Geochemical, Spatial, and Temporal Drivers of Microbial Community Heterogeneity in the Meltwater Ponds of Antarctica

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Abstract

Antarctic meltwater ponds are an abundant, dynamic and sensitive yet poorly understood ecosystem. In this thesis, bacterial communities from surface waters, the water column and benthic zone in geochemically variable meltwater ponds in the Ross Sea Region of Antarctica were investigated. The primary objective of this research was to provide a detailed description of the community composition and to determine the temporal, geochemical and geomorphological drivers of community structure.

A coordinated comparable analysis method was used for all samples so that, although separated into the previously mentioned zones, findings could be directly compared across studies. Bacterial community structure between samples was initially investigated by Automated Ribosomal Intergenic Spacer Analysis (ARISA) of the 16S rRNA gene which, combined with *in-situ* collected geochemistry data was used to identify trends requiring high throughput sequencing (454 pyrosequencing of the V5-V6 hypervariable region of the 16S rRNA gene) analysis coupled with nutrient and elemental data.

A preliminary study in December 2009 compared the water columns of five geochemically distinct ponds. Communities between ponds were distinct, their structure driven primarily by pH and conductivity. One geochemically stratified pond formed distinct surface and bottom clusters with increasing diversity and changes to phyla structure with depth.

Temporal and geomorphological (Bratina Island and Miers Valley) drivers of variation in the microbial community structure between the surface waters of 41 ponds were examined. Conductivity was identified as the most significant driver across all ponds for the dominant cosmopolitan community, however trace elements were more significant drivers of community structure for the unique community (those sequences absent in at least one pond). Pronounced variation was identified between December and January samples and although the bacterial components of the community were similar between January 2012 and 2013 the community structure varied significantly. Despite different environments,

communities from the Miers Valley were not well differentiated from Bratina Island, suggesting biological exchange between locations.

The stratified water column of six Bratina Island and two Miers Valley ponds was investigated. Strongly geochemically stratified ponds exhibited a heterogeneous vertical community structure related to conductivity and dissolved oxygen. Variation in community structure was primarily driven by the abundance of a small number of cosmopolitan OTUs that changed with depth. Although the biological constituents were the same, minor variation in community structure was identified within Huey pond between years (2012 and 2013). Variation between Huey pond (Bratina Island) and Morepork pond (Miers Valley) was correlated with variation of iron and mercury concentration.

Lastly the benthic zone of six ponds from Bratina Island and six from the Miers Valley was examined. The community structure was highly heterogeneous and diverse with 21 phyla identified. No distinction was identified between the two locations with the majority of pyrosequencing reads shared. Potassium, sodium and cobalt were identified as the most significant explanatory variables to the cosmopolitan community and aluminium, uranium and magnesium to the unique community.

This study has granted an unprecedented understanding of the bacterial communities in the meltwater ponds of the Ross Sea Region. Geochemical inter pond heterogeneity is matched with an equally heterogeneous bacterial communities, primarily driven by conductivity. Although harboring a complex and diverse community the majority is comprised of a small number of shared OTUs across spatial and temporal scales.

Statement of Authorship

I was the primary author and contributed the majority of work on all chapters presented in this thesis. Co-authorship forms and prefaces are included for all chapters outlining collaborator contributions.

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Chapter 1 - Introduction and Literature Review

1.1 Introduction

Understanding the dynamics within aquatic ecosystems is fundamental to diverse biological fields including microevolution (Morris *et al.*, 2014; Ray & King, 2006), gene expression and biogeochemical cycling (Chen *et al.*, 2014; Kutovaya *et al.*, 2013; Thottathil *et al.*, 2013; Yang *et al.*, 2014), population dynamics (Bellakhal *et al.*, 2014; Mehnert *et al.*, 2014), chemical pollution (Jackson, 2013; Valinia *et al.*, 2014), and the potential effects of climate change on biological communities (Mulvaney *et al.*, 2014; Xuan & Chang, 2014). Most aquatic investigations are conducted in temperate environments due to logistical ease and anthropological significance; however, the biological simplification, lack of anthropogenic impacts and sensitivity of polar ecosystems to environmental changes (Maxwell & Barrie, 1989; Walther *et al.*, 2002; Yergeau & Kowalchuk, 2008) provides ideal systems for ecological modeling (Archer *et al.*, 2014; Bonilla *et al.*, 2005; Bottos *et al.*, 2008; Comeau *et al.*, 2012; Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; Smol & Douglas, 2007; Villaescusa *et al.*, 2013; Vincent & James, 1996). With predicted global climate changes in the near future, understanding the current state of these sentinel systems is critical so that biodiversity may be captured and ecosystem shifts can be monitored and modeled to predict changes to more complex, temperate environments (Maxwell & Barrie, 1989; Nielsen & Wall, 2013; Walther *et al.*, 2002).

The first sections of this chapter describe temperate and polar aquatic ecosystems. The Chapter then discusses the annual temporal variation within Antarctic aquatic environments and environmental drivers that structure the resident microbial communities. Then the unique biological communities found in Antarctic meltwater ponds, the water column, and benthic zone are described as well as the study sites used in this investigation (Bratina Island and the McMurdo Dry Valleys). Finally, an overview of current methodology relevant to this investigation and an outline of the thesis are presented.

1.2 Aquatic ecosystems

Limnological research is constantly advancing our understanding of how organisms interact and respond to changes in their surrounding environment (Hansson *et al.*, 2013; Manca & Bertoni, 2014). Although simpler than marine or other terrestrial environments, the biota of temperate freshwater ponds contains multiple trophic levels that have dynamic biotic and abiotic interactions. The lowest trophic level reported in many studies of freshwater biology is phytoplankton as primary producers and the food source for planktivorous fish (Boukal, 2014; Hansson *et al.*, 2013; Hessen & Kaartvedt, 2014; Hortal *et al.*, 2014; Manca & Bertoni, 2014). However, increasing focus has been placed on bacteria in aquatic ecosystems in recent years as they are integral in the mineralization of organic matter, and produce significant effects on the ecosystem (Hader *et al.*, 1998; Hessen & Kaartvedt, 2014).

1.3 Antarctic aquatic ecosystems

The majority of ice-free continental Antarctica is comprised of desert soils characterized by low water availability, ultra oligotrophy and extremely cold temperatures (Cary *et al.*, 2010; Hopkins *et al.*, 2006). Aquatic ecosystems found in these areas serve as relative oases amongst the inhospitable conditions, and although exposed to significant environmental pressures, harbor relatively diverse microbial communities (Bowman *et al.*, 2000; Peeters *et al.*, 2012; Sjoling & Cowan, 2003; VanTrappen *et al.*, 2002) that new sequencing technologies are beginning to accurately characterize (Comeau *et al.*, 2012; Villaescusa *et al.*, 2013; Tang *et al.*, 2013; Archer *et al.*, 2014). Environmental pressures present in these ecosystems reduce diversity and restrict the trophic complexity of foodwebs compared to temperate climates (Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; James *et al.*, 1995; Safi *et al.*, 2012; Vincent & James, 1996). Bacteria, flagellates and ciliates dominate Antarctic aquatic ecosystems with the highest trophic level typically being represented by bacteriovorous ciliates with a limited number or tardigrade, nematode and rotifer species previously identified in algal mats (Howard-Williams et al. 1990; Safi et al. 2012; Suren, 1990; Vincent

and James 1996). This causes a greater significance of abiotic variables to microbial community structure.

Antarctica is host to diverse ponds, streams and lakes derived and sustained from snow, ice and glacial melt during the austral summer (De Mora *et al.*, 1994; Howard-Williams & Hawes, 2007; Howard-Williams & Hawes, 2005; Vincent & James, 1996). Local physicochemistry, which structures the biology (Jungblut *et al.*, 2005; Peeters *et al.*, 2012), is the result of the water body's evolution including its persistence, local geology, size, marine influence and surrounding climate (De Mora *et al.*, 1994; Howard-Williams & Hawes, 2007; Schmidt *et al.*, 1991; Stanish *et al.*, 2012; Vincent & James, 1996). Within and along the margins of these aquatic systems are microbial mats thought to contribute the majority of primary productivity to the Dry Valleys (Dore & Priscu, 2001; Fernandez-Valiente *et al.*, 2001; Howard-Williams *et al.*, 1990; Moorhead *et al.*, 2003; Parker & Simmons, 1985; Parker *et al.*, 1982; Smol & Douglas, 2007; Wood *et al.*, 2008).

Small meltwater ponds are found throughout Antarctica. They range from freshwater to hypersaline, ephemeral to perennial, and can be found on the ice shelf, glaciers and scattered among terrestrial systems (Vincent & James, 1996). Lakes are larger, perennial inland bodies of water, which typically have a permanent ice cover and a large reservoir of liquid water year round (Howard-Williams & Hawes, 2007; Vincent & James, 1996). Freshwater input in summer typically floats on top of the denser brine at depth and under the ice cover, which shelters the water column from wind mixing, allowing strong stratification to form (Comeau *et al.*, 2012; Glatz *et al.*, 2006; Hawes *et al.*, 2001; Miller & Aiken, 1996). Streams vary greatly in size and can be glacially or snow-fed with highly intermittent flow, occurring for only a few weeks during the summer months (Howard-Williams & Hawes, 2007). Despite the highly variable conditions, these streams maintain microbial communities ranging from epilithic crusts and films to cohesive mats like those found in lakes and ponds (Vincent & James, 1996; Vincent & Howard-Williams, 1986).

1.4 Antarctic environments as model ecosystems

Antarctic microorganisms experience an array of harsh environmental conditions including extreme cold and aridity with seasonal periods of complete darkness and strong dry katabatic winds (Bergstrom *et al.*, 2006; Hughes *et al.*, 2006). However, far from being lifeless polar deserts, Antarctic ice-free regions have been identified as having diverse microbial communities (Cary *et al.*, 2010; Sjoling & Cowan, 2003; Villaescusa *et al.*, 2013) capable of rapid response to environmental changes (Tiao *et al.*, 2012). To withstand the extreme and dynamic environment, microorganisms that persist require a number of survival mechanisms including the production of stress proteins, and the ability to enter viable but inactive states (cysts and spores) (Chattopadhyay, 2000; Deming, 2002; Gilbert *et al.*, 2004; Laybourn-Parry *et al.*, 2002). These selective pressures limit trophic structure complexity, resulting in greater biological effects from climatological, topographic, spatial and geochemical influences (Cannone *et al.*, 2008; Cary *et al.*, 2010; Chong *et al.*, 2013; Hawes *et al.*, 2013).

As simple ecosystems with restricted exogenous inputs and human interference (Pearce *et al.*, 2009) Antarctica provides an excellent study site to investigate microbial biogeography and can provide great insight into the global distribution of microorganisms (Chong *et al.*, 2013; Horner-Devine *et al.*, 2004; Jungblut *et al.*, 2010; Lee *et al.*, 2012a; Lindstrom & Langenheder, 2012), particularly whether the current geochemical (Miletto *et al.*, 2008; Sommaruga & Casamayor, 2009) or historical biological legacy (Foissner, 2008; Papke *et al.*, 2003) have the greatest significance to the current microbial community. Identifying the resident microbiology and dominant drivers of variation is crucial to understanding potential shifts caused by climatological changes and impacts of future anthropogenic interference (Cowan *et al.*, 2011b; Ellis-Evans & Walton, 1990; Franzmann *et al.*, 1997; Pearce *et al.*, 2010; Wynn-Williams, 1990).

1.5 Temporal variation in Antarctic aquatic environments

Due to its latitudinal position, Antarctica experiences an exaggerated polar light regime including several months of uninterrupted darkness during winter and several months of uninterrupted daylight during summer. Average summer and winter temperatures vary widely, causing significant variation in the physical, chemical and biological conditions in Antarctic environments. Frequent freeze-thaw cycles due to significant diurnal temperature changes occur in Dry Valley mineral soils (20°C changes in a single day are not unusual), resulting in the necessity for robust organisms and relatively slow growth rates (Schmidt *et al.*, 2009; Yergeau & Kowalchuk, 2008).

Several studies have investigated the transition of Antarctic aquatic ecosystems from summer melt to winter freeze conditions, highlighting the rapid physicochemical and biological changes that occur during this period (Foreman *et al.*, 2010; Hawes *et al.*, 1999; Hawes *et al.*, 2011a; Safi *et al.*, 2012; Schmidt *et al.*, 1991; Webster-Brown *et al.*, 2012). Meltwater ponds in particular have exaggerated transition effects due to their tendency to completely freeze in winter and thaw in summer. During the peak of the austral summer (December-January) 24 hour sunlight and increased ambient temperatures allow for ponds to have several weeks of open water and a burst of photosynthetic life and biomass (Hitzfeld *et al.*, 2000; James *et al.*, 1995; Vincent & James, 1996; Wood *et al.*, 2008). Towards winter (late January-April) daylight hours shorten, temperatures drop, and ponds begin to develop ice from the surface down (Foreman *et al.*, 2010; Schmidt *et al.*, 1991). The rapid physicochemical changes during this period are matched by an equally rapid microbial response. Oxygen concentration, pH, biomass and microbial community structure change in just a few weeks, with a net shift from autotrophy to heterotrophy observed between February and April (Hawes *et al.*, 2011a; Safi *et al.*, 2012). It is believed that over winter these organisms enter inactive states, and then during the summer melt become active (Chattopadhyay, 2000; Laybourn-Parry, 2002). The annual cycle of microbial change within meltwater ponds can provide new insights into the responsiveness of microorganisms in Antarctica to the environment, and new methodology is allowing a greater insight into these changes.

1.6 Meltwater ponds

Although lakes and streams have been the subject of the majority of Antarctic aquatic research to date (Howard-Williams & Hawes, 2007; Miller & Aiken, 1996; Moorhead *et al.*, 2003; Parker *et al.*, 1982; Rojas *et al.*, 2009; Stanish *et al.*, 2012; Takacs & Priscu, 1998; Tang *et al.*, 2013; Villaescusa *et al.*, 2013; Vincent & James, 1996; Vincent & Howard-Williams, 1986), the most common aquatic feature across continental Antarctica are meltwater ponds (Vincent & James, 1996) (Figure 1.1). Unlike lakes, which frequently maintain an ice cover through summer (Comeau *et al.*, 2012; Howard-Williams & Hawes, 2007), most ponds go through an annual complete freeze-thaw cycle (Hawes *et al.*, 2011b; Schmidt *et al.*, 1991; Wait *et al.*, 2006) and are typically intermittently fed by melting of nearby snow deposits as opposed to glacial melt, which generally feeds the larger lake systems. The low volume, variable snow input and homogenising effects of wind mixing allow conditions within ponds to change rapidly between seasons based on the local climate (degree days above zero, snow deposition, wind strength and storm frequency) (Hawes *et al.*, 2013).

Even within a study site these ponds can harbour extremely heterogeneous geochemical (Hawes *et al.*, 2014; Howard-Williams & Hawes, 2005; Schmidt *et al.*, 1991) and biological environments (Archer *et al.*, 2014; de los Rios *et al.*, 2004; Fernandez-Valiente *et al.*, 2001; Hawes *et al.*, 1999; Hawes *et al.*, 2011a; Hitzfeld *et al.*, 2000; Jungblut *et al.*, 2005; Safi *et al.*, 2012; Sjoling & Cowan, 2003) through variation in salt and nutrient input, local geology and biology, age, water balance and water source. Ponds are small, ranging from one to several thousand square metres, and are often lined with *Cyanobacteria* dominated mats (Vincent & James, 1996). Due to their large surface area to volume ratio (most ponds are less than 4 m deep), and open water in summer, many ponds have a homogeneous water column created by continued wind-driven turnover, although stratification is possible in those ponds with a relative depth ratio greater than two (Archer *et al.*, 2014; Castro & Moore, 2000; Wait *et al.*, 2006). Relative depth is defined as follows:

$$Relative\ Depth = 50\ \text{Maximum Depth}\sqrt{\frac{\pi}{Surface\ Area}}$$

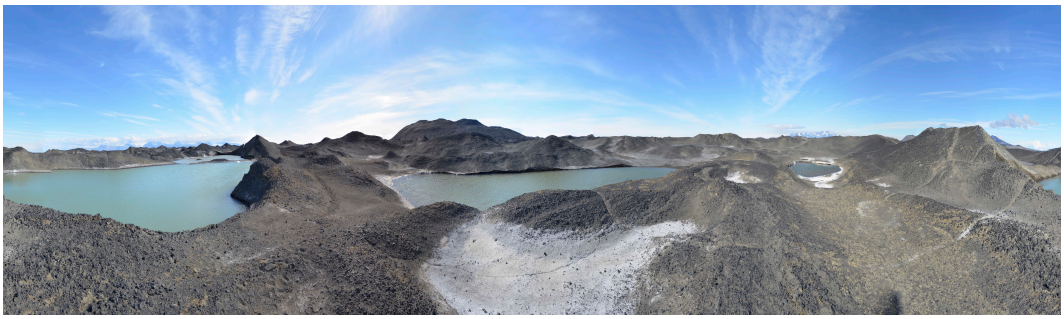

Figure 1. 1 360-degree panoramic view of ponds (from left) Fresh, Brack and Salt on the McMurdo Ice Shelf adjacent to Bratina Island

1.6.1 Pond surface

Although the variable meltwater input received by ponds results in significant annual geochemical variation within individual ponds, most research conducted over the past two decades has been limited to single (or two) sampling periods (Archer *et al.*, 2014; De Mora *et al.*, 1994; DeMora *et al.*, 1996; Healy *et al.*, 2006; James *et al.*, 1995; Lyons *et al.*, 2012; Matsumoto *et al.*, 1992; Mountfort *et al.*, 1999; Peeters *et al.*, 2011; Sorrell *et al.*, 2013; Wait *et al.*, 2006), making it difficult to separate true geochemical heterogeneity from annual variation. Findings from a recent two-decade survey of Bratina Island meltwater ponds has provided valuable insight into the temporal variability of geochemistry that occurs in pond surface waters during the austral summer. Broad average ranges of conductivities (0.12 - 56.3 mS cm^{-1}) were found between ponds studied, which could change significantly within a pond between years based on climatological influences, with a certain degree of consistency over longer timescales. pH (biologically controlled) typically exhibited a narrower range (9 - 11) with less annual variation (Hawes *et al.*, 2013).

Surface waters contain a simple trophic structure, with bacteriovorous ciliates dominating the highest trophic level while a broad diversity of bacteria, flagellates and a few archaea are the dominant components of the lower trophic

level (Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; James *et al.*, 1995; Safi *et al.*, 2012; Vincent & James, 1996). This simplified food web exaggerates the effects of abiotic variation found between ponds, for instance strong conductivity gradients will have a direct effect on the microbial community structure (Archer *et al.*, 2014; Howard-Williams *et al.*, 1989; Jungblut *et al.*, 2005; Sorrell *et al.*, 2013). Biological variability between ponds within a region such as those adjacent to Bratina Island, located <1 km apart, has been previously shown to manifest as variation in the abundance of key groups (Hawes *et al.*, 2011a; James *et al.*, 1995; Safi *et al.*, 2012).

1.6.2 Stratified pond water column

Depending on the surrounding topography (which influences wind mixing) and the ponds relative depth ratio, meltwater ponds may exhibit varying degrees of geochemical and biological stratification (Archer *et al.*, 2014; Hawes *et al.*, 2011a; Healy *et al.*, 2006; Wait *et al.*, 2006). The geochemical profile from Huey pond provides an excellent example of stratification of pH, conductivity and dissolved oxygen (Figure 1.2). The geochemically homogenised surface zone (via wind mixing) extended from the surface to 30-80 cm in depth until a sharp transition zone is reached where conductivity and associated ions increase significantly with depth. These vertical gradients are formed in early winter by downward freeze concentration, which results in high-density basal cryobrine (Grasby *et al.*, 2013; Horita, 2009). During the summer lower density freshwater input from local snow melt layers on top of this brine, ultimately forming a highly stratified water column (Wait *et al.*, 2006). The variation in geochemical and biological variables with depth is similar to that seen in large Dry Valley lakes, although across much smaller scales (Archer *et al.*, 2014; Comeau *et al.*, 2012).

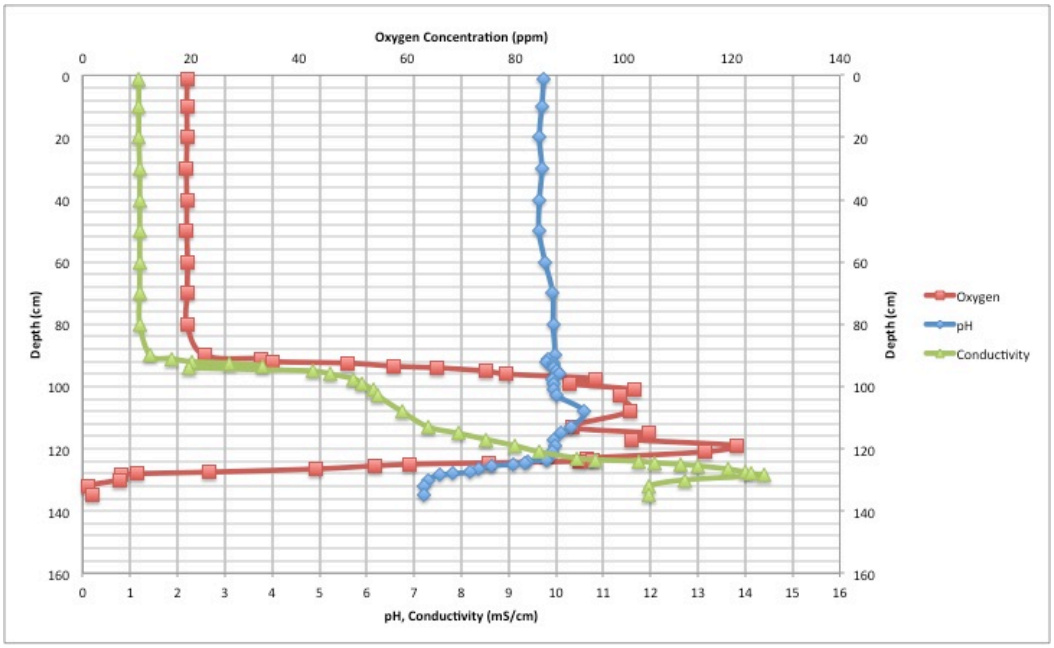

Figure 1. 2 Water column of Huey pond (2012) exhibiting a homogenous mixed zone to 80 cm then extreme stratification of pH, dissolved oxygen and conductivity to 135 cm.

1.6.3 Benthic microbial community

The thick *Cyanobacteria* dominated mats and underlying sediment (Figure 1.3) lining Antarctic aquatic environments have been well studied due to their environmental sensitivity and significance to the surrounding ecosystem (Dore & Priscu, 2001; Fernandez-Valiente *et al.*, 2001; Howard-Williams *et al.*, 1990; Moorhead *et al.*, 2003; Parker & Simmons, 1985; Parker *et al.*, 1982; Smol & Douglas, 2007; Wood *et al.*, 2008). The typical mat structure is a filamentous matrix of *Cyanobacteria* with various diatoms, heterotrophic bacteria and a low abundance of archaea (Brambilla *et al.*, 2001). Cyanobacterial groups frequently detected include *Chroococcales*, *Phormidium, Oscillatoria,* and *Nostoc* species (Brambilla *et al.*, 2001; Wood *et al.*, 2008). *Leptolyngbya* is particularly common throughout all the benthic mats and soils of Victoria Valley (Adams *et al.*, 2006; Aislabie *et al.*, 2006; Wharton *et al.*, 1983), leading to speculation that its presence throughout the soils is a direct result of wind dispersal from mats within the aquatic ecosystems in the valleys (Aislabie *et al.*, 2006; Hopkins *et al.*, 2006). The majority (97%) of algal diversity identified throughout continental Antarctica

belongs to the *Chlorophyta* and *Heterokontophyta* occurring in both aquatic and soil environments (Adams *et al.*, 2006). The underlying sediments have highly diverse bacterial communities dominated by *Proteobacteria* and *Bacteroidetes* (Bowman *et al.*, 2000; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002). As studies have increased sequencing depth, a greater diversity of phyla in these sediments has been identified including *Actinobacteria*, *Firmicutes*, *Verrucomicrobia* and *Acidobacteria* (Brambilla *et al.*, 2001; Peeters *et al.*, 2012; Rojas *et al.*, 2009).

Both mats and sediments have vertical structures likely related to changing conditions with depth (oxygen concentration, temperature and redox potential) (Howard-Williams *et al.*, 1989; Jungblut *et al.*, 2005; Shivaji *et al.*, 2011; Sjoling & Cowan, 2003; Ye *et al.*, 2009). The geochemically heterogeneous overlying water column contributes to a highly variable mat bacterial community between and within locations (Jungblut *et al.*, 2005; Peeters *et al.*, 2012; VanTrappen *et al.*, 2002), and variable local microbiology, surrounding geology, local climate and pond geochemistry further alter the mat structure (de los Rios *et al.*, 2004).

A large amount of biomass is created by primary production each year within the aquatic ecosystems of the Dry Valleys. This biomass accumulates around the edges of these water bodies, is freeze-dried and can be blown throughout the valley, distributing viable organisms and providing a valuable source of nutrients to the oligotrophic soils (Barrett *et al.*, 2006b; Elberling & Brandt, 2003; Moorhead *et al.*, 2003; Nkem *et al.*, 2006; Parker *et al.*, 1982; Wood *et al.*, 2008; Hopkins *et al.*, 2006). The relative size of these systems within a valley, as well as wind intensity and direction, will impact the importance of these sources on the valley-wide nutrient cycling and productivity (Hopkins *et al.*, 2006). In fact, it has been suggested there was a correlation between an absence of major lakes and low glacial melt in Beacon Valley and low cyanobacterial diversity (Wood *et al.*, 2008). Although a recent study suggests dispersal distances may be limited (<3 km) for *Cyanobacteria* (Sokol *et al.*, 2013), the detection of *Cyanobacteria* known to be ubiquitous in benthic mats in soils across the valleys indicates the ability of biological matter to broadly disperse (Aislabie *et al.*, 2006; Barrett *et al.*, 2006b; Wood *et al.*, 2008).

Although there is evidence of increased organic carbon within the vicinity

of lakes (Elberling & Brandt, 2003), ponds (Moorhead *et al.*, 2003) and streams (Aislabie *et al.*, 2006), some believe that contemporary water bodies do not provide as much carbon as that from 'legacy carbon' from ancient lake mats (Figure 1.3B) (Burkins *et al.*, 2000; Burkins *et al.*, 2001). This would explain the presence of organic matter in soils from valleys lacking any contemporary water bodies (Hopkins *et al.*, 2006); however, stable isotope signatures indicate that C and N in contemporary lake sediments provide the bulk of organic matter to the valleys, especially for nearby low elevation areas (Barrett *et al.*, 2006a)

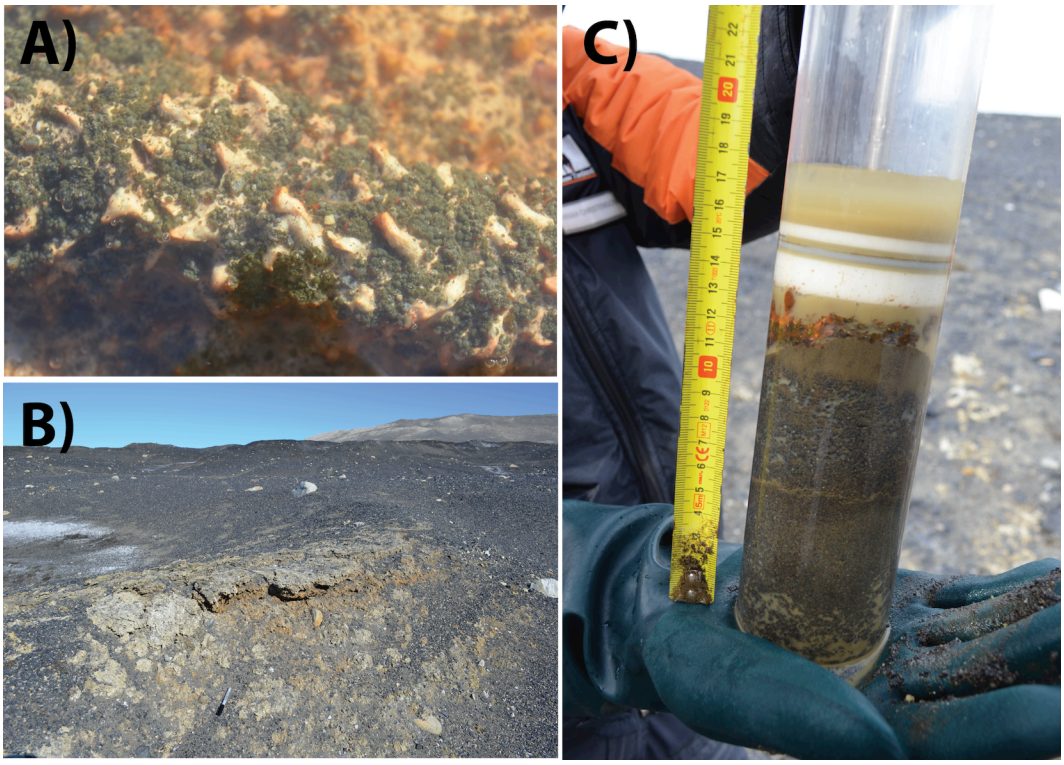

Figure 1. 3 A) The microbial mat lining a meltwater pond, B) an exposed section of ancient dried mat slowly being blown throughout the valley, C) the vertical structure of a mat and underlying sediment

1.7 Study Sites

Located <40 km apart, the study sites selected in this investigation represent ponds situated on the McMurdo ice shelf (Bratina Island) and in a terrestrial valley (the Miers Valley). These locations represent two geomorphically (the processes that form the landscape) distinct environments on

which the ponds form. Most studies of Antarctic aquatic environments are restricted to a single location with little work comparing systems, using consistent methodology, between locations (James *et al.*, 1995; Jungblut *et al.*, 2005; Villaescusa *et al.*, 2013). Given the close proximity of locations in this investigation and the ability of bacteria to move across great distances into and within Antarctica (Bottos *et al.*, 2014; Chong *et al.*, 2013; Jungblut *et al.*, 2010; Sokol *et al.*, 2013), spatial studies are useful to evaluate the extent of endemism between locations and the role local physicochemical structuring plays to the resident community.

Bratina Island (E165.55, S78.01) is a small mass of land at the northern tip of Brown Peninsula on the McMurdo Ice Shelf, southern Victoria Land, located approximately 30 km from New Zealand's Scott Base (Figure 1.4). A permanent camp was established in 1989 to support research of the many meltwater ponds adjacent to the facility resulting in more than two decades of research, with many ponds being sampled frequently over this period (Hawes *et al.*, 2013). The ponds are located on top of the 10-50 m thick floating ice shelf adjacent to Bratina Island. The ice shelf is covered with a 10-20 cm layer of marine sediments that have been integrated into the underside of the ice shelf and are deposited via surface ablation over hundreds of years (Fitzsimons *et al.*, 2012; Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988). More than 40 unofficially named ponds have been identified in the immediate area ranging in size, depth and geochemistry. Meteorological conditions in this region are comparable to those at Scott Base, based on a recent 20 year report on the area (Hawes *et al.*, 2013), with mean winter temperatures from -17°C to -22°C, and 0 to 20 degree-days above zero per year.

The McMurdo Dry Valleys are the largest ice-free region of Antarctica (0.03% of the continent or 4,800 square kilometers). Most valleys are lined with a <30 cm thick layer of arid mineral soil derived from weathering of glacial tills and bedrock (granite, sandstone, basalt and metamorphic rocks) on top of permafrost (Healy *et al.*, 2006; McLeod *et al.*, 2009). Coastal valleys have mean winter temperatures that range from -40°C to -60°C, and degree-days above freezing from 25 to 75 (Cary, *et al*., 2010). The Miers Valley is one of the southern most valleys in the McMurdo Dry Valley system that opens directly onto the McMurdo

Sound. The site comprises a west to east glacially carved valley with walls ranging from 170-1075 m in altitude and two glaciers at the western most point, Miers Glacier to the north and Adams Glacier to the south with melt streams feeding Miers Lake, a permanently ice covered lake 9 km inland, during the peak of summer. The valley is approximately 11 km long and 1.5 – 2.5 km wide (Cary *et al.*, 2010; Cowan *et al.*, 2011a; Dale *et al.*, 1999). The ponds used in this investigation are located at the eastern most point of the valley, which opens to the McMurdo Sound, to the south side.

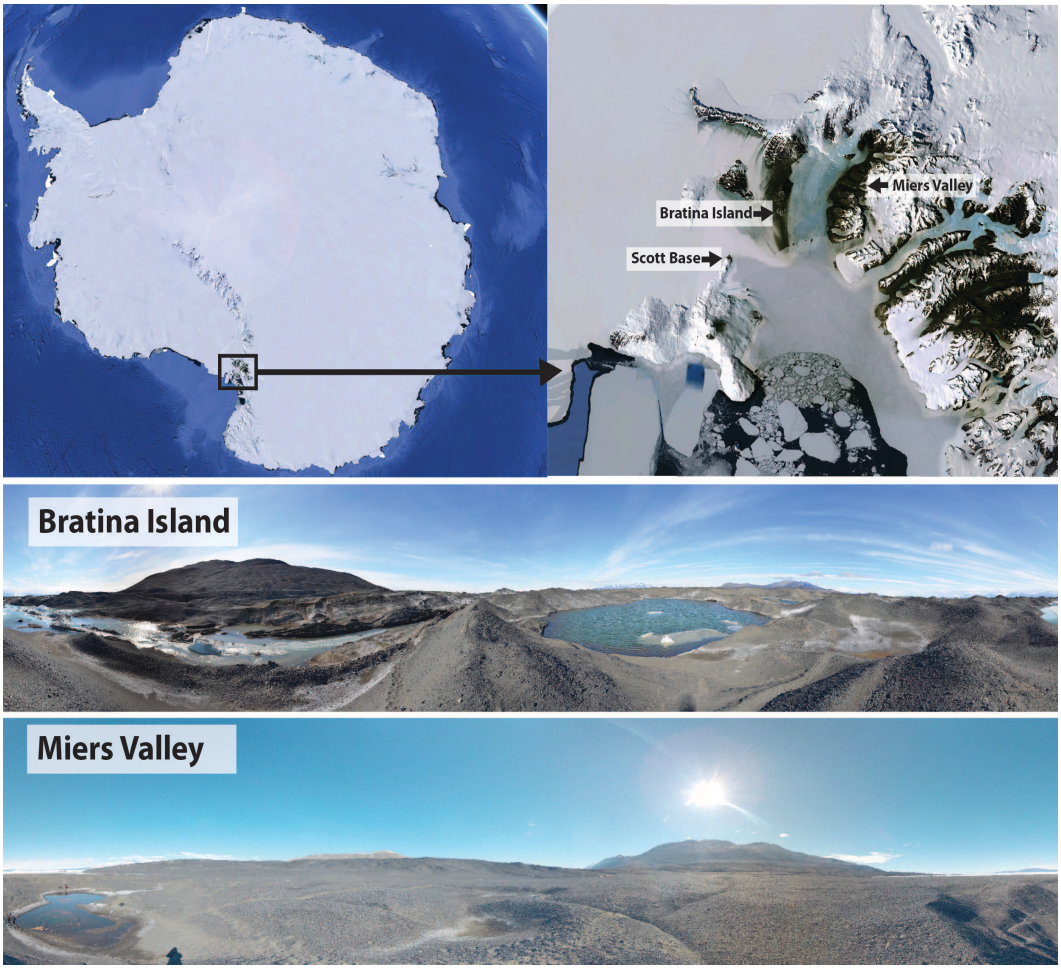

Figure 1. 4 The location of study sites in the Ross Sea Region of Antarctica (top) and typical landscapes presented as 360-degree panoramas. Satellite images gained through Google Earth Pro, Image (C) 2014 DigitalGlobe, U.S. Geological Survey.

1.8 Analysis of microbial communities

A broad variety of techniques have been developed to investigate the microbial communities of environmental samples. Past investigations of microorganisms in extreme environments utilized classical microbiology, involving microscopy and the growth of organisms on artificial media. While cultivation provides the opportunity to comprehensively describe the isolated organism, it is laborious and reduces the diversity of the observed community as only 1% of microorganisms are believed to be readily culturable (Pearce *et al.*, 2003; Torsvik & Ovreas, 2002; Vakhlu *et al.*, 2008; Wagner *et al.*, 1993). The development of Sanger sequencing provided an excellent tool to genetically characterize the microbial community; however, this required the isolation of individual sequences from colonies or the creation of clone libraries. Environmental samples frequently contain hundreds or thousands of individual species, making this method laborious and expensive. Recently, modern genetic tools have allowed the analysis of microbial communities directly from environmental DNA (Anderson & Cairney, 2004). Although subject to under or over-representation of the microbial diversity within a sample (Ashby *et al.*, 2007; Egert & Friedrich, 2003; Isenbarger *et al.*, 2008; Lee *et al.*, 2012b), their use has provided a more comprehensive comparison and/or description of environmental samples than previously attained through cultivation (Christen, 2008; Pearce *et al.*, 2003; Vakhlu *et al.*, 2008).

Genetic methods utilize variation in a gene universally present in the target organism, with conserved regions to allow for PCR primer annealing and amplification, and hypervariable regions to distinguish members of the community. In bacteria the 16S ribosomal RNA gene meets these criteria and has been used extensively in environmental studies (Ashby *et al.*, 2007; Banks *et al.*, 2009; Bernhard & Field, 2000; Bowman *et al.*, 2000; Brambilla *et al.*, 2001; Kemp & Aller, 2004; Lindstrom & Leskinen, 2002; Poitelon *et al.*, 2010; Sjoling & Cowan, 2003; Tringe & Hugenholtz, 2008; VanTrappen *et al.*, 2002; Zeng *et al.*, 2013). By sequencing the 16S rRNA gene from environmental samples

phylogenetic information can be obtained from all bacteria in the community simultaneously (Shokralla *et al.*, 2012).

The first 'next generation' sequencing platform commercially available was the Roche 454 Genome Sequencer in 2005. DNA fragments are amplified with primers including adapter sequences which bind to the surface of either a sepharose or styrofoam bead with complementary binding sequences in an oil-water emulsion, each capable of independent PCR amplification resulting in billions of identical copies adhered to each bead. These beads are distributed across a picotiter plate into individual wells (>1 million wells per plate) with enzyme beads where repeated cycling of PCR reagents containing a single nucleotide (A, T, C or G) per cycle generates the sequence. Detection is based on the pyrophosphate that is released during the incorporation of a nucleotide into the sequence; this is converted to ATP by sulfurylase which is used by luciferase to create light. Each light signal from every well is simultaneously recorded to create individual DNA sequences that, after screening by a series of quality filters, are ready for analysis (Shokralla *et al.*, 2012). Quality checking and processing of this sequencing data is conducted through open-source software such as Mothur (Schloss *et al.*, 2009). This is compared to open-source public databases (GenBank and the Ribosomal Database Project (Benson *et al.*, 1994; Maidak *et al.*, 1996)) to gain phylogenetic insight; however, as more culture independent sequences are added, it is difficult to match target sequences to database sequences with physiological information and to discern high from low quality sequences from previous investigations (Karp, 2001; Lee *et al.*, 2012a; Scholz *et al.*, 2012). The functionality of this method was further expanded by the addition of DNA barcodes to each amplified sequence so that multiple samples could be run on the same plate (Binladen *et al.*, 2007; Hoffmann *et al.*, 2007; Kasschau *et al.*, 2007; Parameswaran *et al.*, 2007). Limitations of the technology compared to Sanger sequencing include limited read length and low accuracy; however, the rapid rate of advancement of this technology (overtaking Moore's law) will soon resolve these issues. Further continual improvements include the minimization of PCR bias, reductions in cost and increased data output and sequencing throughput (Shokralla *et al.*, 2012).

Recently, investigations of microbial communities have shifted focus from describing individual organisms in the community (16S rRNA gene) to how the collective communities genes (its transcriptome) influences its functional activity (Varin *et al*., 2011; Vick-Majors *et al*., 2014). Although some inference of metabolic function can be gained from 16S rRNA gene sequence analysis, this is limited to the knowledge of related organisms in the analysis database (Comeau *et al*., 2012; Langille *et al*., 2013). A number of gene centric techniques have been developed to determine the gene expression of a microbial community based on environmental DNA and RNA samples. The recently released microarray Geochip 4 allows for a rapid and comprehensive analysis of functional gene expression from over 410 gene families covering 141995 coding sequences (Bayer *et al*., 2015; Tu *et al*., 2014). While more labor intensive than microarray technology whole transcriptome shotgun sequencing using next generation sequencing allows for a more detailed and accurate investigation of the transcriptome where genes without probes on a microarray can be identified (Chu *et al*., 2012; Gilbert *et al*., 2008; Morin *et al*., 2008).

DNA fingerprinting techniques enable a quick and cost effective screen of many samples for microbial community relative diversity and composition (Ranjard *et al.*, 2000). They utilize variable sequence size separated via gel electrophoresis to provide a genetic "fingerprint" of the total community, which can be used to compare samples. Automated Ribosomal Intergenic Spacer Analysis (ARISA) has been extensively used for environmental screening as it is rapid, cost effective and highly reproducible (Banks *et al.*, 2009; Cardinale *et al.*, 2004; Chow *et al.*, 2013; Fisher & Triplett, 1999; Kovacs *et al.*, 2010; Lear & Lewis, 2009; Lee *et al.*, 2012a; Popa *et al.*, 2009; Roesch *et al.*, 2009; Smith *et al.*, 2010; Sokol *et al.*, 2013; Soo *et al.*, 2009; Wood *et al.*, 2008). The target sequence is the non-coding, hyper variable Internally Transcribed Spacer (ITS) region between the 16S and 23S ribosomal RNA (rRNA) genes. PCR amplification of this region integrates a labeled fluorescent tag from one of the primers, which can be detected through capillary electrophoresis equipment equipped with a fluorescence detector. The variable sizes of the labeled products create the fingerprint, which can then be compared between samples (Fisher & Triplett, 1999).

The advancement of culture independent molecular microbial ecology has allowed unknown organisms, and the extent of biodiversity in samples previously thought to be biologically constrained by extreme environments, to be identified (Brambilla *et al.*, 2001; Hugenholtz *et al.*, 1998; Pearce *et al.*, 2010; Ranjard *et al.*, 2000). Rapidly advancing DNA sequencing technology has resulted in a paradigm shift in how microbial communities can be investigated. With continually lowering sequencing costs, increased samples per run, and increases in reads per sample, conducting a sequence-based comparative analysis of variation between microbial communities has become commonplace (Laas *et al.*, 2014; Villaescusa *et al.*, 2013; Zeng *et al.*, 2013; Comeau *et al.*, 2012; McDonald *et al.*, 2012), allowing a greater understanding of global microbial communities than ever before.

1.9 Outline

The overall aim of this thesis was to elucidate and comprehensively describe the bacterial structure of meltwater ponds in the surface water, water column and benthic zone across regional (Miers Valley to Bratina Island) and temporal (December 2009, January 2012 to January 2013) scales, and determine the geochemical drivers to this structure using the same high-resolution methodology (Figure 1.5). As extensive research has been conducted on the cyanobacterial component of these ponds (de los Rios *et al*., 2004; Fernandez-Valiente *et al*., 2001; Hitzfeld *et al*., 2000; Jungblut *et al*., 2010; Jungblut *et al*., 2005; Wood *et al*., 2008) this study elected to utilize methodology that would specifically investigate the poorly described non-cyanobacterial component of the microbial community.

Scientific findings are presented in Chapters 2-5, which have been organized as independent papers for publication in peer-reviewed journals. I was the primary researcher involved in all sample collection, processing and analysis of samples as well as the primary author of each chapter. Co-authorship forms and prefaces for each chapter outline contributions made by other researchers.

Chapter 2 describes the bacterial and cyanobacterial community structure within the water columns of five physicochemically distinct meltwater ponds

adjacent to Bratina Island on the McMurdo ice shelf. This work was undertaken as a pilot study and has been expanded to a larger number of ponds at higher resolution in subsequent chapters. ARISA was used to compare microbial communities, which was coupled to *in-situ* collected geochemistry, ICP-MS and nutrient data (NO_2/NO_3, NH_4 and PO_4) to identify drivers to community structure using BEST analysis. Analysis of similarities (ANOSIM), Tukey's honest significance test and Spearmans Coefficient were used to investigate microbial heterogeneity between and within ponds. Based on this data three samples were selected from within a highly stratified pond for 454 pyrosequencing to provide taxonomic information to observed changes in bacterial community structure. This was the most in-depth study of bacterial communities within and between the water columns of Antarctic ponds and identified key drivers of community structure and insight into the bacterial composition of these ponds. This chapter has been published with the following citation:

> **Archer SDJ**, McDonald IR, Herbold CW, Cary SC (2014) Characterisation of bacterioplankton communities in the meltwater ponds of Bratina Island, Victoria Land, Antarctica. FEMS Microbiology Ecology. **89(2):** 451-464.

Chapter 3 describes the microbial and physicochemical heterogeneity between 41 pond surface waters from Bratina Island (McMurdo Ice Shelf) and the Miers Valley (terrestrial dry valleys), as well as community structuring caused by temporal, spatial and physicochemical drivers. ARISA DNA fingerprinting, pH and conductivity data were used to select 17 samples for detailed analysis including high throughput sequencing, nutrient and ICP-MS. BEST, ANOSIM, and Tukey's honest significance test were used to resolve community similarity and physicochemical drivers. Pyrosequencing identified a small number of ubiquitous operational taxonomic units (OTUs) between locations, implying microbial exchange, and across time scales, although seasonal and annual variations in community structure were identified. This chapter has been submitted as a manuscript to *Polar Biology* and is in review.

Chapter 4 describes the microbial community structure across steep physicochemical gradients within six Bratina Island and two Miers Valley meltwater ponds. The persistence of physicochemical and microbial stratification

was also evaluated by sampling two water columns at Bratina Island in both January 2012 and 2013. Sampling intervals within the water column were guided by *in-situ* pH and conductivity across distances as small as 0.5 cm, providing the highest resolution investigation of polar ponds to date. The community structure from the large number of samples was determined by bacterial ARISA, from which representatives were selected for high-throughput 454 sequencing, ICP-MS and nutrient analysis. A comprehensive data set was selected for Huey pond (Bratina Island) in 2012 and three comparative samples each from Huey pond in 2013 and Morepork pond (Miers Valley) in 2013 were sequenced to gain phylogenetic insight into community structure changes with depth between locations and time points. ANOSIM and BEST analysis were used to evaluate community relatedness and physicochemical drivers between and within ponds. Community structure varied significantly with depth linked to strong selective geochemistry within the water columns of these ponds. Trace elements were identified as significant to variations identified between sites although even in deep samples taxa were shared. Community structure varied between ponds; however, dominant taxa were shared across temporal and spatial scales. This manuscript is in preparation for submission to *FEMS Microbiology Ecology*.

Chapter 5 describes the microbial heterogeneity between the benthic communities of six meltwater ponds from Bratina Island and six from the Miers Valley. ARISA DNA fingerprinting of community structure, pH and conductivity of ponds between locations was highly similar, resulting in the selection of three comparable ponds from each location for 454 pyrosequencing. Major phyla in this study were consistent with previous work; however, a far greater diversity was identified due to the high-resolution methodology. BEST analysis identified a number of drivers to individual pond community structure with no causative factors identified separating locations. This manuscript has been published with the following citation:

> **Archer SDJ**, McDonald IR, Herbold CW, Lee CK, Cary SC. (2015) Benthic microbial communities of coastal terrestrial and ice shelf Antarctic meltwater ponds. Frontiers in Microbiology, Terrestrial Microbiology. **6(485):** doi 10.3389/fmicb.2015.00485

Chapter 6 provides a synthesis of results across the pond elements over time including data not previously analyzed from samples collected during the winter freeze up. Future research directions of this work are then presented.

