Influences of Soil Properties on Archaeal Diversity and Distribution in the McMurdo Dry Valleys, Antarctica

A thesis submitted in partial fulfilment

of the requirements for the degree

of

Master of Science in Biological Sciences

By

Ingrid Richter

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

The University of Waikato,

Hamilton, New Zealand

2011

Abstract

The Antarctic Dry Valleys are characterized by extremely low temperatures, arid conditions, high salinity and virtual absence of plants. Therefore, food webs of these microbially dominated soils are among the simplest on earth making these mineral soils a perfect model to study microbial biogeography. This study aims to characterize the distribution and diversity of Archaea within the Dry Valleys as part of the New Zealand Terrestrial Antarctic Biocomplexity Survey (NZTABS). An international multidisciplinary organization focusing on biotic organisms, community structure and their functional linkage to determine what environmental factors drive biocomplexity. Archaea are so far the least known members of the microbial community with only a few successful attempts at detection indicating a patchy distribution and low diversity. A wide range of soil samples, collected from various sites within the Dry Valleys were analyzed using a suite of genetic approaches. DNA fingerprinting techniques (RFLP, T- RFLP) were applied to examine distribution and diversity of archaeal species living in soils of Miers Valley, Marshall Valley, Garwood Valley and Shangri- La. Detailed analysis of physicochemical differences between mineral soils was undertaken in hope to unveil environmental factors driving distribution and biodiversity of archaeal communities present in these soils. Multivariate statistical analysis and ordination of T- RFLP results and physicochemical data revealed a widespread distribution of Archaea across all three valleys, including Shangri- La. Overall, archaeal diversity was relatively low and most of the archaeal communities were composed in majority of one species affiliated with Crenarchaeota Marine Group 1.1b. Archaeal communities that sustain a relatively high diversity appear to be restricted to high elevation ridge areas and coastal moraines. This variation in diversity may be best explained by differences in moisture availability and availability of carbon and nitrogen in mineral soils that harbour these communities. Conversely, soils that harbour high bacterial diversity and primary producers revealed extremely low abundance of Archaea, possibly even total absence of Archaea in these organic rich soils.

Acknowledgements

First and foremost I would like to thank Prof. S. Craig Cary and Assoc. Prof. Ian R. McDonald for their supervision and guidance. I am grateful for all your support and encouragement throughout my time at the TRU.

Also, thank you, Dr. Charles Lee, Dr. Andreas Rueckert, Eric Bottos and Lynne Parker for all your much- needed technical support and help during my research. Many thanks Andreas for teaching me many of the techniques involved in my thesis, and I especially appreciate Charlie' s patience to resolve the many computer issues and Eric' s willingness to supply many of his samples.

I would also like to extend my sincere gratitude to Dr. Craig Herbold who gave valuable criticism and suggestions for interpreting and improving the material presented in this thesis. Thank you, Craig, for your enthusiasm, all the interesting discussions, and help with my work, particularly statistical analyses of T- RFLP data.

I am also grateful to Antarctica New Zealand (Event Case K020) and the Foundation of Research Science and Technology (grant awarded to Professor Craig Cary and Professor Alan Green) for providing funds for this project and Prof. S. Craig Cary for giving me the opportunity to work in such a unique environment.

Last, but certainly not least, I would like to extend my heartfelt gratitude to my family and friends who have gave me continuous support in the many stressful times involved in doing a Masters.

Über allem stehen natürlich meine lieben Eltern, ohne die dieses Studium nie möglich gewesen wäre. Durch ihre moralische und finanzielle Unterstützung ermöglichten sie mir unvergessliche Jahre in Jena, sowie einen einzigartigen, mehrjährigen Aufenthalt in Neuseeland.

„Leider lässt sich eine wahrhafte Dankbarkeit mit Worten nicht ausdrücken."

Johann Wolfgang von Goethe

Table of Contents

List of Figures

List of Tables

Chapter 1: Introductory Review

1.1 Antarctic ecosystems

With a size of approximately 13 million km^2, Antarctica is the fifth largest continent on Earth but has by far one of the most physically and chemically extreme terrestrial environments to be inhabited by microorganisms. Almost the entire continent is covered by glacial ice-sheets, with only a few ice-free areas which are discontinuously distributed around the coastal margins (Cary, et al. 2010).

1.1.1 The McMurdo Dry Valleys

The McMurdo Dry Valleys are located between the Polar Plateau and the Ross Sea in Southern Victoria Land, Antarctica (Figure 8). These valleys have the largest expanse of contiguous ice-free ground in Antarctica, representing over 15% of the ice-free land on the Antarctic continent. Most of this ice-free area is occupied by mineral soils with relatively few lakes and glacial-melt-water streams scattered across the valleys (Figure 1). These soils are considered to be the most 'extreme' ecosystem on Earth due to extreme environmental and physiochemical conditions such as low mean annual temperatures (ranging from -15 to -30℃), low bioavailability of water (< 2% mass water content), high levels of salinity, high incidences of solar radiation and low nutrient availability (Cary, et al. 2010 and references therein).

Figure 1. Miers Valley (78℃6 ′S 164℃0 ′E), McMurdo Dry Valleys, Antarctica.

1.1.2 Microbial activity

Dry Valley soils are inhabited by numerous diverse microorganisms (Smith, et al. 2006). Due to the lack of vascular plants and vertebrates almost the entire soil food web is composed of microorganisms (Adams, et al. 2006), and microbial activity is primarily controlled by environmental factors (Cary, et al. 2010). Variations in physiochemical soil properties, like temperature, soil moisture, pH and salinity, influences the distribution and activity of soil microorganisms. These variations in soil properties are mainly caused by landscape development processes, including geological processes such as weathering and soil formation (Figure 2).

Although the physical environment influences microbial activity and distribution, microbial activity in turn controls the physiochemical properties of the soils (Barrett, et al. 2006). For example, microbial processes such as photosynthesis, soil respiration, denitrification and methanogenesis contribute to changes in carbon and nitrogen concentrations in the soil. Thus, microbial activity and biogeochemical soil processes are influenced by each other.

1.1.3 Carbon turnover in Dry Valleys

In addition to physical constraints, balanced growth of microorganisms is mainly limited by the availability of organic matter (soil organic carbon) and nutrients such as nitrogen and phosphorus. Organic carbon fixation by primary producers is relatively low due to a limited abundance of algae, cyanobacteria (Novis, et al. 2007), lichens (Kappen, et al. 1991) and mosses (Pannewitz, et al. 2005) in Antarctic soils. Carbon accumulation and turnover is estimated to operate over long timescales ranging from 20 to 130 years (Burkins, et al. 2001; Elberling, et al. 2006). Due to this low productivity and climatic limitations on decomposition, soil organic carbon (SOC) concentrations are very low (0.01- 0.5 mg C/g soil) (Burkins, et al. 2001). Thus carbon pools are not only controlled by active carbon cycling by soil communities and contemporary fixation of organic matter but also by carbon input from legacy sources (Figure 2).

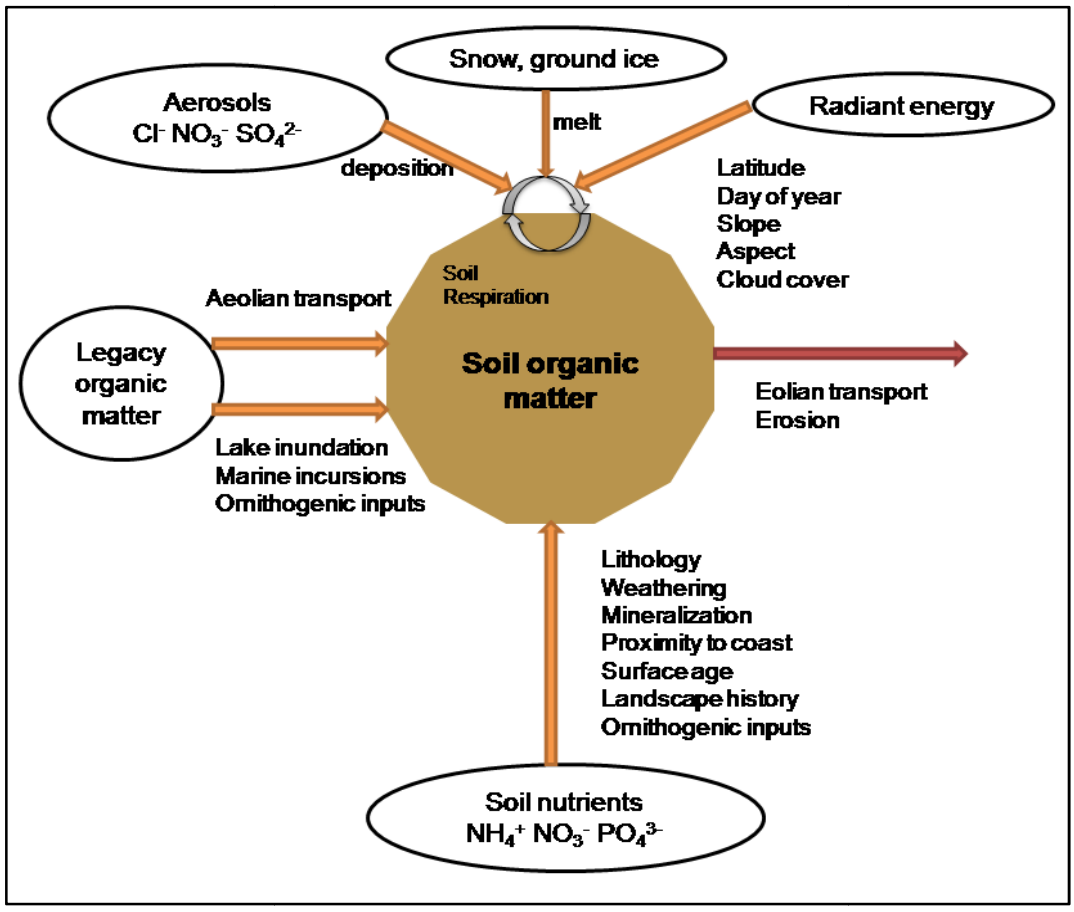


Figure 2. Conceptual model illustrating controls over the accumulation and turnover of organic matter in terrestrial Antarctic ecosystems. Figure modified from Barrett et al. (2006).

The concept of legacy refers to accumulation (during discrete events or continuous inputs) of soil resources in the past, and the potential contribution these materials make to the contemporary functioning of soil carbon turnover (Burkins, et al. 2000; Moorhead, et al. 1999). Based on isotopic evidence it has been proposed that accumulation of organic carbon from ancient lake sediments during the last glacial maximum contributes to the contemporary soil carbon stock (Burkins, et al. 2000). However, it is unclear what contribution legacy carbon makes to contemporary carbon turnover (Hopkins, et al. 2006).

Contemporary processes, such as photosynthesis and respiration may be influenced by legacy inputs, but are also subject to physical environmental factors such as seasonal variation in solar radiation, temperature, moisture availability as well as any contemporary inputs through aeolian redistribution and atmospheric deposition (Figure 2) (Barrett, et al. 2006). For example, carbon may be introduced by aeolian redistribution from cyanobacterial mats that occupy lake margins (Elberling, et al. 2006; Hopkins, et al. 2009). This movement of lake- derived organic material into Dry Valley soils is based on the discovery of a lower amount of SOC and increased turnover times with distance from the lake (Elberling, et al. 2006). Other sources may include contemporary carbon fixation in patches of moss and algae, as well as carcasses of birds and seals that wander inland from the coast (Elberling, et al. 2006). Although, lakes and marine organic matter are likely sources of carbon to soils over large timescales, calculations indicate that aeolian redistribution of organic matter is only a significant source of organic matter to soil biota in areas adjacent to lakes (Barrett, et al. 2006). The estimated carbon turnover is more rapid than can be explained solely on the basis of external inputs indicating that microbial nutrient cycling may contribute to carbon turnover in Dry Valley soils.

1.1.4 Nitrogen cycling in Dry Valleys

High salt imbalances are characteristic of the extremely old Antarctic soils when compared with the salt levels of temperate soils (Bockheim 1997). The resulting nitrogen and biogeochemical gradients may impose strong limitations on the distribution of soil biota (Nkem, et al. 2006; Poage, et al. 2008). Spatial differences were observed between young and old soils. The extremely “old”, usually high elevation regions in continental Antarctica are characterised by high salt concentrations due to a long depositional history of salts from the atmosphere and negligible rates of soil leaching caused by arid conditions (Bockheim 2002). In these saline “old” soils, nitrate concentrations can reach 3% (w/w) exceeding carbon concentrations (Barrett, et al. 2007) whereas “young” soils are typically characterized by low nitrate concentrations (Barrett, et al. 2007). This spatial variability of soil nutrients, such as carbon and nitrogen, presents significant limitations to soil colonization by microorganisms.

1.2 Ecological functions of Archaea in temperate environments

1.2.1 Metabolic diversity

The metabolic diversity and biogeochemical function of Archaea in Antarctica remains largely unknown, although the broad metabolic diversity of Archaea inhabiting other, mostly temperate, ecosystems has been reported. There is evidence that at least some Archaea, i.e., populations of Marine Group 1 Crenarchaeaota, are capable of heterotrophic or mixotrophic growth under certain conditions, which is illustrated by their ability to carry out light- independent carbon fixation (Herndl, et al. 2005; Wuchter, et al. 2003), uptake of amino acids (Herndl, et al. 2005; Ouverney and Fuhrman 2000), and uptake of carbon dioxide (CO_2) (Hallam, et al. 2006). However, most Marine Group 1 Crenarchaeota are believed to be autotrophic and utilise inorganic carbon sources such as CO_2 and bicarbonate (Pearson, et al. 2001). This wide

range of possible substrates for energy production has been further extended by the discovery of a unique gene potentially encoding the key enzyme for nitrification (Venter, et al. 2004) suggesting that Archaea may oxidize ammonia (NH_3) to nitrite (NO_2^-) in marine environments.

1.2.2 Involvement in biogeochemical cycles

Archaea may also play a crucial role in a number of biogeochemical cycles as indicated by their ability to metabolize inorganic substrates like CO_2 and NH_3 which play a central role in the global carbon (C) and nitrogen (N) cycles, respectively. The microbial key players in the global carbon cycle are the methanogens which breakdown organic matter anaerobically by converting a restricted number of carbon substrates, such as CO_2, formate, methanol, methylamines and/or acetate, to methane (Thauer, et al. 2008), thus contributing to soil mineralization. On the other hand, groups of Crenarchaeota termed the Marine Group 1.1a and 1.1b, are believed to play an important role in global nitrogen cycling (Nicol and Schleper 2006). Archaeal ammonia- oxidation is the first and rate- limiting step within the global nitrogen cycle and is crucial to prevent a loss of net nitrogen through subsequent denitrification in soils.

1.3 Detection of Archaea

1.3.1 Culture-dependent approaches

There are two established strategies to investigate microbial diversity in ecosystems, culture- dependant and culture- independent methods (Leuko, et al. 2007). Many archaeal species have been successfully isolated from various thermophilic and mesophilic environments (Blochl, et al. 1997; Koenneke, et al. 2005). However, most Archaea are typically culture recalcitrant, thus limiting their detection to certain environments and species. Therefore, true archaeal diversity is highly underestimated for ecosystem studies based on isolation experiments.

1.3.2 Culture- independent approaches

Due to the limitations of culture- dependent approaches molecular culture- independent studies based on the polymerase chain reaction (PCR) are widely used to assess archaeal diversity in natural environments (Niederberger, et al.). These new DNA- based approaches have unearthed a wide diversity and the ubiquitous presence of Archaea in non- extreme environments such as soils, sediments and oceans (Chaban, et al. 2006), as well as in extreme cold environments like Antarctica (DeLong, et al. 1994; Gillan and Danis 2007; Massana, et al. 1998; Murray, et al. 1998). A variety of genes can be used as targets for these molecular methods but the 16S ribosomal RNA (rRNA) gene, which encodes for the small ribosomal subunit, is typically used for species detection and diversity studies. There are three main reasons why this housekeeping gene is favoured over others: I. it is presumed that the 16S rRNA gene has never been horizontally transferred, II. all cellular life contains at least one copy, and III. some regions within the gene evolve fast and other regions evolve very slowly (1% sequence variation accumulating over 50 Myr (Ochman and Wilson 1987)) allowing phylogenetic relationships to be resolved on various levels (Woese, et al. 1990).

Although, ‘housekeeping’ gene sequences can be used for species detection and identification in the environment, the biological function of species remains unknown when using these ‘housekeeping’ genes. Thus, in order to detect archaeal groups with particular metabolic features, the genes involved in these metabolic pathways must be targeted. One prominent example is the ammonia monooxygenase gene (*amo*) encoding the ammonia monooxygenase (AMO) enzyme, which is responsible for ammonia- oxidation in Bacteria and Archaea. Both the bacterial and archaeal AMO enzyme is composed of three subunits, which are encoded for by the genes *amoA, amoB* and *amoC*. Although, crenarchaeal *amo* sequences are comparatively distant to their bacterial homologues, a 40% sequence similarity can be found at the protein level between both variants indicating similar metabolic functions as they belong to the same protein family (Nicol and Schleper 2006). Comparison of *amo* gene

clusters in soil and marine Crenarchaeota with those of Proteobacteria reveals several differences. The individual proteobacterial genes are larger with a conserved *amoCAB* operon arrangement. In contrast, the gene order of the three *amoA*- like crenarchaeal genes is not conserved and varies between ecologically distinct lineages. For example, in most soil Crenarchaeota *amoC* is not linked to *amoA* and *amoB* but is located elsewhere in the genome (Nicol and Schleper 2006).

These differences in *amo* gene clusters are an important consideration for molecular analysis to distinguish archaeal from bacterial ammonia- oxidizers. Moreover, differences within the ammonia- oxidizing Crenarchaeota reveal a separation into two ecologically distinct lineages- Marine Group 1.1a and 1.1b, mirroring 16S rRNA gene phylogenies (Figure 5). This ecological differentiation into marine (1.1a) and soil (1.1b) ammonia- oxidizing Archaea (AOA) was confirmed by two complementary metagenomic studies of seawater (Venter, et al. 2004) and soil (Treusch, et al. 2005).

PCR- amplified marker genes can be further analysed by fingerprinting techniques providing a rapid initial appraisal of microbial diversity in complex environments (Leuko, et al. 2007). The most widely used marker gene is the 16S rRNA gene, but fingerprints can also be generated using functional genes (Liesack and Dunfield 2002; Prosser 2002), such as *nifH* (Widmer, et al. 1999) and *amoA* (Jin, et al. 2010). The general principle of most molecular fingerprinting techniques is based on the electrophoretic separation of PCR- amplified gene fragments due to differences in the length of their nucleotide sequence (Smalla, et al. 2007). Many fingerprinting techniques are used to investigate species diversity in natural microbial communities including denaturing gradient gel electrophoresis (DGGE) (Muyzer, et al. 1993), temperature gradient gel electrophoresis (TGGE) (Brim, et al. 1999), automated ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett 1999), and terminal restriction fragment length polymorphism (T- RFLP) (Liu, et al. 1997; Marsh 1999).

As shown in many studies, T- RFLP is useful for characterisation and analysis of archaeal communities in various environments (Angel, et al. 2010; Luna, et al. 2009; Moeseneder, et al. 2001; Nishizawa, et al. 2008; Stoica 2009; Takai, et al. 2003), whereas ARISA is not recommended for archaeal fingerprinting, due to the lack of a true intergenic spacer region (ITS) in some marine Archaea (Hewson, et al. 2006; Leuko, et al. 2007). First described by Liu et al. (1997), T- RFLP is an extension of the restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA gene, otherwise known as amplified ribosomal DNA restriction analysis (ARDRA) (Smit, et al. 1997). Briefly, rDNAs are obtained by PCR amplification using universal primers, with one primer fluorescently labelled. The amplified product is digested with restriction enzymes with four base pair recognition sites resulting in terminal restriction fragments (T- RF). Multiple T- RF's of different lengths are obtained based on variations in the position of restriction sites among sequences. The length of fluorescently labelled T- RF's is subsequently determined by high- resolution gel electrophoresis on an automated DNA sequencer (Liu, et al. 1997). Highly precise fragment length determination is achieved by using an internal size standard in each profile, which provides numerical data with high resolution (Dunbar, et al. 2001). Theoretically, each community has a unique fingerprint based on its species composition, thus community fingerprints can be used to assess the similarity of different communities. Moreover, T- RFLP is a sensitive and rapid method to assess community diversity (Liu, et al. 1997) which enables replicated experiments in field- scale studies with statistical analysis to be conducted (Dunbar, et al. 2001). Due to the complexity of obtained T- RFLP fingerprinting patterns, statistical analysis is essential to allow valid comparisons between fingerprints obtained from different microbial communities (Oros-Sichler, et al. 2007). A widely used analysis method is based on the Pearson correlation coefficient to calculate similarity values of pairs of fingerprints. These similarity values are subsequently subjected to hierarchical ranking by applying random permutations analysis to test for significant differences between the similarities of T- RFLP profiles within and between samples (Kropf, et al. 2004). However, for analysis of large data sets ordination methods such

as multidimensional scaling can be applied (Legendre and Legendre 1998; Oros-Sichler, et al. 2007). By grouping and comparing the data, these methods can be used to assess the influence of abiotic factors on microbial community assemblages (Legendre and Legendre 1998). The strength of this method is that community fingerprints are only represented in a few dimensions while the distant relationships among and between fingerprints are preserved (Legendre and Legendre 1998).

While community fingerprinting methods such as T- RFLP 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 it is most likely that the number of peaks detected in T- RFLP assays underestimates the actual richness of any community with a long- tailed rank abundance distribution (Bent, et al. 2007). Indeed, microbial communities are generally found to show a log- normal or log- Laplace species- abundance distribution, which would require sampling of about 80% of the species in any given environment (Gans, et al. 2005). Consequently, the sole use of fingerprinting methods cannot provide reliable diversity estimates. However these methods are of great use for rapid, initial screening of environmental samples, which can be subsequently subjected to more detailed analyses using cloning and sequencing, or modern high- throughput genome sequencing approaches. Although cloning is a very time consuming and cost intensive method (Huse, et al. 2007; Leuko, et al. 2007), it provides detailed information about community composition. Comparisons of obtained sequences determines which phylogenetic groups are present in the community and, moreover, enables species identification (Prosser 2002). Modern high- throughput sequencing approaches are a promising new method in molecular ecology and enable a more extensive sampling of molecular diversity in microbial populations (Huse, et al. 2007). Based on the sequencing- by- synthesis protocol, thousands of short DNA sequence reads can be generated in a few hours, without the time- consuming cloning step (Huse, et al. 2007). These modern sequencing approaches proved to be successful for detection of Archaea in various environments including oceans (Galand, et al. 2009;

Qian, et al. 2010), thermal springs (Mardanov, et al. 2010) and archaeal biofilms (Brazelton, et al. 2010).

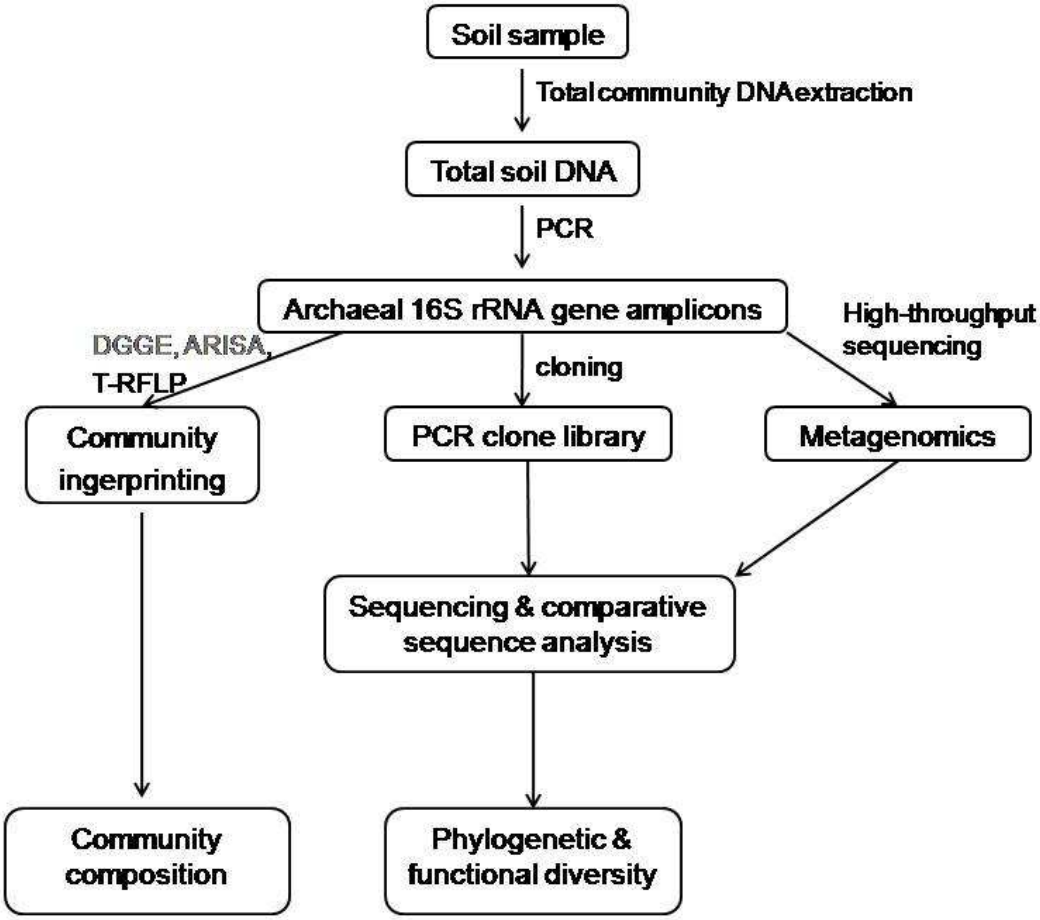


Figure 3. Flow diagram of the culture- independent methods used in this study. Grey colour indicates alternative approaches.

Due to the extreme characteristics of Antarctic soils previous molecular studies failed to detect Archaea in Antarctic soils, including soils from the western Antarctic Peninsula (Yergeau, et al. 2009) and the McKelvey Valley in the McMurdo Dry Valleys (Pointing, et al. 2009). The only evidence that Archaea inhabit soils of the Dry Valleys has been recently reported by Ayton and colleagues (2010) indicating a patchy distribution, low concentration and low diversity of Archaea in Antarctic soils. Despite this it is assumed that Archaea may be present in these soils where they could contribute to nutrient cycling, as they do in temperate environments.

1.4 Involvement in the Nitrogen Cycle

Nitrogen (N) is a critical component of proteins and nucleic acids and is, therefore, fundamental to the structures and biochemical processes that define life. The most abundant form of N is triple- bonded N_2 gas (78% of the atmosphere) which is inaccessible to most microorganisms (Francis, et al. 2007). Thus atmospheric N_2 needs to be fixed first by microorganisms in order to be readily available for use. After initial fixation N is released as NH_4^+, which is a substrate for autotrophic aerobic ammonia- oxidizers which convert NH_4^+ into nitrite (NO_2^-) during the first step of nitrification. Subsequently autotrophic aerobic nitrite- oxidizers oxidize NO_2^- to nitrate (NO_3^-). The N cycle is completed by reduction of NO_3^- to N_2 gas during denitrification under anaerobic conditions (Figure 4).

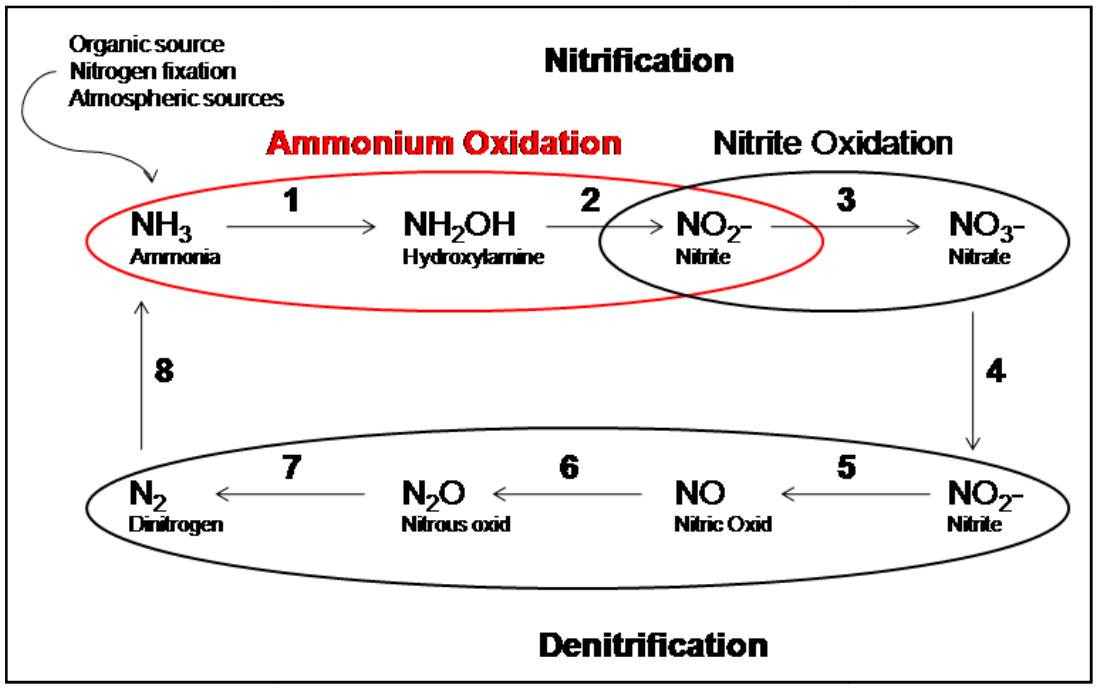


Figure 4. Microbial nitrogen transformation**.** Each step is mediated by the following enzymes: (1) ammonia monooxygenase, (2) hydroxylamine oxidoreductase, (3) nitrite oxidoreductase, (4) nitrate reductase, (5) nitrite reductase, (6) nitric- oxide reductase, (7) nitrous- oxide reductase, (8) nitrogenise.

1.4.1 Ammonia- oxidation

The first step of nitrification, oxidation of ammonia to nitrite ($NH_3 \rightarrow NH_2OH \rightarrow NO_2^-$), is the rate limiting step of nitrification in a wide range of environments and can lead to loss of substantial amounts of net N through subsequent denitrification in soils (Nicol and Schleper 2006). The key enzyme responsible for conversion of ammonia to hydroxylamine (NH_2OH) is ammonia monooxygenase (AMO), which is composed of three subunits (gene products of *amoA*, *amoB* and *amoC*). Interestingly, this obligatory aerobic, chemoautotrophic process is restricted to just a few groups within the Proteobacteria (Kowalchuk and Stephen 2001), but may be widely distributed among Marine Group 1.1 Crenarchaeota.

1.4.2 Phylogeny of ammonia- oxidizing Archaea

As inferred from phylogenetic analysis (Figure 5), the Crenarchaeota are divided into two distinct clades- the Marine Group 1, usually detected in mesophilic marine and terrestrial environments, and the thermophiles. The Marine Group I is placed as sister taxa to all other crenarchaeal groups which are mainly represented by thermophilic sulfur- metabolizing species. Archaeal species capable of ammonia- oxidation are members of the Marine Group 1 Crenarchaeota.

Some researchers consider ammonia- oxidizing Crenarchaeota to be a separate phylum (Thaumarchaea) due to their placement according to concatenated ribosomal protein phylogenies (Brochier-Armanet, et al. 2008). This phylogeny showed that the mesophilic AOA *Cenarchaeum symbiosum* and its relatives are different from hyperthermophilic Crenarchaeota and branch deeper than previously assumed (Brochier-Armanet, et al. 2008). This concept of a third archaeal phylum, Thaumarchaea, is supported by a recent comparative genomic study of the marine ammonia- oxidizers *Nitrosopumilus maritimus* and *Nitrososphaera gargensis*, which confirmed the assignment of mesophilic ammonia- oxidizing Crenarchaeota to a separate phylum and revealed a

Thaumarchaeota- specific set of core informational processing genes (Spang, et al. 2010).

All archaeal ammonia- oxidizers are members of the Marine Group 1.1- a subdivision of the Marine Group 1 Crenarchaeota, or are at least closely related to Marine Group 1.1. Within this group species from marine and terrestrial habitats seem to cluster into two distinct groups according to their environment. For example, almost all marine AOA sequences are placed within group 1.1a, whereas most of the terrestrial AOA sequences cluster within group 1.1b indicating some level of ecological differentiation. Some species, however, cluster within a third group- Marine Group 1.1c with no obvious restriction to certain habitats but seem to be more abundant in grassland, coniferous or boreal forest soils (Figure 5).

As mentioned previously the potentially ammonia- oxidizing species from marine environments fall into monophyletic Marine Group 1.1a. The two isolated marine ammonia- oxidizers *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum* (Hallam, et al. 2006; Koenneke, et al. 2005) cluster with numerous environmental sequences (Figure 5) retrieved from ocean waters, including the Sargasso Sea (Venter, et al. 2004) and Antarctic waters (Kalanetra, et al. 2009). This close phylogenetic relationship of environmental sequences to well known ammonia- oxidizers indicates that ammonia- oxidation may be a common feature of species belonging to Marine Group 1.1a. The high abundance of Marine Group 1.1a Archaea in the world's oceans, where they represent up to 20% of all planktonic prokaryotes (DeLong, et al. 1999), indicates that these species play a central role in ocean nitrification and are thus crucial for N cycling in marine mesophilic and psychrophilic ecosystems such as Antarctica.

On the other hand, most ammonia- oxidizing Archaea present in soils cluster within Group 1.1b. Environmental sequences from various mesophilic and thermophilic environments (Nicol, et al. 2008; Tourna, et al. 2008; Weidler, et al. 2007) are closely related to the thermophilic ammonia- oxidizing *Nitrososphaera gargensis,* as indicated by

phylogenetic analysis (Hatzenpichler, et al. 2008) (Figure 5). Although this group has only one semi- isolated representative (*N. gargensis*), which was obtained from enrichment cultures, its ammonia oxidation activity suggests that all Marine Group 1.1a and 1.1b Archaea may be ammonia- oxidizers and raises the possibility that all Marine Group 1 Archaea are capable of ammonia oxidation. This group is considered to be ubiquitous in most soils, where they make up to 5% of prokaryotic 16S rRNA genes (Ochsenreiter, et al. 2003), indicating that soil Crenarchaeota are the most abundant ammonia- oxidizing organisms in soil ecosystems (Leininger, et al. 2006).

Thermophilic ammonia- oxidizers seem to cluster with various groups of the Marine Group 1 Crenarchaeota, including Group 1.1a and 1.1b (Spear, et al. 2007; Weidler, et al. 2007) indicating a close phylogenetic relationship to mesophilic and psychrophilic ammonia- oxidizing Crenarchaeota. The only thermophilic ammonia- oxidizing isolate *Nitrosocaldus yellowstonii* is however affiliated with a clade basal to the radiation of the Marine Group 1.1 Crenarchaeota (de la Torre, et al. 2008) separating this thermophilic isolate clearly from all other ammonia- oxidizing Archaea (Figure 5).

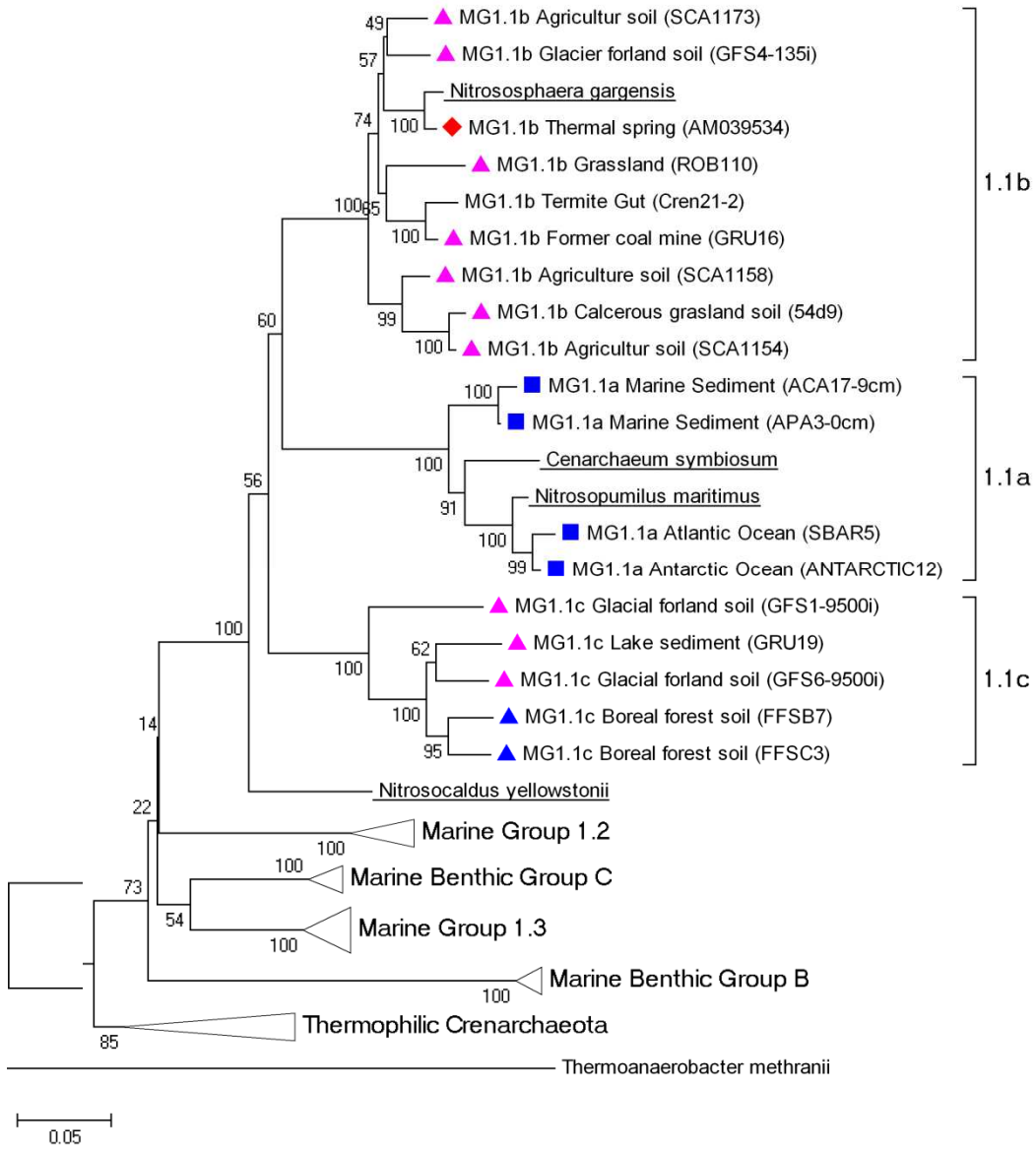


Figure 5. Phylogeny of Marine Group 1 Crenarchaeota based on partial environmental 16S rRNA gene sequences (814bp) and isolates. Isolated species known as ammonia- oxidizers are underlined. Colours and symbols within the Marine Group 1.1 represent different temperature ranges and habitats where clones were detected and are as followed: blue: psychrophilic, pink: mesophilic, red: thermophilic, ■: marine, ▲: terrestrial and ♦: thermal springs. The bacterial isolate *Thermoanaerobacter methranii* is used as outgroup. The evolutionary history was inferred using Neighbor- Joining (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using Maximum Likelihood (Tamura, et al. 2004) and are in the base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 814 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, et al. 2007).

