CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The study of biota diversity in soil environments is an important topic to understand the dynamic interaction between the abiotic and biotic systems. Among various taxonomic groups in soil biosphere, bacteria are the major components of the soil biota in terms of both distribution and abundance (Brussaard, et al., 1997; Ovreas, 2000; Coleman & Whitman, 2005). Compared to the eukaryotes, bacteria are ubiquitously distributed in soils. This included some of the most extreme habitats such as hyperarid Atacama Desert, Antarctic Dry Valleys, etc (Rothschild & Mancinelli, 2001; Cowan, Russell, Mamais, & Sheppard, 2002; Bolter, 2004; Niederberger, et al., 2008; Costello, Halloy, Reed, Sowell, & Schmidt, 2009; Pointing, et al., 2010). It has been estimated that 1 g of soil might contained up to ~10^4 different bacterial species (Torsvik, Ovreas, & Thingstad, 2002). The sheer complexity of the bacterial communities in soil system has allowed different level of biotic interactions including gene transfer, recombinants and competition, which together, contributed to the maintenance and regulations of the ecological functions (Beare, Coleman, Crossley, Hendrix, & Odum, 1995; Torsvik, et al., 2002). Indeed, soil bacteria are known to be heavily involved in biogechemical cycle such as nitrogen, carbon, sulfur and heavy metals cycling (Rosswall, 1982; Beare, et al., 1995; White, Sharman, & Gadd, 1998; Overmann & van Gemerden, 2000). Although it is difficult to directly assess the function of each bacterium in a given soil environment, it is understood that the bacterial diversity will reflect the overall productivity and stability of the ecosystem (Torsvik & Ovreas, 2002; Nannipieri, et al., 2003). For instance, increase diversity is generally correlated with

increase in community level stability to perturbation and elevated primary productivity. The underlying explanation for this might be as follows: community with higher diversity might include greater phenotypic traits, increase competition to allow selection of better lineage and increase chances of including highly productive species (Loreau, et al., 2001; Muradian, 2001).

Hence, as pointed out by Øvreås, (2000), the study of bacterial diversity is therefore important in advancing the understanding of genetic diversity, abundance, distribution patterns, functional role(s) and regulation of the soil bacterial community. In addition, bacterial community patterns can also be used as a proxy to assess the impact of anthropogenic influence, as well as global climate change (Loreau, et al., 2001; Wall, 2005; Caldwell, Bornman, Ballare, Flint, & Kulandaivelu, 2007; Hartman, Richardson, Vilgalys, & Bruland, 2008; Tin, et al., 2009).

1.2 Why molecular approach?

Despite the apparent importance, current knowledge of the bacterial diversity is generally lacking. Traditionally, the diversity of soil bacterial community was measured based on the characteristic of the isolates. Although culturing still serve as an important method to study the morphological characteristics, it has greatly underestimated the soil community diversity because only a small proportion of bacterial population is culturable. This is mainly due to the inability of culture media to reproduce the actual ecological niche, and thus restricted the type of bacteria able to grow on the culture media (Connon & Giovannoni, 2002; Nocker, Burr, & Camper, 2007). As reported by Coleman & Whitman (2005), less than 5% of the total known phylotypes has been cultured and the isolated

bacterial populations are extremely skewed within five major phyla including actinobacteria, bacteroidetes, cyanobacteria, firmicutes, and proteobacteria (from a total of 53 known phyla), which together accounted for approximately 95% of the total cultured and described species. These major limitations have led to the advent of culture independent analyses such as fatty acid, DNA or RNA based methods in the last few decades (Nakatsu, Torsvik, & Ovreas, 2000; Kirk, et al., 2004; Nocker, et al., 2007).

The DNA based molecular techniques usually involved DNA extraction from environmental samples, and DNA amplification using polymerase chain reactions (PCR). Depending on the research target, a wide range of signature genes can be targeted to address specific question, for instance, community or functional gene diversity. The most common gene used in the study of soil bacterial community pattern or diversity is 16S rRNA gene. This is partly due to the establishment of public database such as GenBank (Benson, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2010) and Ribosomal Database Project (Cole, et al., 2009) which greatly facilitated the taxonomic comparison and classification of soil bacterial community diversity.

1.3 Soil bacterial community patterns

The distribution of microorganisms including bacteria have always been assumed to be cosmopolitan owing to the large number, low extinction and high dispersal rate (Vincent, 2000; Torsvik, et al., 2002; Martiny, et al., 2006). Nevertheless, increasing evidences have suggested that the distribution of bacteria in soil environments is not random, but exhibits predictable patterns dependent on the spatial scale and variability of the environments (Wiens, 1989; Ettema & Wardle, 2002; Franklin & Mills, 2003; Nannipieri, et al., 2003;

Coleman & Whitman, 2005; Green & Bohannan, 2006). For example, in small spatial scale such as between a few meters to a few kilometers, the heterogeneous distribution of soil chemical parameters can have profound impact to the soil bacterial community composition by forming distinct microhabitats that preferentially select for suitable bacterial lineages. In contrast, in a larger spatial unit such as comparing site separated by thousands of kilometers, the overall climatic profiles will be more prominent than soil chemical parameters as the total variability in the previous is expected to be normalized across large areas. Therefore, characterizing bacterial community pattern in different spatial scale and environment were important step to understand the dynamic interaction between bacterial community and the natural environments. Furthermore, such study may also facilitate the prediction or assessment on the impact of environmental change (Ettema & Wardle, 2002; Nunan, Wu, Young, Crawford, & Ritz, 2002; Green & Bohannan, 2006).

1.4 Why Antarctica?

Due to the reduced development of major vertebrate and vegetation communities, the terrestrial habitats on Antarctica presented a unique ecosystem which primarily driven by microorganisms. This relatively simple ecosystems (reduced complexity in food webs and biotic interactions) (Vincent, 2000; Hogg, et al., 2006) compare to typical tropical or temperate habitats provided an excellent opportunity for microbiologist to study the fundamental driving forces associated with the bacterial structure, and to assess the direct influence of abiotic factors (e.g. soil moisture, pH, nutrient, etc) to the bacterial communities. In addition, the geographical isolation of Antarctica made it an ideal place to study the bacterial evolution and distribution. It has been hypothesized that if endemism

(genotype distribution limited by geographical isolation) is true for microbial community, then Antarctica is the most probable region to display such distribution patterns (Franzmann, 1996; Vincent, 2000). Together, these might contributed to the increased of interest in Antarctic biodiversity for the past 20 years. However, our current knowledge of bacterial diversity in Antarctic terrestrial environments remain limited and patchy (Tindall, 2004; Frenot, et al., 2005; Wall, 2005; Hogg, et al., 2006; Chown & Convey, 2007).

There is an increasing acceptance that the impact of anthropogenic activities to the climate and biodiversity is not confine to certain region but may present ripple or non-linear effect to ecosystem in other regions (Wall, 2005). For instance, the increased in temperature at around 0.20 – 0.56 ℃ per decade (between 1950 - 2000) observed from Antarctic Peninsula; and a few isolated record from maritime Antarctic Island and continental Antarctica were believed to be due to the increased in the anthropogenic greenhouse gasses (Vaughan, et al., 2003; Turner, et al., 2005). Although the variation in temperature were not linear spatially (i.e. cooling on Eastern continental Antarctic) (Doran, et al., 2002), the warming trend is generally in agreement with the global mean temperature (Vaughan, et al., 2003). Such rapid change in temperature has been predicted to bring profound impact to the Antarctic terrestrial ecosystems (Walther, et al., 2002; Convey, 2003). Convey, (2010) suggested that the warming might lead to the following response to the terrestrial biota diversity: (i) increased colonization rate locally or regionally, (ii) increased local scale population and thus resulted to (iii) increased diversity, biomass, trophic complexity and ecosystem structure, (iv) encouraged biotic interaction and decreased dependency on physical environmental parameters. Nevertheless, the full impact can only be ascertained by first understanding the basic question of “what is there?” (Wall, 2005) Therefore, there is a pressing need to document the bacterial community diversity in Antarctica in order to shed

light on the fundamental functioning of Antarctic ecosystems, and to predict the causation relationship of the climate change and human impact to the Antarctic bacterial communities (Wynn-Williams, 1996; Tindall, 2004; Peck, et al., 2005; Wall, 2005).

1.5 Objectives

The main objectives of this thesis were to assess the soil bacterial communities in ecologically distinct soil habitats on Antarctica, and to understand the link between external influences such as human, animal and vegetation on the underlying soil properties and bacterial assemblage patterns.

In order to achieve the aims and to provide a better coverage of bacterial community patterns at different spatial scales and in disparate terrestrial environments, several individual studies in various Antarctic regions were carried out. These studies were as follows:

Study 1: DGGE fingerprinting of bacteria in soils from eight ecologically different sites around Casey Station, Antarctica (Chong, Tan, Wong, Riddle, & Tan, 2009b).

Study 2: Environmental influences on bacterial diversity of soils on Signy Island, maritime Antarctic (Chong, et al., 2009a)

Study 3: High levels of spatial heterogeneity in the biodiversity of soil prokaryotes on Signy Island, Antarctica (Chong, et al., 2010)

Study 4: Assessment of soil bacterial communities on Alexander Island (in the maritime and continental Antarctic transitional zone)

Study 5: Geographical proximity has less influence on soil bacterial community patterns and taxonomic composition compared to altitude and soil pH

By comparing the data obtained from the molecular analyses and previous published works, this thesis sought to provide insight into the general trend of bacterial diversity and distribution patterns in specific locations in Antarctica. Furthermore, the baseline taxonomic data presented here may also be used to compare with future studies in order to understand and to predict the impact of environment and climate change to the soil bacterial diversity in Antarctica.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antarctic Terrestrial Environments

Antarctica, located at the southernmost part of the world, has among the most extreme terrestrial environments on Earth. Approximately 99.7% of the Antarctic land areas are permanently covered by snow or ice, while the majority of the remaining habitats experienced extended degree of snow or ice cover during austral winter months. Based on the climatic and thermal characteristics, Antarctica can be generally divided into three biogeographical regions, namely sub-Antarctica, maritime Antarctica, and continental Antarctica (Fig 2.1; Table 2.1) (Convey, 2006; Convey, 2010). Despite varying thermal characteristics, all three regions supported primitive trophic systems with the absent of native permanent land animal except invertebrates (e.g. Nematoda, Tardigrada, Rotifera, Acari, Collembola) (Hogg, et al., 2006). The floral diversity on the other hand is relatively more complex in sub-Antarctic with around 40 vascular plant (compare to 900 in Arctic), while only two were found in maritime Antarctic (*Deschampsia antarctica* and *Colobantus quitensis*) and none in the continental Antarctic (Convey, 2003, 2007). Accordingly, the food webs on Antarctic terrestrial environments are almost exclusively comprises of microorganisms such as microalgae, fungi, protozoa, and bacteria (Vincent, 2000; Aislabie, et al., 2006; Barrett, et al., 2006a).

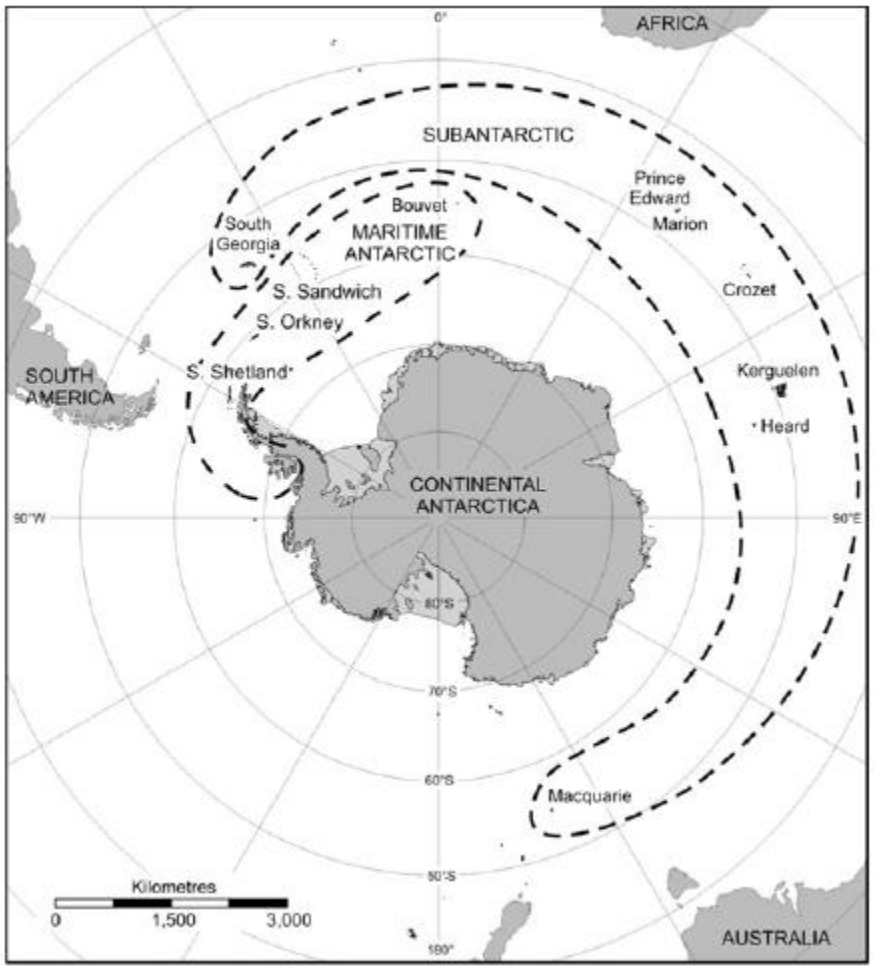

Fig. 2.1 Terrestrial biogeographical zones in Antarctica. Map reproduced from Convey, (2010).

Table 2.1 Generalised thermal characteristic of the major regions of Antarctica, in comparison with those of the high Arctic. Table reproduced from Convey, (2006).

	Months with positive mean air temperature	Air temperature range (℃)		Days above 0℃
		Mean winter to summer	Extreme range	
High Arctic	2 to 4	-34 to +5	-60 to +20	50-350
Sub-Antarctic	6 to 12	-2 to +8	-10 to +25	70-170
Maritime Antarctic	1 to 4	-12 to +2	-45 to +15	6-100
Continental Antarctic coast	0 to 1	-30 to -3	-40 to +10	0
Continental Antarctic inland	0	<-50 to -10	<-80 to -5	0

Except sub-Antarctic and some low altitudes area in maritime Antarctic and coastal continental Antarctic, majority of the soils on Antarctica is classified as gelisol. These soils are generally low in organic input, experiencing cold xeric condition and were subjected to extensive free-thaw cycle (Ugolini & Bockheim, 2008). In comparison, relatively nutrient rich and wetter soil such as histosols, leptosols, regosols and podzols dominated sub-Antarctic islands, and can be found on several biological hotspots (i.e. seal wallows, penguins rookery and moss bank) on maritime Antarctic as well as along the coastline on continental Antarctic (Bolter, Blume, Schneider, & Beyer, 1997; Beyer & Bolter, 2000; Simas, et al., 2007).