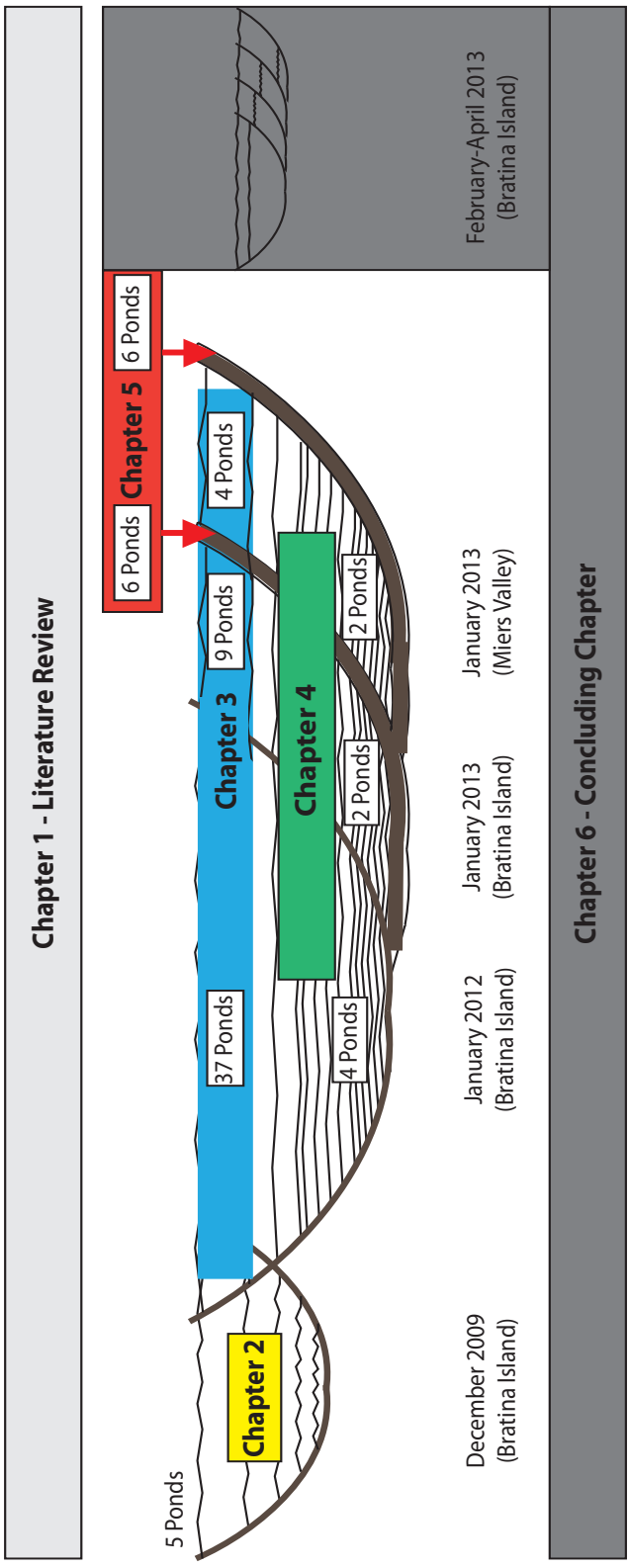

Figure 1. 5 Info-graphic overview of thesis

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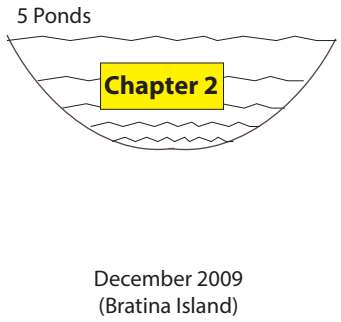
Chapter 2 - Characterization of bacterioplankton communities in the melt-water ponds of Bratina Island, Victoria Land, Antarctica

Preface

This chapter describes the microbial community structure and physicochemical drivers between and within a set of five meltwater ponds collected in the 2009-2010 field season. Sampling, DNA extraction and DNA fingerprinting were originally undertaken as my Masters thesis and was submitted in 2011 at the University of Waikato under the same title. Since submission a pyrosequencing effort was made, all original data were re-analyzed and the entire chapter re-written to gain a publishable manuscript. As the primary author I undertook sample collection, wrote the manuscript and did most of the data analysis. I completed all lab work including sample preparation, however inductively coupled mass spectroscopy (ICPMS) and DNA sequencing were carried out by technicians at the University of Waikato. Craig Herbold earned co-authorship for his part in developing bioinformatics pipelines used to analyse this data and his assistance in statistical analysis. Ian McDonald, my supervisor, prepared Figure 2.6. All authors reviewed and edited this manuscript. This work has been published with the following citation.

> Archer SDJ, McDonald IR, Herbold CW, Cary SC (2014) Characterisation of bacterioplankton communities in the meltwater ponds of Bratina Island, Victoria Land, Antarctica. FEMS Microbiology Ecology. **89(2):** 451-464



Preface Figure 2. 1 Breakdown info-graphic overview of thesis

FEMS MICROBIOLOGY ECOLOGY

RESEARCH ARTICLE

Characterisation of bacterioplankton communities in the meltwater ponds of Bratina Island, Victoria Land, Antarctica

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Abstract

A unique collection of Antarctic aquatic environments (meltwater ponds) lies in close proximity on the rock and sediment-covered undulating surface of the McMurdo Ice Shelf, near Bratina Island (Victoria Land, Antarctica). During the 2009–10 mid-austral summer, sets of discrete water samples were collected across the vertical geochemical gradients of five meltwater ponds (Egg, P70E, Legin, Salt and Orange) for geochemical and microbial community structure analysis. Bacterial DNA fingerprints (using Automated Ribosomal Intergenic Spacer Analysis) statistically clustered communities within ponds based on ANOSIM ($R = 0.766$, $P = 0.001$); however, one highly stratified pond (Egg) had two distinct depth-related bacterial communities ($R = 0.975$, $P = 0.008$). 454 pyrosequencing at three depths within Egg also identified phylum level shifts and increased diversity with depth, *Bacteroidetes* being the dominant phyla in the surface sample and *Proteobacteria* being dominant in the bottom two depths. BEST analysis, which attempts to link community structure and the geochemistry of a pond, identified conductivity and pH individually, and to a lesser extent Ag^{109}, NO_2 and V^{51} as dominant influences to the microbial community structure in these ponds. Increasing abundances of major halo-tolerant OTUs across the strong conductivity gradient reinforce it as the primary driver of community structure in this study.

Introduction

The Antarctic continent offers access to one of the most physically and chemically demanding environments on earth. Antarctic limnetic ecosystems (lakes and ponds), in particular, experience large fluctuations in temperature and light regimes and are characterised by steep chemical gradients, which greatly impact physiological adaptations and life cycle strategies (Wait *et al.*, 2006). These ecosystems, scattered throughout the Antarctic continent and on the surrounding sea ice, provide an extraordinary and tractable opportunity to examine microbial distribution in distinct and environmentally extreme microenvironments. Of the thousands of lakes and ponds throughout Antarctica, most research has focused primarily on lakes due to their size, stability and influence on the surrounding terrestrial system (Craig *et al.*, 1992; Bell & Laybourn-Parry, 1999; Bowman *et al.*, 2000; Pearce *et al.*, 2003; Taton *et al.*, 2003). However, due to their smaller size, ponds can be more susceptible to seasonal (and global) climatic changes, while offering easy access to a broad range of unique pond-specific geochemical conditions.

During the peak of the Antarctic summer up to 20% of the McMurdo Ice Shelf (MIS) in southern Victoria Land is covered with meltwater ponds of various sizes, making them a dominant environment in this area (De Mora *et al.*, 1994). The ponds surrounding Bratina Island on the MIS (Fig. 1) provide a cross-section of the many geochemistries found in aquatic systems throughout Antarctica (Cowan & Tow, 2004). Their existence is dependent on the short period, at the peak of summer, when liquid water from local ice melt collects in the depressions of the rugged landscape of the MIS replenishing pre-existing ponds and forming new ones (Howard-Williams & Hawes, 2005). Marine-derived surface sediments and aerosols, as well as seawater trapped in tide cracks across the ice shelf, provide multiple variable inputs of dissolved salts into ponds. The fine balance between water and salt

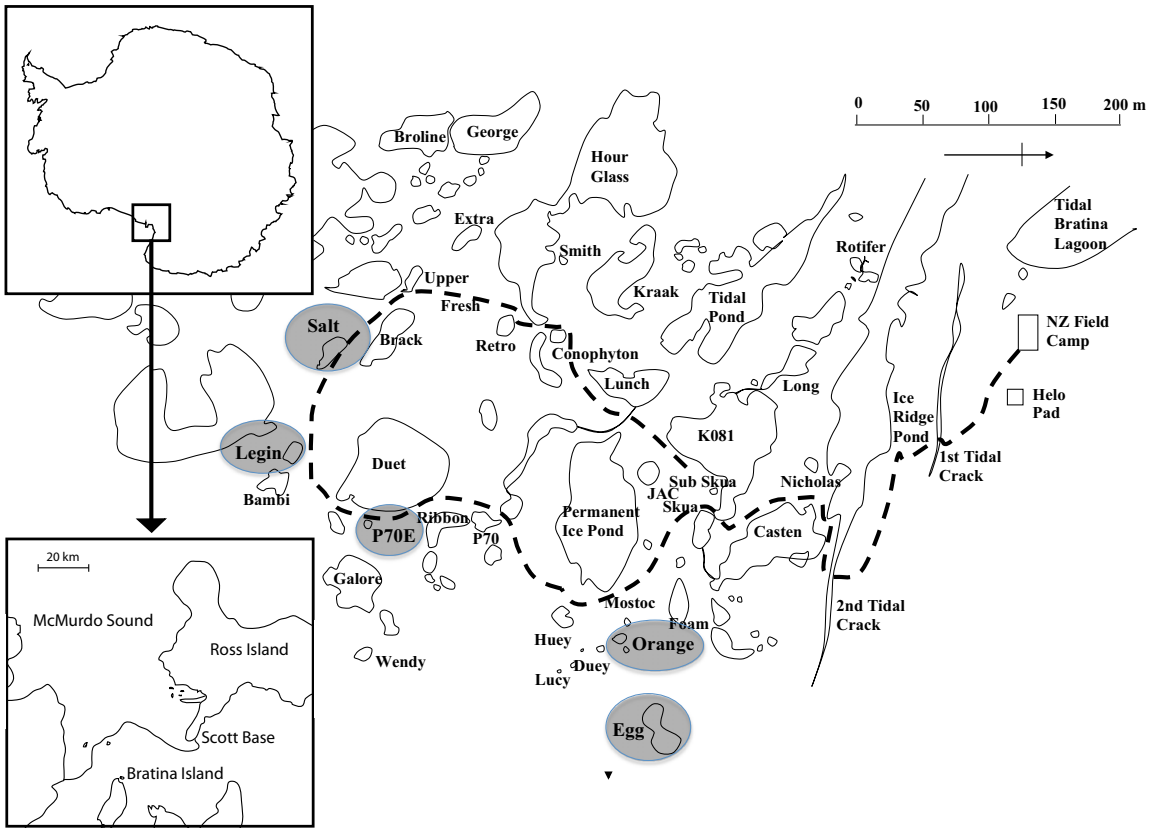

Fig. 1. Map of the MIS Ponds, based on an original map produced by New Zealand Department of Land and Surveys, 1991. Ponds circled were used in this study.

input and evaporation leads to both inter and intraseasonal variation of physicochemistry within a pond (Laybourn-Parry *et al.*, 2002) and variable chemistries between ponds. Ponds in close proximity (metres apart) can vary greatly in size (one to several hundred square metres), depth (< 1 m to around 4 m) and age (1 to at least 30 years old) (Gibson *et al.*, 2006), as well as in metal concentrations, salinity (Schmidt *et al.*, 1991; Matsumoto *et al.*, 1992), pH and dissolved oxygen (Koob & Leister, 1972). The water column within some ponds can be highly stratified, with a unique brine geochemistry derived from the freeze concentration of dissolved salts layering the bottom and newer meltwater input layering the surface. Oxygen, derived from *Cyanobacteria* dominated mats lining the ponds, also exhibits a steep gradient increasing with depth, frequently resulting in oxygen supersaturation in the lower depths of certain highly stratified ponds (James *et al.*, 1995; Wait *et al.*, 2006). Photosynthesis, which causes the oxygen supersaturation, increases pH via the removal of CO_2 from the water reducing carbonic acid (Hendriks *et al.*, 2014), both parameters are constrained by the conductivity density gradients, illustrating the interplay between biotic and abiotic variables along physicochemical gradients that occur with depth in these ponds (Wait *et al.*, 2006).

Past studies of Antarctic ponds, and lakes, have provided some insight into how microbial communities are structured in these extreme aquatic environments. These studies primarily focused on the dense microbial mats and sediments lining the ponds (Fernandez-Valiente *et al.*, 2001; Griffin & Tiedje, 2007) and on the geochemistry of the water column (Matsumoto *et al.*, 1992; De Mora *et al.*, 1994; Wait *et al.*, 2006), with little or no work on the microbiology of the water column itself. Previous research has also been limited by a reliance on microscopic identification of microorganisms, (Vincent & James, 1996; de los Rios *et al.*, 2004; Sabbe *et al.*, 2004), which suggested these pond ecosystems supported low biodiversity, probably due to the presence of cryptic species. However, more recently, the use of culture-independent molecular genetic tools has

allowed microbiologists to identify and investigate a more extensive biodiversity in these systems (Vincent & James, 1996; Brambilla *et al.*, 2001; Sjoling & Cowan, 2003; Hughes *et al.*, 2006; Cary *et al.*, 2010).

This project is the first in-depth study to characterise the microbial communities in the water column of Bratina Island ponds and to describe their relationship to pond geochemistry. Utilising molecular genetic tools (a combination of DNA fingerprinting and high throughput next-gen sequencing) coupled with geochemistry, five ponds were characterised, resulting in the most stratified pond being selected for higher resolution analysis. These data allowed for the identification of possible geochemical drivers, and the specific bacterial groups they control, within a highly stratified water column.

Materials and methods

Field sampling strategy

Water column samples were collected from five separate ponds near Bratina Island 78°01′S latitude, 165°32′E longitude on the MIS (Fig. 1) shortly after most ponds became ice-free at the beginning of the austral summer in December 2009. Sampled pond depths ranged from 0.3 to 0.5 m (Supporting Information, Table S1) and sizes from 3 to 20 m across. Four ponds were ice-free, but a 1-cm surface layer and the middle 25% of Legin pond was still frozen at this time. A customised micromanipulator/sampling apparatus was constructed (Fig. S1) and used to simultaneously collect physicochemical data (*in situ* data used to determine sampling frequency) and water samples from the approximate centre of each pond at 2–10 cm increments (± 1 mm) through a sterilised sampling tube (TYGON® R-3603) that was insulated and capable of mild heating with a nicrome wire heating tape controlled via a rheostat. The tube was flushed with two void volumes (at each depth) before each sample was collected. 8–100 mL of sample was immediately filtered through syringe-mounted 0.22-μm filters (Whatman International Ltd, Kent, UK) until the filter clogged. A small amount of air was pushed through the filter to remove excess water, then gently flooded with a nucleic acid preservative/lysis buffer (CTAB – cetyltrimethylammonium bromide-polyvinyl-pyrrolidone-β-mecaptoethanol) (Coyne *et al.*, 2001) and frozen for transport back to the laboratory. Thirteen millilitre of the filtrate was also collected in 15-mL falcon tubes and frozen immediately for later geochemical analysis. Oxygen concentration and temperature were measured *in situ* using probes attached near the tip of the sampling tube. Oxygen data were collected using a Fibox 3 LCD trace minisensor oxygen metre with data-logger (PreSens Precision sensing, Regensburg, Germany), and temperature data were collected using a Hobo temperature data logger (H09-003-08, Onset Computer Corporation). Finally, a 30 mL unfiltered sample was collected in a 50-mL falcon tube for immediate pH, and conductivity measurements performed in the field using a SPER Scientific water quality meter (Sper Scientific, Arizona).

Nutrient and elemental analysis

NH_4, NO_2 and PO_4 measurements on the filtrate from each sample were carried out at the University of Waikato using an Aquakem 200CD following the manufacturer's instructions (Thermo Fisher Scientific, Waltham). Elemental analysis was performed on each sample by inductively coupled plasma mass spectrometry (ICP-MS) using a Mass Spectrometer ELAN® DRC II (PerkinElmer Inc., Münster, Germany). To prepare samples for ICP-MS, 0.22 μm prefiltered pond water was diluted 1 : 50 with Milli-Q water (Millipore, Billerica, MA). Six samples were diluted 1 : 1000 due to excessive salt concentrations. Once diluted, samples were acidified with 2% $HN0_3$ (Extra pure Nitric Acid, Ajax Finechem, NSW, Australia). Elements analysed were the following: Li^7, B^{10}, Na^{23}, Mg^{24}, Al^{27}, S^{34}, K^{39}, Ca^{43}, V^{51}, Cr^{52}, Fe^{54}, Mn^{55}, Co^{59}, Ni^{60}, Cu^{65}, Zn^{68}, As^{75}, Se^{82}, Sr^{88}, Ag^{109}, Cd^{111}, In^{115}, Ba^{137}, Tl^{205}, Pb^{207}, Bi^{209} and U^{238} (results of all measurable variables are in Table S1).

DNA extraction of samples

DNA was extracted from the filtered biomass using a modified CTAB extraction protocol (Campbell *et al.*, 2001). Briefly, the frozen, sealed 0.22-μm sample filters were first thawed on ice and then connected to a syringe containing 1 mL of fresh CTAB. The exposed filter nipple was parafilmed, and the entire assembly incubated in a Ratek Orbital mixer at 150 r.p.m. and 65 °C for 30 min. The filter assembly was allowed to cool then 0.5 mL of the CTAB in the syringe was pushed through the filter assembly to evacuate the lysate into an eppendorf tube. An equal volume of chloroform/isoamyl alcohol (24 : 1) was then added to the lysate and mixed on the orbital mixer at 150 r.p.m. and 65 °C for a further 30 min. The eppendorf tube was then centrifuged for 15 min at 16 900 ***g***, and the aqueous phase transferred to a new tube. Nucleic acids were precipitated by addition of 1 volume of isopropanol and 0.5 volume of 5 M NaCl and then incubated at −80 °C for at least 1 h. The tube was then centrifuged at 26 000 ***g*** for 30 min, and the supernatant discarded, and the DNA pellet washed with 0.5 mL of 70% EtOH and then centrifuged at 15 500 r.p.m. for 5 s. The pellet was dried and resuspended in 10–50 μL of sterile milliQ H_2O. The extracted

DNA was quantified using a Nanodrop ND-1000 at 260 nm (NanoDrop Technologies, Montchanin, DE).

Automated ribosomal intergenic spacer analysis (ARISA)

ARISA DNA fingerprinting (Fisher & Triplett, 1999) was utilised to resolve bacterial community structure and relative diversity within all ponds, and a comparative subset (Egg, Legin and P70E) was selected to resolve cyanobacterial community structure in Egg pond. From each sample, the bacterial intergenic spacer region (ISR) in the rRNA operon was amplified using PCR. Triplicate 30 μL reactions [to reduce stochastic PCR bias (Wintzingerode *et al.*, 1997)] were run for each sample. Bacterial primers used were ITSReub-Hex (5′-GCCAAGGCATCCACC-3′) and ITSF [5′-GTCGTAACAAGGTAGCCGTA-3′) (Cardinale *et al.*, 2004)] and the cyanobacterial primers were CY-ARISA-F (5′-GYCAYRCCCGAAGTCRTTAC-3′) and 23S30R (5′-CHTCGCCTCTGTGTGCCWAGGT-3′) (Wood *et al.*, 2008)]. Full PCR components and conditions and quality control procedures are described in Supporting Information.

To visualise, compare and interpret the ARISA fingerprints from different samples, the output was processed with an informatics pipeline (modified from (Abdo *et al.*, 2006) First, all peaks with heights exceeding 250 fluorescence units were accepted as true peaks. The remaining peaks were used to calculate model parameters for a log-normal distribution. Iteratively, peaks with an area exceeding the 99.9% cumulative distribution of the calculated log-normal distribution for noise were accepted as true peaks. Fragment lengths < 100 bp for bacteria and 150 bp for cyanobacteria were removed from analysis as these were considered to be too small to be ITS fragments (Cardinale *et al.*, 2004; Wood *et al.*, 2008). Peaks were binned into ARISA Fragment Lengths (AFLs) with width = 2 nt, and total peak area of each bin was used to calculate relative adundance of each AFL in a given data set. The resulting data matrix was imported into PRIMER 6 for statistical analysis (Clarke & Gorley, 2006).

In PRIMER 6, peaks were converted to presence/absence data for further analysis, and the number of peaks was totalled from each sample to be used as a proxy for relative alpha diversity (the number of species in a sample). These were subjected to various statistical tests (Spearman's coefficient and Tukey's honest significant difference test) to elucidate relationships between communities. Beta diversity (the comparison of diversity between sites) was investigated using a resemblance matrix created based on the Bray Curtis similarity index (Bray & Curtis, 1957). From the resemblance matrix, a hierarchical clustering analysis was performed, producing a relational dendrogram. Nonmetric multidimensional scaling (MDS) was performed on the resemblance matrix, which displays relative similarities between communities as distance (i.e. the closer two samples are the more similar community). 2-D MDS plots with a stress value of < 0.2 were used as they were considered to have accurate information. Information from the dendrogram of the communities was overlaid onto the 2-D MDS plots, which provide percentage similarity levels at 20, 40, 60 and 80%, assisting in the evaluation of community structure between samples. ANOSIM analyses and Mantel tests were performed on the resemblance matrix to test specific hypotheses formed from interpretation of MDS plots.

The influence of pond geochemistry on the microbial community was determined using a BEST analysis to find the 'best match' in PRIMER 6. Geochemical data were normalised and a Draftsman plot created to view interrelations between the different variables, so the data could be simplified. Spearman's rank correlation coefficients were then calculated, resulting in a probability (P) of the community differences being explained by the differences in geochemistry. The combination of geochemical variables whose Euclidean distance matrix gave the highest P-value were considered the most likely drivers of community dynamics.

454 pyrosequencing

PCR amplicons containing V5–V6 hypervariable regions of the 16S rRNA gene were utilised to identify specific bacterial community differences in the water column of Egg pond using 454 pyrosequencing. Triplicate 30 μL reactions were run for each sample, each reaction containing 0.4 μM of each unadapted primer Tx9F (5′-GGATTAGAWACCCBGGTAGTC-3′) and 1391R (5′-GACGGGCRGTGWGTRCA-3′) (Ashby *et al.*, 2007), 1x PCR buffer, 0.2 mM dNTPs, 0.02 mg mL^{-1} BSA, 0.02 U Platinum Taq, 2 mM $MgCl_2$, 20 ng bp genomic DNA, and the reaction was made up to 30 μL with milliQ H_2O. Thermal cycling conditions were the following: 94 °C for 2 min, then 30 cycles of 94 °C for 20 s, 55 °C for 10 s (−0.2 °C per cycle), 72 °C for 20 s and a final extension of 72 °C for 3 min. Once amplified, all triplicate PCRs were combined, run on a 2% TAE agarose gel stained with 'SYBR Safe' (Invitrogen Ltd) at 80 V, the bands excised and DNA retrieved using the MO BIO UltraClean 15 DNA Purification Kit (MO BIO Laboratories, Carlsbad, CA) as per manufacturer's instructions, but running the final spin step twice. A second round of triplicate PCR was run as above but with only 10 cycles and using 25 ng of the purified DNA from the previous step per reaction (milli-Q H_2O volume adjusted accordingly). The primers used were adapted for one-way reads

according to the Roche GS Junior System Guidelines for Amplicon Experimental Design Manual (August 2010), including unique MID identifiers for each sample [BacX-Tx9F (5′-CCATCTCATCCCTGCGTGTCTCCGA CTCAG-MID-GGATTAGAWACCCBGGTAGTC-3′) and BacB-1391R (5′-CCTATCCCCTGTGTGCCTTGGCAGTC TCAG-GACGGGCR GTGWGTRCA-3′)]. A second gel extraction was performed as above. Samples went through a final cleanup step using the Agencourt AMPure XP system (Beckman Coulter Genomics, Danvers, MA) as per the manufacturer's instructions. A verification gel was run on a 2% TAE gel to confirm a lack of unwanted bands in the sample. Sample DNA content was quantified using a Qubit Flurometer (Invitrogen Ltd) and was then diluted to 1×10^9 molecules μL^{-1} as per the Roche Amplicon Library Preparation Method Manual [GS Junior Titanium Series, May 2010 (Rev. June 2010)]. QPCR using a KAPA Library Quantification Kit for Roche 454 Titanium/Universal (Kapa Biosystems, Woburn, MA) was used to check the 1×10^9 dilution and was adjusted accordingly for making the amplicon library. The diluted amplicons were mixed together in the desired proportions to create the 1×10^9 amplicon pool. Sequencing was performed using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTiterPlate Kit and GS Junior System according to the manufacturer's instructions (Roche 454 Life Sciences, Branford, CT).

454 pyrosequencing data processing

454 PCR amplicon pyrosequencing data were first processed using AmpliconNoise v1.0 (Quince *et al.*, 2011). Briefly, raw flowgrams (sff files) with perfectly matching primer and barcode sequences were filtered for a minimum flowgram length of 360 cycles (including primer and barcode sequences) before the first noisy signal (i.e. 0.5–0.7 or no signal in all four nucleotides). All flowgrams were then truncated at 360 bases and clustered to remove sequencing noise using PyroNoise (Quince *et al.*, 2009, 2011). Noise introduced by PCR was removed using SeqNoise (Quince *et al.*, 2011), and PCR chimeras were removed using Perseus (Quince *et al.*, 2011). The resulting de-replicated sequences were processed using Mothur 1.17.0 (Schloss *et al.*, 2009) to create a unique sequence and names file. Pairwise alignments and distance were calculated using Espirit (Sun *et al.*, 2009). Mothur was then used to cluster sequences into operational taxonomic units (OTUs) defined at the furthest neighbour Jukes–Cantor distance of 0.03 ($OTU_{0.03}$). Rankabundance data were generated for each sample as well as Venn diagrams and Good's nonparametric coverage estimator was used to evaluate sequencing coverage for each sample. For phylogenetic assignments, representative sequences of all identified $OTU_{0.03}$ were analysed using the Classifier function provided by the Ribosomal Database Project (RDP) Release 10, Update 15 (Wang *et al.*, 2007). Taxonomic assignment threshold was set at 80%. Phylogenetic analysis of sequences was performed with representative sequences of the top 101 $OTUs_{0.03}$ (> 0.1% overall abundance in pyrosequencing data sets, representing 12 376 reads). Taxonomic trees were constructed using ARB (Ludwig *et al.*, 2004), with DNADIST and neighbour joining analysis. Sequence length ranged from 244 to 392 bp, with an average of 384.9 bp.

Results

Physicochemical analysis

Inter and intrapond physicochemical properties were found to be extremely variable, with dissolved oxygen concentrations from 4.4 to 27.3 ppm (Fig. 3a), conductivity ranging from 0.43 to 110.9 mS cm^{-1} (Fig. 3b), pH from 7.3 to 10.1 (Fig. 3c), and temperature from −3.5 to 5.1 °C (Fig. 3d). A notable geochemical gradient was identified throughout the water column of most ponds. Oxygen concentration varied through the water column, but generally it was highest at the deepest point (increasing from 9.7 at the top to 17.0 ppm in the bottom of Legin). Conductivity was also found to increase with depth in each pond (from 8.2 mS cm^{-1} at the top to 93.6 mS cm^{-1} at the bottom of Egg). Although there are significant intrapond variations, the geochemistry MDS analysis (Fig. 2) clearly shows that each pond is geochemically distinct, with Egg forming two distinct clusters.

Egg pond exhibits the most extreme physicochemical stratification observed in this study (Fig. 3a–d). The pH was stable between 9.4 and 9.5 in the upper 38 cm, dropping to 8.8 at 40 cm and to 8.1 at the bottom (48 cm). The drop in pH corresponds with an increase in conductivity, which rose modestly from 8.2 mS cm^{-1} at 0 cm to 10.4 mS cm^{-1} at 38 cm, with a steep increase between 40 and 46 cm, up to 93.6 mS cm^{-1} at 48 cm deep. Dissolved oxygen within Egg increased from 11.5 ppm at the surface, to 17.1 ppm at 36 cm, and 27.3 ppm at 44 cm. The temperature profile was relatively stable, decreasing from 1.6 °C at the surface to −1.1 °C at 46 cm, with a notable increase at the bottom (48 cm) to 0.6 °C.

NH_4 was only detected throughout the water column in Egg and Salt pond (which also have the greatest conductivities) and at the top of Orange and at the bottom of P70E. No NH_4 was detected in Legin. NO_2 was found at extremely low concentrations in all ponds, except P70E where it was 10 times higher in the upper region (0.032 ppm compared with 0.002 ppm in most other ponds). ICPMS analsis revealed that most resolved

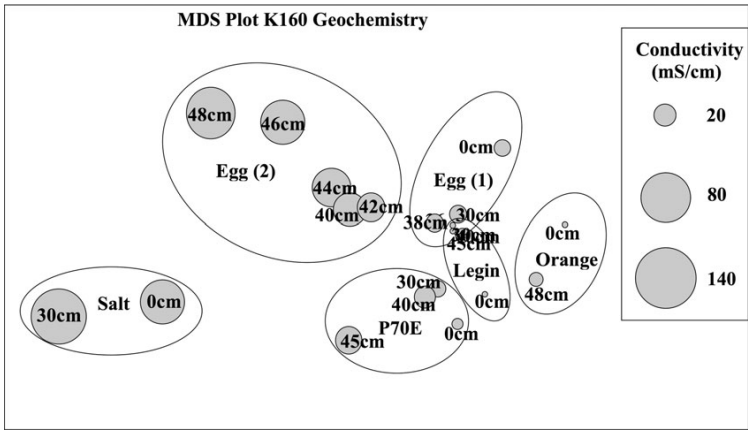

Fig. 2. MDS plot of geochemistry comparing all ponds studied, numbers represent sample depth. The greater the distance between two points, the greater the difference between their geochemistries. Circles surrounding samples represent relative conductivity concentration (the larger the circle the higher the conductivity). MDS plot 2-D stress: 0.08. Outlining circle used to identify pond clusters.

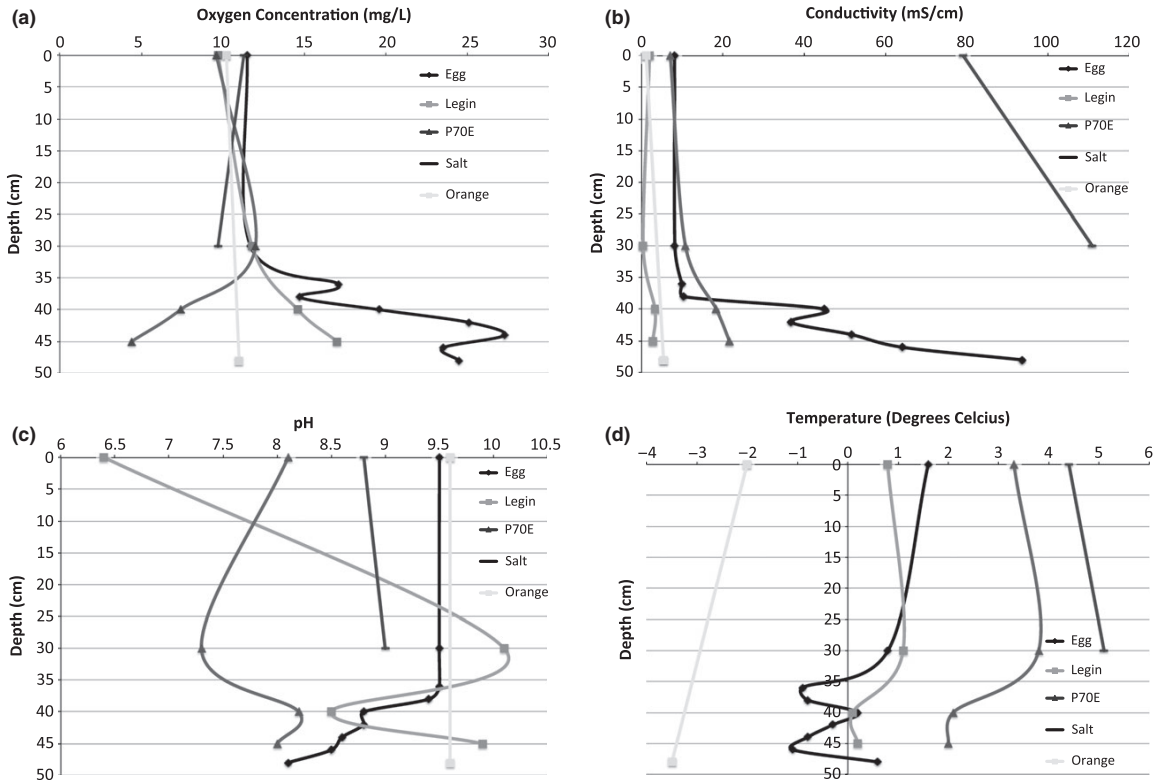

Fig. 3. Geochemistry depth profile showing changes in oxygen concentration (ppm) (a) conductivity (mS cm^{-1}) (b), pH (c) and temperature (°C) (d).

elements increased along conductivity gradients, notable observations were that Co^{59} was only detected in the bottom of Egg (46–48 cm) and throughout Salt pond. Ni^{60} was below the detection limit in Legin and Orange and the upper 38 cm of Egg. Legin also had a significantly reduced amount of Cu^{65} and Mn^{55} compared with other ponds, and Orange had a reduced level of Mn^{55}.

DNA fingerprinting

The number of AFLs was used as a proxy for alpha diversity (Fig. S2). There are no significant differences between alpha diversity of *Bacteria* from different ponds; however, P70E has significantly higher *Cyanobacteria* diversity (29–39 AFLs per sample compared with a mean of 16.6 over

all ponds) than Legin and Egg according to Tukey's Honest Significant Difference Test ($P \leq 0.001$ for Egg and $P \leq 0.001$ for Legin). A Spearman's rank correlation coefficient showed no significant correlations between alpha diversity and depth for *Bacteria* or *Cyanobacteria*. Lowest diversity occurred in Salt pond (12–25 AFLs) containing the highest overall conductivity of the sample set; however, when removed as an outlier, there was a positive correlation between average conductivity and average bacterial ARISA AFLs but with only a marginal significance ($P = 0.04$).

Beta diversity comparisons were visualised using a 2-D MDS plot for bacterial communities from five different ponds (Fig. 4a) and cyanobacterial communities from three ponds (Fig. 4b). Microbial communities were identified as significantly dissimilar between ponds at 40% similarity: cyanobacterial ANOSIM $R = 0.8605$, $P = 0.001$; bacterial ANOSIM $R = 0.766$, $P = 0.001$ (Fig. 4 a and b). P70E and Egg ponds were exceptions to this general observation. In P70E, the bacterial community observed at 0 cm depth differs from the bacterial communities observed at all other depths (40% similarity). A Mantel test confirmed that bacterial communities at similar depths in Egg pond were more comparable than those further apart ($R = 0.8934$, $P = 0.002$). Bacterial communities from Egg pond formed two major clusters based on depth; Egg (1), the upper 0–38 cm and Egg (2), the lower depths, 40–48 cm (Fig. 4a), while a similar clustering structure is apparent in cyanobacterial communities at 60% similarity (Fig. 4b). These observations were reflected in an ANOSIM test for bacterial ($R = 0.975$, $P = 0.008$) and cyanobacterial communities ($R = 0.4438$, $P = 0.026$). This combined data resulted in the selection of three samples from Egg pond, which represented the greatest variation in community structure down the stratified water column. Bacterial communities from Salt pond stood out as being unique from all other ponds (20% similarity).

BEST analysis was used to correlate bacterial and cyanobacterial community structure to differences in geochemical profiles (Table 1), with both appearing to be well correlated to conductivity as a major contributing factor. Conductivity and Ag^{109} were the best explanatory variables for cyanobacterial communities (Spearman's $\rho = 0.662$), and temperature, conductivity and V^{51} were the major explanatory variables for bacterial communities (Spearman's $\rho = 0.698$). When Egg was analysed on its own, higher probabilities were achieved for community

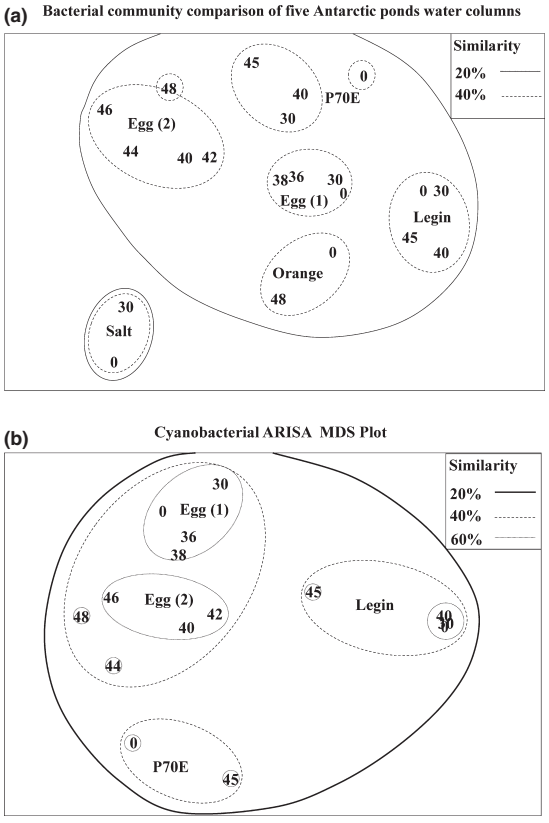

Fig. 4. Bacterial and Cyanobacterial ARISA MDS Plots showing percentage similarities of pond communities (a) bacterial ARISA, data normalised, converted to presence or absence points, and subjected to a Bray Curtis similarity matrix, MDS plot 2-D stress 0.15. (b) cyanobacterial ARISA, data normalised, converted to presence or absence points, and subjected to a Bray Curtis similarity matrix, MDS plot 2D stress 0.09.

Table 1. Geochemistry linked to community structure using BEST analysis for cyanobacterial and bacterial DNA fingerprints (ARISA). A Spearman's rank correlation of 1.0 would indicate a 100% linkage between the variable/s selected and the differences in communities

	Cyanobacterial ARISA		Bacterial ARISA	
	Correlation	Variable	Correlation	Variable
All ponds	0.662	4, 11	0.698	2, 4, 10
	0.639	2, 4, 11	0.697	2, 4
	0.635	4, 7, 11	0.675	4, 10
	0.623	2, 4, 7, 11	0.654	4
	0.591	4, 5, 11	0.652	2–4, 10
Only egg	0.734	3, 11	0.927	5
	0.732	5	0.927	5, 7
	0.732	5, 7	0.926	4
	0.730	4, 11	0.926	4, 7
	0.714	3, 5	0.925	4, 5

Variable designation: 1 = depth, 2 = temperature (C + 10), 3 = dissolved oxygen (ppm), 4 = conductivity (mS cm^{-1}), 5 = pH, 6 = total phosphate (ppm), 7 = NO_2 (ppm), 8 = NH_4 (ppm), 9 = Al^{27}, 10 = V^{51}, 11 = Ag^{109}.

linkages to geochemical variables within Egg. Cyanobacterial communities of Egg appear moderately influenced by pH, which had the highest probability as a single driver of diversity (Spearman's ρ of 0.732). Both pH and conductivity were determined to be strongly correlated to bacterial diversity differences within Egg (Spearman's ρ = 0.927 for pH and ρ = 0.926 for conductivity).

454 pyrosequencing

454 pyrosequencing of three representative samples from Egg pond created a data set of 12 995 reads that passed quality scoring, which clustered into 583 OTUs at a 97% sequence identity ($OTU_{0.03}$; Fig. 5). Good's nonparametric coverage estimator indicates that sampling coverage was 98.6% for 0 cm, 97.4% for 40 cm and 93.5% for 48 cm. Each sample contained a high number of unique OTUs, the number increasing with depth; however, the percentage of reads represented by sample-specific unique OTUs was < 7% in the surface and middle (40 cm) depths and 14.2% in the bottom (48 cm) depth. A majority of reads present in the surface (93.1%) were represented by OTUs present at all depths. A majority of reads in the middle and bottom depths (83.6% and 84.2%, respectively) were represented by OTUs shared between those depths, but absent in the surface.

Phylum/class level investigation revealed dramatic shifts in the bacterial communities with depth through the water column of Egg (Fig. 6). At the surface (0 cm), the community was least diverse, with six phyla well represented. This sample was dominated by *Bacteroidetes* (68%), with one OTU contributing 64.8% [the sequence of this OTU has 100% identity with *Algoriphagus yeomjeoni*, isolated from a marine solar saltern (Yoon *et al.*, 2005)], and β-*Proteobacteria* (18.9%), with one OTU contributing 18.6% [which has 100% sequence identity to *Hydrogenophaga taeniospiralis* isolated in the Arctic, and to a number of Antarctic lake isolates (VanTrappen *et al.*, 2002)]. At 40 cm, community diversity increased, with 12 phyla including the six seen at the surface. At this depth, the community had also shifted to a γ-*Proteobacteria* (66.1%) dominated community with the reads from one OTU contributing 60.8% [this OTU has 99% identity to *Psychromonas* sp. isolated from Arctic sea ice (Auman *et al.*, 2006)], the *Bacteroidetes* were again a major phyla but only 10.9% of the community. In the deepest sample (48 cm), diversity increased dramatically, with 18 phyla including all those seen in the 40 cm sample. Again the γ-*Proteobacteria* were the dominant phyla (50.8%), with one OTU contributing 46.1% (the same OTU dominant at 40 cm), the only other major group were uncharacterised, although this contained only one OTU (16.8%) with a [96–98% identity to environmental sequences from a hypersaline microbial mat (Isenbarger *et al.*, 2008)] The *Bacteroidetes* (5.6%) were the third most represented phyla.

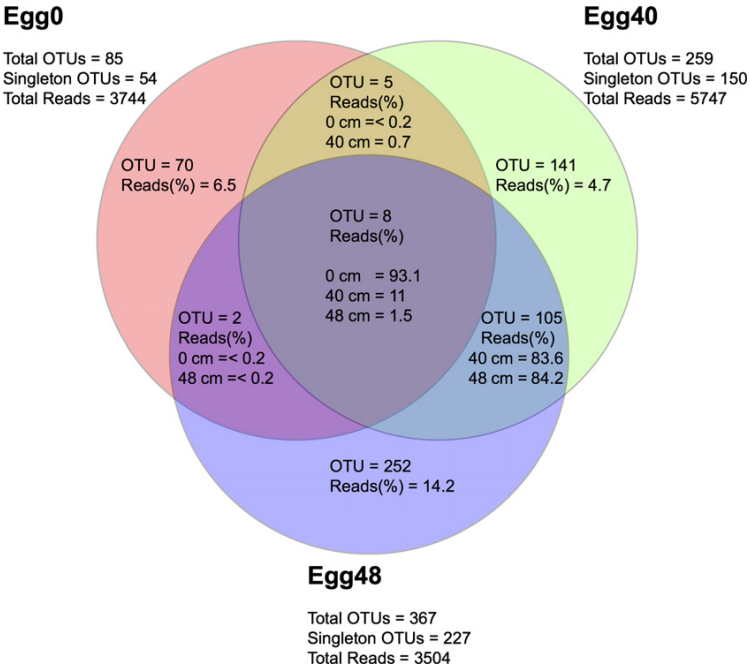

Fig. 5. Summary of 454 sequencing data (at a distance of 0.03), and similarities between depths of Egg pond. The total OTU richness of all the groups is 583, and total read number is 12 995.

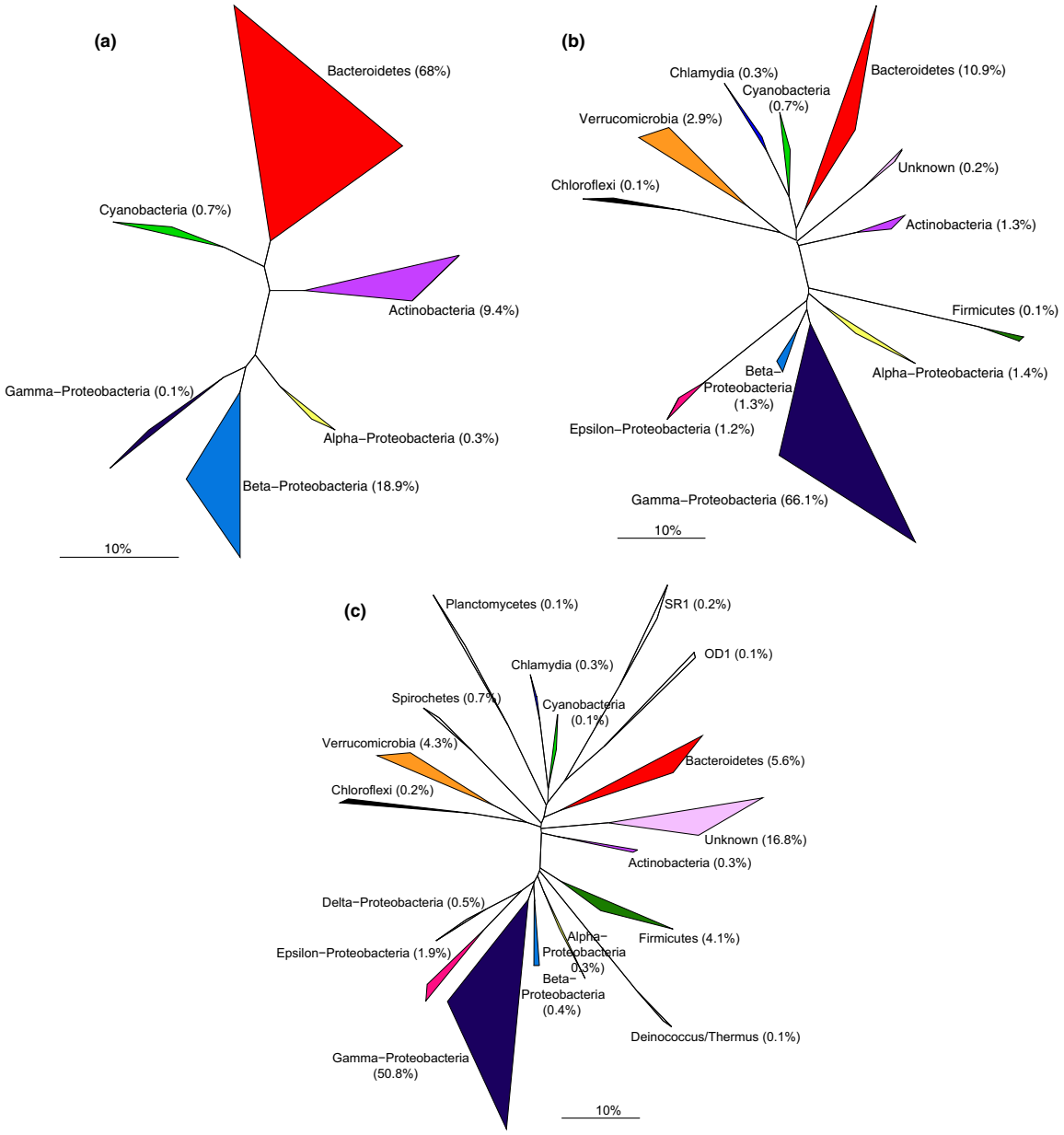

Fig. 6. Phylum-level diversity of bacterial 16S rRNA gene sequences from different depths of Egg pond. Percentage abundance of phyla within each site are shown (in parentheses) for the (a) surface sample, (b) 40 cm deep sample and (c) 48 cm deep sample. 454 pyrosequencing reads were denoised and clustered at 97% (farthest neighbour) to obtain 583 $OTUs_{0.03}$ in total, of which 120 $OTUs_{0.03}$ were shared. The top 101 $OTUs_{0.03}$ with > 0.1% abundance in any sample are used in this analysis.

Discussion

The focus of this study was to determine whether the unique geochemistry previously reported in the water columns of Bratina ponds supports unique bacterioplankton communities (Matsumoto *et al.*, 1992; Wait *et al.*, 2006; Webster-Brown *et al.*, 2012). Early studies of pond sediments from Bratina meltwater ponds have identified highly diverse (Sjoling & Cowan, 2003) and variable microbial communities between ponds (Kemp & Aller,

2004); however; the environmental factors driving these differences were not determined, and overlying waters were never investigated. Analysis of ARISA fragments in this study has revealed a statistically significant difference (ANOSIM) between pond microbial communities for *Bacteria* ($R = 0.766$, $P = 0.001$) and *Cyanobacteria* ($R = 0.8605$, $P = 0.001$). The highly geochemically stratified Egg pond formed two distinct bacterial communities ($R = 0.975$, $P = 0.008$), with BEST analysis indicating pH and conductivity as major contributors to community differences along this steep gradient. Pyrosequencing of representative samples from Egg pond identified a diverse community of 583 unique OTUs, which exhibit notably different community structures with depth reinforcing the conclusions based on ARISA results. Although sequencing coverage was not 100% in any sample for this investigation, this would not affect conclusions formed from this data, only resulting in a slight underestimation of diversity.

The relative beta diversity of *Cyanobacteria* and *Bacteria* (ARISA) was generally pond-specific (visualised using a MDS plot, confirmed statistically by ANOSIM). This observation is consistent with microscopic analysis of planktonic communities (James *et al.*, 1995), which classified three groups of ponds based on size, conductivity, and the presence and abundance of various planktonic groups. Each of the three groups included ponds sampled in this study (P70E, Legin and Salt). This suggests that these ponds consistently harbour different geochemical environments and biological communities. The exception to this observation was Egg pond, which formed two depth-related microbial groupings (Fig. 4a, confirmed by ANOSIM) consistent with a steep geochemical gradient of conductivity and pH, both found to be drivers of community shifts in other studies (Sabbe *et al.*, 2004; de Figueiredo *et al.*, 2010).

BEST analysis has previously been successfully utilised to link geochemical variability to variation in biological community profiles from fingerprinting data (Wood *et al.*, 2008; Soo *et al.*, 2009; Smith *et al.*, 2010). In this study, BEST analysis indicated a strong correlation between differences in conductivity contributing to cyanobacterial and bacterial community structure for all five ponds studied. In addition, independent analysis of Egg indicated that both bacterial and cyanobacterial community structure was significantly correlated to differences in pH and conductivity. The pH and conductivity in Egg show an inverse relationship to one another, so neither can be singled out as the primary variable responsible for community structure changes; however, both have previously been identified as drivers of community structure in aquatic environments (James *et al.*, 1995; Sabbe *et al.*, 2004; Kaartokallio *et al.*, 2005; de Figueiredo *et al.*, 2010; Jiang *et al.*, 2010; Laque *et al.*, 2010). Examination of major OTU abundances between depths reinforces conductivity as the dominant driver to community structure. The Genus *Hydrogenophaga* was identified as a major OTU in the surface sample, decreasing significantly with depth along the increasing conductivity gradient. Several species of this genus have been previously isolated from a number of Arctic and Antarctic environments (Newdell & Rutter, 1994; VanTrappen *et al.*, 2002) and found to have a maximum NaCl tolerance of 1% (Yoon *et al.*, 2008) explaining this observation. *Algoriphagus yeomjeoni,* previously identified in a cold marine environment and in Antarctic lake mats (Bowman *et al.*, 2003; Van Trappen *et al.*, 2004), has a broader salt tolerance of up to 9% NaCl and an optimum of 1% (Yoon *et al.*, 2005). Its abundance is highest at the surface then decreases significantly with depth; however, it remains at higher concentrations than *Hydrogenophaga* in the 40 and 48 cm samples likely due to its greater salt tolerance. The genus *Psychromonas,* previously isolated from pond sediments at Bratina Island (Mountfort *et al.*, 1998) and Arctic ice (Auman *et al.*, 2006), was dominant at both 40 and 48 cm and was barely detected at 0 cm, reflective of its ability to grow well between 1 and 10% NaCl (Auman *et al.*, 2006). The second most abundant OTU at 48 cm further reinforces halophilic dominance with depth, sharing a high identity to sequences gained from a hypersaline solar saltern (Isenbarger *et al.*, 2008).

