Investigating the Biodiversity of Microbial Communities in the McMurdo Dry Valleys, Antarctica: An Inter-Valley Comparison Study.

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Master of Science in Biological Sciences

at

The University of Waikato

by

Béatrice A. Barbier

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

The University of Waikato

2009

Abstract

Extreme environments provide a unique source of often highly adapted and tolerant organisms. Research on organisms in these habitats has led to the discovery of novel and useful compounds and may assist in understanding the impact of global change on biodiversity. The Dry Valleys of Eastern Antarctica are vast, ice-free regions believed to be the coldest, driest desert on Earth. Despite these harsh conditions, there is an increasing amount of evidence demonstrating that the soil ecosystems of the Dry Valleys sustain a wide diversity of microorganisms. The research presented is an inter-valley comparison study which aims to scrutinize microbial communities and environmental factors driving their distribution in the Dry Valleys. Automated ribosomal intergenic spacer analysis (ARISA) was used to provide a "snapshot" of bacterial and cyanobacterial communities living in the mineral sands in Miers Valley, Beacon Valley, Upper Wright Valley and at Battleship Promontory. Rigorous analysis of physico-chemical differences between the soils of these four valleys was undertaken in hope to understand the environmental parameters driving the distribution and biodiversity of microbial communities present. Multivariate statistical analysis and ordination of ARISA and physico-chemical data revealed that bacterial communities from each valley form distinctive clusters. Conversely, cyanobacterial communities showed less diversity and a more even distribution between valleys.

Acknowledgements

I would like to thank my supervisors, Professor Craig Cary and Dr. Ian McDonald for their guidance, support and invaluable advice throughout this project. I would also like to acknowledge Dr. Charles Lee, Dr. Andreas Rueckert and Ariff Khalid for their valuable help and advice. Thank you to Lynne Parker and Colin Monk for their much-needed technical and logistical assistance. I would also like to extend my sincere gratitude to Dr. Susie Wood who gave valuable criticism and suggestions for interpreting and improving the material presented in this thesis. I would also like to thank Antarctica New Zealand (Event Case 023) and the Foundation of Research Science and Technology (grant awarded to Professor Craig Cary and Professor Alan Green) for providing funds for this project. Last, but certainly not least, I would like to extend my heartfelt gratitude to all those around me who have given me continuous support in immeasurable ways, starting with my Parents whom I cannot thank enough for their unconditional faith and encouragement.

"I can no other answer make, but, thanks, and thanks." ~William Shakespeare

Table of Contents

List of Tables

List of Figures

Chapter 1: Introductory Review

1.1. Microbial life at extremes

1.1.1. Extremophiles

Microbial life adapted to extreme environments has long been a source of interest and wonder in the scientific community. Extreme environments can be described as harsh and challenging with conditions of climate and landscape far outside the range of what could comfortably be tolerated from an anthropocentric perspective (Satyanarayana et al., 2005). However, there are living organisms referred to as extremophiles that inhabit such spaces and thrive in these difficult environments. The term ‘‘extremophile’’ was first proposed by MacElroy in 1974 as a broad grouping for organisms which live optimally under extreme conditions. Depending on their optimal growth conditions, extremophiles are named psychrophiles, thermophiles, barophiles, acidophiles, alkalophiles and halophiles. These extremophiles can thrive in various extreme environments, such as the Antarctic and Arctic, hot springs and volcanic areas, hydrothermal vents and pressurized abyssal waters, acidic and alkaline environments, saturated salt brines, the upper atmosphere and even outer space, respectively (Stetter, 1999).

1.1.2. Applications of extremophiles

Extremophiles are at the centre of a large web of research, and hold many current or potential applications (Figure 1). Scientific researchers generally hope to find resources via the exploration of extreme environments and investigation into the mechanisms of adaptation of extremophiles to their surroundings (Wilson & Brimble, 2009) especially their unique proteins, and their applications in various fields. Indeed, extremophiles produce unique biocatalysts that function under extreme conditions comparable to those prevailing in various industrial processes (Niehaus et al., 1999). Such enzymes have the potential for use in food,

chemical and pharmaceutical industries and in environmental biotechnology (Demirjian et al., 2001). Their unique characteristics include resistance against chemical detergents, organic solvents, extremes of pH and temperature. These biocatalysts can consequently be used as a model for designing and constructing proteins with new properties that have great potential for industrial applications (Niehaus et al., 1999).

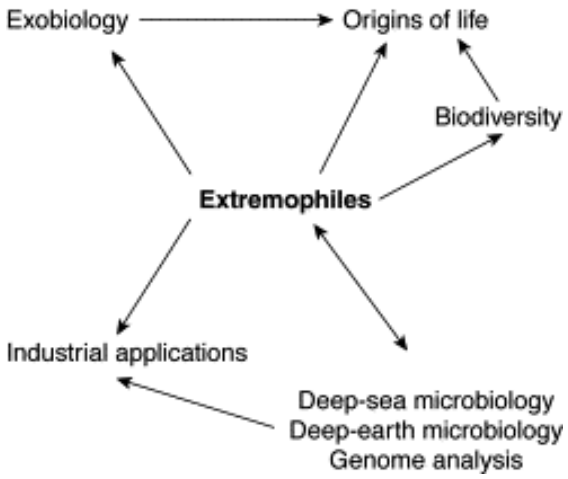

Figure 1. Extremophilic microorganisms as the centre of a web of research activity (Cowan, 1998)

1.1.3. Psychrophiles

Microorganisms adapted to growth at low temperatures include both psychrophilic organisms (having an optimal growth temperature at or below 15ºC, and a maximum growth temperature below 20ºC) (Eddy, 1960) and psychrotrophic organisms (able to grow at temperatures below 15ºC but exhibiting maximum growth rates at temperature optima above 18ºC) (Morgan-Kiss et al., 2006). The study of psychrophiles is of particular importance in fields such as environmental microbiology, geomicrobiology, astrobiology, biotechnology and various industries. For example, in recent years, the study of sea-ice organisms has intensified based on the realization that the physiological mechanisms and adaptations of these salt-tolerant psychrophiles may have considerable potential for biotechnological applications (Thomas & Dieckmann, 2002). The properties of cold-active enzymes make them ideal candidates for certain industrial applications, such as the production of poly-unsaturated fatty

acids for aquaculture and livestock feed and the use of cold-adapted enzymes for a wide range of industries, from cleaning detergents to food processing, as well as agricultural and medical processes (Cavicchioli et al., 2002; Thomas & Dieckmann, 2002).

1.2. Cold desert ecosystems

More than 70% of the earth exists as cold ecosystems that have a stable temperature below or close to the freezing point of water (Feller & Gerday, 2003). Cold habitats include deep ocean, alpine, and polar environments (Morgan-Kiss et al., 2006). Psychrophilic microorganisms represent the most abundant cold-adapted life-forms on earth at the level of species diversity and biomass (Feller & Gerday, 2003). In addition to very low temperatures, these microorganisms are often subjected to other extreme environmental parameters. Many microbial communities associated with the Antarctic and Arctic sea ice, for example, are subjected to salt concentrations several orders of magnitude higher than that of seawater (halopsychrophiles) (Palmisano et al., 1985; Vincent et al., 2004). Most of the terrestrial polar microorganisms are also exposed to osmotic stress and desiccation due to the low water content in their environment, strong dry winds and high Ultra-Violet irradiation (Eicken, 1992; Vincent et al., 2000).

1.2.1. Biodiversity studies of cold desert ecosystems

The notion that bacterial diversity declines with latitude, i.e. increased climatic severity, is widely accepted in the field of environmental microbiology (Willig et al., 2003). This is because the environment is believed to select for organisms with suitable physiology (Hughes Martiny et al., 2006; Oline, 2006; Whitaker et al., 2003), meaning that as environmental constraints get higher, fewer microorganisms possess the necessary adaptations to find the environment suitable for growth. Although soil-borne microorganisms are thought to represent the world's greatest source of biological diversity, extreme environments such as polar deserts are commonly believed to harbour a much lower concentration of adapted microorganisms. However, based on recent studies by Yergeau and

colleagues (2007b) the assumption that extreme environmental conditions such as those found in the Antarctic Dry Valleys result in reduced soil-borne microbial diversity is being challenged.

Microbial communities thriving in desert environments are commonly described as having a high degree of spatial structure and exhibiting an irregular distribution (Barrett et al., 2004; Sjogersten et al., 2006). These characteristics have been supported by recent studies in which the authors have demonstrated that microbial community structures are subject to spatial variability (Cho & Tiedje, 2000; Hughes Martiny et al., 2006; Oline, 2006; Papke & Ward, 2004; Whitaker et al., 2003). However, only a few studies have examined patterns of spatial variation in polar microbial communities (Aislabie et al., 2006; Barrett et al., 2006a; Yergeau et al., 2007a; Yergeau et al., 2007b). The climatic conditions and nutritional stress endured by these communities are such that it is unclear whether a sufficient environmental gradient would be present to support spatial variability. Yergeau and colleagues (2007b) showed in their study that the unusually harsh environmental conditions of terrestrial Antarctic habitats resulted in ecosystems with simplified trophic structures. Microbial processes in these habitats were found to be especially dominant as drivers of soil-borne nutrient cycling.

1.3. Antarctica and the McMurdo Dry Valleys.

1.3.1. Antarctica: the coldest, driest place on Earth.

With its severe katabatic winds and intensely dry and cold climate, Antarctica is considered the most extreme continent on Earth (Hansom & Gordon, 1998). Antarctica's geographic isolation and climatic severity has allowed it to remain one of the most pristine and least studied continents (Hansom & Gordon, 1998). Extreme conditions such as low temperatures, moisture and organic matter availability, high salinity and paucity of metazoan biodiversity limit the development of biotic communities and ecosystem dynamics in terrestrial Antarctica (Niederberger et al., 2008).

1.3.2. The McMurdo Dry Valleys, Antarctica

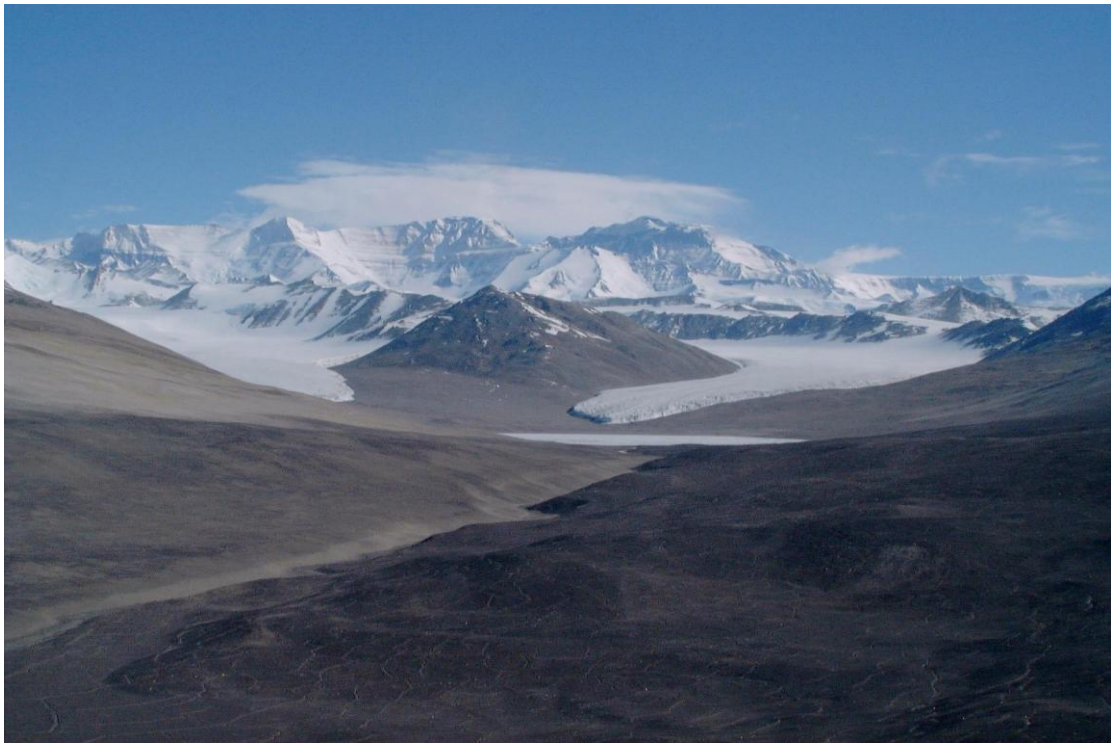

Figure 2. Miers Valley (78°6′S 164°0′E), McMurdo Dry Valleys, Antarctica. (photo provided by Craig Cary).

The McMurdo Dry Valleys are located in southern Victoria Land along the western coast of McMurdo sound, southern Ross Sea, between 160° and 164°E longitude and 76°30’ and 78°30’S latitude. An area of approximately 15,000 km2 is designated as an Antarctic Specially Managed Area to manage human activities in the valleys, for the protection of scientific, wilderness, ecological, and aesthetic values. These Valleys are a 4800 km^2 ice-free region, which accounts for less than 2% of the Antarctic landmass. They are free of ice primarily because glacial flow from the polar plateau is obstructed by the Transantarctic Mountains causing a precipitation shadow (Andersen et al., 1992). It is believed by many to be the coldest, driest desert on Earth (Stonehouse, 2002) with strong winds, precipitation of <10 cm $year^{-1}$ and an average annual air temperature near -20ºC (the temperature usually varies annually between -55ºC to 5ºC) (Clow et al., 1988; Doran et al., 2002). The Dry Valleys receive about 6 months each of sunlight, and darkness. They consist of a pristine mosaic of perennially ice-covered lakes, intermittent streams, arid soils, barren mountains, and surrounding glaciers (Figure 2) (Moorhead et al., 1999; Virginia & Wall, 1999).

The McMurdo Dry Valleys contain unique surface deposits including glacially deposited and modified sediments, sand dunes, desert pavement, glaciolacustrine sediments, and marine fjord sediments containing valuable records of planetary change. The soil, rock, water, and ice environments and their associated biota are of scientific value as model ecosystems that allow deep insights into natural processes operating throughout the biosphere (Management Plan for Antarctic Special Management Area No. 2, 2004). The biogeochemistry of the Dry Valley mineral soils is likely to be an important driver of biodiversity. The physical and chemical characteristics of the mineral soils of the McMurdo Dry Valleys include low total organic matter (from 0.0 to 0.43%), high incident radiation, low humidity and osmotic stress (Cowan et al., 2002; Cowan & Tow, 2004). These soils are characterized by an absence of structure, cohesion, leaching and localized salt accumulation (Campbell & Claridge, 2000). The reduced organic matter in the soil available in the McMurdo Dry Valleys nourishes unique terrestrial microbial assemblages (Burkins et al., 2000). The origin of this organic material is still not clear, given the absence of higher plants in this arid ecosystem (Burkins et al., 2000). Some areas, notably Beacon Valley, have high amounts of copper present in the soil which is a potential contributing factor in reduced biodiversity (Wood et al., 2008), as copper has been shown to interfere with key cellular processes and disrupt plasma membrane integrity (Avery et al., 1996; Suroszl & Palinska, 2004).

1.3.3. Astrobiological perspective: Mars analogy

Scientists carrying out research on psychrophiles often also hope to use their findings on the evolution and mechanisms of adaptation of life at extremes to help understand the environments of other planets such as Mars or Europa. For example, although there are some important differences between the environmental conditions encountered in the Antarctic Dry Valleys and those found on Mars, the many similarities and particularly the field science activities, make the Dry Valleys a useful terrestrial field site to study conditions of possible life on Mars (Andersen et al., 1992). There are no vascular plants or vertebrates and no established insects in the Dry Valleys and microorganisms dominate life in

the area. The surface of Mars is nowadays recognized as being hostile to all known life forms (Hansen et al., 2005), nevertheless in the past the climate on Mars was probably much wetter and warmer than it is today and could have possibly offered conditions suitable for some form of life (McKay & Stocker, 1989). Furthermore, if life ever evolved on Mars, extant life forms may be found in subsurface habitats where liquid water may be present in the form of hypersaline brine, or in permafrost soils (Hansen et al., 2005). This is based on the assumption that Martian life has the same biological basis as terrestrial life surviving in extreme environments (Cabrol & Grin, 1995). Based on these characteristics, the Dry Valleys have been considered the closest Earth analogues to conditions that exist on other icy worlds such as the planet Mars (Doran et al., 2003 ; McKay et al., 2005; Priscu, 1998).

Endolithic cyanobacteria have been found in the McMurdo Dry Valleys under the crust of sandstone and granite (Friedmann, 1982). Analogy with Mars is in accord with the supposition that hypothetical microorganisms existed on the Red Planet and could have migrated into suitable rocks as the amount of available liquid water decreased (Cabrol & Grin, 1995). The endolithic microbial ecosystems of the Dry Valleys are therefore a good terrestrial model of the last stages of early life on Mars (Wierzchos et al., 2006). Furthermore, in several zones of the Dry Valleys, microbial life is found to expire (Wierzchos et al., 2006). The extinction of such cryptoendolithic microorganisms leaves behind inorganic traces of microbial life, and this process is described by Wierzchos and co-authors (2006) to be the best terrestrial analogue of the disappearance of possible early life on Mars.

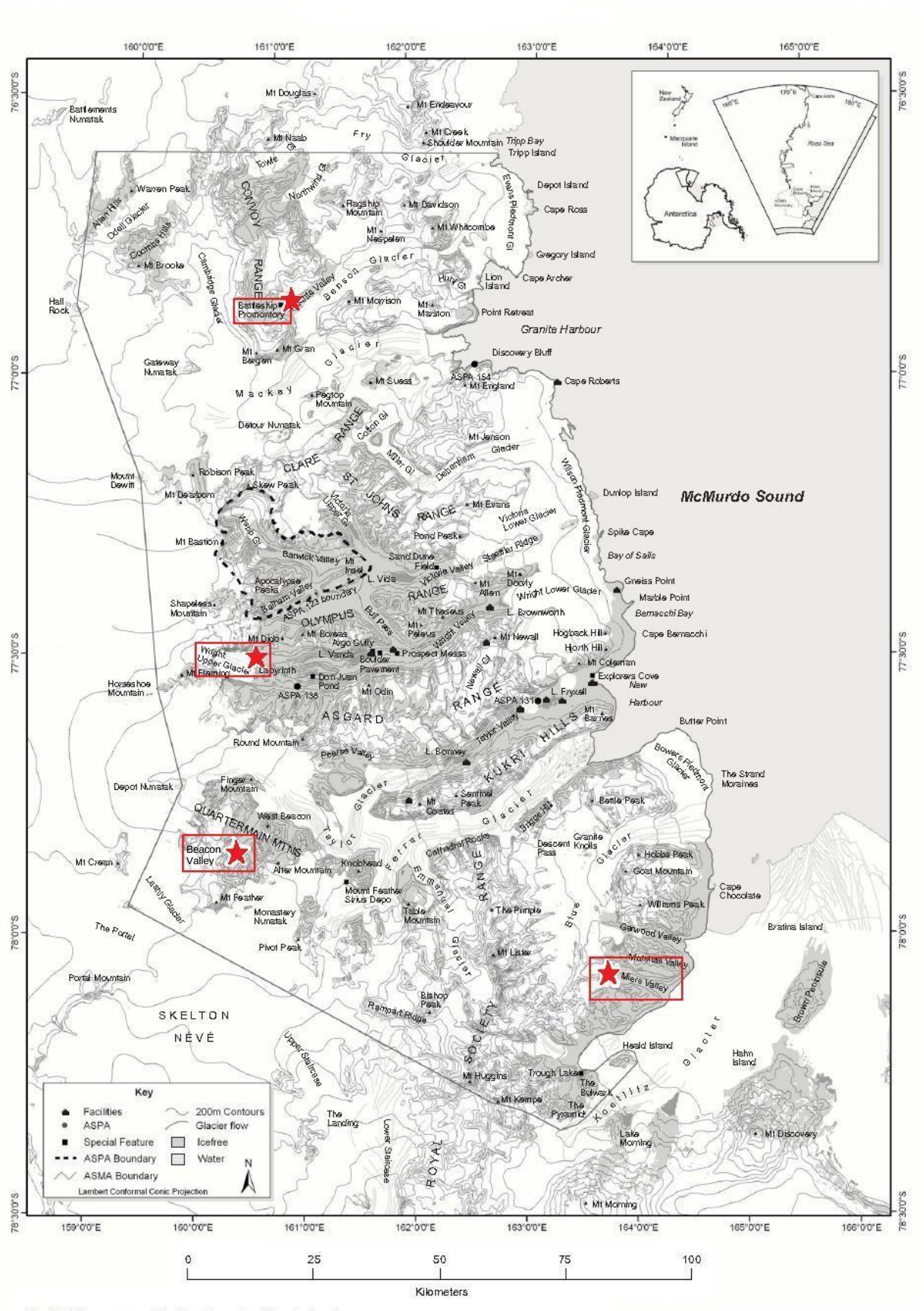

Figure 3. Map of the McMurdo Dry Valleys and our sampling sites.

