Microbial communities of Antarctic soil and lithic habitats

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Abstract

Global climate change is predicted to significantly alter extreme terrestrial environments. The disturbance of desert ecosystems is predicted to profoundly alter key biological processes that are thought to be mediated by micro-organisms in these delicate biomes.

The Antarctic Dry Valleys are a series of hyperarid polar deserts which are highly oligotrophic, experience near-constant below-freezing temperatures, and are critically low in bioavailable moisture. However, increases in surface temperatures and ultra-violet irradiation are predicted to supply endemic microbial communities with previously unattainable levels of moisture and nutrients as ice melt intensifies. Understanding the responses of local microbial populations to changes in moisture content is the critical focus of this study. Here microbial fingerprinting and pyrosequencing in combination with multivariate statistical analyses were utilised to address this knowledge deficit.

This study presents evidence supporting the concept of ecological niche partitioning between local desert habitats (Pointing *et al.*, 2009). Multivariate analysis of bacterial 16S rRNA gene-defined communities generated using T-RFLP showed that edaphic niches; hypoliths, endoliths, soils and mat communities, were distinct in structure. However, local Cyanobacterial populations were not delineated by habitat. Pyrosequencing data revealed that soil communities were highly diverse and are predicted to 'seed' development of specialised communities, such as hypoliths and endoliths, which supports the concept of species recruitment from soils in desert systems (Makhalanyane *et al.*, 2013b).

The role of moisture content was less significant in determining local bacterial diversity patterns according to the fingerprinting techniques applied here. However, pyrosequencing data suggested that Cyanobacterial abundance and diversity was greater in communities exposed to higher levels of moisture content. These data suggest that increases in local moisture content may influence Cyanobacterial population abundance and diversity in this desert environment. The increase in bioavailable moisture has the potential to lead to increased proliferation of the phylum as has been predicted previously (Wood *et al.*, 2008). Taken together, these results appear to suggest that deterministic processes supersede stochastic events in determining diversity patterns across this polar desert. This may be critical in terms of global climate changes as rapidly changing environmental parameters may lead to detrimental changes in local desert community structures.

Declaration

I, Marc Warwick Van Goethem, declare that the thesis/dissertation 'Microbial communities of Antarctic soil and lithic habitats' which I hereby submit for the degree Magister Scientiae (M.Sc.) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution, and that all the sources I have used or quoted have been indicated and acknowledged by complete references throughout.

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Amy, I love you. Thank you for everything, forever and always.

Dedication

This thesis is dedicated to my parents, Jake and Amy. I love you always.

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List of Abbreviations

Abbreviation	Definition
g	acceleration due to gravity
α	Alpha
ANOSIM	Analysis of Similarities
T_m	annealing temperature
bp	base pairs
BLAST	Basic Local Alignment Search Tool
β	Beta
BSC	Biological Soil Crusts
BSA	Bovine Serum Albumin
FAM	carboxyfluorescein
cm	centimetres
°C	Degrees Celsius
df	Degrees of Freedom
dNTP	deoxynucleotide triphosphate
DNA	Deoxyribonucleic Acid
DTT	dithiothrietol
et al	*et alia*
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
EPS	Exopolysaccharides
γ	Gamma
g	gram
kb	kilobases
λ	Lambda
$MgCl_2$	Magnesium Chloride
m	meter
µ	Micro
ml	millilitre
mM	millimolar
min	minutes
M	Molar
ng	nanograms

nMDS	non-metric Multidimensional Scaling
n	number
OTU	Operational Taxonomic Unit
%	Percentage
PERMANOVA	Permutational Multivariate Analysis of Variance
PAR	Photosynthetically Active Radiation
PCR	Polymerase Chain Reaction
PVPP	Polyvinylpolypyrrolidone
P/PET	Precipitation to Potential Evapotranspiration
rfu	relative fluorescent units
rRNA	ribosomal ribonucleic Acid
s	seconds
H’	Shannon Diversity Index
SSU	small sub-unit
NaCl	Sodium Chloride
SDS	Sodium dodecyl sulphate
spp.	species
km^2	Square Kilometres
SD	Standard Deviation
T-RF	Terminal Restriction Fragment
T-RFLP	Terminal Restriction Fragment Length Polymorphism
Tris	tris (hydroxymethyl)-aminomethane
TAE	tris-acetic acid-EDTA
Tris-HCl	Tris-Hydrochloride
UV	Ultra-Violet
V	Volts
v/v	volume per volume
w/v	weight per volume

Chapter 1: Literature Review

1.1 Antarctica

The Antarctic continent is characterised by episodic katabatic winds, physical disturbance (Wynn-Williams, 1990), and high levels of incident radiation, namely ultraviolet irradiation (UVA and UVB), and photosynthetically active radiation (PAR), comprising some of the most extreme environmental conditions on the planet (Cary *et al.*, 2010). The majority of the continent is ice-covered; approximately 0.4% total Antarctic land mass is ice-free. These exposed ice-free regions represent some of the coldest and driest hyperarid deserts on Earth (Cowan and Ah Tow, 2004) (Figure 1).

The extreme environmental conditions of Antarctica are exacerbated by numerous stresses which include near-constant below-zero temperatures, extremely low levels of bioavailable moisture and low nutrient status. These conditions were once thought to render these environments sterile and devoid of life (Horowitz *et al.*, 1972), although recent studies have invalidated these hypotheses. A surprisingly vast microbial diversity has since been described across a range of habitable Antarctic terrestrial niches (Cowan *et al.*, 2002, Smith *et al.*, 2006, Pointing *et al.*, 2009, Lee *et al.*, 2012a, Makhalanyane *et al.*, 2013a, Bottos *et al.*, 2014). Vascular plants and higher eukaryotes are entirely absent from the Dry Valleys of Antarctica, although microbial communities persist and constitute major biological components of the Dry Valleys.

Due to the extreme environmental conditions that shape the continent, initial studies likened the McMurdo Dry Valleys of Antarctica to extra-terrestrial environments. This notion led to a program of Martian analogue research on the continent on the basis that Earth's terrestrial extremes might shed light on the limits of life (Vishniac and Mainzer, 1972, Andersen *et al.*, 1995, Gibson *et al.*, 1983). This 'quasi-Martian' analogue, the McMurdo Dry Valleys (also known as the Dry Valleys), remains a hub of investigation, with research focused on Antarctic microbial diversity and aimed at understanding microbial community ecology and the environmental factors that influence patterns of dispersal across spatial and temporal scales.

The endemic microbial diversity of the Antarctic continent is nearly exclusive to ice-free regions, such as the Dry Valleys, which represent the largest polar desert on the continent at roughly 4800 km^2 (Cowan and Ah Tow, 2004, Hopkins *et al.*, 2009). The Dry Valleys comprise a variety of non-homogenous terrestrial biotopes; including pristine glacial formations, exposed desert pavements, rock structures, melt-water streams and ice-covered lakes, which provide exploitable niches for microbial colonisation (Cary *et al.*, 2010). Surface topography is highly variable in terms of altitude, ranging from 0 m (sea-level) to above 2000 m (Doran *et al.*, 2003).

Figure 1. The Onyx river bed shows the typical desert pavement of the McMurdo Dry Valleys. Ice-free regions of the continent are rare, yet multiple colonisable niches exist where exposed desert pavements are present (Onofri *et al*. 2004). (Photograph courtesy of Prof. Don A. Cowan).

1.1.1 Antarctic Climatic Conditions

The extreme xeric stress experienced in the McMurdo Dry Valleys categorises these valleys as hyperarid deserts. Annual precipitation (rainfall-equivalent) in the Dry Valleys is extremely low, approximately 10 cm per annum (Witherow *et al.*, 2006), falling exclusively as snow, most of which sublimes (Cowan and Ah Tow, 2004). Water content in Dry Valley surface soils is critically low, between 0.5% – 2% weight (Cowan and Ah Tow, 2004). Low water bioavailability is further exacerbated by high levels of salinity, wind-driven evaporation and sublimation (Chinn, 1993).

According to the current measure of aridity, the Precipitation to Potential Evapotranspiration (P/PET) index, the Dry Valleys are classified as hyperarid deserts with a value of < 0.01. This aridity index classifies all environmental systems with a value < 1 as deserts and is commonly used to reflect the moisture deficiency of a terrestrial system. Desert regions are further delineated using this index as sub-humid (0.5 – 0.65), semi-arid (0.2 – 0.5), arid (0.05 – 0.2) and hyperarid (< 0.05) (Pointing and Belnap, 2012). Hyperarid deserts are widely distributed across all continents, including the Negev Desert, Israel; the Namib Desert, Namibia and the Atacama Desert, Chile. These deserts are classified as hot hyperarid deserts, with average annual temperatures > 18°C (Peel *et al.*, 2007).

Extremely low temperatures define polar deserts. Cold temperatures are the most common global abiotic stress factor with over 80% of the earth's biosphere in a permanently cold state (Russell *et al.*, 1990). The Dry Valleys are a series of polar deserts that experience extremely low temperatures, typically below-freezing, for the majority of the year. Temperatures in the Dry Valleys range from maxima of above 9°C in summer to minima of below -55°C in winter months (Doran *et al.*, 2002). Mean annual temperatures have been recorded in the range of -30°C to -15°C (Wynn-Williams, 1988, Doran *et al.*, 2002). In addition, daily temperature fluctuations lead to multiple freeze-thaw events (Aislabie *et al.*, 2006), temperature changes may exceed 20°C within 24 hours. Rapid temperature deviations have been documented in the Ross Desert, where surface soil temperatures warmed by 42.5°C (from -15°C to 27.5°C) in a three hour period (Cameron, 1974). Temperature fluctuations also limit the amount of available atmospheric moisture, resulting in low relative humidity (Bargagli, 2006).

Polar winds greatly influence surface soil and air temperatures. Katabatic winds are low relative humidity winds that move from the Antarctic plateau into valley depressions and are capable of reaching hurricane-force speeds (Nylen, 2004, Cary *et al.*, 2010). These winds evaporate most of the atmospheric moisture while marginally increasing overall air temperature (Nylen, 2004).

The range of stresses that persist in the Dry Valleys limit the survival capacity of most micro-organisms. The highest forms of life typically include eukaryotic mosses or bryophytes, as well as invertebrates such as nematodes, rotifers, protozoa and springtails that survive through sub-lithic colonisation which is predicted to mitigate stress levels (Adams *et al.*, 2006, Cary *et al.*, 2010). By contrast, micro-organisms such as bacteria and fungi are diverse in the Dry Valleys and have been studied extensively across numerous colonisable niches (Barrett *et al.*, 2006, Smith *et al.*, 2006, Pointing *et al.*, 2009, Cowan *et al.*, 2010, Makhalanyane *et al.*, 2013a). Interestingly, Archaeal signatures are lower in abundance than other domains, although a high diversity of this guild has been documented in chasmoendolithic (within-rock) communities (Yung *et al.*, 2014).

1.1.2 Antarctic Microbial Niches

The Dry Valleys are colonised by highly adapted microbial communities typically found in cryptic and refuge niches which are thought to provide varying levels of protection from abiotic and environmental stress. This avoidance strategy has been observed across all desert pavements (Bahl *et al.*, 2011) and the communities adapted to extreme environments, termed extremophiles, are predicted to be of functional importance in desert ecology, particularly in the absence of vascular plants (Cary *et al.*, 2010, Chan *et al.*, 2013). Dry Valley microbiota, including photoautotrophic members such as Cyanobacteria, are thought to be critical mediators of

numerous biological processes in desert ecosystems including nitrogen regulation and nitrification (Chan *et al.*, 2013) as well as local carbon cycling (Högberg *et al.*, 2001).

A fundamental aspect of Antarctic survival is the mitigation of stress levels experienced. Niche colonisation appears to circumvent the effects of hyperaridity, physical abrasion and temperature fluctuations (Cowan and Ah Tow, 2004, Warren-Rhodes *et al.*, 2006). Cryptic and refuge niches, such as hypolithic and endolithic rock substrates as well as sub-surface soils, reduce the harsh conditions experienced by the associated community. Stress reduction is also achieved through the formation of Cyanobacteria-dominated mat communities whereby internal members are protected from excessive UV-irradiation, low temperatures and wind abrasion.

Hypoliths are microbial assemblages that colonise the ventral sides of translucent rocks, such as quartz and marble, at the soil-rock interface (Figure 2 A) (Golubic *et al.*, 1981). The overlying lithic substrate protects the associated community from excessive solar radiation (Schlesinger *et al.*, 2003) and physical disturbance (Wong *et al.*, 2010). This type of colonisation is also thought to provide improved moisture content relative to open soils as water condenses below the rock (Smith *et al.*, 2000, Warren-Rhodes *et al.*, 2006), and reduce the severity of freeze-thaw events (Cowan *et al.*, 2010). This niche type is common across most, if not all, hot (Schlesinger *et al.*, 2003, Pointing *et al.*, 2007, Makhalanyane *et al.*, 2013b, Stomeo *et al.*, 2013) and cold (Cockell and Stokes, 2004, Pointing *et al.*, 2009, Cowan *et al.*, 2010, Khan *et al.*, 2011) deserts.

Hypoliths are typically dominated by Cyanobacteria (Tracy *et al.*, 2010, Bahl *et al.*, 2011, Caruso *et al.*, 2011, Chan *et al.*, 2012), and may serve as basal units for desert ecosystems as members of the phylum have the genetic capacity to mediate numerous nutrient cycling processes (Chan *et al.*, 2012). However the contributions of other photoautotrophic members, such as Chloroflexi, mosses and lichens, to ecological processes cannot be discounted (Yung *et al.*, 2014). Recent studies have proposed that bacterial phyla other than Cyanobacteria may be dominant hypolith members - these include Proteobacteria (Makhalanyane *et al.*, 2013a) and Actinobacteria (Khan *et al.*, 2011).

As photoautotrophs may have the capacity to support heterotrophic colonisers, the most common trophic scenario in desert systems typically features a two-level trophic community of heterotrophic bacteria or fungi utilising photoautotrophs as carbon and nitrogen sources (Pointing *et al.*, 2009). However food webs consisting of three levels are rare in the Dry Valleys (Barrett *et al.*, 2006). Due to the important roles played by Cyanobacteria as photoautotrophs, both in terms of nutrient cycling and establishing microbial communities, this project is highly focused on describing and evaluating members of the phylum.

Antarctic hypoliths have been distinguished into three distinct types on the basis of community composition and which photoautotroph dominates the community (Cowan *et al.*, 2010,

Makhalanyane *et al.*, 2013a). Type I hypoliths are Cyanobacteria-dominated, observed as a green biofilm layer on the underside of translucent rocks, and are highly abundant across the Dry Valleys. Type II hypoliths are fungal-dominated, while Type III hypoliths are moss- or bryophyte-dominated communities (Cowan *et al.*, 2010). It has been shown that Type I hypoliths form basal communities which may undergo successional community development to Type II and possibly Type III hypoliths over time by species recruitment and habitat filtering (Makhalanyane *et al.*, 2013a).

The microbial diversity of hypoliths has been well documented previously. Cyanobacteria have been shown to dominate hypolithons across all deserts and often include *Chroococcidiopsis*-like members and Oscillatorian genera (Pointing *et al.*, 2009, Chan *et al.*, 2012). Heterotrophs such as Actinobacteria, α-Proteobacteria and λ-Proteobacteria are important taxa that are ubiquitous to polar desert hypolith communities (Pointing *et al.*, 2009, Cary *et al.*, 2010, Makhalanyane *et al.*, 2013a). Less abundant phyla include Acidobacteria, Bacteroidetes as well as fungi and mosses (Khan *et al.*, 2011). The diversity of bacterial groups differs between hypolith types. For example, Proteobacteria were shown to be more abundant in hypolith Types I and II, whereas Actinobacteria and Acidobacteria were more prevalent in Type III hypoliths as well as soils (Makhalanyane *et al.*, 2013a). However, lithic colonisers are not limited to the ventral surfaces of rocks as habitable niches exist both within and upon rock substrates. These communities are termed endoliths and epiliths, respectively.

Endoliths are microbial communities that inhabit interior pores, cavities and fissures within rocks (Figure 2 B). Typical endolithic substrates include sandstone, granite and gypsum, all of which possess translucent grains allowing for moderate levels of photosynthetically active radiation (PAR) to reach the microbial community while filtering out the majority of harmful ultra-violet (UV) irradiation (Golubic *et al.*, 1981). Endolithic communities are observed as coloured bands of colonisation a few millimetres below the rock surface. Due to the abundance of photoautotrophic colonisers, endolithic communities are typically green in colour, although black (Figure 2 C), grey and red endolith communities have been observed in the Dry Valleys (Yung *et al.*, 2014). Dark colouration may be due to the presence of pigmented fungi and bacteria as dominant members of the community which may provide protection for UV-sensitive photoautotrophs found below this layer (Ruisi *et al.*, 2007, Pointing and Belnap, 2012). High concentrations of scytonemin produced by Cyanobacteria, thought to act as a ‘sunscreen’, may also result in dark colouration (Vincent, 2007). Chasmoendoliths colonise cracks or fissures in granite rocks that reach the surface. Granite rocks are common in the Dry Valleys and are often exploited by bacterial communities (De Los Ríos *et al.*, 2007, Yung *et al.*, 2014), whereas porous interstices within sandstone rocks are utilised by guilds termed cryptoendoliths (Friedmann and Ocampo, 1976). Endolithic classification is based on the location of the microbial assemblage within the rock substrate, whether the community

colonises cracks or pores (Golubic *et al.*, 1981). Internal lithic colonisation creates an improved environment for micro-organisms to proliferate as PAR remains available while moisture content is improved and the levels of wind abrasion are reduced. Cryptoendoliths are found within the rock pores of sandstone rocks and represent one of the best characterised niches in the McMurdo Dry Valleys (Cary *et al.*, 2010).

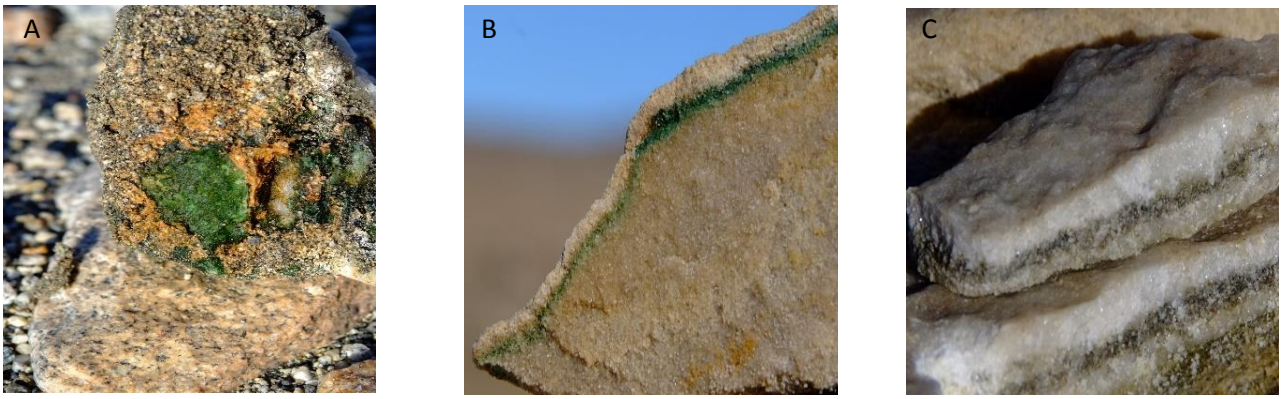


Figure 2. A – An upturned hypolithic community. The green biofilm layer is distinctive of Cyanobacteria-dominated Type I hypoliths. **B** – Green cryptoendoliths colonise porous rocks, such as sandstone, a few millimetres below the rock surface. **C** – Black endoliths - pigmented fungal colonisers and heterotrophic bacteria provide the observed colouration (Ruisi *et al*. 2007). (Photographs courtesy of Prof. Don A. Cowan).

Wierzchos *et al.* (2013) reviewed the microbial diversity of Antarctic endolithic communities, concluding that highly complex communities exist in the McMurdo Dry Valleys compared to other hyperarid endolith communities. A variety of algal and fungal taxa (Ascaso and Wierzchos, 2002) and lichens (Ascaso and Wierzchos, 2003) have been found to colonise granite rocks in the Dry Valleys, although Cyanobacteria may represent the most abundant phylum in both sandstone- and granite-colonising endolith communities (Cary *et al.*, 2010, Yung *et al.*, 2014). Cyanobacterial members of the *Chroococcidiopsis* genus have been found in high abundance (Pointing *et al.*, 2009). Members of the photoautotrophic phylum Chloroflexi have also been detected in endolithic communities, and are predicted to represent alternative primary producers to Cyanobacteria (Pointing *et al.*, 2009). The crypto- and chasmoendolithic communities have been shown to be colonised by significantly different bacterial consortia suggesting that the bacterial communities capable of colonising each niche type are distinct (Chan *et al.*, 2013, Yung *et al.*, 2014). Chasmoendoliths have shown greater microbial diversity than cryptoendolithic communities and greater community similarity to hypoliths than to soils (Chan *et al.*, 2013). Archaea are not frequently detected in these communities (De La Torre *et al.*, 2003), although Archaeal diversity has been described in chasmoendoliths (Yung *et al.*, 2014). Epilith communities are not typically

found in the Dry Valleys and are restricted to more temperate locations of the Antarctic coasts such as the Mars Oasis (Vincent and Howard-Williams, 1986, Edwards *et al.*, 2004).

Cyanobacteria-dominated mat communities are found exclusively in melt-water streams, lakes and ponds in Antarctic desert environments and diversity appears to be strongly influenced by moisture inputs in the Dry Valleys. Mat communities are thought to represent some of the most complex bacterial consortia that survive in self-sustaining assemblages in extreme environments (Paerl *et al.*, 2000). Aggregation of multiple Cyanobacteria allows for the generation of a vast and complex microbial community. Stress resistance is also enhanced in these communities through the production of metabolites including exopolysaccharides (EPS) that are predicted to protect members of the community by conferring resistance to low temperatures, high salinity and excessive UV irradiation (Sutherland, 2001).

Mat communities typically exist as one of two seasonal forms, either as desiccated or wet 'inundated' mats (Paerl *et al.*, 2000). Desiccated mat communities are remnants of previously wetted/inundated mats that had proliferated in ephemeral streams generated by summer glacial ice-melt in the Dry Valleys. Microbial mats have been shown to recover rapidly from desiccation upon rewetting (Vincent and Howard-Williams, 1986), suggesting that viable dormant cells are maintained during periods of extreme xeric stress. Cyanobacterial emergence from dormancy has been shown to occur in desert biological soil crusts (BSC) (Rajeev *et al.*, 2013) as well as in desert soils (Garcia-Pichel and Pringault, 2001). Mat consortia appear to contribute directly to Cyanobacterial diversity in the Dry Valleys, as wind-blown cells have the potential to colonise both lithic and soil substrates upon aeolian deposition (Cary *et al.*, 2010, Hopkins *et al.*, 2009, Bottos *et al.*, 2014).

Differences in stream flow determines the microbial composition that is observed in the mat community. Water at the stream-edge flows much slower than water at the centre of the stream which is favourable for *Nostoc* colonisation, whereas fast flowing stream water is more amenable to *Oscillatoria* spp. or *Phormidium* spp. colonisation (Mcknight *et al.*, 1998). Cyanobacteria found in Lake Fryxell mats include *Phormidium, Oscillatoria, Leptolyngbya* and *Nostoc* species, which may represent cosmopolitan mat signatures (Taton *et al.*, 2003). The use of light microscopy has revealed that particular lineages, such as *Nodularia* and *Hydrocoryne* species, are detected exclusively using morphology-based studies but not in phylogenetic surveys (Taton *et al.*, 2003). This suggests that a polyphasic approach, i.e. utilising both morphological and molecular techniques, is required for the interrogation of Cyanobacterial communities (Taton *et al.*, 2003).

Soils are the most extensive colonisable desert habitat and bacterial community composition has been shown to be significantly different from lithobiontic niches (Pointing *et al.*, 2009,

Makhalanyane *et al.*, 2013a) and mat communities (Wood *et al.*, 2008). Pointing *et al.* (2009) found no Cyanobacterial phylotypes in desiccated McKelvey Valley soils, whereas Acidobacteria and Actinobacteria were detected at high abundances. Similar results found by Makhalanyane *et al.* (2013b) indicate that Actinobacteria and Cyanobacteria represent keystone taxa in soil and hypolith communities, respectively. Smith *et al.* (2006) analysed three unique Dry Valley mineral soils and detected Cyanobacteria in a sample collected from high altitude soil, while Actinobacteria were ubiquitous across all samples. Conversely, Wood *et al.* (2008) found a high diversity of Cyanobacteria in maritime Miers Valley soils (between four and 27 species depending on sample), whereas fewer signals were found in the high-altitude inland Beacon Valley. The higher diversity of Cyanobacteria in Miers Valley is possibly due to the wind-driven dispersal of the phylum present in lake mats, and colonisation of other niches including soils and hypoliths (Wood *et al.*, 2008). Actinobacteria may be dominant members of Dry Valley communities on the basis of 16S rRNA gene sequence data (ca 20.5% of sequences are inferred to the phylum), while Acidobacteria (ca 15.5%) and *Gemmatimonas* (ca 12.5%) may constitute major components of Dry Valley communities (Cary *et al.*, 2010, Cowan *et al.*, 2014). By contrast members of the α-Proteobacteria (ca 39%) may be dominant at locations along the Antarctic Peninsula, including Mars Oasis and Fossil Bluff, while Firmicutes are found here but not in the Dry Valleys (Cary *et al.*, 2010).

The Dry Valley aerosphere was shown to be distinct in bacterial composition from soil communities and has been proposed as a novel niche in this environment (Bottos *et al.*, 2014). The microbial communities housed in this putative niche are thought to be strongly influenced by stochastic (random) wind dispersal events which may introduce diversity to edaphic communities (Chan *et al.*, 2012). Much of the bacterial diversity present in soils and mobile structures, such as desiccated mats, contribute to aerosphere populations in the Dry Valleys (Bottos *et al.*, 2014).

Dry Valley soil bacterial communities are greater in diversity at the wetted sub-surface zone that exists below the soil surface and above the permafrost layer - typically 5 cm to 30 cm below the soil surface (Cary *et al.*, 2010). Permafrost layers limit micro-organisms from deep sub-surface soil colonisation. Physical stability of the overlying soil plays a role in moisture movement along desiccation gradients. Hypoliths have also been suggested to improve moisture content of underlying soils (Warren-Rhodes *et al.*, 2006). Mummified seal carcasses found in the Dry Valleys may act as physical stabilisers of surface soils and also reduce wind disturbance locally (Tiao *et al.*, 2012). This permits moisture released from the permafrost layer to condense and accumulate in the underlying soils beneath the carcass (Tiao *et al.*, 2012). Highly diverse microbial communities are often found in the moist gravels that underlie dry surface soils, where colonisation conditions are improved, particularly with regard to reductions in temperature fluctuations, wind abrasion and increases in moisture content.

Few studies have comprehensively assessed the microbial diversity of Antarctic desert niches. Dry Valley studies have typically focused on describing particular niches in depth, such as the bacterial communities present in surface soils (Smith *et al.*, 2006) and hypolith communities (Cowan *et al.*, 2010). Research into the abiotic factors that may govern changes in bacterial community composition and microbial community structures requires the assessment of all Dry Valley niches.

1.2 Dry Valley Cyanobacterial Diversity

The Cyanobacterial diversity of Dry Valley habitats has been well documented (Table 1). The Cyanobacteria phylum is ubiquitous to all Antarctic desert habitats including soils, hypoliths, crypto- and chasmoendoliths and mat communities. Members of this phylum are thought to be critical functional components of both hot and cold desert ecosystems (Chan *et al.*, 2013). Understanding the ecology of these bacteria, and the factors that influence patterns of dispersal, remains a key focus in desert microbial studies. Recent work has found extensive Cyanobacterial diversity across a range of habitats in the Dry Valleys (Smith *et al.*, 2006, Wood *et al.*, 2008, Pointing *et al.*, 2009) (Table 1). Bottos *et al.* (2014) detected four Cyanobacterial phylotypes in aerosphere samples which suggests limited dispersal of the phylum across the Dry Valleys via airborne transport.

Table 1. Cyanobacterial diversity from five distinct Dry Valley niches. Compiled using data from (Friedmann *et al.*, 1988, De La Torre *et al.*, 2003, Taton *et al.*, 2003, Cowan and Ah Tow, 2004, Jungblut *et al.*, 2005, Adams *et al.*, 2006, Smith *et al.*, 2006, Wood *et al.*, 2008, Pointing *et al.*, 2009, Cary *et al.*, 2010, Wong *et al.*, 2010, Bahl *et al.*, 2011, Cowan *et al.*, 2011a, Khan *et al.*, 2011, Chan *et al.*, 2012, Wierzchos *et al.*, 2013).

	Niche				
Cyanobacterial taxon	**Hypolith**	**Chasmoendolith**	**Cryptoendolith**	**Soil**	**Mat**
Acaryochloris spp.		*			*
Anabaena spp.				*	*
Blastomonas spp.			*		
Chroococcidiopsis spp.	*	*	*	*	*
Cylindrospermum spp.				*	
Gloeocapsa spp.		*	*		
Hormathonema spp.			*		
Leptolyngbya frigida	*	*	*	*	*
Lyngbya spp.				*	*
Methyloshaera hansonii					*
Microcoleus vaginatus				*	
Nodularia spp.					*
Nostoc spp.	*	*	*	*	*
Oscillatoria spp.	*			*	*
Phormidium spp.			*	*	*
Plectonema spp.			*		
Schizathrix spp.					*
Synechococcus spp.		*		*	
Trichormus azollae				*	

The diversity of Cyanobacteria found in the Dry Valleys is vast and lineages may be exclusive to particular habitats. Hypoliths appear to be colonised by a limited number of Cyanobacterial species including cosmopolitan *Chroococcidiopsis*, *Leptolyngbya frigida* and *Nostoc* spp. (Bahl *et al.*, 2011). Chan *et al.* (2013) revealed that polar hypoliths are nearly exclusively dominated by Oscillatoriales. Oscillatorian members appear to be ubiquitous across all Dry Valley niches, except for *Oscillatoria* spp. which appears to be absent from endolithic communities entirely in the Dry Valleys. Soils have been shown to be diverse in Cyanobacterial signals, this is surprising as 'specialised' lithic communities are thought to be major sources of Cyanobacterial diversity in depauperate environments. Soil Cyanobacterial diversity has been characterised by unique species including *Cylindrospermum* spp., *Microcoleus vaginatus* and *Trichormus azollae*.