Combinations of pH and conductivity with temperature, V, NO_2, Ag and dissolved oxygen are all highly correlated to microbial community structure. Each identified variable has previously reported to have some influence on planktonic microorganisms. Vanadium can interfere with iron uptake (Baysse *et al.*, 2000); Ag has well-known antimicrobial properties (Throback *et al.*, 2007); nitrogen limitation alters primary productivity in phytoplankton population in these ponds (Sorrell *et al.*, 2013); hypervariation in oxygen concentration with depth selects for zones of aerobic, anaerobic (Bell & Laybourn-Parry, 1999) and hyperoxic tolerant microorganisms (Fridovich, 1998) and due to variable optimum growth temperatures for microorganisms minor variations in temperature, especially at near-freezing temperatures can dramatically influence community structure (Mountfort *et al.*, 2003). Regardless of biological mechanism, the extent of differences in V, Ag and NO_2 concentration are well below those found to have biological significance (Yamanaka *et al.*, 2005; Sorrell *et al.*, 2013), and their low correlation scores individually indicate that it is unlikely that any of these variables will strongly influence microbial community structure compared with pH and conductivity. DO and temperature are capable of influencing community structure; however, they too provide weak correlations

individually. Interestingly although PO_4 is an essential nutrient, it was not identified as a driver of community structure in this analysis. It is likely the interplay between these, and other unknown factors, produces the net effects in the microbial communities.

Pyrosequencing data indicated an increase in diversity with depth in Egg, inconsistant with bacterial ARISA, which showed no significant correlation between diversity and depth (Spearman's rank correlation coefficient). This could be due to the dominance of a few major groups at the surface (86.9% for the top two abundant groups compared with 66.8% at 48 cm), while the large number of minor groups seen at the bottom in 454 data could have been below the detection limit for ARISA. The dominance of a few taxa in bacterial communities is not uncommon (Massana *et al.*, 2000; Tyson *et al.*, 2004; Zeng *et al.*, 2013; Laas *et al.*, 2014; Lee *et al.*, 2014) however rarely to this extent. The annual extreme freeze thaw process this pond goes through could skew the community towards bacteria that remain metabolically active over winter or those that could rapidly re-emerge from their inactive states (Foreman *et al.*, 2010). The fact that the dominant OTU at each depth had increasing salt tolerances along the conductivity gradient shows the biological response to increasing selective pressures. A large proportion of reads were shared between the middle and bottom depths but not with the surface sample. The middle depth geochemically represented the beginning of the brine layer, which due to its density would impede direct mixing of the surface waters with the lower layers of the pond (Wait *et al.*, 2006); however, the large proportion of shared OTUs indicates there is mixing within this brine layer or that certain taxa share broader tolerances to transcend these extreme gradients. Due to the dynamic nature of these ponds, over the course of a season many groups are likely to emerge from the rare biosphere to become dominant within a community, only to be outcompeted once again when conditions change.

Both ARISA and 454 data separate the surface (whose dominant phyla is *Bacteriodetes*), from the 40 cm and 48 cm samples (where the dominant phyla is *γ-Proteobacteria*). The increase in *γ-Proteobacteria* and decrease in *Bacteriodetes*, with depth correlates to the increase in conductivity and decrease in pH down the water column. These differences in dominant phyla agree with the BEST analysis, which indicated these two factors were the major cause for community shifts. Although many other minerals associated with the conductivity also differed, none of those elements revealed through ICPMS analysis appeared to be significant drivers of community diversity.

An important consideration in any study of meltwater ponds is that the time of sampling can dramatically influence geochemistry (Matsumoto *et al.*, 1992; James *et al.*, 1995; Hawes *et al.*, 1999; Wait *et al.*, 2006), which will in turn influence the resident microbiology. Samples for this study were collected early in the summer (early December 2009), so ponds were not at their maximal biological activity compared with those previously collected in January (James *et al.*, 1995; Wait *et al.*, 2006), when ponds would have been melted for longer, were probably warmer and may have undergone greater mixing due to the lack of surface ice and significant summer winds. As the ponds are meltwater fed, the amount of ice and snow input each year is important to the overall soluble ion concentration of the ponds. In 2009, ponds were significantly shallower than in other years, for example, P70E was 0.85 m deep in January 1992 (James *et al.*, 1995), at least 0.8 m deep in October 2003 and at least 1 m deep in January 2004 (Wait *et al.*, 2006), compared with a significantly shallower 0.45 m in this study. Although differences in meltwater input can rapidly alter geochemical variables during and between seasons (Schmidt *et al.*, 1991; Hitzfeld *et al.*, 2000; Jungblut *et al.*, 2005), comparison with previous work reveals that for a given pond, certain geochemical parameters can remain stable over considerable time scales (De Mora *et al.*, 1994; James *et al.*, 1995; Wait *et al.*, 2006).

Conclusions

The water column of five Bratina Island meltwater ponds was examined to understand how local geochemistry effects the structure of the resident microbial community, using fine scale biological and geochemical sampling. Biological samples were genotyped using ARISA, which allowed systematic comparison of bacterial and cyanobacterial communities supported in each pond through the entire depth profile. Significant differences between and within pond geochemistry and microbial diversity were detected. Specifically, when examining Egg pond separately from other ponds, clear links between the bacterial and cyanobacterial communities with pH and conductivity were identified as major variables that appear to influence microbial diversity and community structure. These results were verified by utilising 454 pyrosequencing to identify specific phylum-level differences throughout the water column. This reinforces the belief that geochemistry can be a driver of community structure. However, the microbial communities of a single Bratina Island pond may be found to have individual drivers, different to those of other ponds. This makes these ponds extremely valuable as a scientific resource, as they can be considered to be completely isolated extreme aquatic ecosystems sharing the same climate, but representative of the geochemical diversity of aquatic ecosystems (both ponds and lakes) scattered across the Antarctic continent.

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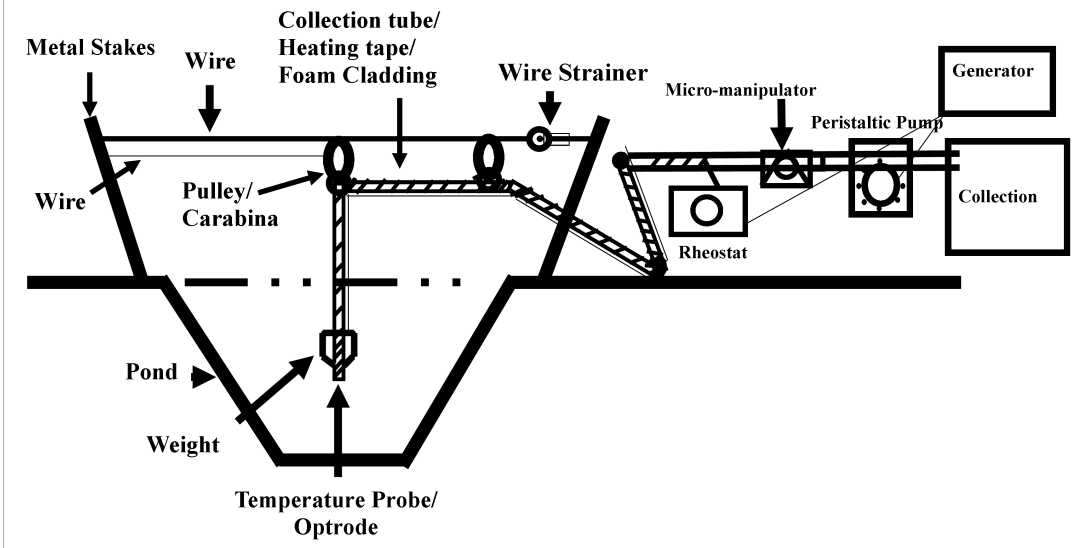
Supporting Information

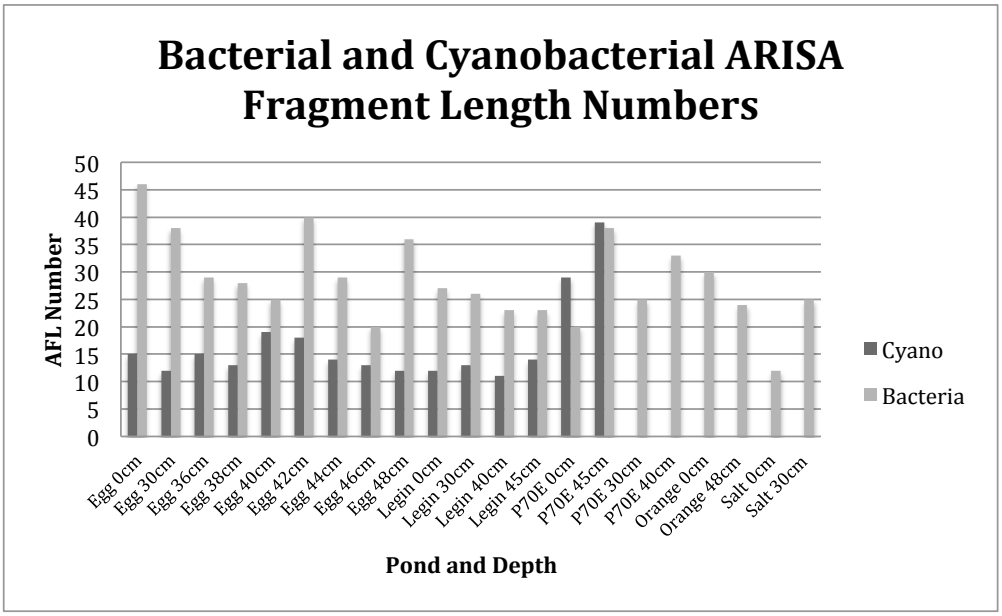
Additional Supporting Information may be found in the online version of this article:

Fig. S1. Diagram showing equipment used to obtain central pond water column samples.
Fig. S2. Summary of bacterial and Cyanobacterial ARISA fragment lengths (AFL) number from each pond sample.
Table S1. Summary geochemistry from Bratina Island ponds December 2009.
Data S1. Automated Ribosomal Intergenic Spacer Analysis (ARISA).

Supplementary Information



Supplementary Figure 2. 1 (Fig. S1) Diagram showing equipment used to obtain central pond water column samples.



Supplementary Figure 2. 2 (Fig. S2): Summary of bacterial and cyanobacterial ARISA fragment lengths (AFL) number from each pond sample.

Supplementary Table 2. 1 (Table S1): Summary geochemistry from Bratina Island ponds December 2009

	Depth (cm)	Temp (C)	DO (ppm)	Conductivity (mS/cm)	pH	Phosphate (ppm)	NO2 (ppm)	NH4 marine (ppm)	Li 7 (ppb)	Na 23 (ppb)	S 34 (ppb)
Egg 0	0	1.6	11.5	8.2	9.5	1.83374	0.00192	0.01218	26.55	608578	33383
Egg 30	30	0.8	11.7	8.2	9.5	0.01139	0.00195	0.00162	25.3	1042225	50442
Egg 36	36	-0.9	17.1	9.9	9.5	0.01313	0.002	0.00684	25.2	1092516	51350
Egg 38	38	-0.8	14.7	10.4	9.4	0.00396	0.00195	0.00427	33.1	1085431	43723
Egg 40	40	0.2	19.6	45.0	8.8	0.0175	0.00191	0.02708	69.85	7844427	256301
Egg 42	42	-0.3	25.1	36.7	8.8	0.01045	0.00202	0.01887	63	6081635	191391
Egg 44	44	-0.8	27.3	51.6	8.6	0.01978	0.0018	0.02802	84.95	8010980	306793
Egg 46	46	-1.1	23.5	64.1	8.5	0.01302	0.002	0.03422	98.85	10265408	374318
Egg 48	48	0.6	24.5	93.6	8.1	0.23069	0.00202	1.38791	124.7	15648235	460124
Legin 0	0	0.8	9.7	2.0	6.4	0.01725	0.00522	0	15.85	52815.65	0
Legin 30	30	1.1	11.8	0.4	10.1	0.0117	0.00482	0	15.6	53272	9235
Legin 40	40	0.1	14.6	3.3	8.5	0.00436	0.00212	0	18.95	196895.7	39578
Legin 45	45	0.2	17.0	2.8	9.9	0.00165	0.0021	0	22.7	316490.5	58851
P70E 0	0	3.3	9.6	7.0	8.1	0.00167	0.03246	0	28.55	665577.4	64381
P70E 30	30	3.8	12.0	10.8	7.3	0.0355	0.01846	0	30.55	501026.2	76305
P70E 40	40	2.1	7.4	18.4	8.2	0.00489	0.02458	0	29.45	986136	103099
P70E 45	45	2.0	4.4	21.6	8.0	0.00916	0.00317	0.00911	37.65	2095524	225878
Salt 0	0	4.4	11.3	79.1	8.8	0.03173	0.00203	0.03804	93.85	24289453	10826266
Salt 30	30	5.1	9.7	110.9	9.0	0.06473	0.00234	0.07429	109.8	33450433	17512464
Orange 0	0	-2.0	10.2	1.1	9.6	0.42872	0.00298	0.10008	19	271116.3	22853
Orange 48	48	-3.5	11.0	5.4	9.6	0.0572	0.00237	0	25.6	754638.4	60368

	K 39 (ppb)	Ca 43 (ppb)	V 51 (ppb)	Mn 55 (ppb)	Co 59 (ppb)	Ni 60 (ppb)	Sr 88 (ppb)	Ag 109 (ppb)	Cd 111 (ppb)	In 115 (ppb)	Ba 137 (ppb)	U 238 (ppb)
Egg 0	29768	19224	6.05	12	0	0	297.14	0	0.25	0	12.25	0.43
Egg 30	49705	25380	7.77	19.4	0	0	437.15	0.05	0.44	0	5.75	0.67
Egg 36	55175	26481	6.17	27.06	0	0	460.87	0.2	0	0	11	0.76
Egg 38	53159	25226	5.3	25.86	0	0	489.5	0	0	0	24.05	0.62
Egg 40	229620	65776	0.37	385.7	0	2.92	2273.61	0.34	0	0	48.4	1.76
Egg 42	186787	57097	6.27	285.55	0	2.09	1818.62	0	0	0	39.95	1.47
Egg 44	248265	75073	0.58	462.03	0	3.93	2535.48	0.98	0.05	0	51.4	2.08
Egg 46	326472	78247	1.84	539.15	0.51	4.67	2916.47	0.54	0	0	83.3	2.74
Egg 48	396131	94337	2.21	498.79	1.03	6.63	3774.11	0.88	0	0	65.1	3.06
Legin 0	2363	3533	4.87	0.2	0	0	60.18	1.04	0	0	3.4	0.05
Legin 30	2701	3966	4.42	0.35	0	0	61.86	0.94	0	0	8.25	0.05
Legin 40	79434	12218	5.82	0.86	0	0	283.08	0.59	0	0	12.15	0.24
Legin 45	13942	19189	8.51	2.79	0	0	483.08	0.34	0	0	5.85	0.43
P70E 0	29186	81021	3.25	56.37	0	0.41	1681.89	1.74	0.2	0	16.45	1.71
P70E 30	21580	80882	2.44	82.61	0	1.17	1544.86	0.35	0	0	11.45	1.22
P70E 40	41436	130098	1.73	262.38	0	3.01	3102.28	1.44	0	0	20.25	2.19
P70E 45	78003	292081	0.26	1091.97	0	9.87	7749.36	1.93	0.2	0	31.75	3.5
Salt 0	500162	196405	11.7	111.23	1.13	14.59	14918.7	0	0.05	0	117.2	13.29
Salt 30	542672	241616	18.16	281.31	1.49	14.2	16489	0	0	0	156.5	12.33
Orange 0	12014	9350	18.77	7.33	0	0	174.9	1.44	0.8	0.35	12.1	1.04
Orange 48	29643	7461	13.4	6.18	0	0	230.09	3.83	0	0.05	16.95	1.92

Supplementary Methods

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

For Bacterial ARISA each reaction contained 0.3 μM of each primer (hexachlorofluorescein (HEX) fluorescent dye labelled ITSReub-Hex (5`-GCCAAGGCATCCACC-3`) and ITSF (5`-GTCGTAACAAGGTAGCCGTA-3`) (Cardinale *et* al., 2004)), 1x PCR buffer (Invitrogen Ltd, New Zealand), 0.2 mM dNTPs (Roche Diagnostics, New Zealand), 0.02 mg/mL bovine serum albumin (BSA), 1 U Platinum Taq (Invitrogen Ltd), 0.5 μM $MgCl_2$, 20 ng genomic DNA and the reaction was made up to 30 μL with milli-Q H_2O. Thermal cycling conditions were: 94°C for 5 min, then 30 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min.

For the cyanobacterial-specific ARISA, triplicate 25 μL reactions were run for each sample. Each reaction contained 0.5 μM of each primer (6-carboxy-flurescine (FAM) fluorescent dye labelled CY-ARISA-F (5`-GYCAYRCCCGAAGTCRTTAC-3`) and 23S30R (5`-CHTCGCCTCTGTGTGCCWAGGT-3`) (Wood e*t al.,* 2008)), 1x PCR buffer, 0.2 mM dNTPs, 1 U Platinum Taq, 5 mM $MgCl_2$, 0.032 mg/mL BSA, 20 ng template DNA and the entire reaction was made up to 25 μL with milli-Q H_2O. Thermal cycling conditions were: 94°C for 2 minutes, then 35 cycles of 95°C for 20 s, 55°C for 15 s and 72°C for 1.5 min, followed by 7 min at 72°C.

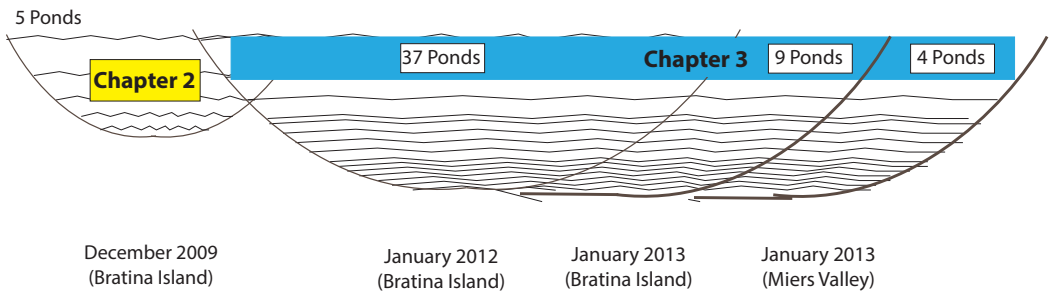
All ARISA PCR reactions were run on a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, CA). Once amplified all triplicate PCR reactions were combined, run on a 1% agarose gel to ensure amplification success and then purified using a 5M Quickclean PCR Purification Kit (Genscript Corporation, New Jersey, USA). The DNA concentrations of purified products were quantified using a NanoDrop™ 1000 Spectrophotometer, diluted to 10 ng/μL using Milli-Q H_2O and fragment lengths were resolved on a 3130XL DNA sequencer using Liz-1200 internal size standard (Applied Biosystems, New York, USA) at the University of Waikato DNA Sequencing Facility.

Chapter 3 - Temporal, spatial and geochemically driven variation of microbial communities in the melt ponds of the Ross Sea region, Antarctica

Preface

This chapter describes the geochemical, spatial and temporal influences to the microbial communities of 41 meltwater ponds surface waters. As the primary author of this chapter, I was involved with all fieldwork over three seasons (December 2009 (Chapter 2), January 2012 and January 2013), and completed the majority of lab work, data analysis and manuscript preparation. Inductively coupled mass spectroscopy (ICPMS) and DNA sequencing were carried out by technicians at the University of Waikato. Craig Herbold and Charles Lee earned co-authorship for their part in developing bioinformatics pipelines used to analyse this data. All authors reviewed and edited this manuscript.

This chapter has been submitted to *Polar Biology* and is in review.



Preface Figure 3. 1 Breakdown info-graphic overview of thesis

Temporal, regional and geochemical drivers of microbial community variation in the melt ponds of the Ross Sea region, Antarctica

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Key words: Antarctic, planktonic, microorganism, community, meltwater

3.1 Abstract

Diverse and dynamic microbial communities inhabit the geochemically heterogeneous surface waters of Antarctic meltwater ponds. A total of 41 ponds, 37 from the McMurdo Ice Shelf (Bratina Island) and four from a terrestrial locale (Miers Valley), were sampled during three austral summers to characterize the poorly understood geochemical, temporal and regional influences on the resident microbial communities. DNA fingerprinting coupled with *in-situ* pH and conductivity was used to select 17 pond samples for in-depth nutrient and chemical analysis and high throughput sequencing of the bacterial V5-V6 hypervariable region of the 16S rRNA gene. Conductivity was the strongest driver of community structure of dominant cosmopolitan OTUs across all ponds and time points; however other influential factors (pH, climatological, Hg, Fe, and PO_4) were also identified. The unique community, those sequences absent in at least one pond, represented a small percentage of total reads but a large proportion of pond biodiversity and was strongly driven by different environmental variables (Si, B and S). December 2009 samples were highly dissimilar to January samples and exhibited a low diversity, with three OTUs representing >80% of total reads. Significant temporal variation in community structure was identified within the same ponds (January 2012 and 2013) although major taxa remained present. Miers Valley ponds exhibited greater similarity to Bratina Island ponds than between each other, suggesting regional movement of microorganisms. Throughout all sampling times and locations 10 cosmopolitan OTUs were identified as dominant components, their variable relative abundances driving the major dissimilarities in community structure.

3.2 Introduction

Ubiquitous throughout Antarctica, aquatic environments serve as microbiological oases where *Cyanobacteria*-dominated microbial mats provide one of the few visible forms of life on the continent (Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; James *et al.*, 1995; Safi *et al.*, 2012; Vincent & James, 1996). Meltwater ponds, the most common aquatic feature, are typically characterized as small bodies of water that undergo annual freeze-thaw cycles (Vincent & James, 1996). These ponds are found wherever liquid water, from glacial or snow melt during the austral summer (Stanish *et al.*, 2012), is able to collect in topographically suitable locations ranging from the surface of marine sediment-covered sea ice (Kellogg & Kellogg, 1987) to the arid mineral soil landscapes of the Dry Valleys (McLeod *et al.*, 2009). Due to the large differences in local geomorphology, historical and recent snow input, local microclimate and age (Kellogg & Kellogg, 1987) highly heterogeneous inter-pond geochemical conditions are observed even between ponds in close proximity (Hawes *et al.*, 2014).

Some 40 meltwater ponds adjacent to Bratina Island (E165.55, S78.01) have been extensively studied over the past three decades due to their geochemical heterogeneity, and close proximity (<1 km) to a permanent New Zealand field camp (Kellogg & Kellogg, 1987; Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; Hawes *et al.*, 2014). These ponds are located on the surface of the McMurdo Ice Shelf in the southwest corner of the Ross Sea (Figure 3. 1). Ice shelf thickness in this area ranges from 10 to 50 m, and is often covered with a 10 to 20 cm layer of marine derived sediment rich in diatoms and occasional mummified higher marine organisms (Fitzsimons *et al.*, 2012; Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988). Meltwater from local snow and sea ice accumulates in depressions formed on the undulating ice, creating ponds ranging from one to several thousand square meters in area. Pond size and depth are dictated by the balance between meltwater input and evaporation, resulting in broad ranges of chemistry, even within the same pond over time (Cowan & Tow, 2004; De Mora *et al.*, 1994; Gibson *et al.*, 2006). Previous investigations of planktonic pond communities at Bratina Island have revealed a highly diverse and

productive environment dominated by Bacteria, flagellates and ciliates, with the ciliates (predominately bacteriovorous in nature) representing the highest trophic level in this simplified food web (Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; James *et al.*, 1995; Safi *et al.*, 2012; Vincent & James, 1996). With a lack of biotic controls, abiotic factors such as salinity (Howard-Williams *et al.*, 1989; Jungblut *et al.*, 2005) and nutrient content (Sorrell *et al.*, 2013), which are typically extremely variable between ponds (Hawes *et al.*, 2014), are postulated to be the primary influence on polar microbial communities which is in contrast to the biotic controls observed in complex temperate communities (Cannone *et al.*, 2008; Cary *et al.*, 2010; Lee *et al.*, 2012).

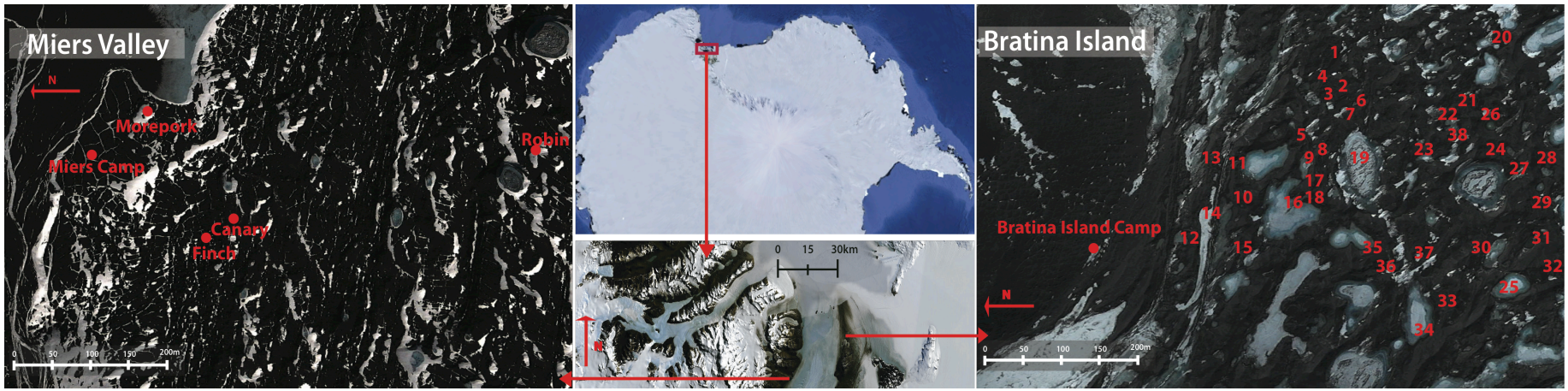

Figure 3. 1 Map of the study sites. Satellite imagery Bratina Island Ponds: 1=Egg(K160), 2=Duey, 3=Orange, 4=Nostoc, 5=Foam, 6=Luey, 7=Huey, 8=iMac, 9=Skua, 10=Nicholas, 11=Casten, 12=Rotifer, 13=Archers, 14=Ice Ridge Pond, 15=Long, 16=K081, 17=Skua, 18=JAC, 19=Permanent Ice Pond, 20=Hawes, 21=Wendy, 22=Bibby, 23=P70, 24=P70E, 25=Fresh, 26=Galore, 27=Fogghorne, 28=Bambi, 29=Legin, 30=Brack, 31=Salt, 32=Russell, 33=Upper, 34=Extra, 35=Lunch, 36=Conophyton, 37=Retro, 38=Ribbon. Satellite images from Google Earth Pro, Image © 2014 DigitalGlobe, U.S. Geological Survey.

Although abundant throughout Antarctica, limited research has been conducted on terrestrial Antarctic ponds in favor of the larger permanent ice covered lakes of the Dry Valleys (Bell & Laybourn-Parry, 1999; Bowman *et al.*, 2000; Roberts *et al.*, 2004; Sabbe *et al.*, 2004; Squyres *et al.*, 1991; Tyler *et al.*, 1998; VanTrappen *et al.*, 2002). To date, most studies on terrestrial ponds have involved the description of the geochemistry and microbial mats lining the ponds with little regard given to the planktonic communities (Lyons *et al.*, 2012; Matsumoto *et al.*, 1992; Moorhead *et al.*, 2003; Peeters *et al.*, 2012; Peeters *et al.*, 2011; Vincent & James, 1996). Terrestrial ponds are physically analogous to McMurdo Ice Shelf ponds with similar pH and conductivity profiles (Hawes *et al.*, 2014; James *et al.*, 1995; Lyons *et al.*, 2012; Sabbe *et al.*, 2004; Schmidt *et al.*, 1991; Vincent & James, 1996), however elemental compositions derived from the local surrounds are well defined between terrestrial and coastal environments (Lyons *et al.*, 2012). The mineral soils on which Dry Valley ponds form are developed from the weathering of granite, sandstone, basalts and metamorphic rocks originating from the continent's bedrock and glacial tills. These soils are typically <30 cm thick and sit on top of permafrost (Cary *et al.*, 2010; McLeod *et al.*, 2009). A number of poorly described terrestrial ponds are located in the Miers Valley, 30 km SW of Bratina Island. Unlike the marine microbiological influence at Bratina Island (Fitzsimons *et al.*, 2012; Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988), the source of the microbiological communities residing in the Miers Valley ponds remains unidentified (Cary *et al.*, 2010).

Although pond summer conditions can be somewhat similar to those found in temperate regions, annual freeze thaw cycles cause a cascade of geochemical and biological changes characteristic to the Polar ecosystem (Hawes *et al.*, 2011a; Hawes *et al.*, 2011b; Safi *et al.*, 2012; Wait *et al.*, 2006; Webster-Brown *et al.*, 2012). Over several weeks of freezing there is a shift from net autotrophy to heterotrophy, causing dissolved oxygen and pH to decrease (Hawes *et al.*, 2011a; Safi *et al.*, 2012) until all life enters presumably inactive forms, with the process reversing during the subsequent summer thaw cycle whereby biological activities recommence (Foreman *et al.*, 2010). These cycles place extreme stress on the endemic planktonic community resulting in rapid temporal variation in community structure (Hawes *et al.*, 2011a; Hawes *et al.*, 2011b; Safi

et al., 2012; Wait *et al.*, 2006; Webster-Brown *et al.*, 2012) and presumably selecting for eurytolerant microbial inhabitants. In addition to physical changes caused by summer ice melt, freshwater inputs to the pond can result in a drastic lowering of the conductivity in surface waters (in ponds lacking wind mixing) (Safi *et al.*, 2012; Schmidt *et al.*, 1991).

The objective of this study was to expand the understanding of the poorly described planktonic bacterial communities inhabiting meltwater ponds of both Miers Valley and Bratina Island. Specifically, the study encompassed the investigation of: (1) the environmental drivers influencing microbial community structure; (2) the biological temporal variation between three representative ponds during the 2012 and 2013 summer sampling periods, and variation between early summer (December) and late summer (January); and (3) comparing microbial communities between contrasting terrestrial ponds situated in Miers Valley and the marine-influenced ice-shelf ponds on Bratina Island. A total of 41 ponds were characterised using ARISA DNA fingerprinting and preliminary *in-situ* collected pH and conductivity. Seventeen representative ponds were selected for in-depth next-generation (454) sequencing of the V5-V6 hypervariable region of the 16S rRNA gene coupled with geochemical surveys to be compared between times and locations. These data identified a small number of shared dominant cosmopolitan OTUs consistent with previous findings, and provide the first in-depth investigation of the intra-seasonal and regional variation of the microbial communities inhabiting these ponds.

3.3 Materials and Methods

3.3.1 Field sampling strategy

Surface water samples (the homogeneous first 10 cm of the water column at the edge of each pond) were collected from 37 meltwater ponds (Table 3. 1) near Bratina Island (78° 01' S, 165° 32' E) on the McMurdo Ice Shelf, and four ponds in Miers Valley (78° 07' S, 164° 12' E) situated within the McMurdo Dry Valleys. Samples were collected in December 2009 as previously outlined in Archer *et al.,* (2014) and January 2012 (Bratina Island) and January 2013 (Miers Valley and Bratina Island). For biological samples, surface water was

immediately filtered through syringe-mounted 0.22 μm filters (Whatman International Ltd, Kent, UK) until the filter clogged (between 3 and 200 mL, due to the ease of filtration). Excess water was removed and the filter then flooded with a nucleic acid preservative/lysis buffer (CTAB - cetyltrimethylammonium bromide-polyvinylpyrrolidone-β-mercaptoethanol) (Coyne *et al.*, 2001) and frozen for transportation. For geochemical analysis, filtrate from biological sample collection was collected into 15 mL conical centrifuge tubes (Falcon™), and frozen immediately for later analysis. Nutrient (NH_4, NO_3/NO_2 and PO_4) and elemental analysis (ICP-MS) were carried out at the University of Waikato using a Lachat Quickchem 8500 series II FIA system as per manufacturer's instructions (Hach Company, Loveland, CO) and a Mass Spectrometer ELAN® DRC II (PerkinElmer Inc., Münster, Germany). Prefiltered pond water for ICP-MS analysis was diluted 1:50 with Milli-Q water (Millipore, Billerica, MA, USA) and subsequently acidified with 2% HNO_3. *In-situ* pH and conductivity measurements were determined from 30 mL of unfiltered sample in a sterile 50 mL conical centrifuge tubes (Falcon™). pH and conductivity were measured using a SPER Scientific water quality meter (Sper Scientific, Arizona, USA) in 2009, by an Orion 4 Star bench top meter (Thermo Fisher Scientific Inc, USA) in 2012 and by a HQ40d portable multi-parameter meter (Hach Company, CO, USA) in 2013. Differences in pH between years were investigated using Tukeys Honest Significant Difference Test (R Core Team, 2013). Environmental data (pH, conductivity, nutrient and elemental) were transformed by a square root followed by log (X+1) and normalization in Primer 6 (Clarke & Gorley, 2006) and MDS plots were generated in R and Primer 6 from these data based on an Euclidean distance matrix.

3.3.2 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

DNA from filtered biomass was extracted using a modified CTAB extraction protocol (Dempster *et al.*, 1999) as used in Archer *et al*. (2014). Bacterial community structure was resolved and trends of interest for further, high-resolution (high-throughput sequencing) analysis identified using ARISA DNA fingerprinting (Fisher & Triplett, 1999). The intergenic spacer region (ISR)

in the bacterial rRNA operon was amplified via the polymerase chain reaction (PCR) using 0.3 μM of each primer ITSReub-Hex (5`- HEX-GCCAAGGCATCCACC-3`) and ITSF (5`-GTCGTAACAAGGTAGCCGTA-3`) as described previously (Cardinale *et al.*, 2004) and 1X PCR buffer (Invitrogen Ltd, New Zealand), 0.2 mM dNTPs (Roche Diagnostics, New Zealand), 0.02 mg/mL bovine serum albumin (BSA), 1 U Platinum *Taq* (Invitrogen Ltd), 0.5 μM $MgCl_2$ (Invitrogen), 20 ng genomic DNA and Milli-Q H_2O to bring the reaction volume to 30 μL. Thermal cycling conditions consisted of: 94°C for 5 min, 30 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min. All ARISA PCR reactions were conducted on a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, CA). Following thermocycling, triplicate PCR reactions were combined and run on a 1% agarose gel to evaluate amplification success. Amplicons were diluted 1:20 in Gibco® ultrapure water and fragment lengths resolved on a 3130XL DNA sequencer (Applied Biosystems, New York, USA) using Liz-1200 internal size standard (Applied Biosystems) at the University of Waikato DNA Sequencing Facility.

3.3.3 ARISA data analysis

ARISA data were processed within an informatics pipeline (modified from Abdo *et al.*, 2006). Peaks exceeding 200 fluorescence units and that were between 50 bp and 1200 bp were used to calculate model parameters for a log-normal distribution. Iteratively, peaks with an area exceeding the 99.9% cumulative distribution of the calculated log-normal distribution for noise were accepted as true peaks. Peaks were then binned into ARISA Fragment Lengths (AFLs) within 5 bp of one another. Beta diversity was investigated via a two-dimensional ordination based on a Bray Curtis community dissimilarity matrix of presence/absence transformed ARISA peaks performed in Primer 6 (Clarke & Gorley, 2006). Data were visualized in R (R Core Team, 2013) with a non-metric multidimensional scaling (NMDS) plot, overlaying vectors of environmental variables, fit onto the ordination in R using the 'vegan' library (Oksanen, 2011). Analysis of Similarity (ANOSIM) analyses were performed on the resemblance

matrix to test specific hypotheses formed from interpretation of MDS plots. Furthermore, relationships between geochemistry and microbial community structure were determined using BEST analysis within Primer 6 (Clarke & Gorley, 2006) with environmental data transformed via square root and a subsequent log (X+1) transformation and data normalization.

3.3.4 454 pyrosequencing

High-throughput 454 pyrosequencing (Roche 454 Life Sciences, Branford, CT, USA) was undertaken on a subset of samples (17, Table 3. 1) chosen to encompass the geochemical, temporal, biological and spatial variation within the data set. The V5-V6 hypervariable region of the 16S rRNA gene was utilized to identify variation in bacterial community diversity and structure using primers and conditions described previously (Archer *et al.*, 2014). Briefly, triplicate 30 µL PCRs were run for each sample using unadapted primers Tx9F (5'-GGATTAGAWACCCBGGTAGTC-3') and 1391R (5'-GACGGGCRGTGWGTRCA-3') (Ashby *et al.*, 2007). The triplicates were pooled and gel extracted (2% TAE agarose stained with "SYBR Safe") and DNA retrieved using the UltraClean 15 DNA Purification Kit (MO BIO Laboratories, Carlsbad, CA, USA) as per manufacturer's instructions. A second 10 cycle round of triplicate PCR was run as above using 25 ng of the purified DNA as a template per reaction (Milli-Q H_2O volume adjusted accordingly). The primers used were adapted for one-way reads according to the Roche GS Junior System Guidelines for Amplicon Experimental Design Manual (August 2010), including unique MID identifiers for each sample (BacX-Tx9F (5`-CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID-GGATTAGAWACCCBGGTAGTC-3`) and BacB-1391R (5`-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-GACGGGCRGTGWGTRCA-3`). After a further gel extraction as outlined above, samples went through a final cleanup step using the Agencourt AMPure XP system (Beckman Coulter Genomics, Danvers, MA, USA) as per manufacturer's instructions. DNA concentration was quantified using a Qubit Flurometer (Invitrogen Ltd) and diluted to 200 pg/µL. DNA concentration and quality verification was performed

using a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and then subsequently diluted to ~$1x10^9$ molecules/μL. The diluted amplicons were combined to create the $1x10^9$ amplicon pool and sequencing performed using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTiterPlate Kit and GS Junior System according to the manufacturer's instructions (Roche 454 Life Sciences, Branford, CT, USA).

3.3.5 454 pyrosequencing data processing

454 PCR amplicon pyrosequencing data were denoised using AmpliconNoise v1.0 (Quince *et al.*, 2011), PyroNoise (Quince *et al.*, 2009; Quince *et al.*, 2011) and SeqNoise (Quince *et al.*, 2011) and PCR chimeras were removed using Perseus (Quince *et al.*, 2011). Resulting de-replicated sequences were processed using Mothur 1.17.0 (Schloss *et al.*, 2009) to create a unique sequence and name file. Pairwise alignments and distances were calculated using Espirit (Sun *et al.*, 2009). Mothur was then used to cluster sequences into operational taxonomic units (OTUs) defined at the average neighbor Jukes-Cantor distance of 0.05 ($OTU_{0.05}$). For phylogenetic assignments, representative sequences of all identified $OTU_{0.05}$ were analyzed using the Classifier function provided by the Ribosomal Database Project (RDP) Release 10, Update 15 (Wang *et al.*, 2007). Taxonomic assignment threshold was set at 80%. A Bray Curtis similarity matrix was produced based on relative pyrosequencing abundances and a BEST analysis (Clarke & Gorley, 2006) conducted to compare the pyrosequencing with the environmental data. Unique sequences, those absent in at least one pond, were separated and a BEST analysis conducted. An NMDS plot with overlaid environmental variables was created using the 'vegan' library in R (R Core Team, 2013) to visualize this information. Good's nonparametric coverage estimator was calculated to evaluate sequencing coverage for each sample and Shannon's biodiversity index (Primer 6) to compare biodiversity between pooled samples. To gain a visual representation of community similarity based on sequencing data, dendrograms were created using a resemblance matrix based on the Bray Curtis community similarities (Primer 6).

3.4 Results

3.4.1 Physicochemical environment and drivers of community structure

Overall physicochemical properties varied between ponds (Table 3. 1 and 2). *In-situ* pH and conductivity were combined with bacterial ARISA community similarity (Figure 3. 2) to provide a framework for the selection of representative samples. ARISA NMDS plots show that, with the exception of 2009 samples, there is little grouping based on year or location. BEST analysis identified conductivity as a strong contributing explanatory variable across the entire ARISA data set (Spearman's p of 0.344), and within data subsets separated based on location (Miers or Bratina) or year (2009, 2012, 2013) (Spearman's rank p in Bratina Island samples of 0.876 in 2009, 0.363 in 2012, 0.669 in 2013, and 0.265 in the Miers Valley Ponds). Representatives were selected from samples collected in 2012 to encompass the full range of conductivity and pH. <1 mS cm^{-1} (Casten) to >40 mS cm^{-1} (Salt), and pH 8.9 (Retro) to 10.5 (Legin and Bambi) (Table 3. 1). Ponds resampled in 2013 and ponds located in Miers Valley were selected based on similarity of bacterial ARISA community profiles (Figure 3. 2) and conductivity (Table 3. 1). BEST analysis identified pH as a weak explanatory variable (spearman's p of 0.121) across the ARISA dataset, however it appeared to separate December from January samples (Figure 3. 2). A Tukey Honest significance difference test confirmed that pH values from Bratina samples in December 2009 were significantly lower than any January samples, ($P< 0.001$) for Bratina 2012 and 2013, and ($P=0.006$) for Miers 2013. Representative samples from 2009 were therefore selected to encompass this pH range (Legin and Egg (K160)).

The relationship between physicochemical profiles (pH, conductivity, ICP-MS and nutrient data) from representative samples provided no clustering based on year or location (Figure 3. 3). Congruent with ARISA data, conductivity was identified as the most significant driver of community structure (BEST Spearman's p of 0.395), however pH was poorly correlated in this analysis (Spearman's p of -0.010). Other factors such as Hg, Fe and PO_4 had vectors perpendicular to conductivity across NMDS axis 1 (Supplementary Figure 3. 1),

but low BEST correlation scores (Spearman's p of -0.036, 0.104 and 0.089 respectively).

The effect of conductivity on community structure in individual ponds can be witnessed in a comparison of three ponds sampled in 2012 which span the range of conductivities, Salt (40.6 mS), P70E (7.1 mS) and Casten (0.8 mS) (Table 3. 2, Figure 3. 4). Analysis of sequence abundance showed there was less similarity between Salt and P70E, than Salt and Casten (Figure 3. 4), with visibly different phyla compositions for all three. Total OTU numbers were lower in Salt than Casten or P70E (157, 238 and 251 respectively), with four of the ten most abundant OTUs across all samples absent in Salt pond, more than in any other pond (Table 3. 3). Conductivity driven inter-pond community variation was identified in the abundance of one specific OTU (100% identity to *Flavobacterium segetis*), which was present at 0% abundance in Salt, 0.3% in P70E and 11.2% in Casten (Table 3. 3).

A BEST analysis of the unique community, sequences absent in at least one pond, represented 1.05, 9.32, 5.96 and 5.92% of total sequences in Bratina 2009, Bratina 2012, Bratina 2013 and Miers 2013 respectively and 1219 of the 1230 (99.1%) total OTUs from this study (Figure 3. 5), identified Si, B and S as strong drivers of the unique community structure (Spearman's p of 0.723, 0.576 and 0.568 respectively).

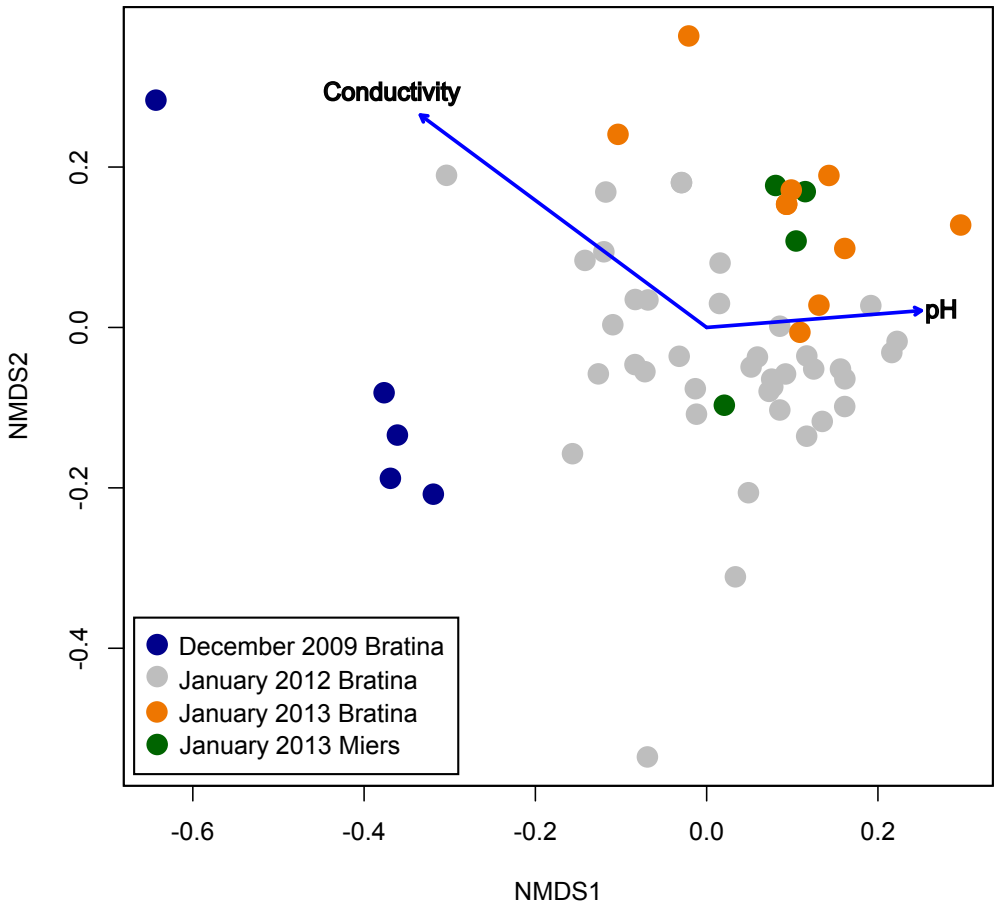

Figure 3. 2 Non-metric multidimensional scaling (NMDS) ordination (stress 0.2) of bacterial ARISA community composition in surface water of meltwater ponds from Bratina Island and the Miers Valley based on Bray Curtis distances. Overlaid vectors indicate strength and direction of environmental variables based on Euclidean distances (where complete data coverage exists).

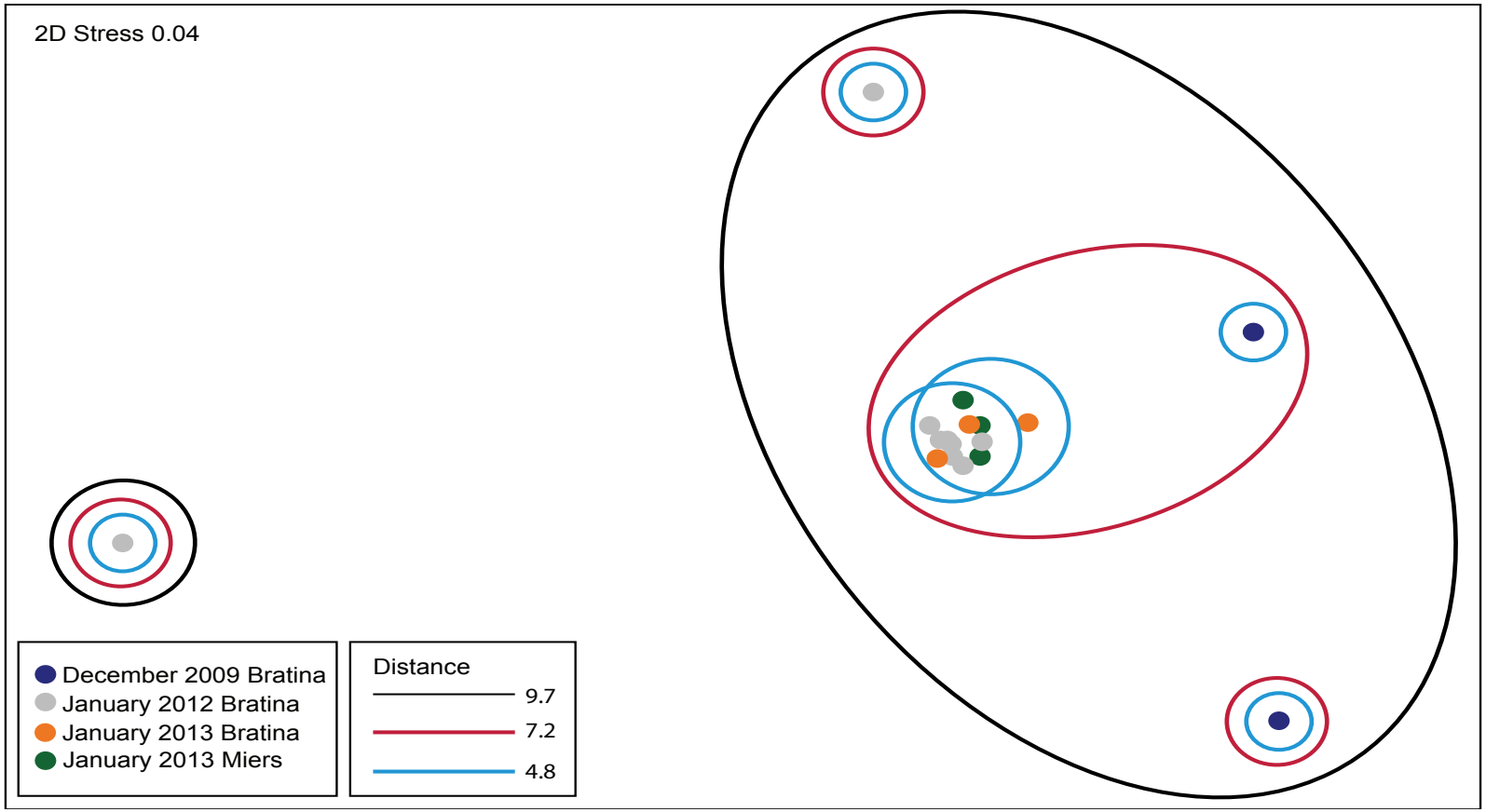

Figure 3. 3 MDS plot of representative pond physicochemical profiles based on a Euclidean distance matrix.

