figure 2.1 Chemical, and physical attributes of the ice cores: A- Measured bulk and calculated brine salinity, B- Texture of ice crystals. The arrow indicates the start of a new layer of columnar ice, marking the annual growth line between 125-130 cm depth. Scale bar in cm.

2.4.2 MYI has three distinct bacterial assemblages: top, middle, and bottom corresponding to the occurrence of three layers of ice, melt pond, old ice and new ice

Based on θyc measure of dissimilarity the microbial assemblages grouped into three statistically significant ($p<0.001$) clusters: top (0-30 cm), middle (30-150 cm), and bottom (150-236 cm) (Figure 2.2, left panel). These sections match the melt pond, old ice, and new ice sections predicted by ice texture and salinity analysis. Within each group, there appears to be clustering of samples by depth (Figure 2.2); however, these clusters were not statistically significant. This apparent clustering indicates that there may be preliminary adaptations to local conditions; however, the ice origin exerts a stronger influence on the microbial assemblage composition.

A phylogeny-based comparison of the assemblages (Lozupone *et al.*, 2006) largely agreed with the OTU-based approach (Table 2.1). The phylogeny-based comparison, however, indicates that the section from 150-180 cm is not significantly different from either the 120 cm-150 cm section or the 180 cm-210 cm section and thus “bridges” the difference between the old ice and new ice sections (Table 2.1). SW1 and SW2 formed a unique group which excluded all ice samples, supporting the notion that the ice assemblage is distinct from the water assemblage despite the predicted permeability of the bottom 90 cm of ice to seawater (Figure 2.2).

Table 2.1 Un-weighted UniFrac scores for all pairwise comparisons of samples:

	0-30cm	30-60cm	60-90cm	90-120cm	120-150cm	150-180cm	180-210cm	210-236cm	SW1	SW2
0-30cm		0.832	0.823	0.85	0.881	0.906	0.942	0.952	0.958	0.916
30-60cm	0.832		0.602	0.654	0.712	0.764	0.835	0.867	0.912	0.898
30-90cm	0.832	0.602		0.616	0.674	0.740	0.827	0.857	0.902	0.894
90-120cm	0.85	0.654	0.616		0.665	0.721	0.798	0.862	0.896	0.9
120-150cm	0.881	0.712	0.674	0.665		0.668	0.758	0.831	0.893	0.879
150-180cm	0.906	0.764	0.740	0.721	0.668		0.693	0.77	0.856	0.848
180-210cm	0.942	0.835	0.827	0.798	0.758	0.693		0.707	0.859	0.89
210-236cm	0.952	0.867	0.857	0.862	0.831	0.77	0.707		0.87	0.898
SW1	0.958	0.912	0.902	0.896	0.893	0.856	0.859	0.87		0.757
SW2	0.916	0.898	0.894	0.9	0.879	0.848	0.89	0.898	0.757	

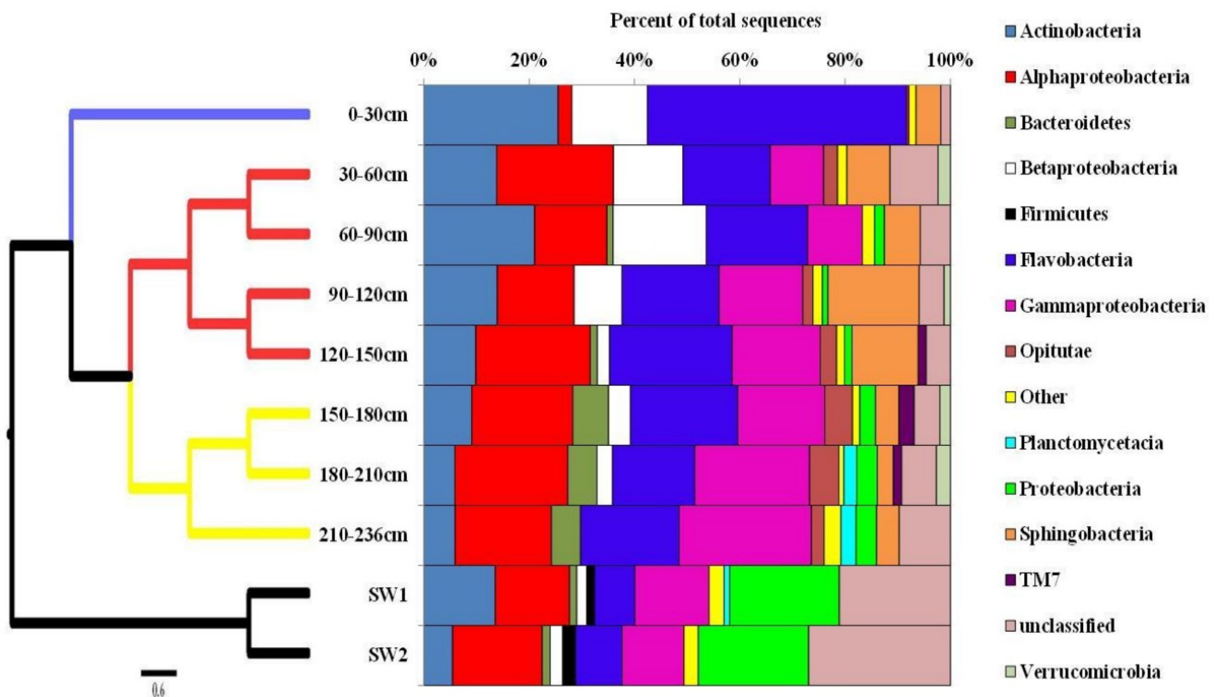


Figure 2.2 Similarity and composition of different ice core sections: Samples that are not significantly different ($p < 0.001$) based on weighted Unifrac scores have the same color lines in the clusters. Assemblage composition at the class level is shown for each 30 cm section.

Proportion is represented as percent of total sequences per section. Classes that amounted to less than 1% of the total sequences per section were pooled as “other”.

2.4.3 Three layers, three assemblage compositions: in-depth look at membership

The melt pond assemblage was dominated by *Bacteroidetes*, which accounted for more than 50% of all sequences. Within this phylum, 90% of the sequences belonged to the class *Flavobacteria* and ~7% belonged to *Sphingobacteriia* (Figure 2.2). Members of the phylum *Actinobacteria* and *Betaproteobacteria* were the second and third most abundant groups, with 27% and ~15% of all sequences, respectively (Figure 2.2).

In contrast, the assemblage of the old ice layer included a high abundance of *Alphaproteobacteria* from the family *Rhodobacteraceae* (ranging from ~20-35% of sequences, Figure 2). Several other groups were also more abundant in this layer when compared to the melt pond, including *Gammaproteobacteria* (~5-15% of total sequences) and *Sphingobacteriia* (~15% of total sequences). *Verrucomicrobia*, mainly of the class *Opitutae* (~3% of total sequences) were found in the old ice layer, but were absent from the melt pond ice. Members of the phylum *Actinobacteria* (~10% of sequences) and the classes *Flavobacteria* (~20% of sequences) were less abundant than in the melt pond layer, where as members of the class *Betaproteobacteria* were similar in abundance between the two layers (Figure 2.2). Members of the candidate division TM7 where detected at levels > 1% of sequences only at the bottom section of the old ice.

Similar to the old ice assemblage, the new ice assemblage was predominantly comprised of *Alphaproteobactera* from the family *Rhodobacteraceae* (~30-35% of all sequences, Figure

2.2), *Flavobacteria* (~15% of sequences), and *Gammaproteobacteria* (~15-25% of sequences). Verrucomicrobia of the class Opitutae (about 3% of sequences) and members of the candidate division TM7 (~1-2% of sequences) were more abundant in the bottom layer of ice than the layers above. Sphingobacteriia (~1% of sequences), Betaproteobacteria (ranging from 3% to <1% of sequences), and Actinobacteria (ranging from ~6% to 2% of sequences) had lower representation in the new ice assemblage than in the melt pond and old ice assemblages (Figure 2.2).

At finer phylogenetic scales, the layers differ further (Table 2.2S, Figure 2.3S). OTUs belonging to dominant phylogenetic groups differ between the three layers. For example, *Flavobacteria* from the melt pond assemblage were comprised of sequences belonging almost exclusively (>98% of sequences classified as *Flavobacteria*) to OTU #1, classified to the genus level as *Flavobacterium*. While this OTU was a dominant member of *Flavobacteria* in the old ice assemblage (~30% of sequences classified as *Flavobacteria*), there were other OTUs associated with the *Flavobacteria* order and sequences closely related to the genus *Ulvibacter* (~20% of the sequences classified as *Flavobacteria*, OTU #7) and the genus *Polaribacter* (~20% of the sequences classified as *Flavobacteria* OTU #17) were abundant. Furthermore, OTU #1 was represented by only four sequences at sections belonging to the new ice assemblage; which was dominated by OTUs #7 (30% of the sequences classified as *Flavobacteria*) and #17 (15% of the sequences classified as *Flavobacteria*), along with sequences closely related to the genus *Maribacter* (30% of the sequences classified as *Flavobacteria*, OUT #16), sequences closely related to the genus *Winogradskyella* and an unclassified *Flavobacteria* (10% of the sequences classified as *Flavobacteria* each OUT #21 & #23 respectively). OUT# 23 also appeared in the seawater (data not shown) samples and classified by NCBI blast as related to the genera

Flexibacter, Flexithrix, and Microscilla (91% identity e-value 7e-81). All dominant phylum-level groups showed similar variability in the OTU-level composition between layers, even where phylum level groups were similar (Supplementary Table 2, Supplementary Figure 4).

The seawater microbial assemblage was distinct from the sea-ice microbial assemblage (Figure 2). SW1 and SW2 were approximately 98% similar to each other. Both were dominated by bacteria unclassified at the phylum level, which represented ~20% and ~25% of the total sequences, respectively. Of the classifiable sequences, *Proteobacteria*, with *Alphaproteobacteria* as the major class, dominated seawater (~20% of the sequences). However, unlike in the sea-ice assemblages, where *Rhodobacteracaea* were the dominant *Alphaproteobacteria*, the majority of the seawater Alphaproteobacteria sequences belonged to the *Pelagibacteraceae* family (data not shown). Unlike in the ice samples, *Bacteroidetes* in seawater were only represented by *Flavobacteria* (~5% of the sequences) and unclassified groups (~3% of the sequences). TM7, *Opitutae*, and other *Verrucomicrobia* were absent from water samples. *Firmicutes* were identified in the water samples (~5% of sequences), but not in the ice (Figure 2.2).

Although Good's coverage estimate shows that sequence coverage was high, with the lowest coverage at 84% (bottom 30 cm of the ice, data not shown), both rarefaction analysis and the ratio of observed OTU to the number of OTU predicted by the Chao1 estimator show that with the exception of 0-30cm the samples were not sequenced to saturation (Figure 2.2S A). Thus, these analyses of microbial assemblage should be viewed with some caution as relative abundances might shift with increased taxon coverage.

2.4.4 Taxon richness and overall diversity vary with ice source

The observed OTU richness and estimated OTU richness based on the ACE non-parametric richness estimator shows three assemblages: top, middle and bottom, with OTU richness increasing with depth (Figure 2.3 A). However, the Chao1 non-parametric richness estimator shows only the bottommost section to be significantly higher in OTU richness than the rest of the core. For most of the core, the average number of OTUs in the ice was slightly lower than that of the underlying seawater; however, the bottom section of the ice had a higher number of observed OTUs than that of the seawater (Figure 2.3 A).

The melt pond assemblage shared ~55% of its observed OTUs with the old ice assemblage and ~28% of its observed OTUs with the new ice assemblage (Figure 2.4S). The old ice assemblage shared ~35% of its observed richness with the new ice assemblage. The new ice assemblage showed the highest proportion endemism with over 75% of its OTU being unique, the assemblages of the old ice and melt pond layers had ~60% and ~41% of their OTUs being unique(Figure 2.4S). The proportion of unique OTUs from both of these assemblages is approximately a third of the total MYI observed richness (Figure 2.4S).

Overall diversity, which accounts for both OTU richness and OTU evenness, was estimated using two commonly utilized non-parametric diversity indexes: the Shannon diversity index (H') and inverse Simpson's index (1/D) (Figure 2.3 B). Both indexes showed that diversity increases with depth in the ice core corresponding to the three ice layers. There was no significant difference between the mean diversity of the ice microbial assemblages and the mean diversity of water microbial assemblages.

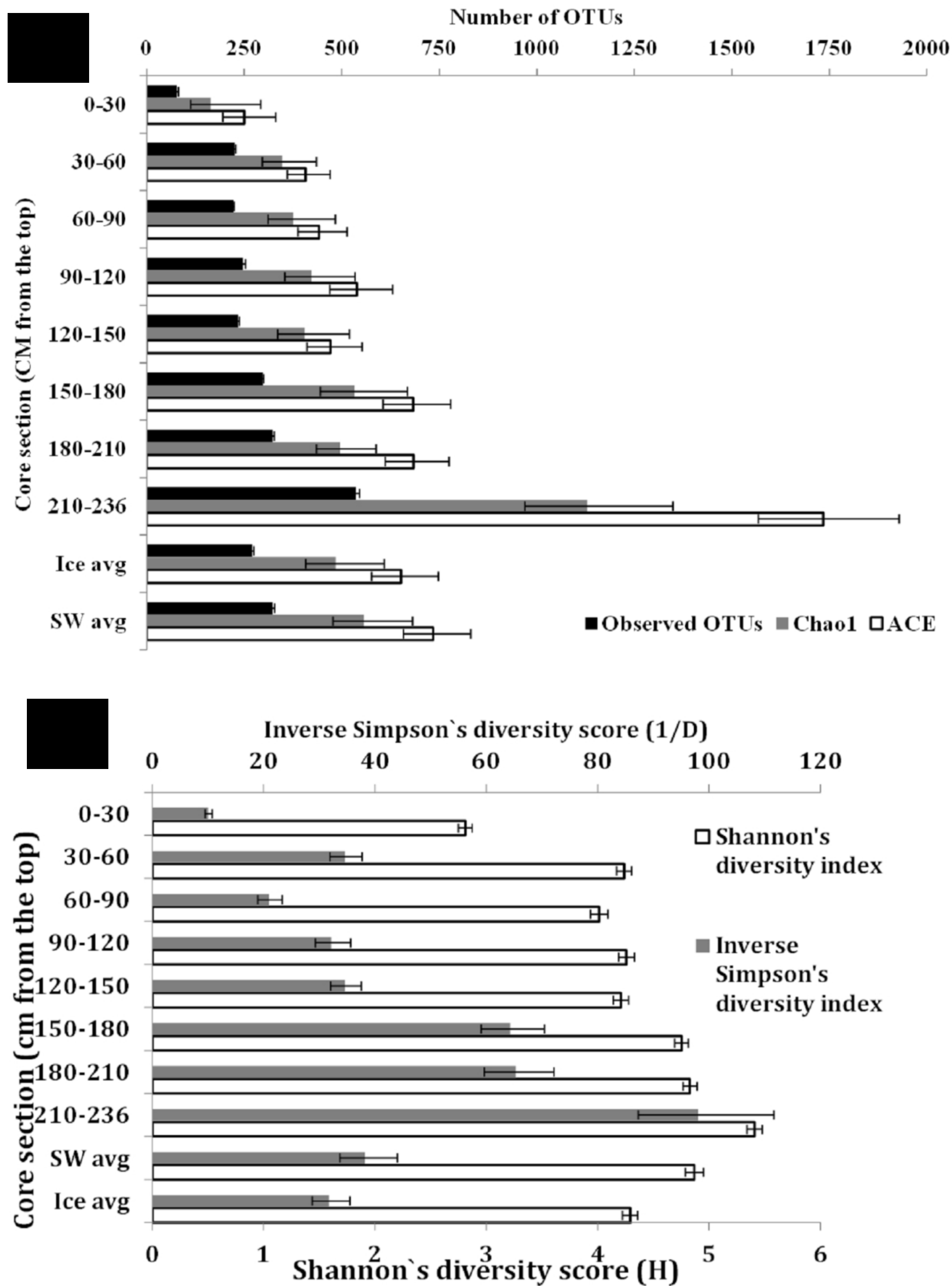


Figure 2.3 Richness and diversity measurements along the core: A- Observed and estimated (Chao1, ACE) OTU richness, B- inverse Simpson's & Shannon's diversity indices for each sample. OTU definition set at 97% similarity.

2.5 Discussion

The core we analyzed had three distinct assemblages: top (0-30 cm), middle (30-150 cm) and bottom (150-236 cm). Based on the bulk salinity profile of the core we analyzed, the uppermost section was clearly a melt pond. The texture of ice crystals in the core indicated that there was a break point at approximately 125 cm depth, marking a separation between the two year old ice and the ~110 cm of new ice below that was added during the winter. The ice layering therefore matches well with our observed three layers in the microbial assemblage: the first layer (0-30 cm) is the melt pond assemblage; the second layer is the old ice assemblage (30-150 cm); and the third layer is the new ice assemblage (150-236 cm).

Texture analysis indicates that the ice from 120-150 cm contains both new and old ice; however, its assemblage composition clusters solely with the old ice. It is possible that as a new ice layer is formed, the temperature near the freezing front is still warm enough to allow mixing of seawater with the brines; therefore, it is likely that the established assemblage of the old ice influenced the composition of the new ice assemblage. That influence diminishes as the freezing front advances further away from the old ice, leading to the development of a distinct microbial assemblage. Another possible explanation is that DNA corresponding to members of the old ice assemblage was more easily extracted or PCR amplified. Samples with higher resolution of sectioning, especially near the annual growth mark, are needed to differentiate between these explanations.

If the *in-situ* physical and chemical gradients play a major role in structuring these bacterial assemblages, we would expect strong clustering of samples within ice sections. While the bacterial assemblages form distinct clusters related to depth within each layer, this clustering

is not statistically significant. Thus, adaptation to environmental gradients was weak at best. The statistical uniformity in the structure of the old ice microbial assemblages may result from homogenization due to melt water and gravity-driven flushing of the ice and increased permeability during the summer. The new ice, on the other hand, is likely homogenized because it is permeable and therefore exchanges microbes freely between the sections and with the underlying seawater. Therefore, it appears that the water source of the ice is the driver of the assemblage structure and that the gradient in abiotic conditions plays a less significant role.

There is a top-to-bottom increase in observed taxon richness and diversity, with a clear separation of the values corresponding to the melt pond, old ice, and new ice layers (Figure 2.3). Estimated taxon richness also increases with depth, with a very large increase at the bottom layer. While the cause of the increased taxon richness and diversity with depth is unknown, it may be due to the fact that condition such as ice permeability, temperature, salinity and light levels are more permissive to biological activities. Therefore while it appears that the *in-situ* physical and chemical gradients may play a less significant role in structuring the composition of the MYI bacterial community they may never the less play a role in structuring other aspects of the community.

Though comparing our work to others was not a main objective of this paper and was done superficially we still found some striking similarities to other studies of sea ice bacterial communities: **A)** Several past studies have quantified the taxon richness and diversity of bacterial assemblages from sea ice (Bowman *et al.*, 1997; Brown & Bowman, 2001; Petri & Imhoff, 2001; Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003a; Bowman *et al.*, 2012) . While it is problematic to compare richness estimators since they tend to inflate with increased sequencing depth, the Shannon and Simpson diversity indexes tend to stabilize at a relatively low sequencing

depth and thus are more suitable for such a comparison (Gihring *et al.*, 2012) . Our mean Shannon diversity index values were similar to those of Bowman and colleagues (2011), who also analysed a MYI sample using high throughput sequencing of 16S rRNA genes; however, both values were approximately three-fold higher than for studies that did not use high throughput sequencing. Thus, higher sequencing depth shows significantly higher diversity in the sea ice, but at similar sequencing depths, MYI from different sites has similar levels of diversity. **B)** The composition of bacterial assemblages in our sample was similar to those observed previously (Bowman *et al.*, 1997; Petri & Imhoff, 2001; Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003a; Bowman *et al.*, 2012) . Alphaproteobacteria , Gammaproteobacteria, Bacteroidetes, and Verrucomicrobia were prevalent components of the assemblage. However, unlike in other sea ice samples, we found that Actinobacteria and Betaproteobacteria were also prevalent in our samples and we did not find sequences related to the phylum Chlamydia (Brown & Bowman, 2001) . Thus, taxon richness, overall diversity, and community composition were similar between our sample and other sea ice samples, possibly indicating a shared role of sea ice microbial communities in MYI globally.

Our results show a clear separation of the MYI bacterial assemblage to three groups corresponding to the three ice layers, both in composition and in diversity. Maslanik and colleagues (2011) showed a decrease of ~40% in the extent of MYI cover over the past three decades, with older, thicker ice cover lost more rapidly. Boè and colleagues (2009) predict the Arctic Ocean will be MYI free by the end of the century. Melt pond ice and old ice will be absent from an FYI covered Arctic Ocean. Though these three layers share many of the most abundant OTUs (Figurs 2.3S & Figure 2.4S), our data suggests that about one third of the observed taxon richness of the MYI core we sampled is found exclusively in the melt pond and

old ice layers and therefore will be lost. Due to the high degree of functional redundancy of bacterial communities it is unclear what effect losing these unique bacterial taxa will have on the ecological functioning of sea ice bacterial communities. However even if these unique taxa do not perform unique ecological functions, loss of biodiversity and as consequence functional redundancy reduces the resilience of an ecosystem and its ability to rebound from disturbance (i.e. the insurance hypothesis) (Yachi & Loreau, 1999) . As the Arctic Ocean shifts towards being MYI free calls of exploiting its vast resources are being voiced in the media frequently (Dodds, 2010). This will expose an ecosystem, already in limbo, to a potential multitude of anthropogenic disturbances. Therefore, further research is needed to understand the ecological functioning of the communities from each layer as well as those coming from newly formed ice sheets to understand its importance to the ecosystem.

2.5.1 Conclusions

Sea ice is a complex environment. Our study had a very modest sampling scheme, as we only sampled one site of landfast MYI; however, taxon richness, overall diversity, and assemblage composition all were similar to previous sea ice studies, possibly indicating broad applicability of our findings. It is clear that at this site, the bacterial assemblage that colonises MYI differs between each layer of ice (i.e. melt pond, old ice, new ice). These differences in the structure could imply differences in function of the assemblages in each of the layers. Furthermore, with the depletion of MYI the Arctic will lose the old and melt pond ice layers and their associated bacterial assemblages. The loss of these layers, which have unique bacterial taxa, could impact the ecological functioning of the sea ice bacterial communities and potentially the Arctic Ocean as a whole in unknown ways.