Endolithic Cyanobacterial diversity appears to be dependent on whether the community is a coloniser of sandstone or granite substrates. Chasmoendoliths have shown distinct Cyanobacterial community composition from cryptoendoliths, although *Gloeocapsa* spp. appear to be endolith-specific. Cryptoendoliths were characterised by three niche-specific Cyanobacteria; *Blastomonas* spp., *Hormathonema* spp. and *Plectonema* spp. (Cowan and Ah Tow, 2004, De La Torre *et al.*, 2003). Chasmoendoliths may possess Cyanobacteria absent from cryptoendoliths (*Acaryochloris* and *Synechococcus* spp.) that have also been detected in mat and soil communities respectively (Yung *et al.*, 2014).

Dry Valley mat communities have shown vast Cyanobacterial diversity (Taton *et al.*, 2003, Cowan and Ah Tow, 2004, Jungblut *et al.*, 2005, Adams *et al.*, 2006, Wood *et al.*, 2008). Cyanobacteria unique to mat communities include *Methyloshaera* spp. (Cowan and Ah Tow, 2004), *Nodularia* spp. (Jungblut *et al.*, 2005) and *Schizathrix* spp. (Taton *et al.*, 2003). Desiccated mats may undergo dispersal through aeolian redistribution (Wood *et al.*, 2008). *Anabaena* spp., *Lyngbya* spp., and *Oscillatoria* spp. also contribute to the community structures of mats, although these species have also been detected in soil samples.

Many of the Cyanobacterial taxa listed in Table 1 have also been described in Tibetan cold desert soils and hypoliths (Wong *et al.*, 2010) including *Chroococcidiopsis* spp., *L. frigida*, *Nostoc* spp., *Oscillatoria* spp. and *Phormidium* spp. By comparison, hot desert hypolith Cyanobacteria are almost exclusively limited to *Chroococcidiopsis* spp. This genus has been found in hypoliths from many hot deserts including the Namib (Makhalanyane *et al.*, 2013b), Mojave (Schlesinger *et al.*, 2003) and Atacama (Warren-Rhodes *et al.*, 2006) deserts.

1.3 Moisture Regimes

Deserts are characterised by low water bioavailability, either continually or seasonally (Pointing and Belnap, 2012). Water is critical for all biological activities and the limitation of this resource is among the harshest stressors for organisms to overcome. Understanding the mechanisms by which microbial communities have adapted to severe desiccation stress may shed light on the terrestrial limits of life on Earth.

Major sources of water exist in the McMurdo Dry Valleys, although high sublimation rates render precipitation in the form of snow negligible. Precipitation levels are extremely low in the Dry Valleys and snow rapidly evaporates or sublimes before surface soils become wetted to more than 0.5 – 1 cm (Cowan, unpublished results). However, the melting of both buried permafrost and glacial ice supplies communities with high quantities of water during the austral summer. The melting of subsurface ice is predicted to supply most microbial communities with the majority of their moisture budgets (Cowan and Ah Tow, 2004). Increased ice melt, as a result of greater levels of solar radiation and local temperatures over the Antarctic continent (Cowan and Ah Tow, 2004, Pointing *et al.*, 2009), is predicted to lead to the wetting of previously hyperarid soils (Fountain *et al.*, 2014), the effects of which remains unknown. Microbial community dynamics are expected to change due to increased moisture content as Cyanobacterial proliferation may occur, which may lead to a reduction in local diversity as less-abundant taxa are out-competed. The mobilization of salts from deep-lying soils may provide previously unattainable nutrients to microbial communities which is likely to directly alter carbon inputs in these communities.

The effect of increased solar radiation is expected to affect the melting of glacial and buried ice in the McMurdo Dry Valleys (Cowan *et al.*, 2014). During the austral summer increases in radiation and temperature cause melting of ice from glaciers which generates ephemeral melt-water streams (Niederberger *et al.*, 2012), although these streams are compromised by hypersalinity. These events lead to the increase of, in particular, inundated mat communities (Cowan and Ah Tow, 2004). The effect of melt streams as water sources in the Dry Valleys is restricted to the local soil and lithic microbial communities within the stream because of the highly limited spatial influence (Esposito *et al.*, 2006).

Multiple factors have been suggested to have deterministic roles in shaping soil bacterial composition in the Dry Valleys including soil pH (Wynn-Williams, 1990) and soil chemistry (Lee *et al.*, 2012a). Additionally, the evaporative processes in ephemeral Dry Valley streams have been linked to differences in bacterial diversity patterns (Zeglin *et al.*, 2010). The bacterial diversity of wetted Dry Valley soil communities appears to be strongly influenced by the salinity of the soil substrate but not by sediment moisture content alone (Zeglin *et al.*, 2010).

It has been shown that certain Cyanobacteria, such as *Oscillatoria*, are capable of actively tracking moisture within arid soils (Garcia-Pichel and Pringault, 2001). By measuring surface spectral reflectance, Cyanobacterial migration was tracked across a soil matrix after inducing wetting and drying periods (Garcia-Pichel and Pringault, 2001). Greening of the surface was noted soon after inundation as previously dormant Cyanobacteria relocated to the moisture-rich surface using hydrotaxis (Pringault and Garcia-Pichel, 2004). The hydrotactic response of the Cyanobacterial species *Microcoleus vaginatus* was illustrated in biological soil crusts and the mechanisms underlying this adaption were suggested (Rajeev *et al.*, 2013). The activation of genes implicated in DNA repair and regulation was revealed to occur rapidly after wetting, while photosynthesis was recovered within 1 hour of the hydration event. These results suggest that behavioural adaptation is critical in moisture exploitation, and survival, within desert soils via xeric stress mitigation (Pringault and Garcia-Pichel, 2004). Tactic responses to both light and water stimuli in the events of dehydration and rehydration show that Cyanobacteria are capable of actively migrating through soils and are predicted to respond rapidly to sporadic moisture inputs in desert systems.

Antarctic soil microbial communities have the capacity to respond to changes in environmental conditions (Van Horn *et al.*, 2013), although responses to environmental perturbations are generally not uniform for all species (Drakare and Liess, 2010). The composition of microbial communities is rapidly altered by increasing local moisture availability and organic resources (Van Horn *et al.*, 2013). The implication of the study was that the metabolic activity of these systems is likely to shift dramatically as both carbon and nitrogen become available in the event of increased moisture (Van Horn *et al.*, 2013). An assessment of changes to bacterial composition of multiple Dry Valley niches from unique moisture regimes is predicted to provide information about the sensitivity of these systems to environmental change, and to what extent increased moisture content would influence general bacterial and Cyanobacterial community composition between habitats. It is hypothesised that events leading to an increase in moisture content will directly alter bacterial community composition by favouring generalist colonisers, microbes that are found across a range of habitats, and positively influence Cyanobacterial population size as proliferation occurs across habitats (Wood *et al.*, 2008). Although these responses have not yet been empirically tested, it is predicted that more extensive Cyanobacterial populations will lead to a dramatic increase in carbon turn-over and nitrogen cycling in the Dry Valleys (Rajeev *et al.*, 2013, Chan *et al.*, 2013). Additionally, increased Cyanobacterial abundance may lead to the dominance of cyanotoxin-producing species in local communities, as has been suggested for freshwater polar ecosystems (Kleinteich *et al.*, 2012). Elevated cyanotoxin concentrations, and the emergence of Cyanobacteria, are predicted to lead to the extinction of rare taxa within these communities which may greatly alter patterns of diversity across this delicate environment (Kleinteich *et al.*, 2014).

1.4 Assessments of Microbial Diversity

It is estimated that between 0.1% and 1% of micro-organisms within a sample are recovered by 'traditional' culturing approaches - the consequence being that the majority of natural microbial diversity has not yet been cultivated (Amann *et al*., 1995). Traditional approaches aiming to describe microbial diversity with culture-independent methods typically use metagenomic DNA-based data for microbial community assessments. The so-called 'metagenome' (Rondon *et al*., 1999) of a sample is obtained by isolating DNA directly from a complex microbial sample and performing downstream analyses on the entire genetic component. The use of conserved structural genes, such as bacterial 16S small sub-unit rRNA (ribosomal) genes, allows for the identification and characterisation of the species within a sample independent of culturing. Metagenomic approaches are essential in revealing the microbial diversity and community structures of environmental samples. Assessments of microbial diversity are achieved by analysing specific gene regions that are universal to the microbial guild of interest but possess high levels of inter- and intra-species variability in order to resolve identities sufficiently. These include the universal bacterial 16S rRNA gene region (Liu *et al*., 1997) and the nuclear ribosomal internal transcribed spacer region which has been used for community fingerprinting of fungi (Schoch *et al*., 2012).

The bacterial 16S rRNA gene comprises nine hyper-variable gene regions, V1 – V9, which show very high levels of sequence variability between lineages (Van De Peer *et al*., 1996). These gene regions are flanked by highly conserved domains that have been targets for oligonucleotide universal primers (Baker *et al.*, 2003), making the gene region a useful marker in bacterial phylogenetic studies (Blackwood *et al*., 2003). The 16S rRNA ribosomal gene is of importance during transcription and is universal to all bacterial species (Van De Peer *et al*., 1996).

Metagenomic analyses of microbial communities can be performed using multiple approaches. Commonly implemented techniques include microbial fingerprinting, selecting phylogenetically informative genes for cloning and sequencing and using next generation sequencing technologies to provide sequence information regarding genetic markers in order to infer phylogenies.

The use of fingerprinting techniques in microbial ecology has greatly improved the accuracy and efficiency of microbial diversity assessments (Fierer and Jackson, 2006). Various fingerprinting technologies applied in microbial ecology are briefly discussed.

1.4.1 Microbial Fingerprinting

Molecular fingerprinting technologies such as terminal restriction fragment length polymorphism analysis (T-RFLP) (Liu *et al*., 1997), denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*., 1993), amplified rDNA restriction analysis (ARDRA) (Vaneechoutte *et al*., 1992), and

automated amplified ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999) have made use of amplicon or restriction fragment differences between species to characterise the overall microbial diversity of environmental samples. T-RFLP analysis was used exclusively in this project to describe microbial fingerprinting of soil, mat and lithic-based communities and is discussed in detail below. Critically, these techniques provide a 'snapshot' of overall community composition and require sequence data to provide information regarding diversity. However these techniques are robust, reproducible and are capable of distinguishing closely-related OTUs (Operational Taxonomic Units) from each other, providing an accurate microbial community fingerprint of each sample (Osborn *et al*., 2000).

T-RFLP analysis is a fingerprinting technique commonly used for the description of microbial communities. The T-RFLP technique differs from other fingerprinting approaches in that one or both primers used during PCR amplification are labelled with a fluorescent dye, such as 6-carboxyfluorescein (6-FAM). Labelled PCR amplicons are digested with restriction enzymes that target four base pair regions, generating unique terminal restriction fragments (T-RFs) for each OTU in the sample. Labelled T-RFs are then detected using capillary electrophoresis and interpreted as an electrophorogram indicating a profile of detected peaks for each sample. This reveals the overall abundance and diversity of all OTUs within each sample (Liu *et al*., 1997). Sequence polymorphisms within species create fragments of differing lengths which allows for discrete phylogenetic resolution to be made between populations. The size of each T-RF peak generated is representative of OTU abundance. Samples can then be characterised by the unique patterns to describe the microbial composition of the population. The T-RFLP approach provides greater sensitivity than other fingerprinting technologies, particularly for the distinction of similar species within complex microbial communities (Osborn *et al*., 2000, Blackwood *et al*., 2003).

The T-RFLP approach was selected for this project because of the high resolution and sensitivity of this technique. Additionally, T-RFLP analysis is sufficiently sensitive to assess unique phyla such as Cyanobacteria through the use of phylum-specific primers. For the assessment of T-RFLP data, the following programs are frequently used; GeneMapper 4.1 (available at SeqServe, University of Pretoria, (http://seqserve.bi.up.ac.za/) Strawberry Perl, the R package and Primer-E 6 software (PRIMER-E). These programs allow for the detection of labelled peaks and the application of appropriate filtering to each sample (GeneMapper 4.1). Multivariate statistical analyses of the bacterial diversity of each sample can be performed and interpreted using scripts (Perl and R). Finally, the comparison of overall microbial diversity between samples can be determined to assess differences in community patterns (Primer 6).

Despite improvements in the techniques designed for characterising microbial diversity, there remain limitations to the use of fingerprinting techniques exclusively. PCR bias, for example, has been well documented in members of Verrucomicrobia and Firmicutes (Blackwood *et al*., 2003), although this may be reduced by pooling multiple PCR reactions from the same sample. Variability in DNA isolations from soil samples is also common as cells may be more recalcitrant to physical or chemical lysis. The incorporation of multiple treatments during metagenomic DNA isolations facilitates the isolation of DNA from environmental soil samples (Miller *et al*., 1999). Pooling multiple DNA isolation products together is also performed to improve overall yield and reduce isolation bias (Blackwood *et al*., 2003).

The digestion patterns generated by restriction enzymes are unique - consideration must be given as to which enzyme best represents the microbial diversity of a community. The T-RFLP technique distinguishes OTUs by the unique terminal restriction fragments generated, although peaks of identical size are impossible to distinguish (Blackwood *et al*., 2007). For this reason multiple restriction enzymes may be utilised in order to best resolve the diversity of each community. The process of restriction enzyme selection can be facilitated by using online tools which allow *in silico* digestions of sequences to be performed online to assign putative T-RFs for the dataset. Online digestions can be performed in programs such as Restriction Endonuclease Picker (REPK) which allows users to determine the restriction enzymes with the greatest resolving accuracy for their sequences (http://rocaplab.ocean.washington.edu/tools/repk) (Collins and Rocap, 2007).

Additional issues regarding the use of fingerprinting techniques include the overestimation of highly abundant OTUs and the loss of rare OTUs from the dataset (Bent and Forney, 2008). These issues may be reduced by using multiple phylogenetic markers, which may include functional genes such as gyrase B (*gyrB*) (Wang *et al.*, 2007a). The use of the *gyrB* gene sequences is a useful alternative to 16S rRNA genes as single phylogenetic markers, although rare biota will remain underappreciated if single OTUs are significantly dominant (Wang *et al.*, 2007a). Functional and protein-coding genes undergo a higher rate of molecular evolution than 16S rRNA genes (Yamamoto and Harayama, 1995), and may be superior phylogenetic markers, in terms of resolving power, for assessing taxonomic relationships at the species level (Wang *et al.*, 2007a). However, the 16S rRNA gene remains a fundamental marker to assess bacterial ecology as comparisons can be made to previous phylogenetic studies based on 16S rRNA diversity patterns.

T-RFLP analysis has been used extensively to assess Dry Valley microbial communities across a suite of distinct habitats (Pointing *et al*., 2009, Makhalanyane *et al*., 2013a). Application of this technique has revealed that hypolithic and soil communities are structurally different in the Namib Desert (Stomeo *et al*., 2013, Makhalanyane *et al*., 2013b).

Fingerprinting techniques are useful for revealing ecological patterns and to describe microbial community structure and changes in microbial diversity on spatial and temporal scales. However, accurate sequence data are required to assess microbial communities in terms of phylogenetic diversity, in terms of species richness, identity and evenness; particularly with regard to less abundant species that are under-represented in fingerprinting approaches (Bent and Forney, 2008). The use of both microbial fingerprinting and pyrosequencing have revealed coherent patterns of diversity (Gobet *et al.*, 2013, Makhalanyane *et al.*, 2013a).

1.4.2 Next-Generation Sequencing (NGS) Technology

The use of next-generation sequencing (NGS) technologies such as 454-pyrosequencing is necessary for high resolution analysis of complex microbial communities (Petrosino *et al.*, 2009). While fingerprinting microbial communities is useful as a 'snapshot' of microbial diversity within a sample, the addition of sequencing data greatly improves the assessments of bacterial community composition (Mardis, 2008). NGS has progressed rapidly since the advent of Sanger sequencing (Sanger *et al*., 1977) and represents a rapid and cost-effective method for confident identification of microbial phyla within a community. The use of 16S rRNA genes as phylogenetic markers has been maintained in microbial ecology and has subsequently allowed for comparisons regarding bacterial diversity to be made between studies.

Available NGS technologies provide greatly improved sequencing throughputs which allow for large sequence datasets to be obtained. NGS is widely used for applications including genome re-sequencing and metagenomic community sequencing in microbial ecology (Mardis, 2008). The major sequencing platforms commercially available are HiSeq 2500 (Illumina, Inc.), GS-FLX+ Titanium 454 pyrosequencing (Roche Diagnostics, CT, USA), Ion Torrent semiconductor sequencing (Life Technologies) and the PacBio RS II (Pacific Biosciences®).

The selection of a sequencing platform is dependent on numerous factors, and is typically project dependant. These include the cost associated with high-throughput sequencing as well as number of reads required for assessments. The trade-off between read length and sequencing accuracy must be considered. This is usually project-specific and is influenced directly by the sequencing platform selected. Sequencing platforms are also unique in terms of the DNA amplification technique used - emulsion PCR used in GS-FLX+ Titanium pyrosequencing, Ion Torrent sequencing and Bridge/Cluster PCR used during Illumina sequencing. Sequencing yields and read lengths are variable across platforms and consideration must be given to the type of sequence data required prior to selecting an appropriate platform.

This project utilises the Roche GS-FLX+ platform (454 pyrosequencing) to provide sequence data of representative habitat samples. Microbial ecology studies have frequently relied on 454

pyrosequencing for phylogenetic studies of metagenomic data (Lee *et al.*, 2012b) as the platform provides greater read lengths than the Illumina platform (1 kb compared to a maximum of 300 bp).

The introduction of multiplex barcode sequences or nucleotide tags has enhanced metagenomic DNA sequencing (Dowd *et al.*, 2008). The incorporation of sample-specific barcodes in a multiplex PCR amplification allows for the sequencing of multiple samples to be performed simultaneously after pooling. The identities of the unique samples are maintained after assigned sequence tags are attached to PCR primers. Computational classification allows for sequence reads to be re-assigned to respective samples from which they were extracted. 454 multiplexing has been used to show that hypoliths might follow a successional development pathway from Type I (Cyanobacteria-dominated) to Type III (bryophyte-based) communities (Makhalanyane *et al*., 2013a).

1.5 Data Assessments in Microbial Ecology

Data analyses performed after the acquisition of fingerprinting or sequence data are critical to understand and interpret findings. Typical tests aim to describe within (α-diversity) and between (β-diversity) sample variation using multi- or univariate statistical models. Commonly employed tests aimed to describe α-diversity include the Shannon, Simpson and Chao1 diversity indices (Chao, 1984).

In terms of ecological theory, α- and β-diversity estimates are typically related to the overall abundance of OTUs within a sample and community differences between samples on the basis of OTU data, respectively. These data can be linked to observed patterns of microbial dispersal across habitats (spatial scale) and over time periods (temporal scale). Numerous processes influence diversity and typically include ecological drift, selection, dispersal and diversification, although spatial variability may account for β-diversity differences globally (Martiny *et al.*, 2011).

Differences in α-diversity are thought to reflect species abundance variability, which may be governed by deterministic factors, such as environmental or abiotic factors. Alternatively stochastic or random events may cause changes to local microbial abundance, which has been suggested to lead to higher productivity in communities driven more by stochastic than deterministic processes (Chase, 2010), although both factors are thought to interact and mediate microbial diversity on the global scale (Caruso *et al.*, 2011). The Dry Valleys have been shown to be low in bacterial and general microbial abundance, which is predicted to be a result of the extreme environmental parameters that govern this system on the regional scale (Cary *et al.*, 2010).

Through metagenomic approaches it is possible to compare entire microbial communities and gain an understanding of the factors that drive differences in community structure on the local, regional and global scales. Differences in diversity patterns can be linked to distinct environmental

parameters, such as habitat type or moisture regimes, or dispersal processes. Applying fingerprinting analysis and sequencing to metagenomic DNA data is commonly performed to reveal differences in community assembly patterns across environments and habitats. Metagenomic theory has been successfully used to distinguish between unique habitats in the Dry Valleys (Pointing *et al.*, 2009) and to reveal patterns of hypolith community assembly in hot and cold desert systems (Makhalanyane *et al.*, 2013a, Makhalanyane *et al.*, 2013b). Fingerprinting and sequencing approaches have been shown to reveal similar ecolgical patterns (Gobet *et al.*, 2013). These are well-suited to describe community structures and remain useful tools for assessing microbial ecology (Fierer and Jackson, 2006).

Fingerprinting data are typically interpreted in diversity matrices. Matrices simplify OTU data into rows and columns so that the community variation between samples can be inferred by comparing each OTU with the relative frequency it constitutes to each sample. Due to the fact that multiple factors may be included in ecological studies, including abiotic variables and OTU numbers, these are termed multivariate analyses as multiple outcome variables may be analysed.

A matrix of T-RFLP information can be used to generate a resemblance matrix using, for example, Bray-Curtis similarities (Bray and Curtis, 1957) or Euclidean distance. These resemblance data are often visualised as non-metric multidimensional scaling (nMDS) ordination plots (Figure 3) in order to observe differences in community structure between samples, or by Principle Component Analyses (PCA) to understand the abiotic factors that drive differences in microbial diversity.

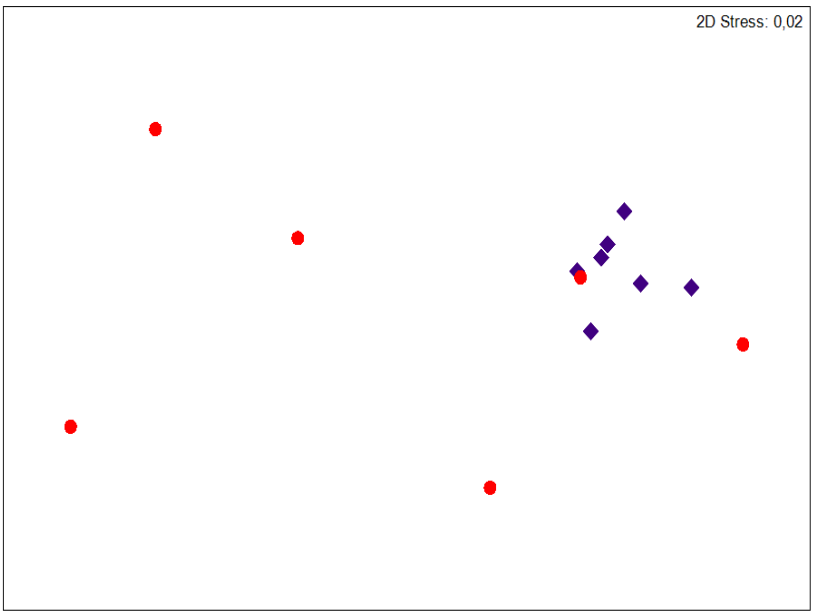


Figure 3. An example of a 2-Dimensional non-metric multidimensional scaling (nMDS) ordination plot. Differences in community structure can be easily viewed in these plots. Each point represents a complex microbial community and the distance between points directly correlates with community similarity. The stress value (0.02 on this plot) is indicative of plot accuracy. ♦ – Community A, ● – Community B.

nMDS plots are effective means of viewing the relationships between complex communities and are widely used to visualise differences in populations from distinct habitats or treatments assigned *a priori*. The nMDS plots are generated after dissimilarity matrices are employed (e.g. Bray-Curtis or Euclidean) in which ranks are assigned to the OTU data that describes the distances between all community members. Each community is then plotted in a non-linear fashion after multiple iterations of the ordination utilising the assigned rank data (Clarke, 1993, Ramette, 2007). In the ordination, each complex microbial community is plotted as a single point and the rank dissimilarity between two points is indicated by the distance between those points. The generated ordination is interpreted in terms of sample proximity reflecting relative similarity. Closer communities are less dissimilar than communities that are plotted further apart in the ordination. For this reason it is important to assess the quality of the ordination, i.e. the goodness of fit, prior to making inferences from the observed data. The stress value assigned to the ordination is indicative of its accuracy. Ordinations with stress values below 0.2 are considered to be accurate representations of the data with low chance of misinterpretation (Clarke, 1993). Assigned stress values below 0.1 correspond to the ideal representation of the ordination (Clarke, 1993) (Figure 3).

In addition to visual means of interpreting nMDS ordinations, it is possible to include multivariate statistical analyses to quantify observed differences in communities after assigning the communities to groups *a priori*. The Analysis of Similarities (ANOSIM) represents a method of

analysis in which β-diversity group variation is compared by testing the null hypothesis that groups are not significantly different (Anderson, 2001). ANOSIM is calculated to provide the significance of community clustering where $P < 0.05$ indicates a significant difference between the communities present in each group - the communities differ more significantly in composition than is expected by chance alone. ANOSIM also generates an *R* value that indicates how distinct communities cluster apart using the rank data (Clarke, 1993). The *R* test value reveals the community separation, and a common value used to describe sufficient separation of communities is $R > 0.4$. Values below this threshold suggest that the communities show a high level of overlap (similarity) and are not completely separated.

Incorporation of additional statistical tests is important to reveal trends in community diversity that may not be accurately evaluated using ANOSIM. Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2005), for example, has been used to reveal significant differences in bacterial community structures across multiple desert environments (Stomeo *et al.*, 2013, Makhalanyane *et al.*, 2013b). The PERMANOVA test is typically performed using the *adonis* function in R and provides greater statistical robustness than ANOSIM, and accounts for unequal sample numbers in the event that assigned groups have unique community members (Anderson and Walsh, 2013).

While α-diversity estimates are used to describe within-community variation through OTU enumeration and species evenness, β-diversity compares the observed diversity between samples by analysing differences in OTU abundance and diversity. β-diversity analyses are useful to assess spatial and temporal differences that exist between environmental samples or time points respectively. Venn diagrams and heat maps are commonly used to visualise differences between samples, typically as qualitative data (presence/absence), although numerous other tests rely on quantitative data.

Venn diagrams are useful for the description of shared and unique OTUs or species between samples. Typically, each community is represented by an oval and overlapping regions between multiple ovals show similarities between groups. The value within each unique oval represents the number of sample-specific OTUs or species. The values in overlapping regions denote the number of shared OTUs or species between those samples. The central area of overlap (of all samples) represents the group of fully shared taxa, cosmopolitan OTUs. Community relatedness can be inferred from Venn diagrams by comparing shared abundances across distinct samples.

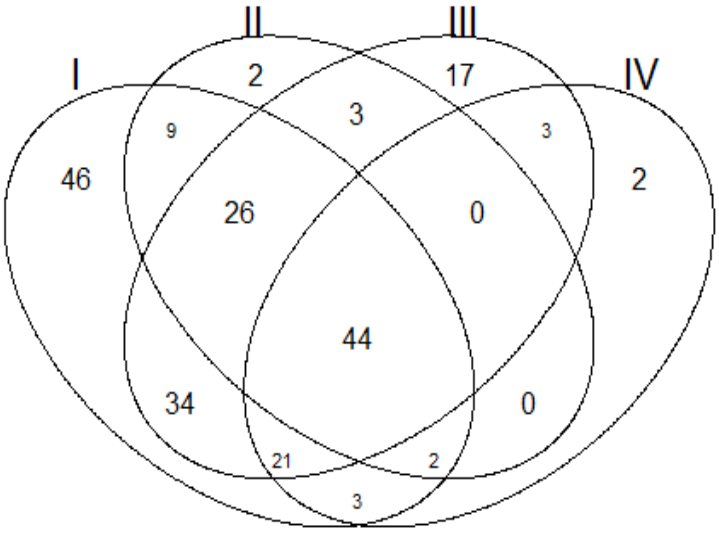


Figure 4. Venn diagram with four ovals. Numbers within each area represent OTU numbers. These diagrams are useful to illustrate the number of similar OTUs between groups as well as unique OTUs that exist within single samples only. I – Community A, II – Community B, III – Community C, IV – Community D.

Assessments of 454 Pyrosequencing data require numerous filtering steps to be implemented prior to inferences of sequence data so that only high-quality reads are assessed. Programs such as MOTHUR (Schloss *et al.*, 2009) and Quantitative Insights into Microbial Ecology (QIIME) (Caporaso *et al.*, 2010) combine various algorithms for the ordering and filtering of complex pyrosequencing data from raw sequence reads. The MOTHUR pipeline is available online (http://www.mothur.org/wiki/454_SOP) which guides users through the numerous filtering steps as well as making community assessments from the normalised sequence data. These programs are highly flexible, allowing the user to set parameters throughout the protocol to best interpret data. Common methodologies involve removal of poor quality reads, long-length homopolymers, chimeric sequences, contaminants and typically ends with alignment to an applicable reference database such as SILVA or Greengenes. Taxonomic affiliations can then be obtained after which it becomes possible to perform multivariate statistical analyses to describe the OTU diversity.

The MOTHUR pipeline utilised in this study has the additional benefit of performing various functions to describe the filtered sequence data. Commands allow for assessments of both α- and β-diversity and may provide information regarding each sample so that accurate community comparisons can be made.

Phylogenetic trees are generated to visualise assignments of sequence data through branches and nodes so that evolutionary distance and the clustering of groups can be interpreted. Branches indicate a specific genetic lineage and the length indicates the number nucleotide changes that have occurred since divergence (Saitou and Nei, 1987). Nodes are points at which two or more

distinct lineages are separated in evolutionary time based on sequence data. Every new lineage included in the tree is represented by a new node during reconstruction. The branch accuracy during tree reconstruction is dependent on multiple factors including the quality of the sequence data, the tree alignment program employed and long-branch attraction. Bootstrap values can be assigned to reflect the confidence of each node in the tree as the percentage of times the node is detected in a number of random tree iterations.