Soil heavy metals distribution in Antarctica is closely related to the soil parent type and external input. For instance, accumulation of heavy metals has been observed in Antarctic mosses and marine vertebrate excreta (Bargagli, Sanchez-Hernandez, Martella, & Monaci, 1998; Yin, Xia, Sun, Luo, & Wang, 2008). These sources of heavy metals will eventually be deposited on the soil and take part in the mineral cycling. Over the past few decades of

increase anthropogenic activities in Antarctica, elevated level of heavy metals were detected in the vicinity of several research stations. Most of the contaminants were introduced to the soil in the form of waste dump, incinerated waste, paint and fuel (Claridge, Campbell, Powell, Amin, & Balks, 1995; Sheppard, Claridge, & Campbell, 2000; Bargagli, 2008; Tin, et al., 2009).

2.2 Antarctic soil bacteria communities

The dispersal of bacteria in Antarctica can occur through several mechanisms including human or animal vectors, atmospheric circulation and ocean currents (Vincent, 2000; Hughes, McCartney, Lachlan-Cope, & Pearce, 2004; Frenot, et al., 2005; Pearce, et al., 2009). Although the transport mediums seems similar to the temperate or tropical counterpart, the Antarctic soil bacteria need to face relatively more severe environmental conditions such as cold weather and total darkness during winter, broad temperature fluctuations during summer, and constantly experiencing high salt and desiccation stress (Vincent, 2000; Niederberger, et al., 2008).

The harsh conditions might have reduced the diversity and limited the spectrum of soil bacteria that are able to establish viable populations in soil environments on Antarctica. Comparing the Shannon diversity index (H') of 16S rRNA clone libraries, it was apparent that the species richness of soil bacteria (H' ranging from 1 to 4) derived from various soil studies in Antarctica (Saul, Aislabie, Brown, Harris, & Foght, 2005; Aislabie, et al., 2006; Smith, Tow, Stafford, Cary, & Cowan, 2006; Niederberger, et al., 2008; Pointing, et al., 2010) were lower than that obtained from tropical and temperature regions (H' ranging from 3.8 to 7) (Dunbar, Ticknor, & Kuske, 2000; Hartmann & Widmer, 2006; Kim,

Sparovek, Longo, De Melo, & Crowley, 2007). Possible explanations for such impoverishment in diversity might be that the Antarctica bacteria possessed higher metabolic flexibility than the soil bacteria in temperature or tropical regions (Wynn-Williams, 1996). Thus, relatively lower number of bacteria was required to carry out most of the necessary functions in soil. Additionally, recent studies suggested also that the Antarctic potentially harbour a great variety of novel species endemic to Antarctica as the retrieved ribotypes normally showed less than 97% homology to the isolated species elsewhere (Franzmann, 1996; Tindall, 2004; Smith, et al., 2006; Yergeau, Newsham, Pearce, & Kowalchuk, 2007b; Niederberger, et al., 2008; Vyverman, et al., 2010).

In contrast to the culture-independent studies, culturable taxa isolated from Antarctica were mainly composed of cosmopolitan genera such as *Achromobacter*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Planococcus*, and *Pseudomonas* which were commonly found in other soil environments (Aislabie, et al., 2006; Smith, et al., 2006). Interestingly, most of the cultured soil bacteria from Antarctica are psychrotrophs rather than true psychrophiles. The psychrotolerant species can be active at low temperatures (4 - 8℃) but will exhibit optimum growth at temperatures >15 ℃, whereas psychrophilic bacteria usually grow at temperature <5 ℃ and have optimum temperature at <15 ℃ (Vincent, 2000; Moosvi, McDonald, Pearce, Kelly, & Wood, 2005). This might be due to that a portion of the isolated species was opportunist that dormant during unsuitable condition but formed transient soil bacterial community at summer months where the soil temperature can reach up to 20 ℃ (Barrett, et al., 2006b; Aislabie, Jordan, & Barker, 2008).

2.3 16S rRNA based molecular methods

Over the last two decades, the PCR-based 16S rDNA molecular approaches were the main analytical methods used in the field of soil bacterial ecology and diversity (O'Donnell & Görres, 1999; Kirk, et al., 2004; Nocker, et al., 2007). The main reasons of the popularity for the PCR based methods are that it requires only very little starting material and are relatively faster than the culture-based methods. More importantly, PCR amplification strategy can be carried out without the need of obtaining pure isolates. This provided a better estimation of the overall diversity as uncultured members can be incorporated into the diversity assessments.

Although a variety of genes can be use as standard in elucidating the soil community composition and bacterial taxonomic relationship, the 16S rRNA gene was normally chosen as it is ubiquitously exists in all prokaryotes. In addition, the 16S rRNA gene is relatively large (~1500 bp) and consists of both conserve and variable region. These features permitted the design of "universal" primer to target the conserve region of the overall or specific community DNA. Subsequently, taxonomic relationship can be separated by comparing the differences in the nucleotide sequences of the variable regions (Head, Saunders, & Pickup, 1998; Hill, et al., 2000).

Despite the popularity and the conspicuous advantages of the PCR-based 16S rDNA molecular methods against the conventional isolation strategies, it is not without its own limitations. For instance, it is understood that some bacteria possessed more than one copy of 16S rRNA genes (Klappenbach, Dunbar, & Schmidt, 2000) while reports have suggested that the 16S rRNA gene might not variable enough to correctly resolve the bacteria down to species level (Franzmann & Dobson, 1993; Vincent, 2000). Additionally, the PCR

amplification can be affected by the presence of inhibitor such as humic acid while sequences with higher affinity to the primers might be preferentially amplified (Kirk, et al., 2004; Nocker, et al., 2007).

Notwithstanding the limitations, 16S rRNA gene remained as the most commonly described gene in bacteria diversity studies. To date, approximately 2 million nucleotide sequences were deposited in GenBank database (assessed on November, 2010). Although marker genes such as bacterial internal transcribed spacer region (ITS) (Gurtler & Stanisich, 1996) and DNA gyrase subunit B (*gry*B) (Watanabe, Nelson, Harayama, & Kasai, 2001) showed relatively higher evolutionary rate, the databases were significantly less complete in comparison to the 16S rRNA genes. Furthermore, by assuming that the DNA and PCR biases were evenly distributed among the studies using similar DNA extraction strategy and PCR primers, the 16S rRNA gene data or profiling permitted direct comparison of community overlap and disparity in soil community structures and taxonomic compositions.

Following PCR amplifications, the 16S amplicons can be separated using a few different approaches. In the studies presented in this thesis, three 16S based molecular methods were used. DGGE was applied in Studies 1, 2, 3 and 4 while T-RFLP and clone libraries were carried out in Studies 4 and 5. Although direct comparison of DGGE data obtained from Studies 1,2, 3 and 4 was difficult due to possible gel to gel variations (Powell, Riddle, Snape, & Stark, 2005a), consistent denaturant gradient and PCR primers were selected for all DGGE analyses.

2.3.1 Denaturing gradient gel electrophoresis (DGGE)

DGGE separates the PCR amplicons by exploiting the differences in the melting behavior of DNA fragments. Theoretically, double stranded DNA with higher G-C content will be more resistance to denaturant in comparison to fragments with higher A-T content as there are three hydrogen bonds between the former while only two between the latter. In DGGE, the PCR products containing a mixture of DNA fragment were resolved using polyacrylamide gel electrophoresis with linear gradient of increasing denaturant such as urea and formamide. Across the polyacrylamide gel, fragment with higher A-T content will be retarded by lower level of denaturant as opposed to fragment with higher G-C content. In order to prevent band smearing resulted by complete strand separation, a 40-50 bp GC-clamp is attached to the 5' of the DNA fragment during PCR amplification. For typical soil bacterial profiles, multiple DGGE bands will be produce when fragments with different melting behaviours migrated to different positions. In addition, each distinct DGGE banding position is regarded as separate ribotypes or OTUs (operational taxonomic units) (Muyzer, de Waal, & Uitterlinden, 1993; Muyzer, 1999; Nakatsu, 2007; Solaiman & Marschner, 2007).

As any molecular profiling method, DGGE also succumbed to several limitations (Muyzer & Smalla, 1998; Muyzer, 1999). For example, only dominant species in the community which occupied > 1% proportion of the total diversity will be captured by DGGE. Furthermore, several bands can be migrated to the same or similar positions (co-migration), hampering the interpretation of community overlap. Nevertheless, the co-migration issue can be overcome by reducing the range of denaturants. Alternately, instead of assessing a full complex picture of community diversity in one DGGE profile, the assessment can be stratified by running several taxon specific DGGE in complementary. The main advantage

of DGGE over other profiling methods such as RISA and T-RFLP is that the DGGE bands can be excised and sequenced to reveal the taxonomic identity of the community (Nakatsu, 2007; Nocker, et al., 2007).

2.3.2 Terminal restriction fragment length polymorphism (T-RFLP)

T-RFLP is another widely used approach for community profiling (Schütte, et al., 2008). This method is usually applied to compare community patterns of a set of communities based on the detection of T-RFLP peaks or T-RFs (terminal restriction fragments). In addition, the relative height of the T-RFs can also be used to represent the relative abundance of each OTUs (Culman, Bukowski, Gauch, Cadillo-Quiroz, & Buckley, 2009).

For bacterial diversity assessment, the T-RFs are obtained by PCR amplification using specific marker genes such as 16S rRNA gene. One or both of the primers will be attached with florescence dye and subsequently digested with 4-base cutter restriction enzymes. Then, the digested and labeled terminal fragment will be detected by using DNA sequencer to produce electropherograms. During the peak detection, the size of each T-RF is corrected using a size standard to assign the fragment length down to one nucleotide resolution (Mengoni, Giuntini, & Bazzicalupo, 2007; Nocker, et al., 2007). Since the same principle can be applied to any other genes, T-RFLP has also been used to study functional gene diversity (Schütte, et al., 2008; Enwall & Hallin, 2009).

In contrast to DGGE, the taxonomic assignment of the T-RFs can be performed by either comparing to T-RFs obtained in a parallel cloning or direct reference to database created by *in-silico* digestion (e.g. MiCA) (Shyu, Soule, Bent, Foster, & Forney, 2007). However, such data need to be handled with care as any one T-RF can be shared by over 15 other

sequences. Further, the T-RFs length is sometimes inconsistent due to "T-RF drift" (Kaplan & Kitts, 2003). Nonetheless, T-RFLP does not suffer from gel to gel variation as experience by DGGE and it is relatively high throughput as compared to cloning, SSCP and DGGE analyses. It is interesting also to note that despite obvious differences in the OTUs definition and assignment, T-RFLP has shown to produce well correlated results with other profiling methods (Smalla, et al., 2007; Enwall & Hallin, 2009).

2.3.3 Clone library construction

Other than using profiling methods such as DGGE and T-RFLP, the PCR amplified DNA sequences can also be studied using cloning. This is carried out through ligation of PCR amplicons containing overhanging-A to the vector with complementing overhanging-T. Theoretically, each vector can only bind to one fragment and thus separating the ligated sequence from the mixture of amplicons. Further, the vector will be transformed into a plasmid for cultivation and selection. Depending on the objectives and budget of the study, an optional dereplication of selected clones using RFLP can be carried out prior to sequencing to produce databases consists of the sequenced clones (Nocker, et al., 2007).

The construction of clone library via cloning and sequencing is also known as metagenomic analysis (Riesenfeld, Schloss, & Handelsman, 2004). The main advantage of this approach in comparison to DNA profiling methods is that it provides a greater taxonomic resolution between species and offer a more detail estimation of heterogeneity in species diversity and composition. For example, the community overlap and variation can be inferred by comparing the number of common and rare sequences while the variation in the species population can be deduce via number of individual having the same sequence or RFLP

pattern. Moreover, phylogenetic tree can be drawn to illustrate the taxonomic relationship of each representing sequence (Head, et al., 1998), and the sequence information can be also used in references to the public database such as GenBank (Benson, et al., 2010) to understand the taxonomic assignment and sampling origin of the closely related sequences.

Normally, one clone library in soil study will involve cloning and sequencing of hundreds of clones. This is laborious and involves relatively higher cost than T-RFLP or DGGE, therefore, impractical to construct clone libraries for multiple replicates, and to compare large numbers of samples. In addition, the interpretation of data from clone library is heavily influenced by the sample coverage. Low sample coverage might result to underestimation of overall diversity and miss-interpretation of the abundance information.

2.3.4 Measures for diversity

For most of the soil community and diversity studies, among the main objectives were to elucidate the diversity within a community (α-diversity) and to detect the overlap or variation between communities (β-diversity). The conclusion is sometimes dependent on the diversity measures applied.

The commonly used diversity measures can be coarsely divided into two types: species-based or divergence-based (Lozupone & Knight, 2008). The species-based analyses relied on clear definition of species. This is routinely defined by using 97% similarity cutoff when comparing a set of 16S rRNA sequences from a community (Stackebrandt & Goebel, 1994). Alternately, when using molecular methods such as DGGE, T-RFLP or RFLP profiling, the species can also be defined as each unique DGGE band, T-RF or RFLP pattern. The diversity is normally inferred as the number of species while the community overlap can be

assessed by comparing the number of species shared by any two communities. Furthermore, the community profiles/patterns can be transformed into similarity/dissimilarity matrices and applied in statistical ordination such as clustering analysis, principle component analysis (PCA) or non-metric multidimensional scaling (nMDS) to illustrate the relationship between communities. In addition, significant differences between communities can also be calculated using non-parametric multivariate analyses including analysis of variance (ANOVA), analysis of similarity (ANOSIM) or permutational analysis of variance (PERMANOVA) (Ramette, 2007).

In comparison, the divergence-based measures exploit the genetic variation within or between communities. A community with more deep branching lineages (highly variable sequences) is considered to be more diverse than community consists of shallow branching of lineages (closely related sequences). On the other hand, the proportion of lineage shared by communities is used as an indicator to compare similarity between communities. In this case, significant differences in genetic variation can be tested using Monte-Carlo type testing procedures such as UniFrac, Libshuff or Fst (Lozupone & Knight, 2008; Schloss, 2008).

As suggested by Martin, (2002) the main differences between the species–based and divergence-based analyses can be shown by using an example of two hypothetical communities (Fig. 2-2). Let's say the two communities contain no same species, but every species found in community A has a closely related species in community B. In this scenario, the divergence-based measures will be able to detect the similarity between the two communities but the species-based measures will regard that the two communities are 100% dissimilar to each other. Despite the advantages, the resolution of divergence-based

measures is highly dependent on the DNA sequence length. Additionally, it is relatively sensitive to the sampling coverage compared to the species-based profiling methods.

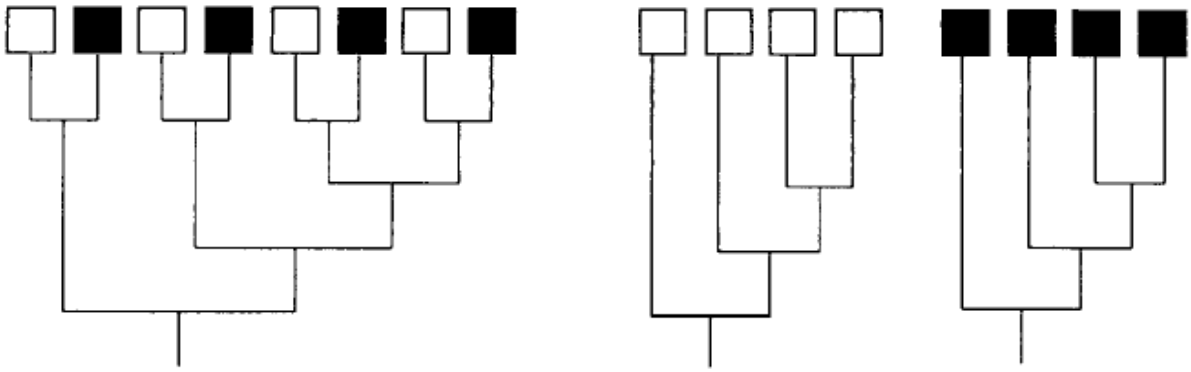

Fig 2.2 Two hypothetical communities: open boxes and solid boxes. The branch lengths of each tree are proportional to the differences in DNA sequences. As shown in the tree on the left, when the two communities combined, none of the species overlap. Nonetheless, when the communities are examined individually, each contains identical phylogenetic diversity (Figure reproduced from Martin, 2002).

