Resilience of microbial mats in Antarctic ponds to climate-relevant environmental disturbance

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ABSTRACT

Continental Antarctica is a cold desert, where the hydrologic system is dependent on melting of snow and ice. In a warming climate it is projected that there will be a significant change in precipitation, evaporation, cloud formation, all affecting the ice-water dynamic. Hydrology is considered vulnerable to climatic change. Hydrological change cascades through the environment affecting Antarctic ponds which are important centres for inland microbial biodiversity. An understanding of community vulnerability to anticipated change can be developed through assessing organism and functional resilience to climate-related and other forms of disturbance. The aim of this study is to identify the effects of disturbance on microbial communities, specifically cyanobacterial mats, with a particular focus on changes that may occur resultant to anthropogenic climate change.

This study undertook three experiments, which identified impacts of disturbance on three key cyanobacterial mat functions – nitrogen fixation, photosynthesis/respiration, and recovery after a physical disturbance. Sampling was undertaken in the McMurdo Ice Shelf (MIS) meltwater ponds in late January 2019. The design for the nitrogen fixation experiment used a natural gradient of conductivity across five ponds (Fresh, Casten, P70, Brack and Salt) in a space-for-time approach that compared microbial composition and nitrogen fixation rates. The light disturbance study completed in New and P70 ponds tested mat ability to retain photosynthesis and respiration under a pulsed disturbance – covering microbial mats with shade cloth for 12 months. In this study the light-photosynthesis response and mat composition were analysed when shaded and not shaded (control plots). The third experiment examined short-term response to physical disturbance by observing the recovery of mat structure and community composition for 2 years was also completed in New and P70 ponds.

It was identified that there was no significant change in acetylene reduction (as a proxy for nitrogen fixation) over the salinity range. Acetylene reduction ranged between 22.4 ± 3.4

µMol/m^2/h in Brack Pond (conductivity = 10.5 mS/cm) and 49.6 ± 17.1 µMol/m^2/h in Casten Pond (conductivity = 2.3 mS/cm). The acetylene reduction difference was statistically significant between Brack Pond and Casten Pond (F = 0.006 (Bonferroni Correction significance of $F \leqslant 0.01$)). Microbial mat composition changed across the conductivity gradient, but significant proportions of different N-fixing and mat-forming genera were evident along the salt gradient, suggesting functional resilience through species turnover.

It was also identified that when receiving a 95-96% decrease in ambient light photosynthetic bacteria were able to maintain photosynthesis and respiration at similar rates within the microbial mats. However, in shaded plots the maximum net oxygen production occurred at an average irradiance between 350 – 450 µMol photons $m^{-2}s^{-1}$ and was inhibited at higher levels. In control plots the maximum net oxygen production was $\geqslant$900 µMol photons $m^{-2}s^{-1}$. In control samples net photosynthesis exceeds zero (photosynthesis exceeds respiration) at ~275 µMol photons $m^{-2}s^{-1}$, in shaded samples this occurred at ~100 µMol photons $m^{-2}s^{-1}$. Rapid shifts in the appearance of mats under, and after shading led to the conclusion that the acclimation may be based on vertical migration of cyanobacteria within the mats, which showed no significant changes in relative abundance of taxa.

After physical removal of microbial mat, communities were shown to reform to similar relative abundances as control samples within two years of the disturbance, within this experiment a successional change in species abundance was observed.

This research highlights the resilience of microbial mat populations on the MIS to the types of change that are anticipated to accompany climate change. It supports conclusions previously identified that cyanobacterial mats are functionally resilient to short-term and long-term disturbances. Ongoing research will improve the knowledge on the resilience of other functions required in these environments.

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1 INTRODUCTION

1.1 CLIMATE CHANGE IN POLAR REGIONS

Global climate variability on Earth is well established, and for most of the history of the planet temperatures have been warmer than today (Link, 2009). “Icehouse” and “greenhouse” phases are recognised, with icehouse defined as when one or both polar regions have ice cover, at present we are within an icehouse phase. Cyclic patterns over thousands of years have caused cooling and warming, termed glacial (when ice cover expands) and interglacial periods (when ice contracts) (Hoffman, Kaufman, Halverson, & Schrag, 1998; Hoffman & Shrag, 2000). An extreme example of an icehouse earth is seen in the theorised snowball Earth period during the Cryogenian (~717 to 636 Ma), where most of the planet was ice covered and biological activity in the epipelagic zone collapsed for millions of years (Hawes, Jungblut, Matys, & Summons, 2018; Hoffman et al., 1998; Hoffman & Shrag, 2000; Vincent et al., 2000).

At present, the Earth is in an interglacial, with an expectation of low ice cover, but current measurements suggest that climatic warming exceeds that expected from natural variability and that this must be at least partially the result of anthropogenic increases in the concentrations of greenhouse gases (Randel et al., 2017; Smith & Polvani, 2017). Globally, the last three consecutive decades have each been warmer than the preceding decade and warmer than any decade since 1850; consequently, this may also be the warmest 30-year period of the last 1,400 years in the Northern Hemisphere (IPCC, 2014). Models project that the climate will continue to warm, and that polar regions are particularly at risk from climate change. The IPCC (2014) 5th assessment models show increased precipitation and temperature increases in the high latitudes exceeding that of the lower latitudes under all representative concentration pathway (RCP 2.6 to 8.5) (Figure 1).

This “Polar Amplification” of climate change is mostly due to feedback effects of atmospheric properties and, particularly, local albedo where loss of ice cover increases absorption of energy by the relatively dark land and ocean surfaces (Smol & Douglas, 2007a; Stuecker et al., 2018). In the ice-dominated high latitudes, temperature increases affect snow and ice dynamics and hence water availability. The Arctic and Antarctic Peninsula regions have in recent years experienced warming at 3-4 times global mean level (Hansen, Ruedy, Sato, & Lo, 2010). For this reason the Arctic and Antarctic are often referred to as the ‘canary in the coal mine’ with respect to climate change as the effects are significantly worse in these areas and subsequently, these areas act as an early warning system for future global change (Smol & Douglas, 2007a). Current climate models are also predicting widespread trends of warming and increases in precipitation in the Antarctic, with large spatial variability throughout the continent (Bracegirdle & Stephenson, 2012; Bronselaer et al., 2018; Dinniman, Klinck, Hofmann, & Smith, 2018; IPCC, 2014). How these broad scale changes will impact on small scale processes such as ice melt, which directly impact on ecosystems, is as yet uncertain.

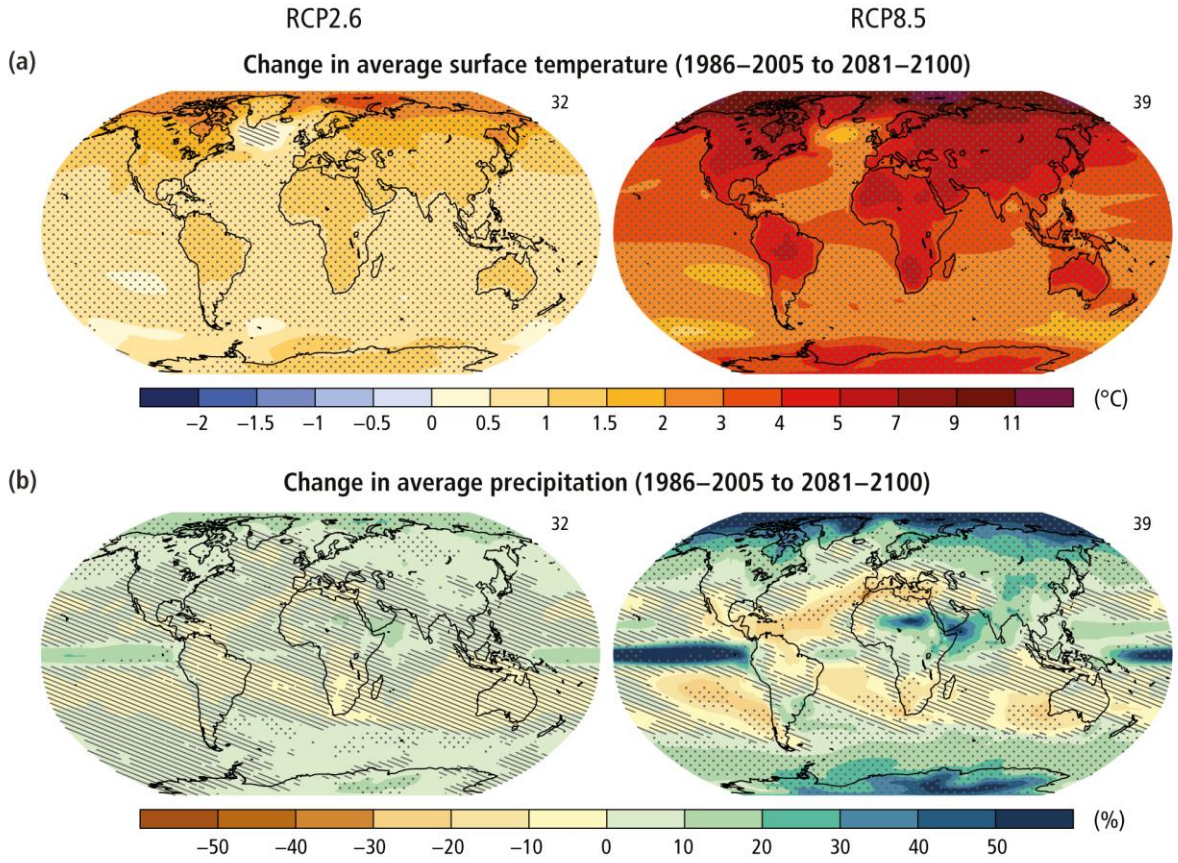


Figure 1. IPCC 5th Assessment report modelled global temperature and precipitation in 2081 - 2100 in comparison to 1986 – 2005. Both increase disproportionately at the poles.

1.2 The Polar Cryosphere and Hydrosphere

Previous work in the Arctic has shown that 20th century climate warming has had an effect on lakes in the region through changes to the hydrosphere and cryosphere caused by increasing temperatures and variable precipitation (K. Griffiths, Michelutti, Sugar, Douglas, & Smol, 2017; Paterson, Rühland, Anstey, & Smol, 2017; Roberts, Jones, Allen, & Huntley, 2015; Rühland et al., 2014; Rühland, Paterson, & Smol, 2015). This effect has also been reported in other hydrologic systems in the high Arctic (Rühland et al., 2015). Recent work in shallow systems has provided evidence of geochemical and biological responses to environmental warming (Hadley et al., 2019; Smol & Douglas, 2007b). Within shallow systems changes include diversification of benthic taxa and increases in whole lake production which are consistent with increased ice-free periods and expanding growing seasons (Hadley et al., 2019).

The impacts of climate change are particularly clear in Arctic ponds where reduced winter snowfall fails to offset increased summer evaporation (Smol & Douglas, 2007b). One Arctic study found that July conductivity had increased by up to 50% over a 24-year period from the 1980's – 2000's (Smol & Douglas, 2007b). Ponds were also reported to have reduced in water level due to increased evaporation caused by warmer summer temperatures, in some cases this occurred to the extent that some previously permanent ponds became ephemeral (Smol & Douglas, 2007b).

The Antarctic climate has been modelled to show a trend similar to that seen in the Arctic with increasing warming and levels of precipitation, this trend is particularly clear in the Antarctic Peninsula (Figure 1) (IPCC, 2014). At present continental Antarctica has warmed more slowly, likely due to a combination of the Southern Ocean absorbing large amounts of energy and the loss of heat through the ozone hole (Randel et al., 2017). Modelling and observations of the climatic response to ozone depletion from 1979 – 1997 and partial recovery of the ozone hole occurring 1998 – 2014 show significant warming in the lower stratosphere of Antarctica after 1998 (Randel et al., 2017).

Recent research, however, is indicating widespread net loss of ice from much of Antarctica (Slater et al., 2021). IPCC modelling also suggests that average ice loss rate from Antarctica has increased from 30 (±60) Gt yr^{-1} during the period from 1992 - 2001 to 147 (±75) Gt yr^{-1} during the period from 2002 – 2011 (IPCC, 2014). At local levels, increasing glacial melt is driving increased discharge in streams, which is resulting in rising levels in endorheic (closed basin) inland water bodies to which these streams drain (Castendyk, Obryk, Leidman, Gooseff, & Hawes, 2016).

Changes in precipitation and temperature have important implications in Antarctic water bodies as small changes in the hydrologic system can have large impacts on the pond environment. In

terrestrial Antarctica, meltwater ponds represent the most common aquatic ecosystem, and are host to a high proportion of terrestrial biodiversity (Hawes, Howard-williams, Gilbert, & Joy, 2021; Hawes, Howard-Williams, & Fountain, 2008; Hawes, Safi, Sorrell, Webster-Brown, & Arscott, 2011; Speirs, Steinhoff, McGowan, Bromwich, & Monaghan, 2010; Vincent & James, 1996). Understanding the consequences which are anticipated to accompany climate change including changes in hydrology and ice phenology are critical for threat diagnosis and the development of future-proofed management plans of pond ecosystems.

1.2.1 The McMurdo Ice Shelf

As discussed above, in the Arctic, small water bodies have proven particularly vulnerable to hydrological change. In continental Antarctica, while much of the limnological research has focussed on the unusual large, ice-covered lakes, small water bodies are overwhelmingly dominant in terms of numbers, and are widely distributed across landscapes (Hawes et al., 2021). One of the best-known areas of ponds in Antarctica is the McMurdo Ice Shelf (MIS) (Figure 2) and these will be the focus of work described in this thesis.

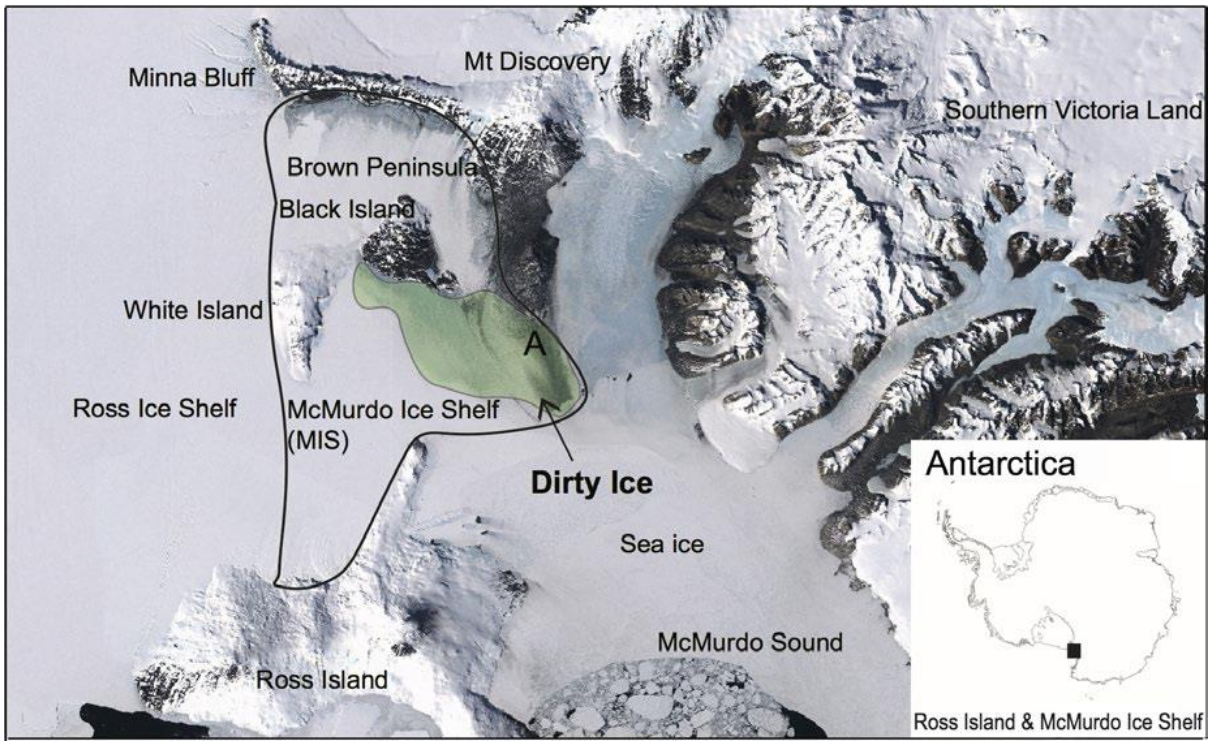


Figure 2. The McMurdo Ice Shelf is a part of the Ross Ice Shelf and located in the south-west corner of the Ross Sea bounded to the south by Minna Bluff and the west by the coast of Southern Victoria. The letter A indicates the location of Bratina Island The inset shows the location of the McMurdo Ice Shelf (MIS) in Antarctica (inset). Image from (Hawes et al., 2018).

The MIS ponds were first explored during the Heroic Age (Debenham, 1919) and have since been regarded as a natural laboratory, and one of the richest areas of ponds and small lakes in Antarctica. The MIS is unusual in that it is an ice shelf largely covered by sediment, which forms an undulating terrain that accumulates surface meltwater as ponds (Hawes et al., 2008; Hawes, Safi, Sorrell, et al., 2011; Speirs et al., 2010; Vincent & James, 1996). Due to sediment reducing the albedo of the snow and ice in these areas, there is ice melting is accelerated during seasonal melting periods, which causes the formation of ponds within the undulating terrain (Hawes et al., 2008; Hawes, Safi, Sorrell, et al., 2011; Speirs et al., 2010; Vincent & James, 1996).

The first theory of how the sediment reached the ice surface was proposed by Mr H. T. Ferrar, the geologist from the British National Antarctic Expedition (1901-1904). Ferrar initially proposed that sediment of marine origin worked its way to the surface of the ice shelf

(Debenham, 1919). The theory of sediment reworking was revisited many times, along with other explanations forming to contradict Ferrar's such as windblown sediment. Further evidence supporting the marine origin was provided through analysis of surface diatoms which discounted the sole introductory method as windblown sediment (D. E. Kellogg & Kellogg, 1987). The current understanding of the system is that sediment is integrated into the base of the ice shelf at the seabed and transported upwards to the ice shelf surface driven by net ice loss at the surface via sublimation and net ice gain onto the base of the ice shelf. When at the surface, such sediment is known as supraglacial debris (Debenham, 1919; D. E. Kellogg & Kellogg, 1987).

On the MIS, two topographic sediment-ice formations exist: (1) "undulating ice" which has thick supraglacial debris cover (tens of cm) and features rolling variations in surface relief of 10 metres and (2) "pinnacle ice" which is flatter, has sparser debris cover (mm-cm) and features ice columns (Hawes et al., 2018; Hawes, Safi, Sorrell, et al., 2011; Howard-Williams, Pridmore, Broady, & Vincent, 1990; D. E. Kellogg & Kellogg, 1987; Vincent & James, 1996). Pinnacle ice areas tend to form surface hydrologic features such as streams and temporary pools, however semi-permanent and permanent ponds ranging in size from 1 – 30,000 m^2 are more common within undulating ice areas (Howard-Williams et al., 1990; Vincent & James, 1996). Ponds in this environment are important for providing habitat to biota and biological refugia during glacial periods (Howard-Williams et al., 1990; Vincent & James, 1996). Water bodies in both the undulating and pinnacle ice are dominated by cyanobacterial mats which typically cover the entire floor of the longer-lasting ponds (Howard-Williams et al., 1990). Ponds within the undulating ice have significantly higher dissolved reactive phosphorus, dissociated ammonium (NH_4 – N), dissolved organic phosphorus and dissolved organic nitrogen in comparison with pinnacle ice waterways (Howard-Williams et al., 1990).

Although there are a range of physiochemical conditions within ponds in the pinnacle ice, most studies to date have focused on the ponds in the undulating ice region, which are larger, more long-lived, and present an enormous range of habitat from freshwater to hypersaline (Howard-Williams, Pridmore, Downes, & Vincent, 1989; Wait, Webster-Brown, Brown, Healy, & Hawes, 2006). Vertical water density stratification within individual ponds is common on the MIS, due to salt exclusion during freezing causing highly saline basal brines and pond temperatures in liquid water reported as low as -20°C (Archer, McDonald, Herbold, & Cary, 2014; Archer, McDonald, Herbold, Lee, & Cary, 2015; de los Ríos, Ascaso, Wierzchos, Ferna, & Quesada, 2004; Dinniman et al., 2018; Hawes, Howard-Williams, & Sorrell, 2014; Hawes, Safi, Sorrell, et al., 2011; Hawes, Safi, Webster-Brown, Sorrell, & Arscott, 2011; Hawes, Smith, Howard-Williams, & Schwarz, 1999; Jungblut et al., 2005; T. B. Kellogg & Kellogg, 1988; Sjöling & Cowan, 2003; Taton et al., 2006; Wait et al., 2006). Gas exclusion during freezing causes supersaturation of pond dissolved oxygen concentration, representing an additional challenge to microorganisms living in the ponds (Hawes, Safi, Sorrell, et al., 2011; Hawes, Safi, Webster-Brown, et al., 2011). Ponds show clear interaction with climate, with increased and decreased winter snowfall consequently resulting in reduced and increased pond electrical conductance, respectively, within ponds (Hawes et al., 2014; Hawes, Safi, Sorrell, et al., 2011; Hawes, Safi, Webster-Brown, et al., 2011).

Long term studies showed that biologically mediated variables within ponds tended to show temporal consistency but spatial variability, with ponds in close proximity showing coherent interannual variations (Hawes et al., 2014) that are inferred to reflect variability in water balance. Prominent ponds have shown irregularities in water level with some desiccating completely. Although this can be related to changes in hydrology and water loading, ice shelf drainage processes also contribute to this variability (Wait et al., 2006). Long term studies estimate organisms living in MIS ponds experience one month of open water, two months in

partially frozen water with surface ice cover – during this time organisms experience cold, liquid and near dark conditions, eight months frozen solid, and one month with ice thaw (Hawes, Safi, Sorrell, et al., 2011).

1.3 MICROBIAL MATS

Microbial mats are macroscopic, laminated communities of microbes and are the dominant habitat in continental Antarctic ponds (Vincent et al., 2000). They usually occur in aquatic benthic environments exhibiting fine scale vertical habitat stratification with different strata favouring different microbial functional groups (Nadeau, Howard-Williams, & Castenholz, 1999). Stratification can consist of cyanobacteria, mineral sediments, extracellular polymeric substances, and void spaces filled with water (de los Ríos et al., 2004; Sjöling & Cowan, 2003; Vincent & James, 1996). Vertical laminations are thought to form based on irradiance, oxygen and redox gradients, favouring a vertical sequence of organisms with physiologies best able to exploit conditions prevailing at depth within the mat (Sjöling & Cowan, 2003). Microbial mats are able to maintain stability over long timeframes in ponds on the MIS due to a low disturbance regime. In particular, bioturbation is absent due to the lack of large invertebrates and vertebrates, and physical disturbance is minimised by the long periods of shelter under ice cover. This favours dominance by perennial cyanobacterial mats, which accumulate slowly in an environment where biological activity is low due to short growing seasons and low ambient temperatures (Castello & Rogers, 2005; Hawes et al., 2008; Vincent et al., 2000). Within MIS mats, motile trichomatous cyanobacteria, particularly members of the Oscillatoreaceae, play a key function in creating the 3-dimensional matrix that structure the mat.

The first published descriptions of the McMurdo Ice Shelf microbial communities occurred in the heroic age of Antarctic exploration in 1901. Cyanobacteria in the Bratina Island ponds were described by members of the National Antarctic Discovery Expedition 1901-1904, who noted large meltwater ponds on the debris covered ice which maintained a high biomass of microbial

mats primarily comprised of cyanobacteria (Fritsch, 1912). Realising the significance of these communities, samples of the McMurdo Ice Shelf microbial mats were archived by the expedition and recently re-analysed in the 2017 studies of their cyanobacterial composition (Jungblut & Hawes, 2017) and their toxin content (Jungblut, Wilbraham, Banack, Metcalf, & Codd, 2018). As the oldest polar samples of cyanobacteria, the 'Discovery' samples represent baseline conditions prior to human activity within Antarctica, prior to the formation of the Antarctic ozone hole and prior to anthropogenic climate change (Jungblut & Hawes, 2017; Jungblut et al., 2018). These authors found little change in community assemblages between historic samples and those taken from similar locations in modern times.

The species composition and genetics of the microbial mats have been widely studied. Microbial mats in the ponds around Bratina Island are made up of a wide range of organisms which have had their physiochemical tolerances and morphological parameters studied (Howard-Williams et al., 1990; Jungblut et al., 2005; Sjöling & Cowan, 2003). Organisms identified in MIS microbial mats include cyanobacteria, protozoa, rotifers, tardigrades and nematodes (Fernández-Valiente, Quesada, Howard-Williams, & Hawes, 2001; Jungblut et al., 2005; Laybourn-Parry, Marahiel, Haymet, Quick, & Benson, 2002; Vincent & James, 1996). Due to the high density of microorganisms within these mats, it is suggested that biological interactions are strong, with symbiotic associations and complementary functionalities (Castello & Rogers, 2005).

Pond conditions are challenging for photoautotrophic growth, with months of darkness, low nutrient levels, low and variable temperature, frequent dehydration, raised UV radiation and regular freezing and thawing of the surrounding pond environment (Hawes, Safi, Webster-Brown, et al., 2011; Jungblut, Lovejoy, & Vincent, 2010; Jungblut & Neilan, 2010; Vincent & James, 1996). Microbial mat organisms have become well adapted to their extreme, climate-driven environment in this area. It is thought that a broad tolerance for physical and chemical

fluctuations was advantageous and that an ability for rapid growth has been lost in developing this tolerance (Nadeau & Castenholz, 2000). This is shown in warming experiments where growth rates remained slow in Antarctic cyanobacteria, suggesting an inability to take advantage of more preferable growth conditions (Nadeau & Castenholz, 2000). Furthermore, a reduction of pond temperature from 1°C to -2°C caused reductions in photosynthesis (11% reduction) and respiration (40% reduction) although the efficiency of light harvesting remained unchanged (Hawes et al., 1999). This has important implications when there is competition for resources. Additional research shows that microbial mats were able to remain photosynthetically active under high salinities (electrical conductivities between 0.04 and 80 mS/cm^{-1}) and that they acclimated to lower irradiance caused by surface ice formation (Hawes et al., 1999).

In the MIS, two essential nutrients for life, biologically utilizable carbon and nitrogen, are limited due to the virtual absence of carbon and nitrogen fixation within their barren catchments (Paerl, Pinckney, & Steppe, 2000). Instead, carbon and nitrogen accrual are primarily autochthonous. cyanobacterial mat populations on the MIS have been shown to fix both nitrogen and carbon in their environments and current research suggests that the pond microbial mats provide the dominant source of nitrogen inputs to the ponds (Fernández-Valiente et al., 2001; Hawes et al., 2014). Carbon fixation by cyanobacteria was shown by Novis et al. (2007), which identified that carbon fixation rates were highly correlated with temperature and irradiance when water content of the mat was greater than 30%.

Nitrogen fixation studies performed in the Vestfold Hills, Princess Elizabeth Land, Antarctica provided insight into the nitrogen fixation rates of Antarctic cyanobacteria and microbial mat communities. Mats containing *Nostoc commune* and *Calothrix* had rates of acetylene reduction (a proxy for nitrogen fixation as the enzyme nitrogenase has affinity for both acetylene and di-nitrogen) ranging between 0.82 to 211.2 p mol ethylene µg $chla^{-1}hr^{-1}$ at temperatures of - 4 to

9 °C (Davey, 1983). This rate was indicated to be highest during the early afternoon. Similarly, a diel pattern in nitrogen fixation rates was shown in MIS ponds, which was likely related to pond temperature, with higher activity at midday and minimal activity during early morning (Fernández-Valiente et al., 2001). However this trend may also be related to sun height in the sky because although irradiance requirements were low, activity was inhibited in dark conditions, suggesting nitrogen is predominantly fixed by cyanobacteria, in the oxic layer of the mat (Fernández-Valiente et al., 2001). Lab studies indicate that *N. commune* is more active at higher temperatures with the rate of acetylene reduction 19% higher at 20°C than at 0 °C in laboratory studies (Davey, 1983). Multiple studies which analysed the effects of desiccation on cyanobacterial nitrogen fixation rates found that, once rehydrated, there was little demonstrable reduction in nitrogen fixation levels (Davey, 1983; Paerl et al., 2000).