2.5.2 Acknowledgements

BDL and CH were supported by the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant program and the Polar Continental Shelf Program (PCSP). CH was an Alberta Innovates – Technology Futures Fellow. IH, BL, and JB were supported by the Northern Scientific Training Program (NSTP) and Circumpolar-Boreal Arctic Research (C-BAR) programs. We would like to thank and acknowledge the PCSP support staff and CFS Alert for their support while in the field.

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3 Hatam I, Lange B, Beckers J, Haas C, Lanoil B (2015) Bacterial communities from Arctic seasonal sea ice are more compositionally variable than those from multi-year sea ice

3.1 Abstract

Arctic sea ice can be classified into two types: seasonal ice (FYI) and multi-year ice (MYI). Despite striking differences in the physical and chemical characteristics of FYI and MYI, and the key role sea ice bacteria play in biogeochemical cycles of the Arctic Ocean, there are a limited number of studies comparing the bacterial communities from these two ice types. Here we compare the membership and composition of bacterial communities from FYI and MYI sampled north of Ellesmere Island, Canada. Our results show that communities from both ice types were dominated by similar class-level phylogenetic groups. However, at the operational taxonomic unit level, communities from MYI and FYI differed in both membership and composition. Communities from MYI sties had consistent structure, with similar membership (presence/absence) and composition (OTU abundance) independent of location and year of sample. By contrast, communities from FYI were more variable. While FYI bacterial communities from different locations and different years shared similar membership they varied significantly in composition. Should these findings apply to sea ice across the Arctic, we predict increased compositional variability in sea ice bacterial communities resulting from the ongoing transition from predominantly MYI to FYI, which may impact nutrient dynamics in the Arctic Ocean.

3.2 Introduction

The Arctic Ocean has a vast sea ice cover, which spans an average area of 15 million km^2 at its maximal extent (Wadhams, 2000). Sea ice can be classified into two main types: A) seasonal ice, which forms during the freeze-up period and undergoes complete melt during the spring and summer, a.k.a. first year ice (FYI); and B) perennial or multi-year ice (MYI), which is ice that survives at least one melt season (Wadhams, 2000). MYI is typically much thicker than FYI; especially along the northern coast of Canada and Greenland where MYI is generally thicker than 3.5 m (Haas et al, 2006; Haas et al, 2010). Until recently, MYI dominated the Arctic sea ice cover; however, the proportion of MYI has dwindled from >70% of the total sea ice cover to under 45% over the past three decades (Serreze et al, 2007; Maslanik et al, 2011). Furthermore, the remaining MYI tends to be younger and therefore thinner (Maslanik et al, 2011). Thus, Arctic perennial sea ice cover is not only shrinking in extent, it is also losing volume. These trends are expected to continue and it is conservatively predicted that the Arctic Ocean will be largely ice-free during summer by the end of the 21st century (Boé et al, 2009).

The loss of MYI has had a significant impact on Arctic climate and ecosystems. Larger open water areas in summer have contributed to the accelerated warming in the Arctic and to potential changes in atmospheric circulation (Overland et al, 2012). Decline in summer sea ice has severely impacted animals that are dependent on ice for feeding, dispersal and mating (see review by (Post et al, 2013)). Phytoplankton blooms in the Arctic Ocean are normally associated with sea ice edge and retreat areas and are reliant on nutrient release from melting sea ice. Over the past fifteen years, massive under-ice phytoplankton blooms have occurred frequently underneath pack ice in the western Arctic Ocean (Lowry et al, 2014; Arrigo et al, 2012). These recent massive under ice blooms are associated with the increase in a much thinner FYI cover

which allows higher light penetration. Furthermore, it has been suggested that sea ice algae productivity would increase with the transition to an Arctic Ocean exclusively covered byFYI (Wassmann & Reigstad, 2011). However a recent study suggests that the productivity of thik MYI has been underestimated and that loss of MYI will not result in increased productivity in Arctic sea ice (Lange et al, 2015).

Arctic sea ice is a habitat for a diverse community of microorganisms that primarily inhabit liquid brine channels within the ice (Junge et al, 2001; Mock & Thomas, 2005). The most recognizable feature of this community is the diatom bloom in the bottom 5-10 cm of the ice during the spring and early summer (Gosselin et al, 1997; McMinn & Hegseth, 2007; McMinn et al, 2007). However, sea ice also hosts diverse communities of heterotrophic bacteria. Constituents of these communities are metabolically active throughout the year and are key regulators of the spring Arctic Ocean diatom bloom through the regeneration of nutrients via the microbial loop (Poltermann, 2001; Junge et al, 2004; Junge et al, 2006; Deming, 2007). Moreover, during the polar winter, heterotrophic production and growth fertilizes the ice in preparation for the spring and summer bloom and supplies the ice and underlying water column with a small but stable subsidy of organic matter through secondary production (Riedel et al, 2008; Wing et al, 2012).

The physical and chemical environment in MYI and FYI are quite different and can therefore be viewed as distinct microbial habitats. The decreased thickness of FYI relative to MYI results in steeper gradients in temperature, light, brine volume, and salinity in FYI, as well as increased light transmission to the underlying water (Eicken, 2008). Due to gravitational drainage of brine and flushing of the ice with fresh water from snowmelt during summer, MYI has lower brine volumes and bulk salinity and therefore has lower porosity than FYI (Wadhams,

2000; Eicken, 2008). MYI can also include layers of refrozen melt pond ice and newly formed sea ice at the bottom that are absent from FYI. These melt pond and newly formed ice layers were recently reported to host bacterial assemblages distinct from those in the older ice layers (Hatam *et al.*, 2014). Furthermore, though MYI undergoes seasonal fluctuations in many chemical and physical parameters, it still provides a more stable habitat than FYI due to the fact that it survives for longer periods. The difference in age between MYI and FYI was speculated to be a key factor in the establishment and development of sea ice microbial community (Rysgaard & Glud, 2004).

Here we describe the first comparative study of bacterial community structure and composition of Arctic MYI and FYI using high throughput sequencing. We found that MYI and FYI bacterial communities collected over three field seasons from the Lincoln Sea were distinct from each other and that MYI bacterial community membership and composition was less variable than for FYI bacterial communities. These results indicate that the ongoing shift from MYI to FYI will lead to shifts in the bacterial community of the Arctic Ocean and to increased variability in these communities which potentially impact the ecosystem functions they provide.

3.3 Material and Methods

3.3.1 Site description and sample collection

Samples for this study were taken from ten sites; five land fast MYI sites and five adjacent land fast FYI sites located off northern Ellesmere Island, Nunavut, Canada (Table 3.1S). This geographical location is characterized by dominance of consolidated MYI flows with patches of first year ice in-between resulting from refrozen open leads. Sampling was conducted during the beginning of May 2010, 2011, and 2012. All MYI samples were collected from

depressions in the ice , indicating the presence of a freshwater melt pond in summer. An accurate age of the MYI was not determined; however, ice thickness ranged from 250 to 260 cm, which is typical of MYI and thicker than most FYI (Table 3.1S). Furthermore, the bulk salinity profile showed low overall salinity and an increase in salinity with depth, which is characteristic of MYI (Figure 3.1S). FYI samples were collected from level floes identified from satellite radar images. For these sites cores ranged in thickness from 90 to 180 cm, which is also characteristic of FYI (Table 3.1S). Bulk salinity for FYI cores was higher overall than for MYI and showed the characteristic 'C'-shaped FYI salinity profile with higher salinity values at the top and bottom portions of the ice, and lower salinity in the middle (Figure 3.1S).

For each site, duplicate ice cores were sampled 1 m apart using a Kovacs Mark II 9 cm-diameter corer (Kovacs Enterprise, Roseburg, Oregon) powered by a DeWalt 36V electric hand drill. Prior to drilling, the core barrel was thoroughly rinsed with sterile deionized water (Milli-Q Integral Water Purification System, EMD Millipore Corporation, Billerica, MA, USA). Ice cores were immediately sectioned on site at 30 cm intervals and placed in polypropylene bags (ULINE, Edmonton, Alberta, Canada). Cutting was done using a hand saw that was rinsed in deionized water and wiped with an ethanol wipe.

At four sites, additional surface sea water samples were collected (Supplementary Table 3.1). Duplicate 2 liter water samples were pumped into polypropylene bags from the coring hole using a manual peristaltic pump (Cole Palmer, Montreal, Quebec, Canada). Both water and ice samples were covered, chilled, and processed within 4 hours of sampling.

3.3.2 Sample processing

To avoid contamination and dilution of samples, ice samples were directly thawed at room temperature in the dark, rather than isotonically (Kaartokallio et al, 2005; Kaartokallio et al, 2008). Melted ice samples and seawater samples were filtered individually through sterile 0.22 µm pore size polyethersulfone membrane filters (Pall, Mississauga, ON, Canada). Between samples, glassware used for filtration and sample measurements was sterilized using 10% household bleach solution followed by thorough rinsing in sterile deionized water. Each filter was placed in a microfuge tube, submerged in RNAlater® solution (Life Technologies, Burlington, ON Canada), and stored at -20°C for later DNA extraction.

3.3.3 DNA extraction amplification and sequencing

DNA was extracted from preserved filters by bead beating using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) as instructed by the manufacturer. Filters from the two corresponding 30 cm sections of the duplicate ice cores were combined prior to DNA extraction in order to ensure sufficient DNA yield. DNA concentration was quantified fluorometrically with SYBR Green (Life Technologies) in a NanoDrop3300 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. DNA extraction products from sections from the same core were combined in equal concentrations prior to amplification and sequencing. The V1-V3 regions of the bacterial 16S rRNA gene were PCR amplified in triplicate using 27F-518R primers (Table 3.2S), mixed in equal concentrations, and purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, MA, USA) as previously described (Hatam et al. 2014). Molecular Research LP (Shallowater, Texas, USA) sequenced the resulting products using FLX-Titanium amplicon pyrosequencing on the the Genome Sequencer FLX System (Roche, Branford, CT) as previously

described (Dowd *et al.*, 2008; Hatam *et al.*, 2014). A list of all primers and barcodes is provided in Table 3.2S.

3.3.4 Pre-processing and quality control of raw sequences

All pre-processing and sequence quality control steps were performed using Mothur v. 1.33.3 following the Mothur 454 SOP for sff & flow files(http://www.mothur.org/wiki/454_SOP access date 08/13/14) (Schloss et al, 2009). In brief, raw flowgram data were used to discard sequences not in the range of 360-720 flows, as recommended (Quince et al, 2011). Noise reduction was performed using the Mothur implementation of the PyroNoise algorithm which corrects PCR- and pyrosequencing- generated errors (Quince et al, 2009; Schloss et al, 2011). Sequences shorter than 200 base pairs (bp) or those containing primer/barcode mismatches or homopolymers longer than 8 bp were discarded. The remaining sequences, which were all 250 - 350bp long, were aligned against the SILVA reference dataset (version 110), and then pre-clustered for further noise reduction as recommended (Pruesse et al, 2007; Huse et al, 2010). Chimeras were detected and removed using the Mothur implementation of UCHIME (Edgar et al, 2011). OTUs were assigned using the average-neighbor clustering algorithm for an OTU definition of 97% similarity. Global singleton OTUs, represented as just one sequence in the dataset, were excluded and the remaining OTUs were assigned to taxonomic classification using the Mothur implementation of the Ribosomal Database Project classifier (train set 10) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). Using this threshold we were able to classify 75%, 85% and 82% of the sequences to below the domain bacteria and 37%, 51% and 18% of the sequences to the genus level in FYI, MYI and SW respectively. Based on this classification, sequences of mitochondrial, chloroplast, eukaryotic or unknown origin (sequences that could not be classified at the domain level) were removed from the data set. Pre-analysis

data clean-up processes eliminated ~30% of the total raw sequences. The sequences were subsampled to the smallest library size (n = 1100 for individual samples, n = 8000 for merged FYI, MYI and SW groups) to allow identical sampling efforts for each sample before further analyses (Gihring et al, 2012). Sequences are available from the National Center for Biotechnology Information Sequence Read Archive under bioproject PRJNA288609.

3.3.5 Community analysis

Mothur v. 1.33.3 was used for all community analyses and statistics as previously described (Schloss et al, 2009). Alpha diversity was estimated using the Chao1 and ACE nonparametric richness estimators, reciprocal Simpson's diversity index (invsimpson), and Shannon diversity index (Shannon, 1947; Simpson, 1949; Chao, 1984; Chao & Shen, 2003). Community evenness was estimated using HEIP (Heip, 1974). Coverage was calculated as the ratio between observed and estimated richness. Based on this calculation all our samples showed ~90% coverage, which indicates a good sequencing effort (Figure 3.1A). Furthermore, rarefaction curves both for individual and merged samples (i.e. 8000 and 1100 sequences) approached an asymptote, further indicating a high level of coverage of the bacterial community diversity (Supplementary Figure 2 A &B). Yue & Clayton's theta measure of dissimilarity (θyc) and Jaccard's dissimilarity coefficient (Jclass) were used to compare the composition (i.e., dissimilarity of communities based on OTU relative proportion) and membership (i.e., similarity of communities based on shared OTUs), respectively, of the different communities in an OTU-based, phylogeny-independent manner (Jaccard, 1912; Yue & Clayton, 2005). Principal coordinates analysis (PCoA) was used to ordinate the different samples in multidimensional space, based on the distance matrices generated by θyc and Jclass. The nonparametric analysis of molecular variance (AMOVA) was used to test the significance of the grouping based on the

PCoA ordination: statistical significance of grouping was tested for $p \leq 0.05$ with Bonferroni correction according to the number of groups tested (Excoffier et al, 1992; Anderson, 2001; Martin, 2002). Based on the distance matrices generated by Mothur, the distances of group members from each sample type to the group centroid were calculated using the betadispr function from the R package vegan (R version 3.1.1, www.r-project.org), to compare between sample type homogeneity in composition and membership. Statistical significance of differences between the distances to the centroid were calculated using ANOVA and pairwise comparisons were calculated posthoc using the TukeyHSD test. The differences were considered statistically significant at $p \leq 0.05$. Mothur v 1.33.3 was used to visualize shared observed richness using Venn diagrams (Schloss et al, 2009). Mathlab v 7.13 was used to plot PCoAs. A neighbor joining phylogenetic tree of representative OTU sequences was constructed for OTUs found either in all MYI, in all SW, or in all FYI samples using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/). Tree visualization and the addition of OTU abundance to each branch was done in R version 3.1.1 (www.r-project.org) with the phyloseq package (McMurdie & Holmes, 2013). Sequences of these OTUs where deposited to the NCBI sequence repository under accession numbers (xxxxxx).

Of the OTUs that were part of the core communities, those found exclusively in MYI were further identified using the nucleotide BLAST search tool (BLASTn) against the NCBI non-redundant (nr) database with the exclusion of model and uncultured organisms to identify the closest cultured relative. Based on this classification, the hypothetical ecological function of the OTU was assumed. We also used a similar BLASTn search but including uncultured bacteria to identify the environments the most similar sequences originated from. For both BLASTn searches, a maximum expect value of e^{-90} was accepted.

3.4 Results

Salinity profiles for the sites in this study were done in 10cm intervals and were taken from Lange et al (Figure 3.1S) (Lange et al, 2015). For each site, mean Chl*a* concentrations as well as snow and ice depth, previously reported by Lange et al. (2015) are also reported in Supplementary Table 1. No statistical differences in these parameters were found between FYI and MYI (Lange et al, 2015).

3.4.1 Communities from FYI have higher taxon richness and diversity than MYI

Taxon richness in FYI (observed and estimated; Figure 3.1A) and diversity (Figure 3.1B) in FYI was significantly higher than for MYI and SW (repeated measure ANOVA, $p < 0.05$). Although observed and estimated OTU richness for bacterial communities from MYI were lowest of all three sample types, overall diversity was higher for MYI than SW (Figure 3.1B). The discrepancy between OTU richness and overall diversity is because OTUs were more unevenly distributed in SW (HEIP evenness 0.186) than FYI (HEIP evenness 0.233) or MYI (HEIP evenness 0.282).

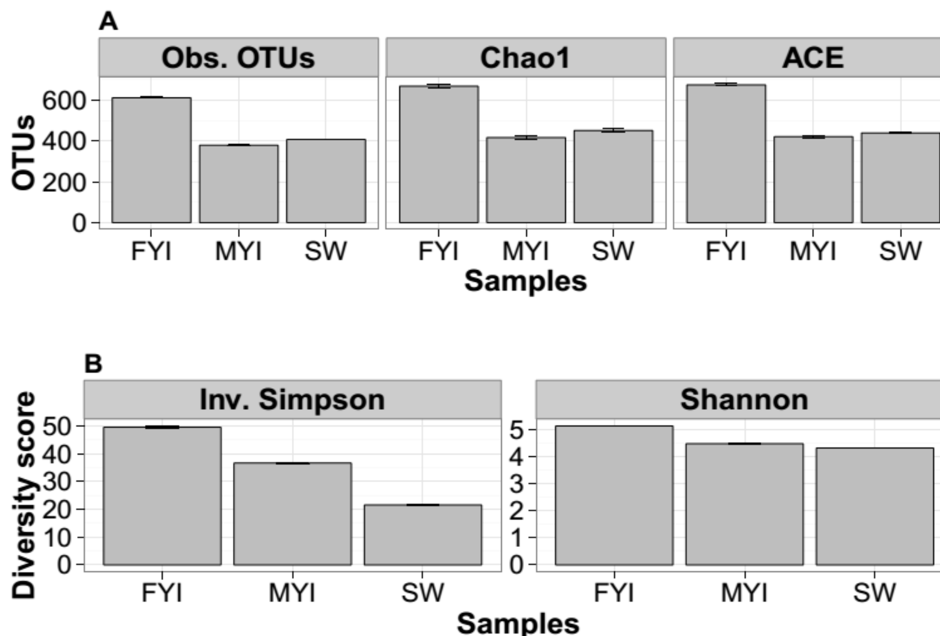


Figure 3.1 Richness (observed and predicted) and diversity measurements: A Observed OTUs, estimated richness (ACE and Chao1) and B diversity (Shannon and inverse Simpson indices) for pooled FYI, MYI, and SW samples. OTU definition set at 97% sequence similarity, error bars ± SEM (n=1000 iterarations of sub-sampling to 8000 sequences and calculating indecies and estimators).

3.4.2 FYI bacterial communities have endemic, MYI-like, and SW-like components

Communities from all sample types showed a high number of OTUs not found in the other sample types (i.e. unique OTUs); however, FYI and SW communities had more unique OTUs (47.5% & 48%, respectively) than MYI communities (36% unique OTUs) (Figure 3.2). MYI bacterial communities shared twice the fraction of OTUs with FYI (59%) as with SW (30%) (Figure 3.2); FYI bacterial communities share similar proportions of their OTUs with MYI and SW communities (36% and 32%, respectively; Figure 3.2). Furthermore, 33% of OTUs from MYI are shared exclusively with FYI, which is eight times higher than the fraction MYI shares exclusively with SW (Figure 3.2). This differential stands in contrast to communities from FYI, which share similar fractions of their OTUs exclusively with both MYI and SW (20% and 16%, respectively; Figure 3.2). Thus, FYI and SW bacterial communities are more similar to each other than MYI and SW.

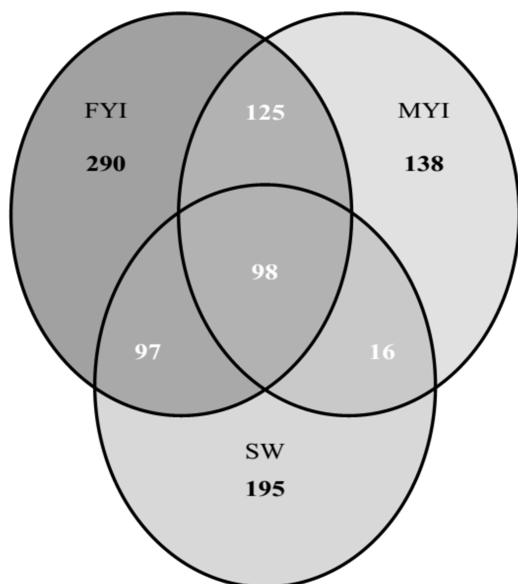


Figure 3.2 Shared OTUs: Venn diagram of shared OTUs between MYI, FYI and SW. OTU definition set at 97% sequence similarity.

3.4.3 Composition of major class level groups shows minimal differences between ice types or seawater

The majority of OTUs from both ice types could be classified to class-level taxonomic groups; those that were not, are represented at the phylum level (Figure 3.3). Most of the class/phylum level groups were similar between the two ice types (Figure 3.3). The predominant class-level groups in both FYI and MYI communities were *Alphaproteobacteria* (~10% in FYI, ~15% in MYI), *Gammaproteobacteria* (~15% in both sample types), *Flavobacteria* (~10% in both sample types) and the class-level group *Actinobacteria* within the phylum *Actinobacteria* (~10% in both sample types) (Figure 3.3). Class level groups representing less than 1% of the total sequences were grouped together as “Other”. The class-level group *Opitutae* represented a fraction greater than 1% of the sequences only in MYI communities (Figure 3.3).

At the class/phylum level, ice communities were minimally different from SW communities. Within the major class/phylum level groups, only sequences closely related to the class *Sphingobacteria* and unclassified *Bacteroidetes* had relative abundance greater than 1% in ice and lower than 1% in SW (Figure 3.3). Sequences classified as closely related to the phylum-

level group *Firmicutes* appeared only in SW (albeit with relative abundance lower than 1%; data not shown) and not in ice.

