Resilience and recovery of Bratina Island meltwater pond microbial communities to environmental change

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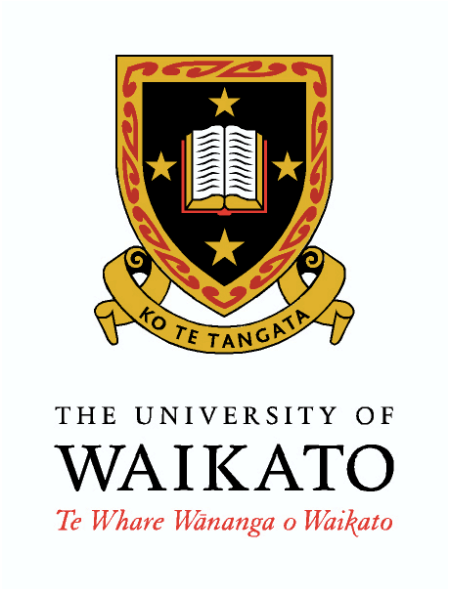

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Abstract

16S rRNA sequencing of three Bratina Island meltwater ponds has revealed the response of microbial communities within cyanobacterial mats exposed to shading and desiccation as environmental stressors. The resilience of these communities is perhaps not surprising considering the extreme environmental conditions they consistently face in these habitats. It was postulated that cyanobacteria will remain the dominant primary producers across the ponds when exposed to shading and desiccation, because they are known to possess adaptations to these stresses. Similar to research in other studies, it was found that three phyla: Cyanobacteria, Bacteroidota and Proteobacteria make up the majority of the diversity in these communities, but their dominance shifted between shading and desiccation samples. The most obvious changes occurred in desiccation samples, where the abundance of cyanobacteria dropped to less than 10% in some samples. Predicted functional changes in gene sets involved in photosynthesis and methanogenesis were also investigated, and unexpectedly, predicted functional changes in methanogenesis only occurred in one pond.

This thesis describes the microbial ecology of Bratina Island meltwater ponds, and the effect of desiccation and shading as environmental stressors on the diversity. It also addresses potential functional changes within these communities. Although, more extensive research into archaeal and eukaryotic communities alongside changes in physiochemical parameters within each pond is needed to fully understand how shading and desiccation effects the resilience and resistance of microbial communities within meltwater ponds.

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Abbreviations

ASV.	Amplicon sequence variants
cm	Centimetre(s)
DNA	Deoxyribonuclease acid
dsDNA	Double-stranded deoxyribonuclease acid
g	Gram(s)
ml	Millilitre(s)
mm	Millimetre(s)
MPa	Megapascal(s)
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PCR	Polymerase chain reaction
OTU	Operational taxonomic unit
µl	Microlitre(s)
UV	Ultra-violet

Chapter 1: Literature Review

This literature review explores scientific literature related to microorganism resilience and recovery from desiccation and shading to mimic environmental changes in Bratina Island, Antarctica. Section 1.1 covers extreme ecosystems, Bratina Island and the ecology of meltwater ponds in polar regions while section 1.2 covers the diversity, growth and resilience and recovery of extremophiles in the polar regions. Section 1.3 covers microbial response to environmental change and past studies investigating the effects of desiccation and shading on microbial mat communities.

1.1 Extreme ecosystems

1.1.1 Extreme ecosystems and extremophiles

An ecosystem is considered extreme if its physical, geochemical or geographical characteristics restrict the growth and development of higher-level life forms. By definition, organisms capable of growth in extreme environments are called extremophiles (Manicinelli, 2001). Extremophiles include members from all three domains of life. Although Archaea are the main group to thrive in extreme environments, a small number of eukaryotes such as protists (fungi, algae and protozoa) and multicellular organisms are also capable of living in extreme environments (Rampelotto, 2013). Another group of organisms which may be thought of as extremophiles are those that exhibit cryptobiosis, entering a stage of dormancy when they can no longer sustain metabolic activity due to environmental conditions (Wharton, 2002).

Present day extreme ecosystems are believed to reflect the Earths early conditions, in which the earliest organisms evolved and adapted for many years (Pearl, 2000). What is considered to be an extreme environment is often a set of conditions based on our experience of the environment. For example, conditions that deviate from the normal limits for human life (4-35°C, neutral pH) are considered extreme (Manicinelli, 2001). Microorganisms living within the optimal conditions for humans are considered to be mesophiles or neutrophils. Those that survive outside the normal ranges are deemed extremophiles (Wharton, 2002).

Extreme conditions can be physical (temperature, pressure, radiation) or geochemical (salinity, pH, metals and gases, desiccation). Microorganisms may be exposed to a single extreme condition or multiple extreme conditions depending on the habitat they occupy (Pearce, 2012). While Archaea are the main group to thrive in extreme conditions, frequently out-performing other domains of life, some of the

most well studied extremophiles are the thermophilic bacteria associated with the high temperatures of hot springs and deep sea hydrothermal vents (Wharton, 2002). It is arguable that a third category of extremes should be taken into consideration when understanding how microbes survive in extreme environments. These being, biological extremes such as population densities, parasites and nutritional extremes (Hawes et al., 2011).

Present day extreme ecosystems include both hot and cold deserts such as the Arctic, Antarctic, the Atacama and Sahara Deserts, volcanoes, geothermal vent systems, deep ocean trenches and the upper atmosphere. However, not all extreme environments are the result of natural processes or representative of earths early ecosystems (Pearl, 2000). Urbanization, industrialization and large-scale conversion of terrestrial ecosystems has had a major impact on earths landscape, contributing to a range of man-made extreme environments (Western, 2001). This includes space stations, space flight equipment and laboratory simulations of space impacts and conditions (Javaux, 2005).

The use of enzymes from extremophiles in laundry detergents, agriculture and pharmaceuticals has created many multi-million dollar industries (Rampelotto, 2013). Furthermore the study of extremophiles has become a key research area for astrobiology (Manicinelli, 2001; Rampelotto, 2013). Due to their biology and ability to adapt to extreme environments, extremophiles serve as model organisms when exploring the existence of life on other planets within the solar system. The study of extremophiles has provided information that challenges the traditional concepts of biology. This has led us to rethink questions such as “what is life?” and “what are the limits of life” (Rampelotto, 2013), posing some challenging new insights into what extra-terrestrial life could be like.

1.1.2 Bratina Island and the McMurdo Ice Shelf

The McMurdo Ice Shelf of Antarctica is home to Bratina Island, a small Antarctic island located on tip of Brown Peninsula.

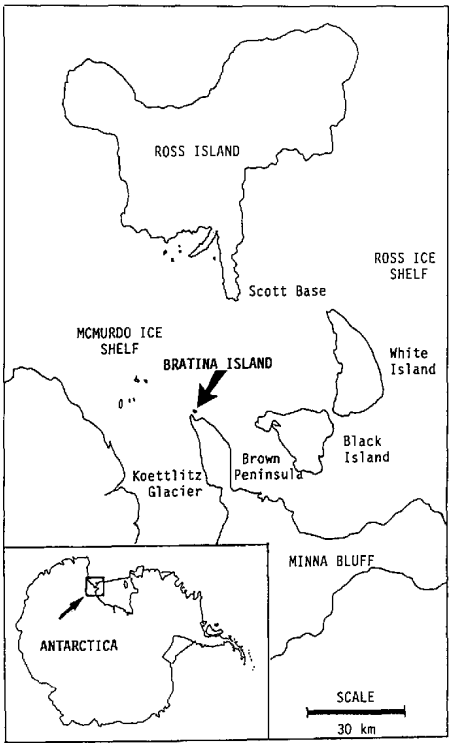

Figure 1: Location of Bratina Island on the northern tip of Brown Peninsula 33km south, south west of Scott Base

Bratina Island is located in the southwest corner of the Ross Sea, at the northern tip of Brown Peninsula with the Victoria Coast of southern Victoria Land to the west. The landscape surrounding Bratina Island is created by the compression of the ice shelf, providing the terrain which has formed thousands of meltwater ponds across the ice shelf near Bratina Island. The top layer of sediment is approximately 10-30 cm thick and contains sediment originating from the basal freezing of marine sediments to the underside of the floating ice shelf and deposition by surface ablation (Archer et al., 2015).

The surface debris is derived from the seafloor below the ice shelf. In many places beneath the surface the ice shelf touches the ocean floor. When the seawater freezes to the underside of the ice shelf it locks sediment and small marine organisms into the ice. As the surface ice is lost by melting and ablation the sediments at the bottom of the ice shelf are driven upward, gradually moving ice upward carrying the material from the ocean floor which is trapped in it. At the surface the marine sediments are released by melting, forming ponds in regions that topographically allow for it (Howard-Williams, 2003). Cyanobacteria form the basis of the microbial communities found within the meltwater ponds, forming thick mat communities. There are large variations in pond conditions across the ice shelf as the conductivity and nutrient content of some ponds is influenced by the tidal marine system. The meltwater system on Bratina Island is part of the

McMurdo Ice Shelf which has the most extensive microbial growth and largest non-marine biota in Victoria Land (Vincent, 2003).

1.1.3 Microbial ecology of meltwater ponds and cyanobacterial mats

Meltwater ponds act as oases whereby cyanobacterial mat communities are one of the only visible forms of life in Antarctica. They are characterised as small bodies of water that undergo annual freezing and thawing cycles, forming in glacial ablation zones where liquid water collects during the summer months (Safi, 2012; Vincent, 2003). The ponds, some only meters apart, differ in physicochemical properties, shape and size determined by the balance between meltwater input and evaporation (Howard-Williams, 2003; Javaux, 2005; Seckbach and Oren, 2010). The microorganisms that inhabit these environments need to be able to endure a number of environmental variables such as high salinity, temperature and UV radiation (Safi, 2012). The meltwater system on Bratina Island has the most extensive microbial growth and largest non-marine biota in Victoria Land (Vincent, 2003).

Cyanobacteria are found in meltwater ponds in the form of microbial benthic mats (Jungblut et al., 2005). Microbial mats represent a vertically stratified environment of physicochemical conditions (Bolhuis, 2014). Microbial cells of different species are distributed in space and time (meaning distribution is described due to variation in location and how it changes over time) according to their functions and physicochemical tolerances, allowing more effective energy distribution and self-protection (Pikuta, 2008). The distinguishing feature of most microbial mat communities in polar regions is an orange or red surface layer rich in carotenoids, followed by a blue-green layer consisting of high concentrations of chlorophyll and phycocyanin (Vincent, 2000).

The mats at Bratina Island harbour a variety of microorganisms. In the top layers of the mat, cyanobacterial taxa make up the majority of the biomass (de los Rios et al., 2004), alongside other basic biofunctional groups such as green algae, diatoms, methanogenic archaea and sulfate reducing and oxidizing bacteria (Prieto-Barajas, 2018). Cyanobacteria act as the dominant phototrophs which in previous studies have been identified as *Oscillatoriales* and *Nostacales*, the most motile of the cyanobacteria phyla (Javaux, 2005). This is further supported in a study conducted by Sutherland (2009) which found that all sampled ponds from the McMurdo Ice Shelf were dominated by filamentous cyanobacteria of the order of *Oscillatoriales* (Sutherland, 2009), accounting for more than 70% of the total biomass within the microbial mats in which they are found (Safi, 2012). The extracellular polymeric substances produced by cyanobacteria protect the mat from desiccation and UV

radiation as well as trapping moisture and nutrients inside the mat (Prieto-Barajas, 2018). Although cyanobacteria are one of the most dominant species, they are often accompanied by *Proteobacteria* and *Bacteroidetes* and a small portion of diatoms (Seckbach and Oren, 2010). The layers beneath the mat are generally anoxic, providing ideal conditions for sulfate reducers, methanogens and nitrate reducers to take advantage of the decomposed organic material produced by cyanobacteria (Seckbach and Oren 2010). The oxygen profile within microbial mats follows the penetration of light, which is quickly depleted in the first few centimetres (Javaux, 2005). The most oxic layers of the mat contain species with different trophic preferences such as *Rotifers, Nematoda* and *Tardigrades* (Almela, 2019).

Electron microscopy conducted by Rios (2004) in several ponds on Bratina Island revealed several layers of small mineral particles in between layers of mats. Some ponds consisted of a single cyanobacterium layer with thin layers around the mineral parts, others had thicker more definite layers throughout the mat. This, along with soil aggregation are said to contribute to the stability of microbial mats (Wilpiszeski et al., 2019). The relationship between soil particle and microbial communities is believed to play a role in the stratification of species (de los Rios, 2004). Factors including water supply, organic carbon content and niche availability have an effect on the stratification of microbes within the community (Wilpiszeski et al., 2019). The incorporation of darker coloured sediments into the mat absorbs UV radiation contributing to localised melting and heating of water (Olsen et al., 1998), potentially creating small microhabitats.

Earlier studies of microbial ecology in the benthic marine sediments of Bratina Island showed a vertical stratification of bacterial species reflective of a gradient of variables including temperature, oxygen content and redox potential of the sediment (Jungblut et al., 2005; Cowan, 2003). A study conducted by Cowen & Sjoling (2003) focusing on the diversity of benthic marine sediment found that almost half of the sequenced samples were from the Proteobacterial domain, of those δ-Proteobacteria were detected exclusively in the first 2 cm of sediment, compared to those of α-Proteobacteria and γ- Proteobacteria which were found between 17-22 cm of depth (see figure 2). The same study also found that *Cytophaga*, *Flavobacterium* and *Bacteriodes* group were derived from sediment samples ranging between 7-17 cm in depth. A similar study on benthic lake sediment collected from Ardley Island in west Antarctica showed similar patterns to that of Cowen & Sjoling's (see figure 3).