A large proportion of research concerning the MIS cyanobacteria involves comparisons of populations across salinity gradients. Despite the large pool of research on this issue there is still disagreement on the influence that salinity has on community structure. Jungblut et al. (2005), using a morphological and genetic approach, suggested that lowered diversity in more saline ponds may be due to fewer species being capable of surviving the conditions. A secondary explanation is that as the more saline ponds are usually smaller, they can support less diversity (Jungblut et al., 2005). Another study using genetic, morphological and molecular approaches found that water chemistry was unlikely to dictate pond community structure as the species present in all ponds are adapted to tolerate a range of conditions (Wood et al., 2008). A third study using microscopic and molecular approaches found salinity and light intensity seemed to influence cyanobacteria community composition, although several operational taxonomic units (OTUs) possessed an ability to survive a greater range of environmental conditions, allowing these to become ubiquitous (Taton et al., 2006). Other studies identify conductivity as well as other geochemical variables as key drivers impacting microbial

community structure, these include pH, potassium and aluminium and to a lesser extent, silver-109, nitrous oxide and vanadium-51 (Archer et al., 2014, 2015), though these studies rely primarily on correlative inferences. Although evidence suggests major ion geochemistry may be a driver of community structure, there are physical drivers within ponds that are implicated, including ice cover and hydrology (Sutherland, Howard-Williams, & Hawes, 2020). Although cyanobacteria display large adaptability to environmental conditions, they demonstrate reduced genetic divergence, a contraction of cold climate habitats through climate warming is likely to force organisms into more localized habitats or alternately extinction (Jungblut et al., 2010). Potential vulnerabilities to changing climate and apparent contradictions amongst studies on the controls of cyanobacteria population highlight the need for additional research in this area to assess population response to geochemical variation more accurately.

1.4 Biological Resistance and Resilience

When considering the vulnerability of the microbial mat communities to disturbance by changing climate, the concepts of ecological resistance and resilience are helpful. Both are thought to be related to internal factors such as structure and diversity of the microbial community as well as external variables (B. S. Griffiths & Philippot, 2013). Definitions of resilience vary based on their context: a study analysing definitions of the term resilience identified over 120 distinct definitions from peer-reviewed academic literature (Stevenson, Vargo, Ivory, Bowie, & Wilkinson, 2015). The Stevenson et al. (2015) study revealed that the most common concepts within the definitions related to an event such as disturbance, and contained words such as ability, adaptation, capacity, recover function. Disturbance is often subdivided, the commonly used examples being a pulse or press disturbance. Pulse refers to a disturbance that is applied and then removed, while press is a disturbance that is applied, and a ramp disturbance continues to increase over time. A further challenge with defining resistance and resilience is that there are many levels to consider, such as habitat, composition, function

etc, and consequently definitions should be used based on requirement of the study – resilience of what to what. In this study we focus on the retention of functional and compositional aspects of the microbial mat communities when challenged with environmental disturbance, which will be inferred from comparisons to other samples and controls. It will use previously proposed definitions of disturbance, resistance, and resilience appropriate for this study.

- **Disturbance** is defined as, "*A cause such as a physical force, process or agent which may be abiotic or biotic that causes a perturbation and stress in an ecological system or component relative to a specified reference state*" (Rykiel, 1985)..
- **Resistance** is defined as, "*The ability of a system to withstand a disturbance*" (B. S. Griffiths & Philippot, 2013).
- **Resilience** is defined as, "*capacity of a system to absorb disturbance and reorganize while undergoing change so as to retain essentially the same function, structure, identity, and feedbacks*" (Folke et al., 2004; Gutt et al., 2012; Walker, Holling, Carpenter, & Kinzig, 2004).

While the comparison of genetic composition of MIS microbial mat from the early 20th Century with modern equivalents described above indicates long term stability, in other locations there is evidence of change in response to disturbance. Over a decadal warming period in the McMurdo Dry Valleys it was found that as a response to environmental change the dominant microbial taxa reduced in abundance while previously less common taxa expanded and increased in abundance (Andriuzzi, Adams, Barrett, Virginia, & Wall, 2018). The Andriuzzi et al. (2018) study showed that over two distinct climate periods the disturbance generated by increased ice melt caused the largest response.

Existing information from the ponds on the MIS provides guidance on what are relevant disturbances, given the likely overarching impact of climate change, as well as what metrics of

resistance or resilience should be used. Ponds are known to undergo several changes intra- and inter-annually. These changes occur naturally due to changes in the ice shelf, atmospheric conditions, hydrology, irradiance and many other variables (Hawes et al., 2014). Pond water and ice level change is thus a credible disturbance, given that most ponds are endorheic and water levels vary with evaporation and influx of water. Within individual ponds, water level can be highly variable independent of other ponds and, in any given year some ponds will fill while others dry up (Hawes et al., 2014), and long term directional changes in evaporation result in shifts in pond salinity (Sutherland 2009). Ice cover can also vary from year to year (from seasonally ice-free to persistent ice cover) which has significant effects on irradiance and gas exchange (Hawes et al., 2014). The three disturbances to be used in this study are thus salinization, irradiance, and physical disturbance.

In an attempt to survive changing conditions, species and communities must be resistant or resilient to changes in the environment. In this study we explore how function (e.g., nitrogen fixation, respiration, and mat formation) is maintained under a disturbance challenge and recognise several levels at which a disturbance can be accommodated, through behavioural responses, physiological acclimation or through species compositional changes. We note also that these accommodation responses have different timelines, increasing in duration from behavioural < acclimation < compositional change (Quesada et al 2008).

1.5 STUDY SITE

The McMurdo Ice Shelf (MIS) is part of the Ross Ice Shelf, bounded between Minna Bluff to the south and Ross Island and McMurdo Sound to the north (T. B. Kellogg & Kellogg, 1988) (Figure 3). The MIS covers an area of 1500 to 2000 km² at a thickness of 10 to 50 metres (Howard-Williams et al., 1990; Vincent, Castenholz, Downes, & Howard-Williams, 1993). As discussed in Chapter 1, the MIS is an unusual ice shelf due to the fact that in this area sediment is entrained in ice shelf transport systems and is brought to the ice surface from the seafloor

and the surface of the ice shelf is more or less covered by sediment (Hawes et al., 2018; D. E. Kellogg & Kellogg, 1987; T. B. Kellogg & Kellogg, 1988). This ice conveyor belt functions in the shallow marine environment due to ice shelf mass balance causing changes in buoyancy and subsequently depositing sediment cover to areas of the MIS (Hawes et al., 2018; D. E. Kellogg & Kellogg, 1987; T. B. Kellogg & Kellogg, 1988). The MIS is currently ablating, primarily losing mass at the ice-air interface through ablation and evaporation process, and gaining mass at the ice-ocean interface through refreezing processes (D. E. Kellogg & Kellogg, 1987; T. B. Kellogg & Kellogg, 1988). Facilitated by the varying sediment coverage, a change in snow albedo causes melt water ponds to form on the ice shelf surface (Hawes et al., 2008; D. E. Kellogg & Kellogg, 1987). Materials transferred between the marine and ice shelf systems provide a mechanism for inorganic and organic exchange between the ice shelf and sea floor (Hawes et al., 2018; D. E. Kellogg & Kellogg, 1987).

The undulating ice ponds provide habitat for the largest non-marine aquatic ecosystem in the McMurdo Sound Region dominated by benthic communities of cyanobacteria (Howard-Williams et al., 1990; Vincent et al., 1993). Several studies isolate the MIS ponds as providing a unique study area with a series of discrete medium-sized ephemeral and semi-permanent ponds. The variation of ponds in this area act as a natural laboratory of meltwater ponds, over a short distance, the ponds have varying characteristics such as size, shape, depth, and salinity which causes each pond to have a unique microbiome. Due to the marine origin of transported ice ponds can contain salts and variable evaporation rates of ponds enable pond salinity to be highly variable from fresh to hypersaline over small areas (Archer et al., 2014; Hawes et al., 2008). The varying characteristics of the ponds enable gradient studies of a range of variables including water conductivity, salinity, temperature, depth, pond size, and ice cover which differ between ponds, these characteristics subsequently impact species composition (Hawes et al., 2018). Within ponds, a salinity gradient often occurs within the water column as seasonal

freezing excludes salts from surface ice resulting in stratified ponds (Hawes et al., 2008). Ponds can also have stratification of oxygen and dissolved materials such as nutrients (Hawes et al., 2008). Due to the variability of meteorological, glaciological, and geological conditions in this area ponds are unstable and regularly change in shape, water depth and conductivity as inputs and outputs change.

This area also has a long-established history of being studied with the first description of the MIS/ Bratina Island ponds dating back over 100 years and annual studies occurring for over 30 years establishing long-term variability within the area (Hawes et al., 2014). Monitoring within the ponds is enabled through a meteorological station located near Salt Pond, long-term return observations monitor pond shape and size related to hydrological variability. Consequently, this location provides benefits for longitudinal studies (studies completed in one pond over many years) as well as cross-sectional studies (studies completed in multiple ponds at one time).

The MIS experiences relatively little wind in comparison with other areas of Antarctica (Coggins, McDonald, & Jolly, 2014; Monaghan, Bromwich, Powers, & Manning, 2005; Speirs et al., 2010), although it can be impacted by foehn winds originating from near the pole (Speirs et al., 2010). The seasonal average wind speed for Bratina Island is <6 ms^{-1} annually, the highest wind speeds tend to be experienced in Autumn (Monaghan et al., 2005). The predominant wind direction for the area blows from the south-east to south-west quarter. As in the rest of Antarctica there is large seasonal change in the temperature between summer and winter. Long term data from 1990–99 and a more recent one-year dataset (2002-03) show the average seasonal temperatures as winter -29.0°C (-33.6°C), autumn -19.6°C (-19.2°C), summer -7.7°C (-7.1°C) and spring -26.6°C (-24.1°C) while the average annual temperature over that period was -20.7°C (-21.0°C) (Monaghan et al., 2005). The ponds receive very little annual precipitation, the annual average is <50 mm (water equivalent) which is distributed with no

seasonal influence (Monaghan et al., 2005). This area experiences a moderate positive mean sea level pressure (hPa) anomaly, common around the Antarctic which acts as a boundary to the subpolar low-pressure belt at 50 – 60 °S (Coggins et al., 2014). In this region low annual snowfall and persistent dry foehn winds frequently remove snowfall and cause net ablation of ice from the surface (Monaghan et al., 2005).

All ponds within this study were located on the MIS within 3 km of Bratina Island (78° 0'28.85"S , 165°32'49.57"E). The studied ponds range in size from 100 – 2000 m² in diameter. They include AMS, Brack, Casten, Fresh, New, Seventy[1] P70 and Salt ponds, locations shown in Figure 3. Ponds were sampled between 19 – 26 January 2019. Three huts located on Bratina Island, 600 metres from the ponds are used as living quarters and laboratories.

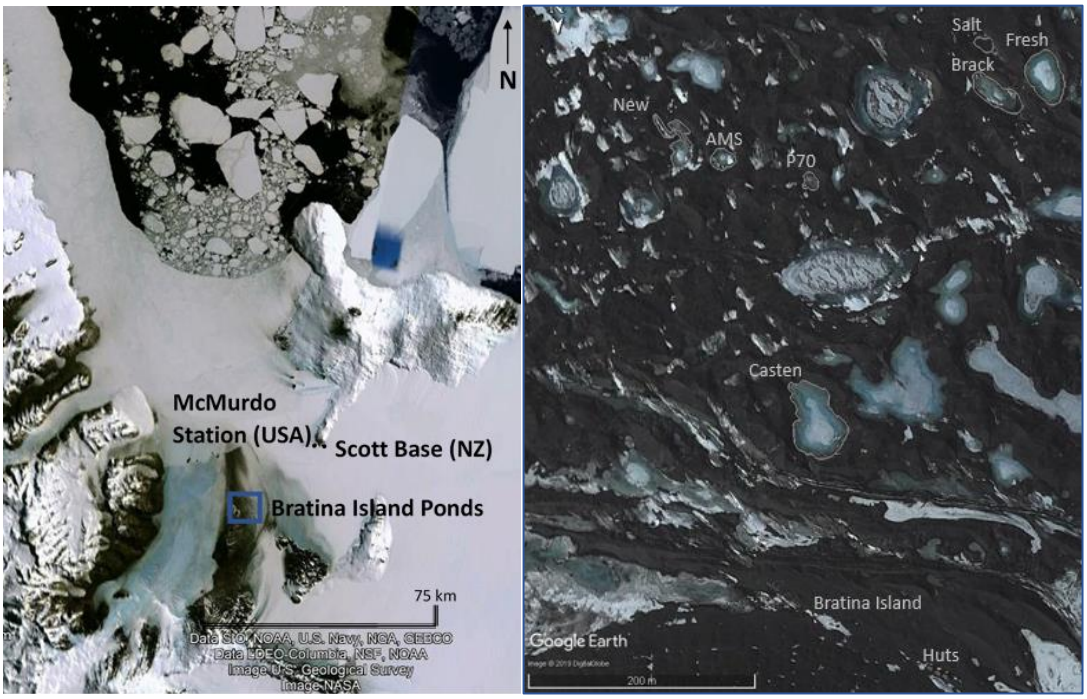


Figure 3. Left image shows the location of Bratina Island ponds on the McMurdo Ice Shelf in relation to Scott Base and McMurdo Station. Right image shows the Bratina Island huts used as laboratories in relation to the ponds. Ponds identified in this study including AMS, Brack, Casten, Fresh, New, P70 and Salt ponds are labelled on this image. Image scale is found on the bottom left of each image, and in both images, north is at the top of the image.

[1] The name Seventy Pond was officially recognised and gazetted in December 2019 by Land Information New Zealand; however, the pond has previously been colloquially known as, and discussed in research as, P70. For consistency to other research this study will use the name P70 in future references.

Samples were collected and transferred to Waikato University under the Ministry of Agriculture and Forestry permit number 2018070802. Samples were taken close to the waters edge of ponds due to accessibility reasons, usually in approximately 50 – 100 mm of water depth. Samples were selected from areas which, to the eye, looked representative of the surrounding pond.

The following sections will describe the methodologies used in the three experiments: Nitrogen Fixation (Section 2.1), Light Disturbance (Section 2.2) and Physical Disturbance (Section 2.3). Each experiment was designed to consider the manipulation of the environment to change a variable in the environment and control outside factors from influencing the study outcome. This study uses randomisations and replications to minimise bias and error in the experiments.

1.6 RESEARCH OBJECTIVES

- Identify whether microbial mats are able to maintain the function of nitrogen fixation over a long-term change in salinity through inferred ramp disturbance.
- Identify how microbial mats are able to maintain the function of photosynthesis over a short-term change in ambient light acting as a press disturbance.
- Identify whether cyanobacteria are able to recover the function of microbial mat formation after physical disturbance.

1.7 HYPOTHESIS

This research hypothesises that:

1. Resilience to pulse and press disturbances in microbial mat communities in Antarctic ponds allows key functions of photosynthesis, respiration, nitrogen fixation and mat structural integrity to be maintained at comparable levels to before disturbance.
2. Due to the slow turnover of species in Antarctic microbial mats, resilience to pulse disturbance is accomplished through a combination of behavioural and physiological accommodation.
3. Response to long lasting press and ramp disturbance is accommodated through a change in community composition with taxa better able to tolerate the new conditions, but filling the same function, therefore replacing existing taxa.

To address these hypotheses a set of experiments were carried out to:

- Identify whether microbial mats are able to maintain the function of nitrogen fixation over a long-term change in salinity, simulating a prolonged period of negative water balance, and whether this is accompanied by a shift in bacterial and cyanobacterial composition.
- Identify whether microbial mats are able to maintain the function of photosynthesis and respiration at prior levels over a 12-month pulse disturbance of ambient light, simulating a year of prolonged ice and snow cover.
- Identify whether cyanobacteria are able to recover the function of microbial mat formation, with no change in community composition, after physical disturbance, simulating a pulse disturbance of reduced water level and loss of mat cover.

This research aims to identify the effects of a short-term disturbance which may occur as a result of anthropogenic climate change. Given a short-term disturbance occurs, a shift of community composition and community function is expected.

2 METHODS

2.1 NITROGEN FIXATION EXPERIMENT

2.1.1 Experimental Design

The nitrogen fixation experiment analysed the nitrogen fixation rates within five ponds, over a conductivity gradient from highly saline (Salt pond) to very low salinity (Fresh pond). Within these ponds, five replicate samples of microbial mat underwent an acetylene reduction assay to assess nitrogen fixation potential. Samples were then returned to NZ for DNA extraction and sequencing of the 16S rRNA gene to characterise microbial populations. The experimental design for this experiment is summarised in Figure 4.

The rationale of the nitrogen fixation experiment is to compare nitrogen fixation potential in microbial mats across a range of pond conductivities to determine if mats are functionally resilient or resistant to changes in the conductivity. If cyanobacteria are functionally resistant to conductivity change, nitrogen fixation (measured by acetylene reduction) will be maintained across conductivities. If cyanobacteria are functionally resilient to conductivity change, then coherent shifts in species composition, particularly for those related to nitrogen fixation, would indicate that functional resilience for nitrogen fixation is accomplished through species turnover along the conductivity gradient. This is a space-for-time approach and relies on an assumption that conductivity evolves over time such that highly saline ponds were once fresh. As the pond conductivity in this experiment is considered to be the dependent variable, ponds were selected to have known conductivities over a conductivity gradient for inter-pond comparison.

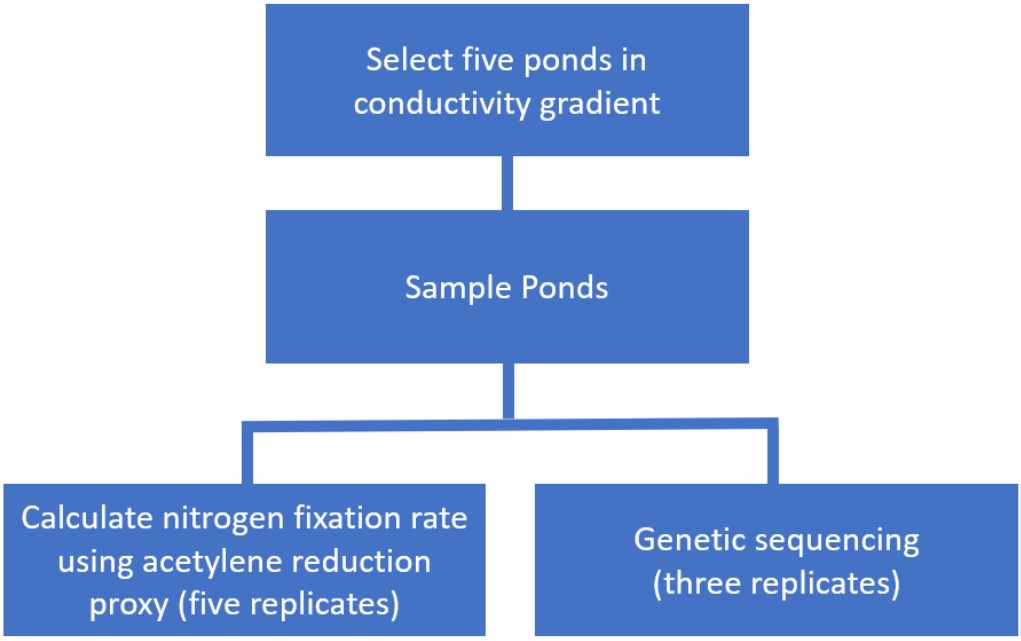


Figure 4. Experimental design for nitrogen fixation experiment.

2.1.2 Pond Selection

The presence of a microbial mat within the pond was deemed as an essential feature, as was pond longevity and stability of the pond to ensure that there was good reason to assume that the microbial communities represented pond conditions. Prior to commencing field work, a literature review (Hawes, Safi, Sorrell, et al., 2011; Wait, Nokes, & Webster-Brown, 2009) of the Bratina Island ponds was completed to understand which ponds would meet these requirements. The ponds selected were ponds in which sufficient data was available to be sure that the ponds were long lived and temporally stable in terms of conductivity, this provided a list of 17 ponds. On arrival to the MIS each pond was assessed for suitable mature mats within the sampling depth, conductivity, pH and Temperature measured using a freshly calibrated YSI meter (Table 1**)**, to select those that met the conductivity gradient requirement. Ponds were thus initially selected on the basis of the historical presence of well-developed microbial mats, long term stability and historic conductivities, with conductivity confirmed in 2019. Five ponds were selected (Table 2) with expected conductivity ranging from 1.2 to 41.2 mS cm^{-1} in 2019.

Table 1. Comparison of McMurdo Ice Shelf Pond geochemistry.

Pond	**Coordinates (WGS 1984)**	**2019 Conductivity (mS/cm)**	**pH (pH unit)**	**Pond temperature (°C)**	**Mat suitability yes/no**
AMS	78° 0'54.10"S, 165°33'23.07"E	3.3	9.6	4	Yes
Brack	78° 0'56.10"S, 165°32'43.64"E	10.5	9.3	4	Yes
Casten	78° 0'46.66"S, 165°33'8.34"E	2.3	9.4	4	Yes
Conophyton*	78° 0'51.97"S, 165°32'39.85"E	1.5	9.2	-	-
Duet	78° 0'55.56"S, 165°32'58.56"E	0.9	10.2	3	Yes
Fogghorne*	78° 0'57.34"S, 165°33'6.29"E	0.6	9.9	-	-
Fresh	78° 0'56.64"S, 165°32'35.90"E	1.2	9.0	4	Yes
Legin*		2.9	9.5	-	-
New	78° 0'54.28"S, 165°33'28.62"E	2.1	9.4	6	Yes
Nostoc		3.6	9.4	6	Yes
Permanent Ice	78° 0'50.57"S, 165°33'4.35"E	0.1	-	1.5	No
P70	78° 0'53.24"S, 165°33'9.80"E	6.2	9.9	3	Yes
P70E*	78° 0'56.68"S, 165°33'5.79"E	10.4	9.6	-	-
Orange		3.2	9.7	6	No
Roger*	78° 0'53.33"S, 165°32'36.89"E	1.3	10.2	-	-
Salt	78° 0'57.59"S, 165°32'44.39"E	41.2	9.4	4	Yes
Skua	78° 0'47.92"S, 165°33'6.00"E	2.2	9.7	3	No

** 2019 conductivity was not available, for these ponds therefore 2018 conductivity was used*

Ponds were selected for qualities such as their permanence in the environment, size, depth, salinity, and prominence of cyanobacterial mat. The nitrogen fixation experiment required ponds with a conductivity gradient, to examine the relationship between the nitrogen fixation function and climate affected conductivity changes. From the information collected in the 2019 survey a conductivity gradient was identified between ponds. In general, ponds which were more established tended to have conductivities in the lower conductivity range, with fewer ponds at the higher conductivities. Considering the three criteria required for the nitrogen fixation experiment: the longevity of the pond, the presence of a microbial mat within the pond and the conductivity of the pond the five ponds in this experiment were selected; Brack, Casten, Fresh, P70 and Salt Pond. As Salt, Brack and P70 had the highest conductivities and suitable microbial mats these three ponds were used. As Duet pond was still ice covered near to the pond edge, this pond was not used as this factor may have affected nitrogen fixation. Subsequently, the next lowest suitable salinity pond, Fresh Pond was included, along with Casten Pond which had the median conductivity of the remaining ponds. Characteristics of the ponds included in the nitrogen fixation experiment are shown in Table 2.

Table 2. Pond characteristics of the nitrogen fixation ponds. Pond area was estimated from Google Earth imagery.

Pond Name	**Area (m^2)**	**Perimeter (m)**	**Approximate dimensions (m)**	**Conductivity (mS)**	**Temperature (°C)**
Casten	2,200	200	70 x 50	2.3	4
P70	90	35	15 x 7	6.2	5
Salt	130	45	15 x 10	41.2	4
Brack	750	125	50 x 20	10.5	4
Fresh	1,200	140	50 x 30	1.2	4

2.1.3 Field Sampling

Acetylene (C_2H_2) reduction to ethylene (C_2H_4) was quantified as a proxy for N-fixation. An acetylene reduction assay (also known as ARA) was used to measure nitrogen fixation of the cyanobacteria mat samples using the procedures developed by Fernández-Valiente et al. (2001) and Stewart, Fitzgerald, & Burris (1967). Acetylene reduction assay calculations are based on each serum bottle containing two times 12 mm cores, 10 ml acetylene water and 2 ml acetylene gas at 4°C using an air density of 1.25 mg/ml as seen in Figure 5.

Five replicate microbial mat samples were taken from visually similar, representative areas, evenly spaced around each of the five selected ponds. Each sample contained comprised two, 12 mm diameter cores of microbial mat and each sample pair was collected within a 5 cm of the other. When collecting, mats samples were taken with as little sediment as possible and placed into 38-mL glass vials and returned to the field laboratory.

In addition, 500 ml of pond water was collected into a plastic bottle to be used in setting up experiments and for analysis of dissolved nutrients. Samples for nutrients were filtered (0.2 μm) and frozen at -20C for return to New Zealand.

On return to the field laboratory 10 ml of pond water was added to each glass vial and the serum bottle was sealed and crimped, two controls of pond water with no mat were included. 2 ml of acetylene was injected into each vial. Acetylene was produced using water and calcium carbide which reacted to form calcium hydroxide and acetylene, $CaC_2 + 2H_2O \rightarrow C_2H_2 + Ca(OH)_2$. Vials were incubated at ambient temperature and irradiance in a water bath at ambient irradiance and temperature (Figure 5) for 6 hours. At the end of each incubation, gas (10 mL) was removed from each sample by syringe and injected into evacuated exetainers. Exetainers were stored at 4^o C under water during return to New Zealand.

For estimation of mat areal biomass, the cores were left in the serum vials and frozen (-20^{o}C) for return to New Zealand.

At three of the mat sampling sites, selected randomly, an additional sample was placed in a 1.5^{o}ml eppendorf tube for analysis of microbial composition. Samples for microbial composition were flash frozen in liquid nitrogen, then stored frozen (-20 and -80 C) until analysed.

Figure 5. Vials used in the acetylene reduction experiment were incubated for 6-hours in a water bath at close to ambient pond water temperature and irradiance conditions.

2.1.4 Genetic Sequencing

The three samples of microbial mat taken from each of the nitrogen fixation ponds were extracted using a methodology established at the Waikato laboratory and designed to maximise recovery of nucleic acids from low biomass samples. It used a modified cetyltrimethylammonium bromide; hexadecyltrimethylammonium bromide (CTAB) extraction protocol following that outlined by Barrett et al. (2006). DNA extraction was quantified using an agarose gel and NanoDrop ND-1000 spectrophotometer developed by Thermo Fisher Scientific at 260 nm in line with the design specifications and using the same methodology as Barrett et al. (2006) (Thermo Scientific, 2010). DNA templates were amplified

using the universal eubacterial (EUB) primers (Votek & Ward, 1995, Earth Microbiome Project, 2021, Appendix C – EUB B/A - PCR Competency Test Protocol). Sequal-Prep was completed by adding 25 ng of DNA per well using the methodology outlined by the Thermo Fisher kit manual (Invitrogen, 2008).

Once sequenced, taxonomy was identified using the Classification Resources for Environmental Sequence Tags (CREST) software. Taxonomy was analysed using RStudio packages 'vegan', 'ggplot2', 'DT', 'phyloseq', 'picante', ' viridis', 'tibble', 'grid', 'gridExtra', 'randomForest', 'plyr' and 'ape'.

2.1.5 Acetylene Reduction Assays

On return to New Zealand, gas samples (10 ml) were analysed at Lincoln University using gas chromatography. Concentration of ethylene was estimated from standard curves prepared using ethylene standards in duplicates at the concentrations of 0.5, 1, 5, 10, 20, 100 ppm. Acetylene reduction was calculated by the amount of ethylene produced in each vial (µmols). This value was determined based on:

$$\left(\left(\frac{Concentration\ (ppb)\ x\ \text{volume (mL) x density (mg/mL)}}{\text{molecular weight of ethylene (g/mol)}}\right)\right.\text{/ (length of incubation (h) x area of cores (cm}^2\text{))}.$$

Areal biomass of core samples was estimate as Ash Free Dry Mass (AFDM). For this, cores from each vial were freeze-dried for 48 hours (or until dry) and subsequently weighed (dry weight). Samples were then placed in a muffle furnace at 400°C for 4 hours to remove organic matter, cooled in a desiccator and were reweighed to allow calculation of the quantity of organic matter as AFDM.

2.1.6 Water Analysis

Water samples were analysed for nutrient concentrations using the technique outlined in Table 3 and included in full in Appendix D – Hill Laboratory Results.

Table 3. Hill Laboratories methodology.