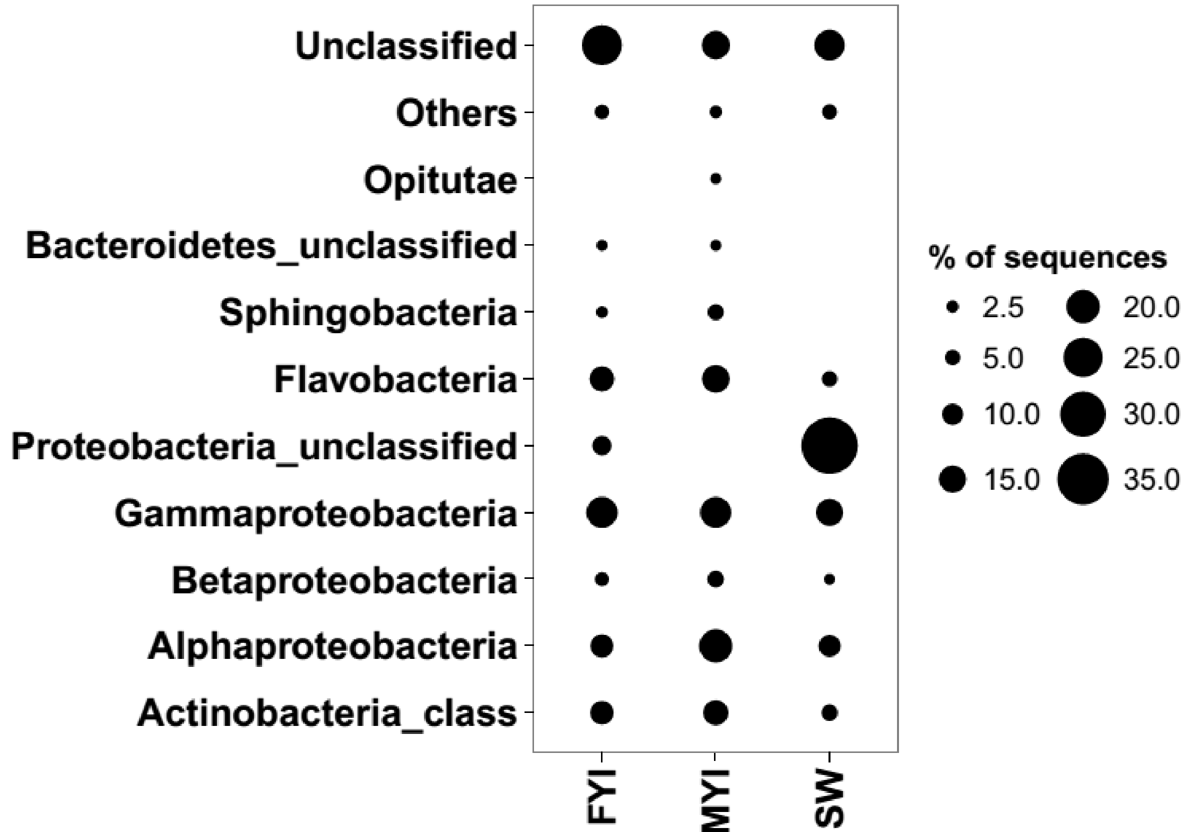
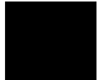


Figure 3.3 Class level composition of the three sample types: Proportion is represented as percent of total sequences per sample type. Classes that amounted to less than 1% of the total sequences per sample type were pooled as "Others". "Unclassified" sequences could not be classified below the level of domain "Bacteria". If classifiable below the domain Bacteria, OTUs that cannot be classified to the class level are identified as *phylum levle group – unclassified*.

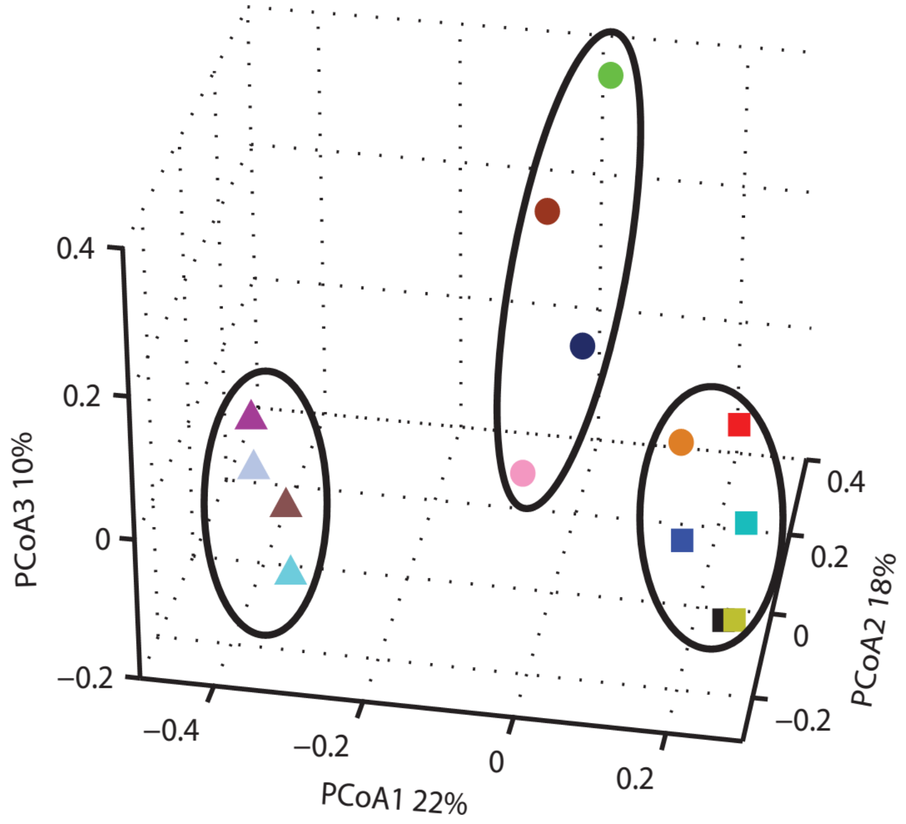
3.4.4 MYI and FYI have distinct membership, but not composition

Despite the high similarity in the composition of FYI, MYI, and SW bacterial communities at higher taxonomic levels, examination of the community membership and compositions at finer scales show significant differences. The J-class index of dissimilarities between memberships of different communities showed clustering of the samples to three statistically significant groups (max pair-wise p-value $p < 0.004$) based on sample type (i.e. FYI, MYI, and SW; Figure 3.4A). An alternate dissimilarity measure, Yue & Clayton's θ measure of dissimilarity (θ_{yc}), which accounts for relative abundance of taxa and thus measures composition, also showed three statistically significant groups (max pair-wise p-value $p < 0.01$; Figure 3.4B); however, the membership of these groups was not determined by sample type. While the SW and MYI mostly maintained their grouping, FYI samples grouped with either SW or MYI samples: two of the FYI samples formed a cluster with four of the five MYI samples; one FYI sample grouped with the SW samples, and two FYI samples grouped with the remaining MYI sample. The difference between clustering by membership and composition indicates that the FYI bacterial communities share mainly low abundance OTUs, while MYI and SW share both low and high abundance OTUs.

The higher variability between FYI samples is also apparent by the spread of the samples in the ordination space. This reflects a lower homogeneity in the composition and membership of communities from FYI compared to MYI which was tested to be statistically significant (ANOVA with post-hoc TukeyHSD on the distance of group members to centroieds $p \leq 0.01$).



F10A
F11A
F11B
F12A
F12B
M10A
M11A
M11B
M12A
M12B
SW1
SW2
SW3
SW4



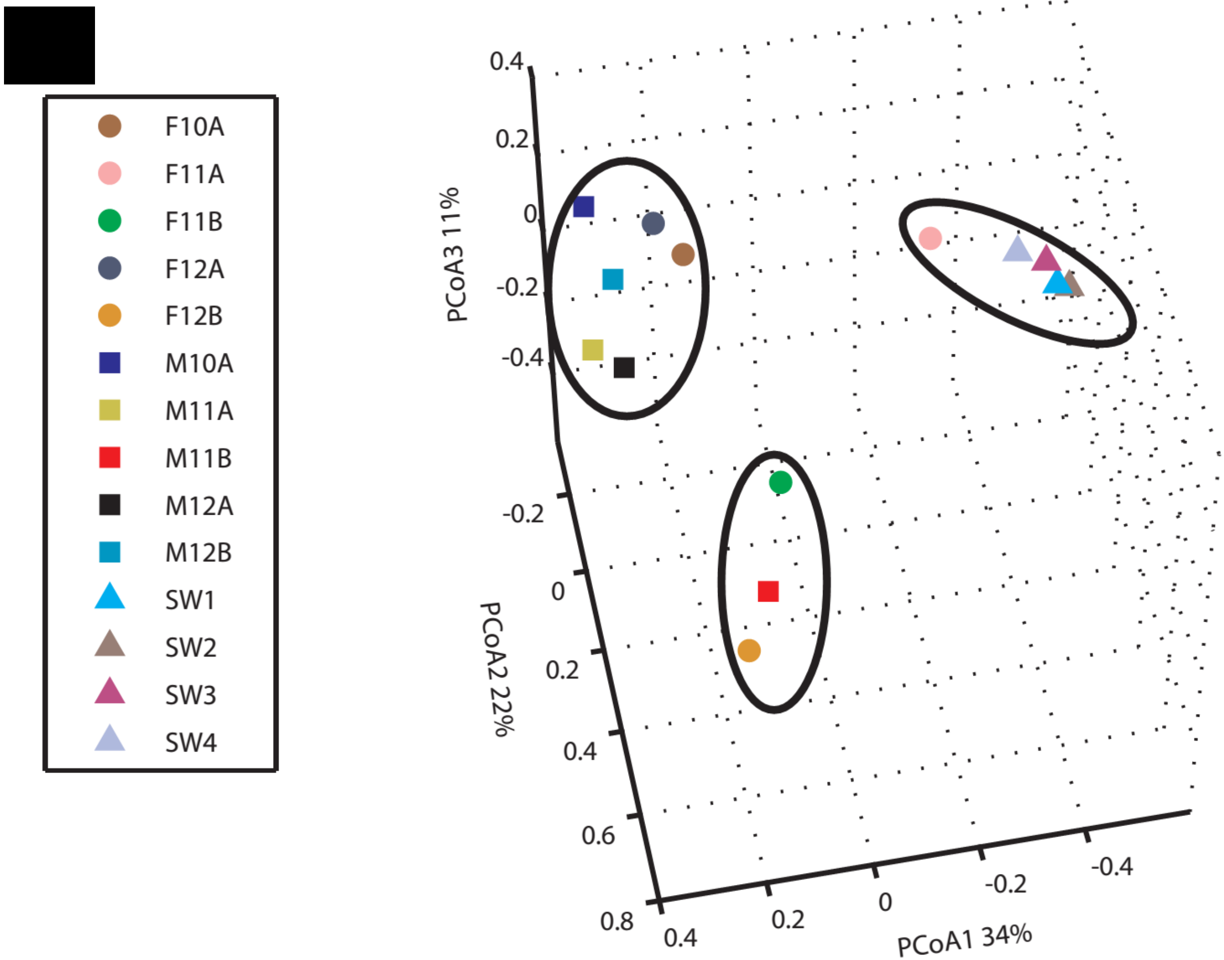


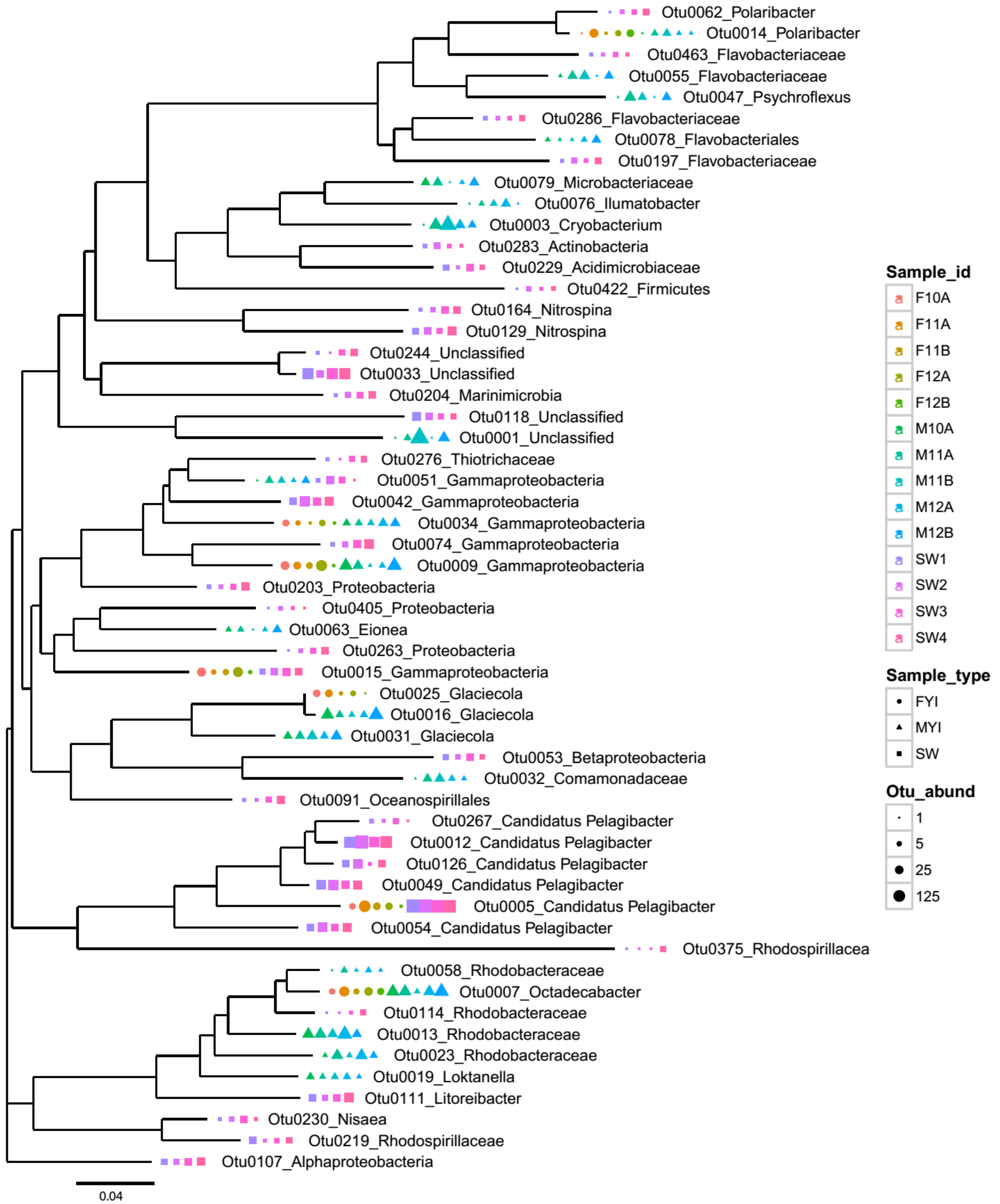
Figure 3.4 Membership and composition at the OTU level: Clustering of the communities based on (A) membership (J-class dissimilarity index; r2 = 0.82; AMOVA scores MvF F(1,9)2.78 p < 0.001, MvSW F(1,6) 1.77 p < 0.002, FvSW F(1,9) 1.42 p < 0.004); and (B) composition (θyc dissimilarity index; r2 = 0.89; AMOVA scores MvF F(1,6) 8.3 p < 0.01, MvSW F(1,10) 9.24 p < 0.001, FvSW F(1,8) 4.14 p < 0.01). Statistically significant clustering based on AMOVA is circled in black. OTU definition set at 97% sequence similarity; sequencing depth was normalized to 1100 reads. 'F' = first year ice, 'M' = multi-year ice, 'S' = surface seawater; numbers represent year sampled; 'A' & 'B' = sites sampled/year.

3.4.5 Communities from MYI have a broader core membership than FYI

To further investigate the core OTUs of the bacterial communities from different sample types, we compared the number of OTUs that are found in all MYI samples, all SW samples and those that are found in all FYI samples (Figure 3.5). Note that these OTUs are not necessarily exclusive to one sample type, but are found in all samples of that type. All MYI samples shared 20 OTUs, representing ~5% of the observed OTUs in MYI; these core MYI OTUs accounted for an average of ~25% of the total sequences from MYI samples (Figure 3.5). Similarly, all SW samples shared 34 OTUs, representing ~8% of the observed OTUs for SW; these core SW OTUs accounted for an average of ~55% of the total sequences from SW samples (Figure 3.5). , all FYI samples shared only seven OTUs, representing ~1% of observed OTUs for FYI; these core FYI OTUs accounted for 11% of the total sequences from FYI samples (Figure 3.5). Furthermore, of those seven core FYI OTUs, four were also shared with all of the MYI samples and two with all of the SW samples, meaning only one OTU was shared between all FYI samples exclusively. In contrast, many more OTUs were shared between all MYI samples exclusively or all SW samples exclusively (15 and 32, respectively; Figure 3.5, Figure 3.3S).

The closest relatives of the 15 OTUs exclusive to all MYI samples with NCBI BLAST were found primarily in cold environments (marine, fresh water, or terrestrial) (Table 3.3S). Four of these OTUs belong to the Rhodobacteraceae family with two of the four in the genera *Loktanella* and *Roseobacter*. Both of these genera are known to play a role in the degradation of DMSP originating from marine algae (Gonzalez et al, 1999; Moran et al, 2007). Other OTUs were members of the genus *Glacicola* (Supplementary Table 3.3) known for their role in hydrolyzing complex organic carbon molecules such as cellulose and chitin originating from algae (Qin et al, 2014). An OTU related to *Psychroflexus torques*, which is known to associate

with ice algae (Bowman et al, 1998) and may oxidize DMSP, was also exclusive to the core MYI group. One of the OTUs was closely related to *Glacicola nitratireducens* (Supplementary Table 3), which is involved in the nitrogen cycle through denitrification (Qin et al, 2014). The fact that these *Glacicola* sequences are found in high abundance in all MYI samples may explain a previous observation that denitrification occurs much more strongly in MYI than FYI (Rysgaard et al, 2008). *Psychroflexus torques* may also contribute to the primary productivity of sea ice via proteorodopsin-mediated carbon fixation and the secretion of EPS (Feng et al, 2013). Taken together, the groups found exclusively in the MYI core community are cold adapted organisms that consume algal products and transform nitrogen and carbon within the ice.



Otu0062_Polaribacter
Otu0014_Polaribacter
Otu0463_Flavobacteriaceae
Otu0055_Flavobacteriaceae
Otu0047_Psychroflexus
Otu0286_Flavobacteriaceae
Otu0078_Flavobacteriales
Otu0197_Flavobacteriaceae
Otu0079_Microbacteriaceae
Otu0076_Ilumatobacter
Otu0003_Cryobacterium
Otu0283_Actinobacteria
Otu0229_Acidimicrobiaceae
Otu0422_Firmicutes
Otu0164_Nitrospina
Otu0129_Nitrospina
Otu0244_Unclassified
Otu0033_Unclassified
Otu0204_Marinimicrobia
Otu0118_Unclassified
Otu0001_Unclassified
Otu0276_Thiotrichaceae
Otu0051_Gammaproteobacteria
Otu0042_Gammaproteobacteria
Otu0034_Gammaproteobacteria
Otu0074_Gammaproteobacteria
Otu0009_Gammaproteobacteria
Otu0203_Proteobacteria
Otu0405_Proteobacteria
Otu0063_Eionea
Otu0263_Proteobacteria
Otu0015_Gammaproteobacteria
Otu0025_Glaciecola
Otu0016_Glaciecola
Otu0031_Glaciecola
Otu0053_Betaproteobacteria
Otu0032_Comamonadaceae
Otu0091_Oceanospirillales
Otu0267_Candidatus Pelagibacter
Otu0012_Candidatus Pelagibacter
Otu0126_Candidatus Pelagibacter
Otu0049_Candidatus Pelagibacter
Otu0005_Candidatus Pelagibacter
Otu0054_Candidatus Pelagibacter
Otu0375_Rhodospirillacea
Otu0058_Rhodobacteraceae
Otu0007_Octadecabacter
Otu0114_Rhodobacteraceae
Otu0013_Rhodobacteraceae
Otu0023_Rhodobacteraceae
Otu0019_Loktanella
Otu0111_Litoreibacter
Otu0230_Nisaea
Otu0219_Rhodospirillaceae
Otu0107_Alphaproteobacteria
0.04
Sample_id
F10A
F11A
F11B
F12A
F12B
M10A
M11A
M11B
M12A
M12B
SW1
SW2
SW3
SW4
Sample_type
FYI
MYI
SW
Otu_abund
1
5
25
125

Figure 3.5 Neighbor joining phylogenetic tree of core OTUs: Relationship of OTUs found in all MYI samples, all FYI samples, or both. OTU definition set at 97% sequence similarity; shapes correspond to ice type (FYI or MYI); symbol size corresponds to OTU abundance and colors correspond to sample id. Sample naming as in Figure 3.4.

3.5 Discussion

Class-level taxonomic groups from the bacterial communities in our sea ice samples are similar to groups previously shown to dominate sea ice in both polar and sub-polar seas. Most of the OTUs that were present in all sea ice samples in our study were most similar to previously identified sea ice bacteria from the Arctic, Antarctic and the Baltic Sea (Figure 5) (Petri & Imhoff, 2001; Junge et al, 2001; Brinkmeyer et al, 2003; Bowman et al, 2012; Hatam et al, 2014; Eronen-Rasimus et al, 2014; Han et al, 2014). Furthermore, MYI bacterial communities from the sites we sampled had comparable OTU richness and diversity values to those shown in previous studies of sea ice bacterial communities (Bowman *et al.*, 2012; Hatam *et al.*, 2014). It is worth noting that communities from FYI showed the highest OTU richness and overall diversity by a large margin. This is the first study to use high throughput sequencing of 16S rRNA genes with Arctic FYI bacterial communities; therefore, there is no point of reference to compare our values to in that geographical location. However, our diversity indices were comparable to those recently published for Antarctic seasonal pack ice (Torstensson et al, 2015).

Although our study had a modest geographic scope, focusing exclusively on landfast ice from the Lincoln Sea, our richness and diversity trends were similar to studies conducted in summer pack ice (Han et al, 2014; Bowman et al, 2012). Furthermore, the OTU composition from our ice samples was similar to these studies at the class level and lower (if the sequences

could be resolved to lower taxonomic levels). Thus, although land fast ice is more affected by riverine input and sediment upwelling than pack ice (Wadhams, 2000), our findings may be broadly applicable to both types of ice and the Arctic as a whole.