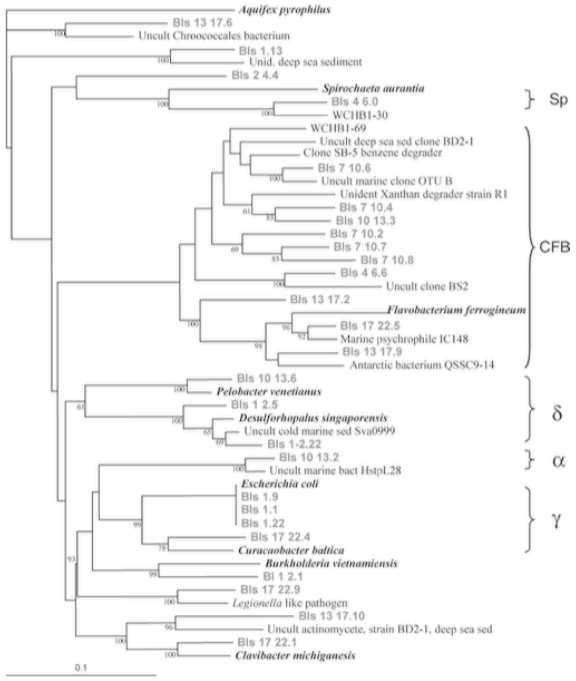

Figure 2: Phylogenetic tree of bacterial 16S rDNA clones derived from Bratina Island pond sediment. Spirochaeta (Sp), Cytophagales– Flavobacterium–Bacteroides (CFB), α-Proteobacteria (α), -γProteobacteria (γ), δ-Proteobacteria (δ) (Cowan, 2003).

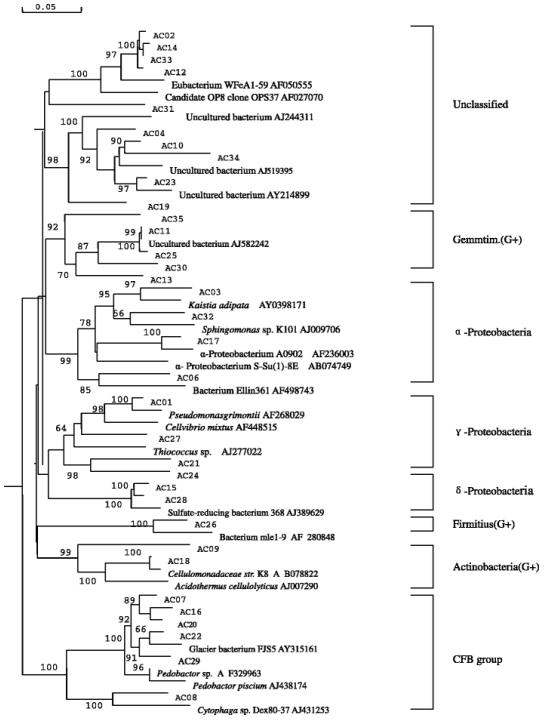

Figure 3: Phylogenetic tree showing the relationship of retrieved clones from the benthic sediment of Lake Ardly, west Antarctica. (Li, 2006).

Characterisation of bacterial communities within the water column of Bratina Island ponds is still a relatively new concept when compared with other studies which focus on sediment and mat communities. At the water's surface bacterial diversity is least diverse, dominated by *Bacteriodetes* and *Proteobacteria*. In the deeper parts of the water column, bacterial diversity starts to increase (see figure 4), although it remains dominated by two phyla, *Proteobacteria* and *Bacteriodetes* (Archer et al., 2014). The overall community structure within the water column varied between ponds, and was dependent on individual drivers such as pH, and conductivity. Similar studies (McDonald et al., 2015; Van Trappen et al., 2002; Ye et al., 2009).

focusing on the bacterial communities across a variety of ponds also found that *Proteobacteria* and *Bacteriodetes* were the most abundant phyla among others (see figure 5) that were less abundant. Aeolian transportation and katabatic winds are possible explanations for this reoccurring pattern (Archer et al., 2015).

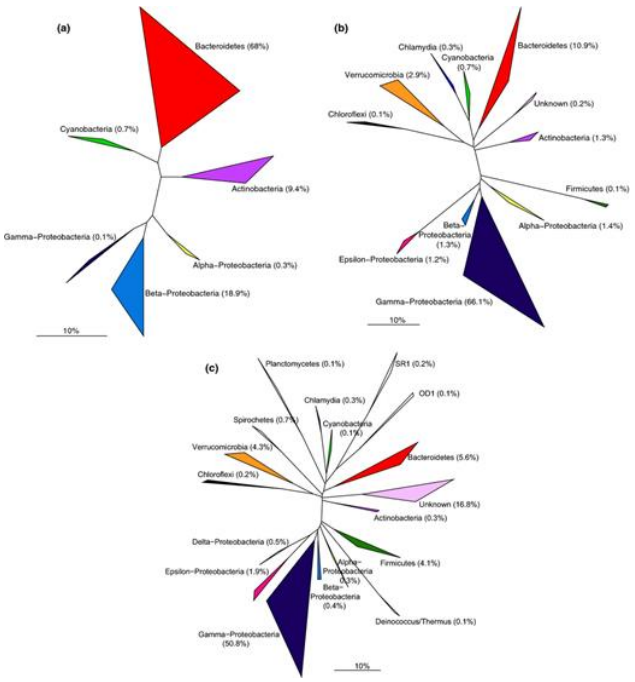

Figure 4: Phylum-level diversity of bacterial 16S rRNA gene sequences from different depths of Egg pond. Percentage abundance phyla within each site are shown for the (a) surface sample, (b) 40 cm deep sample and (c) 48 cm deep sample. (Archer et al., 2014)

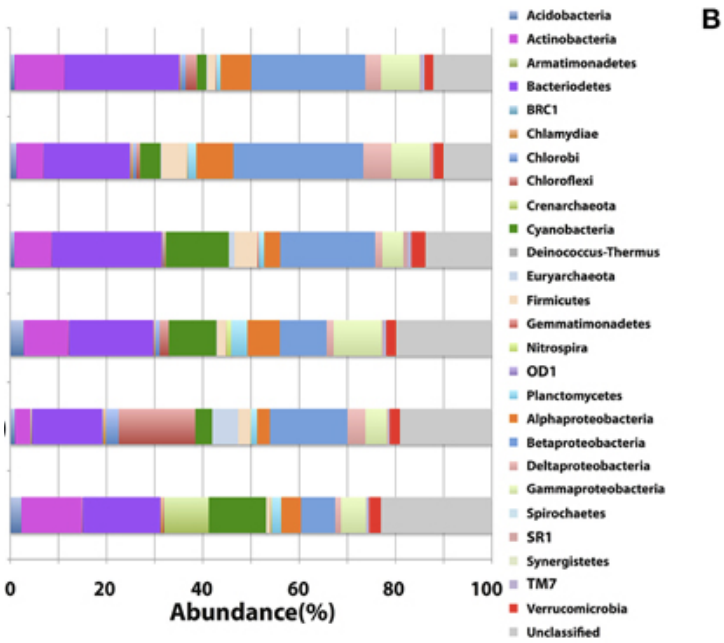

Figure 5: Phylum-level distribution of bacterial 16S rRNA OTUs assigned using the Ribosomal Database Project (RDP), assignment confidence threshold >80%. Ponds from top to bottom; P70E, Huey, canary, Finch, Morepork and Legin (McDonald et al., 2015).

In most polar communities the mats contain a variety of microorganisms including archaea, viruses and other bacteria acting as a consortium (McDonald et al., 2015). These communities rely on nutrient re-cycling and scavenging metabolisms to cope with the low input of nutrients (Jungblut and Vincent, 2012) caused by the lack of input from higher food web organisms such as crustacean zooplankton (Safi, 2012). A number of microbial processes degrade the organic matter into substrates which can be used by other microorganisms (Archer et al., 2014).

1.2 Extremophiles

1.2.1 Diversity and growth of extremophiles

Extremophiles are microorganisms that thrive in extreme ecosystems (see Section 1.1.1.). Extremophiles are dispersed widely across the phylogenetic tree of life; present in all three domains (Madigan, 2000). Often, species diversity decreases as the environment becomes more extreme (Hreggvidsson, 1995). Extremophiles in Antarctica are adapted to localized extremes such as regions of volcanic activity, hypersaline lakes, subglacial lakes, meltwater ponds and within the ice (Pearce, 2012). Many extremophiles have adapted to one or more extreme environments, these are known as polyextremophiles.

Psychrophiles are capable of growth and reproduction in permanently cold regions such as the Arctic and Antarctic, in a temperature range between -15°C to 10°C. To be able to survive in such conditions they have had to overcome key challenges faced by a number of other organisms also living in cold temperatures (Pearce, 2012). Psychrophiles have been found in liquid veins of water within ice, in the depths of the Southern Ocean and within the ice itself which remains frozen for most of the year. *Polaromonas vacuolata* has been characterized as living in frozen ice amongst other organisms such as diatoms and algae (Madigan, 2000).

Deep sea habitats account for the largest fraction of biosphere in terms of volume. Piezophiles (or barophiles in older text) are defined as organisms with optimal growth rates at pressures over 0.1 MPa, while hyperpiezophiles have optimal growth rates over 60 MPa (Bartlett, n.d). In addition to deep sea habitats, high pressure can be found in lakes and within ice formations. The MT41 strain is one of the more extreme piezophiles, found at the bottom of the Mariana Trench where the organism requires at least 50 MPa to maintain growth (Vincent, 2003). Halophiles, found in each of the three taxonomic domains of life, are adapted to life at high salt concentrations. Their hypersaline range can vary from that of the ocean at approximately 3-5% to as much as the Dead Sea with a salt concentration of 31% (Madigan, 2000). Most known halophiles such as *Halobacterium*, *Haloferax* and *Haloarcula* are well studied and have been used as models for studying archaeal domains (Ma et al., 2010).

One of the first acidophiles to be discovered, *Cyanidium caldarium,* a red alga, was found growing in conditions where the pH was around zero. Other acidophiles such as *Picrophilus oshimae* grow in conditions where the pH is equal or less than two (Pearce, 2012). This is well below the pH tolerance of other organisms such as fish which can be found in water with a pH as low as four, and plants or insects which are generally around a pH of two to three (Pikuta, 2008). On the opposite end of the pH scale, there are alkaliphiles. Some, such as *Natronobacterium grego* exhibit optimal growth in environments where the pH is over nine (Pearce, 2012). Like halophiles, alkaliphiles are found in all three domains. The most well studied of the alkaliphiles are the Bacillus and Pseudomonas species, most commonly found in soda lakes such as those in the Kenya rift (Horikoshi, 1999). Both acidophiles and alkaliphiles are found in Antarctic lakes including Blood Falls, Lake Bonney.

The diversity of extremophiles is not limited to those stated in the previous paragraphs. Thermophiles are organisms capable of growth at temperatures ranging between 45°C and 80°C. Thermophiles such as *Thermus aquaticus* and *Pyrolobus fumarii* are most commonly found in regions with high levels of geothermal activity (Pearce, 2012). *Deinococcus radiodurans* is an example of a

radiation resistant organism that can withstand more than a thousand times the radiation needed to harm human life. Little is known about the more uncommon types of extremophile because laboratory cultures are often difficult to establish and maintain (Madigan, 2000).

1.2.2 Resilience and Recovery of extremophiles

The concept of recovery and resilience was introduced into theoretical biology as the rate at which a microbial community returns to its original composition after a disturbance. Resistance is defined as the level to which a community is insensitive to a disturbance while resilience is defined as the rate at which a community returns to its pre-disturbance state (Shade et al., 2012). An ecosystems resilience is influenced by a number of factors, including the magnitude and duration of disturbance, the strength of interaction between the affected organism and its ecosystem and the elasticity of food webs (Muller, 2016).

The functional and taxonomic diversity of microbes allows them to rapidly adapt to changing environmental conditions making them of particular interest in the context of climate change. Anthropogenic activity (species introductions, land conversions and carbon emissions) increases the rate and intensity of environmental change to unknown levels (Oliver et al., 2015), creating weather extremes never before witnessed. Previous studies have shown that both environmental and man-made disturbances can shift a community towards alternative equilibrium states with relatively minor changes to the overall functioning of the community (Baho, 2012; Enwall et al., 2007). Such studies have been conducted on large ecosystems including forests, farmland and marine systems, but to date, the Arctic and Antarctic have not had the same attention, leading to little understanding of resilience and recovery in these systems (Muller, 2016).

If a microbial community is sensitive to a disturbance, they might still be able to recover quickly and return to their predisturbance composition. Some features of microorganisms including fast growth rates and high physiological tolerance suggest that resilience is common within microbial communities (Martiny, 2008). Some bacteria will enter a state of dormancy when faced with a disturbance. This behaviour may act as a buffer for the effects of the disturbance and allow the community to maintain diversity (Baho 2012). If immediate physiological adaption is not an option, then evolution through mutations or horizontal gene transfer might allow some microbes to recover from disturbances. These factors assume that microbial abundance decreases as a result of the disturbance. However, it is

possible for taxa to benefit from the new conditions created by the disturbance and increase in abundance instead (Martiny, 2008).

There have been a number of studies which attempt to investigate whether microbial communities display any forms of resistance or resilience to disturbances. One study conducted by Enwall (2007), which had been comparing fertilized and unfertilized soil plots since 1956 found that the composition of bacteria between the plots remained the same even after the experiment had been running for fifty years (Enwall et al., 2007). While a separate study by Stark et al., (2007) comparing inorganic and organic forms of nitrogen on soil plots found that after ten days the composition differed between the soil treatments but after ninety-one days the soil composition differed only for one species of bacteria. This suggests that the microbial community displayed some degree of recovery and resilience. The dispersal of microbial populations between habitats within a landscape can also enhance resistance and resilience against disturbances (Baho, 2012; Martiny, 2008). A laboratory simulation was conducted to mimic the effects of dispersal into a refuge against salinity as the environmental disturbance. Baho (2012) observed that bacterial communities in the refuge treatment were similar to those of the regional pool, indicating that the dispersal of some species from the original population into the refuge allowed them to somewhat resist the disturbance.