1.4.3 Discovery and distribution of ammonia- oxidizing Archaea

Archaeal ammonia- oxidation was first observed in marine environments. Venter and colleagues first suggested that high nitrite concentrations in the surface of the Sargasso Sea could be the result of ammonia oxidation by Archaea (Venter, et al. 2004) but the definite link between Archaea and ammonia oxidation was established by Könneke et al. (2005) who successfully isolated the first marine, mesophilic ammonia- oxidizing Crenarchaeota, designated *Nitrosopumilus maritimus*, from a saltwater aquarium. Cultivation experiments showed a near- stoichiometric conversion of ammonia to nitrite confirming Venter's results. Extended molecular studies showed that these AOA are widely distributed in oceans especially in areas that are critical for the global nitrogen cycle, including the euphotic zone, suboxic water columns and estuarine coastal sediments. Each of these individual habitats had unique archaeal *amoA* sequences indicating distinct AOA communities within different habitats (Francis, et al. 2005). In detail, all *amoA* sequences retrieved from the water column fell into different clusters depending on depth. The largest of these clusters contained > 70% of all water column sequences, including Sargasso Sea sequences, whereas all sequences retrieved from soil fell within the large soil/sediment cluster. These results reflect the wide habitat range of AOA throughout the ocean and sediments.

Almost simultaneously with the discovery of marine AOA, archaeal ammonia- oxidation was reported in terrestrial habitats indicating a widespread occurrence of AOA (Treusch, et al. 2005). First evidence for archaeal ammonia- oxidation in soils was obtained by retrieval of an *amoA* sequence from calcareous grassland soil which was highly similar to *amoA* from Sargasso Sea plankton indicating that mesophilic terrestrial and marine Crenarchaeota are capable of ammonia- oxidation (Treusch, et al. 2005). Furthermore, molecular studies focusing on *amoA* abundance revealed high abundance of AOA in soils from three different climatic zones including Polar, Temperate and Mediterranean zones (Leininger, et al. 2006), supporting the idea of a global distribution of AOA. Moreover, the ammonia- oxidizing crenarchaeal Group 1.1b is the most widespread

crenarchaeal group in soils (Ochsenreiter, et al. 2003), where they outnumber their bacterial counterparts (Leininger, et al. 2006).

All microbiological and molecular studies mentioned above focused on the distribution of AOA in moderate aquatic and terrestrial environments. Only recently a number of studies showed that archaeal ammonia- oxidation can also occur at extremely high temperatures. First indications for archaeal ammonia- oxidation at elevated temperatures were based on molecular studies showing potential ammonia- oxidation in thermophilic environments such as geothermal mine adit (Spear, et al. 2007) and radioactive thermal springs (Weidler, et al. 2007).

The first thermophilic ammonia- oxidizer for which ammonia- oxidation has been verified on a cellular level was *Nitrososphaera gargensis*, which showed oxidation of ammonia to nitrite when incubated at 46℃ (Hatzenpichler, et al. 2008) . Concurrently, la Torre and colleagues isolated the first truly thermophilic AOA from a terrestrial hot spring in Yellowstone National Park (de la Torre, et al. 2008). The new isolate, designated *Nitrosocaldus yellowstonii,* grows autotrophically at temperatures up to 74℃ by stoic hiometric oxidation of ammonia to nitrite. These data significantly extend the upper temperature limit of nitrification and increase the known habitat range of ammonia- oxidizing Archaea from mesophilic to hyperthermophilic ecosystems.

Extreme cold environments, like Polar regions, are the least studied areas on Earth regarding distribution of ammonia- oxidizing Crenarchaeota, as previous studies focused mostly on environments with moderate to extremely hot temperatures. However, it has been reported that species of the Marine Group I Crenarchaeota are widely distributed in waters from the Arctic Ocean (Galand, et al. 2009; Kirchman, et al. 2007) and Antarctic Ocean (Church, et al. 2003; DeLong, et al. 1994; Massana, et al. 1998; Murray, et al. 1998), thus indicating a potential for ammonia- oxidation in polar waters due to previous discoveries of ammonia- oxidation in Marine Group I Crenarchaeota in other environments. This hypothesis was confirmed recently by Kalanetra and

colleagues who successfully showed that ammonia- oxidizing Crenarchaeota may be highly abundant in Arctic and Antarctic water bodies (Kalanetra, et al. 2009). Surprisingly, a higher abundance was detected in Antarctic waters compared to Arctic waters and, moreover, phylogenetic analysis revealed two distinct phylotypes suggesting a depth- dependant distribution within Antarctic waters (Kalanetra, et al. 2009).

However, diversity and distribution of Crenarchaeota in terrestrial ecosystems in Antarctica still remains unknown, but should be taken into serious consideration. The high abundance of soil Crenarchaeota in moderate and extreme temperature environments and their affiliation with ammonia- oxidation (Treusch, et al. 2005) indicates global distribution of AOA with no obvious restriction to certain cold environments such as Antarctica. It is possible that Crenarchaeota have undergone adaptation and diversification at the genome level to facilitate their survival and persistence in cold terrestrial and marine Antarctic ecosystems (Murray and Grzymski 2007). Although numerous studies have shown that Archaea are highly abundant in Antarctic lakes (Bowman, et al. 2000; Glatz, et al. 2006; Karr, et al. 2006), the only evidence that Archaea inhabit soils of the Dry Valleys has been recently reported by Ayton and colleagues (2010) indicating a patchy distribution, very low concentration and low diversity of Archaea in Antarctic soils.

1.4.4 Activity of ammonia- oxidizing Archaea

Ammonia- oxidizing Archaea are not only highly abundant in oceans worldwide, but are actively involved in ocean nitrification through ammonia- oxidation. For example, ammonia- oxidation was reported for the epi- and mesopelagic waters of the Pacific Ocean by quantification of *amoA* expression levels (Church, et al. 2010). The highest activity of ammonia- oxidation was observed in the upper ocean (< 100 m), which decreased with depth as indicated by a decrease in *amoA* transcripts. This depth- dependant response of crenarchaeal activity may derive from changes in substrate availability, temperature and sunlight.

It has also been shown that external factors like temperature, ammonia concentrations and pH play a role in regulating crenarchaeal *amoA* transcription in terrestrial soils (Nicol, et al. 2008; Tourna, et al. 2008; Treusch, et al. 2005). Temperature dependant ammonia- oxidation has been shown for members of both, marine and soil group Crenarchaeota (Tourna, et al. 2008). Interestingly, most soil sequences that exhibited a temperature response were associated with marine and subsurface Crenarchaeota, rather than soil Crenarchaeota supporting the idea of temperature- dependant *amoA* expression in oceans (Church, et al. 2010).

External substrate concentrations may also play a role in regulation of *amoA* expression. For example, very low substrate concentrations of less than 1mM ammonium resulted in an increased activity of *N. gargensis*, whereas partial inhibition was observed when incubated at > 3mM ammonium (Hatzenpichler, et al. 2008). This high substrate sensitivity may be the result of adaptation to a thermophilic environment.

Another important factor influencing activity of ammonia- oxidation in soils is acidity (pH). Generally, reduced growth and decreased activity of ammonia- oxidizing microorganisms is observed in acidic soils due to the exponential reduction of NH_3 availability with decreasing pH (Frijlink, et al. 1992). A phylogenetic study conducted by Nicol and colleagues (2008) revealed a decrease of AOA abundance and activity with increasing soil pH. Moreover, incubation experiments at different pH's revealed a greater relative activity of species if incubated at their native soil pH (Nicol, et al. 2008). These results highlight the potential of AOA to adapt to their environment (i.e., low soil pH).

1.5 Involvement in the Carbon Cycle

Methane (CH_4) is the most abundant organic species in the Earth's atmosphere and the second most important anthropogenic greenhouse gas after carbon dioxide (CO_2). Most of the atmospheric CH_4 is formed through metabolic activity of methanogenic Archaea demonstrating the important role of these organisms in the global carbon cycle (Figure 6). The global carbon cycle is complex and includes CH_4 and CO_2 cycling in oxic and anoxic environments. The initial step is fixation of carbon into biomass by photosynthesis. Approximately 2% of the net CO_2 that is fixed annually ends up primarily as CH_4. After carbon fixation, biomass in the form of animal and plant polymers is successively degraded into smaller intermediate compounds by aerobic and anaerobic oxidation. These monomers are fermented by syntrophic bacteria, protozoa and fungi to acetic acid, CO_2 and H_2 which are in turn substrates for methanogenic Archaea (Thauer, et al. 2008). Methanogenic Archaea produce large amounts of methane during methanogenesis under anaerobic conditions which is consumed by anaerobic methane oxidizing Bacteria and/or Archaea. Methane can be oxidized under anaerobic conditions if coupled to nitrification or sulfate reduction. Excessive methane that reaches the oxic environment is oxidized to CO_2 by aerobic methanotrophic bacteria, which is then released into the atmosphere completing the cycle (Figure 6).

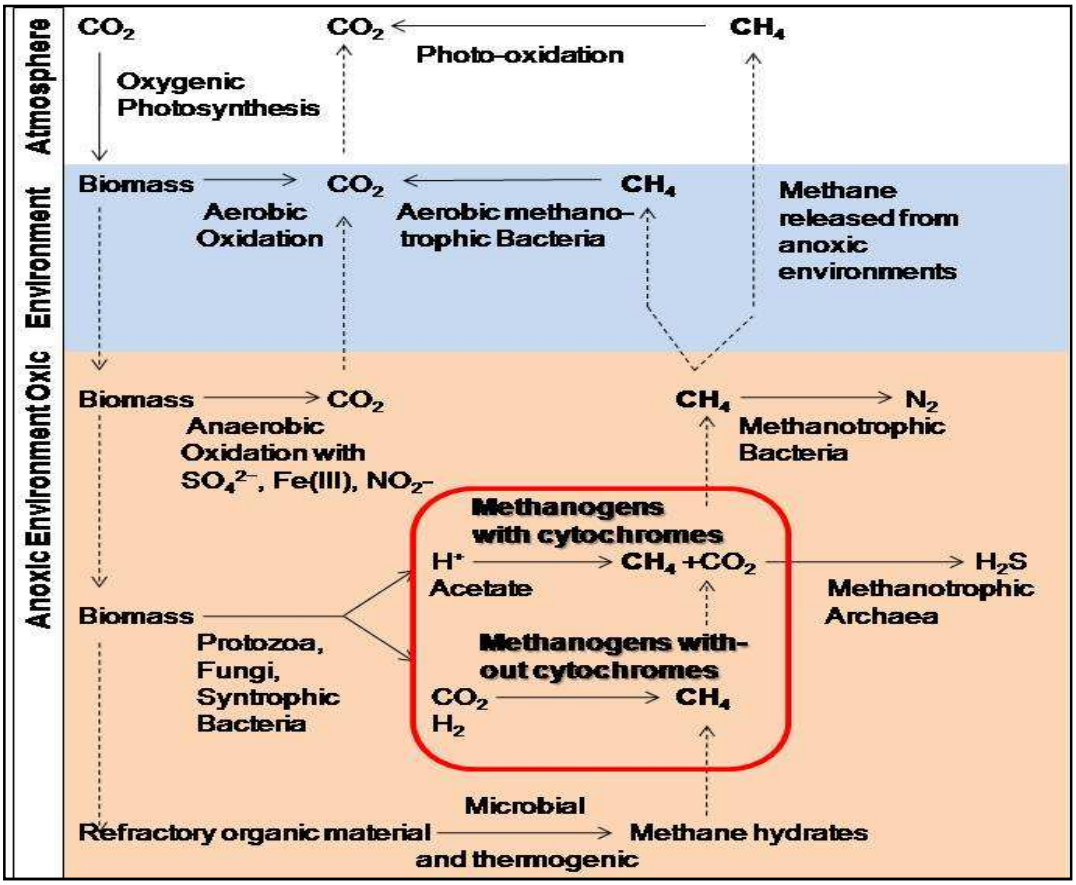


Figure 6. Simplified global carbon cycle. The central role of methanogenesis is highlighted by the red rectangle. Continuous arrows indicate a reaction and dashed arrows indicate diffusion and/or convection (modified from Thauer et al. 2008).

1.5.1 Methanogenesis

Methanogenesis represents the terminal step in the anaerobic breakdown of organic matter under sulfate- limiting conditions (Deppenmeier, et al. 1996) and is, therefore, crucial for mineralization in soils. All methanogenic Archaea derive their metabolic energy from the conversion of a restricted number of C_1 substrates. These substrates are degraded via the CO_2- reducing, methylotrophic or aceticlastic pathway, either with or without cytochromes. The metabolic activity of methanogenic Archaea results in extremely high methane concentrations in anoxic environments such as freshwater sediments, swamps, paddy fields and landfills indicating an important role of methanogens in global carbon cycling.

All organisms capable of methanogenesis belong to a group of phylogenetically diverse, strictly anaerobic Euryarchaeota. The methanogens are by far the most studied group of Archaea with five identified orders to date including Methanosarcinales, Methanopyrales, Methanococcales, Methanobacteriales and Methanomicrobiales (Figure 7). All members of these groups metabolize a restricted number of C_1 substrates to methane. However, energy conservation within the cell can be quite different in distinct species. For example, members of the Methanosarcinales contain cytochromes and show a broad substrate spectrum, whereas members of the other four groups lack cytochromes and reduce CO_2 and/or formate with H_2 to CH_4 via the CO_2-reducing pathway (Thauer, et al. 2008).

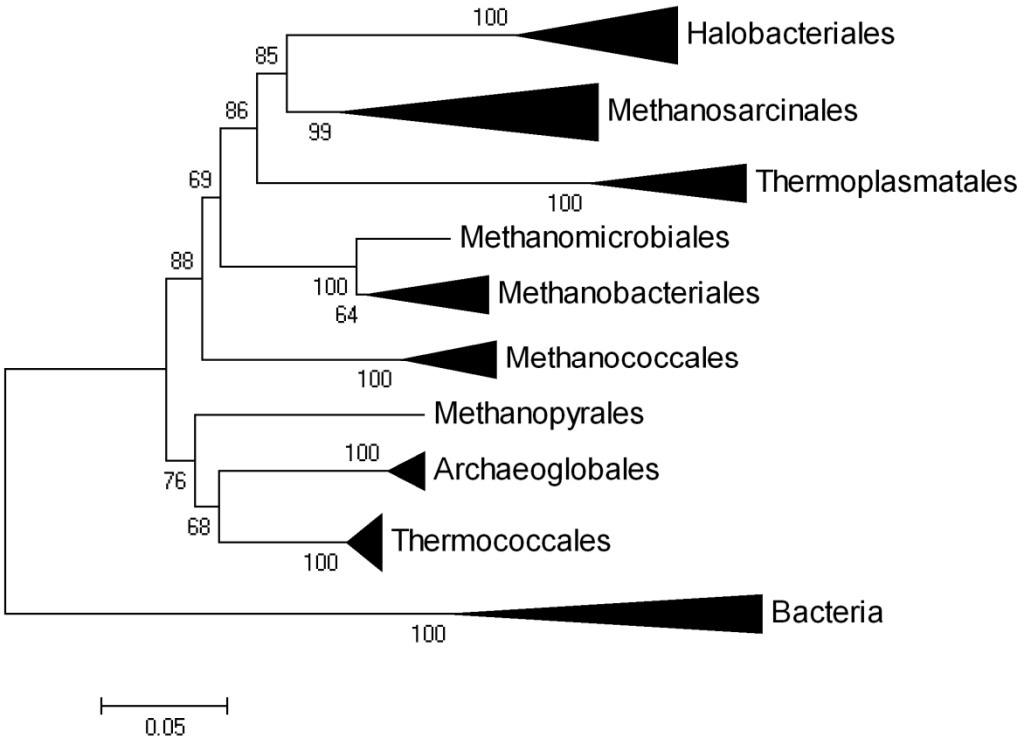


Figure 7. Euryarchaeal rRNA tree based on 16S rRNA gene sequences (1303 bp) of isolates representing the main phylogenetic lineages of Euryarchaeota. Two species from the domain Bacteria were used as outgroup. For analyses details see Figure 5.

Methanosarcinales species utilise methanol and methylamines by the methylotrophic pathway and some species use acetate via the acetoclastic pathway. Interestingly, representatives of Methanosarcinales have only been found in moderate to cold temperate environments with no known hyperthermophilic species, which explains their broad substrate spectrum as these substrates are readily available in non- hyperthermophilic environments which are rich in organic carbon due to high levels of biomass.

Alternatively, the methanogenic groups using CO_2 and H_2 (Methanopyrales, Methanococcales, Methanobacteriales and Methanomicrobiales) as main substrates are represented by many hyperthermophilic species thriving in hydrothermal systems (Jeanthon, et al. 1999; L'Haridon, et al. 1998) where possible substrates are more limited (Thauer, et al. 2008).

1.5.2 Distribution of methanogens

Although, methanogens are restricted to anaerobic environments, it has been shown that these organisms are highly abundant in a broad range of ecosystems in temperate environments. They are typically found in areas where organic matter is decomposed in the absence of oxygen or other oxidants, such as nitrate, sulfate or ferric iron (Conrad 2009). For example, wetland soils are one of the major habitats of methanogens due to the high concentration of organic debris at depth, which favors methanogenesis via the CO_2- reduction pathway. In the shallow subsurface of wetland soils, on the other hand, high concentrations of simple, labile organic compounds promote methanogenesis via the acetate fermentation pathway (Hornibrook, et al. 1997).

This spatial distribution of methanogenic pathways in wetland soils is similar to methanogenesis in ocean sediments, another main source of CH_4 production (Inagaki, et al. 2006). Most of the described species of methanogens are of marine origin and include acidophiles and alkaliphiles, which can grow at pH 5.0 and 9.0, respectively. Further habitats of

methanogens range from lake sediments and rice field soils to digestive systems of many animals (Conrad 2009).

The first psychrophilic methanogen was isolated in 1991 by Zhilina and Zavarzin, followed by the discovery of two new species, *Methanococcoides burtonii* and *Methanofenium frigidum,* isolated from methane- saturated, anaerobic bottom waters of Ace Lake, Antarctica (Franzmann, et al. 1997; 1992). Over the last decade extensive studies have revealed the widespread occurrence of methanogens in cold environments, including marine sediments in Alaska and the Baltic Sea, a freshwater lake in Switzerland and Arctic permafrost (Chong, et al. 2002; Kendall, et al. 2007; Kotsyurbenko, et al. 2007; Morozova and Wagner 2007; Simankova, et al. 2003; Singh, et al. 2005; von Klein, et al. 2002). Not only are methanogens highly abundant in these cold environments they contribute up to 34% to the global methane flux from wetlands (Bartlett and Harriss 1993) indicating an important role of methanogens within cold, polar regions.

Most of the studies on molecular ecology of methanogens in Antarctica have focused on sediments either from the Antarctic Ocean or Antarctic freshwater lakes (Bowman, et al. 2003; Bowman, et al. 2000; Purdy, et al. 2003). The comparative study by Purdy and colleagues revealed a dominance of C_1- utilizing species belonging to *Methanolobus* and *Methanococcoides* in marine sediments whereas most of the clones from freshwater sediments were closely related to the obligate acetate-utilising *Methanosaeta concilii* (*Methanosarcinales*) (Purdy, et al. 2003). Interestingly, archaeal RNA extracted from the sediment represented only 0.2% of the total prokaryotic RNA in the marine sediment but high numbers of bacterial species belonging to the group of sulfate- reducing bacteria (SRB) were obtained. SRB are thought to outcompete methanogenic Archaea in environments where sulfate is freely available because both SRB and methanogens can be seen as ecological equivalents due to their terminal role in mineralization as anaerobic oxidizers of organic matter. The high archaeal abundance (34% of the total prokaryotic signal) observed in sediments of Lake Heywood was expected due to sulfate- limitation, which is common to freshwater

environments. The previous discovery of clones related to *Methanosaeta* from temperate environments suggests a global dominance of these acetoclastic methanogens in freshwater sediments indicating a widespread importance of these organisms (Purdy, et al. 2002).

This high abundance of *Methanosarcinales* species in Antarctic lakes was further confirmed by the discovery of *Methanosarcinales*- like methanogens in the permanently frozen Lake Fryxell (Karr, et al. 2006). Additionally, three other distinct archaeal clusters were obtained including the crenarchaeal Marine Benthic Group C (Figure 5), *Methanomicrobiales* and *Methanoculleus*. Interestingly, a *Methanoculleus*- like 16S rRNA gene was previously detected in cyanobacterial mats that develop in the peripheral melt waters of this lake indicating that methanogens are not restricted to lake sediments but can also occur in surface waters (Brambilla, et al. 2001; Taton, et al. 2003).

So far, no methanogens have been described in Antarctic soils in contrast to Arctic soils where high abundance of methanogens has been reported (Ganzert, et al. 2007; Rivkina, et al. 2007). This is mainly due to significant differences in soil properties between both polar ecosystems. Although, temperature has been reported to influence methanogenesis by inhibiting hydrogenotrophic methanogens, which leads to a shift in the sedimentary metabolism toward acetogenesis and acetoclastic methanogenesis (Schulz and Conrad 1996), the lack of organic carbon and most of all the lack of water in the extremely dry soils of Antarctica may be the primary limiting factors.

However, uptake of CH_4 by desert soils has been reported in the Mojave Desert, Nevada (Striegl, et al. 1992) where CH_4 consumption was obtained to a depth of up to two meters. Antarctic soils, especially those in the Dry Valleys are considered to be very similar to desert soils and it may be possible that as long as a downward transport pathway for CH_4 is available, unfavorable conditions for consumption near the soil surface may allow atmospheric CH_4 to diffuse to some greater depth, where microbial CH_4 consumption can proceed under more favorable conditions (Striegl, et al. 1992). Assuming methylotrophic organisms are present in

these soils to metabolize CH_4 one could assume that primary CH_4 producers, like the methanogens, are present and active in cold desert soils.

1.6 Evolutionary origin of ammonia-oxidizing Crenarchaeota

A small selection of genes are widely used to build phylogenetic trees to reconstruct evolutionary history. To understand the evolution of ammonia-oxidizing Archaea, the use of 16S rRNA genes may not be appropriate as it is not known whether all Marine Group 1 Archaea possess the ability to oxidize ammonia or if this ability is restricted to crenarchaeal groups 1.1a and 1.1b. Consequently, use of the 16S rRNA gene may falsely place some species as ammonia--oxidizers and miss other true ammonia-oxidizers. Nevertheless, most archaeal sequences from soil fall within the 1.1b lineage (Buckley, et al. 1998; Ochsenreiter, et al. 2003) and marine sequences within the 1.1a lineage (Koenneke, et al. 2005; Venter, et al. 2004). Therefore, 16S rRNA-based analysis may target largely *amoA*-possessing organisms assuming ammonia monooxygenase is present in organisms throughout these lineages (Nicol, et al. 2008).

Despite the increasing knowledge about ammonia-oxidizing Crenarchaeota their evolutionary history still remains largely unknown. The question whether ammonia-oxidizing Crenarchaeota are of thermophilic or mesophilic origin is heavily debated. Phylogenetic trees based on 16S rRNA gene sequences (Figure 5) do not support either of the two possibilities if analyzed using the parsimony method. Parsimony implies that the most likely tree, is the one in which the fewest number of nucleotide changes are necessary for two lineages to diverge from a common ancestor. That means, assuming the last common ancestor of ammonia-oxidizing Crenarchaeota was thermophilic (thermophilic origin), three nucleotide substitutions must have occurred during divergence of the lineages (Figure 5, red boxes) to obtain the presented relationship of thermophilic and mesophilic ammonia-oxidizers. The same amount of nucleotide changes is needed assuming a mesophilic origin of

ammonia- oxidation (Figure 5, blue boxes). Therefore, both scenarios are equally likely and none is favored over the other. Three nucleotide substitutions (over 814 bp) is a relatively high number under parsimony definitions, thus both assumptions might not reflect true past events. However, it can be inferred from these analyses that multiple adaptations have occurred during evolution between the two extreme phylotypes.

1.6.1 Thermophilic origin of AOA?

The existence of two thermophilic AOA species, *N. gargensis* and *N. yellowstonii* is indicative for archaeal ammonia- oxidation at elevated temperatures. Further proof for the existence of thermophilic ammonia- oxidizing Crenarchaeota was given by the discovery of the membrane lipid crenarchaeol in terrestrial hydrothermal springs, which was previously thought to be found exclusively in marine Crenarchaeota. (Pearson, et al. 2004).

It has been hypothesized that ammonia- oxidation is an ancient form of energy conservation of thermophilic origin which is consistent with the postulated early earth nitrogen cycle (Canfield, et al. 2006). In this ancient chemically driven nitrogen cycle ammonia is formed by reduction of N_2 at high temperatures (Brandes, et al. 1998), or additionally N_2 can be oxidized by lightning resulting in nitrate and nitrite (Mancinelli and McKay 1988). The latter process was probably the primary source for production of nitrate and nitrite which would then be used by denitrifiers to oxidize organic matters and by anaerobic ammonia- oxidizers to oxidize ammonia to N_2 gas (Canfield, et al. 2006). Once net oxygen increased due to photosynthesis, ammonia could have been oxidized to nitrate by thermophilic Archaea.

Both the discovery of thermophilic ammonia- oxidizing Crenarchaeota and the physicochemical properties of the ‘early earth’, particularly high ammonia concentrations and elevated temperatures argue for a thermophilic origin of ammonia- oxidizing Crenarchaeota. Assuming the last common ancestor was a thermophile, the mesophilic

ammonia- oxidizing Crenarchaeota would be direct descendants of the thermophilic ammonia- oxidizing Crenarchaeota. This assumption is supported by previous studies on hot springs from Yellowstone National Park (Barns, et al. 1994). In these hot springs crenarchaeal 16S rRNA gene sequences were identified that are phylogenetically most related to the low temperature Crenarchaeota (Barns, et al. 1994). However, these results only demonstrate the close relationship between thermophilic and mesophilic ammonia- oxidizers but do not answer the question which of the two lifestyles originated first.

1.6.2 Mesophilic origin of AOA?

Ammonia- oxidation has been reported for two marine crenarchaeal species: the mesophilic isolate *N. maritimus* (Koenneke, et al. 2005) and the sponge symbiont *C. symbiosum* thriving at 10°C (Preston, et al. 1996). Moreover, it was shown that soil AOA are actively growing at temperatures around 10°C by metabolizing ammonia (Tourna, et al. 2008). Although, activity at this low temperature was three orders of magnitude smaller than at 20°C, it indicates that soil Crenarchaeota are capable of ammonia- oxidation at low temperatures.

Evolution from mesophilic to thermophilic ammonia- oxidizers was recently suggested based on the amino acid sequence of ammonia monooxygenase from the thermophile *N. yellowstonii* (de la Torre, et al. 2008). A unique 30 amino acid insertion found in the AmoB protein is believed to be a necessary adaption to maintain enzyme function at high temperatures. The resulting predicted amino acid sequence of this protein is highly divergent (< 50% amino acid sequence identity) from all sequences previously recovered from soil and marine mesophilic habitats indicating adaptation to high temperature environments (de la Torre, et al. 2008). Thus, it is very likely that the last common ancestor of ammonia- oxidizing Crenarchaeota inhabited a cold environment and that thermophilic ammonia- oxidation is a secondary adaptation.

1.7 Evolution of AOA in Antarctica

The 16S rRNA gene is a very slow evolving gene with an estimated 1% sequence variation accumulating over 50 Myr (Ochman and Wilson 1987). Interestingly, this coincides with the timing of the formation of the Antarctic circumpolar current during the Eocene (56 to 34 Myr ago) which resulted in the isolation of the Antarctic continent from warmer waters which allowed cooling and glacier formation on the formerly forested continent (Murray and Grzymski 2007).

It is most likely that ammonia- oxidizing Crenarchaeota inhabited the forest soils of the Antarctic Continent before its separation from South America as it was shown that ammonia- oxidizing Crenarchaeota are present in forest soils of South America (Borneman and Triplett 1997). Due to this physical isolation and subsequently cooling of Antarctica during the Eocene Epoch, the former moderate temperate ammonia- oxidizing Crenarchaeota may have adapted to their new, much colder environment.

Considering the 16S rRNA gene has a length of 1542 nucleotides the estimation of 1% sequence variation over 50 Myr (Ochman and Wilson 1987) would allow for 15 nucleotide changes to have occurred since the Antarctic continent started to cool down. It is unclear if this very slow evolution rate allows for true species diversification, questioning whether the 16S rRNA gene is a suitable marker gene. However, it is assumed that changes in genes involved in metabolism or environmental adaption occur more frequently during the process of adaptation to the cold environment. Taken together, based on the history of the Antarctic continent it is most likely that ammonia- oxidizing Archaea evolved from mesophiles or even thermophiles to psychrophiles in Antarctica. However, this assumption may not apply to evolution of ammonia- oxidizing Archaea in general on a global scale.

1.7.1 Archaeal adaptation to cold environments

All microorganisms are at complete thermal equilibrium with their environment. As a result, all cellular components of microorganisms must be adapted to the habitats' temperature to enable growth and survival (Cavicchioli 2006). Psychrophilic species require numerous adaptations to ensure an overall level of cellular function that is sufficient for survival in cold environments. The recent completion of the genome sequence of the methanogen *Methanococcoides burtonii*, which was isolated from Ace Lake, Antarctica (Franzmann, et al. 1992), gave new insights into the genomic basis and requirements for archaeal growth at low temperatures (Allen, et al. 2009).

1.7.2 Special features in cold-adapted Archaea

Various studies (particularly in Bacteria) have linked cold adaptation to most structural and functional components of the cell, including outer membranes (lipid composition), inner cellular machines (ribosomes), enzymes and nucleic acids (Feller and Gerday 2003). Some features of cold adaptation are characteristic for Archaea.

For example, the isoprenoid lipids in archaeal membranes are thought to maintain the fluidity and hence functionality of the membranes in the cold. The highly unsaturated isoprenoid lipids prevent the membranes becoming rigid at cold temperatures. It has been shown that biosynthesis of these isoprenoid lipids is associated with cold adaptation in *M. burtonii* (Nichols, et al. 2004). A similar relationship between unsaturated lipids and growth temperature was obtained for *H. lacusprofundi* (Gibson, et al. 2005). These results were confirmed by detection of numerous genes encoding enzymes involved in the synthesis of unsaturated lipids in *M. burtonii* during growth at low temperatures (Allen, et al. 2009) indicating that a general feature of cold adaptation in psychrophilic Archaea might be to increase the abundance of unsaturated lipids at low temperatures to ensure membrane fluidity.

Additionally, archaeal membranes contain polysaccharides which are important modifiers of the isoprenoid lipid membrane (Jahn, et al. 2004) and S- layer (Karcher, et al. 1993). These polysaccharides can be secreted as extracellular polymeric substances (EPS) (Paramonov, et al. 1998), which is characteristic for growth of *M. burtonii* in the cold (Reid, et al. 2006). EPS play an important role in archaeal cell aggregation and biofilm formation at low temperature and show increased concentrations if organisms are cultured at low temperatures (Reid, et al. 2006). Interestingly, about 82 protein- coding genes (3.3% of its genome) in *M. burtonii* are involved in polysaccharide biosynthesis. In comparison, in the mesophilic *M. acetivorans* only 30 genes (0.6% of its genome) contribute to polysaccharide biosynthesis (Galagan, et al. 2002). These data, together with the discovery of EPS in the sea- ice bacteria *Colwellia psychrerythraea* (Junge, et al. 2004), clearly suggest that polysaccharide biosynthesis has an important role in the cold adaption of microorganisms and may be a common feature of cold- adapted Archaea.

In order to form EPS, proteins and polysaccharides need to be transported across the membrane to the outer site of the membrane. ABC transporter permeases were previously identified to be involved in lipoprotein export in Gram- negative bacteria (Narita, et al. 2003). Putative novel ABC transporters were detected in the genome of *M. burtonii* which are believed to play an important role in the active transport of proteins leading to the formation of EPS (Allen, et al. 2009). Thus, these novel ABC transporters may be necessary for survival at low temperatures which is supported by the discovery of a gene cluster for an ABC transporter (LIV-I) in *Shewanella sp*. whose expression is up- regulated at low temperatures (Wang, et al. 2009).

Moreover, specific signatures of the genome of *M. burtonii* were shown to be associated with cold adaptation. One example for such signatures are transposases which were significantly different in *M. burtonii* compared to all other Archaea. Genomic characteristics indicate that transposases play an important role in evolving the psychrophilic *M. burtonii* genome (Allen, et al. 2009). Previous studies on the cold- adapted bacterium *Photobacterium profundum SS9* showed a

cold- sensitive inaction of transposases in the deep sea (Lauro, et al. 2008) and DeLong et al. found a relatively high abundance of transposases in metageomic data from cold, deep ocean water (DeLong, et al. 2006) confirming the importance of transposons in cold adaptation. Additionally, Allen and colleagues (2009) observed an exceptional amount of genome plasticity in *M. burtonii,* which had the highest proportion of aberrant sequence composition (51%) among all Archaea. These differences were especially present in genes that are expressed very efficiently which may have a stronger 'psychrophilic' component than genes that are not efficiently expressed. Within these 'psychrophilic' genes a unique amino- acid usage was observed while the same coding bias was not seen in other genes. This may indicate that selection at low temperature may have only significant effects on genes that need to be efficiently expressed in the cell such as high- abundance ribosomal proteins and other proteins critical for growth in the environment. Taken together, *M. burtonii* has evolved cold adaptation through a genomic capacity to accommodate highly skewed amino- acid content while retaining codon usage in common with related mesophilic *Methanosarcina* spp. (Allen, et al. 2009). In contrast, hyperthermophilic and thermophilic Archaea are subject to selection of synonymous codon usage (Lynn, et al. 2002).

When and how evolution from hot to cold environments, or vice versa, took place still remains unknown and will remain open to discussion for some time. However, the genomic studies of *M. burtonii* and other cold- adapted microorganisms clearly indicate that Archaea are capable of adaptation to cold environments and, moreover, successfully colonize these harsh ecosystems, where they may contribute to nutrient cycling.

1.8 Hypothesis, goals of this MSc research project

We hypothesize that Archaea are abundant in soils of the McMurdo Dry Valleys, Antarctica and that their biodiversity and distribution is driven by abiotic parameters such as physicochemical soil properties, lithology and mineralogy. To evaluate this assumption we compared archaeal diversity in four different valleys located in the McMurdo Dry Valleys: Miers Valley (78°6′S 164°0′E), Marshall Valley (78°4′S 164°10′E), Garwood Valley (78°2′S 164°10′E) and Shangri- La (78°3′S 163°10′E). Due to the Valleys' close geographical proximity (Figure 8), they share similar geological, climatic and physicochemical features. Additionally, unique characteristics such as presence or absence of lakes, melt water streams, glacial input and varying microclimates are present in each valley. This makes the Dry Valley soils a perfect model to study archaeal biogeography by assuming that these different characteristics influence diversity and distribution of Archaea. Robust and universal DNA- based polymerase chain reaction (PCR) assays targeting the archaeal 16S rRNA gene were developed, namely: terminated restriction fragment length polymorphism (T- RFLP) and restriction fragment length polymorphism (RFLP). The obtained archaeal distribution and diversity patterns were thoroughly compared with soil properties such as physicochemical composition of soils, geology and geographical location in order to identify potential environmental parameters that govern biodiversity of Archaea in the McMurdo Dry Valleys in the hope of determining a functional linkage of Archaea within the soil microbial communities.

References

Adams, B. J., R. D. Bardgett, E. Ayres, D. H. Wall, J. Aislabie, S. Bamforth, R. Bargagli, C. Cary, P. Cavacini, L. Connell, P. Convey, J. W. Fell, F. Frati, I. D. Hogg, K. K. Newsham, A. O'Donnell, N. Russell, R. D. Seppelt, and M. I. Stevens. 2006. Diversity and distribution of Victoria Land biota. Soil Biology & Biochemistry **38:**3003-3018.

Allen, M. A., F. M. Lauro, T. J. Williams, D. Burg, K. S. Siddiqui, D. De Francisci, K. W. Y. Chong, O. Pilak, H. H. Chew, M. Z. De Maere, L. Ting, M. Katrib, C. Ng, K. R. Sowers, M. Y. Galperin, I. J. Anderson, N. Ivanova, E. Dalin, M. Martinez, A. Lapidus, L. Hauser, M. Land, T. Thomas, and R. Cavicchioli. 2009. The genome sequence of the psychrophilic archaeon, Methanococcoides burtonii: the role of genome evolution in cold adaptation. ISME Journal **3:**1012-1035.

Angel, R., M. I. M. Soares, E. D. Ungar, and O. Gillor. 2010. Biogeography of soil Archaea and Bacteria along a steep precipitation gradient. ISME Journal **4:**553-563.

Ayton, J., J. Aislabie, G. M. Barker, D. Saul, and S. Turner. 2010. Crenarchaeota affiliated with group 1.1b are prevalent in coastal mineral soils of the Ross Sea region of Antarctica. Environmental Microbiology **12:**689-703.

Barns, S. M., R. E. Fundyga, M. W. Jeffries, and N. R. Pace. 1994. Remarkable archaeal diversity in a Yellowstone-National-Park hot-spring environment. Proceedings of the National Academy of Sciences of the United States of America **91:**1609-1613.