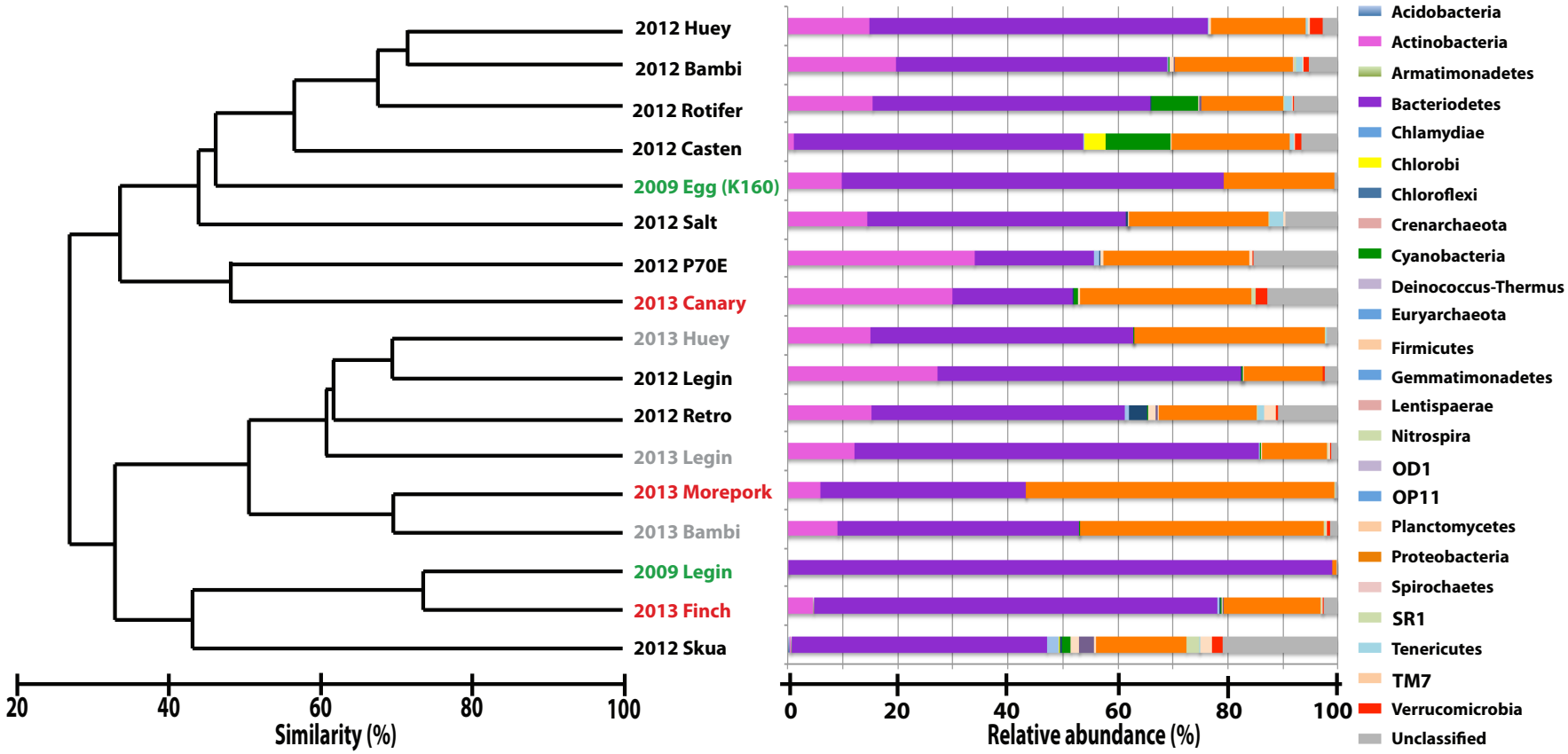

Figure. 4 Classification of 16S rRNA sequencing data using the RDP Classifier (Release 10, Update 15), assignment confidence threshold >80%. Bray-Curtis tree calculated from 16S rRNA gene $OTU_{0.05}$ compositions with no transformation to visualize total relative spatial/temporal similarities. Pond sequences as individual samples. Colors of sample names represent sampling time/location.

Table 3. 1 GPS and geochemical data from meltwater pond sampling. Samples selected for 454 sequencing, nutrient and ICP-MS analysis marked with an asterisk in bold.

December 2009 - Bratina Island				
Pond	**GPS Coordinates**		**Conductivity mScm^{-1}**	**pH**
Orange	S 78.01383	E 165.55515	1.1	9.6
P70E	S 78.01580	E 165.55165	7.0	8.1
***Legin**	**S 78.01624**	**E 165.54903**	**2.0**	**6.4**
***Egg**	**S 78.01395**	**E 165.55847**	**8.2**	**9.5**
Salt	S 78.01608	E 165.54510	79.1	8.8
January 2012 - Bratina Island				
Orange	S 78.01383	E 165.55515	1.9	10.2
***P70E**	**S 78.01580**	**E 165.55165**	**7.1**	**9.2**
***Legin**	**S 78.01624**	**E 165.54903**	**3.3**	**10.5**
***Salt**	**S 78.01608**	**E 165.54510**	**40.6**	**9.9**
Brack	S 78.01569	E 165.54570	10.0	9.8
***Huey**	**S 78.01419**	**E 165.55576**	**3.8**	**9.8**
***Casten**	**S 78.01254**	**E 165.55095**	**0.8**	**9.6**
Nicholas	S 78.01251	E 165.54922	3.8	10.1
K081	S 78.01324	E 165.55084	0.9	9.6
Foam	S 78.01340	E 165.55394	2.0	9.6
Egg (K160)	S 78.01395	E 165.55847	11.5	10.0
Luey	S 78.01426	E 165.55637	2.0	9.1
Duey	S 78.01418	E 165.55710	1.7	10.0
Permanent ice pond	S 78.01420	E 165.55359	0.1	9.8
Ribbon	S 78.01529	E 165.55246	0.5	9.5
Galore	S 78.01579	E 165.55260	5.7	9.4
Bibby	S 78.01564	E 165.55489	3.1	9.1
Wendy	S 78.01597	E 165.55521	1.8	9.8
Russell	S 78.01621	E 165.542224	1.0	9.4
Hawes	S 78.01612	E 165.55963	1.5	9.6
Upper	S 78.01546	E 165.54285	6.0	9.8
Extra	S 78.01489	E 165.54144	6.7	9.3
***Retro**	**S 78.01465**	**E 165.54628**	**4.9**	**8.9**
Conophyton	S 78. 01431	E 165.54500	0.5	9.9
Lunch	S 78.01422	E 165.54628	0.8	9.5
Sub Skua	S 78.01352	E 165.55005	2.5	9.8
Drillbit	S 78.01372	E 165.55185	8.8	8.8
iMac	S 78.01347	E 165.55272	6.8	9.6
Long	S 78.01265	E 165.54625	1.2	10.0
Ice Ridge Pond	S 78.01231	E 165.54587	2.8	8.6
***Rotifer**	**S 78.01225**	**E 165.55063**	**2.6**	**9.8**
Archers	S 78.01194	E 165.55063	1.8	10.1
***Skua**	**S 78.01326**	**E 165.55122**	**1.0**	**9.9**
JAC	S 78.01368	E 165.54967	2.0	9.4
Nostoc	S 78.01368	E 165.55460	1.1	10.2
***Bambi**	**S 78.01649**	**E 165.54936**	**3.4**	**10.5**
Fresh	S 78.01569	E 165.54570	0.6	10.4
P70	S 78.01485	E 165.55193	3.3	10.2
Fogghorne	S 78.01609	E 165.55112	0.7	10.0
January 2013 - Bratina Island				
Orange	S 78.01383	E 165.55515	2.0	9.9
P70E	S 78.01580	E 165.55165	7.7	8.8
***Legin**	**S 78.01624**	**E 165.54903**	**2.2**	**9.7**
Salt Pond	S 78.01608	E 165.54510	40.8	9.6
***Huey**	**S 78.01419**	**E 165.55576**	**4.4**	**9.5**
P70	S 78.01485	E 165.55193	3.8	10.4
Russell Pond	S 78.01621	E 165.542224	1.1	9.5
Skua	S 78.01326	E 165.55122	0.9	9.7
***Bambi**	**S 78.01649**	**E 165.54936**	**3.3**	**10.6**
Fogghorne	S 78.01609	E 165.55112	1.3	10.1
January 2013 - Miers Valley				
***Morepork**	**S 78 07.343**	**E164 12.061**	**4.1**	**9.1**
***Canary**	**S 78 07.433**	**E 164 11.453**	**4.2**	**9.6**
***Finch Top**	**S 78 07.748**	**E 164 11.714**	**1.7**	**9.8**
Robin Top	S 78 07.752	E 164 11.762	0.6	10.1

Table 3. 2 Complete environmental dataset overlaid as vectors in Figure 3 pyrosequencing NMDS plot and used in BEST analysis.

			(ppm)				(ppb)							
Pond	**Conductivity**	**pH**	**Phosphate**	**NH4 marine**	**NO2**	**NO3**	**B 10**	**Al 27**	**Si 28**	**S 34**	**Ca 43**	**V 51**	**Cr 52**	**Fe 54**
Legin 2009	2.0	6.4	0.02	0.00	0.01	0.00	15.2	69.1	27354.7	0.0	3532.9	4.9	0.0	0.0
Egg 2009	8.2	9.5	1.83	0.00	0.01	0.00	262.5	167.7	35480.0	33383.0	19223.9	6.1	0.0	0.0
Bambi 2012	3.4	10.5	0.22	0.00	0.00	0.00	10127.2	171.7	31130.9	82754.3	13385.9	12.2	2.5	0.0
Casten 2012	0.8	9.6	0.10	0.00	0.00	0.00	10633.9	158.6	29227.0	56652.8	10925.7	7.1	1.9	0.0
Skua 2012	1.0	9.9	0.09	0.00	0.00	0.00	10603.3	154.4	28191.7	71909.8	15099.5	6.9	0.7	0.0
Legin 2012	3.3	10.5	0.01	0.00	0.00	0.00	10439.5	126.7	31034.5	77127.2	42677.6	7.8	0.1	0.0
P7OE 2012	7.1	9.2	0.01	0.00	0.10	0.13	10239.7	128.3	28220.2	99432.6	159920.0	0.1	3.1	0.0
Retro 2012	4.9	8.9	0.01	0.00	0.00	0.00	10773.2	128.7	30998.8	149014.7	94448.0	5.5	5.4	0.0
Rotifer 2012	2.6	9.8	0.08	0.00	0.00	0.00	10938.7	140.2	31841.0	74022.1	36815.6	6.1	2.6	0.0
Salt 2012	40.6	9.9	0.02	0.00	0.00	0.00	539580.0	5277.5	1380505.0	1183573.7	167540.2	30.6	33.2	0.0
Huey 2012	3.8	9.8	0.01	0.00	0.00	0.00	11048.0	137.5	30616.1	72176.3	32163.8	9.5	2.9	0.0
Huey 2013	4.4	9.5	0.01	0.03	0.00	0.00	11462.9	175.5	30351.3	81109.2	31250.8	10.1	1.3	0.0
Bambi 2013	3.3	10.6	0.20	0.02	0.00	0.00	11047.1	174.7	33828.2	82953.6	14302.8	14.6	0.0	249.5
Legin 2013	2.2	9.7	0.02	0.00	0.00	0.00	10877.5	166.8	30905.0	78411.8	34647.4	9.1	0.4	194.8
Canary 2013	4.2	9.6	0.15	0.00	0.00	0.00	10855.2	126.7	31199.4	272842.8	25247.0	17.6	0.0	217.3
Finch 2013	1.7	9.8	0.01	0.00	0.00	0.00	10999.5	89.4	29821.7	42482.5	21834.0	6.9	0.0	10.4
Morepork 2013	4.1	9.1	0.01	0.00	0.00	0.00	11193.5	116.7	33450.0	97953.8	48144.9	8.2	4.3	339.8

	(ppb)												
Pond	**Mn 55**	**Co 59**	**Ni 60**	**Cu 63**	**Zn 68**	**As 75**	**Se 82**	**Sr 88**	**Cd 111**	**Ba 137**	**Hg 202**	**Pb 207**	**U 238**
Legin 2009	0.2	0.0	0.0	0.0	0.0	2.2	1.9	60.2	0.0	3.4	0.0	0.0	0.1
Egg 2009	12.0	0.0	0.0	2.9	0.0	11.0	9.3	297.1	0.3	12.3	0.0	0.0	0.4
Bambi 2012	3.7	0.4	0.0	91.3	156.0	6.5	0.0	154.0	0.0	4.5	2.1	2.0	0.6
Casten 2012	4.5	0.2	0.0	81.6	159.3	0.0	0.0	109.2	0.0	4.3	0.0	2.0	0.3
Skua 2012	4.2	0.2	0.0	82.7	222.5	0.0	0.0	146.1	0.0	6.1	0.0	1.3	0.2
Legin 2012	20.0	0.2	0.0	85.4	170.4	0.6	0.0	686.8	0.0	6.0	0.0	6.4	0.4
P7OE 2012	10.8	0.3	0.0	89.5	196.6	2.4	11.8	2039.0	0.0	7.1	0.2	2.0	2.0
Retro 2012	9.2	0.2	0.0	91.3	195.8	0.0	5.3	849.2	0.0	5.5	0.0	1.6	0.9
Rotifer 2012	10.1	0.2	0.0	86.3	183.3	0.0	0.0	405.1	0.1	5.8	0.0	1.2	0.1
Salt 2012	61.2	5.0	400.0	5673.5	22762.8	0.0	0.0	949.0	15.0	190.0	0.0	337.5	0.0
Huey 2012	6.0	0.2	6.9	127.2	430.2	0.0	0.0	341.2	0.0	5.4	0.0	6.2	0.9
Huey 2013	14.2	0.1	0.0	101.6	697.7	1.0	0.0	339.0	0.2	9.5	0.0	1.9	0.8
Bambi 2013	3.4	0.5	0.0	84.4	121.6	8.8	12.3	181.0	0.2	5.0	1.2	0.9	0.5
Legin 2013	7.4	0.0	0.0	79.6	148.0	1.1	3.3	456.3	0.9	4.4	1.4	1.0	0.6
Canary 2013	2.8	0.2	0.0	94.7	286.5	1.1	0.6	383.2	0.5	7.9	1.5	48.8	1.4
Finch 2013	2.5	0.5	0.0	92.9	115.5	0.2	7.7	242.0	0.3	145.2	0.2	0.8	1.1
Morepork 2013	9.4	0.2	0.0	88.4	213.6	5.0	10.2	760.0	0.0	7.8	3.8	0.8	1.6

Table 3. 3 Pyrosequencing results highlighting the ten most abundant OTUs overall, in each individual sample.

Bratina 2009		Bratina 2012									Bratina 2013			Miers 2013			Phylum	Class	Accession #	E-Score	Organism	Description
Egg (K160)	Legin	Legin	P70E	Retro	Rotifer	Salt	Skua	Casten	Huey	Bambi	Bambi	Huey	Legin	Finch	Canary	Morepork						
3.13%	98.07%	20.17%	0.34%	20.05%	8.51%	0.00%	39.63%	11.17%	21.59%	12.26%	34.62%	19.41%	47.87%	72.99%	7.41%	19.83%	Bacteroidetes	Flavobacteria	JX971557.1	5.00E-140	Flavobacterium segetis	South Korea, host mushroom "Pleurotus ostreatus"
																			HM149212.1	5.00E-140	Flavobacterium sp.	South Korea, mesotrophic artificial lake
64.82%	0.15%	3.96%	6.81%	0.19%	15.96%	25.32%	0.83%	18.07%	30.64%	18.69%	2.31%	8.83%	0.25%	0.04%	8.07%	0.00%	Bacteroidetes	Sphingobacteria	FR691439.1	6.00E-133	Algoriphagus sp.	Antarctica:Transantarctic Mountains, Forlidas pond
																			FJ196000.1	3.00E-130	Algoriphagus sp.	Antarctic sandy intertidal Sediments
0.67%	0.20%	4.49%	29.36%	18.72%	35.14%	27.73%	0.94%	24.53%	11.45%	22.87%	4.61%	1.83%	1.15%	0.04%	0.70%	0.07%	Chloroplast	Chloroplast	AB073111.1	2E-140	plastid Geminigera cryophila	Japan, related to shellfish poisoning
																			JQ200103.1	2E-139	uncultured bacterium	USA: CA, Seawater
6.01%	0.00%	16.77%	13.95%	10.30%	7.89%	4.97%	0.05%	0.58%	11.87%	11.53%	7.21%	13.58%	11.68%	3.53%	27.34%	5.81%	Actinobacteria	Actinobacteria	DQ015794.1	1.00E-135	uncultured bacterium	Antarctica:Taylor Valley, Lake Bonney
																			DQ512860.1	3.00E-107	Ornithinimicrobium pekingense	China, from activated sludge
0.08%	0.00%	1.41%	5.67%	7.30%	0.35%	0.99%	0.00%	0.20%	1.53%	7.69%	36.08%	14.13%	3.05%	0.00%	0.00%	54.84%	Proteobacteria	Betaproteobacteria	KF641823.1	7.00E-112	Bordetella sp.	China, Camel rumen
																			KC634248.1	7.00E-112	Castellaniella denitrificans	India, water treatment plant
0.03%	0.04%	16.19%	0.00%	13.04%	0.47%	0.00%	0.10%	0.08%	0.03%	2.52%	1.92%	13.53%	13.94%	0.04%	0.12%	15.87%	Bacteroidetes	Flavobacteria	HQ405608.1	5.00E-107	culture clone	High pressure bioreactor enriching for anaerobic oxidation of methane
																			JQ689940.1	2.00E-105	Tamlana sp.	Korea, Dokdo sea water
18.78%	0.57%	8.68%	0.35%	0.39%	3.22%	0.00%	0.82%	7.73%	2.87%	2.18%	2.70%	12.04%	6.20%	0.19%	10.10%	0.09%	Proteobacteria	Betaproteobacteria	KF051781.1	1E-142	Variovorax paradoxus	China, "Solanum nigrum" plant
																			JQ977446.1	1E-142	Variovorax sp.	China: Tianshan Mountains, rizosphere soil
0.03%	0.00%	0.00%	0.19%	0.39%	0.00%	9.96%	0.12%	0.00%	8.17%	3.43%	1.20%	4.84%	0.29%	15.29%	1.60%	0.81%	Proteobacteria	Alphaproteobacteria	KC702679.1	1.00E-154	Loktanella sp.	India, Pangong Lake water
																			AB681826.1	7.00E-152	Loktanella vestfoldensis	Culture collection = NBRC:102487 Antarctic lake microbial mats
0.03%	0.08%	3.94%	0.01%	1.29%	0.10%	0.00%	0.11%	0.19%	0.08%	0.73%	1.20%	1.46%	9.10%	0.00%	1.87%	1.58%	Bacteroidetes	Sphingobacteria	FR691448.1	1.00E-140	Pedobacter sp.	Forlidas Pond, Antarctic Transantarctic Mountains
																			NR_117785.1	5.00E-144	Pedobacter arcticus	Falcultative psychrophile isolated from Arctic soil
93.56%	99.11%	75.60%	56.69%	71.67%	71.64%	68.96%	42.61%	62.56%	88.22%	81.91%	91.85%	89.65%	93.51%	92.10%	57.22%	98.89%	Total					

3.4.2 Annual Variation

Bratina Island pond samples in both 2012 and 2013 were not clearly separated in the bacterial ARISA NMDS (Figure 3. 2), however ANOSIM identified some variation between time points with low statistical significance R=0.305 P=0.2. Pyrosequencing data were consistent with this finding, with 119 OTUs shared between Bratina samples in 2012 and 2013, representing 96.0% and 98.9% of total pooled reads respectively (Figure 3. 5). Although specific OTU structure varied between years, the 10 most abundant OTUs, each representing >1% of total reads, constitute 75.6 and 93.5% in Legin, 88.2 and 89.7% in Huey, and 81.9 and 91.9% in Bambi, for 2012 and 2013 respectively (Table 3. 3). When individual pond similarity between 2012 and 2013 was compared Huey and Bambi exhibited <40% similarity between years, while Legin exhibited >60% similarity. *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* abundance within ponds was highly variable between years (Figure 3. 4), which was reflected in variable OTU abundances (Table 3. 3). A single OTU found at a consistently higher abundance in Bambi, Huey and Legin in 2012 was a chloroplast sequence (99% identity to the chloroplast of *Geminigera cryophila*) representing 4.6 to 22.9% in Bambi, 1.8 to 11.5% in Huey, and 1.2 to 4.5% in Legin, in 2013 and 2012 respectively. The abundance of this OTU was >20% of total reads in ponds sampled in 2012.

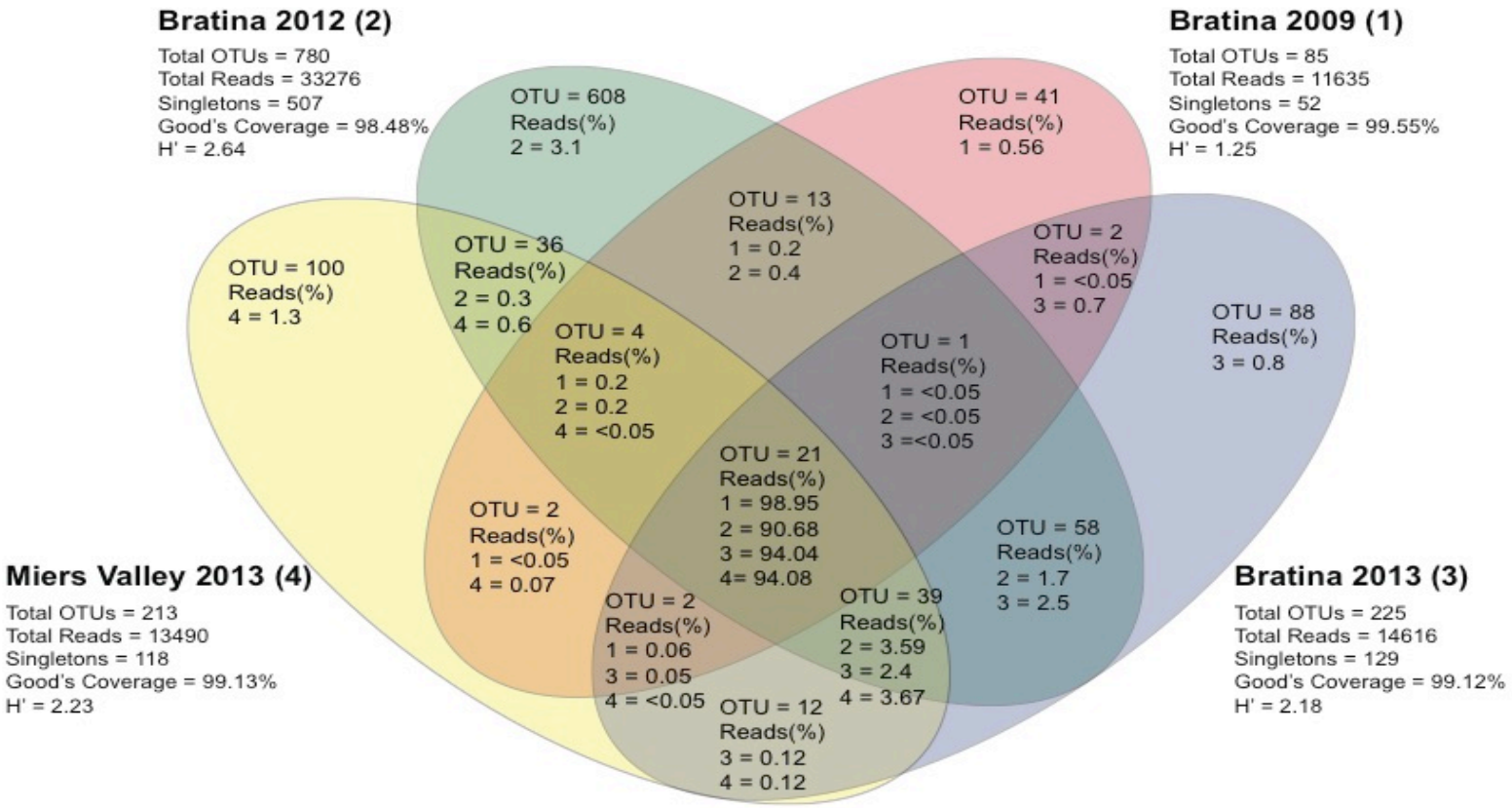

Figure 3. 5 Summary of total 454 sequencing data (total OTUs, Reads, Singletons, Good's Coverage and Shannon's diversity (H') (distance = 0.05)) representing shared OTUs and reads between sampling time/location: Bratina 2009 (Egg and Legin); Miers 2013 (Morepork, Canary and Finch); and three ponds from Bratina sampled in both 2012 and 2013 (Bambi, Legin and Huey). Total OTU richness is 1026, total read number 73017.

3.4.3 Seasonal Variation

Bacterial ARISA data clearly separated December 2009 samples from both January 2012 and 2013 at Bratina Island (Figure 3. 2) (ANOSIM 2009:2012 R=0.847 P=0.01; ANOSIM 2009:2013 R=0.918 P=0.03). This separation is likely linked to lower pH in 2009, although conductivity in Legin was also lower in 2009 (1.99 mS cm^{-1}) than in either 2012 or 2013 (3.34 and 2.2 mS cm^{-1}) (Table 3. 2). Pyrosequencing data identified lower total OTU numbers in 2009 than in 2012 and 2013 (85, 780 and 225 respectively), which was supported by the Shannon diversity index: 1.25, 2.64 and 2.18 for 2009, 2012 and 2013 respectively (Figure 3. 5). The greater diversity in 2012, but similar Shannon diversity index value to 2013, is most likely linked to the higher number of sequenced reads for 2012. Goods coverage estimator scores of >98% for each pooled sample confirmed there was adequate sequencing depth to compare diversity scores. The majority of reads (98.95%) in the 2009-pooled sample were represented by 21 OTUs shared by all pooled samples (Figure 3. 5), with the three most abundant OTUs accounting for >90% of total reads (Table 3. 3). Total OTU numbers from Legin pond samples, which was sampled at all three time points, did exhibit a similar trend (35 in 2009, 233 in 2012 and 76 in 2013). Pyrosequencing data from 2009 samples did not cluster together and both were clear outliers in the dataset (Figure 3. 3). Phyla abundance revealed Legin and Egg 2009 to have simple communities dominated by *Bacteroidetes*-related signatures (98.9 and 69.4% respectively) (Figure 3. 4), however the dominant *Bacteroidetes*-related OTUs were different between ponds. 98.1% of total reads in Legin were 100% identical to *Flavobacterium* sp. previously found in a mesotrophic artificial lake in Korea, and 64.8% in Egg pond were 99% identity to *Algoriphagus* sp. previously found in a pond in the Transantarctic Mountains and Antarctic sandy intertidal sediment (Table 3. 3). Egg pond also contained a *Betaproteobacteria*-related signature that represented 18.8% of its total reads compared to 0.6% in Legin (2009) (100% sequence identity to *Varivorax* sp. previously found in rhizosphere soil in China). All three of these OTUs were also well represented in other ponds (ranging from 0.004 to 73%).

3.4.4 Regional biogeography

The bacterial ARISA NMDS did not differentiate Miers Valley from Bratina Island ponds (Figure 3. 2) (ANOSIM Bratina 2012:Miers R=0.188, P=11.4; ANOSIM Bratina 2013:Miers R=0.07, P=32.4). A Venn diagram illustrating the distribution of unique OTUs was created from pooled pyrosequencing data (Figure 3. 5). All pooled samples (Bratina 2009, Bratina 2012, Bratina 2013 and Miers 2013) shared 21 OTUs, representing 90.68 - 98.95% of total read number. Unique OTUs present in only one set of pooled samples represented 0.6, 3.1, 0.8 and 1.3% in Bratina 2009, 2012, 2013 and Miers 2013 respectively. The 1.3% in the Miers Valley samples represented 176 reads, 100 unique OTUs and 58 singletons. The most abundant unique OTU was a species of *Bacteroidetes* isolated from marine sediment that represents 28% of unique sequences (98% sequence identity to *Lewinella* sp.).

When examined individually the Miers Valley samples (Canary, Morepork and Finch) were more similar to a Bratina Island pond than to one another (Figure 3. 4). Finch (Miers 2013) and Legin (2009) were highly similar (>70%) based on this analysis, and similarly both ponds were dominated (73 and 98.1%) by the same major OTU (*Flavobacterium segetis*) (Table 3. 3). Morepork (Miers 2013) and Bambi (2013) were also highly similar (>60%) (Figure 3. 4) with all but one dominant OTU shared between ponds accounting for 81.9 and 98.9% of total sequences in each sample respectively (Table 3. 3). Canary (Miers 2013) and P70E (2012) were somewhat similar (>40), more so than Canary, Morepork and Finch to one another (<40%). Only three of the 10 most abundant OTUs in this study (*Algoriphagus* sp., *Bordetella* sp., and *Pedobacter* sp.), which are present in all Bratina 2013 samples, are absent from any one of the Miers Valley samples (Table 3. 3). The total sequences represented by these 10 OTUs at both locations are greater than 89% in each pond except Canary (57.2%), which is still a greater proportion than from two ponds sampled in 2012 (P70E to which it is most similar, and Skua pond). Across all individual ponds the cumulative abundance of the top 10 OTUs accounted for an average coverage of 79.6% (sd 16.9%), the lowest being Skua (42.6%), and the highest Legin 2009 (99.2%).

3.5 Discussion

Antarctic meltwater ponds are characterized as having extremely heterogeneous physicochemical habitats, as was reflected in the ponds of this study (Hawes *et al.*, 2014; Healy *et al.*, 2006; Lyons *et al.*, 2012; Wait *et al.*, 2006). As previously documented, all ponds encompassed a wide range of pH (6.4-10.5) and conductivity values (<1-40 mS cm^{-1}), with varied elemental and nutrient compositions (Archer *et al.*, 2014; Hawes *et al.*, 2014; James *et al.*, 1995). As ponds thaw during the brief austral summer, variable fresh water inputs replenished the ponds, causing fluctuations in conductivity (Hawes *et al.*, 2014; Healy *et al.*, 2006). Moreover, biological activity increases rapidly with high levels of photosynthesis continuing into the start of the austral winter. Subsequently, as the ponds commence to freeze from the top down, a shift from net autotrophy to heterotrophy is observed until most ponds freeze solid (Hawes *et al.*, 2011a; Safi *et al.*, 2012). These extreme conditions restrict the trophic complexity of these ecosystems and, as a result, abiotic factors represent the most dominant influence on the endemic microbial community structure (Howard-Williams *et al.*, 1989; Howard-Williams *et al.*, 1990; James *et al.*, 1995; Safi *et al.*, 2012; Vincent & James, 1996).

Consistent with previous studies, conductivity was identified as a key explanatory factor for biological heterogeneity across all samples, with variable significance within a single time/location (Ma *et al.*, 2013; Zhang *et al.*, 2013). As expected, Salt pond (with the highest conductivity of 40.6 mS cm^{-1}) also had the lowest observed diversity of all sampled ponds. However, the phyla structure of this pond was similar to ponds with lower conductivity. This observed similarity in microbial inhabitants across conductivity gradients is most likely due to high tolerance to environmental extremes that would be necessary for survival of these microorganisms in these dynamic and extreme ecosystems (Sabacka & Elster, 2006; Schmidt *et al.*, 2009; Yergeau & Kowalchuk, 2008). For example, the abundance of *Flavobacterium segetis,* a major OTU in most ponds in this study, decreased with increasing conductivity and this organism is known to have optimum growth at low salt (0 %) concentrations (Yi & Chun, 2006). However, many of the dominant OTUs such as *Algoriphagus* sp., *Ornithinimicrobium pekingense*, and *Loktanella* sp. exhibit wide halotolerance which may explain

their detection in ponds with hypervariable conductivities (Liu *et al.*, 2008; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). In addition to conductivity, pH was also proven to correlate with community structure for the December 2009 samples, which is consistent with previous studies within these same pond ecosystems (Archer *et al.*, 2014; Safi *et al.*, 2012). Other environmental parameters (Hg, Fe and PO_4) were identified as weak drivers of community structure across the data set; the low correlation possibly indicative of a smaller influence on community structure perhaps only affecting a subset of ponds due to less pronounced inter-pond variations in these parameters as opposed to the system-wide influence across the broad conductivity gradient (Desai *et al.*, 2012; Dore & Priscu, 2001; Silver & Hobman, 2007). Although the total community was dominated by a small number of OTUs when the unique community, representing the majority (99.1%) of total ecosystem diversity, was analyzed separately a distinct set of environmental drivers, whose influence was much greater than any variable to the total community, were identified. Silicon in particular exerted a strong influence on this community, although it is unclear how it would exert selective control in this ecosystem.

Studies that have investigated variations in Antarctic aquatic ecosystems through seasonal transitions, for example, winter to summer (Pearce, 2005), and summer to winter (Hawes *et al.*, 2011a; Safi *et al.*, 2012), have shown the dynamic nature of the resident microbial communities in Antarctic lakes and ponds. However, detailed comparison of temporal changes in bacterioplankton communities of meltwater ponds remains neglected. In this study, pooled sequences exhibited a high degree of similarity between 2012 and 2013 samples, however, when ponds were examined separately, eliminating any normalization that may occur from pooling heterogeneous ponds, a highly variable community structure was identified in each pond between years. However, a similar "core" of dominant OTUs was present at both time points (2012 and 2013) with a similar total abundance, explaining the high number of shared sequences, yet the relative abundance of many OTUs varied significantly within a pond between the two time points. These results indicate a common microbial core that is well adapted to the environment and may persist in the ponds over long time scales. A number of these OTUs have been previously detected in other Antarctic aquatic

ecosystems, confirming their suitability to this environment and suggesting either a global aeolian source or broad dispersal throughout the continent (Herbold *et al.*, 2014; Sokol *et al.*, 2013; Vincent, 2000; Wood *et al.*, 2008). Within this study, the observed inter-year variation may be due to minor geochemical differences identified between the sampling periods, or due to climatological variation prior to sampling. Also, the higher abundance of chloroplast sequences from samples collected in 2012 may be due to an enrichment of the photoautotrophic component of the microbial community due to a greater number of warmer austral days prior to sampling (Howard-Williams *et al.*, 1989; Lizotte & Priscu, 1992; Moorhead *et al.*, 1997; Vincent & James, 1996; Vincent, 2000).

Microbial communities in December samples (representing early summer) were clearly distinguishable from January samples (representing late summer) for ponds located at Bratina Island. This is consistent with a previous investigation of the transition from winter ice cover to summer melt in three Antarctic lakes which showed that the microbial communities were extremely responsive to variation of physicochemical parameters (Pearce, 2005). Pearce (2005) singled out temperature as the most significant parameter driving changes in community structure, however concurrent seasonal changes such as increased light duration and intensity would have also played a significant role. Interestingly, pH was identified as an important variable separating December and January bacterial community profiles at Bratina Island. Such shifts in pH values within a single pond have been previously documented and are common throughout the year due to changes in biological activities associated with seasonal changes (Safi *et al.*, 2012). In early summer (December), ponds had most likely only thawed for a short period (i.e. days to weeks), thereby reducing the amount of photosynthesis occurring within the pond (also evidenced by the lower chloroplast OTU abundance), which may have led to a drop in pH. The less complex community structure and lower observed diversity is most likely also a reflection of the shorter time since thawing of the ponds where the effects of the extreme winter selection are still visible in the restricted community (Hawes *et al.*, 1999; Hawes *et al.*, 2011a; Safi *et al.*, 2012). The observed community was likely preferentially enriched for microbes with the capability of rapid activation following winter freeze. Variable melt-water input and poor mixing early in the season could also

have influenced the community structure in the December samples, supported by the reduced conductivity values and the selective effects of conductivity on the microbial communities of these ecosystems (Archer *et al.*, 2014; James *et al.*, 1995; Jungblut *et al.*, 2005).

This temporal investigation has shown variability of bacterioplankton communities in these ponds throughout the austral summer and identified environmental drivers for community dynamics. It is, however, difficult to confidently determine how consistent these biological communities are between years given the rapid changes in geochemistry these ponds can experience (Hawes *et al.*, 2014). Yet, an understanding of the natural annual variability, inherent biological processes and drivers of community structure in these ponds is required to utilize these valuable scientific resources for future models of biological change from increasing global temperatures.

This study represents the first comparison of bacterial communities between ice shelf (Bratina Island) and terrestrial ponds (Miers Valley). Regardless of the difference in local soil types, the bacterial communities of the Miers Valley ponds were not unique from those located at Bratina Island, and were in fact more similar to Bratina ponds than to one another, likely due to the similarity of pond geochemical profiles between locations. A small fraction of low abundance OTUs was unique to each location; however the 10 most abundant OTUs represented the majority (42.6-99.1%) of reads from each pond and accounted for most inter-pond heterogeneity. The high percentage of shared sequences and the dominance of similar OTUs may imply an exchange or inoculation from other pools between locations. Bacteria have been previously shown to distribute easily through aeolian mechanisms around and into Antarctica (Bottos *et al.*, 2014; Chong *et al.*, 2013; Jungblut *et al.*, 2010; Sokol *et al.*, 2013; Vincent, 2000) and as such, bacterial communities have been found to be structured by local conditions over geographically disparate locations (De *et al.*, 2012; Sokol *et al.*, 2013). Based on wind movements in the region, it is likely that the ponds in the Miers Valley (as well as ponds in other Dry Valleys) are the biological source of shared organisms to Bratina Island (Atkins & Dunbar, 2009; Bottos *et al.*, 2014; Dunbar *et al.*, 2009). Although the typical surface airflow in the Miers Valley is up-valley, strong katabatic winds and intermittent dust storms could also likely result in

microorganisms being pushed out onto the McMurdo Ice Shelf south-west of Bratina Island (Bottos *et al.*, 2014). Significantly, the strongest winds at Bratina Island originate from a south-east or south-west direction, which are a known transport mechanism for large volumes of dust (Atkins & Dunbar, 2009). As such, this may represent the most important mode of transport from Miers Valley ponds to Bratina Island ponds.

The closest BLAST hit to more than half of the 10 most abundant OTUs in this investigation originates from sequences detected globally, which is consistent with numerous recent Antarctic studies (Bottos *et al.*, 2014; Chong *et al.*, 2013; Herbold *et al.*, 2014; Jungblut *et al.*, 2010; Vincent, 2000). For example, a recent study of geothermal soils in Antarctica has indicated that the soil surface community was dominated by cosmopolitan sequences postulated to have been deposited by global aeolian distribution, with substrata soil containing a unique, yet possibly endemic community (Herbold *et al.*, 2014). With physicochemical gradients found in a number of Antarctic ponds (Healy *et al.*, 2006; Wait *et al.*, 2006) these water columns may have an analogous community structure between the cosmopolitan surface and a distinct and possibly endemic microbial community in the lower water column. Although regional or intra-continental distribution is more likely to occur frequently, and to be the cause of shared sequences between the two locations in this investigation, further studies are required to determine the extent of global aeolian input as the source of many microorganisms in Antarctica.

3.6 Conclusions

Surprisingly, most variation seen between communities was related to changes in the relative abundance of a small number of mostly cosmopolitan OTUs, suggesting a global aeolian-based inoculum source whose presence remained constant across the geochemical, temporal and spatial range of this study. Local geochemistry, particularly conductivity, influenced total community structure however other factors such as pH, Hg, Fe and PO_4 appear to have a pond specific influence, and Si was shown to strongly influence the unique community. Although sampled at the same time of the year, the community structure within

ponds (2012 and 2013) was highly variable, most likely linked to a number of climatologically driven factors. As expected, December samples were distinct from January samples with a lower diversity and pronounced dominance (>80%) of one or two OTUs. Finally, a high degree of community similarity was identified between the terrestrial ponds of the Miers Valley and the Ice Shelf ponds of Bratina Island. The high proportion of cosmopolitan shared sequences and favorable wind direction between locations may hint that the ponds from both the Miers Valley and Bratina Island share similar microbial inhabitants from a shared aeolian-based inoculum source and that propagules are frequently exchanged regionally.

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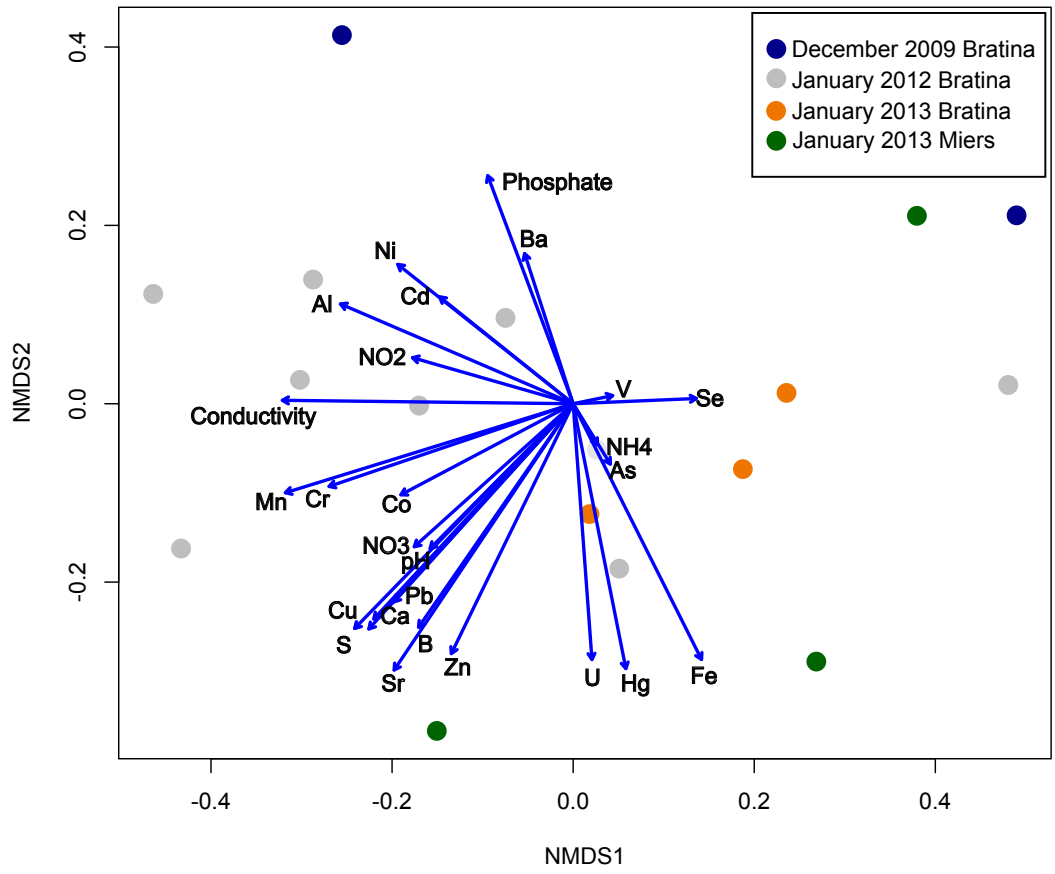
3.9 Supplementary Information



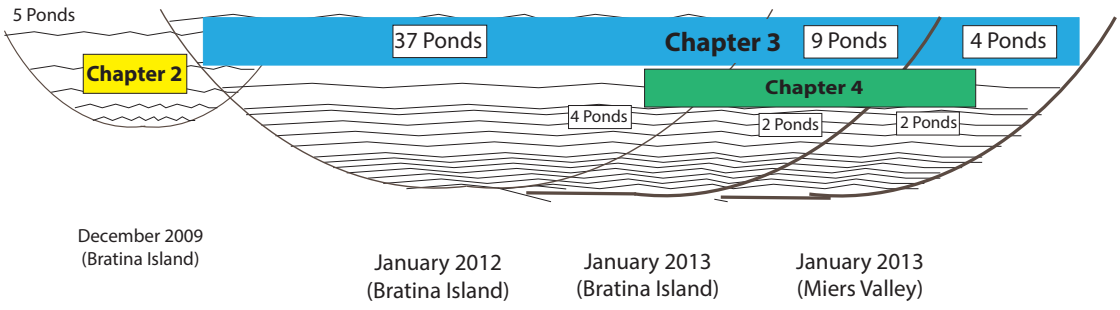
Supplementary Figure 3. 1 Non-metric multidimensional scaling (NMDS) ordination (stress 0.13) based on Bray Curtis distances from total $OTU_{0.05}$ compositions with no transformations. Vectors overlaid include ICP-MS, and nutrient values (Table 2) indicating strength and direction of environmental variables.

Chapter 4 - Microbial community structure across steep geochemical vertical gradients in meltwater ponds of the Ross Sea region, Antarctica

Preface

This chapter describes the drivers to microbial community structure in the highly stratified water columns of meltwater ponds at Bratina Island and the Miers Valley. As the primary author of this chapter, I was involved with all fieldwork and did the majority of lab work, data analysis and manuscript preparation. Inductively coupled mass spectroscopy (ICPMS) and DNA sequencing were carried out by technicians at the University of Waikato. Craig Herbold and Charles Lee earned co-authorship for their part in developing bioinformatics pipelines used to analyse this data. All authors reviewed and edited this manuscript.

This Chapter is in preparation for submission to the *FEMS Microbiology Ecology* Journal.



Preface Figure 4. 1 Breakdown info-graphic overview of thesis

Microbial community structure across steep geochemical vertical gradients in meltwater ponds of the Ross Sea region, Antarctica

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Key words: Antarctic, Planktonic, Microorganism, Community, Melt-water

4.1 Abstract

Meltwater ponds are a ubiquitous feature throughout Antarctica, ranging from terrestrial locations such as the Dry Valleys and across the surface of marine-influenced sediment-covered ice shelves in the Ross Sea Region. Formed in landscape depressions and sustained by local ice and snowmelt, they are geochemically variable, biologically diverse and highly productive. The aim of this study was to determine the variation in bacterial community structure across millimeter-scale vertical geochemical gradients in eight distinct meltwater ponds between two geomorphologically (ice shelf and coastal terrestrial) distinct environments and two consecutive years (2012 and 2013). Bacterial communities from six ponds at Bratina Island and two ponds in the Miers Valley were analysed using DNA fingerprinting and high throughput sequencing of the 16S rRNA gene coupled to elemental and nutrient analysis. Variation in microbial OTU abundances was linked to environmental tolerances of key taxa along chemical gradients that resulted in the formation of three distinct biological communities corresponding to geochemical zones (epilimnion, chemocline and hypolimnion). Conductivity was the dominant selective driver in most ponds with varying importance depending on the strength of the gradient in question. Iron and mercury concentration were identified as dominant explanatory variables for community structure between Miers Valley and Bratina Island. Variation in community structure between years could be attributed to the abundance of the 15 mostly cosmopolitan dominant OTUs that together represented >50% of the total reads for any individual sample. Regardless of extreme annual physicochemical changes, these findings have identified a temporally consistent core of dominant water column bacteria that are strongly affected by extreme gradients and elemental concentration.

4.2 Introduction

Despite the extreme environmental challenges, aquatic environments in Antarctica harbour highly diverse and productive microbial communities (Howard-Williams *et al.*, 1989; James *et al.*, 1995). While the large ice-covered lakes of the Dry Valleys have been comprehensively studied, small meltwater ponds, which are more prevalent across a broad variety of environments, remain comparatively neglected (De Mora *et al.*, 1994; Lyons *et al.*, 2012; Vincent & James, 1996). Pond geochemistry is highly variable with nutrient and element inputs arising from various sources including marine aerosols, trapped seawater on the ice shelf, marine derived sediments and water-rock interactions (De Mora *et al.*, 1994). These ponds are derived from local snow and ice melt collecting in landscape depressions, and the balance between water input and evaporation in ponds may vary considerably, thereby resulting in annual variations of pond diameter and depth as well as elemental and nutrient concentrations (Gibson *et al.*, 2006; Laybourn-Parry *et al.*, 2002). Moreover, as is the case for lake systems, meltwater ponds experience significant seasonal variation in light and temperature regimes. However, due to the small size of pond systems, ponds commonly undergo complete freeze-thaw cycles resulting in a dramatic series of physical, chemical and biological changes (Hawes *et al.*, 2011a; Hawes *et al.*, 2011b; Webster-Brown *et al.*, 2012).

Pronounced stratification of the pond water column is primarily driven by evaporation and freeze concentration of salts, resulting in stratified profiles of dissolved oxygen, pH and various other nutrients and elements (Healy *et al.*, 2006; Lauro *et al.*, 2011; Wait *et al.*, 2006). These gradients result in selective pressures, thereby driving microbial community composition within individual depths of a single pond (Comeau *et al.*, 2012; Lauro *et al.*, 2011). Due to the absence of higher trophic levels, the effects of these abiotic factors are even more pronounced in such biologically constrained Antarctic ecosystems (Roberts *et al.*, 2004). As in polar lake systems, physicochemical stratification in meltwater ponds (Grasby *et al.*, 2013) commences by the formation of a high-density basal cryobrine through evaporation- and freeze-based concentration of the water column (Horita, 2009). Furthermore, local snow and glacial melt during the

Austral summer replenishes the ponds with low density freshwater which, lacking sufficient surface wind to drive mixing of the water column, settles on top of the denser basal cryobrine, resulting in a homogeneous surface layer and highly stratified lower water column (Wait *et al.*, 2006). Unlike many large lake systems that maintain a permanent ice cover preventing significant surface mixing and water column turnover (Comeau *et al.*, 2012), smaller meltwater ponds often have a brief period of complete open water during summer that makes disruption of a defined water column structure more likely (Wait *et al.*, 2006).