Resistance and resilience are said to be enhanced if the community in question contains a variety of individuals with versatile physiologies or physiological plasticity (Sutherland, 2009). In particular bacteria often respond to environmental changes or disturbances by expressing a range of metabolic capabilities allowing the community to confront new conditions (Sutherland, 2009). In contrast mixotrophy, the ability to use different carbon and energy sources, may also lead to increased resilience in microbial communities (Sutherland, 2009).

Resilience has been acknowledged as part of the earths management system for a number of years despite little research into its monitoring and measuring. Most attempts focus on early warning signs as resilience indicators such as the slowing down of an ecosystem (Oliver et al., 2015). However, by the time this is noticed most of the critical ecosystem transitions have already occurred. In order to be successfully monitored, acceptable minimum thresholds and target suits of ecosystem functions in different areas will need to be obtained. One of the biggest challenges will be how to identify the trade-off between the resilience of multiple functions within a species.

1.3 Environmental change and adaption

1.3.1 How microbial communities adapt to life in the polar regions

One of the major challenges in the study of microbial ecology is understanding the mechanisms that determine the structuring of communities, and how they respond to environmental change and stressors (Logares et al, 2013). Most microorganisms are able to adapt to small environmental changes, with timescales ranging from minutes, hours, days to years. Adaptions occur as either behavioural, physiological or structural changes, or as a combination of changes. More often than not, responses are aimed at survival rather than growth (Beales, 2004). In Antarctica and other polar regions, microbial communities must overcome the challenges imposed by freezing conditions, high salinities, UV radiation and dehydration (Vincent, 2003).

One way microbes adapt to the cold environments found in Antarctica is to avoid them completely, by their choice of habitat (Vincent, 2003). Few environments remain warm during the winter months, but these include fumeroles, geothermal sites and solar-heated lakes. As these habitats are considered rare, microbial growth and colonisation in these regions is often very slow. Often microbes seek protection within microbial mats where the dominant species, Cyanobacteria, provide protection to organisms with lower tolerances to temperature change (Prieto-Barajas, 2018). Arguably, when microorganisms can no longer withstand the environmental conditions some species enter a dormant state. In this dormant state respiration and the uptake of substrates is thought to continue at very low rates, but this has yet to be demonstrated under laboratory conditions (Chattopadhyay, 2006).

There are multiple ways microorganisms adapt to chilling and freezing in the polar regions. To adapt to chilling, that is cold environments where temperatures remain just above freezing, there are a number of biochemical adaptation strategies that allow most microorganisms to cope with the stresses of chilling. These include changes in cellular components that perform well in cold temperatures, increases in the cellular concentration of enzymes (Vincent, 2003), and intermediate responses of the metabolic system to minimise the detrimental effects of chilling. Adaptations to freezing, similar to adaptations used to combat chilling, focus on minimising or eliminating ice damage that occurs within cells during the freezing process (Vincent, 2003). One of the most common adaptations for life at freezing temperatures are the addition of anti-freeze proteins which lower the temperature of the freezing point of water, inhibit the formation of ice crystals and prevent the penetration of ice into cellular structures (Margesin, 2007; D'Amico et al., 2006). An increase in the production of extracellular substances alongside an increase in water membrane

permeability reduces the chances of ice formation in the immediate vicinity of the cell and allows the cell to lose water in sites where ice formation is beginning (Russel et al., 1990). Other adaptations include increases in membrane elasticity, allowing the cell to become more resistant to the expansion and contraction stresses by continuous dehydration and rehydration (D'Amico et al., 2006).

The ability to adapt to high salinity environments is of particular importance to microbes inhabiting Antarctica, as the dissolved ions become highly concentrated by the freeze-thaw processes (Vincent, 2003). Compatible solutes which maintain a high internal osmolarity are the universal adaptation to high salinity environments and have been observed in a number of organisms isolated from Antarctica; *Halomonas subglaciescola*, T*ropidonies laevissima* and *Chlamydomonas* (Vincent, 2003; Ma et al., 2010). During the summer months, many microbial communities are exposed to continuous levels of high solar radiation. Some microbial communities show behavioural adaptations to overcome this, by undergoing photosynthesis when light availability is near its minimum (Vincent, 2003). While others such as cyanobacteria produce a large amount of carotenoids protecting them, and the microbes that seek residence in the mats they form, from radiation (Prieto-Barajas, 2018). Mucilage production is said to combat dehydration and often takes place in cyanobacterial species. This extracellular substance prevents the rate of water loss from the cells during desiccation events (Vincent, 2003) and aids in the survival in other extreme conditions such as freezing and high salinities Prieto-Barajas (2018).

1.3.2 The effects of shading and desiccation on microbial mat communities

In recent years, as global climate conditions continue to worsen, the ability of microorganisms to be able to survive periods of unfavourable conditions has been stressed more than usual (Hawes and Vincent, 1992). The effects of desiccation and tolerance to various light availabilities has been investigated in microbial mats from Antarctica since the early 1990's. Some hypothesize that mats from regions of shallow water have a higher tolerance to desiccation and radiation stress than those from deeper ponds. However, further investigation is needed to prove this.

Desiccation tolerance is defined as the ability of an organism to dry to equilibrium with air, and upon rewetting regain normal functions. The diversity of desiccation tolerant species suggests that the potential for organisms to evolve some form of tolerance or resistance is widespread (Alpert, 2005). It is well established that cyanobacterial mats have a high tolerance and recovery rate to desiccation, but this differs among different types of microbial mats (Quesada and Vincent, 2012). One

study conducted by Hawes (1992) found that *Nostoc* dominated mats resumed respiration and photosynthesis within 10 minutes of rewetting, compared to *Phormidium* dominated mats which did not completely recover even after ten days of rewetting. While another study showed that cyanobacterial mats from estuaries that experience sporadic desiccation and rewetting events responded rapidly in two phases to rewetting of the microbial mat. The first phase included the resynthesis of photosynthetic pigments followed by an increase in photosynthetic production and migration toward the surface of the microbial mat (Chennu et al., 2015).

Polar cyanobacterial mat communities are exposed to two extremes of high and low radiation conditions (Quesada and Vincent, 2012). Many species of cyanobacteria are capable of adjusting their position within the microbial mat system according to the availability of light. An experiment where mat samples were brought inside from outside showed a change in colour, becoming darker due to an increase in light capturing pigments (Quesada and Vincent, 2012) but a deficiency in light protecting carotenoids and UV screening pigments. It is expected that this change will also be observed in mats exposed to shade stress. The migration behaviour of cells allows the cells to avoid radiation damage, while still allowing them to migrate to the surface and continue photosynthesis during periods of low radiation or shading conditions such as those observed during freezing of the lake or pond habitat (Quesada and Vincent, 2012). A study conducted by Hawes (1992) also showed that the shading of cyanobacteria led to their persistence deep within the microbial mats which they occupy suggesting that they are able to tolerate prolonged periods of unfavourable growth conditions.

1.3.3 Microbial functional diversity

Microbial communities play key roles in nearly every biogeochemical process on earth, most of these are considered vital as ecosystem processes including primary production, decomposition and nutrient cycling. Functional diversity is described as the diversity of functions performed by organisms in ecosystems. Functional diversity has been deemed the missing link between biodiversity patterns and ecosystem functions. However, its understanding lacks well behind traditional microbial counterparts, even though the first index of functionality was described over thirty-five years ago (Escalas et al., 2019).

The most common approach to understanding functional ecology compares communities directly using their traits, which results in an increase or decrease in the abundance of particular genes. The limitation of this approach is that no specific taxonomic level translates into functional differentiation because in many microorganisms' genes that carry out specific functions are redundant. The second

approach includes that community level functional changes can be linked to community level function. Genotypic traits which encode the functional potential of microbes corresponding to pathways in the genome of microbial taxa or their abundance in a community's' metagenome, expressed as KEGG (Kyoto encyclopaedia of genes and genomes) orthology pathways, are the most common approach for studying microbial traits. This method predicts metagenomes from 16S rRNA data and a reference genome data base inferring the functional contents of a community using its taxonomic composition using software such as PICRUSt or Tax4Fun. Phenotypic traits are used when analysing macroorganisms and are related to ecosystem processes rather than genotypic processes.

Phenotypic traits include but are not limited to shape, motility, cell dimensions and spore formation and are established during the cultivation of organisms under laboratory conditions. However, due to the lack of culturable representative for most microbial taxa, using taxon-phenotypic traits is seen as one of the weaker approaches for understanding functional diversity in microbial communities.

1.3.4 Past and current approaches for investigating microbial communities

Many previous investigations into the effects of environmental change in microbial communities have been hindered by methodological and sampling limitations (Youngblut et al., 2012). In the past taxonomic identification of microbial communities relied on their isolation and culture on different types of media, along with the identification of morphological features and nutrient requirements. Culture-dependent approaches are described as biased because they only capture a limited amount of the existing microbial diversity within a sample. Therefore rarer and more slow growing species are often missed (Abdelfattah et al., 2018). Molecular techniques such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), short tandem repeats or simple sequence repeats have been used as alternatives to more traditional culturing methods (Abdelfattah et al., 2018). However, these methods are unable to provide sophisticated taxonomic information from complex environmental samples. Estimates indicate that more than 99% of the microorganisms present in the environment are not culturable and not accessible for research using these older techniques (Schmitz, 2004).

Current approaches for the analysis of microbial communities includes next generation sequencing. DNA barcoding was an important advancement in the molecular identification of microorganisms. DNA barcoding relies on short DNA fragments from a region of the organisms genome which are then sequenced to

identify different species (Abdelfattah et al., 2018). Clustering reads into OTUs has been the de facto standard for analysing DNA sequence reads since the early 2000's (Caruso et al., 2019). Many large scale projects such as the Human Microbiome Project, the Earth Microbiome project and the International Consensus of Marine Microbes have used the OTU classification method in an attempt to provide a catalogue of the global microbial and taxonomic functional diversity (Abdelfattah et al., 2018). Because the sequence differences are often assumed to be due to variation within the taxonomic group or due to random sequencer noise. This often results in more highly resolved taxa being missed during analysis, altering the perception of community composition (Youngblut et al., 2012).

Recently a new preferred method that resolves amplicon sequence variants (ASV's) has been developed that has the potential to improve on the specificity and sensitivity of 16S rRNA gene sequences compared to OTU's by avoiding assumptions about variation within taxonomic groups, a downfall of OTUs (Caruso et al., 2019). ASV's are said to capture all biological variation present in the data and those from a given data set can be easily reproduced and compared in future datasets (Callahan, 2017). A recent study conducted by Caruso et al., (2019) compared the sequence reads between two OTU methods and three ASV methods to assess their performance on high microbial biomass samples (see figure 6).

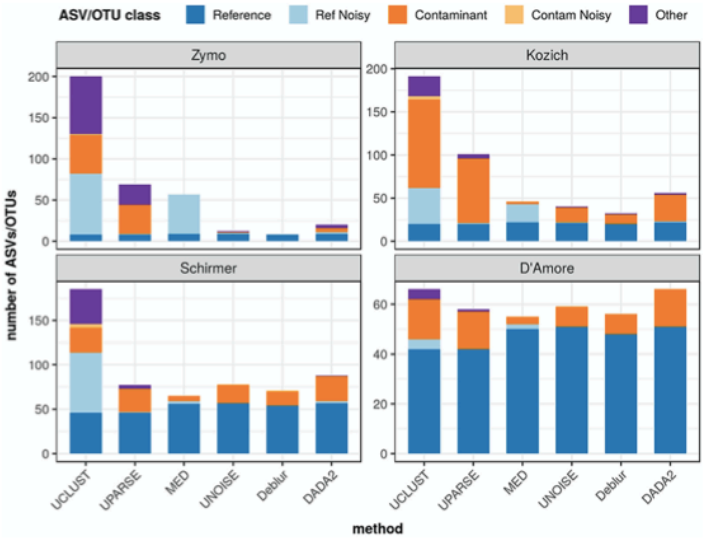

Figure 6: ASV/OTU classification of high biomass samples (Caruso, 2019).

Caruso et al., (2019) found that in general the ASV method outperformed the OTU method in particular when both methods were exposed to technical noise, ASVs were better at eliminating false positives, although they still occurred. A separate study conducted by Callahan et al., (2017) had the same conclusion. Both methods are not sufficiently able to distinguish the target communities from biological contamination, particularly when the sample comes from environments with low biomass (Caruso et al., 2019). The next challenge that needs to be addressed when

focusing on these techniques is more research for removing contamination to reduce the chances of false positives.

1.4 Literature Review Summary

Extreme environments contain more microbial diversity than first thought. However, the mechanisms that determine their community structure and how they respond to environmental stressors in these extreme conditions is not well understood. Although it is clear that some communities are able to recover from such events, as, recent studies have shown when microbial communities of Antarctica were exposed to shading and desiccation, mimicking conditions that have previously remained relatively stable in these environments. As the Earths weather becomes harsher and more unpredictable an obvious next question is: will large disturbances result in shifts in microbial community composition and will the new habitats created by environmental disturbances be recolonised by organisms previously inhabiting the environment or new ones.

To answer these questions, I will undertake a molecular based research project on meltwater ponds from Bratina Island located on the McMurdo Ice Shelf.

1.5 Hypotheses

Where disturbance creates a new habitat to be colonised, the new habitat will be colonised by organisms already abundant in the pond. The new colonists will likely reflect the resident populations within the microbial mats, resisting the turnover of species.

Selective mortality of organisms from vulnerable taxa due to stress events results in a shift in community composition, towards a more terrestrial mat.

1.6 Statement of Research Questions and Objectives

1. To describe how the microbial ecology of ponds New, AMS and P70 changes with environmental stressors

Objective: Use bioinformatics to determine how microbial diversity and abundance changes with desiccation and shading stress. This can be done by producing amplicon sequence variants (ASVs) to show the relative abundance of microbial taxa in cyanobacterial mat communities from ponds New, AMS and P70. Additional samples from each of the ponds at year zero of the experiment will be used as controls.