1.3.4. Microbial biodiversity in the Dry Valleys

Life in the McMurdo Dry Valleys is almost entirely microbial (Horowitz et al., 1972) as the subzero temperatures, prolonged dark periods, extremely low moisture availability and the virtual absence of organic matter limit the growth of most upper life forms. The most studied group of organisms inhabiting the harsh Dry Valley soils are invertebrate animals: springtails, rotifers, tardigrades, collembolan and nematodes (Doran et al., 2002; Stevens & Hogg, 2002, 2003; Treonis et al., 1999; Virginia & Wall, 1999). The enduring presence of relatively large consumers indicates active energy processing and nutrient cycling in the Dry Valleys (Hopkins et al., 2005). Rudimentary vegetation forms such as cryptogams can be found in some areas of the valleys (Yergeau et al., 2007b). Microscopic eukaryotes such as algae, fungi and mosses can also be found, especially a few millimetres inside relatively coarse-grained rocks (Hopkins et al., 2005). Cryptoendolithic microorganisms have been found to colonize the pore spaces of exposed sandstone rocks, forming stratified microbial communities (Friedmann & Ocampo, 1976). Soil microbial communities in the Dry Valleys are remarkably different from those of other terrestrial ecosystems. Freckman and Virginia (1997) showed that Dry Valley soil communities were apparently limited to a small number of phyla, namely rotifers, tardigrades, nematodes, protozoans, fungi, and bacteria. The authors revealed that these soil communities appeared much less diverse and abundant than the soil communities of most terrestrial ecosystems (Freckman & Virginia, 1997)

The environmental factors that determine the patterns of diversity of microbial communities found in the Dry Valleys, such as biogeochemistry, are still being investigated (Barrett et al., 2006a). The prime determinants of species distribution are likely to be soil moisture and temperature (Barrett et al., 2004; Moorhead et al., 1999) resulting in most species having similar growth requirements. Accordingly, the Antarctic Dry Valleys soil environment may be unique in that it could be one of the few examples where abiotic factors (e.g. moisture) are more important than biotic factors (e.g. competition, herbivory, predation) for structuring populations (Convey, 1996; Hogg et al., 2006). Freckman and Virginia

(1997) found in an early study that moisture, carbon, and salinity were important in determining whether habitats were suitable or not to harbour soil communities. Further research by Virginia and Wall (1999) focused on revealing the primary soil factors responsible for the distribution of soil communities in the McMurdo Dry Valleys.

1.3.4.1. Early microbiology studies

Generally speaking, little is known about Antarctic soil microbial communities and their composition. As described in a review by Hogg and co-authors (2006), early culture-based microbial suggested that the soil bacterial diversity and abundance in cold desert areas like the Dry Valleys was very low, as would be predicted by the extreme nature of the ecosystem.

Until recently, hardly anything was known about the microbial biodiversity found in terrestrial ecosystems such as the Dry Valleys, due in large part to the low sensitivity and resolution of early molecular techniques (Barrett et al., 2006a). Previous studies characterized the Antarctic microbial communities by microscopy and by laboratory cultivation of microorganisms (Amann et al., 1995; Siebert et al., 1996). Horowitz and colleagues (1972) described the typical microorganisms of Dry-Valley soils as aerobic, heterotrophic, non-sporulating rods or cocci, often chromogenic in the upper 3 centimetres of the soil and usually colourless or less prominently pigmented below that level. The microorganisms found in these soils were believed to be comprised of only heterotrophs, favouring the conclusion that the ecology of the Dry Valleys was not a complete one, lacking any primary producers of organic matter. Early studies characterized the soils in these valleys as being the coldest, oldest, and driest soils on Earth, generally poorly developed, coarse textured, and low in biological activity (Campbell & Claridge, 1987). These soils were described as being unique among desert soils in having large amounts of soluble salts, high pH, and permafrost at 10–30 cm depth (Pastor & Bockheim, 1980) with such a steep desiccation gradient that the permafrost layer could not furnish adequate liquid water for microbial growth (Wynn-Williams, 1990). In early experiments involving the culture of Antarctic soil microorganisms, large numbers of bacteria such as *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, and *Corynebacterium* were reported,

together with members of the Gram-negative *Eubacteria*. *Firmicutes* isolated included *Bacillus, Micrococcus, Nocardia, Streptomyces*, among others (Cameron et al., 1972; Friedmann, 1993). *Cyanobacteria* are also well-documented inhabitants of Antarctic soil biotopes, especially those with higher percentage moisture (de la Torre et al., 2003; Taton et al., 2003; Wynn-Williams, 2000). A variety of less common genera such as *Beijerinckia*, usually isolated from tropical soils, *Xanthomonas,* a pathogen of higher plants, and *Planococcus*, a marine microorganism, have also been isolated from Antarctic soils (Friedmann, 1993).

1.3.4.2. Recent studies

Until recently, culture-independent studies of Antarctic mineral soils were limited and most culture-independent analyses were reported from Antarctic lakes or ponds (Cowan & Tow, 2004; Morgan-Kiss et al., 2006; Pearce et al., 2003; Sjöling & Cowan, 2003; Taton et al., 2003). Lately however, culture-independent studies employing state of the art molecular genetic tools (Cowan & Tow, 2004; Niederberger et al., 2008) have suggested that the diversity of microorganisms in Dry Valley soils may be considerably higher than previously thought (Hogg et al., 2006).

Recent studies (Aislabie et al., 2006; Babalola et al., 2008; Saul et al., 2005; Smith et al., 2006; Yergeau et al., 2007b) have provided the first in-depth insight into microbial population composition of Antarctic soils based on the analysis of total community nucleic acids. It has been hypothesized that the large population sizes and high distribution potential of microorganisms, as well as their great adaptability, might lead to many cosmopolitan species and generally higher levels of microbial diversity than previously believed (Finlay, 2002; Tindall, 2004; Vincent et al., 2000). In a study by Cowan and colleagues (2002), calculation of biomass levels in desiccated surface soils based on ATP measurements gave microbial population values four orders of magnitude higher in Dry Valley soils than those formerly reported. Previously obtained values were mostly results of culture-based studies which are now widely accepted as poor determinants of true microbial biomass (Cowan et al., 2002). Aislabie and colleagues (2006; 2008) identified ribotypes in soils from the Dry Valleys most closely phylogenetically

affiliated with the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Deinococcus-Thermus*, *Firmicutes* and *Cyanobacteria*. Likewise, Niedergerber et al. (2008) found that a major contribution of signatures within their clone libraries was from *Acidobacteria* and *Actinobacteria*, and members of the *Verrucomicrobia* or *Planctomycetes*, which are recognized as common inhabitants of temperate soils. The authors found members of the *Deinococcus/Thermus* lineage exclusively in dry, low-productivity soils, while *Gammaproteobacteria* of the genus *Xanthomonas* were found exclusively in high-productivity soils (Niederberger et al., 2008). These studies showed that Antarctic Dry Valley soils harbour very diverse bacterial communities and also suggested that many of the phylotypes identified may in fact represent novel, uncultured species (Babalola et al., 2008). Indeed, Niederberger and colleagues (2008) found that a large proportion of signatures they recovered from the Dry Valley soils were low in sequence homology to other environmental sequences. As for the archaeal diversity found in these desert soils, it was found to be severely diminished compared to other soil ecosystems, and limited to the globally ubiquitous Group II low-temperature Crenarchaeotes (Hogg et al., 2006).

1.4. Methods for studying biodiversity in the Dry Valleys

1.4.1. Culture-dependant methods

There are two established strategies to investigate the microbial diversity of a given environment, culture-dependant and culture independent methods (Leuko et al., 2007). Culture-dependant approaches to investigate the diversity of microorganisms in environmental samples are constrained by the difficulty in mimicking actual environmental growth conditions with a culture medium, resulting in the selection and enrichment of certain community representatives only. Culture-dependant techniques are generally acknowledged as being heavily biased, and the cultures obtained are unrepresentative of the distribution of microbial communities in the environment. Colwell and Grimes (2000) demonstrated that only small proportions of soil microbial communities can be cultivated, Amann and colleagues (1995) estimating this number to represent less

than 1% of the microorganisms actually living in the environment. Laboratory trends have therefore shifted in the past decades from culture-based methods to culture-independent methods (Øvreås & Torsvik, 1998).

1.4.2. Culture-independent methods

Recent advances in molecular biology have allowed microbial ecologists to access previously unexplored genetic diversity. The new techniques available are unaffected by the intrinsic bias introduced in culture based experiments (Babalola et al., 2008), but are in turn subject to biases introduced by polymerase chain reaction (PCR), the first step in nearly all molecular biology studies (Polz & Cavanaugh, 1998; von Wintzingerode et al., 1997). Before the development of molecular techniques to estimate genetic diversity, studies on microbial community structure and diversity were restricted to cultivation-based methods. Because cultivating microorganisms selects for some organisms while others are not culturable, these methods grossly underestimate the population diversity present in natural environments (Amann et al., 1995; Torsvik et al., 1990). Advances in culture-independent molecular techniques have been applied to soil ecosystems to study microbial diversity at the molecular level. The 16S rRNA genes is a gene present in all bacteria, and is often used to assess microbial diversity because it contains both conserved regions which can be used to design primers for polymerase chain reaction (PCR) amplification, as well as variable regions that are used to infer taxonomic relationships between microorganisms (Ward et al., 1990). Amplified 16S rRNA can be further analyzed by fingerprinting techniques.

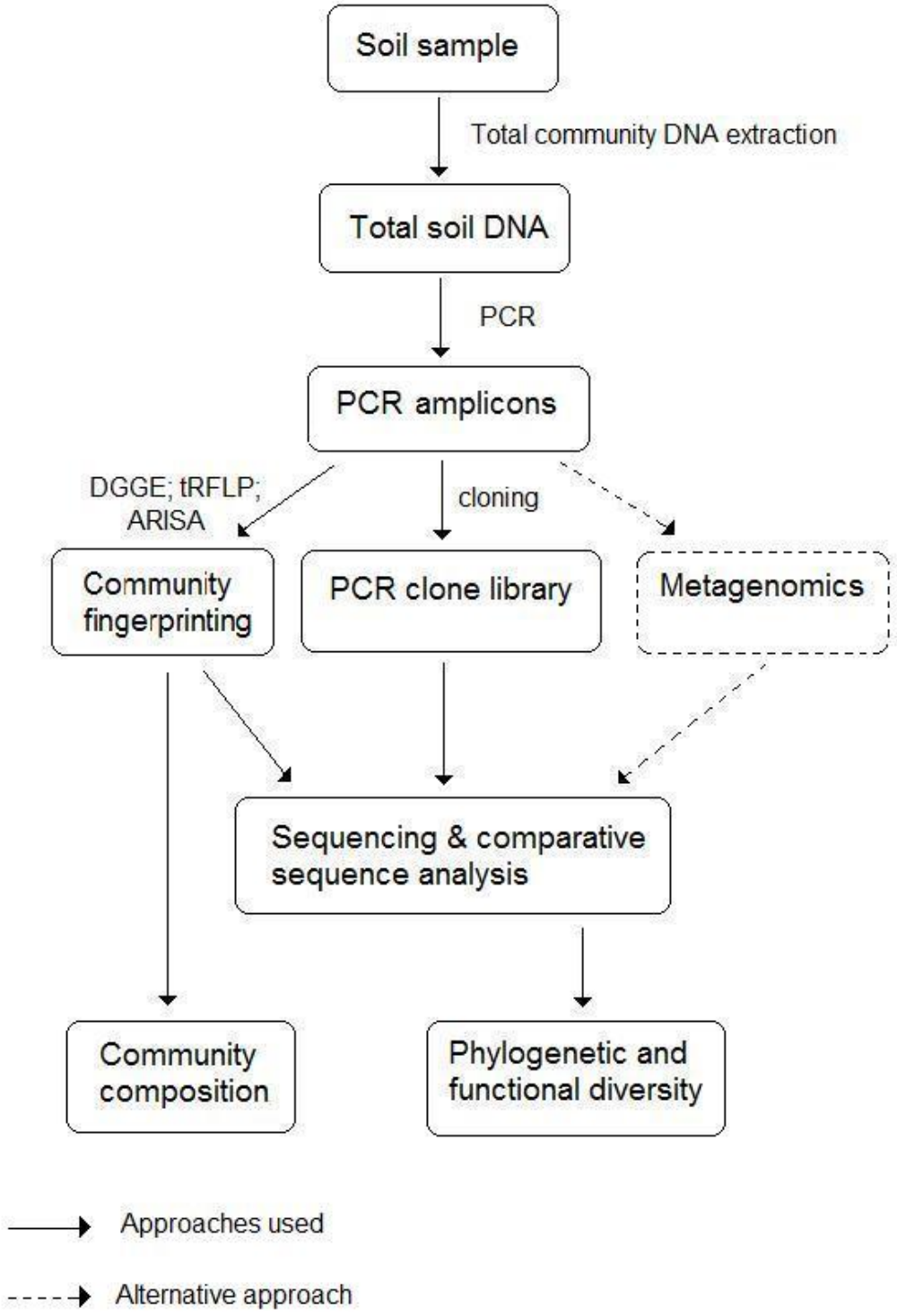

Figure 4. Flow diagram of the culture-independent methods used in this study modified from Hernandez et al. (2008).

1.4.2.1. Community fingerprinting analyses:

The application of molecular biology and genomics to environmental microbiology has enabled the proliferation of in-depth studies of the huge complexity present in natural microbial communities. The surveying of environmental biodiversity, the development of community fingerprinting methods to analyze PCR amplicons from complex environments and functional interrogation of natural microbial populations have become routine practice in environmental microbiology studies, enabled by a range of molecular and bioinformatics techniques (Garcia-Pichel, 2008).

Fingerprinting techniques generally rely on the separation and analysis of PCR products amplified from nucleic acids directly extracted from the environment (Oros-Sichler et al., 2007). Different existing techniques exploit sequence variation of amplified gene fragments. , such as denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993), temperature gradient gel electrophoresis (TGGE) (Brim et al., 1999) terminal restriction fragment length polymorphism (T-RFLP) (Liu et al., 1997; Marsh, 1999), and amplified ribosomal DNA restriction analysis (ARDRA) (Martínez-Murcia et al., 1995; Smit et al., 1997).

Denaturing gradient gel electrophoresis (DGGE) relies on different melting profiles of double stranded PCR products. This method was first introduced in 1993 by Muyzer and colleagues who used 16S rRNA gene fragments amplified from total community DNA extracted from marine environments (1993). Until recently, DGGE was one of the best molecular community fingerprinting techniques in terms of predicting the species richness and distribution (Hui et al., 2008).

Amplified ribosomal DNA restriction analysis (ARDRA) and terminal restriction fragment length polymorphism (tRFLP) exploit the differences in restriction enzymes digestion sites along the investigated gene (Liu et al., 1997). These techniques provide a rough fingerprint that is not always easy to analyze (Oros-Sichler et al., 2007).

Automated ribosomal intergenic spacer analysis (ARISA) is a culture-independent method that was developed by Fisher and Triplett (1999). It has been shown in many studies to be useful for the characterization and analysis of

microbial communities (Brown & Fuhrman, 2005; Fisher & Triplett, 1999; González et al., 2003; Hartmann et al., 2005; Kwon et al., 2005; Leuko et al., 2007; Ranjard et al., 2000; Soo, 2007; Wood et al., 2008). In comparison to the standard 16S rRNA gene cloning approach to investigate microbial diversity, ARISA provides a more rapid initial appraisal of the communities present (Leuko et al., 2007). ARISA targets the intergenic spacer (ITS) region situated between the small subunit (16S) and large subunit (23S) genes of the rRNA operon (Figure 5).

The ITS region is believed to show sufficient heterogeneity in terms of sequence length and composition to differentiate between the various communities present in a given environment (González et al., 2003; Ranjard et al., 2000). ARISA is highly reproducible and considered sensitive enough to detect single nucleotide polymorphisms (Leuko et al., 2007). This method has proven to be a fast and reliable method for analyzing microbial communities found in a vast array of environments such as marine communities (Brown & Fuhrman, 2005), freshwater communities (Fisher & Triplett, 1999), soil communities from diverse geographic locations (Hartmann et al., 2005; Kwon et al., 2005; Ranjard et al., 2000; Soo, 2007; Wood et al., 2008) and even stromatolite samples (Leuko et al., 2007). The use of the internal transcribed spacer (ITS) region has been shown to enable discrimination to the species level and sometimes even within-species (Giovannoni & Stingl, 2005; Guasp et al., 2000; Kwon et al., 2005; Xu & Cotè, 2003) which is not the case with 16S rRNA.

While community fingerprinting methods such as ARISA are useful for comparative analyses, they cannot be used to assess the richness or diversity metrics of complex communities (Dunbar et al., 2000). These methods are limited by their detection threshold, and the number of peaks detected in ARISA can underestimate the actual richness of any community with a long-tailed rank abundance distribution (Bent et al., 2007). Indeed, microbial communities are generally found to approximate long-tailed distributions, such as lognormal or log-Laplace distributions, implying that accurately estimating diversity in a community with a log-normal species-abundance distribution requires sampling about 80% of the species in any given environment (Gans et al., 2005). Consequently, the sole use of fingerprinting methods cannot provide reliable

diversity indices. However, fingerprinting methods like ARISA hold great potential for use when rapid, high-throughput screening for differences or changes in microbial communities is more important than phylogenetic identification of specific organisms (Hartmann et al., 2005).

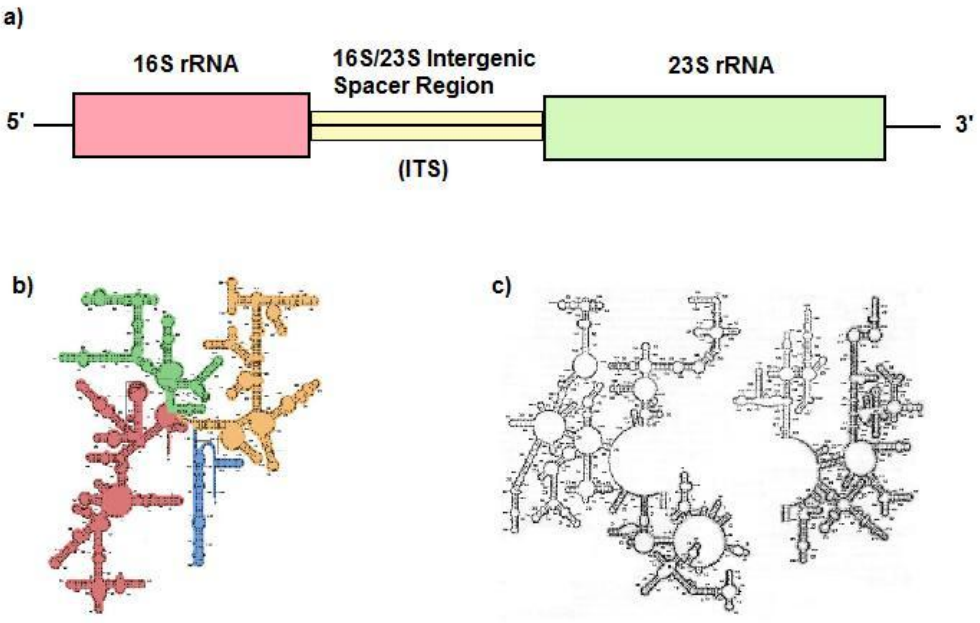

Figure 5. a) Schematic representation of the intergenic spacer region (ITS). Secondary structure of the b) 16S rRNA molecule (Garrett & Grisham, 2005) and c) 23S rRNA molecule from *E. coli* (Freitas & Merkle, 2004).

1.4.2.2. Statistical analysis of community fingerprints.

Community fingerprinting analyses provide snapshots of the microbial community structures present in soils, or qualitatively compare the microbial communities using genomic DNA from different environmental samples. Because of the complexity of the patterns obtained, and the often high number of fingerprints obtained, statistical analysis of ARISA data is essential to allow valid comparisons between the community fingerprints obtained (Oros-Sichler et al., 2007). Similarity values of pairs of fingerprints can be calculated with the Pearson correlation coefficient and subjected to hierarchical ranking by applying random permutations analysis to statistically compare within-sample and between-sample

similarities (Kropf et al., 2004). This can be done, for example, in the PRIMER 6 software package (PRIMER-E, Ltd., UK). Furthermore, ordination methods can help to analyse large data sets and relate abiotic factors to microbial community structures (Oros-Sichler et al., 2007). Multidimensional scaling, for example, is a way of grouping the data and comparing it, answering questions about the influence of abiotic influences on shaping microbial community assemblages (Legendre & Legendre, 1998). This method attempts to represent community fingerprints in a few dimensions while preserving the distance relationships among and between the fingerprints (Legendre & Legendre, 1998).

1.4.2.3. Methods to study phylogenetics and microbial ecology.

Until recently, the most comprehensive way to analyse the biodiversity present in a sample was to build a 16S rRNA clone library and sequence as many of the resulting, unique clones as possible. However, the cost of constructing clone libraries followed by performing capillary-based DNA sequencing is high and often limits the number of sequences included in scientific investigations (Huse et al., 2007). The detection of low abundance taxa demands surveys that are many orders of magnitude larger than most of those reported in the literature (Huse et al., 2007). Recently, there have been advances in technology that have enabled high-throughput genome sequencing to be established in research laboratories using bench-top instrumentation. These new technologies are being used to explore the vast microbial diversity in the natural environment and the untapped genetic variation that can occur in bacterial species (Hall, 2007).