If we define “core community” as OTUs found in all samples of a sample type, then the FYI core community is not robust, i.e. there are few OTUs shared amongst all FYI and they represent a small fraction of the total FYI sequences. Therefore, the ancillary community, i.e. those OTUs found in some but not all of the FYI samples determine the similarity to other sample types based on community composition. In other words, based on composition, FYI does not have a clear core community; rather, it is a composite community that is more similar to either MYI or SW. The lack of a robust core community implies that FYI bacterial communities are highly variable.

In contrast, our results show that bacterial communities from MYI have low sample-to-sample variability in both community membership and composition. Sample grouping was independent of site and year of sampling, indicating a high level of stability of these communities. Their common structure may be the consequence of selection processes resulting from formation and maturation of MYI, including transition from water to ice, freeze and thaw cycles, flushing of the ice with melt water, brine drainage, and the formation of different layers within the ice depending on the original water source (Mock & Thomas, 2005). The low variability and high stability of the MYI bacterial communities is further supported by the high number of OTUs shared by all MYI samples as compared to the low number of OTUs shared by all FYI samples.

One possible explanation for the clustering of FYI based on membership is that the less abundant, unique (i.e. found in at least one FYI sample but not in other sample types) OTUs in FYI are transient and not well adapted to life in sea ice and thus are not found in MYI. These unique OTUs could originate from non-seawater sources such as sediment or the aeolian deposition of particles (Bunch & Harland, 1990; Reimnitz et al, 1992; Laurion et al, 1995), a possibility supported by the presence of members of the genera *Arthrobacter* and *Solirubrobacter* and the Phylum Chloroflexie. These taxa are primarily found in terrestrial and sediment environments and are only found in the FYI samples, not MYI or SW. Alternatively, these OTUs unique to FYI could be present at very low abundance in seawater, but are preferentially scavenged during FYI formation (Garrison et al, 1983; Gradinger & Ikävalko, 1998; Weissenberger & Grossmann, 1998). In this case, bacterial communities from FYI samples group with either SW or MYI based on composition because the most abundant OTUs either originate from SW or are well adapted to survival in sea ice conditions, and are therefore abundant in MYI as well. Thus, the transition from MYI to FYI will result in a more variable and unstable microbial community in Arctic sea ice.

The core OTUs for the MYI bacterial communities are primarily specialized psychrophiles, with relatives found in both cold marine sediments and cold fresh water environments (Supplementary Table 3). Prominent members of the core MYI OTUs seems to be specialized in exploiting the sea ice algal bloom via the oxidation of DMSP or the breakdown of complex organic carbon molecules synthesized by ice algae (Supplementary Table 3). We interpret these findings to indicate a MYI core community acclimated to cold environments and ready to exploit the algal bloom, with implications for the nitrogen, sulfur and carbon cycles. Thus, these biogeochemical cycles might be impacted with the disappearance of MYI and its

stable community. Such losses may lead to changes in nutrient dynamics in sea ice and the Arctic Ocean, such as decreased denitrification in the ice and increased export of organic carbon from the ice (due to lower efficiency of carbon utilization), which in turn may increase nutrient export to the water column and sediments. However, it is unclear whether the increased variability of the FYI community composition might mask such impacts.

3.5.1 Conclusions

To the best of our knowledge, this is the first study comparing the composition of bacterial communities from MYI and FYI using high throughput sequencing. Though of modest scale both geographically and seasonally, our data suggests two distinct phases in the development of bacterial community composition in the sea ice cover of the Lincoln Sea. The first phase is a transient FYI community, with distinct membership but not composition that may reflect the stochastic incorporation of bacteria into the growing ice. The second phase is a mature MYI community with a broad base of shared members found in high relative abundance. As the Arctic warms and MYI transitions to FYI, our results indicate that there will be a shift from a highly stable sea ice bacterial community towards one that is more variable in its composition. Due to the high degree of functional redundancy found in bacterial communities, it is unclear what effect the shift in community structure will have on the ecology of the Arctic Ocean. However, we predict that the variability and instability at the compositional level translates to similar variability and instability at the functional level and may decrease the ability of these communities to adapt to the effects of environmental disturbance and may change nutrient dynamics in the Arctic Ocean.

3.5.2 Acknowledgement

B.D.L. and C.H. were supported by the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant program and the Polar Continental Shelf Program (PCSP). C.H. was an Alberta Innovates – Technology Futures Fellow. I.H., B.L. and J.B. were supported by the Northern Scientific Training Program (NSTP) and Circumpolar-Boreal Arctic Research (C-BAR) programs. We would like to thank and acknowledge the PCSP and CFS Alert staff for their hospitality and logistical support while in the field.

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4 Hatam I, Lange B, Beckers J, Haas C & Lanoil B (2015) Potential lag in response of bacterial communities from seasonal Arctic sea ice to *in-situ* environmental disturbance compared to those from multi-year sea ice

4.1 Abstract

The Arctic Ocean is undergoing a dramatic decrease in the extant of its perennial ice (MYI) cover with some predictions it will be largely covered by seasonal ice (FYI) by the end of the century. Recent studies indicated that Arctic MYI and FYI host bacterial communities with different structure. Since sea ice bacterial communities play a major part in the Arctic Ocean biogeochemical cycles and energy flux, it is important to understand whether this difference in structure also translates to a difference in function. Here we conducted a first *in-situ* study linking the difference in structure between the two communities to response to disturbance. We inverted both MYI and FYI cores, disrupting the sea ice top-to-bottom temperature and salinity gradient for 8 days. Our results show that while the community from the original MYI top sections showed a shift in structure and a marginal increase in OTU richness compared to parallel communities from both disturbed and undisturbed controls, the original bottom community did not respond to the disturbance. Our result also showed no response to the disturbance by the FYI community, indicating that the more mature and structured MYI community may respond quicker to disturbance.

4.2 Introduction

Over the past three decades there has been a dramatic reduction in the extent and volume of Arctic perennial sea ice cover (also known as multiyear ice -MYI), dwindling from 75% to below 45% of the total ice cover (Serreze *et al.*, 2007; Maslanik *et al.*, 2011). Over the past

twelve years, nine record low maximal MYI extant were recorded, with the most recent at 2012. If this trend persists, it is expected that by the end of the 21st century the Arctic Ocean will be covered mostly by seasonal ice (also known as first year ice –FYI) (Boé *et al.*, 2009; Parkinson & Comiso, 2013).

This shift is already affecting the both the Arctic Ocean and the Arctic ecosystem as a whole. The reduction in MYI cover was demonstrated to significantly alter energy flux in the western Arctic Ocean by enabling massive under ice phytoplankton blooms (Arrigo *et al.*, 2012; Lowry *et al.*, 2014). Larger open water areas in the Arctic Ocean serve as a heat sink, alter atmospheric circulation, and contribute to the accelerated warming experienced in the Arctic region (Overland *et al.*, 2012). The decline in perennial ice has also severely impacted animals that are dependent on ice for feeding, dispersal and mating (see review by (Post *et al.*, 2013))

Despite the harsh conditions in sea ice, including low temperatures and high salinity relative to the underlying water column, sea ice bacteria are metabolically active throughout year (Junge *et al.*, 2004; Mock & Thomas, 2005; Junge *et al.*, 2006). Due to their year round metabolic activity, sea ice bacteria are key regulators of the polar ocean nutrient cycling and energy flow (Rysgaard & Glud, 2004; Deming, 2007; Rysgaard *et al.*, 2008). Winter production by heterotrophic sea ice bacteria sustains the ice and subsequently the water column and benthic ecosystems during times when photosynthetic production cannot occur due to low light levels (Wing *et al.*, 2012). This winter production also contributes to the recycling of organic matter, thus seeding the ice in preparation for spring and summer sea ice diatom algae blooms (Riedel *et al.*, 2008). In addition, the feeding of bacteria on organic matter generated during bloom time regulates carbon flux to higher trophic levels in sea ice and the Ocean (Riedel *et al.*, 2007a).

Recently, we reported that the decline in MYI is also expected to affect the bacterial constituents residing within the sea ice brine network. We suggested that MYI hosts more compositionally robust and less variable bacterial communities than FYI sites (Chapter 3). Furthermore, we reported that ice features such as annual growth layers, old ice layers and fresh water melt pond ice layer all exclusive to MYI, host distinct bacterial communities. Thus these communities are thought to be absent from FYI as well (Hatam *et al.*, 2014). Given the important role bacteria play in the Arctic Ocean , it is crucial to understand whether these differences in structure between FYI and MYI bacterial communities also translates to a difference in function.

To date there has been only a handful of studies that tried to link the structure and function of Arctic sea ice bacterial communities. Several studies examined the effect of crude oil and diesel on the membership of bacterial communities from the Arctic sea ice, while others compared the abilities of bacterial communities from FYI and MYI to carry out anaerobic pathways of the nitrogen cycle (Rysgaard & Glud, 2004; Gerdes *et al.*, 2005; Brakstad *et al.*, 2008; Rysgaard *et al.*, 2008). However, none of these studies fully addresses the vertical complexity of bacterial communities from sea ice and most did not perform the experiments *in-situ* (with the exception of Brakstad, et al. 2008). Furthermore in all cases the intricate microstructure of the brine matrix was compromised.

In the present study, we created a mild disturbance in the sea ice environment through inversion transplantation of ice cores (i.e. the reinsertion of an ice core top first) from both FYI and MYI sites north of Ellesmere Island, NU Canada. By doing so we disrupt the inherent top to bottom gradients in temperature and brine salinity while exposing the top communities to much higher temperatures and lower brine salinities and doing the opposite to the bottom communities (Haas *et al.*, 1997). This presents a significantly different temperature and salinity regimes which

may result in changes in permeability for some parts of the inverted cores (Cox & Weeks, 1983; Golden *et al.*, 1998). However it is not expected maintain the intricate microstructure and complexity of the brine network. We predict that since communities in MYI are vertically distinct there will be noticeable changes to the communities of the top and bottom sections but not to those from the middle since these communities face the most significant change in in-situ conditions. Since this is the first research to do so, we provide a description of the vertical distribution of bacterial communities along the depth of FYI . We predict that similar to MYI response to the inversion will reflect in the attributes of the communities from the top and bottom sections.

4.3 Materials and Methods

4.3.1 Site description and sample collection

The study sites were located off northern Ellesmere Island, Nunavut, Canada, where MYI ice floes are located near FYI formed in leads in the ice (MYI site coordinates 82.54,62.40, FYI site coordinates 82.53,62.42). In May, 2013, we drilled four cores at an MYI and four cores at an FYI sites: 1) a time zero core (from here on referred to as Ft0 for FYI and Mt0 for MYI), 2) a time day eight core (from here on referred to as Ft8/Mt8), 3) a coring control, which was removed and then reinserted in the original orientation (from here on referred to as Fcctrl/Mcctrl), and retrieved at the end of the experiment, and 4) an inverted core, which was removed, reinserted in the inverted orientation, and recovered after eight days in that position (from here on referred to as Finv/Minv). The time zero core was used to examine the ice at the onset of the experiment; the day eight core was used to examine the ice at the end of the

experiment; the coring control was used to examine manipulation effects; and the inverted core was the experimental disturbance condition.

Coring was performed using a Kovacs Mark V 14 cm-diameter corer (Kovacs Enterprise, Roseburg, Oregon). Prior to coring, the core barrel was thoroughly rinsed with sterile deionized water (Milli-Q Integral Water Purification System, EMD Millipore Corporation, Billerica, MA, USA). Coring control and inversion cores were wrapped in plastic mesh prior to reinsertion to prevent loss of ice sections while allowing exchange of brine and gas with the surrounding ice sheet and the surface seawater (Industrial Netting 7681 Setzler Pkwy N. Minneapolis, MN). After core reinsertion, the original snow cover was restored to keep thermal insulation and sunlight attenuation close to original conditions.

Upon final retrieval, the cores were immediately sectioned on site at 30 cm intervals using a hand saw (rinsed in deionized water and wiped with an ethanol wipe) and placed polypropylene bags (ULINE). Core sections were covered and processed within 4 hours of sampling. Each 30 cm core section was assigned a number for easy nomenclature starting with 1 as the top 30 cm. To avoid confusion unless stated otherwise stated all cores will be referred to according to pre-inversion order, e.g. section one of MYI time day eight core will be referred to as Mt81 and section one of the FYI inverted core will be referred to as Finv1, both will indicate the top 30 cm of the respective core prior to inversion.

4.3.2 In-situ temperature measurements.

The temperature of the inverted core was measured by drilling holes and inserting a thermometer (Testo 720) immediately after core extraction. Temperatures were measured from top to bottom at intervals of 25 cm. based on the measured temperature profiles a linear

relationship with ice depth demonstrated a good fit (R^2 = 0.99). For the t8 control cores, temperature profile was estimated as a linear increase from the snow-ice interface to the surface seawater. Estimated brine salinity profile was calculated from temperature using the Cox & Weeks equations (Cox & Weeks, 1983).

4.3.3 Sample processing

To avoid nonspecific addition of DNA and dilution of samples, ice samples were directly thawed at room temperature in the dark, rather than isotonically (Kaartokallio *et al.*, 2005; Kaartokallio *et al.*, 2008). For each 30 cm ice section, bulk salinity was measured with an ExStikII® salinity meter (Extech Instruments, Nashua, NH, USA). Two core sections from MYI (Minv8 & Mcctrl6) did not survive this process and are therefore missing from our dataset. Ice melt samples were filtered individually through sterile 0.22 µm pore size Supor (polyethersulfone) membrane filters (Pall, Mississauga, ON, Canada). Between samples, glassware used for filtration and sample measurements was sterilized using 10% household bleach solution followed by thorough rinsing in sterile deionized water. Each filter was placed in a microfuge tube, submerged in RNA*later*® solution (Life Technologies, Burlington, ON Canada), and stored at -20°C for later DNA extraction.

4.3.4 DNA extraction amplification and sequencing

DNA was extracted from preserved filters by bead beating using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) as instructed by the manufacturer. PCR amplification of the V3 variable region of the bacterial 16S rRNA gene for subsequent Ion Torrent™ sequencing was performed using a two-step protocol. First step used 341f primer carrying a universal GLENN tag (developed by Travis Glenn,

http://www.gvsu.edu/dna/universal-primer-tag-6.htm), and a 518r primer carrying a trP1 adapter. The second step used a forward barcoded primer with the matching GLENN universal tag and reverse primer matching the trP1 adapter to allow multiplexing. Conditions for the two-step PCR were as previously described (Berry *et al.*, 2011). All PCR reactions were performed in triplicate to minimize intrinsic biases involved in PCR amplification. A list of all primers and barcodes is provided in Supplementary Table 1.

Prior to sequencing, PCR products were purified using UltraClean GelSpin DNA Extraction Kit according to the manufacturer's instructions (MO BIO Laboratories, Inc). Sequencing was performed using the IonTorrent Personal Genome Machine (PGM) with the Ion PGM^{TM} Sequencing 200 Kit v2 and the Ion 316 Chip v2 according to the manufacturer's instructions (Life Technologies, Carlsbad, CA).

4.3.5 Pre-processing and quality control of raw sequences

All pre-processing and sequence quality control steps were performed using USEARCH (V 7.0.1090 for Linux 32bit) according to the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_pipeline.html, date accessed 1 Oct 2014) (Edgar, 2013) and the Ubuntu 14.04 lts native python installation. In brief, reads were de-multiplexed and sequences containing primer/barcode mismatches discarded. The remaining sequences were screened based on maximum expected error probability and those with a probability higher than 0.25 were discarded (Edgar, 2013). The remaining sequences were then globally trimmed to 100 bp and sequences shorter than the specified length were discarded. Post trimming, the sequences were pre-clustered for further noise reduction as recommended (Huse *et al.*, 2010). OTUs were assigned using the UPARSE greedy algorithm for an OTU definition of 97% sequence similarity.

Though this step also removes chimeras, it is recommended by the USEARCH pipeline to supplement it with a dedicated chimera removal step. Therefore chimeras were additionally detected and removed using UCHIME (Edgar *et al.*, 2011). Global singleton OTUs (those that are represented by a single sequence in the entire dataset) were removed due to their unknown nature. The OTUs were then assigned taxonomic classification using the Ribosomal Database Project classifier (train set 10) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). Samples were sub-sampled to the smallest library size (1100 sequences for FYI, 2200 for MYI) to allow identical sequencing depth for each sample before further alpha and beta diversity analyses (Gihring *et al.*, 2012). Four sections from FYI cores had significantly fewer than 1000 sequences (400-700 sequences) following two rounds of sequencing and thus were eliminated from further analysis (Supplementary Table 1). Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under bioproject XXXXX.

4.3.6 Community analysis

Alpha diversity, including the number of observed OTUs, ACE nonparametric OTU richness estimators and the non-parametric Shannon diversity were calculated in Mothur v.1.33.3 (Shannon, 1947; Chao & Shen, 2003; Schloss *et al.*, 2009). Multivariate community analysis was performed in R version 3.2.0 (www.r-project.org) using the Vegan package (Oksanen *et al.*, 2015). Bray-Curtis and classic Jaccard's dissimilarity measures were used to compare the composition and membership of the different communities respectively (Jaccard, 1912; Bray & Curtis, 1957). Non-metric multi-dimensional scaling (NMDS) was used to ordinate the different samples based on the distance matrixes generated by the dissimilarity measures. Graphical representation of the NMDS was performed in R using the ggplot2 package (Wickham, 2009). The nonparametric analysis of molecular variance (AMOVA) was used to test the significance of

the grouping based on the NMDS ordination; statistical significance of grouping was tested for $p \leq 0.05$ with Bonferroni correction according to the number of groups tested (Excoffier *et al.*, 1992; Anderson, 2001; Martin, 2002).

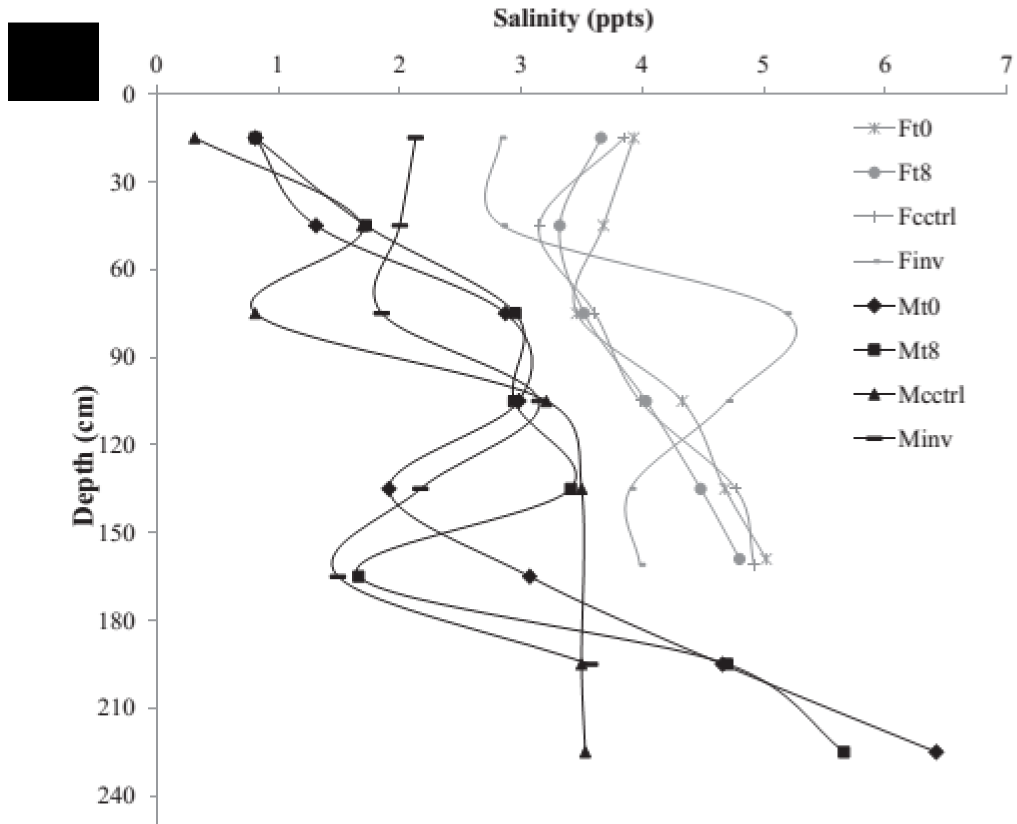
4.4 Results

4.4.1 Inversion disrupts *in-situ* parameters

For both MYI and FYI sites, t0 and t8 cores had similar bulk salinity profiles characteristic of their respective ice type, i.e. C shaped bulk salinity curve for FYI and gradual top to bottom increase in bulk salinity for MYI (Figure 4.1 A). While for the FYI site the Fcctrl core had bulk salinity profile similar to Ft0 and Ft8 cores, the MYI Mcctrl showed no increase in salinity in the last two sections representing the bottom 60cm. This might indicate that some brine drainage probably occurred during on-site processing (Figure 4.1 A). For both sites the inverted cores did not show a complete inversion of the bulk salinity profile. In the Finv core the bulk salinity for sections 5 and 6 (originally at the bottom) and sections 1 and 2 (originally at the top) were slightly lower than that of the all control cores top or bottom sections (Figure 4.1 A). These salinity values were also lower from sections Finv3 and Finv4. For MYI, the bulk salinity profile for Minve do not appear to change much compare to the controls with only sections Minv1-3 show slight increase in bulk salinity values, probably due to contact with the surface seawater (Figure 4.1 A).

In both sites the inverted cores had a completely reestablished temperature profile (i.e. linear increase in temperature from inverted top to inverted bottom). The slope of the temperature profile for the inverted cores was comparable to that of the respective control cores from each site, and showed no statistical difference when examined with a two tailed student's t-

test (pvalue ≤ 0.5), (Figure 4.1 B). As consequence the derived brine salinity profile for the inverted cores from both sites showed complete inversion as well (i.e. linear decrease in brine salinity from inverted top to inverted bottom), (Figure 4.1 B).