Test	Method Description	Detection Limit
Total Ammoniacal-N	Saline sample. Phenol/hypochlorite colorimetry. Flow injection analyser. (NH_4-N = NH_4+-N + NH_3-N). APHA 4500-NH_3 H 23rd ed. 2017.	0.005 g/m^3
Nitrite-N	Saline sample. Automated Azo dye colorimetry, Flow injection analyser. APHA 4500-NO_3 I (modified) 23rd ed. 2017	0.001 g/m^3
Nitrate-N + Nitrite-N	Saline sample. Total oxidised nitrogen. Automated cadmium reduction, Flow injection analyser. APHA 4500-NO_3 I (modified) 23rd ed. 2017.	0.001 g/m^3
Dissolved Reactive Phosphorus	Saline sample. Molybdenum blue colorimetry. Flow injection analyser. APHA 4500-P G 23rd ed. 2017	0.001 g/m^3

2.2 Light Disturbance Experiment

2.2.1 Experimental Design

The light disturbance experiment used short-term manipulations to establish how impacts related to climate change may impact Antarctic Pond microbial communities. This study compared three ponds which had mature microbial mats and similar physical and geochemical pond conditions, particularly considering salinity and pond depth. The intent was that the ponds would act as replicates. In each, three hessian shades, each 30 x 30 cm which reduce irradiance by ~75%, were placed over areas of microbial mat. These areas were returned to one year after shade placement. Controls were delimited alongside these shaded plots.

On return to New Zealand, the functionality of the control and treated mats was assessed by measuring oxygen flux between mat and overlying water in the dark (respiration) and in the

light (net photosynthesis). This was accomplished by following changes in oxygen concentrations in a series of black and clear chambers installed over treatment and control areas. In addition, samples from the three treatments and a single control area for each pond were taken for sequencing of the 16S rRNA gene to assess any change in community composition during the relatively short pulse disturbance.

The experimental design for this experiment is displayed in Figure 6.

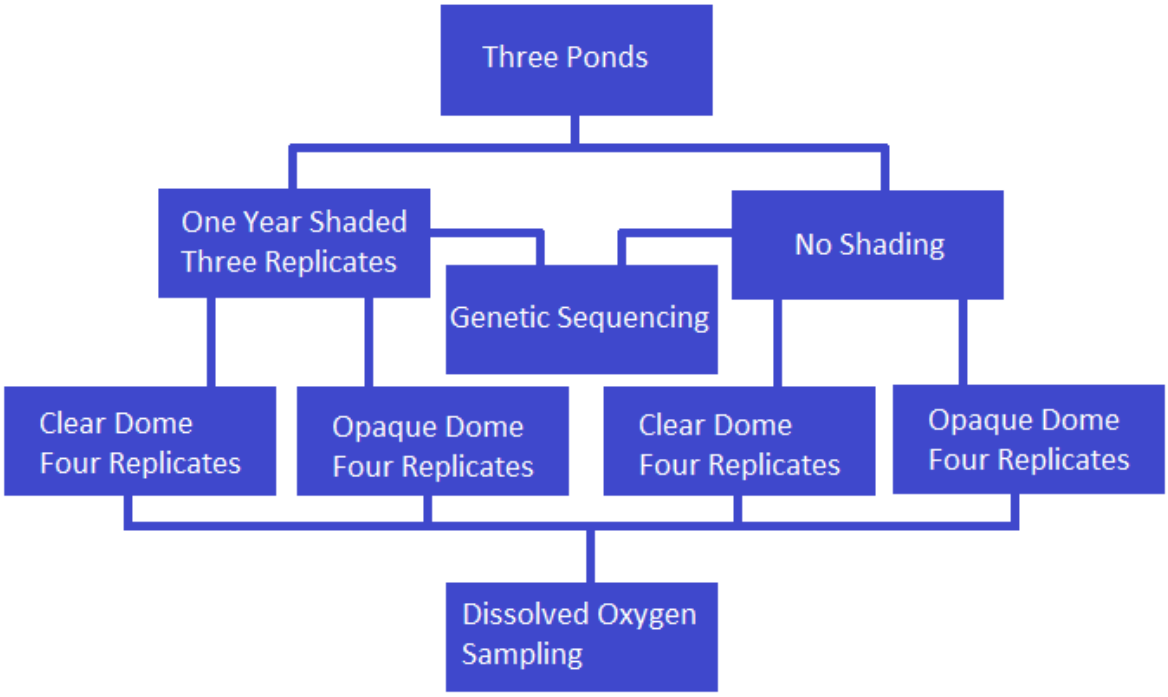


Figure 6. Experimental design for light disturbance experiment.

2.2.2 Pond Selection

Three ponds were selected to be in the disturbance experiment, based on a target of similar of pond conditions to allow them to function as replicates. All three ponds were of a similar size and shape with similar bathymetries and were required to be in close proximity to facilitate experimental manipulations. The three selected ponds, AMS, P70 and New ponds (Table 4**)**, were in the mid-range of the salinity gradient (Table 1). As in the nitrogen fixation experiment, ponds were also required to be permanent in the environment and to have cohesive microbial mat throughout the pond.

Table 4. Pond characteristics of the disturbance experiment ponds.

Pond Name	**Area (m^2)**	**Perimeter (m)**	**Approximate dimensions (m)**	**Conductivity (mS)**	**Temperature (°C)**
New	540*	135	40 x 20	2.1	6
P70	90	35	15 x 7	6.2	5
AMS	160	45	15 x 10	3.3	4

* New pond had two basins, the experimental basin had an area of ~200 m^2

2.2.3 Field Sampling

The light disturbance manipulation was initiated in the Antarctic summer season of 2017/18 (January 21 – 29 2018) through the placement of three 300 mm by 300 mm shade cloth platforms (shades) (Figure 7) into the P70 Pond, AMS Pond and New Pond. These remained in place for sampling and data collection to be completed in the subsequent season – January 2019. Shades were fully submerged, shading areas of microbial mat which looked visually representative of the surrounding pond area. Shades were aimed at areas of the pond where the water depth was similar over the three samples and deep enough to ensure the mats were fully submerged year-round. Shaded areas were assessed January 20, 2019 for visual signs of partial or uneven shading associated with the shading, for example signs of partial submergence or sediment cover.

Despite best intentions, on return to the experimental ponds, the level of AMS Pond had dropped such that the experimental plots were exposed and could not be used. Sampling was undertaken on January 23 (New) and 24 (P70), 2019.

At each pond, oxygen flux measurements involved placing domes made of transparent (clear) and opaque (black) polycarbonate over the experimental and control sites (Figure 7) immediately after the shades had been removed. Domes had weighted flanges that slightly compressed the microbial mats to ensure a good seal. To promote water movement inside the

dome, a motor and battery pack were attached at the base of the dome which encouraged circulation of water and oxygen, and aimed to reduce over saturation of oxygen in the water layer immediately above the mat (motor and battery pack seen in Figure 8). Domes were fitted with a one-way valve and a short length of tubing to allow sampling of the enclosed water with a syringe at 1.5 to 2 h intervals through the incubation. Initially, mixing of the water in the dome was completed using the domes inbuilt pump system, which was then run for one minute prior to each subsequent water sampling. However, during field deployments, in some events pumps did not work and the mixing method was subsequently changed (in samples which the motor was faulty) to manual mixing using the syringe to pump 3 ml of water back and forth from the dome three times prior to sample collection. The tubing and one-way valve linking the chamber to the syringe had an estimated volume of 0.7 ml. To ensure that only chamber water was sampled 2 mL of dead space was removed prior to mixing of the liquid inside the dome or to sampling from pump-mixed domes.

Oxygen concentrations in samples extracted by syringe were estimated using a calibrated PreSens Fibox 4 Fiber Optic Oxygen Meter, with attached Polymer Optical Fiber fixed in a flow-through cell (PreSens Ltd). It was intended to undertake this at the pond site, but the instrument battery did not allow field operation and therefore to overcome the partial equipment failure, samples were measured at the Bratina Island laboratory. To transport samples, a needle was placed on each syringe which was individually sealed by pushing into a rubber stopper to prevent oxygen exchange. Syringes were placed in a bag of pond water to maintain the sampled temperature.

In the laboratory, samples were injected into the PreSens flow cell and the oxygen concentration measurement was accepted when the displayed result plateaued for 5 seconds. Samples were analysed in a vessel that contained pond water, transported to the lab in ziplock bags, to replicate the water temperature to represent the actual dissolved oxygen concentration

more accurately in the samples. Water temperature was measured at the time of sampling using a mercury thermometer, this result was compared to the time of measurement and if appropriate corrections were made to approximate oxygen concentration.

After the experiment was completed two 17 mm cores were removed from each shade for 16S genetic sequencing as described in Section 2.1.3. A control sample was taken from each pond in an area which had not been affected by shading, and these samples were also sequenced. Samples were frozen in dry ice and upon arrival to Scott Base stored at -20°C.

Figure 7. Cloth shades and clear polycarbonate cover and opaque polycarbonate domes in P70 Pond.

Figure 8. Sampling dome with weighted flange to promote sealing and pump and battery pack to circulate water within dome.

2.2.4 Genetic Sequencing

Genetic sequencing was completed using the methodologies outlined in Section 2.1.4.

2.3 PHYSICAL DISTURBANCE EXPERIMENT

2.3.1 Experimental Design

The physical disturbance experiment examined the resilience of microbial mats to disturbances that remove a portion of the mat, and consequently require regrowth from residual populations. This experiment compared three ponds; AMS Pond, New Pond and P70 Pond, which all have matured microbial mats and similar physical and geochemical pond conditions (see section 2.3.2). In each, three replicate sections of microbial mat (approximately 10 cm by 10 cm) were removed in 2017, 2018, and 2019. Sampling of the recolonized area for each time of removal was completed in 2019 providing a 2-year regrowth sample (mat removed in 2017), 1-year regrowth sample (mat removed in 2018) and a 0-year regrowth sample (mat removed

immediately before sampling in 2019) (Table 5**)**. Samples were genetically sequenced to identify the relative abundance and identify recolonizing bacteria.

Mat disturbance similar to that of manual removal occurs naturally through the build-up of gas bubbles under the mat which removes areas of microbial mat through mat lift off as shown in Figure 9. Gas build up to occurs as the sticky, cohesive structure of microbial mats does not allow gases to not be released. The goal therefore was to determine the speed at which mat biota recolonised the area and whether recovery trajectories were likely to reconstitute pre-existing mat, or whether disturbance introduced an element of species turnover.

Table 5. Experimental design for the physical disturbance experiment.

	Number of Ponds	**Year 1 (2017)**	**Year 2 (2018)**	**Year 3 (2019)**
Sample 1 (2-year)	3	Mat removed	No action	Sample
Sample 2 (1-year)	3	No action	Mat removed	Sample
Sample 3 (0-year)	3	No action	No action	Mat removed -> Sample
Sample 4 (Control)	3	No action	No action	Sample

Figure 9. Mat lift-off caused by large gas bubbles forming between the substrate and mat.

2.3.2 Pond Selection

Ponds were selected using the methodology outlined in Section 2.1.2.

2.3.3 Field Sampling

The physical disturbance experiment analysed how cyanobacteria can maintain mat building functions given physical damages to the microbial mat. Areas of microbial mat were removed using a spatula from the pond in annual increments – with one portion removed in 2017, a second section removed in 2018, and a third section in 2019 as described in Table 5 and shown in Figure 10**.** Figure 10 highlights one year of mat regrowth (outlined in blue) and two years of mat regrowth (outlined in yellow). An additional portion of mat was removed to represent the zero-year samples. The microbial mat collected as the zero-year sample was sequenced as the control sample.

Samples in the physical disturbance experiment were extracted using the 16S sequencing methodology outlined in Section 2.1.3.

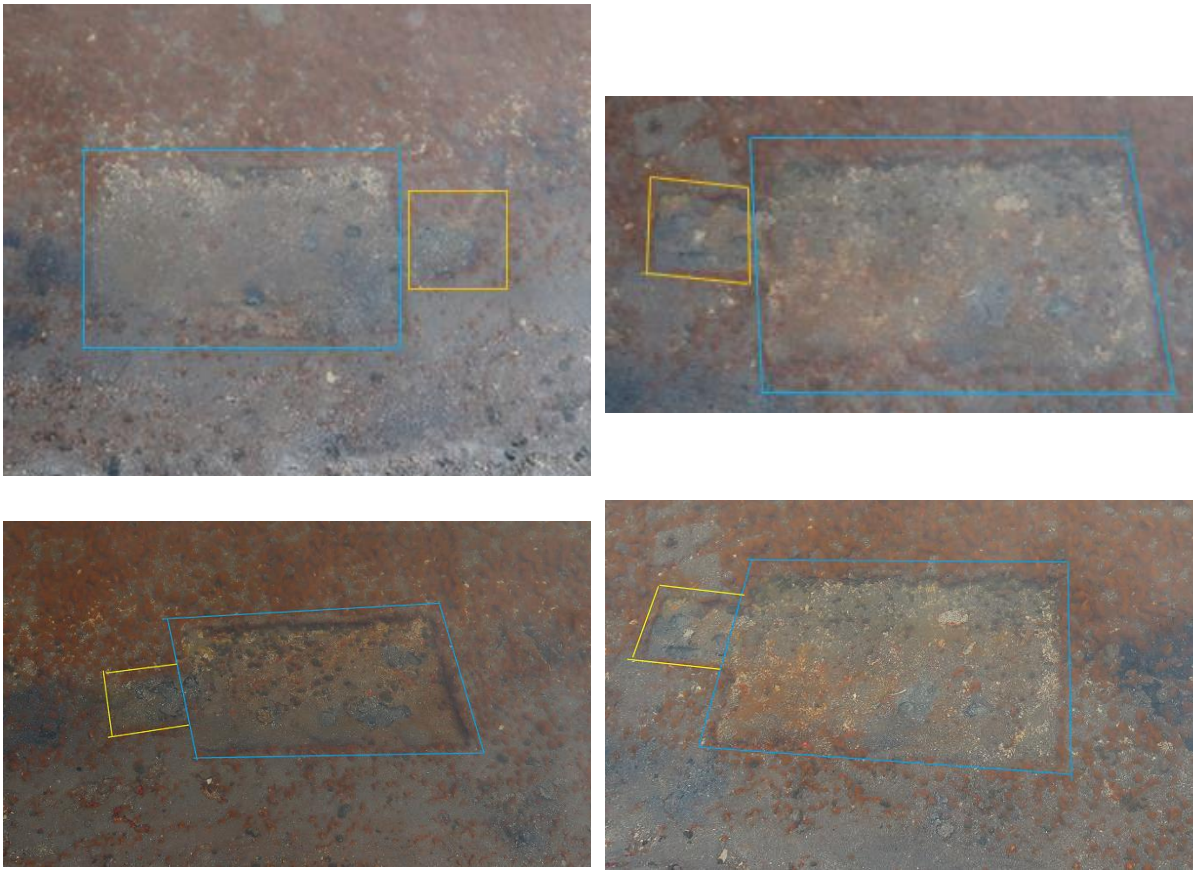


Figure 10. Images of areas of mat removed for the disturbance study, the areas outlined in blue show where two years of mat has regrown, areas highlighted in yellow show areas of one year regrowth. Top left and top right P70 Pond, bottom left and bottom right New Pond.

3 RESULTS

3.1 NITROGEN FIXATION EXPERIMENT

3.1.1 Water Chemistry

Within the five ponds pH was alkaline, ranging from 8.98 (Fresh) to 9.88 (P70), with no apparent relationship to conductivity (Table 6). Nitrite-N was consistently below detection limits, and nitrate-N was also always present at low concentrations. The dominant form of dissolved inorganic nitrogen was ammoniacal-N. In contrast DRP was consistently high with concentrations approaching those of ammoniacal-N. N:P ratios were correspondingly low, ranging from 0.38 (Casten) to 6.44 (P70) by weight. When calculated as molar ratios, all were less than 15 (Table 7).

Table 6. Conductivity and pH of ponds included in the Nitrogen Fixation experiment.

Pond	Conductivity 2019 (mS/cm)	pH
Brack	10.5	9.27
Casten	2.3	9.35
Fresh	1.2	8.98
P70	6.2	9.88
Salt	41.2	9.38

Table 7. Nutrient concentrations measured from nitrogen fixation experiment ponds (g/m^3). Inorganic nitrogen to phosphorous ratios were calculated as the sum of nitrate-N, nitrate-N and ammoniacal-N divided by the dissolved reactive phosphorous concentration.

Pond	Forms of Nitrogen			Dissolved Reactive Phosphorous (g/m^3)	N:P ratio	
	Total Ammoniacal – Nitrogen (g/m^3)	Nitrite – Nitrogen (g/m^3)	Nitrate – Nitrogen (g/m^3)		Inorganic N:P ratio by weight	Molar inorganic N:P ratio
Brack	0.045	<0.001	0.001	0.032	1.4	3.3
Casten	0.048	<0.001	0.001	0.129	0.4	0.8
Fresh	0.105	<0.001	0.005	0.034	3.7	7.2
P70	0.058	<0.001	<0.001	0.009	6.4	13.7
Salt[2]	0.320	<0.002	0.006	0.054	6.6	11.5

3.1.2 Acetylene Reduction Assays

The average mat biomass was significantly lower for Casten and Fresh ponds than the other three ponds (Table 8, Table 9). Ponds which had higher relative abundance of Cyanobacteria also tended to have higher average acetylene reduction (Figure 11) Analysis of variance (ANOVA) indicated that there were significant differences between ponds (Table 18), A Bonferroni corrected post-hoc t-test indicated that Casten and Fresh were significantly lower in areal biomass than the other ponds, which did not differ from each other.

Despite having the lower areal biomass, Casten and Fresh ponds showed the highest areal rates of acetylene reduction, with averages of 49.6 and 40.8 $\mu Mol/m^2/h$, respectively (Table 8). Brack Pond had the lowest average level of acetylene reduction and also the smallest variability between samples (Table 8). Using an ANOVA it was identified that there is a significant difference between samples. A bonferroni corrected post-test t-test indicated that Brack Pond

[2] Insufficient sample required that a dilution be performed prior to analysis of sample, resulting in a detection limit higher than that normally achieved for the NoxN / NO_2N analysis.

has significantly lower acetylene reduction than Casten pond, but that there were no further significant changes within the data. As the low salinity samples had the highest acetylene reduction rates, there seems to be a tendency for lower conductivity ponds to have higher acetylene reduction rates (Figure 12). A negative tendency was identified in the regression analysis, however the relationship was not significant (Figure 13) ($r^2 = 0.7$, $p = 0.19$).

Table 8. Areal biomass (as ash free dry mass) and acetylene reduction rates in five ponds on the McMurdo Ice shelf. Values are mean ± standard deviation (N=5).

Pond Name	**Biomass (kg/m^2)**	**Acetylene reduction rate ($\mu Mol/m^2/h$)**
Brack	4.41 ± 0.62	22.4 ± 3.4
Casten	1.44 ± 0.55	49.6 ± 17.1
Fresh	1.39 ± 0.19	40.8 ± 11.0
P70	3.60 ± 0.16	24.4 ± 7.3
Salt	4.71 ± 0.62	27.8 ± 11.5

Table 9. ANOVA of the areal biomass (ash free dry mass) of microbial mats in five ponds on the McMurdo Ice Shelf. Data are shown in Table 8.

Source of Variation	***SS***	***df***	***Mean Square***	***F***	***P-value***	F_{crit}
Between Groups	52.6932	1	52.7	47.4	<0.001	4.09
Within Groups	42.2691	38	1.1			
Total	94.9623	39				

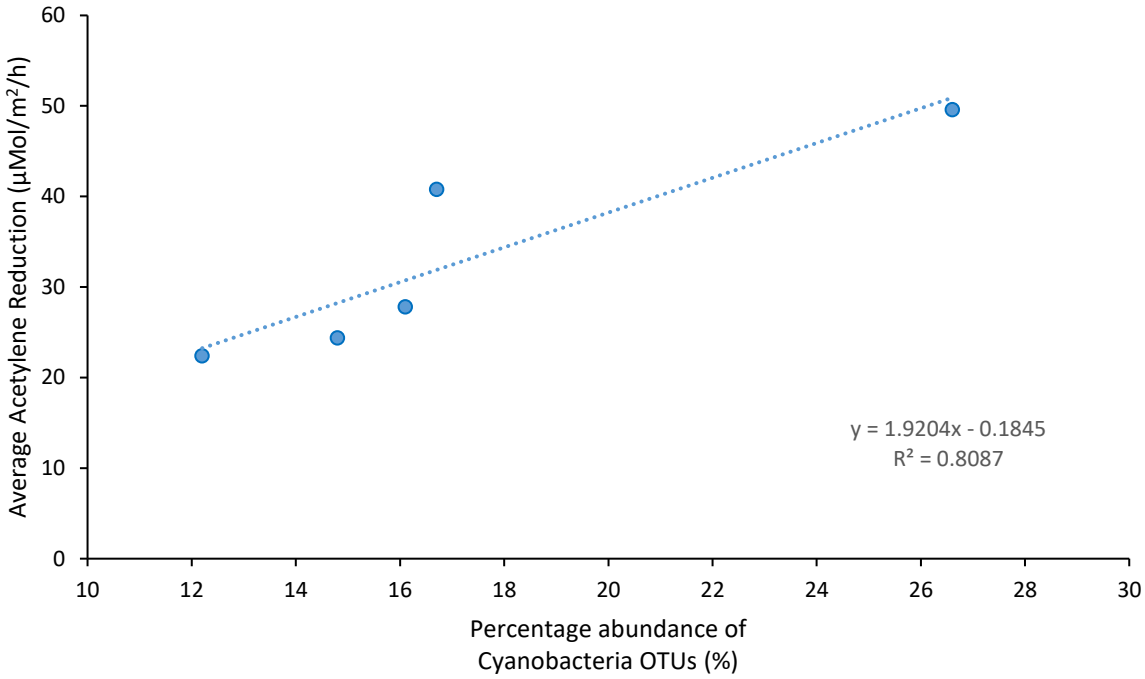


Figure 11. Average acetylene reduction as a function of average percentage abundance of cyanobacteria OTUs in ponds (% abundance of identified cyanobacteria OTUs in comparison to total identified OTUs). Significance P=<0.04.

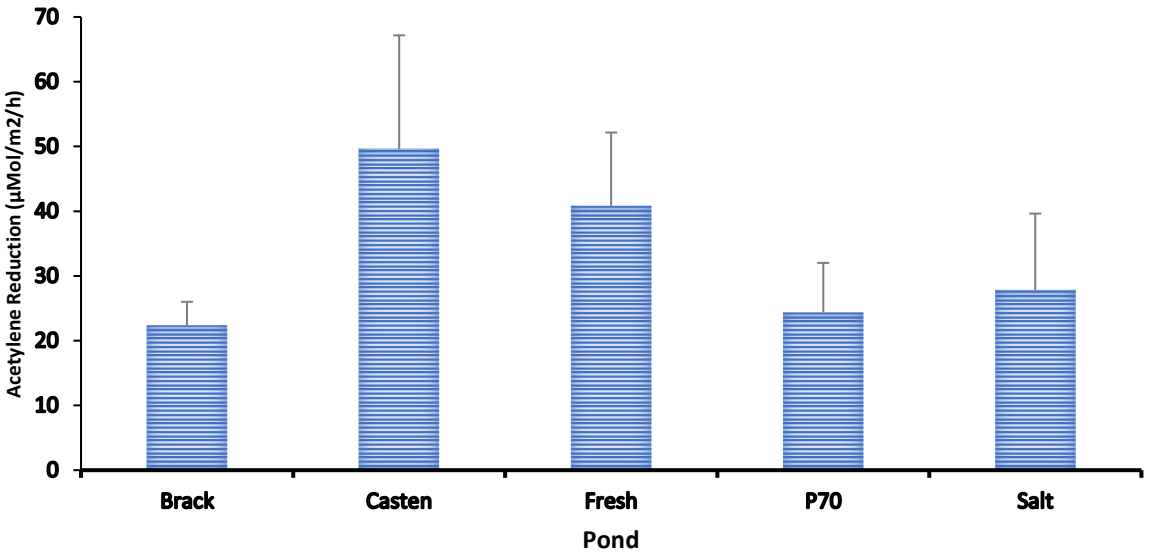


Figure 12. Acetylene Reduction in Bratina Island Ponds. Bars represent mean ± standard deviation.

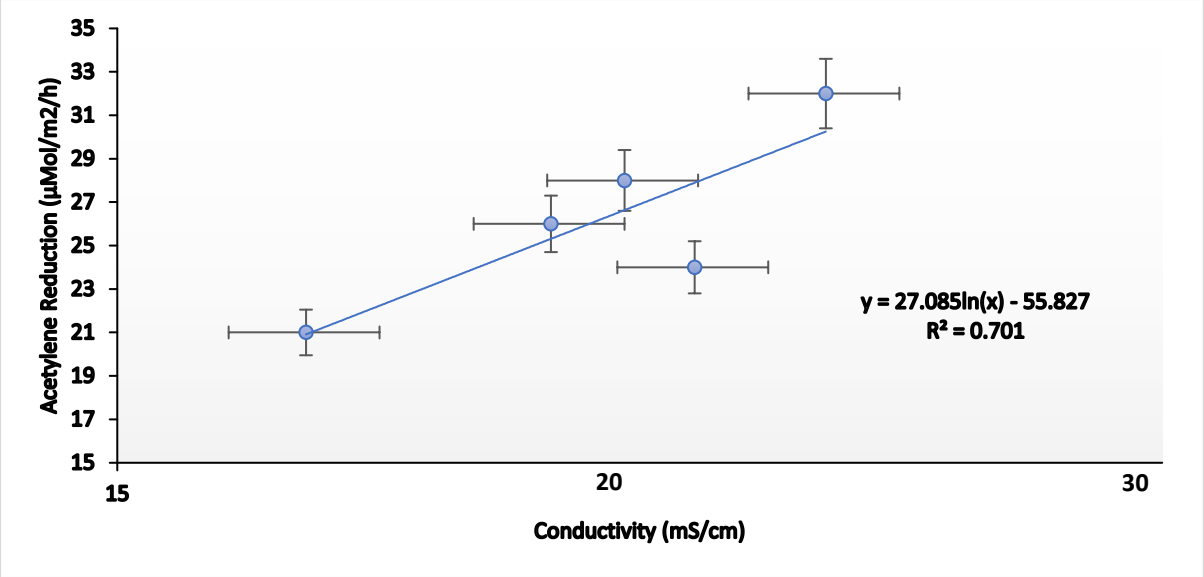


Figure 13. Mean acetylene reduction (5% error) as a function of mean conductivity (5% error) (note log scale) in five ponds of the McMurdo Ice shelf. The line fitted is a logarithmic regression, equation shown, but the slope of this is not significant (p = 0.19).

3.1.3 16S Composition

There was generally good replication when analysing the alpha diversity of samples collected from each pond for the nitrogen fixation experiment (Table 10, Figure 14). Salt Pond had the lowest values in all of the analysed index's, indicating low diversity and evenness, consistent with mats dominated by a few taxa (Table 10). Of the remaining ponds, Casten had the highest observed and ACE index values but had very low Shannon and Simpson values which were comparable to Salt pond, suggesting more taxa but again low evenness. Samples from P70 Pond and Brack Pond tended to group together in all analysis, samples from Fresh Pond were also very similar to these two groups but was slightly offset in each alpha diversity index. Using the Shannon and Simpson analysis Brack and P70 ponds ranked the highest with Fresh Pond slightly behind.

Table 10. Alpha diversity measurements of cyanobacteria in the Brack, Casten, Fresh, P70 and Salt ponds for the nitrogen fixation experiment. Values given are mean ± standard error (N = 3).