One of these new technologies is massively parallel pyrosequencing developed by 454 Life Sciences. This technology offers a means to more extensively sample molecular diversity in microbial populations. Hundreds of thousands for short DNA sequence reads can be generated in a few hours, without the use of the traditional, time-consuming cloning step (Huse et al., 2007). Pyrosequencing was first developed by Ronaghi and colleagues in 1996, when they showed that natural nucleotides could be used to obtain efficient incorporation during a sequencing-by-synthesis protocol. The detection was based on the pyrophosphate (PPi) released during the DNA polymerase reaction (Nyrén,

1987), the quantitative conversion of pyrophosphate to ATP by sulfurylase, and the subsequent production of visible light by firefly luciferase (Ronaghi et al., 1996).

This was applied in a clinical setting by Jonasson and colleagues who showed that provisional classification of bacterial isolates could be performed on a large scale based on 16S rRNA sequence comparisons using Pyrosequencing™, and the concept of signature matching (Jonasson et al., 2002). Binladen et al. (2007) used conventional PCR with 5'-nucleotide tagged primers to generate DNA products from multiple specimens, followed by 454 pyrosequencing. They showed use of coded PCR primers enabled high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing (Binladen et al., 2007).

Parameswaran and colleagues (2007) further rendered multiplexed high-throughput pyrosequencing more efficient in their study by using a novel barcoding approach. This approach enabled pooling of various samples after amplifying each with a primer associated with a specific barcode. Subsequently, the output provided by the pyrosequencer was segregated bioinformatically to analyze each original sample separately. Using the sequencing platform developed by 454/Roche (Roche Diagnostics, Branford, CT, USA), the authors successfully sequenced small RNA libraries simultaneously from 25 diverse samples, and were able to unambiguously assign 99.8% of those sequences (Parameswaran et al., 2007). Meyer and colleagues (2008) developed a similar method of barcoding called "parallel-tagged sequencing", using sample-specific barcoding adapters and enabling the barcoding of pooled PCR products (Meyer et al., 2008). There have been many recent advances in techniques in genomics and bioinformatics that have great potential to help address numerous topics in ecology and evolution (Bouck & Vision, 2007).

1.5. Hypothesis, goals of this MSc research project.

The hypothesis of this study is that the distribution and biodiversity of microorganisms in the McMurdo Dry Valleys, Antarctica are driven by abiotic parameters such as the physico-chemical properties, mineralogy and lithology of the soils. In order to verify this assumption, our approach was to compare and microbial communities in four different valleys located in the McMurdo Dry Valleys: Miers Valley (78°6′S 164°0′E), Upper Wright Valley (77°10′S, 161°50′E), Beacon Valley (77°83'S, 160°66'E) and Battleship Promontory (76°54'S 160°55'E). These four valleys were chosen based on their spread out locations within the Dry Valleys (Figure 3), their varying microclimate and altitude, presence or absence of lakes, diverse physico-chemical characteristics, mineralogy and lithology of their soils. All these characteristics can potentially influence the presence or absence of microbial populations. For the first time, automated ribosomal intergenic spacer analysis (ARISA) was used to characterize the microbial communities living in the soils of four very different Dry Valleys. To explain the biodiversity and distribution obtained, physico-chemical parameters were investigated thoroughly in order to identify potential correlations in hope to gain a better insight on the environmental parameters that govern the biodiversity of the microorganisms inhabiting the McMurdo Dry Valleys. Our aim was to elucidate which environmental factor or combination of factors drive the distribution of the microbial communities in the McMurdo Dry Valleys.

1.6. References

Acinas, S., Klepac-Ceraj, V., Hunt, D., Pharino, C., Ceraj, I., Distel, D., et al. (2004). Fine-scale phylogenetic architecture of a complex bacterial community. Nature, 430, 551-554.

Aislabie, J., Chhour, K., Saul, D., Miyauchi, S., Ayton, J., Paetzol, R., et al. (2006). Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biology and Biochemistry, 38, 3041-3056.

Aislabie, J., Jordan, S., & Barker, G. (2008). Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. Geoderma, 144(1-2), 9-20.

Amann, R., Ludwig, W., & Schleifer, K. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiology and Molecular Biology Reviews, 59(1), 143-169.

Andersen, D., McKay, C., Wharton, R., & Rummel, J. (1992). Testing a Mars science outpost in the Antarctic dry valleys. Advances in Space Research, 12(5), 205-209.

Avery, S., Howlett, N., & Radice, S. (1996). Copper toxicity towards Saccharomyces cerevisiae: dependance on plasma membrane fatty acid composition. Applied and Environmental Microbiology, 62, 3960-3966.

Babalola, O., Kirby, B., Le Roes-Hill, M., Cook, A., Cary, C., Burton, S., et al. (2008). Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environmental Microbiology. Online publication.

Barrett, J., Virginia, R., Wall, D., Cary, S. C., Adams, B., Hacker, A., et al. (2006a). Co-variation in soil biodiversity and biogeochemistry in Northern and Southern Victoria Land, Antarctica. Antarctic Science, 18, 535-548.

Barrett, J., Wall, D., Virginia, R., Parsons, A., Powers, L., & Burkins, M. (2004). Biogeochemical parameters and constraints on the structure of soil biodiversity. Ecology, 85, 3105-3118.

Bent, J., Pierson, J., & Forney, L. (2007). Measuring species richness based on microbial community fingerprints: the emperor has no clothes. Applied and Environmental Microbiology, 73(7), 2399-2401.

Binladen, J., Gilbert, T., Bollback, J., Panitz, F., Bendixen, C., Nielsen, R., et al. (2007). The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. PLoS, 2(2).

Bouck, A., & Vision, T. (2007). The molecular ecologist's guide to expressed sequence tags. Molecular Ecology, 16(5), 907-924.

Brim, H., Heuer, H., Krögerrecklenfort, E., Mergeay, M., & Smalla, K. (1999). Characterization of the bacterial community of a zinc-polluted soil. Canadian Journal of Microbiology, 45(4), 326–338.

Brown, M., & Fuhrman, J. (2005). Marine bacterial microdiversity as revealed by internal transcribed spacer analysis. Aquatic Microbial Ecology, 41, 15-23.

Burkins, M., Virginia, R., Chamberlain, P., & Wall, D. (2000). Origin and distribution of soil organic matter in Taylor Valley, Antarctica. . Ecology, 81(9), 2377-2391.

Cabrol, N., & Grin, E. (1995). A morphological view on potential niches for exobiology on Mars. Planetary and Space Science, 43(1-2), 179-188.

Campbell, I., & Claridge, G. (1987). Antarctica: Soils, weathering processes and environment. United States: Elsevier Science Pub. Co. Inc.,New York, NY.

Campbell, I., & Claridge, G. (2000). Soil temperature, moisture and salinity patterns in Transantarctic Mountain cold desert ecosystems. In W. Davidson, C. Howard-Williams & P. Broady (Eds.), Antarctic ecosystems: models for wider ecological understanding. (pp. 233-240). Christchurch, NZ: Caxton Press.

Cavicchioli, R., Siddiqui, K., Andrews, D., & Sowers, K. (2002). Low-temperature extremophiles and their applications. Current Opinion in Biotechnology, 13(3), 253-261.

Cho, J.-C., & Tiedje, J. (2000). Biogeography and endemicity of fluorescent Pseudomonas strains in soil. Applied and Environmental Microbiology, 66, 5448-5456.

Clow, G., McKay, C., Simmons, G., & Wharton, R. (1988). Climatological observation and predicted sublimation rates at Lake Hoare. Antarctic Journal of Climatology., 1, 715-718.

Colwell, R., & Grimes, D. (2000). Nonculturable microorganisms in the environment. ASM Press, Washington DC, USA.

Convey, P. (1996). The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. . Biological Reviews, 71(2), 191-225.

Cowan, D. (1998). Hot bugs, cold bugs and sushi. Trends in Biotechnology, 16(6), 241-242.

Cowan, D., Russell, N., Mamais, A., & Sheppard, D. (2002). Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. Extremophiles, 6(5), 431-436.

Cowan, D., & Tow, L. (2004). Endangered antarctic environments. Annual Reviews in Microbiology, 58, 649–690.

Daniel, R. (2005). The metagenomics of soil. Nature Reviews in Microbiology, 3, 470-478.

Demirjian, D., Morís-Varas, F., & Cassidy, C. (2001). Enzymes from extremophiles. Current Opinion in Chemical Biology, 5(2), 144-151.

Doran, P., Fritsen, C., McKay, C., Priscu, J., & Adams, E. (2003). Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "ice-

sealed" east Antarctica lake. Proceedings of the National Academy of Sciences, 100, 26-31.

Doran, P., Priscu, J., Lyons, W., Walsh, J., Fountain, A., McKnight, D., et al. (2002). Antarctic climate cooling and terrestrial ecosystem response. Nature 415, 517-520.

Dunbar, J., Ticknor, L., & Kuske, C. (2000). Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology, 66, 2945-2950.

Eddy, B. (1960). The use and meaning of the term 'psychrophillic'. Journal of Applied Bacteriology., 23, 189-190.

Eicken, H. (1992). The role of sea ice in structuring Antarctic ecosystems. Polar Biology, 12, 3-12.

Feller, G., & Gerday, C. (2003). Psychrophilic enzymes: hot topics in cold adaptation. Nature Reviews Microbiology, 1, 200-208.

Finlay, B. (2002). Global dispersal of free-living microbial eukaryote species. Science, 296, 1061-1063.

Fisher, M., & Triplett, E. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Applied and Environmental Microbiology, 65, 4630-4636.

Freckman, D., & Virginia, R. (1997). Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. Ecology, 78(2), 363-369.

Freitas, R., & Merkle, R. (2004). Kinematic Self-Replicating Machines. Georgetown, TX: Landes Bioscience.

Friedman, E. (1982). Endolithic microorganisms in the Antarctic cold desert. Science, 215, 730-737.

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science, 309, 1387-1390.

Garcia-Pichel, F. (2008). Molecular ecology and envrionmental genomics of cyanobacteria. In A. Herrero & E. Flores (Eds.), The cyanobacteria : molecular biology, genomics and evolution Norfolk, UK: Caister Academic Press.

Garrett, R., & Grisham, C. (2005). Biochemistry (3rd ed.). Belmont, California Thomson Brooks/Cole.

Giovannoni, S., & Stingl, U. (2005). Molecular diversity and ecology of microbial plakton. Nature, 437, 343-348.

González, N., Romero, J., & Espejo, R. (2003). Comprehensive detection of bacterial populations by PCR amplification of the 16S-23S rRNA spacer region. Journal of Microbiological Methods, 55(1), 91-97.

Guasp, C., Moore, E., Lalucat, J., & Bennasar, A. (2000). Utility of internally transcribed 16S-23S rDNA spacer regions for the definition of Pseudomonas stutzeri genomovars and other Pseudomonas species. International Journal of Systematic Evolutionary Microbiology, 50, 1629-1639.

Hall, N. (2007). Advanced sequencing technologies and their wider impact in microbiology. Journal of Experimental Biology, 209, 1518-1525.

Hansen, A., Merrison, J., Nornberg, P., Lomstein, B., & Finster, K. (2005). Activity and stability of a complex bacterial soil community under simulated Martian conditions. International Journal of Astrobiology, 4(2), 134-144.

Hansom, J., & Gordon, J. (1998). Antarctic Environments and Resources: A Geographical Perspective. New York: Addison Wesley Longman Ltd.

Hartmann, M., Frey, B., Kölliker, R., & Widmer, F. (2005). Semi-automated genetic analyses of soil microbial communities: comparison of T-RFLP and RISA based on descriptive and discriminative statistical approaches. Journal of Microbiological Methods, 61(3), 349-360.

Hernández, M., Morgante, V., Flores, C., Villalobos, P., González, M., Miralles, P., et al. (2008). Modern approaches for the study of s-Triazine herbicide bioremediation in agricultural soils. Revista de la ciencia del suelo y nutrición vegetal, 8, 19-30.

Hogg, I., Cary, C., Convey, P., Newsham, K., O'Donnell, A., Adams, B., et al. (2006). Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? Soil Biology and Biochemistry, 38(10), 3035-3040.

Hopkins, D. W., Eberling, B., Greenfield, L. G., Gregorich, E. G., Novis, P. M., O'Donnell, A. G., et al. (2005). Soil micro-organisms in Antarctic Dry Valleys: resource supply and utilization. In G. M. Gadd, K. T. Semple & H. M. Lappin-Scott (Eds.), Microorganisms and Earth Systems- Advances in geomicrobiology. (Vol. 65, pp. 71-84). Cambridge: Cambridge University Press.

Horowitz, N., Cameron, R., & Hubbard, J. (1972). Microbiology of the Dry Valleys of Antarctica. Science, 176(4032), 242-245.

Hughes, J., Hellman, J., Ricketts, T., & Bohannan, B. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. Applied and Environmental Microbiology, 67, 4399-4406.

Hughes Martiny, J., Bohannan, B., Brown, J., Colwell, R., Fuhrman, J., & Green, J. e. a. (2006). Microbial biogeography: putting organisms on the map. . Nature Reviews, 4, 102-112.

Hui, L., Ying, Z., Dong-sheng, L., Hui, X., Guan-xiong, C., & Cheng-gang, Z. (2008). Comparisons of different hypervariable regions of rrs genes for fingerprinting of microbial communities in paddy soils. Soil Biology and Biochemistry, In Press, Corrected Proof.

Huse, S., Huber, J., Morrison, H., Sogin, M., & Welch, D. (2007). Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biology, 8(7), R143.

Jonasson, J., Olofsson, M., & Monstein, H.-J. (2002). Classification, identification and subtyping of bacteria based on pyrosequencing and signature matching of 16S rDNA fragments. APMIS, 110(3), 263-272.

Kropf, S., Heuer, H., Grüning, M., & Smalla, K. (2004). Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. Journal of Microbiological Methods, 57(2), 187-195.

Kwon, S., Park, J.-Y., Kim, J.-S., Kang, J.-W., Cho, Y.-H., Lim, C.-K., et al. (2005). Phylogenetic analysis of the genera Bradyhyrzobium, Mesorhizobium, Rhizobium, and Sinorhizobium on the basis of 16S rRNA gene and internally transcribed spacer region sequences. International Journal of Systematic Evolutionary Microbiology, 55, 263-270.

Legendre, P., & Legendre, L. (1998). Ordination in reduced space. In Numerical Ecology (2nd English ed. ed., pp. 387-480). Amsterdam, The Netherlands: Elsevier Science.

Leuko, S., Goh, F., Allen, M., Burns, B., Walter, M., & Neilan, B. (2007). Analysis of intergenic spacer region length polymorphisms to investigate the halophilic archaeal diversity of stromatolites and microbial mats. Extremophiles, 11(1), 203-210.

Liu, W., Marsh, T., Cheng, H., & Forney, L. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of

genes encoding 16S rRNA. Applied and Environmental Microbiology, 63(11), 4516-4522.

MacElroy, R. (1974). Some comments on the evolution of extremophiles. Biosystems, 6, 74-75.

Marsh, T. (1999). Terminal restriction fragment length polymorphism (T-RFLP): An emerging method for characterizing diversity among homologous populations of amplification products. Current Opinion in Microbiology, 2(3), 323-327.

Martínez-Murcia, A., Acinas, S., & Rodriguez-Valera, F. (1995). Evaluation of prokaryotic diversity by restrictase digestion of 16S rDNA directly amplified from hypersaline environments. FEMS Microbiology Ecology, 17(4), 247-255.

McKay, C., Andersen, D., Pollard, W., Heldmann, J., Doran, P., Fritsen, C., et al. (2005). Polar lakes, streams, and spings as analogs for the hydrological cycle on Mars. In T. Tokano (Ed.), Water on Mars and life. (pp. 219-233). Berlin, Germany: Springer-Verlag.

McKay, C., & Stocker, C. (1989). The early environment and its evolution on Mars- implications for life. Review in Geophysics, 27, 189-214.

Meyer, M., Stenzel, U., & Hofreiter, M. (2008). Parallel tagged sequencing on the 454 platform. Nature Protocols, 3(2), 267-278.

Moorhead, D., Doran, P., Fountain, A., Lyons, W., McKnight, D., Priscu, J., et al. (1999). Ecological legacies: impacts on ecosystems of the McMurdo Dry Valleys. Bioscience, 49, 1009-1019.

Morgan-Kiss, R., Priscu, J., Pocock, T., Gudynaite-Savitch, L., & Huner, N. (2006). Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. . Microbial Molecular Biology Review, 70, 222-252.

Muyzer, G., de Waal, E., & Uitterliden, A. (1993). Profiling of complez microbial populations by denaturing gradient gel electrophoresis analyses of polymerrade chain reaction-amplified genes for 16S rRNA. Applied and Environmental Microbiology, 59, 695-700.

Niederberger, T., McDonald, I., Hacker, A., Soo, R., Barrett, J., Wall, D., et al. (2008). Microbial community composition in soils of Northern Victoria Land, Antarctica. Environmental Microbiology, 10(7), 1713-1724.

Niehaus, F., Bertoldo, C., Kähler, M., & Antranikian, G. (1999). Extremophiles as a source of novel enzymes for industrial application. Applied Microbiology and Biotechnology, 51(6), 711-729.

Nyrén, P. (1987). Enzymatic method for continuous monitoring of DNA polymerase activity. Analytical Biochemistry, 167(2), 235-238.

Oline, D. (2006). Phylogenetic comparisons of bacterial communities from serpentine and nonserpentine soils. . Applied and Environmental Microbiology, 72, 6065-6071.

Oros-Sichler, M., Costa, R., Heuer, H., & Smalla, K. (2007). Molecular fingerprinting techniques to analyze soil microbial communities. In J. D. v. Elsas, J. Jansson & J. Trevors (Eds.), Modern Soil Microbiology (2 ed., pp. 355-386). Boca Raton, FL: CRC Press/Taylor & Francis.

Øvreås, L., & Torsvik, V. (1998). Microbial Diversity and Community Structure in Two Different Agricultural Soil Communities. Microbial Ecology, 36(3), 303-315.

Palmisano, A., SooHoo, J., & Sullivan, C. (1985). Photosynthesis-irradiance relationships in sea ice microalgae from McMurdo Sound, Antarctica. . Journal of Phycology., 21, 34.

Papke, R., & Ward, D. (2004). The importance of physical isolation to microbial diversification. . FEMS Microbial Ecology, 48, 293-303.

Parameswaran, P., Jalili, R., Tao, L., Shokralla, S., Gharizadeh, B., Ronaghi, M., et al. (2007). A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. Nucleic Acids Research, 35(19), e130.

Pastor, J., & Bockheim, J. (1980). Soil Development on Moraines of Taylor Glacier, Lower Taylor Valley, Antarctica. Soil Science Society of America Journal, 44(2), 341-348.

Pearce, D., van der Gast, C., Lawley, B., & Ellis-Evans, J. (2003). Bacterioplankton community diversity in a maritime Antarctica lake, determined by culture-dependant and culture-independant techniques. Microbial Ecology, 45, 59-70.

Polz, M., & Cavanaugh, C. (1998). Bias in Template-to-Product Ratios in Multitemplate PCR. Applied and Environmental Microbiology, 64(10), 3724-3730.

Priscu, J. (1998). Ecosystem dynamics in a polar desert: the McMurdo Dry Valleys, Antarctica. (Vol. 72). Washington, D.C.

Ranjard, L., Brothier, E., & Nazaret, S. (2000). Sequencing Bands of Ribosomal Intergenic Spacer Analysis Fingerprints for Characterization and Microscale Distribution of Soil Bacterium Populations Responding to Mercury Spiking. Applied and Environmental Microbiology, 66(12), 5334-5339.

Ronaghi, M., Karamohamed, S., Pettersson, B., Uhlén, M., & Nyrén, P. (1996). Real-Time DNA Sequencing Using Detection of Pyrophosphate Release. Analytical Biochemistry, 242(1), 84-89.

Satyanarayana, T., Raghukumar, C., & Shivaji, S. (2005). Extremophilic microbes: Diversity and perspectives. Current Science, 89(1), 78-90.

Saul, D., Aislabie, J., Brown, C., Harris, L., & Foght, J. (2005). Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. . FEMS Microbiology Ecology, 53, 141-155.

Sjogersten, S., van der Wal, R., & Woodin, S. (2006). Small-scale hydrological variation determines landscape CO2 fluxes in the high Arctic. Biogeochemistry, 80, 205-216.

Sjöling, S., & Cowan, D. (2003). High 16S rDNA bacterial diversity in glcial melt-water sediment, Bratina Island, Antarctica. . Extremophiles, 7, 275-282.

Smit, E., Leeflang, P., & Wernars, K. (1997). Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. FEMS Microbiology Ecology, 23(3), 249-261.