The species-based and divergent-based analyses may reveal different aspects of the overall diversity. Nevertheless, data from the two measures are routinely used to correlate with environmental parameters using Mantel-type correlations to provide insight into the relationship between the soil parameters, external environmental factors and the soil bacterial community patterns (e.g. Saul, et al., 2005; Barrett, et al., 2006b; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Aislabie, et al., 2008).

CHAPTER THREE

GENERAL METHODOLOGIES

3.1 Site descriptions and sampling procedures

3.1.1 Study 1

Soil samples were collected from eight sites around the Casey Station (66° 17'S, 110° 32'E), Newcomb Bay, Windmill Island, East Antarctica during the summer 2005/2006 (Fig 3.1; Table 3.1). The sites can be classified as (1) those with high human impact, (2) those with low impact, and (3) protected areas. Red Shed, Thala Valley, Oil Spill site and Wilkes Tip are considered as sites with high human impact because Red Shed is the main domestic building in the Casey Station while Thala Valley and Wilkes Tip are former waste disposal sites. The Oil Spill site is where an oil spill occurred in September 1999 (winter) when more than 2000 litres of fuel leaked from the fuel tank (Snape, Ferguson, Harvey, & Riddle, 2006).

Browning Peninsula and Mitchell Peninsula are considered sites with low human impact because both are relatively far away from the station and are visited only occasionally by people. Two Antarctic Specially Protected Area (ASPA) sites were included in this study: ASPA 135 is located about 200 metres from the station and has abundant mosses and lichens visible during summer in seasonally ice-free patches. ASPA 136 on Whitney Point, situated north-west of the station, is an Adelie penguin colony. Sterile sampling bottles and tubes were used to collect the surface soil samples (top 4-6cm depth) which were stored at -

20˚C until use. Three replicate samples of approximately 50g each were collected from all the sampling sites.

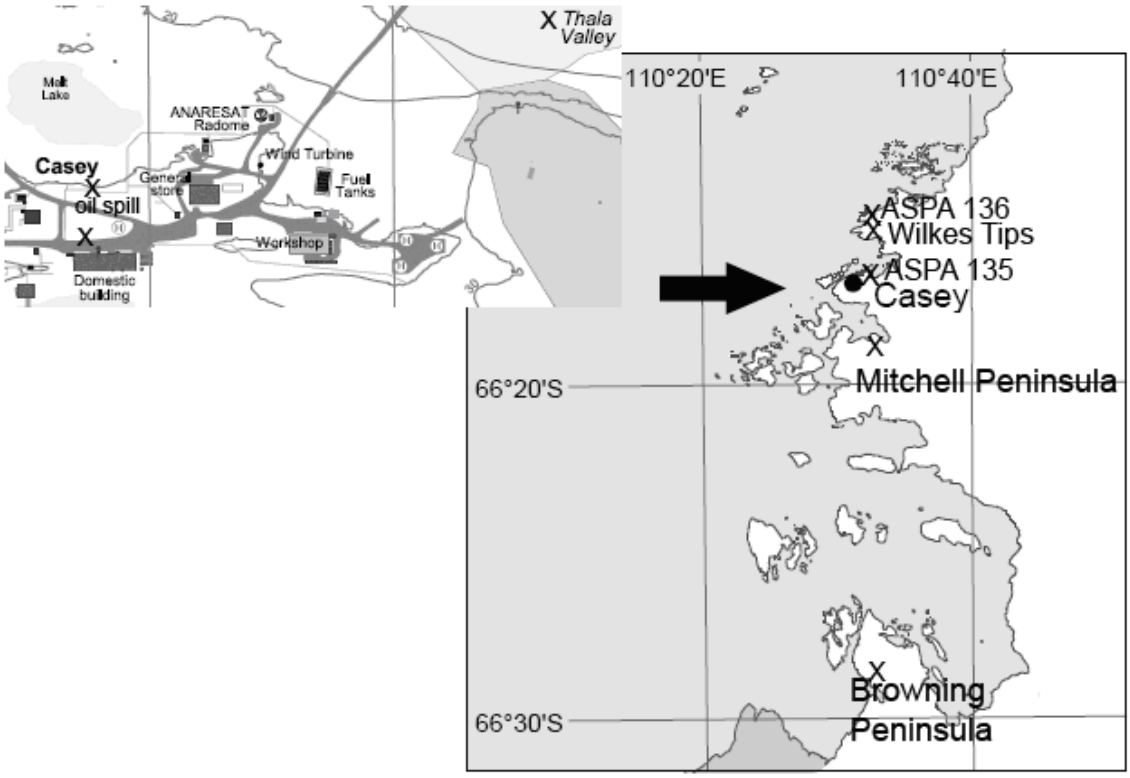

Fig 3.1 Map of Windmill Island. "X" marks the sample collection sites. Inset shows the enlarged map around Casey Station. Source: Australian Antarctic Division Data Center (http://aadc-maps.aad.gov.au/aadc/mapcat/index_new.cfm)

Table 3.1 Location and description of the soil collection sites around Casey Station, Windmill Island

	Site Name	Location		Description
1	Red Shed	66º16' 58''S	110º31' 21''E	Just outside the main domestic building in Casey Station. High human impact
2	Oil Spill site	66º16' 54''S	110º31' 31''E	In the drainage path of an oil spill from the main powerhouse, Casey Station. High human impact.
3	Thala Valley	66º16' 49''S	110º32' 14''E	Former waste disposal site. Close to Casey Station.
4	Wilkes Tip	66º15' 35''S	110º32' 22''E	Former waste disposal site. Further away from Casey Station and close to ASPA 136.
5	Browning Peninsula	66º28' 20''S	110º32' 59''E	Far from Casey Station. Low / no human impact.
6	Mitchell Peninsula	66º18' 51''S	110º32' 55''E	Far from Casey Station and not in close proximity to bird or seal colonies. Low / no human impact.
7	ASPA 135	66º16' 55''S	110º32' 20''E	Relict Adélie penguin colony now supporting abundant mosses and lichens.
8	ASPA 136	66º15' 08''S	110º32' 15''E	Active Adélie penguin rookery

3.1.2 Studies 2 and 3

Signy Island (60° 43'S 45° 36'W) is a maritime Antarctic island within the South Orkney Island archipelago. The annual soil temperature is around -2 ℃ and annual precipitation approximates 400 mm yr^{-1} (Bokhorst, Huiskes, Convey, van Bodegom, & Aerts, 2008). The soils of the island are predominantly gelisols, with a prevalence of psammoturbels, haplorthels, haploturbels, and psammorthels. In addition, histoturbels, historthels, and fibristels are present in low altitude areas, especially along the island's western coast (Guglielmin, Evans, & Cannone, 2008). The vegetation of the island, typical of the maritime Antarctic, is predominantly cryptogramic (Smith, 1990; Bokhorst, Huiskes, Convey, & Aerts, 2007a; Guglielmin, et al., 2008).

For Study 2, eight locations on Signy Island were selected to provide a subjective gradient or ranking of the level of vertebrate impact on their neighbouring terrestrial ecosystem. The studied sites included three associated with penguin rookeries (Gourlay Peninsula, North Point and Cummings Cove), three with seal wallows (Cemetery Flats 1, Cemetery Flats 2 and Elephant Flats), one typical low-altitude vegetated fellfield soil (Berntsen Point) and one more barren high altitude fellfield soil (Jane Col).

North Point is located on the northern coast of the island, whilst Cummings Cove and the Gourlay Peninsula are situated in the south-west and south-east parts of the island, respectively. Three penguin species breed on Signy Island, Adélie (*Pygoscelis adelie*), chinstrap (*P. antarctica*) and gentoo (*P. papua*). Approximately 30,000 pairs of Adélie penguin and 50,000 pairs of chinstrap penguins constitute the majority of this breeding population and, of these, 18, 000 and 13,000 pairs respectively are located on the Gourlay Peninsula (Lynnes, Reid, & Croxall, 2004) while the rest are distributed around North Point,

Cummings Cove and the island's south-west coast (British Antarctic Survey, unpublished data). Small colonies (total ~700 pairs) of gentoo penguins breed exclusively only at North Point. In total, 25% of the Signy breeding penguin population is located at North Point, 26% at Cummings Cove and the south west coast, and 49% at the Gourlay Peninsula. The Gourlay Peninsula was the most heavily impacted site, with soils being obtained within the penguin rookery. The sampling location at North Point provided an intermediate level of penguin impact. Cummings Cove and the south west coast of the island include a large area, with individual sub-colonies being more dispersed, and provided the least impacted of the three rookery sites; the chinstrap penguin rookery adjacent to the sample collection site in Cummings Cove included only 150-180 pairs of birds.

Cemetery Flats and Elephant Flats are coastal supralittoral areas including wallows of elephant seals as well as moulting and resting areas of fur seals during the austral summer. The number of fur seals present on Signy Island increases greatly from mid January each year and peaks at 15-20,000 individuals in February (Smith, 1988b). Sampling sites Cemetery Flats 1 and Cemetery Flats 2 were located ~250 m apart. The former was closer to the shore and wallow location, as was the sampling site at Elephant Flats, while the latter was located more inland and was relatively less impacted, showing visible moss cover.

Berntsen Point is a low altitude vegetated fellfield site located approximately 500 m from the Signy Research Station. Although not hosting permanent colonies of penguins or seals, in common with most such terrestrial habitats on Signy Island, Berntsen Point experiences vertebrate impact in the form of occasional visits of penguins and seals, along with overflight of smaller petrels from nearby nesting cliffs and screes, and nesting skuas. Finally, and contrasting with the other locations, Jane Col is largely free of animal influence, and shows very limited development of fellfield vegetation.

For Study 3, soil samples were collected from 15 locations across the Signy Island, specifically targetting sites expected to maximize spatial heterogeneity. Each location was visually inspected for potential human disturbance and classified according to one of the four major environmental influences that govern soil type on Signy Island: (a) association with vertebrate activity such as penguin rookeries and seal wallows (vertebrates) (Gourlay Peninsula, North Point, Cummings Cove, Elephant Flats, Cemetary Flats 1 & 2); (b) presence of well-developed vegetation (vegetated) (Deschampsia Point, Berntsen Point) or (c) absence (barren) (Skua Terrace, Jane Col, Knob Lake, Pumphouse, Signy Station); (d) close proximity to the coast with low visible animal influence (shore) (Cummings Shore, Factory Shore). Additionally, the spatially restricted consequences of human occupation were taken into consideration.

Signy Station currently operates only during the austral summer period, housing a maximum of 9 personnel, although previously it operated year-round between its establishment in 1944 and 1995, with a larger contingent of up to 25 personnel. Soils within a 5 m radius of the station buildings were considered to be subject to intense human activity. Samples were collected outside the main domestic building (Signy Station) and from the supralittoral area approximately 2 m in front of the station (Factory Shore). Three further sampling locations targeted areas of previously intense human activity. Berntsen Point was in the vicinity of the main buildings of the now-removed wintering Signy Station, Cemetery Flats was a location of whaling activities in the 1920s, and Pumphouse was the location of a whaling era water pumping station, around which corroded metal engine parts and traces of coal remain visible.

For both studies, the soil samples were collected during austral summer season 2006/2007 (Fig 3.2; Table 3.2). At each location, six replicate samples of approximately 50 g were

collected from the top 5 cm of the soil profile using sterile falcon tubes. Samples were kept at 4 ℃ prior to DNA extraction (within 24 hours of collection) and frozen (-20 ℃) at the earliest opportunity after DNA extraction.