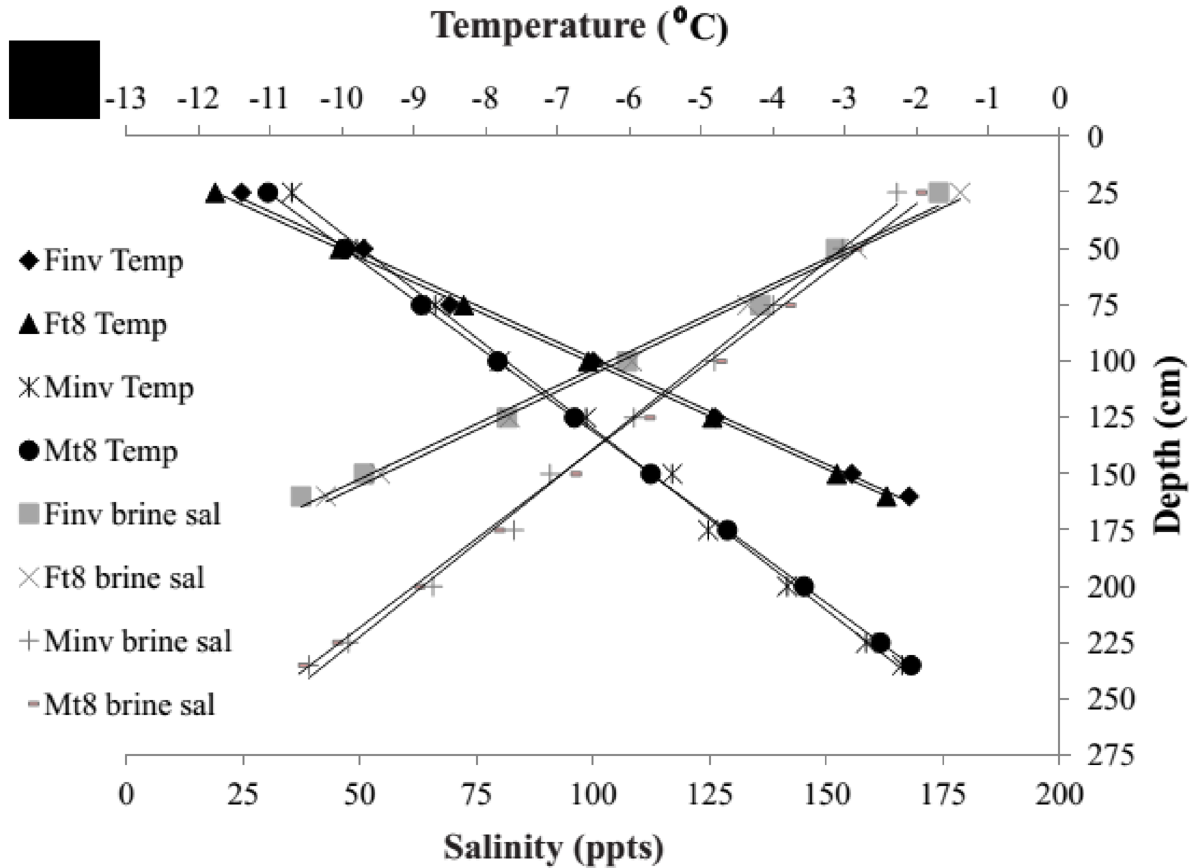


Figure 4.1 *in-situ* conditions for each core: Bulk salinity (A), temperature and brine salinity profiles (B) of inverted and control cores from MYI site. Note that for the temperature and brine salinity profiles the orientation of the inverted core is post inversion. Linear regression (full lines) equations and R^2 : Minv Temp y = 24.803x + 291.26 R^2 = 0.9977, Minv brine y = -1.669x + 305.85 R^2 = 0.9964, Mt8 Temp y = 23.438x + 283.59 R^2 = 1, Mt8 brine y = -1.5779x + 297.07 R^2 = 0.9983, Finv Temp y = 14.477x + 192.78 R^2 = 0.9966, Finv brine y = -0.9787x + 201.14 R^2 = 0.9912 , Ft8 Temp y = 14.433x + 194.85 R^2 = 1, Ft8 brine y = -0.9884x + 204.54 R^2 = 0.9982

4.4.2 Inversion did not change the vertical distribution patterns of major class level groups

Next we tested whether disturbing the brine salinity and temperature gradients changes the vertical distribution patterns of the major class level groups in the ice (Figure 4.2). Our results show that neither the coring process nor the inversion itself changed the vertical distribution patterns in neither sites (Figure 4.2).

All the control cores from MYI showed a top to bottom decrease in the presence of sequences classified as related to members of the class level groups *Flavobacteria* and *Sphingobacteria* along with *β-proteobacteria* (Figure 4.2). Conversely sequences classified as related to members of the class level groups *γ-proteobacteria* and to a lesser extent α-*proteobacteria* and *Actinobacteria* showed an increase in abundance with depth (Figure 4.2). The Minv core sections showed similar trend of sequence distribution to that of their parallel sections from the control cores (Figure 4.2).

Unlike MYI, sequence distribution in none of the cores from the FYI site showed depth related trends (Figure 4.2). This however was not the only difference between the distribution of class level groups in FYI and MYI. Sequence distribution in core sections from FYI was not as even as in the cores from the MYI site. For the most part FYI core sections were heavily dominated by *γ-proteobacteria* accounting for 40% - 80% of the total number of sequences per core section (Figure 4.2). Furthermore, possibly due to the cornucopia of *γ-proteobacteria* related sequences, there was a high degree of variability in the makeup of the groups representing ≥1% of the sequences in cores from the FYI site. This was evident both between

core sections of the same core and between parallel core sections from different cores (Figure 4.2).

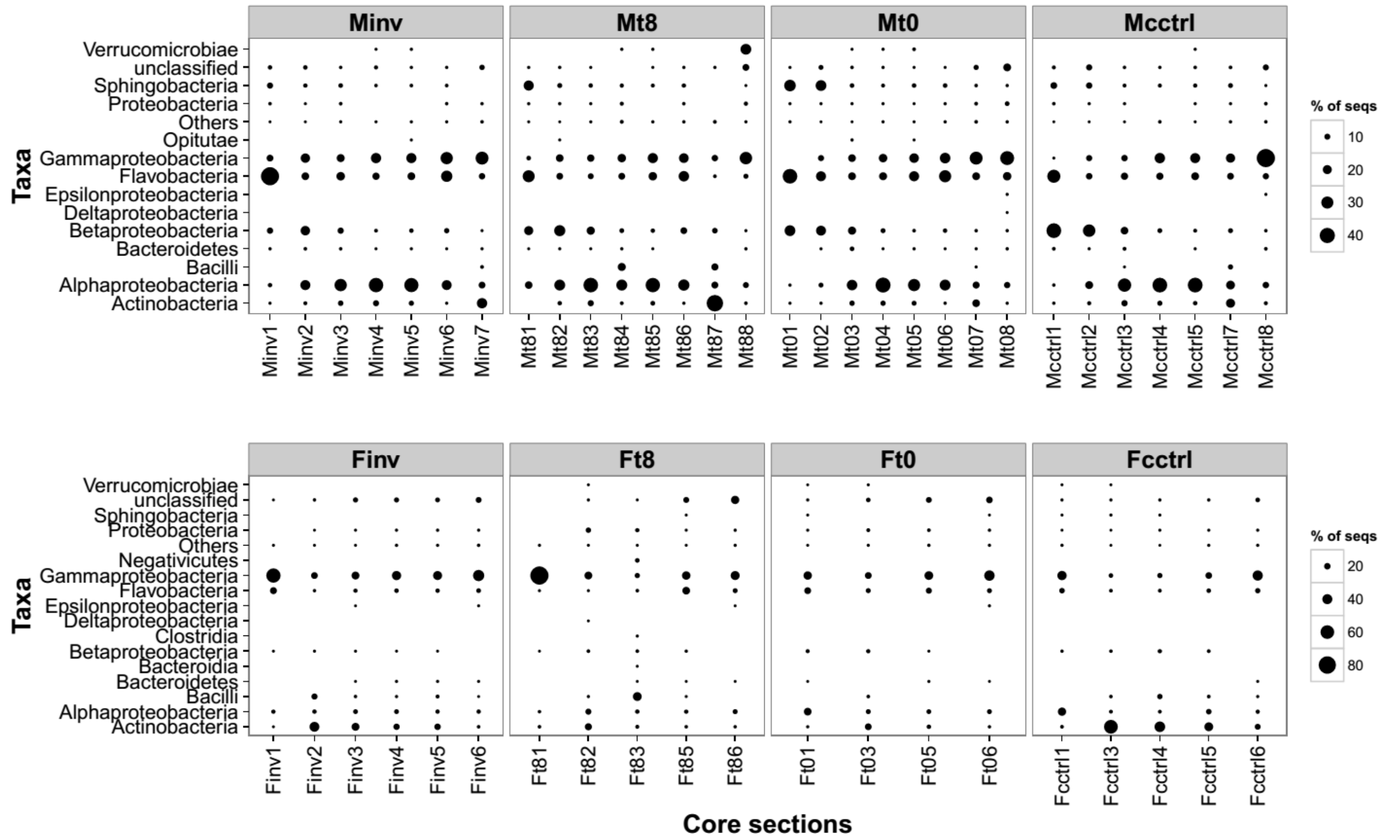


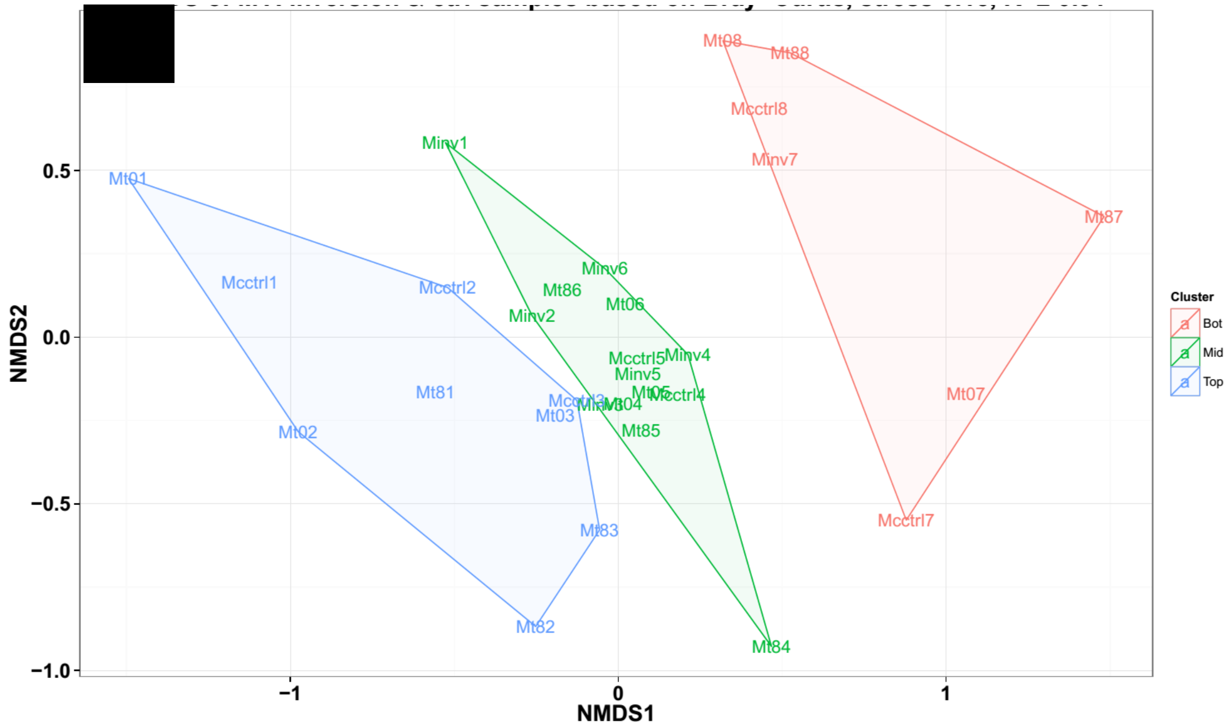
Figure 4.2 Distribution of class level groups: Major class level taxonomic groups from both inverted and control cores from MYI and FYI sites. Group representing less than 1% are represented as “Others”. OTU definition set at 97% sequence similarity.

4.4.3 Inversion changed the composition and membership of inverted MYI original top

Both Composition (Bray-Curtis) and Membership (Jaccard) divided all MYI control cores into three statistically distinct groups: Top corresponding to sections 1-3 based on composition and 1-2 based on membership, Mid corresponding to sections 4-6 based on composition and sections 3-6 based on membership & Bottom corresponding to sections 7-8 based on both dissimilarity measures (Figure 4.3 A&B). For the most part, the Minv core sections clustered with section parallel to their original orientation both with membership and composition. The only exception were sections 1-3 that cluster with Mid based on both dissimilarity measures (1-2 in the case of composition) rather than with the Top as was shown for the controls (Figure 4.3 A&B).

Like MYI, the control cores from FYI were also divided into three statistically significant groups, both for membership and composition. However, unlike MYI, they did not show vertical distribution (Figure 4.4 A&B). Group Top-bot consisted of communities form section of 1 and 6 of most control cores (Figure 4.4 A&B). Group Mid-bot mainly consisted of communities form sections 5 of all control cores, however communities from section Ft86 and Ft03 also clustered with them (Figure 4.4 A&B). Group Mid mainly consisted of communities from sections 2-4 from Ft8 and FCctrl with no representative from the Ft0 core (Figure 4.4 A&B). Communities from sections of the inverted FYI core clustered with the Top-bot & Mid-bot, similarly to communities from the T0 FYI core. The community from core section 2 with the Top-bot group rather than with the Mid groups like parallel section 2 from the Ft8 control (Figure 4 A&B). Furthermore, while section 5 of the inverted core, originally corresponding to section 5 of the control cores, matched its pre-inversion grouping, communities from sections 3 & 4 of the

inverted cores grouped with the mid-bot sections rather than with the Mid much like the Ft0 core and in contrast to parallel sections from Fcctrl and Ft8 cores (Figure 4.4 A&B).



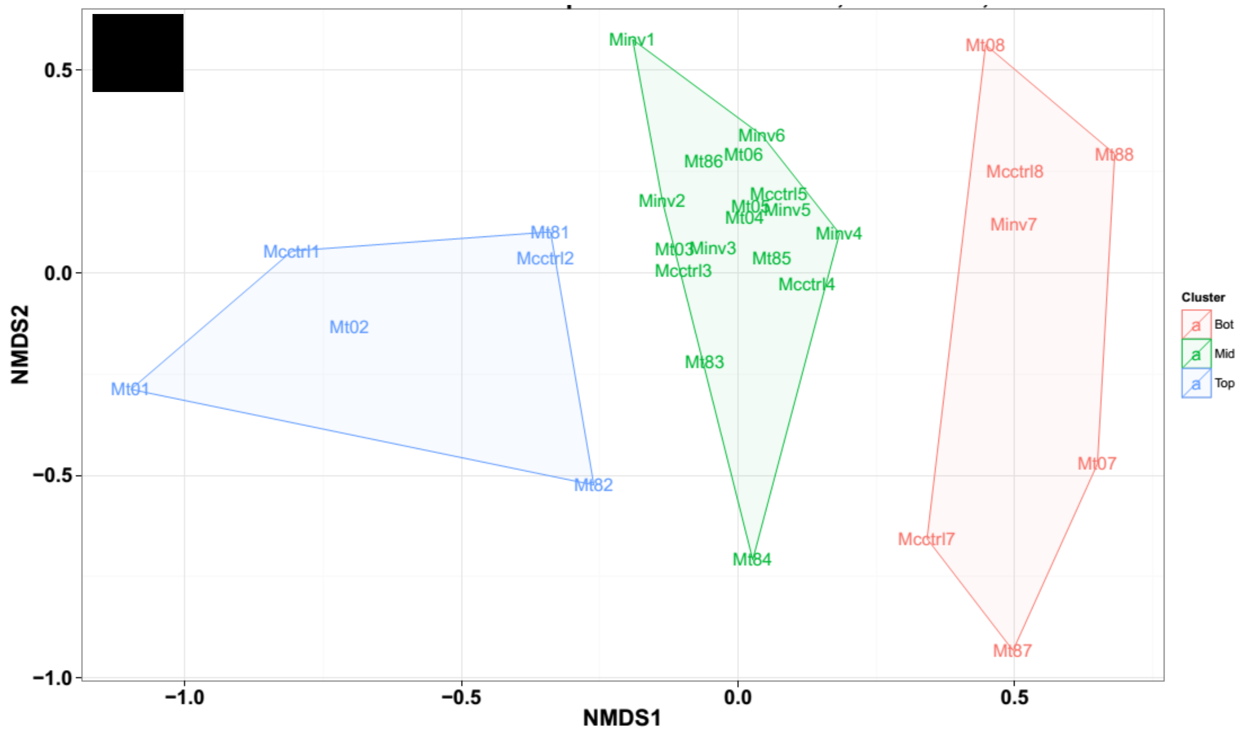
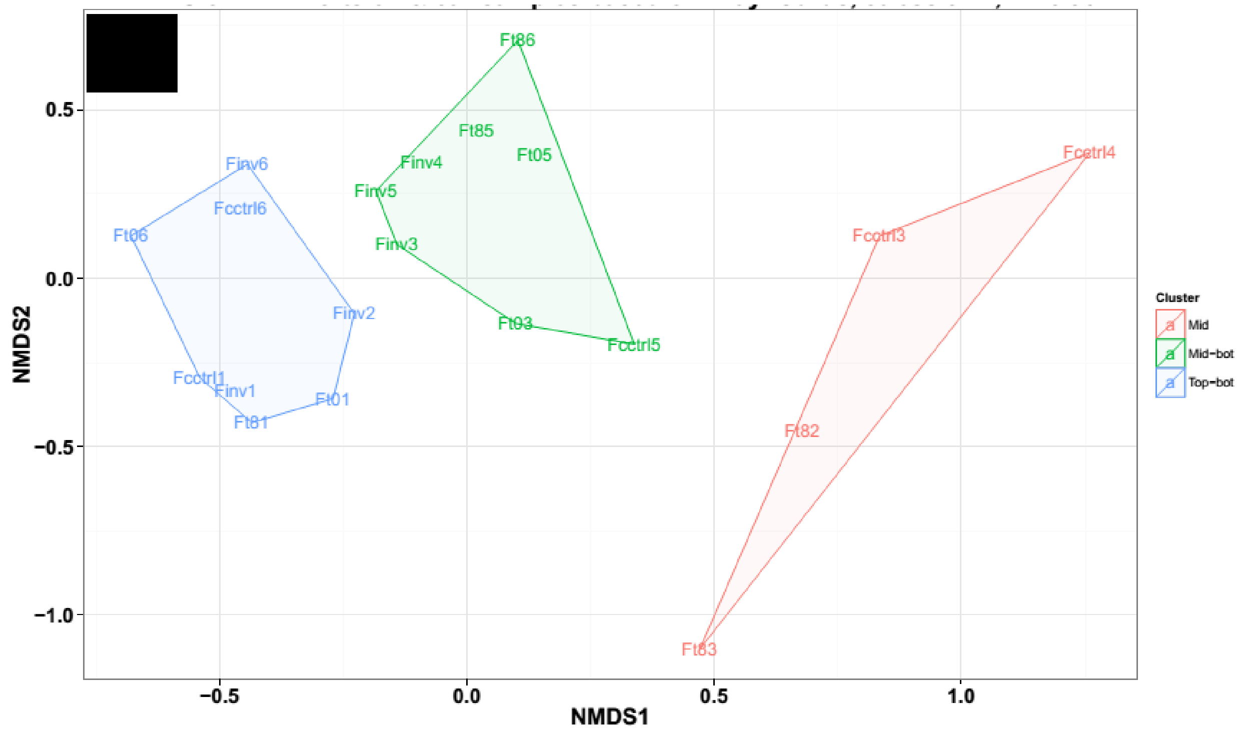


Figure 4.3 composition and membership of bacterial communities from MYI: NMDS ordination based on Bray-Curtis (A) and Jaccard (B) dissimilarity measures of MYI samples. Grouping indicates statistically significant clustering (A: $BotvTop_{(1,14)}$ 4.33 pval <0.001, $BotvMid_{(1,19)}$ 4.84, pval<0.001, $MidvTop_{(1,21)}$ 3.57 pval<0.001 B: $BotvTop_{(1,11)}$ 4.01 pval <0.001, $BotvMid_{(1,22)}$ 5.13, pval<0.001, $MidvTop_{(1,21)}$ 4.97 pval<0.001) based on AMOVA. OTU definition set at 97% similarity. Stress level for A 0.15, r^2 0.91, stress level for B 0.14, r^2 0.92.