Barrett, J. E., R. A. Virginia, D. W. Hopkins, J. Aislabie, R. Bargagli, J. G. Bockheim, I. B. Campbell, W. B. Lyons, D. L. Moorhead, J. N. Nkem, R. S. Sletten, H. Steltzer, D. H. Wall, and M. D. Wallenstein. 2006. Terrestrial ecosystem processes of Victoria Land, Antarctica. Soil Biology & Biochemistry **38:**3019-3034.

Barrett, J. E., R. A. Virginia, W. B. Lyons, D. M. McKnight, J. C. Priscu, P. T. Doran, A. G. Fountain, D. H. Wall, and D. L. Moorhead. 2007. Biogeochemical stoichiometry of Antarctic Dry Valley ecosystems. Journal of Geophysical Research-Biogeosciences **112:**12.

Bartlett, K. B., and R. C. Harriss. 1993. Review and assessment of methane emissions from wetlands. Chemosphere **26:**261-320.

Bent, S. J., J. D. Pierson, and L. J. Forney. 2007. Measuring species richness based on microbial community fingerprints: The emperor has no clothes. Applied and Environmental Microbiology **73:**2399-2399.

Blochl, E., R. Rachel, S. Burggraf, D. Hafenbradl, H. W. Jannasch, and K. O. Stetter. 1997. Pyrolobus fumarii, gen. and sp. nov., represents a novel group of Archaea, extending the upper temperature limit for life to 113 degrees C. Extremophiles **1:**14-21.

Bockheim, J. G. 2002. Landform and soil development in the McMurdo Dry Valleys, Antarctica: a regional synthesis. Arctic, Antarctic and Alpine Research **34:**308-317.

Bockheim, J. G. 1997. Properties and classification of cold desert soils from Antarctica. Soil Science Society of America Journal **61:**224-231.

Borneman, J., and E. W. Triplett. 1997. Molecular microbial diversity in soils from eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology **63:**2647-2653.

Bowman, J. P., S. A. McCammon, J. A. E. Gibson, L. Robertson, and P. D. Nichols. 2003. Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Applied and Environmental Microbiology **69:**2448-2462.

Bowman, J. P., S. M. Rea, S. A. McCammon, and T. A. McMeekin. 2000. Diversity and community structure within anoxic sediment from marine salinity meromictic lakes and a coastal meromictic marine basin, Vestfold Hills, Eastern Antarctica. Environmental Microbiology **2:**227-237.

Brambilla, E., H. Hippe, A. Hagelstein, B. J. Tindall, and E. Stackebrandt. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles **5:**23-33.

Brandes, J. A., N. Z. Boctor, G. D. Cody, B. A. Cooper, R. M. Hazen, and H. S. Yoder. 1998. Abiotic nitrogen reduction on the early earth. Nature **395:**365-367.

Brazelton, W. J., M. L. Sogin, and J. A. Baross. 2010. Multiple scales of diversification within natural populations of Archaea in hydrothermal chimney biofilms. Environmental Microbiology Reports **2:**236-242.

Brim, H., H. Heuer, E. Krogerrecklenfort, M. Mergeay, and K. Smalla. 1999. Characterization of the bacterial community of a zinc-polluted soil. Canadian Journal of Microbiology **45:**326-338.

Brochier-Armanet, C., B. Boussau, S. Gribaldo, and P. Forterre. 2008. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nature Reviews Microbiology **6:**245-252.

Buckley, D. H., J. R. Graber, and T. M. Schmidt. 1998. Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. Applied and Environmental Microbiology **64:**4333-4339.

Burkins, M. B., R. A. Virginia, C. P. Chamberlain, and D. H. Wall. 2000. Origin and distribution of soil organic matter in Taylor Valley, Antarctica. Ecology **81:**2377-2391.

Burkins, M. B., R. A. Virginia, and D. H. Wall. 2001. Organic carbon cycling in Taylor Valley, Antarctica: quantifying soil reservoirs and soil respiration. Global Change Biology **7:**113-125.

Canfield, D. E., M. T. Rosing, and C. Bjerrum. 2006. Early anaerobic metabolisms. Philosophical Transactions of the Royal Society B-Biological Sciences **361:**1819-1834.

Cary, S. C., I. R. McDonald, J. E. Barrett, and D. A. Cowan. 2010. On the rocks: the microbiology of Antarctic Dry Valley soils. Nature Reviews Microbiology **8:**129-138.

Cavicchioli, R. 2006. Cold-adapted Archaea. Nature Reviews Microbiology **4:**331-343.

Chaban, B., S. Y. M. Ng, and K. F. Jarrell. 2006. Archaeal habitats - from the extreme to the ordinary. Canadian Journal of Microbiology **52:**73-116.

Chong, S. C., Y. T. Liu, M. Cummins, D. L. Valentine, and D. R. Boone. 2002. Methanogenium marinum sp nov., a H-2-using methanogen from Skan Bay, Alaska, and kinetics of H-2 utilization. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **81:**263-270.

Church, M. J., E. F. DeLong, H. W. Ducklow, M. B. Karner, C. M. Preston, and D. M. Karl. 2003. Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula. Limnology and Oceanography **48:**1893-1902.

Church, M. J., B. Wai, D. M. Karl, and E. F. DeLong. 2010. Abundances of crenarchaeal amoA genes and transcripts in the Pacific Ocean. Environmental Microbiology **12:**679-688.

Conrad, R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports **1:**285-292.

de la Torre, J. R., C. B. Walker, A. E. Ingalls, M. Konneke, and D. A. Stahl. 2008. Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. Environmental Microbiology **10:**810-818.

DeLong, E. F., C. M. Preston, T. Mincer, V. Rich, S. J. Hallam, N. U. Frigaard, A. Martinez, M. B. Sullivan, R. Edwards, B. R. Brito, S. W. Chisholm, and D. M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science **311:**496-503.

DeLong, E. F., L. T. Taylor, T. L. Marsh, and C. M. Preston. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Applied and Environmental Microbiology **65:**5554-5563.

DeLong, E. F., K. Y. Wu, B. B. Prezelin, and R. V. M. Jovine. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature **371:**695-697.

Deppenmeier, U., V. Muller, and G. Gottschalk. 1996. Pathways of energy conservation in methanogenic Archaea. Archives of Microbiology **165:**149-163.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2000. Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology **66:**2943-2950.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied and Environmental Microbiology **67:**190-197.

Elberling, B., E. G. Gregorich, D. W. Hopkins, A. D. Sparrow, P. Novis, and L. G. Greenfield. 2006. Distribution and dynamics of soil organic matter in an Antarctic Dry Valley. Soil Biology & Biochemistry **38:**3095-3106.

Feller, G., and C. Gerday. 2003. Psychrophilic enzymes: Hot topics in cold adaptation. Nature Reviews Microbiology **1:**200-208.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**.

Fisher, M. M., and E. W. Triplett. 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.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal **1:**19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing Archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America **102:**14683-14688.

Franzmann, P. D., Y. T. Liu, D. L. Balkwill, H. C. Aldrich, E. C. deMacario, and D. R. Boone. 1997. Methanogenium frigidum sp. nov., a psychrophilic, H-2-using methanogen from Ace Lake, Antarctica. International Journal of Systematic Bacteriology **47:**1068-1072.

Franzmann, P. D., N. Springer, W. Ludwig, E. C. Demacario, and M. Rohde. 1992. A Methanogenic archaeon from Ace Lake, Antarctica: Methanococcoides burtonii sp. nov. Systematic and Applied Microbiology **15:**573-581.

Frijlink, M. J., T. Abee, H. J. Laanbroek, W. Deboer, and W. N. Konings. 1992. The bioenergetics of ammonia and hydroxylamine oxidation in Nitrosomonas europaea at acid and alkaline pH. Archives of Microbiology **157:**194-199.

Galagan, J. E., C. Nusbaum, A. Roy, M. G. Endrizzi, P. Macdonald, W. FitzHugh, S. Calvo, R. Engels, S. Smirnov, D. Atnoor, A. Brown, N. Allen, J. Naylor, N. Stange-Thomann, K. DeArellano, R. Johnson, L. Linton, P. McEwan, K. McKernan, J. Talamas, A. Tirrell, W. J. Ye, A. Zimmer, R. D. Barber, I. Cann, D. E. Graham, D. A. Grahame, A. M. Guss, R. Hedderich, C. Ingram-Smith, H. C. Kuettner, J. A. Krzycki, J. A. Leigh, W. X. Li, J. F. Liu, B. Mukhopadhyay, J. N. Reeve, K. Smith, T. A. Springer, L. A. Umayam, O. White, R. H. White, E. C. de Macario, J. G. Ferry, K. F. Jarrell, H. Jing, A. J. L. Macario, I. Paulsen, M. Pritchett, K. R. Sowers, R. V. Swanson, S. H. Zinder, E. Lander, W. W. Metcalf, and B. Birren. 2002. The genome of M-acetivorans reveals extensive metabolic and physiological diversity. Genome Research **12:**532-542.

Galand, P. E., E. O. Casamayor, D. L. Kirchman, M. Potvin, and C. Lovejoy. 2009. Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. ISME Journal **3:**860-869.

Galand, P. E., E. O. Casamayor, D. L. Kirchman, M. Potvin, and C. Lovejoy. 2009. Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. ISME Journal **3:**1116-1116.

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

Ganzert, L., G. Jurgens, U. Munster, and D. Wagner. 2007. Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. FEMS Microbiology Ecology **59:**476-488.

Gibson, J. A. E., M. R. Miller, N. W. Davies, G. P. Neill, D. S. Nichols, and J. K. Volkman. 2005. Unsaturated diether lipids in the psychrotrophic archaeon Halorubrum lacusprofundi. Systematic and Applied Microbiology **28:**19-26.

Gillan, D. C., and B. Danis. 2007. The archaebacterial communities in Antarctic bathypelagic sediments. Deep-Sea Research Part Ii-Topical Studies in Oceanography **54:**1682-1690.

Glatz, R. E., P. W. Lepp, B. B. Ward, and C. A. Francis. 2006. Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-covered Lake Bonney, Antarctica. Geobiology **4:**53-67.

Hallam, S. J., T. J. Mincer, C. Schleper, C. M. Preston, K. Roberts, P. M. Richardson, and E. F. DeLong. 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. Plos Biology **4:**520-536.

Hatzenpichler, R., E. V. Lebedeva, E. Spieck, K. Stoecker, A. Richter, H. Daims, and M. Wagner.2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proceedings of the National Academy of Sciences of the United States of America **105:**2134-2139.

Herndl, G. J., T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler.2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Applied and Environmental Microbiology **71:**2303-2309.

Hewson, I., J. A. Steele, D. G. Capone, and J. A. Fuhrman. 2006. Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. Limnology and Oceanography **51:**1274-1283.

Hopkins, D. W., A. D. Sparrow, B. Elberling, E. G. Gregorich, P. M. Novis, L. G. Greenfield, and E. L. Tilston. 2006. Carbon, nitrogen and temperature controls on microbial activity in soils from an Antarctic Dry Valley. Soil Biology & Biochemistry **38:**3130-3140.

Hopkins, D. W., A. D. Sparrow, E. G. Gregorich, B. Elberling, P. Novis, F. Fraser, C. Scrimgeour, P. G. Dennis, W. Meier-Augenstein, and L. G. Greenfield. 2009. Isotopic evidence for the provenance and turnover of organic carbon by soil microorganisms in the Antarctic Dry Valleys. Environmental Microbiology **11:**597-608.

Hornibrook, E. R. C., F. J. Longstaffe, and W. S. Fyfe. 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. Geochimica Et Cosmochimica Acta **61:**745-753.

Huse, S. M., J. A. Huber, H. G. Morrison, M. L. Sogin, and D. Mark Welch. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biology **8**.

Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M. Lever, A. Lauer, M. Suzuki, K. Takai, M. Delwiche, F. S. Colwell, K. H. Nealson, K. Horikoshi, S. D'Hondt, and B. B. Jorgensen. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments, on the Pacific Ocean Margin. Proceedings of the National Academy of Sciences of the United States of America **103:**2815-2820.

Jahn, U., R. Summons, H. Sturt, E. Grosjean, and H. Huber. 2004. Composition of the lipids of Nanoarchaeum equitans and their origin from its host Ignicoccus sp strain KIN4/I. Archives of Microbiology **182:**404-413.

Jeanthon, C., S. L'Haridon, A. L. Reysenbach, E. Corre, M. Vernet, P. Messner, U. B. Sleytr, and D. Prieur. 1999. Methanococcus vulcanius sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise, and identification of Methanococcus sp. DSM 4213(T) as Methanococcus fervens sp. nov. International Journal of Systematic Bacteriology **49:**583-589.

Jin, T., T. Zhang, and Q. M. Yan. 2010. Characterization and quantification of ammonia-oxidizing Archaea (AOA) and Bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. Applied Microbiology and Biotechnology **87:**1167-1176.

Junge, K., H. Eicken, and J. W. Deming. 2004. Bacterial activity at -2 to -20 degrees C in Arctic wintertime sea ice. Applied and Environmental Microbiology **70:**550-557.

Kalanetra, K. M., N. Bano, and J. T. Hollibaugh. 2009. Ammonia-oxidizing Archaea in the Arctic Ocean and Antarctic coastal waters. Environmental Microbiology **11:**2434-2445.

Kappen, L., M. Breuer, and M. Bolter. 1991. Ecological and physiological investigations in continental Antarctic cryptogams: Photosynthetic production of Usnea sphacelatadiurnal courses, models, and the effect of photoinhibition. Polar Biology **11:**393-401.

Karcher, U., H. Schroder, E. Haslinger, G. Allmaier, R. Schreiner, F. Wieland, A. Haselbeck, and H. Konig.1993. Primary structure of the heterosaccharide of the surface glycoprotein of Methanothermus fervidus. Journal of Biological Chemistry **268:**26821-26826.

Karr, E. A., J. M. Ng, S. M. Belchik, W. M. Sattley, M. T. Madigan, and L. A. Achenbach. 2006. Biodiversity of methanogenic and other Archaea in the permanently frozen Lake Fryxell, Antarctica. Applied and Environmental Microbiology **72:**1663-1666.

Kendall, M. M., G. D. Wardlaw, C. F. Tang, A. S. Bonin, Y. T. Liu, and D. L. Valentine. 2007. Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of Methanogenium boonei sp nov. Applied and Environmental Microbiology **73:**407-414.

Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2007. Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. Limnology and Oceanography **52:**495-507.

Koenneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437:**543-546.

Kotsyurbenko, O. R., M. W. Friedrich, M. V. Simankova, A. N. Nozhevnikova, P. N. Golyshin, K. N. Timmis, and R. Conrad. 2007. Shift from acetoclastic to H-2-dependent methanogenes is in a West Siberian peat bog at low pH values and isolation of an acidophilic Methanobactetium strain. Applied and Environmental Microbiology **73:**2344-2348.

Kowalchuk, G. A., and J. R. Stephen. 2001. Ammonia-oxidizing Bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55:**485-529.

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

L'Haridon, S., V. Cilia, P. Messner, G. Raguenes, A. Gambacorta, U. B. Sleytr, D. Prieur, and C. Jeanthon. 1998. Desulfurobacterium thermolithotrophum gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. International Journal of Systematic Bacteriology **48:**701-711.

Lauro, F. M., K. Tran, A. Vezzi, N. Vitulo, G. Valle, and D. H. Bartlett. 2008. Large-scale transposon mutagenesis of Photobactetium profundum SS9 reveals new genetic loci important for growth at low temperature and high pressure. Journal of Bacteriology **190:**1699-1709.

Legendre, P., and L. Legendre. 1998. Ordination in reduced space, p. 387-480, Numerical Ecology (2nd English edition). Elsevier Science, Amsterdam, The Netherlands.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G. W. Nicol, J. I. Prosser, S. C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature **442:**806-809.

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

Liesack, W., and P. F. Dunfield. 2002. Biodiversity in soils: Use of molecular methods for its characterization, p. 528-544. *In* G. Bitton (ed.), Encyclopedia of Environmental Microbiology. Wiley & Sons Inc., New York.

Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology **63:**4516-4522.

Luna, G. M., K. Stumm, A. Pusceddu, and R. Danovaro. 2009. Archaeal diversity in deep-sea sediments estimated by means of different terminal-restriction fragment length polymorphisms (T-RFLP) protocols. Current Microbiology **59:**356-361.

Lynn, D. J., G. A. C. Singer, and D. A. Hickey. 2002. Synonymous codon usage is subject to selection in thermophilic Bacteria. Nucleic Acids Research **30:**4272-4277.

Mancinelli, R. L., and C. P. McKay. 1988. The evolution of nitrogen cycling. Origins of Life and Evolution of the Biosphere **18:**311-325.

Mardanov, A., V. Gumerov, A. Beletsky, E. Bonch-Osmolovskaya, N. Ravin, and K. Skryabin. 2010. Characteristic of biodiversity of thermophilic microbial community by parallel pyrosequencing method. Doklady Biochemistry and Biophysics **432:**110-113.

Marsh, T. L. 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:**323-327.

Massana, R., L. J. Taylor, A. E. Murray, K. Y. Wu, W. H. Jeffrey, and E. F. DeLong. 1998. Vertical distribution and temporal variation of marine planktonic Archaea in the Gerlache Strait, Antarctica, during early spring. Limnology and Oceanography **43:**607-617.

Moeseneder, M. M., C. Winter, J. M. Arrieta, and G. J. Herndl. 2001. Terminal-restriction fragment length polymorphism (T-RFLP) screening of a marine archaeal clone library to determine the different phylotypes. Journal of Microbiological Methods **44:**159-172.

Moorhead, D. L., P. T. Doran, A. G. Fountain, W. B. Lyons, D. M. McKnight, J. C. Priscu, R. A. Virginia, and D. H. Wall. 1999. Ecological legacies: Impacts on ecosystems of the McMurdo Dry Valleys. Bioscience **49:**1009-1019.

Morozova, D., and D. Wagner. 2007. Stress response of methanogenic Archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. FEMS Microbiology Ecology **61:**16-25.

Murray, A. E., and J. J. Grzymski. 2007. Diversity and genomics of Antarctic marine micro-organisms. Philosophical Transactions of the Royal Society B **362:**2259-2271.

Murray, A. E., C. M. Preston, R. Massana, L. T. Taylor, A. Blakis, K. Wu, and E. F. DeLong. 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Applied and Environmental Microbiology **64:**2585-2595.

Muyzer, G., E. C. Dewaal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal RNA. Applied and Environmental Microbiology **59:**695-700.

Narita, S., K. Kanamaru, S. Matsuyama, and H. Tokuda. 2003. A mutation in the membrane subunit of an ABC transporter LolCDE complex causing outer membrane localization of lipoproteins against their inner membrane-specific signals. Molecular Microbiology **49:**167-177.

Nichols, D. S., M. R. Miller, N. W. Davies, A. Goodchild, M. Raftery, and R. Cavicchioli. 2004. Cold adaptation in the Antarctic archaeon Methanococcoides burtonii involves membrane lipid unsaturation. Journal of Bacteriology **186:**8508-8515.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing Archaea and Bacteria. Environmental Microbiology **10:**2966-2978.

Nicol, G. W., and C. Schleper. 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? Trends in Microbiology **14:**207-212.

Niederberger, T. D., I. R. McDonald, and S. C. Cary. Archaeal microbial diversity in polar environments. *In* L. G. Whyte and M. R. Miller (ed.), Polar Microbiology. American Society of Microbiology Press. (in press)

Nishizawa, T., M. Komatsuzaki, N. Kaneko, and H. Ohta. 2008. Archaeal diversity of upland rice field soils assessed by the terminal restriction fragment length polymorphism method combined with real time quantitative-PCR and a clone library analysis. Microbes and Environments **23:**237-243.

Nkem, J. N., R. A. Virginia, J. E. Barrett, D. H. Wall, and G. Li. 2006. Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. Polar Biology **29:**643-651.

Novis, P. M., D. Whitehead, E. G. Gregorich, J. E. Hunt, A. D. Sparrow, D. W. Hopkins, B. Elberling, and L. G. Greenfield. 2007. Annual carbon fixation in terrestrial populations of Nostoc commune (Cyanobacteria) from an Antarctic Dry Valley is driven by temperature regime. Global Change Biology **13:**1224-1237.

Ochman, H., and A. C. Wilson. 1987. Evolution in Bacteria - Evidence for a universal substitution rate in cellular genomes. Journal of Molecular Evolution **26:**74-86.

Ochsenreiter, T., D. Selezi, A. Quaiser, L. Bonch-Osmolovskaya, and C. Schleper. 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology **5:**787-797.

Oros-Sichler, M., R. Costa, H. Heuer, and K. Small. 2007. Molecular fingerprinting techniques to analyze soil microbial communities, p. 355-386. *In* J. D. van Elsas, J. K. Jansson, and J. T. Trevors (ed.), Modern Soil Microbiology - second edition. CRC Press, Boca Raton, FL, USA.

Ouverney, C. C., and J. A. Fuhrman. 2000. Marine planktonic Archaea take up amino acids. Applied and Environmental Microbiology **66:**4829-+.

Pannewitz, S., T. G. A. Green, K. Maysek, M. Schlensog, R. Seppelt, L. G. Sancho, R. Turk, and B. Schroeter. 2005. Photosynthetic responses of three common mosses from continental Antarctica. Antarctic Science **17:**341-352.

Paramonov, N. A., L. A. S. Parolis, H. Parolis, I. F. Boan, J. Anton, and F. Rodriguez-Valera. 1998. The structure of the exocellular polysaccharide produced by the Archaeon Haloferax gibbonsii (ATCC 33959). Carbohydrate Research **309:**89-94.

Pearson, A., Z. Huang, A. E. Ingalls, C. S. Romanek, J. Wiegel, K. H. Freeman, R. H. Smittenberg, and C. L. Zhang. 2004. Nonmarine crenarchaeol in Nevada hot springs. Applied and Environmental Microbiology **70:**5229-5237.

Pearson, A., A. P. McNichol, B. C. Benitez-Nelson, J. M. Hayes, and T. I. Eglinton. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific Delta C-14 analysis. Geochimica Et Cosmochimica Acta **65:**3123-3137.

Poage, M. A., J. E. Barrettt, R. A. Virginia, and D. H. Wall. 2008. The influence of soil geochemistry on nematode distribution, McMurdo Dry Valleys, Antarctica. Arctic Antarctic and Alpine Research **40:**119-128.

Pointing, S. B., Y. Chan, D. C. Lacap, M. C. Y. Lau, J. A. Jurgens, and R. L. Farrell. 2009. Highly specialized microbial diversity in hyper-arid polar desert. Proceedings of the National Academy of Sciences of the United States of America **107:**1254-1254.

Preston, C. M., K. Y. Wu, T. F. Molinski, and E. F. DeLong. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: Cenarchaeum symbiosum gen nov, sp, nov. Proceedings of the National Academy of Sciences of the United States of America **93:**6241-6246.

Prosser, J. I. 2002. Molecular and functional diversity in soil microorganisms. Plant and Soil **244:**9-17.

Purdy, K. J., M. A. Munson, D. B. Nedwell, and T. M. Embley. 2002. Comparison of the molecular diversity of the methanogenic community at the brackish and marine ends of a UK estuary. FEMS Microbiology Ecology **39:**17-21.

Purdy, K. J., D. B. Nedwell, and T. M. Embley. 2003. Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Applied and Environmental Microbiology **69:**3181-3191.

Qian, P.-Y., Y. Wang, O. O. Lee, S. C. K. Lau, J. Yang, F. F. Lafi, A. Al-Suwailem, and T. Y. H. Wong. 2010. Vertical stratification of microbial communities in the Red Sea revealed by 16S rDNA pyrosequencing. ISME Journal.

Reid, I. N., W. B. Sparks, S. Lubnow, M. McGrath, and J. Livio Valenti. 2006. Terrestrial models for extraterrestrial life: Methanogens and halophiles at Martian temperatures. International Journal of Astrobiology **5:**89-97.

Rivkina, E., V. Shcherbakova, K. Laurinavichius, L. Petrovskaya, K. Krivushin, G. Kraev, S. Pecheritsina, and D. Gilichinsky. 2007. Biogeochemistry of methane and methanogenic Archaea in permafrost. FEMS Microbiology Ecology **61:**1-15.

Saitou, N., and M. Nei. 1987. The Neighbor-Joining Method - A new metahod for reconstructing phylogenetic trees. Molecular Biology and Evolution **4:**406-425.

Schulz, S., and R. Conrad. 1996. Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. FEMS Microbiology Ecology **20:**1-14.

Simankova, M. V., O. R. Kotsyurbenko, T. Lueders, A. N. Nozhevnikova, B. Wagner, R. Conrad, and M. W. Friedrich. 2003. Isolation and characterization of new strains of methanogens from cold terrestrial habitats. Systematic and Applied Microbiology **26:**312-318.

Singh, N., M. M. Kendall, Y. T. Liu, and D. R. Boone. 2005. Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: Description of Methanococcoides alaskense sp nov., and emended description of Methanosarcina baltica. International Journal of Systematic and Evolutionary Microbiology **55:**2531-2538.

Smalla, K., M. Oros-Sichler, A. Milling, H. Heuer, S. Baumgarte, R. Becker, G. Neuber, S. Kropf, A. Ulrich, and C. C. Tebbe. 2007. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? Journal of Microbiological Methods **69:**470-479.

Smit, E., P. Leeflang, and K. Wernars. 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:**249-261.

Smith, J. J., L. A. Tow, W. Stafford, C. Cary, and D. A. Cowan. 2006. Bacterial diversity in three different Antarctic cold desert mineral soils. Microbial Ecology **51:**413-421.

Spang, A., R. Hatzenpichler, C. Brochier-Armanet, T. Rattei, P. Tischler, E. Spieck, W. Streit, D. A. Stahl, M. Wagner, and C. Schleper.2010. Distinct gene set in two different lineages of ammonia-oxidizing Archaea supports the phylum Thaumarchaeota. Trends in Microbiology **18:**331-340.

Spear, J. R., H. A. Barton, C. E. Robertson, C. A. Francis, and N. R. Pace. 2007. Microbial community biofabrics in a geothermal mine adit. Applied and Environmental Microbiology **73:**6172-6180.

Stoica, E. 2009. Application of T-RFLP analysis to the study of the coastal Black Sea bacterioplankton. Romanian Biotechnological Letters **14:**4710-4719.

Striegl, R. G., T. A. McConnaughey, D. C. Thorstenson, E. P. Weeks, and J. C. Woodward. 1992. Consumption of atmospheric methane by desert soils. Nature **357:**145-147.

Takai, K., M. R. Mormile, J. P. McKinley, F. J. Brockman, W. E. Holben, W. P. Kovacik, and J. K. Fredrickson. 2003. Shifts in archaeal communities associated with lithological and geochemical variations in subsurface Cretaceous rock. Environmental Microbiology **5:**309-320.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution **24:**1596-1599.

Tamura, K., M. Nei, and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences **101:**11030-11035.

Taton, A., S. Grubisic, E. Brambilla, R. De Wit, and A. Wilmotte. 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.

Thauer, R. K., A. K. Kaster, H. Seedorf, W. Buckel, and R. Hedderich. 2008. Methanogenic Archaea: Ecologically relevant differences in energy conservation. Nature Reviews Microbiology **6:**579-591.

Tourna, M., T. E. Freitag, G. W. Nicol, and J. I. Prosser.2008. Growth, activity and temperature responses of ammonia-oxidizing Archaea and Bacteria in soil microcosms. Environmental Microbiology **10:**1357-1364.

Treusch, A. H., S. Leininger, A. Kletzin, S. C. Schuster, H. P. Klenk, and C. Schleper.2005. Novel genes for nitrite reductase and amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environmental Microbiology **7:**1985-1995.

Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Y. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers, and H. O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66-74.

von Klein, D., H. Arab, H. Volker, and M. Thomm. 2002. Methanosarcina baltica, sp nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. Extremophiles **6:**103-110.

Wang, F., X. Xiao, H. Y. Ou, Y. B. Gai, and F. P. Wang. 2009. Role and regulation of fatty acid biosynthesis in the response of Shewanella piezotolerans WP3 to different temperatures and pressures. Journal of Bacteriology **191:**2574-2584.

Weidler, G. W., M. Dornmayr-Pfaffenhuemer, F. W. Gerbl, W. Heinen, and H. Stan-Lotter. 2007. Communities of Archaea and Bacteria in a subsurface radioactive thermal spring in the Austrian Central Alps, and evidence of ammonia-oxidizing Crenarchaeota. Applied and Environmental Microbiology **73:**259-270.

Widmer, F., B. T. Shaffer, L. A. Porteous, and R. J. Seidler. 1999. Analysis of nifH gene pool complexity in soil and litter at a Douglas fir forest site in the Oregon Cascade Mountain Range. Applied and Environmental Microbiology **65:**374-380.

Woese, C. R., O. Kandler, and M. L. Wheelis. 1990. Towards a natural sysytem of organisms - Proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences of the United States of America **87:**4576-4579.

Wuchter, C., S. Schouten, H. T. S. Boschker, and J. S. S. Damste.2003. Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiology Letters **219:**203-207.

Yergeau, E., S. A. Schoondermark-Stolk, E. L. Brodie, S. Dejean, T. Z. DeSantis, O. Goncalves, Y. M. Piceno, G. L. Andersen, and G. A. Kowalchuk. 2009. Environmental microarray analyses of Antarctic soil microbial communities. ISME Journal **3:**340-351.

Zhilina, T. N., and G. A. Zavarzin. 1991. Low- temperature methane production by pure culture of Methanosarcina sp. Doklady Akademii Nauk Sssr **317:**1242-1245.

Chapter 2: Influences of soil properties on archaeal diversity in the Antarctic Dry Valleys

2.1 Abstract

The Antarctic Dry Valleys are characterized by extremely low temperatures, arid conditions, high salinity and the virtual absence of plants. Food webs of these microbially dominated soils are among the simplest on earth making these mineral soils a perfect model to study microbial biogeography. This study aims to characterize the distribution and diversity of Archaea within the Dry Valleys as part of the New Zealand Terrestrial Antarctic Biocomplexity Survey (NZTABS):- an international multidisciplinary organisation focusing on biotic organisms, community structure and their functional linkage to determine which environmental factors drive biocomplexity. Archaea are so far the least known members of the microbial community in Antarctic soils with only a few successful attempts at detection that indicate patchy distribution and extremely low diversity. A wide range of soil samples, collected from various sites within the Dry Valleys, were analyzed using a suite of genetic approaches. DNA fingerprinting techniques (RFLP, T- RFLP) were applied to examine distribution and diversity of archaeal species living in soils of Miers Valley, Marshall Valley, Garwood Valley and the Shangri- La region. Detailed analysis of physicochemical differences between mineral soils was undertaken to determine what environmental factors drive distribution and biodiversity of archaeal communities in these soils. Multivariate statistical analysis and ordination of T- RFLP results and physicochemical data suggest a widespread distribution of Archaea across all four valleys. Overall, archaeal diversity was relatively low (average of five OTUs per sample site) and most of the archaeal communities were composed of only species affiliated with Crenarchaeota Marine Group 1.1b. However, relatively high archaeal diversity was identified in several samples revealing a restriction of diverse archaeal communities to high elevation ridge areas and coastal moraines. This variation in diversity may be

explained by differences in availability of moisture, carbon and nitrogen in mineral soils that harbour these communities, suggesting a negative correlation between archaeal diversity and soil carbon concentrations. Congruently, organic rich soils that harbour high bacterial diversity and primary producers, revealed extremely low abundance of Archaea, possibly even total absence of Archaea in these organic rich soils.

2.2 Introduction

The Antarctic continent is one of the harshest and most extreme environments on earth to be inhabited by microorganisms. Almost the entire continent is permanently ice- covered with a limited number of ice- free areas discontinuously distributed around the coastal margins (Cary, et al. 2010).

At 6692km^2 the McMurdo Dry Valleys, located between the Polar Plateau and the western coast of the Ross Sea, Southern Victoria Land (Figure 8), constitute the largest ice- free region on the Antarctic continent, representing over 15% of the ice- free land in Antarctica (Cary, et al. 2010). The absence of a permanent ice cover is primarily due to the presence of the Royal Society Range north west of the valleys which obstruct the glacial flow from the East Antarctic ice sheet (Andersen, et al. 1992; Pewe 1960). Because outlet glaciers from the East Antarctic are literally absent, the Dry Valleys were primarily shaped by Quaternary ice sheets grounded in the Ross Sea. At least four major glacial advances of the McMurdo icecap during Late Wisconsin have been reported (Denton, et al. 2004). Each of these westward flowing ice fluctuations was successively less extensive than the former (Brook, et al. 1995; Denton, et al. 2004) resulting in different types of deposits and landforms within each valley, i.e., different types of glacial push- moraines. In brief, moraine 3 is located around the coastal margins in each Dry Valley and is the result of the youngest glaciation, namely ‘Ross Sea Drift’ (Brook, et al. 1995). ‘Ross Sea Drift’ deposits are composed of till and stratified sediments made up of clasts of dark, volcanic rocks derived from the McMurdo Volcanic Group. Estimated exposure ages of these young deposits range from 8 to

106 kyr (Brook, et al. 1995). Older glacial deposits, like moraine 2 and 1 are located inland on the valley floor and on ridge areas due to farther extension of the McMurdo ice sheet during previous glaciation events. For example, deposits of the 'Marshall Drift' are located along the coastal foothills and have estimated exposure ages of 130 to 190 kyr (Denton, et al. 2004), whereas deposits of the relatively old 'McMurdo Glaciation' (Pewe 1958) can be found on ridges up to 550 m high. These 'McMurdo' deposits are composed mainly of red porphyritic rocks and wind planed granite boulders (Pewe 1960). In addition to glacial push- moraines, soils of the Dry Valleys are also characterized by calcareous sandy aeolian and fluvial sediments and bedrock dominated by dolomite, granite and metamorphosed rocks (Elberling, et al. 2006).

Classified as a hyper- arid desert, the Dry Valley soils are the driest, coldest ecosystems on Earth (Stonehouse 2002). Due to these extreme conditions, snowfall is sublimated almost immediately resulting in minimal liquid- water input to soils (Doran, et al. 2002; Wynn-Williams 1990), causing extremely low bioavailability of water. Soils are characterized by large variations in temperature (ranging from -30 to 25℃) (Aislabie, et al. 2006; Doran, et al. 2002), high levels of salinity (Bockheim 1997), high incidences of solar radiation (Smith, et al. 1992) and low nutrient availability (Vishniac 1993). Additionally, the effects of widely fluctuating physiochemical conditions and steep chemical gradients (Poage, et al. 2008) combine to create a harsh environment for all resident biota. Consequently only a few plants and animals are able to survive. Vascular plants and vertebrates are virtually absent, however, microbes are widely distributed dominating almost the entire soil food web (Adams, et al. 2006).

Bacteria have been well documented within these soil microbial communities (Smith, et al. 2006; Wynn-Williams 1990; Yergeau, et al. 2009). Although a surprisingly high bacterial diversity has been reported (Cary, et al. 2010; Smith, et al. 2006) little is currently known about the presence, diversity and community composition of members of the Archaea within Antarctic soils. In contrast, distribution of Archaea in temperate environments has been the subject to numerous molecular

studies revealing a widespread occurrence of both Crenarchaeota and Euryarchaeota (Bintrim, et al. 1997; Buckley, et al. 1998; Jurgens, et al. 1997; Nicol and Schleper 2006; Ochsenreiter, et al. 2003). Members of the Crenarchaeota are most commonly detected in temperate soils where they account for up to 5% of the total soil prokaryotic community (Buckley, et al. 1998; Ochsenreiter, et al. 2003). Despite their high abundance the Crenarchaeota phylum as a whole is relatively homogeneous compared to Euryarchaeota. Comparisons based on genome size, gene number and metabolic diversity indicate a much higher diversity within the Euryarchaeota then in the Crenarchaeota. Although members of the Crenarchaeota detected in soils tend to be restricted to a few lineages, namely Marine group 1.1b and 1.1c (Jurgens, et al. 1997; Nicol, et al. 2006; Ochsenreiter, et al. 2003), a worldwide distribution is assumed due to their detection in most soil environments including agricultural soils, high and low nutrient grassland soils, woodland soils, limestone soil, soil overlying permafrost and soils developing in deglaciated environments (Nicol, et al. 2006; Nicol, et al. 2005; Ochsenreiter, et al. 2003). However, previous molecular studies failed to detect Archaea in Antarctic soils including soils from the western Antarctic Peninsula (Yergeau, et al. 2009) and from McKelvey Valley in the McMurdo Dry Valleys (Pointing, et al. 2009). The only evidence that Archaea inhabit soils of the Dry Valleys has been recently reported by Ayton and colleagues (2010) indicating a patchy distribution, low concentration and low diversity of Archaea in Antarctic soils.

Due to the low abundance of Archaea in Antarctic soils, detection of Archaea in these soils is very challenging. There are two established strategies to investigate microbial diversity in ecosystems', culture- dependent and culture- independent methods (Leuko, et al. 2007). Most Archaea are typically culture recalcitrant and only certain community representatives can be successfully isolated due to difficulties in mimicking actual environmental growth conditions (Babalola, et al. 2009). Therefore, molecular based culture- independent studies, based on PCR, are widely used to assess archaeal diversity in natural environments (Leuko, et al. 2007). PCR based community fingerprinting techniques such as terminal

restriction fragment length polymorphism (T- RFLP) (Liu, et al. 1997; Marsh 1999) provide a rapid initial appraisal of microbial diversity in complex environments (Leuko, et al. 2007). T- RFLP has been proven to be a fast, sensitive and reliable method to analyse archaeal communities in numerous environments such as marine waters and sediments (Luna, et al. 2009; Moeseneder, et al. 2001), hyper- saline lakes (Stoica 2009), desert and forest soils (Angel, et al. 2010), agricultural pasture (Nishizawa, et al. 2008) and even in Cretaceous rock and sandstone (Takai, et al. 2003).