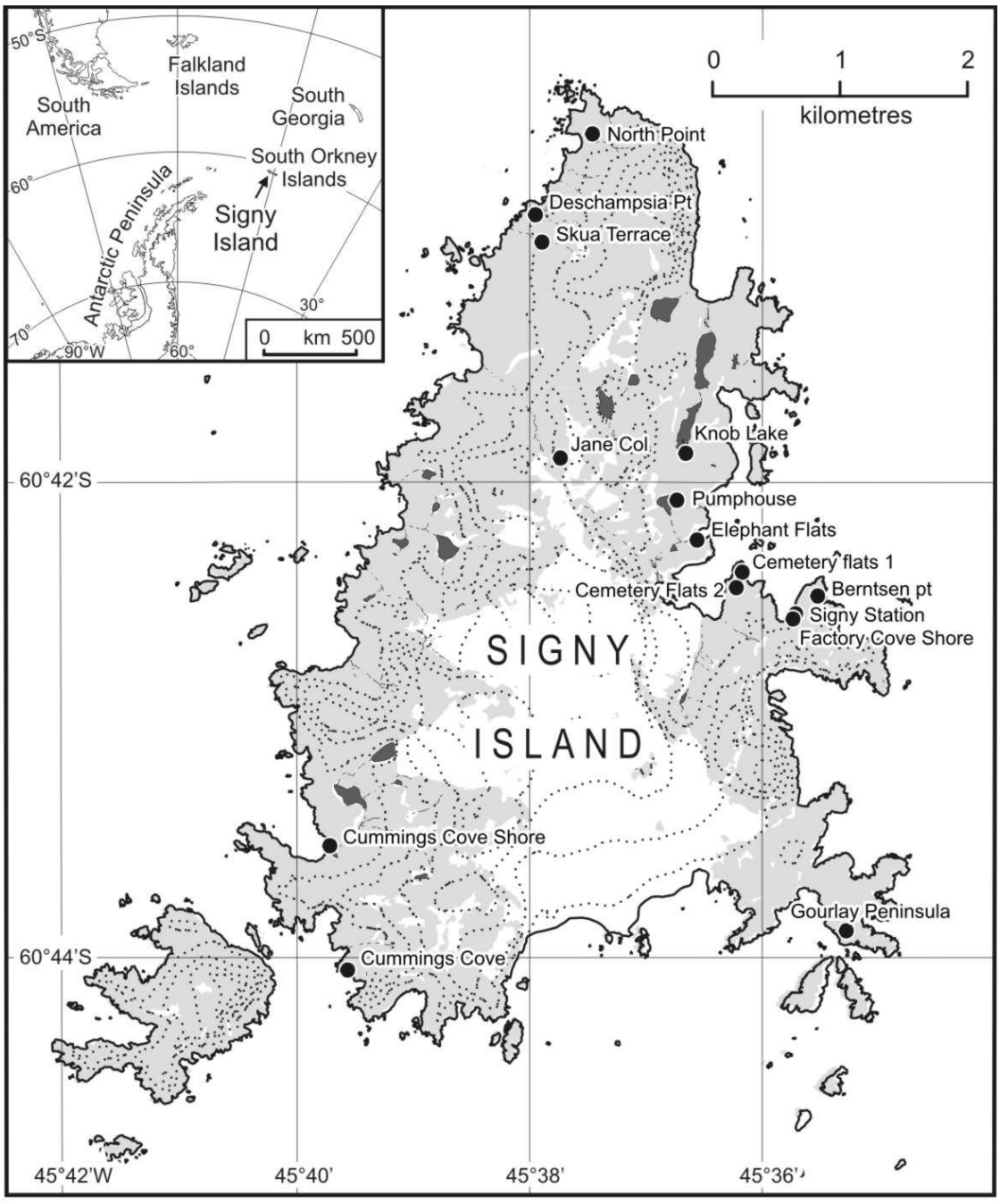

Fig 3.2 Map of Signy Island

Table 3.2 Sampling sites for Study 2 and Study 3

Sites	Primary environmental Influence	History of human impact	GPS	Elevation	Study 2	Study 3
Gourlay Peninsula	Vertebrate (Penguin rookery)	No	60 °43.854'S 45 °35.297'W	22 m	X	X
North Point	Vertebrate (Penguin rookery)	No	60 °40.495'S 45 °37.484'W	14 m	X	X
Cummings Cove	Vertebrate (Penguin rookery)	No	60 °44.020'S 45 °39.593'W	30 m	X	X
Elephant Flats	Vertebrate (Seal wallows)	No	60 °42.207'S 45 °36.584'W	7 m	X	X
Cemetery Flats1	Vertebrate (Seal wallows)	Yes	60 °42.343'S 45 °36.196'W	2 m	X	X
Cemetery Flats 2	Vertebrate (Seal wallows)	Yes	60 °42. 408'S 45 °36.246'W	12 m	X	X
Deschampsia Point	Vegetation presence	No	60 °40.837'S 45 °37.973'W	42 m		X
Berntsen Point	Vegetation presence	Yes	60 °42.442'S 45 °35.547'W	28 m	X	X
Skua Terrace	Barren fellfield	No	60 °40.950'S 45 °37.918'W	67 m		X
Jane Col	Barren fellfield	No	60 °41.861'S 45 °37.760'W	159 m	X	X
Knob Lake	Barren fellfield	No	60 °41.840'S 45 °36.682'W	20 m		X
Pumphouse Lake	Barren fellfield	Yes	60 °42.038'S 45 °36.758'W	28 m		X
Signy Staion	Barren fellfield	Yes	60 °42. 514'S 45 °35. 721'W	9 m		X
Cummings Shore	Shore	No	60 °43.495'S 45 °39.745'W	5 m		X
Factory Shore	Shore	No	60 °42.517'S 45 °35.736"W	30 m		X

3.1.3 Studies 4 and 5

In Study 4, Soil samples were collected during austral summer of 2008/2009 from three sites (~4 km apart) within the Two Step Massif, south-east Alexander Island (western Antarctic Peninsula) to study the intermediate scale spatial heterogeneity in relatively cold and arid environments. The Two Step Massif is generally composed of grey sedimentary rocks such as sandstones and mudstones (Andr é & Hall, 2005). Detailed descriptions including topology and vegetation of Mars Oasis, Viking Valley and Ares Oasis are given by Convey & Smith (1997). Mean monthly air temperatures here are generally below 0 ℃, with the mean maximum (~1 ℃) in December and January and the mean minimum (~-20 ℃) in August (Hughes & Lawley, 2003). The mean daily doses of PAR and UV-B range between 0-300 Wm^{-2} and 0-7 Wm^{-2} respectively.

For Study 5, sampling locations in Alexander Island were grouped and was used to compare with two other environmentally distinct "regions" off the west coast of the Antarctic Peninsula. These incorporated a total of nine sampling locations. Four of these are characterised by considerable vertebrate or marine aerosol influence, being on low altitude islands and a peninsula in Ryder Bay (south-west Adelaide Island). Two were situated on a higher altitude montane ridge (Reptile Ridge) close to Ryder Bay. The remaining three were located in a more extreme ablation area on south-east Alexander Island.

General information on the geological and biological features of the sample collection sites are given by Dewar (1970), Sugden & Clapperton (1981), Moncrieff & Kelly (1993), Convey & Smith (1997) and Maslen & Convey (2006). In brief, samples from Ryder Bay were collected from low altitude islands subjected to constant marine vertebrate and other

direct marine influences (Convey & Smith, 1997; Milius, 2000; Bokhorst, Huiskes, Convey, & Aerts, 2007b). Although situated close to the coast, Reptile Ridge, in contrast, is a narrow mountain ridge undulating between ~250 and 432 m a.s.l. and is relatively free of vegetation other than lichens. Located on the boundary between maritime and continental Antarctica, the ice-free ecosystems of south-east Alexander Island are the most arid environments in the maritime Antarctic (Smith, 1988a; Convey & Smith, 1997). The map and brief description of the sampling locations were shown in Fig 3.3 and Table 3.3.

Table 3.3 Locations and descriptions of soil sample collection sites in Studies 4 and 5.
Note: All sites were analysed in Study 5 but only sites marked with * were used in Study 4.

Sites	Region	GPS	Description
Iron Col (IC)	Reptile Ridge	67 °32.875' S 68 °09.989' W	Mineral soil with no visible plant material elevation = 200 m
Ammo Col (AC)	Reptile Ridge	67 ° 33.268' S 68 °09.319' W	Mineral soil with no visible plant material Elevation = 240 m
Killingbeck Island (KB)	Ryder Bay	67 °34.248' S 68 °04.195' W	Adjacent to shag colonies Elevation = 27.8 m
Lagoon Island (LI)	Ryder Bay	67 °35.469' S 68 °14.748' W	Adjacent to elephant seal wallows Elevation = 13.4m
Anchorage Island (AI)	Ryder Bay	67 °36.178' S 68 °12.550' W	Adjacent to Skua nesting site Elevation = 10.4 m
Rothera Point (RP)	Ryder Bay	67 °34.235' S 68 °07.662' W	Within Rothera Reseach Station Elevation = 4 m
Viking Valley* (VV)	Alexander Island	71 °49.989' S 68 °20.632' W	Maritime-Continental Antarctic transition zone Elevation = 38.2m
Ares Oasis* (AO)	Alexander Island	71 ° 50.329' S 68 °13.487' W	Maritime-Continental Antarctic transition zone Elevation = 41m
Mars Oasis* (MO)	Alexander Island	71 °52.677' S 68 °14.915' W	Maritime-Continental Antarctic transition zone Elevation = 39.2m

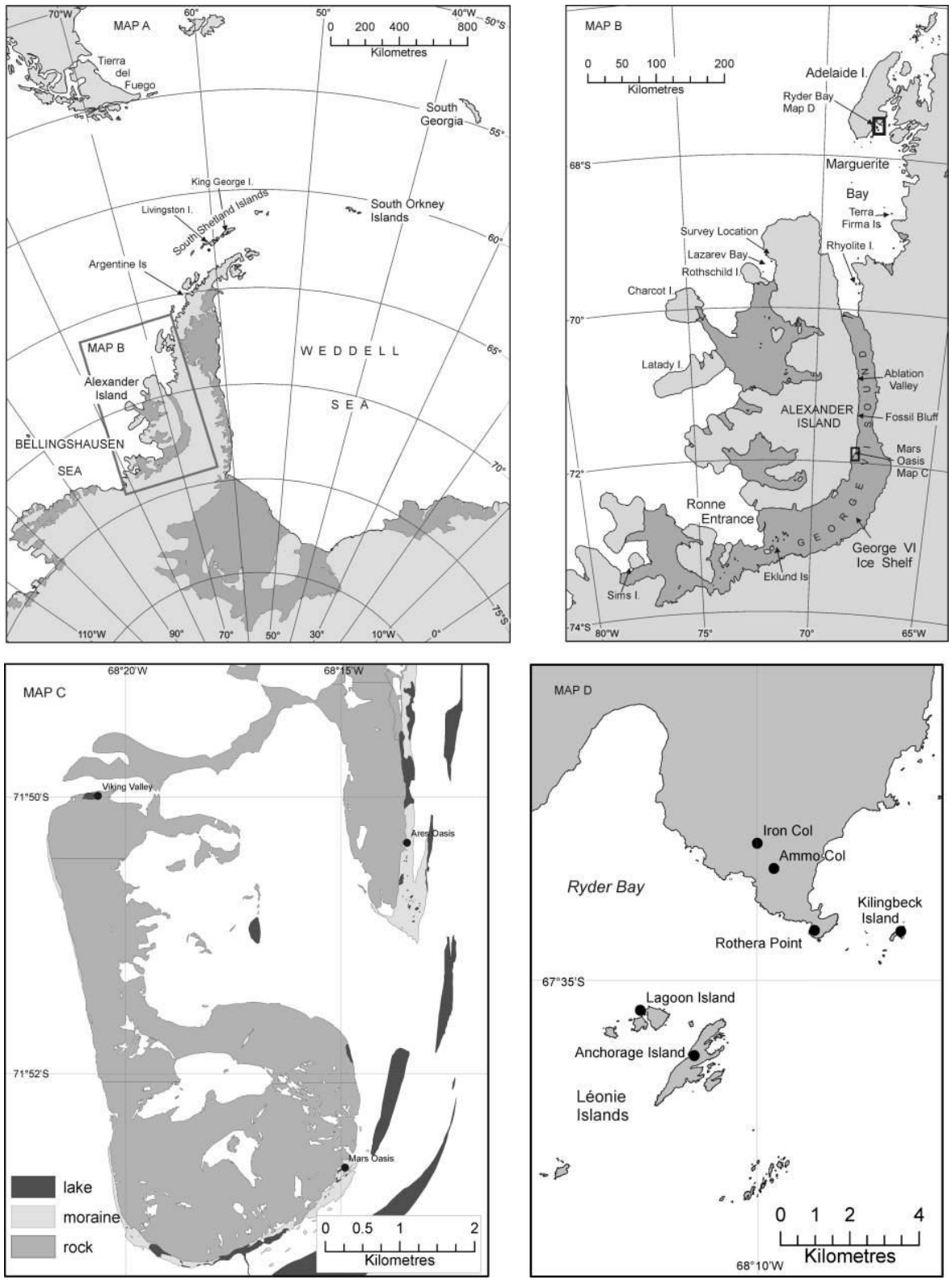

Fig 3.3 Map of the studied sites on Reptile Ridge, Ryder Bay and Alexander Island

At each location, 6 replicate soil samples (from surface to a depth of ~ 5 cm) of approximately 50 g were collected using sterile falcon tubes. Samples were kept at 4℃ prior to soil DNA extraction at the British Antarctic Survey's Rothera Research Station (within 24 h of collection) and then frozen (-20℃).

3.2 Soil chemical analysis

Analysis of the carbon, hydrogen and nitrogen content of the soils were commissioned to the Chemistry Department of the National University of Singapore which used a Perkin-Elmer PE 2400 CHN/CHNS Elemental Analyzer (Perkin-Elmer, USA) and a Euro vector EA3000 Elemental Analyzer (EuroVector, Italy). The soil pH was measured in 1:2 (w/v) suspensions of soil in water; salinity, measured as electrical conductivity(μS/cm), was measured in 1:5 (w/v) suspensions of soil in water; while the water content was determined as weight loss after drying the soil at 70℃ until constant weight.

Five heavy metals were analysed: iron, zinc, lead and copper, and nickel (nickel was not measured in Study 1). The metals were extracted from the soil by digesting 2 g of dry soil in 40ml of 1M HCl for 4 hours in an orbital shaker (Snape, et al., 2004). This extraction method targets only labile or bioavailable metals from soils which are most likely to exert influence on biota (Santos, Silva-Filho, Schaefer, Albuquerque-Filho, & Campos, 2005; Scouller, Snape, Stark, & Gore, 2006). The extracts were then filtered and analysed by a Shimadzu AA6200 atomic absorption spectrometer (Shimadzu, Japan) (Study 1) or Avanta atomic absorption spectrometer (GBC Scientific Equipment, Australia) (Studies 2,3,4 and 5).

Differences in soil chemical properties between locations in each study were analyzed individually using Statistica 7.0 (StatSoft Inc., USA), by carrying out univariate and multivariate analysis of variance (ANOVA/MANOVA), and the Tukey HSD test for *post hoc* analysis.

3.3 Extraction of Total DNA from soil

The UltraClean™ Soil DNA Isolation Kit or PowerSoil® DNA Isolation Kit (MoBio Inc., USA) was used to extract DNA from the collected soil samples. Each soil replicate for each location was extracted individually. Approximately 1.0 g soil (wet mass) was loaded into the bead column and the manufacturer's instructions followed. After a series of washings, the DNA was eluted in 50 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

3.4 DGGE

3.4.1 PCR and DGGE condition

DGGE profiling was carried out in Studies 1, 2, 3, and 4. Same PCR condition was applied in all four studies: For the primary amplification, PCR was first conducted with primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Newberry, et al., 2004). The 50 μL reaction mixture contained 1 μL template (50 x dilution of extracted DNA), 0.5 μM of each primer, 0.25 mM of each dNTP, 1x PCR buffer and 1.25 U *Taq* DNA polymerase. For the secondary amplification, the primer pair 341F-GC (with 40bp GC-clamp) and 907R (Powell, Bowman, Snape, & Stark,

2003; Powell, et al., 2005b) was used and the reaction mixture consisted of 0.5 μM of each primer, 0.40 mM of each dNTP, PCR buffer 1x and 2.5 U Taq DNA Polymerase.

DGGE was performed with a D-Code Universal Mutation Detection System (Bio-Rad, USA). 45 μL of the secondary PCR products were loaded on a 6% acrylamide gel with a denaturing gradient of 35-60% (where 100% denaturant is 7 M urea and 40% formamide). Gels were pre-run at 80 V, 60 ℃ in 1X TAE for 30 min before the samples were loaded, and later at 80 V for 15 h (Powell, et al., 2003; Powell, et al., 2005b).

Gels were stained in 1:10,000 Sybergold in the dark for 60 min and then rinsed with distilled water prior to viewing on a UV transilluminator (Syngene Bio Imaging., UK). Each DGGE profile was at least repeated once to confirm the reproducibility of the banding patterns.

3.4.2 Sequencing of DGGE bands

Dominant bands in the DGGE were excised using a scalpel blade. The excised fragments were incubated at 4 ℃ overnight in sterile distilled water before they were re-amplified with the secondary primers. The positions of the excised bands in the DGGE gel were confirmed with repeated DGGE. Bands showing the expected melting position were amplified with the secondary primer without GC-clamp (341F, 907R). The PCR products were purified using PCRquick-spin^{TM} PCR Product Purification Kit (iNtRON Biotechnology, Korea) and sequenced with ABI Big Dye Terminator V3.1 kit in ABI377-96 upgrade and ABI3100 Genetic Analyzer. Taxonomic identities of the partial 16S rRNA gene sequences were obtained using the Sequence Match search tool in the Ribosomal

Database Project II (RDP)-Release 9 (Cole, et al, 2009) and BLAST search in the GenBank database (Johnson, et al., 2008).

3.4.3 Genetic distance

In Studies 2 and 3, the genetic distance of the sequences within each location was analysed by MEGA 3.1 or MEGA 4 (Kumar, Tamura, & Nei, 2004; Tamura, Dudley, Nei, & Kumar, 2007) under Jukes Cantor calculation model which averages the pairwise genetic distance of "unique" sequences (DGGE bands from different melting positions) detected within six replicates from each location.