1.6 Research Aims and Objectives

Deserts represent key terrestrial biomes. Understanding the factors that influence local microbial ecology remains a major research focus in this field; particularly as environmental climate change may lead to major local and regional perturbations in liquid water availability. The unique communities that colonise Antarctic Dry Valley niches are thought to be crucial contributors to local carbon and nitrogen provenance in these highly oligotrophic soils in the absence of higher photoautotrophs. Critical to the success of microbial consortia are photoautotrophic Cyanobacterial populations which were interrogated in this study. Although recent phylogenetic work has revealed the endemic bacterial diversity of this desert system (Makhalanyane *et al.*, 2013a, Cowan *et al.*, 2014), issues relating to community assembly and the factors that govern local diversity patterns have not yet been fully elucidated for this hyperarid system. As moisture content is predicted to be greatly increased in this environment due to climate change, addressing the effect of moisture regime on community structure, and particularly Cyanobacterial population structure, is the critical focus in this thesis. The aim of this project is to elucidate bacterial and Cyanobacterial diversity patterns across various habitats under distinct moisture regimes across the Dry Valleys under the hypothesis that moisture content would lead to increases in bacterial biomass and particularly cyanobacterial abundance.

Research objectives:

1. To assess bacterial and Cyanobacterial diversity patterns across multiple Dry Valley niches and unique moisture regimes at the microscale using microbial fingerprinting.
2. To investigate distinct bacterial niche compositions using phylogenetic pyrosequencing to gain insights into the effect of moisture regime on local diversity patterns.

Chapter 2: Materials and Methods

2.1 Materials

2.1.1 General procedures

The chemicals and reagents used in this study were of analytical grade and obtained from various suppliers. All glassware, media, Eppendorf tubes and buffers used were sterilised by autoclaving at 121°C for 15 minutes prior to use.

2.1.2 Enzymes

The enzymes used in PCR amplifications, restriction enzyme digestions and for the removal of RNA from metagenomic DNA samples are listed in Table 2.

Table 2. Enzymes used in this study.

Enzyme Name	Enzyme Function	Supplier
DreamTaq™ DNA Polymerase	PCR amplification	Fermentas
*Hae*III (*Bsu*RI)	Restriction digestion	Fermentas
*Msp*I (*Hpa*II)	Restriction digestion	Fermentas
FastDigest *Msp*I	Restriction digestion	Fermentas
RNase A	RNA digestion	Thermo Scientific

2.2 Field Sampling

All samples were collected from the inland Victoria Valley in the McMurdo Dry Valleys (77°20' S, 161°39' E) of Eastern Antarctica during the austral summer of 2013 by Prof. D. A. Cowan (Figure 5). Sites comprised a low elevation glacial melt-water stream running through a gravel stream bed between elevated moranic soils. Edaphic samples were collected from sites defined by two distinct moisture regimes, either exposed to stream water; high moisture content, or dry gravel adjacent to the stream sites; low moisture content (Figure 5). The collection of samples from two distinct moisture regimes served as a proxy for evaluating differences in microbial community structure on the basis of local water availability. Samples collected were representative of four distinct edaphic desert habitats (*n*=33); surface soils (*n*=5), Type I hypoliths (*n*=14), cryptoendoliths (*n*=10) and Cyanobacterial mat communities (*n*=4). Lithic and mat samples were retrieved via aseptic transfer of sample material into sterile Whirl-Pak bags (Nasco International, Fort Atkinson, WI, USA). Soil samples were transferred under sterile conditions to 50 ml conical Falcon tubes (SPL Life Sciences, Gyeonggi-do, South Korea). Samples were stored at -20°C on dry-ice in transit to the

laboratory, University of Pretoria (UP), South Africa. Upon arrival, samples were weighed and aliquots of 2 g were recovered. The remaining sample material was stored at -80°C until required. Sample properties are listed in Appendix A, Table S1.

Figure 5. Photograph of the sampling site in the Victoria Valley. Hypoliths and mat samples were retrieved from wetted sites within or adjacent to the stream. Soils (moraine) and endoliths were collected from dry sites up to 25 m from the stream bed. Photograph courtesy of Prof. Don Cowan.

2.3 DNA Extraction, Purification and Amplification

2.3.1 DNA Extraction

DNA extractions were performed using a modified 50-50-50 buffer-chloroform/phenol protocol (Miller *et al.*, 1999) which included physical bead-beating to lyse bacterial cells. Briefly, 1 ml extraction buffer at pH 8 (50 mM NaCl, 50 mM Tris-HCl at pH 7.6, 50 mM EDTA at pH 8.0 and 5% SDS) (Appendix B) was added to 1 g of sample material in a 2 ml microcentrifuge tube containing 0.4 ml of silica beads. 1 µl of 1 M of dithiothrietol (DTT) was added to the solution and vortexed or bead-beaten for 30 seconds. Centrifugation was then performed at 14, 000 x *g* for 3 min in an Eppendorf Centrifuge 5454 R. The supernatant was recovered and transferred to a new sterile 2 ml Eppendorf tube. Purification was performed by adding half volumes of both phenol (pH 8.0) and chloroform/isoamyl alcohol [24:1] to the supernatant. The solution was vortexed at maximum

speed for 5 s and centrifuged as before. The aqueous phase was transferred to a new sterile 2 ml Eppendorf tube to which an equal volume of chloroform was added. The solution was centrifuged as before and the aqueous phase transferred to a sterile 1.5 ml Eppendorf tube. To the recovered solution, 0.7 volumes isopropanol and 0.1 volumes 3M Sodium Acetate (pH 5.2) were added, and the solution was mixed by inverting the tube which precipitated the DNA. Precipitated DNA was pelleted by 14, 000 x *g* centrifugation at 10°C for 30 min, after which the supernatant was removed. The remaining pellet was washed with ice-cold 70% ethanol (EtOH) and re-pelleted by centrifugation at 14, 000 x *g* for 5 min at 10°C. Remaining EtOH was evaporated at 50°C in a heating block, drying the pellet. Pelleted DNA was resuspended in a 50 µl volume of autoclaved filter-sterilised Millipore water and stored at -20°C until processing. The presence of metagenomic DNA in each sample was confirmed by gel electrophoresis which were resolved as 1% agarose gels. Multiple DNA samples were pooled to reduce extraction bias and to increase overall DNA concentration. This was performed by eluting multiple (up to four) samples in a single 50 µl volume. Polyvinylpolypyrrolidone (PVPP) columns were used to purify low quality DNA samples by removing co-extracted compounds. Samples may have possessed high levels of contaminants such as humic acid or EPS compounds prior to purification.

PVPP columns were constructed by combining filter tips (end cut off) with PVPP solution in a 0.6 ml Eppendorf tube (end and lid cut off) to create a filter column. The column was placed in a sterile 1.5 ml Eppendorf tube and centrifuged at 11 000 x *g* for 30 seconds. The DNA sample was loaded to the dried column and filtered through the PVPP and filter tip by centrifugation into a sterile 1.5 ml Eppendorf tube.

An additional step of improving metagenomic DNA quality included the removal of RNA from samples with RNase A (Thermo Scientific, USA). RNase A (1 µl) [10mg/ml] was added to the DNA sample after completing the DNA isolation protocol and was incubated at 37°C for 1 hour. The absence of RNA from the sample was confirmed by gel electrophoresis.

2.3.2 PCR Amplifications

DNA amplification of 16S rRNA genes was achieved using two primer sets; 341F-908R (Lane *et al.*, 1985, Liu *et al.*, 1997), universal bacterial primers, and 359F-781R (Nübel *et al.*, 1997) for Cyanobacteria specifically (Table 3). These primers were designed to target the hypervariable V3 – V5 sections of the 16S rRNA gene region in bacteria which has been frequently used for bacterial resolution of communities (Van De Peer *et al.*, 1996). All forward primers were 6-FAM labelled, which was handled in low light conditions and stored in complete darkness. PCR amplification was performed using the Bio-RAD Thermal Cycler T100 (Bio-RAD, CA, USA) in 50 µl reaction volumes. Each PCR contained 1X Dream Taq Buffer (Fermentas, USA) containing 20 mM $MgCl_2$, 2 mM

dNTPs, 5 µM of each primer, 0.4 mM bovine serum albumin (BSA), 2.5 units Dream Taq polymerase (Fermentas, USA) and 2 ng template DNA.

Thermal cycling conditions for the 341F-908R primer set were as follows; denaturation at 95°C for 5 minutes; 30 cycles of the following three steps; 95°C denaturation for 30 s; 55°C annealing for 30 s and 72°C elongation for 90 s, with a final elongation step at 72°C for 10 min. Triplicate 50 µl PCR reaction volumes were pooled and purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Hoerdt, France) according to the manufacturer's instructions. Both negative and positive controls were routinely included in the PCR set-up and were treated as previously described. Autoclaved filter-sterilised deionised Millipore water and *E. coli* DNA were included as negative and positive controls, respectively, instead of metagenomic DNA templates. These controls were included to validate the sensitivity of PCR amplification. PCR reactions were prepared on ice and loaded in sterile PCR tubes.

A nested PCR approach was used to amplify Cyanobacterial DNA. Unlabelled PCR products generated from 341F-908R amplification were used as template DNA (1µl) for the 359F-781R primer set reactions. PCR amplification conditions for the 359F-781R primer set were as follows; denaturation at 95°C for 5 min; 30 cycles of the following three steps; 95°C denaturation for 30 s; 64°C annealing for 30 s and 72°C elongation for 90 s; and a final elongation step at 72°C for 10 min (Keyster, 2007). Each sample required a unique dilution of the PCR template; between 1/25 and 1/125 for Cyanobacteria-specific amplification to be observed. Triplicate PCR reactions were performed for each sample and products were pooled and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Hoerdt, France). Labelled PCR products were maintained in complete darkness until required to prevent excitation of the incorporated FAM isomer.

Table 3. Primers used in this project.

Primer name	Sequence (5' – 3')	Reference	Application
27F	CGGACGGGTGAGTAACGCGTGA	Lane 1991	454-pyrosequencing
341F	CCTACGGGAGGCAGCAG	Liu *et al.* 1997	T-RFLP (bacterial)
359F	GGGGAATYTTCCGCAATGGG	Nübel *et al.* 1997	T-RFLP (cyanobacterial)
519R	GTNTTACNGCGGCKGCTG	Lane *et al.* 1985	454-pyrosequencing
781R	GAGTACTGGGGTATCTAATCCCAT	Nübel *et al.* 1997	T-RFLP (cyanobacterial)
908R	CCGTCAATTCMTTRAGTTT	Lane *et al.* 1985	T-RFLP (bacterial)

2.4 General Laboratory Techniques

2.4.1 Agarose Gel Preparation and Electrophoresis

Agarose gels were prepared using SeaKem® LE Agarose powder and were constructed as one percent [w/v] agarose gels using 1 x TAE buffer solution (0.2% [w/v] Tris, 0.5% [v/v] acetic acid, 1% [v/v] 5 M EDTA [pH 8]) unless otherwise stated.

The resolution of DNA was performed by gel electrophoresis. PCR samples were loaded in a 1:3 ratio of GelRed loading dye (Anatech, RSA) to product and in a 1:1 ratio of GelRed loading dye to DNA sample or pooled purified PCR product. Electrophoresis was performed at 90V for between 45 min to 1.5 hours. Fragments were sized according to the size standard marker used (Gene Ruler™ 1kb Ladder, Thermo Scientific, RSA) under UV illumination. UV illumination of agarose gels was achieved using a digital imaging system (Molecular Imager® GelDoc™ XR+, Bio-RAD, RSA) and Image Lab™ Software.

2.4.2 DNA Quantification

Quantification of metagenomic DNA was performed using the NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Concentrations were measured against the elution solution used. This was performed with the addition of 1 µl filter-sterilised autoclaved Millipore water which was used for DNA elution, or 1 µl of NE buffer (Tris-HCl; pH 8) as used in the elution of purified DNA or PCR products to the instrument as a blank measurement.

2.5 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

2.5.1 Restriction enzyme digestion and ABI capillary electrophoresis

Restriction enzyme digestion of 6-FAM labelled purified PCR products was performed using *Hae*III (*Bsu*RI) (Fermentas, USA) and *Msp*I (*Hpa*II) (Fermentas, USA), independently. Amplicons were normalised to 200 ng/µl prior to restriction digestions. Reactions were set up in 20 µl volumes; 2 µl 200 ng PCR product, 2 µl R Buffer/Tango Buffer, 1.0 µl *Hae*III/*Msp*I enzyme, 15 µl ddH_2O. Samples were digested overnight at 37°C. Digested products were purified using the NucleoSpin® Gel and PCR Clean-up Kit and eluted in 15 µl sterile water. The quality of digestions was confirmed using agarose gel electrophoresis.

Preparation for ABI capillary electrophoresis involved adding 4 µl purified digest products to 0.25 µl of GeneScan™ 600 LIZ® Size Standard v2.0 (Applied Biosystems®, USA), for fragment length acquisition, and 6.75 µl Hi-Di™ Formamide (Applied Biosystems®, USA). Reactions were performed for 24 samples per run. The reactions were set-up in Axygen Scientific (CAL, USA) 96 well PCR® Microplates and covered with a Bio-RAD PCR sealer™ Microseal® 'B' film; plates were

incubated at 95°C for 5 minutes for denaturation. Plates were placed immediately on ice after incubation and spun down using an Eppendorf Centrifuge 5810 R and stored on ice. Plates were submitted for GeneScan® analysis at SeqServe, UP. Fluorescently labelled fragments were sized by capillary electrophoresis on the ABI3500xl genetic analyser (Applied Biosystems®, CA, USA) after co-injection with GeneScan™ 600 LIZ® Size Standard v2.0.

2.5.2 T-RFLP Data Analyses

The terminal restriction fragment (T-RF) profile data generated from the ABI3500xl genetic analyser were examined using GeneMapper® v4.1 software (Applied Biosystems®, CA, USA). Individual samples were analysed as electrophorograms and background noise was filtered at 5 rfu for all samples. Similar fragments were binned using a 2 bp window at incremental 0.1 bp size shifts to retrieve 'true' peaks. Raw data regarding peak heights and the number of peaks per sample were exported. This data was analysed using Strawberry Perl and R (v 3.1.0) software packages.

The separation of background noise from true peaks and binning was performed as described by Abdo *et al.* (2006) using the R statically environment (http://www.r-project.org/) and the Strawberry Perl programming language (www.perl.org). Unique T-RFs were classified as OTUs and these terms are used interchangeably in this manuscript. The assessment of T-RFLP data was performed in R using the packages *permute*, *lattice*, *gplots* and *vegan* (Oksanen *et al.*, 2013). Interpretation of the distance matrix was performed in Primer 6. Peaks falling within the noise baseline of 3 standard deviations were removed from the dataset. Retained T-RFs were used to generate a distance matrix that compares T-RF relative abundance for each unique peak across all samples analysed.

Multivariate analyses of the fingerprinting data were performed using a suite of software programs including Primer-E 6 v 6.1.11 (Primer-E Ltd, Devon, UK) and R. OTU data were directly imported into the package *vegan* (Oksanen *et al.*, 2013) in R prior to importing scripts. T-RF data were analysed using Bray-Curtis dissimilarity matrices (Bray and Curtis, 1957) of biotic data after appropriate transformations (square-root-transformed phylotype richness) (Clarke, 1993, Legendre and Gallagher, 2001) in Primer 6. Multivariate patterns of community data (sample dissimilarities) were visualised as non-metric multidimensional scaling (nMDS) ordination plots (Bray and Curtis, 1957). A minimum stress value of 0.01 was selected and ordination data were permutated 9999 times. Unique and shared OTUs were illustrated using Venn diagrams constructed in R using the packages *permute*, *lattice*, *vegan* and *gplots*. Significant clustering of communities in the ordinations were tested using analysis of similarities (ANOSIM) in Primer 6 and Permutational Multivariate Analysis of Similarities (PERMANOVA) (Anderson, 2005) in R using the *adonis*

function in the *vegan* library. Groups were defined as significantly different when $P < 0.05$. Species richness was estimated using the DIVERSE function in Primer 6, in addition both Simpson and Shannon Indices were calculated for each sample as an assessment of alpha-diversity.

Differences in α-, β-, and γ-diversity across habitat and moisture content were tested using R which include two-way ANOVA (Analysis of Variances) and Kruskal-Wallis tests, which are one-way analyses of variance. ANOVA is able to assess the effect of two or more pre-defined factors (variables) separately. The Kruskal-Wallis test uses a null hypothesis that all populations will have identical distributions and does not assume normal distributions of the data and is thus a non-parametric test.

The Kruskal-Wallis test was used to test for differences in diversity, and significant differences were further supported using *post-hoc* Wilcoxon-Mann-Whitney tests. This rank-sum test is used to calculate whether two independent sample observations have identical distributions by assuming that differences between each are not normally distributed (non-parametric).

The function *betadisper* was implemented in R under the *vegan* package in order to assess the homogeneity of group dispersions (Anderson *et al.,* 2006). The significance of these results was tested using *permutest*. A canonical redundancy analysis (RDA) was performed in R to assess the effect of both niche habitat and moisture content on microbial community structure (Peres-Neto *et al.*, 2006). This test assesses variation partitioning by calculating the amount of community variation that can be attributed to both the environment, *E*, and space, *S.* Testing the significance of results was performed with 999 Monte Carlo permutations of the data, the assigned R^2 values were adjusted as prescribed (Peres-Neto *et al.*, 2006).

2.6 454 Pyrosequencing

2.6.1 16S rRNA Amplicon Barcoded Pyrosequencing

Metagenomic DNA samples were prepared to 50 µl volumes following extractions using a prescribed protocol with modifications as listed previously for pyrosequencing (Miller *et al.*, 1999). Representative DNA samples were submitted to a commercial supplier, MrDNA, USA (www.mrdnalab.com/) at -20°C for pyrosequencing on the Roche GS-FLX+ platform (Roche Diagnostics, CT, USA). DNA concentrations varied from 16 ng/µl to 34 ng/µl, DNA quality ranged from 1.6 – 1.8 (A_{260}/A_{280}) which was sufficient to generate approximately 3 000 reads per assay. Representative samples of four niches were; Type I Cyanobacterial-dominated hypolith (H3), moranic surface soil (S1), black fungal-dominated cryptoendolith (E2) and green Cyanobacteria-dominated cryptoendolith (E5.1) (Table 4). Multiplex pyrosequencing was performed using the

bacteria-specific primer set 27F – 519R (Lane *et al.*, 1985, Lane, 1991) (Table 3) for the V1 – V3 hypervariable regions of the small sub-unit 16S rRNA gene.

Amplicons were subsequently sequenced under a modified bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP®) protocol (Dowd *et al.*, 2008). Firstly, a HotStarTaq *Plus* Master Mix Kit (Qiagen, CA, USA) was used under the following conditions with untagged primers; denaturation at 94°C for 3 min; then 28 cycles of 94°C denaturation for 30 s; 53°C annealing for 40 s and 72°C elongation for 1 min with a final elongation step of 72°C for 5 min. Following PCR amplifications, amplicons from unique samples were pooled in equal concentrations and purified using an Agencourt® AMPure® XP PCR Purification system (Agencourt Bioscience Corporation, Beverly, MA, USA). A second round of PCR amplification was performed using 100 ng of purified PCR amplicons as templates with primers incorporating sample-specific tags (8 bases in length) (Table 4) for 10 cycles. Products were then purified as before and used for 454-pyrosequencing on the Roche GS FLX+ Titanium pyrosequencing platform (Roche Diagnostics, CT, USA) as per the manufacturer's instructions. Sequence data were exported as a single .sff file with all generated sequences, primer and barcode data attached.

Table 4. Adaptor sequences and sample tags used for the Roche 454 sequencing platform.

Sample Name	Adaptor (5' - 3')	Tag Sequence (5' - 3')
Black Endolith (E2)	CGGACGGGTGAGTAACGCGTGA	AGGGTGGC
Green Endolith (E5.1)	CGGACGGGTGAGTAACGCGTGA	AGGTAAAG
Hypolith (H3)	CGGACGGGTGAGTAACGCGTGA	AGGTAAGT
Soil (S1)	CGGACGGGTGAGTAACGCGTGA	AGGTGGCC

2.6.2 Pyrosequencing Data Analyses

The sequence data generated were analysed using the MOTHUR software package (v.1.33.1) following an existing pipeline (Schloss *et al.*, 2009) (http://www.mothur.org/wiki/454_SOP) in order to remove barcodes, primers and sequence artefacts. Briefly, the sffinfo command was implemented for the extraction of FASTA, flow data and quality data from the available flowgrams. The shhh.flows command allowed for the removal of low quality (< 35) sequences, reducing the background noise in the dataset. Implementation of the unique.seqs command simplified the dataset by providing only unique sequences. Primer redundancies were removed from the dataset (pdiffs=0), although 1 nucleotide difference in the barcode sequences was allowed (bdiffs=1). Reads containing homopolymers of a length of 8 nucleotides or less were retained (maxhomop=8), while reads below 200 nucleotides in length were removed (minlength=200). Taxonomic

alignments using the SILVA 16S ribosomal reference database (Pruesse *et al.*, 2007) (http://www.arb-silva.de/download/arb-files) were performed using the align.seqs command with the flip=t prompt implemented. Chimeras were screened and removed with UCHIME v4.2 (Edgar *et al.*, 2011), the command chimera.uchime (http://drive5.com/uchime) was implemented in MOTHUR. Remaining OTUs were assigned phylum-level taxonomies using the cluster.split command according to a Bayesian rRNA classifier. Sequences that were unaligned to the reference database were removed prior to further analysis. A confidence of 80% was selected for these comparisons, duplicate sequences were filtered using remove.seqs. Putative contaminant sequences, including eukaryal and fungal signals, were subsequently removed using remove.lineages, although sequences of chloroplastic provenance were retained in the dataset due to high similarity with Cyanobacterial targets. Remaining OTUs were classified at a Jukes-Cantor distance of 0.03, the most stringent OTU definition applicable ($OTU_{0.03}$), to account for both sequencing and PCR errors as well as 16S ribosomal variability (Schloss *et al.*, 2009).

Sequence alignments were performed using nucleotide data of high-quality reads from all samples. Sequences were aligned using ClustalW (http://www.clustal.org), and phylogenies were inferred after comparisons to the SILVA Incremental Aligner (SINA) online (v1.2.11) (Pruesse *et al.*, 2012) at a 70% identity threshold. Phylogenetic analyses and alignments were performed in ARB (Ludwig *et al.*, 2004) by comparing the assigned reads to the SINA 16S database to generate phylogenetic trees. In addition, the free software iTOL (Interactive Tree of Life) (http://itol.embl.de) was implemented for sequence alignments and phylogenetic tree reconstruction (Letunic and Bork, 2007) using the ARB output files.

OTU-based analyses of sequence data was performed in MOTHUR using the collect.single command to calculate α-diversity estimates of richness using Simpson, Shannon and Chao1 tests. Estimates of sequencing depth were made using the rarefaction.single command of Chao1 richness and UniFrac-based metrics (Lozupone and Knight, 2005). Estimates of β-diversity were performed in MOTHUR with the venn command to generate Venn diagrams documenting shared OTUs between samples.

Phylogenetic Cyanobacterial tree reconstruction was performed in MEGA v6.06. Closely-related Cyanobacterial sequences were retrieved from the GenBank nucleotide database from NCBI online using BLASTn searches (http://www.ncbi.nlm.nih.gov). Sequence similarity of 97.5% was employed as prescribed by Taton *et al.* (2003). Maximum likelihood trees were generated (1000 replications) and confidence was obtained from bootstrap values, presented as percentages of nodes for 1000 resampled datasets. Dendogram tree reconstruction was performed in MOTHUR with the tree.shared command at an OTU definition of 97%. Trees were generated to describe

sample similarities using the jclass and thetayc calculators and were visualised using MEGA. Clustering of sequenced communities utilised a Bray-Curtis dissimilarity measure that had been averaged per sample.

Chapter 3: Microbial community structure of Victoria Valley edaphic niches under varying moisture regimes

3.1 Introduction

Victoria Valley forms part of a pristine set of continental Antarctic landscapes owing to a lack of direct anthropological impacts in the region. These hyperarid systems have remained an exciting area for research since the 1970's (Friedmann and Ocampo, 1976). The Antarctic continent represents an ideal environment for assessing the factors that drive microbial diversity and community structure on both the local and regional scale. However, the research focus has shifted from describing microbial diversity and community structure to understanding which factors act deterministically on these communities and drive patterns of dispersal. Assessing microbial communities along environmental gradients has highlighted the limits of bacterial diversity in deserts and has contributed to understanding the roles of deterministic factors in shaping local microbial populations (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007, Warren-Rhodes *et al.*, 2007, Zeglin *et al.*, 2010, Van Horn *et al.*, 2013). Factors such as pH and water availability have been implicated in shifts in bacterial community structures on a regional and local scales (Fierer and Jackson, 2006, Pointing *et al.*, 2007). Critically, increases in moisture content in the Dry Valleys are predicted as higher levels of temperature and UV irradiation due to climate change have led to greater ice melt in the region (Cowan *et al.*, 2014, Fountain *et al.*, 2014). Understanding the influence of increased moisture content on microbial consortia is essential, especially since desert soils globally are estimated to store almost a third of total terrestrial carbon (Trumper *et al.,* 2008). The simplicity of the trophic system in Antarctic soils presents a viable model for understanding how perturbations, as a result of climate change for instance, may affect microbial communities and system processes by applying ecological theory to metagenomic information.

Pointing *et al.* (2007) showed that hypolithic community structure was altered as a result of increased moisture content in China's hyperarid deserts. Additionally, Yergeau *et al.* (2007a) have suggested that both bacterial and fungal communities were more susceptible to changes in community structure under improved nutrient status. Gibson *et al.* (2006) have shown that Cyanobacterial diversity, in particular, was influenced similarly by nutrient status. Interestingly, increased wetting in the Dry Valleys is predicted to provide communities with both higher levels of water content and nutrients as deep-lying minerals and salts reach surface communities (Cowan *et al.,* 2014), although the long-term effects of augmented temperature extremes and fluctuations

remain unclear as yet (Benhua *et al.*, 2014). It has been shown that particular Cyanobacteria use a hydrotactic response in desert environments during wetting events and are thus capable of tracking moisture in soil (Garcia-Pichel and Pringault, 2001). The Cyanobacterium *Microcoleus vaginatus*, present in biological soil crusts (BSCs), was shown to respond to wetting events by emerging from dormancy and migrating towards the wetted surface (Rajeev *et al*., 2013). It remains unclear, however, how microbial communities in specialised niches (i.e. hypoliths, endoliths, mats) will respond to increased wetness.

Current research has shown that, despite the harsh environmental conditions that persist in the Dry Valleys, the local microbial diversity is vast and often exclusive to the region (Cowan and Ah Tow, 2004, Cary *et al.*, 2010). Communities present in specialised niches are thought to be supported by pioneer members of the Cyanobacteria phylum (Friedmann *et al.*, 1988), and in some instances by Actinobacteria (Griffiths and Mccormick, 1984). The ability of Cyanobacteria to adapt to environmental and climatic challenges is evidenced by the detection of the phylum across all major terrestrial niches in the Dry Valleys (Jungblut *et al.*, 2005, Pointing *et al.*, 2009, Lee *et al.*, 2012a) as well as in soils at maritime locations of the Antarctic continent, including Mars Oasis and Fossil Bluff (Yergeau *et al*., 2007b). Cyanobacteria may be mediators of numerous critical biologically-driven ecological processes in the Dry Valleys including primary productivity, nitrogen fixation and carbon sequestration, especially in the absence of higher plants (Cowan *et al.*, 2011a, Chan *et al.*, 2013).

Previous studies have suggested that improved moisture content in the Dry Valleys may lead to an overall increase in Cyanobacterial abundance and biomass on the regional scale (Wood *et al.*, 2008, Van Horn *et al.*, 2013). Additionally, changes in biologically available water may alter bacterial diversity patterns as earlier studies have revealed in other hyperarid deserts *(*Pointing *et al*., 2007, Zeglin *et al.,* 2010). Melt-water streams represent a simple yet elegant model for assessing the effect of distinct moisture regimes on local microbial communities in the Dry Valleys.

This study assesses the bacterial and Cyanobacterial diversity of various cryptic niches as well as moranic surface soils using terminal restriction fragment length polymorphism (T-RFLP) fingerprinting analysis (Liu *et al.*, 1997). T-RFLP analysis has been used previously to interrogate differences in bacterial community structure between Dry Valley niches (Pointing *et al.*, 2009), and to illustrate the effect of quartz rocks on the underlying bacterial communities, that is the hypolith and sub-lithic guilds, in the hyperarid Namib desert (Makhalanyane *et al.*, 2013b). T-RFLP analysis was selected to assess the community structure of bacterial and Cyanobacterial consortia as the technique represents a robust and discreet method of describing community fingerprints *(*Liu *et al.*,

1997, Osborn *et al*., 2000, Blackwood *et al.*, 2003). The hypothesis that Cyanobacterial diversity patterns are shaped by moisture content was tested in this chapter.

3.2 Results and Discussion

3.2.1 Metagenomic DNA isolations

The bacterial and Cyanobacterial community structures of four distinct Dry Valley niches were evaluated using T-RFLP fingerprinting (Liu *et al.*, 1997) with the aim of testing whether moisture content and habitat influences local community structure and diversity patterns. Samples included hypolithic (*n*=14) and endolithic (*n*=10) cryptic habitats as well as surface soils (*n*=5) and microbial mat communities (*n*=4) from Victoria Valley (Chapter 2; section 2.2). Detailed descriptions of each sample are provided in Appendix A.

High quality metagenomic DNA was extracted from all samples (*n*=33) using a modified 50-50-50 buffer-chloroform/phenol method (Miller *et al.*, 1999) (Figure 6) (Chapter 2; section 2.3.1). Triplicate DNA isolations were pooled prior to further community analyses.