Smith, J., Tow, L., Stafford, W., Cary, C., & Cowan, D. (2006). Bacterial Diversity in Three Different Antarctic Cold Desert Mineral Soils. Microbial Ecology, 51(4), 413-421.

Sogin, M., Morrison, H., Huber, J., Welch, D., Huse, S., Neal, P., et al. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of the National Academy of Sciences, 103(32), 12115-12120.

Soo, R. (2007). Microbial biodiversity of thermophilic communities in hot mineral soils of Tramway Ridge, Mt. Erebus, Antarctica. Waikato University, Hamilton.

Stetter, K. (1999). Extremophiles and their adaptation to hot environments. FEBS Letters, 452(1-2), 22-25.

Stevens, M., & Hogg, I. (2002). Expanded distributional records of Collembola and Acari in southern Victoria Land, Antarctica. Pedobiologia, 46(5), 485-495.

Stevens, M., & Hogg, I. (2003). Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail Gomphiocephalus hodgsoni from Victoria Land, Antarctica. Molecular Ecology, 12(9), 2357-2369.

Stonehouse, B. (2002). Encyclopedia of Antarctica and the Southern Oceans: John Wiley and Sons.

Suroszl, W., & Palinska, K. (2004). Effects of heavy-metal stress on cyanobacterium Anabeana flos-aqua. Archives in Environmental Contamination Toxicology, 48, 40-48.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R., & Wilmotte, A. (2003). Cyanobacterial diversity in natural and artificial microbial mats of lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology, 69, 5157-5169.

Thomas, D., & Dieckmann, G. (2002). Antarctic sea ice--a habitat for extremophiles. Science, 295(5555), 641-644.

Tindall, B. (2004). Prokaryotic diversity in the Antarctic: the tip of the iceberd. Microbial Ecology, 47, 271-283.

Torsvik, V., Goksoyr, J., & Daae, F. (1990). High diversity in DNA of soil bacteria. Applied and Environmental Microbiology, 56(3), 782-787.

Treonis, A., Wall, D., & Virginia, R. (1999). Invertebrate biodiversity in antarctic Dry Valley soils and sediments. Ecosystems, 2(6), 482-492.

Venter, C., Remington, K., Heidelberg, J., Halpern, A., Rusch, D., Eisen, J., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304, 66-74.

Vincent, W., Gibson, J., Pienitz, R., Villeneuve, V., Broady, P., Hamilton, P., et al. (2000). Ice shelf microbial ecosystems in the high Arctic and implications for life on snowball earth. . Naturwissenschafter, 84, 137-141.

Vincent, W., Mueller, D., & Bonilla, S. (2004). Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. Cryobiology, 48, 103-112.

Virginia, R., & Wall, D. (1999). How soils structure communities in the Antarctic Dry Valleys. Bioscience, 49, 973-983.

von Wintzingerode, F., Gobel, U., & Stackebrandt, E. (1997). Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiology Research, 21, 213-229.

Ward, D., Weller, R., & Bateson, M. (1990). 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature, 345(6270), 63-65.

Whitaker, R., Grogan, D., & Taylor, J. (2003). Geographical barriers isolate endemic populations of hyperthermophilic archaea. Science, 301, 976-978.

Wierzchos, J., Ascaso, C., Ager, F., García-Orellana, I., Carmona-Luque, A., & Respaldiza, M. (2006). Identifying elements in rocks from the Dry Valleys desert (Antarctica) by ion beam proton induced X-ray emission. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 249(1-2), 571-574.

Willig, M., Kaufman, D., & Stevens, R. (2003). Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annual Review of Ecology, Evolution, and Systematics, 34(1), 273.

Wilson, Z., & Brimble, M. (2009). Molecules derived from the extremes of life. Natural Product Reports, 26, 44-71. 10.1039/b800164m

Wood, S., Rueckert, A., Cowan, D., & Cary, C. (2008). Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME J, 2(3), 308-320.

Wynn-Williams, D. (1990). Ecological aspects of Antarctic microbiology. Advances in Microbial Ecology, 11, 71-146.

Xu, D., & Cotè, J.-C. (2003). Phylogenetic relatonshops between Bacillus species and related genera inferred from comparison of 3' and 16S rDNA and 5' end 16S-23S ITS nucleotide sequences. International Journal of Systematic Evolutionary Microbiology, 53, 695-704.

Yergeau, E., Bokhorst, S., Huiskes, A., Boschker, H., Aerts, R., & Kowalchuk, G. (2007a). Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. FEMS Microbiology Ecology, 59(2), 436-451.

Yergeau, E., Newsham, K., Pearce, D., & Kowalchuk, G. (2007b). Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environmental Microbiology, 9(11), 2670-2682.

Chapter 2: Inter-Valley Comparison Study of the Microbial Biodiversity Present in Four of the McMurdo Dry Valleys, Antarctica.

2.1. Abstract

Extreme environments provide a unique source of often highly adapted and tolerant organisms. Research on organisms in these habitats has led to the discovery of novel and useful compounds and may assist in understanding the impact of global change on biodiversity. The Dry Valleys of Eastern Antarctica are vast, ice-free regions believed to be the coldest, driest desert on Earth. Despite these harsh conditions, there is an increasing amount of evidence demonstrating that the soil ecosystems of the Dry Valleys sustain a wide diversity of microorganisms. The research presented is an inter-valley comparison study which aims to scrutinize microbial communities and environmental factors driving their distribution in the Dry Valleys. Automated ribosomal intergenic spacer analysis (ARISA) was used to provide a "snapshot" of bacterial and cyanobacterial communities living in the mineral sands in Miers Valley, Beacon Valley, Upper Wright Valley and at Battleship Promontory. Rigorous analysis of physico-chemical differences between the soils of these four valleys was undertaken in hope to understand the environmental parameters driving the distribution and biodiversity of microbial communities present. Multivariate statistical analysis and ordination of ARISA and physico-chemical data revealed that bacterial communities from each valley form distinctive clusters. Conversely, cyanobacterial communities showed less diversity and a more even distribution between valleys.

2.2. Introduction

Microbial life adapted to extreme environments has long been a source of interest and wonder in the scientific community. Examples of extreme environments include the Antarctic and Arctic, hot springs and volcanic areas and hydrothermal vents. Organisms referred to as "extremophiles" inhabit these habitats and thrive in these difficult environments.

Antarctica's geographic isolation and climatic severity has allowed it to remain one of the most pristine and least studied continents (Hansom & Gordon, 1998). Biotic communities and ecosystem dynamics in terrestrial Antarctica are limited by an array of extreme conditions including low temperatures, moisture and organic matter availability, high salinity and paucity of biodiversity at higher trophic levels to facilitate key ecological processes.

The McMurdo Dry Valleys, located between 160° and 164°E longitude and 76°30' and 78°30'S latitude, are a 4800 km^2 ice-free region. They are free of ice primarily because glacial flow from the polar plateau is obstructed by the Transantarctic Mountains (Andersen et al., 1992). They are believed by many to be the coldest, driest desert on Earth (Stonehouse, 2002). The microorganisms able to thrive in such an environment are remarkable in terms of evolution and distribution. The Dry Valleys can be divided into three microclimate zones: a coastal thaw zone (e.g. Miers, Garwood and Marshall Valleys), an inland mixed zone (e.g. Taylor Valley, Victoria Valley, Battleship Promontory and Bull Pass) and a stable upland zone (e.g. Beacon Valley and Upper Wright Valley). These zones are defined on the basis of temperature measurements, soil moisture, and relative humidity. Valleys can also differ in geomorphology, lithology and mineralogy. The physico-chemical characteristics of the mineral soils of the McMurdo Dry Valley include low total organic matter (from 0.0 to 0.43%), high incident radiation, low humidity range and high osmotic stress (Cowan et al., 2002; Cowan & Tow, 2004). Soil communities in the Dry Valleys are markedly different from those of other soil ecosystems because are almost completely isolated from human influence on soils (Virginia & Wall, 1999). The Dry Valleys soil environments may be unique in that it is one of the few examples where abiotic factors (e.g., moisture) are more important than biotic factors (e.g., competition, herbivory, predation) for structuring populations (Convey, 1996;

Hogg et al., 2006). Two particularly important determinants of species distribution are likely to be soil moisture availability and temperature (Barrett et al., 2004; Moorhead et al., 1999) resulting in most species having similar growth requirements. Most of these microorganisms are likely unique to this environment. Investigating the adaptations of these microbial organisms to their surroundings has important implications for the understanding of life adapted to extreme environments and the field of exobiology. Because of the singularity of this cold desert, the McMurdo Dry Valleys were classified as the first official Antarctic Specially Managed Area (ASMA) in June 2004. The ASMA covers an area roughly 15000 km^2 which contains cold desert soils millions of years old, special geological features, and unusual communities of plants and microorganisms (http://www.mcmurdodryvalleys.aq/, 2004).

Recently, culture-independent studies employing molecular genetic tools (Aislabie et al., 2006; Babalola et al., 2008; Cowan & Tow, 2004; Niederberger et al., 2008; Saul et al., 2005; Smith et al., 2006; Yergeau et al., 2007) demonstrated that the diversity of microorganisms in Dry Valley soils may be considerably higher than previously thought (Hogg et al., 2006).

There are two established strategies to investigate microbial diversity of a given environment, culture-dependant and culture independent methods (Leuko et al., 2007). Culture-dependant approaches constrained by the difficulties in mimicking actual environmental growth conditions with a culture media, resulting in the selection and enrichment of only certain community representatives. A myriad of new, culture-independent techniques are now available and are unaffected by the intrinsic bias of culture based techniques (Babalola et al., 2008). One of these techniques is automated ribosomal intergenic spacer analysis (ARISA). ARISA is a community fingerprinting method that was developed by Fisher and Triplett (1999). This method has proven to be a fast and reliable method for analyzing microbial communities found in a vast array of environments such as marine communities (Brown & Fuhrman, 2005), freshwater communities (Fisher & Triplett, 1999), soil communities from diverse geographic locations (Hartmann et al., 2005; Kwon et al., 2005; Ranjard et al., 2000; Soo, 2007; Wood et al., 2008) and even stromatolite samples (Leuko et al., 2007). In comparison to the 16S rRNA gene cloning approach, ARISA provides a rapid initial appraisal of the community structure (Leuko et al., 2007). ARISA targets

the intergenic spacer (ITS) region situated between the 16S and 23S genes of the rRNA operon. The ITS region has marked heterogeneity in both sequence length and composition to differentiate between the various communities present in a given environment (González et al., 2003; Ranjard et al., 2000). ARISA is highly reproducible due to automation and considered sensitive enough to detect single nucleotide polymorphisms (Leuko et al., 2007).

The research presented here is an inter-valley comparison study between four very different Dry Valleys. The goal of this study was to investigate bacterial and cyanobacterial diversity in the mineral soils of Miers Valley, Beacon Valley, Upper Wright Valley and Battleship Promontory. Community composition and structure assessed using ARISA, in concert with physicochemical measurements, were used to elucidate the parameters driving differences in microbial diversity between valleys.

2.3. Materials and Methods

2.3.1. Sample collection

Mineral soils were collected from four different valleys in the McMurdo Dry Valleys (Figure 3): Miers Valley (78°6′S 164°0′E, altitude 153 m) , Upper Wright Valley (77°10′S, 161°50′E, altitude 940 m), Beacon Valley (77°83’S, 160°66’E, altitude 1500 m) and Battleship Promontory (76°54'S 160°55'E, altitude 1000 m). Two 50 m transects that each radiated out from a central sampling point (X) were established at each sampling site (Figure 6). At the end of each transect and at the centre point 1 m^2 sampling sites were marked out. Four scoops of soil were collected from 2-4 cm depth sampling area with a sterile spatula and combined into a Whirlpak® (Fisher Scientific Ltd., Ontario, Canada). Samples were kept at -20°C until further analysis.

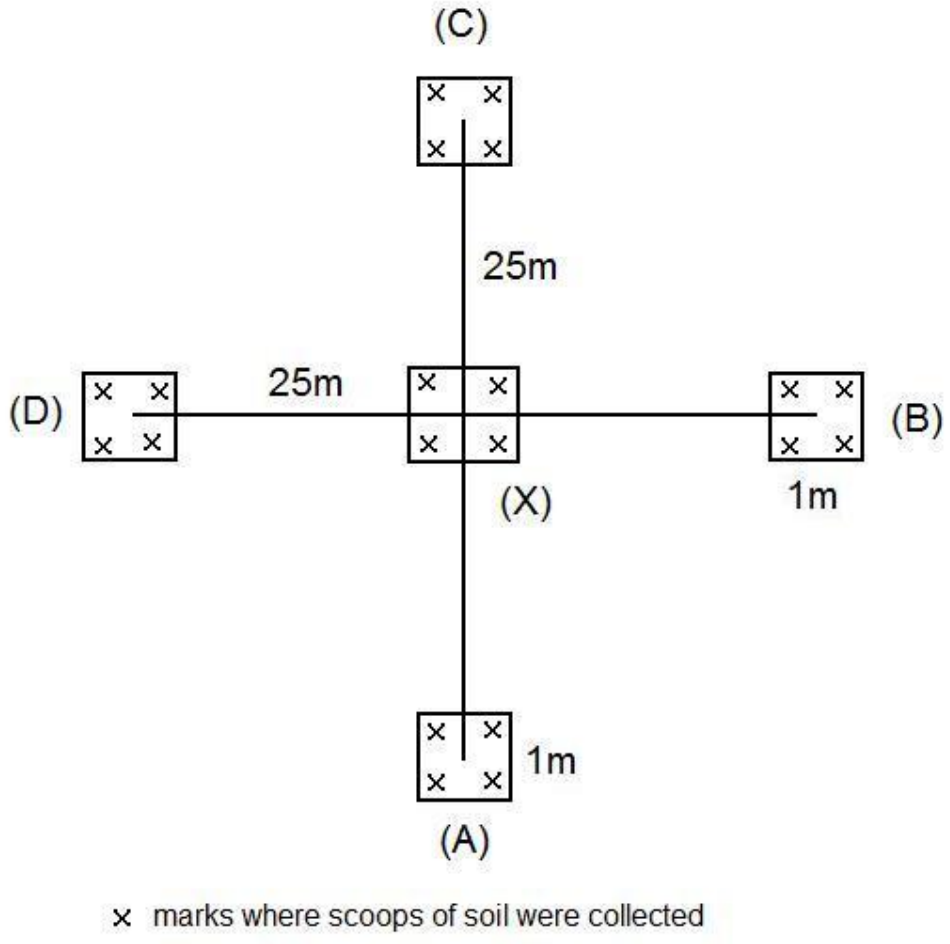

Figure 6. Schematic diagram showing the layout of transects and samples sites. This protocol was used in each of the four study valleys.

2.3.2. Soil physicochemical analysis

Moisture content of soils was determined by gravimetrically drying 40 g of each soil sample at 105°C until samples reached a constant weight (Soo, 2007).

Conductivity and pH were measured using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd, Singapore) following the slurry technique which consists in mixing 1:2.5 mass ratio of samples and de-ionized water (Edmeades et al., 1985).

The total percentage carbon and nitrogen of the soils was measured on a LECO Truspec (LECO Corporation, St. Joseph, Michigan). Samples were prepared for stable isotope analysis by grinding to a fine powder in a ball-grinder.

Ground soil (0.2 g) was weighed out precisely and analyzed by combustion in the LECO Truspec.

Samples were prepared for inductively coupled plasma mass spectrometry (ICP-MS) by acid digestion, as adapted from US EPA analytical methods (2002-2). The digestion involved adding 4 ml of concentrated HNO_3 diluted 1:1 with type I water and 10 ml concentrated HCl diluted 1:4 to 1 g of soil in a 50 ml beaker. A procedural blank was included, containing no soil but treated exactly the same as the soil samples. Beakers were covered with parafilm and allowed to stand (30 min). They were then transferred to a hot plate (preheated to 95°C), left to digest (30 min) before being allowed to cool. The samples were transferred to 100 ml volumetric flasks. Type I de-ionized water was added up to the 100 ml mark and the samples were mixed by repeated inversion. Sub-samples (30 ml) were transferred to 50 ml Falcon tubes (BD Biosciences, Franklin Lakes, New Jersey, USA) and centrifuged for 10 min at 800 x g. The supernatant (20 ml) was transferred to 100 ml volumetric flasks and 1.6 ml of concentrated HNO_3 added (to adjust the concentration of the sample to 2% conc. HNO_3 to match the ICP-MS matrix). Type I de-ionized water was added up to the 100 ml mark and samples mixed well by repeated inversion. Samples were then filtered (0.45 µm) into 15 ml Falcon tubes. ICP-MS analysis was performed using a Mass Spectrometer ELAN® DRC II (PerkinElmer Inc., Münster, Germany). Elements analysed were: Ag^{109}, Al^{27}, As^{75}, B^{10}, Ba^{137}, Bi^{209}, Ca^{43}, Cd^{111}, Co^{59}, Cr^{52}, Cu^{63}, Fe^{54}, Hf^{180}, Hg^{202}, In^{115}, K^{39}, Li^{7}, Mg^{24}, Na^{23}, Ni^{60}, P^{31}, Pb^{207}, Se^{82}, Si^{28}, Sr^{88}, Ti^{49}, Tl^{205}, U^{238}, V^{51}, Zn^{68} and Zr^{90}.

2.3.3. Extraction of genomic DNA

DNA was extracted from 0.8 g of soil using the CTAB/bead-beating method described in Coyne et al., (2001). Soil samples (0.8 g) were measured into screw-capped tubes containing 0.5 g each of 0.1 mm and 2.5 mm silica-zirconia beads. Phosphate buffer (100 mM NaH_2PO_4) and SDS lysis buffer (100mM NaCl, 500mM Tris, 10% SDS) were added and the tubes were shaken at 4.2 ms^{-1} for 30 sec (Mini-Beadbeater, Glenmills inc, Clifton, NJ). The aqueous phase was extracted by centrifugation (16,000 × *g*) for 3 min at room temperature. Then, cetyltrimethylammonium bromide (CTAB) containing 0.4% (v/v) β-

mercaptoethanol was added to the tubes, which were incubated at 60°C for 30 minutes. The aqueous phase containing nucleic acids was separated by centrifugation (16,000 × *g*) for 5 min at 4°C. The aqueous phase was then extracted by mixing with an equal volume of chloroform-isoamyl alcohol (24:1) followed by centrifugation (16,000 × *g*) for 5 min at 4°C. This step was repeated, allowing the samples to incubate at room temperature on a rocking bed for 20 min before centrifugation. Total nucleic acids were subsequently precipitated from the extracted aqueous layer with 7 M ammonium acetate to a final concentration of 2.5 M, followed by centrifugation (16,000 × *g*) for 5 min at 4°C. Ice cold isopropyl alcohol (0.54 volumes) was added to the supernatant and incubated at -20°C overnight. The precipitated nucleic acids were centrifuged (16,000 × *g*) at 4°C for 20 min. Pelleted nucleic acids were washed in ice cold 70% (vol/vol) ethanol and air dried prior to resuspension in 20 µl of ultrapure TE (10 mM Tris base and 1 mM EDTA.). DNA was stored at -20°C for further use in PCR analyses.

2.3.4. Automated rRNA intergenic spacer analysis (ARISA)

The bacterial ARISA PCR reactions were performed in duplicate 50 µl volumes containing; between 10 to 50 ng of DNA, 1.5 µl of each bacterial primer ITSF labelled with the fluorescent dye hexachlorofluorescein (HEX) (5'-HEX-GTCGTAACAAGGTAGCCGTA-3') and ITSReub (5'-GCCAAGGCAATC CCACC-3'; 10 µM) (Cardinale et al., 2004), 1X Platinum Taq PCR buffer (Invitrogen Ltd, New Zealand), 3 µl $MgCl_2$ (50 mM), 1U of Platinum Taq DNA polymerase (Invitrogen Ltd, New Zealand), 5 µl bovine serum albumine (BSA, 0.2mg/ml), 5 µl dNTPs (2 mM; Roche Diagnostics, New Zealand) and Milli-Q PCR-grade water (Millipore, Billerica, MA, USA) to 50 µl. Thermal cycling conditions were; initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 45 sec, 55°C for 30 sec, 72°C for 2 min, and a final extension at 72°C for 7 min.

The cyanobacterial ARISA PCR reactions were performed as previously described (Wood et al., 2008). PCR reactions were performed in duplicate 50 µl volumes containing; between 10 to 50 ng of DNA, 1 µl of each cyanobacterial primer CY-ARISA-F labelled with the fluorescent dye 6-carboxy-fluorescine

(FAM) (5'-FAM-GYCAYRCCCGAAGTCRTTAC-3') and 23S30R (5'-CHTCGCCTCTGTGTGCCWAGGT-3'; 10 µM), 1X Platinum Taq PCR buffer (Invitrogen Ltd, New Zealand), 3 µl $MgCl_2$ (50 mM), 1U of Platinum Taq DNA polymerase (Invitrogen Ltd, New Zealand), 5 µl bovine serum albumine (BSA, 0.2mg/ml), 5 µl dNTPs (2 mM) (Roche Diagnostics, New Zealand) and Milli-Q PCR-grade water (Millipore, Billerica, MA, USA) to 50 µl. Thermal cycling conditions were; initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 50°C for 30 sec, 72°C for 2 min, and a final extension at 72°C for 7 min.