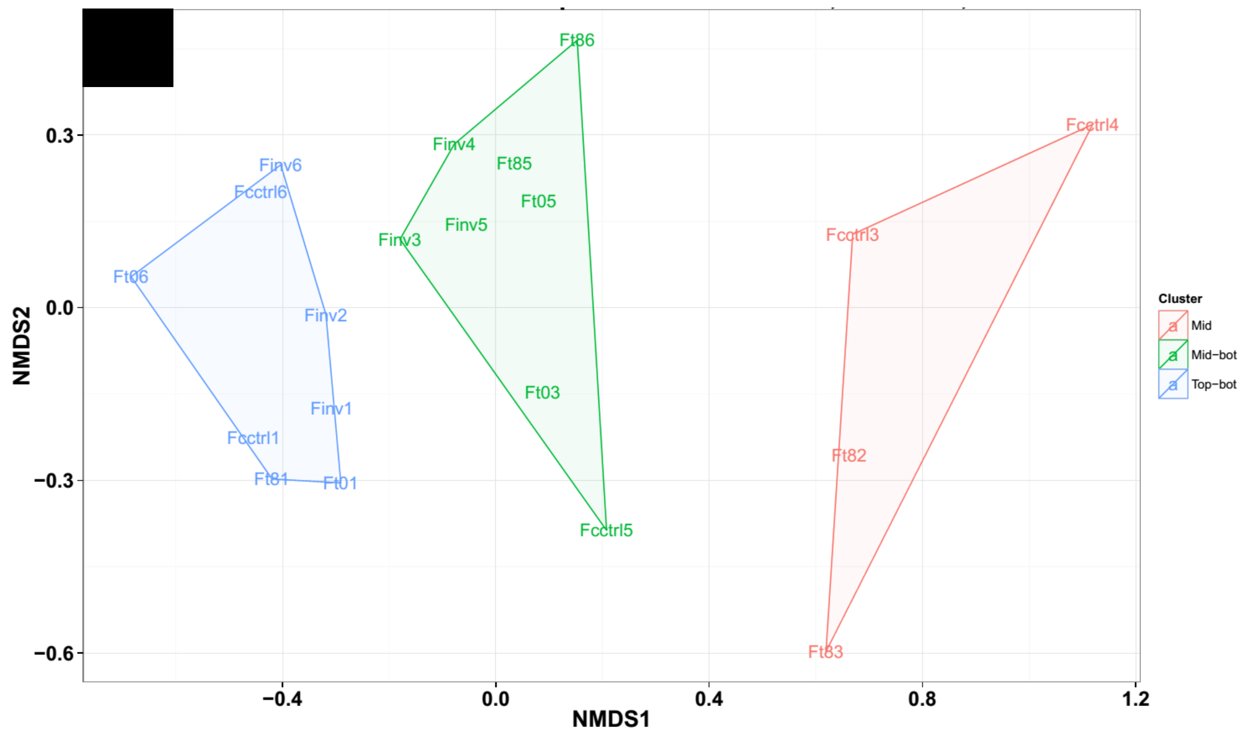


Figure 4.4 composition and membership of bacterial communities from FYI: NMDS ordination based on Bray-Curtis (A) and Jaccard (B) dissimilarity measures of FYI samples. Grouping indicates statistically significant clustering (A: $TBvM_{(1,10)}$ 4.2 pval <0.001, $TBvMB_{(1,14)}$ 3.18, pval<0.001, $MvMB_{(1,10)}$ 2.44, pval<0.001 B: $TBvM_{(1,10)}$ 3.62 pval <0.003, $TBvMB_{(1,14)}$ 3, pval<0.001, $MvMB_{(1,10)}$ 2.91 pval<0.001) based on AMOVA. OTU definition set at 97% similarity. TB=Top-Bot, MB=Mid-Bot, M=Mid

4.4.4 Inversion marginally increases observed and expected richness in MYI

All MYI control cores showed similar apparent trend in observed richness were there was an apparent increase from top to middle and a decrees from middle to bottom with a spike at section 8 (Figure 4.5 A, Figure 4.1S). For the most part, the expected richness based on the

ACE richness estimator agreed with the trend (Figure 4.5 A, Figure 4.1S). Diversity in the MYI control cores showed an apparent top to bottom increase throughout all the sections (Figure 4.5 B, Figure 4.1S). Diversity estimation in the Minv core showed the same apparent trend as the control core. However unlike with the diversity, the Minv core showed higher expected and observed richness values in sections 1 and 2 both compared to other sections of Minv and compared to parallel section in the control cores (Figure 4.5 B, Figure 4.1S).

Since sections 1 and 2 in the controls clustered as Top both based on membership and composition but clustered as mid for Minv, we wanted to test whether this increase in richens is significant and also part of the effect of the inversion. In order to do so we averaged the OTU observed and estimated richness values of sections 1 and 2 from the Minv and each of the controls. We than compared the averaged from each of the avareged groups with Kruskal–Wallis H test. The Kruskal-Wallis test showed only marginal statistical significance to the difference between all the groups ($p<0.07$). We followed it with a post-hoc Mann-Whitney U test that the majority of the marginal difference comes from the difference between the averages 1 and 2 sections from the Minv cores and each of the averaged 1and 2 sections from the other cores (not shown).

For the most part controls and inverted cores from FYI showed similar apparent trend in observed and expected richness, where OTU numbers decrease from top to middle and increase from middle to bottom (Figure 4.5 A &B, Figure 4.2S). Two exceptions to these are section Ft86 which had much lower OTU count than the rest of the parallel sections and section Finv2 which had higher. However due to the low number of sections we could not test this statistically. Diversity estimation was inconsistent in trend with respect to FYI. With the exception of section 1 Fcctrl and Finv showed similar apparent trend of increase in diversity from top t bottom. Ft8

also showed an increase from top to bottom though mainly from section 1 to section 2. Ft0 showed the most different profile in diversity with all sections showing similar values (Figure 4.5 A &B, Figure 4.2S).

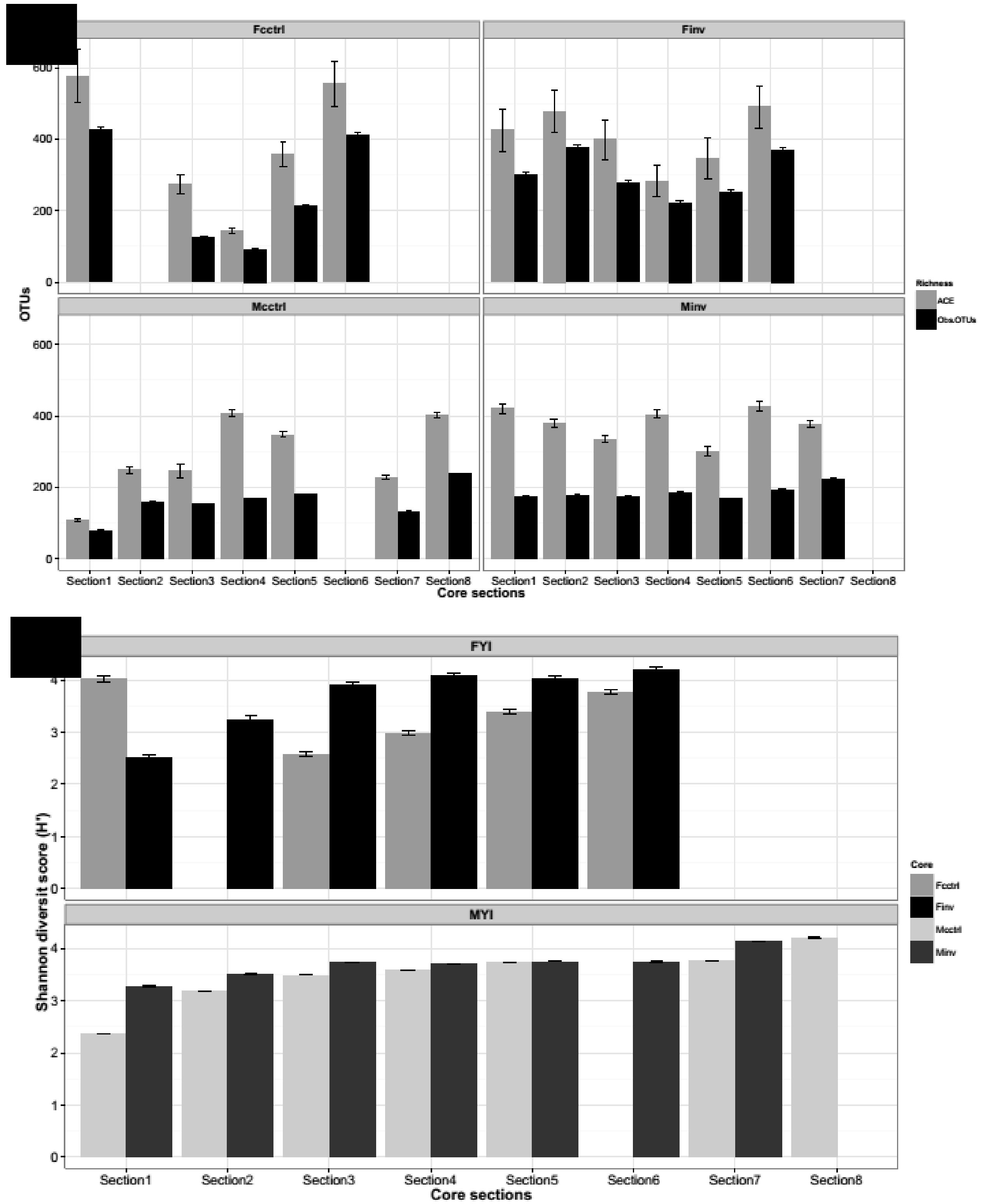


Figure 4.5 Estimated richness and diversity for MYI and FYI inv and cctrl core sections: observed number of OTUs, expected number of OTU (ACE) (A) and Shannon diversity index (B). OTU definition set at 97% sequence similarity. Samples where sub-samples to 2200 sequences for MYI and 1100 sequences for FYI, error bars represent ±SE from the mean of 1000 iterations of sub-sampling and value measurement.

4.5 Discussion

The primary goal of this study was to compare the response of bacterial communities from seasonal and perennial ice to in-situ environmental disturbance. To achieve this we chose to perform an inversion transplantation of cores from both MYI and FYI site. The incentives to perform this specific type of disturbance were that it is relatively easy accomplish, does not carry any long term ecological implications and most importantly preserves the intricate structure of the brine network. There were several challenges with regards to this type of disturbance; first and foremost it is inherently dependent on the reversal of in-situ gradients in temperature and temperature related parameters. Therefore we a-priori assumed that the eight day duration given for this experiment will be sufficient for the establishment of a new top to bottom temperature gradient. Second, however equally important, depth dependent segregation of bacterial communities was not studied in FYI up-until now, thus we a-priori assumed this was the case. Furthermore, although it was recently demonstrated by Hatam and colleagues that MYI holds vertically distinct bacterial communities, this was only shown for one site and still needed to be reconfirmed (Hatam *et al.*, 2014). Lastly, this sort of experiment involves the reconstitution of

the cores pre reinsertion, a process which might present a disturbance within itself and also may result in brine loss and consequently the loss of bacterial cells.

With respect to the first challenge, our results clearly demonstrate that the 8 days duration were sufficient for both sites to establish a new top to bottom temperature gradient and as consequence a brine salinity gradient as well (Cox & Weeks, 1983). Thus we demonstrated that a disturbance of the in-situ conditions was achieved both in FYI and MYI sites. Our results indicated vary little brine movement in the inverted cores from both sites. The most conspicuous one is the gain of bulk salinity at the inverted bottom (originally the top sections of the ice). This is probably due the increase in temperature which allowed for increased permeability of the core and led to exchange of dissolved ions nutrients, ions and gases with the surface seawater (Cox & Weeks, 1983; Golden *et al.*, 1998). Our results also indicated possible reduction in bulk salinity from section 2 of the core from the MYI site. This could be the result of brine rejection due to the colder temperature the sections was exposed to in its new position (Cox & Weeks, 1975). Alternatively, this could stem from loss of brine during the initial stages of coring and experiment setup. Both of these may also explain the apparent decrease in brine salinity in the top and bottom sections and the disruption of the c-shape bulk salinity profile of the inverted core from the FYI site.

Unlike the inversion process, it was clear that the coring process itself did not present a significant added stress and did not significantly alter the measured attributes of the respective bacterial communities. This was a bit surprising since the MYI coring control core did show possible loss of brine from its bottom two sections. However the fact that the apparent loss of brine did not result in significant changes to the attributes of the bacterial communities from

these sections could be since the majority of bacterial cells found at the bottom portions of sea ice brine matrix were reported to be associated with surfaces (Junge *et al.*, 2004).

The results of inverting the MYI core somewhat fell in line with our predictions. The duration of the experiment did not allow for drastic changes in the vertical distribution of the major class level groups in the inverted MYI core, which resembled in distribution and taxonomic classification both all control cores and MYI core from a Hatam and colleagues (Hatam *et al.*, 2014). At a finer resolution, as expected, sections 1-2 of the inverted MYI core showed a marginally significant increase in OTU richness as well as changes in in composition and membership compared to parallel sections of the control cores. It is likely that these changes to the communities from these sections are due to both an increase in temperature and possibly increase in DOM concentration due to exchange with from the surface seawater due to the inversion (Martin *et al.*, 2011; Eronen-Rasimus *et al.*, 2014a). The increase in DOM and temperature could enable members of dormant seed population, normally adjusted to the milder conditions, such as those found in lower sections, to increase in proportion and influence OTU count and representation. A response along these lines was also reported for bacterial communities form the top layers of thick Antarctic sea ice when exposed seawater interface conditions (Martin *et al.*, 2011). Unlike the top sections of Minv the bottom sections showed no changes in attributes. This is probably due to the dramatic increase in salinity and decrease in temperature which may inhibit or decrease bacterial activity (Deming, 2007). It is possible that given longer incubation time a greater response would have been detected at Minv bottom sections, however, a similar procedure in the Antarctic lasting 18 days showed similar trend (Martin *et al.*, 2011).

Much like in MYI, FYI sections hosted bacterial communities with members belonging to taxonomic groups previously reported in arctic sea ice ((Bowman *et al.*, 2012; Hatam *et al.*, 2014). However, unlike MYI there was no apparent top to bottom segregation of the communities from sections of the control cores, which does not agree with our a-priori assumption. Most interestingly, communities from sections 1 and 6 from all cores, i.e. sections with the most dramatic difference in temperature and brine salinity clustered as one group both based on membership and composition. This might indicate that temperature and brine salinity might not be the dominating factors in structuring the bacterial communities from FYI. Alternatively this might indicate that the segregation to different structures based on vertical location takes longer than the lifespan of FYI.

Unlike in MYI samples, the composition and membership of corresponding sections from all control cores of the FYI site was not similar. Core sections from Ft0 were completely absent from the FYI Mid group, which consisted only from communities of Ft8 and Fcctrl sections indicating a possible trigger that caused a change in the community structure which took place during the 8 days incubation. Whatever this trigger was, it would appear that the communities from the inverted FYI core sections did not respond to it as they grouped similarly to the Ft0 core sections. This might indicate that the inversion had other effects, not directly measured by us, with added stress to the system in a yet unknown and unforeseen way that cause a lag in the response to the trigger changing the Ft8 and Fcctrl communities. Support to this could be found in the diversity profile of Finv which looked more like that of Fcctrl and to lesser extent Ft8 than to Ft0. This might indicate that a change might be just starting to progress and that given more incubation time Finv will look like Fcctrl and Ft8.

4.5.1 Conclusions

Here we demonstrated that communities from perennial and seasonal sea ice have a different distribution of communities along the depth of the ice. To the best of our knowledge this is the first study to do so. We also demonstrated that this difference in structure corresponds with a different response to a similar environmental disturbance, thus tying community structure to community function in Arctic sea ice using an in-situ experiment. To the best of our knowledge this is also a first. Our data, therefore, joins and support recent studies reporting that the shift toward an Arctic Ocean covered by seasonal sea ice will result in a shift from a well structured sea ice bacterial communities with components shaped and segregated in to niches by succession processes to a more structurally variable one (Hatam *et al.*, 2014). Furthermore, our results provide first support that FYI communities might be more prone to seasonal changes which might render them incapable to respond to environmental disturbance than MYI. However given the modest sampling scheme of our study and the reported high variability of bacterial communities from FYI further research in needed.

4.5.2 Acknowledgments

B.D.L. and C.H. were supported by the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant program and the Polar Continental Shelf Program (PCSP). C.H. was an Alberta Innovates – Technology Futures Fellow. I.H. and J.B. were supported by the Northern Scientific Training Program (NSTP) and Circumpolar-Boreal Arctic Research (C-BAR) programs. B.L. was supported by Helmholtz Young Investigators Group "Iceflux" (VH-NG-800). We would like to thank and acknowledge the PCSP and CFS Alert staff for their hospitality and logistical support while in the field.

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5 Synthesis and conclusions

Three objectives stood at the heart the studies presented as part of this thesis: 1) Characterizing the vertical distribution of bacterial communities along FYI and MYI ice. 2) Determining whether MYI and FYI host microbial communities with overall different composition and membership. 3) Determine whether any detectable differences in the membership and composition also translate to a difference in response to environmental disturbance, thus linking difference in structure to difference in function.

Results from chapters 2 & 4 show that MYI host vertically distinct bacterial communities corresponding to the occurrence of different ice layers within the ice sheet. On the other hand chapter 4 shows that while there are different communities within FYI they are not vertically distinct. The results presented as part of this thesis also demonstrate that FYI and MYI have overall significant differences in membership and composition which translate to a different response to environmental disturbance in the form of inversion transplantation (chapters 3&4).

There are however, additional significant findings not addressed within the chapters themselves along with additional research directions that will discuss in the following paragraphs.

5.1 Bacterial communities from sea ice are rich and diverse

Recently it was reported that the addition of DOM to a mesocosm containing seawater samples led to a two fold increase in the richness and diversity of the bacterial communities (Landa et al., 2013). However, despite much higher DOM concentrations present in brine compared to seawater, one does not expect that the harsh conditions occurring within the brine network will allow for the development of a rich and diverse bacterial community. Nevertheless

chapters 2-4 show that bacterial communities from sea ice are more diverse than once thought. The results in these chapters join other studies of sea ice covered regions, reporting that not only does sea ice have comparable or greater cell concentrations compared to seawater (Junge et al., 2002), but also that bacterial communities from sea ice have OTU richness and diversity values comparable or greater than those of seawater from the Arctic Ocean (Bowman et al., 2012; Hatam et al., 2014; Torstensson et al., 2015).

Given that surface seawater is considered the main inoculum source for sea ice and that not all bacterial OTUs found in the water column can survive the incorporation into the ice, one can ask what is (or are) the origin of this OTU richness and diversity ? Possible explanations could be that incorporation of bacterial cells from the sediment (Reimnitz *et al.*, 1992) and snow (Harding *et al.*, 2011) are more prominent than previously considered. Alternatively, since the Arctic Ocean receives vast quantities of river discharge prior to and during the early stages of sea ice formation (Wadhams, 2000), it is possible that this enriches the surface seawater with bacterial OTUs that are normally not found in the water column of the Arctic Ocean. Another possible explanation could be that somehow the environmental conditions in the brine as well as the relative higher complexity of sea ice as a habitat enable the success of OTUs undetected in seawater while inhibit more abundant OTUs found in the initial inoculum thus allowing for a higher and more even OTU count.

5.2 Bacterial communities from the Lincolns Sea sea ice resemble communities from other sea ice regions

At the class level, sea ice samples from all chapters had comparable membership, though the composition of each group changed between samples: that however is to be expected due to natural variability (Chapters 2-4). Our findings are in agreement with previous work done in the

Arctic and other sea ice cover regions which reported the presence of similar taxonomic groups at class level and below (Petri & Imhoff, 2001; Junge *et al.*, 2001; Brinkmeyer *et al.*, 2003; Bowman *et al.*, 2012; Hatam *et al.*, 2014; Eronen-Rasimus *et al.*, 2014; Torstensson *et al.*, 2015).

For the most part, direct comparison of the sequences belonging to OTUs from similar groups coming from different studies is problematic due to the use of different variable regions of the 16S rRNA gene, or due to the use of fingerprinting methods rather than sequencing. However it is still remarkable that such similar results were obtained from studies across different geographical areas, times of sampling and methodologies. This speaks volumes regarding the reproducibility of research in the field as well as for the validity of the findings presented here and their relevance to other polar and sub-polar regions, both in the northern and southern hemispheres.

5.3 Bacterial communities from sea ice are dynamic but changing in a predictable fashion - sea ice as a possible model system for short term succession

With the exception of Collins and colleagues (2010), all studies comparing the bacterial communities of sea ice and surface seawater, including work done in chapters 2-4, found the membership (and composition if tested) of the two to be markedly different (Junge *et al.*, 2002; Collins *et al.*, 2010; Bowman *et al.*, 2012; Hatam *et al.*, 2014). In a study performed in the Baltic Sea, it was reported that differences in membership between the surface seawater and sea ice are noticeable even at the early stages of sea ice consolidation (Eronen-Rasimus *et al.*, 2014).

By comparing all the data presented here, along with that of other studies, a pattern of directional change emerges. It starts with the early stages of sea ice formation and the assembly of ice bacterial communities with similar membership to that of the seawater at the site of

sampling (Eronen-Rasimus *et al.*, 2014). The second stage is the formation of semi-mature, seasonal ice communities which begin as the ice consolidates to sheets. At this stage, bacterial communities share similar proportion of constituents with the surface seawater and with more mature sea ice, however the membership is different from both. This stage can also be characterized by high variability between sites, mainly in composition but also in the membership of the bacterial communities. Lastly, at this stage, vertical gradients in the physical and chemical parameters within the brine network do not seem to play a role in structuring the bacterial communities (Chapters 2, 4, (Eronen-Rasimus *et al.*, 2014). The final stage is the formation of mature MYI communities. This stage is characterized by the formation of a broad core membership of abundant OTUs shared between communities from different MYI sites, thus these communities show much lower between site variability than communities from FYI. This mature MYI community is also characterized by the partitioning of sub-communities within the ice based on the different ice layers in MYI (Chapters 2 and 4).

This directional change in membership and composition with a known beginning and end point makes the Arctic sea ice a good model system to study processes of bacterial succession under extreme conditions. Furthermore, because the communities from FYI are so well structured it is relatively easy to detect changes to their membership and composition after relatively short periods of time (Chapter 4). This also makes MYI a good natural system for studying sensational processes post disturbance.

5.4 Implications of findings, proposed future progress and challenges

The work presented as part of this thesis clearly demonstrates that as the Arctic shifts to being MYI free, it stands to lose a stable and well structured bacterial community which will be

replaced by a young unstructured and variable community lacking several components found exclusively in MYI. Furthermore, though a response to a short term environmental disturbance was detected in MYI, we were unable to detect it in FYI. This might indicate that a young unstructured community can lag in response in structure to environmental disturbance as was shown in chapter 4. Thus the transition from MYI to FYI might have significant effects on the Arctic Ocean ecosystem as a whole. Therefore, an immediate direction towards which the research presented here could be expanded is to better understand the linkage between the structure and function of bacterial communities from sea ice. In other words, do changes seen between the membership and composition of communities from MYI and FYI, or between communities from manipulated and control sea ice samples, also translate to a difference in function. Since marker gene based metagenomic approaches such as the one employed in this thesis are limited with regard to the function of bacterial communities due to a high degree of functional redundancies, one way to approach this question is by the use of shotgun metagenomics. The use of shotgun metagenomics could shed light on any differences in the presence or enrichment of metabolic pathways linked to biogeochemical cycles and ecological services provided by bacterial communities from FYI and MYI. Furthermore since such a study is not limited by the choice of a marker gene, it could also shed light on the interactions between bacteria and other components of the microbial loop such as phages, thereby enhancing our understanding of their involvement in bacterial mediated geochemical cycles. Shotgun metagenomics studies could be then complimented by metatranscriptomics and metaproteomics studies which would shed light on the active components both within the bacterial communities and the pathways that are utilized.