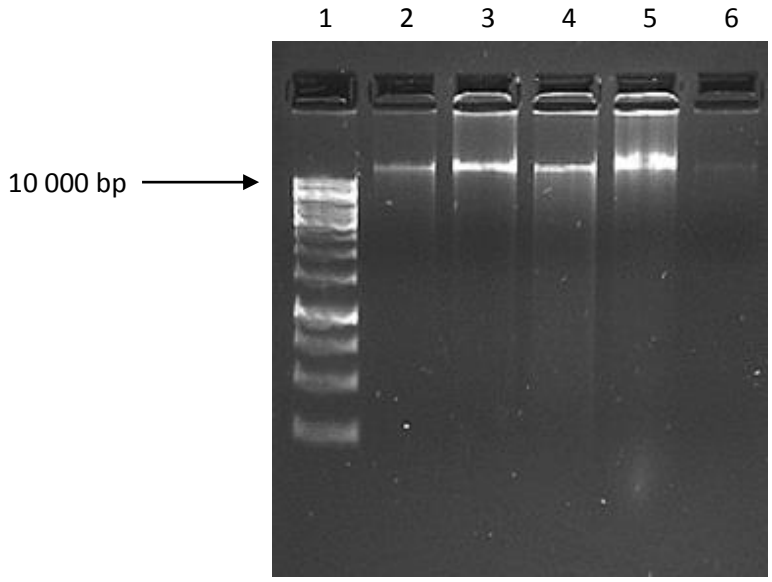


Figure 6. Agarose gel (1%) confirming the presence of metagenomic DNA following extractions. Lane 1: GeneRuler™ 1 kb Ladder. Lanes 2 – 6: Metagenomic DNA from environmental niche samples.

3.2.2 T-RFLP Fingerprinting Analysis

The total bacterial community and the Cyanobacterial population of each sample was assessed through T-RFLP fingerprinting analysis. Cyanobacteria-specific small sub-unit 16S rRNA genes were amplified by nested PCR reactions using the fluorescently-labelled Cyanobacteria-specific primer set; 5' 6-FAM 359F – 781R. These reactions incorporated unlabelled universal bacterial PCR amplicons as nested templates (universal 16S rRNA gene products; 341F-908R; Figure 7) and required sample-specific optimisations (data not shown). Cyanobacterial amplicons were generated for all samples indicating the ubiquity of the phylum across all niches analysed. Repeated DNA isolations and nested PCR amplifications showed that this result was reproducible.

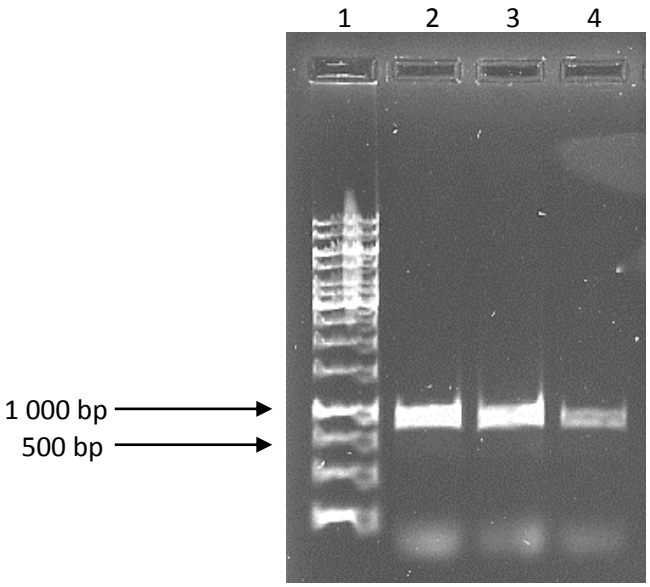


Figure 7. Agarose gel (1%) showing PCR amplification using the 5' 6-FAM 341F – 908R primer set. Lane 1: GeneRuler™ 1 kb Ladder. Lanes 2 – 4: Amplification of the expected fragment size (567 bp) was observed for all samples.

3.3 Bacterial Community Structure of Dry Valley cryptic niches

Restriction enzyme digestions, performed using *Msp*I and *Hae*III endonucleases independently, revealed a high level of congruency in terms of bacterial community patterns observed and a high level of similarity in terms of OTU numbers generated for each sample. Although *Msp*I digestions presented higher levels of α-diversity on average this difference was not significant ($P > 0.05$). Only the data generated from *Msp*I digestions of labelled 341F – 908R amplicons are shown here. Duplicate T-RFLP runs were performed and revealed highly similar diversity patterns and levels of OTUs per sample.

3.3.1 Total Bacterial Niche Community Composition

Bacterial 16S ribosomal gene amplicon digestion yielded a total of 163 unique bacterial OTUs for the 33 samples analysed (Figure 8). It is noted that the OTUs found here are likely to represent only dominant bacterial members of each sample, an inherent bias of the T-RFLP technique and 16S rRNA gene PCR amplifications (Blackwood *et al*., 2007). Figure 8 shows the distribution of bacterial OTUs across four Victoria Valley niches in a Venn diagram. Overlapping areas represent shared OTUs while non-overlapping lobes represent niche-specific OTUs. OTUs (α-diversity) ranged from 13 – 68 T-RFs per sample, at an average of 41.2 across all niches. These levels of bacterial abundance would be considered low for temperate soils (Fierer and Jackson, 2006), although the results are consistent with previous estimates of bacterial abundance in Antarctic environments (Wood *et al.,* 2008, Pointing *et al.,* 2009, Khan *et al*., 2011, Makhalanyane *et al*., 2013a). Average OTUs ($\bar{\alpha}$) of distinct niches revealed that cryptic communities, hypoliths and

endoliths, consistently generated higher numbers of OTUs than soils and mat communities, although this difference was not significant ($P > 0.05$). These diversity trends were also found in γ-diversity estimates, the total number of bacterial species within an ecological habitat (Table 5).

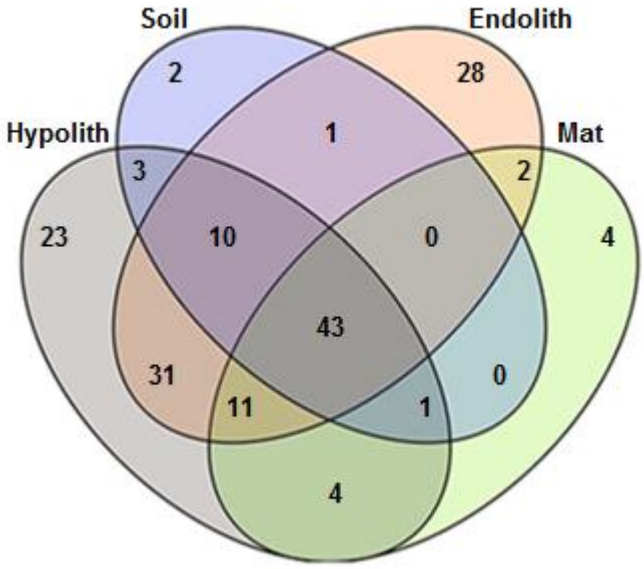


Figure 8. Venn diagram showing the distribution of bacterial OTUs (T-RFs) between the four distinct Dry Valley niches.

Rarefaction curves of Chao1 estimates of sampling suggest that only hypoliths and endoliths had reached an asymptote after sampling despite the relatively low number of samples analysed here, 14 and 10, respectively (data not shown). Chao1 is a non-parametric diversity estimate which allows for the comparison of total diversity between locations or habitats and is thus complementary to microbial ecology studies (Chao, 1987). This result is unsurprising as cold desert systems have been characterised by very low phylogenetic diversity (Cary *et al.*, 2010) and relatively low microbial biomass compared to temperate environments (Cowan *et al*., 2002). The high number of niche-specific OTUs may be explained, at least in part, by undersampling estimates for both soil and mat communities. It is likely that many of the unique OTUs detected for the lithic communities may be present in mats and/or soils, although their relative abundance lies below the detection threshold. However, niche specialisation has been recorded in the Dry Valleys which suggests that bacteria are non-homogenously distributed between habitats (Pointing *et al.*, 2009) which may explain the increase in niche-specific OTUs with sampling depth observed here.

Interestingly, only six of the 163 bacterial phylotypes detected here were entirely absent from lithic habitats. This is similar to previous results which have also shown that many bacterial species are capable of persisting in lithic consortia (Makhalanyane *et al.*, 2013a). This suggests that both endoliths and hypolith communities have greater bacterial richness than either soils or mats in

desert systems (Cary *et al.*, 2010), although undersampling of these habitats may have influenced this result. Lithic niches were shown to have multiple niche-specific OTUs as well as more OTUs per sample on average than soils and mats according to both ᾱ- and γ-diversity estimates. Estimates of abundance are not necessarily related to estimates of richness and vice versa, for example polar hypoliths have been shown to support a high abundance of Oscillatorian Cyanobacterial morphotypes (Chan *et al.*, 2012), although these communities typically exhibit low species richness (bacterial diversity) relative to the surrounding soils (Cockell and Stokes, 2004, Pointing *et al*., 2009).

The β-diversity, defined as species differences between habitats, of the dataset revealed that many OTUs were unique to each community, suggesting that niches may be unique in bacterial composition despite sharing many bacterial species. This reinforces the substantial volume of data that has revealed a non-homogenous distribution of bacteria across the Dry Valleys (Pointing *et al.*, 2009, Cary *et al.*, 2010, Lee *et al.*, 2012a, Cowan *et al.*, 2014). In total, 57 OTUs (~35.0%) were unique to specific habitats and the number of cosmopolitan OTUs (*n*=43), shared between all habitats, represented a total of 26.4% of total T-RFs (Figure 8).

Both hypoliths and endoliths harboured more niche-specific bacterial OTUs, possibly attributed to greater α-diversity and sampling effort. These results appear to support conventional theories proposing that hypoliths (Smith *et al.*, 2000, Pointing *et al*., 2009, Makhalanyane *et al*., 2013a) and endoliths (Pointing and Belnap, 2012, Yung *et al*., 2014) serve as 'hotspots' of microbial, and particularly Cyanobacterial, abundance in the Dry Valley deserts (Cary *et al*., 2010). The number of shared OTUs suggests that communities do not develop in isolation but are colonised by many cosmopolitan bacteria capable of persisting across multiple Dry Valley niches.

β-diversity, interpreted as a function of total niche diversity divided by the average niche diversity, was calculated as: $\beta = \gamma/\bar{\alpha}$, where γ (gamma-diversity) represents the overall number of OTUs in each niche, and ᾱ (average alpha) represents the average OTU abundance for each habitat. Calculations revealed that hypoliths and endoliths presented with the highest levels of β-diversity, 3.05 and 2.88, respectively. Soils and mats showed lower β-diversity on average, 1.80 and 1.81, respectively (Table 5) which suggests more homogenous distribution in the Victoria Valley. Processes affecting bacterial β-diversity have been shown to include dispersal limitation on the local scale (Graham and Fine, 2008, Martiny *et al.*, 2011) as well as niche conservatism (Graham and Fine, 2008). These results suggest that deterministic processes are likely to strongly govern the diversity patterns of soils and mats. By contrast hypoliths and endoliths are more likely to be influenced by stochastic events in this environment with a weaker deterministic role (Chase, 2010).

Table 5. Descriptions of each niche type in terms of averaged OTU abundance and diversity.

Niche	Average OTUs (ᾱ)	Total OTUs (γ)	β-diversity	Unique OTUs	Shared OTUs (%)
Endolith	43.7 [±17.1 (SD)]	126	2.88	28	77.8
Hypolith	41.3 [±11.7 (SD)]	126	3.05	23	81.7
Mat	35.8 [±2.4 (SD)]	65	1.81	4	93.8
Soil	33.4 [±5.8 (SD)]	60	1.80	2	96.6

In order to link sample heterogeneity and estimates of diversity, both within and between each niche, the species diversity of each sample was calculated. Species diversity (d) was calculated from Margalef's d = (S-1)/Log(N). S represents the total species present (richness), while N represents the total number of individuals in the sample (abundance). Species diversity was found to be highly variable, between 9.47 and 23.69, and endoliths were shown to have the highest species diversity on average (đ = 17.06). These results support the quantitative measures of α-diversity which suggest a greater bacterial richness in both lithic communities (Table 5). However, the trend of higher estimates of diversity in the lithic communities was not observed after implementing the Shannon Index (H'), which estimates phylotype richness and diversity, and the Simpson (1-λ') Diversity Index, which quantifies the biodiversity of each habitat. Despite the quantitative measures employed, T-RFLP data only describes dominant community members. Moreover, this methodological bias results in simplified fingerprints and estimates of total diversity (Bent and Forney, 2008).

Interestingly, soil communities were characterised as the most diverse niche according to both Shannon and Simpson indices applied. This is consistent with contemporary findings that have shown that soil communities have greater bacterial diversity, although rarely greater abundance, than specialised edaphic niches (Wood *et al*., 2008, Pointing *et al.*, 2009, Khan *et al.*, 2011, Makhalanyane *et al*., 2013b), and may serve as reservoirs of bacterial diversity in arid environments (Stomeo *et al*., 2013). Both Pointing *et al*. (2009) and Khan *et al*. (2011) found species abundance to be higher in open soils than hypoliths in the Dry Valleys, the result was supported by Chao1 and Shannon estimates of diversity, respectively. Evidence has been provided that suggests that hypolithic development occurs through species recruitment from surrounding soils in the hyperarid Namib desert (Makhalanyane *et al*., 2013b). Taken together, these results suggest that Dry Valley soils act as reservoirs of generalist microbial species (cosmopolitan bacteria) from which hypolith, and possibly endolith, specialised communities develop over time. This is in contrast to reports suggesting that hypoliths develop independently from the surrounding soil communities (Warren-Rhodes *et al*., 2006, Pointing *et al.*, 2007). Only 3 OTUs present in the soil communities were absent from the hypolithic consortia.

Table 6. Univariate models estimating the phylotype diversity for each niche.

Niche	S (richness)	N (abundance)	đ (diversity)	Shannon (H')	Simpson (1-λ')
Endolith	45,20	13,01	17,06	3,68	1,06
Hypolith	42,79	13,41	16,04	3,67	1,05
Mat	35,25	11,66	13,94	3,48	1,06
Soil	30,80	10,03	12,84	3,32	1,07

The samples from each habitat are predicted to show high levels of community relatedness by virtue of the high number of shared OTUs on the global scale (β-diversity), although differences in α-diversity between individual samples suggest unique bacterial communities exist. The overall community structure of habitats with greater evenness - soils and mats - are predicted to be more similar as composition is more limited, possibly due to high levels of environmental selection that govern these habitats (Chase, 2010). Hypolith and endolith communities are expected to show greater within-niche variability on the basis of the high number of unique OTUs present in those communities as well as high estimates of β-diversity, which suggests habitat differences. Lithic niches are predicted to be constituted by multiple unique bacterial consortia which may lead to greater within-sample variability and thus show greater community distinctness.

In order to visualise the separation of bacterial communities, non-metric multidimensional scaling (nMDS) was applied in Primer 6 following Bray-Curtis comparisons of assigned T-RFLP rank data (Bray and Curtis, 1957, Clarke, 1993) (Figure 9). Data transformations, square-root-transformed proportional abundance of OTUs, were implemented prior to community assessments (Abdo *et al.,* 2006). The distinctness of community fingerprints is determined by the proximity of samples in the ordination, thus separation can be visually interpreted as relative differences in community composition. The Bray-Curtis matrix calculates the dissimilarity of samples to plot the ordination in a non-linear manner, thus samples are plotted further apart as relative dissimilarity increases. The ordinations were run 9999 times at a minimum stress value of 0.01 (Figure 9).

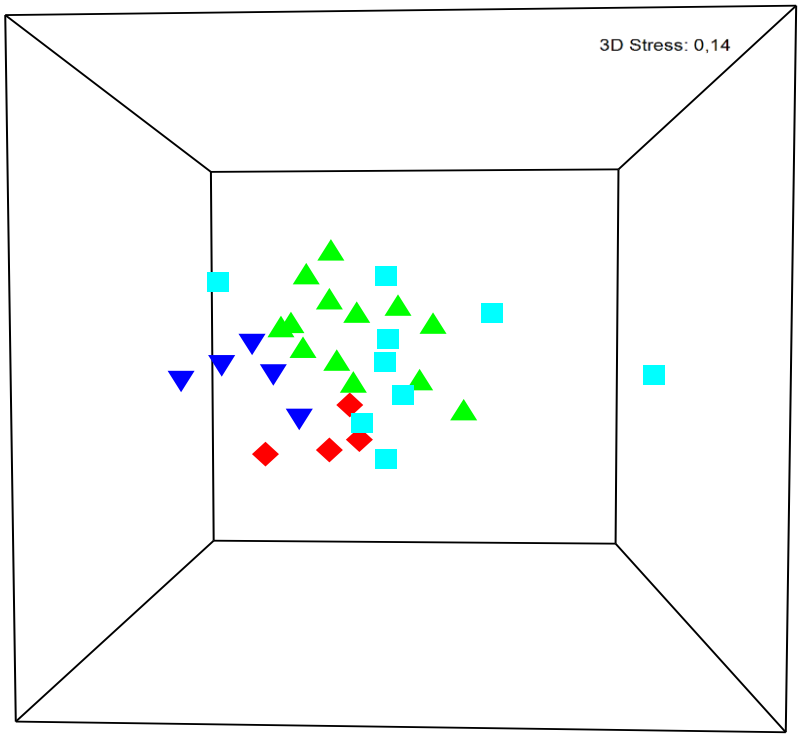


Figure 9. Nonmetric multidimensional scaling (nMDS) ordination plot of Bray-Curtis dissimilarities showing the relative differences in total bacterial community distribution on the basis of 16S rRNA gene-defined structure. The low stress value shows the quality of the ordination. Each point in the plot represents a complex microbial community and the distance between points represents sample dissimilarity. Proximal samples are less dissimilar in community composition than distal samples. ▲Hypolith, ■ Endolith, ♦ Mat, ▼ Soil.

The significance of sample grouping was tested according to *a priori* defined habitats; hypoliths, endoliths, soils and mats. Tests included ANOSIM function (Analysis of Similarities) implemented in Primer 6, and PERMANOVA (Permutational Analysis of Variance) (Anderson, 2005) using an *adonis* function in R under the *vegan* software package (Oksanen *et al.*, 2013). Both tests revealed strongly significant differences in community structure between the four habitats analysed (Global ANOSIM, $P < 0.005$; PERMANOVA, $P < 0.001$). This result was anticipated as different Dry Valley niches have previously been shown to harbour structurally distinct microbial communities on the basis of 16S rRNA genes (Wood *et al.*, 2008, Pointing *et al.*, 2009, Makhalanyane *et al.*, 2013a, Yung *et al*., 2014). This result suggests that habitat may drive local bacterial diversity and species composition in Dry Valley environments and supports the concept of ecological niche partitioning.

The significance of the observed grouping of niches was confirmed by ANOSIM after 999 random permutations of the ordination ($P < 0.005$), although groups showed high similarity, defined as overlap, according to the assigned *R*-value ($R = 0.272$).

ANOSIM calculates community distinction for all treatments or parameters analysed using pairwise comparisons. Niches revealed unique levels of distinction and grouping significance from each other according to pairwise comparisons of biotic Bray-Curtis data. Mat and soil communities revealed the greatest inter-niche composition differences (R = 0.713), although the significance of this grouping was low (P < 0.02). These niches share 44 of 81 OTUs (54.3%), although no OTUs were shared between mats and soils exclusively. Hypolithic communities showed strongly significant grouping from the endolithic guilds (R = 0.242, P < 0.005) but less so compared to mat communities (R = 0.374, P < 0.05) and to soils (R = 0.302, P < 0.05). This is consistent with previous findings which have described differences in microbial community assembly on the basis of 16S rRNA genes between hypoliths and surrounding soils (Khan *et al*., 2011, Makhalanyane *et al*., 2013a) as well as from mats (Wood *et al*., 2008) and endoliths (Pointing *et al*., 2009) in the Dry Valleys. Pairwise comparisons of endolith and mat communities revealed no significant differences in terms of structure (R = -0.028, P > 0.05). A possible reason for the high similarity found between these communities may be, in part, due to the high number of shared OTUs. The mat communities share a vast majority of OTUs with endoliths, whereas only 6 OTUs were unique to mat consortia according to the T-RFLP data. Niche habitats are proposed to be colonised by unique bacterial consortia, which suggests that they may possess unique functional capacities on the basis of distinct compositions (Chan *et al*., 2013).

The lack of distinction between the communities may be due to numerous inclusive factors. Firstly, the T-RFLP technique is limited by the fact that highly abundant taxa are over-represented, while rare taxa are often completely missed by fingerprinting analyses (Osborn *et al*., 2000, Blackwood *et al*., 2007). Low resolving capacity may suggest a higher community similarity than exists in reality (Blackwood *et al*., 2007, Bent and Forney, 2008). It is thus challenging to accurately predict differences in absolute microbial diversity of bacterial communities through the implementation of microbial fingerprinting technologies exclusively. Secondly, the level of aeolian dispersal processes and redistribution in the Dry Valley deserts are predicted to be significant, particularly with regard to the migration of bacteria, spores and extant cells, from soil and mat communities to other regional habitats (Bottos *et al*., 2014, Pointing and Belnap, 2014). High levels of dispersion of bacterial spores in the Dry Valley aerosphere has been shown to be a major factor of local migration of numerous bacterial phyla on the regional scale (Bottos *et al.*, 2014). Wind events may reduce the effect of environmental selection and are predicted to influence soil microbial communities stochastically rather than in a continuous manner, leading to increased community similarity over time (Bottos *et al*., 2014, Pointing and Belnap, 2014).

PERMANOVA analysis was performed with the *adonis* function in R in order to validate ANOSIM results. PERMANOVA is more statistically robust than ANOSIM in terms of heterogeneity in

dispersion and differences in correlation structure (Anderson and Walsh, 2013), as well as accounting for unequal sample numbers (Anderson and Walsh, 2013). When implementing PERMANOVA, niche type was found to have a strongly significant effect on bacterial community structure (R^2 = 0.19; P < 0.001). Habitat type alone explained 19.3% of the variance of the entire bacterial dataset according to this test. This suggests that other abiotic factors are also important in explaining the variation that exists between communities on the local scale, although niche type appears to be a major delineator of bacterial patterns here. Multivariate statistical analyses were implemented in order to explain differences in microbial community structure between habitats.

No significant differences in α-diversity were found between habitats (Kruskal-Wallis, P > 0.05). The Kruskal-Wallis test is employed to test for differences between groups, particularly if the data fails to follow a normal distribution. The average niche community structure was assessed and β-diversity was then calculated in order to assess the variability between each niche.

Estimates of β-diversity (dissimilarity between communities from a single group) suggest that lithic niches show significantly greater β-diversity than mats and soil communities in this environment (P < 0.05) (Figure 10). Communities with higher β-diversity are predicted to be greatly influenced by stochastic processes but less by deterministic factors, and are thought to show more variability in terms of evenness (Chase, 2010). Highly diverse communities (high β-diversity) are typically found in locations that experience less severe environmental stresses, at least in desert ecosystems. These communities are typically of greater functional importance than communities structured as a result of deterministic abiotic or environmental factors (Chase, 2010). This model predicts that stochastic effects will shape hypoliths or endoliths more than soil and mat communities which are more homogenously distributed across the environment and are shaped by the environment. This includes deterministic factors and habitat type. This is congruent with the observed patterns of diversity visualised in Figure 10.

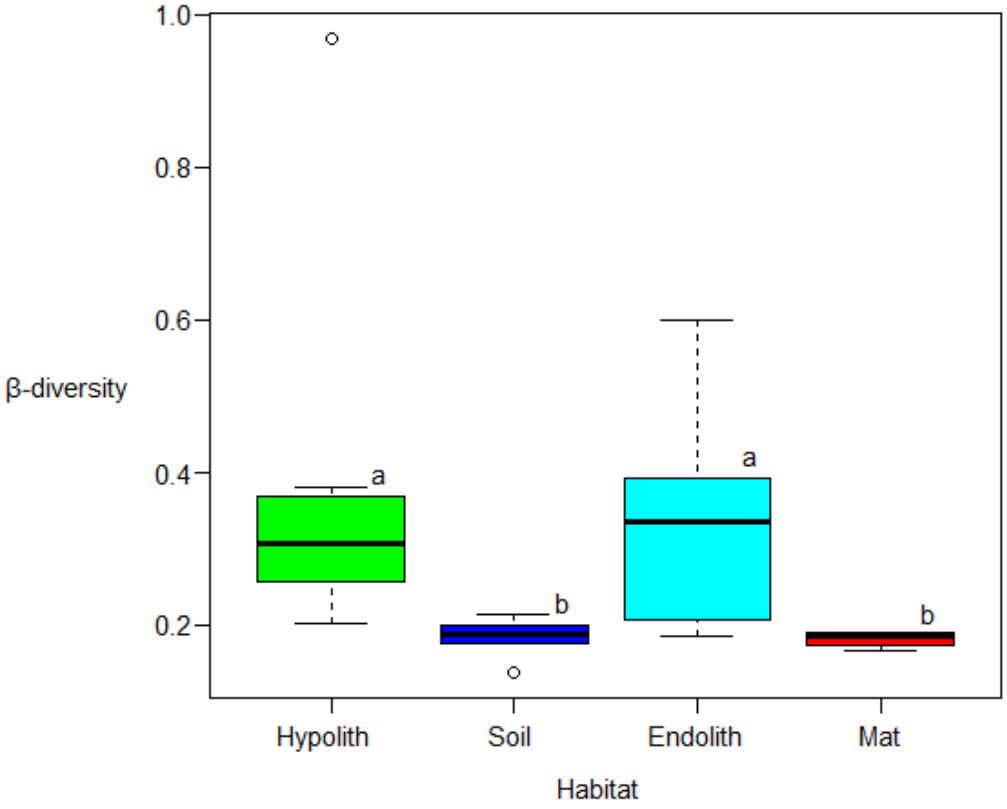


Figure 10. The variability of β-diversity for each habitat type is shown as a function of pair-wise comparisons. Hypoliths and endoliths (a) were consistently found to have significantly higher β-diversity than soil and mat communities (b) (Kruskal-Wallis test, $P < 0.05$).

Hypoliths and endoliths are also predicted to be colonised by more specialised communities than mats and soils and have been suggested to be at greater risk of extinction than generalist communities (Tilman, 1994).The Kruskal-Wallis test was implemented in R and confirmed that β-diversity was significantly influenced by niche type ($P < 0.002$). A *post-hoc* Wilcoxon-Mann-Whitney test was performed in order to test sample dispersion according to β-diversity. Pairwise comparisons revealed that niche type had a significant effect overall on β-diversity ($P < 0.05$). Significant differences in β-diversity were observed between hypoliths and soils ($P < 0.02$) as well as between hypoliths and mat communities ($P < 0.05$) following Bonferroni corrections of the *P*-value statistic (Figure 10). These results suggest that habitat type significantly influences bacterial community patterns and diversity, resulting in distinct microbial communities. β-diversity estimates appear to increase in communities predicted to have higher productivity. Differences in β-diversity between hypoliths and soils have also been observed in the Namib Desert system (Stomeo *et al*., 2013). Niche type has been shown to be a good predictor of β-diversity for general bacterial communities in the Dry Valleys.

The nMDS ordination generated (Figure 9) illustrates that while hypolith, soil and mat communities show proximal grouping, endolithic and hypolithic communities are characterised by greater

dispersal within the ordination. The function *betadisper* was implemented in R in order to test the multivariate homogeneity of group dispersions, or variances, of each habitat (Anderson *et al.,* 2006). The test calculates the distance to the centroid sample of each niche within the ordination and averages this distance between all other samples in the group. All comparisons made using this test were performed after Hellinger transformations of the data (Legendre and Gallagher, 2001) and were compared as a Bray-Curtis distance matrix of relative abundances. Endolithic communities showed the greatest dispersal from the mean (0.3894) according to *betadisper*. By contrast, niches characterised by less β-diversity presented with less group variation; hypoliths (0.3501), mats (0.2383), and soils (0.2191) (Table 7). This result suggests that endolithic and hypolithic communities may show greater variability across the environment than either soils or mat communities, although the function *permutest*, performed in R, revealed that no significant differences existed ($P > 0.05$).

Table 7. Diversity indices and tests calculated for each habitat type, including the results of significance tests.

Diversity metrics	**Hypolith**	**Endolith**	**Mat**	**Soil**	***P* value**	**F value (ANOVA)**
$\bar{\alpha}$	41.3[±11.7]	43.7[±17.1]	35.8[±2.4]	33.4[±5.8]	0.286	0.954
γ	126	126	65	60	-	-
β (γ/$\bar{\alpha}$)	3.05	2.88	1.81	1.80	0.002	0.812
Tests	**Hypolith**	**Endolith**	**Mat**	**Soil**	***P* value**	
betadisper	0.3501	0.3894	0.2383	0.2191	0.069	
ANOSIM		$P < 0.005$				
adonis		$P < 0.001$				

In summary, the effect of habitat on bacterial diversity, and to a lesser extent community composition, has been shown. Hypolith and endolith communities have shown greater microbial abundance and diversity than soils and mats, although soil communities were shown to possess greatest species richness overall according to univariate statistical tests. Lithic niches had higher levels of β-diversity and were more dispersed in the ordination, suggesting that these consortia may be more disparate in composition than mats or soils which show greater homogeneity across the environment. This is consistent with previous studies which have proposed that the structures of desert microbial communities are shaped both by deterministic and stochastic events (Caruso *et al.*, 2011, Makhalanyane *et al.*, 2013a). The high levels of similarity observed between the niches is suggestive that community development of each habitat does not occur independently in the Dry Valley environment, but rather that development is a constant process in which specialised communities 'recruit' species from surrounding soils (Makhalanyane *et al.*, 2013b).

In terms of ecological implications of these results, lithic niches are predicted to be at greater risk of climate change events according to the concept proposed by Chase (2010) which suggests that stochastic processes may influence environments of higher productivity more than low productivity habitats which are predicted to be governed by deterministic factors. The effect of predicted moisture increases in the Dry Valleys was assessed in order to better understand whether water content is a significant driver of bacterial community structure in this desert.

3.3.2 Bacterial Composition of communities under distinct Moisture Regimes

The degree of bacterial community relatedness and structure was tested according to moisture regimes defined *a priori*. The aim was to assess the degree to which moisture content shapes community structure and influences bacterial diversity in Dry Valley niche consortia. Hypoliths and mat communities were collected from sites within the wetted stream bed and serve as 'wet' communities (*n*=18). Endoliths and soil samples were obtained from dry sites (*n*=15). Soils in particular were collected from hyperarid moranic sites up to 25 meters from the stream bed.