Index	Brack	Casten	Fresh	P70	Salt
Observed OTUs	20 ± 1.5	24.3 ± 2.0	23.3 ± 42.3	19.7 ± 1.7	15.7 ± 2.7
Chao1	22.2 ± 1.2	27.7 ± 2.2	26.7 ± 2.9	21.0 ± 2.1	18.0 ± 3.2
ACE	23.8 ± 0.9	31.5 ± 3.1	26.0 ± 3.1	23.2 ± 2.6	22.2 ± 5.0
Shannon	2.2 ± 0.14	1.6 ± 0.22	2.0 ± 0.10	2.2 ± 0.08	1.4 ± 0.10
Simpson	0.9 ± 0.02	0.7 ± 0.09	0.8 ± 0.02	0.9 ± 0.01	0.7 ± 0.04

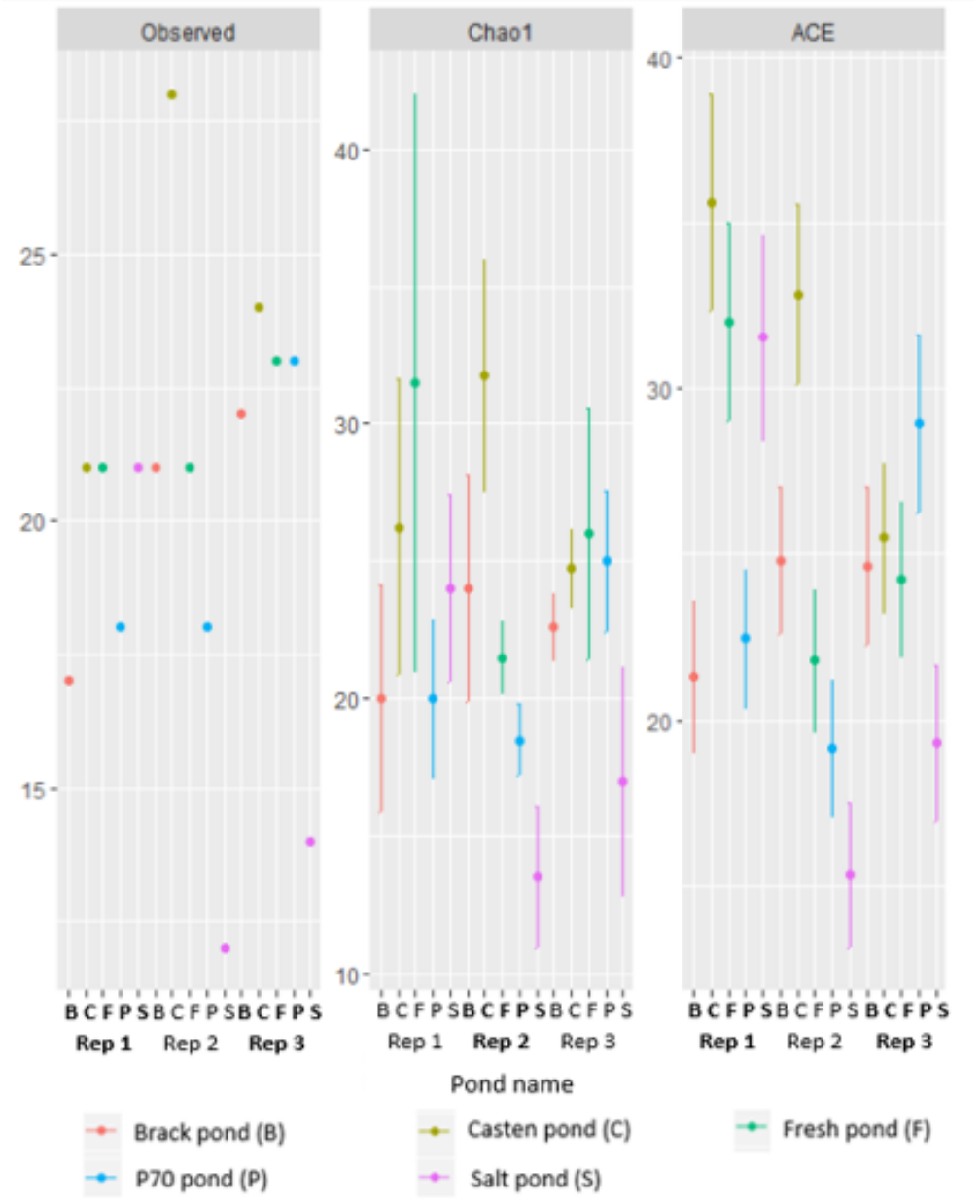


Figure 14. Alpha diversity measurements of cyanobacteria in the Brack, Casten, Fresh, P70 and Salt ponds for the nitrogen fixation experiment.

Phylogenetic diversity is the minimum total length of all the phylogenetic branches required to span a given set of taxa on the phylogenetic tree originally posed by Faith (1992). Phylogenetic trees show the evolutionary relationships between samples. In this experiment it seems that lower salinity ponds tended to contain a more evolutionarily diverse range of organisms. Saline ponds tended to show least phylogenetic diversity, indicating a narrower range of phylogenies were able to colonise and thrive in this environment.

Casten and Salt ponds had the highest number of identified OTUs, however P70 Pond had the most unique OTUs (Table 11). Most samples returned 15-25 unique cyanobacterial OTUs, with similar total cyanobacterial OTU counts of between 2500 and 5000. Brack and P70 had the lowest total number of identified OTUs and Salt Pond had the lowest number of unique OTUs (Table 11). The number of unique cyanobacteria OTUs and the total number of cyanobacteria OTUs had a weak, positive correlation (Figure 15, Figure 16).

Table 11. Identified total and unique Bacteria and cyanobacteria OTUs identified in the Nitrogen Fixation study. Table show average and standard deviation (s.d.) N=3 in each case.

Pond Name	Total number of OTUs Identified	Number of unique OTUs identified	Total number of cyanobacteria OTUs Identified	Number of cyanobacteria unique OTUs identified	Percentage of cyanobacteria OTUs in sample
Brack	22,041 (3254)	604 (113)	2,680 (47)	21 (3)	12.2 (2.2)
Casten	25,411 (3905)	519 (58)	6,762 (2010)	25 (4)	26.6 (9.5)
Fresh	23,478 (2913)	564 (83)	3,922 (227)	22 (1)	16.7 (3.1)
P70	22,254 (4591)	797 (115)	3,304 (532)	20 (3)	14.8 (0.7)
Salt	25,502 (5902)	395 (29)	4,106 (255)	16 (5)	16.1 (4.9)

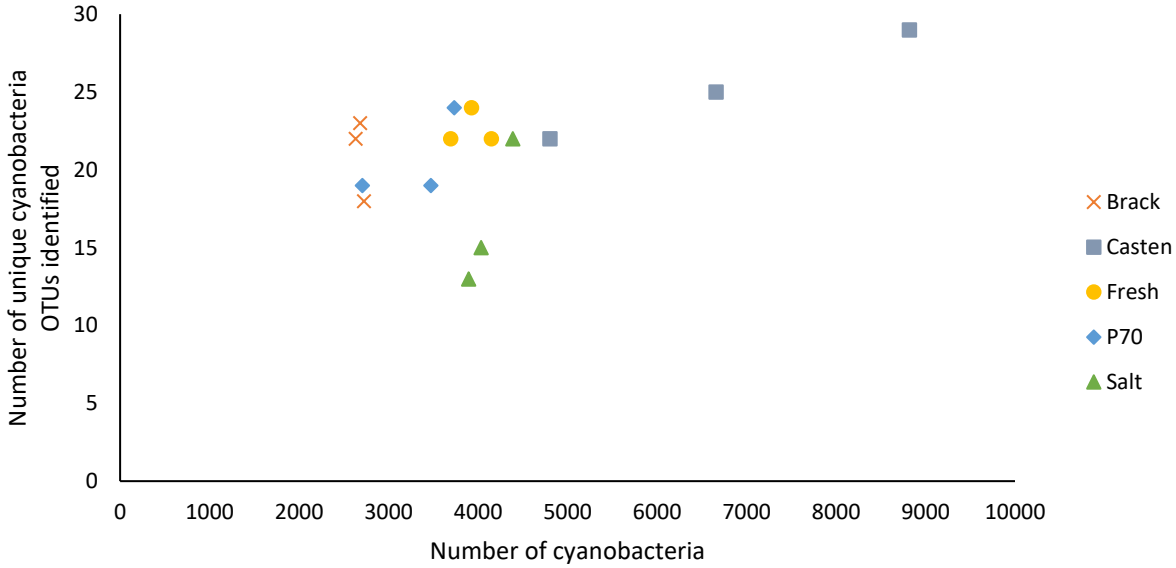


Figure 15. Number of unique cyanobacteria OTUs found in the nitrogen fixation pond samples as a function of total cyanobacteria present per sample. Red symbols indicate the average for each pond and blue individual replicates.

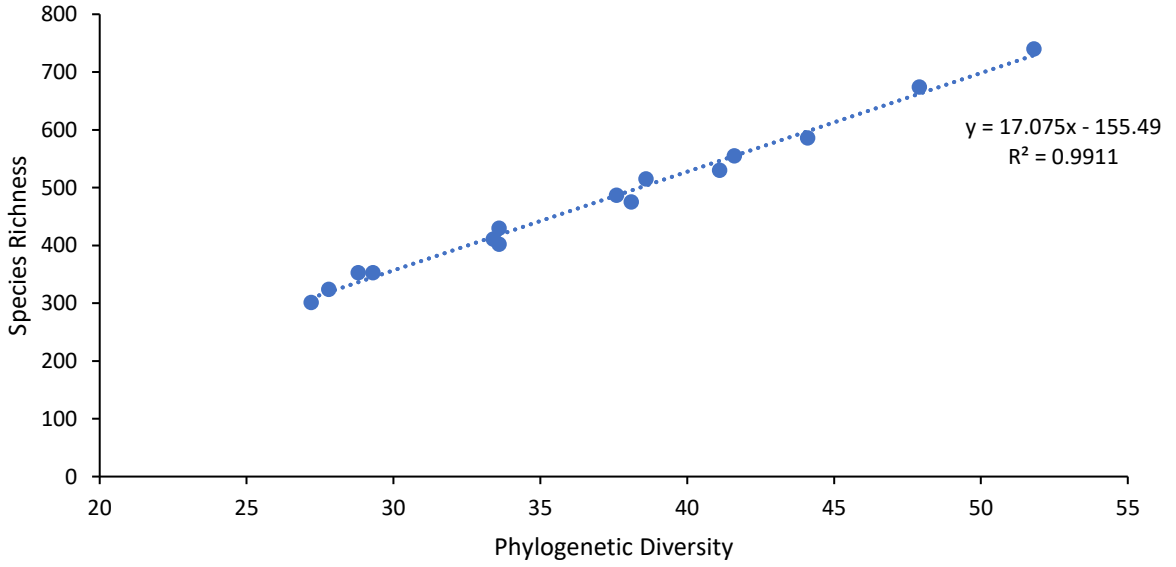


Figure 16. Species richness of the nitrogen fixation pond samples as a function of the phylogenetic diversity of each sample (p-value = 0.04).

Phylogenetic allocation of 16S sequences by CREST showed that, in general, all five ponds had very similar microbial composition at a phylum level (Figure 17). Pond composition in all ponds were numerically dominated by Cyanobacteria, Bacteroidota, and Proteobacteria, and

within each pond samples tended to have good replication at this level. Within the cyanobacteria, at the family level, pond samples also tended to show good replication (Figure 18). The Casten 1 sample appears more similar to the Salt Pond samples than to other Casten samples, it is possible that this represents contamination. Other than the Casten 1 sample each pond otherwise appears distinct to each other. All ponds primarily contained representatives of the families *Pseudobaenaceae*, *Cyanobacteriaceae*, *Nostocaceae*, *Phormidiaceae*, *Oscillatoriaceae* and *Nodosilineaceae*. More saline ponds had a higher abundance of *Oscillatoriaceae*, and fresher ponds had a higher abundance of *Pseudobaenaceae*. All ponds contained substantial numbers of OTUs allocated to the *Nostocaceae*, the family of heterocyte-containing cyanobacteria most commonly associated with nitrogen fixation.

The same trends occurred at the genus level, with good replication in ponds, again with exception of Casten 1 (Figure 19).

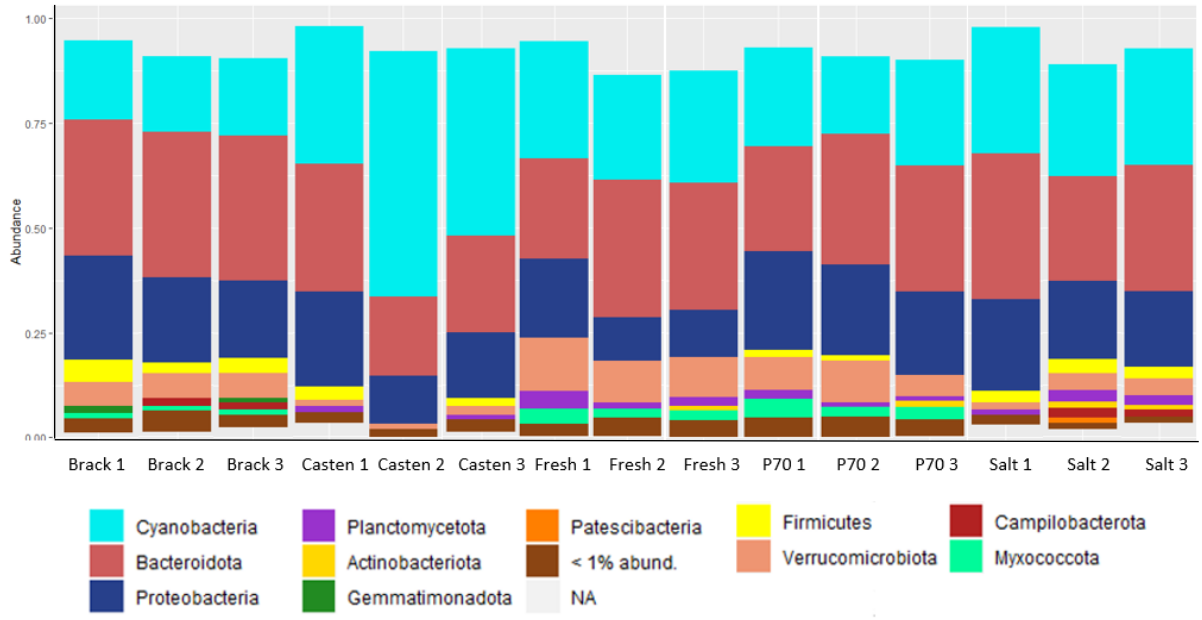


Figure 17. Proportion abundance of specific bacterial OTUs at a phylum level, allocated by CREST through 16S sequencing.

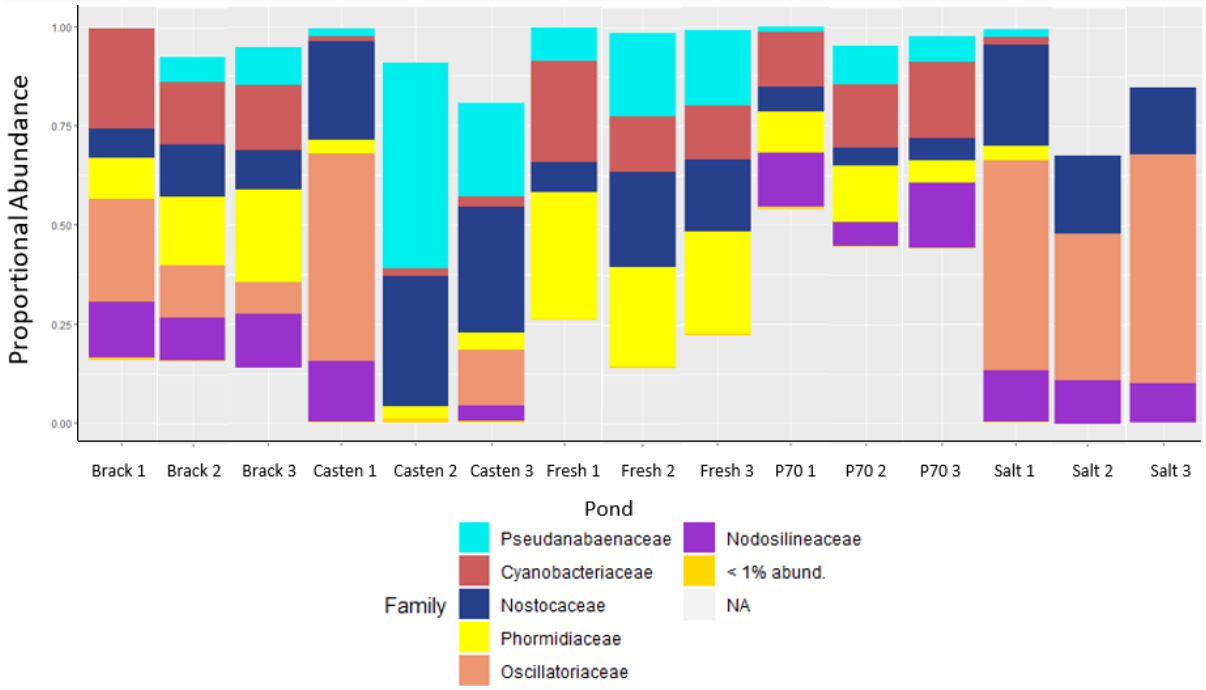


Figure 18. Proportion abundance of specific cyanobacterial OTUs at a family level, allocated by CREST through 16S sequencing.

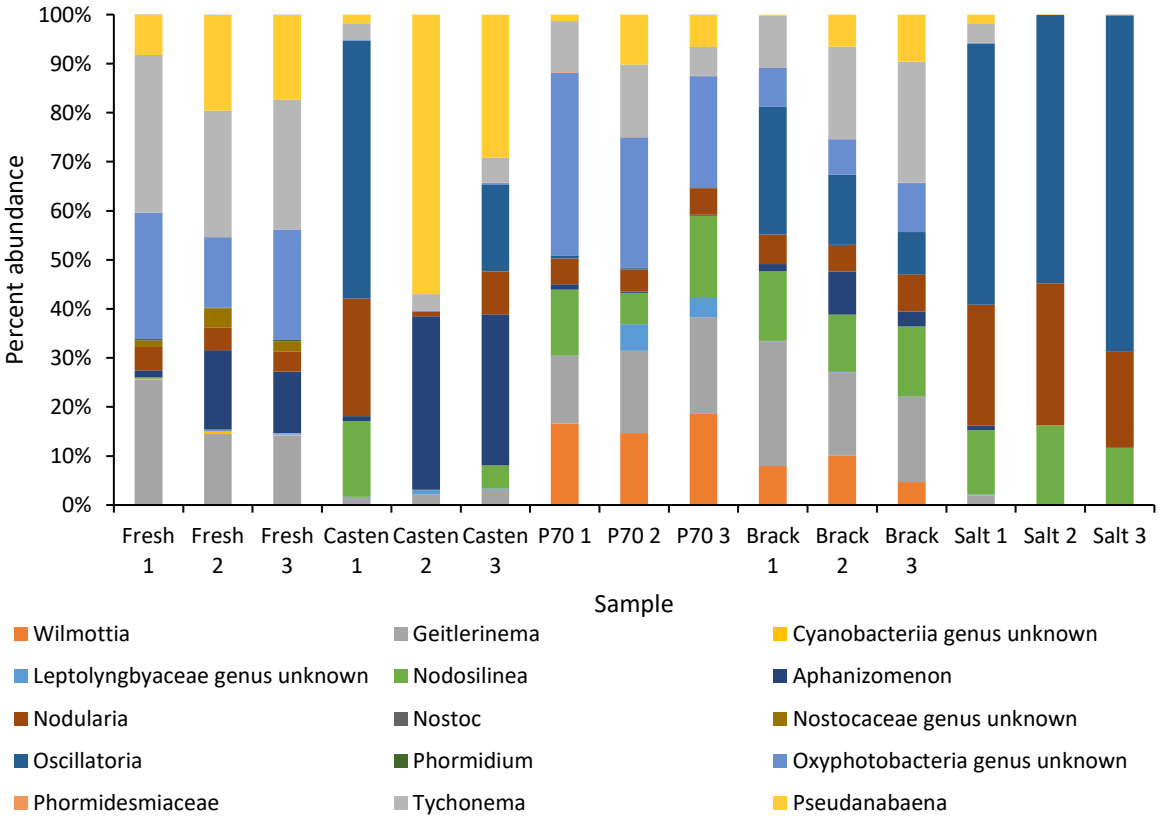


Figure 19. Proportion abundance of specific cyanobacterial OTUs at a genus level, identified by CREST through 16S sequencing.

Analysis using non-metric multidimensional scaling of all bacteria (Figure 20) and cyanobacteria only (Figure 21) further illustrates the similarity of samples from each pond. In this analysis, which is based on a resemblance matrix, the closer two samples or groups are, the more similar their communities are. Stress levels in the analysis are low, with values much less than 0.20 which is generally accepted as indicating an accurate representation within the axes presented. Samples tended to show strong replication by pond. Once again, the Casten samples do not follow these trends as accurately, as the Casten 1 sample is very similar to the Salt Pond samples, as seen in other analysis and possibly indicative of sample contamination or confusion. With the exception of Casten Pond, changes in the community highlighted in Figure 20 and Figure 21 are consistent with a change in community along a conductivity gradient, which correlates well with axis 1 of both NMDS plots, while axis 2 primarily separates samples within ponds.

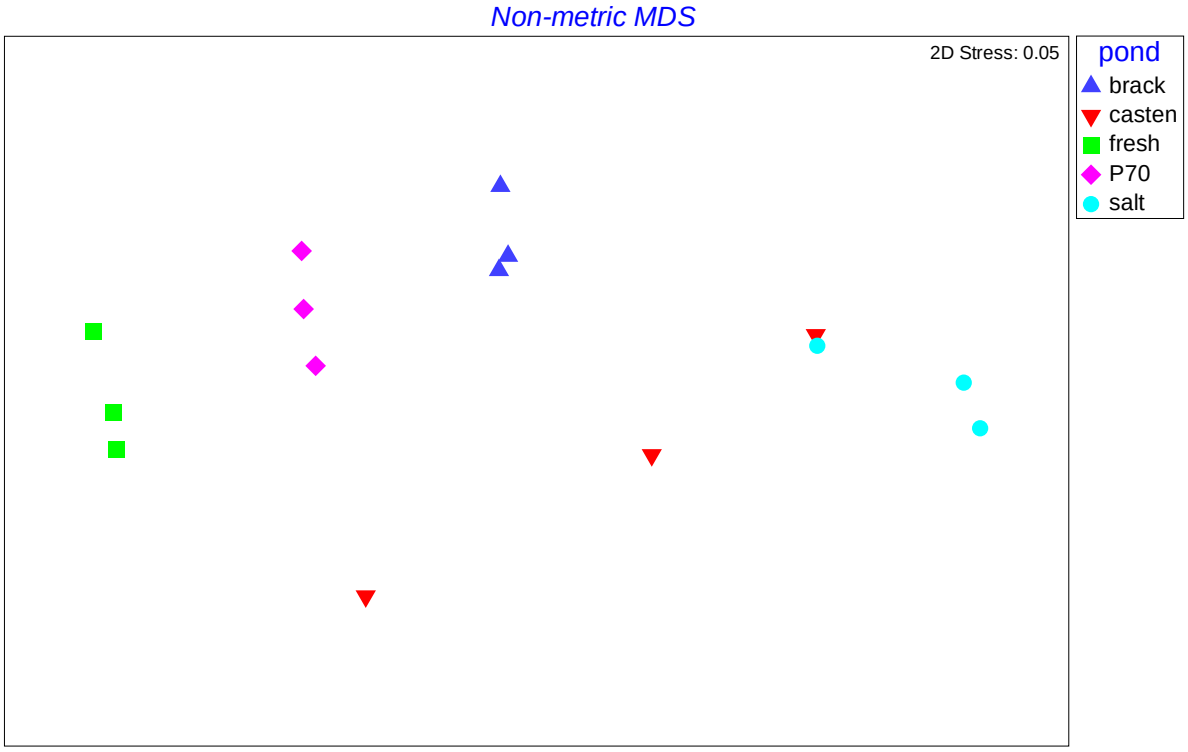


Figure 20. Non-metric multidimensional scaling plot of resemblance for all bacteria OTUs (square root transformed) in the five ponds used for the N-fixation experiment.

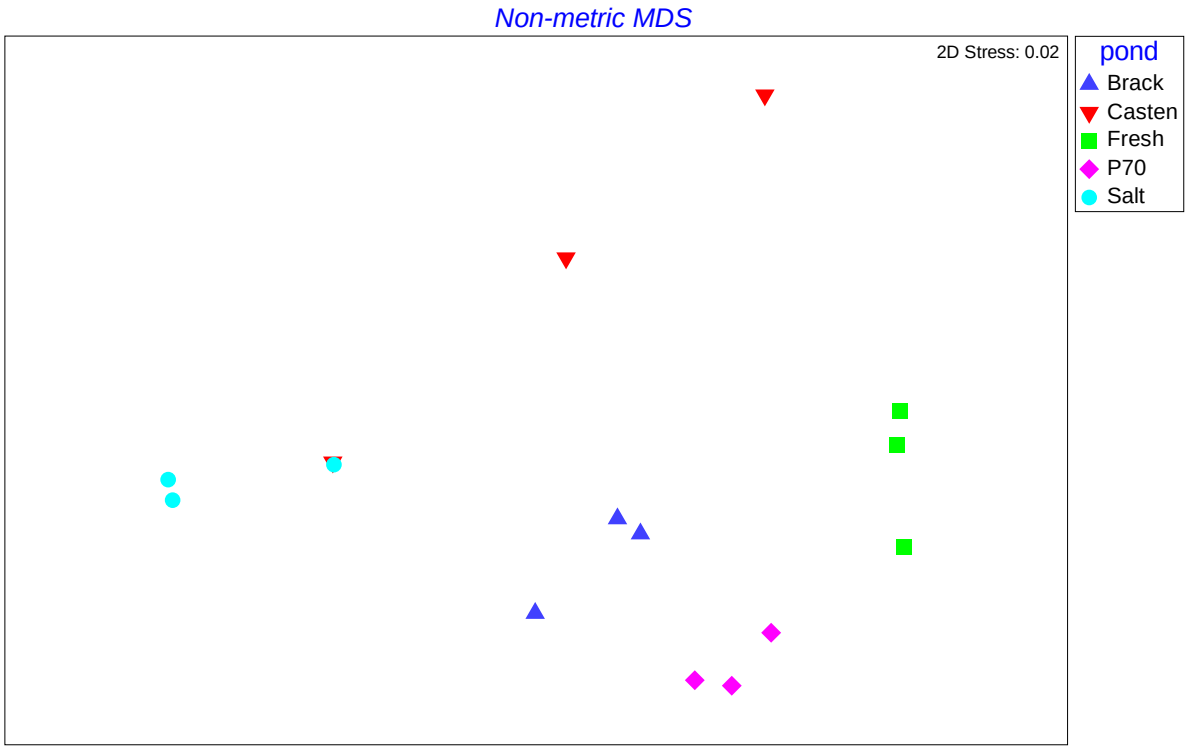


Figure 21. Non-metric multidimensional scaling plot of resemblance for cyanobacteria OTUs (square root transformed) in the five ponds used for the N-fixation experiment.

3.2 Light Disturbance Experiment

The study design for the disturbance experiments originally considered three ponds for the light disturbance experiment however due to water level fluctuations the experiment was compromised as the shades in AMS Pond were brought above the waterline by water level drop. Of the three AMS Pond shades initially deployed below water level, only one remained submerged after being left for a year, one was partially submerged, and the third was completely out of the water (Figure 22A). Shaded plots in AMS Pond were too shallow to allow the oxygen flux chambers to be deployed. All three shades in New Pond (Figure 22B) had sediment build-up on top of the shade causing uneven and additional shading. There were no notable issues with the shades placed in P70 Pond (Figure 22C). Samples from P70 Pond and New pond, the two least compromised ponds, were used in the light disturbance experiment and were sampled for DNA extraction.

Figure 22. Images taken January 2019 of shades in: A) AMS Pond B) New pond C) P70 Pond.

During initial observations, significant colour changes were noted in the shaded areas of microbial mat (Figure 23). Figure 23 displays the area of the mat which was shaded and shows a distinct green pigmentation where the mat has previously been covered, which is visually different from the orange mat colour seen in the remainder of the pond. The microbial mats tended to be the darkest shades of green at the centre of the shaded areas and have lighter green pigmentation at the edge of the shade extent. Visually this appeared as a dark green centre surrounded by a ring of light yellowy-green which transitioned into an orange pigmented microbial mat. This colouring pattern was seen under all three ponds in which the experiment was undertaken but was less pronounced in AMS Pond where the shades were only partially submerged. The bright red areas seen on the tips of mat pinnacles are clusters of the rotifer *Philodina gregaria*.