Hundreds of geochemically heterogeneous ponds, many of which are highly stratified, are located adjacent to Bratina Island at the tip of Brown Peninsula on the McMurdo Ice shelf in Victoria Land, and at the mouth of the nearby (<40 km) Miers Valley (McMurdo Dry Valleys). Although physically similar, the substrata on which the ponds have formed at these two disparate locations are geomorphologically distinct. Ponds near Bratina Island are located on a layer of marine derived sediment on the surface of the McMurdo Ice Shelf (Kellogg & Kellogg, 1988), whilst ponds in Miers Valley are located on locally derived mineral soils on top of permafrost (Healy *et al.*, 2006; McLeod *et al.*, 2009). Although studies of both terrestrial and marine-derived ponds (such as those from the Miers Valley and Bratina Island, respectively) have been conducted (Healy *et al.*, 2006; Safi *et al.*, 2012; Wait *et al.*, 2006), direct comparison of the associated biology in the water column has not been undertaken.

To enable high-resolution sampling of the subtle transitions that occur over centimetre scales in these highly stratified ponds, a specially designed sampling rig was utilized, as previously descibed in Archer *et al.*, (2014). Subsequently, in this study, a dual-pronged genetic approach was undertaken whereby DNA-based fingerprinting data were initially used to identify trends of interest and samples were then chosen for community profiling via pyrosequencing, thereby providing deeper insight into the microbial composition of pond planktonic communities. The objectives of this study were to characterise the highly variable planktonic microbial communities across strongly stratified ponds, to identify geochemical-based drivers of community structure (including

trace elements), and to compare the microbial communities between different pond substrates (i.e. terrestrial vs. marine) and sampling years (2012 and 2013).

4.3 Materials and Methods

4.3.1 Field sampling, nutrient and elemental analysis

Water column samples were collected from six meltwater ponds (Huey, Brack, Fresh, Salt, P70E, Legin) near Bratina Island (78° 01' S, 165° 32' E) on the McMurdo Ice Shelf in January 2012. Ponds Huey and P70E were resampled in January 2013 and ponds Morepork and Canary sampled in the Miers Valley (78° 07' S, 164° 12' E) in January 2013. The size of each pond was determined and the relative depth ratios calculated according to (Castro & Moore, 2000) (Supplementary Table 4.1). Biomass and filtered water samples were collected using a sterilized sampling tube from the approximate center of the pond using a micromanipulator sampling apparatus (Archer *et al.*, 2014) at increments ranging from 0.5 to 80 cm, manually guided in real-time by changes in pH and conductivity in the water column. Up to 40 samples were collected from a single pond depending on the severity of the gradient, and then frozen for transport back to the laboratory. During the 2012 sampling expedition, *in situ* measurements of pH and conductivity were taken by an Orion 4 Star bench top pH, conductivity meter (Thermo Fisher Scientific Inc, USA) and dissolved oxygen (DO) measured by a Fibox 3 LCD trace minisensor oxygen meter with data-logger (PreSens Precision sensing, Regensburg, Germany). During the 2013 sampling expedition, measurement of pH, DO and conductivity were conducted with an HQ40d portable multi-parameter meter (Hach Company, CO, USA). All in-field meters were freshly calibrated before operation. NH_4, NO_3/NO_2 and PO_4 concentration and elemental analysis (ICP-MS) were carried out at the University of Waikato using a Lachat Quickchem 8500 series II FIA system (Hach Company, Loveland, CO) and a Mass Spectrometer ELAN® DRC II (PerkinElmer Inc., Münster, Germany) respectively, as per manufacturer's instructions. To prepare samples for ICP-MS, 0.22 μm prefiltered pond water was diluted 1:50 with Milli-Q water for surface samples and 1:2500 for samples found to have conductivity readings >19

mS cm^{-1} (Millipore, Billerica, MA, USA). Once diluted, samples were acidified with 2% HNO_3 (Extra pure Nitric Acid, Ajax Finechem, NSW, Australia).

4.3.2 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

DNA was extracted from the filtered biomass using a modified CTAB extraction protocol (Dempster *et al.*, 1999, for full details see Archer *et al.*, 2014). ARISA DNA fingerprinting (Fisher & Triplett, 1999), using PCR primers ITSReub-Hex (5`-Hex-GCCAAGGCATCCACC-3`) and ITSF (5`-GTCGTAACAAGGTAGCCGTA-3`) as used previously (Cardinale *et al.*, 2004), was utilized to resolve structure within bacterial communities and identify trends and samples of interest. All ARISA reactions were run in triplicate on a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, CA) as described previously (Archer *et al.* 2014). Following amplification, reactions were combined and run on a 1% agarose gel to ensure amplification success. Amplicons were then diluted 1:20 in Ultrapure™ water (Gibco) and fragment lengths resolved on a 3130XL DNA sequencer (Applied Biosystems, New York, USA) using Liz-1200 internal size standard (Applied Biosystems) at the University of Waikato DNA Sequencing Facility.

ARISA fingerprints were processed within an informatics pipeline as modified from Abdo *et al.*, (2006) as previously descibed (Sokol *et al.*, 2013). Peaks between 50 bp and 1200 bp and exceeding 200 fluorescence units were accepted *a priori* as true peaks and used to calculate model parameters for a log-normal distribution. Iteratively, peaks with an area exceeding the 99.9% cumulative distribution of the calculated log-normal distribution for noise were accepted as true peaks. Peaks were then binned into ARISA Fragment Lengths (AFLs) within 5 bp of one another. The resulting data matrix was analysed using a combination of Primer 6 (Clarke & Gorley, 2006) and R for statistical analysis (Team, 2013) as described below.

4.3.3 ARISA data analysis

Within Primer 6, beta diversity was investigated using a resemblance matrix created based on the Bray Curtis community dissimilarities.

These were examined in a two-dimensional ordination using non-metric multidimensional scaling (NMDS) overlaying vectors of environmental variables, fit onto the ordination in R using the 'vegan' library (Oksanen, 2011). Analyses of Similarity (ANOSIM) were performed on resemblance matrices to test specific hypotheses formed from interpretation of MDS plots. Relationships between pond geochemistry and the microbial community were determined using a BEST analysis to find the 'best match' in PRIMER 6. Available environmental data (where complete with ARISA data) were transformed using a square root followed by a log (X+1) transformation and normalization of data.

4.3.4 454 pyrosequencing

A subset of 14 samples was selected based on DNA-fingerprinting and geochemical data for high-throughput 454 pyrosequencing from the 138 water column samples: eight samples were selected from Huey pond, which was the most highly stratified pond in 2012; three comparative samples were selected from Huey in 2013 for the temporal analysis; and three from a geochemically similar pond in the Miers Valley (2013). The V5-V6 hypervariable regions of the 16S rRNA gene were utilized to identify variation in bacterial community diversity and structure. Triplicate 30 µL reactions were run for each sample, each reaction containing 0.4 µM of each unadapted primer Tx9F (5′-GGATTAGAWACCCBGGTAGTC-3′) and 1391R (5′-GACGGGCRGTGWGTRCA-3′) (Ashby *et al.*, 2007), 1x PCR buffer, 0.2 mM dNTPs, 0.02 mg/mL BSA, 0.02 U Platinum *Taq*, 2 mM $MgCl_2$, 20 ng genomic DNA and MilliQ H_2O to make the reaction up to 30 µL. Thermal cycling conditions were: 94°C for 2 min, then 30 cycles of 94°C for 20 s, 55°C for 10 s (-0.2° C per cycle), 72°C for 20 s, and a final extension of 72°C for 3 min. Following amplification, all triplicate PCRs were combined, run on a 2% TAE agarose gel stained with "SYBR Safe" (Invitrogen Ltd) at 80V, and the bands excised and DNA retrieved using the MO BIO UltraClean 15 DNA Purification Kit (MO BIO Laboratories, Carlsbad, CA, USA) as per manufacturer's instructions, with the exception of an extra final centrifugation step. A second round of triplicate PCRs was run as above but with only 10 cycles and using 25

ng of the purified DNA from the previous step per reaction (volume adjusted accordingly with Milli-Q H_2O). The primers used were adapted for one-way reads according to the Roche GS Junior System Guidelines for Amplicon Experimental Design Manual (August 2010), including unique MID identifiers for each sample (BacX-Tx9F (′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID-GGATTAGAWACCCBGGTAGTC-3′) and BacB-1391R (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-GACGGGCRGTGWGTRCA-3 ′)). A second gel extraction was performed as above and samples put through a final cleanup step using the Agencourt AMPure XP system (Beckman Coulter Genomics, Danvers, MA, USA) as per the manufacturer's instructions. A verification gel was run on a 2% TAE gel to confirm purity and DNA content quantified using a Qubit Flurometer (Invitrogen Ltd). Finally, DNA was diluted to ~ $1x10^9$ molecules/μL as per the Roche Amplicon Library Preparation Method Manual (GS Junior Titanium Series, May 2010 (Rev. June 2010)). qPCR using a KAPA Library Quantification Kit for Roche 454 Titanium/Universal (Kapa Biosystems, Woburn, MA, USA) was used to check the $1x10^9$ dilution, and was adjusted accordingly for making the amplicon library. The diluted amplicons were mixed together in the desired proportions to create the $1x10^9$ amplicon pool. Sequencing was performed using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTiterPlate Kit and GS Junior System according to the manufacturer's instructions (Roche 454 Life Sciences, Branford, CT, USA).

4.3.5 454 pyrosequencing data processing

454 PCR amplicon pyrosequencing data were first processed using AmpliconNoise v1.0 (Quince *et al.*, 2011). Briefly, raw flowgrams (sff files) with perfectly matching primer and barcode sequences were filtered for a minimum flowgram length of 360 cycles (including primer and barcode sequences) before the first noisy signal (i.e., 0.5-0.7 or no signal in all four nucleotides). All flowgrams were then truncated at 360 bases and clustered to remove sequencing noise using PyroNoise (Quince *et al.*, 2009; Quince *et al.*, 2011). Noise introduced by PCR was removed using SeqNoise (Quince *et al.*, 2011), and PCR

chimeras removed using Perseus (Quince *et al.*, 2011). The resulting de-replicated sequences were processed using Mothur 1.17.0 (Schloss *et al.*, 2009) to create a unique sequence and names file. Pairwise alignments and distances were calculated using Espirit (Sun *et al.*, 2009). Mothur was then used to cluster sequences into operational taxonomic units (OTUs) defined at the average neighbor Jukes-Cantor distance of 0.05 ($OTU_{0.05}$). Rank-abundance data were generated for each sample as well as comparative Venn diagrams. A Venn diagram was created by pooling sequences into three groups: Bratina 2012, Bratina 2013 and Miers 2013, identifying shared and unique sequences. For phylogenetic assignments, representative sequences of all identified $OTU_{0.05}$ were analyzed using the Classifier function of the Ribosomal Database Project (RDP) Release 10, Update 15 (Wang *et al.*, 2007). Taxonomic assignment threshold was set at 80%. Environmental data including nutrient and elemental results but excluding pH were transformed using a square root followed by a log ($x + c$) transformation, where c represents the first percentile value. Within Primer 6, a Draftsman plot was created to view interrelations between different variables, particularly to conductivity, so that data could be simplified. A Bray-Curtis similarity matrix was created based on relative OTU abundances from pyrosequencing data in Primer 6 and visualized on an NMDS plot with overlaid environmental variables using the 'vegan' library of R. BEST analysis between the normalized environmental data and the pyrosequencing data was then conducted in Primer 6. Community similarity based on sequencing data was visually presented using dendrograms created by a resemblance matrix based on Bray Curtis community similarities.

4.4 Results

4.4.1 Geochemical structuring of bacterial communities

Although all eight ponds sampled in this study were <1.5 m deep (Supplementary Table 4.1), six ponds (Legin, Salt, Huey, P70E, Morepork and Canary) exhibited sharp geochemical gradients through the water column (Figure 4.1a-c). Huey exhibited the most pronounced stratification with conductivity increasing with depth from 3.8 to 132.4 mS cm^{-1} (Figure 4.1a), dissolved oxygen

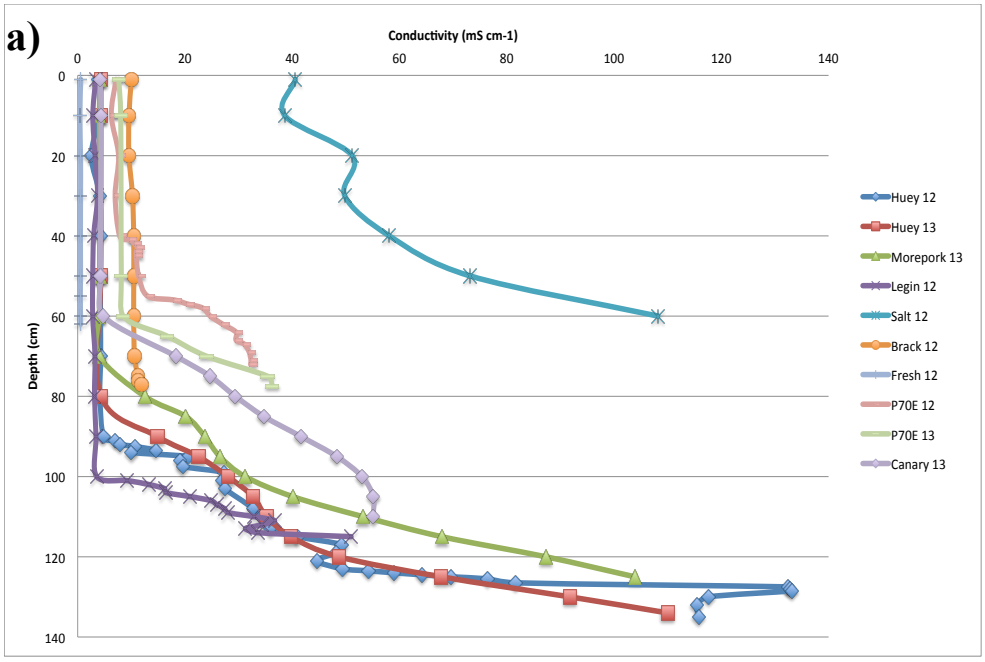
(DO) from 19.2 ppm at the surface to 121 ppm in the middle and 1.2 ppm at the bottom (Figure 4.11b) with pH ranging from 9.8 to 7.2 (Figure 4.1c).

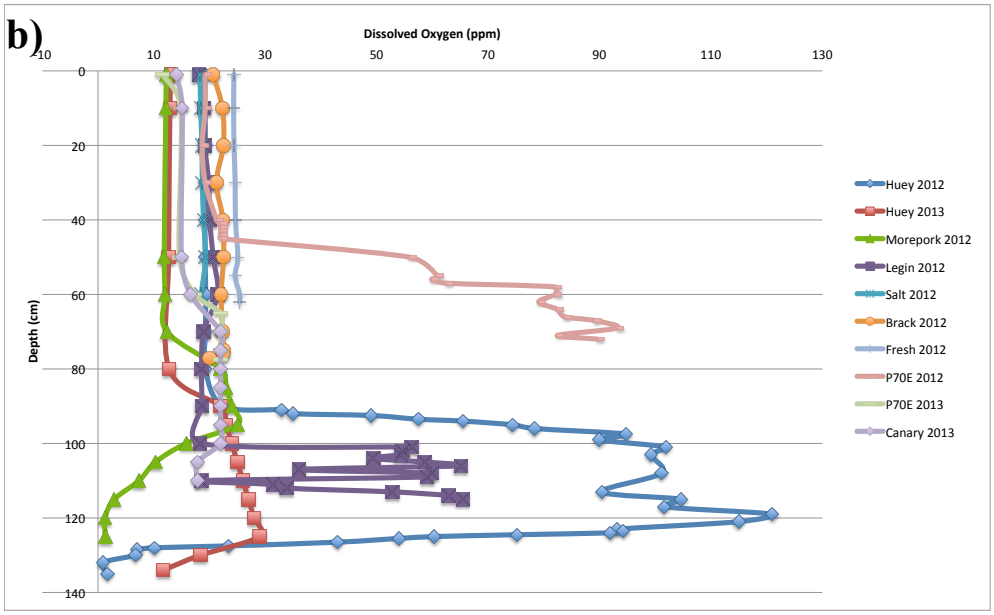
Bacterial community structure across these geochemical gradients was compared using ARISA visualized on a two-dimensional NMDS ordination, with comparative geochemical data overlaid (Figure 4.2). For all samples, an inverse relationship between pH and conductivity was identified along NMDS axis 1, with DO having a perpendicular relationship with both factors (Figure 4.2). BEST analysis identified conductivity as the most significant single driver of microbial community structure across all ponds (Spearmans P of 0.383). Moreover, stronger correlations were observed when data from individual ponds were analyzed separately to determine pond-specific geochemical drivers (Supplementary Table 4.2). All ponds with strong conductivity stratification, i.e., Huey, Legin and Salt in 2012 and all the 2013 samples, were identified as having conductivity as the major single driver of community structure (Supplementary Table 4.2). Interestingly, P70E switched from pH to conductivity as its major bacterial community driver between sampling years. The Brack and Fresh ponds both had a geochemically homogeneous water column with weak BEST correlations; however, although Brack-associated ARISA data formed a tight cluster, ARISA data varied across NMDS axis 2 for Fresh, suggesting a relationship with DO (Figure 4.2).

A total of 14 samples were selected for pyrosequencing data analysis, resulting in the recovery of 133,071 high quality sequences (Supplementary Table 4.3). The Goods coverage ratio ranged between 97.27 and 99.53%. Eight points across the stratified water column in Huey 2012 were selected to encompass total community dissimilarity based on ARISA data (Figure 4.2), and geochemical range based on pH, DO and conductivity (Figure 4.1a-c). Three samples from each of Huey 2013 and Morepork were also selected at comparative depths and geochemistry. Community similarity based on pyrosequencing OTU abundance was represented on a two-dimensional NMDS ordination with comparative geochemical data overlaid (Figure 4.3). Examination of correlation scores based on BEST analysis identified Fe and Hg as dominant geochemical drivers (Spearmans P of 0.582 and 0.572); however, these elements were only detected in Morepork (Supplementary Table 4.4). pH and DO had strong vectors across the

NMDS2 axis, sharing this directionality with depth in the chemocline (BEST analysis Spearmans P of 0.476 and 0.444 respectively) (Figure 4.3). Conductivity showed a moderate relationship with the NMDS1 axis (BEST analysis Spearmans P of 0.470), with samples collected lower in the chemocline, with higher conductivity exhibiting a consistent trend across this axis (Figure 4.3).

Abundance of the 15 major unique OTUs (those contributing >1% of total sequences) across the data set was also examined to provide insight into the dominant bacterial components, their contribution to the total community, their variability between ponds and depth related community structure (Table 4.1). Congruent with ARISA data the abundance of dominant OTUs based on pyrosequencing data also exhibited depth structure (Table 4.1). For example, the abundance of *Bordetella* sp. signatures decreased with depth in 2013 water columns, *Marinobacter daqiaonesis*-related signatures abundance increased with depth in all water columns and the abundance of *Variovorax paradoxus*–related signatures decreased with depth in the Huey 2013 water column (Table 4.1).


a)
Conductivity (mS cm-1)
0
20
40
60
80
100
120
140
Depth (cm)
0
20
40
60
80
100
120
140
Huey 12
Huey 13
Morepork 13
Legin 12
Salt 12
Brack 12
Fresh 12
P70E 12
P70E 13
Canary 13


b)
Dissolved Oxygen (ppm)
-10
10
30
50
70
90
110
130
Depth (cm)
0
20
40
60
80
100
120
140
Huey 2012
Huey 2013
Morepork 2012
Legin 2012
Salt 2012
Brack 2012
Fresh 2012
P70E 2012
P70E 2013
Canary 2013

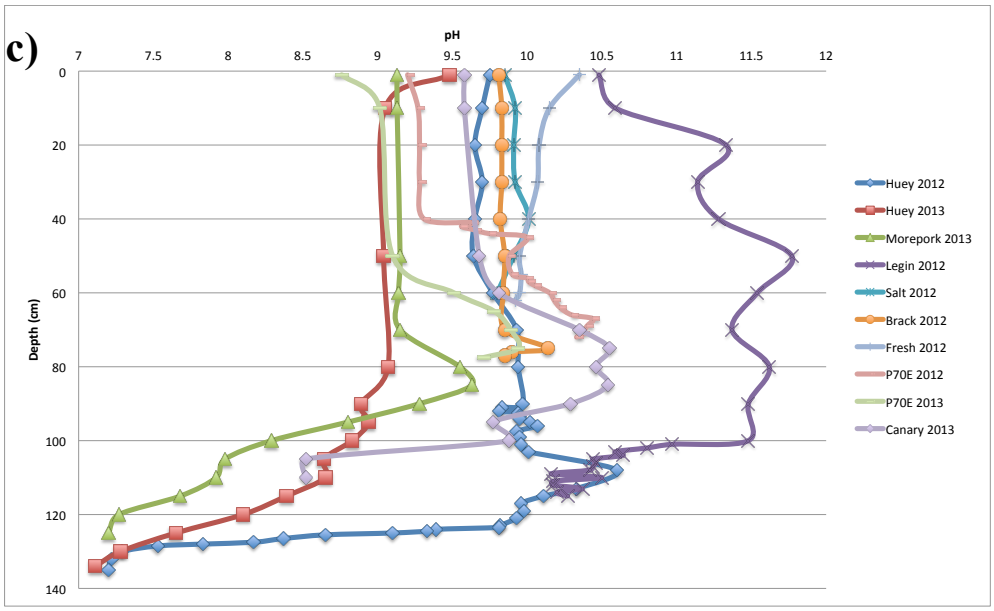


Figure 4. 1 Geochemical depth profile of eight meltwater ponds from Bratina Island (January 2012 and 2013) and the Miers Valley (January 2013) showing changes in conductivity (a), dissolved oxygen concentration (ppm) (b), and pH (c).

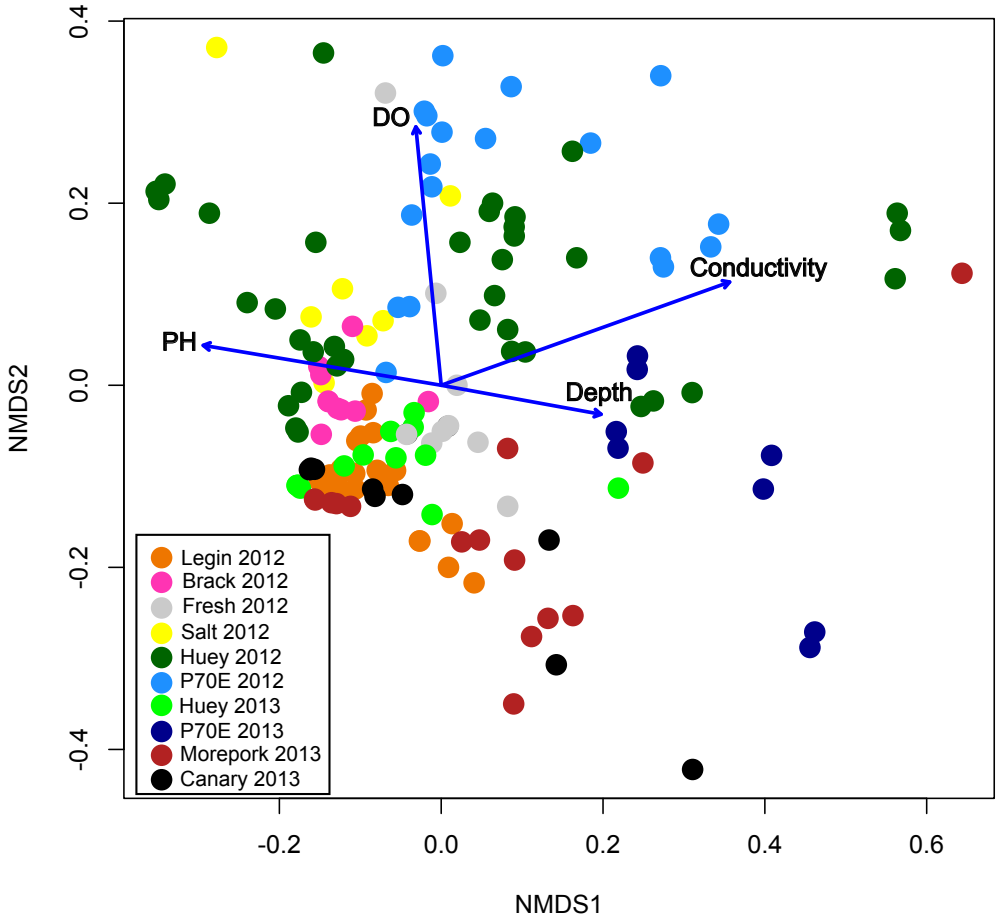


Figure 4. 2 Non-metric multidimensional scaling (NMDS) ordinations of bacterial ARISA community compositions in top to bottom water columns of meltwater ponds in January 2012 and 2013 from Bratina Island and the Miers Valley based on Bray Curtis distances (stress 0.18). Overlaid vectors indicate strength and direction of environmental variables based on Euclidean distances (where complete data coverage exists).

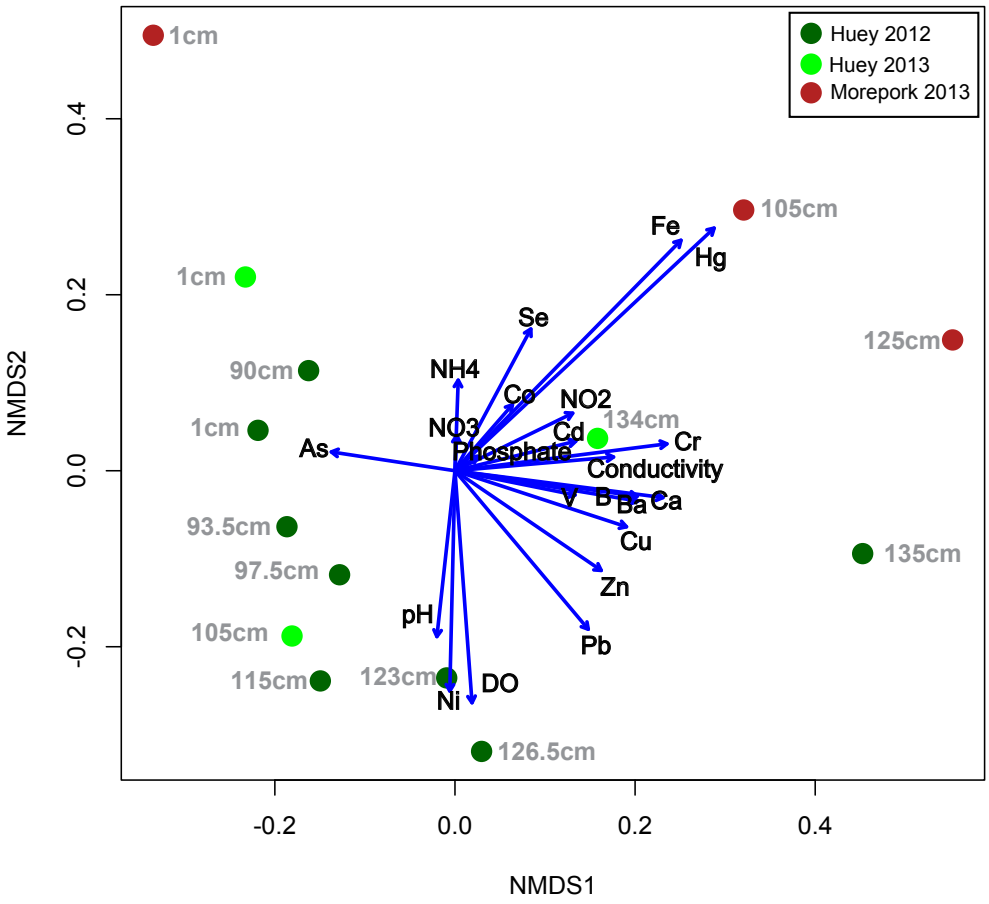


Figure 4. 3 Non-metric multidimensional scaling (NMDS) ordination based on Bray Curtis distances (stress 0.11) calculated from total $OTU_{0.05}$ compositions with no transformation. Samples collected across the geochemical gradient in Huey pond (Bratina Island, eight samples in 2012, three in 2013) and Morepork Pond (Miers Valley). Overlaid vectors indicate strength and direction of environmental variables (including ICP-MS, and nutrient values) based on Euclidean distances. Environmental data were transformed using a square root followed by a log (x+c) transformation and normalization of data.

Table 4. 1 Fifteen most abundant OTUs (based on total sequenced number across all samples) illustrating relative abundance between samples, identity of sequence, and description of known isolates.

OTU	Bratina Island Huey Pond 2012								Bratina Island Huey Pond 2013			Miers Valley Morepork Pond 2013		
	1 cm	90 cm	93.5 cm	97.5 cm	115 cm	123 cm	126.5 cm	135 cm	1 cm	105 cm	134 cm	1 cm	105 cm	125 cm
1	35.32%	23.58%	24.63%	46.83%	60.22%	27.64%	10.86%	1.14%	9.17%	49.92%	18.90%	0.00%	0.51%	0.02%
2	9.42%	20.66%	25.21%	19.59%	0.04%	1.52%	0.35%	1.91%	5.03%	2.67%	46.63%	0.81%	5.61%	1.46%
3	24.89%	21.36%	0.71%	0.44%	0.64%	0.53%	0.37%	0.28%	20.18%	0.05%	0.02%	19.84%	0.61%	0.06%
4	1.76%	2.55%	1.95%	1.96%	1.20%	0.88%	0.42%	0.96%	14.69%	1.53%	0.24%	54.89%	0.97%	0.21%
5	13.69%	15.23%	6.76%	8.40%	2.95%	1.20%	1.31%	0.59%	14.12%	2.19%	0.50%	5.81%	1.88%	0.13%
6	0.03%	0.04%	0.26%	3.03%	9.90%	12.83%	49.31%	1.37%	0.11%	18.36%	0.01%	0.00%	0.14%	0.01%
7	0.13%	0.53%	1.04%	0.40%	0.11%	1.70%	0.16%	0.36%	0.00%	0.94%	0.84%	0.00%	34.30%	1.23%
8	0.01%	0.00%	0.19%	0.00%	0.60%	4.81%	1.60%	29.86%	0.00%	0.02%	1.52%	0.00%	0.64%	7.68%
9	0.54%	0.64%	0.97%	1.11%	0.75%	4.56%	0.72%	1.65%	0.36%	1.43%	2.18%	0.02%	17.61%	7.24%
10	0.00%	0.00%	0.00%	0.00%	0.00%	0.18%	0.06%	0.43%	0.00%	0.00%	3.20%	0.00%	10.76%	19.76%
11	3.31%	4.65%	1.04%	0.88%	3.92%	3.82%	1.19%	0.57%	12.51%	5.57%	0.90%	0.09%	0.23%	0.15%
12	0.03%	0.03%	0.13%	0.07%	0.00%	0.00%	0.00%	0.01%	14.06%	0.05%	0.03%	15.88%	0.32%	0.01%
13	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	14.80%	0.00%	0.00%	0.91%	0.00%	0.07%	7.96%
14	0.01%	0.03%	0.13%	0.00%	0.11%	1.41%	0.96%	11.16%	0.01%	0.00%	1.89%	0.00%	0.39%	4.43%
15	0.72%	1.12%	0.97%	0.64%	1.05%	9.01%	0.30%	2.18%	0.33%	0.94%	0.34%	0.00%	5.10%	1.02%
Total	89.87%	90.43%	64.00%	83.34%	81.47%	70.10%	67.62%	67.28%	90.55%	83.67%	78.12%	97.35%	79.14%	51.38%

Table 4.1 continued

OTU	Phylum	Class	Accession #	E-Score	Organism	Description
1	Bacteroidetes	Sphingobacteria	FR691439.1	6.00E-133	*Algoriphagus* sp.	Antarctica:Transantarctic Mountains, Forlidas pond
			FJ196000.1	3.00E-130	*Algoriphagus* sp.	Antarctic sandy intertidal Sediments
2	Proteobacteria	Alphaproteobacteria	KC702679.1	1.00E-154	*Loktanella* sp.	India, Pangong Lake water
			AB681826.1	7.00E-152	*Loktanella vestfoldensis*	Culture collection = NBRC:102487 Antarctic lake microbial mats
3	Bacteroidetes	Flavobacteria	JX971557.1	5.00E-140	*Flavobacterium segetis*	South Korea, host mushroom "Pleurotus ostreatus"
			HM149212.1	5.00E-140	*Flavobacterium* sp.	South Korea, mesotrophic artificial lake
4	Proteobacteria	Betaproteobacteria	KF641823.1	7.00E-112	*Bordetella* sp.	China, Camel rumen
			KC634248.1	7.00E-112	*Castellaniella denitrificans*	India, water treatment plant
5	Actinobacteria	Actinobacteria	DQ015794.1	1.00E-135	uncultured bacterium	Antarctica:Taylor Valley, Lake Bonney
			DQ512860.1	3.00E-107	*Ornithinimicrobium pekingense*	China, from activated sludge
6	Proteobacteria	Gammaproteobacteria	KF384124.1	1.00E-141	*Marinobacter* sp.	Lake Vida ice-entrained cryobrine collected at depth of 18.4 m
			NR_074735.1	1.00E-141	*Marinobacter* sp.	Arctic Ocean: Canada Basin water depth of 1568 m
7	Proteobacteria	Alphaproteobacteria	FJ196047.1	0	*Sulfitobacter* sp.	Antarctic Sea ice
			JN248462.1	0	*Sulfitobacter* sp.	Arctic Ocean: Beaufort Sea 3m depth
8	Verrucomicrobia	Verrucomicrobiae	NR_026266.1	8.00E-108	*Verrucomicrobium spinosum*	
			HG529123.1	5.00E-147	Uncultured bacterium	Finland Sphagnum associated methanotrophic communities along a peatland
9	Actinobacteria	Actinobacteria	NR_109611.1	4.00E-137	*Pontimonas salivibrio*	Korea, inlet seawater of solar saltern
			NR_042275.1	5.00E-136	*Microcella alkaliphila*	Non-saline alkaline groundwater, Portugal
10	Proteobacteria	Gammaproteobacteria	NR_108457.1	2.00E-71	*Marinobacter daqiaonensis*	halophile isolated from a Yellow Sea salt pond
			NR_026517.1	2.00E-71	*Acidithiobacillus caldus*	Moderately thermophilic acidophile
11	Proteobacteria	Betaproteobacteria	KF051781.1	1.00E-142	*Variovorax paradoxus*	China, "Solanum nigrum" plant
			JQ977446.1	1.00E-142	*Variovorax* sp.	China: Tianshan Mountains, rizosphere soil
12	Bacteroidetes	Flavobacteria	HQ405608.1	5.00E-107	bacterium enrichment culture clone	High pressure bioreactor enriching for anaerobic oxidation of methane
			JQ689940.1	2.00E-105	*Tamlana* sp.	Korea, Dokdo sea water
13	Firmicutes	Clostridia	NR_024886.1	2.00E-84	*Fusibacter paucivorans*	an anaerobic, thiosulfate-reducing bacterium from an oil-producing well
			AM943575.1	1.00E-134	Uncultured bacterium	Brazil:Lagoa Vermelha, carbonaceous sediments from a hypersaline lagoon
14	OD1	OD1	AY193293.1	5.00E-134	Uncultured candidate division OD1	sulphur river filaments
			NR_102470.1	2.00E-61	*Bdellovibrio bacteriovorus*	Italy, River tiber
15	Proteobacteria	Alphaproteobacteria	NR_117979.1	8.00E-157	*Tabrizicola aquatica*	Iran: Tabriz city, Qurugol lake
			NR_115057.1	8.00E-157	*Rhodobacter ovatus*	India: Hyderabad, industrially polluted pond water

4.4.2 Biological variation across geochemical gradients

A dendrogram based on pyrosequencing data (Figure 4.4) indicates that the Huey 2012 samples separated into three main groups: 1-90 cm (representing the epilimnion, the homogeneous mixed zone), 93.5-123 cm (the chemocline), and the variable bottom samples 126.5 and 135 cm (the hypolimnion) (Figure 4.4). The abundance of most major bacterial groups varied between samples with some exhibiting depth related trends. For example, *Actinobacteria* represented up to 16.5% of total sequences in the epilimnion, and <9.6% in the remaining water column; *Bacteroidetes* abundance remained >37% in the epilimnion reaching its peak in the chemocline (67.7%), then decreasing with depth to 5.6% in the hypolimnion. *Proteobacteria* abundance varied from 13.6 to 60.7% with no obvious depth related pattern, but at finer taxonomic resolution (Supplementary Figure 4.1) the *Gammaproteobacteria* increased with depth in the chemocline, reaching a maximum of 56.3% of total reads at 126.5cm until the bottom sample (hypolimnion) where abundance suddenly dropped to 3.2%, *Epsilonproteobacteria* were absent or at <0.5% abundance until mid- chemocline (123 cm) increasing to 1% through the hypolimnion, *Deltaproteobacteria* were at <0.5% until an observed increase to 1.2% at the bottom sample, and *Alphaproteobacteria* were more abundant in the upper water column. Unclassified sequences increased with depth from 2.8% in the epilimnion to 8% at the bottom sample. Chloroplast sequences were initially <12% of the total community signatures in the epilimnion with a peak 71.8% in the chemocline, remaining between 30-55% until the bottom sample where abundance dropped to <1% (Supplementary Figure 4.2).

The presence and abundance of every OTU varied across samples, although the cumulative abundance remained >51% in each pond (Table 4.1). Many OTUs exhibited dominance at a particular depth and either a gradual or sharp decrease in abundance from this point. For example, OTUs with closest BLAST matches to *Loktanella* sp. and *Bordetella* sp. were highest at 1 and 90 cm depths (epilimnion) in the Huey 2012 profile, *Geminigera cryophilla* plastid and *Ornithinimicrobium pekingense* were dominant in the chemocline, and *Sulfitobacter* sp., *Tamlana* sp. and *Fusibacter paucivorans* were dominant in the hypolimnion. *Algoriphagus* sp., the most abundant OTU across this study

(ranging from 60.2 to 0 % of the total communities), maintained a high abundance throughout the upper water column, decreasing from its peak at 115 cm to the bottom sample.

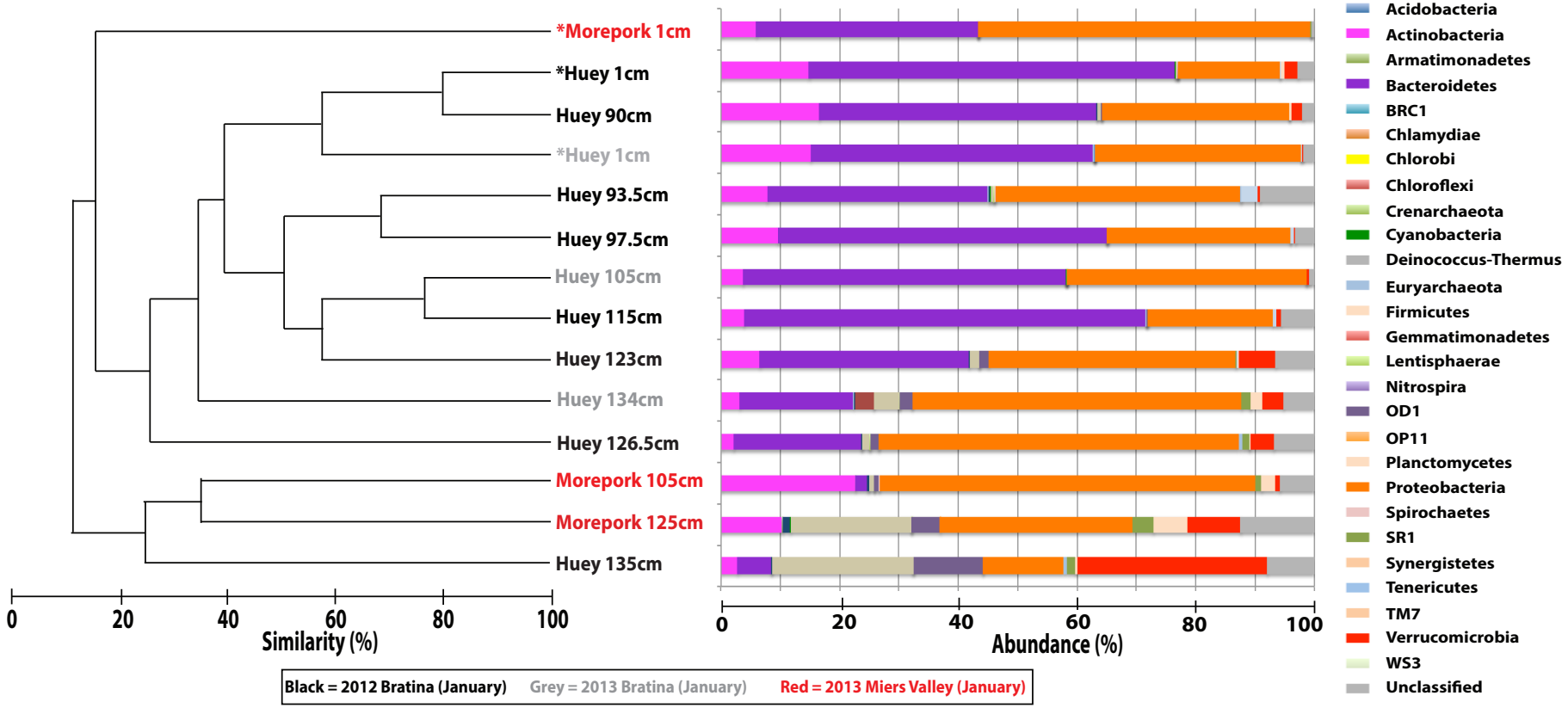


Figure 4. 4 Relative community similarities, summary sequencing statistics and taxonomic distributions of geochemically stratified meltwater pond water columns. Bray-Curtis tree calculated from total $OTU_{0.05}$ compositions with no transformation to visualize total relative spatial/temporal similarities between water columns (Left). Phylum-level distribution of bacterial 16S rRNA $OTUs_{0.05}$ assigned using the Ribosomal Database Project (RDP) Release 10, Update 15 Classifier, assignment confidence threshold >80% (Right). Note: * symbol denotes samples redrawn from Chapter 3.

4.4.3 Temporal variation

Huey, representing the most significant geochemical and biological stratification from 2012 samples, and P70E, that appeared physically consistent implying similar biotic environments between years (Supplementary Table 4.1), were sampled in January 2012 and 2013 to examine temporal variation within the ponds. DO and pH values were higher in both ponds in 2012 in the surface mixed layers; however both parameters were similar at the bottom of Huey (Figure 4.1b and c). The conductivity profiles for P70E and Huey were similar between years, although stratification began 10-20 cm deeper in both ponds in 2013. Analysis of pyrosequencing data from Huey showed 94.1 and 97.7% of total reads were shared between pooled samples from 2012 and 2013 respectively. Chloroplast abundance was much higher in 2012 than 2013 (Supplementary Figure 4.2). Samples from Huey broadly clustered into the three previously identified zones (epilimnion, chemocline and hypolimnion) (Figure 4.4). Epilimnion samples between years had a similar abundance of *Actinobacteria*, however abundance of *Bacteroidetes* and *Verrucomicrobia* was higher in 2012, and *Proteobacteria* was higher in 2013. The 105 cm sample (in the chemocline) from 2013 was highly similar (>70%) to the comparable 2012 sample, however hypolimnion samples were <30% similar and contained a visibly different abundance of most dominant phyla, with the 2013 bottom sample sharing greater similarity to the 2012 mid water column than to the bottom sample (Figure 4.4). All 15 major OTUs detected in all ponds were detected in both years, many with similar abundances; however there was some notable variation (Table 4.1). For example, *Sulfitobacter* sp., *Tamalana* sp. and *Fusibacter paucivorans* were proportionally higher in 2012, while *Flavobacterium segetis* and *Variovorax paradoxus* were higher in 2013.

4.4.4 Spatial variation

Morepork, in the Miers Valley, had the most similar pH and conductivity profile to Huey from Bratina Island (Figure 4.1a-c and Supplementary Figure 4.3), and was thus chosen to investigate possible regional influences on the microbial community. ANOSIM results based on the bacterial ARISA data showed a high degree of similarity between Huey 2013 and Morepork 2013,

although with no statistical significance (ANOSIM R=0.165 P=1). Moderate similarity, but strong statistical significance, was identified between Huey 2012 and Morepork 2013 (ANOSIM R=0.308 P=0.01). Bacterial community structure in the three water columns was highly correlated to conductivity (Spearman's p of 0.832 for Huey 2012, 0.791 for Morepork, and 0.761 for Huey 2013) (Supplementary Table 4.2).

Pooled pyrosequencing data identified a high degree of shared sequences (>96.9%) between Huey 2013 and Morepork 2013 water columns. Pyrosequencing data from individual depths show Morepork and Huey samples were distinctly separated (<30% between any two samples from the two locations) (Figure 4.4). The clear outlier, the Morepork surface sample (1 cm), had one of the simplest communities, dominated (99.2% of the total community) by three phyla (being *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*) and four OTUs. All of the 15 most abundant OTUs from all pools were present at both locations; however the abundance of each one varied (Table 4.1). BEST analysis indicated Hg and Fe as strong contributing factors to bacterial community structure between locations, however these elements were only detected in Morepork (Supplementary Table 4.4).

4.5 Discussion

4.5.1 Geochemical structuring of bacterial communities

Correlations between geochemical data and microbial community structure (ARISA DNA fingerprinting) in this study identified conductivity, pH and dissolved oxygen (DO) as key environmental drivers of bacterial community structure. The effects of pH, DO and conductivity on microbial communities have been previously identified in other Antarctic aquatic environments (Archer *et al.*, 2014; Bell & Laybourn-Parry, 1999; Howard-Williams *et al.*, 1990; Jungblut *et al.*, 2005; Lentini *et al.*, 2012; Safi *et al.*, 2012) and, given the magnitude of change throughout the water column and a lack of higher trophic structure, it is not surprising that geochemical variables are significant influencers of pond biology.

Conductivity, which increased with depth in most ponds, was the most significant driver in seven of the 10 pond water columns studied. The abundance of several major OTUs was related to depth, however only one could be exclusively linked to conductivity as many parameters (notably pH and DO) changed significantly within these water columns. The abundance of OTU 3 (100% identity to *Flavobacterium sergetis*) decreased with increasing salt concentration consistently in all three water columns sequenced (Huey 2012, 2013 and Morepork 2013), indicating salt concentration in the ponds was exerting the greatest selective pressure on this OTU. *F. sergetis* has a known optimal growth at 0% NaCl (growth between 0-3% or 0-48.6 mS cm^{-1}), and a broad tolerance to pH (6-11) (Yi & Chun, 2006). This conductivity range was only present in the epilimnion and upper chemocline where the concentration of *F. sergetis* related signatures was at its highest. Consistent with this investigation, a halophilic bacterium (*Marinobacter* sp*.,* OTU 6) was found at higher abundances in the deeper (saline) water column of a meromictic lake in East Antarctica (Naganuma *et al.*, 2005). This lake had a similar conductivity profile to Huey and Morepork, however the abundance of OTU 6 decreased in the bottom (anoxic) sample of Huey 2012. This trend exhibits the multiple selective pressures (in this case conductivity and DO) that influence the distributions of bacteria in these environments.

The dominant driver in P70E switched from pH in 2012 to conductivity in 2013. The conductivity profile in this pond was fairly similar between years; however, there was a consistently lower pH throughout the water column in 2013 and a corresponding shift in microbial community structure likely related to the different geochemical conditions. These changes corroborate recent studies that show inter-seasonal to annual biological responses to environmental/ climatological variation in various Antarctic ecosystems (Hawes *et al.*, 2011a; Tiao *et al.*, 2012; Yergeau & Kowalchuk, 2008), although further understanding of inherent annual variation within ponds is necessary (Hawes *et al.*, 2013). In contrast, Huey also exhibited a similar change in pH, yet conductivity remained the dominant driver. It is however likely that the selective pressure of a more extreme conductivity profile in Huey, compared to P70E, over-rode the effects of

minor variations in pH. These observations suggest that the strength of one gradient can mask the effect of other significant biological drivers in these ponds.

Analogous to Lake Limnopolar in the South Shetland Islands, Antarctica, a water column lacking strong chemical stratification (as identified in Fresh pond) was capable of having a biologically heterogeneous community structure with depth (Villaescusa *et al.*, 2013). Heterogeneous community structure in Lake Limnopolar was related to variable biological inputs from meltwater at the surface and benthic interaction at the bottom (Villaescusa *et al.*, 2013). However, as with this study it is possible that another driving variable with consistent depth relationship (such as irradiance) was present but not measured (Lizotte & Priscu, 1992). This finding shows that while across strong gradients geochemistry is significant in structuring the biological community, other physical and biological influences do exert an effect that is of greater significance in homogenous water columns.

Pyrosequencing data from representative samples throughout the water column of Huey (Bratina Island, 2012 and 2013) and Morepork (Miers Valley, 2013), coupled to a high resolution environmental dataset (elemental analysis and key nutrients), identified iron and mercury as major drivers of spatial variation in microbial community structure. Iron is an essential trace element in nearly all organisms (Desai *et al.*, 2012), while mercury is toxic and, although resistance in bacteria is widely observed, the varying effectiveness (and energy requirements) of resistance mechanisms could introduce selective pressures to the Morepork community not seen in Huey (Silver & Hobman, 2007). Major nutrients such as NH_4 and PO_4 also affect the microbial community; however, their influence is less than would be expected from previous studies, likely due to the greater geochemical gradients experienced in this investigation (Dore & Priscu, 2001; Fernandez-Valiente *et al.*, 2001; Sorrell *et al.*, 2013). NH_4 has previously been identified as a limiting factor for plankton primary production in McMurdo Ice Shelf ponds (Sorrell *et al.*, 2013), and phosphate limitation of microbial communities has also been reported for McMurdo Dry Valley lakes (Dore & Priscu, 2001). Consistent with the ARISA data, pH, DO and conductivity were still identified as having intra-pond influences, with the directionality of these

variables with depth in the upper water column suggesting that many drivers may have variable impacts at different points within the water column.