Assessments of bacterial diversity were performed using Primer 6 and R software. Communities from wet and dry niches had highly similar levels of α- and γ-diversity estimates ($P > 0.05$). The overall number of OTUs generated from wet and dry samples (γ-diversity) showed a high level of similarity independent of restriction enzyme utilised, although wet communities were consistently higher in abundance across all samples. Phylotype richness and diversity were not significantly influenced by moisture content after implementing Shannon and Simpson indices. Species diversity was not influenced by moisture content ($P > 0.05$), calculated as d = (S-1)/Log(N).

β-diversity, calculated as $\beta = \gamma/\bar{\alpha}$, was highly similar for both wet and dry communities, 3.66 and 3.67, respectively. The β-diversity similarity was unsurprising as both γ and $\bar{\alpha}$ estimates were very similar irrespective of moisture content. These results suggest that moisture content is not a major delineator of bacterial abundance or species richness (α- and γ-diversity estimates) nor of bacterial community diversity or structure (β-diversity) in Dry Valley environments (Table 8).

Table 8. Diversity indices and statistical tests calculated for each moisture class, including the results of significance.

Diversity metrics	Dry	Wet	*P* value	F value (ANOVA)
$\bar{\alpha}$	40.2[±14.9]	40.1[±10.5]	0.962	0.002
γ	132	132	-	-
β (γ/$\bar{\alpha}$)	3.67	3.66	0.986	0
Tests	**Dry**	**Wet**	***P* value**	
betadisper	0.3954	0.3533	0.359	
ANOSIM	$P < 0.05$			
adonis	$P > 0.05$			

Species diversity revealed that a high proportion of bacterial OTUs (62.0%) were shared between wet and dry communities (*n*=101), while 31 OTUs were unique to each moisture class. This indicates that, as expected, generalist bacteria are capable of persisting across varied moisture regimes, although each moisture class also has unique bacterial species, putatively Cyanobacteria in the wet communities. Members of Cyanobacteria, such as *Microcoleus vaginatus*, have been shown to exhibit hydrotaxis as a means of facilitating bioavailable water acquisition (Garcia-Pichel and Pringault, 2001, Rajeev *et al.*, 2013). Although this mechanism has not been shown in the Dry Valleys it is plausible that Cyanobacteria employ responses to water inputs in this environment.

In order to assess differences in bacterial diversity according to moisture content, nMDS was implemented in Primer 6. Samples were analysed following square-root data transformations (Figure 11). The significance of the observed grouping was tested by employing the ANOSIM function (999 random permutations), and by applying PERMANOVA in R with the *adonis* function.

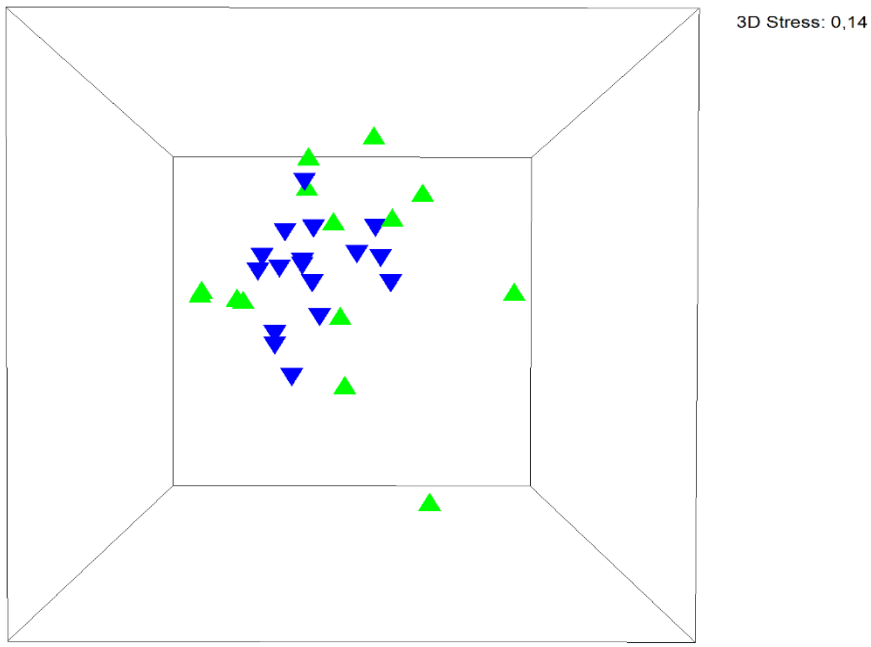


Figure 11. A 3D nMDS ordination plot showing the dispersal of bacterial communities according to moisture class. Ordination quality is shown by the low stress value. ▲Low moisture content community (Dry), ▼High moisture content community (Wet).

Significant grouping (ANOSIM, R = 0.08, P < 0.05) of bacterial communities based on moisture content was calculated for the global nMDS after regenerating the ordination 9999 times (Figure 11). However, the low level of community separation (R = 0.08) calculated suggests a high degree

of similarity in terms of community composition for wet and dry samples. This is congruent with β-diversity estimates of shared OTUs globally. The *adonis* function implemented in R (PERMANOVA) indicated that no significant differences exist between communities on the basis of moisture content (R^2 = 0.028; P > 0.05). These contrasting results illustrate the requirement to incorporate statistically relevant tests in order to better understand the observed data trends. The role of moisture content in shaping the community structure was estimated to be ~3% according to PERMANOVA which suggests that other abiotic factors are more dominant in explaining the observed variation.

The Kruskal-Wallis test was implemented to test for variation between communities under distinct moisture content regimes. No significant differences were noted for either α- or β-diversity tests in relation to moisture content (P > 0.05).

In order to test for differences in variability between the two groups *betadisper* was used in R. This test revealed no significant differences between dispersal patterns observed for wet (0.353) and dry (0.395) bacterial communities (P > 0.05).

Finally, a canonical redundancy analysis (RDA) was performed in order to assess the effect of both moisture content and habitat type simultaneously on the variation partitioning observed. Habitat alone was a significant factor in shaping bacterial community structures following Hellinger transformations of the Bray-Curtis matrix (P < 0.01). Habitat type was predicted to explain 14.5% of the variation observed, R^2 = 0.145, after adjustment of the statistic as previously suggested (Peres-Neto *et al.*, 2006). The effect of moisture in shaping bacterial communities, predicted to be 5.6%, was not significant (P > 0.05). Combined, these environmental variables are able to explain only 20.1% of total variation, suggesting that unexplained variation is approximately 80%. However, high levels of unexplained variation are inherent to ecology studies as species responses to environmental parameters may not be uniform (Drakare and Liess, 2010) and species abundances may also be highly disparate across similar environments (Dumbrell *et al.*, 2009). The unexplained variation in this study may be explained by physicochemical data (such as pH, total carbon and total nitrogen) and stochastic processes or interspecies interactions which were not assessed in this study.

3.4 Cyanobacterial composition of cryptic Dry Valley niches

The Cyanobacterial population of each community was assessed in order to link differences in phylum structure and patterns of diversity with distinct niches and unique moisture regimes. The effect of water availability on Cyanobacterial population structure has been shown in China's hyperarid deserts (Pointing *et al*., 2007) as well as across Antarctic soil biotopes (Yergeau *et al.*, 2007a, Van Horn *et al.*, 2013). Wood *et al.* (2008) predict that improved water content may lead to increased Cyanobacterial proliferation in Antarctic soils and cryptic niches. These changes in community structure may have implications in nutrient cycling and microbial diversity. The effect of moisture content on major edaphic niches has not yet been assessed in detail in the Dry Valley system.

3.4.1 Cyanobacterial distribution between distinct habitats

Cyanobacterial amplicons were analysed as before after restriction digestions with *Msp*I and *Hae*III endonucleases independently. The data generated from these digestions were highly congruent in terms of OTU abundances and diversity patterns generated, consequently only *Msp*I data are presented. The number of OTUs detected (α-diversity) was in the range of 1 – 8 OTUs per sample, this result was reproducible for replicate T-RFLP runs.

Cyanobacteria have been shown to be major colonisers of hypolithic and endolithic niches (Wood *et al*., 2008, Pointing *et al*., 2009, Makhalanyane *et al*., 2013a, Yung *et al.*, 2014) and are critical components of mat communities worldwide (Nübel *et al*., 1999, Paerl *et al*., 2000, Jungblut *et al.*, 2005). The β-diversity estimates of Cyanobacterial distribution between four habitats suggests that, as predicted, hypoliths and endoliths have a higher number of Cyanobacterial signals than soils and mat communities (Figure 12). The proportion of Cyanobacterial populations in relation to the overall bacterial communities was low, constituting between 10.9% - 18.6% of communities on average. Only 5 Cyanobacterial phylotypes were shared globally; approximately 23% of the 22 Cyanobacterial phylotypes observed. Cyanobacterial members including *Chroococcidiopsis* spp., *Nostoc* spp. and *Leptolyngbya frigida* have been shown to be ubiquitous across the Dry Valleys cryptic niches (Taton *et al*., 2003, Jungblut *et al*., 2005, Adams *et al*., 2006, Smith *et al*., 2006, Wood *et al*., 2008, Pointing *et al*., 2009, Bahl *et al*., 2011, Khan *et al*., 2011, Yung *et al*., 2014).

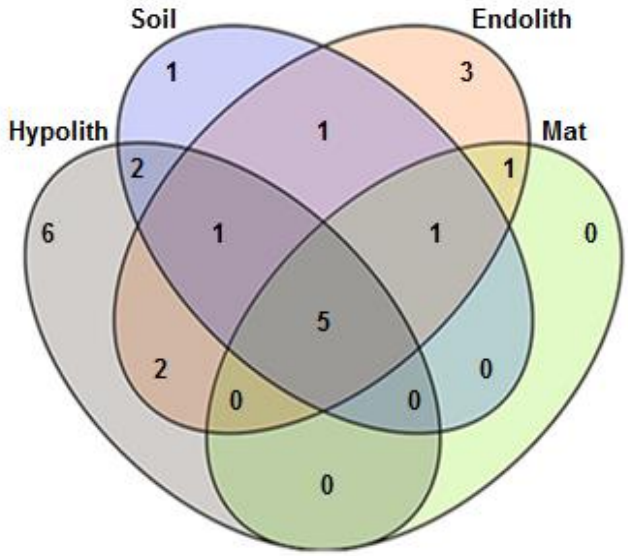


Figure 12. Venn diagram showing the distribution of Cyanobacterial OTUs across the four niche habitats (β-diversity).

The Kruskal-Wallis test revealed that no significant differences in α-diversity were found between habitats ($P > 0.05$), although β-diversity was significantly different ($P < 0.05$). A *post-hoc* Wilcoxon-Mann-Whitney test suggested that significant differences in β-diversity exist between hypoliths and soil Cyanobacterial populations as well as between endoliths and soil populations ($P < 0.05$). A direct interpretation of these results is that Cyanobacterial consortia present in lithic-based communities are more diverse than either soils or mat communities which appear to be constituted by limited niche-specific Cyanobacteria. Observationally, mat community biomass is nearly exclusively constituted by *Nostoc* species. However the detection of multiple Cyanobacterial lineages is not uncommon for mat communities in the Dry Valleys as members of *Leptolyngbya* and *Phormidium* (Oscillatoriales) have also been charcterised in these communities (Wood *et al.*, 2008, Taton *et al.*, 2003, Jungblut *et al.*, 2005, Adams *et al.*, 2006).

In order to link Cyanobacterial abundance with differences in community structure, nMDS ordination plots (Figure 13) were applied. The significance of niche grouping was determined using the ANOSIM function in Primer 6 and PERMANOVA in R.

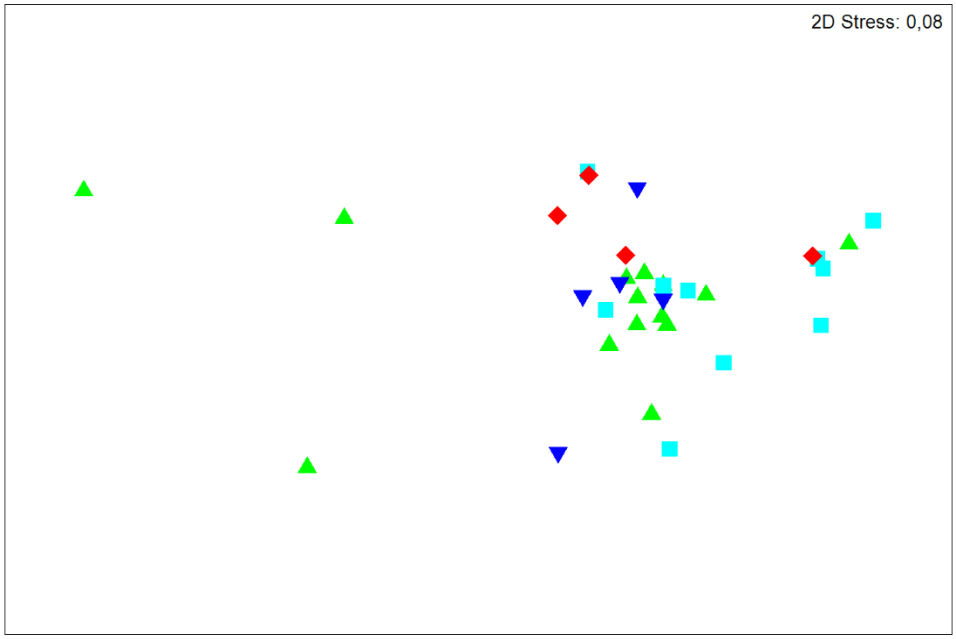


Figure 13. The nMDS ordination plot of hypoliths, endoliths, soils and mat communities on the basis of Cyanobacteria-specific 16S rRNA gene community-defined fingerprints. The quality of the ordination is illustrated by the low stress value. ▲Hypolith, ■ Endolith, ♦ Mat, ▼ Soil.

Cyanobacteria were assessed according to habitat (Figure 13), and no significant differences in bacterial community structure were detected according to the assigned ANOSIM values (R = 0.028, P > 0.05) or *adonis* in R (P > 0.05). Niche type is predicted to explain only 12.6% of the community variation overall. This value is lower than the estimate obtained for the global bacterial communities (R^2 = 0.19). The observed grouping (Figure 13) suggests that Cyanobacterial populations are highly similar across niche habitats in the Dry Valleys which implies a limited local diversity. Lithic communities showed greatest variability in terms of dispersal which indicates major differences between individual hypolith and endolith Cyanobacterial populations. By contrast, Cyanobacterial communities present in the remaining niches were consistent in terms of grouping and did not show the same level of dispersal.

The variability of dispersion within the ordination was tested using *betadisper* in R. The test revealed similar levels of dispersion that were obtained when bacterial variability was assessed. All Cyanobacterial populations had greater levels of dispersion than bacterial communities. However, the levels of group dispersion remained consistent; endoliths (0.3972) and hypoliths (0.3930) showed greater variation than mats (0.3138) or soils (0.3053) which were more homogenously dispersed according to niche type. No significant differences in dispersion were detected when using the test *permutest* (P > 0.05) (Table 9).

Table 9. Values of diversity for each habitat type on Cyanobacterial populations, including the results of significance tests.

Diversity metrics	Hypolith	Endolith	Mat	Soil	*P* value	F value (ANOVA)
ᾱ	4.6±1.8	4.1±1.6	3.8±1.5	5.2±1.1	0.596	0.791
γ	16	14	7	11	-	-
β (γ/ᾱ)	3.47	3.41	1.84	2.12	0.009	1.508
Tests	**Hypolith**	**Endolith**	**Mat**	**Soil**	***P* value**	
betadisper	0.3930	0.3972	0.3138	0.3053	0.864	
ANOSIM			$P > 0.05$			
adonis			$P > 0.05$			

Taken together, these results suggest that niche type is not a dominant factor in determining Cyanobacterial abundance, although β-diversity is significantly influenced. High productivity niches (lithic communities) have been shown to be more variable in the Victoria Valley suggesting that unique constitutions exist, possibly as a result of unique local assembly patterns.

3.4.2 The effect of Moisture Content on Cyanobacterial populations

The effect of moisture content on the Cyanobacterial populations was assessed. The average within-sample diversity (ᾱ-diversity) was calculated for both moisture classes and revealed that higher moisture content samples had slightly lower levels of Cyanobacterial abundance [4.42 (mean) ±1.8 (SD)] than samples from dry sites (4.5±1.5), although this difference was not significant ($P > 0.05$).

The γ-diversity estimates revealed that dry sites had marginally lower levels of Cyanobacterial diversity (17 OTUs) than wet sites (18 OTUs), with 52.2% of OTUs (n=12) ubiquitous across both moisture regimes. Moisture regimes may delineate Cyanobacterial species in the Dry Valleys as preferences for specific moisture content have been suggested by these results. The response of specific Cyanobacterial phylotypes during wetting, such as *Oscillatoria* spp. (Garcia-Pichel and Pringault, 2001) and *M. vaginatus* (Rajeev *et al*., 2013), is predicted to lead to higher Cyanobacterial diversity and abundance as members migrate to the surface.

A Kruskal-Wallis test was used to assess the effect moisture content on α- and β-diversity. The test revealed neither α-diversity nor β-diversity were significantly influenced by moisture content ($P > 0.05$). β-diversity, calculated here as $\beta = \gamma/\bar{\alpha}$, of Cyanobacterial populations in wet conditions was greater than the dry communities, 5.402 and 4.419, respectively (Table 10). A *post-hoc* Wilcoxon-Mann-Whitney test revealed that moisture content does not lead to significant differences between the Cyanobacterial populations following Bonferroni corrections of the p value statistic, $P > 0.05$.

Table 10. Values of diversity and statistical tests for each moisture class for Cyanobacterial populations, including the results of significance tests.

Diversity metrics	Dry	Wet	*P* value	F value (ANOVA)
ᾱ	4.5±1.6	4.4±1.8	0.97	0.001
γ	17	18	-	-
β (γ/ᾱ)	4.49	5.30	0.47	0.527
Tests	**Dry**	**Wet**	***P* value**	
betadisper	0.3989	0.4049	0.96	
ANOSIM		*P* > 0.05		
adonis		*P* > 0.05		

The effect of moisture content on Cyanobacterial populations was visualised as an nMDS plot generated in Primer 6 after square-root-transformations of phylotype abundances and compared as a Bray-Curtis dissimilarity matrix (Figure 14).

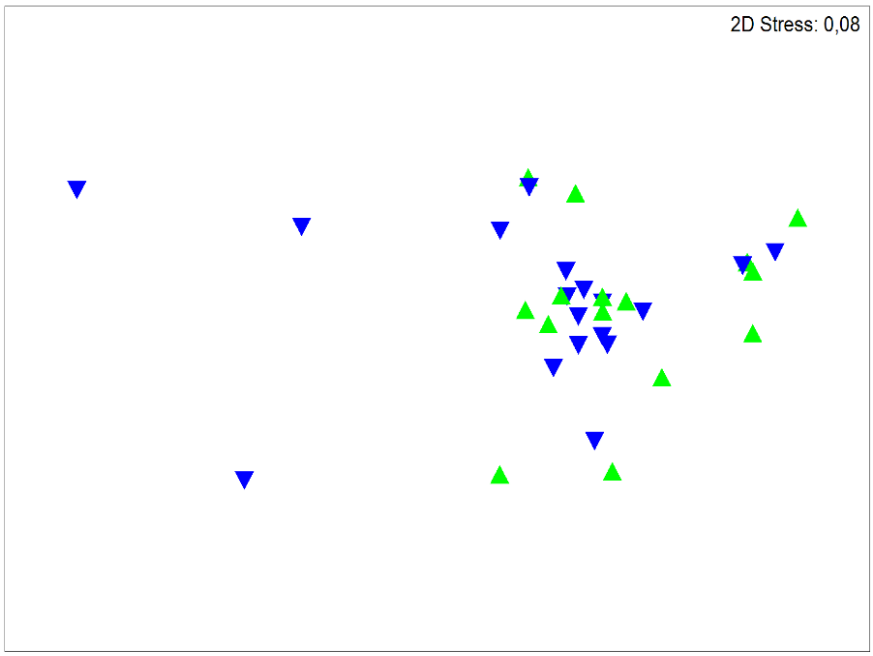


Figure 14. A 2-D nMDS ordination plot showing grouping of Cyanobacteria according to moisture content. The quality of the ordination is represented by the low stress value. ▲Low moisture content community (Dry), ▼High moisture content community (Wet).

The significance of grouping was assessed using multivariate statistical analyses as before. Cyanobacterial community structure was not significantly influenced by moisture content (ANOSIM, $P > 0.05$), and the separation of communities within the ordination was very slight ($R = -0.004$) (Figure 14). The significance of grouping was also tested using the *adonis* function in R, which

revealed no significant differences between moisture classes ($P > 0.05$) and predicted that moisture content alone explains 3.29% of the community variation observed.

High moisture content samples (n=18) showed similar grouping to dry samples (n=15). Both classes showed moderate levels of dispersal within the ordination. High similarity of the communities was predicted on the basis shared OTUs. Both classes may be colonised by multiple unique Cyanobacterial species (richness), but may have similar species abundance (evenness).

The homogeneity of multivariate dispersal was tested using *betadisper* in R (Anderson *et al*., 2006), and revealed that samples showed similar levels of variation within the ordination (Table 10). The significance of the variability was tested using *permutest*, no significant difference between Cyanobacterial communities under distinct moisture content were found ($P > 0.05$).

3.5 Conclusion

Microbial fingerprinting analysis using T-RFLP revealed that habitat type, but not moisture content, influences overall bacterial community composition on the local scale. It was shown that specialised niches, hypoliths and endoliths, typically have higher bacterial richness and relative number of species, than either soils or mat communities. However, soils were shown to have higher bacterial diversity in this environment, supporting hypotheses proposing that soils serve as reservoirs of bacterial diversity in deserts (Makhalanyane *et al*., 2013b, Stomeo *et al*., 2013). The high number of shared OTUs (cosmopolitan bacteria) may colonise multiple habitats which further supports the concept of bacterial species recruitment from open soils (Makhalanyane *et al.*, 2013b). This finding is in contrast to reports of hypolithic development independently from surrounding soils (Warren-Rhodes *et al.*, 2006). Cryptic niches were characterised with significantly higher β-diversity suggesting that these communities increase in variability over time, possibly due to habitat filtering during assembly as community complexity increases. Soil and mat communities appear to be more homogenously distributed across the environment. Niche habitats were analysed together and were shown to be significantly distinct in community composition which suggests that niche specialisation is a major factor that influences local patterns of diversity in the Dry Valleys. This corroborates previous phylogenetic studies performed in the Dry Valleys that have demonstrated evidence for niche specialisation (Wood *et al.*, 2008, Pointing *et al.*, 2009, Makhalanyane *et al.*, 2013a). Moisture content did not influence bacterial community composition, diversity or abundance, and was predicted to explain less variation than habitat alone.

The ubiquitous detection of Cyanobacteria in all samples analysed here counters the finding that Dry Valley soils are devoid of Cyanobacteria entirely (Pointing *et al.*, 2009). In this study it is suggested that moisture content alone is not a significant driver of either bacterial or Cyanobacterial community structure. Overall, no correlations were found linking changes in

diversity or composition with moisture regime. Moreover, the effect of stochastic changes in the Dry Valleys are predicted to have a greater influence on both endolith and hypolith communities by virtue of the higher productivity of these sites, estimated with β-diversity (Chase, 2010). Finally, habitat type was not a major delineator of Cyanobacterial populations in the Dry Valleys, although β-diversity was significantly influenced by niche and was notably higher in specialised populations.

Chapter 4: Phylogenetic analysis of bacterial diversity across distinct niche habitats

4.1 Introduction

The Dry Valleys of Antarctica comprise a unique set of ice-free landscapes that are colonised by diverse microbial communities (Cary *et al.*, 2010). Across this hyperarid environment microbial life is found in edaphic habitats including soils, hypoliths, endoliths and mat communities. Critical to ecosystem functioning of this desert system are photoautotrophic Cyanobacteria which mediate the majority of primary productivity in the absence of higher plants. Cyanobacteria are predicted to be important members of microbial communities found underneath or within lithic substrates such as quartz, marble, granite and sandstone rocks (Friedmann and Ocampo, 1976). Carbon turnover and nitrogen fixation by Cyanobacteria are predicted to contribute to local nutrient budgets as Antarctic soils are typically highly oligotrophic (Cary *et al.*, 2010). Benefits of lithic colonisation have been proposed to include improved physical stability, moderation of excessive PAR and harmful UV radiation as well as thermal buffering (Warren-Rhodes *et al.*, 2006, Wong *et al.*, 2010, Cowan *et al.*, 2014). An additional benefit of the hypolithic lifestyle is proposed to be increased moisture content as condensation occurs on the underside of the rock at the soil interface (Warren-Rhodes *et al.*, 2006). This is critical for the associated hypolithon as Antarctic conditions are highly xeric and all precipitation is in the form of snow, most of which sublimes (Chinn, 1993).

Alternative moisture sources exist in the forms of glacial and permafrost ice-melt which takes place exclusively during the austral summer. These events supply a major portion of local moisture budgets to microbial communities, although the roles of seasonal moisture inputs have yet to be fully elucidated. Moisture levels in the Dry Valleys are predicted to increase dramatically as a result of rising UV irradiation and local temperatures over Antarctica (Fountain *et al.*, 2014). Critically, the effects of climate change may have detrimental implications on the unique communities that persist in the Dry Valleys. Wood *et al.* (2008) predict that increased moisture content will lead to a marked increase in Cyanobacterial abundance, particularly in soil communities. Increased Cyanobacterial abundance may alter regional community structures and nutrient processing mechanics. Additionally, upturns in Cyanobacterial proliferation may lead to the production of excessive amounts of cyanotoxins which may be harmful to local assemblages (Kleinteich *et al.*, 2012, Neilan *et al.*, 2013). This is an issue that is likely to influence Dry Valley consortia as *Nostoc* spp. are both major producers of cyanotoxins (namely microcystin) and are widely distributed across this environment (Wood *et al.*, 2008). Hypolith community structures have also been shown to be significantly influenced by moisture content in China's hot and cold hyperarid deserts (Pointing *et*

al., 2007). Understanding microbial community responses to environmental alterations remains the critical focus in desert ecology.

Cyanobacteria are capable of supporting a large heterotrophic component which has been postulated to span numerous bacterial phyla (Cary *et al.*, 2010). Additional heterotrophic colonists of Dry Valley communities include both fungal (Ruisi *et al.*, 2007, Rao *et al.*, 2012) and, to a lesser extent, Archaeal populations (Yung *et al.*, 2014). Moreover, soil communities, though not as protected as hypoliths or endoliths, have been suggested to serve as reservoirs of heterotrophic bacterial diversity (generalists) from which more specialised communities are 'seeded' (Makhalanyane *et al.*, 2013b, Stomeo *et al.*, 2013). However this concept requires further support, at least in the Dry Valleys, as studies have revealed distinct community structures between soils and specialised lithic-based consortia (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007, Pointing *et al.*, 2009).

The distinct niche communities studied here are briefly described below. Polar hypoliths have been characterised as low in diversity, near-exclusively dominated by Oscillatorian Cyanobacteria, but high in microbial abundance (Cowan *et al.*, 2011b, Chan *et al.*, 2012). Water bioavailability has been implicated as a delineator of hypolith bacterial diversity in the Dry Valleys (Barrett *et al.*, 2006). Recent studies have revealed that heterotrophic components of hypoliths may be more diverse than previously thought (Pointing *et al.*, 2009, Cowan *et al.*, 2010, Khan *et al.*, 2011, Chan *et al.*, 2012, Makhalanyane *et al.*, 2013a). Similarly, cryptoendoliths are typically dominated by Cyanobacteria, often of the genus *Chroococcidiopsis* (Friedmann *et al.*, 1988), although fungal-dominated cryptoendoliths are also abundant across the Dry Valleys (Ruisi *et al.*, 2007). Mat communities are free-forming Cyanobacteria-dominated assemblages that proliferate in the presence of flowing streams, characteristically from glacial melt-water. Desiccated mats are dormant remnants of these communities and typically form in the austral winter as water availability decreases. Dry Valley mats are highly complex communities and may also possess Archaea, Eukaryal members, viruses and heterotrophic bacteria (Paerl *et al.*, 2000). Overall, Cyanobacteria are critical members of many niches, particularly from a functional perspective, and understanding their ecology remains a fundamental focus in microbial ecology research.

Hypolithon assembly is predicted to be influenced by both stochastic and deterministic processes on the global scale (Caruso *et al.*, 2011). Bacterial community composition is also known to depend on habitat locally (Wood *et al.*, 2008, Pointing *et al.*, 2009), although unique moisture regimes appear to influence hypolithons (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007) and endoliths (Yung *et al.*, 2014) locally. Differing geochemistries drive soil diversity patterns on the regional scale (Lee *et al.*, 2012a) and water content has been suggested to influence soil

community structure as the migration of moisture to the soil surface transports deep-lying nutrients and salts (Van Horn *et al.*, 2013). However, as Dry Valley moisture regimes become altered in the near future, particularly through supplemented ice-melt in the region, communities are predicted to receive previously unattainable quantities of bioavailable water and associated nutrients.

In this study, the bacterial biodiversity of distinct niche habitats from Victoria Valley were characterised. The Victoria Valley represents a relatively unexplored edaphic system within the Dry Valleys. This study represents the first phylogenetic survey of the endemic bacterial diversity in this region using culture-independent techniques. Samples from 'wet' and 'dry' sites along a stream bed were analysed. The bacterial diversity and relative abundances of each moisture class were compared in order to reveal whether moisture regimes influence community structure locally. The overarching aim was to elucidate the effect of moisture content differences on community composition, with an emphasis on Cyanobacterial populations, through the use of next-generation tagged-pyrosequencing. This information may reveal the effects of moisture inputs on the local ecology of these critical desert communities. The bacterial composition and phylogenetic diversity of four distinct niche communities is described on the basis of small sub-unit ribosomal genes.

4.2 Results

4.2.1 16S rRNA gene amplicon sequencing

The dominant bacterial diversity of samples from four distinct desert habitats were assessed based on phylogenetic microbial community characterisation; H3 (Cyanobacteria-dominated hypolith), S1 (moranic surface soil), E2 (fungal-dominated black cryptoendolith) and E5.1 (Cyanobacteria-dominated green cryptoendolith).