3.4.4 Statistical analysis

The DGGE bands were detected and transformed into an absence/presence binary matrix using Quantity One 4.6.5 (Bio-Rad, USA) or Cross Checker V2.91 (*http://www.dpw.wau.nl/pv/pub/CrossChecker/zips/downl5.html*). The banding patterns of different gels were normalized with respect to marker and sequencing results of bands.

In Study 1, the soil bacterial assemblage pattern was illustrated using non-metric multidimensional scaling (NMDS) ordination (Clarke, 1993). The plot was drawn using Bray-Curtis resemblance data created from occurrence frequency of bands within the replicates. For instance, a score of 1 was given to band which appeared 1 time at a specific position from three replicates in the DGGE profile, 2 for bands which appeared 2 times at the same position and 3 for bands which showed up at the same position in all three

replicates in the DGGE profile. The same data was also used to calculate Shannon diversity index (H').

In Study 2, the absence/presence binary data were aggregated by including a "factor column" representing different major environmental influences experienced in the studied locations (i.e. vertebrate influences, vegetated fellfield or barren fellfield). The similarity between sites and between each factor was compared using analysis of similarity (ANOSIM) (Clarke, 1993) in which an *R* value of 1 indicates maximum variation between sites while an *R* value of 0 shows no difference between sites.

In order to measure the variation within sampling locations, multivariate dispersion indices (MVDISP) were calculated. In addition, the bacterial richness in each site was described using H'.

In Study 3, the binary data were aggregated using a nested approach. First level of grouping includes two factors: absence or presence of human impact. The second level grouping divided the samples into four groups according to the greatest environmental influences present at each location.

The presence of clustering effect between presence of human disturbance history and environmental influences were assessed by using permutational multivariate analysis of similarity (PERMANOVA) (Anderson, 2001; Anderson, Gorley, & Clarke, 2008) based on the Jaccard similarity coefficient. Further pairwise t-test comparisons of environmental influences were made to investigate the similarity among the four clustered environmental influences. A test of homogeneity of dispersion (PERMDISP) was carried out to test for significant differences in within-group multivariate dispersion among factors (Anderson, et al., 2008). For both PERMANOVA and PERMDISP, the *P* value was estimated by a

random 9999 permutation test. Unconstrained ordinations such as NMDS plots, and constrained ordinations such as canonical analysis of principal coordinates (CAP), were created to illustrate the distribution of bacterial assemblages across sites or groupings. Pearson correlations of DGGE banding positions were superimposed on the CAP to illustrate the DGGE sequences which established linear relationships with the different environmental influence locations.

In Study 4, spatial clustering effects between the three study locations in Alexander Island were assessed using PERMANOVA and NMDS.

All NMDS, ANOSIM, MVDISP, PERMDISP, CAP and PERMANOVA routines were carried out using the PRIMER 6 and PRIMER 6 PERMANOVA add on multivariate data analysis package (Plymouth Marine Laboratory, UK).

3.5 T-RFLP

3.5.1 T-RFLP condition

T-RFLP was carried out in Studies 4 and 5. Template DNA from each replicate soil sample (6 per site) was amplified using the bacteria specific 16S primers, 27F and 1492R with phosphoramidite fluorochrome 5-carboxyfluorescein attached at the 5' end to produce amplicons of ~1500bp. The reaction mixture was as described as in the primary amplification of DGGE.

The amplified DNA was then purified with PCRquick-spin^{TM} PCR Product Purification Kit (iNtRON Biotechnology, Korea) and digested with MSP-I (Fermentas, USA). Electrophoretic separation of restriction fragments was conducted by FirstBase

Laboratories (Selangor, Malaysia) using ABI 3100 and ABI 3730XL genetic analyzers (Applied Biosystems, USA), with ROX-labelled GeneScan 500 control as a size standard (Applied Biosystems, USA).

3.5.2 Statistical analysis

T-RFLP profiles were noise filtered and aligned using the web-based programme T-REX (Smith, et al., 2005; Culman, et al., 2009).

In Study 4, the absence/presence binary data of T-RFLP from three sites on Alexander Island was transformed into a Jaccard similarity matrix. This was later used to detect the presence of significant spatial clustering between location with PERMANOVA routine.

In Study 5, the T-RFLP data was assessed using both qualitative and semi-quantitative approaches. For qualitative analysis, the bacterial richness was estimated as the number of detected peaks, while the Shannon diversity index weighted using relative peak height of the individual T-RFLP profiles was used to give a semi-quantitative estimate. Comparisons of bacterial assemblage patterns using PERMANOVA were also carried out using the absence/presence and relative abundance data matrices. The pairwise PERMANOVA similarity matrix was transformed into a principle coordinate ordination (PCO) to illustrate the similarity of the bacterial assemblage patterns.

3.6 Cloning, sequencing and phylogenetic analyses

3.6.1 Cloning library construction

In Studies 4 and 5, one clone library for each site was constructed by pooling the replicate DNA amplified using primers 27F and 1492R. The pooled PCR products were subsequently purified with PCRquick-spin™ PCR Product Purification Kit (iNtRON Biotechnology, Korea). The products were then ligated into pGEM®-T Easy Vector System (Promega, USA) and transformed into one shot® TOP10 *E. coli* (Invitrogen, USA).

The transformed clones were picked based on blue-white selection and screened by restriction fragment length polymorphism (RFLP) with restriction enzyme MSP-I (Fermentas, USA). Clones with unique RFLP patterns were further purified with DNA-spin™ plasmid DNA purification kit (iNtRON Biotechnology, Korea) and sent to FirstBase Laboratories (Selangor, Malaysia) for bidirectional sequencing using primers T7 promoter (5'-TAA TAC GAC TCA CTA TAG GG-3') and SP6 (5'-ATT TAG GTG ACA CTA TAG-3').

The DNA sequences were manually checked to identify low quality fragments requiring re-sequencing or removal, before being assembled into contigs. Possible chimeric sequences were screened using the Mallard (Ashelford, Chuzhanova, Fry, Jones, & Weightman, 2006) and Pintail (Ashelford, Chuzhanova, Fry, Jones, & Weightman, 2005) programs. Sequences which proved to be chimeric in both programs were excluded from further analysis and the remaining sequences were grouped into operational taxonomic units (OTUs) or ribotypes at a resolution of 97% sequence homology using Mothur 1.11.0 (Schloss, et al., 2009). The riboypes were then compared with the GenBank database through a BLAST-search (Johnson, et al., 2008), and taxonomic assignments made using the RDP classifier tool

(Cole, et al., 2009). Sequences were submitted to the EMBL database under accession numbers FN811185 - FN811267, FR666700, and FR749710 - FR749826.

3.6.2 Phylogenetic and statistical analysis

The sequences were aligned and filtered using Mothur 1.11.0 (Schloss, et al., 2009) and the alignment was then exported into MEGA 4 (Tamura, et al., 2007) to generate UPGMA phylogenetic trees using the Jukes–Cantor distance correction.

In Study 4, a comparison of the differences in divergence-based β-diversity (see section 2.3.4) was carried out using the normalized weighted UniFrac analysis (Hamady, Lozupone, & Knight, 2010). In theory, UniFrac measures the distance between communities based on the lineages they contain (Lozupone, Hamady, & Knight, 2006).

In Study 5, the divergence-based α and β diversity measures was assessed qualitative and semi-quantitatively. For the α-diversity measure, genotypic richness was derived as phylogenetic diversity (PD), which sums the minimum total branch length of a set of taxa from a given community from the phylogenetic tree (Faith, 1992). In short, the greater the number of deep branching lineage, the higher the PD value in the community. PD is regarded as qualitative measure because it is insensitive to the abundance data. For example, the addition of identical sequences into the phylogenetic tree will not increase the tree branch length and hence not affect the PD value. The quantitative measure was inferred as the mean number of pairwise nucleotide differences (θ_π), which estimates the total nucleotide disparity in a sample of sequences (Martin, 2002). The abundance of the phylotypes was the respective number of clones and θ_π was calculated using Arlequin 3.5 (Excoffier, Laval, & Schneider, 2005). In parallel, the qualitative and quantitative β-

diversity comparison was carried out using unweighted UniFrac and normalized weighted UniFrac analyses (http://128.138.212.43/fastunifrac//). The pairwise relationship/distances of each community estimated using UniFrac were subsequently ordinate using PCO analysis.

The taxonomic distinctness within each library in Study 5 was estimated using a net relatedness index (NRI) and nearest taxon index (NTI). NRI measures the overall mean pairwise distance of closely related taxa while NTI measures the terminal clustering of the taxa relative to the total gene pool. NRI and NTI greater than 0 indicate phylogenetic clumping while NRI and NTI lower than 0 suggest phylogenetic overdispersion (Webb, Ackerly, McPeek, & Donoghue, 2002; Horner-Devine & Bohannan, 2006; Vamosi, Heard, Vamosi, & Webb, 2009).

The significance levels of the NRI and NTI indices were obtained using a permutation test (1000 resamplings), and the calculations carried out using Phylocom 4.1 (Webb, Ackerly, & Kembel, 2008) by importing the newick-formatted UPGMA tree file from MEGA 4.

3.7 Correlation of environmental data with bacterial diversity richness and bacterial community patterns

In order to understand the relationship between the environmental factors and soil bacterial assemblage patterns, Mental-type correlation and distance-based redundancy analysis was carried out using Statistica 7.0 (StatSoft Inc.,USA) or/and, PRIMER 6 and PRIMER 6 PERMANOVA add on multivariate data analysis package (Plymouth Marine Laboratory, UK).

In Study 1, the correlations between the environmental variables and Shannon diversity index were carried out on the Log (1+V) transformed data using non-parametric Spearman rank-order correlations.

In Study 2, the BEST procedure was used to identify the strongest relationships (correlation) between measured environmental variables and the bacterial community composition as described by DGGE profiles. Spearman correlations were used and the significance level of the sample statistic was calculated by permutation test (99 permutations).

Distance-based linear models (DISTLM) were used in Studies 3, 4, and 5 to identify the soil properties which showed the strongest correlations with prokaryote community composition. For Study 4 and 5, undetectable or detectable but unmeasurable parameters were omitted to increase the robustness of the analysis.

In Study 3, for the initial marginal test, the soil properties were treated with fourth root transformation and grouped into edaphic (soil pH, water, carbon, nitrogen content, conductivity) and metal (zinc, lead, iron. nickel, and copper) classes. A parallel test on only the human disturbed and non-disturbed locations was also carried out. A further marginal test was carried out using individual measured soil parameters. A total of six (3x2) separated DISTLM were calculated to compare the differences of the best correlated soil factors and single soil variables in all, non-human disturbed and human disturbed locations.

In Study 4, parsimonious models were generated using “An Informative Criterion” (AIC) (Anderson, et al., 2008) to show the simplest combinations of soil parameters which best explained the variation in bacterial assemblage patterns in T-RFLP and DGGE analyses.

Lastly in Study 5, four separate DISTLMs were carried out to correlate the soil chemical properties to the bacterial assemblage patterns generated using different diversity measures

(i.e. species-based versus divergence-based; qualitative versus semi-quantitative). In addition, Spearman rank correlations carried out to calculate the resemblance coefficients of matrices derived from pairwise geological distance with matrices individually inferred using PERMANOVA or UniFrac, and to correlate the environmental paraters to the NRI and NTI indices.

CHAPTER FOUR

STUDY 1. DGGE FINGERPRINTING OF BACTERIA IN SOILS FROM EIGHT ECOLOGICALLY DIFFERENT SITES AROUND CASEY STATION, ANTARCTICA

4.1 Introduction and objectives

Casey Station (66° 17'S, 110° 32'E), which was opened in 1988, is located on a coastal ice-free rock and gravel peninsula in the Windmill Islands, East Antarctica (Snape, et al., 2006; Revill, Snape, Lucieer, & Guille, 2007). There have been two existing stations in the vicinity. The now abandoned Wilkes Station, is located about 3 km from Casey across Newcomb and was operated by the US from 1957 to 1959 and then by Australia until 1969. The Old Casey Station, which has mostly been removed, was adjacent to the current Casey Station and was operated by Australia from 1969 until the current Casey Station was commissioned in 1988.

Most of the bacterial community studies around Casey Station have been focused on marine sediments (Stark, 2000; Powell, et al., 2003; Stark, Riddle, Snape, & Scouller, 2003; Powell, et al., 2005b; Stark, Snape, Riddle, & Stark, 2005) and relatively few studies on terrestrial soil bacterial diversity have been published. Moreover, among the few limited studies on terrestrial soil microbial diversity on Wilkes Land, (Bolter, 1992; Roser, Seppelt, & Ashbolt, 1993; Beyer, Pingpank, Wriedt, & Bolter, 2000) the majority was enumerated using total bacterial count and total cell volume. Despite providing valuable information on the α-diversity (bacterial richness within each locations), these methods did not take into account of the community overlap (β-diversity) between locations.

In order to assess the diversity and community structure of dominant bacteria around Casey Station, eight sites from ecologically distinct environments were studied using DGGE. The selected sites consisted of high human-impacted sites (Red Shed, Oil Spill, Thala Valley and Wilkes Tip), relatively remote sites (Mitchell Peninsula and Browning Peninsula) and protected areas (ASPA 135 and ASPA 136). Additionally, several soil chemical parameters were assessed to study the link between the bacterial diversity and soil heterogeneity.

4.2 Materials and Methods

The methodologies were listed in Chapter 3.

4.3 Results and Discussion

4.3.1 Soil chemical properties

The soil chemical properties and H' of eight sites around Casey Station were listed in Table 4.1. Accordingly, higher conductivity was detected in soil from ASPA 136, Wilkes Tip and Thala Valley compared to other sites. The soil samples around Casey Station generally contain low levels of nutrients. Higher carbon, nitrogen and water contents were observed in soils from ASPA 135 and ASPA 136 compared to the other sites. ASPA 135 is a relict Adélie penguin colony (Wasley, 2004), with the high nutrients deposited by the birds as guano in the past now supporting the growth of mosses and lichens. ASPA 136 is currently inhabited by an active Adélie penguin colony and the nutrients there are continuingly added to the soil in the form of penguin guano and as dead birds.

ASPA 135 and 136 had high levels of copper, iron and zinc compared to the other sites, and this could be attributed to the high organic content which acted as a chelating and binding agent for heavy metal (Gadd & Griffiths, 1977) or simply resulted from guano and plant material input in these two sites. Lead however was highest in Thala Valley and Wilkes Tip, while high level of zinc was detected from Thala Valley, a reflection perhaps of these two being former waste disposal sites (Townsend & Snape, 2008). Terrestrial soil in other contaminated sites in Antarctica, including at the Brazilian Ferraz Antarctic station (Santos, et al., 2005) and in the McMurdo Sound region (Claridge, et al., 1995) were reported to possess 11.5 and 28.5 ppm of lead respectively. Relatively remote sites such as Browning Peninsula and Mitchell Peninsula had lower levels of heavy metals compared to the contaminated sites of Oil Spill, Red Shed and Thala Valley.

Table 4.1 Selected environmental properties of soils samples around Casey Station, Windmill Island. Three replicates (n=3) were analysed from each site except % carbon and % nitrogen where only one was analysed randomly from the three replicates.