2. To describe the functional changes that occur within microbial communities in relation to environmental stressors

Objective: To use bioinformatics to track the functional changes within phyla of the microbial mat community. Samples collected in triplicate for the duration of the experiment that were not subjected to environmental manipulation from AMS, New and P70 ponds will be used as controls.

3. To demonstrate the resilience and resistance of the cyanobacterial mat communities within the three ponds

Objective: Analyse the changes in the microbial communities of cyanobacterial mats across all samples to assess whether the community possesses traits of resistance or resilience.

Chapter 2: Experimental design and methods

2.1 Introduction

The aim of this study is to explore the resilience and recovery of microbial communities from three ponds in Bratina Island (New, AMS and P70) that were exposed to stress in the form of shading and desiccation. Alongside these separate controls were taken from each pond for the duration of the experiment to account for natural variation within the microbial communities in the mats of each pond. The ponds in this study are meltwater ponds formed in glacial ablation zones where liquid water collects during the summer months (Safi, 2012; Vincent, 2003). Within the ponds, cyanobacterial mat communities dominate the benthic sediment areas. This study represents the first investigation of desiccation and shading stressors on the microbial ecology of these ponds. Previous studies of similar environments have shown that when cyanobacterial mat communities are exposed to environmental stressors such as desiccation and light stress, they tend to recover quickly all while maintaining their functional diversity (Chennu et al., 2015; Alpert, 2005; Hawes and Vincent, 1992; Quesada and Vincent, 2012).

To test the differences in community composition in response to treatment between the ponds, samples of cyanobacterial mat were collected for each treatment in triplicate for the duration of the experiment. DNA was extracted from the mat sample, the 16S rRNA gene was amplified via PCR, sequenced and processed using a bioinformatics pipeline in R Studio.

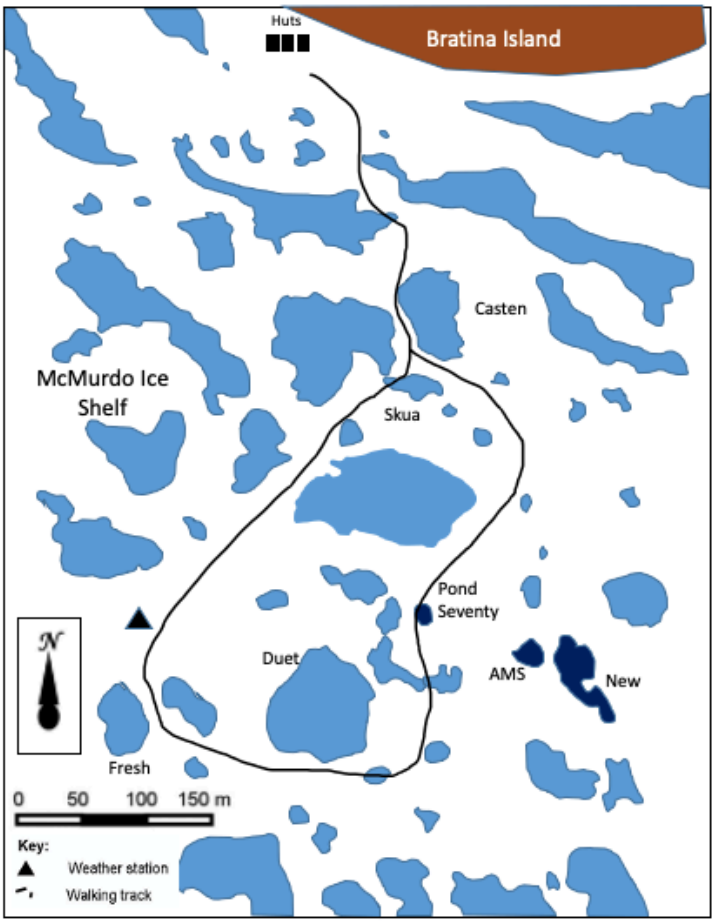

Figure 7: Location of Pond AMS, New and P70 on the Ross Ice Shelf. Ponds are coloured dark blue.

2.2.1 Site description, sample collection and experimental design

The experiment exploring the effects of shading and desiccation on cyanobacterial mat communities from three ponds on Bratina Island took place over three years from 2016 to 2018. Three treatments including shading, desiccation and control samples were taken from each of the three ponds, AMS, P70 and New over the duration of the experiment. These ponds were chosen because they are relatively similar in terms of their environmental conditions and the species composition of the microbial mats within each of the ponds. This allows any changes and observations made throughout the experiment to be compared between the ponds.

Figure 8: Ponds from left to right; AMS, New and P70

During year one of the experiment, control samples were collected from each of the three ponds in triplicate. Shades (cloth that allowed 50% of the light to pass through) were installed in each pond in triplicate to replicate environments of reduced ice transparency. Blocks of microbial mat were cut out and placed on the pond edge to replicate the desiccation of the microbial mat as the pond water level dropped.

Figure 9: Set up of experimental design for year zero. From left to right; Control plot, desiccation and shading.

During year two of the experiment, three treatments were sampled from each of the ponds. These were the sample controls, the shaded mats and the dried mats. The shaded samples were relocated to a second spot in each pond to allow the microbial community to recover from the shading acting as a pulsed stress mechanism. The sample from the previous year where shading blocks were removed became the recovery plots.

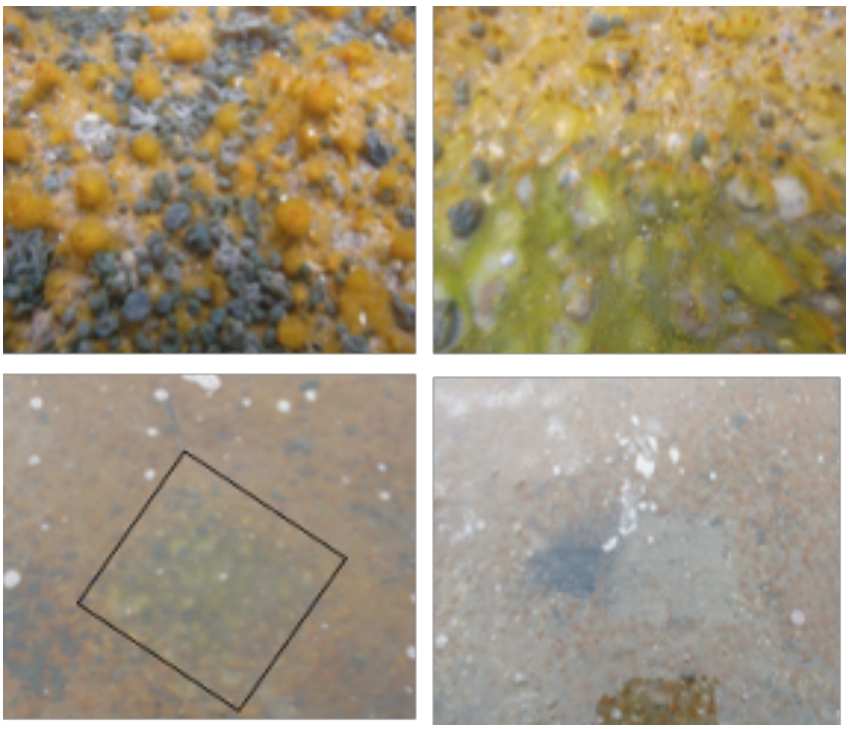

Figure 10: Photographs of experimental plots in AMS pond taken during year two of the experiment. Control plot (top left), shade vs non shade (top right), shade (bottom left) and cut sites for one year and one day (bottom right).

Figure 11: Photographs of experimental plots in New pond taken during year two of the experiment. Control plot (top left), cut site (top right), desiccation plot (bottom left) and shade plot (bottom right).

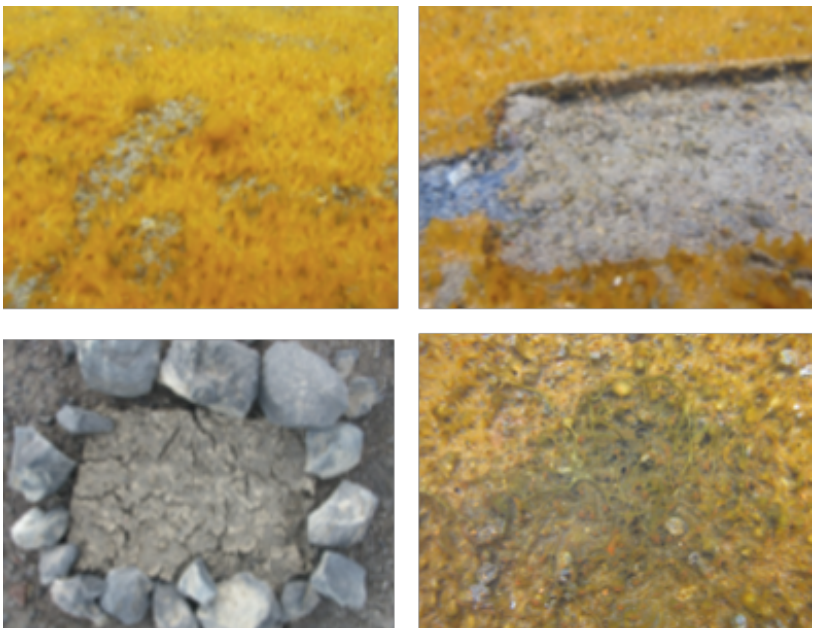

Figure 12: Photographs of experimental plots in P70 pond taken during year two of the experiment. Control plot (top left), cut sites from one year versus one day (top right), desiccation plot (bottom left) and the shading plot (bottom right).

During year three of the experiment there were four variables to be sampled. These were the sample controls, desiccation and shaded plots, and the new recovery plots for each pond. After the final year of the experiment, shade cloths and desiccation plots were removed to allow the microbial mat to return to its normal state. All samples were collected aseptically and preserved either by flash freezing or by flash freezing in Lifeguard.

Table 1: Sampling metadata including sample and sample ID, experimental year, treatment and pond.

Sample	Sample ID	Experimental Year	Treatment	Pond
AMS_2_T0	C1	0	Control	AMS
AMS_3_T0	C2			
AMS_control1_T1	C3	1	Control (in water)	
AMS_control2_T1	C4			
AMS_control3_T1	C5			
AMS_control1_T2	C6	2		
AMS_control2_T2	C7			
AMS_control3_T2	C8			
AMS_dry1_T1	D1	1	Desiccation	
AMS_dry2_T1	D2			
AMS_D1_T2	D3	2		
AMS_D2_T2	D4			
AMS_D3_T2	D5			

AMS_shade1_T1	S1	1	Shade	
AMS_shade2_T1	S2			
AMS_shade3_T1	S3			
AMS_shade1_T2	S4	2		
AMS_shade2_T2	S5			
AMS_shade3_T2	S6			
AMS_recovery1_T2	S7		Recovery	
AMS_recovery2_T2	S8			
New_2_T0	C1	0	Control	New
New_3_T0	C2			
New_control1_T1	C3	1	Control (in water)	
New_control2_T1	C4			
New_control3_T1	C5			
New_control1_T2	C6	2		
New_control2_T2	C7			
New_control3_T2	C8			
New_dry1_T1	D1	1	Desiccation	
New_dry2_T1	D2			
New_dry3_T1	D3			
New_D1_T2	D4	2		
New_D2_T2	D5			
New_D3_T2	D6			
New_shade1_T1	S1	1	Shade	
New_shade2_T1	S2			
New_shade3_T1	S3			
New_shade1_T2	S4	2		
New_shade2_T2_	S5			
New_shade3_T2	S6			
New_recovery1_T2	S7		Recovery	
New_recovery2_T2	S8			
P70_1_T0	C1	0	Control	P70
P70_2_T0	C2			
P70_3_T0	C3			
P70_control1_T1	C4	1	Control (in water)	
P70_control2_T1	C5			
P70_control3_T1	C6			
P70_control1_T2	C7	2		
P70_control2_T2	C8			
P70_control3_T2	C9			
P70_dry1_T1	D1	1	Desiccation	
P70_dry2_T1	D2			
P70_dry3_T1	D3			

P70_D2_T2	D4	2		
P70_D3_T2	D5			
P70_shade1_T1	S1	1	Shade	
P70_shade2_T1	S2			
P70_shade3_T1	S3			
P70_shade1_T2	S4	2		
P70_shade2_T2	S5			
P70_shade3_T2	S6			
P70_recovery1_T2	S7		Recovery	
P70_recovery2_T2	S8			
P70_recovery3_T2	S9			

2.2.2 DNA Extraction and Quantification

DNA was extracted in duplicate from each of the seventy-two samples, for each set of extractions a blank was also used in order to detect contamination which might occur from the reagents or between the samples being processed. The blank is carried through the entire process. 0.5 g of soil was weighed into a 1.5 ml polypropylene tube containing 0.5 g each of 0.1mm and 2.5 mm silica-zirconia beads. 270 µl of both phosphate buffer (Science lab Incorporated) and SDS lysis buffer (Science lab Incorporated) was added to each of the tubes. Samples were then placed on a bead beater for 15 secs followed by 10 min on the vortex genie. 180 µl of a working solution containing CTAB buffer (number of samples x 200 µl + 8 µl of 0.5% BME per 1 ml of CTAB (Science lab Incorporated)) was added to each of the samples, which were vortexed and then incubated at 300 rpm at 60°C for 30 mins.

After incubation, 350 µl of chloroform: isoamyl alcohol (24:1) (Science lab Incorporated). was added. The top aqueous layer was then transferred to a new sterile 1.5 ml tube followed by the addition of 500 µl of chloroform: isoamyl alcohol (24:1) (Science lab Incorporated). Samples are placed on a rocking bed for 20 min. The top aqueous layer was removed and placed in a new sterile 1.5 ml tube. 10M of ammonium acetate (Science lab Incorporated) to a final concentration of 2.5M was then added to each of the samples, again the top aqueous layer was transferred to a new sterile 1.5 ml tube. 0.54 times the recorded volume of isopropyl alcohol is added to the samples which were incubated at -20°C overnight.