4.5.2 Community structure across geochemical gradients

Microbial communities were dominated by a small number of cosmopolitan OTUs, the abundance of which either gradually varied across physicochemical gradients, showing broad environmental tolerances (Schmidt *et al.*, 1991), or were highly restricted, exhibiting clear preference for specific depths. Three distinct microbial communities were identified corresponding to geochemical zones (epilimnion, chemocline and hypolimnion) down the highly stratified water column. Consistent with previous investigations the *Actinobacteria* and *Bacteroidetes* typically decreased with depth, and the hypolimnion samples contained a distinct structure with a larger proportion of unclassified sequences and rare phyla (Archer *et al.*, 2014; Comeau *et al.*, 2012; Villaescusa *et al.*, 2013). The increase of unclassified sequences with depth could represent a more endemic community that has yet to be successfully colonized by the cosmopolitan microbes prevalent in surface waters (Ellis-Evans & Walton, 1990; Pearce *et al.*, 2009; Wynn-Williams, 1990). However, with the inherently dynamic nature of the water column in melt-water ponds, this postulation seems unlikely.

Although not targeted by the primer set used in this investigation, a number of archaeal and chloroplast sequences were classified in RDP. Correspondingly high sequence identities using the NCBI database BLASTn algorithm validated this finding. Successful amplification of chloroplast DNA with cyanobacteria-specific primers has been previously reported (Burja *et al.*, 2001) and photosynthetic eukaryotes are known to be present in these ponds which could explain the existence of these sequences from universal bacterial primers (James *et al.*, 1995; Kellogg & Kellogg, 1987; Safi *et al.*, 2012). They were retained as a useful insight into the dynamics of these groups when compared between samples (although presented separately from 16S data). As photosynthesizing organelles, the increasing abundance of Chloroplasts with increasing oxygen concentration in the water column, which influences the

bacterial community, indicate they are important primary producers in this system. Other than conductivity, oxygen concentration was identified as structuring components of the microbial community.

The abundance of *Proteobacteria* classes corresponded to assumptions about oxygen sensitivities, and previous investigations of polar aquatic ecosystems (Comeau *et al.*, 2012; Lauro *et al.*, 2011; Villaescusa *et al.*, 2013). For example, *Epsilonproteobacteria* and *Deltaproteobacteria*, which are typically microaerophilic or anaerobic (Grote *et al.*, 2008; Sorokin *et al.*, 2008) were only identified at low abundance (<0.5%) in surface samples, increasing in abundance (>1%) at lower depths in line with lower oxygen concentrations in the bottom samples of Huey and Morepork ponds. The most abundant *Alphaproteobacteria* (OTU 2; *Loktanella* sp.) was an obligate aerobe (Van Trappen *et al.*, 2004), the abundance of which generally decreased with depth and oxygen concentration in the 2012 water column. The high abundance of *Alphaproteobacteria* at the bottom sample in 2013 could be explained by the higher oxygen concentration at this depth than in the previous year. *Gammaproteobacteria* abundance decreased in the bottom sample in both Huey water columns, similar to previous lake studies (Comeau *et al.*, 2012; Lauro *et al.*, 2011; Villaescusa *et al.*, 2013). The two most abundant *Gammaproteobacteria* signatures were most closely related to aerobic or facultatively anaerobic *Marinobacter* sp. (Gao *et al.*, 2013; Gauthier *et al.*, 1992), thereby accounting for their persistence in the near anaerobic bottom samples and higher abundance when oxygen was present. Oxygen concentration clearly had a strong selective pressure on bacteria in the water column; however, it was not identified as being strongly correlated to community structure. Unlike conductivity, which increased steadily across the stratified zone, oxygen concentration increased from 15 ppm to > 100 ppm in the column, then decreased to < 2 ppm in the last few centimeters. Increased oxygen concentration with depth is common for these ponds (Archer *et al.*, 2014; Healy *et al.*, 2006; Safi *et al.*, 2012; Wait *et al.*, 2006), however the decreased oxygen in the lower water column is typically only common in (deeper) ice-covered lakes (Comeau *et al.*, 2012; Lauro *et al.*, 2011; Naganuma *et al.*, 2005). These extreme conditions can therefore select for the aforementioned bacteria with different metabolic requirements and oxygen tolerances within each different depth profile. Although

a number of trends in geochemistry were identified throughout the water column, the broad physiological capabilities within the higher taxonomic levels of *Bacteria* (Ferla *et al.*, 2013) make it difficult to infer a community response to the geochemical environment.

4.5.3 Temporal variation

The water columns of Antarctic meltwater ponds are capable of significant annual geochemical changes based on climatological influences (Hawes *et al.*, 2013). Although some work has been conducted following microbial dynamics during seasonal transitions in Antarctica (Foreman *et al.*, 2010; Hawes *et al.*, 1999; Hawes *et al.*, 2011a; Schmidt *et al.*, 1991) little is known regarding temporal variation in bacterioplankton communities. pH and DO were lower in 2013 within ponds Huey and P70E, consistent with observations of lower abundance of phototrophic organisms in 2013 (specifically chloroplast signatures), however conductivity profiles were similar within each pond. Pyrosequencing results showed a high degree of temporal similarity in microbial communities, particularly in the shallower depths. Larger polar lakes have previously shown inter-annual temporal stability (Comeau *et al.*, 2012), however this was not expected in these ponds due to the annual disruption caused by complete freeze thaw cycles (Hawes *et al.*, 2011b). Collectively, these observations suggest that ponds may go through fairly consistent annual cycles, and although the community changes drastically throughout the year as seen previously (Foreman *et al.*, 2010; Hawes *et al.*, 1999; Hawes *et al.*, 2011a; Schmidt *et al.*, 1991), samples collected at the same time of the year can support a similar community, provided climatologically driven variation in factors such as degree days above zero, water balance and sunlight hours has been limited.

4.5.4 Spatial variation

To examine regional variation a pond located in the Miers Valley (Morepork) was compared with one from Bratina Island (Huey). These ponds had similar conductivity, pH and DO profiles. Although located only 30 km apart,

these two ponds represent geomorphologically distinct environments, one primarily marine (Bratina) and the other terrestrial (Miers). Dry Valley soils such as those in the Miers Valley originate from weathering of bedrock and glacial tills (Cary *et al.*, 2010), while soils at Bratina Island have been deposited by sublimation from basal freezing of marine sediments which provides an extremely variable source material to establish ponds (Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988). Given this close proximity it would be expected that biological entities could, through aeolian processes, easily distribute between sites. A recent aerosol study in the Miers Valley identified a highly cosmopolitan microbial community with few identifiable marine members, suggesting restricted exchange between ecosystems due to stochastic dust storm events (Bottos *et al.*, 2014). Pyrosequencing identified a large number of shared sequences between locations, including all 15 of the most dominant OTUs, indicating that microbial exchange between locations, likely driven by dust storms, does occur between locations. However, limited similarity was identified in microbial community structure at comparative depths, caused by variation in the abundance of key phyla and OTUs between locations driven primarily by mercury and iron. Overall, robust cosmopolitan microbes were the major biological constituents in these ponds and were present in both locations; however, the community structure was highly diverse, most likely linked to minor variations in local geochemistry.

4.6 Conclusions

This study of shallow meltwater ponds identified a highly stratified, geochemically influenced, bacterial community. Conductivity was consistently the strongest explanatory variable between and within ponds; however other variables were identified as more significant within particular ponds or populations, and between locations. OTU abundance changed progressively across geochemical gradients, with three niches within the water column identified, the epilimnion, chemocline and hypolimnion. A significant proportion of total reads were shared between years/locations in these ponds, however differences were identified in their abundance. Community structure showed greater temporal similarity (same pond) than spatial (spatial variation was linked

to iron and mercury), although the changing of geochemical drivers between years, as seen in pond P70E, suggests the possibility of significant temporal variation. Stratified meltwater ponds in this study contained a core of dominant cosmopolitan OTUs, but the community structure within and between ponds was strongly influenced by extreme chemical gradients. Temporal microbial stability and the significant influence of local geochemistry can also provide a sensitive model to monitor long-term environmental change. Although a core of cosmopolitan bacteria was identified within these ponds, the examination of analogous ecosystems across continental Antarctica would determine if these communities are endemic to this specific region or are common inhabitants of Antarctic aquatic ecosystems across the entire continent.

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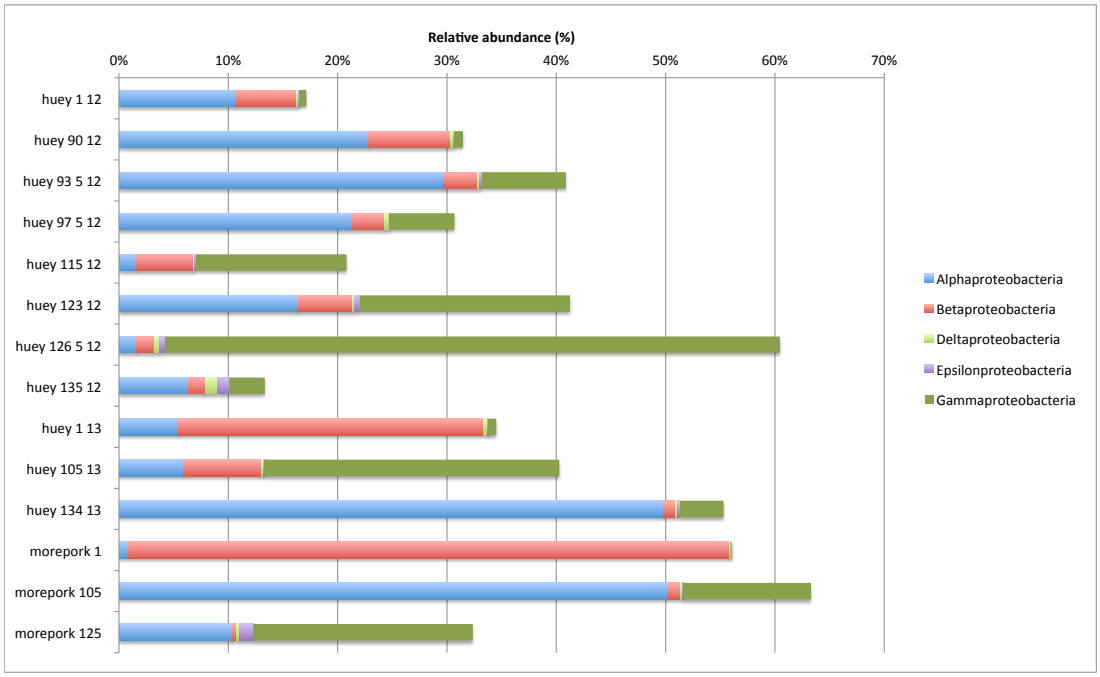
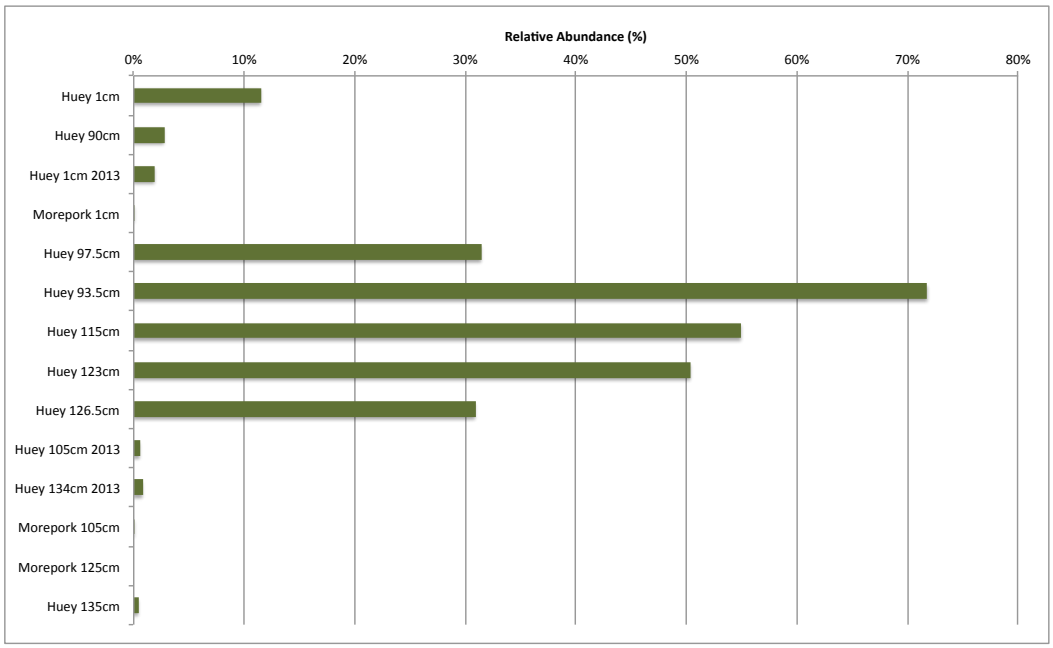
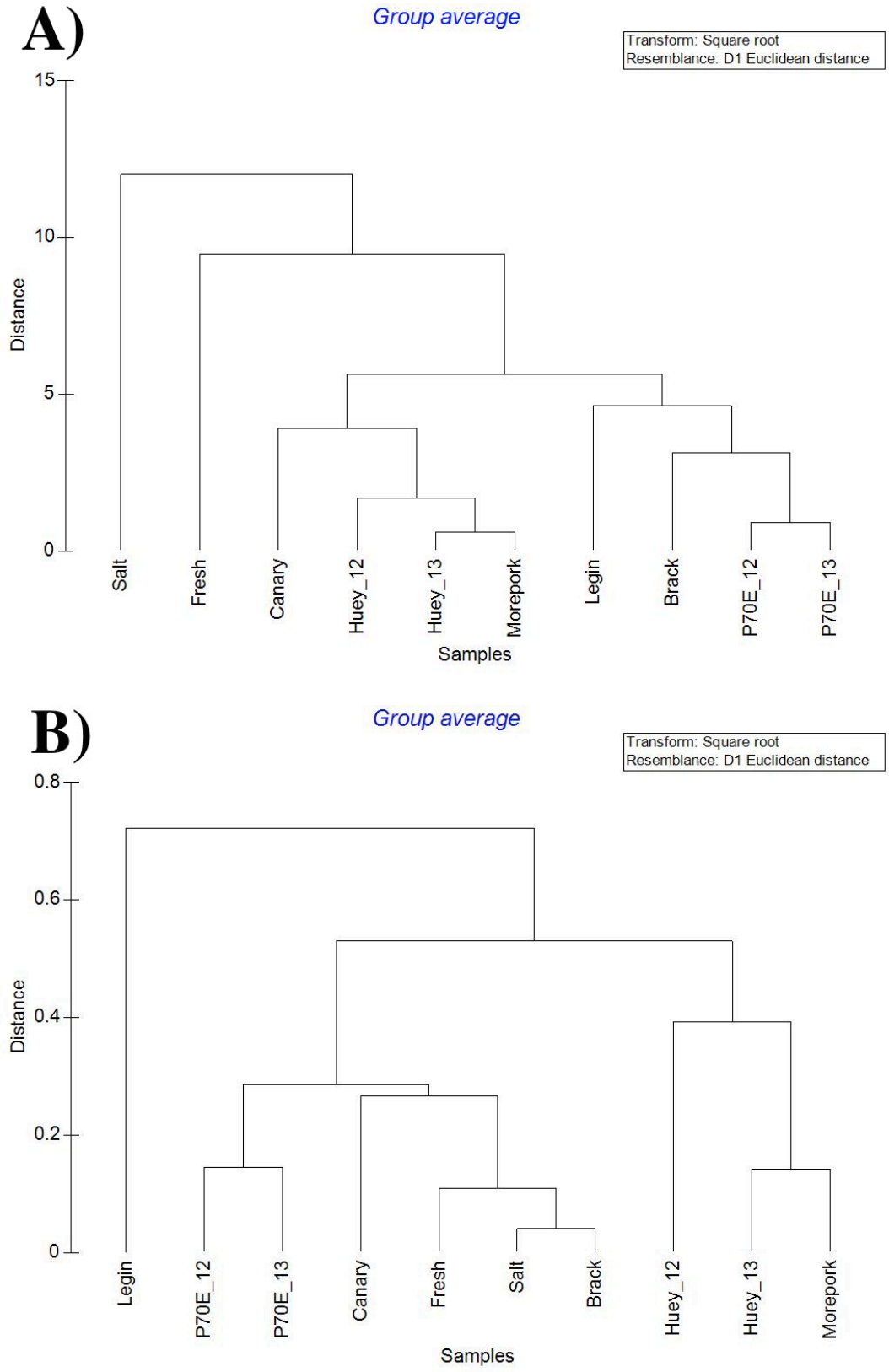
4.9 Supplementary Informaiton



Supplementary Figure 4. 1 Distributions of *Proteobacteria* between samples.



Supplementary Figure 4. 2 Total percentage of sequences assigned to chloroplast signatures (via the RDP database).



Supplementary Figure 4. 3 Dendrogram displaying relative similarity of pond water columns for A) Conductivity, B) pH.

Supplementary Table 4. 1 Summary of physical measurements of sampled ponds.

Pond	Lattitude	Longitude	Surface Area (m^2)	Depth Max (m)	Relative Depth	width (m)	length (m)
Huey 2012	S 78.01419	E 165.55576	47	1.35	17.4	5	12
Huey 2013	S 78.01419	E 165.55576	47	1.34	17.3	5	12
Legin	S 78.01624	E 165.54903	162	1.15	8.0	12	17.2
Brack	S 78.01569	E 165.54570	1166	0.75	1.9	27	55
Fresh	S 78.01549	E 165.54305	1531	0.62	1.4	30	65
Salt	S 78.01608	E 165.54510	184	0.7	4.6	13	18
P70E 2012	S 78.01580	E 165.55165	12	0.72	18.1	3.5	4.5
P70E13	S 78.01580	E 165.55165	12	0.775	19.5	3.5	4.5
Morepork	S 78 07.343	E164 12.061	33	1.24	19.1	7	6
Canary	S 78 07.433	E 164 11.453	63	1.05	11.7	10	8

Supplementary Table 4. 2 Summary of BEST analysis results indicating strength of correlations between ARISA community structure and *in situ* collected physicochemical variables.

2012 / 2013		
Pond	**Correlation**	**Variable(s)**
All 2012/2013	0.406	2,3
	0.356	2,3,4
	0.317	1,2,3
	0.305	2
	0.302	2,4
	0.301	3

Pond	Correlation	Variable(s)	Pond	Correlation	Variable(s)
Huey, P70E 2012 and 2013	0.357	2,3,4	**Huey 2012 and 2013, Morepork 2013**	0.577	2,3,4
	0.351	2,3		0.573	2,3
	0.342	3,4		0.571	3,4
	0.333	ALL		0.519	3
	0.329	3		0.508	ALL
	0.287	4		0.447	4
	0.223	2		0.385	2

2012			2013		
Pond	**Correlation**	**Variable(s)**	**Pond**	**Correlation**	**Variable(s)**
All	0.445	2,3,4	**All**	0.501	3
	0.416	2,3		0.472	2,3
	0.403	2,4		0.454	3,4
	0.383	3		0.446	2,3,4
Huey	0.761	3	**Bratina Island Ponds**	0.576	4
	0.734	3,4		0.563	3,4
	0.719	2,3		0.435	2,3,4
	0.716	2,3,4		0.426	2,4
P70E	0.890	4	**Huey**	0.834	3,4
	0.878	2,4		0.832	3
	0.866	ALL		0.807	2,3,4
	0.865	2,3,4		0.788	2,4
Legin	0.698	1,2,3	**P70E**	0.734	3
	0.696	ALL		0.681	2,3
	0.681	1,3,4		0.680	3,4
	0.666	3		0.676	2,3,4
Brack	0.696	1,2	**Miers Valley Ponds**	0.740	2,3
	0.682	1,2,3		0.699	3
	0.673	2,3		0.690	2,3,4
	0.552	2		0.666	1,2,3
Salt	0.911	3	**Morepork**	0.833	2,3
	0.878	2,3		0.791	3
	0.799	ALL		0.776	2,3,4
	0.792	1,2,3		0.758	2
Fresh	-0.064	2	**Canary**	0.847	3,4
	-0.133	1		0.798	1,3,4
	-0.176	1,4		0.785	3.000
	-0.193	1,2		0.768	2,3,4

Variable Designation: 1=Depth (cm), 2=Dissolved Oxygen (ppm), 3=Conductivity (mS/cm), 4=pH

Supplementary Table 4. 3 Summary of pyrosequencing data including: sequences unique to individual samples, total reads, total OTUs present at a single read and Goods Coverage.

Sample	Year/Location	Unique seq	Reads	Singleton seq	Coverage
Huey 1cm	Bratina 2012	454	18207	300	98.35%
Huey 90cm	Bratina 2012	300	9483	189	98.01%
Huey 93.5cm	Bratina 2012	174	5746	106	98.16%
Huey 97.5	Bratina 2012	132	4433	71	98.40%
Huey 115cm	Bratina 2012	139	6229	83	98.67%
Huey 123cm	Bratina 2012	269	5717	140	97.55%
Huey 126.5cm	Bratina 2012	331	9397	157	98.33%
Huey 135cm	Bratina 2012	524	10040	274	97.27%
Huey 1cm	Bratina 2013	147	8746	80	99.09%
Huey 105cm	Bratina 2013	112	9986	47	99.53%
Huey 134cm	Bratina 2013	417	17050	181	98.94%
Morepork 1cm	Miers 2013	65	8231	39	99.53%
Morepork 105cm	Miers 2013	275	11115	107	99.04%
Morepork 125cm	Miers 2013	403	8685	169	98.05%

Supplementary Table 4. 4 Complete dataset of environmental variables overlaid as vectors in Figure 3 and used in BEST analysis.

Pond	Depth (cm)	DO (ppm)	Conductivity (mS cm^{-1})	pH	Phosphate	NH^4 marine (ppm)	NO^2	NO^3	B 10	Ca 43	V 51	Cr 52
Huey 1cm 2012	1	19.2	3.8	9.8	0.011	0.000	0.000	0.000	11.048	32.164	0.009	0.003
Huey 90cm 2012	90	22.5	4.8	10.0	0.015	0.041	0.000	0.000	11.478	25.499	0.009	0.003
Huey 93.5cm 2012	93.5	57.5	14.6	10.0	0.082	0.000	0.011	0.009	11.315	32.880	0.006	0.004
Huey 97.5cm 2012	97.5	94.8	19.6	9.9	0.066	0.000	0.000	0.000	508.955	597.480	0.021	0.054
Huey 115cm 2012	115	104.7	41.0	10.1	0.136	0.716	0.000	0.000	540.280	170.968	0.000	0.077
Huey 123cm 2012	123	93.2	49.4	9.8	0.178	0.052	0.015	0.016	522.710	255.115	0.039	0.000
Huey 126.5cm 2012	126.5	43.0	81.6	8.4	0.194	1.777	0.032	0.000	573.898	191.919	0.003	0.000
Huey 135cm 2012	135	1.7	115.8	7.2	1.318	11.680	0.036	0.063	556.305	202.888	0.015	0.000
Huey 1cm 2013	1	13.1	4.4	9.5	0.011	0.029	0.000	0.000	11.463	31.251	0.010	0.001
Huey 105cm 2013	105	25.0	32.7	8.6	0.052	0.094	0.000	0.000	580.688	194.308	0.000	0.029
Huey 134cm 2013	134	11.7	110.0	7.1	1.359	9.183	0.016	0.000	568.483	212.530	0.122	0.099
Morepork 1cm 2013	1	12.3	4.1	9.1	0.011	0.000	0.000	0.000	11.193	48.145	0.008	0.004
Morepork 105cm 2013	105	10.3	40.1	8.0	0.019	0.090	0.000	0.000	578.854	237.343	0.000	0.205
Morepork 124cm 2013	125	1.3	103.9	7.2	0.449	3.931	0.061	0.000	577.147	356.553	0.105	0.105

Pond	Fe 54	Co 59	Ni 60	Cu 63	Zn 68	As 75 (ppm)	Se 82	Cd 111	Ba 137	Hg 202	Pb 207
Huey 1cm 2012	0.000	0.000	0.007	0.127	0.430	0.000	0.000	0.000	0.005	0.000	0.006
Huey 90cm 2012	0.000	0.000	0.009	0.127	0.436	0.001	0.003	0.000	0.005	0.000	0.008
Huey 93.5cm 2012	0.000	0.001	0.010	0.149	0.428	0.014	0.031	0.000	0.014	0.000	0.008
Huey 97.5cm 2012	0.000	0.005	0.395	5.720	24.493	0.000	0.000	0.000	0.240	0.000	0.363
Huey 115cm 2012	0.000	0.000	0.000	4.334	10.026	0.000	0.000	0.000	0.240	0.000	0.108
Huey 123cm 2012	0.000	0.000	0.000	4.075	14.269	0.000	0.000	0.000	0.268	0.000	0.123
Huey 126.5cm 2012	0.000	0.005	0.000	4.454	12.741	0.000	0.000	0.000	0.298	0.000	0.088
Huey 135cm 2012	0.000	0.000	0.000	4.398	10.452	0.000	0.000	0.000	0.228	0.000	0.095
Huey 1cm 2013	0.000	0.000	0.000	0.102	0.698	0.001	0.000	0.000	0.010	0.000	0.002
Huey 105cm 2013	0.000	0.000	0.000	4.512	9.933	0.000	0.000	0.010	0.243	0.000	0.083
Huey 134cm 2013	0.000	0.003	0.000	4.140	10.019	0.000	0.000	0.010	0.265	0.000	0.068
Morepork 1cm 2013	0.340	0.000	0.000	0.088	0.214	0.005	0.010	0.000	0.008	0.004	0.001
Morepork 105cm 2013	556.097	0.030	0.000	3.988	6.805	0.000	0.140	0.020	0.248	0.070	0.060
Morepork 124cm 2013	12.647	0.000	0.000	3.803	6.123	0.000	0.000	0.005	0.248	0.058	0.050

Chapter 5 - Benthic bacterial communities of coastal terrestrial and ice shelf Antarctic meltwater ponds

Preface

This chapter describes the benthic microbial community in 12 meltwater ponds, examining interpond variation within and between sampling locations and the influence of the overlying water geochemistry. As the primary author of this chapter, I was involved with all fieldwork and provided guidance for DNA extraction and a preliminary fingerprinting effort by two summer students Natasha Jaques and Keiran Oxton. I completed all pyrosequencing preparation, the reanalysis of data and manuscript preparation. Inductively coupled mass spectroscopy (ICPMS) and DNA sequencing were carried out by technicians at the University of Waikato. Craig Herbold and Charles Lee earned co-authorship for their part in developing bioinformatics pipelines used to analyse this data. All authors reviewed and edited this manuscript. This chapter has been published with the following citation.

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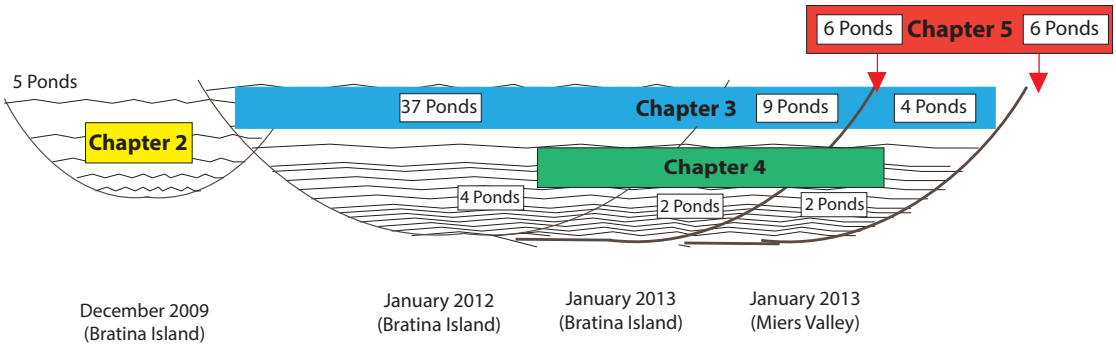

Preface Figure 5. 1 Breakdown info-graphic overview of thesis

Benthic bacterial communities of coastal terrestrial and ice shelf Antarctic meltwater ponds

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Key words: Biogeography, Antarctic, Benthic, Microorganism, community, melt-water, Dry valleys

5.1 Abstract

The numerous perennial meltwater ponds distributed throughout Antarctica represent diverse and productive ecosystems central to the ecological functioning of the surrounding ultra oligotrophic environment. The dominant taxa in the pond benthic communities have been well described, however little is known regarding their regional dispersal and local drivers to community structure. The benthic bacterial communities of 12 meltwater ponds in the McMurdo Sound of Antarctica were investigated to examine variation between pond microbial communities and their biogeography. Geochemically comparable but geomorphologically distinct ponds were selected from Bratina Island (ice shelf) and Miers Valley (terrestrial) (<40 km between study sites), and community structure within ponds was compared using DNA fingerprinting and pyrosequencing of 16S rRNA gene amplicons. More than 85% of total sequence reads were shared between pooled benthic communities at different locations, which in combination with favorable prevailing winds suggests aeolian regional distribution. Consistent with previous findings, *Proteobacteria* and *Bacteroidetes* were the dominant phyla, representing over 50% of total sequences; however, a large number of other phyla (21) were also detected in this ecosystem. Although dominant bacteria were ubiquitous between ponds, site and local selection resulted in heterogeneous community structures and more than 45% of diversity was pond specific. Potassium was identified as the most significant contributing factor to the cosmopolitan community and aluminium to the unique community of individual ponds based on a BEST analysis (Spearman's P of 0.632 and 0.806 respectively). These results indicate that the microbial communities in meltwater ponds are easily dispersed regionally and that the local geochemical environment drives the ponds community structure.

5.2 Introduction

Antarctic aquatic mats and associated sediments harbor diverse microbial communities crucial to nutrient cycling (Bowman *et al.*, 2000) and are the presumed dominant source of terrestrial biomass outside of coastal areas (Moorhead *et al.*, 2003; Wood *et al.*, 2008). Although smaller than their well-studied lake counterparts, meltwater ponds derived from local ice and snow melt are more abundant throughout the continent, providing individually distinct geochemical environments (Doran *et al.*, 1994; Matsumoto *et al.*, 1992; Vincent & James, 1996). Their significance to terrestrial Antarctic processes, coupled with a relatively high biodiversity, productivity and responsiveness to the geochemical environment (Jungblut *et al.*, 2005; Sabbe *et al.*, 2004; Safi *et al.*, 2012; Sutherland, 2009), makes these ponds useful for monitoring future environmental changes in Antarctica. Polar pond mats and sediments have been investigated in the past (de los Rios *et al.*, 2004; Rojas *et al.*, 2009; Sabacka & Elster, 2006; Suren, 1990; Sutherland, 2009; VanTrappen *et al.*, 2002), however few studies have utilized high-throughput sequencing to accurately describe and compare the bacterial community structure between ponds or locations.

The meltwater ponds at Bratina Island, Victoria Land on the McMurdo Ice Shelf (MIS ponds) are probably the best characterized to date (Hawes *et al.*, 2013). Bratina Island is located in the southwest corner of the Ross Sea, at the northern tip of Brown Peninsula with the coast of southern Victoria Land to the west (Figure 5. 1). The undulating landscape, driven by compression of the ice shelf, provides an ideal terrain for the formation of thousands of meltwater ponds (Howard-Williams *et al.*, 1990). A 10-30 cm layer of soil/sediment deposits covers this region, originating from basal freezing of marine sediments to the underside of the floating ice shelf and deposition by surface ablation (Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988). Within 1 km of an established field camp are a series of previously studied ponds of variable size, depth, age and chemistry.

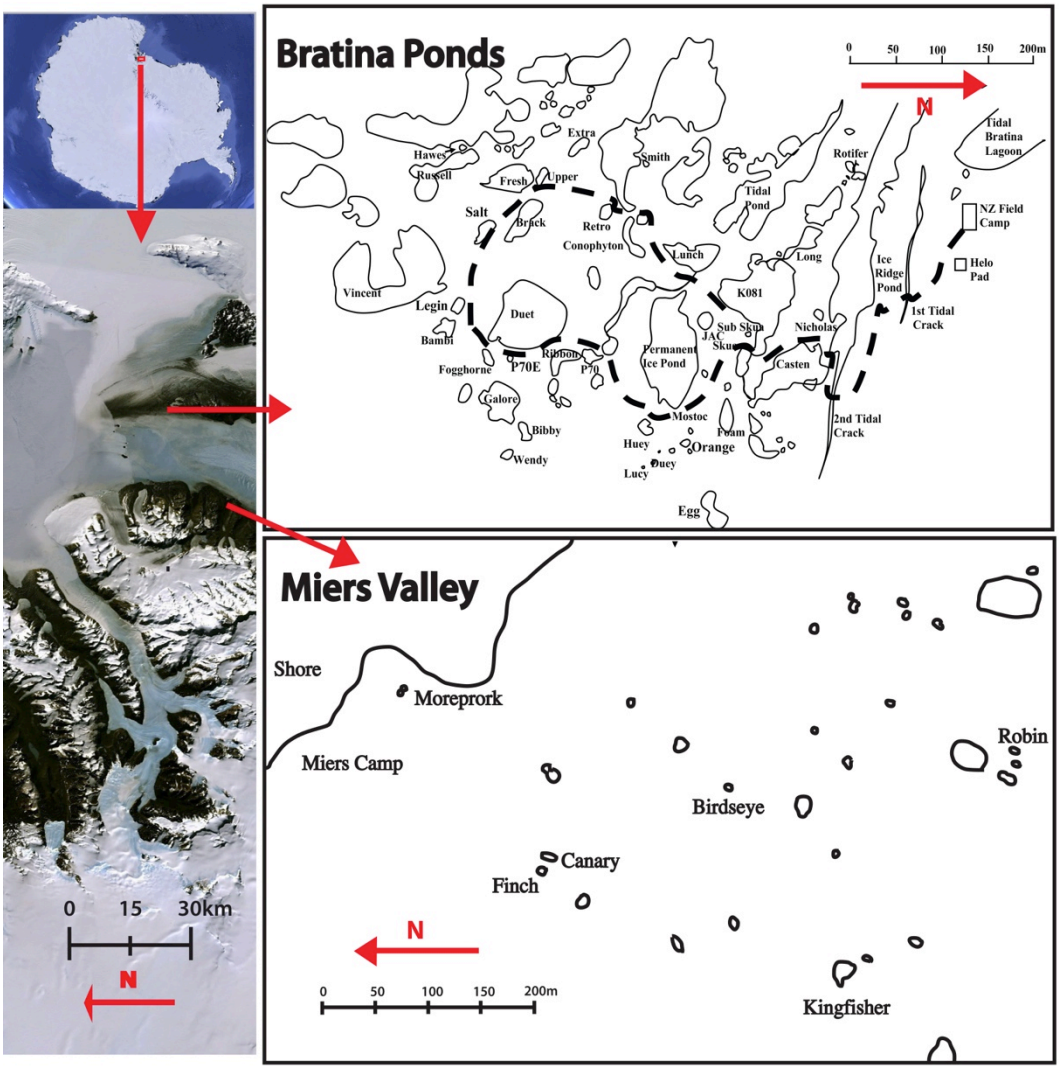

Figure 5. 1 Location of study sites in the Ross sea region of Antarctica (left), and the scale and proximity of ponds at Bratina Island (top right) and the Miers Valley (bottom right).

Although considered a polar desert, dozens of terrestrial meltwater ponds are distributed throughout the Dry Valleys of Antarctica (Vincent & James, 1996). The McMurdo Dry Valleys are the largest ice-free area of the Antarctic continent, encompassing a series of valleys whose soil biological communities are influenced by local geology, chemistry and geographic factors (Cary *et al.*, 2010). A number of poorly studied coastal terrestrial ponds (CT ponds) are located at the eastern most point (the "mouth") of the Miers Valley. Although formed by meltwater accumulation in landscape depressions, as is seen at Bratina Island, the <30 cm soils in the Miers Valley cover continental permafrost and typically originate from bedrock and glacial till erosion (Cary *et al.*, 2010; Healy *et al.*, 2006; McLeod *et al.*, 2009).

The cyanobacterial mats ubiquitous in Antarctic aquatic ecosystems are morphologically complex and variable, their structure dependent on local substrate composition (grain size, deposition rates etc), the dominant *Cyanobacteria* present, climatological influences and pond geochemistry (de los Rios *et al.*, 2004). As in temperate climates, the sediments underlying these pond mats (Mountfort *et al.*, 2003) contain a diverse microbial community with vertical stratification defined by the immediate redox conditions (Shivaji *et al.*, 2011; Ye *et al.*, 2009). These sediments have built up over decades, even through thick ice cover, by repeated layering of aeolian dust from the surrounding environment and mat re-growth (Squyres *et al.*, 1991). The visually dominant *Cyanobacteria* are well studied in Antarctic meltwater pond mats and sediments (Jungblut *et al.*, 2005; Sabbe *et al.*, 2004; Taton *et al.*, 2003; Taton *et al.*, 2006; Wood *et al.*, 2008), however, *Proteobacteria* and *Bacteroidetes* are also consistently identified as significant components of the microbial community (Bowman *et al.*, 2000; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002) with other intermittently identified phyla such as *Firmicutes*, *Actinobacteria*, *Verrucomicrobia* and *Acidobacteria* (Brambilla *et al.*, 2001; Peeters *et al.*, 2012; Rojas *et al.*, 2009).

This study presents a comparative survey of the benthic communities from 12 meltwater ponds in the Ross Sea Region of Antarctica. By utilizing high-throughput sequencing coupled with biogeochemical data, this represents the highest resolution comparison of these communities ever undertaken. The

objectives of this study were to compare the bacterial benthic communities from MIS and CT ponds, to identify and describe the dominant phyla and OTUs across the ponds, and to investigate the geochemical drivers of community structure so that a greater understanding of these unique communities could be gained.

5.3 Materials and Methods

5.3.1 Field sampling strategy

Sediment cores, 4 cm in depth, were collected from around the edge of twelve fully thawed meltwater ponds during the summer season in January 2013 from Bratina Island (6 ponds) (78° 01' S, 165° 32' E) and the Miers Valley (6 ponds) (78° 07' S, 164° 12' E) (Table 5. 1 and Figure 5. 1). Sites were selected to encompass a broad range of surface water geochemistry from ponds at each location. Cores were aseptically collected using a disposable push-corer developed from a 50 mL syringe (BD, Singapore). The corer (with the plunger removed) was inserted 4-6 cm into the sediment, the plunger reinserted and core removed carefully to retain the sediment structure. After excess sediment was removed, each core was sub-sectioned into four one-centimeter samples, placed in sterile 15 oz whirlpack bags (Nasco, WI, USA), then frozen for transportation to the laboratory.

Table 5. 1 *In situ* environmental data from overlying water column. Pond names in bold represent those selected for high-throughput sequencing.

Pond	Location	GPS Coordinates		DO(ppm)	Conductivity(mS/cm)	pH
P70E	Bratina	S 78.01580	E 165.55165	11.61	7.65	8.76
Huey	Bratina	S 78.01419	E 165.55576	13.08	4.35	9.48
Legin	Bratina	S 78.01624	E 165.54903	13.15	2.2	9.65
Salt	Bratina	S 78.01608	E 165.54510	14.81	40.8	9.58
Bambi	Bratina	S 78.01649	E 165.54936	14.79	3.31	10.55
Conophyton	Bratina	S 78. 01431	E 165.54500	12.26	0.617	9.73
Finch	Miers	S 78 07.748	E 164 11.714	13.35	1.742	9.76
Birdseye	Miers			20.18	14.38	9.84
Canary	Miers	S 78 07.433	E 164 11.453	14.07	4.18	9.58
Robin	Miers	S 78 07.752	E 164 11.762	13.94	0.634	10.06
Kingfisher	Miers			14.02	0.987	9.39
Morepork	Miers	S 78 07.343	E164 12.061	12.26	4.06	9.13

5.3.2 Geochemical data

In situ dissolved oxygen, pH, temperature and conductivity of the overlying pond water were measured using an HQ40d portable multi-parameter meter (Hach Company, CO, USA). Thirteen millilitres of 0.22 μm filtered (Whatman International Ltd, Kent, UK) water was collected in 15 mL falcon tubes and frozen for later geochemical analysis. NH_4, NO_2/NO_3 and PO_4 measurements of the overlying pond filtrate from selected samples were carried out at the University of Waikato using an Aquakem 200CD following the manufacturer's instructions (Thermo Fisher Scientific, Waltham, USA). Elemental analysis was performed on each sample by inductively coupled plasma mass spectrometry (ICP-MS) using a Mass Spectrometer ELAN® DRC II (PerkinElmer Inc., Münster, Germany). To prepare samples for ICP-MS, 0.22 μm prefiltered pond water was diluted 1:50 with Milli-Q water (Millipore, Billerica, MA, USA). Once diluted, samples were acidified with 2% $HN0_3$ (Extra pure Nitric Acid, Ajax Finechem, NSW, Australia). Differences in aluminium concentration between sites were investigated using Tukeys Honest Significant Difference Test (in R). In Primer 6 (Clarke & Gorley, 2006) geochemical data were transformed using a square root followed by a log (X+1) transformation and normalization. Two-dimensional ordinations using non-metric multidimensional scaling (NMDS) were performed based on a Euclidean distance matrix to represent the relative distances between individual ponds.

5.3.3 DNA extraction

DNA was extracted from 0.5 g $\pm$ 0.1 g of individual sediment sections using a modified bead-beating method (Coyne *et al.*, 2001). Briefly, sediment was added to 0.5 g each of 0.1 mm and 2.5 mm silica-zirconia beads. To each sample 270 μL of phosphate buffer (100 mM NaH_2PO_4) and 270 μL of SDS lysis buffer (100 mM NaCl, 500 mM Tris pH 8.0, 10% SDS) were added and samples were horizontally shaken on a Vortex Genie 2 (MO BIO Laboratories Inc, Carlsbad, CA, USA) for 15 minutes. Samples were centrifuged at 12,500 rpm for 30 seconds and 180 μL of CTAB extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP and 0.4% BME) was added. Samples were

vortexed for 10 seconds prior to incubation at 60°C and 300 rpm for 30 minutes on a rocking bed. Samples were centrifuged at 12,500 rpm for 30 seconds and then 350 µL of chloroform/isoamyl alcohol (24:1) was added. Samples were again vortexed for 10 seconds and centrifuged for 5 minutes at 12,500 rpm. The aqueous phase was transferred to a new eppendorf tube then 500 µL of chloroform/isoamyl alcohol (24:1) was added. Samples were vortexed and left on a rocking bed HulaMixer (Invitrogen, Carlsbad, CA, USA) for 20 minutes. Samples were centrifuged for 5 minutes at 13,500 rpm, the aqueous phase was removed and 10 M ammonium acetate was added to the samples to achieve a final concentration of 2.5 M. The samples were vortexed and centrifuged for five minutes at 13,500 rpm. The aqueous layer was removed to a new tube and 0.54 volumes of isopropanol was added and mixed. Samples were left overnight at -20°C then centrifuged for 20 minutes at 13,500 rpm. The supernatant was removed, the pellet washed with 1 mL of 70% AR grade ethanol and centrifuged for 1 minute at 13,500 rpm. Ethanol was removed and DNA was re-suspended in 30 µL of sterile TE then quantified using the Qubit 2.0 Fluorometer (Invitrogen). The four individual sectioned samples from each core were diluted to 10 ng/µL, then 10 µL of each was pooled and frozen at -20°C until use.

5.3.4 ARISA community fingerprinting and analysis

Automated Ribosomal Intergenic Spacer Analysis (ARISA) DNA fingerprinting (Fisher & Triplett, 1999) was utilised to resolve relative structure between bacterial communities as a preliminary comparison between ponds and sites (Archer *et al.*, 2014). Briefly, from each sample the bacterial intergenic spacer region (ISR) in the rRNA operon was amplified using PCR primers ITSReub-Hex (5`-GCCAAGGCATCCACC-3`) and ITSF (5`-GTCGTAACAAGGTAGCCGTA-3`) according to (Cardinale *et al.*, 2004). All ARISA PCR reactions were run in triplicate on a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, CA). Thermal cycling conditions were: 94°C for 5 min, then 30 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min. Once amplified, all triplicate PCR reactions were resolved on a 1% agarose gel to ensure

amplification success then pooled. Amplicons were diluted 1:20 in Gibco ultrapure water and fragment lengths were resolved on a 3130XL DNA sequencer (Applied Biosystems, New York, USA) using Liz-1200 internal size standard at the University of Waikato DNA Sequencing Facility.

ARISA fingerprints were processed with an informatics pipeline (modified from (Abdo *et al.*, 2006) (Sokol *et al.*, 2013)). Peaks exceeding 200 fluorescence units, greater than 50 bp and less than 1200 bp, were accepted as true peaks. The remaining peaks were used to calculate model parameters for a log-normal distribution. Iteratively, peaks with an area exceeding the 99.9% cumulative distribution of the calculated log-normal distribution for noise were accepted as true peaks. Peaks were binned into ARISA Fragment Lengths (AFLs) within 5 bp of one another. The resulting data matrix was analysed using a combination of Primer 6 (Clarke & Gorley, 2006) and R for statistical analysis (Team, 2013). In Primer 6, beta diversity was investigated using a resemblance matrix created based on the Bray Curtis community dissimilarities. These were examined in a two-dimensional ordination using non-metric multidimensional scaling (NMDS). ANOSIM analyses were performed on the resemblance matrix to test specific hypotheses formed from interpretation of MDS plots.

5.3.5 DNA Pyrosequencing

The V5-V6 hypervariable region of the 16S rRNA gene was utilized to identify variation in bacterial community diversity and structure using primers and conditions detailed in Archer *et al.* (2014). Briefly, triplicate 30 µL PCR reactions were run for each sample using un-adapted primers Tx9F (5‘-GGATTAGAWACCCBGGTAGTC-3’) and 1391R (5‘-GACGGGCRGTGWGTRCA-3’). The triplicates were pooled then gel extracted on a 2% TAE agarose gel stained with “SYBR Safe” and DNA was retrieved using the UltraClean 15 (MoBio, Inc, Carlsbad, USA) DNA Purification Kit as per manufacturer’s instructions. A second round of triplicate PCR was run as above but with only 10 cycles and using 25 ng of the purified DNA from the previous step per reaction (milli-Q H_2O volume adjusted accordingly). The primers used were adapted for one-way reads as per manufacturer’s instructions,

including unique MID identifiers for each sample (BacX-Tx9F (5`-CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID-GGATTAGAWACCCBGGTAGTC-3`) and BacB-1391R (5`-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-GACGGGCRGTGWGTRCA-3`)). A second gel extraction was performed as above. Samples went through a final cleanup step using the Agencourt AMPure XP system (Beckman Coulter Genomics, Danvers, MA, USA) as per the manufacturer's instructions. Sample DNA content was quantified using a Qubit Fluorometer and diluted to 200 pg/μL. The DNA concentration and quality verification was performed using a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and then diluted to $1x10^9$ molecules/μL. The diluted amplicons were mixed together in the desired proportions to create the $1x10^9$ amplicon pool. Sequencing was performed using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTiterPlate Kit and GS Junior System according to the manufacturer's instructions (Roche 454 Life Sciences, Branford, CT, USA).

The 454 amplicon pyrosequencing data were processed using AmpliconNoise v1.0 for quality filtering, denoising and chimera removal (Quince *et al.*, 2011). Briefly, raw flowgrams (sff files) with perfectly matching primer and barcode sequences were filtered for a minimum flowgram length of 360 cycles (including primer and barcode sequences) before the first noisy signal (i.e., 0.5-0.7 or no signal in all four nucleotides). All flowgrams were then truncated at 360 bases and clustered to remove sequencing noise using PyroNoise (Quince *et al.*, 2009; Quince *et al.*, 2011). Noise introduced by PCR was removed using SeqNoise (Quince *et al.*, 2011), and PCR chimeras were removed using Perseus (Quince *et al.*, 2011). Resulting de-replicated sequences from Perseus were processed using Mothur 1.17.0 (Schloss *et al.*, 2009) to create a unique sequence and names file. Pairwise alignments and distance were calculated using Espirit (Sun *et al.*, 2009). Mothur was then used to cluster sequences into operational taxonomic units (OTUs) defined at the average neighbor Jukes-Cantor distance of 0.05 ($OTU_{0.05}$). Rank-abundance data were generated for each sample.

For phylogenetic assignments, representative sequences of all identified $OTU_{0.05}$ were analyzed using the Classifier function provided by the Ribosomal Database Project (RDP) Release 10, Update 15 (Wang *et al.*, 2007). Taxonomic

assignment threshold was set at 80%. To gain a visual representation of individual sample community similarity based on sequencing data a dendrogram was created in Primer 6 using a resemblance matrix based on the Bray Curtis community similarities. BEST analysis between the normalized geochemical data and the pyrosequencing data was conducted to investigate geochemical drivers of community structure.

5.4 Results

5.4.1 Pond geochemistry

In situ geochemistry varied across all ponds with DO values ranging from 11.6-20.2 ppm, conductivity from 0.6-40.8 mS cm^{-1} and pH from 8.8-10.6 (Table 5. 1). The relationship between in-field collected geochemistry (pH, DO and conductivity) of all ponds is represented in a non-metric MDS plot (Figure 5. 2A). Based on this plot, six ponds, three from each location, were identified for higher resolution geochemical (nutrient and elemental) and genetic analyses. Comparisons of geochemical profiles including nutrient and elemental data (Supplementary Table 5. 1) are represented by a non-metric MDS plot (Figure 5. 2B), in which ponds between locations clustered closer together than within locations.