	Browning Peninsula	**Mitchell Peninsula**	**ASPA 136**	**ASPA 135**	**Wilkes Tip**	**Thala Valley**	**Red Shed**	**Oil Spill**
EC[a]	24.62	14.28	189.70	36.90	290.10	657.50	21.34	37.75
	±10.12	±6.71	±48.91	±14.23	±89.04	±101.23	±4.36	±9.94
pH	6.22	5.28	4.78	5.21	5.31	7.56	7.58	4.69
	±0.44	±0.23	±0.75	±0.99	±0.97	±0.87	±0.91	±0.57
% H_2O	8.99	1.65	24.71	36.20	11.04	6.13	5.84	7.23
	±1.22	±0.70	±12.65	±16.78	±5.67	±2.12	±1.23	±2.54
% C	<0.50	<0.50	2.58	7.26	<0.50	<0.50	<0.50	0.83
% N	U.D.[c]	<0.50	0.52	0.77	<0.50	U.D.[c]	<0.50	<0.50
Iron[b]	1304.60	734.68	2152.47	3998.07	746.87	1982.33	2348.20	5838.53
	±85.20	±65.43	±62.36	±61.77	±66.65	±109.08	±6.70	±60.24
Zinc[b]	7.12	4.65	24.24	48.78	6.03	32.85	16.61	14.62
	±1.17	±1.11	±11.92	±25.05	±1.59	±11.64	±2.05	±3.19
Lead[b]	5.07	6.20	7.33	7.08	11.41	27.15	8.69	7.55
	±0.58	±0.59	±2.55	±2.65	±1.87	±1.56	±1.17	±0.83
Copper[b]	3.25	2.37	22.26	39.96	1.85	8.61	3.62	7.88
	±0.20	±0.89	±15.09	±21.69	±0.59	±1.68	±0.34	±3.13
H' [d]	3.15	2.98	2.90	3.01	3.25	2.73	2.78	2.00

[a] conductivity (µS/cm); [b] unit in ppm; [c] undetected; [d] Shannon diversity index inferred using occurrence frequency of the DGGE bands (see section 3.4.4).

4.3.2 DGGE profiles

A total of 23 bands were excised from the DGGE gels (Figure 4.1), re-amplified and sequenced (Table 4.2). 10 out of 23 excised bands were from the Bacteroidetes group. This phylum was dominant in all sites, showing highest percentage in the Oil Spill site (75%), Mitchell Peninsula (68.41%) and ASPA 136 (65%). The sequences of six other excised bands were affiliated to β-Proteobacteria which made up 6.25% to 16% of the total sequenced bands from all sites except Thala Valley (25%) and Browning Peninsula (38.82%). One representative each from γ-Proteobacteria and Firmicutes was detected from the DGGE profiles. Both were absent in the Oil Spill site. Five sequences remained unclassified and they accounted for 10 to 26% of the excised bands.

Bacteroidetes were found to be dominant and abundant in Victoria Land (Aislabie, et al., 2006; Aislabie, et al., 2008) and in surface lake sediments from Ardley Island, South Shetland Islands (Li, Xiao, Yin, & Wang, 2006). They are known to be capable of degrading a wide range of polymeric substances (Aislabie, et al., 2006; Li, et al., 2006; Aislabie, et al., 2008). This ability might explain the high occurrence of Bacteroidetes (75%) at the Oil Spill site, which showed the least diversity of bacteria. However, no *Pseudomonas* or *Rhodococcus* strains, commonly found in hydrocarbon-contaminated sites, were detected from our DGGE profiles. The low diversity at the Oil Spill site is consistent with results reported by Saul et al. (2005) who found decreased overall bacterial diversity in hydrocarbon contaminated soil from Ross Island, Antarctica.

Three sequences which were affiliated with aromatic compound degraders, *Acinetobacter* (Thangaraj, Kapley, & Purohit, 2008), *Delftia tsuruhatensis* (Shigematsu, et al., 2003) and

Burkholderia fungorum (Coenye, et al., 2001), were found in the DGGE profiles. Band 14 which has 97% sequence homology to *Acinetobacter* was prevalent in almost all sites while Band 20 which has 98% sequence homology to *Delftia tsuruhantensis* was detected only in protected (i.e. ASPA 135, ASPA 136) and relatively remote sites (i.e. Browning Peninsula, Mitchell Peninsula). Band 21 which has 99% sequence similarity to *Burkholderia fungorum* was found only in Wilkes Tip and Browning Peninsula.

Bacteria phyla such as actinobacteria and cyanobacteria were not detected from the DGGE profiles. This may be due to methodological limitations e.g. the primer sets used, the short length of the PCR products, and the fact that only numerically-dominant species are detected as visible bands (Muyzer, 1999; Pearce, 2003; Dorigo, Volatier, & Humbert, 2005).

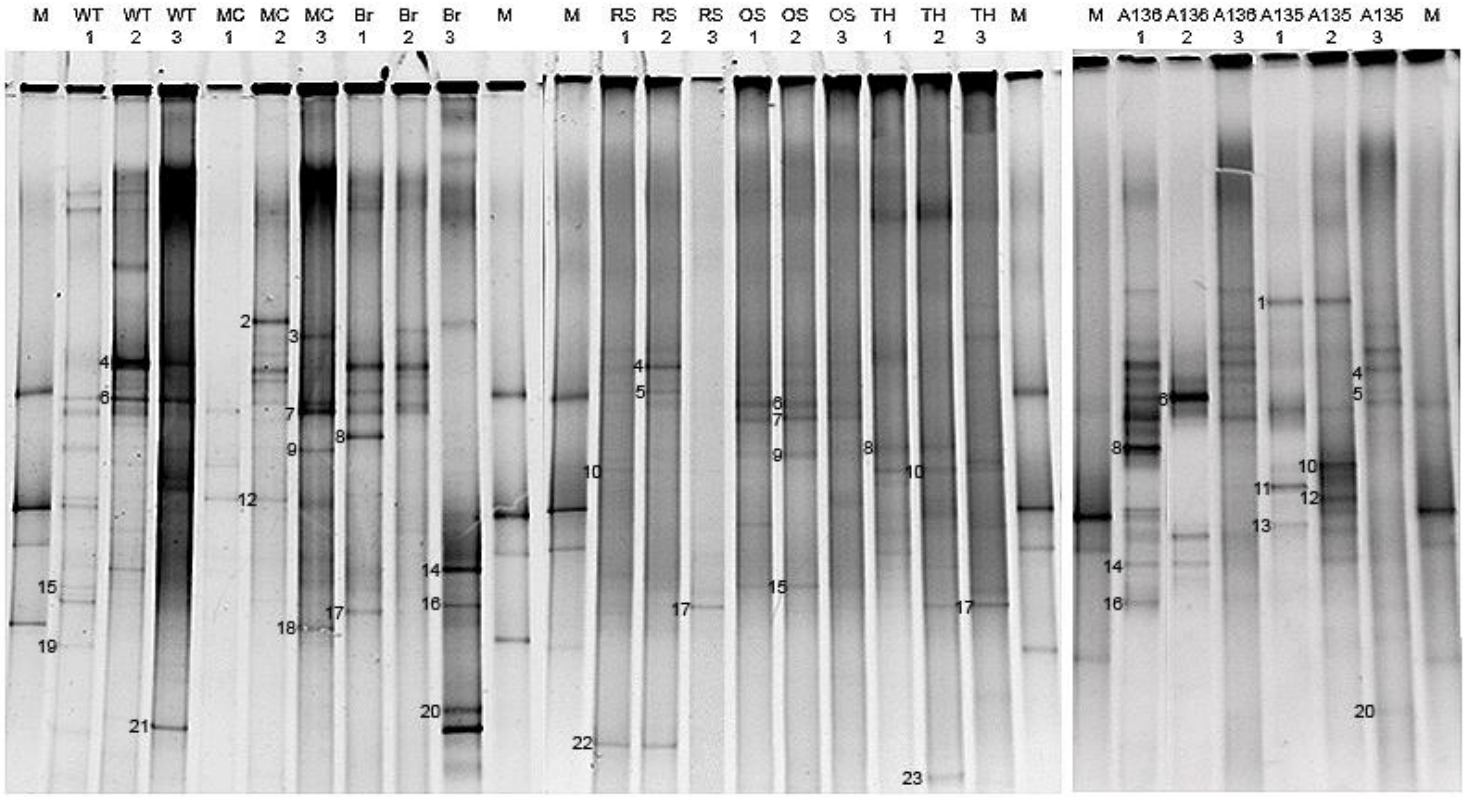

Fig 4.1 DGGE banding patterns of 16S rDNA fragments amplified using bacterial specific primers 341F-GC and 907R. Numbers indicate excised and sequenced bands. The markers were four excised bands with distinct melting position from previous DGGE run. Lane M: marker, WT: Wilkes Tip; MC: Mitchell Peninsula; Br: Browning Peninsula; RS: Red Shed; OS: Oil Spill; TH: Thala Valley; A136: ASPA 136; A135: ASPA 135.

Table 4.2 Identity from BLAST search in GenBank (assessed on October, 2008)

Band	Nearest Match	% homology	Accesion No.	Phlyum or sub phylum	Source
1	Uncultured *Chlorobi* bacterium clone Cart-N3	92	AY118152	Unclassified	Environmental sample
2	Uncultured bacterium clone FCPN496	99	EF516369	Bacteroidetes	Environmental sample
3	Uncultured *Sphingobacteria* bacterium clone GASP-KA1W1_B03	99	EU297187	Bacteroidetes	Environmental sample
4	Uncultured *Crenotrichaceae* bacterium clone Amb_16S_762	98	EF018383	Bacteroidetes	trembling aspen rhizosphere under ambient CO_2 conditions
5	Uncultured bacterium clone Madera675	98	AY647378	Bacteroidetes	Madera soil
6	Uncultured Bacteroidetes bacterium clone AI-1F_E12	98	EF219546	Bacteroidetes	unvegetated soil environments on Anchorage Island
7	Uncultured Bacteroidetes bacterium clone D10_WMSP1	100	DQ450751	Bacteroidetes	saturated alpine tundra wet meadow soil
8	Uncultured *Flavobacteria* bacterium clone GASP-WA1W1_C06 (99%)	99	EF072298	Bacteroidetes	Environmental sample
9	Uncultured bacterium clone KD2-72	98	AY218589	Bacteroidetes	Penguin Dropping, Ardley Island
10	*Algoriphagus antarcticus* clone SE45	93	AY771759	Bacteroidetes	Arctic
11	Uncultured Chlorobi bacterium clone MVS-13	94	DQ676315	Unclassified	suboxic freshwater-pond sediment
12	Uncultured Bacteroidetes bacterium clone 411T3	97	DQ110123	Bacteroidetes	Environmental sample
13	Uncultured proteobacterium clone GASP-KB2S2_G09	94	EU298061	β-Proteobacteria	Environmental sample
14	*Acinetobacter* sp. DJQD16	97	EF694304	β-Proteobacteria	subsurface water from the China Sea
15	Uncultured bacterium clone DC-II-8	96	DQ660863	Unclassified	acid mine drainage
16	*Paenibacillus daejeonensis*	95	AF290916	Firmicutes	Cultures
17	Uncultured bacterium clone KIS.T79	100	EU030493	β-Proteobacteria	west Antarctic ice sheet
18	Uncultured *Oxalobacteraceae* bacterium clone 469C4	99	EU127414	β-Proteobacteria	Cape Cod Aquifer, Subsurface Core C
19	Uncultured *Chloroflexi* bacterium clone FQSS112	97	EF522332	Unclassified	Rocky Mountain endolithic sandstone community
20	*Delftia tsuruhatensis* strain A90	98	EF421404	β-Proteobacteria	Waste water
21	*Burkholderia fungorum* isolate cd-3254	99	EF650018	β-Proteobacteria	Soil
22	Uncultured *Fibrobacteres* bacterium	97	AM690985	Unclassified	Fresh water lake
23	Uncultured beta proteobacterium clone CC_10	99	EF562559	β-Proteobacteria	Paper Pulp Column

4.3.3 Bacterial diversity and environmental variables

The bacterial diversity derived from DGGE banding patterns are converted to Shannon diversity index (Table 4.1). Generally, higher diversity was detected from remote and protected sites (H' = 2.90 – 3.15) in contrast to impacted sites (H' = 2.00 – 2.78). However, the highest bacterial diversity (H' = 3.25) was observed in soils from Wilkes Tip, an old waste disposal site located next to ASPA 136, a penguin rookery. This is not surprising as Wilkes Tip was no longer used after the late 1960s and the area has not been physically disturbed since (Stark, et al., 2003).

The Spearman rank order correlations suggested that no significant relationship was established between H' and soil chemical factor. However, water (P = 0.429) and nitrogen content (P = 0.350) showed positive correlations while the heavy metals showed negative correlations (P = -0.357 to -0.571) to the measured soil bacterial diversity. The trend with enriched heavy metal content and reduced bacteria diversity was observed from the more recently impacted sites (Thala Valley, Red Shed and Oil Spill). Such observation might be due to the detrimental effects of heavy metals to the soil bacteria (Konopka, et al., 1999; Hinojosa, Carreira, Garcá-Ruíz, & Dick, 2005). Yergeau et al. (2007a) had reported significant correlations between water and nitrogen content and abundance of 16S rRNA genes across a latitude gradient in Antarctic soil, and Powell et al. (2003) found high correlations between microbial community structure patterns and a combination of total organic carbon and heavy metals content in nearshore sediment around Casey Station.

4.3.4 NMDS ordination

The relative similarity among replicate samples within sites and among sites, based on individual and pooled banding patterns, were presented as NMDS ordinations in Figure 4.2a and 4.2b respectively. Samples from disturbed sites such as Oil Spill, Wilkes Tip and Thala Valley were generally more homogenous than those from sites with low human impact such as ASPA 135, Browning Peninsula and Mitchell Peninsula. ASPA 136 however, was the only unimpacted site which showed homogenous replicates. Figure 4.2b shows that the most similar sites, in terms of DGGE banding patterns, were ASPA 136, Wikes Tip and Mitchell Peninsula (~60% similarity), followed by Red Shed and Thala Valley (~55% similarity). The Oil Spill site banding pattern was distinct from the other sampling sites. The differences between Figure 4.2a and Figure 4.2b are due to different factors being used to plot the two figures. Figure 4.2a was constructed using only absence and presence binary matrix while Figure 4.2b was plotted using the combined occurrence frequency in three replicates from each site. The low value of the Kruskal stress index in Figure 4.2b (0.05) indicates that the 2-dimensional plot is a very good fit to the data.

The high similarity between ASPA 136 and Wilkes Tip is not unexpected as both are located in close proximity on Clark Peninsula. Although Wilkes Tip was a former waste disposal site, it has not been disturbed for nearly 50 years (Stark *et al*., 2003). Furthermore, soil and bacteria could be transported between these two sites by wind and occasional animal visit from ASPA 136. Browning Peninsula and Mitchell Peninsula are far from each other but both are barren, ice-free and away from regular human disturbances. These factors might have contributed to the bacterial similarity in these two sites. High homology was also observed between Red Shed and Thala Valley (~55%). These two highly impacted sites are located within 1 km radius of Casey station. The Oil Spill site was most dissimilar

to the other sites in the nMDS ordination of pooled samples (Figure 4.2b). There may be selective pressures on the soil microbial community at this site favouring only those strains which are able to survive in the high hydrocarbon environment. Soil bacterial species which are not able to survive or evolve in such an environment would therefore not occur in the Oil Spill site resulting in a lower bacterial diversity compared to the other study sites and a greater dissimilarity between the bacterial community at this site than those at other locations.