All PCR reactions were run on a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Loboratories Inc, Hercules, CA). Duplicate PCR reactions were combined and visualized on 1% agarose gel, then purified using a QuickClean PCR Purification Kit (Genscript Corporation, New Jersey, USA). Purified ARISA PCR products were quantified using a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA), diluted 1:10 (v/v) with Milli-Q H_2O (Millipore, Billerica, MA, USA). Diluted product (2 µl) was mixed with 0.25 µl of ROX-labelled genotyping internal size standard ETR900R (GE Healthcare). Each sample was made to 10 µl with 0.2 v/v Tween-20 in sterile water. Intergenic spacer lengths were determined by capillary electrophoresis using the MegaBACE 500 DNA Analysis system (GE Healthcare).

2.3.5. Statistical analysis

A two-way analysis of variance (ANOVA) followed by a Tukey HSD post-hoc test were performed using Statistica 7 (StatSoft Inc, Tulsa, USA) to identify significant differences in physico-chemical properties between valleys at a 95% confidence interval.

ARISA fragment lengths (AFL) were processed using Genetic Profiler V.2 (GE Healthcare) and transferred to Microsoft Excel (Microsoft Corporation, WA, USA) for alignment. AFLs were aligned using an Excel Macro. AFLs that differed by less than 2 base-pairs (bp) in length were considered identical and only the AFL with the highest florescence was maintained (Wood et al., 2008). ARISA fragment lengths falling below a threshold of 250 Fluorescence Units

were removed since they could not be fully distinguished from instrument "noise". ARISA fragment lengths shorter than 180 bp (Bacteria) and 300 bp (Cyanobacteria) were removed as they were considered to short to be true ITS's (Cardinale et al., 2004; Wood et al., 2008). All AFL information was transposed to presence/absence data for further statistical analysis.

An analysis of similarities (ANOSIM) was performed in Statistica 7 (StatSoft Inc, Tulsa, USA) to compare the four valleys: Miers Valley, Beacon Valley, Battleship Promontory and Upper Wright Valley.

To visualize the relative similarity of AFL profiles, nonmetric multidimensional scaling (MDS) analysis based on the Bray-Curtis similarity index was undertaken using the PRIMER 6 software package (PRIMER-E, Ltd., UK). The relative distance between sites in these ordination plots indicates the relative similarity of AFL profiles (i.e., community structure). Plots with a stress value less than 0.20 provide interpretable information (Clarke 1993). Nonmetric multidimensional scaling analysis was undertaken with 100 random restarts and results plotted in two-dimensions. Agglomerative, hierarchical clustering of the Bray-Curtis similarities was carried out using the CLUSTER method of the PRIMER 6 software. These results were also imposed onto the two-dimensional MDS at similarity levels of 20% and 40%. The influence of physicochemical variables on the community structure of the soil samples was assessed using the BEST analysis in PRIMER 6 (Clarke and Gorley 2006). The BEST procedure calculates the value of Spearman's rank correlation coefficient (ρ) using every possible combination of variables until it finds the 'best' fit (i.e., the combination of parameters whose Euclidean distance matrix gives the highest ρ). The combination of explanatory geochemical variables yielding the highest ρ was chosen for analysis and discussion.

2.4. Results

2.4.1. Geochemistry of soils

Soil physical and chemical properties varied significantly between valleys, but were consistent within valleys. There were two exceptions to this. Cluster analysis based on euclidean distance and MDS ordination showed that Miers Valley sample B (MV-B) and Beacon Valley sample C (BV-C) clustered more closely with samples from other valleys (Figure 7, 8).

The mean water content was higher in samples from Beacon Valley (2.4%) relative to Battleship Promontory and Upper Wright Valley (1.1% in both). Miers Valley samples contained the least amount of moisture (0.5%; Table 1).

The pH was generally found to be around neutral in all mineral sand samples, with the exception of Miers Valley recording a more basic pH of 8.6. The carbon to nitrogen ratio also differed significantly between Miers Valley and the other three valleys (Table 1). Miers had the highest C/N ratio (9.04) compared to Beacon Valley, Battleship Promontory and Upper Wright Valley (1.77, 2.18 and 0.94 respectively).

All four valleys had similar dominant elements: aluminium, boron, calcium, iron, potassium, manganese, sodium, phosphorus, silica and titanium. The analysis of variance showed that the concentration of nearly all trace metal elements varied significantly between valleys, with the exception of silver, aluminium, arsenic, boron, bismuth, cadmium, mercury and uranium (Table 1). The post-hoc Tukey test demonstrated that the elemental analysis showed a similar trend to that of the C/N ratio with the Miers Valleys elemental concentrations often significantly different from the other valleys (which did not differ significantly, Appendix C).

Table 1. Physiochemical properties of the four valleys Results are the average of five samples per valley.

	Miers Valley	**Beacon Valley**	**Battleship Promontory**	**Upper Wright Valley**	**F**	**p**
Altitude (m)	153	1500	1000	940	n/a	n/a
% moisture	0.52	2.36	1.14	1.07	1141448	<0.001
pH	8.6	7.1	7.7	7	25.73	<0.001
Conductivity (mS)	0.3	3.92	0.11	6.13	25.73	<0.001
%C	0.46	0.14	0.1	0.11	23.23	<0.001
%N	0.05	0.08	0.05	0.12	18.48	<0.001
C/N ratio	9.04	1.77	2.18	0.94	3.41	0.043
Ag	0.02	0.02	0.00	0.00	1.76	0.195
Al	23441.25	27272.59	24689.95	20033.00	1.64	0.219
As	1.86	2.20	1.16	1.95	1.63	0.223
B	1010.70	1013.85	1037.81	1028.43	0.39	0.761
Ba	150.50	54.97	24.65	40.06	19.95	<0.001
Bi	0.95	0.08	0.02	0.06	1.07	0.39
Ca	24673.21	16187.29	9812.92	5494.02	26.61	<0.001
Cd	0.25	0.21	0.13	0.17	3.23	0.051
Co	30.88	22.34	16.12	12.53	22.34	<0.001
Cr	51.84	10.96	7.45	8.61	79.67	<0.001
Cu	23.89	147.76	99.72	66.30	52.28	<0.001
Fe	44330.21	48771.24	31943.47	27758.07	8.96	0.001
Hf	0.61	0.40	0.28	0.39	5.23	0.01
Hg	0.04	0.05	0.01	0.02	3.55	0.038
In	0.03	0.02	0.02	0.02	3.79	0.032
K	5157.30	2098.73	1026.13	3752.67	10.06	<0.001
Li	17.10	14.12	8.34	13.38	8.22	0.002

Table 1. (Continued)

	Miers Valley	Beacon Valley	Battleship Promontory	Upper Wright Valley	F	p
Mg	40689.52	10561.11	5524.19	7881.83	101.43	<0.001
Mn	733.50	425.26	317.42	300.56	20.93	<0.001
Na	7767.40	4426.65	2799.42	7030.73	6.33	0.005
Ni	169.63	29.02	22.51	18.15	103.46	<0.001
P	1750.60	974.41	719.47	517.38	35.1	<0.001
Pb	4.21	8.85	3.62	9.14	20.02	<0.001
Se	0.96	1.58	1.16	1.13	4.74	0.015
Si	12387.92	1886.26	1762.82	1888.86	39.82	<0.001
Sr	321.11	70.45	37.21	32.43	52.67	<0.001
Ti	5118.20	834.42	864.55	460.97	58.17	<0.001
Tl	0.09	0.07	0.03	0.08	4.93	0.013
U	1.17	1.09	0.89	1.05	0.77	0.523
V	78.39	155.82	80.72	64.59	9.11	<0.001
Zn	104.44	104.13	63.28	64.61	15.04	<0.001
Zr	30.74	14.45	10.13	18.02	9.23	<0.001

*all trace metal concentrations are in ppb
F is the resulting statistic from the ANOVA
p is the probability that this result is statistically significant. $p < 0.5$ means there is no statistical difference between means compared.

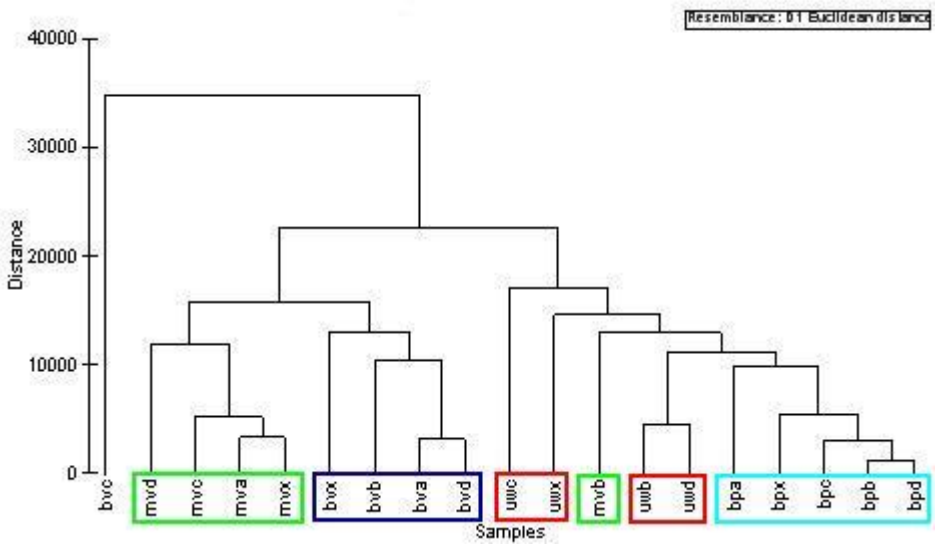

Figure 7. Cluster plot based on Euclidean distances between the physico-chemical properties of the four Dry Valley soils. Miers Valley (mv), green; Beacon Valley (bv), dark blue; Battleship Promontory (bp), light blue; Upper Wright Valley (uw), red.

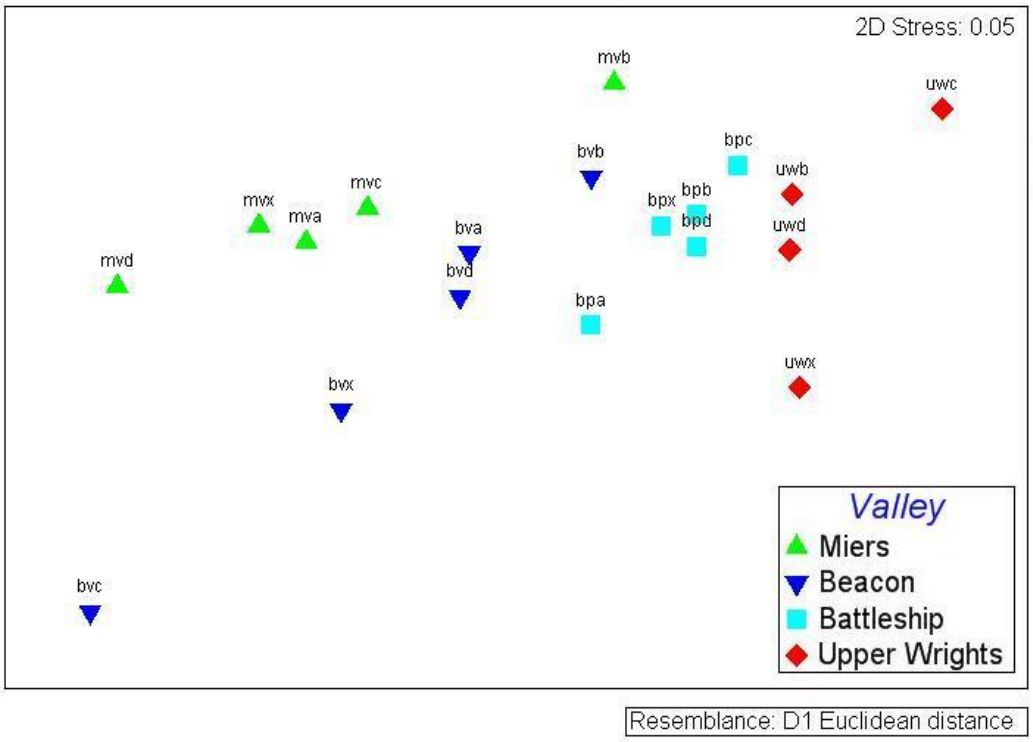

Figure 8. Two-dimensional, non-metric MDS ordination (stress = 0.05) based on Euclidean similarities of physico-chemical properties of four Antarctic Dry Valleys.

2.4.2. DNA fingerprinting analysis:

Analysis of the ARISA data for all samples identified a total of 419 distinct peaks for bacteria and 97 distinct peaks for cyanobacteria. When peak numbers were totalled across each sample (Table 2), the highest diversity and average peak number per sample was observed in Battleship Promontory (181 bacterial peaks and 41 cyanobacterial peaks) followed closely by Miers Valley (122 bacterial peaks and 39 cyanobacterial peaks). Beacon Valley had the lowest number of peaks (56 bacterial peaks and 5 cyanobacterial peaks). Upper Wright sample A UW-A) only had very few ARISA peaks for both bacterial and cyanobacterial ARISA (data not shown). This was possibly due to inhibition or experimental error. These peaks were counted in the total (Table 2, Figure 9), but this sample was excluded from further analysis.

Table 2. Summary of ARISA fragment lengths (AFL) in each sample

Sample location	Total no. of different AFLs in sample type		Average AFL per sample (range)	
	Bacterial	Cyanobacterial	Bacterial	Cyanobacterial
Miers Valley	122	39	35 (5-42)	8 (4-10)
Beacon Valley	56	5	15 (2-21)	2 (1-2)
Battleship Promontory	181	41	44 (35-52)	9 (5-15)
Upper Wright Valley	60	12	9 (1-18)	4 (3-7)

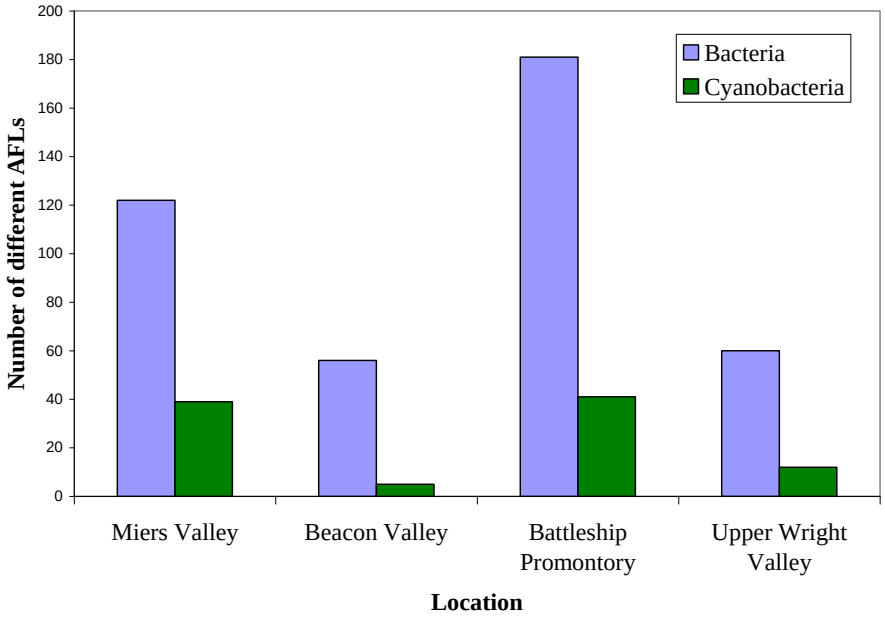

Figure 9. Number of different AFLs in each valley**.**

The clustering patterns of the two-dimensional MDS ordinations varied significantly between bacterial and cyanobacterial communities. With the exception of sample D from Battleship Promontory (BP-D), the samples from Miers Valley, Battleship Promontory, Upper Wright Valley and Beacon Valley formed tight, within-valley clusters when using the bacterial ARISA data (Figures 10,11). The initial cyanobacterial MDS ordination results in a tight un-interpretable clustering of all but three samples (Figure 13). Samples C, D and X from Beacon Valley did not cluster closely to the other samples (Figure 12). These three samples contained only one or no cyanobacterial ARISA peaks each (data not shown). However, they contained multiple, varied peaks when amplified with the ITSF/ITSReub bacterial ARISA primers. It is therefore likely that these results reflect the true cyanobacterial diversity and that the absence of peaks was not due to the presence of PCR-inhibitors. The MDS analysis was repeated excluding these samples to allow visualisation of ordination patterns among other samples (Figure 14). The Cluster analysis of the cyanobacterial ARISA data was in contrast to that of the bacterial data, samples from Battleship Promontory clustering closest to samples from Wrights Valley. Upper Wrights sample C, was the exception clustering with the Miers Valley samples (Figures 12, 14).

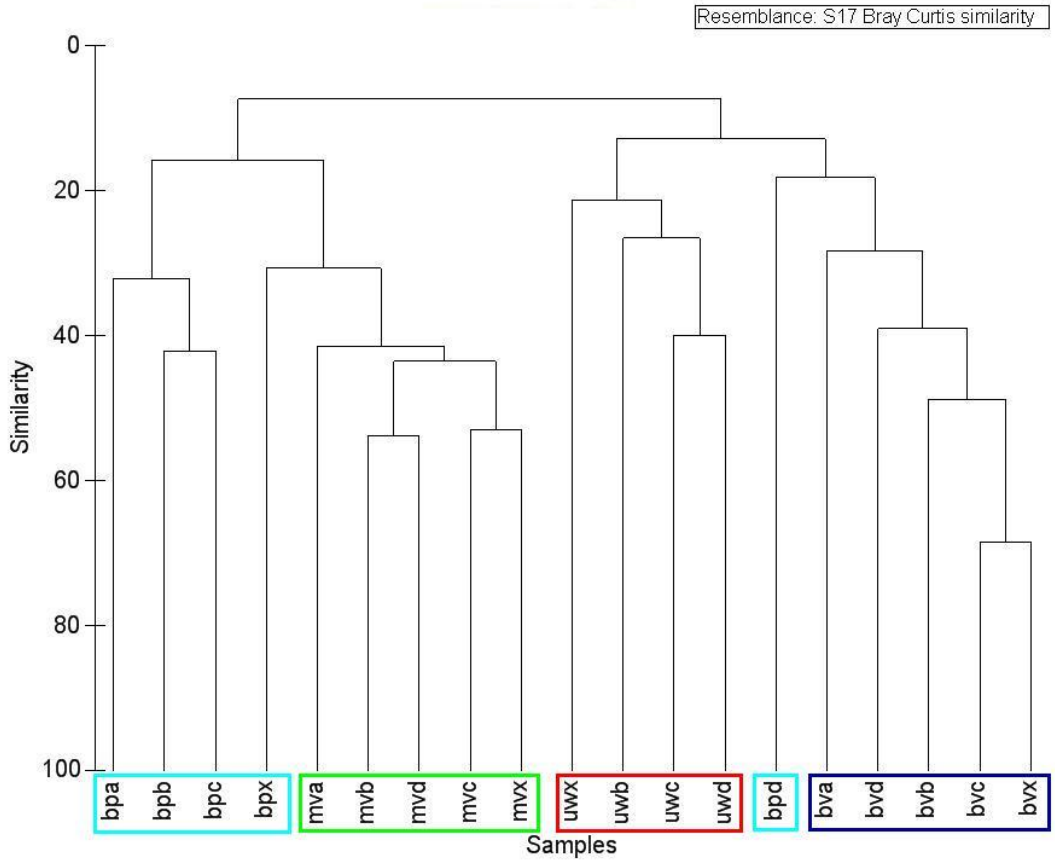

Figure 10. Cluster plot showing Bray-Curtis similarity between bacterial ARISA profiles. Miers Valley (mv), green; Beacon Valley (bv), dark blue; Battleship Promontory (bp), light blue; Upper Wright Valley (uw), red).