Although archaeal diversity in Antarctic soils is still poorly understood, these extreme ecosystems are a perfect model to study archaeal distribution and diversity in conjunction with environmental factors as drivers of diversity. Due to the virtual absence of higher trophic food levels the structure of the soil biotic communities is simple, typically being dominated by microorganisms with only a few metazoan invertebrate taxa present (Adams, et al. 2006). Thus, the Dry Valley soils are one of the few examples where abiotic factors (e.g. moisture, pH or conductivity) have a stronger influence on diversity than biotic factors (e.g. competition, herbivory, predation) (Convey 1996; Hogg, et al. 2006; Pointing, et al. 2009). This means that physical processes and soil biogeochemistry tend to dominate over ecosystem processes (Fountain, et al. 1999), thus influencing the spatial variability of biotic communities in Dry Valley soil ecosystems (Barrett, et al. 2004). For example, temperature, moisture, soil organic carbon and salinity have been suggested to be the main drivers of invertebrate species distribution (Ball, et al. 2009; Barrett, et al. 2004; Moorhead, et al. 1999). This makes the Dry Valley soils a perfect model system to investigate the relationships between biodiversity and ecosystem function which is the main focus of the New Zealand Terrestrial Antarctic Biocomplexity Survey (NZTABS). This study aims to characterize the distribution and diversity of Archaea within the southern part of the Dry Valley system as part of NZTABS. Archaeal distribution and diversity in mineral soils, assessed using T- RFLP, was analyzed in conjunction with physiochemical soil properties

in order to elucidate influences of environmental factors on archaeal community composition and structure.

2.3 Materials and Methods

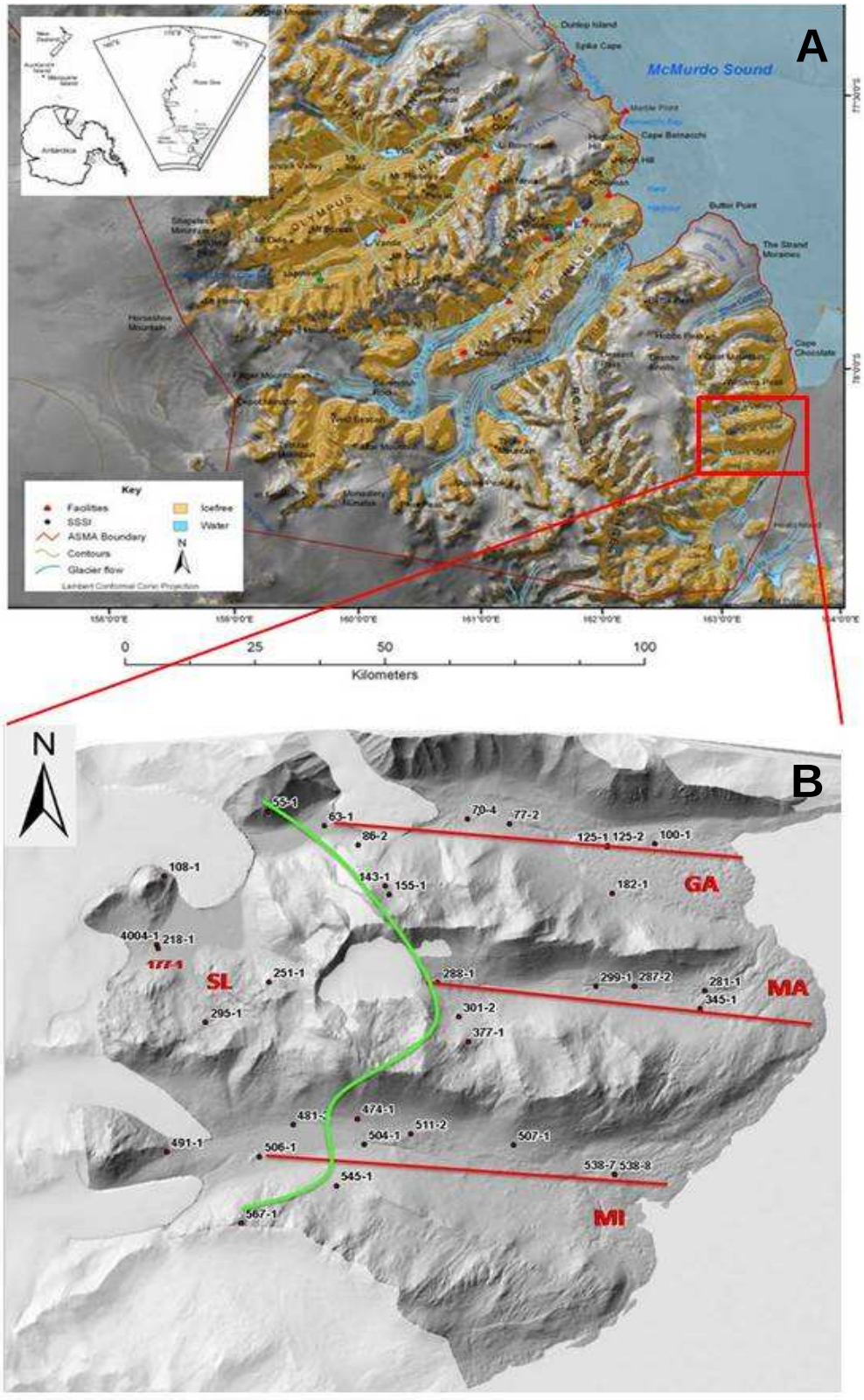


Figure 8. A. Map of the McMurdo Dry Valleys showing our study site (red rectangle). **B**. Location of sample points in Miers Valley (MI), Marshall Valley (MA), Garwood Valley (GA) and Shangri- La (SL). Red lines show moraine transects for each valley and green line shows ridge transect across all three valleys.

2.3.1 Soil geomorphology and sample collection

The geographical attributes of the landscape were derived from the Light Detection and Ranging (LIDAR) dataset freely available online from the U.S. Geological Survey (USGS, www.usgs.gov/). LIDAR is an optical remote sensing technology that measures properties of scattered light to find range and/or other information of each individual sampling location including: aspect, elevation and slope of the sample site.

Basic geology and geomorphology was interpreted using available geological maps and expert interpretation of landforms made during the fieldwork as part of the NZTABS program. All analyses were conducted using the ArcInfo Geographic Information System (GIS) software (Esri, www.esri.com/).

Predicted geographical and geomorphic attributes, such as geology, aspect, elevation and slope were verified on- site at each individual sample location, and changed if necessary, prior to sampling.

Mineral soils were collected from four different valleys in the McMurdo Dry Valleys (Figure 8) located between the Royal Society Range (78°10'S 162°40'E) and the McMurdo Sounds at the lo be of the Koettlitz Glacier: Miers Valley (78°6 ′S 164°0 ′E), Marshall Valley (78°4'S 164°10'E) and Garwood Valley (78°2'S 164°10'E). Shangri- La, an elevated plain located in the north west of Miers Valley and Marshall Valley was also included in the study area. For inter- and intra- valley comparisons, four sampling transects were established (Figure 8). One set of transects were laid across the valley floor running from the coast up to the inland glaciers at the end of each valley (east to west) covering all three types of moraines (moraine 1- 3). The second set of transects spanned across the granite ridges of all three valleys (south to north, Figure 8). A third set of alluvial soil samples was taken from the shores of Lake Miers (Miers Valley), Lake Colleen (Garwood Valley), Lake Buddha (Shangri- La) and a pond in the upper Marshall Valley. In addition four surface samples were collected from beneath a mummified seal located in Miers Valley. One sample was taken from underneath the seal at its original position (OPS1 78°5' 606''S 163°51'368''E) before the seal was mov ed to a new location.

After one year three more samples were collected from underneath the seal at its new location (NPS1-3 78℃5'592''S 163℃5' 137''E).

A total of 34 samples were collected from the top 2 cm of soil in 42 oz stand- up Whirlpak® (Fisher Scientific Ltd., Ontario, Canada) with a sterile spatula. After collecting about 400 g of soil, large stones and pebbles were removed, and the Whirlpak was sealed and shaken to homogenize the sample. Samples were kept at -20℃ until further analysis.

2.3.2 Physicochemical soil analysis

Conductivity and pH were measured in the field using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd, Singapore) using the slurry technique. Briefly, soil samples were dissolved in de- ionized water (mass ratio 1:2.5) prior to measurements (modified from Edmeades et al. 1985).

ATP content was determined in the field using the 3M™ Clean- Trace™ Beverage Test Kit (3M Center, St. Paul, MN, USA) by incubating 100 mg of soil in 100 µl ATP extractant buffer for 1 min. After incubation 75 µl of reconstituted enzyme was added and ATP content (in relative fluorescent units) was measured using the 3M™ Clean-Trace™ NG Luminometer (3M Center, St. Paul, MN, USA).

Moisture content of soils was measured gravimetrically by drying 40 g of each soil sample at 105℃ until samples reached a consistent weight (Soo, et al. 2009). The maximal weight loss is used to calculate water content as % per g soil.

To determine total percentage of soil carbon and nitrogen each soil sample was air dried and ground in a ball mill. 75 mg of dry soil were analyzed on a Carlo Erba 1500 elemental analyzer in the Dartmouth College Environmental Measurements Laboratory (Barrett, et al. 2006).

2.3.3 Extraction of genomic DNA

DNA was extracted from the soil using the CTAB/bead- beating extraction method modified from Niederberger et al. (2008). Briefly, soil samples (0.7 g) were measured into screw- capped tubes containing 0.5 g each of 0.1 mm and 2.5 mm silica- zirconia beads. 300 µl phosphate buffer (100 mM NaH_2PO_4) and 300 µl SDS lysis buffer (100 mM NaCl, 500 mM Tris, pH 8.0, 10% SDS) were added and incubated on a vertical shaker for 10 min at 4.2 ms^{-1} (Vortex-Genie®2, MO BIO Laboratories Inc, Carlsbad, CA) at room temperature. The aqueous phase was transferred to a new tube and centrifuged at 16,000 x g for 3 min. Then, 200 µl cetyltrimethylammonium bromide (CTAB) containing 0.4% (v/v) β- mercaptoethanol was added and incubated at 60℃ o n a rocking bed (500 rpm) for 30 min. Nucleic acids were extracted by adding 350 µl chloroform- isoamyl alcohol (24:1) followed by thoroughly mixing. After centrifugation at 16.000 x g for 5 min the aqueous phase, containing nucleic acids was transferred into a fresh tube and nucleic acid extraction was repeated by adding an equal volume of chloroform- isoamyl alcohol 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 x g) for 5 min. Finally, 0.54 volumes of isopropyl alcohol was added to the supernatant and incubated overnight at -20℃. The precipitated nucleic acids were centrifuged (16.000 x g) for 20 min. After the supernatants were discarded the precipitated nucleic acids were washed in 70% ethanol (v/v) and air dried prior to re- suspension in 20 µl of ultrapure TE (10 mM Tris, 1 mM EDTA). Following quantification using a NanoDrop ™ 1000 Spectrophotometer (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA), DNA was stored at -20℃ until analysis.

DNA was also extracted using a Robotics 4 x- tractor (Corbett Robotics, Bio-Strategy, Queensland, Australia), initially the CTAB/ bead- beating extraction method described above was followed. After the first DNA extraction step using chloroform- isoamyl alcohol (24:1) nucleic acids were extracted from the upper aqueous layer using the Robotics 4 x- tractor following the manufactures manual.

2.3.4 Archaeal T- RFLP

Archaea- specific primers were selected after a virtual "*in- silico*" test against all archaeal 16S rRNA gene sequences (261) from isolates available in the RDP database (http://rdp.cme.msu.edu/). Primers used for PCR were A92F (5'- ACGGCTCAGTAACRC-3') (Buckley, et al. 1998) and A836R (5'- GTGCTCCCCCGCCAATTCCT-3') (Stahl and Amann 1991) which amplify a 794 bp product of the 16S rRNA gene. The forward primer A92F was labeled at the 5' end with the fluorescent dye FAM. The 25 µl PCR reactions contained 200 nM of the forward primer A92F, 100 nM of the reverse primer A836R, 3 mM $MgCl_2$ (Invitrogen Ltd, New Zealand), 1x Platinum Taq PCR buffer (Invitrogen Ltd), 0.04 U of Platinum Taq DNA polymerase (Invitrogen Ltd), 32 µg/ml BSA, 200 µM of each dNTP (Roche Diagnostics, New Zealand), 10 ng of extracted template DNA and UltraPure™ distilled water (Invitrogen Ltd) to 25 µl. To exclude contamination the master mix, containing all reagents except the fluorescently labeled forward primer was treated with 0.1 µg/µl ethidium monoazide bromide (Biotium Inc. Hayward, CA) by incubation in the dark for 1 min followed by exposure to high wattage light for 1 min (Rueckert and Morgan 2007). Negative controls, containing the PCR mixture but no DNA template, were run during each amplification. Positive controls contained 100 pg DNA of an archaeal 16S gene, which was extracted and sequenced from Antarctic soil. Amplification was performed using an initial denaturation step at 94℃ for 2 min followed by 35 cycles of denaturation at 94℃ for 20 sec, annealing at 56℃ for 10 sec, a nd an extension at 72℃ for 40 sec. Cycling was completed by a final extension at 72℃ for 5 min. All PCR reactions were run on a Bio- Rad DNA Engine® (PTC- 200) Peltier Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, CA).

For each sample, three individual PCR reactions were run and visualized on a 1% agarose gel to minimize stochastic PCR biases. Triplicate PCR reactions were subsequently pooled together and purified using the UltraClean™ 15 DNA Purification Kit (MO BIO Laboratories Inc, Carlsbad, CA). Purified T- RFLP PCR products were quantified with the Qubit® fluorometer using the Quant-iT™ dsDNA HS Assay Kit (Invitrogen Ltd). All samples were measured in duplicates. Briefly, fluorescence of

FAM- labeled samples was estimated prior to measurements containing the fluorescent quantification dye.

To increase detection sensitivity, seal samples were analyzed using nested PCR. Briefly, cleaned PCR products amplified from the seal samples using primer pair A92F/A836R were used as template for a second PCR reaction containing 200 nM of primers A324F (5'-CGGGGYGCASCAGGCGCGAA- 3') (Kemnitz, et al. 2005) and A703R (5'-GGACTACVSGGGTATCTAAT- 3') (Takai and Horikoshi 2000). Both primers bind inside of the primer pair FAMA92F/A836R used during the first PCR reaction, thus resulting in a shorter amplification product (420 bp). The PCR reactions and cycling conditions were identical to those described above except of a change in the annealing temperature to 55℃.

The restriction enzyme *Msp*I was selected from a number of 4 bp cutters after a virtual "*in- silico*" T- RFLP run on an in- house program modified from Abdo et al. (2006). 16S rRNA gene sequences of 261 archaeal isolates were cut back to the size of the expected PCR product (794 bp), including primer sites, prior to sequence alignment using ClustalW (Thompson, et al. 1994) using a delay divergent cutoff of 30%. This diverse set of sequences was then virtually restricted with *Alu*I, *Hae*III, *Rsa*I, *Msp*I and *Hha*I based on previous studies (Luna, et al. 2009; Moeseneder, et al. 2001). A random sequence was used as negative control. The number of unique forward restriction fragments, digested by each enzyme, is indicated as frequency in percent of all sequences blotted against fragment size (Appendix B). 100 ng of the purified amplicons were digested at 37℃ for 3 h in 20 µl reactions containing 10- 20 U of *Msp*I, 1x Buffer 4 (both Thermo Fisher Scientific Inc., New Zealand) and UltraPure™ distilled water (Invitrogen Ltd). The enzyme was heat inactivated by incubation at 65℃ for 20 min. All reactions were stored at -20℃ until further analysis.

2 µl of the diluted (1 in 10 diluted in UltraPure™ distilled water, Invitrogen Ltd) restriction digest were denatured in the presence of 17.75 µl Hi- Di™ Formamide at 95℃ for 4 min and then chilled down to 4℃. The samples were loaded onto an ABI 3130 xl sequencer (PE

Applied Biosystems, Foster City, USA) and run under GeneScan mode at 15 kV for 45 min according to the manufactures manual. Each sample contained 0.25 µl of the internal GS1200LIZ ZyStandard (PE Applied Biosystems) to determine the size of fluorescently labeled fragments during analysis. Briefly, this standard contains 68 single- stranded DNA fragments of various sizes ranging from 20 to 1200 bp. Each fragment is labeled with the fluorescent dye LIZ® which allows for detection in a given lane. The detected fragments are used to generate a sizing curve which is subsequently used to estimate fragment sizes within samples.

Data obtained from genotyping was analyzed and interpreted using a range of computational and statistical approaches. T- RFLP electropherograms were processed using the PeakScanner™ software v1.0 (PE Applied Biosystems) as well as an in- house pipeline modified from Abdo et al. (2006) written using Python 2.7.1 (© Copyright 1990-2011, Python Software Foundation) and R (http://www.r-project.org). Analysis was performed using a size cut- off for peaks, where all peaks shorter than 30 bp (minimum length) and longer than 750 bp (maximum length) were excluded from analysis. To distinguish peaks from noise, all signals above 20 relative fluorescent units (RFU) were considered peaks (signal minimum) and peaks below 100 RFU were considered noise (noise maximum). Peaks between these two intensity cut- offs were modelled using the log- normal to test whether they represent noise or signal (alpha value). The smaller the alpha the less peaks are recovered from noise whereas a higher alpha allows for more peaks to be pulled out of the noise. Results were obtained in the form of a table with m rows and n columns where samples are represented in rows (n) and peak lengths in columns (m). Each entry therefore is the relative abundance of a given peak in a given sample.

Dataset 3 (30bp to 750bp long, α 0.001, noise max 100, Table 8) was used to test for significant correlation between two different digestion protocols (10 U versus 20 U enzyme) based on peak number within samples. The non- parametric Mann- Whitney- Test (Mann and Whitney 1947) indicated a significant difference (p value 0.0526) between fully digested (20 U of enzyme) and partially digested (10 U of enzyme)

samples thus only completely digested samples were used for statistical clustering analysis.

Dataset 1 to 8 (Table 8) was subjected to two different tests of independence, namely Chi- square (χ^2) and Pearson Product Moment Correlation. To test for independence, peak frequencies given in matrices from T- RFLP analysis (see above) were subjected to an χ^2 test as implemented in R (chi.sq(…)), assuming that peak frequencies are randomly distributed with no significant correlations between samples (null hypothesis). This null distribution is based on mutual absence and mutual presence of peaks, peaks present in one sample absent in the other and peaks absent in one sample present in the other. To estimate the χ^2 value between two samples, the observed peak frequencies in each sample are calculated which are used to calculate the expected frequencies for each possible category (mutual absence and mutual presence of peaks, peaks present in one sample absent in the other and peaks absent in one sample present in the other). For each category an individual χ^2 statistic is obtained by subtracting the observed peak frequency from the expected. The resulting value is squared before dividing it by expected peak frequency. The resulting χ^2 values for each category are added up to obtain the test χ^2 which is used to estimate significance (ρ- value) of the null hypothesis using the Chi- square distribution. P- value calculation was done in R using Monte Carlo simulation. The number of replicates (B) used in the Monte Carlo simulations was set to 5/Bonferroni where Bonferroni is the adjusted p- value for multiple testing (see below). This ensures that there are adequate counts to calculate valid p- values if the data follow the null distribution.

The significance of pairwise correlations between the abundances of mutually present peaks in each pair of samples was tested using the two- sided Pearson Product Moment Correlation as implemented in R (cor.test(…)). A significant result indicates that a correlation does exist between the relative abundances of organisms found in two samples and thus suggests similar community structure. Pearson's correlation reflects the degree of a linear relationship between two variables, in this case peak abundance in samples.

The tests of independence were used to test for independence between samples and between peaks (peak size) in all datasets (Table 8). Clusters were graphed and lines were drawn between samples using Graphviz (Graph Visualization Software, www.graphviz.org) to visualize different levels of similarity based on Bonferroni corrected α values (Figure 13) where an alpha of 0.05 (black line) indicates significant, strong similarities between samples and an alpha of 0.1 (blue line) a less stringent connection. An alpha of 0.25 (green line) was used as cut- off where all connections with alpha > 0.25 were considered as “un- true” and excluded from further analysis.

To visualize relative similarities between T- RFLP profiles non- metric multi- dimensional scaling (MDS) was undertaken using the PRIMER 6 software package (PRIMER-E Ltd., UK). Peak abundances in samples were transformed using the presence/absence model prior to generating a Bray- Curtis similarity matrix. This resemblance matrix is represented in low- dimensional space (usually 2- d) in ordination plots. The relative distance between sites in these ordination plots indicates the relative similarity of T- RFLP profiles (i.e., community structure). Plots with a stress value less than 0.20 provide interpretable information (Clarke and Ainsworth 1993). Non- metric multi- dimensional scaling analysis was undertaken with 50 random restarts and results were plotted in two dimensions.

A Pearson correlation matrix was used to assess co- variance among soil variables prior to principal- component analysis (PCA) using the PRIMER 6 software package. Pearson’s correlation was visualized in pairwise scatter plots between all variables (Draftsman Plot). Variables showing no linear relationship or a heavily skewed distribution (to the left or to the right) were submitted to transformation (log(10) or square root) and normalisation prior to PCA analysis which was undertaken with a maximum number of five PC’s (principal components). 2- d or 3- d plots of any combination of these PC’s are shown in PCA ordinations. Samples, regarded as points in the high- dimensional variable space are projected onto a ‘best- fitting’ plane (low- dimensional solution). These plots can be

used to characterize distribution of Archaea across soil biogeochemical gradients.

The influence of physicochemical variables on the community structure of the soil samples was assessed using the BEST (Bio-Environment Stepwise) analysis in PRIMER 6. 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 geochemical variables yielding the highest ρ, are the most correlated with archaeal diversity.

2.3.5 PCR, cloning and sequencing

The PCR reactions and cycling conditions were identical to those described above. Following PCR, additional poly- (A) homomers were added to the 3' end of PCR products by incubating the 20 µl reactions, containing 320 ng of PCR product, 1x Platinum Taq PCR buffer, 0.03 U of Platinum Taq DNA polymerase and 1.25 µmol dATP, at 72℃ for 15 min.

40 ng PCR product was used in cloning reactions using the TOPO TA Cloning® Kit for Sequencing (Invitrogen Ltd, New Zealand) following the recommendations of the manufacturer. Insert- containing colonies were selected and directly used in a colony PCR protocol for clone identification.

For colony PCR for clone identification, 25 µl reactions contained 200 nM of primers, M13f (5'- GTAAAACGACGGCCAG- 3') and M13r (5'- CAGGAAACAGCTATGAC- 3') (Invitrogen Ltd, New Zealand), 3 mM $MgCl_2$, 1x Platinum Taq PCR buffer, 0.04 U of Platinum Taq DNA polymerase, 32 µg/ml BSA, 200 µM of each dNTP, UltraPure™ distilled water to 25 µl and the DNA template from a single picked colony. 20 colonies were picked and re- suspended directly in the PCR mixture after streaking onto a fresh LB plate containing 50 µg/ml kanamycin (Invitrogen Ltd, New Zealand). Negative controls, containing the PCR mixture but no DNA template, were run during each PCR. Positive controls

contained 10 pg of the plasmid pUC19 (Invitrogen Ltd, New Zealand). Amplification was performed using an initial denaturation step at 94℃ for 3 min followed by 30 cycles of denaturation at 94℃ for 30 sec, annealing at 55℃ for 1 min, and an extension at 72℃ for 2 m in. Cycling was completed by a final extension at 72℃ for 7 min.

All PCR reactions were run and visualized on a 1% agarose gel to identify positive clones containing the correct size insert based on the size of the expected amplicon (950 bp). The PCR reactions of 20 selected positive clones were then gel purified using the MO BIO UltraClean™ 15 DNA Purification Kit and again visualized on a 1% agarose gel. Purified amplicons were quantified using a NanoDropTM 1000 Spectrophotometer.

The PCR products generated from the clone library were screened for similarity by standard RFLP analysis. Restriction digests contained between 32 and 110 ng of plasmid DNA, 1 U *Alu*I, 1x SuRE/Cut Buffer A (both Roche Diagnostics, New Zealand) and UltraPure™ distilled water (Invitrogen Ltd) to 12.5 µl. After incubation at 37℃ for 3 hours, the enzyme was heat inactivated at 60℃ for 20 min. Restriction digests were visualized on a 2% agarose gel. Clones with unique restriction patterns, were selected for DNA sequencing on a capillary sequencer (PE Applied Biosystems). Sequencing was performed bidirectional using the primer pair M13 forward and reverse. Partial 16S rRNA sequences were matched to those in GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST (June 2010). The analysis was run using the search algorithm blastn and the default algorithm parameters were used except for word size, which was changed to seven.

2.3.6 Phylogenetic analysis

All phylogenetic analyses were performed using MEGA 4 (Molecular Evolutionary Genetics Analysis software version 4.0, Tamura et al. 2007). The evolutionary history was inferred using the Neighbour-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method. All positions containing

alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). Bootstrap values were calculated using 1000 replicates. Archaeal sequences are shown in Appendix H.

2.4 Results

2.4.1 Soil characteristics

Physical and chemical properties of soil samples from all four valleys are shown in Table 1. Common features of soils from the Dry Valleys are very low water content (< 0.9%), low concentration of carbon (0.08%) and nitrogen (0.01%, Table 2). Extremely low water availability was recorded for samples from Miers Valley (0.77%). All mineral soils were alkaline, ranging from moderately alkaline in Marshall and Garwood Valley (pH 8.5) to extremely alkaline in Miers Valley (pH >9). Conductivity was highest in Miers Valley (751 µS/cm) and relatively low in soils from Marshall Valley (139 µS/cm) and Garwood Valley (135 µS/cm). ATP content, a proxy for microbial activity, was highest in Marshall Valley (34595 RFU) and Miers Valley (26705 RFU), whereas Garwood Valley showed the least biological activity (8688 RFU). Organic carbon and total nitrogen were very low in all valleys (average 0.088% and 0.010%, respectively) with highest concentrations of carbon (0.033% to 0.116%) and nitrogen (0.003% to 0.015%) recorded in Marshall Valley.

Although significant differences in chemical and physical properties were recorded in soils from different valleys (Table 2), cluster analysis based on Euclidean distance and MDS ordination revealed no significant differences between and within valleys (Figure 9, Appendix C) due to the limited number of samples analyzed.

Table 1. Selected geomorphic, chemical and physical properties of soil samples analyzed for archaeal diversity studies.

Sample	GM[a]	Aspect	Hight (m)[b]	Slope (°)	Water (% g $^{-1}$)	pH	EM[c] (µS cm^{-1})	ATP (RFU)[f]	Total N (%)	Total C (%)	C/N ratio
Garwood Valley											
63-1	All	Flat	300	Flat	0.99	8.5	83.7	3739	0.0053	0.0540	10.1
143-1	Gra	North	800+	0-20	0.97	8.3	29.4	1155	0.0050	0.0477	9.5
155-1	Gra	North	800+	20+	0.99	8.3	32.6	2466	N/A	N/A	N/A
86-2[+]	Gra	North	300	20+	0.99	9	48	3870	0.0095	0.1142	11.9
55-1	M1	South	300	0-20	0.99	9	56.4	20209	0.0048	0.0378	7.7
65-4	M1	North	100	0-20	0.99	9.5	90.2	10725	0.0025	0.0724	27.9
70-4	M2	North	100	0-20	0.99	9	113.9	11123	0.0048	0.0502	10.2
77-2[+]	M2	North	100	0-20	1	8.9	57.1	6665	0.0093	0.0744	7.9
100-1	M3	East	100	0-20	0.99	7.9	868	10270	0.0049	0.0486	9.9
125-1	M3	North	100	0-20	0.9	8.2	46.1	17240	0.0028	0.0283	9.9
125-2	M3	North	100	0-20	0.98	7.8	66.2	5980	0.0138	0.1297	9.3
182-1	M3	North	100	0-20	0.99	8.6	136.4	10817	0.0021	0.0316	15.0
Marshall Valley											
301-2[+]	All	Flat	500	Flat	1	8.3	60	32991	0.0035	0.0346	9.8
377-1	Gra	East	700	20+	1	9.3	70	38739	0.0031	0.0338	10.7
288-1[+]	M1	East	500	0-20	1.01	9.7	110	38069	0.0031	0.0336	10.6
299-1	M1	North	300	0-20	1.01	8.9	310	75331	0.0161	0.1278	7.9
287-2	M2	South	300	0-20	0.99	9.8	336	21674	0.0062	0.0455	7.3
345-1	M2	North	100	0-20	1.01	8.4	130	41447	0.0768	0.5322	6.9
281-1	M3	South	100	0-20	0.59	7.5	7.89	53	0.0072	0.0751	10.4
378-1	Sch	West	700	0-20	1	9.2	90	28453	0.0050	0.0474	9.3
Miers Valley											
481-3[+]	All	Flat	100	Flat	1	9.8	370	99864	0.0223	0.2566	11.5
545-1	Gne	North	300	0-20	0.45	8.4	29	40034	0.0139	0.0986	7.0
567-1	Gra	East	500	0-20	1	7.8	90	7735	0.0068	0.0446	6.5
491-1	M1	South	300	0-20	0.99	9.2	140	30964	0.0102	0.0825	8.0
506-1	M1	North	100	0-20	0.82	9.7	924	7457	0.0055	0.0526	9.4
504-1[+]	M2	Flat	100	Flat	1	9.2	130	16872	0.0048	0.0391	8.1
511-2	M2	South	100	0-20	0.51	9.7	73	5057	0.0031	0.0209	6.6
507-1[+]	M3	South	300	0-20	0.52	8.3	5130	36519	0.0068	0.0436	6.4
538-7	M3	South	100	0-20	0.75	8.6	670	13319	0.0522	0.3466	6.6
538-8	M3	South	100	0-20	0.75	8.6	670	13319	0.0028	0.0239	8.4
474-1[+]	Scr	South	300	0-20	0.7	9.4	42	22617	0.0047	0.0479	10.0

Sample	GM[a]	Aspect	Hight (m)[b]	Slope (°)	Water (% g $^{-1}$)	pH	EM[c] (µS cm^{-1})	ATP (RFU)[f]	Total N (%)	Total C (%)	C/N ratio
NPSx[d]	M1	South	100	0-20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OPS1[d]	M1	South	100	0-20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Shangri-La											
177-1[e]	All	Flat	Flat	Flat		7.8	60.9		0.0221	0.1592	7.1
4004-1	All	North	300	0-20	0.41	9	58.1	861	0.0041	0.1671	39.9
108-1	Gra	North	700	20+	0.98	8.6	61.3	45384	0.0096	0.0747	7.7
251-1[+]	Gra	North	500	0-20	0.99	9.1	50	39488	0.0095	0.0800	8.3
295-1	Gra	East	500	0-20	0.99	9	101.4	24164	0.0061	0.0487	7.9
218-1	M1	North	300	0-20	0.98	8	754	10286	0.0065	0.0425	6.4

a. Geomorphology (GM): M1: Moraine 1, M2: Moraine 2, M3: Moraine 3, Gra: Granite, Sch: Schist, Scr: Scree, All: Alluvia.
b. Elevation is given in average meter above valley floor: 100m = 0-200m, 300m = 200-400m, 500m = 400-600m, 700 = 600-800m.
c. Electrical conductivity (EM) .
d. These samples were collected from beneath seal carcasses located in Miers Valley (close to Lake Miers).
e. This sample was used for sequencing and phylogenetic analyses.
f. Relative fluorescent units (RFU).
+. These samples were clustered together using Pearson correlation.

Table 2. Soil physicochemical properties (mean ± SD) for soils collected from all three Dry Valleys and from Shangri- La (EM= electrical conductivity).

Parameter	All samples	Miers Valley	Marshall Valley	Garwood Valley	Shangri- La
Water content (%)	0.9 ± 0.18	0.77 ± 0.21	0.95 ± 0.14	0.98 ± 0.02	0.87 ± 0.26
pH	8.8 ± 0.6	9.0 ± 0.6	8.91 ± 0.7	8.6 ± 0.4	8.61 ± 0.5 4
EC (µS/cm)	334 ± 860	751 ± 148	139 ± 119	135 ± 232	181 ± 281
ATP (RFU)	22082 ± 21319	26705 ± 27046	34595 ± 21165	8688 ± 58 58	24036 ± 18845
Total N (%)	0.010 ± 0.014	0.012 ± 0.014	0.015 ± 0.025	0.006 ± 0 .003	0.009 ± 0.006
Total C (%)	0.088 ± 0.100	0.096 ± 0.106	0.116 ± 0.171	0.064 ± 0 .032	0.095 ± 0.05
C/N ratio	10.31 ± 6.26	8.1 ± 1.67	9.15 ± 1.54	11.54 ± 5.51	12 .95± 1.32

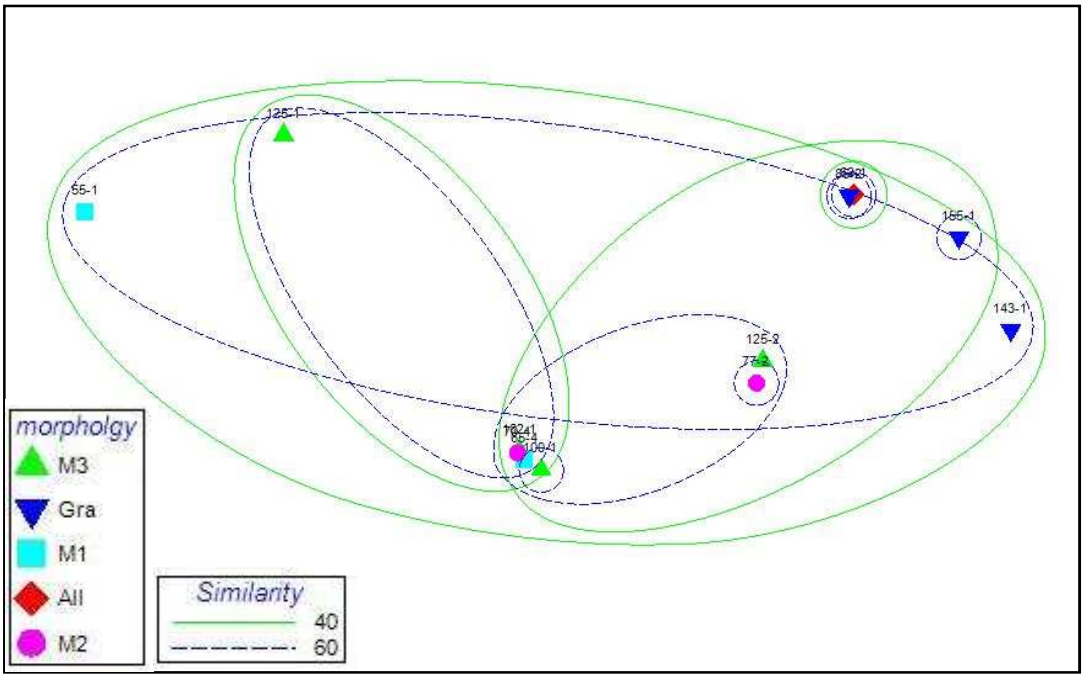


Figure 9. Two- dimensional, non- metric MDS ordination (stress= 0) based on Euclidean similarities of geomorphic and physicochemical properties of Garwood Valley (M3= Moraine 3, M2= Moraine 2, M1= Moraine 1, All= Alluvial).

Similar results were obtained for all sets of transects (Table 3) when analyzed using Euclidean distance and MDS ordination (Appendix C). However, significant differences in soil biogeochemical properties were obtained when comparing transects individually (Table 3).Conductivity was highest in samples from Miers Valley transect (1105 µS/cm) and lowest in samples from the Ridge Transect (53 µS/cm). Samples from Miers Valley transect had the least amount of available water (0.76%) whereas highest water content was recorded for samples from Garwood Valley transects (0.97%). The mean ATP content was relatively similar in samples from all transects (ranging from 11629 RFU to 18133 RFU), except of samples from Marshall Valley transect which had the highest ATP content (35315 RFU).

Table 3. Soil physicochemical properties (mean ± SD) for soils from inter- valley transects (Garwood Valley, Marshall Valley, Miers Valley) and intra- valley transects (Ridge transect).

Parameter	Garwood Transect	Marshall Transect	Miers Transect	Ridge Transect
Water content (%)	0.97 ± 0.03	0.92 ± 0.18	0.76 ± 0.19	0.88 ± 0.2
pH	8.6 ± 0.6	8.9 ± 0.9	9 ± 0.6	8.7 ± 0.5
EC (µS/cm)	179 ± 280	178 ± 139	1105 ± 1805	53 ± 25
ATP (RFU)	11629 ± 4858	35315 ± 27730	17644 ± 11788	18133 ± 16 371
Total N (%)	0.005 ± 0.004	0.021 ± 0.031	0.012 ± 0.017	0.007 ± 0 .003
Total C (%)	0.059 ± 0.033	0.162 ± 0.209	0.087 ± 0.116	0.065 ± 0 .03
C/N ratio	12.27 ± 6.72	8.64 ± 1.74	7.7 ± 1.15	9.24 ± 1.8

Inter- valley comparisons between the different types of moraines revealed minor variations in chemical and physical properties (Table 4), although all samples failed the significance test using MDS ordination based on Euclidean distance (Figure 10, Appendix C).

Table 4.Soil physiochemical properties (mean ± SD) for soils from various moraines from all three Dry Valleys.