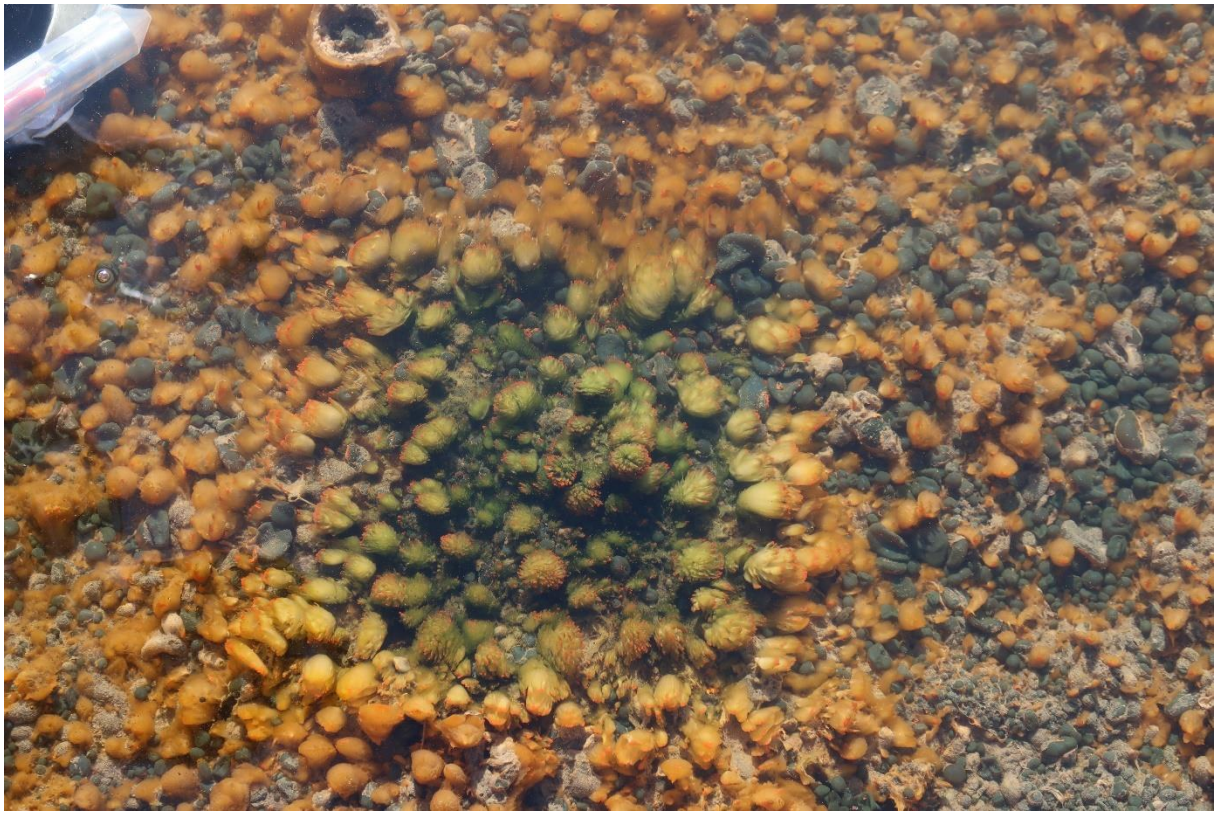


Figure 23. Image showing the area of mat previously shaded (green).

Measurements of irradiance were taken above and under shades to estimate the absorption of photosynthetically available radiation by the shades. Measurements, taken in air, show the shading was between 870 and 930 µMol photons $m^{-2}s^{-1}$. The P70 Pond shades were allowing through approximately 35 to 48 µE/m^2 (Table 12), a 95-96% decrease in ambient light.

Table 12. Irradiance transmission through the shades from P70 and New ponds at the end of the shading treatment.

Pond/ Date/Time	**Ambient irradiance (µMol photons $m^{-2}s^{-1}$)**	**Shaded irradiance (µMol photons $m^{-2}s^{-1}$)**	**% transmission**
New Pond, 23/01/2019/ 15:50	930	45 - 48	4.8 – 5.2
P70 24/01/2019 15:50	870	35 - 40	4.0 – 4.6

Oxygen flux measurements (Table 13, Table 14, Figure 24) show that microbial mats which were covered by opaque domes, and subsequently received no light, had a net oxygen consumption while those covered by clear domes had a net oxygen production as occurs during photosynthesis. This trend was evident irrespective of whether the samples were previously shaded. Samples maintained in clear domes tended to have dissolved oxygen concentrations 12 – 14 mg/L or 90 – 120% air saturation in the initial reading which increased to between 16 and 18 mg/L or 110 – 140% air saturation at the final reading taken six to seven hours later. Samples maintained in opaque domes tended to have dissolved oxygen concentrations of 11 – 12 mg/L or 90 – 100% air saturation in the initial reading which decreased to 4 – 7 mg/L or 30 – 50% air saturation at the final reading six to seven hours later.

When the rates of net photosynthesis in each segment of the incubations are related to the average irradiance during that segment, distinct differences between the shaded and the control treatments were evident (Fig 22-23). Specifically, shaded treatments showed maximum net oxygen evolution at an irradiance of 200-300 µMol photons $m^{-2}s^{-1}$, an irradiance at which the control samples in both ponds were still underwent net respiration. Control samples tended to continue to increase net photosynthesis to the highest irradiances encountered, while for shaded samples the rate of net oxygen evolution was declining as irradiance increased, reaching zero or negative rates at 500-600 µMol photons $m^{-2}s^{-1}$.

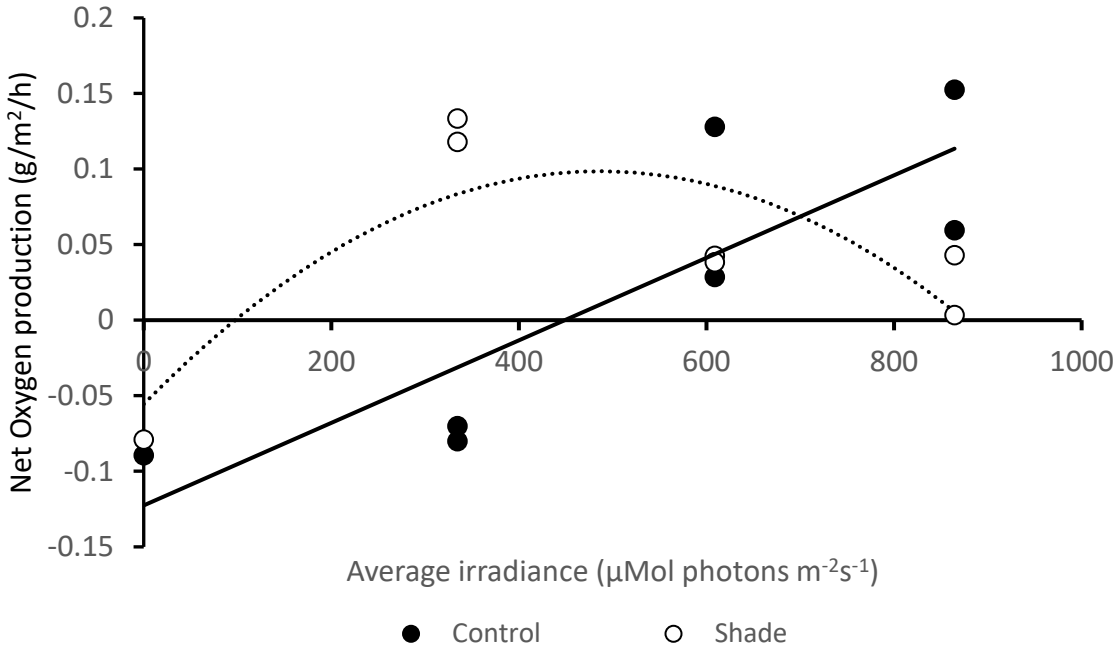
Table 13. Dissolved oxygen concentrations from an oxygen flux experiment at P70 Pond 23 January 2019. Oxygen concentrations in samples withdrawn from clear and opaque flux chambers are given as mg/L and % atmospheric saturation at ambient temperature (AS). Pond-side irradiance and ambient water temperature are also indicated.

Time since start (h)	**0**		**1.5**		**3.5**		**6**	
Water Temperature (°C)	8		8		8		8	
Ambient Irradiance (µMol photons $m^{-2}s^{-1}$)	880		890		720		260	
	mg/L	***%AS***	**mg/L**	***%AS***	**mg/L**	***%AS***	**mg/L**	***%AS***
Control Clear 1	13.34	*109.2*	13.56	*112.2*	15.78	*127.1*	17.52	*138.9*
Control Clear 2	13.62	*112.1*	13.97	*116.3*	16.09	*129.5*	16.18	*130*
Control Opaque 1	12.23	*101.1*	10.58	*87.8*	9.01	*72*	6.57	*52.1*
Control Opaque 2	12.74	*104.8*	11.47	*95.1*	8.65	*70.1*	6.13	*48.7*
Shade Opaque 1	7.55	*61.8*	4.62	*38.5*	7.78	*62.5*	10.05	*79.4*
Shade Clear 2	14.2	*117.2*	14.62	*121.3*	16.75	*135.1*	18.14	*143.8*
Shade Opaque 1	8.68	*70.9*	6.9	*57.1*	5.82	*46.8*	5.2	*34*
Shade Black 2	12.22	*99.5*	9.37	*77.9*	6.97	*56.3*	3.9	*30.8*

Table 14. Dissolved oxygen concentrations from oxygen flux experiment at New pond, 24 January 2019. Oxygen concentrations in samples withdrawn from clear and opaque flux chambers are given as mg/L and % atmospheric saturation at ambient temperature (AS). Pond-side irradiance and ambient water temperature are also indicated.

Time since start (hours)	**0**		**2**		**5**		**7**	
Water Temperature (°C)	**-**		**7**		**7**		**6**	
Ambient irradiance (µMol photons $m^{-2}s^{-1}$)	**930**		**800**		**420**		**250**	
	mg/L	**%AS**	**mg/L**	**%AS**	**mg/L**	**%AS**	**mg/L**	**%AS**
Control Clear 1	13.9	111.2	17.0	138.2	17.8	134.9	16.4	121.0
Control Clear 2	12.2	98.1	13.4	108.7	17.2	129.5	15.6	116.6
Control Opaque 1	11.5	92.5	10.6	86.9	8.8	66.4	6.8	50.9
Control Opaque 2	13.6	108.0	11.7	96.2	8.3	62.2	5.7	42.5
Shade Clear 1	11.9	96.2	12.0	98.5	8.9	142.0	16.0	118.2
Shade Clear 2	13.3	106.8	14.1	114.8	15.3	114.3	17.6	131.0
Shade Opaque 1	7.5	60.2	3.6	29.4	5.8	44.1	4.1	30.8
Shade Opaque 2	11.5	92.5	9.2	75.4	2.5	18.7	4.3	31.8

A.



B.

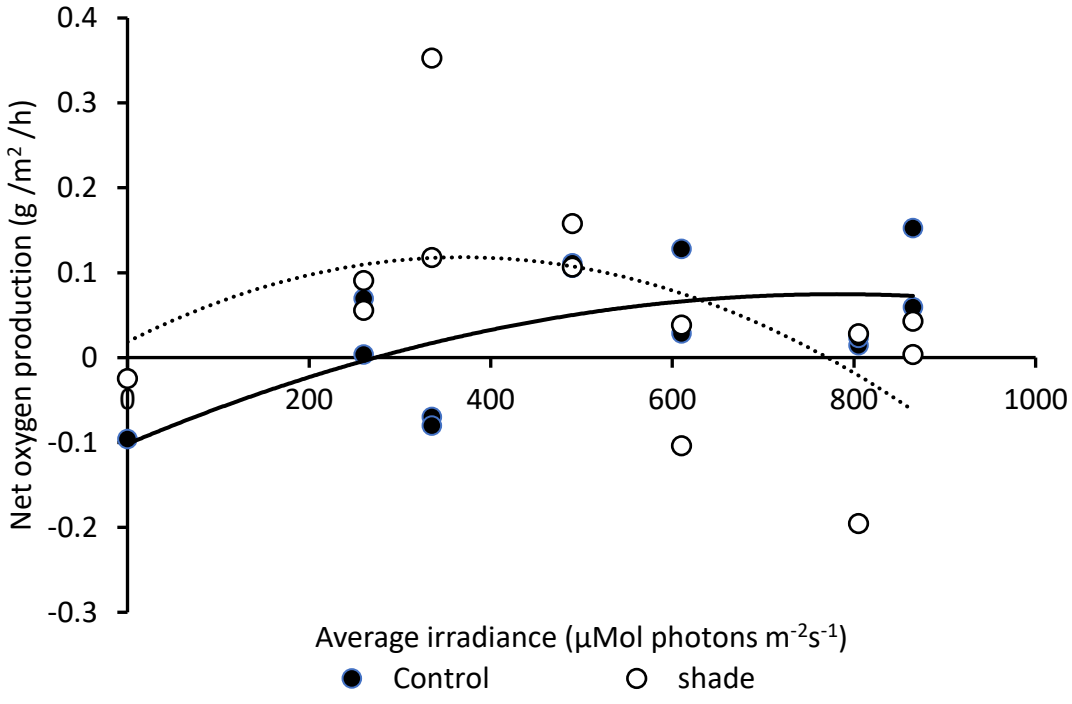


Figure 24. The relationship between the irradiance and flux of oxygen within the chambers deployed on control and shaded microbial mats that had been covered for 12 months by 75-95% shading. Average irradiance is the mean of that at the beginning and end of 1.5-to-2-hour periods (Table 14). Note that the zero is the mean oxygen exchange for all of the dark incubation segments. Plot A is for P70, and Plot B is for New Pond. Curves fitted to these data are second order polynomials and have no intent other than to show the general pattern of the relationships.

Both ponds had similar species richness and phylogenetic diversity (Table 15**)** and there was little evidence of a marked change in either variable during the shading treatments. As seen in other experiments, mats from P70 and New Pond are primarily comprised of cyanobacteria, Bacteroidota and Proteobacteria (Figure 25**,** Figure 26). The control samples of both ponds contain higher proportions of firmicutes, and New B contained a higher proportion of Chloroflexi. Species composition was, however, not seen to vary largely between ponds or between treatments. Mats showed a high level of similarity across treatments when cyanobacteria families were identified, and there was no clear evidence of a shift in composition that accompanied the shade treatment in either pond (Figure 27). New pond contained high abundance of *Pseudobaenaceae* and *Nostocaceae* (Figure 27), while P70 Pond contained primarily *Cyanobacteriaceae.* However, *Phormidiaceae, Nodosilineaceae, Nostocaceae, Oscillatoriaceae* and *Pseudobaenaceae* were also common in various samples.

Table 15. New pond and P70 Pond species richness and phylogenetic diversity– light experiment.

Pond	**Treatment**	**Phylogenetic Diversity**	**OTU abundance**
New	Control	57.6	799
	A	56.3	783
	B	46.9	630
	C	50.9	677
P70	Control	47.7	652
	A	53.5	733
	B	52.8	750
	C	44.7	600

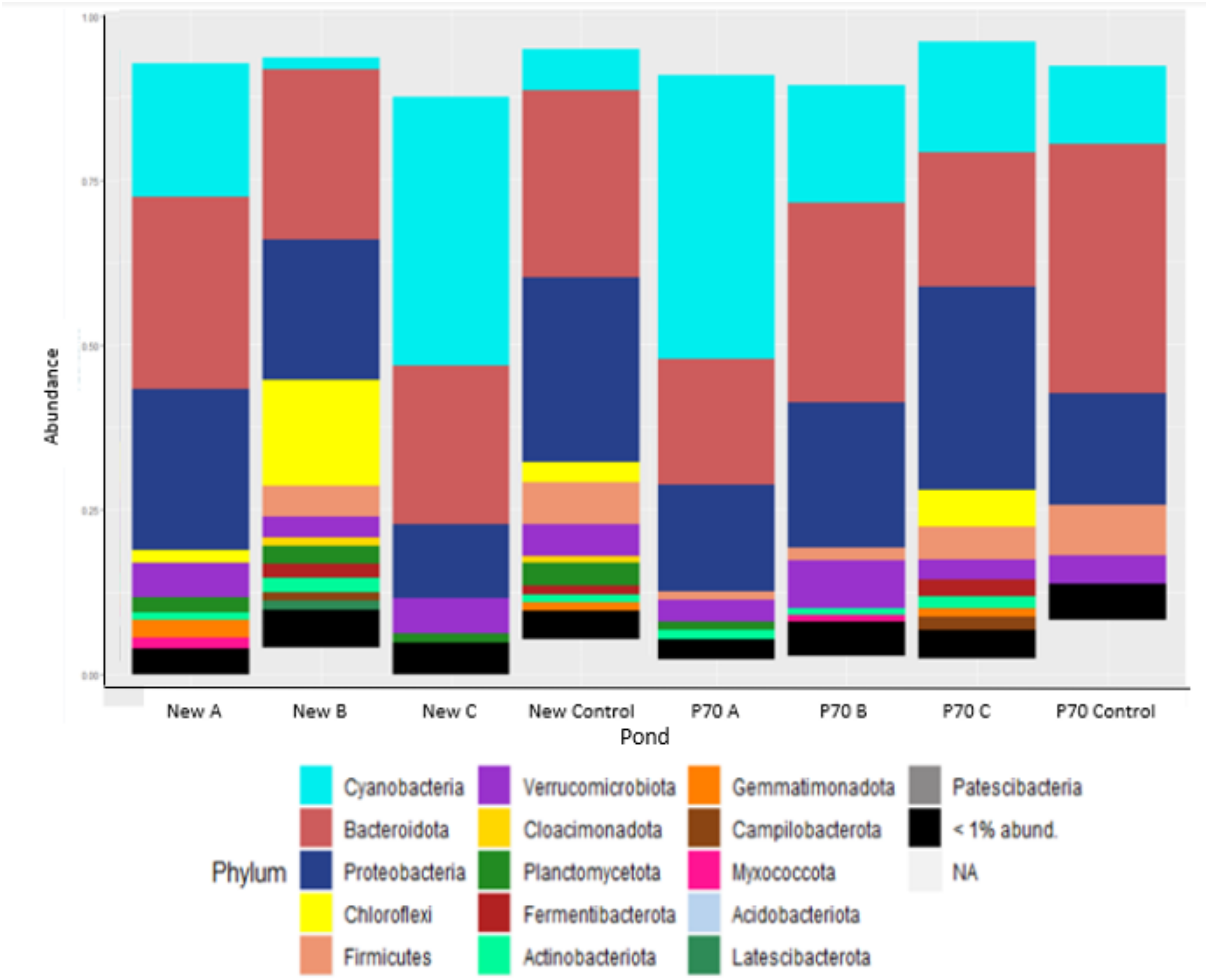


Figure 25. Proportion abundance of bacterial OTUs at phylum level allocated by CREST through 16S sequencing.

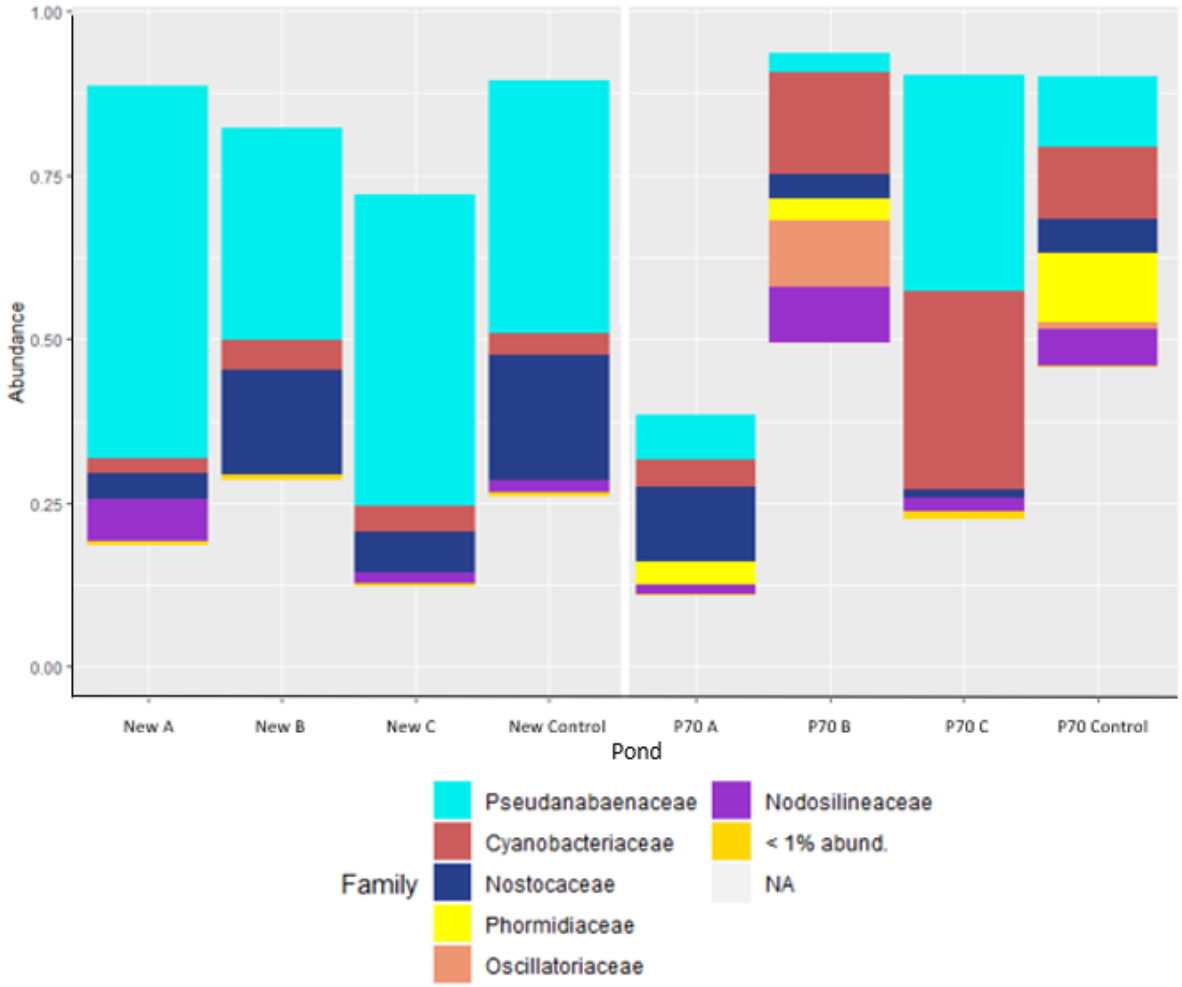


Figure 26. Proportion abundance of cyanobacteria OTUs identified at phylum level in the disturbance experiment allocated by CREST through 16S sequencing.

3.3 PHYSICAL DISTURBANCE EXPERIMENT

Originally, this experiment was planned to take place in three ponds, however it appeared that during the year 2018 – 2019 the pond level had dropped and subsequently desiccated the areas of microbial mat which had been removed in 2017 and 2018. Of the New and P70 samples, samples in general looked more similar to the control microbial mat the longer the area had to regenerate (Figure 27). However, the New 1-year sample could not be distinguished from the surrounding microbial mat. It was also initially planned to sample thee replicate cleared areas from each pond, but this was not possible due to time and sample constraints.

New and P70 Pond samples collected during the physical disturbance experiment have similar alpha diversity traits (Appendix E –Rarefied Species Figures). The 0-year samples, which had just been disturbed, tended to have lower Chao1 and ACE diversity than the 1 and 2-year samples. The New Pond 2-year sample Chao1 diversity was higher than the New Pond 0-year sample, as was the P70 1-year sample to the P70 0-year sample. The P70 2-year sample was higher than the 1-year sample in the ACE analysis but was not outside of error in the Chao1 measure. The Chao1 and ACE index extrapolated the number of rare organisms which may have been missed due to under sampling to predict the number of taxa which could be present in a sample. The Chao1 and ACE indexes generally estimated that the more recently disturbed samples contained fewer rare taxa than more distantly disturbed samples, this trend did not continue with control samples. There was no reduction in species diversity or evenness with change from control to the 0-year samples however there was an observed gradual loss of richness as the mats begin to regrow. Simpson analysis shows that samples have a reasonably high species richness and evenness and therefore high diversity, samples were closely grouped with the majority of samples sitting between 8 and 8.25. Control ponds had the highest value when analysed with the Simpson index, these ponds also had high values when analysed with the Shannon index.

Genetic sequencing shows that in general, New Pond tended to have a higher species richness and phylogenetic diversity than P70 Pond, as shown in Table 16. Genetic sequencing showed that the microbial mats from P70 and New Pond are primarily comprised of Cyanobacteria, Bacteroidota and Proteobacteria (Figure 27). The composition of the two ponds appear very different to each other on a phylum level as New Pond has a much more diverse species makeup while P70 Pond is largely comprised of 5 types of phylum: Cyanobacteria, Bacteroidota, Proteobacteria, Firmicutes and Verrucomicrobiota. However, on a family level both New and

P70 Pond samples primarily contained *Pseudobaenaceae, Nostocaceae* and *Phormidiaceae* (Figure 28).

The New 0-year sample appeared to have very low abundance of Cyanobacteria at the phylum level, but otherwise looked very similar to the 2-year and control samples (Figure 27). Although appearing similar at the phylum level, at the family level the 0-year New pond sample looked very different as it contained higher abundance of *Phormidiaceae* and *Nodosilineaceae* and a lower abundance of *Pseudobaenaceae* (Figure 28).

The P70 samples also looked very similar at a phylum level although the presence of Planctomycetota and Actinobacteria were identified in higher abundances in the 0-year sample than the other samples (Figure 27). Although all of the P70 samples looked similar at the phylum level, the 1-year and 2-year samples looked very similar to each other while the 0-year and control samples were less similar (Figure 27). At the family level the P70 Pond control appeared the most dissimilar to the other samples as it contained a lower abundance of *Pseudobaenaceae* and a higher abundance of *Cyanobacteriaceae* than other samples from that pond (Figure 28). The P70 Pond 0-year sample contained a higher abundance of *Oscillatoriaceae* than the 1-year and 2-year samples but otherwise all three samples appeared fairly consistent with the other samples (Figure 28).

Table 16. Disturbance experiment 16S rRNA gene influences common biodiversity metrics.

Pond	Replication	Phylogenetic Diversity	Species Richness
New	New control	57.6	799
	0-year	55.9	753
	2-year	67.6	968
P70	P70 control	47.7	652
	0-year	52.5	716
	1-year	37.1	501
	2-year	40.3	531

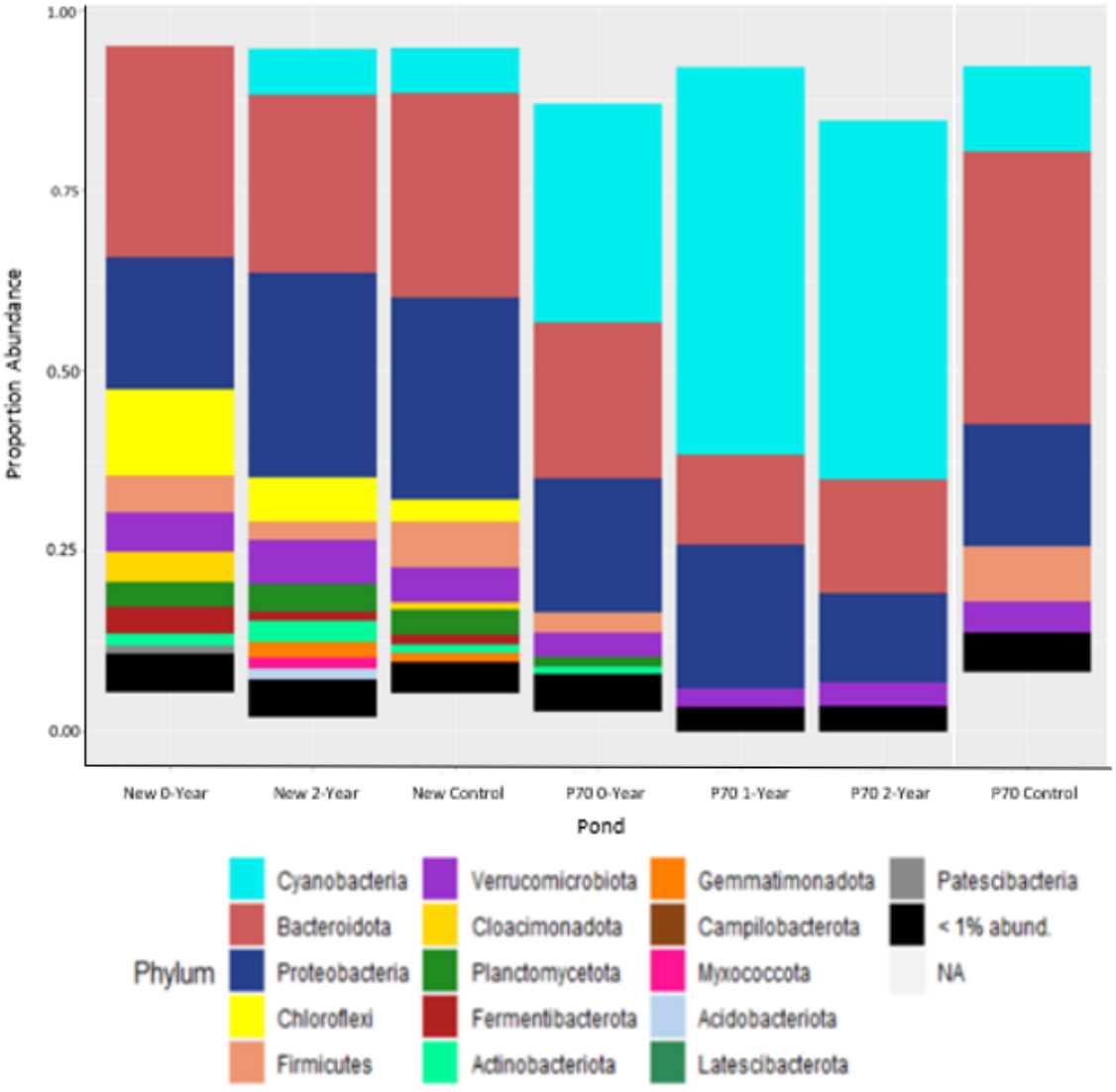


Figure 27. Proportion abundance of bacterial OTUs identified at phylum level in the disturbance experiment allocated by CREST through 16S sequencing.