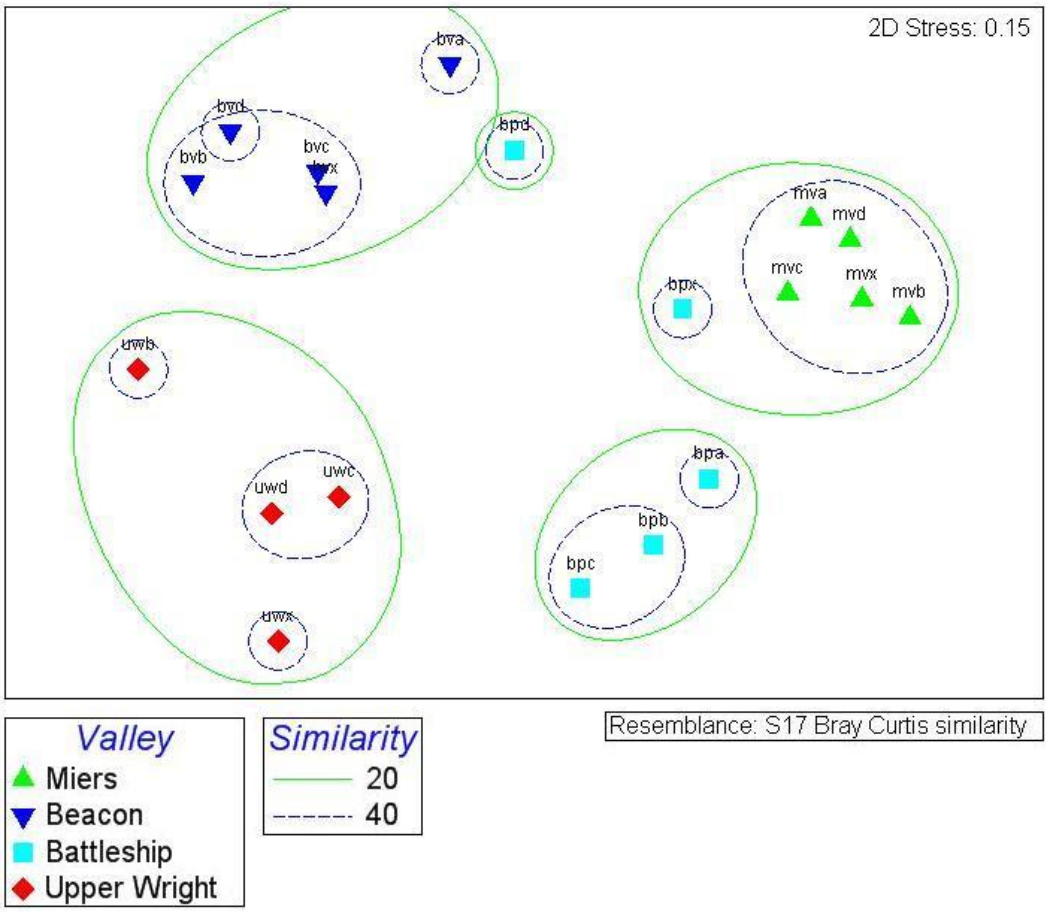

Figure 11. Two-dimensional, non-metric MDS ordination (stress = 0.15) based on Bray-Curtis similarities of ARISA fingerprints of bacterial communities from four Antarctic Dry Valleys. Points enclosed by solid green circles cluster at 20% similarity, points enclosed by dashed blue circles cluster at 40% similarity.

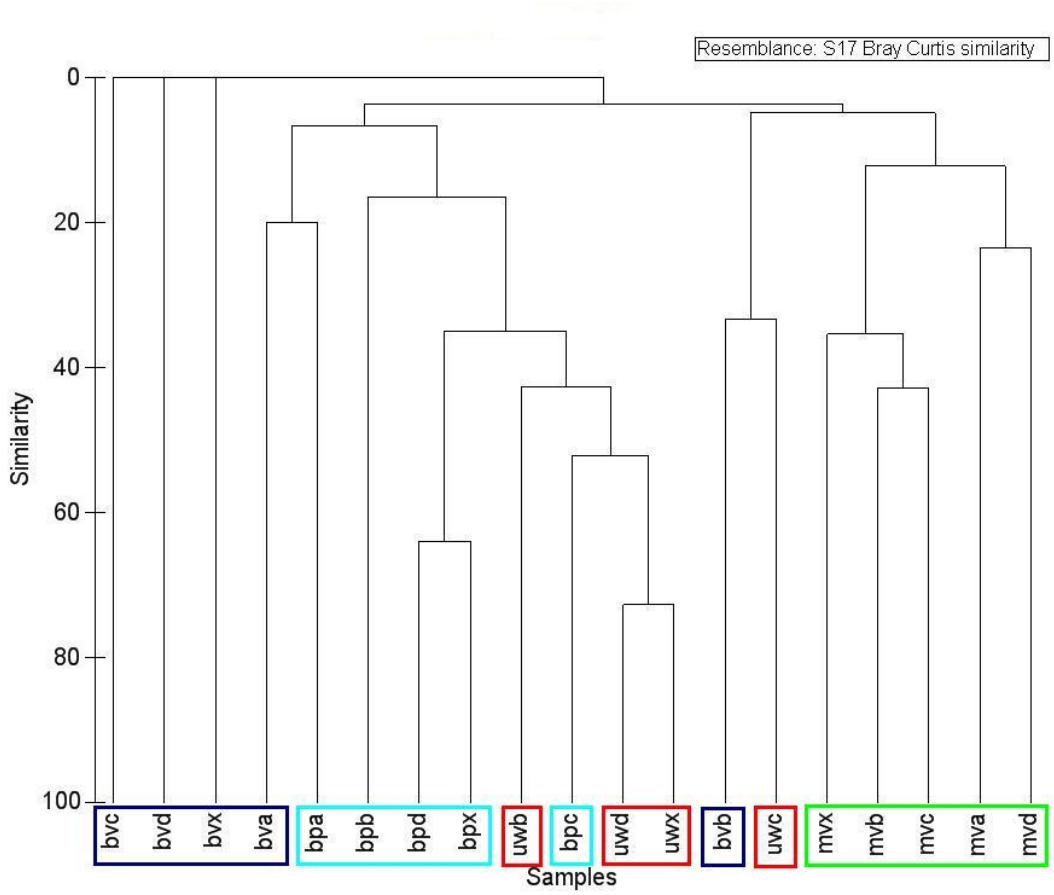

Figure 12. Cluster plot showing Bray-Curtis similarity between cyanobacterial ARISA profiles. Miers Valley (mv), green; Beacon Valley (bv), dark blue; Battleship Promontory (bp), light blue; Upper Wright Valley (uw), red.

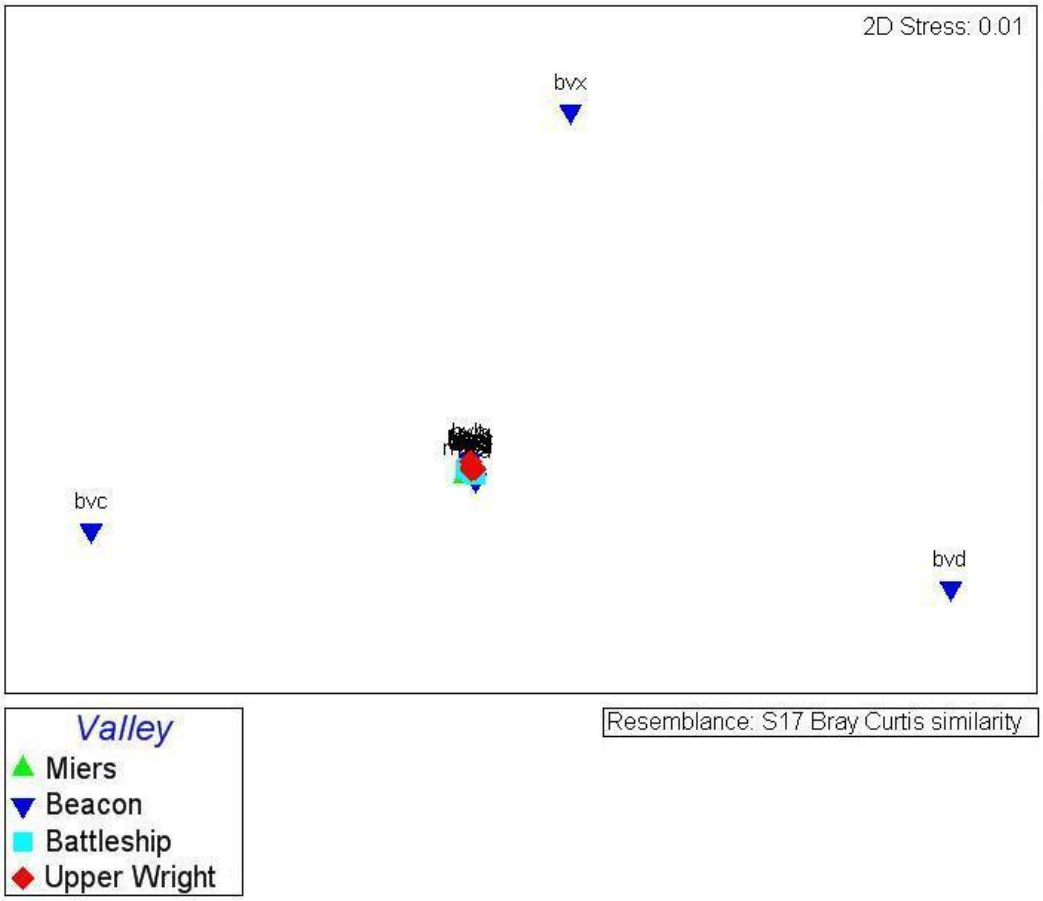

Figure 13. Two-dimensional, non-metric MDS ordination (stress = 0.01) based on Bray-Curtis similarities of ARISA fingerprints of cyanobacterial communities from four Dry Valleys, Antarctica.

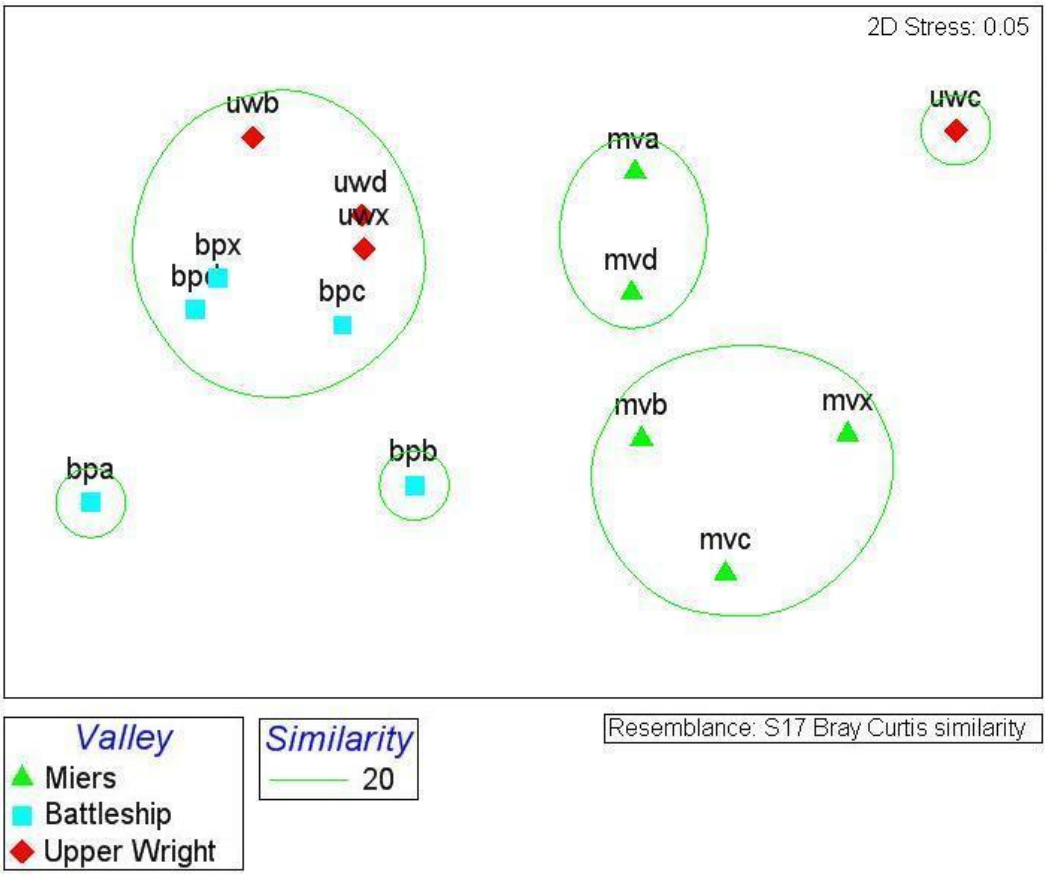

Figure 14. Two-dimensional, non-metric MDS ordination (stress = 0.05) based on Bray-Curtis similarities of ARISA fingerprints of cyanobacterial communities from locations in three Dry Valleys, Antarctica (Beacon Valley excluded). Points enclosed by solid green circles cluster at 20% similarity.

Analysis of similarities (ANOSIM) provides a method to assess statistical differences between groups in multivariate data sets. ANOSIM produces a sample statistic, R, which represents the degree of separation between test groups (Clark, 1993). A value close to 1 indicates the community composition is totally different where a value of 0 indicates no difference. ANOSIM showed a high degree of variability in bacterial ARISA data between valleys (global R = 0.88, significance level = 0.1%). The difference in cyanobacterial community structure was not pronounced (global R = 0.424, significance level = 0.1%). Pair-wise comparisons between valleys revealed significant differences between the bacterial communities of all four different valleys, with the least variation observed between Battleship Promontory and Miers Valley (Table 3). Cyanobacterial communities also showed some variation between Miers Valley and Battleship Promontory, but overall the variation between valleys was not as marked as the bacterial ARISA data. No significant difference was observed between the cyanobacterial communities of Beacon Valley and Upper Wright Valley, and between Battleship Promontory and Upper Wright Valley (Table 3).

Table 3. ANOSIM statistics for tests involving a comparison of all four sampling locations.

	Comparison		R-statistic	Significance level (%)
	Location 1	Location 2		
Bacteria	Battleship Promontory	Miers Valley	0.672	0.8
	Beacon Valley	Miers Valley	1	0.8
	Miers Valley	Upper Wright Valley	1	0.8
	Battleship Promontory	Beacon Valley	0.854	0.8
	Battleship Promontory	Upper Wright Valley	0.797	0.8
	Beacon Valley	Upper Wright Valley	0.878	0.8
Cyanobacteria	Beacon Valley	Miers Valley	0.436	0.8
	Miers Valley	Upper Wright Valley	0.481	2.4
	Battleship Promontory	Miers Valley	0.789	0.8
	Battleship Promontory	Beacon Valley	0.457	0.8
	Battleship Promontory	Upper Wright Valley	0.256	7.1*
	Beacon Valley	Upper Wright Valley	0.12	15.1 *

*not significant

BEST analysis using a Spearman rank correlation with 99 permutations in PRIMER revealed that pH, conductivity and copper concentration (Spearman's ρ = 0.729, significance level = 1%) were the best explanatory variables of the bacterial community fingerprints obtained with ARISA. The same analysis on the cyanobacterial community fingerprints revealed that vanadium and copper concentrations (Spearman's ρ = 0.577, significance level = 1%) were the best explanatory variables for the cyanobacterial ARISA profiles obtained. The second best score obtained to explain these profiles also included total carbon content (Spearman's ρ = 0.560, significance level = 1%).

2.5. Discussion

Soil ecosystems are typically complex environments in which a multitude of biotic and abiotic factors shape diverse communities of microorganisms. The Antarctic soils of the McMurdo Dry Valleys however, are a particular, simpler ecosystem in which the absence of biotic influences (i.e. absence of vascular plants and higher life forms) means that microbial populations may be driven solely by abiotic factors (Convey, 1996; Hogg et al., 2006).

Each valley within the Dry Valleys region is a different ecosystem in itself. They can be divided into three main climatic zones: subxerous (coastal areas), xerous (inland valleys), and ultraxerous (adjacent to the Polar Plateau) (Campbell & Claridge, 1987). Each valley has a combination of distinct characteristics such as geomorphology, the presence or absence of lakes, distance from the coast, altitude, average temperatures, lithology and mineralogy of the soils. All of these different characteristics might directly influence the production and availability of carbon, and the special and temporal availability or water. *Differences in soil mineralogy and lithology might control the presence or absence and distribution of microbial life.* The four valleys in this study were chosen based on such differences.

Miers Valley is a coastal (subxerous), low altitude valley containing a large lake (Lake Miers). Surface sediments are primarily glacial till derived from granitic rocks, which is characteristic of the eastern valleys (Bockheim & McLeod, 2008) Fine-grained deposits blown around by winds, including carbonate and

gypsum, as well as granite and marble mantle the floor of Miers Valley (Clayton-Greene et al., 1988). We found that the soils sampled from this valley had the lowest moisture content out of all four valleys sampled; the sediments were a coarse mix unlikely to support biodiversity (Table 1). The presence of Miers Lake allows for moisture to enter the system and the establishment of primary producers, i.e. cyanobacteria, which in turn can sustain diverse populations of heterotrophs.

In stark contrast to Miers Valley, the two next locations sampled were Beacon and Upper Wright Valleys. In these upland valleys, exposed bedrock consists of generally flat-lying sedimentary rocks of the Beacon Supergroup (sandstones, siltstones, and conglomerates) and Ferrar Dolerite (Elliot & Fleming, 2004). Beacon Valley is an ultraxerous, lakeless, high-altitude valley in the Quartermain Mountains. Sediments in this Western valley are dominated by gypsum-cemented dolerite and sandstone (Bockheim & McLeod, 2008) which we found to have a relatively high salt content (Table 1). Because of its proximity to the Polar Plateau, the valley has extremely low temperatures and strong, desiccating winds. The average soil temperature recorded in Beacon Valley is -22°C, with a high of -5°C in the summer (Marchant & Head, 2007). We found the soils to have a low moisture content (Table 1) creating an overall inhospitable environment for soil microorganisms.

The third valley sampled, Upper Wright Valley, is also an ultraxerous valley. It is at a relatively high altitude, western valley at the margin of the East Antarctic Ice Sheet (Lewis et al., 2006). The soils of Upper Wright can be described as a mix of fine-grained, stratified sandstone grading upward into coarse sand and capped by poorly sorted boulders, cobbles, and gravel (Nichols, 1971) with scarce niches available for microorganisms to become established and lacking any established cyanobacterial mats. Wright Valley also has the largest occurrence of soils with saltpans in Antarctica (Bockheim & McLeod, 2008), which explains why conductivity value we obtained was from the Upper Wright samples was the highest out of the four Dry Valleys studied.

The final set of samples was taken from Battleship Promontory, a valley in the inland mixed (i.e. xerous) zone of the McMurdo Dry Valleys. It shares certain characteristics with Beacon Valley: it is high in altitude and has a similar mineralogy and lithology, the soils and cliff formations being mostly composed of

Beacon sandstone. However, this valley is different in that it has periodically available liquid water from melted snow and transient ponds. This water is absorbed and retained by the sandstone, creating moister, sheltered niches available for microorganisms to colonize.

A comparison of the ARISA fragment lengths among the samples enabled us to make inter-valley comparisons of microbial communities across a range of physico-chemical conditions between the four distinct Dry Valleys: Miers Valley, Beacon Valley, Battleship Promontory and Upper Wright Valley. Out of the four locations we have studied, Miers Valley is virtually the only one where previous research on bacterial diversity has been carried out. Cyanobacterial diversity has been investigated in Miers and Beacon Valley, as well as Battleship Promontory (Wood et al., 2008; Wynn-Williams, 2000). To our knowledge, this is the first study examining cyanobacterial abundance in Upper Wright Valley.

Our ARISA data indicated that there are very diverse microbial communities living in the soils of Miers Valley. This result was comparable to findings by Smith et al. (2006) and Babalola et al. (2008) who observed high bacterial diversity in the Miers soils, mostly dominated by Actinobacteria and Acidobacteria. We also found relatively high bacterial diversity in the moist sandstone dominated soils from Battleship Promontory as well. In contrast, our ARISA data revealed a much lower diversity in the upland valleys of Beacon and Upper Wright. These valleys are under the influence of the Polar Plateau, and have colder year-round temperatures and consequently little available liquid water. We do not know if the few bacterial communities observed are established and active within these valleys, or if they are merely assemblages of organisms attached to mobile particulates that have been blown inland from more hospitable coastal valleys (Wynn-Williams, 1990). Indeed, wind dispersal has been found to being the most likely dispersal agent in this case, given the extremely small amount of precipitation in the Dry Valleys (Broady, 1996; Nkem et al., 2005; Wood et al., 2008).

Smith and colleagues (2006) have shown that Miers Valley soils harbour high cyanobacterial diversity. Their study of eight different sites within the valley brought them to the conclusion that signatures found were from free-living *Cyanobacteria* (Smith et al., 2006). Another study explained their distribution was

driven by wind dispersal from lakes (Aislabie et al., 2006). Wood et al. (2008) used ARISA to study the distribution of cyanobacterial signatures across Miers Valley and found that lake and hydroterrestrial cyanobacterial mats had a significant influence on soil cyanobacterial communities. Interestingly, the authors also sampled soils from five different points in Beacon Valley and found that, despite the higher moisture content of the soil, only a few ARISA fragment lengths could be identified. Confirming this, we found the soils of Miers Valley to harbour high cyanobacterial diversity, while there was very low apparent cyanobacterial diversity in the soils of Beacon Valley. We also found a low abundance of cyanobacterial AFLs in Upper Wright Valley. Given the very low moisture content of the soils, it is likely that the few cyanobacterial species found in the two latter valleys originate from cyanobacteria being blown across the valleys from cyanobacterial mats around lakes (Aislabie et al., 2006) rather than from free-living terrestrial species.