Parameter	Moraine 3	Moraine 2	Moraine 1
Water content (%)	0.8 ± 0.18	0.91 ± 0.19	0.96 ± 0.07
pH	8.2 ± 0.4	9.2 ± 0.5	9.3 ± 0.3
EC (µS/cm)	949 ± 1723	140 ± 100	271 ± 331
ATP (RFU)	13439 ± 10683	17140 ± 13441	30459 ± 24880
Total N (%)	0.011 ± 0.016	0.017 ± 0.029	0.007 ± 0.005
Total C (%)	0.090 ± 0.108	0.127 ± 0.199	0.067 ± 0.035
C/N ratio	9.52 ± 2.69	7.88 ± 1.29	11.97 ± 7.9

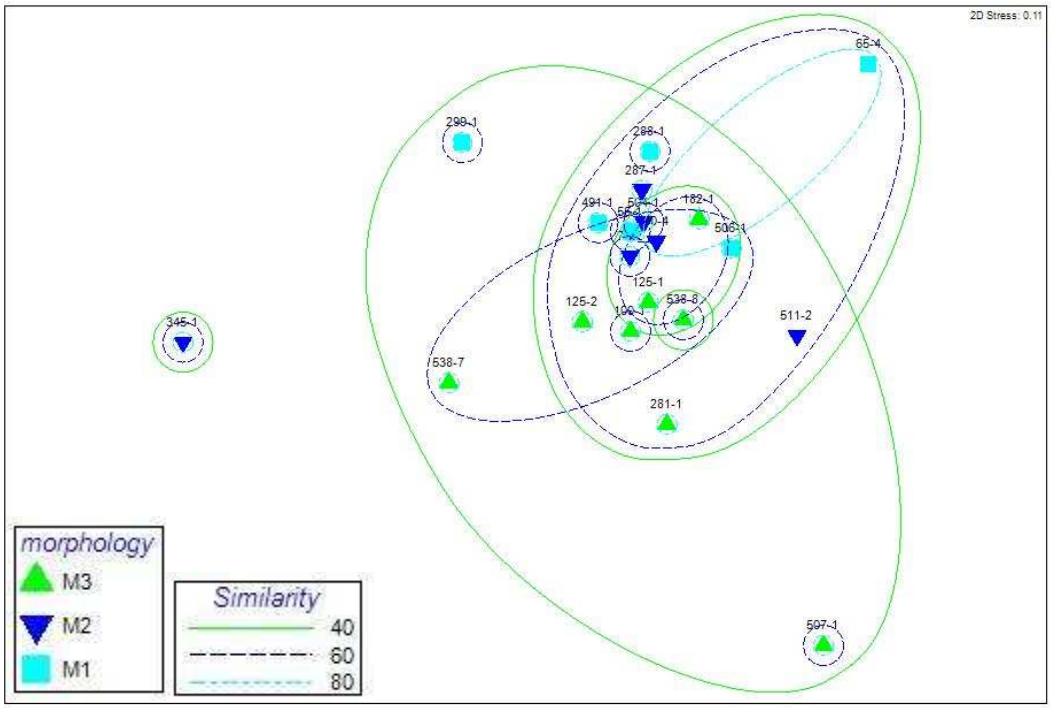


Figure 10. Two- dimensional, non- metric MDS ordination (stress= 0.11) based on Euclidean similarities of geomorphic and physicochemical properties of moraine samples from all three valleys (M3= Moraine 3, M2= Moraine 2, M1= Moraine 1).

2.4.2 Diversity and distribution of soil Archaea

The composition of Archaeal communities was investigated in 36 mineral soil samples from three different Antarctic Dry Valleys. Soil samples were analyzed in duplicates by T- RFLP analysis. The presence of different T- RFLP peaks defined as operational taxonomic units (OTUs) was determined in each soil sample.

T- RFLP measurements detected Archaea in all 36 soil samples yielding a total of 247 distinct OTUs with an average of 6 OTUs in each sample. Although, differences between valleys were only marginal when numbers of unique OTUs were taken across all samples, in general a higher diversity was observed in Miers and Garwood Valley (5 and 4 samples with unique OTUs, respectively, Table 5) closely followed by Marshall Valley (3 unique OTUs). Shangri- La had the lowest number of unique peaks (2 unique OTUs). Samples located on the top of the ridges between valleys were designated to the valley located south of the ridge and are include in total counts per valley.

Table 5. Summary of OTUs in all samples based on T- RFLP fragments.

Sample location	Total no of distinct OTUs in samples	Samples with unique OTUs (range of OTUs)
Miers Valley	83	5 (1-13)
Marshall Valley	70	3 (3-15)
Garwood Valley	63	4 (2-10)
Shangri- La	31	2 (1-14)

Diversity and peak number was relatively similar in all three individual valley transects (Table 6). Highest diversity and highest number of unique OTUs was obtained in Miers Valley (53 total OTUs, 4 unique OTUs) followed by Garwood Valley (47 total OTUs, 3 unique OTUs) and lowest peak number was obtained in Marshall Valley (36 total OTUs, 3 unique OTUs) showing a similar diversity distribution for each valley when compared to total OTU counts of each valley (Table 5). When individual moraines are compared with each other across all three valleys, a slightly higher diversity was observed in moraine 1 (44 total OTUs, 4 unique OTUs), closely followed by moraine 3 (52 total OTUs, 3 unique OTUs) and moraine 2 (40 total OTUs, 3 unique OTUs) which had the lowest number of peaks per sample (Table 6, Figure 11).

Table 6. Summary of OTUs in samples from moraines along transects (Uni= Number of samples with unique OTUs).

Transects	Moraine 3		Moraine 2		Moraine 1		Total OTUs	
	Total	Uni	Total	Uni	Total	Ave	Total	Uni
Miers Valley	21	1	17	1	15	2	53	4
Marshall Valley	3	0	11	1	22	2	36	3
Garwood Valley	28	2	12	1	7	0	47	3
Total OTUs	52	3	40	3	44	4		

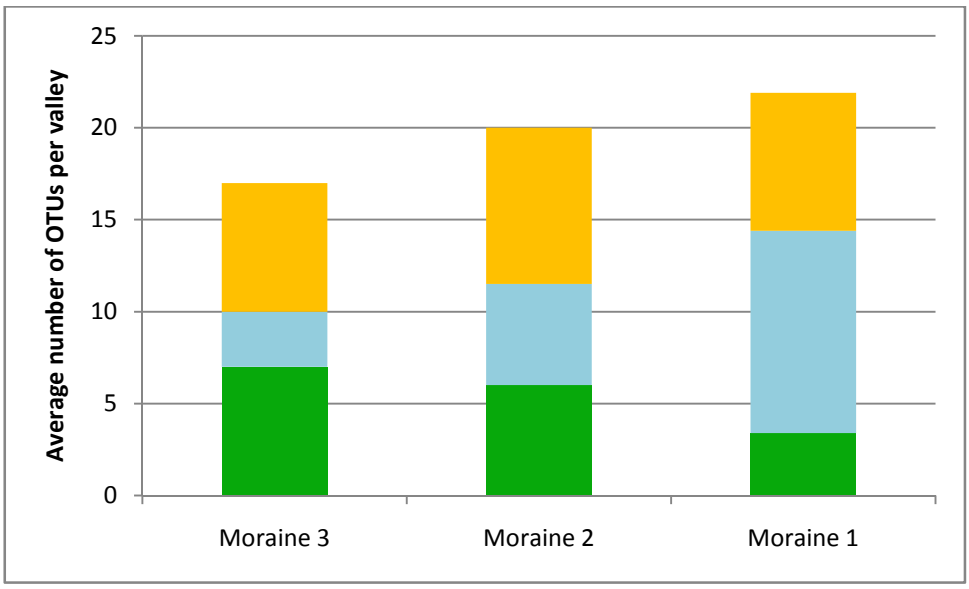


Figure 11. Average number of different OTUs present in moraines from each valley: Garwood Valley (green), Marshall Valley (blue) and Miers Valley (orange).

The south north orientated transect covers the ridges located between the valleys as well as various slopes and valley floors (Figure 8). Although, the total number of OTUs (50) and number of unique OTUs (1) of the Ridge Transect (Table 7) indicates a similar diversity distribution as the individual valley transects (Table 6), a patchy, spatial diversity was observed along this transect. For example, highest diversity was observed at the ridge top (sample 567-1, 10 total OTUs) and valley floor (sample 474-1, 10 total OTUs) of Miers Valley, whereas lowest diversity was observed in a north facing slope (sample 545-1, 1 total OTU) of Miers Valley. Statistical analysis revealed a high diversity at the top of ridges located between the valleys and in samples from Shangri- La (α= 0.06). One Shangri- La sample (295-1) showed very low diversity and was comprised of a very different type of rock (red gravel) compared to all other ridge samples (granite). When excluding this sample from analysis, archaeal diversity was significantly correlated to ridge areas (α= 0.01).

Diversity followed a relatively patchy distribution among samples from Shangri- La, an elevated plain northwest of Marshall Valley. Alluvial samples collected from lake shores were relatively low in diversity (Table 7) with two exceptions: sample 301-2 (11 total OTUs) collected from Lake Marshall and sample 481-3 (9 total OTUs) from Lake Miers.

Table 7. Summary of OTUs in samples from Ridge Transect, Shangri- La and Alluvial origin.

Sample location	Total no of distinct OTUs in sample	Samples with unique OTUs (range of OTUs)
Ridge Transect	50	1 (1-10)
Shangri-La	29	2 (1-15)
Alluvial	24	2 (2-11)

To identify samples with similar archaeal community composition datasets were analyzed using two different tests of independence, namely $\chi 2$ and Pearson correlation (Table 8). Briefly, the $\chi 2$ test is used to determine whether there is a significant difference between observed and expected data, whereas Pearson's correlation is a measure of a linear dependence between two variables. Samples that show significant similarities ($\alpha > 0.05$) are clustered together. The most pronounced clustering of samples was obtained in dataset 3 and 4 for both the $\chi 2$ test and Pearson correlation (Table 8), with dataset 4 showing the most significant connections between samples based on Pearson correlation (Figure 13). Some of the relationships shown in Figure 13 were also obtained in other datasets using different statistical methods (data not shown) supporting the obtained relationships and minimizing the chance that connections between data points were made randomly by chance.

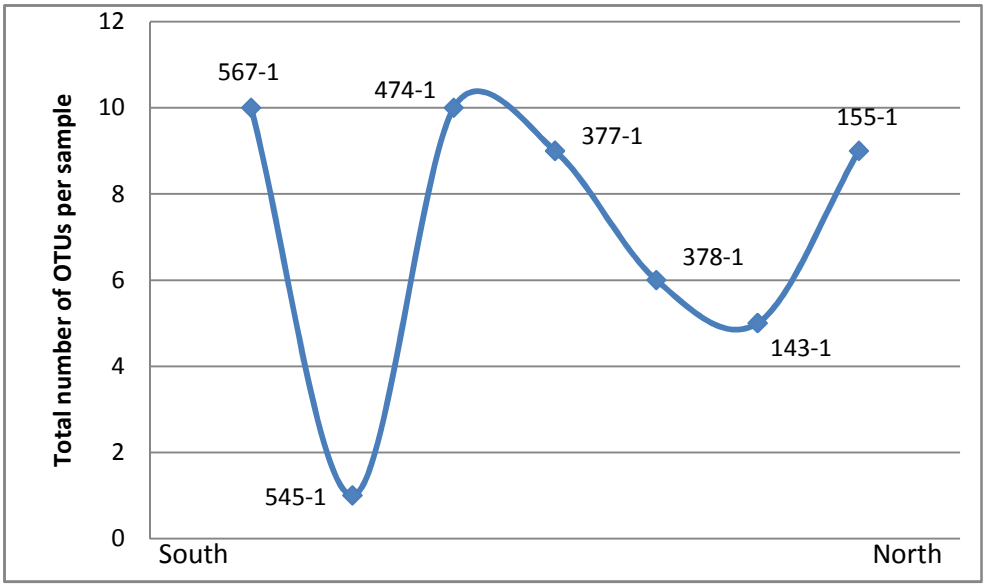


Figure 12. Total number of different OTUs present in each sample along a south north transect. Sample location is as followed: 567-1 Miers Valley Ridge, 545-1 Miers Valley Slope, 474-1 Miers Valley floor, 377-1 Marshall Valley Ridge, 378-1 Marshall Valley Slope, 143-1 Garwood Valley Ridge and 155-1 Garwood Valley Ridge.

Table 8. Different analysis parameters used for T- RFLP data analysis, results of statistical analysis using Chi- Square test and Pearson correlation are shown.

Dataset	minimum length	maximum length	alpha	noise max	Clustering method	Correlations χ2	Pearson's r
1	30bp	750bp	0	200	Samples	none	none
					OTUs	none	none
2	30bp	750bp	0	500	Samples	none	none
					OTUs	none	none
3	30bp	750bp	0.001	100	Samples	yes	yes
					OTUs	yes	yes
4	30bp	750bp	0.001	200	Samples	yes	yes
					OTUs	yes	none
5	50bp	750bp	0	200	Samples	none	none
					OTUs	none	none
6	50bp	750bp	0	500	Samples	none	none
					OTUs	none	none
7	50bp	750bp	0.001	100	Samples	yes	none
					OTUs	none	yes
8	50bp	750bp	0.001	200	Samples	yes	none
					OTUs	none	yes

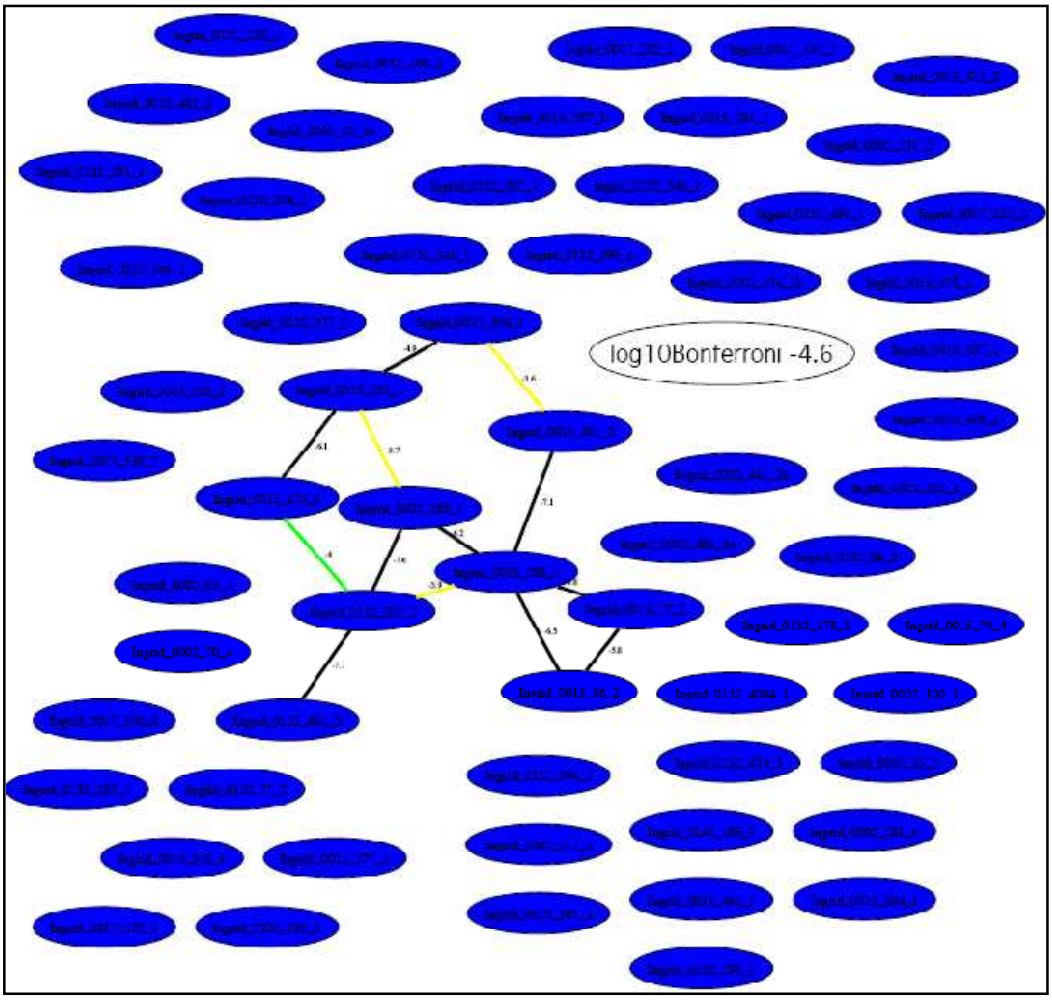


Figure 13. Clustering of samples using dataset 4 (30 bp to 750 bp, alpha 0,001, noise max 200) based on Pearson's r. Lines are drawn between samples that are correlated to each other. Colour code: black= alpha 0.05, blue= alpha 0.1, green= alpha 0.25, yellow= alpha 0.33 (all alpha's are Bonferroni corrected). Sample labels indicate different digestion protocols (0132 and 0002= 10 U enzyme, 0015 and 0017= 20 U enzyme) and name of soil sample (i.e. 377-1).

Location and physicochemical properties of samples that cluster together are listed in Table 1. All samples within this cluster are characterized by high OTU numbers (ranging from 8 to 15 OTUs per sample), indicating highly diverse archaeal communities. Although these samples are very heterogeneous regarding geomorphic properties and sample location, they are all characterized as a homogeneous gravelly type of soil such as moraine gravel, scree gravel and granite gravel. Another common feature between samples is the relatively high water content in all but one sample (507-1, Figure 14) based on Pearson correlation.

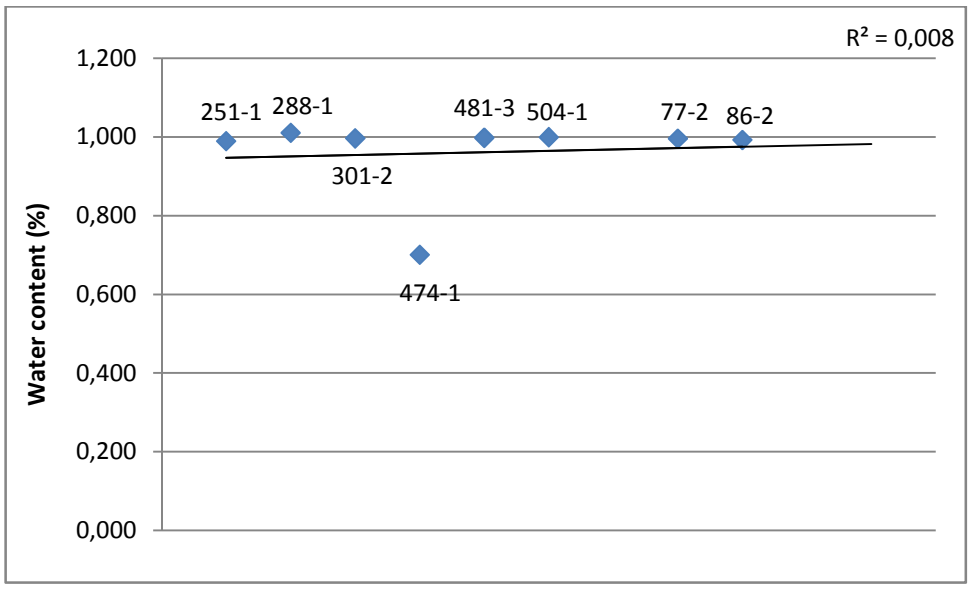


Figure 14. Water content of diverse samples that cluster together based on Pearson correlation (excluding sample 507-1). Coefficient of determination (r^2) is given.

No Archaea were detected in soil samples from underneath a mummified seal located in Miers Valley (Figure 8), including a soil sample from nearby. Both sample sets, taken before and after the seal was moved, did not yield a detectable archaeal signal using PCR (Figure 15, right hand side of marker). When samples were spiked with archaeal DNA, however, the expected 800 bp product was obtained in all seal samples (Figure 15, left hand side of marker) indicating that amplification of archaeal DNA was not inhibited by Bacteria. Bacteria, on the other hand, were successfully detected using bacterial primers (data not shown). Nested PCR, a more sensitive approach also failed to detect any Archaea in all seal samples (Appendix D). Thus, the attempt to recover enriched archaeal signatures from soils from underneath a seal carcass was unsuccessful.

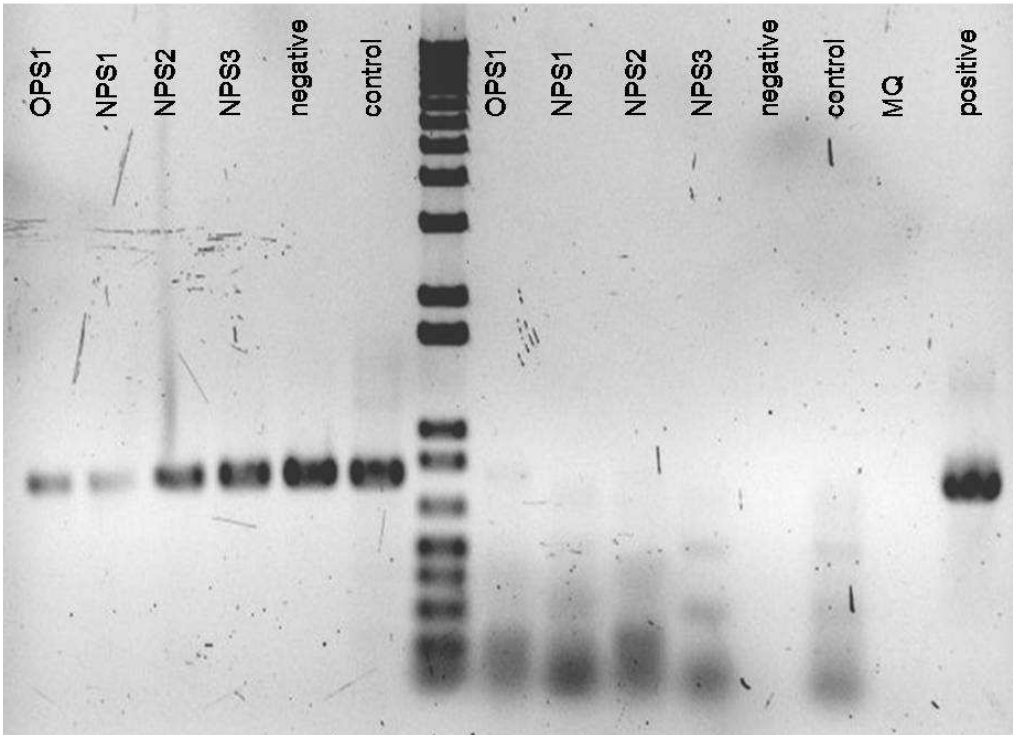


Figure 15. Amplification of archaeal 16S rDNA gene from mummified seal soil samples with (left side of marker) and without (right side of marker) archaeal DNA added. OPS1= original position of seal, NPS1- 3= new position of seal, negative= negative DNA extraction, control= nearby soil sample, MQ= milliQ water, positive= archaeal DNA.

2.4.3 Archaeal community composition and phylogenetic analysis

Sample (177-1) used for the archaeal 16S rRNA gene clone library was collected from the shore at the south east end of Lake Buddha (Shangri- La, Figure 8). A total of 40 clones were screened by RFLP analysis. Based on RFLP profiles (Figure 16), clones were grouped into five RFLP operational taxonomic units (RFLP- OTUs). One representative of each RFLP- OTU was sequenced and matched to their closest neighbours in GenBank. All five archaeal OTUs aligned most closely with members of the phylum *Crenarchaeota*. The two crenarchaeal isolates *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum* were the closest relatives of all clones showing a low 82% and 81% maximum identity, respectively. When searching the nucleotide database for closest neighbours, clones were matched to different environmental archaeal sequences (Table 9). The crenarchaeal clones showed a high level of similarity to existing sequences in the database with all being > 98%

identical over 750 nucleotides. Placement of clones within the *Crenarchaeota* was subsequently supported by phylogenetic analysis (Figure 18). All five clones clustered with *Crenarchaeota* group 1.1b (Marine Group 1.1b) together with clones retrieved from low nutrient grassland soil (Ochsenreiter, et al. 2003), slit loam soil from an agricultural station (Bintrim, et al. 1997), soil developing at a deglaciated site (Nicol, et al. 2006) and soil from a sandy ecosystem (Treusch, et al. 2005) (Figure 18). This group also included the isolate *Nitrososphaera gargensis*, an ammonia- oxidizing *Crenarchaeota* isolated from a hot spring in Russia (Hatzenpichler, et al. 2008). Although sequences of all clones were highly similar, a separation into two clades was supported by high bootstrap values (Figure 18). The first clade contained clones 37 and 11, as well as two environmental sequences retrieved from termite gut (Friedrich, et al. 2001) and freshwater lake sediment (Ochsenreiter, et al. 2003), whereas the second clade contained clones 14, 17 and 19 only. This separation was further supported by RNA/DNA manipulation experiments on soils from Miers Valley (Dr Charles Lee, personal communication). Archaea were detected in extremely low abundance in samples treated with milliQ water and nitrate. Most of the archaeal sequences detected were assigned to the phylum *Crenarchaeota* which were present in the DNA and RNA component of samples. Archaeal sequences that were associated with crenarchaeal sequences recovered from Shangri- La were used for OTU analysis based on pairwise distances. Using different clustering methods (furthest neighbour vs. nearest neighbour) and parameters (0.01 to 0.15 nucleotide substitutions per position) revealed separation of crenarchaeal clones into two distinct OTUs, thus indicating the presence of two different ribotypes in soil sample 177-1.

In- silico digestion of all five sequences using *Msp*I (recognition site C^CGG) was performed to determine the smallest terminal fluorescently labelled fragment (170 bp in all clones). This information was used to analyze community composition within T- RFLP profiles of all samples. The 170 bp fragment was highly abundant in all samples indicating a widespread distribution of this member of the crenarchaeal group 1.1b (Figure 18).

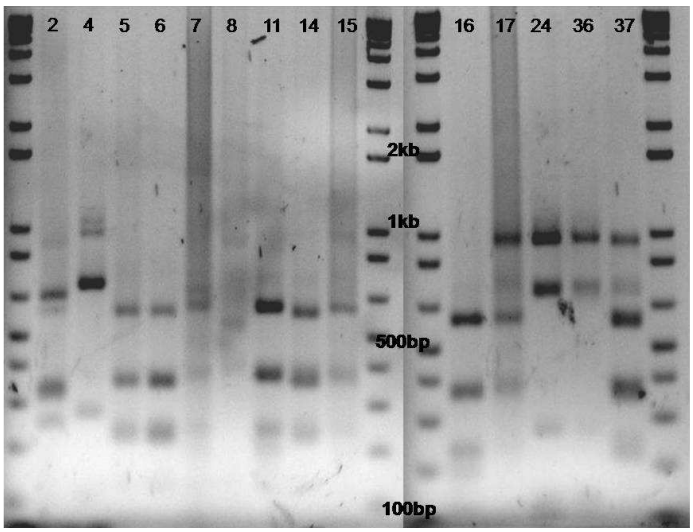


Figure 16. *Alu*I restriction digest of 14 clones generated from soil sample 177-1.

Table 9. Closest relatives of archaeal clones in DNA extracted from Shangri-La (sample 177-1) using environmental sequences retrieved from GenBank.

Clone	Closest match in Nucleotide Database (accession number)	Source	% identity of clone to its closest match
11	Uncultured archaeon clone VA35 (EU423018)	arid lands in North America	99
14	Uncultured crenarchaeote clone Hm5_rock_SK470 (HQ647195)	lichen rocks in SW Norway	99
17	Uncultured archaeon clone TX1C04 (FJ784302)	alkaline saline soil of lake Texcoco(Mexico)	99
19	Uncultured crenarchaeote clone Hm5_rock_SK470 (HQ647195)	lichen rocks in SW Norway	99
37	Uncultured archaeon clone VA35 (EU423018)	arid lands in North America	99

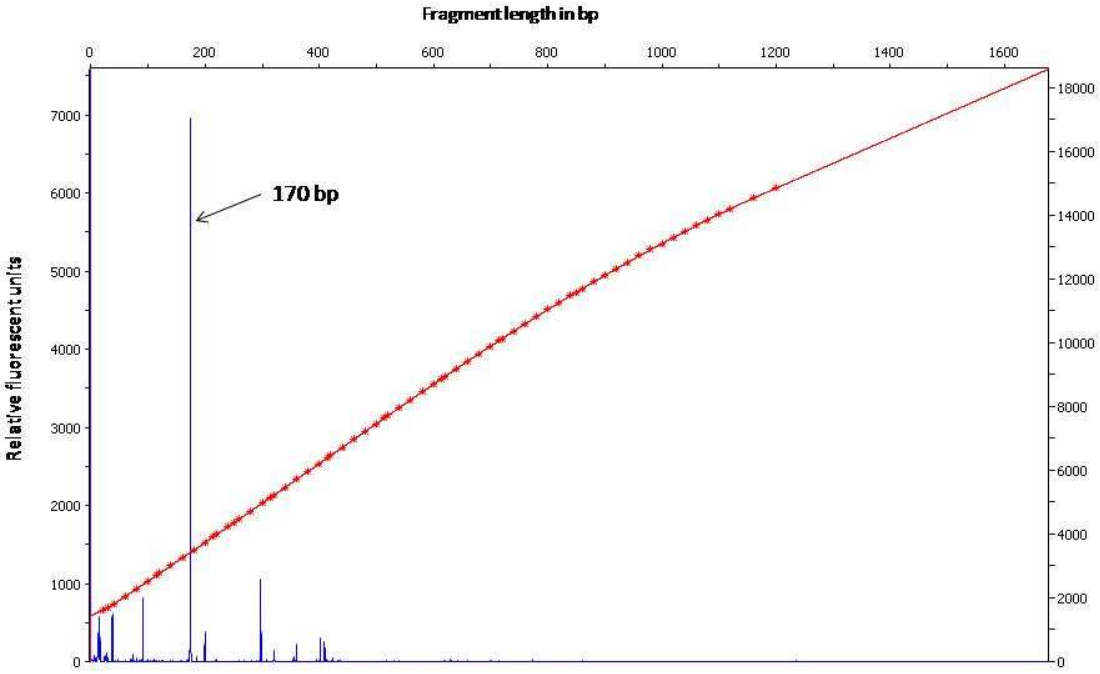


Figure 17. T- RFLP profile of sample 77-2 digested with *Msp*I showing the most abundant OTU (170 bp fragment). The red line shows quality of run based on standard.

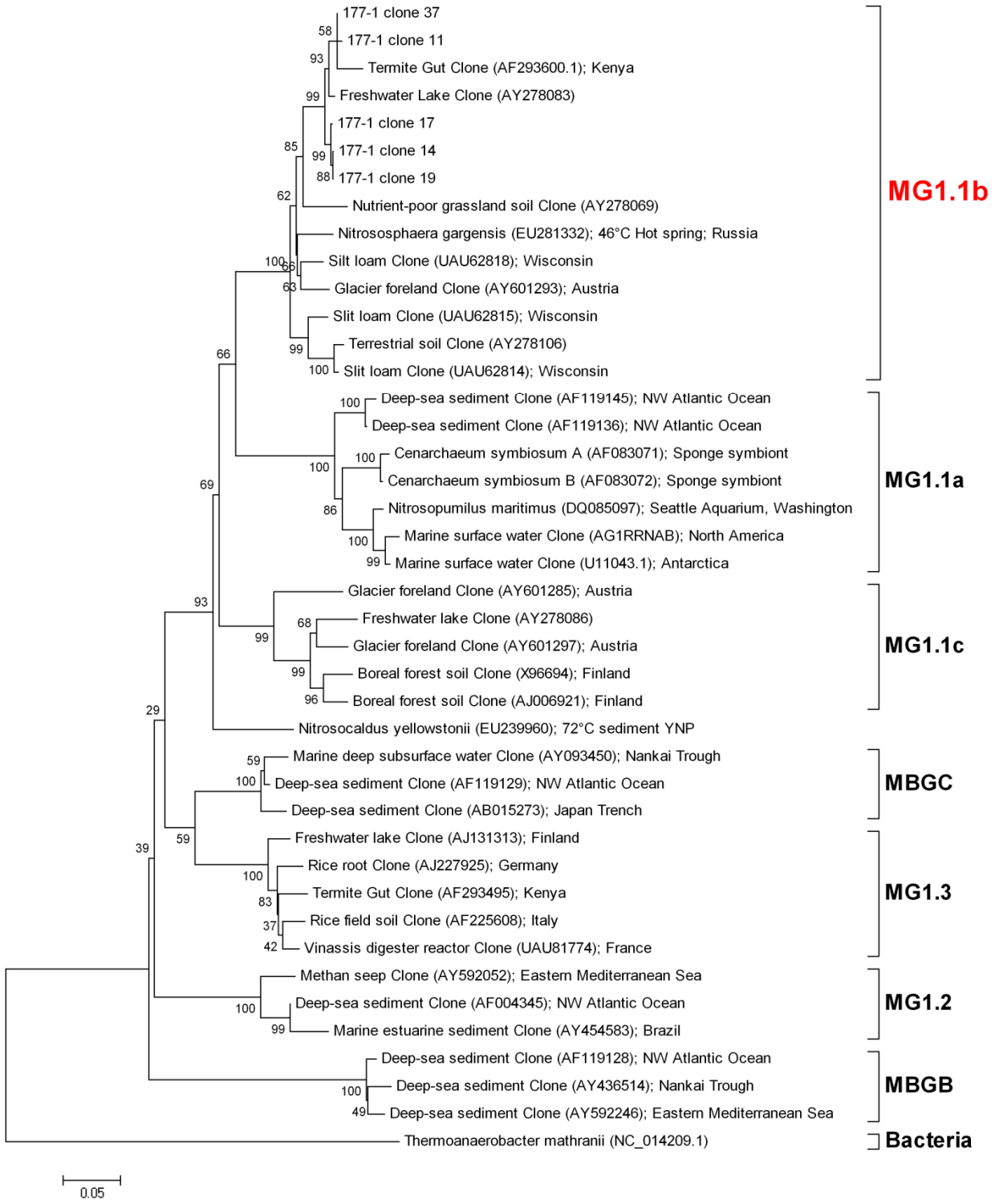


Figure 18. Crenarchaeal phylogeny of clones retrieved from Dry Valley soil.Included partial environmental 16S rRNA gene sequences (814 bp) and crenarchaeal isolates were retrieved from GenBank as representatives of each subgroup: MG1.1b= Marine Group 1.1b, MG1.1a= Marine Group 1.1a, MG1.1c= Marine Group 1.1c, MBGC= Marine Benthic GroupC, MBGB= Marine Benthic Group B, MG1.3= Marine Group 1.3 and MG1.2= Marine Group 1.2. The bacterial isolate *Thermoanaerobacter methranii* was used as outgroup. The evolutionary history was inferred using Neighbor- Joining (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using Maximum Likelihood (Tamura, et al. 2004) and are in the base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 814 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, et al. 2007).

2.4.4 Environmental drivers of Archaeal diversity and distribution

Ordination (PCA) and BEST analysis was used to investigate archaeal distribution across biogeochemical soil properties and their influence on archaeal community structure. The results of the BEST analysis (Table 10) are given as Spearman's rank correlation coefficients (ρ). Using the entire dataset, a very low Spearman's ρ of 0.180 revealed slope, water, carbon and nitrogen as the best explanatory variables of the archaeal community fingerprints obtained. However, when samples were analyzed based on location, and/or morphology, nitrogen was identified as the best explanatory variable for archaeal diversity in Shangri- La (ρ= 0.855). Second best scores obtained to explain archaeal distribution patterns include elevation, slope, carbon and nitrogen for Marshall Valley Transect (ρ= 0.689), water and nitrogen for Ridge Transect (ρ= 0.610) and slope and nitrogen for Moraine 2 (ρ= 0.629). Samples that clustered together based on Pearson's r (see above) revealed a correlation of archaeal diversity with water and conductivity (ρ= 0.398). These results indicate that water and carbon/nitrogen may be the primary drivers of archaeal diversity and distribution as one or more of these factors contributed to the BEST score in 11 sample sets analyzed (out of 13 sample sets).

Table 10. Spearman's rank correlations (ρ) are given for environmental factors that were the best explanatory variables for archaeal diversity in various sample sets.

Sample set analyzed	Environmental variables giving highest ρ	ρ value
All samples	slope, water, carbon, nitrogen	0.180
Miers Valley	water	0.315
Marshall Valley	height, carbon	0.535
Garwood Valley	slope	0.250
Shangri- La	nitrogen	0.855
Miers Transect	water	0.338
Marshall Transect	height, slope, carbon, nitrogen	0.689
Garwood Transect	nitrogen	0.112
Ridge Transect	water, nitrogen	0.610
Moraine 3	conductivity	0.356
Moraine 2	slope, nitrogen	0.629
Moraine 1	pH, carbon, nitrogen	0.521
Diverse sample set*	water, conductivity	0.398

***This sample set showed highest archaeal diversity based on Pearson's r (see above).

Principal component analysis revealed no spatial variation in soil biogeochemical properties over the valley transects. The results of the ordination for the Miers Valley Transect are represented in a 2d- plot (Figure 19), and show a positive correlation of most samples with carbon/nitrogen concentrations and water content. Ordination of the samples, showing highest diversity based on OTU number, indicates an influence of water, conductivity and ATP on archaeal diversity in some samples and a slightly negative influence of C/N and pH on diversity in most of the samples (Figure 20).

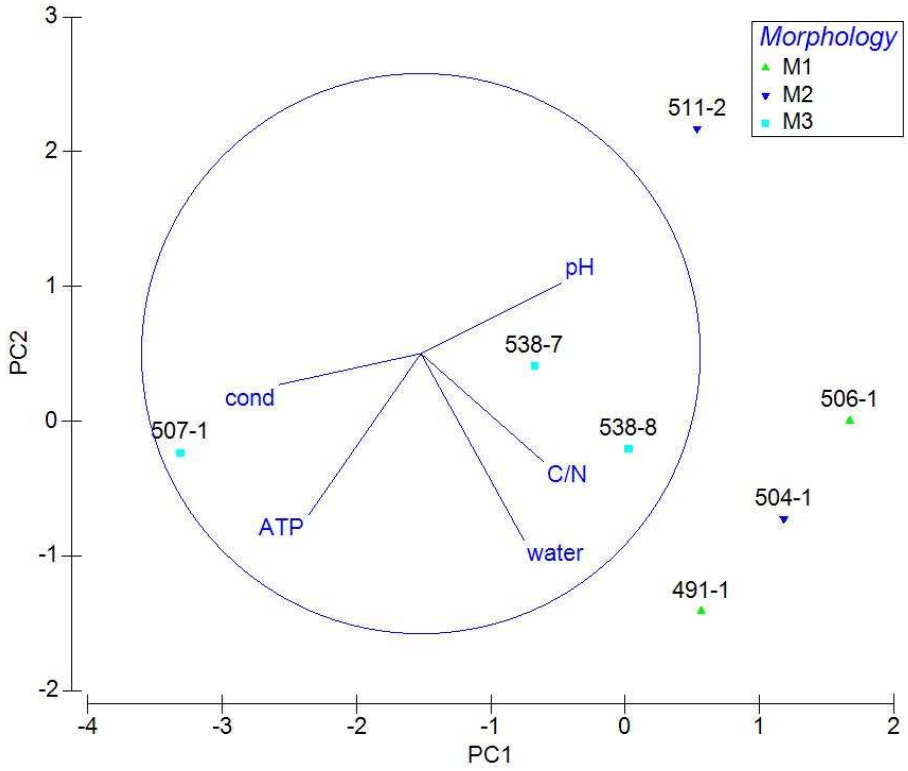


Figure 19. 2d- PCA plot of samples from the Miers Valley Transect representing Moraine1, 2 and 3.