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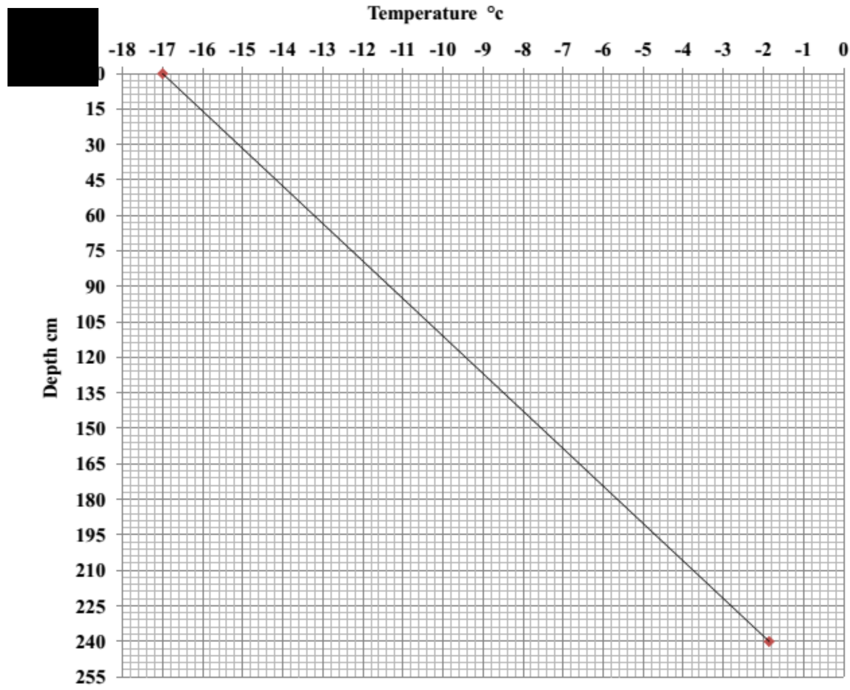
7 Appendix 1 Chapter 2 supplementary material



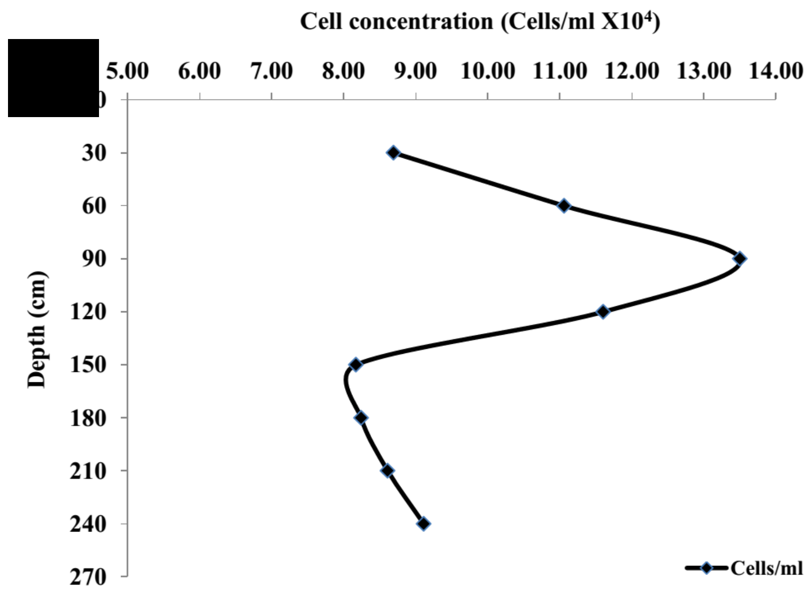


Figure 2.1S Cell concentrations and temperature profile. A- Extrapolated temperature gradient along the ice core, B- cell concentration per ml of thawed 30 cm section of ice.

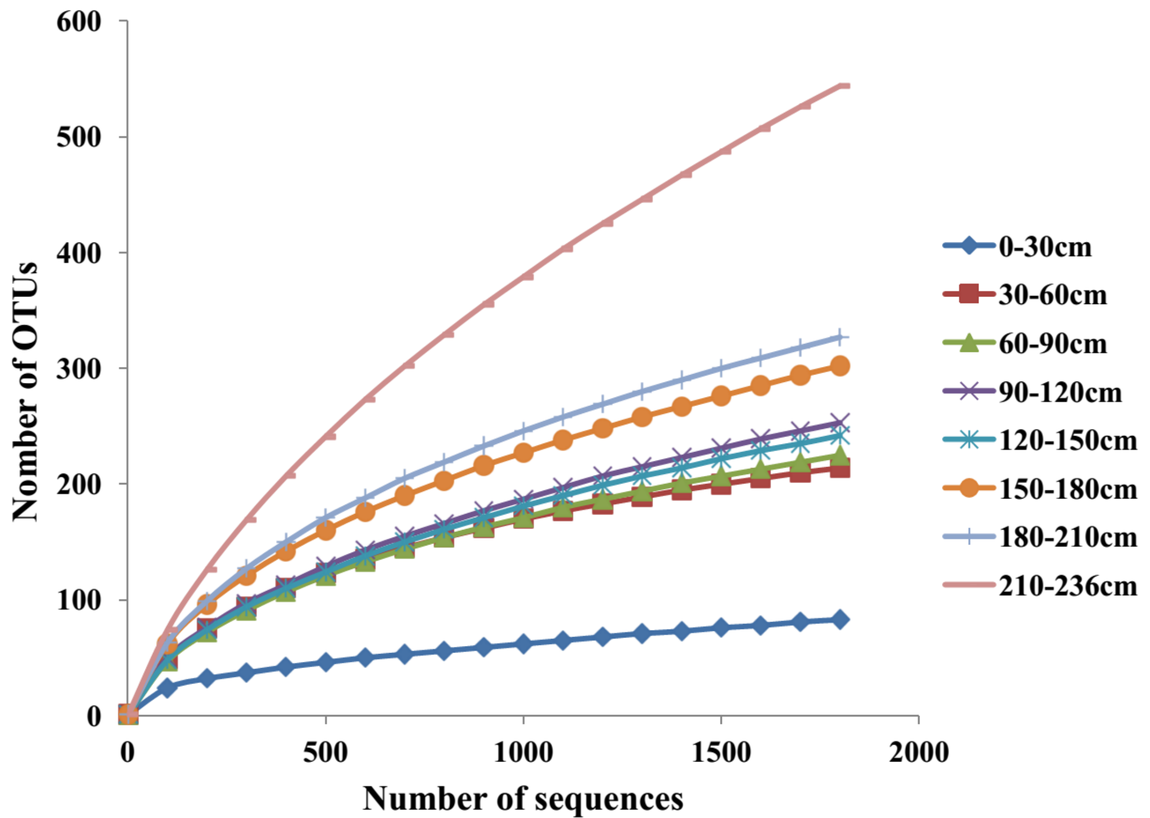


Figure 2.2S Rarefaction curves. Rarefaction curves for each 30cm section of ice, OTU definition set at 97% similarity.

Melt pond
Old ice
New ice
OTU number
Classification
OTU001 Flavobacterium
OTU003 Rhodobacteraceae
OTU004 Microbacteriaceae
OTU002 unclassified
OTU009 Comamonadaceae
OTU006 Gammaproteobacteria
OTU008 Algoriphagus
OTU007 Ulvibacter
OTU010 Glaciecola
OTU011 Octadecabacter
OTU012 Bacteroidetes
OTU016 Maribacter
OTU015 Loktanella
OTU019 Puniceicoccaceae
OTU005 Proteobacteria
OTU017 Polaribacter
OTU020 Ilumatobacter
OTU021 Winogradskyella
OTU014 Frigoribacterium
OTU022 Sphingobacteriales
OTU018 Oxalobacteraceae
OTU024 Haliea
OTU026 Marinobacter
OTU028 TM7
OTU025 Granulosicoccus
OTU023 Flavobacteriales
OTU031 Verrucomicrobiaceae
OTU032 Psychroflexus
OTU027 Oceanospirillales
OTU033 Opitutus
OTU029 Planctomycetaceae
OTU013 Alphaproteobacteria
OTU037 Roseivirga
OTU036 Eionea
OTU042 Persicivirga
OTU038 Albidiferax
OTU046 Oleispira
OTU040 Flammeovirgaceae
OTU035 Polaromonas
OTU030 Actinobacteria
OTU048 Colwellia
OTU043 Sphingobacteriaceae
OTU041 Pedobacter
OTU051 Methylophilaceae
OTU052 Lewinella
OTU047 Ralstonia
OTU050 Ulvibacter
OTU045 Persicirhabdus
OTU058 Salinibacterium
OTU060 Hydrogenophaga

Figure 2.3S Heat map of the 50 most abundant OTUs. Red indicates OTUs that are relatively more abundant where as black indicates ones that are relatively less abundant within each group, white indicates OTU that are not present within each group. OTU definition set at 97% similarity.

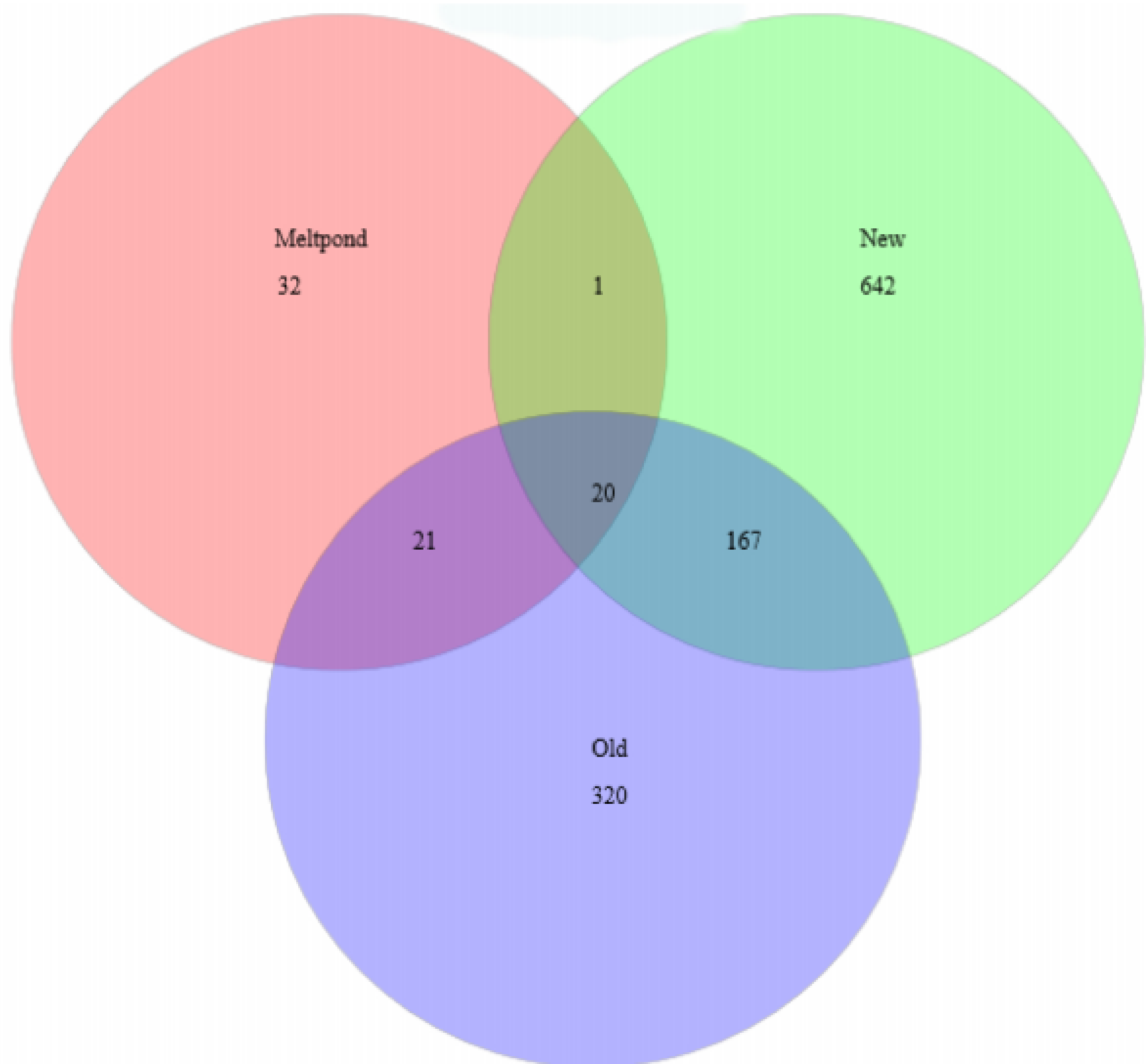


Figure 2.4S Venn diagram of shared observed richness. Melt pond: melt pond ice layer, Old: old ice layer, New: new ice layer. OTU definition set at 97% similarity.

Table 2.1S Oligonucleotide sequences used in this manuscript

Oligo	Sequence
27F	5´-AGRGTTTGATCMTGGCTCAG-3´
519R	5´-GTNTTACNGCGGCKGCTG-3´
Sequencing primer	5'-AGRGTTTGATCMTGGCTCAG-3'
0-30cm barcode	5'-ACCTGATG-3'
30-60cm barcode	5'-ACCTGTAG-3'
60-90cm barcode	5'-ACCTGTTC-3'
90-120cm barcode	5'-ACCTTCCA-3'
120-150cm barcode	5'-ACCTTCGT-3'
150-180cm barcode	5'-ACCTTGCT-3'
180-210cm barcode	5'-ACCTTGGA-3'
210-240cm barcode	5'-ACGAACCT-3'
SW1 barcode	5'-ACCTCAAG-3'
SW2 barcode	5'-ACCTCATC-3'

Table 2.2S Dominant OTUs (>10%) for each dominant class per ice layer including averaged relative proportions per layer within the phylum

	Actinobacteria	Alphaproteobacteria	Betaproteobacteria	Flavobacteria	Gammaproteobacteria	Sphingobacteria
Melt pond ice	OTU 4 (75%) OTU 14 (25%)	OTU 15(85%)	OTU 18 (69%) OTU 35 (10%)	OTU 1 (98%)	N/A	OTU 8 (55%) OTU 41 (33%)
Old ice	OTU 4 (70%) OTU 14 (10%) OTU 20 (10%) OTU 30 (10%)	OTU 3 (62%) OTU 15 (21%) OTU 11 (16%)	OTU 9 (90%)	OTU 1 (30%) OTU 7 (20%) OTU 16 (20%)	OTU 10 (50%) OTU 6 (20%) OTU 26 (10%)	OTU 8 (80%) OTU 22 (15%)
New ice	OTU 20 (40%) OTU 14 (35%)	OTU 3 (68%) OTU 11 (30%)	OTU 9 (50%) OTU 51 (35%)	OTU 16 (30%) OTU 17 (30%) OTU 21 (15%) OTU 23 (10%)	OTU 6 (30%) OTU 10 (20%) OTU 24 (10%) OTU 25 (10%)	OTU 22 (40%) OTU 42 (20%) OTU 37 (10%)

8 Appendix 2 Chapter 3 Supplementary material

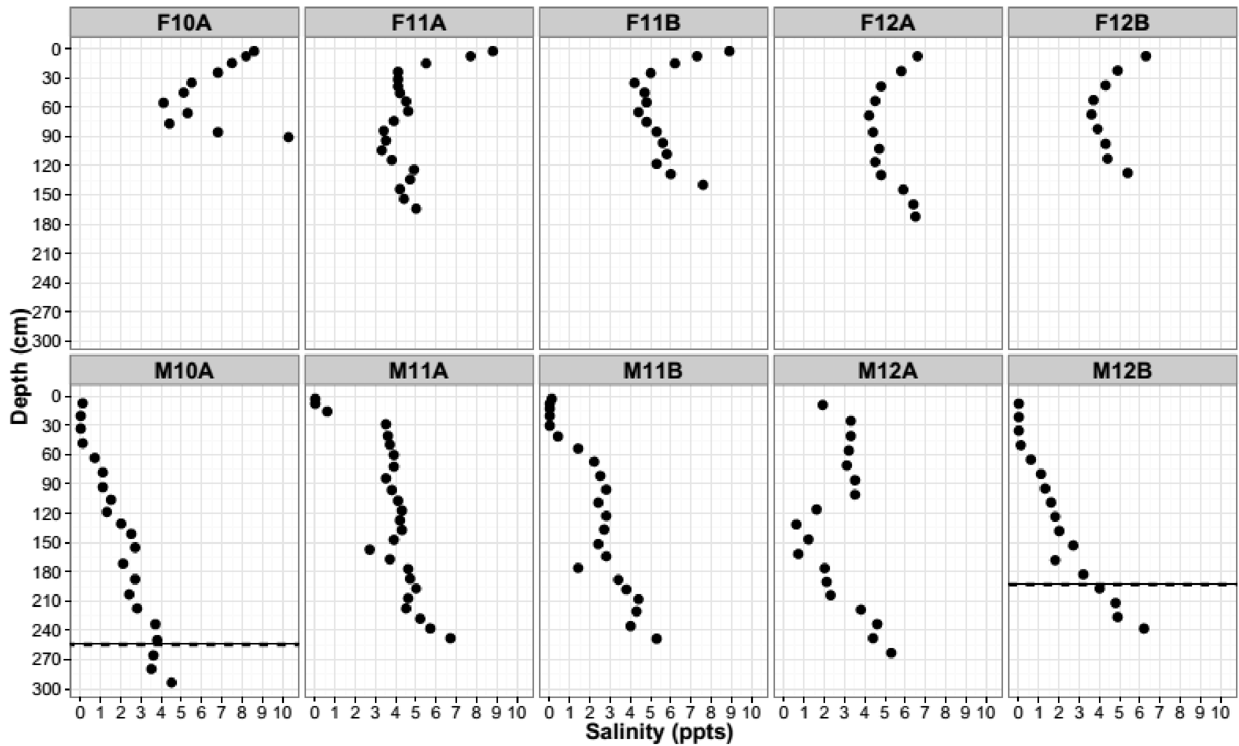
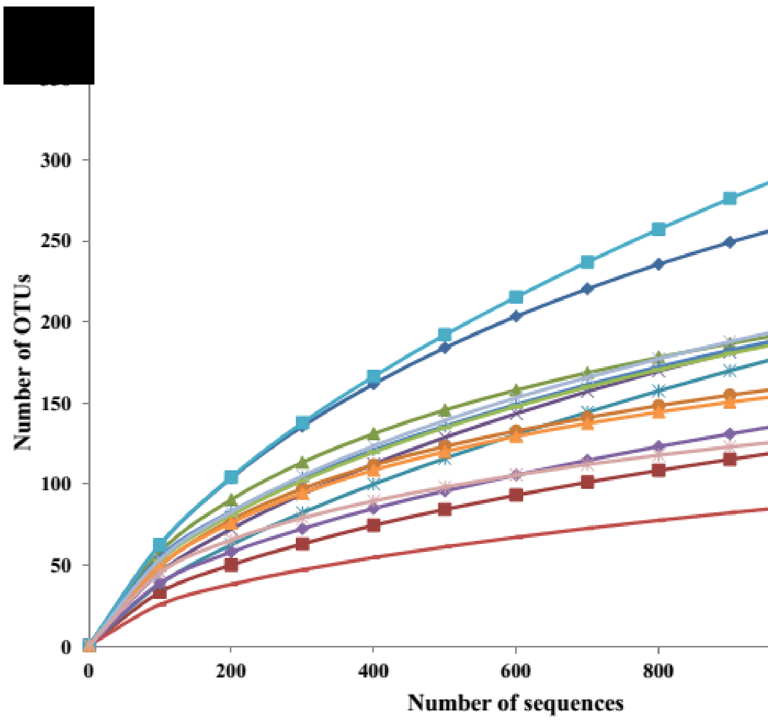


Figure 3.1S Salinity profile: Salinity profile for each core, salinity in parts per thousand. Dashed lines indicate the transition between multi-year ice and new ice in MYI cores. If no dashed line is indicated on an MYI core, no clear transition between the multi-year and new ice lyers could be determined for the MYI core.


Number of OTUs
300
250
200
150
100
50
0
0
200
400
600
800
Number of sequences

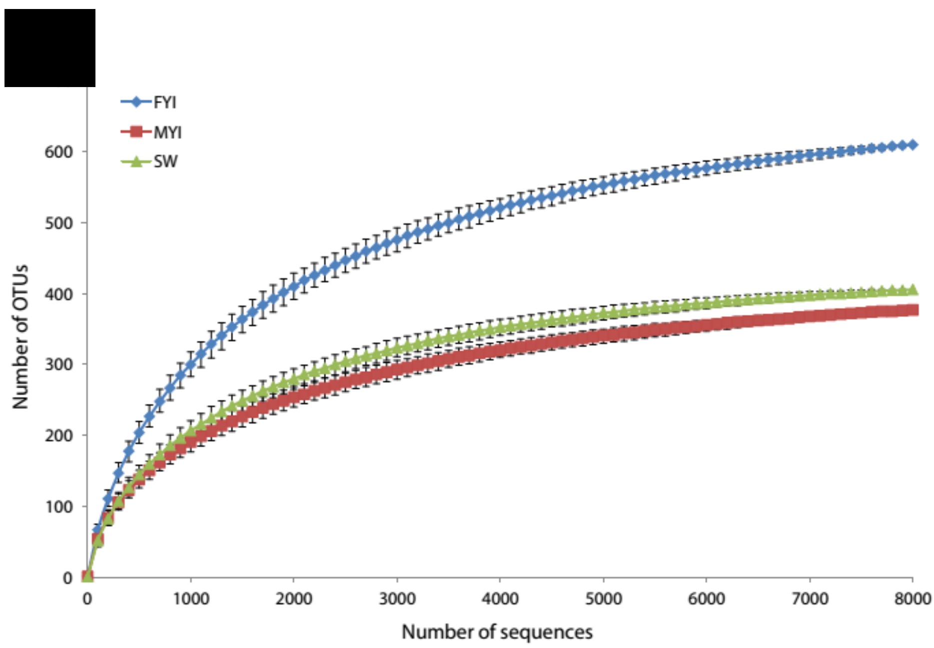

FYI
MYI
SW
600
500
400
300
200
100
0
Number of OTUs
0
1000
2000
3000
4000
5000
6000
7000
8000
Number of sequences

Figure 3.2S: Rarefaction curve of A) individual and B) merged samples. A 1100 sequences B 8000 sequences, OTU definition 97% sequence similarity.