Tagged-pyrosequencing was performed on the Roche 454 GS-FLX+ platform (Roche Diagnostics, CT, USA) following a prescribed protocol (Dowd *et al.*, 2008) by Mr DNA (Shallowater, TX, USA), a commercial supplier. Bacteria-specific primers targeted the V1 – V3 hyper-variable regions of the small sub-unit ribosomal gene (full protocol described in Chapter 2, 2.6). Partial sequencing of the rRNA gene was performed unidirectionally.

Pyrosequencing generated 14 727 raw sequence reads from the representative samples (*n*=4). Reads were an average length of 386 nucleotides prior to flowgram quality filtering. Sequence data were analysed using MOTHUR software (v.1.33.3) (Schloss *et al.*, 2009). The pipeline followed for filtering high-quality reads from the dataset has been described in detail in Chapter 2, 2.6.2. Denoising the dataset included screening and removal of chimeric sequences with UCHIME v4.2 (Edgar *et al.*, 2011). Barcode tags and homopolymers were removed in MOTHUR after binning sequences. Sequences aligned to the SILVA bacterial reference database (Pruesse *et al.*, 2007) were maintained in the data set. OTUs were described at a cut-off of 97% similarity (expressed throughout as $OTU_{0.03}$), the furthest Jukes-Cantor distance which accounts for PCR amplification errors as well as 16S rRNA gene variability (Schloss and Handelsman, 2006). Kunin *et al.* (2010) propose that selecting a sequence identity greater than 97% leads to an overestimation of rare taxa within the community as a result of pyrosequencing error alone. The average length of high-quality reads was 241 nucleotides after filtering, which is slightly lower than the average of length of 250 nucleotides obtained from 454 GS-FLX+ pyrosequencing (Mardis, 2008).

Aligned sequences comprised 724 $OTUs_{0.03}$, only 5 $OTUs_{0.03}$ (0.69%) were common to all niches. Low numbers of shared $OTUs_{0.03}$ are not uncommon for Dry Valley communities (Lee *et al.*, 2012a, Makhalanyane *et al.*, 2013a), particularly as the niches analysed here are predicted to be distinct in composition on the basis of 16S ribosomal gene-defined communities (Pointing *et al.,* 2009). H3 had 65 unique $OTUs_{0.03}$, 24 $OTUs_{0.03}$ were unique to S1, 18 $OTUs_{0.03}$ were unique to E2, and 22 $OTUs_{0.03}$ were exclusively detected in E5.1. The large number of sample-specific and the few shared $OTUs_{0.03}$ may be partly attributed to insufficient sequencing depth, since samples had not reached asymptote according to Chao1 estimates of coverage, resampled to the lowest sequence

number (*n*=963) (Figure 15). Unweighted UniFrac analysis (Lozupone and Knight, 2005) revealed the same trends as Chao1 richness estimates (data not shown).

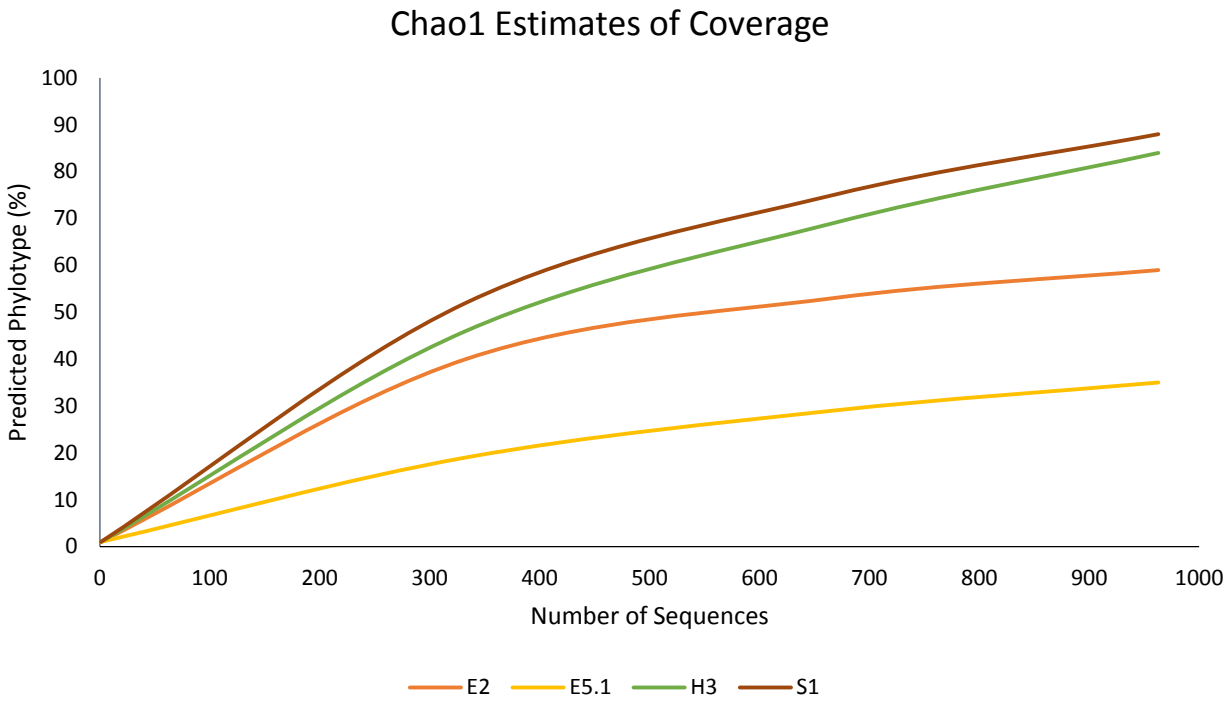


Figure 15. Estimates of pyrosequencing coverage for the four samples on the basis of Chao1 data.

Estimates of species richness were calculated in MOTHUR by subsampling to the lowest sequencing effort (*n*=963). These included α-diversity tests (ANOVA); Chao1 richness diversity estimates, Simpson diversity (1-λ') and the Shannon Index (H'). Species diversity was highest in the soil sample according to both 1-λ' and H' estimates (Table 11). This may be explained by the concept that soils serve as reservoirs of generalist bacterial diversity in desert systems (Stomeo *et al.*, 2013, Pointing and Belnap, 2014) from which more specialised communities, such as hypoliths and endoliths, may develop through species recruitment over time (Makhalanyane *et al.*, 2013b). The H' values of 4.3 and 3.8 obtained for the soil and hypolith, respectively, were much higher than the endoliths; 2.0 and 2.3 for E2 and E5.1, respectively. These findings are consistent with estimates of β-diversity observed for the T-RFLP results and appear to validate the higher levels of diversity in soils and hypoliths (Chapter 3, 3.3.1). The Chao1 value obtained for H3, 305.5, supports the hypothesis that hypolithons possess a higher number of species than other desert communities. Between 83 and 362 $OTUs_{0.03}$ were obtained from 963 and 5089 reads across the four communities (Table 11).

Table 11. The estimates of Chao1 richness, Simpson and Shannon diversity generated for each filtered dataset are presented, including total reads obtained and $OTU_{0.03}$ distribution.

Sample	Reads	Total $OTUs_{0.03}$	Unique $OTUs_{0.03}$	Chao1 richness	Simpson (1-λ')	Shannon (H')
E2	1340	83	18	67.1 [±20.2 (SD)]	0.28	2.0
E5.1	3273	103	22	107.1 [±43.0 (SD)]	0.20	2.3
H3	5089	362	65	305.5 [±94.6 (SD)]	0.06	3.8
S1	963	176	24	165.1 [±53.3 (SD)]	0.02	4.3

Rank-abundance analysis revealed that communities were dominated by a core group of generalist $OTUs_{0.03}$ while the tail comprised numerous rare $OTUs_{0.03}$ (Figure 16). Rarest $OTUs_{0.03}$ were detected only once in the dataset (rarefied singletons), while the most abundant taxon was sequenced 109 times. Overall, over 90% of the bacterial diversity was explained by only 28 of the most abundant $OTUs_{0.03}$. This type of distribution is commonly observed for environmental bacterial communities (Fuhrman, 2009, Pearce *et al.*, 2012).

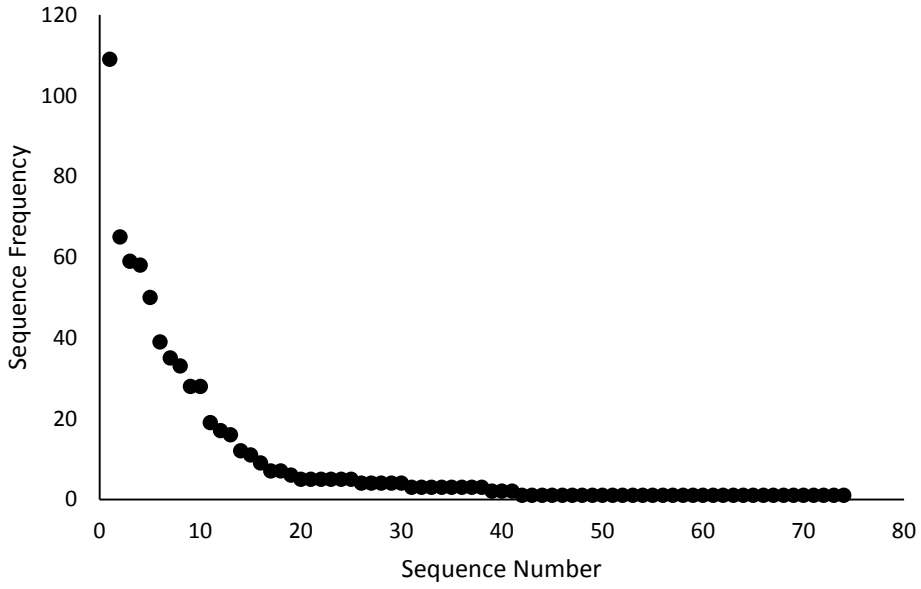


Figure 16. Rank-abundance graph illustrating the diversity found in this study.

4.2.2 Phylogenetic diversity of distinct bacterial habitats

Retained $OTUs_{0.03}$ were assigned phylum-level taxonomies after comparisons to the Ribosomal Database Project (RDP) Release 11.1, online (Wang *et al.*, 2007b) at a confidence of 80%. A total of 11 bacterial phyla were found, excluding unclassified sequences (Figure 17) of which 10 were present in the highly diverse soil sample (Figure 18). This result appears to corroborate previous findings that soils may 'seed' diversity for cryptic communities (Makhalanyane *et al.*, 2013b). The

phyla described here are common inhabitants of soils and lithic niches worldwide (Fierer and Jackson, 2006, Cary *et al.*, 2010, Steven *et al.*, 2013). Phylum-level distributions were unique for each sample, although H3 and E2 were most similar in terms of bacterial diversity at this level. E5.1 was most disparate in terms of relative phylum abundances. The relative abundances of phyla in each community are shown in Figure 18.

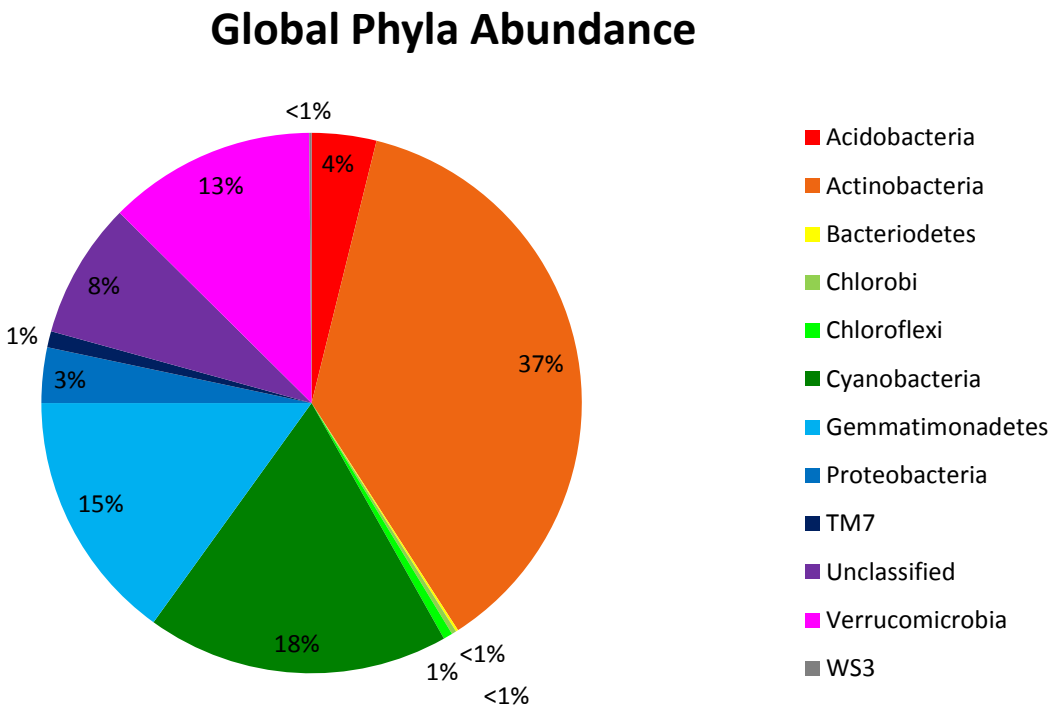


Figure 17. The phylogenetic diversity of bacterial 16S rRNA genes across four Dry Valley niches according to classifications against the RDP classifier (80% confidence).

$OTUs_{0.03}$ of possible anthropogenic origin were not recorded in the dataset after screening bacterial signatures as previously suggested (Bottos *et al.*, 2014). Additionally, no reads associated with fungal or Archaeal provenance were found in the dataset, validating the sensitivity of PCR amplification with the bacteria-specific 27F – 519R primer set and the filtering parameters applied in MOTHUR.

Overall, Actinobacteria was the most abundant phylum, comprising an estimated 37% of the diversity across all samples (Figure 17). The relative abundance of Actinobacteria was between 29.6% and 63.1% of affiliated sequences, and this taxon dominated all communities at the phylum-level. Members of Actinobacteria are frequently found in Dry Valley soils (Smith *et al.*, 2006, Lee *et al.*, 2012a, Tiao *et al.*, 2012) and are thought to be ubiquitous colonists of soils worldwide (Jones *et*

al., 2009). Actinobacteria have been implicated in soil fertility in arid environments (Griffiths and Mccormick, 1984) while numerous physiological adaptations including UV repair mechanisms, rapid sporulation and the production of secondary metabolites are thought to facilitate survival in extreme environments (Wellington *et al.*, 1992, Gao and Garcia-Pichel, 2011). Members of this phylum are generally ubiquitous to hypoliths (Pointing *et al.*, 2007, Pointing *et al.*, 2009) and endoliths (Yung *et al.*, 2014) in desert systems.

Cyanobacteria were pervasive to all samples analysed. Microbial fingerprinting analysis had revealed that Cyanobacteria were present in each community (Chapter 3, 3.4.1). The phylum contributed more than 18% of the bacterial sequences found which comprises 129 $OTUs_{0.03}$. Endolithic communities were surprisingly low in Cyanobacterial abundance, the phylum contributed 16.9% of reads in E2 and 6.8% in E5.1. In direct contrast, the soil community was unexpectedly rich in Cyanobacterial signals (21% of total sequences), while H3 had a substantial Cyanobacterial population contributing 20.2% of the diversity in that community (Figure 18). These results corroborate with estimates of α-diversity according to T-RFLP analysis. This suggests that soils were highest in Cyanobacterial abundance followed by hypoliths, although both were higher than endoliths. Comparisons of bacterial communities on the basis of relative phylum abundance was performed in MOTHUR. Averaged $OTU_{0.03}$ clustering revealed that H3 and E2 were most similar in diversity at the phylum-level, while soil appeared to be an intermediate community from which specialised niches may recruit species. The attached dendogram jclass tree was generated in MOTHUR, and tree visualisation was computed in MEGA v6.06 (Chapter 2, 2.6.2) (Figure 18).

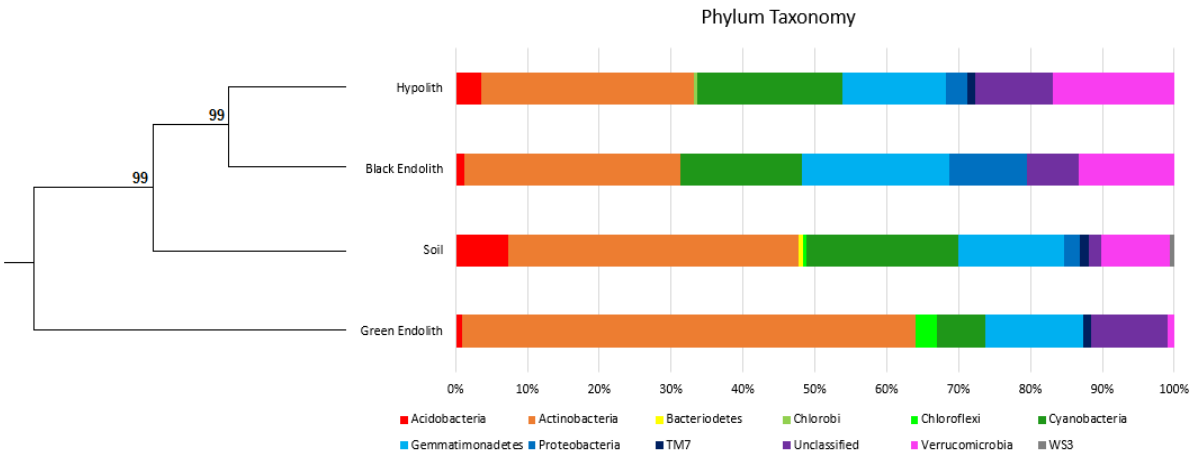


Figure 18. Phylum-level distributions of each community presented as a percentage of overall diversity (relative abundance). Bacterial $OTUs_{0.03}$ were phylogenetically assigned using the RDP classifier at a phylum confidence > 80%. The attached dendogram shows $OTU_{0.03}$ clustering of averaged diversity at each habitat (Bray-Curtis dissimilarity) generated in MOTHUR and visualised in MEGA. Frequently sequenced taxa have been listed in Table 12.

In addition to the detection of Actinobacteria and Cyanobacteria, Verrucomicrobia (90 $OTUs_{0.03}$), Gemmatimonadetes (109 $OTUs_{0.03}$) and Acidobacteria (28 $OTUs_{0.03}$) were ubiquitous phyla across the samples analysed. Verrucomicrobia predominantly belonged to the classes Verrucomicrobiae, Opitutae and Spartobacteria. These bacteria may be functionally important members of these communities given the high abundances at which they were found (Bergmann *et al.*, 2011). With the exception of E5.1, Verrucomicrobia account for more than 10% of phylum diversity. All Gemmatimonadetes members were classified as *Gemmatimonas* (77% confidence) and were shown to be relatively uniform in abundance, in the region of 15%, across the samples. Members of this phylum are highly adapted to low moisture content environments (Debruyn *et al.*, 2011). Interestingly, Gemmatimonadetes have recently been shown to be capable of photoautotrophy and possess functional type 2 photosynthetic reaction centres (Zeng *et al.*, 2014). The capacity for autotrophy has also been documented in Acidobacteria (Norris *et al.*, 2011). Acidobacteria, although low in abundance in the cryptic niches (approximately 2% abundance), were cosmopolitan members of all communities. Acidobacterial prevalence was notably higher in the soil community with approximately 7% of the $OTUs_{0.03}$ in this community confidently assigned to the phylum.

Less abundant phyla included Proteobacteria (24 $OTUs_{0.03}$), Chloroflexi (4 $OTUs_{0.03}$), and the candidate division TM7 (7 $OTUs_{0.03}$). Proteobacteria have been frequently described in Dry Valley soils previously (Cary *et al.*, 2010, Lee *et al.*, 2012a, Cowan *et al.*, 2014) and were shown to be universal to hypolithic communities worldwide (Pointing *et al.*, 2007, Pointing *et al.*, 2009, Wong *et al.*, 2010, Makhalanyane *et al.*, 2013a). Proteobacteria were not highly abundant in the communities analysed, contributing an estimated 3% of total diversity. Interestingly, E5.1 lacked Proteobacteria despite previous studies suggesting that these are important members of crypto- and chasmoendolithic communities (Pointing *et al.*, 2009, Yung *et al.*, 2014). In direct contrast, over 10% of $OTUs_{0.03}$ in E2 were affiliated with Proteobacteria.

Members of β-Proteobacteria have been proposed to be important components of bioreactor functioning and may perform biodegradation of phenolic compounds as a mechanism of carbon acquisition in nitrifying conditions (Manefield *et al.*, 2002). Chloroflexi were found in both S1 (< 1% abundance) and E5.1 (2.9%) and may represent alternatives to Cyanobacteria as primary carbon sources by serving as photoautotrophs. The possible functional importance of Chloroflexi is most apparent in E5.1 as Cyanobacterial abundance is markedly lower than the other communities assessed here. The candidate division TM7 was detected in all samples except E2. TM7 has been distinguished into two classes, TM7-1 and TM7-3, which appear to be pH delineated in polar regions (Winsley *et al.*, 2014). The positive association of TM7-1, a group of facultative anaerobes, with members of Acidobacteria, which are pervasive here, suggests that TM7-1 may be present.

TM7-3, a class of possible pathogenic heterotrophs, have a negative interaction with Acidobacteria and Gemmatimonadetes which were both found here (Winsley *et al.*, 2014).

Figure 19. The global diversity of bacterial 16S rRNA sequences represented at the phylum level for sequences defined at 97% similarity. Neighbour-joining tree was created from sequence alignments to the SILVA database in MOTHUR and generated in iTOL after alignments were made in ARB. Numbers in parenthesis indicate overall percentage abundance, phyla detected < 1% in the dataset are not represented by values. Scale bar indicates nucleotide changes over time.

Niche-specific phyla were expectedly low in abundance, comprising < 2% total bacterial diversity. Chlorobi (2 $OTUs_{0.03}$), a group of obligate anaerobic photoautotrophs, were found in H3, while both candidate division WS3 (1 $OTU_{0.03}$) and Bacteroidetes (1 $OTU_{0.03}$) were exclusive to soil. Chlorobi

may represent an alternative source of primary productivity in the hypolith community. Members of this phylum have not been previously found in Dry Valley soils, although they have been shown to dominate lake communities in the Vestfold Hills of maritime Antarctica (Burke and Burton, 1988). It is likely that the Chlorobi signals recovered in H3 may be lacustrine signatures. The detection of Bacteroidetes in the soil community was expected, although the low abundance of this phylum across all samples was unexpected. A comprehensive phylogenetic review of the Dry Valleys by Cary *et al.* (2010) found that approximately 8% of bacterial diversity is constituted by Bacteroidetes across soils, hypoliths and cryptoendoliths. The Bacteroidetes sequence was most similar to a *Hymenobacter* isolate following NCBI BLASTn comparisons (97%). Members of this genus have been previously described in hyperarid desert soils, including China's hot and cold deserts (Zhang *et al.*, 2007) and Dry Valley soils (Hirsch *et al.*, 1998). Candidate division WS3 has not been previously found in Dry Valley soil communities, although studies have found members in aquatic environments of the Antarctic (Nakai *et al.*, 2012, Powell *et al.*, 2012). It is noted that singleton $OTUs_{0.03}$ may be artefacts of PCR amplification, spurious signatures, and may not represent actual bacterial diversity. However, the stringent filtering pipeline implemented is likely to have prevented 'false' $OTUs_{0.03}$ from persisting in the dataset.

The phylogenetic relationships between the detected phyla were reconstructed in order to reveal the diversity of these sequences on the basis of 16S rRNA genes (Figure 19). All sequences (*n*=724) were assigned to phyla (97% confidence) and aligned in ARB v1.2.11 (Ludwig *et al.*, 2004). ARB tree outputs include only sequences above a 70% rejection threshold. Unrooted neighbour joining trees (Saitou and Nei, 1987) were imported into the Interactive Tree Of Life (iTOL) online (Letunic and Bork, 2007) for visualisation. Colours were used to denote the detected phyla, including unclassified bacterial signatures (Figure 19).

Detailed descriptions of the most frequently found taxa, sequenced at least 5 times in the dataset, are presented in Table 12. The 10 most common groups of the 74 genera described were *Gemmatimonas* (109 $OTUs_{0.03}$), *Iamia* (65), *GpIV* (58), *Luteolibacter* (50), *GpI* (39), *Ilumatobacter* (35), *Aciditerrimonas* (33), *Euzebya* (28), *Nocardioides* (28) and *Verrucomicrobium* (19). In total 33 taxa were only found once, comprising roughly 5% of total sequences, which represent almost half of the taxa found. Family-level descriptions of diversity revealed that soil and hypolith were more similar than at the phylum-level. These communities shared a large number of $OTUs_{0.03}$, whereas the endoliths were constituted by unique groups of $OTUs_{0.03}$, and were more similar at this level (Figure 20).

A total of 59 sequences remained unclassified against the latest RDP release at an 80% confidence threshold. A *post-hoc* comparison of unclassified reads to the SILVA Incremental

Aligner (SINA) online (Pruesse *et al.*, 2012) revealed that 19 $OTUs_{0.03}$ could be reclassified as *Armatimonadetes*, formerly candidate division OP10, at a confidence greater than 96%. Members of *Armatimonadetes* have been described in freshwater lakes as well as geothermally heated soils from temperate environments (Tamaki *et al.*, 2010, Lee *et al.*, 2011). This phylum, under previous nomenclature, was found in Dry Valley soils at low abundance (Cary *et al.*, 2010) and in Fossil Bluff and the Mars Oasis of the Antarctic Peninsula (Yergeau *et al.*, 2007b). Unclassified bacteria still represent a major avenue of investigation in microbial ecology research and may represent functionally important members of terrestrial communities.

Table 12. The most abundant $OTU_{0.03}$ taxa are presented. Sequences were detected at least 5 times in the dataset. Numbers in parenthesis indicate the relative abundance of each genus in the sample. No detection is marked with a 0.

	H3 (%)	E2 (%)	S1 (%)	E5.1 (%)	Overall (%)
Actinobacteria					
Actinobacteria					
Acidimicrobiales					
Acidimicrobineae					
Aciditerrimonas	17 (4.68)	2 (2.33)	5 (2.86)	9 (9.00)	33 (4.56)
Iamiaceae					
Iamia	40 (11.02)	3 (3.49)	12 (6.86)	10 (10.00)	65 (8.98)
Acidomicrobiaceae					
Ilumatobacter	15 (4.13)	5 (5.81)	12 (6.86)	3 (3.00)	35 (4.83)
Actinomycetales					
Nocardioidaceae					
Aeromicrobium	1 (0.28)	0	6 (3.43)	0	7 (0.97)
Marmoricola	3 (0.83)	0	7 (4.00)	6 (6.00)	16 (2.21)
Nocardioides	9 (2.48)	0	16 (9.14)	3 (3.00)	28 (3.87)
Kineosporiaceae					
Angustibacter	2 (0.55)	1 (1.16)	0	2 (2.00)	5 (0.69)
Unclassified Kineosporiaceae	3 (0.83)	2 (2.33)	0	0	5 (0.69)
Micrococcaceae					
Arthrobacter	1 (0.28)	2 (2.33)	1 (0.57)	1 (1.00)	5 (0.69)
Sporichthyaceae					
Sporichthya	2 (0.55)	1 (1.16)	1 (0.57)	1 (1.00)	5 (0.69)
Mycobacteriaceae					
Unclassified Mycobacteriaceae	1 (0.28)	0	2 (1.14)	2 (2.00)	5 (0.69)
Euzebyales					
Euzebyaceae					
Euzebya	7 (1.93)	3 (3.49)	0	18 (18.00)	28 (3.87)
Gemmatimonadetes					
Gemmatimonadetes					
Gemmatimonadales					
Gemmatimonadaceae					
Gemmatimonas	52 (14.33)	18 (20.93)	25 (14.29)	14 (14.00)	109 (15.06)
Verrucomicrobia					
Verrucomicrobiae					
Verrucomicrobiales					
Verrucomicrobiaceae					
Luteolibacter	33 (9.09)	9 (10.47)	8 (4.57)	0	50 (6.91)
Verrucomicrobium	15 (4.13)	0	4 (2.29)	0	19 (2.62)
Opitutae					
Opitutales					
Opitutaceae					

	H3 (%)	E2 (%)	S1 (%)	E5.1 (%)	Overall (%)
Opitutus	7 (1.93)	0	3 (1.71)	1 (1.00)	11 (1.52)
Spartobacteria					
Unclassified Spartobacteria	4 (1.10)	2 (2.33)	0	0	6 (0.83)
Cyanobacteria					
Cyanobacteria					
Family I					
GpI	20 (5.51)	2 (2.33)	13 (7.43)	4 (4.00)	39 (5.39)
Family IV					
GpIV	33 (9.09)	12 (13.95)	13 (7.43)	0	58 (8.01)
Family XIII					
GpXIII	6 (1.65)	0	6 (3.43)	0	12 (1.66)
Unclassified Cyanobacteria	5 (1.38)	0	3 (1.71)	1 (1.00)	9 (1.24)
Acidobacteria					
Acidobacteria Family Gp1					
Gp1	3 (0.83)	0	2 (1.14)	0	5 (0.69)
Acidobacteria Family Gp4					
Gp4	6 (1.65)	0	11 (6.29)	0	17 (2.35)
TM7	4 (1.10)	0	2 (1.14)	1 (1.00)	7 (0.97)
Unclassified Bacteria	40 (11.02)	5 (5.81)	3 (1.71)	11 (11.00)	59 (8.15)
Taxa represented less than					
5 times in the entire dataset	34 (9.37)	19 (22.09)	20 (11.43)	13 (13.00)	86 (11.88)

Comparisons of family-level diversity between samples revealed various interesting trends. The endolithic communities appear to be constituted by distinct bacterial groups relative to each other despite high similarity than at the phylum-level (Figure 20). This was noted for members of Cyanobacteria and the family Nocardioidaceae of the phylum Actinobacteria. The Cyanobacterial population of E2 was dominated by the genus *GpIV* (13.95%), although this taxon was entirely absent from E5.1. Members of *GpIV* include *Leptolyngbya frigida* and *Phormidium* spp. These species have been suggested to have fundamental roles in carbon and nitrogen turn-over in desert communities (Bergman *et al.*, 1997). The absence of this genus from E5.1 suggests the presence of unique nutrient cycling members. E5.1 is constituted by a high proportion of Nocardioidaceae that were not found in E2. These members of filamentous Actinomycetales are of biotechnological use in the production of many antibiotics (Shomura *et al.*, 1979, Cowan *et al.*, 2014).