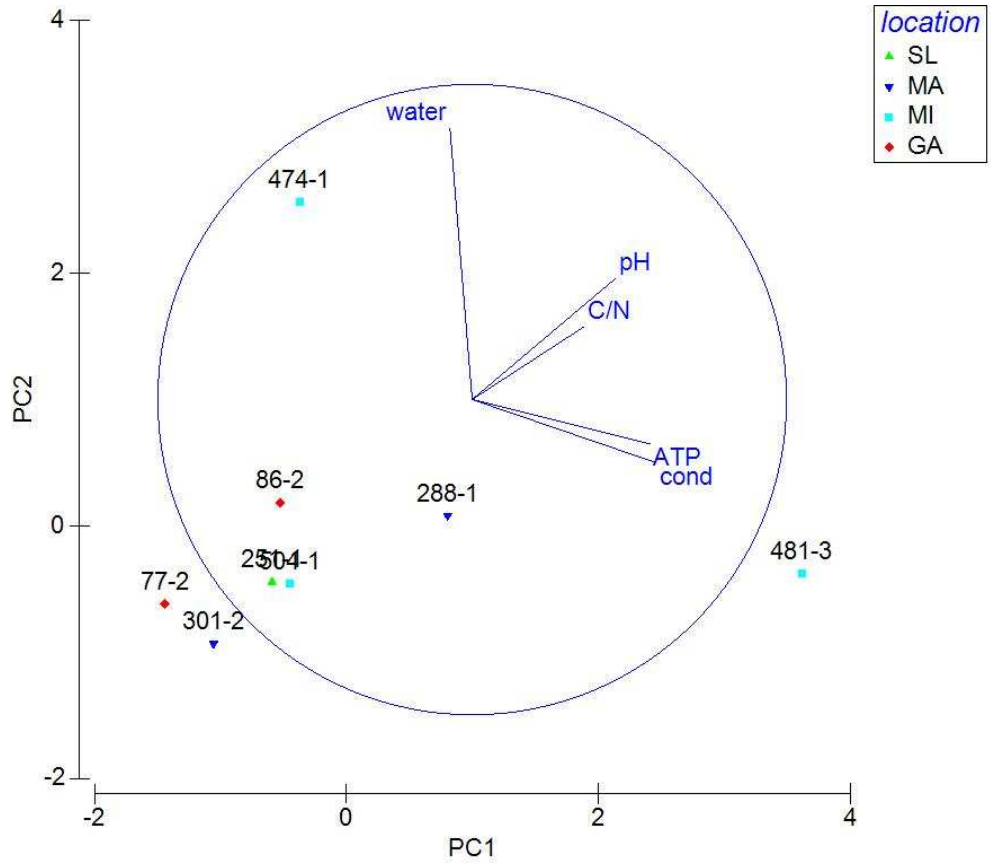


Figure 20. 2d- PCA plot of samples with high archaeal diversity from different locations.

2.5 Discussion

2.5.1 Soil conditions in soils of the McMurdo Dry Valleys

The cold desert soils of the McMurdo Dry Valleys are one of the harshest and most extreme ecosystems on Earth characterized by extreme low temperatures (ranging from -15 to -40℃) and low water content (Doran et al. 2002). As a consequence only a few plants and animals are present and soil food webs are almost entirely composed of microorganisms (Adams, et al. 2006). Due to the absence of biotic factors, Antarctic soils are relatively simple ecosystems dominated by abiotic factors which may be the sole drivers of microbial activity (Convey 1996; Hogg, et al. 2006).

Each valley within the McMurdo Dry Valley region is considered a unique ecosystem (Campbell and Claridge 1987). The area can be divided into three main climatic zones: subxerous (coastal areas), xerous (inland valleys), and ultraxerous (adjacent to the Polar Plateau) (Campbell and Claridge 1987). All three valleys chosen for this study, namely Miers Valley, Marshall Valley and Garwood Valley, are coastal (subxerous zone), low altitude valleys characterized by similar soil geomorphology, lithology and mineralogy. Additionally, each valley has a combination of distinct characteristics such as presence or absence of lakes, fluctuating melt water streams, glacial input and varying microclimate (i.e., average temperature, wind speed and precipitation). All these different characteristics influence the physical and chemical properties of soils such as carbon and nitrogen concentrations (Ugolini and Bockheim 2008). For example, soils and sediments adjacent to waterbodies have higher concentrations of soil organic carbon due to the activity of dense microbial mats (Barrett, et al. 2006; Gregorich, et al. 2006). This spatial availability of organic matter and the spatial and temporal availability of water might be the main control over absence or presence and distribution of microbial life (Aislabie, et al. 2006).

2.5.2 Physicochemical soil properties of the Dry Valley soils

Miers, Marshall and Garwood Valley are U- shaped glacial valleys characterized by calcareous sandy aeolian and fluvial sediments, glacial push- moraines, and bedrock dominated by dolomite, granite and metamorphosed rocks (Elberling, et al. 2006). Soils are permafrost- affected and are primarily Typic Haploturbels developed on Ross Sea drift. Ground ice is present within a meter of the surface and is accompanied by Glacial Haploturbels (Bockheim and McLeod 2008). Soils in the coastal areas are characterized by extensive cryoturbation due to comparatively abundant soil moisture whereas soils in the upper valleys are classified as Typic Hyplorthels (Bockheim and McLeod 2008), which are characterized by dry- frozen permafrost and minimal cryoturbation (Bockheim 2002). These small patches of pre- Ross Sea drift in the upper part of the valleys are distinctly older (104- 567 kyr) than soils in coastal areas which were deposited during the last glacial period (8- 106 kyr, Brook et al. 1995). These differences in surface exposure ages significantly influence local geochemistry and habitat suitability for resident microbes (Barrett, et al. 2007; Bockheim 2002).

Using non- metric MDS analysis only minimal differences in physical and chemical soil properties were identified across all three valleys (Figure 9, Figure 10, Appendix C). These results are supported by a previous study showing that areas within a specific climate zone, such as the subxerous zone, are characterized by homogeneous soil properties (Bockheim 2008). However, Miers Valley soils had different physicochemical properties than those in Garwood and Marshall Valley. Samples from Miers Valley were typically lower in relative water content (0.7% mean), more alkaline (pH= 9 mean) and had higher electrical conductivity (751 µS/cm mean). Salt composition of soils from the same climate zone are known to follow a latitudinal trend with the prevalence of chloride along the coasts, a decreasing trend for sulfates, and an increasing trend for nitrite in the interior (Bockheim 2008). Although, the concentrations of different types of salt were not tested, the highest electrical conductivity was measured in samples from coastal areas (Moraine 3) indicating high salt concentrations were due to marine

influences. Interestingly, the second highest salt concentrations were obtained in inland samples (Moraine 1), and lowest salt concentrations were measured in samples from Moraine 2. Moraine 1 and other older, higher elevation regions in continental Antarctica can reach extremely high salt concentrations. For example, nitrate concentrations can reach 3% w/w, exceeding carbon concentrations, due to a long history of salt deposition and accumulation, and low leaching rates in old soils (Barrett, et al. 2007; Bockheim 2002). High organic carbon concentrations were reported in soils and sediments adjacent to lakes and streams (Burkins, et al. 2001; Elberling, et al. 2006; Gregorich, et al. 2006) due to the presence of primary producers, e.g. cyanobacteria. Our measurements revealed relatively similar carbon concentrations for all valleys excluding Garwood Valley, which had the lowest mean total carbon concentration (mean 0.064%), coinciding with values reported by Elberling et al. (2006). This is somewhat surprising as the highest mean water content was measured in Garwood Valley (mean 0.98%), with the expectation that carbon concentrations would be higher as well. In Antarctic soils, however, organic matter is not solely derived from soils adjacent to lakes. Instead, sources of organic carbon include (i) chemolithotrophic, endolithic and hypolithic microbes; (ii) legacy carbon deposits from ancient lake beds; (iii) spatial subsidies from coastal regions, or microbial mats from modern lakes, carried to the soils by aeolian dispersal (Hopkins, et al. 2005; Wood, et al. 2008); and (iv) presence of mummified seal carcasses (Cary, et al. 2010). Thus, measured carbon concentrations are not representative of present- day nutrient input. Rather, the distribution patterns are only suggestive of a few potential sources known to occur in the present- day landscape (Fritsen, et al. 2000), and are controlled by various factors. Carbon concentrations in samples taken from lake edges and alluvial soils were very inconsistent, with some samples showing high carbon concentrations (0.159- 0.256%), with others relatively low in total carbon (0.034- 0.054%). This may be due to spatial differences in distribution of primary producers around lake margins, different water levels of lakes due to varying microclimates, and distance of the sampling spot from the lake margin (Elberling, et al. 2006; Hopkins, et al. 2006).

Relatively high water content was expected in samples from the Garwood and Miers Valleys as both have glacially fed lakes, and several seasonal meltwater streams running eastwards to the Ross Sea, whereas the Marshall Valley is characterized by only one seasonal meltwater stream and an absence of a permanent lake. Conversely, highest relative water content was obtained in Marshall Valley and Garwood Valley (0.95% and 0.98% respectively), and lowest available moisture was recorded in Miers Valley (0.77%). Due to the above mentioned topographic characteristics of the valleys it is most likely that a bigger sample set is required to make valid assumptions about relative moisture availability within each valley.

2.5.3 Physicochemical soil properties of soils from Shangri- La

The elevated plain of Shangri- La, located inland, northwest of Marshall Valley, is a very different area compared to the three eastern Dry Valleys. Located in the xerous climate zone, this area is characterized by little modern downslope movement which is usually restricted to north- facing slopes with high moisture content (Bockheim 2008). Surface sediments are primarily glacial till derived from granitic rocks similar to the eastern Dry Valleys (Bockheim and McLeod 2008). The presence of a large lake (Lake Buddha), and numerous other lakes and ponds, allows for high moisture input into the system, thus Shangri- La is considered a “hot spot” for microbial activity (Gregorich, et al. 2006), as availability of water is thought to be the primary limiting resource for microbial life (Kennedy 1993). Samples taken from lake shores and ridge areas were surprisingly low in water content (0.87% mean) indicating less availability of moisture than in the three eastern Dry Valleys (Table 2). All samples from Shangri- La, with the exception of one, had relative water contents greater than 0.98%, thus the low mean water content obtained may not be representative of the area due to the limited number of samples analysed. As expected, total carbon concentrations were highest in samples collected from lake edges and lower in ridge samples.

2.5.4 Archaeal distribution and diversity in Dry Valley soils

We used PCR and T- RFLP analysis for a comparative study of archaeal diversity and distribution across three distinct Dry Valleys located in the coastal climate zone namely: Miers Valley, Marshall Valley and Garwood Valley, including the inland plain Shangri- La. Based on PCR analysis we detected Archaea in all samples analyzed, extending previous studies by Ayton et al. (2010) who reported a patchy distribution of Archaea primarily present in coastal areas of the Ross Sea region. In contrast to previous attempts (Pointing, et al. 2009; Yergeau, et al. 2009), this is the first time that Archaea were successfully detected in Antarctic Dry Valley soils from a variety of different landscapes such as coastal moraines, terminal moraines, meltwater stream deltas, lake edges, hill slopes, ridges and polygons. Detection was successful in soils exceeding electrical conductivity of 5 mS/cm and pH greater than 9.5. Soil conditions like these were previously not thought to be colonized by Archaea (Ayton, et al. 2010).

Our results indicate a very low diversity of Archaea in Antarctic Dry Valley soils, which is comparable to low diversity estimates reported from soils of the Ross Sea region and similar environments such as the Canadian High Arctic (Ayton, et al. 2010; Steven, et al. 2008). However, a comparison of the T- RFLP fragment lengths among the samples enabled us to identify several areas that support highly diverse archaeal communities which were scattered across the entire study area. Sample sites showing high diversity varied significantly based on geomorphic features such as different types of rocks (granite, scree) and landscapes (moraines, lake edges, hill slopes, ridges (Table 1)). Thus, no significant correlation between diversity and any geomorphic or topographic factor could be identified. However, different types of moraines were related to different levels of diversity, showing a decrease in diversity from inland moraine 1 to coastal moraine 3 (Figure 12). Statistical analysis using the Mann- Whitney- Test showed the opposite, with highest diversity in moraine 3 and lowest diversity in moraine 1, although none of the correlations was significant (moraine 3: $\alpha = 0.15$, moraine 2: $\alpha = 0.37$, moraine 1: $\alpha = 0.4$). These inconsistent results are primarily due to the

small number of samples analyzed, as stringency of any statistical test increases with increasing sample size. It was expected that soils from moraine 3 would have the highest diversity, as the close proximity of these soils to the Ross Sea may foster introduction of euryarchaeal species, as reported for other coastal areas of the Ross Sea region (Ayton, et al. 2010). A marine influence on coastal soils has been confirmed by Bockheim (2002), who showed that anions in coastal soils were of marine origin. Due to the very low abundance of *Euryarchaea* in soils of Granite Harbour (Ayton, et al. 2010) it is most likely that *Euryarchaea*, although present in soils of moraine 3, are well below detection limit. However, observed archaeal diversity may follow the latitudinal salt gradients, as soils in coastal areas are enriched in chlorides, whereas soils in inland valleys are enriched in SO_4 and/or NO_3 (Bockheim 2002). The higher diversity in inland moraines might indicate a positive selection for Archaea in soils enriched in SO_4 and NO_3 salts rather than chlorides. Additionally, high diversity was observed on the ridges (α= 0.06), which coincides with the concept that Archaea may favour soils high in SO_4 and NO_3 salts. The high elevation ridges are extremely “old” soils, which are characterized by high salt concentrations due to a long depositional history of salts from the atmosphere and negligible rates of soil leaching caused by arid conditions (Bockheim 2002). Moreover, due to the age and location of these old soils they are more heterogeneous than the younger gravel of coastal areas. Heterogeneous soils may facilitate protected environments, such as cracks in rocks, or the underside of translucent rocks, providing a more stable environment for archaeal colonization (Cary, et al. 2010). Taken together, archaeal diversity was relatively low in soils of the Dry Valleys, except ridge areas which may be hot- spots of archaeal diversity. Preliminary phylogenetic analysis identified only one species in soil from Shangri- La, confirming low diversity estimates. However, two ribotypes of the same species were present in the relatively small clone library (40 clones screened) indicating some level of diversity.

2.5.5 Archaeal community composition

All archaeal OTUs retrieved from soils of Shangri- La belonged to the phylum *Crenarchaeota* group 1.1b (Figure 18). Members of this lineage have been recently detected in coastal soils of the Ross Sea region namely: Scott Base, Marble Point, Granite Harbour and Victoria Valley (Ayton, et al. 2010). The majority (89%) of all crenarchaeal clones recovered from these soils grouped together with the Dry Valley soil clones from this study, supported by a high bootstrap value (Appendix G, Figure 25), thus suggesting that members of group 1.1b are the most abundant crenarchaeal species in Antarctic soils. By combining T- RFLP and phylogenetic analysis it was demonstrated that members of group 1.1b are widely distributed in soils of the Dry Valleys: namely Miers Valley, Marshall Valley, Garwood Valley and Shangri- La and are not restricted to coastal areas as previously thought (Ayton, et al. 2010).

Crenarchaeal species belonging to group 1.1b are highly abundant and ubiquitous in most temperate soils, where they make up to 5% of prokaryotic 16S rRNA genes (Ochsenreiter, et al. 2003). Species belonging to group 1.1b were the only *Crenarchaeota* that were successfully detected in low- nutrient grassland soils (Ochsenreiter, et al. 2003), and in pioneer and intermediate soils in the foreland of a receding glacier (Nicol, et al. 2005). Due to similarities between the two environments it can be assumed that members of group 1.1b are particularly adapted to colonize cold, low- nutrient soils devoid of vegetation. Pesaro and colleagues (2003) reported a higher resistance to freeze- thaw cycles for members of group 1.1b than for members of group 1.1a, which may explain their ability to successfully colonize Antarctic soils. Resistance to freeze- thaw is a crucial adaptation for survival as multiple freeze- thaw cycles can occur within a single day in Antarctic soils (Aislabie, et al. 2006) caused by daily temperature fluctuations greater than 20℃.

2.5.6 Physicochemical drivers of archaeal diversity

A comparison of T- RFLP fragment lengths among the samples allowed for inter- valley comparisons of archaeal communities across a range of physicochemical conditions. Detection of diverse archaeal communities, and previously reported high bacterial diversity in soils of Miers Valley (Babalola, et al. 2009; Smith, et al. 2006), indicate that the physiochemical properties of these soils may support diverse microbial communities.

The only physicochemical parameter that was correlated to diversity was relative high water content (Figure 14, Table 10) supporting the theory that water is the primary limiting resource for microbial life (Gregorich, et al. 2006; Kennedy 1993). However, a significant correlation between water content and archaeal diversity was not obtained, questioning the sole role of water as a driver of diversity. It has been suggested recently that soil organic matter, salinity and pH are the best predictors of microbial activity (Zeglin, et al. 2009), supporting previous assumptions that soil organic carbon, nitrogen and phosphorous are the main drivers for microbial activity and diversity in these systems (Gregorich, et al. 2006). Concentrations of these nutrients are locally high, in soils adjacent to lakes or streams, due to the presence of primary producers such as cyanobacteria and algae (Barrett, et al. 2006; Gregorich, et al. 2006; Wood, et al. 2008). CO_2 efflux studies showed an increase in microbial activity in these transition zones between lake and terrestrial ecosystems simply because more nutrients are readily available for indigenous soil heterotrophs (Barrett, et al. 2007; Burkins, et al. 2001; Hopkins, et al. 2006). Although bacterial diversity has been shown to be higher in wetter soils than in dry soils (Aislabie, et al. 2006) similar trends for archaeal diversity were not revealed in this study. In fact, archaeal diversity seems to almost follow the opposite trend, with most of the alluvial soil samples being low in diversity (with two exceptions), and total carbon concentrations not significantly higher in samples supporting a higher diversity of Archaea (Table 1). These findings suggest that archaeal diversity may not be driven by availability of water and/or organic carbon.

This assumption was supported by community analysis of Archaea underneath a mummified seal. Mummified seals are patchily distributed across many of the Dry Valleys and represent a unique microniche to be inhabited by microorganisms. The seal carcasses influence the soil beneath them by stabilizing the physical environment, reducing the immediate dehydrating effects of wind, and by supplying a rich resource of carbon. As a consequence, more moisture accumulates directly under the seal fostering a unique enriching environment for microbial growth. A recent molecular study showed that bacterial communities under the seal are highly diverse and that community composition is different to samples taken from areas 15 meter away without a mummified seal (Cary, et al. 2010). Surprisingly, Archaea seem to be absent from this unique soil ecosystem as molecular methods failed to detect any archaeal signals (Figure 15). This would indicate that Archaea are either present in very low abundance, below our detection limit, or are totally absent from these soils. Assuming archaeal species were present before the seal carcass covered the soil, it is possible that Bacteria may simply outcompete their archaeal counterparts as soon a stable physical environment is established underneath the seal. For example, the absence of rapid freeze- thaw cycles underneath the seal may indicate selection for Bacteria and against Archaea. Interestingly, species that dominated the bacterial community under the seal were found in low abundance in adjacent soils suggesting that the input of organic matter and water by the seal carcass favours their lifestyle resulting in a bacterial bloom of these particular species. In summary, archaeal species are either outcompeted by Bacteria when environmental conditions are favourable, or archaeal diversity and distribution is not driven by physicochemical soil properties such as soil carbon and water content.

This negative correlation between concentrations of organic matter and archaeal diversity was confirmed by a comparative RNA study (Dr. Charles Lee, personal communication). In brief, very low archaeal abundance was measured in high- productivity soils (heterogeneous soil, endoliths present) whereas a stronger archaeal signal was recovered from soils low in organic matter (homogeneous gravel, no obvious primary

producers present) indicating that distribution of Archaea may be negatively correlated to primary production, or where high concentrations of organic carbon occur.

The main geochemical drivers that determine diversity of Dry Valley microbial communities are still being investigated. Early studies suggested that moisture, carbon and salinity were the determining factors as to whether or not habitats were suitable to harbour soil microbial communities (Freckman and Virginia 1997). Barrett and colleagues (2004) identified that temperature and moisture are more likely to determine soil species distribution. Of all the environmental parameters measured in this study, water, and carbon and nitrogen concentrations best explained the prevailing archaeal community (Table 10).

What these findings indicate is that geochemistry may not be the main driver of biodiversity in these Dry Valleys. Rather, the combination of relatively milder temperatures, water availability and geomorphology work together to create appropriate niches for microorganisms to thrive in. The availability of these niches, such as cracks and pans on rock surfaces, and seal carcasses, is the most likely determinant of whether or not a valley will sustain high microbial diversity (Cary, et al. 2010). This coincides with our observations of high archaeal diversity in ridge areas which are characterized by highly heterogeneous soil properties, thus providing suitable microhabitats for microbial colonization.

2.5.7 Limitations of molecular approaches

An important consideration when using T- RFLP as a proxy for diversity is that species diversity may be underestimated because different species may have identical restriction cut sites and therefore restriction fragments appear under the same length. On the other hand, T- RFLP can also overestimate species diversity due to partial digestion, which results in multiple RFL's from one single species. 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). In addition, overestimation of diversity can be caused by extraction and amplification of legacy DNA. Because in this study total genomic DNA was 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), and not an indication of actual viable microorganisms. Indeed, the combination of high salt content and extremely cold temperatures creates an environment in which nucleic acids might be very stable for long periods of time (legacy DNA). Therefore, it is probable that a percentage of the diversity observed is derived from preserved DNA rather than from viable microorganisms. However, recent manipulation experiments using RNA extracts revealed that Archaea are present in both the RNA and DNA component extracted from soils of Miers Valley (Dr. Charles Lee, personal communication). Thus, it can be assumed that at least some of the observed archaeal diversity and distribution patterns were obtained from living organisms and not legacy DNA. Nevertheless, overestimation of diversity due to the presence of legacy DNA is always possible when working with total genomic DNA.

2.5.8 Role of Crenarchaeota in Dry Valley soils

Using molecular studies to understand habitat preferences, probable carbon and energy sources, and physiological properties of Crenarchaeota in soils of the Dry Valleys is a challenge. Speculation of the attributes of Crenarchaeota detected in soils can only be based on close relatives that have been successfully isolated.

It has been shown that soil Crenarchaeota affiliated with group 1.1b contribute to ammonia oxidation in temperate (Hatzenpichler, et al. 2008; Treusch, et al. 2005), Polar and Mediterranean soils (Leininger, et al. 2006). In one study the estimation of *amoA* abundances indicated that ammonia- oxidizing Crenarchaeota outnumber their bacterial counterparts, particularly at depth (Leininger, et al. 2006). Based on results in this study it is proposed that Antarctic soil Crenarchaeota may also be active ammonia- oxidizing Crenarchaeota as the Dry Valley soil OTUs clustered

with the 16S rRNA gene from a Crenarchaeota fosmid54d9 (AJ627422, Appendix G, Figure 26), which contains *amoA* (Treusch, et al. 2005). Similarly, among the closest relatives of our clones was *Nitrososphaera* g*argensis,* a well known ammonia- oxidizing Crenarchaeote recovered from a mesophilic hot spring (Hatzenpichler, et al. 2008). To confirm that the detected *Crenarchaea* are actively involved in nitrification requires further investigation. Results of a recent manipulation experiment (Dr. Charles Lee, personal communication) indicated a positive response of the Crenarchaea to nitrogen addition. In brief, Dry Valley soils sampled after nitrogen addition showed an increase in archaeal abundance based on metagenomic analysis. In support, high archaeal diversity in Dry Valley ridge areas, known for high salt concentrations, gives further proof for an involvement in nitrification. Indeed, potential nitrification activity has been measured in Antarctic soils (Hopkins, et al. 2006; Yergeau, et al. 2007), although only bacterial, not archaeal *amoA* genes were investigated (Yergeau, et al. 2007). Ammonia is derived from mineralization of organic matter or precipitation of volatilized ammonium near bird colonies. Ayton and colleagues (2010) detected *Archaea* in coastal soils of the Ross Sea region with ammonia concentrations ranging from 1.2 to 4.7 mg/kg. These levels are similar to deglaciated soils in Austria which contained Crenarchaea affiliated with group 1.1b (Nicol, et al. 2006). Because of the close phylogenetic relationship of members of group 1.1b and known ammonia- oxidizing Crenarchaeota ammonia- oxidation may be a common feature of all group 1.1b Crenarchaeota. Members of this group have been detected in a number of different soil environments from different climatic zones (Hatzenpichler, et al. 2008; Leininger, et al. 2006; Spear, et al. 2007; Weidler, et al. 2007) indicating a worldwide distribution. Ammonia-oxidizing Crenarchaeota have been recently reported in Antarctic waters (Kalanetra, et al. 2009), supporting the possibility that ammonia- oxidizing Crenarchaeota are present in Antarctic soils. Crenarchaeal clones were recovered from coastal areas of the Ross Sea Region and were closely affiliated with group 1.1b (Ayton, et al. 2010). Extending these previous results it was shown in this study that members of group 1.1b are widely distributed in soils of the McMurdo Dry Valleys where they may contribute to nitrogen cycling via ammonia- oxidation.

2.6 Conclusion

In this study, soils from three eastern McMurdo Dry Valleys, Antarctica, and one adjacent inland plain, were analyzed based on their location along inter- and intra-valley transects. Extracted total genomic nucleic acids of soil samples were used for genotyping and construction of a clone library. The results of T- RFLP and geophysicochemical analyses were subjected to detailed statistical analyses in order to determine which environmental factors drive archaeal diversity and biogeography. To date, this is the first study to characterize and compare the composition of archaeal communities present in various soils of the McMurdo Dry Valleys using T- RFLP fingerprinting analysis. Combining these results with different geomorphic and physicochemical soil properties revealed for the first time what environmental factors shape diversity and distribution of these archaeal communities. Our T- RFLP data showed that archaeal species thrive in all mineral soils indicating a widespread distribution of Archaea across the eastern McMurdo Dry Valleys. Although overall diversity of these archaeal communities was relatively low, significant differences in diversity were identified between distinct landscape features. An inter- valley comparison revealed the presence of highly diverse archaeal communities on the ridge areas which separate the three valleys from each other. It appears that communities in coastal areas (moraine 3) sustain higher archaeal diversity than inland soils (moraine 2 and 1). This variation in diversity seems to be best explained by differences in moisture availability and availability of carbon and nitrogen in mineral soils that harbour these communities. These physicochemical differences of soils are primarily due to differences in lithology, mineralogy and exposure ages of soils. Thus, in support of the original hypothesis, abiotic factors appear to shape the archaeal communities living in Dry Valley mineral soils.The major component of these communities is one single species affiliated with Crenarchaeota Marine Group 1.1b. Members of group 1.1b are globally abundant in soils and are known to be involved in ammonia- oxidation thus suggesting that Antarctic Crenarchaeota may contribute to nitrogen cycling in Antarctic soils. Future studies should aim to extend phylogenetic analysis of highly diverse archaeal communities in

order to unveil true archaeal diversity in Antarctic soils and their functional linkages within this soil ecosystem.

Chapter 3: General Conclusions

The main objective of this study was to compare and contrast the archaeal biodiversity and biogeography in soils of the eastern McMurdo Dry Valleys and to determine the main abiotic, environmental factors driving archaeal diversity in these mineral soils.

The environmental factors that drive biogeography and biodiversity of microbial communities inhabiting the mineral soils of the Dry Valleys are still under investigation (Barrett, et al. 2006). Molecular- based research has shown that microbial communities are characterized by high bacterial diversity (Aislabie, et al. 2006; Hogg, et al. 2006; Niederberger, et al. 2008; Smith, et al. 2006), despite extremely harsh environmental conditions. Archaea, however, are the least known members of the microbial community and have been only recently detected in soils of the Ross Sea region indicating a patchy distribution, low diversity and low abundance (Ayton, et al. 2010). To date, this is the first study to apply T- RFLP community fingerprinting analysis to characterize and compare archaeal populations present in various Dry Valleys and attempt to identify the influences of the geochemical properties of the soils in shaping and maintaining these communities' distributions. Our T- RFLP data showed widespread distribution of Archaea across all three eastern Dry Valleys. To our knowledge, this is the first time that Archaea were successfully detected in a wide range of Dry Valley soils (Pointing, et al. 2009; Yergeau, et al. 2009). Although, overall diversity of these archaeal communities was relatively low, more diverse archaeal communities were detected on ridge areas and in coastal moraines.

The results of T- RFLP and geochemical analyses were combined for rigorous statistical analysis to elucidate the factors driving archaeal biodiversity in these three Dry Valleys. Variation in diversity seems to be best explained by differences in moisture availability, carbon and nitrogen concentrations and geomorphology of soils that harbour these communities. Availability of water and organic matter has been previously reported to support the diversity of bacterial communities detected in Antarctic mineral soils (Aislabie, et al. 2006; Barrett, et al. 2004; Gregorich,

et al. 2006; Zeglin, et al. 2009). Conversely, data from this study indicates a negative correlation between archaeal biodiversity and organic- rich soils.

Some of the physicochemical parameters measured in this study, such as soil water content, pH and even total carbon and nitrogen are likely to vary periodically or seasonally. In order to verify this, it would be useful to sample soils of the Dry Valleys at different time points throughout the year, to study this physicochemical variability. Moreover, spatial differences in carbon concentrations are highly pronounced in these polar desert ecosystems, due to complex interactions of a combination of factors such as geomorphology, chemical and physical soil properties (Hopkins, et al. 2005), thus making valid interpretations difficult.

An important consideration when using community fingerprinting methods is that T- RFLP may be useful for comparative, qualitative analyses of microbial populations, but cannot be used to assess the richness or diversity of complex communities (Dunbar, et al. 2000). Due to the lower detection threshold it is most likely that the number of peaks detected in T- FRLP assays underestimates the actual richness of any community with a long- tailed rank abundance distribution (Bent, et al. 2007). To accurately estimate diversity would require sampling of approximately 80% of the total species present (Gans, et al. 2005). Consequently, the sole use of fingerprinting methods cannot provide reliable diversity indices but does hold potential 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).

T- RFLP results can be linked to sequence data on the microorganisms present, to study their community composition on a species level. Our phylogenetic analysis revealed that the archaeal communities are composed mostly of one single species affiliated with Crenarchaeota Marine Group 1.1b. Members of group 1.1b are present in soils worldwide (Hatzenpichler, et al. 2008; Leininger, et al. 2006; Nicol and Schleper 2006; Spear, et al. 2007) and were shown to be highly

abundant in temperate soils (Leininger, et al. 2006) and the Antarctic ocean (Kalanetra, et al. 2009). Because most members of group 1.1b are capable of ammonia- oxidation, Antarctic Crenarchaeota may contribute to nitrogen cycling in mineral soils of the McMurdo Dry Valleys.

In order to put the fingerprinting results into perspective and to consolidate and extend the phylogenetic relationships, samples were prepared for 454 pyrosequencing analysis. Although, the sequences obtained did not pass the quality check, and were therefore excluded from this study, phylogenetic analysis revealed affiliation to Crenarchaeota group 1.1b. Due to the limited time frame afforded to this study, the pyrosequencing protocol had not undergone sufficient optimization to yield valuable data at time of thesis submission in order to be included here. Thus, future studies should aim to extend phylogenetic analysis of highly diverse archaeal communities in order to unveil true archaeal diversity in Antarctic soils and to determine functional linkages of Archaea within these soil ecosystems.

References

Abdo, Z., U. M. E. Schuette, S. J. Bent, C. J. Williams, L. J. Forney, and P. Joyce. 2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environmental Microbiology **8:**929-938.

Adams, B. J., R. D. Bardgett, E. Ayres, D. H. Wall, J. Aislabie, S. Bamforth, R. Bargagli, C. Cary, P. Cavacini, L. Connell, P. Convey, J. W. Fell, F. Frati, I. D. Hogg, K. K. Newsham, A. O'Donnell, N. Russell, R. D. Seppelt, and M. I. Stevens. 2006. Diversity and distribution of Victoria Land biota. Soil Biology & Biochemistry **38:**3003-3018.

Aislabie, J. M., K. L. Chhour, D. J. Saul, S. Miyauchi, J. Ayton, R. F. Paetzold, and M. R. Balks. 2006. Dominant Bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biology & Biochemistry **38:**3041-3056.

Allen, M. A., F. M. Lauro, T. J. Williams, D. Burg, K. S. Siddiqui, D. De Francisci, K. W. Y. Chong, O. Pilak, H. H. Chew, M. Z. De Maere, L. Ting, M. Katrib, C. Ng, K. R. Sowers, M. Y. Galperin, I. J. Anderson, N. Ivanova, E. Dalin, M. Martinez, A. Lapidus, L. Hauser, M. Land, T. Thomas, and R. Cavicchioli. 2009. The genome sequence of the psychrophilic archaeon, Methanococcoides burtonii: the role of genome evolution in cold adaptation. ISME Journal **3:**1012-1035.

Andersen, D. T., C. P. McKay, R. A. Wharton, and J. D. Rummel. 1992. Testing a Mars science outpost in the Antarctic Dry Valleys, p. 205-209. *In* R. D. Macelroy, M. M. Averner, T. W. Tibbitts, B. B. Bugbee, G. Horneck, and E. H. Dunlop (ed.), Life Sciences and Space Research Xxiv, vol. 12, The Hague, Netherlands.

Angel, R., M. I. M. Soares, E. D. Ungar, and O. Gillor. 2010. Biogeography of soil Archaea and Bacteria along a steep precipitation gradient. ISME Journal **4:**553-563.

Ayton, J., J. Aislabie, G. M. Barker, D. Saul, and S. Turner. 2010. Crenarchaeota affiliated with group 1.1b are prevalent in coastal mineral soils of the Ross Sea region of Antarctica. Environmental Microbiology **12:**689-703.

Babalola, O. O., B. M. Kirby, M. Le Roes-Hill, A. E. Cook, S. C. Cary, S. G. Burton, and D. A. Cowan. 2009. Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environmental Microbiology **11:**566-576.

Ball, B. A., R. A. Virginia, J. E. Barrett, A. N. Parsons, and D. H. Wall. 2009. Interactions between physical and biotic factors influence CO2 flux in Antarctic Dry Valley soils. Soil Biology & Biochemistry **41:**1510-1517.

Barns, S. M., R. E. Fundyga, M. W. Jeffries, and N. R. Pace. 1994. Remarkable archaeal diversity in a Yellowstone-National-Park hot-spring environment. Proceedings of the National Academy of Sciences of the United States of America **91:**1609-1613.

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

Barrett, J. E., R. A. Virginia, D. W. Hopkins, J. Aislabie, R. Bargagli, J. G. Bockheim, I. B. Campbell, W. B. Lyons, D. L. Moorhead, J. N. Nkem, R. S. Sletten, H. Steltzer, D. H. Wall, and M. D. Wallenstein. 2006. Terrestrial ecosystem processes of Victoria Land, Antarctica. Soil Biology & Biochemistry **38:**3019-3034.

Barrett, J. E., R. A. Virginia, W. B. Lyons, D. M. McKnight, J. C. Priscu, P. T. Doran, A. G. Fountain, D. H. Wall, and D. L. Moorhead. 2007. Biogeochemical stoichiometry of Antarctic Dry Valley ecosystems. Journal of Geophysical Research-Biogeosciences **112:**12.

Barrett, J. E., R. A. Virginia, A. N. Parsons, and D. H. Wall. 2006. Soil carbon turnover in the McMurdo Dry Valleys, Antarctica. Soil Biology & Biochemistry **38:**3065-3082.

Barrett, J. E., R. A. Virginia, D. H. Wall, A. N. Parsons, L. E. Powers, and M. B. Burkins. 2004. Variation in biogeochemistry and soil biodiversity across spatial scales in a Polar Desert Ecosystem. Ecology **85:**3105-3118.

Bartlett, K. B., and R. C. Harriss. 1993. Review and assessment of methane emissions from wetlands. Chemosphere **26:**261-320.

Bent, S. J., J. D. Pierson, and L. J. Forney. 2007. Measuring species richness based on microbial community fingerprints: The emperor has no clothes. Applied and Environmental Microbiology **73:**2399-2399.

Bintrim, S. B., T. J. Donohue, J. Handelsman, G. P. Roberts, and R. M. Goodman. 1997. Molecular phylogeny of Archaea from soil. Proceedings of the National Academy of Sciences of the United States of America **94:**277-282.

Blochl, E., R. Rachel, S. Burggraf, D. Hafenbradl, H. W. Jannasch, and K. O. Stetter. 1997. Pyrolobus fumarii, gen. and sp. nov., represents a novel group of Archaea, extending the upper temperature limit for life to 113 degrees C. Extremophiles **1:**14-21.

Bockheim, J. G. 2008. Functional diversity of soils along environmental gradients in the Ross Sea region, Antarctica. Geoderma **144:**32-42.

Bockheim, J. G. 2002. Landform and soil development in the McMurdo Dry Valleys, Antarctica: a regional synthesis. Arctic, Antarctic and Alpine Research **34:**308-317.

Bockheim, J. G. 1997. Properties and classification of cold desert soils from Antarctica. Soil Science Society of America Journal **61:**224-231.

Bockheim, J. G., and M. McLeod. 2008. Soil distribution in the McMurdo Dry Valleys, Antarctica. Geoderma **144:**43-49.

Borneman, J., and E. W. Triplett. 1997. Molecular microbial diversity in soils from eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology **63:**2647-2653.

Bowman, J. P., S. A. McCammon, J. A. E. Gibson, L. Robertson, and P. D. Nichols. 2003. Prokaryotic metabolic activity and community

structure in Antarctic continental shelf sediments. Applied and Environmental Microbiology **69:**2448-2462.

Bowman, J. P., S. M. Rea, S. A. McCammon, and T. A. McMeekin. 2000. Diversity and community structure within anoxic sediment from marine salinity meromictic lakes and a coastal meromictic marine basin, Vestfold Hills, Eastern Antarctica. Environmental Microbiology **2:**227-237.

Brambilla, E., H. Hippe, A. Hagelstein, B. J. Tindall, and E. Stackebrandt. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles **5:**23-33.

Brandes, J. A., N. Z. Boctor, G. D. Cody, B. A. Cooper, R. M. Hazen, and H. S. Yoder. 1998. Abiotic nitrogen reduction on the early earth. Nature **395:**365-367.