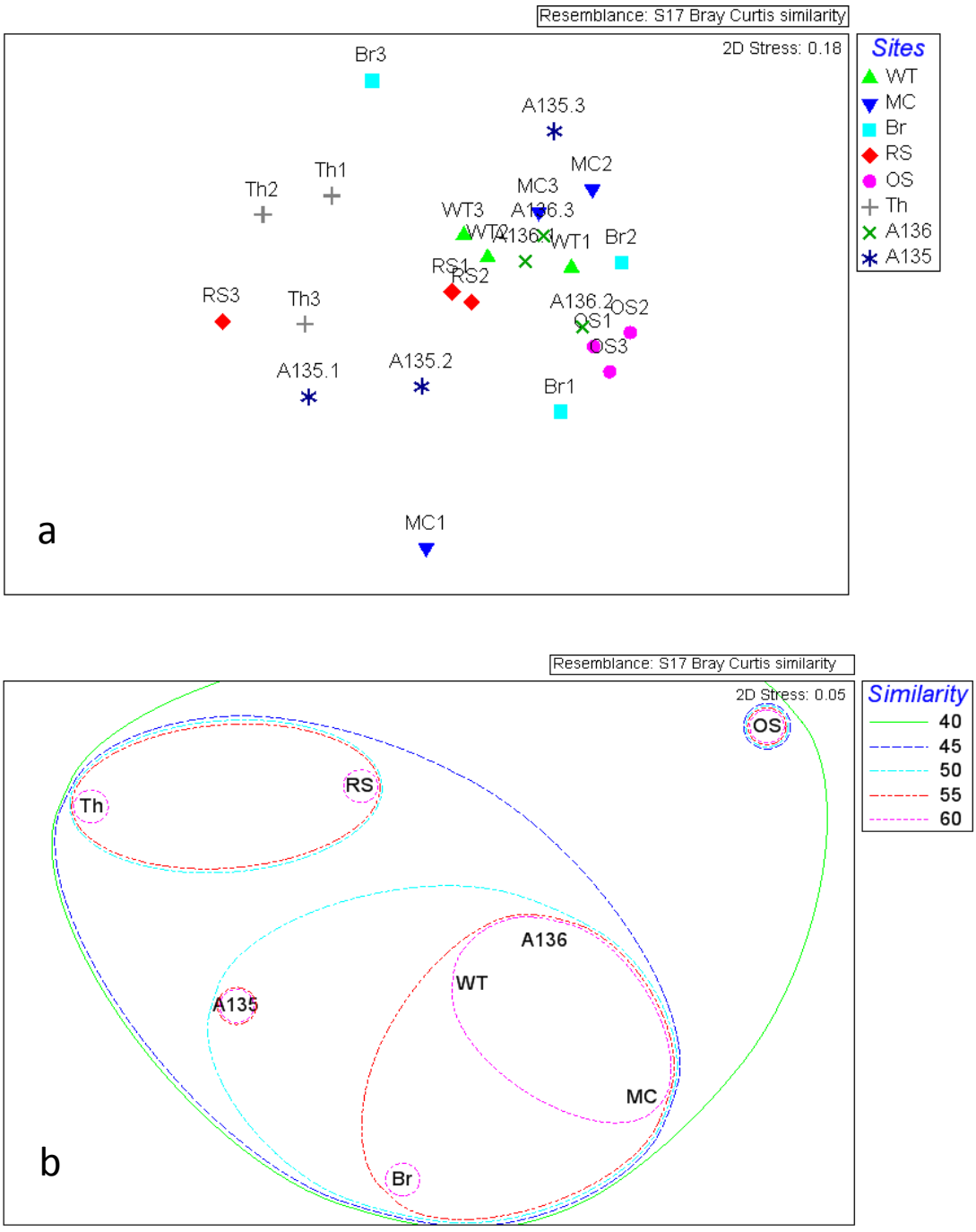

Fig 4.2 Non-metric Multidimensional Scaling Plots (NMDS) of DGGE profiles based on presence/absence binary matrix: a. individual samples; b. pooled samples–The bounding lines were derived from hierarchical agglomerative clustering. WT: Wilkes Tip; MC: Mitchell Peninsula; Br: Browning Peninsula; RS: Red Shed; OS: Oil Spill; TH: Thala Valley; A136: ASPA 136; A135: ASPA 135.

4.4 Summary

The bacterial diversity in soil samples collected from eight sites around Casey Station indicated that protected (ASPA 136 and ASPA 135) and pristine/remote sites (Browning and Mitchell Peninsulas) have higher diversity compared to impacted sites (Oil Spill, Red Shed and Thala Valley). Although Wilkes Tip was a former waste disposal site, the soil bacterial community could have rejuvenated as dumping ceased 50 years ago and since then the site has seen very little human disturbance, it being close to ASPA 136. Although no significant correlations between bacterial diversity and soil chemical properties were obtained, a trend of higher soil bacterial diversity with elevated water and nitrogen content; and lower diversity in soil with greater heavy metal content (with the exception of ASPAs sites) was observed. Despite high levels of copper and zinc being detected in ASPA 135 and 136, the soil condition might have been ameliorated by enriched moisture and nitrogen input from mosses and birds respectively which led to higher bacterial diversity in these two sites. Higher levels of heavy metals (copper, zinc and lead) in the soil samples from Thala Valley and the Oil Spill site (which also has high hydrocarbon content) might have limited the range of bacteria which were able to survive in such environments.

CHAPTER FIVE

STUDY 2. ENVIRONMENTAL INFLUENCES OF BACTERIAL DIVERSITY OF SOILS ON SIGNY ISLAND, MARITIME ANTARCTIC

5.1 Introduction and objectives

Signy Island harbours a wide range of terrestrial habitats (see Smith, (1972) and Holdgate, (1977) for detailed descriptions of terrestrial habitats and vegetation). The island's geology is predominantly quartz-mica-schist with some outcrops of marble and amphibolites (Caulkett & Ellis-Evans, 1997). Being a small island experiencing typically strong winds, the entire island is considered to receive nutrient inputs through sea-spray (Bokhorst, et al., 2007b). Additionally, most terrestrial lowland habitats on the island are impacted by marine vertebrate activities, notably ornithogenic input (guano, etc) from birds and in particular penguin rookeries, along with elephant seal (*Mirounga leonina*) wallows in certain coastal locations, and from resting and moulting fur seals (*Arctocephalus gazella*) in most accessible coastal areas. Thus, levels of vertebrate impact can be considered along a 'gradient' from the most impacted sites at the centre of dense penguin rookeries and seal wallows, to the relatively few 'non-impacted' locations mostly restricted to higher altitude areas, and areas not directly accessible from the coast.

To date, studies of bacterial diversity on Signy Island have focused almost entirely on the freshwater environment (Pearce, 2003; Pearce, van der Gast, Lawley, & Ellis-Evans, 2003). Only Yergeau et al. (2007a,b) have addressed the bacterial communities of certain terrestrial habitats, as part of a larger microbial diversity study spanning the wide environmental gradient between the Falkland Islands and southern Antarctic Peninsula.

Nevertheless, a variety of bacteria has been reported in these studies from different ecosystems on Signy Island (see also Moosvi, et al., 2005).

The primary objective of this study was to provide baseline understanding of the dominant contributions to soil bacterial diversity across soils with widely varying levels of vertebrate influence on Signy Island. In addition, 10 soil chemical parameters were assessed in order to elucidate the abiotic factors having the greatest influence on the structure of bacterial communities across an environmental gradient.

5.2 Materials and Methods

The methodologies were listed in Chapter 3.

5.3 Results

5.3.1 Soil chemical properties

The pH values of the eight studied soils were generally acidic (Table 5.1), with the most alkaline condition being found in barren inland soil from Jane Col. Significantly higher carbon, nitrogen, water and copper content were measured from the densely populated penguin rookeries (North Point, Gourlay Peninsula) in contrast to other locations. Although not statistically significant, samples from penguin rookeries and seal wallows contained higher levels of copper and lead than Jane Col and Berntsen Point. Due to the close proximity of most study locations to sea, most of the soils registered high electrical conductivity, particularly those with strong ornithogenic influence (North Point and

Gourlay Peninsula) and Cemetery Flats 1. Conversely, Jane Col which is situated at an elevated inland location with much lower vertebrate influence, generated the lowest carbon, nitrogen and conductivity levels.

Table 5.1 Selected environmental properties (mean ± SE) of soil samples from the study sites (n=6 for each site)

Sites	%Water	pH	E.C.	% Carbon	% Nitrogen	Copper (ppm)	Lead (ppm)	Zinc (ppm)	Iron (ppm)	Nickel (ppm)
Gourlay Peninsula	44.15[b] ± 16.23	5.53[a,c,d] ± 0.27	439.17[c] ± 223.12	16.17[b] ± 5.10	3.17[b] ± 1.14	188.21[b] ± 47.58	12.04[a,b] ± 2.76	74.30[c] ± 8.22	6673.30[a,b] ± 546.96	8.56[a] ± 2.72
North Point	56.81[b] ± 11.76	4.99[a,d] ± 0.44	219.68[a,c] ± 89.75	19.14[b] ± 4.92	3.18[b] ± 0.88	176.52[b] ± 59.81	16.13[b] ± 2.84	78.48[c] ± 17.94	13508.53[c] ± 796.72	7.00[a,b] ± 3.67
Cummings Cove	27.98[a] ± 8.74	4.79[a] ± 0.55	121.73[a] ± 40.89	6.20[a] ± 7.78	0.91[a] ± 0.91	28.87[a] ± 14.76	12.50[a,b] ± 1.32	22.35[a,b] ± 10.89	12202.90[c] ± 1283.29	0.84[b] ± 1.34
Elephant Flats	18.98[a] ± 3.58	5.26[a,c,d] ± 0.29	122.15[a,b] ± 75.67	1.91[a] ± 1.18	0.54[a] ± 0.10	7.59[a] ± 2.02	11.72[a,b] ± 1.24	13.40[a,b] ± 4.51	7064.85[a,b] ± 224.05	6.33[a,b] ± 2.17
Cemetery Flats 1	13.03[a] ± 3.98	5.97[b,c] ± 0.26	391.00[a,c] ± 273.51	1.47 [a] ± 0.99	0.52 [a] ± 0.05	6.10[a] ± 3.07	12.01[a,b] ± 5.73	16.37[a,b] ± 6.58	3877.15[a] ± 2687.62	5.85[a,b] ± 4.45
Cemetery Flats 2	17.20[a] ± 6.95	5.66[b,d] ± 0.32	280.67[a,b,c] ± 221.14	3.01[a] ± 2.12	0.66 [a] ± 0.26	15.80[a] ± 16.83	11.68[a,b] ± 3.41	29.55[b] ± 16.42	7208.75[a,b] ± 4238.88	4.18[a,b] ± 6.59
Berntsen Point	14.64[a] ± 4.14	5.09[a,d] ± 0.69	60.23[b] ± 31.96	3.80 [a] ± 1.85	0.76 [a] ± 0.28	2.88 [a] ± 0.42	8.70[a] ± 2.70	5.35 [a] ± 2.25	7624.42[b] ± 1264.51	[j]N.D.[b]
Jane Col	13.06 [a] ± 1.94	6.30[b] ± 0.30	27.05[b] ± 5.14	0.57[a] ± 0.17	<0.5[a] ± 0.00[i]	2.81[a] ± 1.62	10.98[a,b] ± 4.08	18.34[a,b] ± 4.90	5691.87[a,b] ± 1102.05	8.85[a] ± 6.96

Superscripts [a,b,c,d] denote significant differences among means. Sites with different letter are significantly different ($P < 0.05$).

[i] All six replicates showed result lower than 0.5%

[j] Not Detected

Note: For the ease of calculation, value <0.5 was defined as 0.5 and not detected was defined as 0 in ANOVA

Table 5.2 Diversity indices, multivariate dispersion indices, genetic distance and taxonomic composition derived from DGGE banding patterns

	Gourlay Peninsula	North Point	Cummings Cove	Elephant Flats	Cemetery Flats 1	Cemetery Flats 2	Berntsen Point	Jane Col
Distance[a]	0.236	0.203	0.236	0.236	0.217	0.245	0.276	0.283
S[b]	14.83 ± 3.54	7.83 ± 2.32	7.00 ± 3.90	10.67 ± 2.34	9.50 ± 2.26	9.00 ± 2.37	8.67 ± 5.39	9.67 ± 6.19
H'[c]	2.68 ± 0.22	2.02 ± 0.33	1.76 ± 0.72	2.35 ± 0.23	2.23 ± 0.23	2.17 ± 0.27	1.97 ± 0.69	2.00 ± 0.90
Dispersion[d]	0.346	0.853	1.401	0.836	1.342	0.722	1.142	1.387
Bacteroidetes	81.82% (18)	82.35% (14)	78.95% (15)	77.27% (17)	83.33% (20)	81.25% (13)	72.72% (16)	70.83% (17)
Firmicutes	4.55% (1)	5.88% (1)	10.52% (2)	9.09% (2)	8.33% (2)	6.25% (1)	13.64% (3)	12.5% (3)
Cyanobacteria	9.09% (2)	5.88% (1)	10.52% (2)	4.55% (1)	4.17% (1)	0% (0)	9.09% (2)	8.33% (2)
Proteobacteria	4.55% (1)	5.88% (1)	0% (0)	4.55% (1)	4.17% (1)	6.25% (1)	0% (0)	0% (0)
Acidobacteria	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	4.17% (1)
Unclassified	0% (0)	0% (0)	0% (0)	4.55% (1)	0% (0)	6.25% (1)	4.55% (1)	4.17% (1)

[a] Genetic Distance by Jukes Cantor Model; [b] Total Species; [c] Shannon Diversity Index; [d] Multivariate Dispersion indices

Note: number in parentheses refers to number of "unique" bands in each phylum detected from six replicates within each location

5.3.2 DGGE profiling and bacterial diversity

High within site variability was observed from the DGGE profiles (Fig 5.1; Table 5.2 - Dispersion), with Cummings Cove showing the highest variation and Gourlay Peninsula the least. Although no clear pattern in this intra-site variability was apparent, a trend of lower variation in sites with greater nutrient content (carbon and nitrogen – Table 5.1) was observed when comparison was made within the three penguin rookeries (lowest variation in Gourlay Peninsula, followed by North Point and Cummings Cove) and within the three seal wallows (lowest variation in Elephant Flats, followed by Cemetery Flats 2 and Cemetery Flats 1).

A total of 31 DGGE band positions were detected across all sampling sites, and the identities of 29 bands were determined (Table 5.3). The majority of the identified bands (21 sequences) were assigned to the Bacteroidetes group. This class of bacteria was dominant in all sites, with higher abundance in vertebrate-influenced sites (77.27% - 83.33%) compared to Bernsten Point (72.72%) and Jane Col (70.83%). Most of the sequences affiliated to the Bacteroidetes showed high homology to uncultured clones extracted from ornithogenic sites in Antarctica (Table 5.3). In addition, representatives from Firmicutes (3 bands), Cyanobacteria (2 bands), Acidobacteria (1 band), Proteobacteria (1 band) and an unclassified group (1 band) were also retrieved. Acidobacteria were identified only from Jane Col.