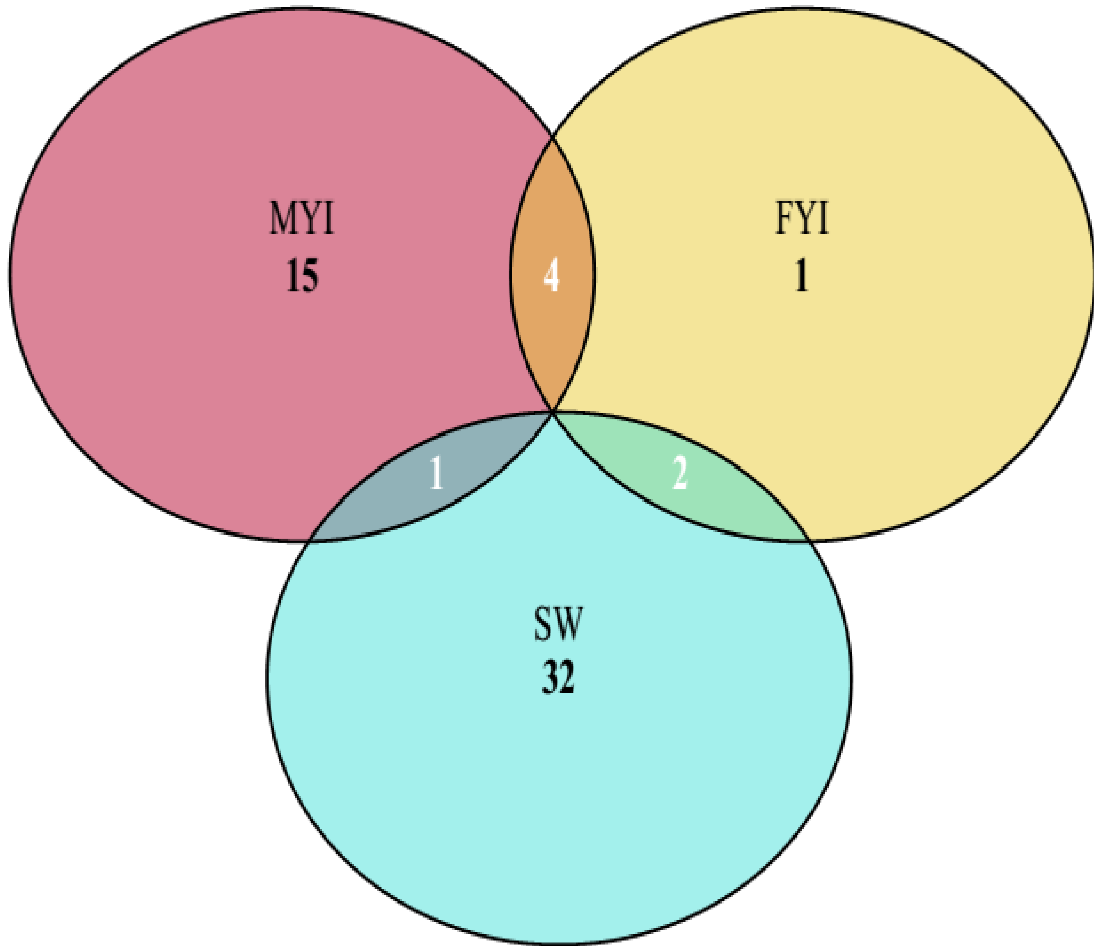


Figure 3.3S Shared Core OTUs per sample type: Venn diagram of core OTUs (OTUs that are found in all samples of a sample type) per sample type, OTU definition set at 97% sequence similarity.

Table 3.1S Mean core length and GPS coordinates of duplicate sea ice core sites. 'M' denotes MYI sample; 'F' denotes FYI sample; numbers denote year of sampling; 'A' & 'B' denotes first and second sample site of sampling year, respectively.

Sample	Averaged core length	Snow cover	Averaged total ice thickness	Dist from ice	Chl *a* mg m^{-3}	Date d/m/y	GPS coordinates
M10A	250 cm	50 cm	254 cm	NA	0.3	12/5/10	82.54241, -62.61735
M11A	253 cm	32 cm	448 cm	NA	0.5	7/5/11	82.55621, -62.15554
M11B	257 cm	30 cm	302 cm	NA	0.1	8/5/11	83.54237, -65.9644
M12A	260 cm	47 cm	369 cm	NA	0.8	5/5/12	82.54287, -62.68143
M12B	260 cm	31 cm	323 cm	NA	0.2	5/5/12	82.92446, -58.62613
F10A	90 cm	9 cm	93 cm	NA	2.1	7/5/10	82.56173, -61.95383
F11A	164 cm	38 cm	167 cm	NA	1.4	3/5/11	82.5591, -62.34562
F11B	143 cm	17 cm	152 cm	NA	0.1	6/5/11	82.57041, -62.15123
F12A	184 cm	20 cm	200 cm	NA	0.1	2/5/12	82.53749, -62.70666
F12B	135 cm	33 cm	151 cm	NA	0.1	3/5/12	82.54908, -62.37007
SW1	NA*	NA	NA	50 cm	NA	7/5/10	82.56173, -61.95383
SW2	NA	NA	NA	50 cm	NA	8/5/11	83.54237, -

							65.9644
SW3	NA	NA	NA	50 cm	NA	5/5/12	82.54287, -62.68143
SW4	NA	NA	NA	50 cm	NA	2/5/12	82.53749, -62.70666

Table 3.2S Oligonucleotide sequences used in this manuscript

Oligo	Sequence
27F	5´-AGRGTTTGATCMTGGCTCAG-3´
519R	5´-GTNTTACNGCGGCKGCTG-3´
Sequencing primer	5'-AGRGTTTGATCMTGGCTCAG-3'
M10A barcode	5'-ACAGAGAC-3'
M11A barcode	5'-ACCTACCT-3'
M11B barcode	5'-ACCTACGA-3'
M12A barcode	5'-TCAGGTCC-3'
M12B barcode	5'-TCAGTAAG-3'
F10A barcode	5'-ACCTAGGT-3' & 5’-ACAGAGTG-3’
F11A barcode	5'-ACAGAGAC-3'
F11B barcode	5'-ACAGAGTG-3'
F12A barcode	5’-TCAGGAGG-3’
F12B barcode	5’-TCAGGATA-3’
SW1 barcode	5'-ACCTCAAG-3'
SW2 barcode	5'-ACCTCATC-3'
SW3 barcode	5’-ACCTCTTG-3’

SW4 barcode	5'-ACCTGAAC-3'

Table 3.3S: Classification of MYI core 15 OTUs

OTU id	Class	Lowest RDP classification (60% confidence)	NCBI classification (cultured organisms only when possible, min e-value e^{-90})	Environment found based on survey of 16S sequences from both cultured and uncultured organisms found in the NCBI database	Function
OTU 13	Alphaproteobacteria	Rhodobacteraceae	Roseobacter sp. ARCTIC-P4	Arctic marine environments	Sulfur cycle (DMSP oxidation) Carbon cycle (Carbon fixation, aromatic compounds degradation)
OTU 19		Rhodobacteraceae	Loktanella salsilacus	Arctic glaciers	
OTU 23		Rhodobacteraceae	Rhodobacteraceae	Various cold marine environments	
OTU 58		Rhodobacteraceae	Rhodobacteraceae	Cold marine and sediment environments	
OTU 16	Gammaproteobacteria	Glaciecola	Glaciecola from various sp. ANT	Sea ice	Carbon cycle (Cellulose, xylan, chitin hydrolysis) Nitrogen cycle (denitrification)
OTU 31		Glaciecola	Glaciecola nitratireducens		
OTU63		Eionea	Eionea	Cold marine	NA
OTU32	Betaproteobacteria	Comamonadaceae	Various Hydrogenophaga	Cold alkaline lakes and sediment	Hydrogen oxidation Carbon cycle (Aromatic compounds

					degradation)
OTU 47	Flavobacteria	Psychroflexus	Psychroflexus torquis	Sea ice	Algae associated psycrofilic suprophyet involved in the sulfer cycle via DMSP degredation and possible carbon cycle via EPS secretion.
OTU 55	Flavobacteria	Flavobacteriaceae	Flavobacteriaceae	Sea ice	NA
OTU 78		Flavobacteriales	Cryomorphace-uncultured	Cold marine	NA
OTU 76	Actinobacteria	Ilumatobacter	Ilumatobacter	Cold marine/sediment/estuaries	NA
OTU 79		Microbacteriaceae	Microbacteriaceae	Snow	NA
OTU 3		Cryobacterium	Candidatus Aquiluna	Cold marine and fresh water bacterioplankton	NA
OTU 1	NA	Bacteria	Uncultured marine clone	Marine	NA

9 Appendix 3 chapter 4 supplementary

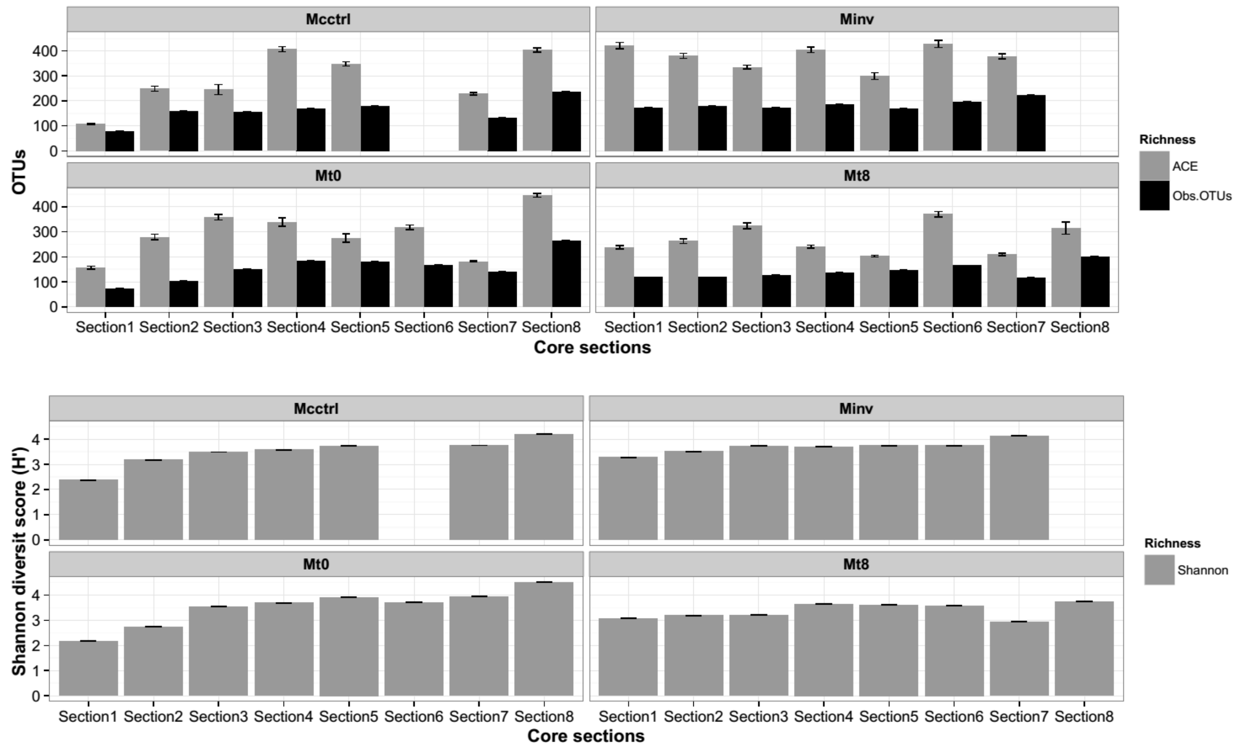


Figure 4.1S Richness and diversity of all MYI core sections: Estimated richness and diversity for MYI samples based on observed number of OTUs, expected number of OTU (ACE) and Shannon diversity index. OTU definition set at 97% sequence similarity. Samples where sub-samples to 2200 sequences, error bars represent ±SE from the mean of 1000 iterations of sub-sampling and value measurement.

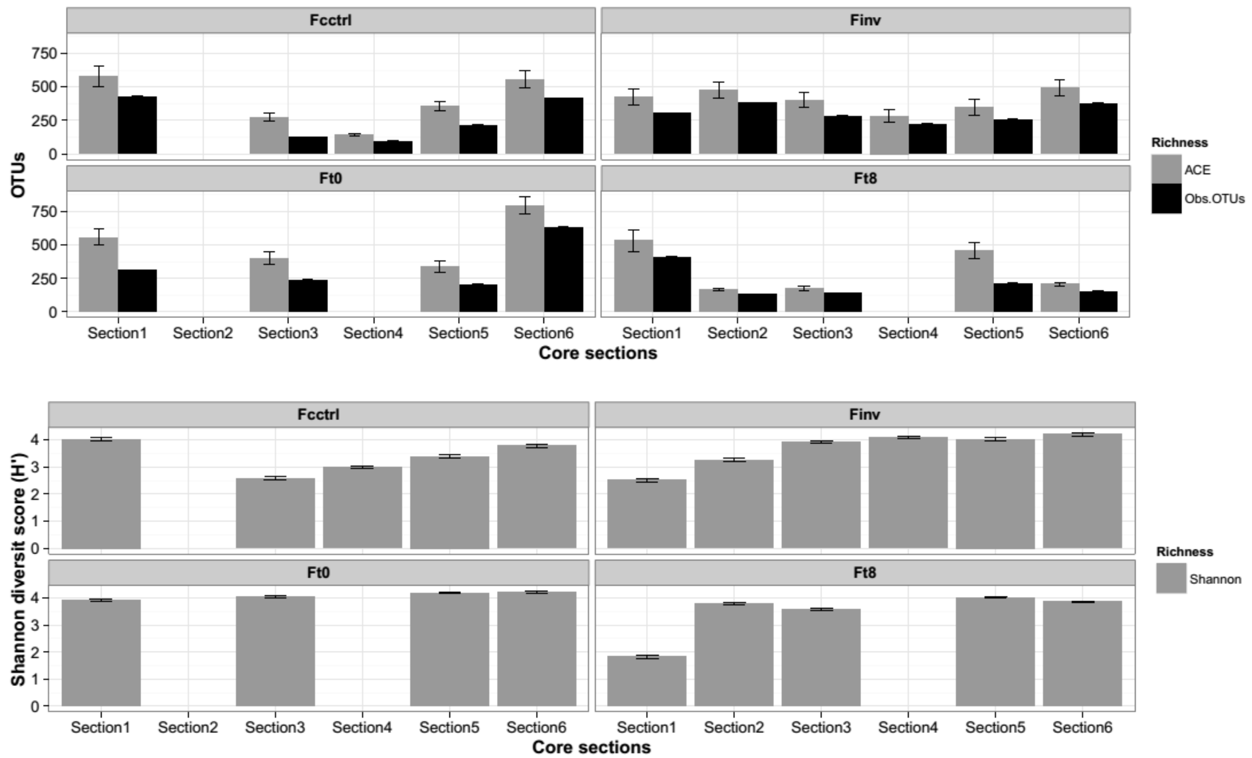


Figure 4.2S Richness and diversity of all FYI core sections: Estimated richness and diversity for FYI samples based on observed number of OTUs, expected number of OTU (ACE) and Shannon diversity index. OTU definition set at 97% sequence similarity. Samples where sub-samples to 1100 sequences, error bars represent ±SE from the mean of 1000 iterations of sub-sampling and value measurement.

Table 4.1S Barcodes and primers sequences highlighted rubrics represent samples with less than 1000 sequences which were omitted from the analysis

Sample / Primer	Oligo sequence 5'-3'
341F	CCTACGGGAGGCAGCAG
581R	ATTACCGCGGCTGCTGG
A adaptor and sequencing primer	CCATCTCATCCCTGCGTGTCTCCGACTCAG
Gleen adaptor	CAGTCGGGCGTCATCA
trP1	CCTCTCTATGGGCAGTCGGTGAT
Fcctrl1	ATCCGGAATC
Fcctrl2	TCGACCACTC
Fcctrl3	CGAGGTTATC
Fcctrl4	TCCAAGCTGC
Fcctrl5	TCTTACACAC
Fcctrl6	TTCTCATTGAAC
Finv1	AACCATCCGC
Finv2	TTACAACCTC
Finv3	CCTGAGATAC
Finv4	AACCTCATTC
Finv5	TGCCACGAAC
Finv6	TTCGAGACGC
Ft01	TGAGCGGAAC
Ft02	CTGACCGAAC
Ft03	TCCTCGAATC
Ft04	TAGGTGGTTC
Ft05	TCTAACGGAC

Ft06	TTGGAGTGTC
Ft81	TCTGGATGAC
Ft82	TCTATTCGTC
Ft83	AGGCAATTGC
Ft84	TTAGTCGGAC
Ft85	CAGATCCATC
Ft86	TCGCAATTAC
Mcctrl1	TCCTAGAACAC
Mcctrl2	TCCTTGATGTTC
Mcctrl3	TCTAGCTCTTC
Mcctrl4	TCACTCGGATC
Mcctrl5	TTCCTGCTTCAC
Mcctrl7	CCTTAGAGTTC
Mcctrl8	CTGAGTTCCGAC
Minv1	TCTGGCAACGGC
Minv2	CAGCATTAATTC
Minv3	TCCACCTCCTC
Minv4	CCGGAGAATCGC
Minv5	CTGGCAATCCTC
Minv6	CCGCATGGAAC
Minv7	TTGAGCCTATTC
Mt01	CTAAGGTAAC
Mt02	TAAGGAGAAC
Mt03	AAGAGGATTC
Mt04	TACCAAGATC
Mt05	CAGAAGGAAC
Mt06	CTGCAAGTTC

Mt07	TTCGTGATTC
Mt08	TTCCGATAAC
Mt81	CTTGACACCGC
Mt82	TTGGAGGCCAGC
Mt83	TGGAGCTTCCTC
Mt84	TCAGTCCGAAC
Mt85	TAAGGCAACCAC
Mt86	TTCTAAGAGAC
Mt87	TCCTAACATAAC
Mt88	CGGACAATGGC

10 Appendix 4 analysis of a mock bacterial community

For each of each dataset presented as part of this thesis a mock community of 18 bacterial strains was prepared. This procedure was done using the same DNA extraction and PCR protocols and reagents as the experimental samples (Chapters 2-4). The mock communities were analyzed together with the experimental samples using similar criteria. The mock communities where than used as positive controls assessing the accuracy of richness estimation as well as membership analysis preformed on the experimental samples. Entailed here is an example of the analysis of the mock community generated for Chapter 4.

Both observe and estimated richness gave an OTU/strain ratio of 0.94 (used 18 strains estimated to have minimal richness of 17 OTUs) which indicates that the OTU estimation made for the environmental samples is not likely to be an over estimation (figure 9.1S A). This is also supported by the rarefaction curve which reached an asymptote at 17 OTUs (figure 9.1S B).

List of the members of the mock community are found in table 9.1. The analysis demonstrates that the sequences belonging to the mock community where classified as relating to groups closely related to those used to construct it (table 9.1).

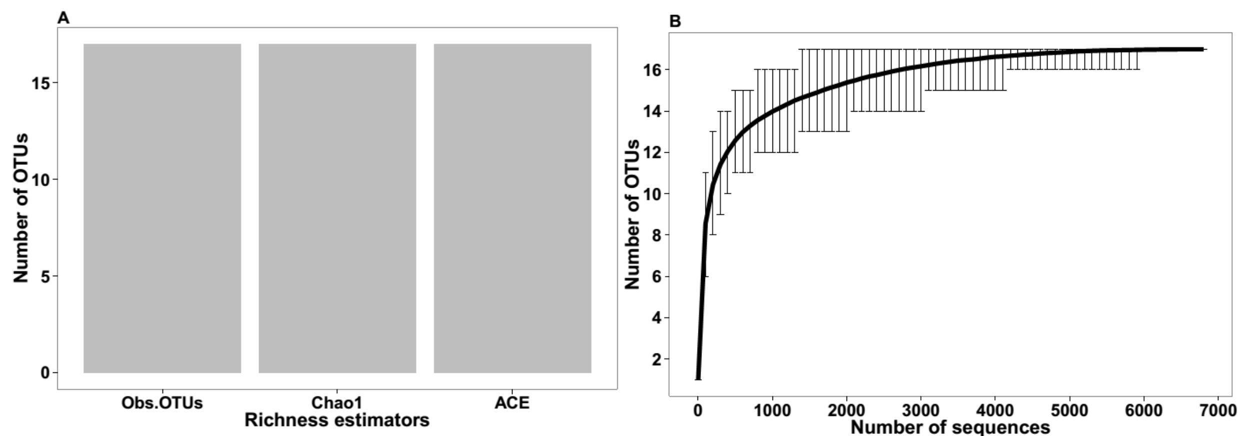


Figure 10.1 Richness of the mock bacterial community: A- Estimated richness and for the mock community sample based on observed number of OTUs, expected number of OTU (Chao 1 &ACE). B- Rarefaction curve for the mock community sample. OTU definition set at 97% sequence similarity, library was subsampled to 6600 sequences. Error bars represent high and low confidance intervals.

Table 10.1S Membership of constructed library strains used vs. classification of OTUs by RPD clasiffier at 60% confidence

Srain used	OTU id	RDP classification 60% confidence
Desulfomicrobium sp.	OTU_1	Bacteria;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfomicrobiaceae;Desulfomicrobium;
P.vulgaris	OTU_2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Proteus;
E. coli	OTU_3	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Escherichia/Shigella;
Thaurea	OTU_4	Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;Thauera;
S.aureus	OTU_5	Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Staphylococcus;
E.aerogenes	OTU_6	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;
Anabena	OTU_7	Bacteria;Cyanobacteria/Chloroplast;Cyanobacteria; Nostocales
P.aeruginosa	OTU_8	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;
E.faecelis	OTU_9	Bacteria;Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;
Leprolegbia	OTU_10	Bacteria;Cyanobacteria/Chloroplast;Cyanobacteria;unclassified_Cyanobacteria;
Synechocystis	OTU_11	Bacteria;Cyanobacteria/Chloroplast;Cyanobacteria;Oscillatoriophycideae;
M.sporium	OTU_12	Bacteria;Proteobacteria;Alphaproteobacteria
M.Capsulatus	OTU_13	Bacteria;Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;Methylosarcina;
N.europea	OTU_14	Bacteria;Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Nitrosomonas;
M.trichosporium	OTU_15	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;
N.communis	OTU_16	Bacteria;Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Nitrosomonas;
	OTU_17	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Proteus;
Thermatoga cell12	NA	
T.affectus	NA	