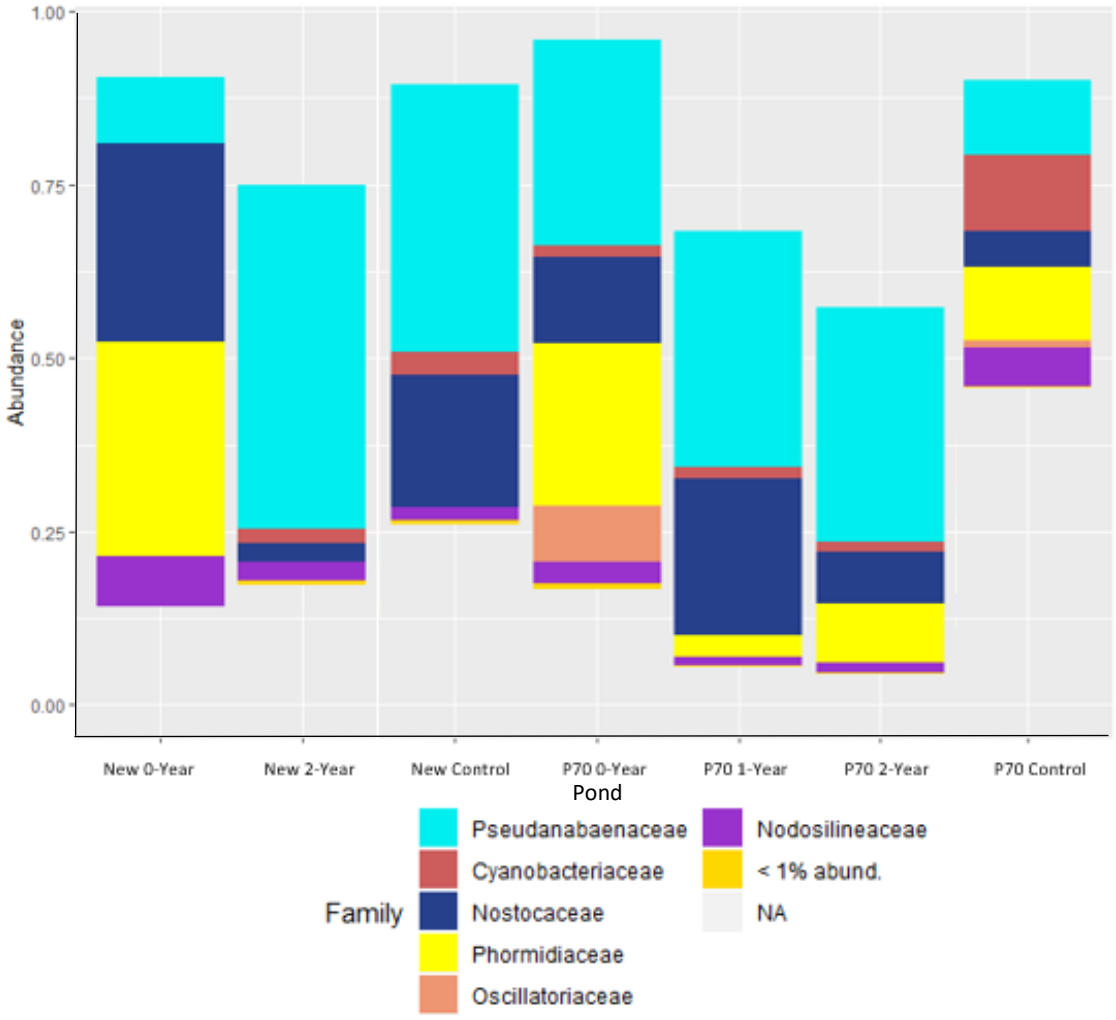


Figure 28. Proportion abundance of cyanobacterial OTUs identified at phylum level in the disturbance experiment allocated by CREST through 16S sequencing.

4 DISCUSSION

In this study, I investigated the response of microbial mats in seven ponds on the MIS to disturbance. As discussed in Chapter 1, pond systems in Antarctica, including the MIS ponds studied in this research, are vulnerable to a range of impacts associated with climate change, particularly where those changes impact on hydrology. As climate change in the polar regions becomes more prominent of an issue, these impacts are anticipated to intensify, and this study aims to identify whether cyanobacteria dominated microbial mats, which are the pre-eminent biota in most cases, are able to show resilience of key functions and retain their character. This research addressed three likely climatic forcings; geochemical (salinity) changes due to hydrological shifts resulting in pond dilution or salinisation, physical disturbance such as damage due to mat lift-off and irradiance disturbance likely to accompany changes in pond depth, cloudiness, and ice cover.

The importance of the hydrological cycle in the Antarctic is overarching as this cycle controls the cryosphere impacting the organisms that inhabit the area as changes to precipitation, ice melt and cloud cover change habitat. The three disturbance studies aimed to identify changes in microbial mats to each of a ramped, press and pulse disturbance. Based on the results of this study, it seems that microbial mats can maintain functional resilience given a climate impacted environmental disturbance occurs, but that the mechanisms employed vary between the nature of disturbance.

The first experiment was designed around an inferred, long term ramped disturbance, which compared ponds with varying salt content. The assumption was that ponds on the MIS are likely to begin as accumulations of dilute meltwater, and that salinization is a long slow process during which pond biota gradually adjust to increased salinity. This assumption remains untested. However, our expectation was that if functional attributes were sustained (in this case

the ability to develop a high biomass microbial mat that was capable of fixing nitrogen) across a strong salt gradient, then the community was resistant or resilient to this stressor. If functional retention were to be accompanied by shifts in species composition, this would be consistent with functional resilience by the selection of tolerant taxa as conditions change.

The second experiment explored the ability of mats to maintain respiration, mat formation, and photosynthesis under changing irradiance conditions. Here we anticipated that mats could accommodate such changes either by adapting their physiology or by rapid selection of more suitable species, both again indicators of different mechanisms of resilience. The third experiment focussed on the ability to tolerate physical disturbance, and the ability of the pre-existing mat community to re-establish after disturbance, or, alternatively, whether such disturbance could enhance taxonomic turnover.

Using these three experiments I tested whether cyanobacteria in microbial mats show resilience or resistance to a disturbance. The implications of species turnover in this context are that if species have an ecological niche at which they can be at peak productivity, outside of this niche the productivity will reduce until the species is unable to compete with other taxa which are better able to accommodate to environmental changes. In this context turnover is inherent to functional resilience. Where no turnover occurs in response to disturbance, we can infer that other mechanisms are in play (resistance mechanisms such as behavioural or physiological acclimation) if functional and taxonomic conservation is evident. Whether mats showed resilience or resistance was to be assessed based on taxonomic composition of samples as well as chapter specific experiments to identify the extent of function change. In this study the null hypothesis was that when microbial mats were impacted by a disturbance there would be no change in community composition or function as a result of the disturbance. The alternative hypothesis was that taxonomic composition would change as a result of a disturbance, however the change in community would not cause a loss of key functions.

Within the ponds studied in the nitrogen fixation experiment, low nitrogen and phosphorus levels are typical due to few natural sources of the nutrients from their catchments. Interannual seasonal variability within the MIS cause regular fluctuations of pond depth, size, and shape. Analysis of previous works identifies inter-annual variability in both dissolved reactive phosphorus (DRP) and nitrate-nitrogen (NO3-N) in the ponds (Table 17). Comparison of the interannual pond nutrients showed that although concentrations fluctuated, all ponds remained low in nitrogen-containing compounds; in most ponds nitrate/nitrite-N was below detectable limits within the ponds. The nitrogen available within ponds is predominantly in the form of ammonium, a recycled form of nitrogen. 'New' biologically available nitrogen in the form nitrate/nitrite is uncommon in these ponds driving the requirement for nitrogen fixation. Similarly, ammonium concentrations are also low in the MIS ponds, the average pond ammoniacal-nitrogen concentration was 0.12 which is significantly lower than pond concentrations generally are.

To analyse the effect of a ramped, multi-annual disturbance, the nitrogen fixation experiment utilised a space for time substitution, along a conductivity (i.e., salinity) gradient. To analyse the effect of a disturbance over a 1-year period, microbial mats were studied in two similar ponds to assess the effect of increased shading (decreased irradiance). The third study physically removed microbial mats to determine the time scales (i.e., days, one year, two years) over which the communities were able to recover from disturbance. Due to the taxonomic interdependencies within microbial mats, maintenance of functions has additional importance as a loss of a key function may either be the cause or the result of community collapse.

The three studies analysed three critical functions which cyanobacteria provide to microbial ponds, nitrogen fixation, photosynthesis, and accrual of structural material. As a response to a disturbance, it was hypothesised that microbial mats would show functional resilience by absorbing the disturbance and within the mat, reorganising and undergoing changes to retain

essentially the same function. Within this study microbial mat changes identified were characterised as either compositional changes or functional changes.

Many previous studies have identified correlations between N-fixation (as measured by acetylene reduction) and pond geochemical variables such as the N:P ratio (Fernández-Valiente et al., 2001; Howard-Williams et al., 1990; Wait et al., 2006). Unlike in other published research this study could not identify any correlation between pond inorganic N:P ratio and N-fixation (Fernández-Valiente et al., 2001; Howard-Williams et al., 1990). Prediction of the nitrogen fixation rate using the N:P ratios accurately estimated that Casten Pond (49.6 µMol/m2/h) and Fresh Ponds (40.8 µMol/m2/h) had the highest nitrogen fixation rates. However, given that a low N:P ratio should benefit nitrogen fixers, it would be expected that Brack Pond would also have a high measured nitrogen fixation rate. In this study Brack Pond had the lowest acetylene reduction rate (Salt Pond - 27.8, P70 Pond - 24.4, Brack Pond – 22.4 µMol/m2/h) suggesting that N-fixation rate may also be influenced by other factors. Furthermore, there was no correlation between pH and N-fixation, as Casten Pond which had the median pH (9.35) in this experiment had the highest N-fixation rates. P70 Pond had low rates of N-fixation and had the highest measured pH in this study (9.88). The other samples showed no correlation between the two variables (Table 17).

In this study, a significant positive correlation ($P = 0.04$) between the abundance of cyanobacteria OTUs and N-fixation was found (Figure 11). This suggests that functional resilience is sufficient to overcome a change in conductivity over a long time period. N-fixation in this experiment ranged from a low of 22.4 µMol/m^2/h measured in Brack Pond, which also had the lowest percent abundance of cyanobacteria OTUs (12.2%) and lowest N-fixation rate, to a high in Casten Pond (49.6 µMol/m^2/h) which had the highest N-fixation rate and cyanobacteria percent abundance (26.6%). This trend was continued across the range of ponds: P70 (24.4 µMol/m^2/h, 14.8%), Salt (27.8 µMol/m^2/h, 16.1%) and Fresh (40.8 µMol/m^2/h,

16.7%) (Figure 11). However, the N-fixation rate correlated negatively with the relative proportion of N-fixing cyanobacteria OTUs over total cyanobacterial community OTUs.

Overall, the results of the three experiments indicated the communities could respond to retain function in response to a change. This was shown through: (1) a community shift across the conductivity gradient in the nitrogen fixation experiment, (2) the vertical migration of cyanobacteria in the light disturbance experiment and (3) the stabilisation of microbial communities to control abundances in the physical disturbance experiment. These results suggest that cyanobacteria within microbial mats are functionally resilient to climate-related changes to their environment. I will further discuss these results under three headings: (1) community compositional change, (2) behavioural and physiological change and (3) functional change.

Table 17. Nutrient concentrations of nitrogen fixation ponds.

	NO_3-N (mg/m^3)*			**Dissolved Reactive Phosphorus (mg/m^3)**		
	This study	January 1998, 2000 (Fernández-Valiente et al., 2001)	(Wait et al., 2006) *(mg/kg)	This study	January 1998, 2000 (Fernández-Valiente et al., 2001)	January 1988 (Howard-Williams et al., 1989; Wait et al., 2006)
Brack	<1	3.3	<0.1	32	85.8	14.1
Casten	<1	1.5	-	129	145	-
Fresh	<1	2.0	-	34	67.1	24.3
P70	<1	3.4	<0.1	9.3	17.9	0.7
Salt	<2	8.4	<0.1	54	120.8	56.0

4.1 COMPOSITIONAL CHANGES

Within this study compositional change was identified in two of the experiments, suggesting that function was maintained in responses to the disturbances. These changes only occurred at a significant level within the long-term studies. Genetic sequencing successfully identified over 528 unique OTUs within the three experiments, including 31 unique cyanobacteria OTUs. This is thought to be the majority of taxa in the environment (Figure 29, Figure 30).

In the physical disturbance experiment, a successional change of species was observed over the timeframe of the experiment. Immediately after the disturbance there was a large presence of *Phormidiaceae* within both the New Pond and P70 Pond. The high abundance of *Phormidiaceae* is not unexpected as this taxon is common basally on mats and was the quickest taxon to reinhabit the area. Within the 1-year samples, successional change was seen within the P70 pond as an increase in *Nostocaceae* (which fix nitrogen), *Oscillatoriaceae* and motile taxa that support early mat development. After 1 year, succession processes had enabled the formation of a fully developed microbial mat with organisms capable of supporting all required functions embedded. In the New pond, 1-year sample, the first-year sample could not be distinguished from the control suggesting rapid mat re-development at least in terms of taxa present. Two years after the disturbance it was observed that, in both ponds, taxa had attained similar levels of abundance as the controls. This was particularly clear in P70 Pond as OTU evenness increased over time post- disturbance.

After a disturbance, in order to restore community cyanobacteria taxa mobilise within the pond. Changes in taxonomic abundance and composition occurred locally within the area of disturbance to provide the required function. Immediately after the disturbance of the sample, mat development is a useful function; however, over time as the microbial mat forms, this function may be less important than nutrient cycling. In the experiment, maintenance of function seemed to be achieved through rapid colonisation by organisms already present in the

pond. Although all cyanobacteria were present immediately after the disturbance, the reforming mat communities were saturated with the species able to perform useful functions. Over time, as overall cyanobacteria abundance increased, *Pseudanabaenaceae* also became more dominant. Change in species composition within the ponds enabled a shift in community composition from a dominance of mat building cyanobacteria to a dominance of nutrient cycling cyanobacteria.

Similarly, within the ponds studied for nitrogen fixation, species were able to adapt to a long-term disturbance through changes in species relative abundance and composition, which have likely occurred over time to maintain the required functions. As in the physical disturbance experiment, the full range of cyanobacteria and other bacterial phylla are present in the ponds which enable the microbial mat to adjust to disturbance and environmental changes over time.

The number of unique cyanobacteria OTUs was substantially higher in the least saline pond (Fresh Pond; 22 unique cyanobacteria OTUs) than the most saline pond (Salt Pond; 16 unique cyanobacteria OTUs) despite both returning similar abundance of cyanobacteria OTUs. The ability to persist at high salt content requires use of energy to move ions against electrochemical gradients (Pade & Hagemann, 2015) and it is likely that this ability is insufficient in many bacteria and cyanobacteria from the fresher waters and likely explains why P70 Pond, with its slightly elevated salinity has the highest biodiversity. As it is more energetically favourable for saline-acclimated organisms to transition to fresher environments, this likely explains why P70 Pond has the highest biodiversity. Saline ponds had higher proportions of the *Oscillatoria* genus, which were less prevalent in fresher ponds, however in low salinity ponds higher abundance of *Pseudanabaena* were found, and in an analogous way to N-fixation, this turnover of mat-forming taxa at contrasting salt contents may have facilitated resilience to enable the ability to form thick mat communities. Genetic sequencing showed a community shift from

species of *Nostoc* to *Nodularia* over the salinity range, and it seems likely that this allowed maintenance of the function of nitrogen fixation in the pond.

4.2 PHYSIOLOGICAL CHANGES

Within this study physiological change was identified in only the light disturbance experiment. These changes were interpreted as the microbial mat response to the disturbance which enabled function to be maintained. From the irradiance and dissolved oxygen data there seems to be a change in photosynthesis between shaded samples and control samples. From visual inspection of the samples there was obvious pigment variation related to where the microbial mat was shaded (Figure 23), however, despite the visual changes to the microbial mat there did not seem to be substantial differences in the OTU composition (Figure 25). We also noted that on removal of dark chambers placed on control mat areas, the greening of mat surfaces was visible suggesting it is a rapid transitional (<6 h) process.

Analysis of the data collected of the short-term response of a microbial mat to a change in light levels indicated that microbial mats were able to maintain photosynthetic function despite changes in light levels. After the one-year, shaded mats were better adapted to a lower irradiance environment and poorly adapted to high irradiance. As the microbial mat-maintained photosynthesis despite a change in irradiance it is indicated that cyanobacteria were either resilient or resistant to this change in light. Considering the lack of change in species composition (Figure 25**)**, and the change in mat appearance, the mat may have undergone reorganisation. This would suggest behavioural and/or physiological resilience, occurring in less than one year. Visual inspection of the microbial mat after the experiment showed that shaded samples returned to control sample pigmentation gradually and were unnoticeable within approximately one week of removal of shading. Mat reorganisation in this way has previously been documented by Vincent, Downes, Castenholz, & Howard-Williams (1993) who proposed that microbes are able to migrate within the layers of the microbial mat to attain

the conditions which they require, thereby maintaining their function. To remain photoadapted, photosynthesising organisms migrate within the microbial mat, moving upwards in low irradiance scenarios and downwards to the sediment interface of the mat in high irradiance scenarios to mitigate irradiance effects (Vincent, Downes, Castenholz, & Howard-Williams, 1993; Nadeau et al., 1999).

Due to the extremely high light conditions generally experienced within Antarctica during summer, photosynthetic organisms are thought to have photoadapted either by shading within microbial mat structures or by lowering chlorophyll concentrations to reduce the efficiency of light absorption. Generally, photosynthesis in Antarctic ponds occurs deeper within the mat (Nadeau et al., 1999) but in the shaded mats photosynthesis could occur closer to the mat surface, through a behavioural response, and resulting visual change to the cyanobacteria mats.

In the shading experiment the photosynthetic response occurred within minutes to hours. Mats enclosed in opaque domes quickly reduced net DO production, while respiration occurred. In clear domes, photosynthesis occurred, maintaining net DO production. Of the clear-dome samples which had been previously shaded, half responded quickly to sunlight, increasing the dissolved oxygen in the dome within 1.5 to 2 hours. However, the other half of the shaded clear-dome samples experienced a reduction in dissolved oxygen concentration initially. Reduction of dissolved oxygen shows either that respiration rates were higher than photosynthesis rates, or that sampling error occurred where the dome was not properly sealed from the outside pond water. Furthermore, over the course of the experiment the mats themselves were still saturated with oxygen, which continued to diffuse into the water, resulting in ponds showing higher starting photosynthesis rates or lower respiration rate than the actual rates. An additional challenge was that there may have been small inconsistencies in measured DO concentrations due to temperature changes in water samples between collection and measurement.

Along with the visual changes in samples which had been shaded, there was also a shift in peak irradiance levels for photosynthesis between the shaded and control groups. In the experiment, mats which were shaded maximised photosynthesis at lower ambient light levels at the expense of peak performance at high irradiance levels. This study identified that mats which had been shaded photosynthesised at much lower light conditions and, therefore, reduced the irradiance at which net respiration occurs. Thus, shaded samples were not adapted to photosynthesize at higher ambient light levels. Such photoadaptation is likely to benefit mat production rates at the beginning and end of the summer season when 24-hour sunlight conditions cease.

Similarly, through analysis of the species composition data and visual analysis of the images of the visual disturbance experiment, it was apparent that mat formation was maintained despite the disturbance. Whole mat removal from the bed of the pond, which was mimicked in this study, is common in the pond environment, and subsequently resilience to this disturbance is crucial for microbial mat survival. There did not seem to be any substantial visual or abundance changes in microbial mat development between the New Pond and P70 Pond.

4.3 FUNCTIONAL CHANGES

Maintaining functional resilience is important for recovery from a disturbance. In this research cyanobacteria showed rapid adaptation to a disturbance to maintain function within the microbial mat. Resilience in this study was sufficient to maintain functions after a shading disturbance, salinity disturbance and after the total removal of the microbial mat from the bed of the pond. The key functions identified in this study are cohesiveness of the mat, nitrogen accrual and carbon accrual through photosynthesis as opposed to carbon usage through respiration. In this study there were significant changes in composition and physiology to maintain function. The findings of this study were roughly consistent with other research which identified functional resilience in cyanobacteria and microbial communities.

Importantly, over the range of salinities N-fixation, mat formation and carbon accrual remained high. This study identified that although climate change may have a range of impacts on the environment, at a basic level microbial mat communities exhibit a level of resilience to these changes. This study identified that cyanobacteria communities are able to show resilience by not only changing community composition over a medium to long-term timescale, but also through physiological and behavioural adaptations to adjust to the ecological niche within the short term.

4.4 LIMITATIONS

One of the prominent issues which affected this study were the issues with AMS Pond samples (Figure 22). In AMS Pond, shades and physical disturbance plots did not remain submerged over the course of the experiment due to exceptional change in water level from the previous year. As the conditions affecting the AMS samples had varied so significantly between replicates and the success of the shading could not be confirmed these samples were not included in the experiment. Subsequently it was not possible to have triplicate samples for these two experiments. Furthermore, changes were also noticed in New pond where sediment build-up occurred on top of the shades, subsequently resulting in variance in the amount of irradiance reaching the microbial mat between ponds. The slight additional shading was considered to be acceptable in this study as varying water and ice levels between ponds may also impact irradiance reaching the samples and a light disturbance was achieved. As there was sediment build up on the shade this suggests that under the shade sedimentation may have been reduced relative to control areas and impacts of this on mat activity cannot be further accounted for.

Additional constraints to the study were environment related equipment challenges. Due to the cold environment items which require a battery tended to be temperamental in this environment. Subsequently, field sampling of DO concentrations using the PreSens was not possible, to overcome this samples were tested in the field camp laboratory located approximately 15-minutes' walk from the pond location, this limitation may have increased the sampling variability however if this occurred it was without prejudice and therefore this cannot be rectified. The same issue also occurred in the pumps for mixing dome water as there was a failure of some of the motors included in the samples. Sampling error may have occurred due to inconsistencies in mixing of the water column related to the partial, or in some cases complete, failure of the motor and battery pack which was used to dislodge bubbles and circulate the water within the domes. The failure of battery power is likely ascribed to the low ambient temperatures (water temperature ~7°C and air temperature ~-1°C).Sampling error was reduced through manual pumping to disturb and mix the water and oxygen within the dome to prevent gradients within samples and enable equilibrium to be reached through diffusive methods. It is unknown which method was more effective at mixing the water in the samples.

In addition, general sampling errors also occurred including the potential for domes not completely sealing to the pond floor and therefore allowing for sample contamination. In the nitrogen fixation experiment it was not possible to consider the variances in pond geochemistry however it is unlikely that concentrations of minor nutrients will be a prime driver of nitrogen fixation potential as this was not discovered in reviews of available literature.

In this study there was a difficulty in accessing OTU Classification Resources that could accurately diagnose cyanobacterial OTU identity. There was no mitigation for this, but the OTU data received provides a large limitation on the research as in many cases species are not identified down to genus and family level. Although other classifiers identified over 2000 unique OTUs present in the samples, many were not identified past phylum level. The CREST

database was used to provide the highest visibility on cyanobacterial species, but this proved inadequate.

Sampling variation seen within Casten Pond was much higher than in the other ponds and may be due to sampling error or geographic variability of samples due to micro changes in the pond ecosystem. It is impossible to definitively determine if sampling variance in Casten Pond is natural or related to human error in the field and/or laboratory. Although a larger sample size may have levelled variation between samples, only a fixed number of samples could be analysed from each pond due to budgeting constraints, logistical effort to retrieve samples from Antarctica and to preserve the integrity of this environment.

4.5 IMPLICATIONS AND FUTURE RESEARCH

This study will contribute to a wider study identifying microbial mat resilience on the MIS a largescale project important for determining the potential effects of changing climate in high latitude environments. On a larger scale it is hoped that this study will contribute to the research on biotic resilience to climate change effects as this issue is prominent globally. Research in this area is moving rapidly, achieving goals set by previous researchers, particularly focussing on furthering techniques of the current research, and providing longer term studies. Although the research in this area is rapidly moving forward, prominent gaps still exist.

The key factor which would improve this study is increased replication and more extensive experimentation to identify trends more accurately. In the Antarctic isolation, cost and accessibility are key factors. It would, however, be of value to study nitrogen fixation rates and photosynthesis rates on a seasonal scale. Particularly considering the finding that shaded mats can photoadapt and may be active at low irradiance, for example when ice first begins to melt in spring prior to usual period of field access. Identification of key relationships affecting nitrogen fixation may be used to settle contradicting opinions in research. Other key

disturbances which are not fully understood include the effects of freezing and desiccation disturbance on the mats.

Antarctic field research is expensive, and field experiments are difficult to control and predict. Complementary laboratory-based studies may provide further information regarding the resilience of taxa, without the confounding factor of natural environmental variability. In addition, a laboratory study considering the possible correlation between cyanobacteria abundance and nitrogen fixation would assist with interpretation of my results. Furthering the light disturbance study by examining effects of increasing ambient light may provide further details on the effects of ambient light levels. Distinction between resilience and resistance may also be important and could be used to provide more insight to this study and other related studies.

5 CONCLUSIONS

The terrestrial ponds of the MIS support a range of cyanobacteria that provide key functions to enable the survival of the microbial mat community. This study investigated cyanobacterial mats in six meltwater ponds with a range of salinities to assess their ability to sustain these functions when challenged by disturbances that are projected to occur under modelled climate change impacts. The results of the three experiments in this study suggest that cyanobacterial mats possess functional resilience to climate change-related variations.

The three experiments examined the effects of salinization on nitrogen fixation, irradiance reduction on photosynthesis and respiration and physical disturbance on mat composition. This study identified that after a disturbance, microbial communities were able to maintain key functions.

The nitrogen fixation experiment used a space for time substitution to develop a salinity gradient representing a long-term ramped change in salinity. This experiment identified no significant change in nitrogen fixation over the conductivity range, but suggested that this functional resilience is accompanied by turnover of the community. Mat-forming and nitrogen-fixing cyanobacteria turned over along the salinity gradient.

The irradiance experiment used a 12-month pulse disturbance that reduced irradiance reaching the microbial mats by approximately 90-95%. Shaded communities sustained similar maximum net photosynthesis and dark respiration rates as control samples, but peak photosynthesis was achieved at lower irradiance than controls and inhibited at high irradiance. No species turnover was evident in this experiment and the ability to retain high rates of photosynthesis appeared to be related to physiological and/or behavioural responses of cyanobacteria.

The physical disturbance experiment identified that when complete removal of the mat occurred, microbial communities were able to reach community compositions similar to the control sample within two years of the disturbance.

This research appears to confirm the hypothesis that species are functionally resilient, at least within the ranges included in these experiments, to climate change related disturbances but that the mechanisms varied under different types and duration of disturbance.

The hydrological system in the Antarctic is sensitive to climatic change and therefore environmental conditions in the pond ecosystems that support substantial inland biodiversity are at risk due to changes in pond water balance due to varying ice and snow melt, precipitation, and evaporation, as well as irradiance shifts due to changes in ice and cloud coverage in the region. Communities are pre-adapted to a variable habitat and can be expected to retain existing characteristics and key functions at least during the early phases of climate-induced change.

Further research is required to develop a comprehensive understanding of potential effects to microbial communities from climatic change. Future work may identify the resilience of additional key functions such as the ability to rehydrate after desiccation to increase resilience in this variable environment. As the impacts from climate change become increasingly threatening in these environments the race is on to identify the resilience of cold adapted biota.