After the incubation period samples were placed in a centrifuge at 13,000 rpm at 4°C of 20 min, the supernatant was discarded, and the pellet left behind. The pellet was washed with 1 ml of cold AR grade 70% ethanol (Remel, Lenexa, KS, USA), centrifuged and the ethanol pipetted off. Samples were then dried in a speed

vacuum for approximately 4-10 minutes on manual, medium drying depending on the nature of the individual samples. When the pellet was completely dry, and all the residual ethanol had evaporated the DNA was pooled into corresponding duplicates. The DNA was then re-suspended in 20 µl sterile LO-TE by pipetting up and down and scaping the pipette tip on the side of the microfuge tube.

DNA was quantified using the Qubit Fluorometer high sensitivity dsDNA protocol (as per manufacturer's instructions). 2µl of DNA from each sample was loaded on the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Massachusetts, USA) and the Denovix DS-11 pectrophotometer/ fluorometer (Denovix Incorporated, Delaware, USA) and the average DNA in ng/µl was recorded.

2.2.3 PCR and Sequencing

The V4 region of the 16S rRNA gene was amplified in triplicate reactions using the IonXpress primers 515F (5'CCATCTCATCCCTGCGTGTCTCCGACTCAGXXXXXXXXXXXXGATGTGCCAGCMGCCGCGGTAA'3) and 926R (5'CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATCCGYCAATTYMTTTRAGTTT '3). One forward primer with a specific barcode at the end is used per sample to uniquely identify it.
PCR master mix per triplicate reaction consisted of: H2O, 0.4 mg/ml BSA, 2 mM dNTPs, 10x PCR buffer, 50 mM MgCl2, 10 mM forward primer, 10 mM reverse primer, 5U/ µl Platnium Taq and 1 ng/µl of DNA.

The Proflex PCR system (Thermo Fisher Scientific) was used to amplify the 16S rRNA amplicons. The PCR protocol was as followed; Initial denaturation was achieved at 94°C for 3 min, followed by a second denaturation at 94°C for 45 seconds. Annealing was undertaken at 50°C for 1 min, extension at 72°C for 1 min 30 seconds and a final extension at 72°C for 10 min. These conditions ran for a total of 30 PCR cycles. Once completed, triplicates were held at 4°C, until they were removed. To confirm amplification of the PCR product before sequencing proceeded, amplicon bands were visualised by gel electrophoresis using a 1% Agarose medium using the Alpha Innotech imaging system (Alpha Innotech, California, USA).

Triplicate PCR products from each sample were pooled. A total of 25 µl of each sample was treated with Invitrogen SequalPrep Normalization (Thermo Fisher Scientific) to normalize the PCR products, remove DNA fragments smaller than 100 base pairs and to purify the samples. 2 µl of each PCR triplicate was pooled together to generate the sequence library. The sequence library was quantified

using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) to determine the concentration of DNA in ng/µl. The number of amplifiable molecules was determined and treated with Ion PGM™ Template IA 500 Kit (Thermo Fisher Scientific). Ion PGM™ Hi-Q™ View Sequencing Kit (Thermo Fisher Scientific) was then used to prepare the library for sequencing. The library was added to an Ion 318 Chip Kit v2 BC (Thermo Fisher Scientific). Samples were sequenced on an Ion Torrent PGM (Thermo Fisher Scientific).

2.2.4 DNA sequence processing

Raw data reads obtained for each sample from the Ion Torrent PGM (Thermo Fisher Scientific) were processed using the DADA2 pipeline (v 1.16) which utilises functions in R studio. Quality profiles of the sequence reads were inspected. Sequences were trimmed and filtered according to the recommendations for Ion Torrent PGM and the error rates for each sample were examined. The DADA2 algorithm inferred 1191 sequence variants from 5159 input unique sequences. Taxonomy was assigned to ASVs using the RDP classifier against the SILVA 16S rRNA database (138.1), an ASV count table and taxonomy table was generated for each sample and exported as tsv files for analysis.

2.2.5 Statistical Analyses Methods

All statistical analyses and visualisations were performed in R (v4.0.1) using Phyloseq (v1.33), ggplot (v3.3.1) and Vegan (v2.5) packages. Taxonomy was assigned to ASVs using the RDP classifier against the SILVA 16S rRNA database (138.1) with a minimum confidence score of 0.8 (80%). A phyloseq object was then created containing the sample information, taxonomy table, ASV counts and a phylogenetic tree which was used throughout R to analyse the data. Sample counts were transformed to relative abundance using the transform_sample_counts function in Phyloseq. Relative abundance of the top ten most abundant phyla for each sample was then presented as stacked bar graphs for each pond.

Alpha diversity metrics were calculated using the plot_richness function in the phyloseq package and plotted as a graph using ggplot2. The richness shown as Observed ASVs and evenness shown as Shannon diversity index were plotted for each sample across the ponds. Beta diversity using the plot_ordination function in phyloseq was used to calculate Bray-Curtis dissimilarity and non-metric multidimensional scaling to ordinate samples in space. Next a multidimensional scaling principle coordinate analysis was simultaneously performed alongside a weighted UniFrac analysis to visualise similarities and dissimilarities between

treatments across ponds and treatments within ponds. The MDS (PCoA) weighted UniFrac was then used in conjunction with the ANOSIM (analysis of similarities) to calculate an R value and a significance value to test the differences between the microbial communities in this study. A significance value less than 0.05 is considered to be statistically significant. An R value close to 1.0 suggests dissimilarity between tested groups.

To determine functional changes in the microbial community 16S rRNA sequences were run through a bioinformatics software package known as PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2) to predict gene families for microorganisms using their sequences relatives as a reference. Gene predictions are given as a KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway references which correspond to a set of genes. The counts for each sample were transformed to relative abundance using Microsoft excel.

2.3 Results: Microbial Community Composition

2.3.1 Results: Microbial Community Composition

To understand the microbial mat community composition of ponds AMS, New and P70, total DNA from the sampled mats was extracted and sequenced using 16S rRNA primers (see section 2.2.4). Downstream analysis of the 16S rRNA gene was performed in order to produce an ASV table. After ASV sequences were assigned to taxonomy, the mean relative abundance (0.1%) was calculated to show diversity. This was also used for alpha and beta diversity measures.

The community composition of the ponds AMS, New and P70 is displayed (Figures 12, 13 and 14) and the most abundant phyla (n=10, > 0.1% average relative abundance) in the entire ASV dataset were: *Actinobacteria, Bacteroidota, Cyanobacteria, Desulfobacteria, Firmicutes, Gemmatimonadota, Myxococcota, Planctomycetota*, *Proteobacteria* and *Verrucomicrobiota.* Across the ponds and treatments, *Bacteroidota, Proteobacteria, Verrucomicrobiota* and *Cyanobacteria* display the highest abundance of all the phyla.

Differences in community composition are more noticeable between ponds with the same treatments, than between treatments in the same pond. P70 pond has a larger abundance of cyanobacteria in desiccation samples compared to those in New and AMS pond. *Firmicutes* is more prevalent across all samples in AMS and P70 ponds, whereas *Planctomycetota* is most prevalent across samples in New pond.

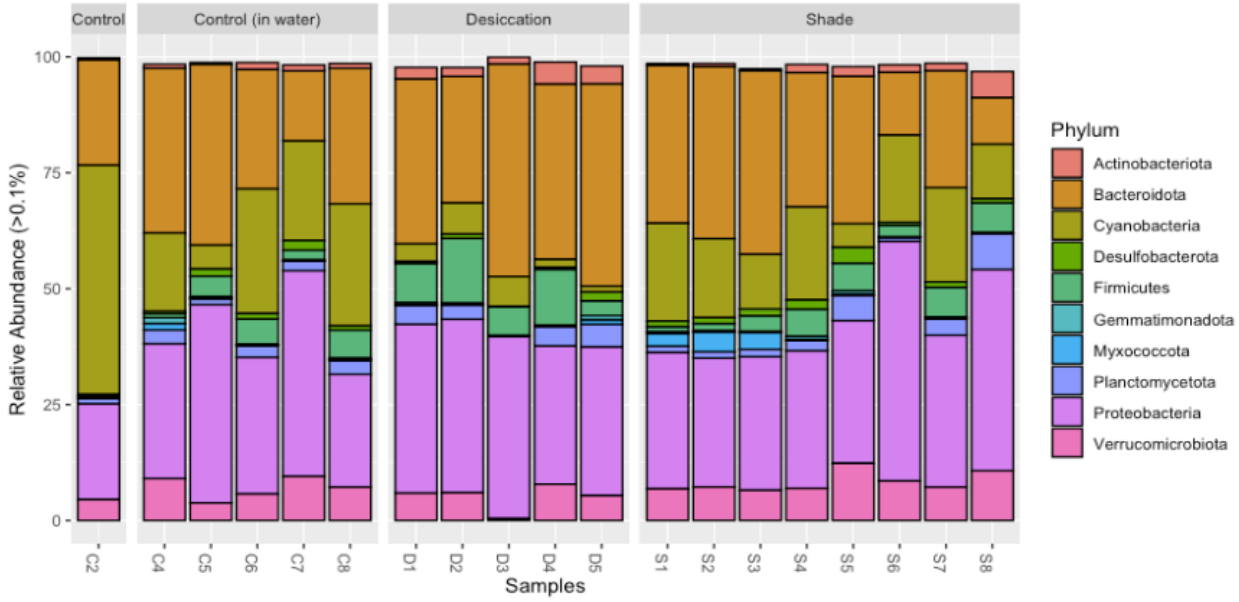

Figure 13: Bar graph of ten most abundant phyla in AMS pond, displayed per treatment. Community compositions are split according to treatment (control, control (in water), desiccation and shade, shown as biological triplicates) and year (0, 1 or 2), refer to table 1 for sample names. Relative abundance is based on bacterial and archaeal ASVs of the V4 region from 16S rRNA gene sequencing grouped into respective phyla.

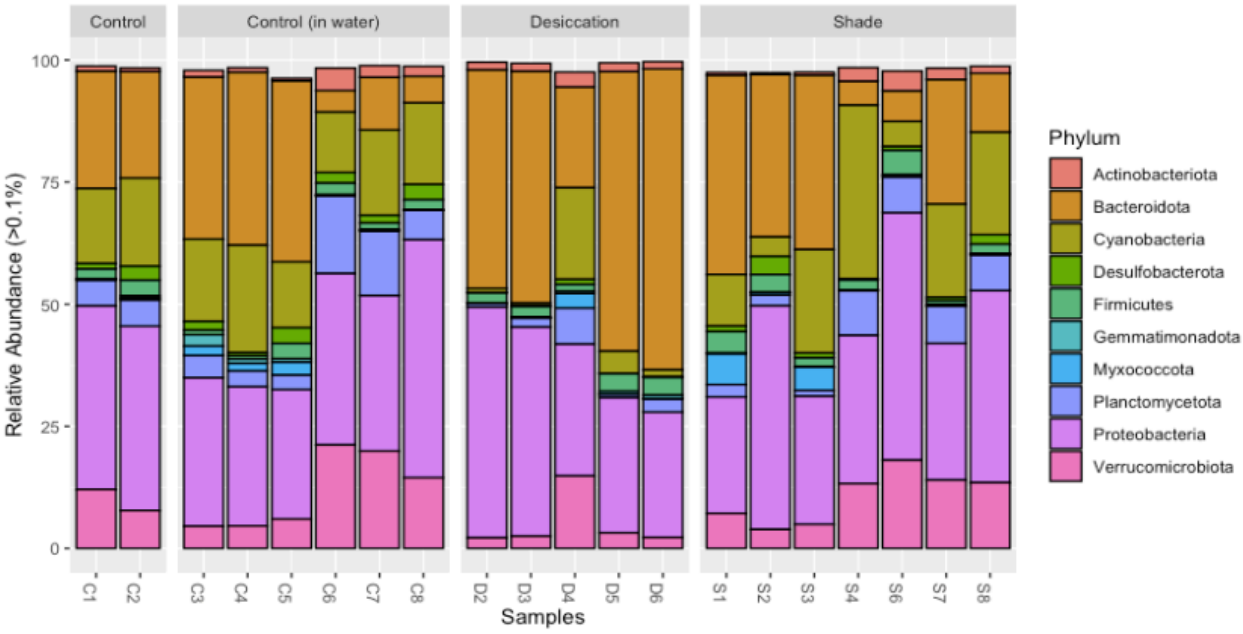

Figure 14: Bar graph of top ten most abundant phyla in New pond, displayed per treatment. Community compositions are split according to treatment (control, control (in water), desiccation and shade, shown as biological triplicates) and year (0, 1 or 2), refer to table 1 for sample names. Relative abundance is based on bacterial and archaeal ASVs of the V4 region from 16S rRNA gene sequencing grouped into respective phyla.