An important consideration when analyzing ARISA results is that some of the apparent diversity obtained is possibly due to the presence of intragenomic heterogeneity between multiple rRNA operons from single organisms (Brown & Fuhrman, 2005). Conversely, ARISA can also underestimate species diversity because different species may have identical ITS lengths and therefore appear under the same AFL (Wood et al., 2008). Furthermore, microbial communities are found to approximate long-tailed distributions, implying that to accurately estimate diversity requires sampling approximately 80% of the total species present (Gans et al., 2005) which is beyond the resources available for most studies.

Water availability is frequently positively correlated with microbial activity, as water is the main component of the protoplasm and necessary for microbial physiology (Edwards et al., 1999). However, such a correlation is not apparent in our findings. One reason for this could be that only a fraction of the moisture content is actually available to microorganisms. Quick response to intermittent water supplies and utilisation of transient dew is a characteristic adaptation of cyanobacteria in desert environments (Wynn-Williams, 2000). This may account in part for cyanobacterial abundance in moister desert niches such as Miers Valley

and Battleship Promontory (Wood et al., 2008; Wynn-Williams, 2000). During favourable, warmer conditions when water becomes more available, these cyanobacteria make a contribution to the net primary carbon productivity in the system. They add nutrients to nutrient-depleted soils, supporting heterotrophs. We found that the soils studied from all four valleys were generally low in carbon and nitrogen. This finding is consistent with previous studies of Dry Valley soils (Barrett et al., 2006b; Barrett et al., 2004; Wood et al., 2008), and can be explained by the absence of vascular plants in the Valleys (Aislabie et al., 2006). Mineral soils from Miers Valley and Battleship Promontory showed a significantly higher carbon to nitrogen ratio (C/N) than the soils from the other valleys. This can be explained by the presence of a large, central lake in Miers Valley, and moisture absorbent sandstone in Battleship Promontory. The cyanobacterial communities thriving in the beacon sandstone cliffs of Battleship Promontory explains the higher C/N found in this area (Wynn-Williams, 2000).

The MDS ordination and analysis of similarities (ANOSIM) between the four Dry Valley ARISA profiles indicates a clear delineation in bacterial community structure between samples. This finding correlates with other Dry Valley studies (Smith et al., 2006; Soo, 2007; Wood et al., 2008).

The BEST analysis indicates that pH, conductivity and traces of copper in the soils were the most likely explanatory variable for the variation in bacterial diversity. The same analysis of cyanobacterial distribution indicates that cyanobacterial diversity was best explained by the traces of vanadium and copper present in the soils, as well as total percentage of carbon. Copper has been hypothesized to contribute to the low cyanobacterial biodiversity in Beacon Valley (Wood et al., 2008), as copper appears to be toxic to certain key cellular processes and disrupts plasma membrane integrity (Avery et al., 1996; Suroszl & Palinska, 2004). This, and the absence of a lake or ponds in this valley could account in part for the low cyanobacterial diversity observed in the soils from Beacon Valley.

The main geochemical factors that determine the patterns of diversity of Dry Valley microbial communities are still being investigated. Freckman and Virginia (1997) found in an early study that moisture, carbon and salinity were determining factors as to whether or not habitats were suitable to harbour soil microbial communities. Barrett and colleagues (2004) found that soil moisture and

temperature were more likely to determine soil species distribution, while Eichorst and colleagues (2007) identified pH as having a crucial influence on microbial community composition. Of all the environmental parameters measured in this study, conductivity and copper concentration best explained the prevailing microbial community. High conductivity (i.e. high-ionic-strength conditions) seems to have a negative impact on the biodiversity present in the soils, as observed in the mineral soils of Beacon Valley and Upper Wright Valley.

The ANOVA revealed little or no significant variation in the trace metal concentrations between the four valleys. These results do not enable us to make clear conclusions about the significant differences between geochemical parameters of the valleys. However, by performing a post-hoc Tukey test on the ANOVA results, we were able to see a trend, in which Miers Valley often stood out compared to the other three valleys.

What our findings indicate is that geochemistry may actually not be the main driver of biodiversity in these Dry Valleys. Rather, the combination of relatively milder temperatures, water availability and lithology work together to create appropriate niches for microorganisms to thrive in, protected from the external harsh environment. The availability of such niches is the most likely determinant in whether or not a valley will sustain a high microbial diversity, as we have observed in coastal or mixed inland Miers Valley and Battleship Promontory, versus upland Beacon and Upper Wright Valleys.

An important consideration when analyzing any of these results is that we have worked with total genomic DNA extracted directly from the soils. The apparent diversity observed could in fact be amplified genes or gene fragments of environmental DNA that are preserved in the soils (Adams et al., 2006), not an indication of actual viable microorganisms. Indeed, the combination of high salt content, high concentration of silica (as shown in Table 1) and extremely cold temperatures of the soils create an environment in which nucleic acid might be very stable for long periods of time. Therefore, it is probably that a percentage of the diversity observed is derived from preserved DNA rather than from viable microorganisms. Future studies need to take this into consideration, and advances have to be made, to find a way to discriminate between “active” and preserved sequences.

2.6.Conclusion

In this study, soils from four different Dry Valleys were sampled on the basis of their location within the McMurdo Dry Valleys and their physico-chemistry. Total genomic nucleic acids were extracted from each Dry Valley soil samples and were genotyped. The results of the ARISA and geochemical analyses were combined for rigorous statistical analysis in order to elucidate the factors driving the microbial biodiversity in these four Dry Valleys. To date, this is the first study to apply ARISA community fingerprinting analysis to characterize and compare the microbial populations present in various Dry Valleys and attempt to identify the influences of the soils' lithology and geochemical properties in shaping and maintaining these communities' distributions. *S*ignificant differences in bacterial community structures were identified between valleys. Our ARISA results showed that multiple bacterial and cyanobacterial species thrive in the Dry Valley mineral sands. All four valleys appear to harbour a high bacterial biodiversity, with communities from coastal Miers Valley and inland Battleship Promontory sustaining more diverse populations than upland Beacon and Upper Wright Valleys. Our results show that bacterial communities vary significantly from one valley to another, and this variation appears to be explained in large part by the varying microclimatic conditions, moisture availability and lithology of the mineral soils that harbour these populations. Cyanobacterial communities were also analysed and were found to heavily colonize the moister soils of Miers and the sandstone of Battleship Promontory. These cyanobacteria likely play a crucial role in net primary production of the Dry Valley soils. In support of our original hypothesis, abiotic factors appear to shape the microbial communities living in Dry Valley mineral soils. These communities are composed in majority of varied bacterial species, with a subset composed of cyanobacteria. Future studies should look into the actual phylogeny of the species making up these varied communities.

2.7. References

Adams, B., Bardgett, R., Ayres, E., Wall, D., Aislabie, J., & Bamforth, S. e. a. (2006). Diversity and distribution of Victoria Land biota. *Soil Biology and Biochemistry, 38*, 3003-3018.

Aislabie, J., Chhour, K., Saul, D., Miyauchi, S., Ayton, J., Paetzol, R., et al. (2006). Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. *Soil Biology and Biochemistry, 38*, 3041-3056.

Andersen, D., McKay, C., Wharton, R., & Rummel, J. (1992). Testing a Mars science outpost in the Antarctic dry valleys. *Advances in Space Research, 12*(5), 205-209.

Avery, S., Howlett, N., & Radice, S. (1996). Copper toxicity towards *Saccharomyces cerevisiae:* dependance on plasma membrane fatty acid composition. *Applied and Environmental Microbiology, 62*, 3960-3966.

Babalola, O., Kirby, B., Le Roes-Hill, M., Cook, A., Cary, C., Burton, S., et al. (2008). Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. *Environmental Microbiology*.

Barrett, J., Virginia, R., Hopkins, D., Aislabie, J., Bargagli, R., Bockheim, J., et al. (2006b). Terrestrial ecosystem processes of Victoria Land, Antarctica. *Soil Biology and Biochemistry, 38*(10), 3019-3034.

Barrett, J., Wall, D., Virginia, R., Parsons, A., Powers, L., & Burkins, M. (2004). Biogeochemical parameters and constraints on the structure of soil biodiversity. *Ecology, 85*, 3105-3118.

Bockheim, J., & McLeod, M. (2008). Soil distribution in the McMurdo Dry Valleys, Antarctica. *Geoderma, 144*(1-2), 43-49.

Broady, P. (1996). Diversity, distribution and dispersal of Antarctic terrestrial algae. *Biodiversity and Conservation, 5*, 1307-1335.

Brown, M., & Fuhrman, J. (2005). Marine bacterial microdiversity as revealed by internal transcribed spacer analysis. *Aquatic Microbial Ecology, 41*, 15-23.

Campbell, I., & Claridge, G. (1987). *Antarctica: Soils, weathering processes and environment*. United States: Elsevier Science Pub. Co. Inc.,New York, NY.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.-M., Rizzi, A., et al. (2004). Comparison of Different Primer Sets for Use in Automated Ribosomal Intergenic Spacer Analysis of Complex Bacterial Communities. *Applied and Environmental Microbiology, 70*(10), 6147-6156.

Clayton-Greene, J., Hendy, C., & Hogg, A. (1988). Chronology of a Wisconsin age proglacial lake in the Miers Valley, Antarctica. *New Zealand Journal of Geology & Geophysics, 31*(3), 353-361.

Convey, P. (1996). The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. . *Biological Reviews, 71*(2), 191-225.

Cowan, D., Russell, N., Mamais, A., & Sheppard, D. (2002). Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles, 6*(5), 431-436.

Cowan, D., & Tow, L. (2004). Endangered antarctic environments. *Annual Reviews in Microbiology, 58*, 649–690.

Edmeades, D., Wheeler, D., & Clinton, E. (1985). The chemical composition and ionic strength of soil solutions from New Zealand topsoils. *Australian Journal of Soil Research, 23*(2), 151-165.

Edwards, K., Gihring, T., & Banfield, J. (1999). Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Applied and Environmental Microbiology, 65*(8), 3627-3632.

Eichorst, S., Breznak, J., & Schmidt, T. (2007). Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the Phylum Acidobacteria. *Applied and Environmental Microbiology, 73*(8), 2708-2717.

Elliot, D., & Fleming, T. (2004). Occurrence and dispersal of magmas in the Jurassic Ferrar Large Igneous Province, Antarctica. *Gondwana Research, 7*(1), 223-237.

Fisher, M., & Triplett, E. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology, 65*, 4630-4636.

Freckman, D., & Virginia, R. (1997). Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. *Ecology, 78*(2), 363-369.

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science, 309*, 1387-1390.

González, N., Romero, J., & Espejo, R. (2003). Comprehensive detection of bacterial populations by PCR amplification of the 16S-23S rRNA spacer region. *Journal of Microbiological Methods, 55*(1), 91-97.

Hansom, J., & Gordon, J. (1998). *Antarctic Environments and Resources: A Geographical Perspective*. New York: Addison Wesley Longman Ltd.

Hartmann, M., Frey, B., Kölliker, R., & Widmer, F. (2005). Semi-automated genetic analyses of soil microbial communities: comparison of T-RFLP and RISA based on descriptive and discriminative statistical approaches. *Journal of Microbiological Methods, 61*(3), 349-360.

Hogg, I., Cary, C., Convey, P., Newsham, K., O'Donnell, A., Adams, B., et al. (2006). Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? *Soil Biology and Biochemistry, 38*(10), 3035-3040.

http://www.mcmurdodryvalleys.aq/. (2004). Management Plan for Antarctic Specially Managed Area No. 2. McMurdo Dry Valleys, Southern Victoria Land. Retrieved May 2nd, 2008

Kwon, S., Park, J.-Y., Kim, J.-S., Kang, J.-W., Cho, Y.-H., Lim, C.-K., et al. (2005). Phylogenetic analysis of the genera *Bradyhyrzobium, Mesorhizobium, Rhizobium,* and *Sinorhizobium* on the basis of 16S rRNA gene and internally

transcribed spacer region sequences. *International Journal of Systematic Evolutionary Microbiology, 55*, 263-270.

Leuko, S., Goh, F., Allen, M., Burns, B., Walter, M., & Neilan, B. (2007). Analysis of intergenic spacer region length polymorphisms to investigate the halophilic archaeal diversity of stromatolites and microbial mats. *Extremophiles, 11*(1), 203-210.

Lewis, A., Marchant, D., Kowalewski, D., Baldwin, S., & Webb, L. (2006). The age and origin of the Labyrinth, western Dry Valleys, Antarctica: Evidence for extensive middle Miocene subglacial floods and freshwater discharge to the Southern Ocean. *Geology, 34*(7), 513-516.

Marchant, D., & Head, J. (2007). Antarctic dry valleys: Microclimate zonation, variable geomorphic processes, and implications for assessing climate change on Mars. *Icarus, 192*(1), 187-222.

Moorhead, D., Doran, P., Fountain, A., Lyons, W., McKnight, D., Priscu, J., et al. (1999). Ecological legacies: impacts on ecosystems of the McMurdo Dry Valleys. *Bioscience, 49*, 1009-1019.

Nichols, R. (1971). Glacial geology of the Wright valley, McMurdo Sound, Antarctica. In L. Quam (Ed.), *Research in the Antarctic* (pp. 293–340). Washington, DC: AAAS.

Niederberger, T., McDonald, I., Hacker, A., Soo, R., Barrett, J., Wall, D., et al. (2008). Microbial community composition in soils of Northern Victoria Land, Antarctica. *Environmental Microbiology*, *10*(7), 1713-1724.

Nkem, J. N., Wall, D., Virginia, R., Broos, E., Barrett, J., & al, P. D. e. (2005). Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. *Polar Biology, 29*, 346-352.

Ranjard, L., Brothier, E., & Nazaret, S. (2000). Sequencing Bands of Ribosomal Intergenic Spacer Analysis Fingerprints for Characterization and Microscale Distribution of Soil Bacterium Populations Responding to Mercury Spiking. *Applied and Environmental Microbiology*, *66*(12), 5334-5339.

Saul, D., Aislabie, J., Brown, C., Harris, L., & Foght, J. (2005). Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. . *FEMS Microbiology Ecology, 53*, 141-155.

Smith, J., Tow, L., Stafford, W., Cary, C., & Cowan, D. (2006). Bacterial Diversity in Three Different Antarctic Cold Desert Mineral Soils. *Microbial Ecology, 51*(4), 413-421.

Soo, R. (2007). *Microbial biodiversity of thermophilic communities in hot mineral soils of Tramway Ridge, Mt. Erebus, Antarctica.* Waikato University, Hamilton.

Stonehouse, B. (2002). *Encyclopedia of Antarctica and the Southern Oceans*: John Wiley and Sons.

Suroszl, W., & Palinska, K. (2004). Effects of heavy-metal stress on cyanobacterium *Anabeana flos-aqua. Archives in Environmental Contamination Toxicology, 48*, 40-48.

Virginia, R., & Wall, D. (1999). How soils structure communities in the Antarctic Dry Valleys. *Bioscience, 49*, 973-983.

Wood, S., Rueckert, A., Cowan, D., & Cary, C. (2008). Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. *ISME J, 2*(3), 308-320.

Wynn-Williams, D. (1990). Ecological aspects of Antarctic microbiology. *Advances in Microbial Ecology, 11*, 71-146.

Wynn-Williams, D. (2000). Cyanobacteria in deserts- Life at the limit ? In B. Whitton & M. Potts (Eds.), *The Ecology of Cyanobacteria* (pp. 341-365). Dordrecht, the Netherlands: Kluwer Academic Publishers.

Yergeau, E., Newsham, K., Pearce, D., & Kowalchuk, G. (2007). Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environmental Microbiology, 9*(11), 2670-2682.

Chapter 3: General Conclusions

The main objective of this study was to compare and contrast the microbiology of four McMurdo Dry Valleys chosen on the basis of their varying climate, mineralogy, lithology and physico-chemical soil properties.

The environmental factors that determine the distribution patterns and biodiversity of microbial communities inhabiting the mineral soils of the Dry Valleys are still under investigation (Barrett et al., 2006a). Recent molecular-based research (Aislabie et al., 2006; Barrett et al., 2006a; Cowan et al., 2002; Hogg et al., 2006; Niederberger et al., 2008; Smith et al., 2006) has shown that the Dry Valley soils contain a much more diverse community assemblage than was expected given the extremely harsh environmental conditions. To date, this is the first study to apply ARISA community fingerprinting analysis to characterize and compare the microbial populations present in various Dry Valleys and attempt to identify the influences of the soils' geochemical properties in shaping and maintaining these communities' distributions. The results of the ARISA and geochemical analyses were combined for rigorous statistical analysis in order to elucidate the factors driving the microbial biodiversity in these four Dry Valleys. We found that the water availability and lithology of the soils were likely to be the main determinants of microbial diversity in the McMurdo Dry Valleys, rather than the geochemistry, as had previously been reported in various studies (Barrett et al., 2004; Eichorst et al., 2007; Freckman & Virginia, 1997).

Some of the physico-chemical parameters measured in our study, such as soil water content, pH and even total carbon and nitrogen are likely to vary periodically or seasonally. In order to consolidate our findings, it would be useful obtain samples from the Dry Valleys at different time points in the year, to study this variability.

An important consideration is that community fingerprinting methods such as ARISA may be useful for comparative, qualitative analyses of microbial populations, but they cannot be used to assess the richness or diversity metrics of complex communities (Dunbar et al., 2000). These methods are limited by their detection threshold, and the number of peaks detected in ARISA can underestimate the actual richness of any community with a long-tailed rank

abundance distribution (Bent et al., 2007). Accurately estimating diversity in a community with a log-normal species-abundance distribution would require sampling about 80% of the species in any given environment (Gans et al., 2005). Consequently, the sole use of fingerprinting methods cannot provide reliable diversity indices. However, fingerprinting methods like ARISA hold great potential for use when rapid, high-throughput screening for differences or changes in microbial communities is more important than phylogenetic identification of specific organisms (Hartmann et al., 2005). They can then be consolidated by obtaining sequence data on the microorganisms present, to study their taxonomy infer phylogenetic relationships.

In order to put our fingerprinting results into perspective and study the ecology and phylogenetics of the four Dry Valleys under investigation, our samples were prepared for pyrosequencing analysis. However, due to the limited time frame accorded for this study, the sequence data obtained had not undergone sufficient analysis at the time of submission in order to be included in this manuscript. However, the sample preparation procedure and materials used are included in Appendix D, and the combined results of ARISA and pyrosequencing will be submitted for publication in the near future.

References:

Aislabie, J., Chhour, K., Saul, D., Miyauchi, S., Ayton, J., Paetzol, R., et al. (2006). Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. *Soil Biology and Biochemistry*, *38*, 3041-3056.

Barrett, J., Virginia, R., Wall, D., Cary, S. C., Adams, B., Hacker, A., et al. (2006a). Co-variation in soil biodiversity and biogeochemistry in Northern and Southern Victoria Land, Antarctica. Antarctic Science, 18, 535-548.

Bent, J., Pierson, J., & Forney, L. (2007). Measuring species richness based on microbial community fingerprints: the emperor has no clothes. Applied and Environmental Microbiology, 73(7), 2399-2401.

Cowan, D., Russell, N., Mamais, A., & Sheppard, D. (2002). Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. Extremophiles, 6(5), 431-436.

Dunbar, J., Ticknor, L., & Kuske, C. (2000). Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology, 66, 2945-2950.

Eichorst, S., Breznak, J., & Schmidt, T. (2007). Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the Phylum Acidobacteria. *Applied and Environmental Microbiology, 73*(8), 2708-2717.

Freckman, D., & Virginia, R. (1997). Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. *Ecology, 78*(2), 363-369.

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science, 309*, 1387-1390.

Hartmann, M., Frey, B., Kölliker, R., & Widmer, F. (2005). Semi-automated genetic analyses of soil microbial communities: comparison of T-RFLP and RISA based on descriptive and discriminative statistical approaches. Journal of Microbiological Methods, 61(3), 349-360.

Hogg, I., Cary, C., Convey, P., Newsham, K., O'Donnell, A., Adams, B., et al. (2006). Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? Soil Biology and Biochemistry, 38(10), 3035-3040.

Niederberger, T., McDonald, I., Hacker, A., Soo, R., Barrett, J., Wall, D., et al. (2008). Microbial community composition in soils of Northern Victoria Land, Antarctica. Environmental Microbiology, 10(7), 1713-1724.