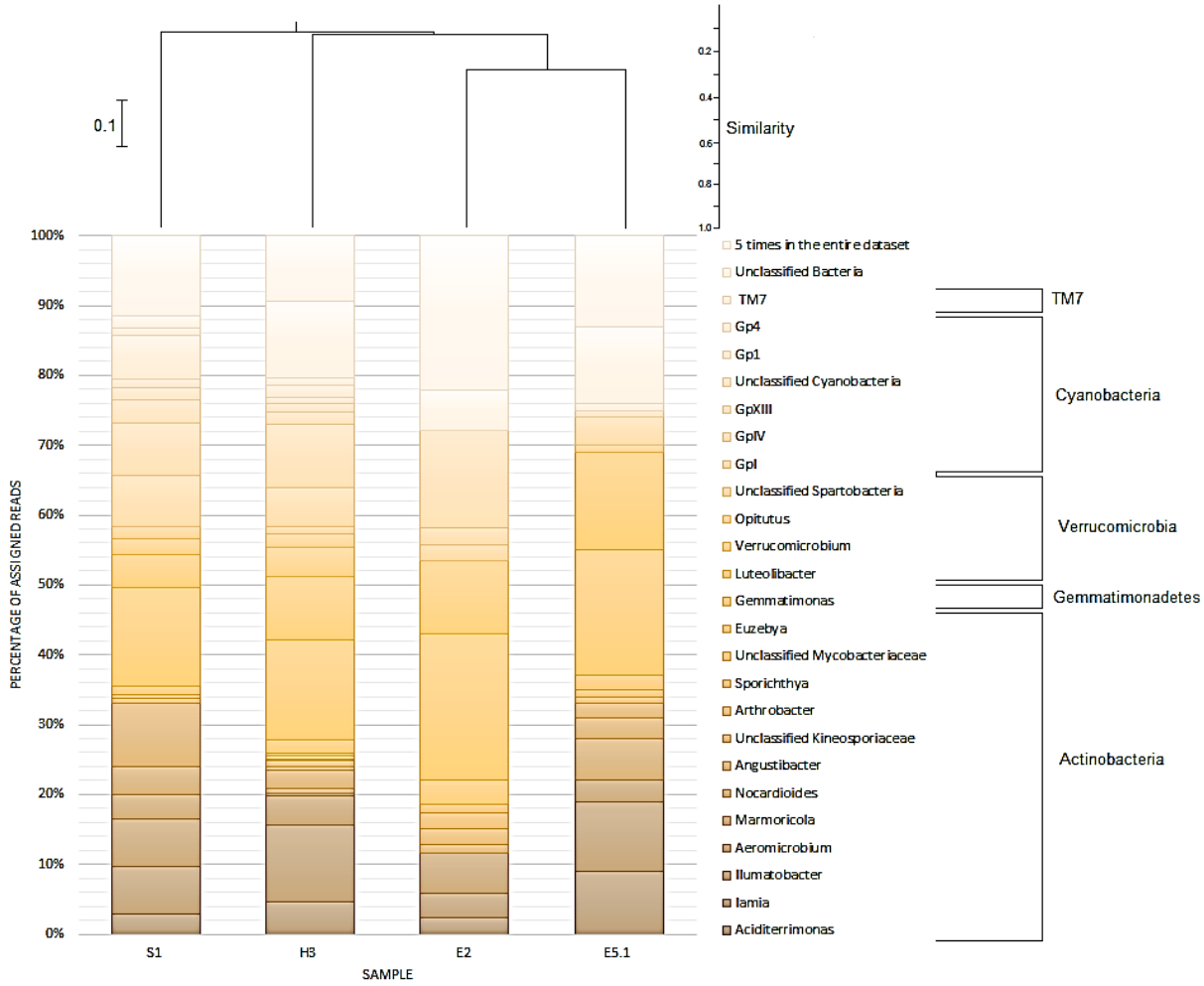


Figure 20. Bacterial communities from distinct Victoria Valley habitats differentiated on both vertical and horizontal axes at the family-level. Relative abundances of sequences from each community are plotted according to bacterial family assignments, phylum groups have been indicated. Attached dendogram shows the clustering of Bray-Curtis dissimilarities (averaged per sample) of $OTU_{0.03}$ data by habitat.

The high similarity between soil and hypolith at the $OTU_{0.03}$ level supports the concept that hypoliths develop from surrounding soil communities (Cowan *et al.*, 2011b, Makhalanyane *et al.*, 2013b), rather than independently (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007, Tracy *et al.*, 2010). Furthermore these results corroborate previous findings of niche specialisation in the Dry Valleys on the basis of small sub-unit 16S ribosomal genes (Pointing *et al.*, 2009).

Overall, the distribution of phyla described is congruent with diversity described in the Dry Valleys previously (Cary *et al.*, 2010). The local bacterial diversity of Victoria Valley soils and edaphic niches has not been previously explored; this study represents the first molecular analysis of the bacterial populations from this Dry Valley region. The major differences in relative bacterial abundances across the communities was predicted based on previous Dry Valley niche characterisations (Pointing *et al.*, 2009, Makhalanyane *et al.*, 2013a). However the low abundance

of Cyanobacteria found in the cryptoendolith E5.1 was unexpected. Chlorobi and WS3 may represent novel findings of this study. Chlorobi are capable of photosynthesis and represent important functional members of the hypolith community (Bryant *et al.*, 2012). Similarly, Chloroflexi may be responsible for local carbon and nitrogen cycling in endoliths.

Improved sequencing depth is likely to reveal that diversities have been slighty underestimated, Chao1 estimates confirmed that an asymptote of sequencing was not saturated. Bent and Forney (2008) reviewed numerous diversity metrics and techniques commonly used to describe community richness and concluded that increasing sampling depth is required to better assess complex microbial communities. Rare taxa are often, if not always, excluded from datasets by virtue of methodological bias which is inherent to PCR amplification (Blackwood *et al.*, 2007). Well-documented PCR-recalcitrant bacterial phyla include members of Firmicutes and Verrucomicrobia (Blackwood *et al.*, 2003, Bergmann *et al.*, 2011, Lee *et al.*, 2012b). Despite the incomplete characterisation of niche diversity presented here, these data suggest a greater bacterial diversity than has been previously documented for soils, hypoliths and airborne populations of the Dry Valleys after appropriate sequence validations (Khan *et al.*, 2011, Lee *et al.*, 2012a, Makhalanyane *et al.*, 2013a, Bottos *et al.*, 2014).

4.3 Cyanobacterial population structures

Cyanobacterial populations were compared in order to reveal differences in diversity and relative abundances of members of the phylum across distinct moisture regimes. Moisture gradient studies have suggested that Cyanobacterial diversity decreases with increasing aridity in desert environments (Warren-Rhodes *et al.*, 2006, Yung *et al.*, 2014). Additionally, local Cyanobacterial abundance is predicted to increase as water content is improved in the Dry Valleys (Wood *et al.*, 2008). The aim of this component of the study was to determine the effect of moisture content on Cyanobacterial consortia at the microscale.

Cyanobacterial lineages have been shown to be indicator species of hypoliths (Makhalanyane *et al.*, 2013b), and are often dominant bacterial phyla of specialised edaphic niches worldwide (Bahl *et al.*, 2011, Cowan *et al.*, 2014). Sequences affiliated with Cyanobacteria were retrieved from the dataset by implementing the remove.lineage command in MOTHUR. Retained reads were inferred Cyanobacterial phylogenies at a 97.5% confidence, as detailed by Taton *et al.* (2003), on the NCBI nucleotide database with the BLASTn function. Order, family and taxon assignments are listed in Table 13.

Table 13. Assignments of Cyanobacterial lineages following NCBI BLASTn comparisons (97.5% confidence). Numbers in parenthesis denote the relative abundance of each lineage to the total Cyanobacterial population, ubiquitous lineages shown in bold. No detection is denoted by a 0.

	Number of Cyanobacterial $OTUs_{0.03}$				
	H3 (%)	**E2 (%)**	**S1 (%)**	**E5.1 (%)**	**Total (%)**
Cyanobacteria					
Cyanobacteria					
Oscillatoriales					
Spirulina	0	0	0	1 (14.29)	1 (0.78)
Pseudanabaenaceae					
Leptolyngbya frigida	**36 (50.00)**	**7 (53.85)**	**12 (32.43)**	**1 (14.29)**	**56 (43.41)**
Pseudoanabaena	0	0	2 (5.41)	0	2 (1.55)
Phormidiaceae					
Phormidium	21 (29.17)	5 (38.46)	10 (27.03)	0	36 (27.91)
Microcoleus vaginatus	0	0	5 (13.51)	0	5 (3.88)
Gomontiellaceae					
Crinalium epipsammum	1 (1.39)	0	1 (2.70)	0	2 (1.55)
Unclassified Oscillatoriales	4 (5.56)	1 (7.69)	0	0	5 (3.88)
Synechococcales					
Synechococcaceae					
Synechococcus	0	0	0	1 (14.29)	1 (0.78)
Chroococcales					
Xenococcaceae					
Chroococcidiopsis	2 (2.78)	0	1 (2.70)	0	3 (2.33)
Unclassified family					
Acaryochloris	0	0	0	2 (28.57)	2 (1.55)
Plectolyngbya	1 (1.39)	0	0	0	1 (0.78)
Unclassified Cyanobacteria	7 (9.72)	0	6 (16.22)	2 (28.57)	15 (11.63)

Overall, populations were dominated by filamentous Oscillatorian Cyanobacteria with approximately 80% of signals assigned to the taxon (Figure 21). Members of the order Oscillatoriales found in this study include; *Crinalium epipsammum*, *Leptolyngbya frigida*, *Microcoleus vaginatus*, *Phormidium* spp. and *Spirulina* spp. The Oscillatoriaceae family is commonly found in extreme environments, including sulphide-rich springs (Castenholz, 1977), hypersaline mats (Nübel *et al.*, 2000) as well as hyperarid chasmoendolithic (Yung *et al.*, 2014) and hypolithic niches (Chan *et al.*, 2012) of the Dry Valleys.

Oscillatorian non-heterocystous Cyanobacteria are predicted to be critical to Dry Valley functioning and are capable of performing N_2 fixation in microbial mats (Paerl *et al.*, 2000), CO_2 fixation by photosynthesis (Smith, 1983). This taxon has also been implicated in fermentation in microbial communities (Stal and Moezelaar, 1997). The detection of these lineages in soil and cryptic niches

suggests functional roles in local Dry Valley ecology. Antarctic soils and cryptic niche communities have been shown to possess the potential to drive important biological processes as a direct result of photoautotrophic community members (Cowan *et al.*, 2011b, Niederberger *et al.*, 2012, Chan *et al.*, 2013). However, it is unknown whether photoautotrophic taxa found in these environments represent members driving ongoing soil processes or are ancestral fingerprints of previous colonisation events (Cowan *et al.*, 2014).

The high diversity and abundance of Oscillatorian lineages in the 'wet' hypolith suggests a greater genetic capacity, as a direct result of increased Cyanobacterial diversity, for photoautotrophic fixation than in the communities exposed to hyperarid conditions which consistently had fewer signals associated with the taxon. Hypoliths have been suggested to be important mediators of carbon and nitrogen turnover in the Dry Valleys (Hopkins *et al.*, 2009), and the inundated hypolith community assessed here appears to possess a larger Cyanobacterial population than has previously been found at hyperarid locations of the Dry Valleys (Pointing *et al.*, 2009, Khan *et al.*, 2011, Chan *et al.*, 2012, Makhalanyane *et al.*, 2013a). A direct interpretation of these data suggests that improved moisture conditions may increase the genetic capacity of hypoliths, on the basis of increased diversity, to perform local nutrient turnover by virtue of more diverse Oscillatorian cyanobacterial populations. The augmented moisture content may also lead to the association of a complex heterotrophic assemblage, which is supported in the sample analysed here.

The hypolith community comprised 7 unique Cyanobacterial taxa, including 4 Oscillatorian lineages, and had the highest Cyanobacterial abundance of the samples analysed here with 72 $OTUs_{0.03}$. Surprisingly, both endolithic communities had fewer Cyanobacterial phylotypes than the soil population, which was predicted to be low in Cyanobacterial signals on the basis of previous studies (Pointing *et al.*, 2009, Chan *et al.*, 2012, Makhalanyane *et al.*, 2013a). H3, S1 and E2 populations were consistent in Cyanobacterial diversity and were supported by a co-dominance of Oscillatorian Cyanobacteria; *Phormidium* spp. (27.91% total Cyanobacterial sequences) and *L. frigida* (43.4%). These anaerobic non-heterocystous (Type II Nitrogen-fixing) Cyanobacteria are commonly found in Dry Valley hypolithic (Pointing *et al.*, 2009, Caruso *et al.*, 2011, Cowan *et al.*, 2011b, Khan *et al.*, 2011), endolithic (De La Torre *et al.*, 2003, Yung *et al.*, 2014) and microbial mat communities (Jungblut *et al.*, 2005, Adams *et al.*, 2006), as well as in maritime soils of the continent such as Miers Valley (Wood *et al.*, 2008). Warren-Rhodes *et al.* (2006) described an increase in hypolith-associated *Phormidium* signals at wetter sites of the Atacama Desert than at more xeric sites. Similarly, Yung *et al.* (2014) showed that Oscillatorian phylotypes were more abundant in chasmoendoliths retrieved from North-facing, warmer and wetter, slopes of the Miers Valley than communities retrieved from South-facing, colder and drier, slopes. Both studies report

declines in Cyanobacterial, and to a lesser extent bacterial, diversity with increasing aridity. These findings are highly congruent with diversity trends observed here which show that H3 supports a more diverse Cyanobacterial population than the hyperarid communities (Figure 21).

A review on hypolith biodiversity by Chan *et al.* (2012) reported that polar hypoliths are nearly exclusively dominated by Oscillatoriales compared to non-polar hypolithic communities that may span up to six Cyanobacterial orders. The hypolith community assessed here appears to be more diverse than polar hypoliths retrieved from xeric locations and spans three orders; Pleurocapsales, Oscillatoriales and Nostocales as well as Unclassified Cyanobacteria.

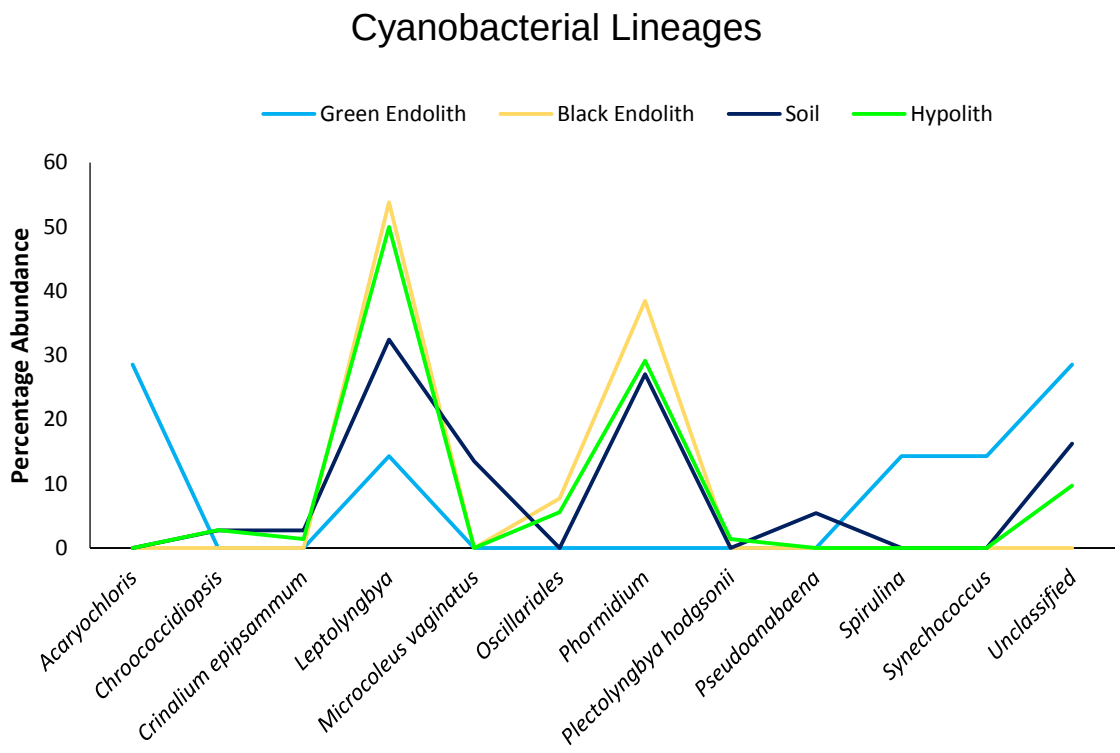


Figure 21. The taxonomic classifications of representative Cyanobacteria from four distinct niche habitats. Relative abundances were determined from specific taxa identified from BLASTn comparisons at a 97.5% confidence and are detailed in Table 13.

E5.1 was low in Cyanobacterial diversity and lacked functionally important *Phormidium* while *L. frigida* was found once (14.29%). Moreover, Oscillatorian members of E5.1 are limited to single *Leptolyngbya* and *Spirulina* signals. The contributions of Oscillatoriales to the remaining populations were markedly higher, representing between 76 and 100% of Cyanobacterial sequences. Endolithic contributions to Cyanobacterial diversity were highly disparate from each other despite the low geographical distance between them, the highly similar environmental

conditions and colonisation of similar sandstone rocks. Whether these diversity dissimilarities are a result of interactions with fungal or eukaryal species, which are predicted to be non-homogenously distributed across these communities remains unclear. Alternatively, these differences may be due to differing chemistries which have been shown to drive soil diversity patterns on the regional scale (Lee *et al.*, 2012a). Microbial fingerprinting revealed that β-diversity estimates of Cyanobacterial populations was highest in high productivity niches, particularly endoliths - this is supported by the contrasting structures of endolith communities observed here. The unique community constitutions found here may reflect differences in endolith community assembly. Community development in endoliths and hypoliths is predicted to be highly variable on the basis of T-RFLP and pyrosequencing data presented here which suggests a strong role for stochastic community assembly. These data appear to support the concept that high productivity niches are more influenced by stochastic processes during community development (Chase, 2010).

Chroococcidiopsis-like sequences were low in abundance across all samples, and were found exclusively in hypolith and soil populations. *Chroococcidiopsis* are unicellular Cyanobacteria that are highly adapted to desiccation stress and have been shown to persist in some of the most xeric environments on Earth including the Dry Valleys (Yung *et al.*, 2014), and the Atacama desert, Chile (Warren-Rhodes *et al.*, 2006). A recent study by Bahl *et al.* (2011) analysed *Chroococcidiopsis* isolates from numerous arid locations worldwide to demonstrate the global distribution of desert-associated Cyanobacteria and concluded that contemporary distributions are due to ancient evolutionary divergence patterns. This and other studies have described *Chroococcidiopsis* as important functional members of hypolithic communities (Caruso *et al.*, 2011, De Los Ríos *et al.*, 2014).

Microcoleus vaginatus (Oscillatoriales) was found exclusively in soil and constituted 13.56% of Cyanobacterial signals in that community. The hydrotactic response of this Cyanobacterium was shown by wetting desert surface soils and biological soil crusts which caused a visible ‘greening’ of the surface (Garcia-Pichel *et al.*, 2001, Rajeev *et al.*, 2013). *M. vaginatus* has not been previously found in Dry Valley soils, although members have been described in the maritime soils of Schirmacher Oasis (Pankow *et al.*, 1991) and Windmill Hills (Ling and Seppelt, 1998). *M. vaginatus* is frequently found in hot desert soils and biological soil crusts (Pointing and Belnap, 2012, Steven *et al.*, 2013).

The phylogenetic relationships of retained Cyanobacterial sequences were tested using a maximum likelihood tree (1000 replications) to closely-related environmental Cyanobacteria sequences retrieved from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) (Figure 22). This was performed to reveal similarities of the sequences retrieved here with Cyanobacterial

isolates from the Dry Valleys and other arid environments. Sequences from a phylogenetic study by Pointing *et al.* (2009) were included for Dry Valley community comparisons. *Clostridium perfringens*, an anaerobic member of the phylum Firmicutes, was included as the outgroup. Visualisation of reconstructed trees was performed in MEGA using a non-protein coding substitution model (Figure 22). A Jukes-Cantor distance model was applied with a Gamma Distributed (G) substitution rate.

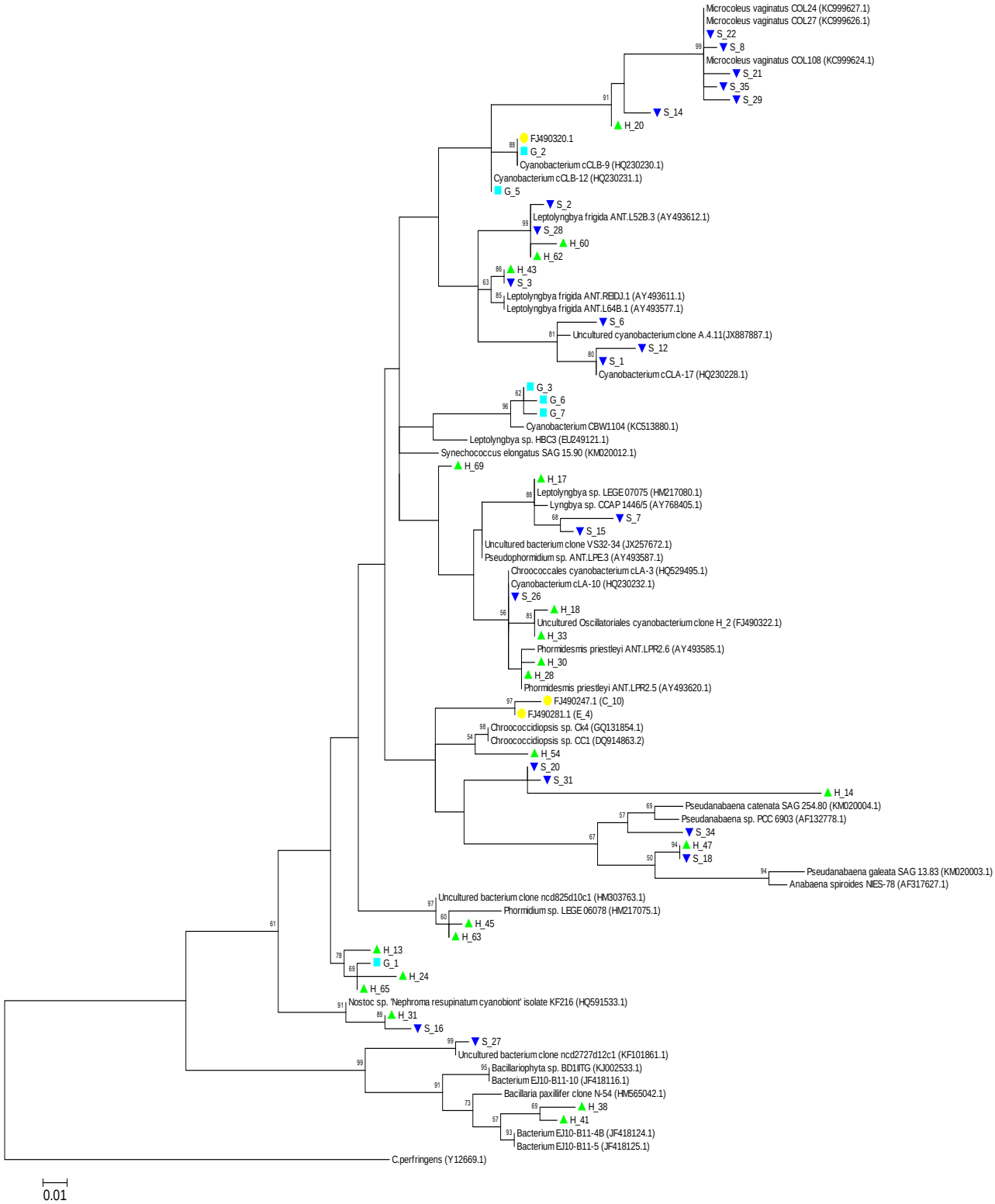


Figure 22. Maximum Likelihood tree showing the phylogenetic relationships of Cyanobacterial phylotypes recovered from hypolith, soil and endolith samples ($OTUs_{0.03}$) from Victoria Valley on the basis of 16S rRNA genes. Closest species and genotype sequences are provided from the GenBank database, which includes cultured and environmental sequences. Accessions numbers are provided in parenthesis. Nodes are supported by bootstrap values reported as percentages for 1000 resampled datasets in which the branches were supported in >50% of trees. ▲ Hypolith (H), ■ Endolith (G), ▼ Soil (S) and ● Pointing *et al.* (2009). (Scale bar, 0.01-nucleotide changes per position).

Figure 22 shows that 5 sequences from S1 clustered into a *M. vaginatus* clade (99% confidence) with sequences from North American desert biocrusts (Garcia-Pichel *et al.*, 2013); neither H3 nor the endoliths provided reads associated with this lineage. Overall, Cyanobacteria grouped into the orders Oscillatoriales, Pleurocapsales and Nostocales, with Oscillatoriales dominating globally. However, endolithic sequences were not confidently clustered with retrieved lineages beyond the phylum level (Figure 22), despite high similarity with *L. frigida* and *Phormidium* spp. sequences on the RDP database.

Cyanobacterial sequences (*n*=124) are listed in Appendix D, Table S2. Table S2 provides additional information about the retained cyanobacterial signatures including the closest homologous sequence on the GenBank database, the percentage identity and source of the related sequence.

4.4 Conclusion

This aspect of the study shows that the distinct habitats of the Victoria Valley have demonstrably different bacterial diversity patterns and relative abundances of phyla on the basis of tagged 16S rRNA gene-based pyrosequencing. This is largely consistent with the results obtained by Pointing *et al.* (2009) which used ribosomal RNA gene-defined community assembly to show ecological niche specialisation between distinct Dry Valley habitats.

In this study, soils were characterised by high bacterial diversity relative to specialised cryptic communities present in the hypolith and endoliths. However, the hypolith community had a greater bacterial abundance compared to soil, while both endolith communities were phylum-poor and generally low in abundance. Hypolith bacterial diversity was shown to be greater than previous estimates from polar deserts (Pointing *et al.*, 2009, Chan *et al.*, 2012), and is predicted to be the result of high moisture content on the basis of these results. The heterotrophic component of the hypolithic community was extensive, spanning 8 bacterial phyla, while Cyanobacterial signals were confidently assigned to three orders. Most Cyanobacterial populations were dominated by both *Phormidium* spp. and *L. frigida*, which are functional members of Oscillatoriales.

Many bacterial species were shared between soil and specialised niches which appears to support the hypothesis that hypolith and endolith communities do not develop independently of surrounding soils (Makhalanyane *et al.*, 2013b). The bacterial diversity within Victoria Valley appears to be greater than other Dry Valley regions based on the phylogenetic information obtained here (Cary *et al.*, 2010), this trend was noted across all habitats analysed. However the cause of the high bacterial diversity estimates remains unclear and may in fact be due to multiple inclusive factors such as superior sequencing depth and accuracy relative to previous studies. These results suggest that the bacterial diversity of Dry Valley habitats, notably in specialised niches, have been underestimated.

Photosynthetic processes may be driven by both Cyanobacteria and Chlorobi in the hypolith community, while Chloroflexi may represent an alternative source of photoautotrophy in endoliths and soil communities. Moisture content is predicted to increase the genetic diversity (and possibly genetic capacity) of communities to drive carbon and nitrogen turn-over as functionally important taxa may increase in abundance locally. Alterations to local and regional moisture regime may have effects on the local nutrient cycling, particularly carbon and nitrogen turn-over based on these results (Hopkins *et al.*, 2009, Chan *et al.*, 2013). These critical processes may be solely mediated by photoautotrophs, primarily Cyanobacteria, in desert environments. Hypolithic communities in the Dry Valleys have been predicted to supply 0.38 kg N yr^{-1} (Cowan *et al.*, 2014). This is a significant contribution to local nitrogen budgets as exogenous sources of this nutrient are nearly entirely

absent. Autotrophic carbon fixation is also driven by hypoliths in cold deserts (Hopkins *et al.*, 2009) and is estimated at 0.8±0.3 g m^{-2} yr^{-1} in a polar Arctic desert (Cockell and Stokes, 2004). The roles of these communities in nitrogen and carbon sequestration is substantial due to the highly oligotrophic nature of Dry Valley soils. Increased water content, through augmented glacial and permafrost ice melt, is predicted to alter local microbial structures as these communities are thought to be active and responsive to environmental changes. Based on these results Cyanobacterial abundance may be predicted to increase locally as a result of increased moisture content (Wood *et al.*, 2008).

Additionally, increased Cyanobacterial prevalence has important implications in terms of cyanotoxin production. Cyanotoxins are a group of natural toxins of which microcystin is the most diverse in terms of structure. Microcystin has been shown to be produced in microbial mat communities by *Leptolyngbya* and *Phormidium* members (Kleinteich *et al.*, 2014). Environmental changes are predicted to influence Cyanobacterial abundance which may directly augment the number of cyanotoxin-producing Cyanobacteria in those communities (Kleinteich *et al.*, 2012).

However, these results require further support through more extensive sequencing, improving the sampling effort as well as long-term monitoring. Importantly, the diversity described here is from a single time point and details the most abundant phylotypes within each community. Applying temporal data to ecological studies is critical to evaluate community changes as environmental parameters are altered. However, ecological studies performed along environmental gradients may reveal the limits of microbial colonisation and differences in community structure as a result of shifting environmental conditions.