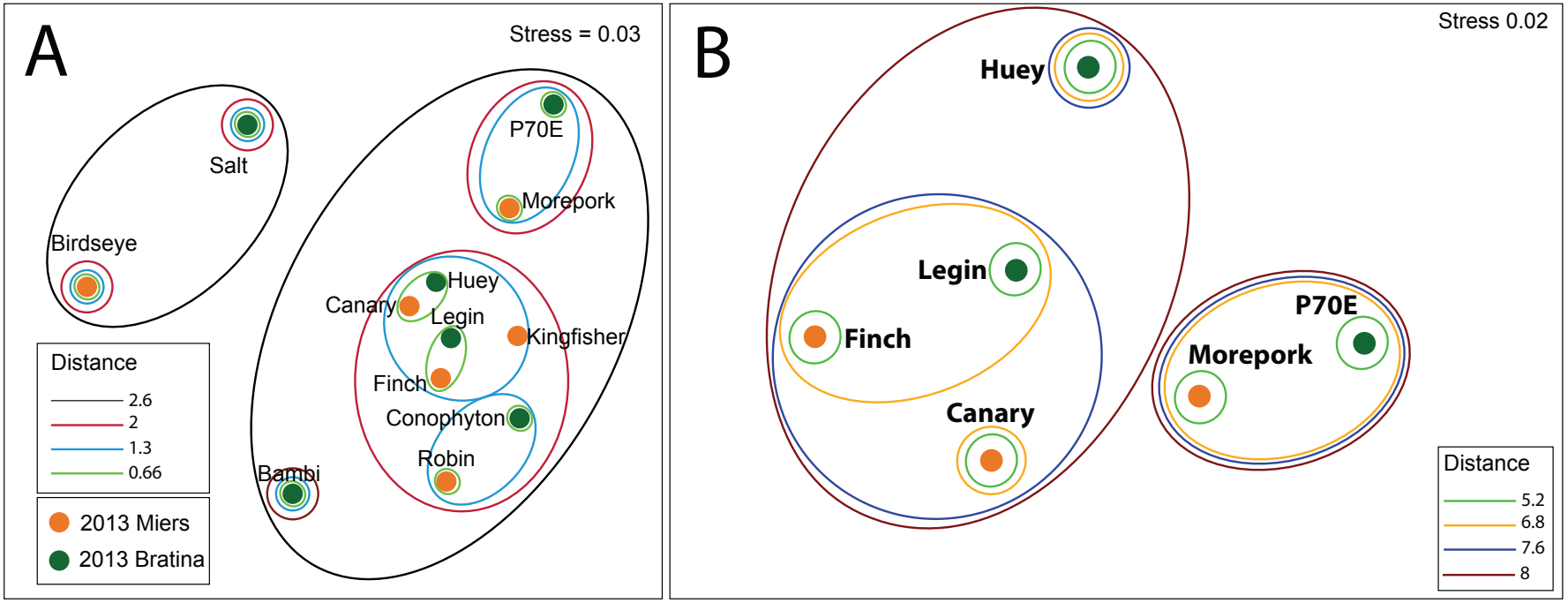

Figure 5. 2 MDS ordinations of sediment geochemical profiles. A Euclidean distance matrix was calculated using geochemical data that was square root, log (X+1) transformed and normalized. Preliminary geochemistry, pH, DO and conductivity from all ponds (A). Preliminary geochemistry, ICP-MS, and nutrient values of selected subset of ponds (Supplementary Table 2) (B).

5.4.2 Benthic microbial community structure in meltwater ponds

Inter-pond benthic bacterial ARISA community structure based on a non-metric MDS plot (Figure 5. 3) had a broad distribution with little clustering based on location (Bratina or Miers). Six benthic samples (three from each location) were selected for high-throughput sequencing (Supplementary Table 5. 2). A venn diagram was created to represent unique and shared OTUs and reads from pooled data between locations (Bratina 2013 and Miers Valley 2013) and between individual ponds. A total of 753 shared OTUs represented 86.3 and 85.2% of total reads in the Miers Valley and Bratina Island, respectively (Figure 5. 4). Benthic community structure of individual ponds based on OTU abundance was heterogeneous (<50% similarity between all ponds except P70E and Huey), although phyla compositions appeared similar (Figure 5. 5). The large proportion of shared sequences and similar phyla compositions, but low community structure similarity, between ponds resulted from variable abundances of dominant OTUs (Table 5. 2). The most biologically similar ponds among the six ponds examined were P70E and Huey, however, multiple variations in phyla and OTU abundance were identified (Figure 5. 5, Table 5. 2). The relative abundances of *Actinobacteria* (10.2% vs. 5.5%) and *Bacteroidetes* (23.6% vs. 17.8%) were higher in P70E, whereas *Proteobacteria* (41.8% vs. 49.0%), *Firmicutes* (1.6% vs. 4.2%), and *Cyanobacteria* (1.9% vs. 4.2%) were less abundant in P70E. This variation extended to the abundance of individual OTUs (Table 5. 2). OTUs unique to individual ponds represented 7.5 to 17.3% of total pond reads, however accounted for 45.2 to 54.0% of total OTUs from each pond (Figure 5. 4).

A total of 23 phyla were identified across all ponds sampled (Figure 5. 5), with variable relative abundance between ponds, however those representing >1% of total reads from all samples were present in all samples (*Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Chloroflexi*, *Cyanobacteria*, *Euryarchaeota*, *Firmicutes*, *Planctomycetes* and *Verrucomicrobia*). *Chloroflexi* (6 to 1.2%), *Crenarchaeota* (<0.1 to 1.2%), *Euryarchaeota* (2.2 to 0.01%) and *Cyanobacteria* (8.8 to 3.8%) exhibited the greatest phyla differences between pooled Miers Valley and Bratina Island benthic communities. Some phyla were detected at particularly high relative abundance in individual ponds, such as *Chloroflexi* in

Morepork, and *Crenarchaeota* in Legin pond (almost 10-fold higher than from any other pond). Sequences affiliated with *Bacteroidetes* and *Proteobacteria* were dominant across all ponds, accounting for 14.6-23.6% and 19.3-49.0% of sequences, respectively. Variability between samples was also examined at class level for *Proteobacteria*, the dominant class being *Betaproteobacteria* followed by *Gammaproteobacteria* and *Alphaproteobacteria*.

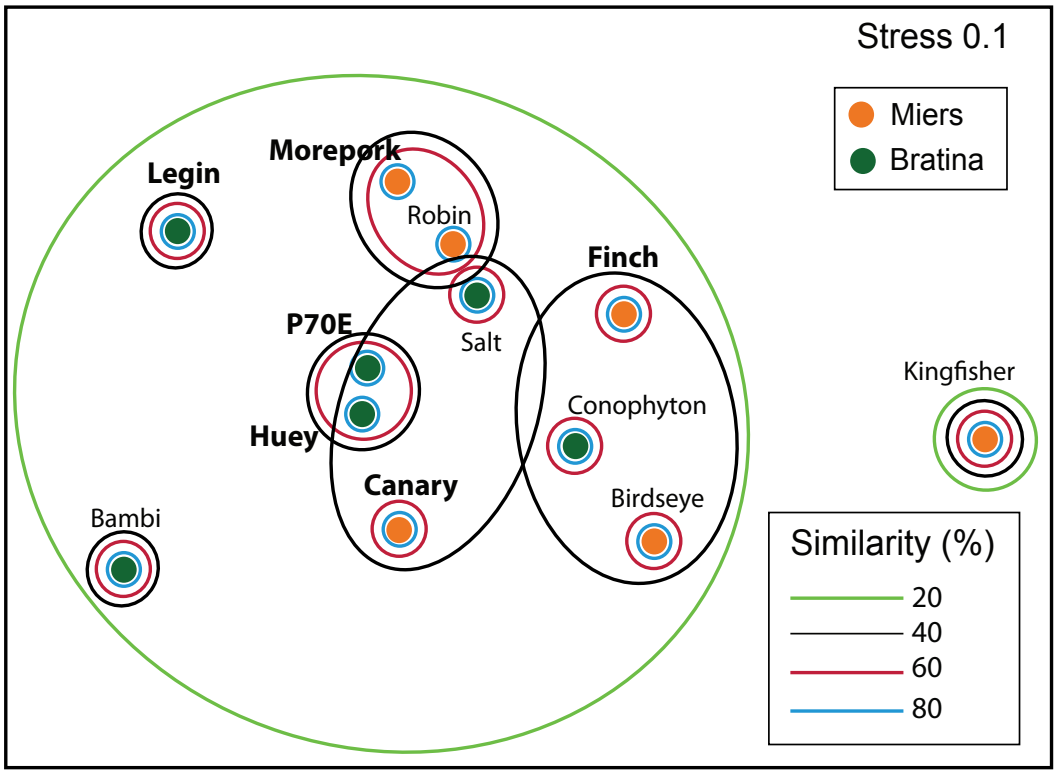

Figure 5. 3 Non-metric multidimensional scaling (NMDS ordinations of bacterial ARISA community compositions based on Bray Curtis distances (Stress=0.1). Samples collected in January 2013 from Bratina Island (6 samples) and the Miers Valley (6 samples). Larger bold names represent samples selected for high-throughput sequencing.

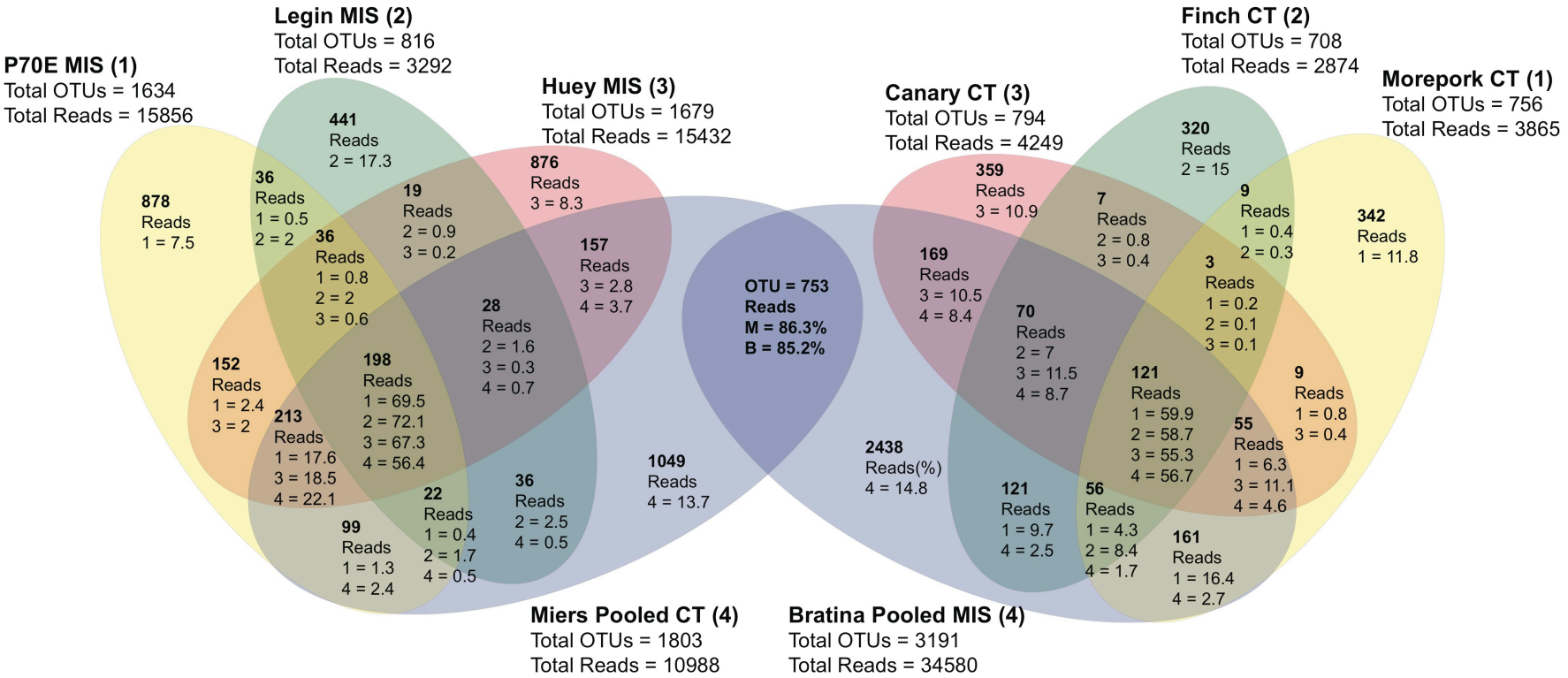

Figure 5. 4 Summary of 454 sequencing data (distance = 0.05) representing shared OTUs and reads from individual ponds and pooled data between sample locations.

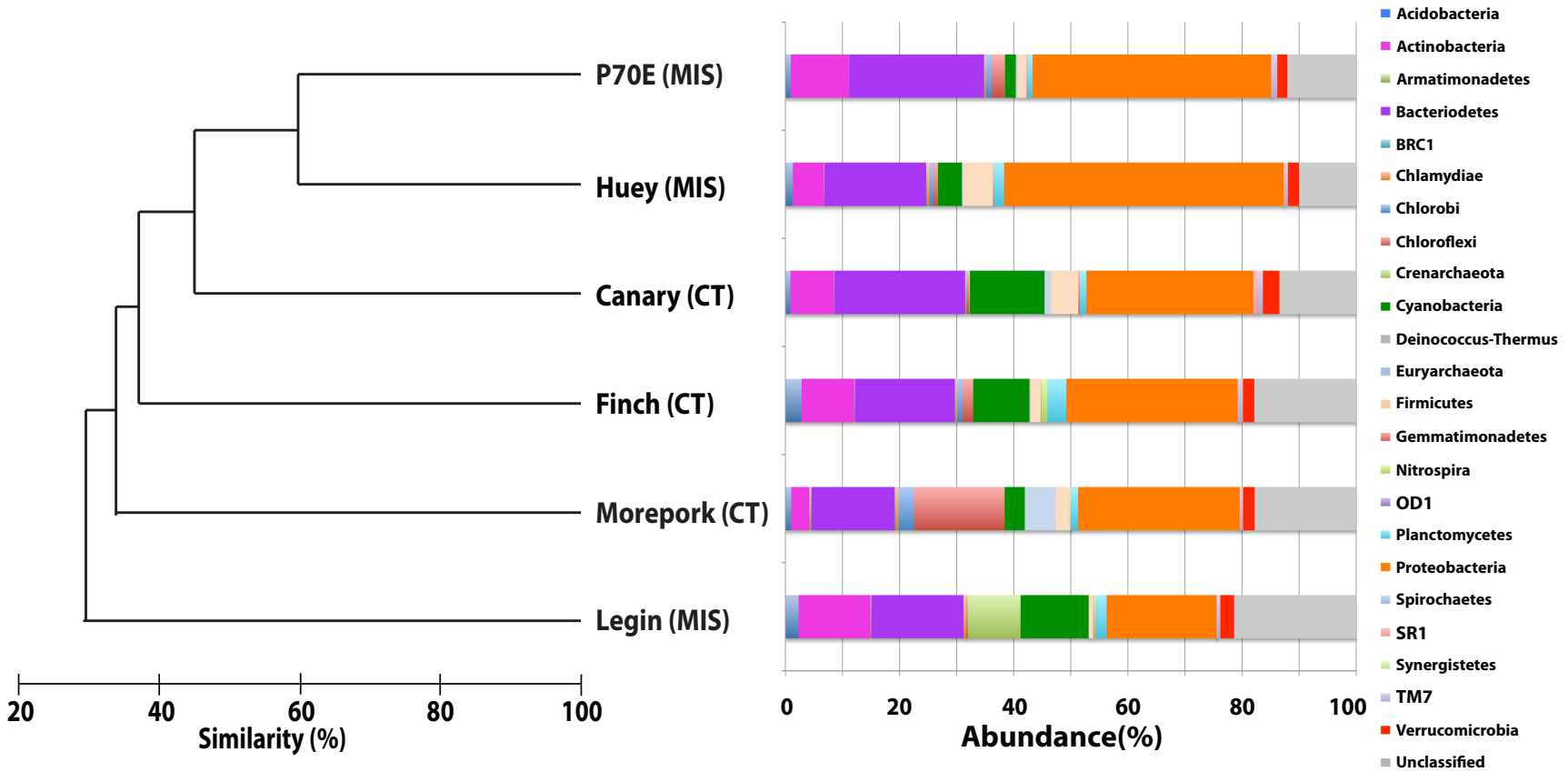

Figure 5. 5 Bray-Curtis tree calculated from total $OTU_{0.05}$ compositions with no transformation to visualize total relative spatial/temporal similarities between water columns (left). Phylum-level distribution of bacterial 16S rRNA $OTUs_{0.05}$ assigned using the Ribosomal Database Project (RDP) Release 10, Update 15 Classifier, assignment confidence threshold >80% (right).

Table 5. 2 Heatmap of abundance of the 15 most abundant OTUs based on sequencing analysis. The color intensity represents a larger fraction of total sequences. Data representative of total reads represented by the top 15 OTUs in each sample is in Purple, location based totals of each OTU (Green) and individual sample (Red high, Blue low).

	Miers Valley			Bratina Island				Pooled	
OTU	Morepork	Canary	Finch	Huey	Legin	P70E	Total	Pooled Bratina	Pooled Miers
1	4.58%	14.64%	4.24%	14.98%	3.22%	8.70%	7.19%	6.72%	7.82%
2	5.77%	0.49%	0.07%	4.70%	0.12%	9.38%	2.93%	3.55%	2.11%
3	0.62%	11.41%	2.33%	2.02%	2.92%	2.05%	3.05%	1.75%	4.79%
4	1.11%	0.14%	1.15%	1.71%	0.73%	5.81%	1.52%	2.06%	0.80%
5	2.10%	2.40%	4.63%	2.46%	2.16%	3.32%	2.44%	1.98%	3.04%
6	3.10%	1.93%	2.47%	3.39%	0.79%	2.85%	2.08%	1.76%	2.50%
7	0.93%	1.01%	1.57%	3.91%	0.70%	2.24%	1.48%	1.71%	1.17%
8	1.37%	0.33%	0.24%	3.66%	0.24%	1.81%	1.09%	1.43%	0.65%
9	0.88%	0.75%	1.91%	1.54%	0.49%	2.80%	1.20%	1.21%	1.18%
10	0.88%	1.60%	3.27%	1.42%	2.25%	1.78%	1.60%	1.36%	1.92%
11	0.67%	1.84%	2.37%	1.46%	1.18%	1.98%	1.36%	1.16%	1.62%
12	0.00%	4.14%	0.14%	0.41%	1.37%	0.32%	0.91%	0.52%	1.43%
13	0.21%	0.28%	0.03%	1.89%	1.37%	0.88%	0.67%	1.04%	0.17%
14	11.25%	0.12%	0.24%	0.15%	0.00%	0.89%	1.81%	0.26%	3.87%
15	0.39%	5.70%	0.00%	0.25%	2.00%	0.16%	1.21%	0.60%	2.03%
Total	33.87%	46.79%	24.67%	43.97%	19.53%	44.97%	30.54%	27.12%	35.11%

5.4.3 Geochemical drivers of community structure

BEST analysis was performed to examine how geochemistry affects benthic community structure, measured using pyrosequencing, between ponds. Potassium, sodium and cobalt were the strongest explanatory variables to total community structure (Spearman's P value of 0.632, 0.436 and 0.428 respectively) and to cosmopolitan OTUs (those present in at least one pond at each location) (Spearman's P value of 0.607, 0.368 and 0.392). BEST analysis of unique OTUs (those present in only one of the six ponds) identified aluminium, uranium and manganese as the strongest explanatory variables to community structure (Spearman's P value of 0.806, 0.435 and 0.415 respectively). A Tukey Honest significance difference test between locations confirmed a significantly higher concentration of aluminium in the MIS ponds (P=0.006).

5.5 Discussion

The meltwater ponds included in this study represent the high degree of geochemical heterogeneity typically found in Antarctic meltwater ponds (Hawes *et al.*, 2013; Healy *et al.*, 2006; Lyons *et al.*, 2012; Wait *et al.*, 2006). The study sites include two distinct underlying substrate types, which are known to influence cyanobacterial mat structure (de los Rios *et al.*, 2004). The sediments from the McMurdo Ice Shelf (MIS) at Bratina Island are marine derived while the Coastal Terrestrial (CT) ponds in the Miers Valley are derived from glacial weathering of bedrock (Cary *et al.*, 2010; Kellogg & Kellogg, 1987; Kellogg & Kellogg, 1988; McLeod *et al.*, 2009). Similar geochemical pond profiles were identified between locations, with little coherence between ponds from the same location. The geochemical similarity between locations is surprising as many meltwater ponds reside in closed basins, their geochemical properties reflective of their immediate environment and often exhibiting marked variations in elemental composition within (Hawes *et al.*, 2013; Matsumoto *et al.*, 1992) and particularly between sites (Healy *et al.*, 2006).

Community DNA profiling of benthic bacterial communities using ARISA revealed little clustering based on location. High throughput pyrosequencing of representative ponds identified a diverse community, yet more

than 85% of total sequences from all samples were shared between locations, suggesting movement and redistribution of microorganisms at a regional scale. Given the close proximity (<40 km) between Bratina Island and the Miers Valley, aeolian transportation of microorganisms between locations likely takes place. This has been demonstrated for invertebrates and *Cyanobacteria* throughout the McMurdo Dry Valleys (Nkem *et al.*, 2006; Wood *et al.*, 2008) and suggested for Bacteria across large (>800 km) distances along the Transantarctic Mountains (Sokol *et al.*, 2013). Katabatic winds in the Miers Valley move down from the Polar Plateau onto the ice shelf (Bottos *et al.*, 2014), where storms transport large volumes of dust, mostly from a southerly direction (Atkins & Dunbar, 2009; Dunbar *et al.*, 2009). Bratina Island is located northeast of Miers Valley (Figure 5. 1) and receives its strongest winds from the southeast or southwest (Hawes *et al.*, 2013), providing a mechanism for transportation.

Variations in community structure between pond benthic zones were identified, even between Huey and P70E, the most biologically similar ponds in this study. Community variation was manifested primarily in the abundance of several key phyla and OTUs, as has been seen previously (Bowman *et al.*, 2000), and was likely the result of the highly variable geochemistry of each pond (Jungblut *et al.*, 2005). The small number of pond specific sequences identified in this study represented a large localized diversity. Although of low abundance at the sampling time point, a number of these taxa may have been more significant throughout the year due to the extreme seasonality experienced by these ponds (Hawes *et al.*, 1999; Laybourn-Parry, 2002).

The depth of sequencing coverage in this study resulted in the detection of 23 phyla, a far greater diversity than previously reported in comparable ecosystems (Bowman *et al.*, 2000; Peeters *et al.*, 2012; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002). Although there was a large core of shared sequences, inter-site variation in community structure (abundance of individual phyla and OTUs) was identified, as seen between *Crenarchaeota* and *Euryarchaeota*. *Crenarchaeota*, a highly abundant phylum in marine environments (Karner *et al.*, 2001) was present at a 10-fold higher abundance at Bratina Island, consistent with previous findings (Sjoling & Cowan, 2003). *Euryarchaeota* abundance was 200-fold higher in the Miers Valley, and was

previously the only *Archaea* identified in a study of six Antarctic Dry Valley lakes (Vestfold Hills, Princess Elizabeth Land) (Bowman *et al.*, 2000). This shows that although the bulk microbial community is shared between locations there are microorganims closely linked to a specific biome.

Several high abundance phyla were ubiquitous in all pond sediments in this and previous studies, the most abundant being *Proteobacteria* (Bowman *et al.*, 2000; Peeters *et al.*, 2012; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002). Similar to past studies, *Alpha* and *Gammaproteobacteria* were present in all ponds (Bowman *et al.*, 2000; Peeters *et al.*, 2012; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002), however *Betaproteobacteria,* most abundant in this study (Bowman *et al.,* 2000; Sjoling & Cowan, 2003), and *Deltaproteobacteria* (Vantrappen *et al.,* 2002; Peeters *et al.,* 2012) were not detected in all studies. *Bacteroidetes* was the only other phylum present across all studies with *Actinobacteria, Firmicutes, Planctomycetes, Verrucomicrobia, Acidobacteria* and *Chloroflexi* intermittently identified at low abundance in the other studies, consistent with our findings (Bowman *et al.*, 2000; Peeters *et al.*, 2012; Sjoling & Cowan, 2003; Tang *et al.*, 2013; VanTrappen *et al.*, 2002). These results indicate that previous investigations had likely detected the most abundant portion of the ecosystem, however, given the high number of previously undetected phyla, a large part of the diversity was missed.

Surprisingly, *Cyanobacteria* were poorly represented in the pyrosequencing data, a result of low PCR primer matching (<1%) to known cyanobacterial sequences (https://rdp.cme.msu.edu/probematch/search.jsp). However, as this bias would exert an equal effect between samples, cyanobacterial sequence abundances are comparable within this study. Variable abundances of cyanobacterial OTUs were identified between locations and within ponds. This was likely related to selection pressures caused by source material (for example grain size) (de los Rios *et al.*, 2004) and conductivity (variable within ponds) (Jungblut *et al.*, 2010). The continual layering of aeolian dust causes surface cyanobacterial mats to be covered and thus form sediments (Squyres *et al.*, 1991), therefore difference in cyanobacterial mat communities is likely to have a significant impact on the formation of the underlying sediment

communities. Cyanobacterial mats, with a complicated associated bacterial community (Barrett *et al.*, 2006; Moorhead *et al.*, 2003; Wood *et al.*, 2008), are a possible vector for microbial transportation between the Miers Valley and Bratina Island (Vincent, 2000). These mats have been shown to be capable of distribution throughout the McMurdo Dry Valleys and remain viable (de los Rios *et al.*, 2004), although a recent study suggests that distribution distances for *Cyanobacteria* in Antarctica may be limited to <3 km (Sokol *et al.*, 2013).

BEST analysis identified potassium as most significantly correlated with differences in the cosmopolitan community between ponds. Potassium is an essential intracellular cation in most bacteria (Epstein, 2003; Malek *et al.*, 2012) that can cause osmotic stress in freshwater environments (Wetzel & Likens, 2000). The variable potassium concentration in ponds is likely reflective of local geology (Campbell & Claridge, 1977). A BEST analysis on location unique sequences provided a distinct set of correlated variables, the strongest of which, more significant to structuring the unique community than potassium to the cosmopolitan community, was aluminium. Although poorly soluble in alkaline water, aluminium is an abundant metal in the Earth's crust with recognized anti-microbial effects (Pina & Cervantes, 1996). Higher aluminium concentrations in all MIS ponds is likely a contributing factor to community variation between locations.

Although habitat type is known to affect community structure of Antarctic microbial mats (Peeters *et al.*, 2012), the communities of the coastal terrestrial (Miers Valley) and MIS (Bratina Island) ponds appear to share the majority of the dominant OTUs, likely due to close geographic proximity and favorable wind direction between locations. Variation in communities between ponds and locations was related to differences in major OTU abundances and the presence of pond specific diversity likely driven by local geochemistry. These factors should be taken into account when investigating regional community variation in future studies.

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5.8 Supplementary Information

Supplementary Table 5. 1 Complete geochemistry dataset including ICPMS and nutrient analysis. NO_2 and NO_3 were below detection limit for all samples so are not presented in the table.

Pond	Year/Location	GPS Coordinates		Temp(Celcius)	DO (ppm)	Cond(mS/cm)	pH	Phosphate (ppm)	NH4 (ppm)	B 10(ppb)	Na 23(ppb)
P70E	2013 Bratina	S 78.01580	E 165.55165	10.1	11.6	7.7	8.8	0.01049	0	11018.15846	771854.5
Huey	2013 Bratina	S 78.01419	E 165.55576	1.4	13.1	4.4	9.5	0.01104	0.02885	11462.9	850613.1871
Legin	2013 Bratina	S 78.01624	E 165.54903	4.5	13.2	2.2	9.7	0.02212	0	10877.5108	468474.65
Finch	2013 Miers	S 78 07.748	E 164 11.714	5.5	13.4	1.7	9.8	0.01204	0	10999.45815	317448.95
Canary	2013 Miers	S 78 07.433	E 164 11.453	6.5	14.1	4.2	9.6	0.145	0	10855.16129	940154.2
Morepork	2013 Miers	S 78 07.343	E164 12.061	7.2	12.3	4.1	9.1	0.01098	0	11193.48039	630021.7
					ppb						
	Mg 24	**Al 27**	**Si 28**	**S 34**	**K 39**	**Ca 43**	**V 51**	**Cr 52**	**Fe 54**	**Mn 55**	**Co 59**
P70E	165225.75	177.85	30378.3	134618.129	37649.45	186094	2.25	3.15	177.606469	13.45	0.1
Huey	115385.6872	175.5	30351.3	81109.17799	34075.15	31250.83813	10.1001381	1.346685083	0	14.24378453	0.1
Legin	38805.2	166.75	30904.95	78411.76927	21580.9	34647.4	9.05	0.35	194.8339582	7.35	0
Finch	19886.75	89.4	29821.65	42482.4542	23650.4	21833.95	6.85	0	10.43057051	2.45	0.5
Canary	28365.55	126.65	31199.35	272842.7581	35445.85	25246.95	17.55	0	217.3105857	2.75	0.2
Morepork	84642.6	116.65	33450	97953.77389	41060.35	48144.85	8.2	4.3	339.7551889	9.4	0.15
					ppb						
	Ni 60	**Cu 63**	**Zn 68**	**As 75**	**Se 82**	**Sr 88**	**Cd 111**	**Ba 137**	**Hg 202**	**Pb 207**	**U 238**
P70E	0.85	100.1	114.55	2.2	15.7	1972.821199	0.81905782	8.7	0.9	0.6	1.059957173
Huey	0	101.571133	697.73826	1.019457205	0	338.9502762	0.2	9.5	0	1.85	0.75
Legin	0	79.6	148	1.1	3.25	456.3174946	0.87473002	4.4	1.35	1	0.583153348
Finch	0	92.85	115.45	0.15	7.65	242.0439914	0.28969957	145.2	0.15	0.8	1.06223176
Canary	0	94.7	286.45	1.05	0.6	383.1774194	0.48387097	7.9	1.5	48.75	1.403225806
Morepork	0	88.4	213.55	4.95	10.15	760.0490196	0	7.8	3.75	0.75	1.617647059

Supplementary Table 5. 2 Summary of pyrosequencing statistics of individual sediment samples.

Sample	Location	Unique seq	Reads	Singelton seq	Coverage
Canary	Miers	794	4249	482	88.66%
Finch	Miers	708	2874	407	85.84%
Huey	Bratina	1679	15432	979	93.66%
Legin	Bratina	816	3292	510	84.51%
Morepork	Miers	756	3865	454	88.25%
P70E	Bratina	1634	15856	929	94.14%
Total		4703	45568	2792	93.87%

Chapter 6 – Integrated Analyses and Conclusions

6.1 Data synthesis

Exposed to extreme cold temperatures, low precipitation and light regimes, including 3 months annually of total 24 hr sunlight and darkness, Antarctic environments are considered some of the most extreme on Earth (Cary *et al.*, 2010; Wynn-Williams, 1996). Regardless of the inhospitable environment, perennial terrestrial and ice-bound meltwater ponds are ubiquitously present throughout the continent, residing in landscape depressions and replenished during the austral summer from snow and ice melt (De Mora *et al.*, 1994; Howard-Williams & Hawes, 2007; Howard-Williams & Hawes, 2005; Vincent & James, 1996). Their productivity, abundance, trophic simplicity and hypervariable inter-pond geochemistry provide tractable ecosystems to investigate biogeography. In addition, their responsiveness and vulnerability to environmental changes and lack of anthropogenic interference makes them valuable scientific resources for climate change modeling that require protection and management (Hawkins, 2001; Hillebrand, 2004; Howard-Williams *et al.*, 1990; Maxwell & Barrie, 1989; Nielsen & Wall, 2013; Vincent & James, 1996; Vincent, 2000; Walther *et al.*, 2002).

With few exceptions (Bonilla *et al.*, 2005), studies of Antarctic aquatic ecosystems have typically focused on either the benthic community (Bowman *et al.*, 2000; Hawes *et al.*, 2001; Moorhead *et al.*, 1997; Sabbe *et al.*, 2004), the surface water (Hawes *et al.*, 2014; James *et al.*, 1995) or the stratified water column (Hawes *et al.*, 2011a; Healy *et al.*, 2006; Villaescusa *et al.*, 2013; Wait *et al.*, 2006). The work presented in this thesis provides insight into each of these zones using recently introduced high-resolution methodologies. These data have provided the first opportunity to compare the biological communities between zones, allowing a greater understanding of the melt-water pond microbial community as a whole. As previous work has focussed on the cyanobacterial component of these systems, these studies were the first comprehensive, coordinated investigation of the non-cyanobacterial bacterial community of Bratina Island and Miers Valley ponds and the first to describe their relationship over time, between locations and with local geochemistry. The studies presented

in this thesis have identified significant microbial community heterogeneity between and within the McMurdo meltwater ponds, the temporal heterogeneity within ponds, and the importance of geochemistry, particularly conductivity, in structuring the biological community.

Bacterial ARISA and pyrosequenced samples presented across all studies in this thesis were combined (Figure 6.1, 6.2 and 6.3) and sequencing statistics are presented in Table 6. 1. Consistent across all zones was the dominance of *Bacteroidetes, Proteobacteria* and *Actinobacteria*, although the specific OTUs from each phylum differed between samples (Table 6. 2). A large proportion of the microbial community in each study was represented by a small number of cosmopolitan OTUs, the variable abundances of which drive the most significant differences in community structure. While many OTUs were identified in the different zones, no single OTU was identified in every sample in this study. When the Miers Valley samples were excluded, two OTUs representing approximately 22% of total sequences were shared between all Bratina Island water column and sediment samples. When sediment samples were also excluded, only one additional OTU was shared between all Bratina Island water column samples. Dominance of single OTUs was more prevalent in water column samples (up to 98.1%), compared to sediment samples (up to 20.2%). Diversity was highly variable between samples, but was statistically higher in sediment samples based on Tukey's honest significance test ($P< 0.001$) for Bratina 2012 and 2013 and Miers 2013, and ($P=0.02$) for extended season samples. Conductivity was consistently identified as a dominant driver of community structure, particularly across strong intra-pond conductivity gradients; however trace elements and nutrients had significant effects within pond zones. These dominant components were also present at different time points with highly variable abundances. This suggests a relatively consistent surface, stratified and sediment biological community regardless of extreme annual physical changes.

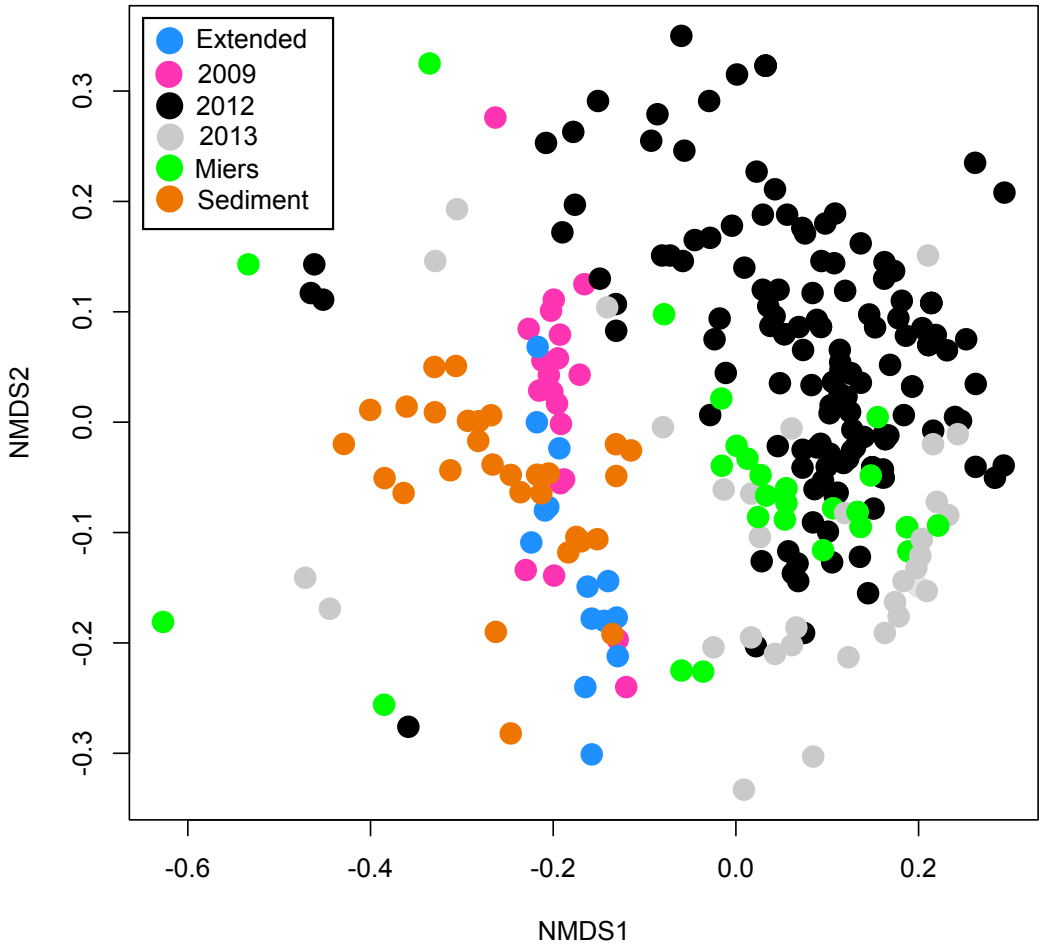


Figure 6. 1 NMDS ordination (stress=0.22) of bacterial ARISA community compositions with a presence/absence transformation based on Bray Curtis distances. Samples as described in Chapters 2-5, additional samples include 14 Extended Season samples collected between February and April in 2008 during the winter freeze. Conductivity was the only geochemical parameter measured across all samples; a BEST analysis was conducted with sediment samples removed to compare conductivity to water column community structure (Spearman's rank P of 0.274).

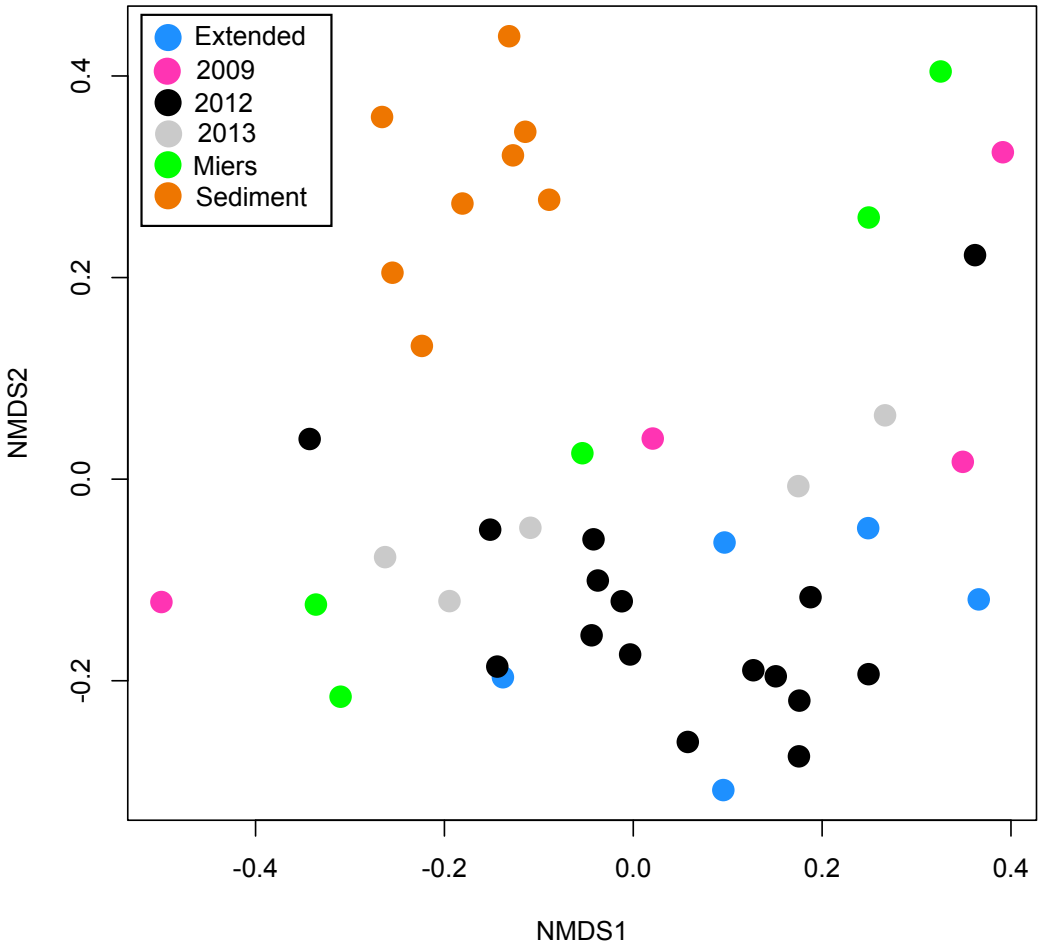


Figure 6. 2 NMDS ordination of community structure based on Bray Curtis distances (stress 0.18) calculated from total pyrosequencing $OTU_{0.05}$ compositions with no transformation. Total OTUs =8295, Total Reads=349260 (Table 1). BEST analysis conducted on conductivity as only variable measured across all water column samples resulted in a moderate correlation score (Spearman's rank P of 0.571).

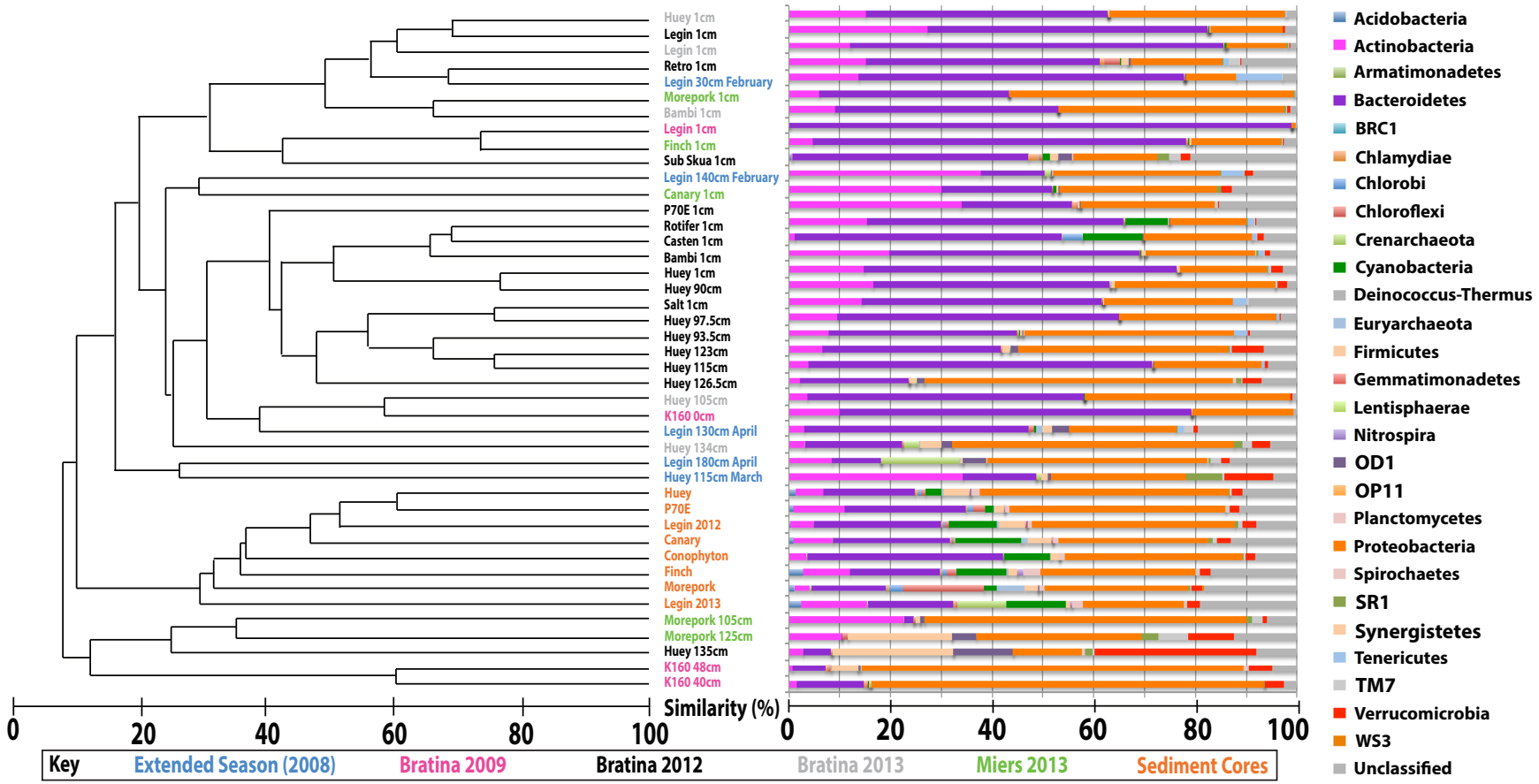
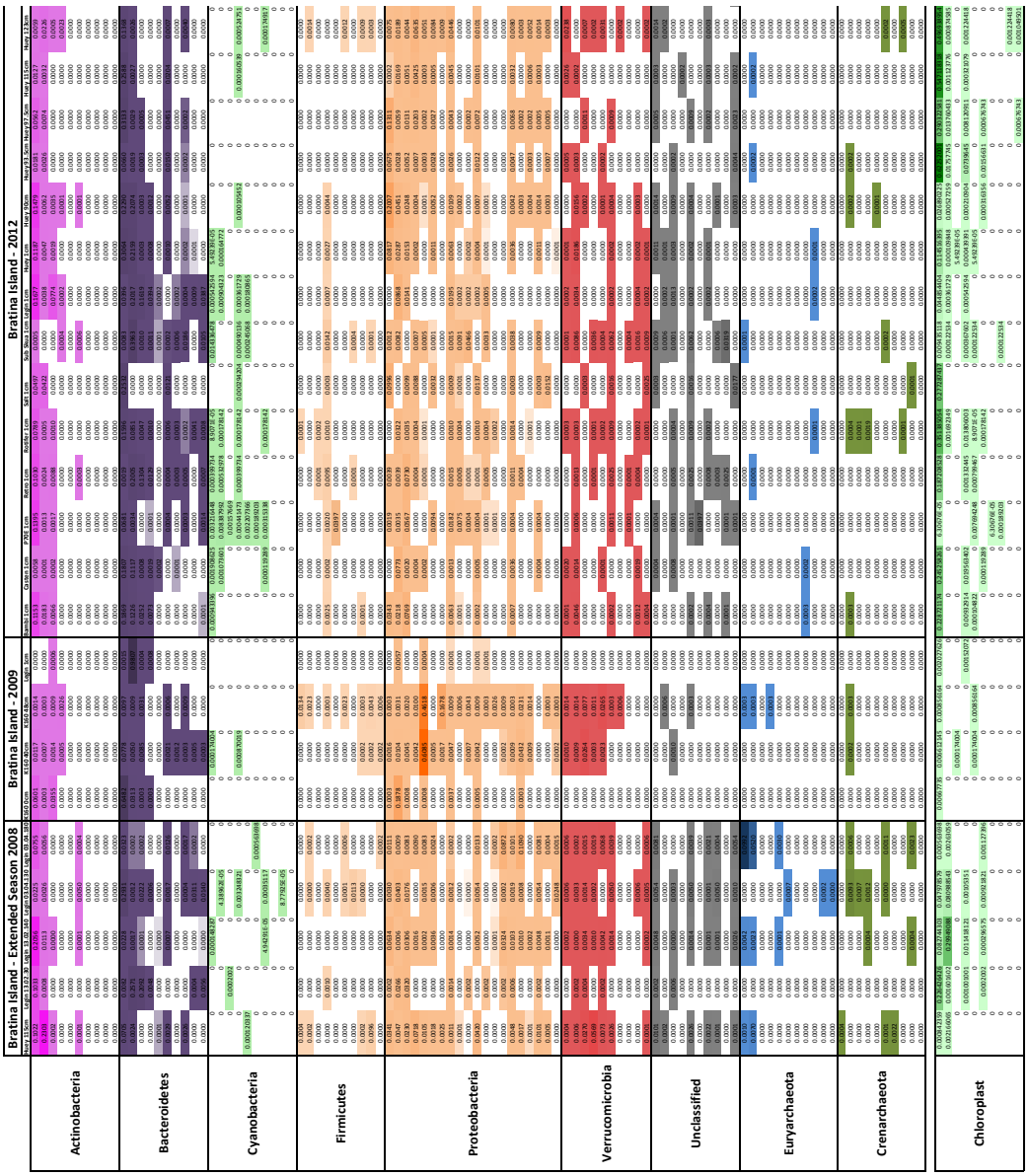


Figure 6. 3 A Bray-Curtis similarity tree calculated from total $OTU_{0.05}$ compositions with no transformation (Left). Phylum-level abundance of $OTUs_{0.05}$ assigned using the RDP Release 10, Update 15 Classifier, assignment confidence threshold >80% (Right).

Table 6. 1 Pyrosequencing summary statistics including total read number (after quality control), unique sequences (total OTUs identified in that sample), singleton sequences (unique sequences present as a single copy) and Goods coverage statistic.