Appendix

A. Complete geochemistry results

I] Total carbon and nitrogen content

	SAMPLE	%N	%C
Miers	mva	0.05	0.27
	mvb	0.05	0.46
	mvc	0.05	0.47
	mvd	0.06	0.41
	mvx	0.06	0.70
Beacon	bva	0.06	0.11
	bvb	0.08	0.20
	bvc	0.09	0.17
	bvd	0.08	0.11
	bvx	0.08	0.11
Battleship	bpa	0.04	0.10
	bpb	0.05	0.10
	bpc	0.04	0.09
	bpd	0.05	0.10
	bpx	0.04	0.10
Upper Wright	uwa	0.15	0.10
	uwb	0.09	0.11
	uwc	0.11	0.13
	uwd	0.13	0.11
	uwx	0.10	0.10

II] ICP-MS

Complete results of the ICP-MS. All trace-element concentrations are in part per billion (ppb)

1) Miers Valley

Analyte	mva	mvb	mvc	mvd	mvx
Li	18.923	11.947	15.629	20.036	18.962
B	1029.125	1080.898	1012.135	974.687	956.644
Na	7615.442	5886.483	7362.35	9681.155	8291.594
Mg	42724.53	29478.33	41811.32	48057.06	41376.36
Al	24602.74	16071.96	22145.9	29281.29	25104.35
Si	13739.24	9046.157	12929.2	17651.65	8573.345
P	1314.745	1573.195	1724.623	2169.054	1971.405
S	-858.087	-866.136	-753.698	-887.889	-545.527
K	5375.359	3539.767	4525.189	6395.321	5950.873
Ca	24663.46	16804.91	23436.45	30856.43	27604.81
Ti	5805.522	3418.161	5093.574	6981.998	5274.956
Ti	5674.467	3448.283	4754.812	6794.958	4918.478
V	78.494	54.802	76.503	98.62	83.519
Cr	53.308	36.867	55.932	64.095	48.985
Cr	82.979	58.874	81.136	97.845	80.267
Fe	45971.43	30961.85	43262.72	54330.99	47124.06
Mn	742.187	518.718	727.168	892.113	787.332
Co	31.902	20.949	32.168	37.474	31.906
Ni	174.811	117.409	185.824	200.448	169.659
Cu	24.78	15.222	23.466	29.916	26.06
Cu	27.799	17.027	26.621	33.325	29.194
Zn	123.734	102.294	111.37	108.505	115.566
Zn	114.506	96.341	103.566	100.225	107.558
As	0.871	1.2	1.298	4.378	1.541
Hf	0.873	0.342	0.58	0.728	0.519
Se	17.534	11.587	17.233	23.496	19.548
Se	0.624	0.905	1.061	1.334	0.875
Sr	328.088	196.06	299.734	415.312	366.36
Zr	35.653	16.32	29.684	41.956	30.109
Ag	0.033	-0.032	-0.013	0.095	0.001
Cd	0.29	0.175	0.233	0.285	0.27
In	0.035	0.017	0.023	0.043	0.032
Ba	150.297	95.375	133.861	200.444	172.505
Hg	0.063	0.035	0.034	0.052	0.032
Tl	0.102	0.043	0.074	0.114	0.113
Pb	4.324	2.884	4.25	6.436	5.629
Pb	3.796	2.603	3.766	5.837	5.065
Pb	4.279	2.869	4.2	6.338	5.6
Bi	0.135	0.024	0.045	0.113	4.415
U	3.215	3.043	3.114	4.346	3.775
U	1.012	0.847	1.041	1.559	1.371

2) Beacon Valley

Analyte	bva	bvb	bvc	bvd	bvx
Li	12.554	10.591	20.309	12.67	14.474
B	944.415	1010.808	1075.935	975.178	1062.908
Na	3316.579	3219.939	6827.886	4354.557	4414.27
Mg	9136.123	9283.76	14285.56	9670.574	10429.54
Al	24230.63	18844.32	38396.4	25558.47	29333.16
Si	2002.716	1913.983	1857	1858.109	1799.48
P	1008.128	1237.784	1000.759	782.912	842.451
S	11374.04	693.01	9111.495	8981.195	6252.638
K	1291.345	1349.675	3466.624	2095.382	2290.632
Ca	17984.44	9099.23	21134.72	16395.5	16322.57
Ti	850.343	356.819	1247.365	718.317	1021.495
Ti	842.923	357.489	1242.876	720.339	1008.472
V	122.367	82.767	215.146	148.248	210.555
Cr	8.658	6.944	16.358	11.015	11.848
Cr	38.46	38.595	51.881	37.082	41.31
Fe	41521.27	40086.2	66271.29	43673.53	52303.92
Mn	417.192	323.335	584.29	379.907	421.558
Co	20.824	20.713	27.726	20.393	22.039
Ni	24.426	24.86	38.256	27.667	29.871
Cu	123.123	150.439	189.993	134.869	140.36
Cu	124.461	149.877	188.97	136.25	139.786
Zn	97.317	83.655	145.672	102.99	118.673
Zn	92.694	79.441	137.738	99.188	111.588
As	2.185	1.704	2.995	1.907	2.188
Hf	0.406	0.252	0.627	0.313	0.4
Se	17.656	16.944	19.63	14.763	16.959
Se	1.195	1.306	1.953	1.409	2.03
Sr	60.066	41.933	94.539	74.762	80.938
Zr	14.684	8.16	22.6	12.428	14.402
Ag	-0.061	-0.058	0.137	0.03	0.05
Cd	0.133	0.144	0.307	0.248	0.202
In	0.019	0.013	0.037	0.024	0.025
Ba	26.177	19.647	115.236	73.712	40.057
Hg	0.031	0.026	0.102	0.038	0.036
Tl	0.039	0.045	0.113	0.09	0.075
Pb	7.751	7.681	13.057	9.351	9.771
Pb	7.274	7.121	11.991	8.687	9.158
Pb	7.568	7.504	12.931	9.233	9.622
Bi	0.057	0.052	0.101	0.096	0.079
U	2.737	3.429	3.627	2.914	2.915
U	1.01	0.961	1.42	0.964	1.119

3) Battleship Promontory

Analyte	bpa	bpb	bpc	bpd	bpx
Li	10.28	7.365	7.562	7.874	8.623
B	1011.429	1066.089	1096.962	1014.178	1000.374
Na	3513.845	2709.95	2446.864	2831.777	2494.662
Mg	6629.989	5155.959	4618.984	5494.16	5721.86
Al	29820.62	23813.09	23162.74	24991.45	21661.85
Si	1895.406	1797.639	1855.805	1610.416	1654.846
P	892.974	744.504	616.473	620.594	722.81
S	-1021.1	-1214.22	-913.699	-1730.2	-1683.47
K	1234.609	922.622	915.679	979.78	1077.937
Ca	11552.11	10078.24	8940.829	9862.026	8631.405
Ti	923.046	808.578	776.818	953.06	861.238
Ti	921.205	812.193	764.648	957.544	876.264
V	88.562	73.975	72.464	92.636	75.953
Cr	8.632	7.376	7.147	7.128	6.985
Cr	36.728	36.225	32.209	32.124	35.234
Fe	37354.94	30446.37	27730.78	30070.54	34114.71
Mn	358.635	291.032	257.065	315.414	364.959
Co	19.592	14.951	13.051	15.599	17.392
Ni	27.745	22.047	19.162	22.338	21.266
Cu	121.748	96.276	77.567	98.158	104.832
Cu	123.135	97.264	77.665	98.078	105.775
Zn	72.16	59.256	52.791	72.29	75.321
Zn	68.52	57.303	49.622	68.556	72.414
As	1.397	1.278	0.688	1.125	1.328
Hf	0.329	0.269	0.199	0.278	0.313
Se	15.146	14.719	11.602	14.065	13.347
Se	1.121	1.055	0.971	1.556	1.081
Sr	44.538	36.53	32.896	38.956	33.143
Zr	10.993	9.936	6.669	11.597	11.471
Ag	-0.076	-0.048	-0.072	-0.091	-0.066
Cd	0.152	0.082	0.105	0.166	0.159
In	0.023	0.014	0.017	0.015	0.018
Ba	28.271	21.913	19.311	27.989	25.758
Hg	0.017	0.007	0.01	0.016	0.024
Tl	0.047	0.014	0.027	0.026	0.036
Pb	4.753	3.503	3.301	3.918	4.188
Pb	4.309	3.236	3.032	3.642	3.877
Pb	4.694	3.439	3.218	3.861	4.074
Bi	0.029	0.012	0.013	0.022	0.02
U	2.629	2.057	2.305	1.949	2.502
U	1.096	0.846	0.779	0.874	0.872

4) Upper Wright Valley

Analyte	uwa	uwb	uwc	uwd	uwx
Li	12.701	13.129	9.819	15.229	16.012
B	976.824	1060.32	1032.469	1043.777	1028.769
Na	6920.164	4318.822	3800.437	7395.778	12718.43
Mg	9631.336	7680.432	6344.371	8952.059	6800.972
Al	19038.46	18678.92	13653.22	21619.86	27174.52
Si	1534.659	2075.079	1851.26	2044.628	1938.679
P	636.176	593.099	370.902	503.575	483.155
S	844.353	-376.968	-912.899	621.032	5495.231
K	2472.141	2700.961	2252.891	4000.766	7336.592
Ca	5504.799	5041.135	2944.202	5521.004	8458.982
Ti	599.483	508.606	360.808	439.759	396.183
Ti	601.312	511.005	361.054	440.238	398.409
V	71.441	82.3	53.297	58.991	56.937
Cr	8.606	9.588	6.908	10.364	7.595
Cr	34.111	40.767	33.362	39.881	38.216
Fe	29648.2	31110.01	21054.85	29985.89	26991.41
Mn	271.772	283.093	191.07	314.401	442.486
Co	14.786	14.314	9.511	13.612	10.405
Ni	19.817	19.751	14.504	20.303	16.385
Cu	78.351	73.478	52.644	71.154	55.866
Cu	78.015	73.64	52.946	70.78	56.503
Zn	67.624	76.44	50.942	69.818	69.559
Zn	65.348	73.76	48.116	69.048	66.786
As	1.952	1.767	2.163	2.175	1.705
Hf	0.31	0.376	0.289	0.451	0.514
Se	14.261	15.69	11.36	14.666	16.129
Se	1.381	1.013	1.022	1.083	1.148
Sr	34.676	28.928	20.789	36.8	40.933
Zr	13.362	13.471	12.023	24.381	26.848
Ag	0.012	-0.051	-0.047	-0.041	-0.004
Cd	0.302	0.123	0.101	0.132	0.2
In	0.014	0.017	0.01	0.02	0.02
Ba	42.407	36.887	28.746	42.728	49.54
Hg	0.026	0.014	0.012	0.019	0.042
Tl	0.087	0.068	0.044	0.093	0.122
Pb	9.618	9.516	7.16	12.295	10.555
Pb	9.074	8.78	6.691	11.453	9.703
Pb	9.413	9.32	6.991	11.999	10.35
Bi	0.044	0.063	0.041	0.061	0.074
U	1.92	2.173	1.954	3.143	5.348
U	0.831	0.909	0.596	1.119	1.791

B. Photos

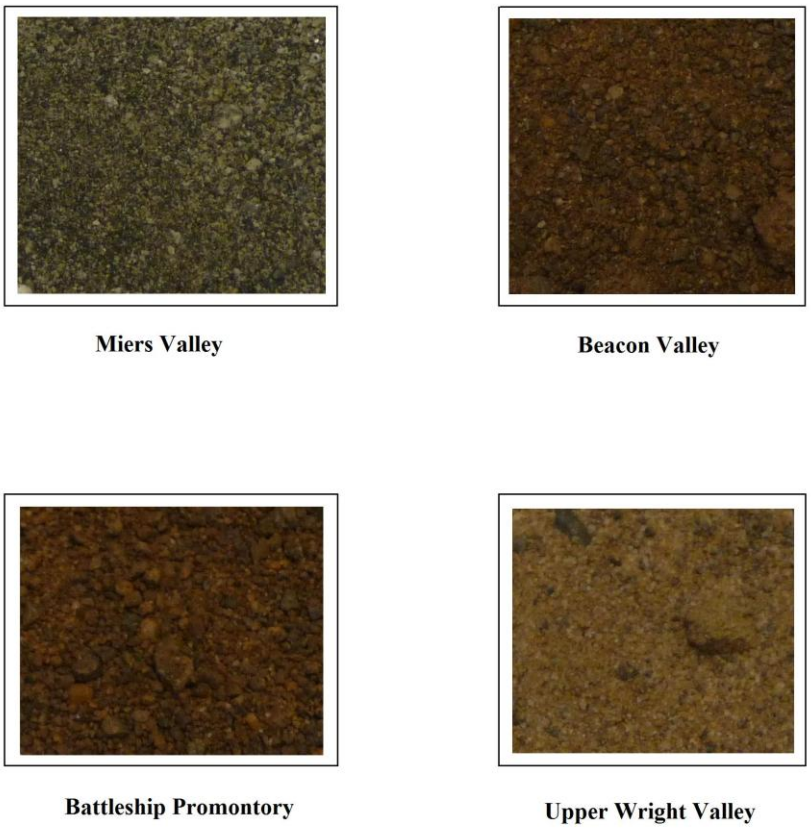

Figure 15. Appearance of mineral sands sampled from the four valleys studied.

Figure 16. Upper Wright Valley, McMurdo Dry Valleys, Antarctica. (photo provided by Craig Cary)

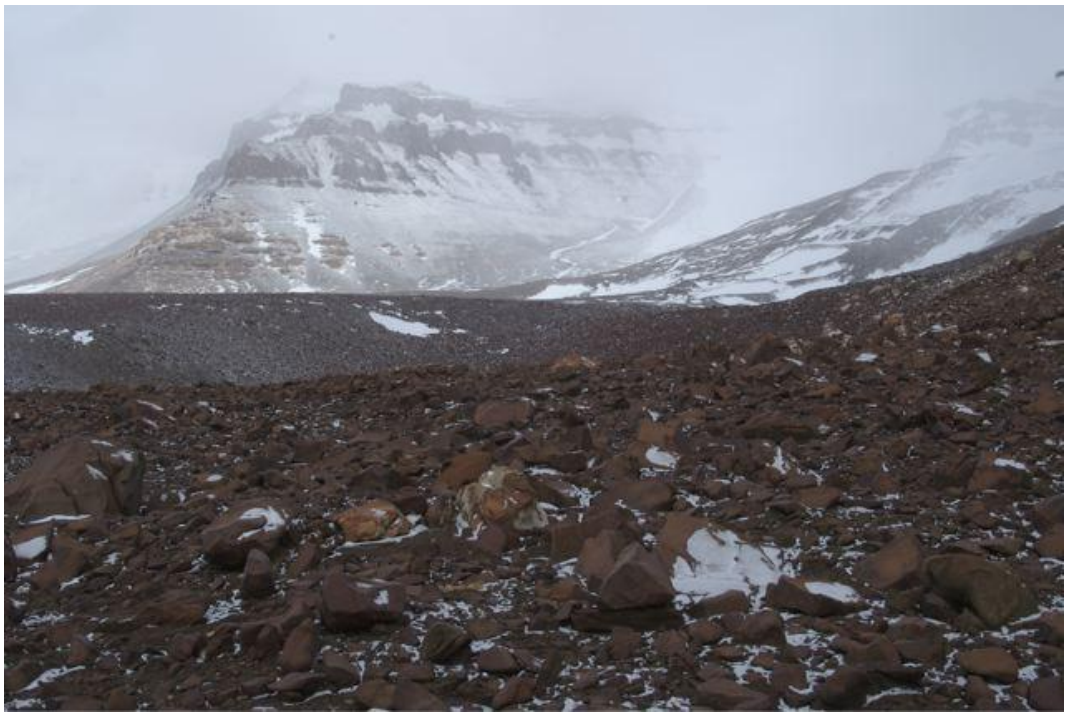

Figure 17. Beacon Valley, McMurdo Dry Valleys, Antarctica. (photo provided by Craig Cary)

C. Post-hoc Tukey test results

Table 4. Post Hoc Tukey test to show geochemical variation between valleys. X indicates a statistically significant difference between the two valleys across.

pH

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			X
UW	X		X	

% Moist

	MV	BV	BP	UW
MV		X	X	X
BV	X		X	X
BP	X	X		
UW	X	X		

%C

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

%N

	MV	BV	BP	UW
MV		X		X
BV	X		X	X
BP		X		X
UW	X	X	X	

C/N ratio

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Li

	MV	BV	BP	UW
MV			X	
BV			X	
BP	X	X		
UW				

B

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Na

	MV	BV	BP	UW
MV			X	
BV				
BP	X			X
UW			X	

Mg

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Si

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

P

	MV	BV	BP	UW
MV		X	X	X
BV	X			X
BP	X			
UW	X	X		

K

	MV	BV	BP	UW
MV		X	X	
BV	X			X
BP	X			
UW			X	

Ti

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

V

	MV	BV	BP	UW
MV		X		
BV	X		X	X
BP		X		
UW		X		

Cr

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Fe

	MV	BV	BP	UW
MV				X
BV			X	X
BP		X		
UW	X	X		

Mn

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Al

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Ca

	MV	BV	BP	UW
MV		X	X	X
BV	X			X
BP	X			
UW	X	X		

Co

	MV	BV	BP	UW
MV		X	X	X
BV	X			X
BP	X			
UW	X	X		

Ni

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Cu

	MV	BV	BP	UW
MV		X	X	X
BV	X		X	X
BP	X	X		X
UW	X	X	X	

Zn

	MV	BV	BP	UW
MV			X	X
BV			X	X
BP	X	X		
UW	X	X		

As

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Hf

	MV	BV	BP	UW
MV			X	
BV				
BP	X			
UW				

Se

	MV	BV	BP	UW
MV		X		
BV	X			
BP				
UW				

Sr

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Zr

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Ag

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Cd

	MV	BV	BP	UW
MV			X	
BV				
BP	X			
UW				

Ln

	MV	BV	BP	UW
MV				X
BV				
BP				
UW	X			

Ba

	MV	BV	BP	UW
MV		X	X	X
BV	X			
BP	X			
UW	X			

Hg

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

Ti

	MV	BV	BP	UW
MV			X	
BV				
BP	X			X
UW			X	

Pb

	MV	BV	BP	UW
MV		X		X
BV	X		X	
BP		X		X
UW	X		X	

Bi

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

U

	MV	BV	BP	UW
MV				
BV				
BP				
UW				

D. Pyrosequencing

Once an overview of the microbial communities present in the environment has been obtained, the "fingerprints" can be assigned to their owners by combining the ARISA data with high-throughput sequencing. Massively parallel pyrosequencing was developed by 454 Life Sciences. This technology offers a means to more extensively sample molecular diversity in microbial populations as hundreds of thousands for short DNA sequence reads can be generated in a few hours, without the use of the traditional, time-consuming cloning step (Huse et al., 2007).

454 pyrosequencing using bar-coded primers was undertaken in order to identify the majority of species representatives in each of the four valleys. The sequencing data confirmed the presence of *such and such species, genera, phyla, etc* and also identified some new phylotypes that seem to be unique to this selective environment.

DNA extraction and PCR product preparation for 454 pyrosequencing

DNA to be used as a template for 454 pyrosequencing was extracted using PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc.) Each of the subsamples (A, B, C, D, X) were extracted, for each valley. These subsamples were mixed at equal volumes, and 15ng of each mix used as a template for PCR amplification. PCRs were performed in triplicate 30µl reactions containing 1x PrimeSTAR buffer (Mg^{2+} plus, 5mM) (Takara Bio Inc, Japan), 200 µM each dNTP mixture, 0.3 µM forward and reverse primers (Table 5), 0.75 U PrimeSTAR HS DNA polymerase (Takara Bio Inc, Japan) and the final volume adjusted to 30µl with MilliQ H_2O. Cycling was performed using a Bio-Rad DNA Engine® (PTC-200) Peltier Thermal Cycler (Bio-Rad Loboratories Inc, Hercules, CA) with the following cycle program: initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 20 sec, 55°C for 5 sec decreasing by 0.2°C per cycle, 72°C for 30 sec, and a final extension at 72°C for 5 min. PCR reactions were visualized on 1% agarose. To purify the single band corresponding to the V3V4 16SrRNA region, the PCR products were run on a 1.7% agarose gel at 70V for 90min, and the 500bp bands cut out. Amplified DNA was removed from the

gel matrix and cleaned up using a GenScript 5M Gel Extraction purification kit (Genscript Corporation, NJ, USA). The integrity of the PCR products were checked using gel electrophoresis, and quantification was performed using a NanoDrop^{TM} 1000 Spectrophotometer (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) and Qubit fluorometer (Invitrogen Corporation, Carlsbad, CA, USA). All four samples were pooled at equal abundance for sequencing.

Sequencing was carried out on a Genome Sequencer FLX (Roche Diagnostics, Mannheim, Germany).

Table 5. Primers used for 454 pyrosequencing

Miers Valley	Forward	5'-GCCTCCCTCGCGCCATCAG**AGAC**ACTCCTACGGGAGGCAGCAG -3'
	Reverse	5'-GCCTTGCCAGCCCGCTCAG**AGAC**GGACTACCAGGGTATCTAAT -3'
Beacon Valley	Forward	5'-GCCTCCCTCGCGCCATCAG**ACAT**ACTCCTACGGGAGGCAGCAG -3'
	Reverse	5'-GCCTTGCCAGCCCGCTCAG**ACAT**GGACTACCAGGGTATCTAAT -3'
Battleship Promontory	Forward	5'-GCCTCCCTCGCGCCATCAG**AGTC**ACTCCTACGGGAGGCAGCAG -3'
	Reverse	5'-GCCTTGCCAGCCCGCTCAG**AGTC**GGACTACCAGGGTATCTAAT -3'
Upper Wright Valley	Forward	5'-GCCTCCCTCGCGCCATCAG**ACGT**ACTCCTACGGGAGGCAGCAG -3'
	Reverse	5'-GCCTTGCCAGCCCGCTCAG**ACGT**GGACTACCAGGGTATCTAAT -3'