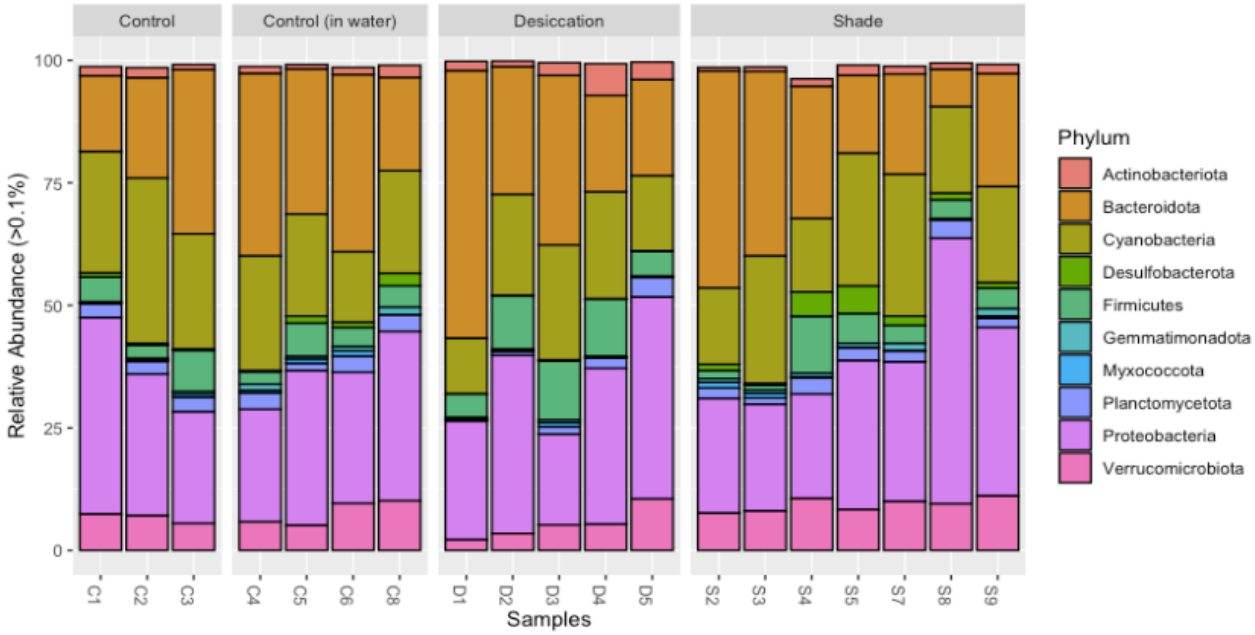

Figure 15: Bar graph of top ten most abundant phyla in P70 pond, displayed per treatment. Community compositions are split according to treatment (control, control (in water), desiccation and shade, shown as biological triplicates) and year (0, 1 or 2), refer to table 1 for sample names. Relative abundance is based on bacterial and archaeal ASVs of the V4 region from 16S rRNA gene sequencing grouped into respective phyla.

To examine the diversity between treatments within the same pond, alpha diversity metrics (Shannon diversity index and Observed ASV richness) were calculated using the original dataset of ASVs. No filtering was applied to the sequence data before alpha diversity indices were measured. The main trend for ASV richness (Figure 15) is that in each pond, ASV richness is lower in samples that underwent desiccation stress. Shannon diversity index (H) values are generally higher in the shading and in water controls in each pond indicating that increased diversity and evenness exist in these treatments compared to the others. Values for the control samples are more variable between ponds (P70, New, AMS).

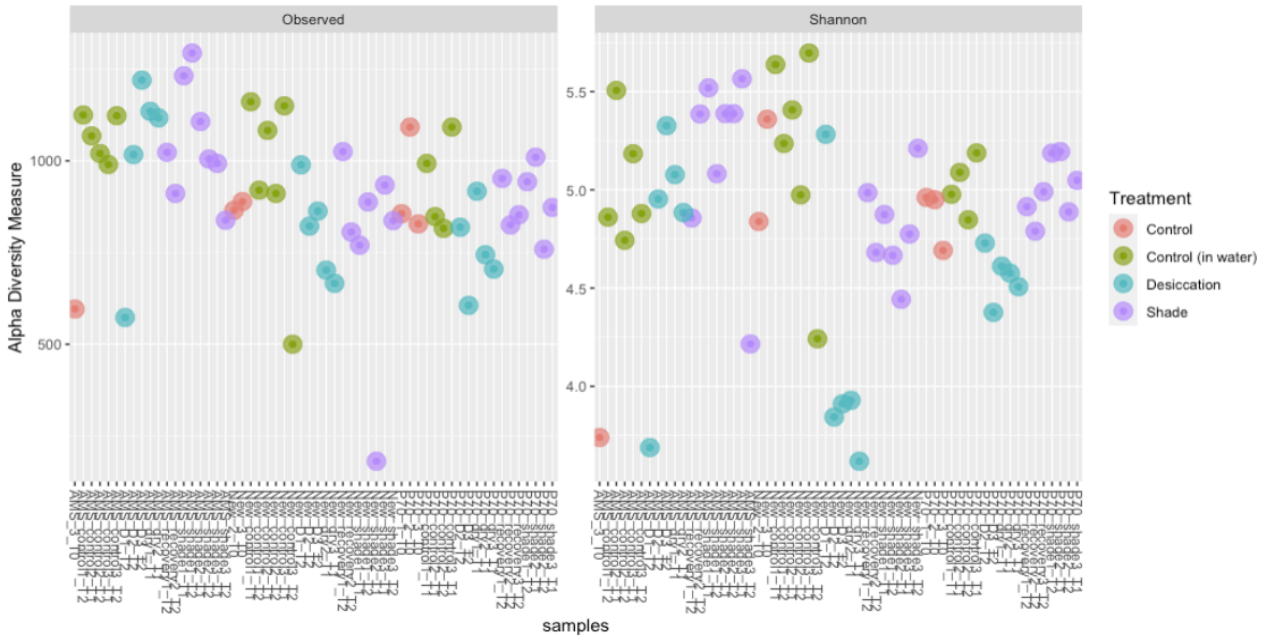

Figure 16: Alpha diversity measures of samples. Diversity is shown by both ASV richness (Observed ASVs) and evenness (Shannon diversity index, H) for each pond and treatment. Samples are coloured according to the treatment they received.

To determine if there were compositional differences between the sample sites (beta diversity), Bray-Curtis dissimilarity was calculated. To visualise Bray-Curtis dissimilarity in space, a NMDS (Non-metric multidimensional scale) ordination plot was used (Figure 16). This shows the clustering (or similarity) of ponds and treatments. The NMDS plot shows that samples from the same pond tend to group in space, meaning they have similar beta diversity values (or similar community composition). Samples also tend to cluster by treatment (shown by polygons). Overall beta diversity is similar between ponds indicating that the ratio of diversity at each site is similar. All statistical methods are shown in section 2.2.5.

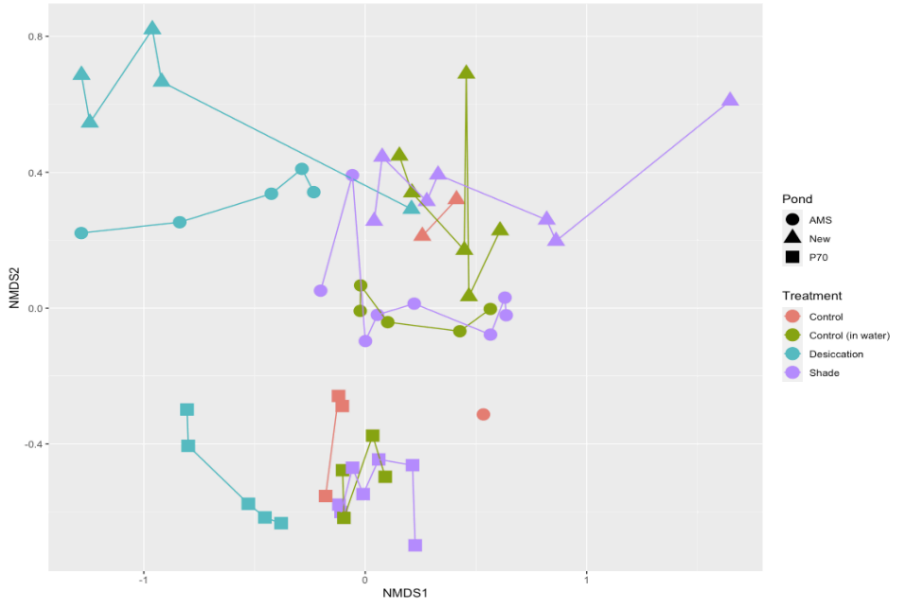

Figure 17: Beta diversity of all mat sample communities, calculated using Bray-Curtis dissimilarity and non-metric multidimensional scaling to ordinate samples in space. No filtering of ASVs was applied to the data prior to calculating Bray-Curtis dissimilarities. Samples are coloured according to the treatment they received (control, shading, desiccation and controls (in water)). Samples are clustered by site using polygons.

To examine whether the differences observed in community composition were statistically significant in treatments across ponds a PCoA analysis was performed alongside the ANOSIM analysis (Figure 17). Ponds of significance were AMS and New which had significance values less than 0.05 indicating there is a significant difference between the microbial groups found across the treatments within these ponds. P70 had a significance value higher than 0.05 indicating that there was no significant different in the microbial communities for each of the treatments.

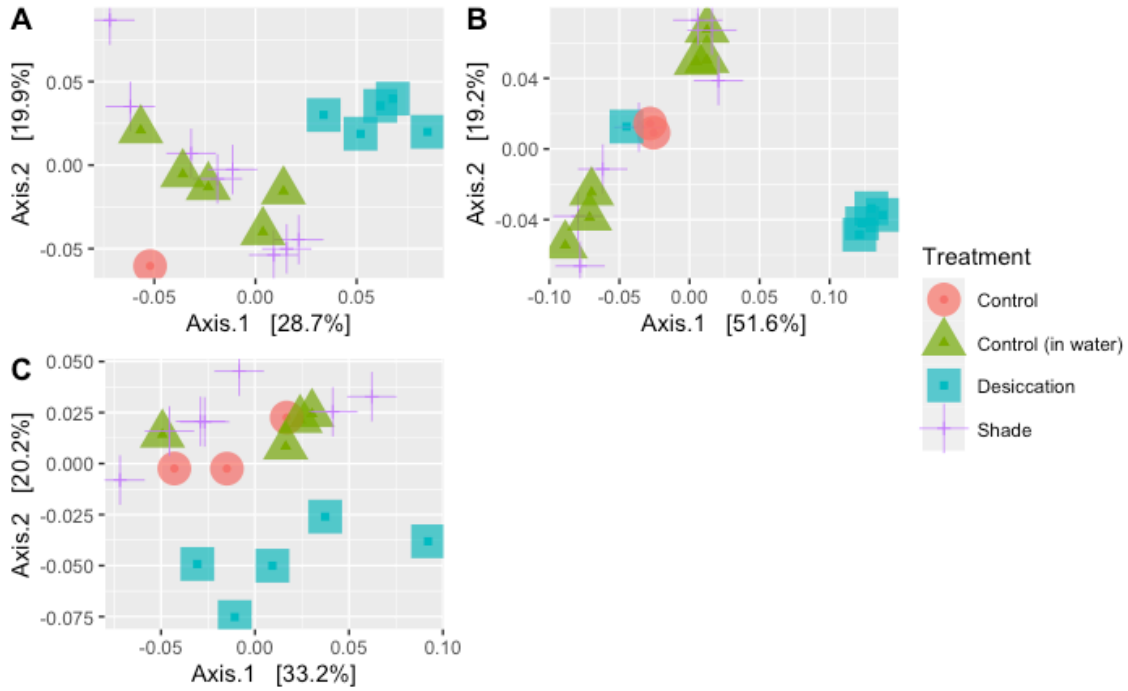

Figure 18: Principle coordinates analysis (PCoA) of beta diversity of treatments within ponds. (A) AMS (B) New, (C) P70. AMS (0.001) and New (0.01) had statistically significant significance values.

To examine whether the differences observed in community composition were statistically significant across treatments within ponds a PCoA analysis was performed alongside the ANOSIM analysis (Figure 18). Treatments of significance include the control, desiccation and shading samples indicating that difference in community composition between the treatments in the different ponds was significant. There was no significant difference in community composition for the control (in water) treatment.

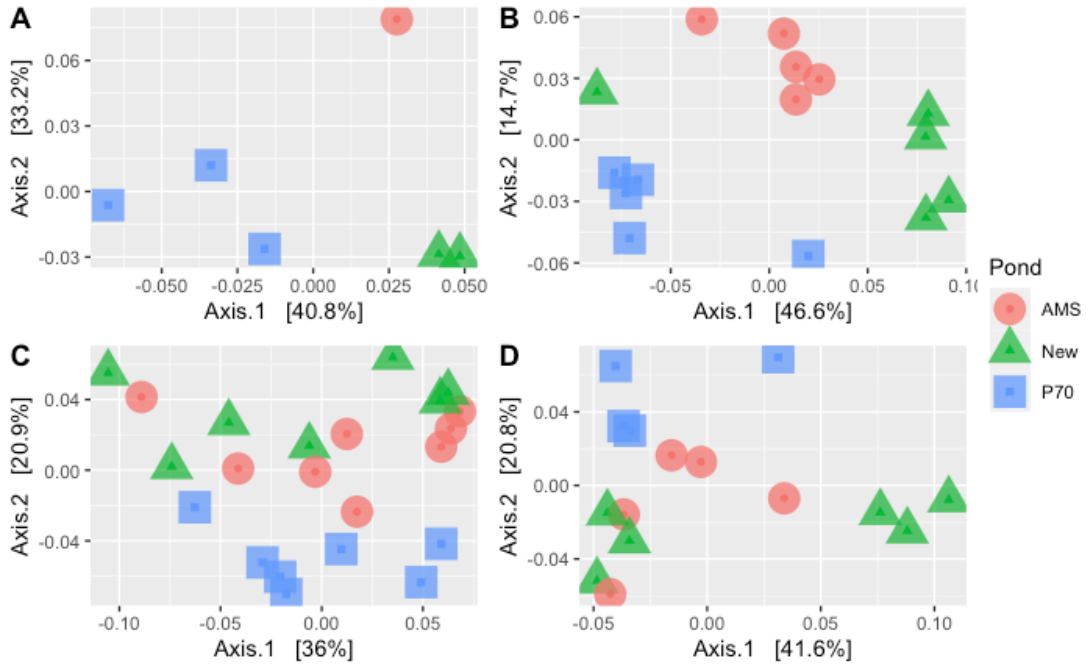

Figure 19: Principle coordinates analysis (PCoA) of beta diversity of treatments across ponds. (A) Control samples, (B) Desiccation samples, (C) Shading samples, (D) Control (in water) samples. Control (0.033), Desiccation (0.01) and Shading (0.001) samples had statistically significant significance values.