Chapter 5: Thesis Synthesis

5.1 Summary of the Results

Increased water availability in continental Antarctica as a result of climate change is predicted to have dramatic consequences on the local microbial diversity (Convey, 2006, Convey, 2011, Cowan *et al.*, 2014, Fountain *et al.*, 2014). In this study, culture-independent approaches were applied to elucidate the effect of distinct moisture regimes on local bacterial consortia. Samples from two distinct moisture regimes were interrogated; defined *a priori* as hyperarid and moisture sufficient. Hypolithic and mat communities were sampled from a high moisture regime - within a melt water stream. These consortia were compared to soils and endolithic communities that were retrieved from hyperarid sites adjacent to the stream, which were critically low in bioavailable moisture content. Differences in community structure may result in changes to local microbial functionality on the basis of alterations to regional and local diversity (Stomeo *et al.*, 2012, Chan *et al.*, 2013, Van Horn *et al.*, 2013). Rapid wetting of previously arid soils may have detrimental consequences on local bacterial communities (Wood *et al.*, 2008, Fountain *et al.*, 2014). Addressing changes to bacterial and cyanobacterial diversity as a result of liquid water availability was the focus of this research presented here.

Highly congruent patterns of bacterial community dispersal emerged from data resolved through microbial fingerprinting and pyrosequencing techniques. Ecological patterns generated using traditional microbial fingerprinting and next-generation pyrosequencing of 16S rRNA genes have previously revealed coherent estimates of bacterial diversity (Gobet *et al.*, 2013). The analyses performed in this study revealed that niche type was a significant delineator of bacterial communities, as has been proposed previously in the Dry Valleys (Wood *et al.*, 2008, Pointing *et al.*, 2009, Khan *et al.*, 2011, Makhalanyane *et al.*, 2013a). However, this data suggests that Cyanobacterial populations were not significantly influenced by niche. These results are consistent with previous findings suggesting that Cyanobacterial and heterotrophic bacterial community composition are not driven by similar ecological processes (Drakare and Liess, 2010). Additionally, the soil communities of Victoria Valley are proposed to be more diverse in bacterial signatures relative to cryptic niches, hypoliths and endoliths of this region, on the basis of the data provided here. A direct interpretation of these data suggests the soils may serve as reservoirs of general bacterial diversity in this desert system which has been suggested as a mechanism of cryptic community development in hyperarid deserts (Makhalanyane *et al.*, 2013b). Pyrosequencing data revealed that 11 bacterial phyla were found in the diverse soil community.

Within-community differences were higher in lithic communities (higher β-diversity); mats and soils appear to be more homogenously distributed across this system. High β-diversity estimates have

been linked with more productive communities which are thought to be governed less by environmental selection than by stochasticity (Chase, 2010). Specialised communities are also predicted to more susceptible to extinction events than generalist consortia (Tilman, 1994) and, on the basis of these results, a loss of diversity by sudden environmental changes is more likely to influence lithic communities than soils or mat communities.

Cyanobacteria were found in all samples analysed yet strikingly, relative abundance was shown to be highest in the soil community, followed by hypoliths and then endoliths according to both approaches implemented here. The ubiquity of cyanobacteria across all habitats is in alignment with the suggestion by Wood *et al.* (2008) but counters suggestions that the most hyperarid Dry Valley soils are devoid of Cyanobacterial signatures entirely (Pointing *et al.*, 2009). Cyanobacteria showed low relative abundances in endolithic consortia which have previously been suggested to be dominated by the phylum (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007, Cowan *et al.*, 2010, Khan *et al.*, 2011, Makhalanyane *et al.*, 2013a). However, the hypolith community was highly diverse in Cyanobacterial taxa according to the pyrosequencing data. The hypolithic community was shown to be more diverse in Cyanobacterial signatures than similar communities retrieved from hyperarid sites in polar deserts (Cockell and Stokes, 2004, Wong *et al.*, 2010, Chan *et al.*, 2012). This difference is thought to be the result of high water availability.

Despite the expected relaxation of regional hyperaridity and temperature stress in continental Antarctica (Kleinteich *et al.*, 2012, Fountain *et al.*, 2014), increases in UV irradiation levels, possible changes in freeze-thaw frequency and cooling in some regions (Doran *et al.*, 2002) are predicted to augment desiccation stress (Convey, 2011). This is likely to compromise colonisation in the Dry Valleys (Convey, 2006). These conflicting environmental changes make Dry Valley ecology an exciting avenue of research as both positive and negative factors influencing diversity can be assessed across various temporal and spatial scales. Predicting the responses of microbial communities to multiple environmental perturbations is challenging without long-term monitoring programs and may require the continued assessment of microbial guilds to reveal the effects of climatic changes on community structure and function. In line with this aim, this study has revealed the positive consequence of changes in moisture content on local cyanobacterial communities. This supported the hypothesis that increased liquid water availability would significantly alter bacterial community structure.

The accumulation of data from this research suggests that increased moisture content alone may lead to local Cyanobacterial proliferation in continental Antarctica. These changes have the potential to alter microbial community composition, productivity and expansion (Convey, 2006). Increased Cyanobacterial abundance is likely to positively influence biological productivity in the

Dry Valley system by increasing carbon and nitrogen provenance locally (Cowan *et al.*, 2011b). By contrast, larger Cyanobacterial populations may to lead to an increased production of cyanotoxins. Environmental perturbations, including temperature fluctuations, are thought to affect Cyanobacterial diversity in freshwater polar ecosystems by promoting cyanotoxin-producing species in local populations (Kleinteich *et al.*, 2012). This is predicted to be detrimental to local desert ecosystems, as has been proposed for microbial communities from maritime locations of the continent (Kleinteich *et al.*, 2014).

5.2 Limitations of the study

Environmental microbial communities are influenced by numerous factors that interact to drive global dispersal patterns (Fierer and Jackson, 2006). This combination of variables make microbial ecology studies challenging, particularly when addressing single factors in spatial or temporal contexts. Although both habitat and moisture content were assessed independently in this study, several microenvironmental variables are likely to have contributed to the observed diversity patterns found here. For example, soil pH has been shown to be a major driver of dispersal patterns globally (Fierer and Jackson, 2006). Similarly, soil geochemistry has been implicated as a major delineator of Dry Valley soil communities at the regional scale (Lee *et al.*, 2012a).

Microbial ecology studies have typically focused on explaining spatial variation, although microbial communities are known to change rapidly over short temporal scales as well (Febria *et al.*, 2012). Incorporating spatial data, with continual monitoring of communities over time (temporal information), may reveal the rate at which communities are able to respond and adapt to changes in habitat parameters, such as moisture content and UV irradiation.

Methodological constraints may have limited the scope of results obtained in this project. Inherent biases associated with PCR amplification, DNA isolation, next-generation sequencing and unequal sample numbers were noted - the robustness of each test was improved where possible. These issues were addressed throughout the experimental design by introducing replicates and removing chimeras. Additionally the statistical tests implemented here account for unequal sample and sequence numbers by calculating to the lowest effort. The use of null models that predict random or non-normal distributions of data were implemented to increase the power of observations. The community fingerprints and sequence data presented here are inevitably over-simplified, although these techniques still represent the most viable methods of describing and interpreting ecological microbial patterns of diversity (Fierer and Jackson, 2006, Lee *et al.*, 2012b, Gobet *et al.*, 2013). Finally the sampling strategy employed limits the power of the observations drawn here and would have been greatly improved had we been able to compare moisture regimes within habitats (i.e. soil) rather than between distinct niches.

Addressing ecological variation remains a fundamental and contemporary avenue of desert microbial research - particularly as notable climatic and anthropogenic threats to the Antarctic and other desert regions emerge. It is critically important to better understand microbial responses to environmental changes as local and regional parameters shift as a result of climate change (Cowan *et al.*, 2014). The shift in local and regional environmental parameters as a result of climate change is expected to alter microbial responses in the delicate and pristine Dry Valleys. Researching these intricate factors and interactions lays the groundwork for future knowledge expansion in desert microbial ecology.

Appendices

Appendix A

Table S1. Data regarding the properties of each sample utilised in this study including niche type, sampling co-ordinates and date.

Sample name	Sample type	Sampling date	Sampling co-ordinates	Mineral type	Community type
H1	Hypolith	21 January 2013	S 77° 20.130 E 161° 38.049	Quartz	Type I
H2	Hypolith	21 January 2013	S 77° 20.366 E 161° 38.773	Quartz	Type I
H3	Hypolith	21 January 2013	S 77° 20.505 E 161° 39.291	Quartz	Type I
H4	Hypolith	21 January 2013	S 77° 20.509 E 161° 39.268	Quartz	Type I
H5	Hypolith	21 January 2013	S 77° 20.497 E 161° 39.261	Quartz	Type I
H6	Hypolith	21 January 2013	S 77° 20.516 E 161° 39.304	Quartz	Type I
H7	Hypolith	21 January 2013	S 77° 20.513 E 161° 39.311	Marble	Type I
H8	Hypolith	21 January 2013	S 77° 20.533 E 161° 39.446	Marble	Type I
H9	Hypolith	21 January 2013	S 77° 20.533 E 161° 39.447	Marble	Type I
H10	Hypolith	21 January 2013	S 77° 20.533 E 161° 39.448	Quartz	Type I
H11	Hypolith	21 January 2013	S 77° 20.513 E 161° 39.311	Quartz	Type I
H12	Hypolith	21 January 2013	S 77° 20.527 E 161° 39.399	Quartz	Type I
H13	Hypolith	18 January 2013	S 77° 20.527 E 161° 39.376	Marble	Type I
H14	Hypolith	18 January 2013	S 77° 20.527 E 161° 39.376	Quartz	Type I
S1	Soil	21 January 2013	S 77° 20.533 E 161° 39.466		
S2	Soil	21 January 2013	S 77° 20.509 E 161° 39.279		
S3	Soil	21 January 2013	S 77° 20.509 E 161° 39.280		
S4	Soil	21 January 2013	S 77° 20.509 E 161° 39.281		
S5	Soil	21 January 2013	S 77° 20.509 E 161° 39.282		
E1	Endolith	21 January 2013	S 77° 20.515 E 161° 39.302	Sandstone	Cyanobacteria
E1b	Endolith	21 January 2013	S 77° 20.515 E 161° 39.302	Sandstone	Cyanobacteria
E2	Endolith	21 January 2013	S 77° 20.509 E 161° 39.272	Sandstone	Fungal
E3	Endolith	21 January 2013	S 77° 20.492 E 161° 39.240	Sandstone	Fungal
E4	Endolith	21 January 2013	S 77° 20.521 E 161° 39.333	Sandstone	Cyanobacteria
E4.1	Endolith	21 January 2013	S 77° 20.521 E 161° 39.330	Sandstone	Cyanobacteria
E4.2	Endolith	21 January 2013	S 77° 20.521 E 161° 39.331	Sandstone	Cyanobacteria
E4.3	Endolith	21 January 2013	S 77° 20.521 E 161° 39.332	Sandstone	Cyanobacteria
E5.1	Endolith	21 January 2013		Sandstone	Cyanobacteria
E5.2	Endolith	21 January 2013		Sandstone	Cyanobacteria
M1	Mat	21 January 2013	S 77° 20.517 E 161° 39.318	Wet mat	Mat
M2	Mat	21 January 2013	S 77° 20.517 E 161° 39.319	Wet mat	Mat
M3	Mat	21 January 2013	S 77° 20.517 E 161° 39.320	Wet mat	Mat
M4	Mat	21 January 2013	S 77° 20.517 E 161° 39.321	Wet mat	Mat

Appendix B

Media

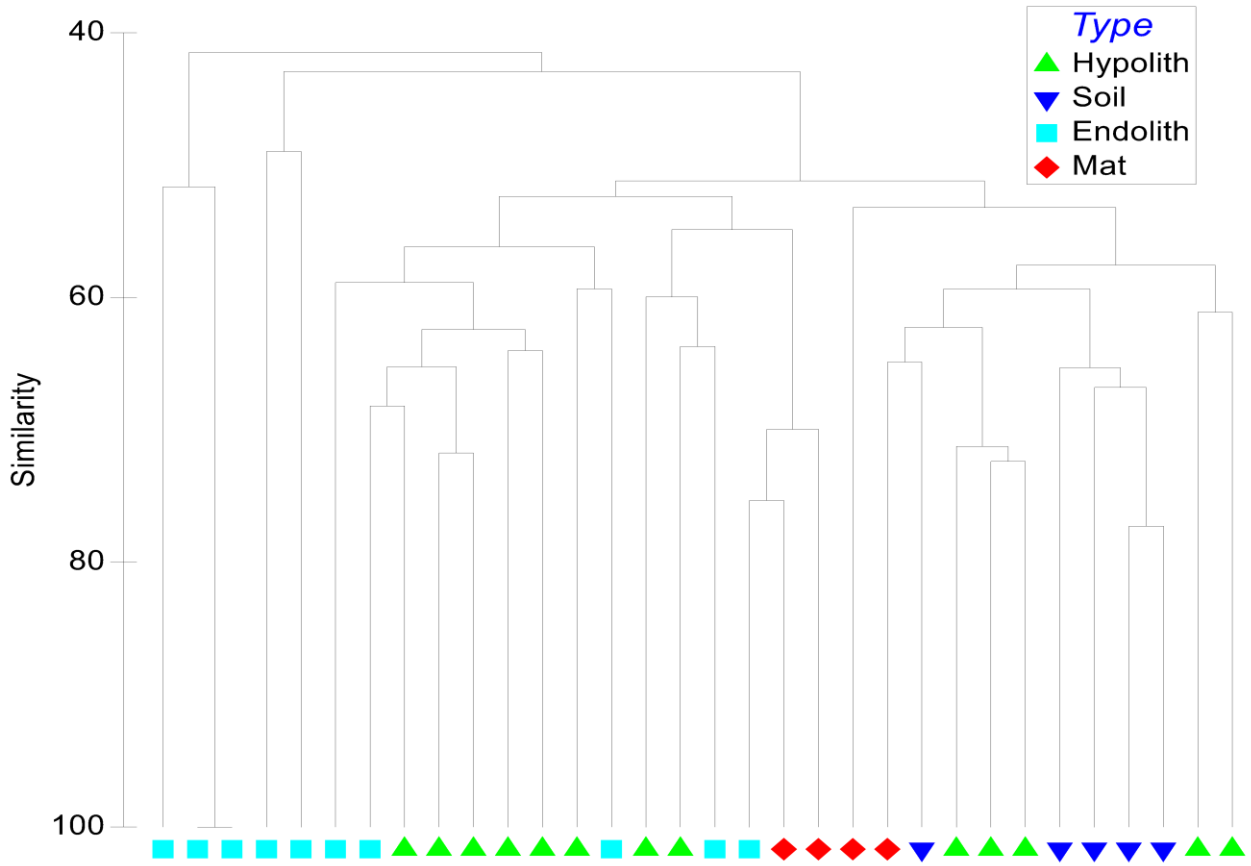
1. DNA Extraction Buffer (values in ml/L)

1M Tris-HCl (pH 7.6)	50
1M EDTA (pH 8.0)	50
20% SDS	25
5M NaCl	10

The total volume was made up to 1 L by adding distilled water (ddH_2O). The pH of the solution was adjusted to 8.0 and stirred until all components were dissolved. Solutions were then autoclaved after which 25 ml SDS was added.

Appendix C

Hierarchical clustering analysis of group averages performed in Primer 6. Clustering revealed that habitats with lower β-diversity, mats and soils, grouped together, in comparison to hypoliths and endoliths which grouped apart despite showing similarity.



Appendix D

Table S2. Retrieved sequences from the NCBI nucleotide database (GenBank) using the BLASTn function. Only Cyanobacterial sequences are included here, sample source (this study and) isolation source (closest reference sequence) are provided.

Sequence ID	Sample source	Accession number	Closest Homolog in GenBank	% Identity	Origin	Isolation source
E_1	Endolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
E_2	Endolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	100	Antarctica	Benthic mats
E_3	Endolith	HQ832919.1	*Pseudanabaena* sp. LEGE 07190	99	Portuguese coast	Soil
E_4	Endolith	EU078511.1	*Phormidium* sp. LMECYA 214	94	Portugal	Freshwater
E_5	Endolith	AY493579.1	*Phormidesmis priestleyi* ANT.L52.6	97	Antarctica	Benthic mats
E_6	Endolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	97	Antarctica	Benthic mats
E_7	Endolith	AY493586.1	*Phormidesmis priestleyi* ANT.LACV5.1	96	Antarctica	Benthic mats
E_8	Endolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	94	Antarctica	Benthic mats
E_9	Endolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	96	Antarctica	Benthic mats
E_10	Endolith	HQ832919.1	*Pseudanabaena* sp. LEGE 07190	97	Portugal	Freshwater
E_11	Endolith	EU078511.1	*Phormidium* sp. LMECYA 214	94	Portugal	Freshwater
E_12	Endolith	AY493586.1	*Phormidesmis priestleyi* ANT.LACV5.1	99	Antarctica	Benthic mats
G_1	Endolith	KF690274.1	*Synechococcus* sp. CALU 1739	93	Lake Ladoga	Freshwater
G_2	Endolith	HQ230230.1	Cyanobacterium cCLB-9	99	Arctic	Snow
G_3	Endolith	KC513880.1	Cyanobacterium CBW1104	98	Chesapeake estuary	Freshwater
G_4	Endolith	GQ859652.1	*Leptolyngbya tenuis* PMC304.07	99	Sub-Saharan Africa	Freshwater
G_5	Endolith	HQ230231.1	Cyanobacterium cCLB-12	99	Arctic	Snow
G_6	Endolith	KC513880.1	Cyanobacterium CBW1104	98	Chesapeake estuary	Freshwater
G_7	Endolith	KC513880.1	Cyanobacterium CBW1104	97	Chesapeake estuary	Freshwater
H_1	Hypolith	AB015059.1	*Synechococcus* sp. PCC 7003	93	Marine various	Marine
H_2	Hypolith	JQ029935.1	*Oscillatoria* sp. Ind77	92		
H_3	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
H_4	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	98	Antarctica	Benthic mats
H_5	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	97	Antarctica	Benthic mats

Sequence ID	Sample source	Accession number	Closest Homolog in GenBank	% Identity	Origin	Isolation source
H_6	Hypolith	HQ230227.1	Cyanobacterium cCLA-11	99	Arctic	Snow
H_7	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	96	Antarctica	Benthic mats
H_8	Hypolith	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
H_9	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	99	Antarctica	Benthic mats
H_10	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	99	Antarctica	Benthic mats
H_11	Hypolith	GQ859652.1	*Leptolyngbya tenuis* PMC304.07	99	Sub-Saharan Africa	Freshwater
H_12	Hypolith	EU078511.1	*Phormidium* sp. LMECYA 214	94	Portugal	Freshwater
H_13	Hypolith	KF690274.1	*Synechococcus* sp. CALU 1739	92	Lake Ladoga	Freshwater
H_14	Hypolith	AY493615.1	*Plectolyngbya hodgsonii* ANT.LG2.1	100	Antarctica	Benthic mats
H_15	Hypolith	DQ493874.1	*Phormidium autumnale* Ant-Ph68	100	Antarctic peninsula	Soil
H_16	Hypolith	AY493581.1	*Phormidesmis priestleyi* ANT.L66.1	100	Antarctica	Benthic mats
H_17	Hypolith	HM217080.1	*Leptolyngbya* sp. LEGE 07075	100	Portugal	Freshwater
H_18	Hypolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	98	Antarctica	Benthic mats
H_19	Hypolith	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
H_20	Hypolith	JQ687337.1	*Phormidium autumnale* CYN79	99	Antarctica	
H_21	Hypolith	JQ687337.1	*Phormidium autumnale* CYN79	98	Antarctica	
H_22	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
H_23	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	97	Antarctica	Benthic mats
H_24	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	97	Antarctica	Benthic mats
H_25	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
H_26	Hypolith	JQ687335.1	*Phormidesmis priestleyi* CYN71	96	Antarctica	
H_27	Hypolith	HQ230227.1	Cyanobacterium cCLA-11	99	Arctic	Snow
H_28	Hypolith	AY493620.1	*Phormidesmis priestleyi* ANT.LPR2.5	100	Antarctica	Benthic mats
H_29	Hypolith	AF170758.1	*LPP*-group cyanobacterium QSSC8cya	100	Antarctica	Hypolith
H_30	Hypolith	AY493620.1	*Phormidesmis priestleyi* ANT.LPR2.5	99	Antarctica	Benthic mats
H_31	Hypolith	NR_112218.1	*Crinalium epipsammum* strain SAG 22.89	98		
H_32	Hypolith	AY493579.1	*Phormidesmis priestleyi* ANT.L52.6	96	Antarctica	Benthic mats
H_33	Hypolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	98	Antarctica	Benthic mats
H_34	Hypolith	JQ687337.1	*Phormidium autumnale* CYN79	99	Antarctica	
H_35	Hypolith	KC463233.1	*Leptolyngbya subtilissima* Ep_Yyy1300	99	South Africa	BSC

Sequence ID	Sample source	Accession number	Closest Homolog in GenBank	% Identity	Origin	Isolation source
H_36	Hypolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	96	Antarctica	Benthic mats
H_37	Hypolith	EU078511.1	*Phormidium* sp. LMECYA 214	93	Portugal	Freshwater
H_38	Hypolith	JF706701.1	Diatom EJ10-B11-11A	99	Iceland	Lava Flows
H_39	Hypolith	JQ687337.1	*Phormidium autumnale* CYN79	99	Antarctica	
H_40	Hypolith	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
H_41	Hypolith	JF418124.1	Bacterium EJ10-B11-4B	98	Iceland	Lava Flows
H_42	Hypolith	AF170758.1	*LPP*-group cyanobacterium QSSC8cya	99	Antarctica	Hypolith
H_43	Hypolith	AY493576.1	*Leptolyngbya frigida* ANT.L53B.2	99	Antarctica	Benthic mats
H_44	Hypolith	AY493584.1	*Leptolyngbya* sp. ANT.L52.1	97	Antarctica	Benthic mats
H_45	Hypolith	JN382221.1	*Phormidium terebriforme* UAM 409	97	Spain	Mat
H_46	Hypolith	JQ687337.1	*Phormidium autumnale* CYN79	99	Antarctica	
H_47	Hypolith	AM259268.1	*Pseudanabaena* sp. 0tu30s18	95	Lake Tuusulanjarvi	Freshwater
H_48	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	98	Antarctica	Benthic mats
H_49	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	97	Antarctica	Benthic mats
H_50	Hypolith	AY493579.1	*Phormidesmis priestleyi* ANT.L52.6	98	Antarctica	Benthic mats
H_51	Hypolith	HQ832915.1	*Leptolyngbya* cf. *halophila* LEGE 06152	97	Portuguese coast	Soil
H_52	Hypolith	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
H_53	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	98	Antarctica	Benthic mats
H_54	Hypolith	DQ914863.2	*Chroococcidiopsis* sp. CC1	96	China	Hypolith
H_55	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	98	Antarctica	Benthic mats
H_56	Hypolith	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
H_57	Hypolith	AY493581.1	*Phormideismis priestleyi* ANT.L66.1	98	Antarctica	Benthic mats
H_58	Hypolith	GQ859652.1	*Leptolyngbya tenuis* PMC304.07	99	Sub-Saharan Africa	Freshwater
H_59	Hypolith	EF150780.1	*Chroococcidiopsis* sp. HSC19	94	Australia	Stromalite
H_60	Hypolith	AY493612.1	*Leptolyngbya frigida* ANT.L52B.3	99	Antarctica	Benthic mats
H_61	Hypolith	AF170758.1	*LPP*-group cyanobacterium QSSC8cya	98	Antarctica	Hypolith
H_62	Hypolith	AY493612.1	*Leptolyngbya frigida* ANT.L52B.3	100	Antarctica	Benthic mats
H_63	Hypolith	JN382221.1	*Phormidium terebriforme* UAM 409	99	Spain	Mat
H_64	Hypolith	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	98	Antarctica	Benthic mats
H_65	Hypolith	KF690274.1	*Synechococcus* sp. CALU 1739	95	Lake Ladoga	Freshwater

Sequence ID	Sample source	Accession number	Closest Homolog in GenBank	% Identity	Origin	Isolation source
H_66	Hypolith	HQ230227.1	Cyanobacterium cCLA-11	99	Arctic	Snow
H_67	Hypolith	JQ687335.1	*Phormidesmis priestleyi* CYN71	97	Antarctica	
H_68	Hypolith	GQ859652.1	*Leptolyngbya tenuis* PMC304.07	98	Sub-Saharan Africa	Freshwater
H_69	Hypolith	AF170758.1	*LPP*-group cyanobacterium QSSC8cya	99	Antarctica	Hypolith
S_1	Surface soil	HQ230228.1	Cyanobacterium cCLA-17	100	Arctic	Snow
S_2	Surface soil	AY493612.1	*Leptolyngbya frigida* ANT.L52B.3	99	Antarctica	Benthic mats
S_3	Surface soil	AY493576.1	*Leptolyngbya frigida* ANT.L53B.2	99	Antarctica	Benthic mats
S_4	Surface soil	AY493580.1	*Phormidesmis priestleyi* ANT.LG2.4	99	Antarctica	Benthic mats
S_5	Surface soil	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	98	Antarctica	Benthic mats
S_6	Surface soil	HQ230228.1	Cyanobacterium cCLA-17	95	Arctic	Snow
S_7	Surface soil	HM217080.1	*Leptolyngbya* sp. LEGE 07075	96	Portugal	Freshwater
S_8	Surface soil	KC463217.1	*Microcoleus vaginatus* EucYyy1000	98	South Africa	BSC
S_9	Surface soil	AY493611.1	*Leptolyngbya frigida* ANT.REIDJ.1	99	Antarctica	Benthic mats
S_10	Surface soil	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	98	Antarctica	Benthic mats
S_11	Surface soil	AY493580.1	*Phormidesmis priestleyi* ANT.LG2.4	99	Antarctica	Benthic mats
S_12	Surface soil	HQ230228.1	Cyanobacterium cCLA-17	97	Arctic	Snow
S_13	Surface soil	JQ687330.1	*Phormidium* sp. CYN64	100	Antarctica	
S_14	Surface soil	GQ451414.1	*Phormidium autumnale* CYN55	100	New Zealand	Benthic mats
S_15	Surface soil	HM217080.1	*Leptolyngbya* sp. LEGE 07075	97	Portugal	Freshwater
S_16	Surface soil	CP003620.1	*Crinalium epipsammum* PCC 9333	97		
S_17	Surface soil	AY493579.1	*Phormidesmis priestleyi* ANT.L52.6	97	Antarctica	Benthic mats
S_18	Surface soil	AM259268.1	*Pseudanabaena* sp. 0tu30s18	95	Lake Tuusulanjarvi	Freshwater
S_19	Surface soil	JQ687330.1	*Phormidium* sp. CYN64	98	Antarctica	
S_20	Surface soil	HQ230240.1	Cyanobacterium cWHL-32	100	Arctic	Snow
S_21	Surface soil	KC463217.1	*Microcoleus vaginatus* EucYyy1000	99	South Africa	BSC
S_22	Surface soil	JQ687332.1	*Microcoleus* sp. CYN66	100	Antarctica	
S_23	Surface soil	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
S_24	Surface soil	AY493589.1	*Leptolyngbya antarctica* ANT.LACV6.1	99	Antarctica	Benthic mats
S_25	Surface soil	AY493580.1	*Phormidesmis priestleyi* ANT.LG2.4	97	Antarctica	Benthic mats
S_26	Surface soil	HQ230235.1	Cyanobacterium cLA-3	100	Arctic	Snow

Sequence ID	Sample source	Accession number	Closest Homolog in GenBank	% Identity	Origin	Isolation source
S_27	Surface soil	GQ243436.1	*Bacillariophyta* sp. GSL075	98	Utah, USA	Saline Lake
S_28	Surface soil	AY493612.1	*Leptolyngbya frigida* ANT.L52B.3	99	Antarctica	Benthic mats
S_29	Surface soil	KC463217.1	*Microcoleus vaginatus* EucYyy1000	99	South Africa	BSC
S_30	Surface soil	JQ687330.1	*Phormidium* sp. CYN64	99	Antarctica	
S_31	Surface soil	HQ230240.1	Cyanobacterium cWHL-32	99	Arctic	Snow
S_32	Surface soil	AY493607.1	*Leptolyngbya antarctica* ANT.L18.1	98	Antarctica	Benthic mats
S_33	Surface soil	KC463233.1	*Leptolyngbya subtilissima* Ep_Yyy1300	98	South Africa	BSC
S_34	Surface soil	AM709632.1	*Pseudanabaena* sp. PCC 6903	96		Freshwater
S_35	Surface soil	KC463217.1	*Microcoleus vaginatus* EucYyy1000	99	South Africa	BSC
S_36	Surface soil	AY493580.1	*Phormidesmis priestleyi* ANT.LG2.4	98	Antarctica	Benthic mats

Publications and Research outputs originating from this Thesis

Publications

1. Makhalanyane, T. P., Valverde, A., Velázquez, D., Gunnigle, E., Van Goethem, M. W., Quesada, A. & Cowan, D. A. (2015). Cyanobacterial diversity, ecology and biogeochemistry of polar habitats. *Submitted*.

International Travel

1. The 15th International Society for Microbial Ecology conference. August 2014. Seoul, South Korea.

Poster/Presentations outputs

1. **Van Goethem, M. W., Makhalanyane, T. P. & Cowan, D. A. (2013).** The role of moisture sufficiency in Antarctic microbial communities. *ISFM 2013.* Pretoria, South Africa. **Poster**
2. **Van Goethem, M. W., Makhalanyane, T. P. & Cowan, D. A. (2013).** The role of moisture sufficiency in Antarctic microbial communities. *SASM 2013*. Bela-Bela, South Africa. **Poster**
3. **Van Goethem, M. W., Makhalanyane, T. P., S. C. Cary & Cowan, D. A. (2014).** Moisture sufficiency shapes Cyanobacterial communities in a Polar Hyperarid desert. *SANAP Symposium 2014*. Grahamstown, South Africa. **Poster**
4. **Van Goethem, M. W., Makhalanyane, T. P., S. C. Cary & Cowan, D. A. (2014).** Moisture sufficiency shapes Cyanobacterial communities in a Polar Hyperarid desert. *ISME 15 Conference 2014*. Seoul, South Korea. **Poster**

Chapter 6: References

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