	Sample	Unique seq	Reads	Singelton seq	Coverage
Bratina Island - 2008	Egg 115cm	276	8310	138	98.34%
	Legin 13.02.30	149	4995	100	98.00%
	Legin 13.02.140	378	20231	219	98.92%
	Legin 03.04.130	1094	22781	590	97.41%
	Legin 03.04.180	380	5322	203	96.19%
	K160 0cm	64	3744	43	98.85%
Bratina Island - 2009	K160 40cm	209	5747	109	98.10%
	K160 48cm	314	3504	177	94.95%
	Legin 1cm	35	7891	17	99.78%
Bratina Island - 2012	Bambi 1cm	266	9540	159	98.33%
	Casten 1cm	238	8383	130	98.45%
	P70E 1cm	251	9353	178	98.10%
	Retro 1cm	373	7505	215	97.14%
	Rotifer 1cm	337	11227	191	98.30%
	Salt 1cm	157	6798	94	98.62%
	Sub Skua 1cm	824	8161	447	94.52%
	Legin 1cm	233	5529	169	96.94%
	Huey 1cm	454	18207	300	98.35%
	Huey 90cm	300	9483	189	98.01%
	Huey 93.5cm	174	5746	106	98.16%
	Huey 97.5cm	132	4433	71	98.40%
	Huey 115cm	139	6229	83	98.67%
	Huey 123cm	269	5717	140	97.55%
	Huey 126.5cm	331	9397	157	98.33%
	Huey 135cm	524	10040	274	97.27%
Bratina Island - 2013	Huey 1cm	147	8746	80	99.09%
	Huey 105cm	112	9986	47	99.53%
	Huey 134cm	417	17050	181	98.94%
	Bambi 1cm	76	3079	45	98.54%
	Legin 1cm	76	2791	42	98.50%
Miers Valley 2013	Morepork 1cm	65	8231	39	99.53%
	Morepork 105cm	275	11115	107	99.04%
	Morepork 125cm	403	8685	169	98.05%
	Canary 1cm	104	2564	45	98.24%
	Finch 1cm	99	2695	63	97.66%
Sediment 2013	P70E	1646	15856	933	94.12%
	Conophyton	594	4322	364	91.58%
	Huey	1691	15432	992	93.57%
	Legin 2012	673	6155	317	94.85%
	Legin 2013	816	3292	511	84.48%
	Morepork	764	3865	458	88.15%
	Finch	716	2874	415	85.56%
	Canary	804	4249	493	88.40%
Total		8295	349260	4760	98.64%

Table 6. 2 Heatmap where color intensity represents a larger fraction of total sequences. Data representative of the most abundant sequences from each phylum (color based on Figure 6.3 phyla), the far right column represents total abundance across all samples (Blue).



		Bratina Island - 2013					Miers Valley 2013					Sediment 2013									
Huey 126.5cm	Huey 135cm	Huey 1cm	Huey 105cm	Huey 134cm	Bambi 1cm	Legin 1cm	Morepork 1cm	Morepork 105cm	Morepork 125cm	Canary 1cm	Finch 1cm	P70E	Conophyton	Huey	Legin 2012	Legin 2013	Morepork	Finch	Canary	Total	Class
0.0087	0.0059	0.1358	0.0217	0.0049	0.0721	0.1168	0.0581	0.0188	0.0013	0.2734	0.0353	0.0000	0.0000	0.0003	0.0002	0.0003	0.0000	0.0000	0.0000	0.05677146	Actinobacteria
0.0048	0.0164	0.0034	0.0142	0.0216	0.0039	0.0011	0.0002	0.1760	0.0724	0.0055	0.0000	0.0003	0.0002	0.0004	0.0016	0.0003	0.0000	0.0007	0.0035	0.01864227	Actinobacteria
0.0002	0.0003	0.0056	0.0003	0.0021	0.0065	0.0011	0.0007	0.0238	0.0075	0.0043	0.0111	0.0011	0.0030	0.0027	0.0042	0.0018	0.0013	0.0031	0.0019	0.00408005	Actinobacteria
0.0000	0.0014	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0037	0.0165	0.0000	0.0000	0.0194	0.0072	0.0147	0.0078	0.0118	0.0067	0.0237	0.0184	0.00306648	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0155	0.0005	0.0068	0.0039	0.0103	0.0005	0.0059	0.0078	0.00132566	Actinobacteria
0.0000	0.0004	0.0003	0.0000	0.0036	0.0000	0.0000	0.0000	0.0018	0.0058	0.0000	0.0000	0.0005	0.0000	0.0006	0.0000	0.0000	0.0028	0.0000	0.0000	0.00083892	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0107	0.0000	0.0027	0.0052	0.0015	0.0028	0.0000	0.0040	0.00079024	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0064	0.0000	0.0025	0.0032	0.0158	0.0008	0.0063	0.0038	0.0007158	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0056	0.0000	0.0024	0.0021	0.0103	0.0000	0.0042	0.0026	0.00056119	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0065	0.0000	0.0021	0.0019	0.0067	0.0003	0.0038	0.0019	0.00054401	Actinobacteria
0.0720	0.0114	0.0883	0.4960	0.1872	0.0231	0.0025	0.0000	0.0051	0.0002	0.0807	0.0004	0.0287	0.0317	0.0159	0.0193	0.0058	0.0088	0.0191	0.0078	0.11930081	Sphingobacteria
0.0024	0.0028	0.1941	0.0005	0.0002	0.3462	0.4787	0.1983	0.0061	0.0006	0.0741	0.7299	0.0204	0.1242	0.0202	0.0408	0.0295	0.0062	0.0230	0.1144	0.09812747	Flavobacteria
0.0000	0.0001	0.1353	0.0005	0.0003	0.0192	0.1394	0.1587	0.0032	0.0001	0.0012	0.0004	0.0213	0.1136	0.0058	0.0018	0.0003	0.0052	0.0097	0.0005	0.02227853	Flavobacteria
0.0000	0.0002	0.0146	0.0001	0.0000	0.0120	0.0910	0.0158	0.0005	0.0001	0.0187	0.0000	0.0000	0.0000	0.0003	0.0002	0.0009	0.0000	0.0003	0.0007	0.00317815	Sphingobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0201	0.0067	0.0150	0.0370	0.0279	0.0088	0.0334	0.0165	0.0031667	Sphingobacteria
0.0024	0.0006	0.0011	0.0183	0.0002	0.0000	0.0000	0.0001	0.0022	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00284029	Sphingobacteria
0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0351	0.0211	0.0118	0.0075	0.0073	0.0060	0.0017	0.0007	0.0027229	Flavobacteria
0.0087	0.0019	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0085	0.0000	0.0201	0.0047	0.0000	0.0010	0.0003	0.0125	0.00249671	Flavobacteria
0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0043	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00224761	Flavobacteria
0.0000	0.0000	0.0024	0.0000	0.0001	0.0000	0.0043	0.0000	0.0001	0.0000	0.0016	0.0000	0.0006	0.0000	0.0001	0.0002	0.0003	0.0000	0.0000	0.0000	0.00209013	Flavobacteria
0	0.0001992	0	0	0	0	0	0	0	0	0.005460218	0.001855288	0.000320753	0.08537714	0.003888025	0.000487409	0.013669502	0	0.001391788	0.041186161	0.00253393	Cyanobacteria
0	9.9602E-05	0	0	0.000117302	0	0	0	0	0	0.000390016	0	0	0.000231374	0.019051322	0.030869212	0.013669502	0.002069858	0.000347947	0.002824194	0.0020386	Cyanobacteria
0	0	0	0	0	0	0	0	0	0.000115141	0	0	0	0.000462749	0.002527216	0.031844029	0.020048603	0.003880983	0	0.056954578	0.0016807	Cyanobacteria
0.000212834	0	0	0.001001402	0	0	0	0	0.000179937	0	0.000780031	0	0	0	0.003304821	0.000974817	0.020959903	0.01759379	0.084551148	0.004236291	0.00164634	Cyanobacteria
0	0	0	0	0	0	0	0	0	0	0.000390016	0	0	0.000231374	0.001231208	0.000812348	0.035236938	0	0.008698678	0.016709814	0.00079311	Cyanobacteria
0	0	0	0	0	0	0	0	0.000179937	0	0	0	0	0.001156872	0.000324002	0.021445979	0.004252734	0.000776197	0.000695894	0.000941398	0.00049533	Cyanobacteria
0	0	0	0	0	0.000324781	0	0	0	0	0	0	0	0.000694123	0.001360809	0.001137287	0.002126367	0.000258732	0.001739736	0.003530242	0.00022333	Cyanobacteria
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005523964	0	0	0	0	9.7349E-05	Cyanobacteria
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.000517464	0	0	1.1453E-05	Cyanobacteria
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.000706048	8.5896E-06	Cyanobacteria
0.0000	0.1472	0.0000	0.0000	0.0090	0.0000	0.0000	0.0000	0.0007	0.0796	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00682013	Clostridia
0.0000	0.0035	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0013	0.0508	0.0000	0.0000	0.0020	0.0007	0.0019	0.0000	0.0000	0.0008	0.0000	0.0005	0.00242513	Clostridia
0.0002	0.0481	0.0000	0.0000	0.0009	0.0000	0.0000	0.0000	0.0000	0.0037	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00153181	Clostridia
0.0000	0.0000	0.0005	0.0000	0.0003	0.0000	0.0011	0.0000	0.0001	0.0000	0.0008	0.0007	0.0015	0.0021	0.0005	0.0000	0.0003	0.0005	0.0007	0.0000	0.0014316	Clostridia
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00106225	Clostridia
0.0002	0.0033	0.0000	0.0000	0.0043	0.0000	0.0000	0.0000	0.0006	0.0150	0.0000	0.0000	0.0007	0.0000	0.0032	0.0013	0.0000	0.0005	0.0000	0.0031	0.00099926	Clostridia
0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00076161	Clostridia
0.0000	0.0031	0.0000	0.0000	0.0046	0.0000	0.0000	0.0000	0.0034	0.0120	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00075302	Clostridia
0.0061	0.0055	0.0000	0.0004	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00064422	Bacilli
0.0000	0.0050	0.0001	0.0000	0.0012	0.0000	0.0000	0.0000	0.0001	0.0169	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00063849	Clostridia
0.0023	0.0190	0.0484	0.0265	0.4619	0.0120	0.0029	0.0081	0.0561	0.0146	0.0160	0.1529	0.0079	0.0067	0.0056	0.0054	0.0033	0.0013	0.0153	0.0024	0.05040944	Alphaproteobacteria
0.0079	0.0057	0.1204	0.0554	0.0089	0.0270	0.0620	0.0009	0.0022	0.0015	0.1010	0.0019	0.0858	0.0877	0.1464	0.2015	0.0319	0.0442	0.0418	0.1457	0.03912844	Betaproteobacteria
0.0028	0.0096	0.1413	0.0152	0.0023	0.3608	0.0305	0.5484	0.0097	0.0021	0.0000	0.0000	0.0002	0.0000	0.0005	0.0000	0.0000	0.0008	0.0000	0.0000	0.03298975	Betaproteobacteria
0.3272	0.0136	0.0010	0.1824	0.0001	0.0000	0.0000	0.0000	0.0014	0.0001	0.0000	0.0000	0.0033	0.0012	0.0029	0.0003	0.0009	0.0010	0.0073	0.0007	0.01929508	Gammaproteobacteria
0.0293	0.0038	0.0006	0.0120	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.01654069	Gammaproteobacteria
0.0011	0.0036	0.0000	0.0093	0.0083	0.0003	0.0000	0.0000	0.3428	0.0123	0.0023	0.0000	0.0022	0.0019	0.0011	0.0000	0.0000	0.0000	0.0017	0.0000	0.01329955	Alphaproteobacteria
0.0004	0.0043	0.0000	0.0000	0.0317	0.0000	0.0000	0.0000	0.1075	0.1976	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.01180782	Alphaproteobacteria
0.0020	0.0217	0.0032	0.0093	0.0033	0.0016	0.0036	0.0000	0.0510	0.0102	0.0998	0.0015	0.0226	0.0160	0.0398	0.0294	0.0070	0.0093	0.0153	0.0101	0.01075417	Alphaproteobacteria
0.0000	0.0001	0.0011	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0998	0.0032	0.0488	0.0023	0.0012	0.0577	0.0007	0.0049	0.00797687	Betaproteobacteria
0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0001	0.0008	0.0000	0.0324	0.0687	0.0241	0.0110	0.0213	0.0210	0.0459	0.0219	0.00584951	Gammaproteobacteria
0.0062	0.0007	0.0008	0.0556	0.0004	0.0013	0.0000	0.0000	0.0014	0.0000	0.0367	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00510794	Gammaproteobacteria
0.0000	0.0000	0.0010	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0003	0.0035	0.0007	0.0282	0.0053	0.0336	0.0255	0.0079	0.0310	0.0247	0.0191	0.00433202	Betaproteobacteria
0.0000	0.0024	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0012	0.0000	0.0000	0.0185	0.0134	0.0372	0.0260	0.0024	0.0137	0.0024	0.0033	0.00349596	Deltaproteobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00323541	Gammaproteobacteria
0.0026	0.0010	0.0031	0.0102	0.0023	0.0006	0.0000	0.0000	0.0014	0.0001	0.0062	0.0000	0.0062	0.0037	0.0014	0.0044	0.0009	0.0000	0.0003	0.0009	0.00311802	Gammaproteobacteria
0.0005	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00279734	Gammaproteobacteria
0.0016	0.0056	0.0002	0.0050	0.0097	0.0000	0.0000	0.0000	0.0067	0.0382	0.0051	0.0000	0.0004	0.0000	0.0001	0.0000	0.0012	0.0000	0.0000	0.0000	0.00223043	Alphaproteobacteria
0.0054	0.0142	0.0000	0.0019	0.0003	0.0000	0.0004	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.00180381	Epsilonproteobacteria
0.0004	0.0012	0.0000	0.0014	0.0020	0.0000	0.0000	0.0000	0.0244	0.0076	0.0000	0.0000	0.0016	0.0025	0.0003	0.0002	0.0006	0.0000	0.0010	0.0002	0.00169215	Alphaproteobacteria
0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00166638	Gammaproteobacteria
0.0106	0.2971	0.0000	0.0002	0.0150	0.0000	0.0000	0.0000	0.0064	0.0768	0.0004	0.0000	0.0000	0.0002	0.0005	0.0005	0.0000	0.0000	0.0000	0.0002	0.01233465	Verrucomicrobiae
0.0006	0.0010	0.0005	0.0002	0.0001	0.0062	0.0025	0.0000	0.0000	0.0000	0.0195	0.0000	0.0073	0.0081	0.0097	0.0156	0.0131	0.0060	0.0063	0.0087	0.0039054	Verrucomicrobiae
0.0006	0.0005	0.0001	0.0036	0.0163	0.0000	0.0000	0.0000	0.0007	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00247094	Verrucomicrobiae
0.0004	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0004	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016263	Verrucomicrobiae
0.0105	0.0093	0.0002	0.0001	0.0007	0.0000	0.0000	0.0000	0.0003	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00154899	Verrucomicrobiae
0.0002	0.0003	0.0014	0.0013	0.0009	0.0000	0.0000	0.0001	0.0002	0.0009	0.0000	0.0000	0.0007	0.0000	0.0003	0.0000	0.0003	0.0000	0.0000	0.0000	0.00107943	Verrucomicrobiae
0.0000	0.0037	0.0000	0.0000	0.0021	0.0000	0.0000	0.0000	0.0000	0.0063	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00037222	Subdivision5
0.0000	0.0000	0.0000	0.0000	0.0012	0.0003	0.0004	0.0001	0.0019	0.0046	0.0000	0.0007	0.0005	0.0000	0.0008	0.0002	0.0000	0.0005	0.0000	0.0000	0.00032927	Verrucomicrobiae
0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0004	0.0006	0.0002	0.0008	0.0008	0.0003	0.0013	0.0000	0.0007	0.00030923	Opitutae
0.0004	0.0005	0.0000	0.0003	0.0022	0.0000	0.0000	0.0000	0.0003	0.0001	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00030923	Verrucomicrobiae
0.0054	0.0142	0.0000	0.0019	0.0003	0.0000	0.0004	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.00180381	Epsilonproteobacteria
0.0000	0.0003	0.0000	0.0000	0.0016	0.0000	0.0000	0.0000	0.0000	0.0035	0.0000	0.0000	0.0146	0.0000	0.0051	0.0003	0.0000	0.0217	0.0052	0.0019	0.00140583	Anaerolineae
0.0000	0.0000	0.0097	0.0010	0.0000	0.0010	0.0018	0.0002	0.0005	0.0000	0.0004	0.0000	0.0021	0.0012	0.0106	0.0013	0.0088	0.0114	0.0010	0.0038	0.00134857	Cyanobacteria
0.0000	0.0001	0.0000	0.0000	0.0033	0.0003	0.0000	0.0000	0.0198	0.0174	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00123404	Anaerolineae
0.0002	0.0003	0.0014	0.0013	0.0009	0.0000	0.0000	0.0001	0.0002	0.0009	0.0000	0.0000	0.0007	0.0000	0.0003	0.0000	0.0003	0.0000	0.0000	0.0000	0.00107943	Verrucomicrobiae
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00106225	Clostridia
0.0006	0.0017	0.0002	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0983	0.0000	0.0000	0.0007	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.00097635	OD1_genera_incertae_sedis
0.0000	0.0004	0.0003	0.0000	0.0036	0.0000	0.0000	0.0000	0.0018	0.0058	0.0000	0.0000	0.0005	0.0000	0.0006	0.0000	0.0000	0.0028	0.0000	0.0000	0.00083892	Actinobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0001	0.0009	0.0000	0.0000	0.0000	0.0000	0.0007	0.0000	0.00083605	OD1_genera_incertae_sedis
0.0001	0.0001	0.0000	0.0001	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00079311	Sphingobacteria
0.0000	0.0000	0.0000	0.0001	0.0299	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.003244	Thermoprotei
0.0000	0.0004	0.0000	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00114242	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0005	0.0002	0.0926	0.0000	0.0000	0.0012	0.00093054	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0004	0.0009	0.0000	0.0000	0.0003	0.0002	0.0002	0.0000	0.0006	0.0003	0.0010	0.0024	0.00012025	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.4401E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.5811E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	3.1495E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.4316E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.1453E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.1453E-05	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	8.5896E-06	Thermoprotei
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0014	0.0000	0.0008	0.0003	0.0003	0.0473	0.0007	0.0087	0.00075016	Methanomicrobia
0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0002	0.0000	0.0008	0.0000	0.0000	0.00067571	Methanomicrobia
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0005	0.0000	0.0001	0.0003	0.0000	0.0135	0.0000	0.0000	0.00023192	Methanobacteria
0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00016893	Thermoplasmata
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0005	0.0036	0.0000	0.0005	0.0000	0.0026	0.00014602	Methanomicrobia
0.0002	0.0023	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001088	Thermoplasmata
0.0000	0.0000	0.0000	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	8.017E-05	Methanobacteria
0.0024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	7.7306E-05	Archaeoglobi
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	6.0127E-05	Halobacteria
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0049	0.0000	0.0000	5.4401E-05	Methanomicrobia
																					Host
0.307332127	0.00109562	0.018294077	0.002603645	0.000527859	0.04611887	0.011465425	0.000728952	0.000269906	0	0.007020281	0.000371058	0.293595638	0	0	0	0.000303767	0	0	0	0.10102789	Cryptomonadaceae
0.000106417	0	0.000114338	0.000901262	0.007917889	0	0.000358295	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02374735	Chlorophyta
0	0	0	0	0	0	0	0	0	0	0	0	0.280444777	0	0	0	0	0	0	0	0.00751589	Bacillariophyta
0.000212834	9.9602E-05	0.001029042	0.002603645	0	0.000649562	0.000716589	0	8.99685E-05	0	0.049141966	0	0.000213835	0	0	0	0	0	0	0	0.00385673	Bangiophyceae
0.000319251	0.00338645	0.000114338	0	5.8651E-05	0	0	0	0	0	0	0	0.000106918	0.000231374	0.001036807	0.00357433	0.027339004	0	0	0.000470699	0.000879	Bacillariophyta
0	0	0	0	0.000117302	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00015461	Bacillariophyta
0	0	0	0	0	0	0	0	0	0	0	0	0.000106918	0	0.000388802	0.000324939	0.003037667	0	0	0.002353495	8.5896E-05	Bacillariophyta
0	0	0	0	0	0	0	0	0	0	0	0	0	0.000231374	0.000194401	0	0.000607533	0	0.000347947	0	3.1495E-05	Chlorophyta
0.000319251	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.8632E-05	Cryptomonadaceae
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5769E-05	Cryptomonadaceae

6.2 Temporal changes to meltwater ponds

Antarctic ponds experience annual complete freeze-thaw cycles (Hawes *et al.*, 1999), resulting in a continually changing physical, chemical and biological environment (Hawes *et al.*, 2011a; Hawes *et al.*, 2011b; Webster-Brown *et al.*, 2012). Towards the onset of the Austral winter, between late January and April, light levels reduce to near darkness and temperatures drop below 0°C, causing downward ice growth in the water column and reduced light penetration (Foreman *et al.*, 2010; Schmidt *et al.*, 1991). These physical changes result in biologically mediated effects such as oxygen super saturation (Koob & Leister, 1972; Lyons *et al.*, 2006) then reduction to anoxic conditions, a decrease in inorganic carbon and increase of pH (Hawes *et al.*, 2011a). Over this period there is a net shift in microbial communities from autotrophy to heterotrophy (Safi *et al.*, 2012) as photosynthetic species are outcompeted (Bell & Laybourn-Parry, 1999) by organisms able to tolerate the harsh winter conditions without the reliance on sunlight as an energy source (Chattopadhyay, 2000; Laybourn-Parry, 2002). Ponds begin to thaw once ambient temperatures rise above 0°C, most ponds becoming completely thawed by December (Chapter 2). Biological activity increases from November to January to the point where oxygen bubbles can appear from the microbial mats lining the ponds.

By integrating all available data from the studies presented in this thesis it was possible to view these pond environments as a whole over time, gaining insight into rapid annual changes that occur in these ponds. Bacterial ARISA data clustered samples into two groups, those from January (2012 and 2013), and those collected in December 2009, February – April 2008 (Extended), and sediment samples (Figure 6. 1). Analysis based on pyrosequencing data clusters sediment samples into a unique group, while water column samples from each time period lacked significant differentiation (Figure 6. 2). The clustering of sediment samples into a unique group with pyrosequencing data is likely related to the comparatively high number of low abundance sequences that may have been below the detection limit during the ARISA analysis. Examination of individual samples using Bray-Curtis similarities based on pyrosequencing data (Figure 6. 3) clustered surface water column samples from all time points, although significant variation was identified between samples (see Chapter 3). Similarity between

water column samples would likely have been the result of shared dominant components across the time periods in this investigation. The abundance of chloroplast sequences detected was also related to this clustering, being much higher in the January samples and lower in the April, deep-water column and sediment samples (Supplementary Figure 6. 1).

Samples from Legin Pond, which had the most comprehensive dataset covering all time points in this investigation, were separated so that temporal trends could be easily visualized (Figure 6. 4 and 6. 5). Bacterial ARISA data formed distinct groups based on sampling year (Figure 6. 4); however, the pyrosequencing data clustered samples into three distinct groups at 20% similarity: surface, bottom and sediment samples (Figure 6. 5). Surface samples from January 2012 and 2013, and February 2008 were >50% similar, with the surface sample from 2009 being <40% similar to this cluster (Figure 6. 5). The abundance of major phyla varied between times and with depth (Figure 6. 3), as has been seen previously (Bell & Laybourn-Parry, 1999; Comeau *et al.*, 2012), however the same dominant phyla were always present. *Actinobacteria* were almost undetectable in December (2009), increasing in abundance in January (12.1% in 2013 and 27.2% in 2012) and February (13.7% in the surface of the pond and 37.8% in the bottom) then decreasing in April (3% in the top and 8.5% in the bottom). Conversely, *Bacteroidetes* was strongly dominant in December, accounting for almost the entire community (98.9%), then declined in January (55.2 in 2013 and 73.5% in 2012), February (64% in the top and 12.5% in the bottom) and April (44.2% in the top and 1% in the bottom). *Proteobacteria* and a large number of minor phyla were more abundant in the April sample.

Due to the great variation in populations between samples it was difficult to find trends for all samples by examining the major OTUs from each phylum (Table 6.2). Once again samples from all Legin pond timepoints were examined separately, identifying significant temporal variation of each OTU (Table 6. 3). For example, the most abundant *Actinobacteria* OTU in January (16.8% in 2012 and 11.7% in 2013) and February (10.3% in the top sample and 20.6% in the bottom) was found at lower abundance in April (2.3% in the top sample and 7.6% in the bottom), and was absent in 2009. The most abundant *Bacteroidetes* OTU was found in high abundance in April (2.3% in the top sample and 29.3% in the

bottom), and was a minor component (≤ 4%) at all other times. The second most abundant *Bacteroidetes* was at high abundance in the February surface sample (25.7%), almost the only OTU found in 2009 (98.1%), and was at high abundance in the January samples (20.2% in 2012 and 47.9% in 2013). This OTU could be the first to proliferate in December, being slowly outcompeted over summer and moving into an inactive state at the onset of winter. Archaea were absent in all December samples, with traces detected in January samples, however abundance increased markedly in the deep February and April samples. It is important to note that although community structure was presented as relative abundance, there was a net decrease in cell numbers in ponds sampled between February and April (Hawes *et al.*, 2011a; Safi *et al.*, 2012).

The similarity of February and January samples was likely the result of downward ice growth in February concentrating the communities in the surface water column that was established in January (Wait *et al.*, 2006). After the complete freezing of the water column over winter and summer melting the surface communities in December, microorganisms will have emerged from their inactive states, changing the community markedly from that in January (Foreman *et al.*, 2010; Mountfort *et al.*, 2003). The remaining samples from February and April show a high degree of differentiation from this surface cluster, reflective of a rapidly changing physical environment (Hawes *et al.*, 1999). The similarity between January 2012 and 2013 indicates that populations go through fairly consistent annual biological changes. The variation of the time of ice and snow melt in spring has been found to significantly affects primary production in mat communities (Moorhead *et al.*, 1997), which explains the minor variation between samples collected between years at the same point in the season. These results show that bacterial communities in Antarctic meltwater ponds appear to experience rapid, however relatively consistent, seasonal succession. Although many of the major OTUs present in these ponds during the summer season have likey been identified, further research involving frequent sampling across several years and locations is required to gain a more comprehensive understanding of these biological changes.

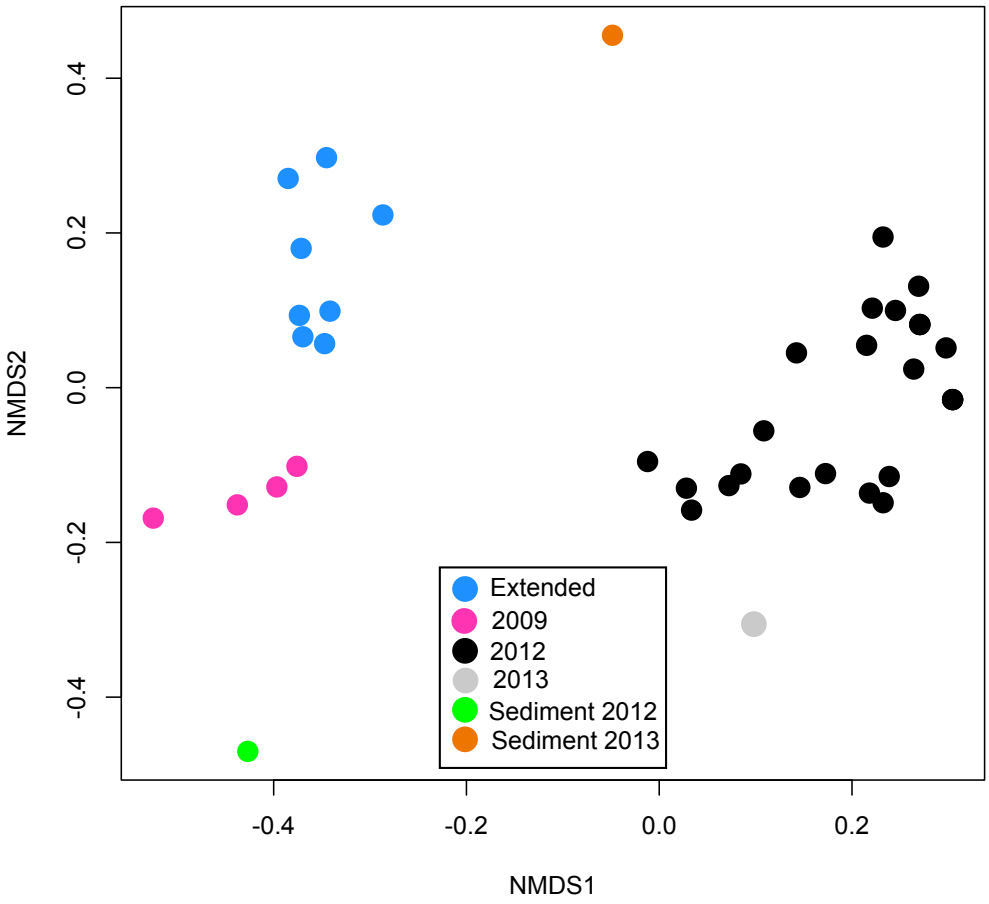


Figure 6. 4 NMDS ordination (Stress=0.13) of bacterial ARISA community compositions from Legin pond (subset from Figure 1) with a presence/absence transformation based on Bray-Curtis distances.

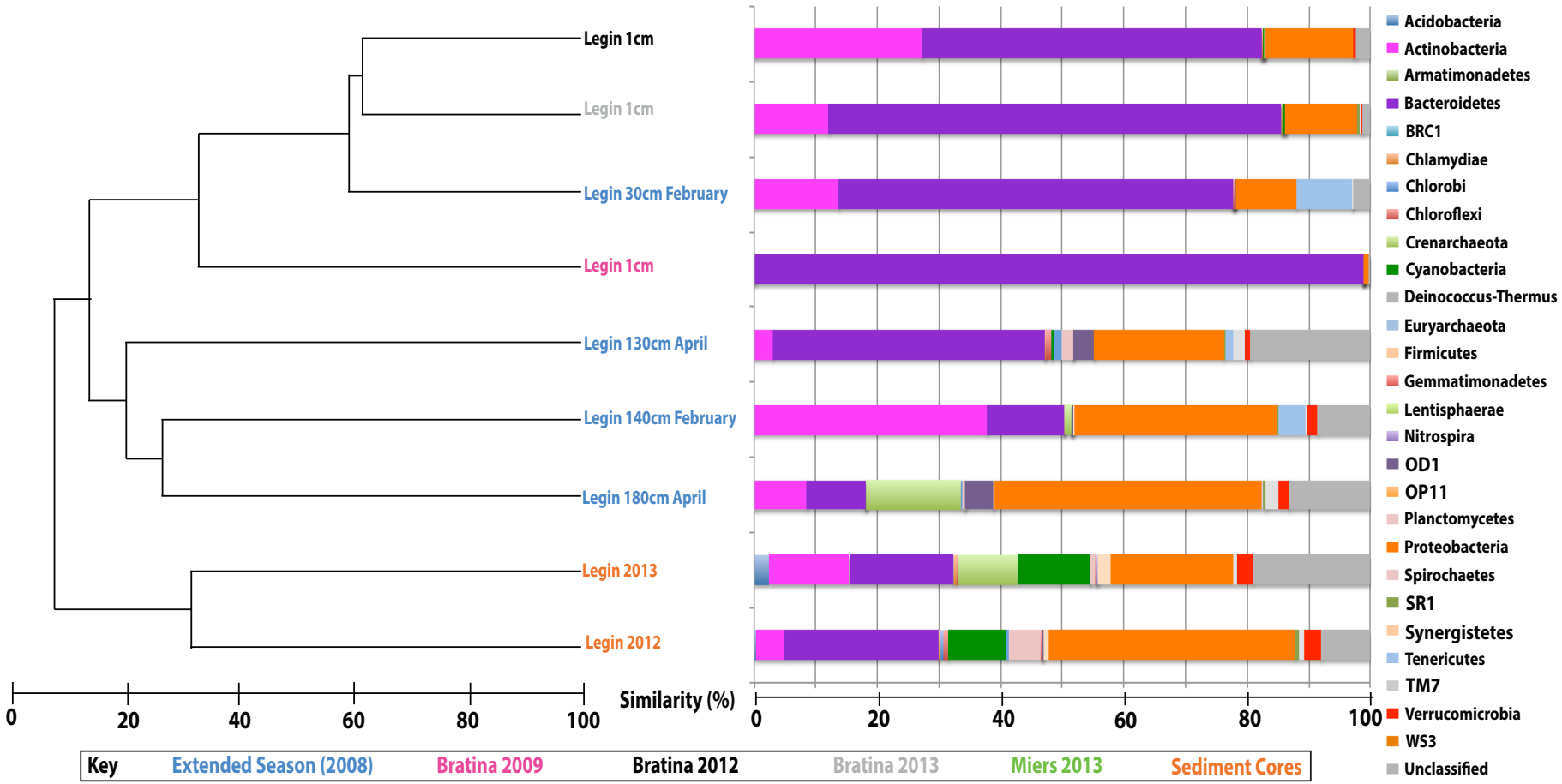


Figure 6. 5 A Bray-Curtis similarity tree calculated from total $OTU_{0.05}$ compositions from Legin pond (subset from Figure 3) with no transformation (Left). Phylum-level abundance of $OTUs_{0.05}$ assigned using the RDP Release 10, Update 15 Classifier, assignment confidence threshold >80% (Right).

Table 6. 3 Heatmap where color intensity represents a larger fraction of total sequences. Data representative of the most abundant sequences from each phylum in Legin pond samples.

Phyla	Extended				2009	2012	2013	Sediment		Class
	13.02.30	13.02.140	03.04.130	03.04.180	1cm	1cm	1cm	2012	2013	
Actinobacteria	10.3%	20.6%	2.3%	7.6%	0.0%	16.8%	11.7%	0.0%	0.0%	Actinobacteria
	0.1%	1.1%	0.3%	0.6%	0.0%	0.4%	0.1%	0.2%	0.0%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.1%	7.7%	0.1%	0.4%	0.2%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.8%	1.2%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	1.0%	Actinobacteria
	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	0.2%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	1.6%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	1.0%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.7%	Actinobacteria
Bacteriodetes	0.8%	2.3%	29.3%	3.2%	0.2%	4.0%	0.3%	1.9%	0.6%	Sphingobacteria
	25.7%	0.2%	0.1%	0.0%	98.1%	20.2%	47.9%	4.1%	2.9%	Flavobacteria
	20.9%	0.0%	2.2%	0.0%	0.0%	16.2%	13.9%	0.2%	0.0%	Flavobacteria
	0.5%	0.0%	0.1%	0.0%	0.1%	3.9%	9.1%	0.0%	0.1%	Sphingobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.7%	2.8%	Sphingobacteria
	0.0%	0.1%	0.2%	1.3%	0.0%	0.0%	0.0%	0.0%	0.0%	Sphingobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.7%	Flavobacteria
	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.5%	0.0%	Flavobacteria
	0.0%	0.0%	3.1%	0.0%	0.0%	0.1%	0.4%	0.0%	0.0%	Flavobacteria
	0.6%	0.0%	1.4%	0.0%	0.0%	3.9%	0.4%	0.0%	0.0%	Flavobacteria
Cyanobacteria	22.6%	8.3%	4.8%	0.1%	0.2%	4.5%	1.1%	0.0%	0.0%	Chloroplast
	0.2%	29.9%	8.1%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	Chloroplast
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Chloroplast
	0.1%	1.1%	0.1%	0.0%	0.2%	0.1%	0.1%	0.0%	0.0%	Chloroplast
	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	1.4%	Cyanobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	3.1%	1.4%	Cyanobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.2%	2.0%	Cyanobacteria
	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.1%	2.1%	Cyanobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Oscillatoriophycideae
	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.2%	0.1%	0.9%	Cyanobacteria
Firmucutes	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.1%	0.0%	0.4%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	Clostridia
	0.0%	0.0%	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Bacilli
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia

Table 6.3 cont

Proteobacteria	0.0%	6.3%	0.3%	1.1%	0.0%	0.0%	0.3%	0.5%	0.3%	Alphaproteobacteria
	2.7%	0.1%	4.0%	0.1%	0.6%	8.7%	6.2%	20.1%	3.2%	Betaproteobacteria
	3.2%	3.1%	1.8%	0.8%	0.0%	1.4%	3.0%	0.0%	0.0%	Betaproteobacteria
	0.0%	0.2%	0.0%	0.9%	0.0%	0.0%	0.0%	0.0%	0.1%	Gammaproteobacteria
	0.0%	0.0%	0.1%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	Gammaproteobacteria
	0.0%	0.4%	0.1%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	Alphaproteobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Alphaproteobacteria
	0.1%	0.1%	0.1%	0.0%	0.0%	2.0%	0.4%	2.9%	0.7%	Alphaproteobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	Betaproteobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	2.1%	Gammaproteobacteria
Verrucomicrobia	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.0%	0.3%	0.0%	0.0%	0.3%	0.3%	1.6%	1.3%	Verrucomicrobiae
	0.0%	0.3%	0.1%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.4%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.1%	0.5%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Subdivision5
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	Opitutae
	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
Unclassified	0.0%	0.5%	0.5%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	Epsilonproteobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Anaerolineae
	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.2%	0.1%	0.9%	Cyanobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Anaerolineae
	0.0%	0.1%	0.5%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	Verrucomicrobiae
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Clostridia
	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	OD1_genera_incertae_sedis
	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Actinobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	OD1_genera_incertae_sedis
	0.0%	0.3%	0.1%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	Sphingobacteria
Euryarchaeota	0.0%	0.4%	0.0%	9.9%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.2%	0.0%	5.2%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.3%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	Thermoprotei
	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoprotei
Crenarchaeota	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Methanomicrobia
	0.0%	0.0%	0.9%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	Methanomicrobia
	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Methanobacteria
	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoplasmata
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	Methanomicrobia
	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	Thermoplasmata
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Methanobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Archaeoglobi
	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	Halobacteria
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	Methanomicrobia

6.3 Future directions

This investigation has provided the most comprehensive characterization of the bacterial communities within the McMurdo Sound meltwater ponds, and represents the first study to compare the microbial communities and determine the geochemical drivers responsible for variation between coastal terrestrial (Miers Valley) and McMurdo Ice shelf (Bratina Isalnd) meltwater ponds. The findings from this thesis identified a large proportion of shared OTUs between locations, suggesting regional redistribution of microorganisms and raising questions about the extent of biological exchange between environments in Antarctica. A comparative survey of bacterial communities across a greater geographic region (McMurdo Sound, Antarctica, polar, or global) would be essential to assist in understanding the full extent of diversity, distribution and endemism of the biological communities in Antarctic meltwater ponds. Additionally, although partially representative of the broad geochemical heterogeneity found in similar systems (lakes and ponds) across Antarctica, the inclusion of more geochemically distinct ponds would assist in determining universal and site-specific drivers of community structure and likely identify a large number of species not detected in this investigation.

Given the complexity of interactions within an environment it is difficult to capture all factors that may have an effect on the microbial community. The strong influence of geochemical parameters on microbial community structure identified in this investigation indicates they have a dominant effect, however future studies could expand the environmental data collected to gain a better insight into other explanatory variables. Conductivity was consistently identified as a strong contributing factor across broad gradients (Chapters 2, 3 and 4), however trace elements, derived from local geology, were also shown to influence community structure. An investigation of surrounding minerals and biological materials that are deposited into the ponds would provide another important dimension in understanding factors that structure the current microbial community. Furthermore, although the concentrations of a number of nutrients were determined, previous work has revealed the importance of other key trace nutrients, whose concentration is highly variable between sites, in structuring the biological community (Hawes *et al*., 2011; Hawes *et al*., 2013; Sorrell *et al*.,

2013). Determining the environmental drivers that underpin the dynamics of community structure in these most simple ecosystems is essential for future management efforts and to understand microbial ecology in temperate environments.

When the datasets from each chapter were combined, interpolations could be drawn concerning the biological succession that occurs across the highly variable seasonal cycle. The findings identified significant temporal shifts in community structure congruent with previous work (Hawes *et al.*, 2011a; Hawes *et al.*, 2011b; Webster-Brown *et al.*, 2012) and also consistent community components within the same niche between years. However, as these findings are restricted to a limited number of time points the persistence of these groups is still unclear. A coordinated sampling effort of the water column at greater frequency throughout the year and across several years would allow a greater fundamental understanding of the biological changes that occur throughout the season and multitude of survival strategies utilized by the resident microbiology (Chattopadhyay, 2000; Laybourn-Parry, 2002).

This study utilized DNA-based technologies to describe the bacterial communities of these ponds, however it shed little light on Archaea and Eukarya. The increase in the proportion of Archaea, unintentionally amplified in this study, in the Legin water column as winter progressed raises questions about the persistence of these groups throughout the highly active summer period and the relationship to the Bacterial component of this system. A dedicated study of Archaea and Eukarya and their abundance relative to Bacteria throughout the year would be useful to clarify the role of these organisms in the ecological functioning of the meltwater ponds and provide a more holistic understanding of the microbiology within these ponds.

The research presented in Chapters 2 and 4 identified massive geochemical and biological stratification similar to that seen in the extensively studied meromictic Antarctica lakes (Comeau *et al.*, 2012; Glatz *et al.*, 2006; Hawes *et al.*, 2001; Lauro *et al.*, 2011; Mosier *et al.*, 2006; Naganuma *et al.*, 2005; Villaescusa *et al.*, 2013). This stratification provides an excellent model to study biogeochemical processes within the pond water columns. A metagenomic analysis would provide insights into functional changes, in relation to

environmental transitions. Aditionally, the extreme conditions Antarctic ecosystems are useful to investigate psychrophiles survival strategies including the viable but non-culturable state and enzyme activity at low temperatures (De Maayer *et al*., 2014). Identifying the metabolic processes occuring in an ecosystem allows those functionally important populations and processes to be identified, monitored and modelled for microbial responses to future climate change and can be the first step in identifying previously undiscovered enzymes for industrial applications (Hess *et al.,* 2011; Raies *et al.,* 2011; Schloss & Handelsman., 2003; Simon & Daniel*.,* 2011).

6.4 Final Conclusions

The meltwater ponds of the McMurdo Sound contain diverse and dynamic microbial communities. The similarity between communities depends on many factors including location, geochemistry, size, stratification profiles and climatic effects. Three primary zones within ponds, the homogeneous mixed surface waters (epilimnion), stratified heterogeneous zone (chemocline and hypolimnion), and the mats and sediments were investigated. Bacterial communities within pond samples from each zone, even between years, typically exhibit a higher degree of similarity to one another than to other pond zones. The components within these zones were consistent through most time points, however the community structure (abundance of key components) varied significantly. Conductivity was the overarching geochemical factor that structured many bacterial pond communities, although other variables significantly influenced ponds across local (within pond) and spatial (between locations) scales. A large fraction of the microbial community was shared between Bratina Island and Miers Valley ponds in surface waters, the stratified water column and sediment samples. This finding suggests biological exchange which, based on previous work, is the result of dust storm transportation from the Miers Valley to Bratina Island (Atkins & Dunbar, 2009; Bottos *et al.*, 2014; Dunbar *et al.*, 2009). Given the ability of microorganisms to redistribute within the region and the similarity of the pond environments to Antarctic lakes, the lack of shared signatures identified throughout the studies presented in this thesis was surprising. This was likely related to the previously

described distinct annual cycles each system undergoes. While lakes maintain an ice cover and typically maintain a liquid reservoir throughout the austral winter, ponds freeze solid, providing an extreme selective pressure for the resident microbial community. While this investigation has provided significant insight into the bacterial communities of these ponds, they still represent a relatively unknown and valuable scientific resource.

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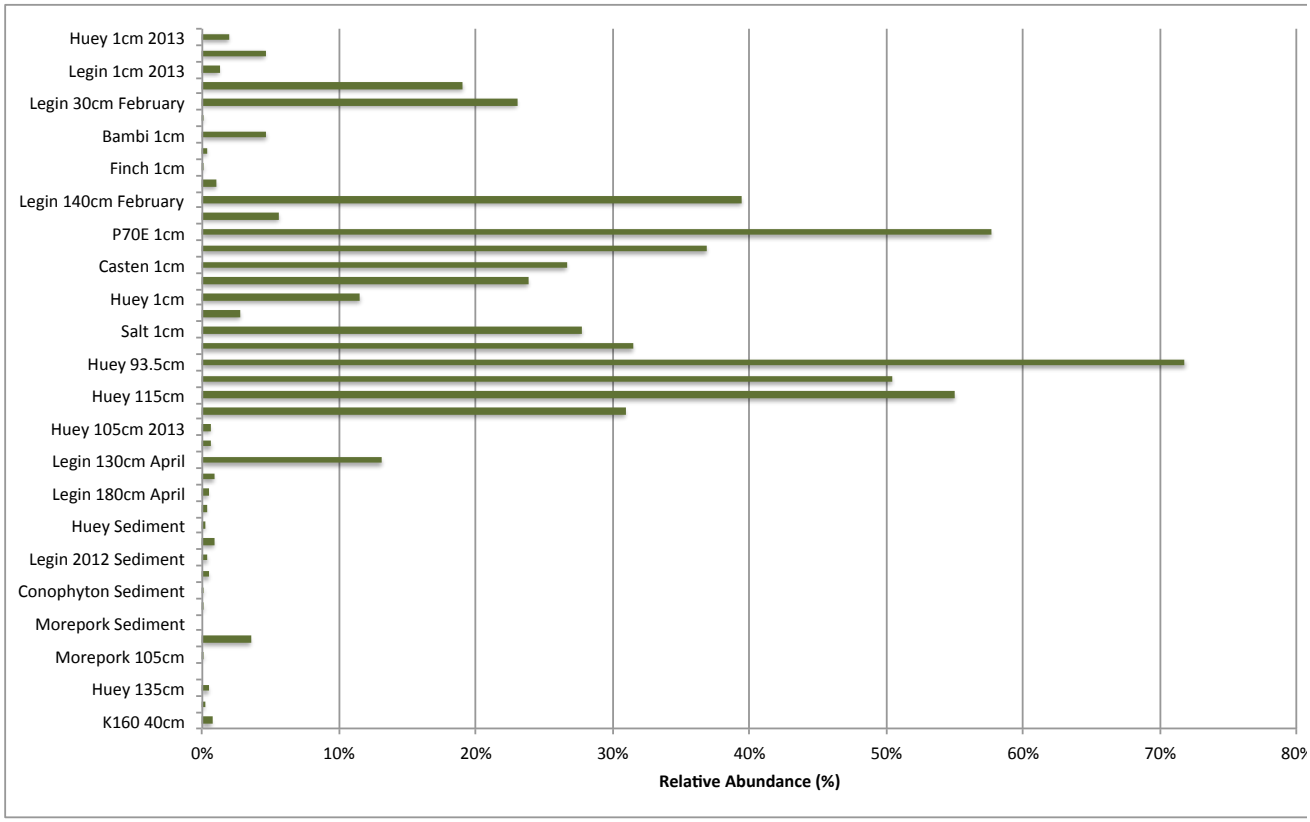
6.6 Supplementary Information



Supplementary Figure 6. 1 Relative abundances of chloroplast sequences from pyrosequenced samples.

Appendix 1 – Co-Authorship Forms

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Co-Authorship Form

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Thesis chapter 2
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Nature of contribution by PhD candidate	**Sample collection, experimental work, data analysis, manuscript preparation**
Extent of contribution by PhD candidate (%)	**80**

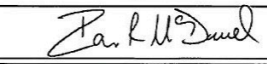
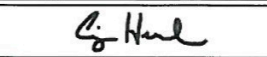
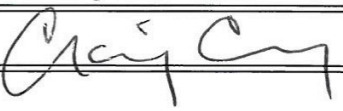
CO-AUTHORS

Name	Nature of Contribution
IR McDonald	Supervision, manuscript preparation
CW Herbold	Data analysis, manuscript preparation
SC Cary	Supervision, manuscript preparation

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
IR McDonald		Dec 16, 2014
CW Herbold		Sept. 16, 2014
SC Cary		17/12/2014

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Thesis chapter 3
This chapter has been submitted to the Polar Biology Journal

Nature of contribution by PhD candidate	**Sample collection, experimental work, data analysis, manuscript preparation**
Extent of contribution by PhD candidate (%)	**80**

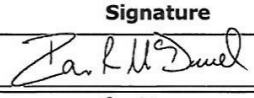
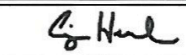
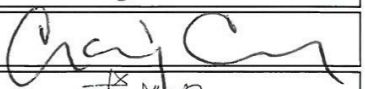
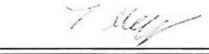
CO-AUTHORS

Name	Nature of Contribution
IR McDonald	Supervision, manuscript preparation
CW Herbold	Analytical pathway preparation
SC Cary	Supervision, manuscript preparation
C Lee	analytical pathway preparation
T Niederberger	Manuscript preparation

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
IR McDonald		Dec 16, 2014
CW Herbold		Sept. 16, 2014
SC Cary		17/12/2014
C Lee		4-Dec-14
T Niederberger		01 Dec 2014

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Thesis chapter 4
In preparation to be submitted for review

Nature of contribution by PhD candidate	**Sample collection, experimental work, data analysis, manuscript preparation**
Extent of contribution by PhD candidate (%)	**80**

CO-AUTHORS

Name	Nature of Contribution
IR McDonald	Supervision, manuscript preparation
CW Herbold	analytical pathway preparation
SC Cary	Supervision, manuscript preparation
C Lee	Analytical pathway preparation
T Niederberger	Manuscript preparation

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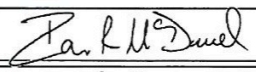
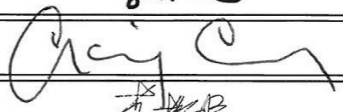
IR McDonald		Dec 16, 2014
CW Herbold		Sept. 16, 2014
SC Cary		17/12/2014
C Lee		4-Dec-214
T Niederberger		01 Dec 2014

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Thesis chapter 5
This chapter has been submitted to the Frontiers: Microbiology Journal and is in review

Nature of contribution by PhD candidate	**Sample collection, experimental work, data analysis, manuscript preparation**
Extent of contribution by PhD candidate (%)	**80**

CO-AUTHORS

Name	Nature of Contribution
IR McDonald	Supervision, manuscript preparation
CW Herbold	analytical pathway preparation
SC Cary	Supervision, manuscript preparation
C Lee	analytical pathway preparation, manuscript preparation

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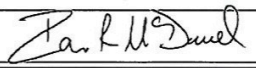
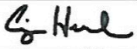
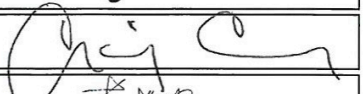
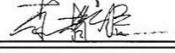
IR McDonald		Dec 16, 2014
CW Herbold		Sept. 16, 2014
SC Cary		17/12/2014
C Lee		4-Dec-2014