6 REFERENCES

Andriuzzi, W. S., Adams, B. J., Barrett, J. E., Virginia, R. A., & Wall, D. H. (2018). Observed trends of soil fauna in the Antarctic Dry Valleys: early signs of shifts predicted under climate change. *Ecology*, *99*(2), 312–321. https://doi.org/10.1002/ecy.2090

Archer, S. D. J., McDonald, I. R., Herbold, C. W., & Cary, S. C. (2014). Characterisation of bacterioplankton communities in the meltwater ponds of Bratina Island, Victoria Land, Antarctica. *FEMS Microbiology Ecology*, *89*(2), 451–464. https://doi.org/10.1111/1574-6941.12358

Archer, S. D. J., McDonald, I. R., Herbold, C. W., Lee, C. K., & Cary, C. S. (2015). Benthic microbial communities of coastal terrestrial and ice shelf Antarctic meltwater ponds. *Frontiers in Microbiology*, *6*(MAY), 1–11. https://doi.org/10.3389/fmicb.2015.00485

Barrett, J. E., Virginia, R. A., Wall, D. H., Cary, S. C., Adams, B. J., Hacker, A. L., & Aislabie, J. M. (2006). Co-variation in soil biodiversity and biogeochemistry in northern and southern Victoria Land, Antarctica. *Antarctic Science*, *18*(4), 535–548. https://doi.org/10.1017/S0954102006000587

Bracegirdle, T. J., & Stephenson, D. B. (2012). Higher precision estimates of regional polar warming by ensemble regression of climate model projections. *Climate Dynamics*, *39*(12), 2805–2821. https://doi.org/10.1007/s00382-012-1330-3

Bronselaer, B., Stouffer, R. J., Winton, M., Griffies, S. M., Hurlin, W. J., Russell, J. L., … Sergienko, O. V. (2018). Change in future climate due to Antarctic meltwater. *Nature*, *564*(7734), 53–58. https://doi.org/10.1038/s41586-018-0712-z

Castello, J. D., & Rogers, S. O. (2005). *Life in ancient ice*. Retrieved from https://books.google.co.nz/books?hl=en&lr=&id=iQCKum_U02AC&oi=fnd&pg=PR9&dq=Castello+and+Rogers,+2005&ots=qX6xOpJDWR&sig=dLT9YyVMqRernqG6QIw6_dhueeI#v=onepage&q=ice age&f=false

Castendyk, D. N., Obryk, M. K., Leidman, S. Z., Gooseff, M., & Hawes, I. (2016). Lake Vanda: A Sentinel for Climate Change in the McMurdo Sound Region of Antarctica. *Global and Planetary Change.*, *144*, 213–227. https://doi.org/Doi: 10.1016/j.gloplacha.2016.06.007.

Coggins, J. H. J., McDonald, A. J., & Jolly, B. (2014). Synoptic climatology of the Ross Ice Shelf and Ross Sea region of Antarctica: K-means clustering and validation. *International Journal of Climatology*, *34*(7), 2330–2348. https://doi.org/10.1002/joc.3842

Davey, A. (1983). Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. *Polar Biology*, *2*(2), 95–100. https://doi.org/10.1007/BF00303174

de los Ríos, A., Ascaso, C., Wierzchos, J., Ferna, E., & Quesada, A. (2004). Microstructural Characterization of cyanobacterial Mats from the. *Applied and Environmental Microbiology*, *70*(1), 569–580. https://doi.org/10.1128/AEM.70.1.569

Debenham, F. (1919). A New Mode of Transportation by Ice: the Raised Marine Muds of South Victoria Land (Antarctica). *Quarterly Journal of the Geological Society*, *75*(1–4), 51–76. https://doi.org/10.1144/GSL.JGS.1919.075.01-04.08

Dinniman, M. S., Klinck, J. M., Hofmann, E. E., & Smith, W. O. (2018). Effects of projected changes in wind, atmospheric temperature, and freshwater inflow on the Ross Sea. *Journal of Climate*, *31*(4), 1619–1635. https://doi.org/10.1175/JCLI-D-17-0351.1

Earth Microbiome Project. (2021). 16S Illumina Amplicon Protocol.

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, *61*(1), 1–10.

Fernández-Valiente, E., Quesada, A., Howard-Williams, C., & Hawes, I. (2001). N2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. *Microbial Ecology*, *42*(3), 338–349. https://doi.org/10.1007/s00248-001-1010-z

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Lance, G., & Holling, C. S. (2004). Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 557–581. Retrieved from https://www.jstor.org/stable/pdf/30034127.pdf

Fritsch, F. E. (1912). Freshwater algae. In F. J. Bell (Ed.), *National Antarctic expedition 1901–04* (pp. 1–60). London: British Museum.

Griffiths, B. S., & Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews*, *37*(2), 112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x

Griffiths, K., Michelutti, N., Sugar, M., Douglas, M. S. V., & Smol, J. P. (2017). Ice-cover is the principal driver of ecological change in High Arctic lakes and ponds. *PLoS ONE*, *12*(3). https://doi.org/10.1371/journal.pone.0172989

Gutt, J., Adams, B., Bracegirdle, T., Cowan, D., Cummings, V., Di Prisco, G., … Xavier, J. (2012). Antarctic thresholds - Ecosystem resilience and adaptation: A new SCAR-biology programme. *Polarforschung*, *82*(2), 147–150. https://doi.org/10.013/epic.42531.d001

Hadley, K. R., Paterson, A. M., Rühland, K. M., White, H., Wolfe, B. B., Keller, W., & Smol, J. P. (2019). Biological and geochemical changes in shallow lakes of the Hudson Bay Lowlands: a response to recent warming. *Journal of Paleolimnology*, *61*(3), 313–328. https://doi.org/10.1007/s10933-018-0061-9

Hansen, J., Ruedy, R., Sato, M., & Lo, K. (2010). Global surface temperature change. *Reviews of Geophysics*, *48*(4), 1–29. https://doi.org/10.1029/2010RG000345

Hawes, I., Howard-williams, C., Gilbert, N., & Joy, K. (2021). Towards an environmental classification of lentic aquatic ecosystems in the McMurdo Dry Valleys , Antarctica. *Environmental Management.*, *2*, 1–54. https://doi.org/10.1007/s00267-021-01438-1

Hawes, I., Howard-Williams, C., & Sorrell, B. (2014). Decadal timescale variability in ecosystem properties in the ponds of the McMurdo Ice Shelf, southern Victoria Land, Antarctica. *Antarctic Science*, *26*(3), 219–230. https://doi.org/10.1017/S0954102013000576

Hawes, I., Howard-Williams, C., & Fountain, A. G. (2008). Ice-based freshwater ecosystems. In W. F. Vincent & J. Laybourn-Parry (Eds.), *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems* (pp. 103–118). Retrieved from https://books.google.co.nz/books?hl=en&lr=&id=ppUSDAAAQBAJ&oi=fnd&pg=PA103&dq=Microbial+communities+of+an+unusual,+inland+tidal+lagoon+on+the+McMur

do+Ice+Shelf:+composition,+biomass+and+productivity+HAWES&ots=jndN6mirsA&sig=DT52QH-TR7759Hg8W-fpsNhuS10#v=o

Hawes, I., Jungblut, A. D., Matys, E. D., & Summons, R. E. (2018). The "Dirty Ice" of the McMurdo Ice Shelf: Analogues for biological oases during the Cryogenian. *Geobiology*, *16*(4), 369–377. https://doi.org/10.1111/gbi.12280

Hawes, I., Safi, K., Sorrell, B., Webster-Brown, J., & Arscott, D. (2011). Summer-winter transitions in Antarctic ponds I: The physical environment. *Antarctic Science*, *23*(3), 235–242. https://doi.org/10.1017/S0954102011000046

Hawes, I., Safi, K., Webster-Brown, J., Sorrell, B., & Arscott, D. (2011). Summer-winter transitions in Antarctic ponds II: Biological responses. *Antarctic Science*, *23*(3), 243–254. https://doi.org/10.1017/S0954102011000058

Hawes, I., Smith, R., Howard-Williams, C., & Schwarz, A.-M. (1999). Environmental conditions during freezing, and response of microbial mats in ponds of the McMurdo Ice Shelf, Antarctica. *Antarctic Science*, *11*(02), 198–208. https://doi.org/10.1017/s0954102099000267

Hoffman, P. F., Kaufman, A. J., Halverson, G. P., & Schrag, D. P. (1998). A Neoproterozoic Snowball Earth. *Science*, *281*(August), 1342–1346. https://doi.org/10.1126/science.281.5381.1342

Hoffman, P. F., & Shrag, D. P. (2000). Snowball Earth. *Scientific American*, *282*(1), 68–75. https://doi.org/10.4135/9781446247501.n3578

Howard-Williams, C., Pridmore, R. D., Broady, P. A., & Vincent, W. F. (1990). Environmental and Biological Variability in the McMurdo Ice Shelf Ecosystem. In K. Kerry & G. Hempel (Eds.), *Antarctic Ecosystems. Ecological Change and Conservation* (pp. 23–31). https://doi.org/10.1007/978-3-642-84074-6

Howard-Williams, C., Pridmore, R., Downes, M. T., & Vincent, W. F. (1989). Microbial biomass, photosynthesis and chlorophyll a related pigments in the ponds of the McMurdo Ice Shelf , Antarctica. *Antarctic Science*, *1*(2), 125–131.

Invitrogen. (2008). *SequalPrep ™ Normalization Plate (96) Kit*. Retrieved from https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fsequalprep_platekit_man.pdf&title=U2VxdWFsUHJlcCBOb3JtYWxpemF0aW9uIFBsYXRlICg5NikgS2l0

IPCC. (2014). Climate Change 2014: Synthesis Report; Chapter Observed Changes and their Causes. In *Ipcc*. https://doi.org/10.1046/j.1365-2559.2002.1340a.x

Jungblut, A. D., & Hawes, I. (2017). Using captain scott's discovery specimens to unlock the past: Has antarctic cyanobacterial diversity changed over the last 100 years? *Proceedings of the Royal Society B: Biological Sciences*, *284*(1857). https://doi.org/10.1098/rspb.2017.0833

Jungblut, A. D., Hawes, I., Mountford, D., Hitzfeld, B., Dietrich, D. R., Burns, B. P., & Neilan, B. A. (2005). Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environmental Microbiology*, *7*, 519–529. https://doi.org/10.1111/j.1462-2920.2004.00717.x

Jungblut, A. D., Lovejoy, C., & Vincent, W. F. (2010). Global distribution of cyanobacterial

ecotypes in the cold biosphere. *ISME Journal*, *4*(2), 191–202. https://doi.org/10.1038/ismej.2009.113

Jungblut, A. D., & Neilan, B. A. (2010). NifH gene diversity and expression in a microbial mat community on the McMurdo Ice Shelf, Antarctica. *Antarctic Science*, *22*(2), 117–122. https://doi.org/10.1017/S0954102009990514

Jungblut, A. D., Wilbraham, J., Banack, S. A., Metcalf, J. S., & Codd, G. A. (2018). Microcystins, BMAA and BMAA isomers in 100-year-old Antarctic cyanobacterial mats collected during Captain R.F. Scott's Discovery Expedition. *European Journal of Phycology*, *53*(2), 115–121. https://doi.org/10.1080/09670262.2018.1442587

Kellogg, D. E., & Kellogg, T. B. (1987). Diatoms of the McMurdo ice shelf, Antarctica: Implications for sediment and biotic reworking. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *60*(C), 77–96. https://doi.org/10.1016/0031-0182(87)90025-3

Kellogg, T. B., & Kellogg, D. E. (1988). Antarctic cryogenic sediments: Biotic and inorganic facies of ice shelf and marine-based ice sheet environments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *67*(1–2), 51–74. https://doi.org/10.1016/0031-0182(88)90122-8

Laybourn-Parry, J., Marahiel, M. A., Haymet, T., Quick, P., & Benson, E. (2002). Survival mechanisms in Antarctic lakes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1423), 863–869. https://doi.org/10.1098/rstb.2002.1075

Link, P. K. (2009). "Icehouse" (Cold) Climates. *Encyclopedia Paleoclimatology and Ancient Environments.*, 1275–1279. https://doi.org/10.1007/978-1-4020-4411-3_110

Meeks, J. C. (2007). *Physiological Adaptations in Nitrogen-fixing Nostoc–Plant Symbiotic Associations*.

Monaghan, A. J., Bromwich, D. H., Powers, J. G., & Manning, K. W. (2005). The climate of the McMurdo, Antarctica, region as represented by one year of forecasts from the Antarctic Mesoscale Prediction System. *Journal of Climate*, *18*(8), 1174–1189. https://doi.org/10.1175/JCLI3336.1

Nadeau, T. L., & Castenholz, R. W. (2000). Characterization of psychrophilic oscillatorians (cyanobacteria) from antarctic meltwater ponds. *Journal of Phycology*, *36*(5), 914–923. https://doi.org/10.1046/j.1529-8817.2000.99201.x

Nadeau, T. L., Howard-Williams, C., & Castenholz, R. W. (1999). Effects of solar UV and visible irradiance on photosynthesis and vertical migration of Oscillatoria sp. (Cyanobacteria) in an Antarctic microbial mat. *Aquatic Microbial Ecology*, *20*(3), 231–243. https://doi.org/10.3354/ame020231

Pade, N., & Hagemann, M. (2015). Salt acclimation of cyanobacteria and their application in biotechnology. *Life*, *5*(1), 25–49. https://doi.org/10.3390/life5010025

Paerl, H. W., Pinckney, J. L., & Steppe, T. F. (2000). cyanobacterial-bacterial mat consortia: Examining the functional unit of microbial survival and growth in extreme environments. *Environmental Microbiology*, *2*(1), 11–26. https://doi.org/10.1046/j.1462-2920.2000.00071.x

Paterson, A. M., Rühland, K. M., Anstey, C. V., & Smol, J. P. (2017). Climate as a driver of increasing algal production in Lake of the Woods, Ontario, Canada. *Lake and Reservoir Management*, *33*(4), 403–414. https://doi.org/10.1080/10402381.2017.1379574

Randel, W. J., Polvani, L., Wu, F., Kinnison, D. E., Zou, C. Z., & Mears, C. (2017). Troposphere-Stratosphere Temperature Trends Derived From Satellite Data Compared With Ensemble Simulations From WACCM. *Journal of Geophysical Research: Atmospheres*, *122*(18), 9651–9667. https://doi.org/10.1002/2017JD027158

Roberts, S., Jones, V. J., Allen, J. R. M., & Huntley, B. (2015). Diatom response to mid-Holocene climate in three small Arctic lakes in northernmost Finnmark. *Holocene*, *25*(6), 911–920. https://doi.org/10.1177/0959683615572853

Rühland, K., Hargan, K., Jeziorski, A., Paterson, A., Keller, W., & Smol, J. (2014). A multi-trophic exploratory survey of recent environmental changes using lake sediments in the Hudson Bay Lowlands, Ontario, Canada. *Arctic, Antarctic, and Alpine Research*, *46*(1), 139–158. https://doi.org/10.1657/1938-4246-46.1.139

Rühland, K., Paterson, A., & Smol, J. P. (2015). Lake diatom responses to warming: reviewing the evidence. *Journal of Paleolimnology*, *54*(1), 1–35. https://doi.org/10.1007/s10933-015-9837-3

Rykiel, E. J. J. (1985). Towards a definition of ecological disturbance. *Australian Journal of Ecology*, *10*, 361–365.

Sjöling, S., & Cowan, D. A. (2003). High 16S rDNA bacterial diversity in glacial meltwater lake sediment, Bratina Island, Antarctica. *Extremophiles*, *7*(4), 275–282. https://doi.org/10.1007/s00792-003-0321-z

Slater, T., Lawrence, I. R., Otosaka, I. N., Shepherd, A., Gourmelen, N., Jakob, L., … Nienow, P. (2021). Earth's ice imbalance. *The Cryosphere*, *15*(1), 233–246. Retrieved from https://tc.copernicus.org/articles/15/233/2021/

Smith, K. L., & Polvani, L. M. (2017). Spatial patterns of recent Antarctic surface temperature trends and the importance of natural variability: lessons from multiple reconstructions and the CMIP5 models. *Climate Dynamics*, *48*(7–8), 2653–2670. https://doi.org/10.1007/s00382-016-3230-4

Smol, J. P., & Douglas, M. S. V. (2007a). From controversy to consensus: Making the case for recent climate change in the Arctic using lake sediments. *Frontiers in Ecology and the Environment*, *5*(9), 466–474. https://doi.org/10.1890/060162

Smol, J. P., & Douglas, M. S. V. (2007b). Crossing the final ecological threshold in high Arctic ponds. *Proceedings of the National Academy of Sciences*, *104*(30), 12395–12397. https://doi.org/10.1073/pnas.0702777104

Speirs, J. C., Steinhoff, D. F., McGowan, H. A., Bromwich, D. H., & Monaghan, A. J. (2010). Foehn winds in the McMurdo Dry Valleys, Antarctica: The origin of extreme warming events. *Journal of Climate*, *23*(13), 3577–3598. https://doi.org/10.1175/2010JCLI3382.1

Stal, L. J., & Wolfgang, K. E. (1985). Nitrogenase activity in the non-heterocystous cyanobacterium Oscillatoria sp. grown under alternating light-dark cycles. *Archives of Microbiology*, *143*, 67–71.

Stevenson, J. R., Vargo, J., Ivory, V., Bowie, C., & Wilkinson, S. (2015). Resilience Benchmarking and Monitoring Review. *National Science Challenges*, *6*, 51. Retrieved from https://www.resiliencechallenge.nz/wp-content/uploads/2018/03/1ResilienceBenchmarkMonitoring-Deliverable1-1.pdf

Stewart, B. Y. W. D. P., Fitzgerald, G. P., & Burris, R. H. (1967). In Situ Studies On N2 Fixation Using The Acetylene Reduction Technique. *Proceedings of the National Academy of Sciences of the United States of America*, *58*(5), 2071–2078.

Stuecker, M. F., Bitz, C. M., Armour, K. C., Proistosescu, C., Kang, S. M., Xie, S. P., … Jin, F. F. (2018). Polar amplification dominated by local forcing and feedbacks. *Nature Climate Change*, *8*(12), 1076–1081. https://doi.org/10.1038/s41558-018-0339-y

Sutherland, D., Howard-Williams, C., & Hawes, I. (2020). Environmental drivers that influence microalgal species in meltwater pools on the McMurdo Ice Shelf, Antarctica. *Polar Biology*, (43), 467–482.

Taton, A., Grubisic, S., Balthasart, P., Hodgson, D. A., Laybourn-Parry, J., & Wilmotte, A. (2006). Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiology Ecology*, *57*(2), 272–289. https://doi.org/10.1111/j.1574-6941.2006.00110.x

Thermo Scientific. (2010). *NanoDrop 1000 Spectrophotometer V3.8 User's Manual*. Retrieved from http://tools.thermofisher.com/content/sfs/manuals/nd-1000-v3.8-users-manual-8 5x11.pdf

They, N. H., Amado, A. M., & Cotner, J. B. (2017). Redfield ratios in inland waters: higher biological control of C:N:P ratios in tropical semi-arid high water residence time lakes. *Frontiers in Microbiology*, *8*(AUG). https://doi.org/10.3389/fmicb.2017.01505

Van Gorkom, H. J., & Donze, M. (1971). Localization of Nitrogen Fixation in Anabaena. *Nature*, *234*, 231–232. Retrieved from https://www.nature.com/articles/234231b0

Vincent, W. F., Castenholz, R. W., Downes, M. T., & Howard-Williams, C. (1993). Antarctic cyanobacteria: Light, Nutrients, and Photosynthesis in the Microbial Mat Environment. *Journal of Phycology*, *29*(6), 745–755.

Vincent, W. F., Gibson, J. A. E., Pienitz, R., Villeneuve, V., Broady, P. A., Hamilton, P. B., & Howard-Williams, C. (2000). Ice shelf microbial ecosystems in the high arctic and implications for life on snowball earth. *Naturwissenschaften*, *87*(3), 137–141. https://doi.org/10.1007/s001140050692

Vincent, W. F., & James, M. R. (1996). Biodiversity in extreme aquatic environments: Lakes, ponds and streams of the Ross Sea sector, Antarctica. *Biodiversity and Conservation*, *5*(11), 1451–1471. https://doi.org/10.1007/BF00051987

Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., … Sprent, J. I. (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry*, *57–58*(McKey 1994), 1–45. https://doi.org/10.1023/A:1015798428743

Votek, M. A., & Ward, B. B. (1995). Detection of Ammonium-Oxidizing Bacteria of the Beta-Subclass of the Class Proteobacteria in Aquatic Samples with the PCR. *Applied and Environmental Microbiology*, *61*(4), 1444–1450.

Wait, B. R., Webster-Brown, J. G., Brown, K. L., Healy, M., & Hawes, I. (2006). Chemistry and stratification of Antarctic meltwater ponds I: Coastal ponds near Bratina Island, McMurdo Ice Shelf. *Antarctic Science*, *18*(4), 515–524. https://doi.org/10.1017/S0954102006000563

Walker, B., Holling, C. S., Carpenter, S. R., & Kinzig, A. (2004). Resilience, adaptability and transformability in social-ecological systems. *Ecology and Society*, *9*(2).

https://doi.org/10.5751/ES-00650-090205

Wood, S. A., Mountfort, D., Selwood, A. I., Holland, P. T., Puddick, J., & Cary, S. C. (2008). Widespread distribution and identification of eight novel microcystins in antarctic cyanobacterial mats. *Applied and Environmental Microbiology*, *74*(23), 7243–7251. https://doi.org/10.1128/AEM.01243-08

APPENDIX A – SAMPLE REGISTER

BRATINA ISLAND Sample Name	Longitude	Latitude	Type	Analysis	Container Type	MAF Permit No.	Collected By Researcher	Date Collected	No. of Units	Unit Weight (g)
New Pond control	165.55769	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
New Pond recovery	165.55769	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
New Pond shaded	165.55769	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
New Pond control	165.55769	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond recovery	165.55769	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond shaded	165.55769	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond control	165.55769	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond recovery	165.55769	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond shaded	165.55769	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS Pond control	165.55551	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
AMS recovery	165.55551	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
AMS shaded	165.55551	-78.01514	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
AMS Pond control	165.55551	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS recovery	165.55551	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS shaded	165.55551	-78.01514	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS Pond control	165.55551	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS recovery	165.55551	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
AMS shaded	165.55551	-78.01514	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 control	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
P70 recovery	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
P70 shaded	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	9	1
P70 control	165.55168	-78.01485	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 recovery	165.55168	-78.01485	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 shaded	165.55168	-78.01485	mixed microbial mat	microscopy	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 control	165.55168	-78.01485	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 recovery	165.55168	-78.01485	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
P70 shaded	165.55168	-78.01485	mixed microbial mat	RNA	15 mL falcon	2018070802	IH/MS/FM	25/01/2019	3	1
New Pond Mat	165.55769	-78.01514	mixed microbial mat	Experimental material	Tupperware	2018070802	IH/MS/FM	25/01/2019	3	10
P70 Mat	165.55168	-78.01485	mixed microbial mat	Experimental material	Tupperware	2018070802	IH/MS/FM	25/01/2019	3	5
Casten Mat	165.55074	-78.01299	mixed microbial mat	Experimental material	Petri Dish	2018070802	IH/MS/FM	25/01/2019	9	3
New Pond disturbance	165.55769	-78.01514	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	22/01/2019	2	2
New Pond disturbance	165.55769	-78.01514	mixed microbial mat	DNA	60 ml falcon	2018070802	MS/FM	22/01/2019	4	2
New Pond disturbance	165.55769	-78.01514	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	22/01/2019	18	2
P70 disturbance	165.55168	-78.01485	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	22/01/2019	7	2
P70 disturbance	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	22/01/2019	21	2
Fresh Nfix DNA	165.54236	-78.01547	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Brack Nfix DNA	165.54503	-78.01579	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Salt Nfix DNA	165.54527	-78.01608	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
P70 Nfix DNA	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Casten Nfix DNA	165.55074	-78.01299	mixed microbial mat	Biomass/pigments	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Fresh Nfix BP	165.54236	-78.01547	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Brack Nfix BP	165.54503	-78.01579	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Salt Nfix BP	165.54527	-78.01608	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
P70 Nfix BP	165.55168	-78.01485	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Casten Nfix BP	165.55074	-78.01299	mixed microbial mat	DNA	15 ml falcon	2018070802	MS/FM	23/01/2019	3	2
Fresh Nfix microscopy	165.54236	-78.01547	mixed microbial mat	microscopy	eppendorf	2018070802	MS/FM	23/01/2019	3	0.2
Brack Nfix microscopy	165.54503	-78.01579	mixed microbial mat	microscopy	eppendorf	2018070802	MS/FM	23/01/2019	3	0.2
Salt Nfix microscopy	165.54527	-78.01608	mixed microbial mat	microscopy	eppendorf	2018070802	MS/FM	23/01/2019	3	0.2
P70 Nfix microscopy	165.55168	-78.01485	mixed microbial mat	microscopy	eppendorf	2018070802	MS/FM	23/01/2019	3	0.2
Casten Nfix microscopy	165.55074	-78.01299	mixed microbial mat	microscopy	eppendorf	2018070802	MS/FM	23/01/2019	3	0.2
Fresh water sample	165.54236	-78.01547	water	nutrients	60 ml Falcon	2018070802	MS/FM	23/01/2019	1	40
Brack water sample	165.54503	-78.01579	water	nutrients	60 ml Falcon	2018070802	MS/FM	23/01/2019	1	40
Salt water sample	165.54527	-78.01608	water	nutrients	60 ml Falcon	2018070802	MS/FM	23/01/2019	1	40
P70 water sample	165.55168	-78.01485	water	nutrients	60 ml Falcon	2018070802	MS/FM	23/01/2019	1	40
Casten water sample	165.55074	-78.01299	water	nutrients	60 ml Falcon	2018070802	MS/FM	23/01/2019	1	40
Fresh microbial mat (NFIX - glass jar)	165.54236	-78.01547	mixed microbial mat	Biomass/pigments	30 ml vial	2018070802	MS/FM	23/01/2019	5	2
Brack microbial mat (NFIX - glass jar)	165.54503	-78.01579	mixed microbial mat	Biomass/pigments	30 ml vial	2018070802	MS/FM	23/01/2019	5	2
Salt microbial mat (NFIX - glass jar)	165.54527	-78.01608	mixed microbial mat	Biomass/pigments	30 ml vial	2018070802	MS/FM	23/01/2019	5	2
P70 microbial mat (NFIX - glass jar)	165.55168	-78.01485	mixed microbial mat	Biomass/pigments	30 ml vial	2018070802	MS/FM	23/01/2019	5	2
Casten microbial mat (NFIX - glass jar)	165.55074	-78.01299	mixed microbial mat	Biomass/pigments	30 ml vial	2018070802	MS/FM	23/01/2019	5	2

APPENDIX B – CTAB PROTOCOL

Cary Lab Antarctic Dry Valley Soils Default Extraction Protocol

Potentially cleaning with water / (pps) – for brack and salt samples?

1. Place **0.5**g soil in a **2.0mL** screw-capped conical bottomed polypropylene tube containing **0.5g each of 0.1 mm and 2.5 mm silica-zirconia beads**

⇩

2. Add 270 µL phosphate buffer (100mM NaH_2PO_4), 270 µL SDS lysis buffer (100mM NaCl, 500mM Tris pH 8.0, 10% SDS) to tube

⇩

3. Bead beat samples for 15 seconds each and then shake horizontally on a vortex genie for 10 min. (Difficult samples may require further bead beating)

⇩

4. Centrifuge at 10,000rpm for 30 seconds to compact samples minutes.

⇩

5. Make up a working solution of CTAB buffer + 0.5% BME, (Working solution= (CTAB buffer= No. samples x 200uL) + 8uL of 50% BME per 1mL of CTAB buffer)). Add 180 uL CTAB buffer, vortex 10 seconds. Incubate at 300rpm at 60°C for 30 minutes

⇩

6. Centrifuge at 10,000rpm for 30 seconds to reduce bubbles.

⇩

7. Add 350 µL chloroform:isoamyl alcohol (24:1) vortex for 15 sec then centrifuge at 10,000rpm for 5 minutes. Remove upper aqueous layer into a new 1.5mL sterile microfuge tube.

⇩

8. Add 500 µL chloroform:isoamyl alcohol (24:1). Vortex the sample for 15 seconds.

Place on rocking bed for 20 minutes. Centrifuge the sample at 13,200rpm for 5 min. Remove upper aqueous layer into a new 1.5mL sterile microfuge tube.

⇩

Make notes about how much is transferred and how much needs to be added

9. Add 10M ammonium acetate to a final conc. of 2.5 M (e.g divide the above volume by 3 and add this amount in uL). Vortex for 10 seconds. There may or may not be a ppt. Centrifuge at 13,200rpm for 5 min and transfer the upper layer to a new sterile microfuge tube. Record the volume.

⇩

10. Add 0.54 times the recorded volume of isopropyl alcohol *(lower concentration and higher quantities, if it doesn't work first time*), mix by repeated inversion (20 times) then incubate at -20°C for at least an hour, overnight is best.