Brazelton, W. J., M. L. Sogin, and J. A. Baross. 2010. Multiple scales of diversification within natural populations of Archaea in hydrothermal chimney biofilms. Environmental Microbiology Reports **2:**236-242.

Brim, H., H. Heuer, E. Krogerrecklenfort, M. Mergeay, and K. Smalla. 1999. Characterization of the bacterial community of a zinc-polluted soil. Canadian Journal of Microbiology **45:**326-338.

Brochier-Armanet, C., B. Boussau, S. Gribaldo, and P. Forterre. 2008. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nature Reviews Microbiology **6:**245-252.

Brook, E. J., M. D. Kurz, R. P. Ackert, G. Raisbeck, and F. Yiou. 1995. Cosmogenic nuclide exposure ages and glacial history of Late Quaternary Ross Sea Drift in McMurdo Sound, Antarctica. Earth and Planetary Science Letters **131:**41-56.

Buckley, D. H., J. R. Graber, and T. M. Schmidt. 1998. Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. Applied and Environmental Microbiology **64:**4333-4339.

Burkins, M. B., R. A. Virginia, C. P. Chamberlain, and D. H. Wall. 2000. Origin and distribution of soil organic matter in Taylor Valley, Antarctica. Ecology **81:**2377-2391.

Burkins, M. B., R. A. Virginia, and D. H. Wall. 2001. Organic carbon cycling in Taylor Valley, Antarctica: quantifying soil reservoirs and soil respiration. Global Change Biology **7:**113-125.

Campbell, I. B., and G. G. C. Claridge. 1987. Antarctica: Soils, weathering processes and environment. Elsevier Science Pub. Co. Inc., New York.

Canfield, D. E., M. T. Rosing, and C. Bjerrum. 2006. Early anaerobic metabolisms. Philosophical Transactions of the Royal Society B-Biological Sciences **361:**1819-1834.

Cary, S. C., I. R. McDonald, J. E. Barrett, and D. A. Cowan. 2010. On the rocks: the microbiology of Antarctic Dry Valley soils. Nature Reviews Microbiology **8:**129-138.

Cavicchioli, R. 2006. Cold-adapted Archaea. Nature Reviews Microbiology **4:**331-343.

Chaban, B., S. Y. M. Ng, and K. F. Jarrell. 2006. Archaeal habitats - from the extreme to the ordinary. Canadian Journal of Microbiology **52:**73-116.

Chong, S. C., Y. T. Liu, M. Cummins, D. L. Valentine, and D. R. Boone. 2002. Methanogenium marinum sp nov., a H-2-using methanogen from Skan Bay, Alaska, and kinetics of H-2 utilization. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **81:**263-270.

Church, M. J., E. F. DeLong, H. W. Ducklow, M. B. Karner, C. M. Preston, and D. M. Karl. 2003. Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula. Limnology and Oceanography **48:**1893-1902.

Church, M. J., B. Wai, D. M. Karl, and E. F. DeLong. 2010. Abundances of crenarchaeal amoA genes and transcripts in the Pacific Ocean. Environmental Microbiology **12:**679-688.

Clarke, K. R., and M. Ainsworth. 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series **92:**205-219.

Conrad, R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports **1:**285-292.

Convey, P. 1996. The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. Biological Reviews of the Cambridge Philosophical Society **71:**191-225.

de la Torre, J. R., C. B. Walker, A. E. Ingalls, M. Konneke, and D. A. Stahl. 2008. Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. Environmental Microbiology **10:**810-818.

DeLong, E. F., C. M. Preston, T. Mincer, V. Rich, S. J. Hallam, N. U. Frigaard, A. Martinez, M. B. Sullivan, R. Edwards, B. R. Brito, S. W. Chisholm, and D. M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science **311:**496-503.

DeLong, E. F., L. T. Taylor, T. L. Marsh, and C. M. Preston. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Applied and Environmental Microbiology **65:**5554-5563.

DeLong, E. F., K. Y. Wu, B. B. Prezelin, and R. V. M. Jovine. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature **371:**695-697.

Denton, G. H., J. G. Bockheim, S. C. Wilson, and M. Stuiver. 2004. Late Wisconsin and early Holocene glacial history, inner Ross Embayment, Antarctia. Quaternary Research **31:**151-182.

Deppenmeier, U., V. Muller, and G. Gottschalk. 1996. Pathways of energy conservation in methanogenic Archaea. Archives of Microbiology **165:**149-163.

Doran, P. T., J. C. Priscu, W. B. Lyons, J. E. Walsh, A. G. Fountain, D. M. McKnight, D. L. Moorhead, R. A. Virginia, D. H. Wall, G. D. Clow, C. H. Fritsen, C. P. McKay, and A. N. Parsons. 2002. Antarctic climate cooling and terrestrial ecosystem response. Nature **415:**517-520.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2000. Assessment of microbial diversity in four southwestern United States soils by 16S

rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology **66:**2943-2950.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied and Environmental Microbiology **67:**190-197.

Elberling, B., E. G. Gregorich, D. W. Hopkins, A. D. Sparrow, P. Novis, and L. G. Greenfield. 2006. Distribution and dynamics of soil organic matter in an Antarctic Dry Valley. Soil Biology & Biochemistry **38:**3095-3106.

Feller, G., and C. Gerday. 2003. Psychrophilic enzymes: Hot topics in cold adaptation. Nature Reviews Microbiology **1:**200-208.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**.

Fisher, M. M., and E. W. Triplett. 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.

Fountain, A. G., W. B. Lyons, M. B. Burkins, G. L. Dana, P. T. Doran, K. J. Lewis, D. M. McKnight, D. L. Moorhead, A. N. Parsons, J. C. Priscu, D. H. Wall, R. A. Wharton, and R. A. Virginia. 1999. Physical controls on the Taylor Valley ecosystem, Antarctica. Bioscience **49:**961-971.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal **1:**19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing Archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America **102:**14683-14688.

Franzmann, P. D., Y. T. Liu, D. L. Balkwill, H. C. Aldrich, E. C. deMacario, and D. R. Boone. 1997. Methanogenium frigidum sp. nov., a psychrophilic, H-2-using methanogen from Ace Lake, Antarctica. International Journal of Systematic Bacteriology **47:**1068-1072.

Franzmann, P. D., N. Springer, W. Ludwig, E. C. Demacario, and M. Rohde. 1992. A Methanogenic archaeon from Ace Lake, Antarctica: Methanococcoides burtonii sp. nov. Systematic and Applied Microbiology **15:**573-581.

Freckman, D. W., and R. A. Virginia. 1997. Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. Ecology **78:**363-369.

Friedrich, M. W., D. Schmitt-Wagner, T. Lueders, and A. Brune. 2001. Axial differences in community structure of Crenarchaeota and Euryarchaeota in the highly compartmentalized gut of the soil-feeding termite Cubitermes orthognathus. Applied and Environmental Microbiology **67:**4880-4890.

Frijlink, M. J., T. Abee, H. J. Laanbroek, W. Deboer, and W. N. Konings. 1992. The bioenergetics of ammonia and hydroxylamine

oxidation in Nitrosomonas europaea at acid and alkaline pH. Archives of Microbiology **157:**194-199.

Fritsen, C. H., A. M. Grue, and J. C. Priscu. 2000. Distribution of organic carbon and nitrogen in surface soils in the McMurdo Dry Valleys, Antarctica. Polar Biology **23:**121-128.

Galagan, J. E., C. Nusbaum, A. Roy, M. G. Endrizzi, P. Macdonald, W. FitzHugh, S. Calvo, R. Engels, S. Smirnov, D. Atnoor, A. Brown, N. Allen, J. Naylor, N. Stange-Thomann, K. DeArellano, R. Johnson, L. Linton, P. McEwan, K. McKernan, J. Talamas, A. Tirrell, W. J. Ye, A. Zimmer, R. D. Barber, I. Cann, D. E. Graham, D. A. Grahame, A. M. Guss, R. Hedderich, C. Ingram-Smith, H. C. Kuettner, J. A. Krzycki, J. A. Leigh, W. X. Li, J. F. Liu, B. Mukhopadhyay, J. N. Reeve, K. Smith, T. A. Springer, L. A. Umayam, O. White, R. H. White, E. C. de Macario, J. G. Ferry, K. F. Jarrell, H. Jing, A. J. L. Macario, I. Paulsen, M. Pritchett, K. R. Sowers, R. V. Swanson, S. H. Zinder, E. Lander, W. W. Metcalf, and B. Birren. 2002. The genome of M-acetivorans reveals extensive metabolic and physiological diversity. Genome Research **12:**532-542.

Galand, P. E., E. O. Casamayor, D. L. Kirchman, M. Potvin, and C. Lovejoy. 2009. Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. ISME Journal **3:**860-869.

Galand, P. E., E. O. Casamayor, D. L. Kirchman, M. Potvin, and C. Lovejoy. 2009. Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing (vol 7, pg 860, 2009). Isme Journal **3:**1116-1116.

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

Ganzert, L., G. Jurgens, U. Munster, and D. Wagner. 2007. Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. FEMS Microbiology Ecology **59:**476-488.

Gibson, J. A. E., M. R. Miller, N. W. Davies, G. P. Neill, D. S. Nichols, and J. K. Volkman. 2005. Unsaturated diether lipids in the psychrotrophic archaeon Halorubrum lacusprofundi. Systematic and Applied Microbiology **28:**19-26.

Gillan, D. C., and B. Danis. 2007. The archaebacterial communities in Antarctic bathypelagic sediments. Deep-Sea Research Part Ii-Topical Studies in Oceanography **54:**1682-1690.

Glatz, R. E., P. W. Lepp, B. B. Ward, and C. A. Francis. 2006. Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-covered Lake Bonney, Antarctica. Geobiology **4:**53-67.

Gregorich, E. G., D. W. Hopkins, B. Elberling, A. D. Sparrow, P. Novis, L. G. Greenfield, and P. Rochette. 2006. Emission of CO2, CH4 and N2O from lakeshore soils in an Antarctic Dry Valley. Soil Biology & Biochemistry **38:**3120-3129.

Hallam, S. J., T. J. Mincer, C. Schleper, C. M. Preston, K. Roberts, P. M. Richardson, and E. F. DeLong. 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental

genomic analyses of marine Crenarchaeota. Plos Biology **4:**520-536.

Hartmann, M., B. Frey, R. Kolliker, and F. Widmer. 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:**349-360.

Hatzenpichler, R., E. V. Lebedeva, E. Spieck, K. Stoecker, A. Richter, H. Daims, and M. Wagner. 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proceedings of the National Academy of Sciences of the United States of America **105:**2134-2139.

Herndl, G. J., T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler. 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Applied and Environmental Microbiology **71:**2303-2309.

Hewson, I., J. A. Steele, D. G. Capone, and J. A. Fuhrman. 2006. Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. Limnology and Oceanography **51:**1274-1283.

Hogg, I. D., S. C. Cary, P. Convey, K. K. Newsham, A. G. O'Donnell, B. J. Adams, J. Aislabie, F. Frati, M. I. Stevens, and D. H. Wall. 2006. Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? Soil Biology & Biochemistry **38:**3035-3040.

Hopkins, D. W., B. Eberling, L. G. Greenfield, E. G. Gregorich, and P. Novis. 2005. Soil micro- organisms in Antarctic Dry Valleys: Resource supply and untilization., p. 71-84. *In* G. M. Gadd, K. T. Semple, and H. M. Lappin-Scott (ed.), Microorganisms and Earth Science: Advances in Geomicrobilogy, SGM Symposium 65. Cambridge University Press, Cambridge, UK.

Hopkins, D. W., A. D. Sparrow, B. Elberling, E. G. Gregorich, P. M. Novis, L. G. Greenfield, and E. L. Tilston. 2006. Carbon, nitrogen and temperature controls on microbial activity in soils from an Antarctic Dry Valley. Soil Biology & Biochemistry **38:**3130-3140.

Hopkins, D. W., A. D. Sparrow, E. G. Gregorich, B. Elberling, P. Novis, F. Fraser, C. Scrimgeour, P. G. Dennis, W. Meier-Augenstein, and L. G. Greenfield. 2009. Isotopic evidence for the provenance and turnover of organic carbon by soil microorganisms in the Antarctic Dry Valleys. Environmental Microbiology **11:**597-608.

Hornibrook, E. R. C., F. J. Longstaffe, and W. S. Fyfe. 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. Geochimica Et Cosmochimica Acta **61:**745-753.

Huse, S. M., J. A. Huber, H. G. Morrison, M. L. Sogin, and D. Mark Welch. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biology **8**.

Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M. Lever, A. Lauer, M. Suzuki, K. Takai, M. Delwiche, F. S. Colwell, K. H. Nealson, K. Horikoshi, S. D'Hondt, and B. B. Jorgensen. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments, on the Pacific Ocean

Margin. Proceedings of the National Academy of Sciences of the United States of America **103:**2815-2820.

Jahn, U., R. Summons, H. Sturt, E. Grosjean, and H. Huber. 2004. Composition of the lipids of Nanoarchaeum equitans and their origin from its host Ignicoccus sp strain KIN4/I. Archives of Microbiology **182:**404-413.

Jeanthon, C., S. L'Haridon, A. L. Reysenbach, E. Corre, M. Vernet, P. Messner, U. B. Sleytr, and D. Prieur. 1999. Methanococcus vulcanius sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise, and identification of Methanococcus sp. DSM 4213(T) as Methanococcus fervens sp. nov. International Journal of Systematic Bacteriology **49:**583-589.

Jin, T., T. Zhang, and Q. M. Yan. 2010. Characterization and quantification of ammonia-oxidizing Archaea (AOA) and Bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. Applied Microbiology and Biotechnology **87:**1167-1176.

Junge, K., H. Eicken, and J. W. Deming. 2004. Bacterial activity at -2 to -20 degrees C in Arctic wintertime sea ice. Applied and Environmental Microbiology **70:**550-557.

Jurgens, G., K. Lindstrom, and A. Saano. 1997. Novel group within the kingdom Crenarchaeota from boreal forest soil. Applied and Environmental Microbiology **63:**803-805.

Kalanetra, K. M., N. Bano, and J. T. Hollibaugh. 2009. Ammonia-oxidizing Archaea in the Arctic Ocean and Antarctic coastal waters. Environmental Microbiology **11:**2434-2445.

Kappen, L., M. Breuer, and M. Bolter. 1991. Ecological and physiological investigations in continental Antarctic cryptogams: Photosynthetic production of Usnea sphacelatadiurnal courses, models, and the effect of photoinhibition. Polar Biology **11:**393-401.

Karcher, U., H. Schroder, E. Haslinger, G. Allmaier, R. Schreiner, F. Wieland, A. Haselbeck, and H. Konig. 1993. Primary structure of the heterosaccharide of the surface glycoprotein of Methanothermus fervidus. Journal of Biological Chemistry **268:**26821-26826.

Karr, E. A., J. M. Ng, S. M. Belchik, W. M. Sattley, M. T. Madigan, and L. A. Achenbach. 2006. Biodiversity of methanogenic and other Archaea in the permanently frozen Lake Fryxell, Antarctica. Applied and Environmental Microbiology **72:**1663-1666.

Kemnitz, D., S. Kolb, and R. Conrad. 2005. Phenotypic characterization of Rice Cluster III Archaea without prior isolation by applying quantitative polymerase chain reaction to an enrichment culture. Environmental Microbiology **7:**553-565.

Kendall, M. M., G. D. Wardlaw, C. F. Tang, A. S. Bonin, Y. T. Liu, and D. L. Valentine. 2007. Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of Methanogenium boonei sp nov. Applied and Environmental Microbiology **73:**407-414.

Kennedy, A. D. 1993. Water as a limiting factor in the Antarctic terrestrial environment- A biogeographical synthesis. Arctic and Alpine Research **25:**308-315.

Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2007. Standing stocks and activity of Archaea and

Bacteria in the western Arctic Ocean. Limnology and Oceanography **52:**495-507.

Koenneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437:**543-546.

Kotsyurbenko, O. R., M. W. Friedrich, M. V. Simankova, A. N. Nozhevnikova, P. N. Golyshin, K. N. Timmis, and R. Conrad. 2007. Shift from acetoclastic to H-2-dependent methanogenes is in a West Siberian peat bog at low pH values and isolation of an acidophilic Methanobactetium strain. Applied and Environmental Microbiology **73:**2344-2348.

Kowalchuk, G. A., and J. R. Stephen. 2001. Ammonia-oxidizing Bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55:**485-529.

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

L'Haridon, S., V. Cilia, P. Messner, G. Raguenes, A. Gambacorta, U. B. Sleytr, D. Prieur, and C. Jeanthon. 1998. Desulfurobacterium thermolithotrophum gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. International Journal of Systematic Bacteriology **48:**701-711.

Lauro, F. M., K. Tran, A. Vezzi, N. Vitulo, G. Valle, and D. H. Bartlett. 2008. Large-scale transposon mutagenesis of Photobactetium profundum SS9 reveals new genetic loci important for growth at low temperature and high pressure. Journal of Bacteriology **190:**1699-1709.

Legendre, P., and L. Legendre. 1998. Ordination in reduced space, p. 387-480, Numerical Ecology (2nd English edition). Elsevier Science, Amsterdam, The Netherlands.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G. W. Nicol, J. I. Prosser, S. C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature **442:**806-809.

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

Liesack, W., and P. F. Dunfield. 2002. Biodiversity in soils: Use of molecular methods for its characterization, p. 528-544. *In* G. Bitton (ed.), Encyclopedia of Environmental Microbiology. Wiley & Sons Inc., New York.

Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology **63:**4516-4522.

Luna, G. M., K. Stumm, A. Pusceddu, and R. Danovaro. 2009. Archaeal diversity in deep-sea sediments estimated by means of different terminal-restriction fragment length polymorphisms (T-RFLP) protocols. Current Microbiology **59:**356-361.

Lynn, D. J., G. A. C. Singer, and D. A. Hickey. 2002. Synonymous codon usage is subject to selection in thermophilic Bacteria. Nucleic Acids Research **30:**4272-4277.

Mancinelli, R. L., and C. P. McKay. 1988. The evolution of nitrogen cycling. Origins of Life and Evolution of the Biosphere **18:**311-325.

Mann, H. B., and D. R. Whitney. 1947. On a test of whether one of two random variables is stochastically larger than the other. The Annals of Mathematical Statistics **18:**50-60.

Mardanov, A., V. Gumerov, A. Beletsky, E. Bonch-Osmolovskaya, N. Ravin, and K. Skryabin. 2010. Characteristic of biodiversity of thermophilic microbial community by parallel pyrosequencing method. Doklady Biochemistry and Biophysics **432:**110-113.

Marsh, T. L. 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:**323-327.

Massana, R., L. J. Taylor, A. E. Murray, K. Y. Wu, W. H. Jeffrey, and E. F. DeLong. 1998. Vertical distribution and temporal variation of marine planktonic Archaea in the Gerlache Strait, Antarctica, during early spring. Limnology and Oceanography **43:**607-617.

Moeseneder, M. M., C. Winter, J. M. Arrieta, and G. J. Herndl. 2001. Terminal-restriction fragment length polymorphism (T-RFLP) screening of a marine archaeal clone library to determine the different phylotypes. Journal of Microbiological Methods **44:**159-172.

Moorhead, D. L., P. T. Doran, A. G. Fountain, W. B. Lyons, D. M. McKnight, J. C. Priscu, R. A. Virginia, and D. H. Wall. 1999. Ecological legacies: Impacts on ecosystems of the McMurdo Dry Valleys. Bioscience **49:**1009-1019.

Morozova, D., and D. Wagner. 2007. Stress response of methanogenic Archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. FEMS Microbiology Ecology **61:**16-25.

Murray, A. E., and J. J. Grzymski. 2007. Diversity and genomics of Antarctic marine micro-organisms. Philosophical Transactions of the Royal Society B **362:**2259-2271.

Murray, A. E., C. M. Preston, R. Massana, L. T. Taylor, A. Blakis, K. Wu, and E. F. DeLong. 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Applied and Environmental Microbiology **64:**2585-2595.

Muyzer, G., E. C. Dewaal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal RNA. Applied and Environmental Microbiology **59:**695-700.

Narita, S., K. Kanamaru, S. Matsuyama, and H. Tokuda. 2003. A mutation in the membrane subunit of an ABC transporter LolCDE complex causing outer membrane localization of lipoproteins against their inner membrane-specific signals. Molecular Microbiology **49:**167-177.

Nichols, D. S., M. R. Miller, N. W. Davies, A. Goodchild, M. Raftery, and R. Cavicchioli. 2004. Cold adaptation in the Antarctic archaeon Methanococcoides burtonii involves membrane lipid unsaturation. Journal of Bacteriology **186:**8508-8515.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing Archaea and Bacteria. Environmental Microbiology **10:**2966-2978.

Nicol, G. W., and C. Schleper. 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? Trends in Microbiology **14:**207-212.

Nicol, G. W., D. Tscherko, L. Chang, U. Hammesfahr, and J. I. Prosser. 2006. Crenarchaeal community assembly and microdiversity in developing soils at two sites associated with deglaciation. Environmental Microbiology **8:**1382-1393.

Nicol, G. W., D. Tscherko, T. M. Embley, and J. I. Prosser. 2005. Primary succession of soil Crenarchaeota across a receding glacier foreland. Environmental Microbiology **7:**337-347.

Niederberger, T. D., I. R. McDonald, and S. C. Cary. Archaeal microbial diversity in polar environments. *In* L. G. Whyte and M. R. Miller (ed.), Polar Microbiology. American Society of Microbiology Press. (in press)

Niederberger, T. D., I. R. McDonald, A. L. Hacker, R. M. Soo, J. E. Barrett, D. H. Wall, and S. C. Cary. 2008. Microbial community composition in soils of Northern Victoria Land, Antarctica. Environmental Microbiology **10:**1713-1724.

Nishizawa, T., M. Komatsuzaki, N. Kaneko, and H. Ohta. 2008. Archaeal diversity of upland rice field soils assessed by the terminal restriction fragment length polymorphism method combined with real time quantitative-PCR and a clone library analysis. Microbes and Environments **23:**237-243.

Nkem, J. N., R. A. Virginia, J. E. Barrett, D. H. Wall, and G. Li. 2006. Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. Polar Biology **29:**643-651.

Novis, P. M., D. Whitehead, E. G. Gregorich, J. E. Hunt, A. D. Sparrow, D. W. Hopkins, B. Elberling, and L. G. Greenfield. 2007. Annual carbon fixation in terrestrial populations of Nostoc commune (Cyanobacteria) from an Antarctic Dry Valley is driven by temperature regime. Global Change Biology **13:**1224-1237.

Ochman, H., and A. C. Wilson. 1987. Evolution in Bacteria - Evidence for a universal substitution rate in cellular genomes. Journal of Molecular Evolution **26:**74-86.

Ochsenreiter, T., D. Selezi, A. Quaiser, L. Bonch-Osmolovskaya, and C. Schleper. 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology **5:**787-797.

Oros-Sichler, M., R. Costa, H. Heuer, and K. Small. 2007. Molecular fingerprinting techniques to analyze soil microbial communities, p. 355-386. *In* J. D. van Elsas, J. K. Jansson, and J. T. Trevors (ed.), Modern Soil Microbiology - second edition. CRC Press, Boca Raton, FL, USA.

Ouverney, C. C., and J. A. Fuhrman. 2000. Marine planktonic Archaea take up amino acids. Applied and Environmental Microbiology **66:**4829-+.

Pannewitz, S., T. G. A. Green, K. Maysek, M. Schlensog, R. Seppelt, L. G. Sancho, R. Turk, and B. Schroeter. 2005. Photosynthetic responses of three common mosses from continental Antarctica. Antarctic Science **17:**341-352.

Paramonov, N. A., L. A. S. Parolis, H. Parolis, I. F. Boan, J. Anton, and F. Rodriguez-Valera. 1998. The structure of the exocellular polysaccharide produced by the Archaeon Haloferax gibbonsii (ATCC 33959). Carbohydrate Research **309:**89-94.

Pearson, A., Z. Huang, A. E. Ingalls, C. S. Romanek, J. Wiegel, K. H. Freeman, R. H. Smittenberg, and C. L. Zhang. 2004. Nonmarine crenarchaeol in Nevada hot springs. Applied and Environmental Microbiology **70:**5229-5237.

Pearson, A., A. P. McNichol, B. C. Benitez-Nelson, J. M. Hayes, and T. I. Eglinton. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific Delta C-14 analysis. Geochimica Et Cosmochimica Acta **65:**3123-3137.

Pesaro, M., F. Widmer, G. Nicollier, and J. Zeyer. 2003. Effects of freeze-thaw stress during soil storage on microbial communities and methidathion degradation. Soil Biology & Biochemistry **35:**1049-1061.

Pewe, T. L. 1960. Multiple glaciation in the McMurdo Sound region, Antarctica: A progress report. The Journal of Geology **68:**498-514.

Pewe, T. L. 1958. Quaternary glacial geology of the McMurdo Sound region, Antarctica- a progress report. IGY Glaciological Rept. **1:**1-4.

Poage, M. A., J. E. Barrettt, R. A. Virginia, and D. H. Wall. 2008. The influence of soil geochemistry on nematode distribution, McMurdo Dry Valleys, Antarctica. Arctic Antarctic and Alpine Research **40:**119-128.

Pointing, S. B., Y. Chan, D. C. Lacap, M. C. Y. Lau, J. A. Jurgens, and R. L. Farrell. 2009. Highly specialized microbial diversity in hyper-arid polar desert. Proceedings of the National Academy of Sciences of the United States of America **107:**1254-1254.

Preston, C. M., K. Y. Wu, T. F. Molinski, and E. F. DeLong. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: Cenarchaeum symbiosum gen nov, sp, nov. Proceedings of the National Academy of Sciences of the United States of America **93:**6241-6246.

Prosser, J. I. 2002. Molecular and functional diversity in soil microorganisms. Plant and Soil **244:**9-17.

Purdy, K. J., M. A. Munson, D. B. Nedwell, and T. M. Embley. 2002. Comparison of the molecular diversity of the methanogenic community at the brackish and marine ends of a UK estuary. FEMS Microbiology Ecology **39:**17-21.

Purdy, K. J., D. B. Nedwell, and T. M. Embley. 2003. Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Applied and Environmental Microbiology **69:**3181-3191.

Qian, P.-Y., Y. Wang, O. O. Lee, S. C. K. Lau, J. Yang, F. F. Lafi, A. Al-Suwailem, and T. Y. H. Wong. 2010. Vertical stratification of

microbial communities in the Red Sea revealed by 16S rDNA pyrosequencing. ISME Journal.

Reid, I. N., W. B. Sparks, S. Lubnow, M. McGrath, and J. Livio Valenti. 2006. Terrestrial models for extraterrestrial life: Methanogens and halophiles at Martian temperatures. International Journal of Astrobiology **5:**89-97.

Rivkina, E., V. Shcherbakova, K. Laurinavichius, L. Petrovskaya, K. Krivushin, G. Kraev, S. Pecheritsina, and D. Gilichinsky. 2007. Biogeochemistry of methane and methanogenic Archaea in permafrost. FEMS Microbiology Ecology **61:**1-15.

Rueckert, A., and H. W. Morgan. 2007. Removal of contaminating DNA from polymerase chain reaction using ethidium monoazide. Journal of Microbiological Methods **68:**596-600.

Saitou, N., and M. Nei. 1987. The Neighbor-Joining Method - A new metahod for reconstructing phylogenetic trees. Molecular Biology and Evolution **4:**406-425.

Schulz, S., and R. Conrad. 1996. Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. FEMS Microbiology Ecology **20:**1-14.

Simankova, M. V., O. R. Kotsyurbenko, T. Lueders, A. N. Nozhevnikova, B. Wagner, R. Conrad, and M. W. Friedrich. 2003. Isolation and characterization of new strains of methanogens from cold terrestrial habitats. Systematic and Applied Microbiology **26:**312-318.

Singh, N., M. M. Kendall, Y. T. Liu, and D. R. Boone. 2005. Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: Description of Methanococcoides alaskense sp nov., and emended description of Methanosarcina baltica. International Journal of Systematic and Evolutionary Microbiology **55:**2531-2538.

Smalla, K., M. Oros-Sichler, A. Milling, H. Heuer, S. Baumgarte, R. Becker, G. Neuber, S. Kropf, A. Ulrich, and C. C. Tebbe. 2007. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? Journal of Microbiological Methods **69:**470-479.

Smit, E., P. Leeflang, and K. Wernars. 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:**249-261.

Smith, J. J., L. A. Tow, W. Stafford, C. Cary, and D. A. Cowan. 2006. Bacterial diversity in three different Antarctic cold desert mineral soils. Microbial Ecology **51:**413-421.

Smith, R. C., B. B. Prezelin, K. S. Baker, R. R. Bidigare, N. P. Boucher, T. Coley, D. Karentz, S. Macintyre, H. A. Matlick, D. Menzies, M. Ondrusek, Z. Wan, and K. J. Waters. 1992. Ozone depletion-ultraviolet-radiation and phytoplankton biology in Antarctic waters. Science **255:**952-959.

Soo, R. M., S. A. Wood, J. J. Grzymski, I. R. McDonald, and S. C. Cary. 2009. Microbial biodiversity of thermophilic communities in hot mineral soils of Tramway Ridge, Mount Erebus, Antarctica. Environmental Microbiology **11:**715-728.

Spang, A., R. Hatzenpichler, C. Brochier-Armanet, T. Rattei, P. Tischler, E. Spieck, W. Streit, D. A. Stahl, M. Wagner, and C. Schleper. 2010. Distinct gene set in two different lineages of ammonia-oxidizing Archaea supports the phylum Thaumarchaeota. Trends in Microbiology **18:**331-340.

Spear, J. R., H. A. Barton, C. E. Robertson, C. A. Francis, and N. R. Pace. 2007. Microbial community biofabrics in a geothermal mine adit. Applied and Environmental Microbiology **73:**6172-6180.

Stahl, D. A., and R. Amann. 1991. Development and application of nucleic acid probes in bacterial systematics. John Wiley & Sons Ltd., Chichester, England.

Steven, B., W. H. Pollard, C. W. Greer, and L. G. Whyte. 2008. Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. Environmental Microbiology **10:**3388-3403.

Stoica, E. 2009. Application of T-RFLP analysis to the study of the coastal Black Sea bacterioplankton. Romanian Biotechnological Letters **14:**4710-4719.

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

Striegl, R. G., T. A. McConnaughey, D. C. Thorstenson, E. P. Weeks, and J. C. Woodward. 1992. Consumption of atmospheric methane by desert soils. Nature **357:**145-147.

Takai, K., and K. Horikoshi. 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Applied and Environmental Microbiology **66:**5066-+.

Takai, K., M. R. Mormile, J. P. McKinley, F. J. Brockman, W. E. Holben, W. P. Kovacik, and J. K. Fredrickson. 2003. Shifts in archaeal communities associated with lithological and geochemical variations in subsurface Cretaceous rock. Environmental Microbiology **5:**309-320.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution **24:**1596-1599.

Tamura, K., M. Nei, and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences **101:**11030-11035.

Taton, A., S. Grubisic, E. Brambilla, R. De Wit, and A. Wilmotte. 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.

Thauer, R. K., A. K. Kaster, H. Seedorf, W. Buckel, and R. Hedderich. 2008. Methanogenic Archaea: Ecologically relevant differences in energy conservation. Nature Reviews Microbiology **6:**579-591.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. Clustal-W - Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research **22:**4673-4680.

Tourna, M., T. E. Freitag, G. W. Nicol, and J. I. Prosser. 2008. Growth, activity and temperature responses of ammonia-oxidizing Archaea and Bacteria in soil microcosms. Environmental Microbiology **10:**1357-1364.

Treusch, A. H., S. Leininger, A. Kletzin, S. C. Schuster, H. P. Klenk, and C. Schleper. 2005. Novel genes for nitrite reductase and amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environmental Microbiology **7:**1985-1995.

Ugolini, F. C., and J. G. Bockheim. 2008. Antarctic soils and soil formation in a changing environment: A review. Geoderma **144:**1-8.

Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Y. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers, and H. O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66-74.

Vishniac, H. S. 1993. Antarctic Microbiology, p. 297-341. Wiley-Liss, New York.

von Klein, D., H. Arab, H. Volker, and M. Thomm. 2002. Methanosarcina baltica, sp nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. Extremophiles **6:**103-110.

Wang, F., X. Xiao, H. Y. Ou, Y. B. Gai, and F. P. Wang. 2009. Role and regulation of fatty acid biosynthesis in the response of Shewanella piezotolerans WP3 to different temperatures and pressures. Journal of Bacteriology **191:**2574-2584.

Weidler, G. W., M. Dornmayr-Pfaffenhuemer, F. W. Gerbl, W. Heinen, and H. Stan-Lotter. 2007. Communities of Archaea and Bacteria in a subsurface radioactive thermal spring in the Austrian Central Alps, and evidence of ammonia-oxidizing Crenarchaeota. Applied and Environmental Microbiology **73:**259-270.

Widmer, F., B. T. Shaffer, L. A. Porteous, and R. J. Seidler. 1999. Analysis of nifH gene pool complexity in soil and litter at a Douglas fir forest site in the Oregon Cascade Mountain Range. Applied and Environmental Microbiology **65:**374-380.

Woese, C. R., O. Kandler, and M. L. Wheelis. 1990. Towards a natural sysytem of organisms - Proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences of the United States of America **87:**4576-4579.

Wood, S. A., A. Rueckert, D. A. Cowan, and S. C. Cary. 2008. Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME Journal **2:**308-320.

Wuchter, C., S. Schouten, H. T. S. Boschker, and J. S. S. Damste. 2003. Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiology Letters **219:**203-207.

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

Yergeau, E., S. Kang, Z. He, J. Zhou, and G. A. Kowalchuk. 2007. Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. ISME Journal **1:**163-179.

Yergeau, E., S. A. Schoondermark-Stolk, E. L. Brodie, S. Dejean, T. Z. DeSantis, O. Goncalves, Y. M. Piceno, G. L. Andersen, and G. A. Kowalchuk. 2009. Environmental microarray analyses of Antarctic soil microbial communities. ISME Journal **3:**340-351.

Zeglin, L. H., R. L. Sinsabaugh, J. E. Barrett, M. N. Gooseff, and C. D. Takacs-Vesbach. 2009. Landscape distribution of microbial activity in the McMurdo Dry Valleys: Linked biotic processes, hydrology, and geochemistry in a cold desert ecosystem. Ecosystems **12:**562-573.

Zhilina, T. N., and G. A. Zavarzin. 1991. Low- temperature methane production by pure culture of Methanosarcina sp. Doklady Akademii Nauk Sssr **317:**1242-1245.

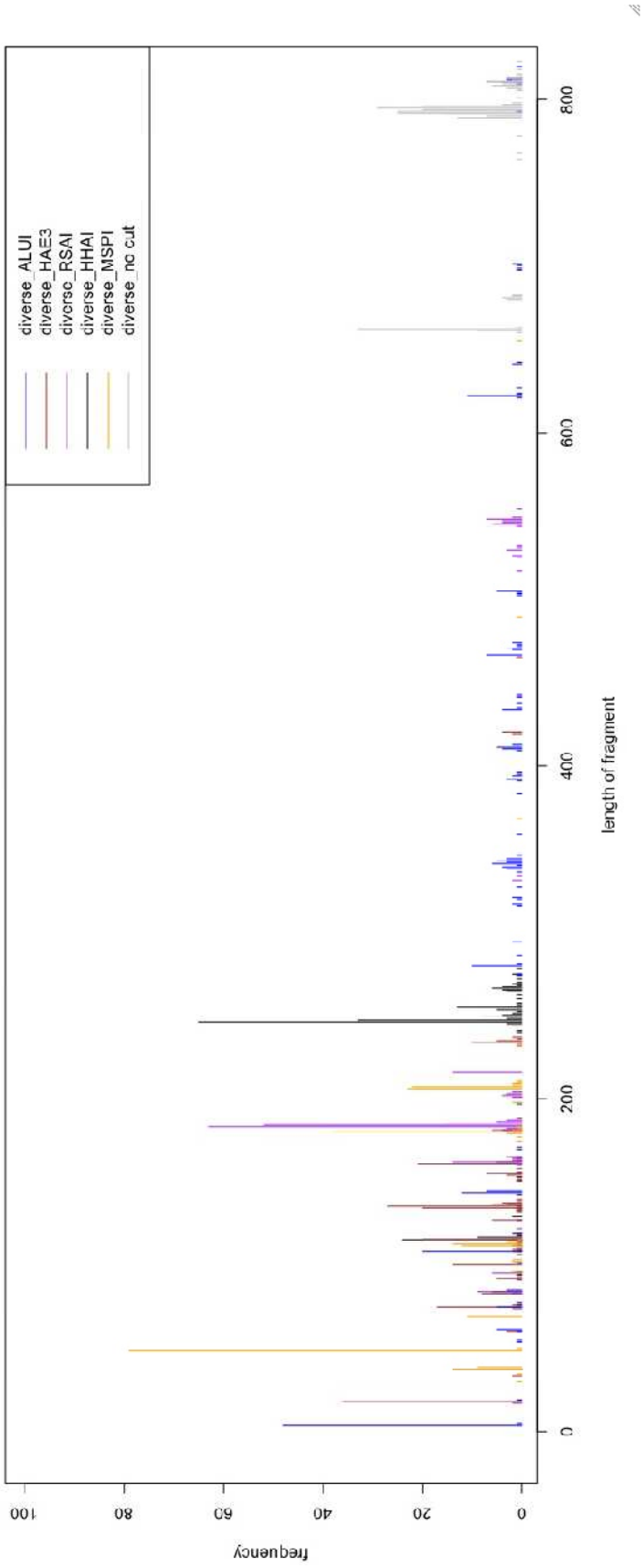
Appendix

A. Photos

Figure 21. Upper Marshall Valley, McMurdo Dry Valleys, Antarctica.

Figure 22. Shangri-La, McMurdo Dry Valleys, Antarctica.