2.3.2 Results: Predicting microbial functional changes

This section will investigate whether a shift in microbial composition across treatments and ponds will result in a change in function, or whether functionality is maintained throughout treatments and ponds. To answer this 16S rRNA sequences were analysed using a bioinformatics software package known as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), which infers the functional profile of a microbial community based on the diversity and abundance of a phylogenetic marker gene. Functional changes relating to photosynthesis, carbon dioxide fixation and methanogenesis will be investigated through the microbial communities in the three ponds (AMS, New and P70) as these microbial processes are likely to be affected by shading and desiccation stress.

To investigate the effects of shading and desiccation on community functional changes, genes involved in photosynthesis were examined. Genes encoding photosystem I and II were examined, however, in all samples across all ponds there was an absence of genes present for photosystem I. Figures 19, 20 and 21 show the predicted relative abundance of genes encoding for the chlorophyll apoprotein, reaction center D2 protein and reaction center D1 protein in photosystem II for

each sample, in each pond. The genes were predicted to be most abundant in the control samples AMS pond, compared to the other ponds where the gene abundance was more evenly distributed across a number of samples including the controls, recovery and desiccation (see figures 18 and 19). In AMS pond the gene was least abundant in year 2 of the desiccation samples and year 1 of the desiccation samples in New pond.

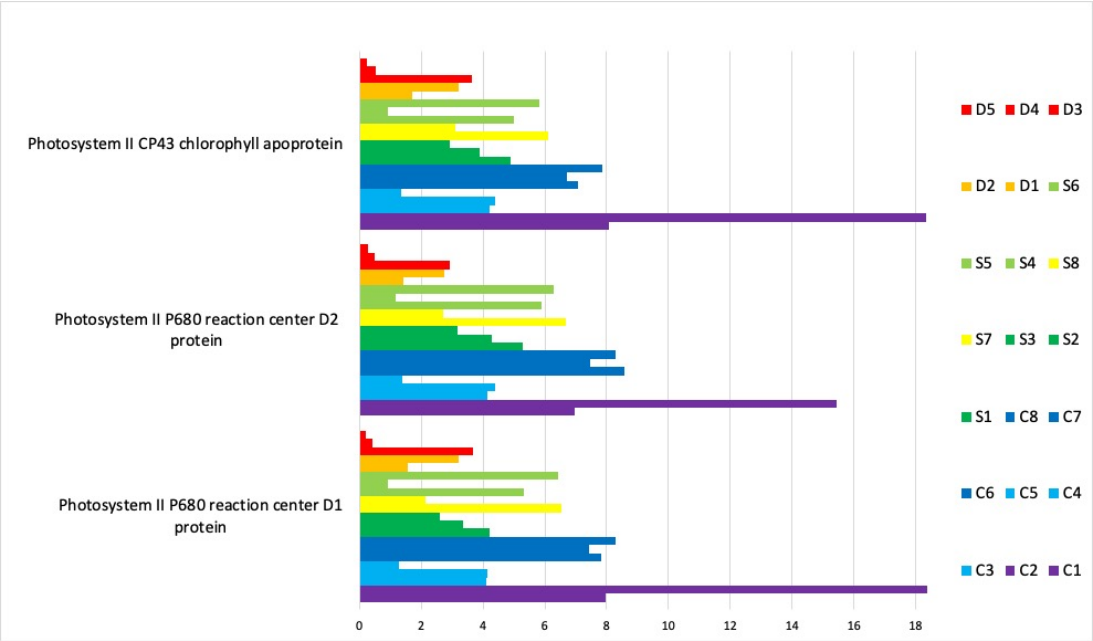

Figure 20: Predicted relative abundance of gene sets in photosystem II, AMS Pond.

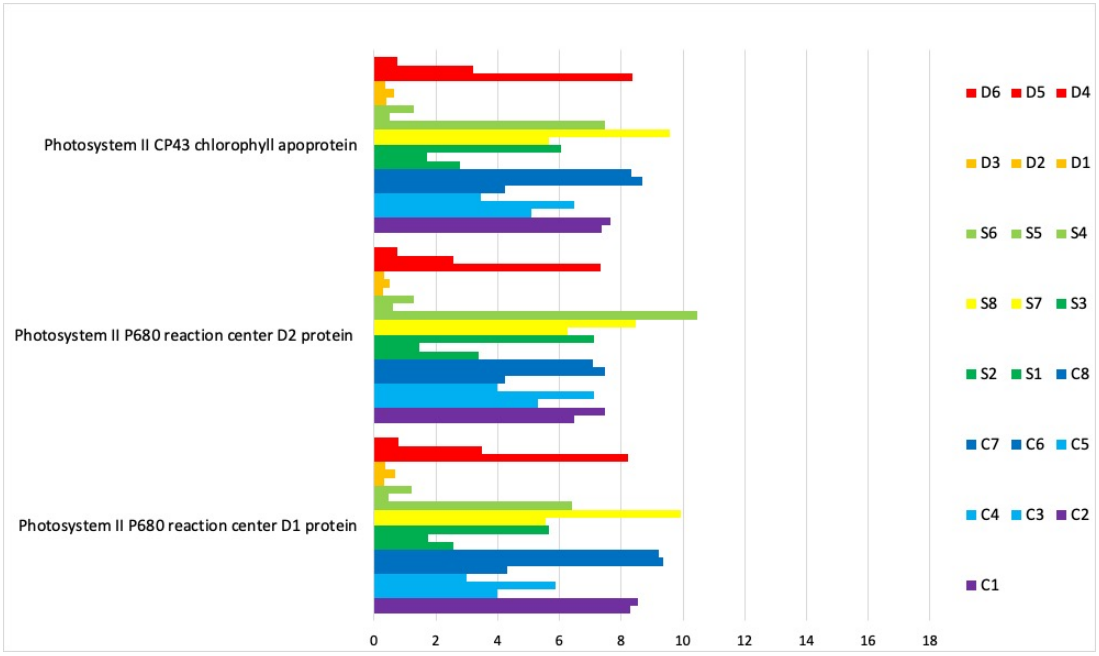

Figure 21: Predicted relative abundance of gene sets in photosystem II, New Pond

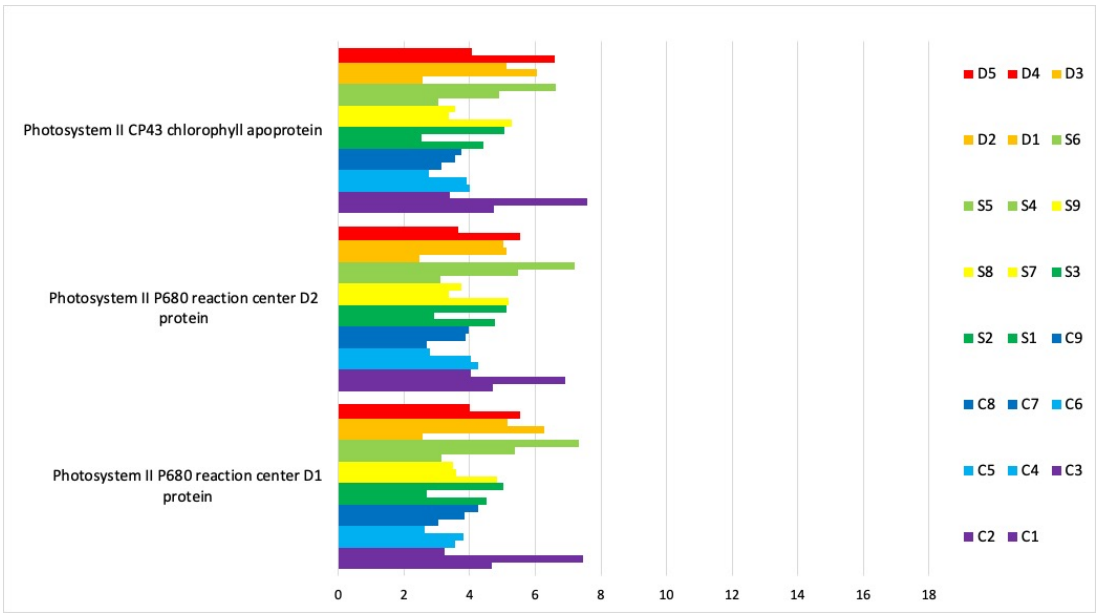

Figure 22: Predicted relative abundance of gene sets in photosystem II, P70 Pond.

To investigate the effects of shading and desiccation on CO_2 fixation via the Calvin cycle, three genes involved in the Calvin cycle were examined (Figures 22, 23 and 24). The gene encoding for phosphoribulokinase has the highest predicted abundance in the control (year 0) and shading (year 1) samples across all three ponds and was least abundant in all of the desiccation samples (both year 1 and 2). The predicted abundance of transketolase and phosphoglycerate kinase remained relatively consistent throughout all ponds and samples.

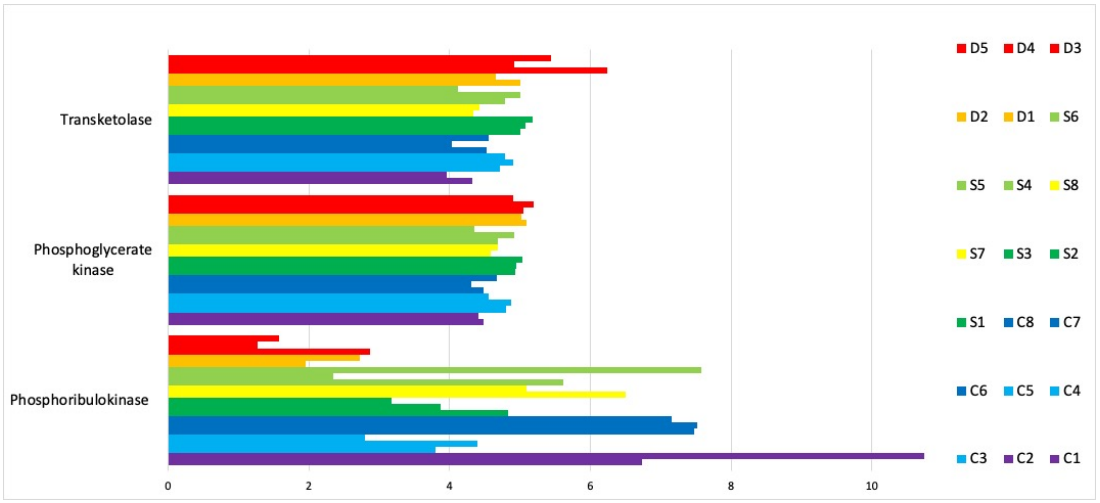

Figure 23: Predicted relative abundance of gene sets in the Calvin cycle, AMS pond.

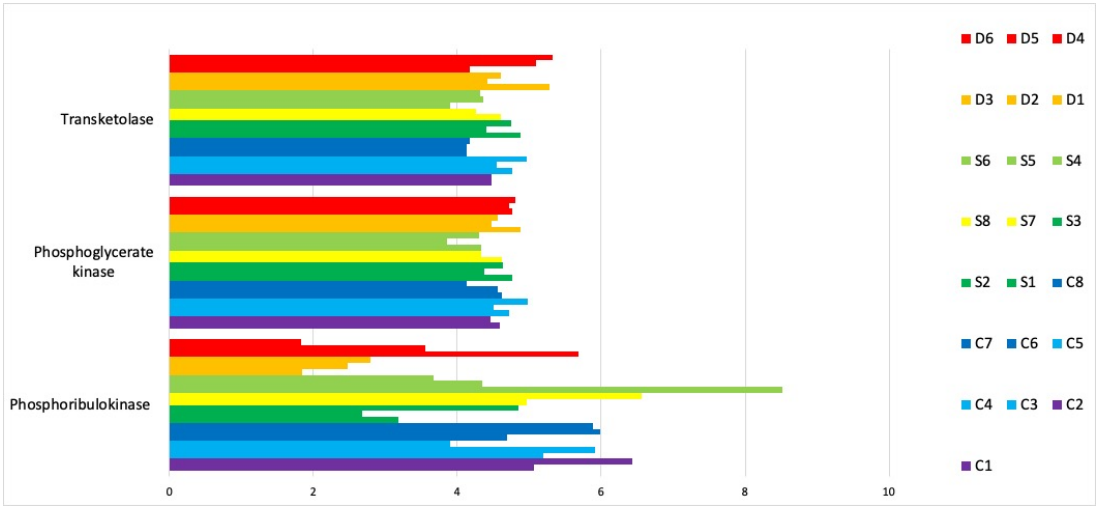

Figure 24: Predicted relative abundance of gene sets in the Calvin cycle, New pond.

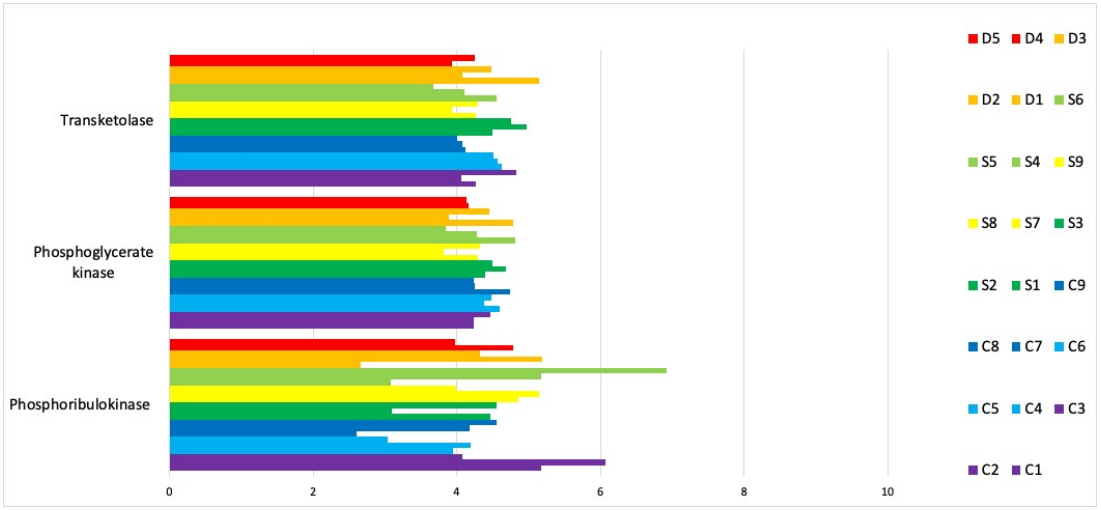

Figure 25: Predicted relative abundance of gene sets in the Calvin cycle, P70 pond.