⇩

11. Centrifuge at 13,200rpm for 20 min, discard supernatant, leave pellet (Note: pellet may not be visible)

⇩

12. Wash the pellet with 1mL cold 70% AR grade ethanol. Centrifuge 13,200rpm for 1 min to wash pellet. Pipette off ethanol.

⇩

13. Dry in a speed vacuum for approx. 4-15 min on manual, medium drying. **(5 min first then check samples every 2minutes).**

⇩

14. Re-suspend DNA in 20 µL sterile LO-TE 20 µL sterile LO-TE by pipetting up and down and scraping the pipette tip on the side of the microfuge tube. Vortex for 10 seconds.

If pellet does not dissolve heat at 55°C for 10min. Store at -80°C until use.

APPENDIX C – EUB B/A - PCR COMPETENCY TEST PROTOCOL

EUB (Eub) B/A - PCR Competency Test Protocol

Master Mix Reagents: Recommended reaction volume 15 µL

Concentration	Reagents	25 µL	20 µL	15 µL
50mM	MgCl2	1.5	1.2	0.9
2mM	dNTPs	2.5	2	1.5
10x	buffer	2.5	2	1.5
0.4mg/mL	BSA	1.5	1.2	0.9
5U/uL	Taq	0.2	0.16	0.12
10mM	EUB A	0.625	0.5	0.375
10mM	EUB B	0.625	0.5	0.375
UVed	H2O	14.55	11.44	8.33
1.0 – 5.0 ng/ µL	DNA	1	1	1
Total Vol. µL		25.0	20.0	15.0

Use DNA at a concentration of 1.0 to 2.0 µL (of 1.0 to 5.0ng/ µL). If more volume of DNA is desired, e.g. the sample if of low concentration, adjust the volume of water used to maintain the correct PCR reaction volume, 15 µL, 20 µL, 25µL

PCR reactions should be done is duplicate when testing for PCR competency. For purposes of sequencing, it is recommended that samples be tested in triplicate.

Remember to make enough master mix for x2 number of samples, + negative control, + positive control + couple more to allow for pipetting error.

Instructions

Working in the PCR cabinet
UV treat all PCR tubes (0.2 µL), 1.5mL centrifuge tubes, MiliQ H_20
Defrost, vortex and spin down all reagents.
Make up the Master Mix in the PCR cabinet.
Remember to leave out the Taq.

Move to the Biosafety Cabinet
Add DNA in biosafety cabinet. Add Taq.
Vortex Master mix,
Aliquot Master mix out to all PCR reaction tubes and close lids.
Add water to your negative control first and close the lid.
Vortex and spin your DNA samples.
Add your DNA to your PCR reaction tubes. Have only one tube open at a time.
Make sure all lids are closed then add your positive control DNA.
Spin down PCR tubes using the microfuge by the Thermo cyclers in PC1.
Place in Thermo Cycler, being carefully not to knock reaction tubes.

Clean up
Leave by the thermo cycler a PCR rack and tinfoil, with your name, date and PCR type. Include in special instructions, e.g. light sensitive.

Important Notes
Please book the Thermo cycler and book for longer than you will need.
Remember to include a negative control, and a positive control.

Remember to UV treat your MiliQ H_20. (Use enough to make up to a reaction volume of 15 µL, typically 7.33 µL with 2.0 µL DNA template).

Add DNA to a concentration of 5-10ng/uL. Aim for 2.0 uL of 5ng/uL or 5 uL of <5ng/ µL for low concentration DNA templates, this is sufficient in most instances! More DNA can be used if desired, BUT remember more DNA is not necessarily better. PCR does not always work the first time so make sure you conserve the amount of DNA you use.

Trouble shooting
Contamination: If contamination is expected, e.g., amplification is present in your negative control, try EMA treatment on your master mix. This occurs prior to addition of your DNA.

To EMA treat your Master mix add EMA to the equivalent of 1/100 of the total volume your Master mix, to your Master mix. E.g. Master Mix = 500 µL add 5 µL of diluted EMA to the Master mix. Vortex. Place in ice in complete darkness for 1minute. Place tube on side on ice under a strong light for 1 minute.

Program used

EUB A/B – (Most commonly used)
1. 94°C for 2min
2. 94°C for 45s
3. 55°C for 30s
4. 72°C for 2min
5. go to step 2, 29 times
6. 72°C for 7min
7. Hold at 4°C indefinitely

Remember to come back and take your PCR reactions out of the machine and Exit from the program before turning the Thermo cycler off.
Store your PCR samples in the fridge well covered for up to a month. Freeze only for long-term storage.

APPENDIX D – HILL LABORATORY RESULTS

Hill Laboratories
TRIED, TESTED AND TRUSTED

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Hamilton 3240 New Zealand

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Certificate of Analysis

Client:	University of Waikato	**Lab No:**	2264927 SPv1
Contact:	Ian Hawes C/- University of Waikato Private Bag 3105 Hamilton 3240	**Date Received:**	25-Oct-2019
		Date Reported:	04-Nov-2019
		Quote No:	101294
		Order No:	127 1117
		Client Reference:	
		Submitted By:	Ian Hawes

Sample Type: Saline

Sample Name:		Brack 2019 0.45 Nitrate	Eastern 2019 0.45 Nitrate	Fresh 2019 0.45 Nitrate	P70 2019 0.45 Nitrate	Salt 2019 0.45 Nitrate
Lab Number:		2264927.6	2264927.7	2264927.8	2264927.9	2264927.10
Total Ammoniacal-N	g/m³	0.045	0.048	0.105	0.058	0.32
Nitrite-N	g/m³	< 0.0010	< 0.0010	< 0.0010	< 0.0010	< 0.002 #1
Nitrate-N	g/m³	0.0013	0.0011	0.0052	< 0.0010	0.006
Nitrate-N + Nitrite-N	g/m³	0.0015	0.0013	0.0057	0.0013	0.006 #1
Dissolved Reactive Phosphorus	g/m³	0.032	0.129	0.034	0.0093	0.054
Phosphate	g/m³	0.098	0.39	0.105	0.028	0.166

Sample Type: Aqueous

Sample Name:		KH Moat Nuts	KH If Nuts 22-Oct-2019	Candle Ward Nuts 23-Oct-2019	Ward Moat 7 Nuts 23-Oct-2019	Adams If Ward Nuts 23-Oct-2019
Lab Number:		2264927.1	2264927.2	2264927.3	2264927.4	2264927.5
Total Ammoniacal-N	g/m³	0.015	0.042	< 0.005	< 0.005	0.006
Nitrite-N	g/m³	< 0.0010	< 0.0010	< 0.0010	< 0.0010	0.0011
Nitrate-N	g/m³	0.0063	0.034	0.0037	0.0118	0.52
Nitrate-N + Nitrite-N	g/m³	0.0068	0.034	0.0040	0.0121	0.52
Dissolved Reactive Phosphorus	g/m³	< 0.004	0.005	< 0.004	< 0.004	< 0.004
Phosphate*	g/m³	< 0.013	0.015	< 0.013	< 0.013	< 0.013

Analyst's Comments

#1 Insufficient sample required that a dilution be performed prior to analysis of sample, resulting in a detection limit higher than that normally achieved for the NoxN / NO2N analysis.

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.
Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Saline

Test	Method Description	Default Detection Limit	Sample No
Total Ammoniacal-N	Saline sample. Phenol/hypochlorite colorimetry. Flow injection analyser. (NH4-N = NH4+-N + NH3-N). APHA 4500-NH_3 H 23rd ed. 2017.	0.005 g/m³	6-10
Nitrite-N	Saline sample. Automated Azo dye colorimetry, Flow injection analyser. APHA 4500-NO_3^- I (modified) 23rd ed. 2017.	0.0010 g/m³	6-10
Nitrate-N + Nitrite-N	Saline sample. Total oxidised nitrogen. Automated cadmium reduction, Flow injection analyser. APHA 4500-NO_3^- I (modified) 23rd ed. 2017.	0.0010 g/m³	6-10
Dissolved Reactive Phosphorus	Saline sample. Molybdenum blue colorimetry. Flow injection analyser. APHA 4500-P G 23rd ed. 2017.	0.0010 g/m³	6-10

Sample Type: Aqueous

Test	Method Description	Default Detection Limit	Sample No
Total Ammoniacal-N Trace	Phenol/hypochlorite colorimetry. Flow injection analyser. (NH4-N = NH4+-N + NH3-N). APHA 4500-NH_3 H 23rd ed. 2017.	0.005 g/m³	1-5
Nitrite-N Trace	Automated Azo dye colorimetry, Flow injection analyser. APHA 4500-NO_3^- I (modified) 23rd ed. 2017.	0.0010 g/m³	1-5

Sample Type: Aqueous			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Nitrate-N	Calculation: (Nitrate-N + Nitrite-N) - NO2N. In-House.	0.0010 g/m^3	1-10
Nitrate-N + Nitrite-N Trace	Total oxidised nitrogen. Automated cadmium reduction, flow injection analyser. APHA 4500-NO_3^- I (modified) 23rd ed. 2017.	0.0010 g/m^3	1-5
Dissolved Reactive Phosphorus	Filtered sample. Molybdenum blue colourimetry. Flow injection analyser. APHA 4500-P G (modified) 23rd ed. 2017.	0.004 g/m^3	1-5
Phosphate from DRP*	Calculation: from Dissolved Reactive Phosphorus * 3.065.	0.004 g/m^3	1-10

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Carole Rodgers-Carroll BA, NZCS
Client Services Manager - Environmental

APPENDIX E –RAREFIED SPECIES FIGURES

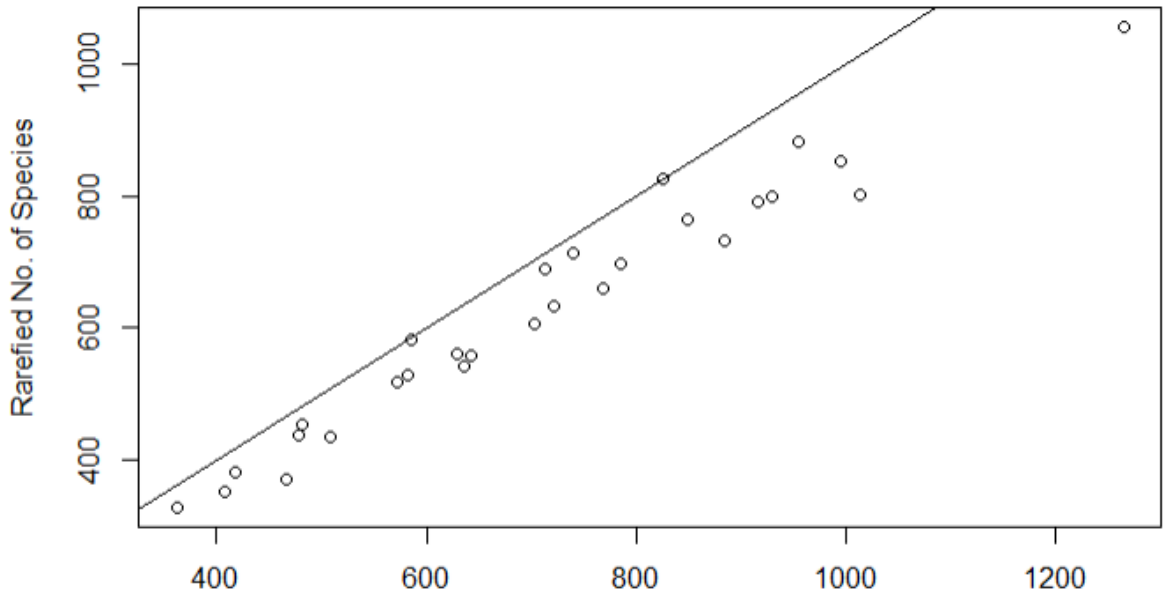

Figure 29. Total number of Rarefied OTUs.

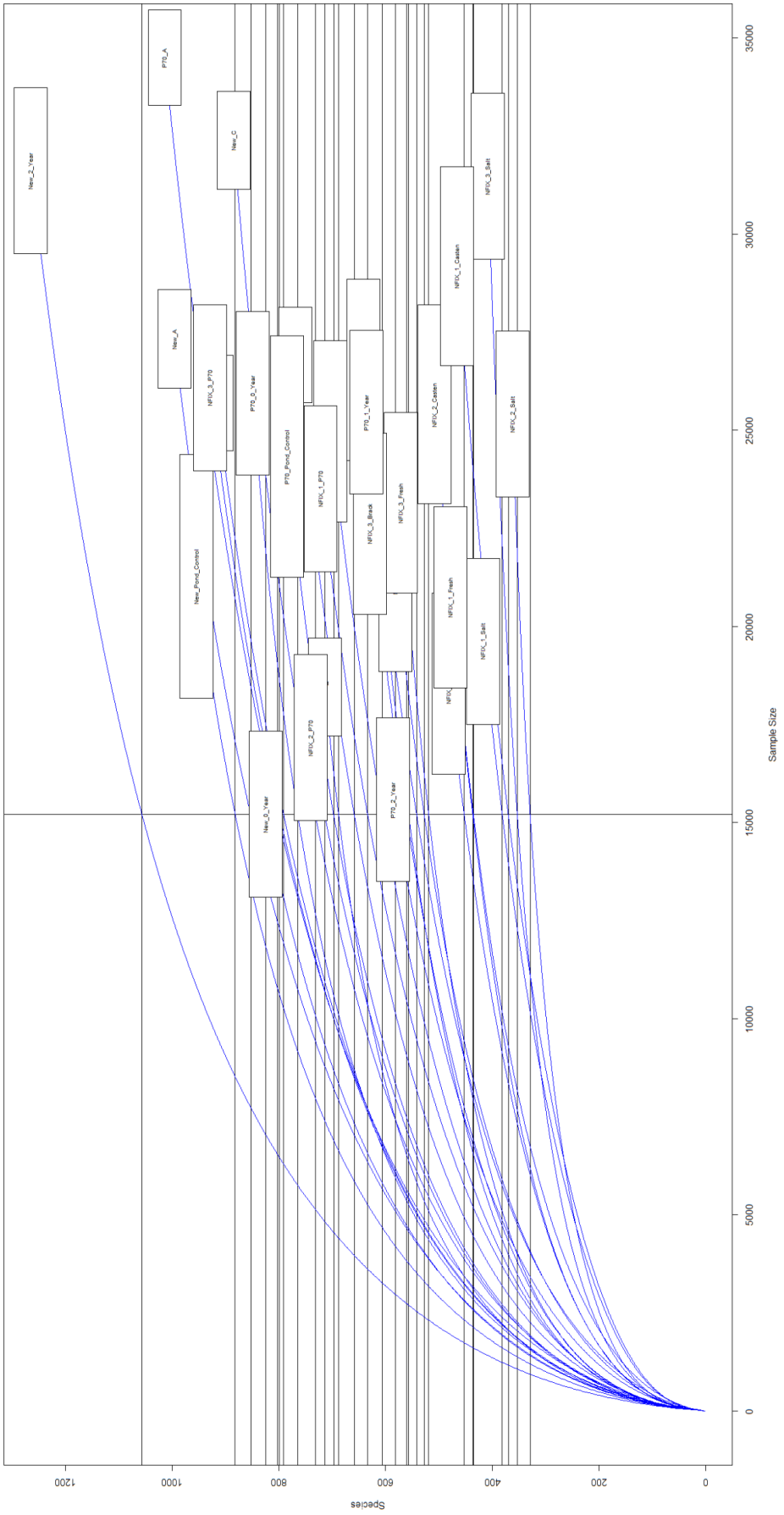

Figure 30. Rarefaction curve of all samples which were genetically sequenced.

APPENDIX F – ALPHA DIVERSITY FIGURES

APPENDIX G – PHYLOGENETIC TREE

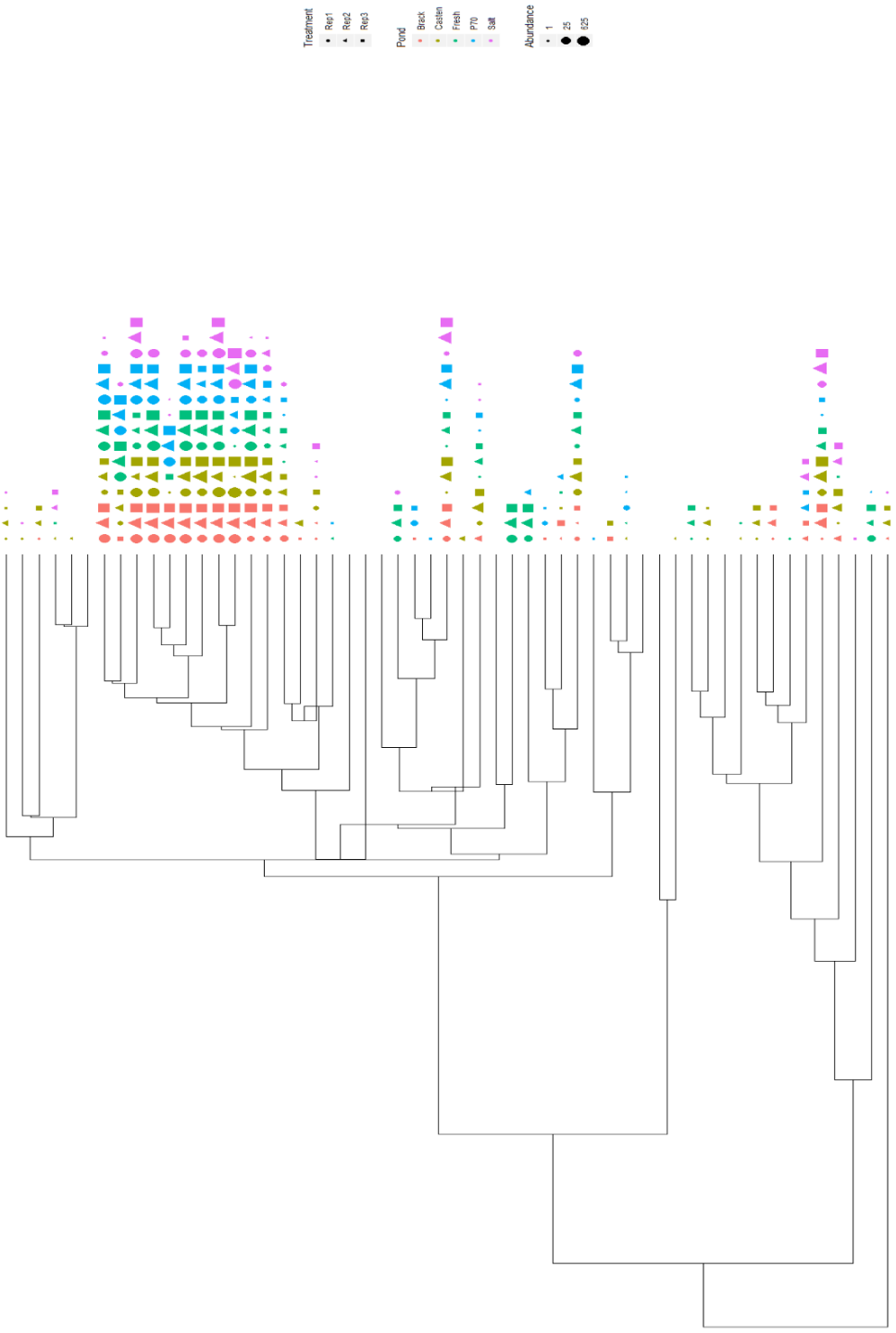

Figure 31. Phylogenetic tree for the nitrogen fixation experiment ponds. The lines of the tree represent evolutionary relationships between organisms. The branching shows an evolution to a new species, subsequently lines with fewer branches are older species. In this figure points which have fewer branches between them are more closely related than points with more branching. We can see from this figure lower salinity ponds contain more older species while more saline ponds tend to contain more recently evolved species. Ponds tended to be more similar in composition with more recently evolved species and very dissimilar based on salinity in older species.

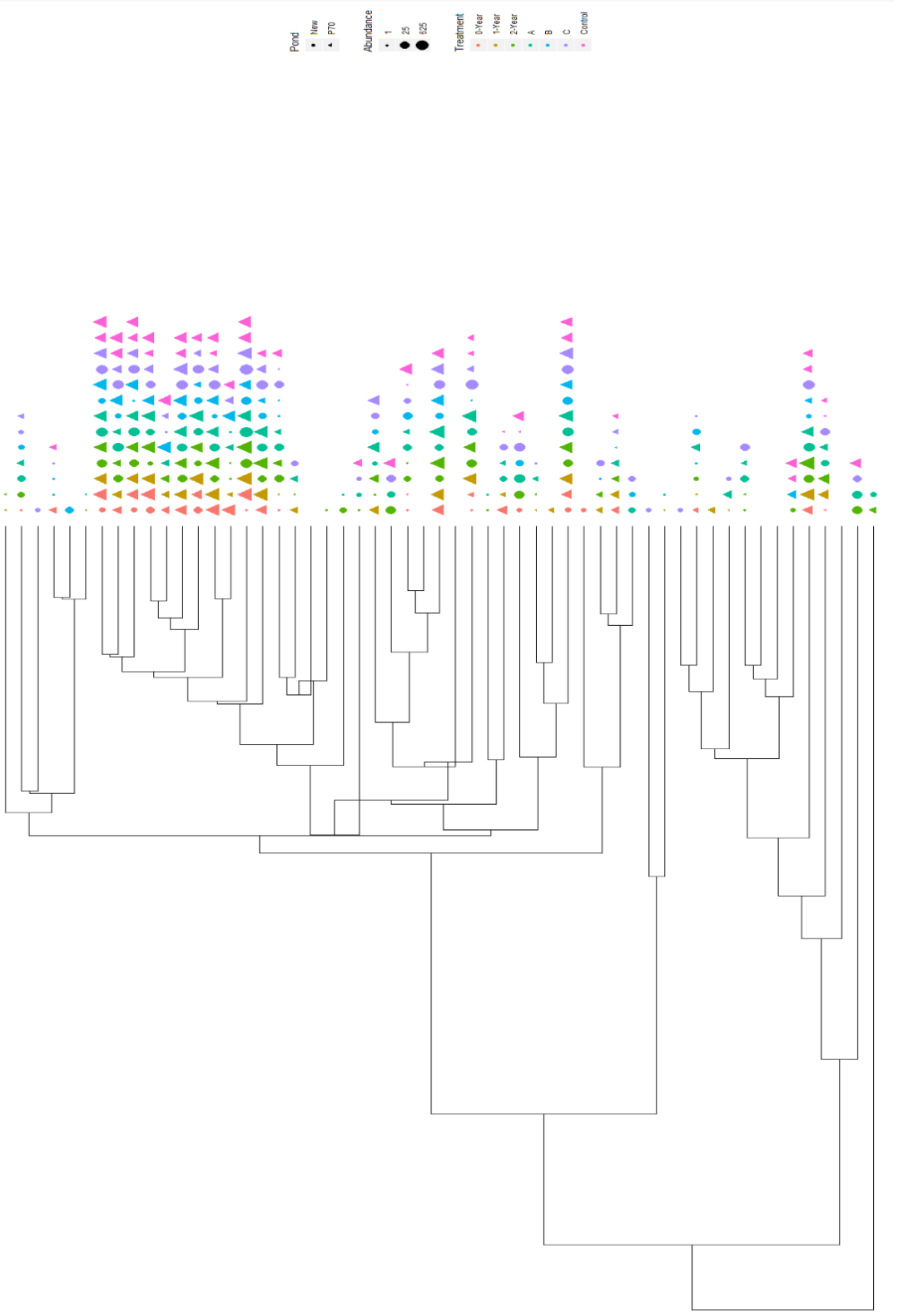

Figure 32. Phylogenetic tree for the disturbance experiment ponds. The lines of the tree represent evolutionary relationships between organisms. The branching shows an evolution to a new species, subsequently lines with fewer branches are older species. In this figure points which have fewer branches between them are more closely related than points with more branching. We can see from this figure both P70 and New ponds primarily contain more recently evolved species and have fewer older species present. In general ponds tend to be very similar in species presence.

APPENDIX H – ANOVA SIGNIFICANCE

Table 18. ANOVA Test for the 99.5 %ile.

Source of Variation	**Sum of Squares (SS)**	**Degrees of Freedom (df)**	**Mean Squares (MS)**	**F Ratio (F)**	**P-value**	**F critical Value**
Between Groups	2756	4	689	5.32	0.004	2.87
Within Groups	2592	20	129			
Total	5349	24				

Table 19. Summary of two-way T-Test results.

	Brack	**Casten**	**Fresh**	**P70**	**Salt**
Brack		**0.006****	0.018*	0.615	0.282
Casten	**0.006****		0.367	*0.013	*0.037
Fresh	*0.018	0.367		*0.042	0.138
P70	0.615	*0.013	*0.042		0.528
Salt	0.282	*0.037	0.138	0.528	

* Significant with ANOVA value of significance - $F \leq 0.05$
****** Significant with Bonferroni Correction value of significance - $F \leq 0.01$

t-Test: Two-Sample Assuming Equal Variances for Brack pond

	Brack	Casten	Fresh	P70	Salt
Mean	19.7	45.0	36.6	22.0	25.5
Variance	58.9	352.3	209.8	78.9	129.7
Observations	7	7	7	7	7
Pooled Variance		205.6	134.4	68.9	94.3
Hypothesized Mean Difference		0	0	0	0
df		12	12	12	12
t Stat		-3.306	-2.732	-0.516	-1.126
P(T<=t) one-tail		0.003	0.009	0.307	0.141
t Critical one-tail		1.8	1.8	1.8	1.8
P(T<=t) two-tail		0.006	0.018	0.615	0.282
t Critical two-tail		2.2	2.2	2.2	2.2

t-Test: Two-Sample Assuming Equal Variances for Casten pond

	Brack	Casten	Fresh	P70	Salt
Mean	19.7	45.0	36.6	22.0	25.5
Variance	58.8	352.3	209.8	78.9	129.7
Observations	7	7	7.0	7.0	7.0
Pooled Variance	205.6		281.1	215.6	241.0
Hypothesized Mean Difference	0		0.0	0.0	0.0
df	12		12.0	12.0	12.0
t Stat	-3.306		0.938	2.936	2.349
P(T<=t) one-tail	0.003		0.183	0.006	0.018
t Critical one-tail	1.8		1.8	1.8	1.8
P(T<=t) two-tail	0.006		0.367	0.012	0.037
t Critical two-tail	2.2		2.2	2.2	2.2

t-Test: Two-Sample Assuming Equal Variances for Fresh pond

	Brack	Casten	Fresh	P70	Salt
Mean	19.7	45.0	36.6	22.0	25.5
Variance	58.9	352.3	209.8	78.9	129.7
Observations	7.0	7.0	7.0	7.0	7.0
Pooled Variance	134.4	281.1		144.4	169.8
Hypothesized Mean Difference	0.0	0.0		0.0	0.0
df	12.0	12.0		12.0	12.0
t Stat	-2.732	0.938		2.278	1.591
P(T<=t) one-tail	0.009	0.183		0.021	0.069
t Critical one-tail	1.8	1.8		1.8	1.8
P(T<=t) two-tail	0.018	0.367		0.042	0.138
t Critical two-tail	2.2	2.2		2.2	2.2

t-Test: Two-Sample Assuming Equal Variances for P70 pond

	Brack	Casten	Fresh	P70	Salt
Mean	19.7	45.0	36.6	22.0	25.5
Variance	58.9	352.3	209.8	78.9	129.7
Observations	7.0	7.0	7.0	7.0	7.0
Pooled Variance	68.9	215.6	144.4		104.3
Hypothesized Mean Difference	0.0	0.0	0.0		0.0
df	12.0	12.0	12.0		12.0
t Stat	-0.516	2.936	2.278		0.651
P(T<=t) one-tail	0.307	0.006	0.021		0.264
t Critical one-tail	1.8	1.8	1.8		1.8
P(T<=t) two-tail	0.615	0.012	0.042		0.528
t Critical two-tail	2.2	2.2	2.2		2.2

t-Test: Two-Sample Assuming Equal Variances for Salt pond

	Brack	Casten	Fresh	P70	Salt
Mean	19.7	45.0	36.6	22.0	25.5
Variance	58.9	352.3	209.8	78.9	129.7
Observations	7.0	7.0	7.0	7.0	7.0
Pooled Variance	94.3	241.0	169.8	104.3	
Hypothesized Mean Difference	0.0	0.0	0.0	0.0	
df	12.0	12.0	12.0	12.0	
t Stat	-1.126	2.349	1.591	0.651	
P(T<=t) one-tail	0.141	0.018	0.069	0.264	
t Critical one-tail	1.8	1.8	1.8	1.8	
P(T<=t) two-tail	0.282	0.037	0.138	0.528	
t Critical two-tail	2.2	2.2	2.2	2.2	