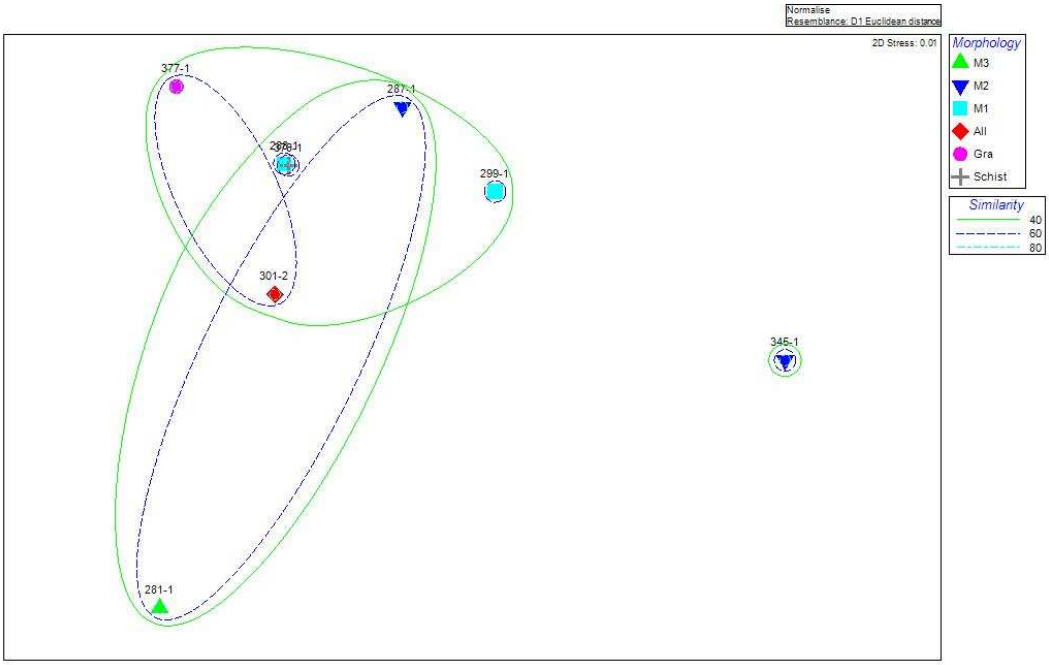
B. *In-silico* T- RFLP of a diverse set of archaeal sequences from isolates (N= 261)



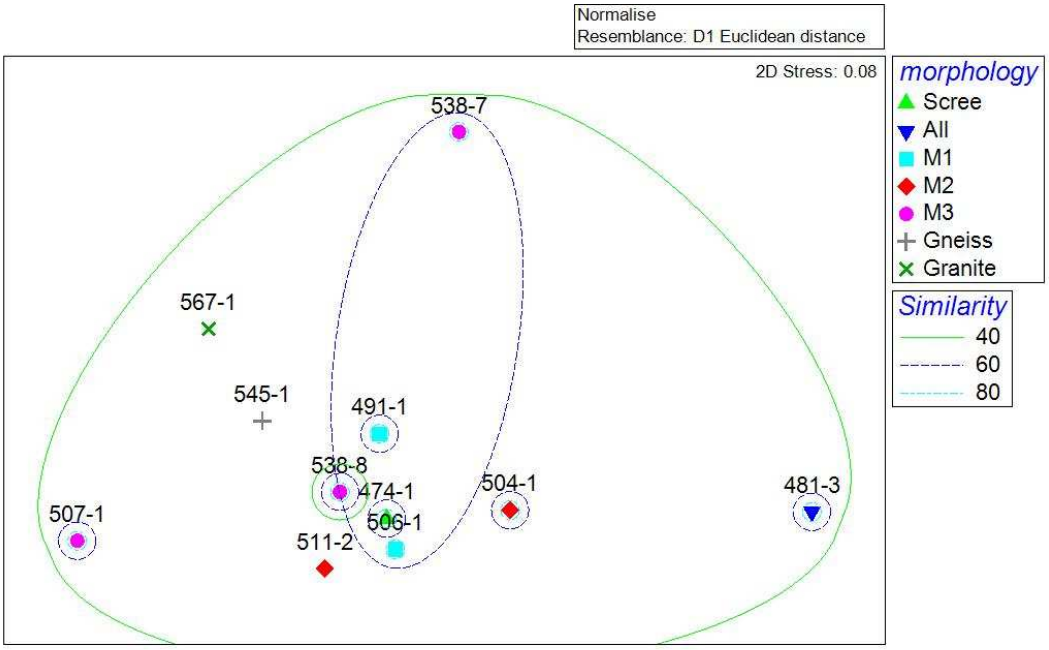
C. Complete statistical analysis

Two- dimensional, non-metric MDS ordination based on Euclidean similarities of geomorphic and physicochemical properties.

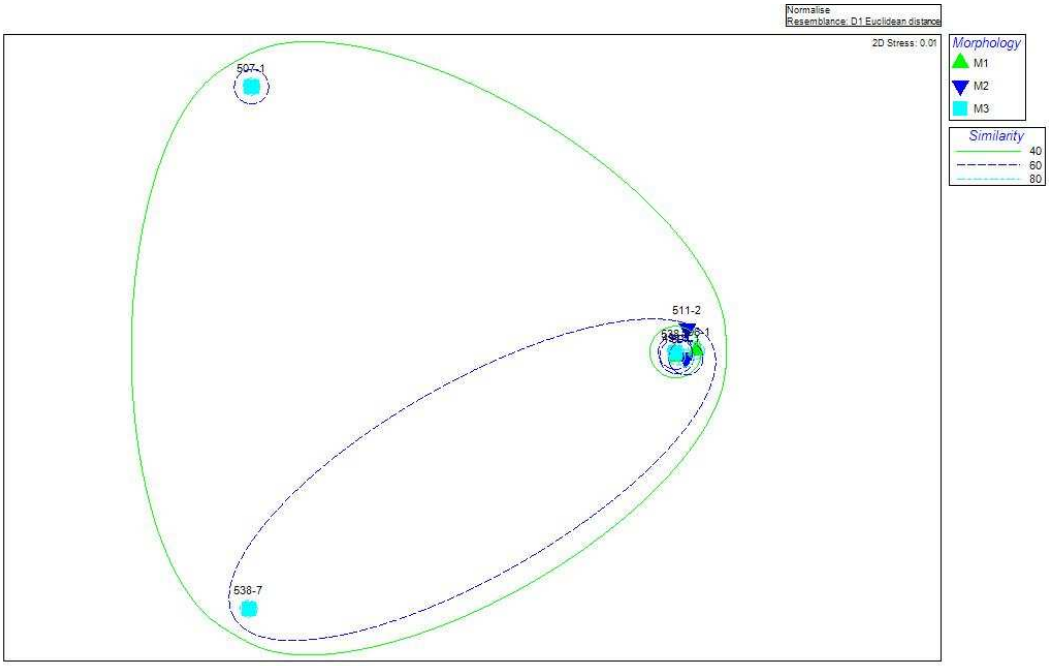
a. *Marshall Valley*



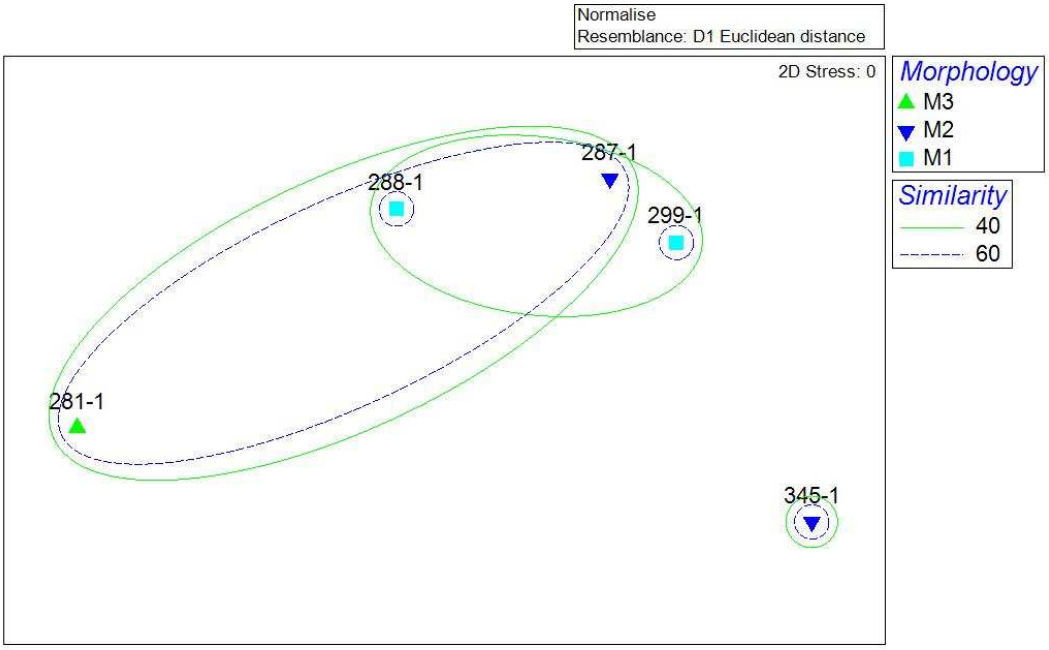
b. *Miers Valley*



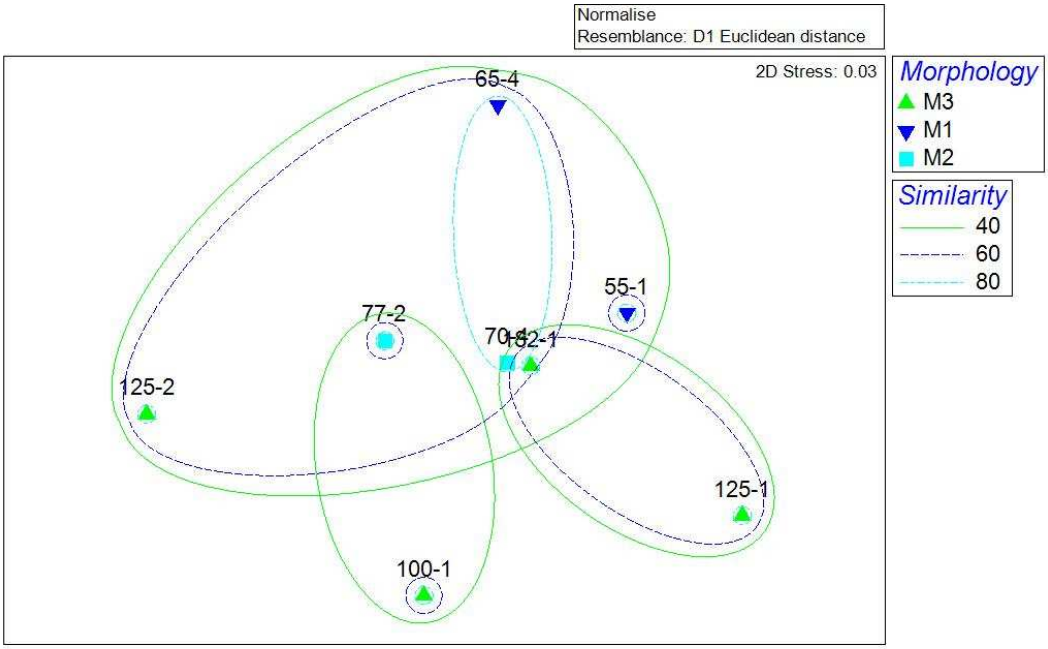
c. *Miers Valley Transect*



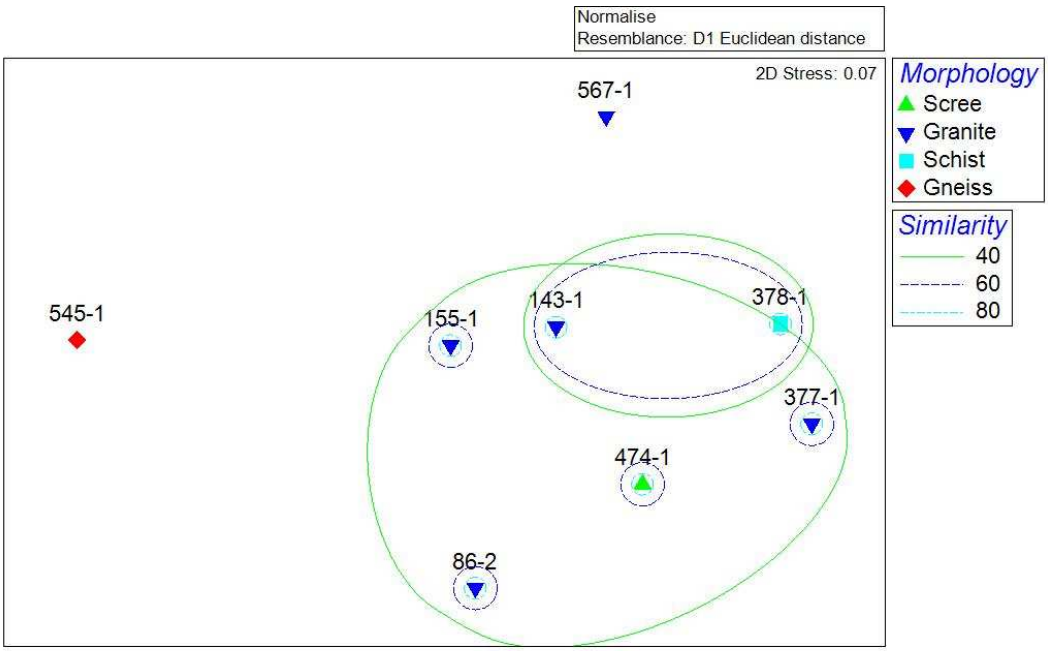
d. *Marshall Valley Transect*



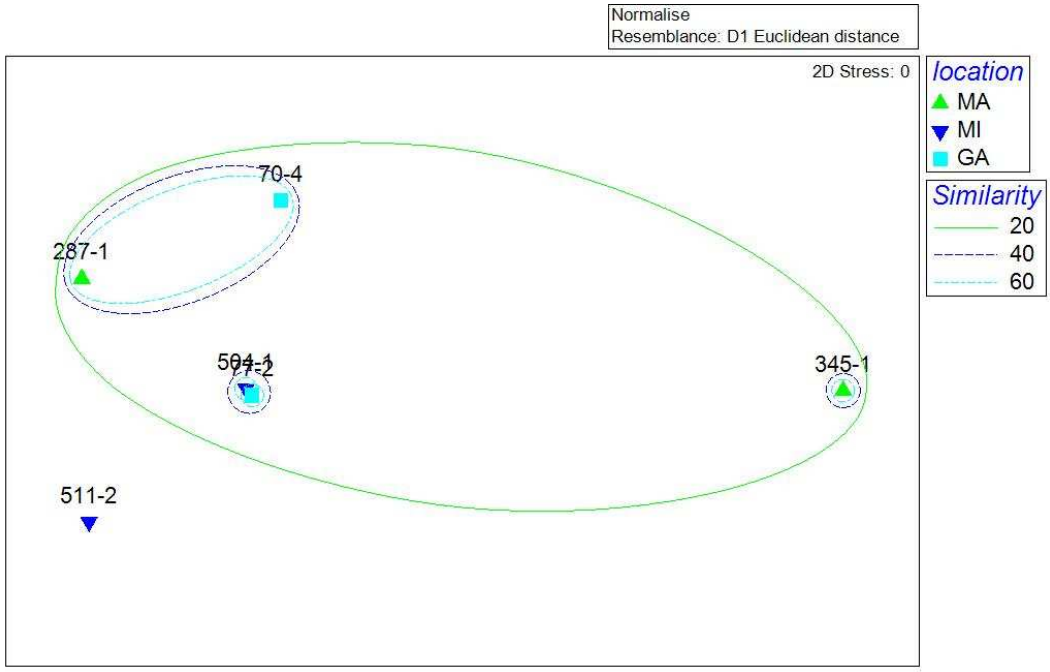
e. *Garwood Valley Transect*



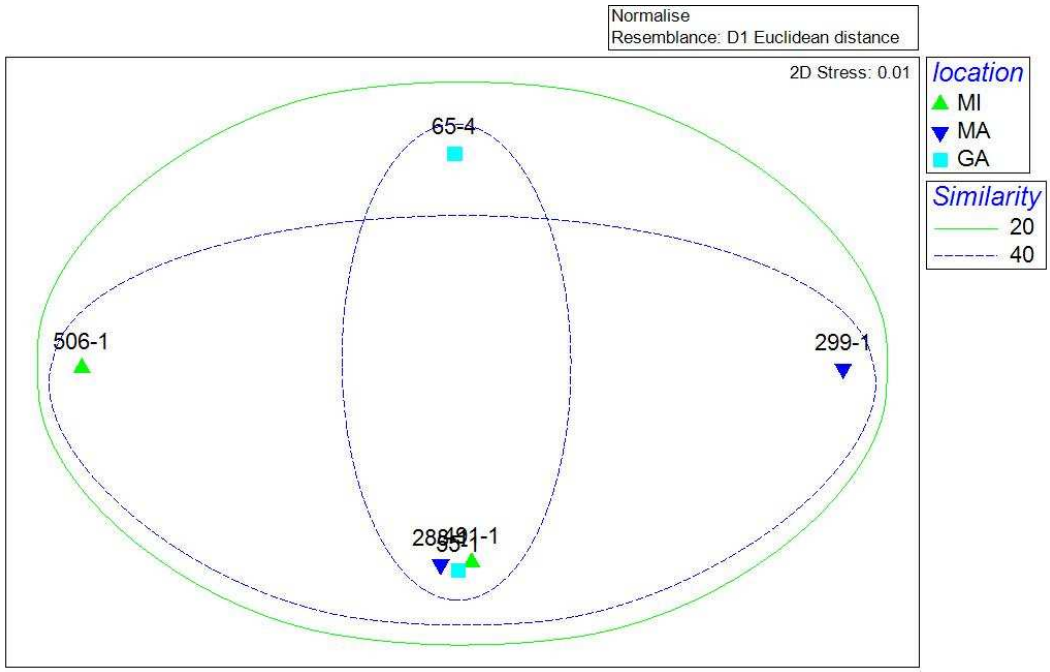
f. *Ridge Transect*



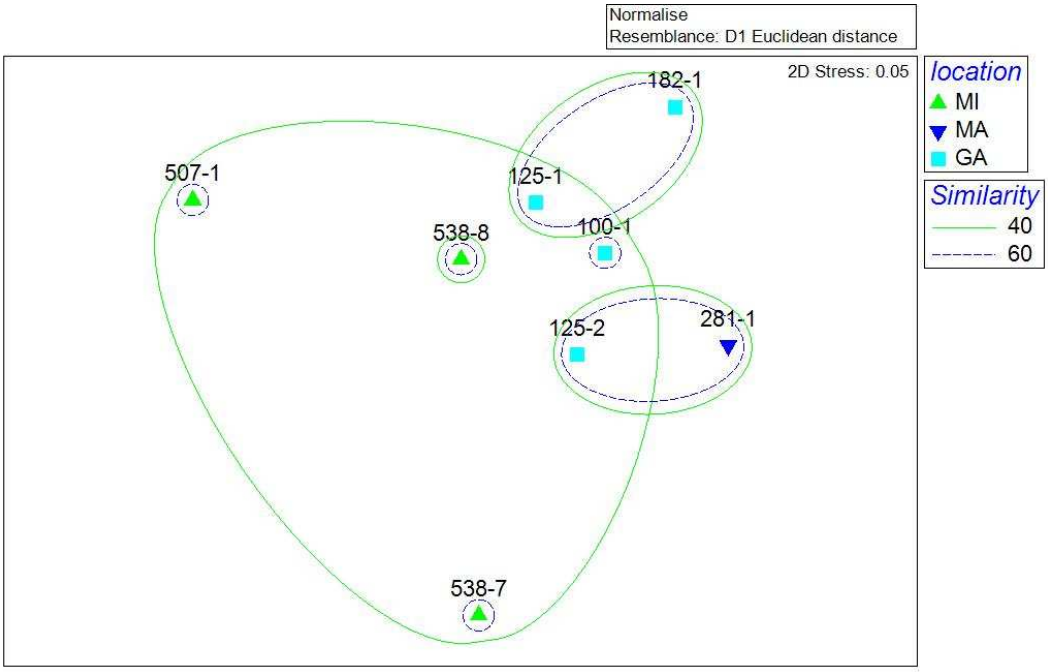
g. *Moraine 2*



h. *Moraine 1*



i. *Moraine 3*



D. Nested PCR

Of soil samples collected from underneath a mummified seal

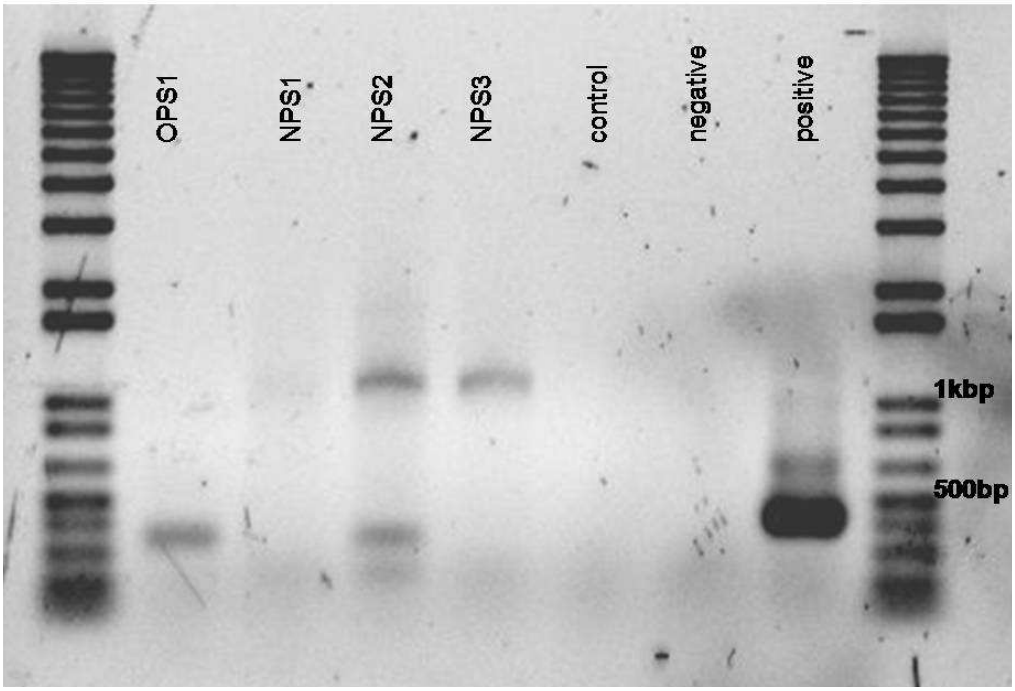


Figure 23. Amplification of archaeal 16S rDNA gene from mummified seal soil samples: OPS1= original position of seal, NPS1-3= new position of seal, negative= negative DNA extraction, control= nearby soil sample, MQ = milliQ water, positive= archaeal DNA.

E. Size standard used in the thesis

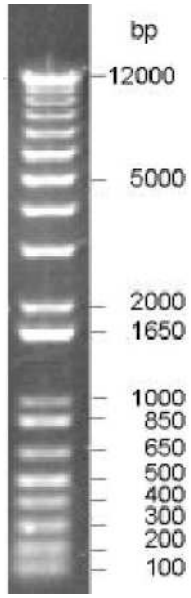


Figure 24. 1Kb Plus DNA Ladder™ (Invitrogen Ltd, New Zealand) (used in agarose gels)).

F. Phylogenetic tree of crenarchaeal clone 177-1

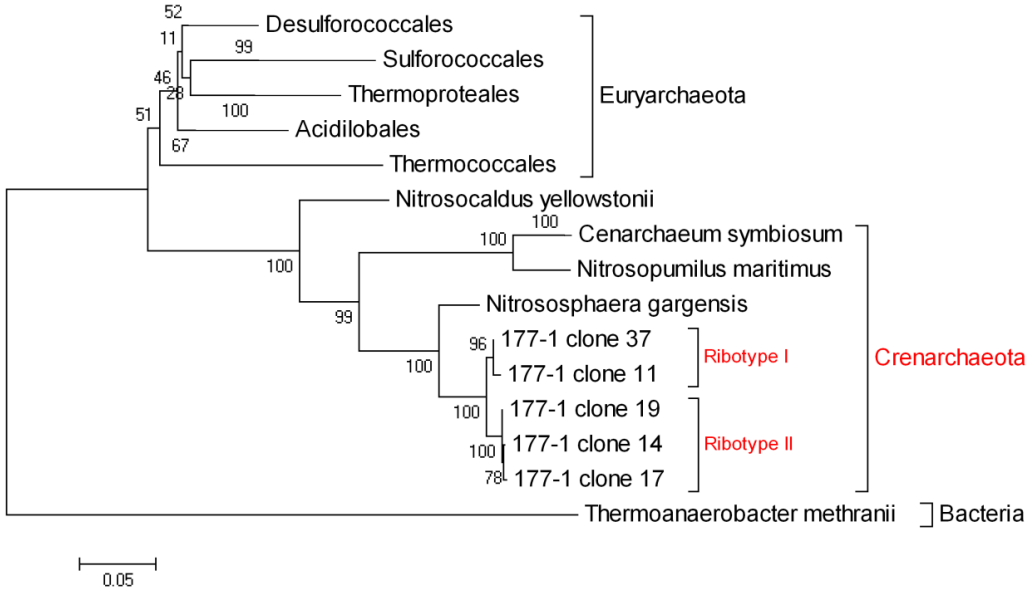


Figure 25. Phylogenetic tree of archaeal clones retrived from Dry Valley soil (sample 177-1) and reference sequences of archaeal isolates retrieved from GenBank. For analysis details see **Figure 5**.

G. Phylogenetic tree of Antarctic soil clones

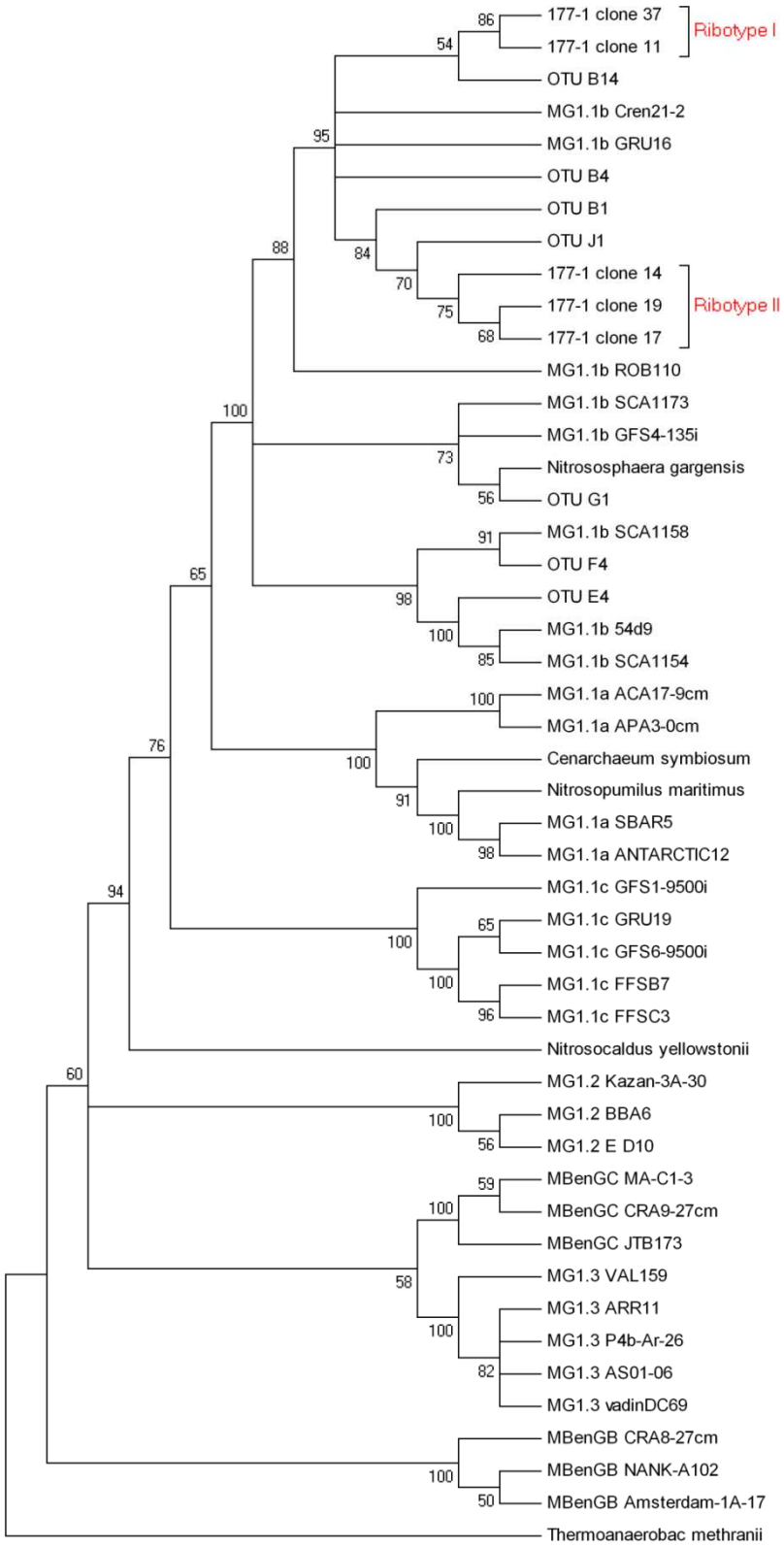


Figure 26. Phylogenetic tree of archaeal clones retrieved from Dry Valley soil (sample 177-1) including partial environmental 16S rRNA gene sequences (814bp) and crenarchaeal isolates retrieved from GenBank. Clones named OTUB1-J1 were recovered from soil of the Ross Sea region (Ayton, et al. 2010). For analysis details see **Figure 5**.

H. Alignment of 16S rRNA gene sequences (5’- 3’) recovered from soil sample 177-1 located in Shangri-La.

```
c_37    GTAGTCAACATGCCCAGGGG-ACGTGGATAA-CCTCGGGAAACTGAGGAT
c_11    GTAGTCAACATGCCCAGGGG-ACGTGGATAA-CCTCGGGAAACTGAGGAT
c_17    GTAGTCAACATGCCCAGGGG-ACGTGGATAM-CCTCGGGAAACTGAGGAT
c_14    GTAGTCAACATGCCCAGGGGGACGTGGATAAACCTCGGGAAACTGAGGAT
c_19    GTAGTCAACA TGCCCAGGGG-ACGTGGATAA-CCTCGGGAAACTGAGGAT

c_37    AAA-CCGCGATAGGTCACTACTT-CTGGAATGGGTAATGACCCAAA-CCT
c_11    AAA-CCGCGATAGGTCACTACTT-CTGGAATGGGTAATGACCCAAA-CCT
c_17    AAA-CCGCGATAGGTCATCACNTTCTGGAATGGGTAATGACTTAAA-TCT
c_14    AAAACCGCGATAGGTCATCACTT-CTGGAATGGGTAATGACTTAAAATCT
c_19    AAA-CCGCGATAGGTCATCACTT-CTGGAATGGGTAATGACTTAAA-TCT

c_37    ATATGGCCCCTGGATTGGACTGCGGCCGATCAGGCTGTTGGTGAGGTAAT
c_11    ATATGGCCCCTGGATTGGACTGCGGCCGATCAGGCTGTTGGTGAGGTAAT
c_17    ATATGGCCCCTGGATTGGACTGCGGCCGATCAGGCTGTTGGTGAGGTAAT
c_14    ATATGGCCCCTGGATTGGACTGCGGCCGATCAGGCTGTTGGTGAGGTAAT
c_19    ATATGGCCCCTGGATTGGACTGCGGCCGATCAGGCTGTTGGTGAGGTAAT

c_37    GGCCCACCAAACCTGTAACCGGTACGGGCTCTGAGAGGAGGAGCCCGGAG
c_11    GGCCCACCAAACCTGTAACCGGTACGGGCTCTGAGAGGAGGAGCCCGGAG
c_17    GGCCCACCAAACCTGTAACCGGTACGGGCTCTGAGAGGAGGAGCCCGGAG
c_14    GGCCCACCAAACCTGTAACCGGTACGGGCTCTGAGAGGAGGAGCCCGGAG
c_19    GGCCCACCAAACCTGTAACCGGTACGGGCTCTGAGAGGAGGAGCCCGGAG

c_37    ATGGGCACTGAGACAAGGGCCCAGGCCCTATGGGGCGCAGCAGGCGCGAA
c_11    ATGGGCACTGAGACAAGGGCCCAGGCCCTATGGGGCGCAGCAGGCGCGAA
c_17    ATGGGCACTGAGACAAGGGCCCAGGCCCTATGGGGCGCAGCAGGCGCGAA
c_14    ATGGGCACTGAGACAAGGGCCCAGGCCCTATGGGGCGCAGCAGGCGCGAA
c_19    ATGGGCACTGAGACAAGGGCCCAGGCCCTATGGGGCGCAGCAGGCGCGAA

c_37    ACCTCTGCAATAGGCGAAAGCTTGACAGGGTTACTCTGAGTGATTTCCGT
c_11    ACCTCTGCAATAGGCGAAAGCTTGACAGGGTTACTCTGAGTGATTTCCGT
c_17    ACCTCTGCAATAGGCGAAAGCTTGACAGGGTTACTCTGAGTGATTTCCGT
c_14    ACCTCTGCA-TAGGCGAAAGCTTGACAGGGTTACTCTGAGTGATTTCCGT
c_19    ACCTCTGCAATAGGCGAAAGCTTGACAGGGTTACTCTGAGTGATTTCCGT

c_37    TAAGGAGATCTTTTGGCACCTCTAAAAATGGTGCAGAATAAGGGGTGGGC
c_11    TAAGGAGATCTCTTGGCACCTCTAAAAATGGTGCAGAATAAGGGGTGGGC
c_17    TAAGGAGATCTTTTGGCACCTCTAAAAATGGTGCAGAATAAGGGGTGGGC
c_14    TAAGGAGATCTTTTGGCACCTCTAAAAATGGTGCAGAATAAGGGGTGGGC
c_19    TAAGGAGATCTTTTGGCACCTCTAAAAATGGTGCAGAATAAGGGGTGGGC

c_37    AAGTCTGGTGTCAGCCGCCGCGGTAATACC-AGCACCCCGAGTGGTCGGG
c_11    AAGTCTGGTGTCAGCCGCCGCGGTAATANCCAGCMCCCCGAGTGGTTGGG
c_17    AAGTCTGGTGTCAGCCGCCGCGGTAATACC-AGCACCCCGAGTGGTCGGG
c_14    AAGTCTGGTGTCAGCCGCCGCGGTAATACC-AGCACCCCGAGTGGTCGGG
c_19    AAGTCTGGTGTCAGCCGCCGCGGTAATACC-AGCACCCCGAGTGGTCGGG

c_37    ACGTTTATTGGGCCTAAAGCATCCGTAGCCGGTTCTACAAGTCTTCCGTT
c_11    ACGTTTATTGGGCCTAAAGCATCCGTAGCCGGTTCTACAAGTCTTCCGTT
c_17    ACGTTTATTGGGCCTAAAGCATCCGTAGCCGGTTTTACAAGTTTTCCGTT
c_14    ACGTTTATTGGGCCTAAAGCATCCGTAGCCGGTTCTACAAGTCTTCCGTT
c_19    ACGTTTATTGGGCCTAAAGCATCCGTAGCCGGTTCTACAAGTCTTCCGTT

c_37    AAATCCACCTGCTCAACAGA GGGCTGCGGAAGATACTATGGAGCTAGGA
c_11    AAATCCACCTGCTCAACAGATGGGCTGCGGAAGATACTATGGAGCTAGGA
c_17    AAATCCACCTGCTTAACAGATGGGCTGCGGAAGATACTATAGAGCTAGGA
c_14    AAATCCACCTGCTTAACAGATGGGCTGCGGAAGATACTATAGAGCTAGGA
c_19    AAATCCACCTGCTTAACAGATGGGCTGCGGAAGATACTATAGAGCTAGGA

c_37    GGCGGGAGAGGCAAGCGGTACTCGATGGGTAGGGGTAAAATCCGTTGATC
c_11    GGCGGGAGAGGCAAGCGGTACTCGATGGGTAGGGGTAAAATCCGTTGATC
c_17    GGCGGGAGAGGCAAGCGGTACTCGATGGGTAGGGGTAAAATCCGTTGATC
```

```
c_14    GGCGGGAGAGGCAAGCGGTACTCGATGGGTAGGGGTAAAATCCGTTGATC
c_19    GGCGGGAGAGGCAAGCGGTACTCGATGGGTAGGGGTAAAATCCGTTGATC

c_37    CATTGAAGACCACCAGTGGCGAAGGCGGCTTGCCAGAACGCGCTCGACGG
c_11    CATTGAAGACCACCAGTGGCGAAGGCGGCTTGCCAGAACGCGCTCGACGG
c_17    CATTGAAGACCACCAGTGGCGAAGGCGGCTTGCCAGAACGCGCTCGACGG
c_14    CATTGAAGACCACCAGTGGCGAAGGCGGCTTGCCAGAACGCGCTCGACGG
c_19    CATTGAAGACCACCAGTGGCGAAGGCGGCTTGCCAGAACGCGCTCGACGG

c_37    TGAGGGATGAAAGCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCC
c_11    TGAGGGATGAAAGCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCC
c_17    TGAGGGATGAAAGCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCC
c_14    TGAGGGATGAAAGCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCC
c_19    TGAGGGATGAAAGCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCC

c_37    CAGCTGTAAACGATGCAGACTCGGTGATGAGTTGGCTATATGCCAACTCA
c_11    CAGCTGTAAACGAGGCAGACTCGGTGATGAGTTGGCTATATGCCAACTCA
c_17    CAGCTGTAAACGATGCAGACTCGGTGATGAGTTGGCTTATTGCTAACTCA
c_14    CAGCTGTAAACGATGCAGACTCGGTGATGAGTTGGCTTATTGCTAACTCA
c_19    CAGCTGTAAACGATGCAGACTCGGTGATGAGTTGGCTTATTGCTAACTCA

c_37    GTGCCGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGG-AGTACGGTCGCAA
c_11    GTGCCGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGG-AGTACGGTCGCAA
c_17    GTGCCGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGG-AGTACGGTCGCAA
c_14    GTGCCGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGG-AGTACGGTCGCAA
c_19    GTGCCGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGGGAGTACGGTCGCAA

c_37    GACTGAAACT TAA
c_11    GACTGAAACT TAA
c_17    GACTGAAACT TAA
c_14    GACTGAAACT TAA
c_19    GACTGAAACT TAA
```