Next, the effects of shading and desiccation on methanogenesis were examined (Figure 25). The methyl coenzyme M reductase (Mcr) gene was only predicted to be present in AMS Pond in some of the samples. The Mcr gene was predicted to be in the highest abundance in the desiccation (year 1) samples and was completely absent in the control samples.

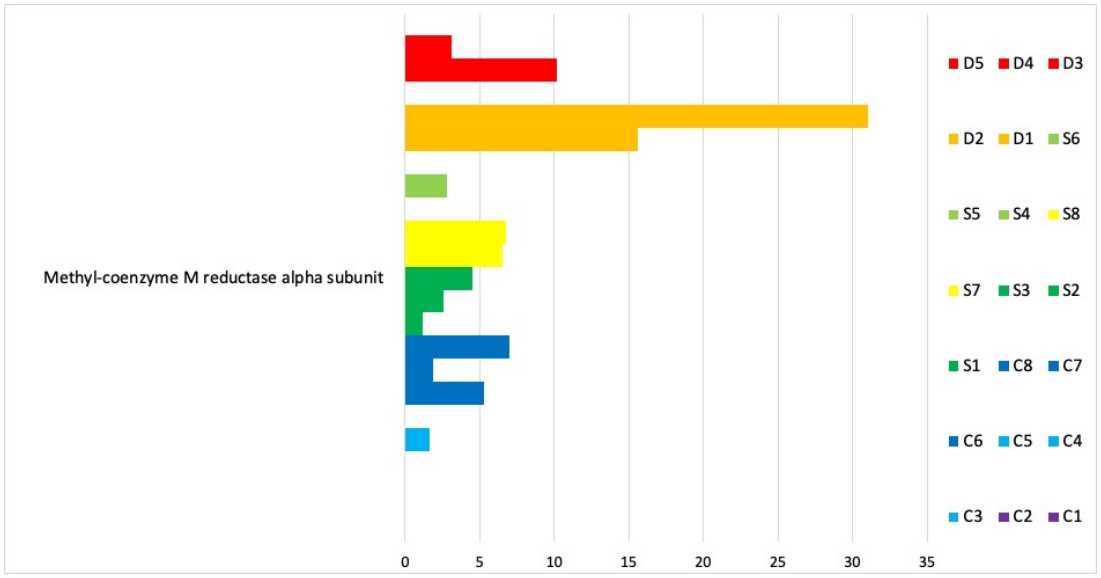

Figure 26: Predicted relative abundance of gene sets used in methanogenesis. Note that this gene was only found in AMS pond.

Chapter 3: Discussion and conclusions

3.1 Microbial community composition and functional changes

The most abundant phyla observed across all samples and ponds in decreasing abundance were *Proteobacteria* (66.9 %), *Bacteroidota* (58.3 %), *Cyanobacteria* (34 %), *Verrucomicrobiota* (14 %) *Firmicutes* (8.9 %), *Planctomycetota* (7.2 %), *Actinobacteria* (3 %), *Desulfobacterota* (2.5 %), *Myxococcota* (1.5 %) *and Gemmatimonadota* (1 %). These phyla are commonly detected in microbial mat communities (see Figure 4), especially those from the Antarctic region (Seckbach and Oren, 2010). Others that were identified but weren't at any significant abundance included *Acidobacteriota, Bdellovibrionota, Chloroflexi, Patescibacteria and Spirochaetota*. Of the detected phyla the Cyanobacteria, Actinobacteria and Firmicutes possess adaptations to extreme environmental conditions such as desiccation, high salinities and UV radiation and/or carryout oxygenic photosynthesis allowing them to colonise a wide range of habitats (Hedges, 2009). A low abundance of methanogenic archaea was also found in AMS pond (supported by figure 21).

Although there appears to be some changes within the microbial communities of meltwater ponds on Bratina Island, there is no definitive answer to whether desiccation and shading were solely responsible for these changes. The response of microbes to environmental stressors is often due to a combination of factors making it hard to determine the exact cause and magnitude of change within a microbial community (Logue and Langenheder, 2015). The microbial communities of ponds New, AMS and P70 are dominated by three major phyla: *Proteobacteria, Bacteroidota* and *Cyanobacteria*. With the effects of shading and desiccation in mind, it is interesting to note that the relative abundance of Cyanobacteria decreases across the desiccation samples in AMS and New ponds (supported by figure 17) compared to their respective controls, and remains at a constant level of abundance throughout all samples in P70 pond. Cyanobacteria are the dominant primary producers in the Bratina Island meltwater system and exhibit many adaptations to combat desiccation, such as the secretion of extracellular polysaccharides and flagellar mobility allowing them to move throughout the mat (Karsten, 2013), so this is somewhat unexpected. However, the actual abundance of the cyanobacteria may not have changed just that increases in other phyla may have causes the relative abundance of the cyanobacteria to change.

Further investigation into the desiccation samples revealed that ponds AMS and New are almost solely dominated by *Oscillatoria* while P70 is dominated by a combination of *Oscillatoria, Phormidium* and *Nostocales*. Numerous studies across

varying regions of Antarctica agree that *Oscillatoria* are one of the most dominant orders of Cyanobacteria (Pearl, 2000; Pessi et al., 2018; Martiny, 2008). While *Nostocales*, is found less frequently. The decrease in the relative abundance of Cyanobacteria across the desiccation samples described above could be due to a number of factors. Extracellular polymeric substances secreted by some species of *Nostocales* have a negative effect on cell lysis (Pessi et al., 2018), potentially inhibiting their detection in molecular studies such as those conducted in this study. It is also known that low levels of phosphorus available to microbial communities also negatively impacts *Nostocales* as nitrogen fixation activity decreases (Pessi et al., 2018). Some species of *Nostocales* form akinetes which are resistant to cold and desiccation (Sufkenik, 2019), and could explain why the desiccation samples in P70 pond maintain the relative abundance of Cyanobacteria compared to ponds AMS and New. While investigating the *Bacteroidota* phyla revealed that *Flavobacteria* and *Chitinophagia* remain equally dominant across all ponds and samples exhibiting little to no change in their abundance with shading and desiccation. Similar to this pattern, the majority of the Proteobacteria phylum, comprised of two families; *Rhodobacteraceae* and *Comamonadaceae*, have relative abundances that also remain consistent across ponds and samples indicating these phyla appear to be somewhat more resistant to desiccation and shading then Cyanobacteria.

Recovery samples were included in the shading data set to investigate whether the microbial communities in AMS, New and P70 pond were resilient to shading. Recovery samples of ponds New and P70 reflect what is seen in the control samples (Figure 13 and 14) while the recovery samples of AMS pond do not reflect what is seen in the control sample (Figure 12). These recovery samples show a higher relative abundance of *Bacteroidota*, and a much lower abundance of Cyanobacteria compared to the control sample. This could be due to only having one control sample for AMS pond or a combination of other factors specific to this pond that were not investigated.

To determine if shading and desiccation had an effect on the functionality of the microbial communities present in the meltwater ponds of Bratina island, PICRUSt was used to predict gene sets linked to metabolic capacities in photosystem II, the Calvin cycle and methanogenesis. These pathways were chosen because they were deemed the most likely to change with shading and desiccation manipulations, given that the Antarctic meltwater pond ecosystem is driven by the primary productivity of cyanobacteria. The three genes involved in photosystem II all decrease in predicted abundance in the desiccation samples of ponds AMS and New but remain constant across the samples in p70 pond. The control samples of AMS pond have an unusually high predicted abundance of all three genes; chlorophyll apoprotein and D1 and D2 reaction centres in comparison to all other

samples. Furthermore, shading and desiccation had no obvious predicted effect on the gene sets involved in the Calvin cycle. The relative predicted abundance of all genes remained constant across all ponds and samples except for the recovery samples which appeared to have a predicted abundance slightly higher than their experimental counter parts (see figures 22, 23, 24). These trends are reflections of those for the relative abundance of Cyanobacteria (see figures 12, 13, 14), as explained in the previous paragraphs.

The last gene set investigated was the Mcr gene involved in methanogenesis. This gene is present in all methanogens but was only predicted to be present in AMS pond, indicating that there are potentially metabolically active Archaea in at least one pond (see figure 25). The Mcr gene was predicted to be most abundant in the first-year desiccation samples and absent in the controls. It was also predicted to be present in other samples within the AMS pond at low abundance. However, when examining archaeal community structure, it is clear that methanogens were also present in New and P70 ponds. Although detected (see supplementary), they were a very minor component of the microbial communities in the meltwater ponds. It is possible that their detection was poor due to the choice of region for amplification, and perhaps a more specific primer set for archaea would have resulted in a much higher detection rate.

Changes in these microbial communities may be more subtle than first thought. While the diversity in the ponds was relatively similar, with the only obvious differences seen in the desiccation samples, there was a significant difference in the microbial communities both across treatments but within the same ponds and in treatments across ponds (Figures 17 and 18). Overall, this study has shown that at least three phyla, Cyanobacteria, Proteobacteria and Bacteroidota are resistant and/or resilient to the environmental stressors used in this research (figures 12, 13, 14). This is perhaps not surprising as many other studies have shown these phyla to be dominant in a variety of environments (Cowan, 2003; Archer et al., 2014; Archer et al., 2015). However, it is possible that changes in the microbial communities of ponds AMS, New and P70 were not able to be detected by 16S rRNA amplification alone and further research is needed to fully understand the responses of those communities to shading and desiccation. It also remains to be determined if the same outcomes would be seen if experiment was carried out for a longer period of time.

3.2 Research Significance

This study is the first investigation of the microbial ecology of the cyanobacterial mats of ponds New, AMS and P70 ponds on Bratina Island. It gives a preliminary insight into the composition and functional capabilities of the microbial communities present in these ponds. This research indicates the resilience and resistance of some bacteria to shading and desiccation, which has potentially evolved over many years adapting to regular exposure to these stresses. This research also corroborates other studies from Antarctica which have investigated the microbial communities from other meltwater ponds.

3.3 Future Directions

There are several limitations of this research. Firstly, the microbial community composition of the meltwater ponds was only examined using primers to target the V4 region 16S rRNA gene. This meant that only the bacterial and archaeal composition of the microbial mats was combined, resulting in a poor detection of archaeal phyla. For a more extensive investigation, primers targeting the V1 and V2 sub regions of the 16S rRNA gene could have been used to get a better understanding of how Archaea are affected by shading and desiccation as suggested in previous research (Bahram et al., 2019). Eukaryote diversity using 18S rRNA could also be investigated, as was initially intended in this study but was dropped due to shortened research time, so that the overall results are reflective of the changes in community composition across all three domains and not confined to one domain.

Additionally, the length of the experimental manipulation of desiccation and shading as environmental stressors may not be long enough to see any significant changes in community composition. Antarctica exhibits a short growing season with summer typically lasting from November to February, so a two to three year experiment may not have been long enough given the short growth season. Previous studies using environmental manipulations in Antarctica have typically conducted their experiments for the same duration as this research, so this is a limitation for numerous Antarctic studies that needs to be addressed in the future.

It may also be important to investigate the physicochemical properties of each pond. This will give new insights into the similarities and differences in each of the ponds and may provide a greater understanding of patterns in microbial community composition. Also, the use of SILVA software means that taxonomic classifications of the 16S rRNA sequences from the samples at Bratina Island are only accurate to the genus level, and if there were any significant changes within phyla at the species level they would have been overlooked.

In future studies, the addition of metagenomics and transcriptomics should be used to investigate the function of a community instead of using a bioinformatics software programme such as PICRUSt which relies on predicting metagenome function. The use of metagenomes would give a more accurate understanding of the functional capability of the microbial communities in the three meltwater ponds on Bratina Island. Approaches using transcriptomics, which have been used in many recent studies on microbial communities from different environments, would give insights into the changes in gene expression caused by the environmental manipulations of shading and desiccation.

3.3 Final conclusions

The microbial mats of meltwater ponds on Bratina Island harbour biodiversity similar to other studies conducted on meltwater ponds in Antarctica. It is almost certain that cyanobacteria are not as resistant to desiccation as first thought, especially compared to *Bacteroidota* and *Proteobacteria* which maintain their relative abundances throughout most of the samples. It remains unclear what effect, if any, shading and desiccation had on the gene sets involved in photosynthesis and methanogenesis. Although gene sets involved in photosystem II decreased in the desiccation samples of AMS and New ponds, further investigation is needed to rule out other parameters which may also have an effect on their predicted abundance.

In conclusion, this study is the first to explore the microbial community composition of ponds New, AMS and P70 on Bratina Island. The microbial composition of these ponds is comparable to other mat communities found in the aquatic systems of Antarctica (Cowan, 2003; Archer et al, 2014; Archer et al., 2015). Overall, this thesis provides an insight into how microbial communities change in relation to desiccation and shading as environmental stressors. Like all preliminary studies, there is much research left to do for conclusions to be made in higher resolution.

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Supplementary Figures

Supplementary Figure 1 | Relative abundance of Archaea in ponds New, AMS and P70.