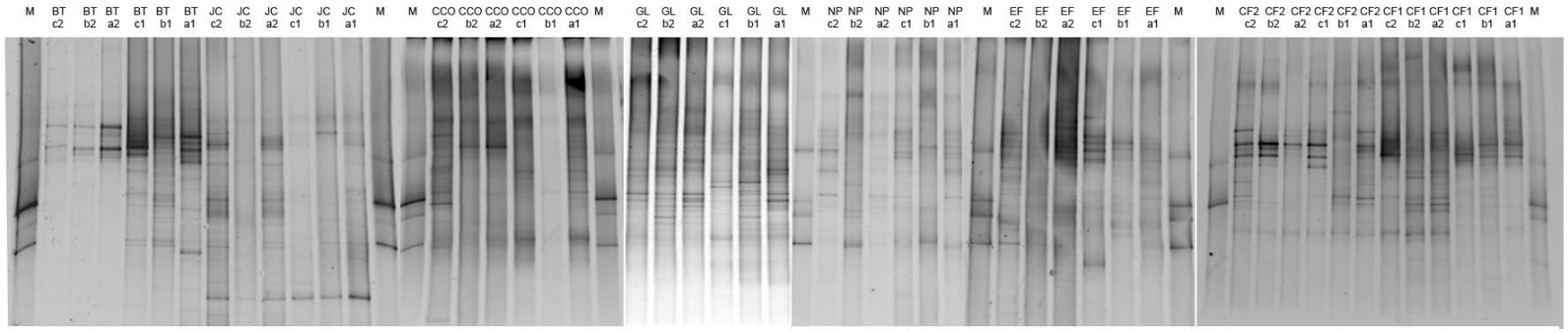

Fig 5.1 DGGE banding patterns of 16S rDNA fragments amplified using bacterial specific primers 341F-GC and 907R. The markers were four excised bands with distinct melting position from previous DGGE run. Lane M: marker, BT: Berntsen Point; JC: Jane Col; CCO: Cummings Cove; NP: North Point; GL:Gourlay Peninsula; EF: Elephant Flats; CF2: Cemetery Flats 2; CF1: Cemetery Flats

Table 5.3 Identity of excised and sequenced DGGE bands from BLAST search in GenBank (assessed on November, 2008)

Band	Closest relative from GenBank BLAST search	% Hit	Phylum/ Sub-phylum	Accession No	Origin
B1	Uncultured cyanobacterium clone A822	99%	Cyanobacteria	EU283559	Anderson Lake, USA
B2	Uncultured bacterium; KD8-75	99%	Bacteroidetes	AY218692	Penguin Droppings Sediments, Ardley Island
B3	Uncultured bacterium; KD2-72	97%	Bacteroidetes	AY218589	Penguin Droppings Sediments, Ardley Island
B4	Uncultured bacterium; KD6-86	99%	Bacteroidetes	AY188323	Penguin Droppings Sediments, Ardley Island
B5	Uncultured bacterium clone KD7-36	99%	Bacteroidetes	AY218708	Penguin Droppings Sediments, Ardley Island
B6	Uncultured bacterium; UOXD-e10	98%	Bacteroidetes	EU869765	Onyx River, Antarctica
B7	Uncultured bacterium; KD1-64	98%	Bacteroidetes	AY218564	Penguin Droppings Sediments, Ardley Island
B8	Uncultured Bacteroidetes; AI-1F_E12	99%	Bacteroidetes	EF219546	Unvegetated soil environments, Anchorage Island
B9	Uncultured bacterium; KD8-102	99%	Bacteroidetes	AY218677	Penguin Droppings Sediments
B10	Uncultured soil bacterium; E08_bac_con	100%	Bacteroidetes	EU861850	Dry meadow surface soil
B11	Uncultured bacterium clone KD5-100	100%	Proteobacteria	AY218723	Penguin Droppings Sediments, Ardley Island
B12	Uncultured bacterium; KD3-93	99%	Bacteroidetes	AY218622	Penguin Droppings Sediments
B13	Uncultured bacterium; zEL27	97%	Bacteroidetes	DQ415821	Frasassi sulfidic cave stream biofilm
B14	Pedobacter kribbensis strain PB93	96%	Bacteroidetes	EF660752	Soil
B15	Lewinella nigricans, strain ATCC 23147T	90%	Bacteroidetes	AM295255	Beach Sediment
B16	Uncultured bacterium; BFA_075	95%	Bacteroidetes	EF444134	freshwater wetland soils
B17	Uncultured bacterium; KD3-67	97%	Bacteroidetes	AY188307	Penguin Droppings Sediments
B18	Uncultured bacterium; KD3-67	98%	Bacteroidetes	AY188307	Penguin Droppings Sediments
B19	Uncultured soil bacterium; TIIA5	98%	Bacteroidetes	DQ297951	Hydrocarbon contaminated soil
B20	Uncultured bacterium; SRRB35	98%	Bacteroidetes	AB240509	Environmental sample
B21	Ginsengisolibacter sp. NP11	99%	Bacteroidetes	EU196345	Cold Saline Sulfidic Spring, Canadian High Arctic
B22	Uncultured bacterium clone FCPT767	97%	Unclassified	EF516481	Grassland soil
B23	Uncultured Firmicutes; GASP-MA1W3_H06	97%	Firmicutes	EF662740	Cropland
B24	Uncultured bacterium; KD4-4	97%	Bacteroidetes	AY218633	Penguin Droppings Sediments
B25	Phormidium autumnale CCALA 697	99%	Cyanobacteria	AM778710	Cultures
B26	Uncultured Clostridiaceae; pCOF_65.7_F11	94%	Firmicutes	EU156149	Hot Spring
B27	Uncultured Firmicutes; GASP-MB1S2_E01	91%	Firmicutes	EF664607	Forest Soil
B29	Uncultured bacterium; 1/2/3B	99%	Bacteroidetes	FJ380137	Antarctica: Cape Hallett
B31	Uncultured bacterium; Elev_16S_1335	85%	Acidobacteria	EF019956	Trembling aspen rhizosphere

No statistically significant difference in diversity (H') was apparent between sampling sites (Table 5.1). However, higher values of H' were observed from penguin rookeries and seal wallows (with the exception of Cummings Cove). Nevertheless, as shown in Table 5.2, this might simply be due to more bands being detected in DGGE from those sites. The diversity index was not correlated with the estimate of genetic distance, with the site with lowest H' (Jane Col) showing the greatest genetic distance while that with the highest H' (Gourlay Peninsula) showed only moderate genetic distance.

Analysis of Similarity (ANOSIM) between sampling locations (Table 5.4a), indicate significant differences in bacterial community assemblages between locations (ANOSIM, Global $R = 0.393$, $P = 0.001$). The highest discrepancy was observed between North Point and Cemetery Flats 2, while considerable difference was seen between Gourlay Peninsula and North Point, and between Gourlay Peninsula and the three wallow sites. In addition, ANOSIM was performed on groups of aggregated data: Cemetery Flats 1, Cemetery Flats 2 and Elephant Flats were grouped as seal wallows; North Point, Gourlay Peninsula and Cummings Cove as penguin rookeries ($R = 0.324$; $P = 0.001$). Comparison was then made between these two groupings and Jane Col (barren soil) and Berntsen Point (vegetated fellfield soil). Notwithstanding the overall differences indicated by ANOSIM, this analysis also indicated that the dominant bacterial groups clearly overlapped within both the animal influenced sites and between animal influenced sites and vegetated soil, while barren soil community from Jane Col was again significantly distinct from the other locations (Table 5.4b).

Table 5.4a Pairwise ANOSIM of DGGE derived bacteria community structure (Global R = 0.393; P = 0.001)

	BT	JC	CCO	GL	NP	EF	CF2	CF1
BT								
JC	0.334^{*}							
CCO	0.206^{ns}	0.235^{*}						
GL	0.560^{**}	0.530^{**}	0.404^{**}					
NP	0.496^{**}	0.514^{**}	0.260^{*}	0.791^{**}				
EF	0.357^{*}	0.524^{**}	0.460^{**}	0.706^{**}	0.387^{**}			
CF2	0.468^{**}	0.629^{**}	0.447^{**}	0.743^{**}	0.859^{**}	0.443^{**}		
CF1	0.091^{ns}	0.420^{**}	0.23^{ns}	0.376^{**}	0.254^{*}	0.020^{ns}	0.126^{ns}	

Note: ^{ns} not significant; * P<0.05; ** P<0.01; BT=Berntsen Point; JC=Jane Col; CCO=Cummings Cove; GL=Gourlay Peninsula; EF=Elephant Flats; CF2=Cemetery Flats 2; CF1=Cemetery Flats1

Table 5.4b Grouped pairwise ANOSIM of sites with and without animal influence (Global R = 0.324; P = 0.001)

	Vegetated	Barren	Rookeries	Wallows
Vegetated[a]				
Barren[b]	0.334^{*}			
Rookeries[c]	0.262^{*}	0.362^{*}		
Wallows[d]	0.285^{**}	0.609^{**}	0.261^{**}	

Note: [a] Berntsen Point; [b] Jane Col; [c] Gourlay Peninsula, North Point and Cummings Cove; [d] Cemetery Flats 1, Cemetery Flats 2 and Elephant Flats

In order to clarify the relationship between soil variables and bacterial community structure as indicated by DGGE banding patterns, the bacterial assemblage pattern and soil variable data were subjected to the BEST routine from the Primer 6 package (Table 5.5). The highest correlated single variables to the DGGE banding patterns were pH, followed by conductivity and Cu, while the combination of environmental variables with strongest explanatory value included pH, conductivity, Cu and Pb (global $R = 0.361$, $P = 0.0001$).

Table 5.5 Spearman rank correlations of environmental variables with the bacterial DGGE banding profiles (Global $R = 0.316$; $P = 0.0001$)

	Correlation Coefficients	**Variables**
Single variables	0.230	pH
	0.145	E.C.
	0.136	Cu
Multiple variables	0.316	pH, E.C. Cu and Pb
	0.313	pH, E.C. and Pb
	0.309	pH and Pb

5.4 Discussion

High within site variation was observed in both the environmental variables and DGGE banding patterns of soil samples collected from Signy Island. Analogous observations including variations in soil inorganic nutrient (Arnold, Convey, Hughes, & Wynn-Williams, 2003), soil physical and chemical properties (Holdgate, 1977; Davey & Rothery, 1993), and patchiness in terrestrial flora and fauna (Holdgate, 1977; Usher & Booth, 1986) have been reported previously from Signy Island and also from Alexander Island (southern maritime Antarctic) (Engelen, Convey, Hodgson, Worland, & Ott, 2008).

Ornithogenic soils (Gourlay Peninsula, North Point, Cummings Cove) were generally enriched in nutrients, water, salinity and heavy metals compared to non-ornithogenic soils (Jane Col, Berntsen Point) (Table 5.1) (Melick, Hovenden, & Seppelt, 1994; Barrett, et al., 2006b; Michel, et al., 2006; Simas, et al., 2007; Aislabie, et al., 2008). The higher level of carbon and nitrogen content may be due to constant deposition of guano and moulted feathers (Mizutani & Wada, 1988). Soil pH was consistently slightly acidic in penguin rookeries, which can be attributed to mineralization processes acting on guano to produce nitric and sulphuric acid (Bolter, et al., 1997; Simas, et al., 2007).

Despite not being statistically evident in the current study, seal wallows have been reported to possess greater nutrient levels in comparison to mineral soil in Antarctica (Smith, 2005). In this study, the total carbon and nitrogen levels measured in the Signy Island seal wallows were greater than those found at Jane Col (Table 5.1) and in mineral soil elsewhere in Antarctica (Aislabie, et al., 2006; Chong, et al., 2009a). The elevated nutrient contents measured at Berntsen Point may also be attributed to vertebrate influence as although this site does not support breeding colonies, it is clearly close to sites of bird activity (petrel spp.

and skua) and is regularly transited by these birds as well as by penguins and seals. The potential importance of nutrient input from overflying birds was noted by Bokhorst et al.(2007b).

A trend of increasing zinc and copper were observed in seal wallows and penguin rookeries. Krill, which contains enriched levels of trace metals (Table 5.6), is likely to be the main source of copper and zinc in penguin and seal diet, before ultimately being deposited in the soil. Diet data (between 2004 and 2006), obtained from the routine CCAMLR monitoring programme, indicated that gentoo penguins from North Point, together with chinstrap and Adélie penguins from both North Point and Gourlay Peninsula, mainly consume crustaceans consisting of around 98% Antarctic krill, *Euphausia superba* (data not shown). Krill had also been reported to be one of the main diet components in Antarctic seal populations (Green & Williams, 1986; Reid, 1995). Other diets including fish (Table 5.6) might also contribute to the elevated metal content. The copper content in the densely populated penguin rookeries (North Point and Gourlay Peninsula) was about 10 fold greater than in seal wallows. This might be related to the difference in metal content in animal excrement (10 times more copper in penguin excrement compared to seal excrement) (Yin, et al., 2008). The elevated iron content in vegetated sites (North Point, Cummings Cove, Cemetery Flats 2 and Berntsen Point) as opposed to non-vegetated sites (Gourlay Peninsula and Jane Col) might have originated from local plant material (algae and moss) (Table 5.6). Yergeau et al. (2007a) reported that soil iron content in vegetated sites was approximately twice that in fellfield locations on Signy Island.

As suggested by Kowalchuk et al. (2006), the data obtained from DGGE profiling is more accurately considered as the "structure of dominant populations" rather than a general measure of bacterial diversity, due to the fact that only numerically abundant phylotypes

will be detected (Nakatsu, 2007). Thus, the H' reported here more accurately describes the diversity of dominant bacteria. No significant differences were observed from H' values obtained from all eight studied sites (Table 5.2). Nevertheless, vertebrate influenced sites (with the exception of Cummings Cove) showed a tendency for greater H' values.

Table 5.6 Heavy metal content (μg/g dry weight) of representative Antarctic organisms

Species	Cu	Zn	Fe	Pb	Ni	Reference
Algae						
Palmaria decipiens n=10	-	37.8	4450	-	-	dos Santos et al., 2006
Macrocystis spp. n=10	-	27.7	91	-	-	dos Santos et al., 2006
Desmarestia spp. n=10	-	39.6	460	-	-	dos Santos et al., 2006
Bryophytes						
Bryum spp. n=5	-	18.1	3040	-	-	dos Santos et al., 2006
Polytrichum spp. n=5	-	28.0	4348	-	-	dos Santos et al., 2006
Lichen						
Usnea spp. n=10	-	5.6	139	-	-	dos Santos et al., 2006
Crustacea						
Euphausia superba n=76	12.7	9.6	3.6	0.04	0.45	Yamamoto et al., 1987
E. superba n=76	54.1	44.5	14.4	0.45	1.87	[a]Honda et al., 1987
E. superba n=20	36.0	122.0	140	0.019	3.0	Deheyn et al., 2005
Fish						
Trematomus scotti n=12	2.0	27.0	90	1.0	13.0	Deheyn et al., 2005
Champsocephalus gunnari n=5	12.0	86.0	800	8.0	1020.0	Deheyn et al., 2005
Trematomus bernacchii n=30	0.29	7.78	2.12	-	0.07	[a]Honda et al., 1987
Pagothenia borchgrevinki n=22	0.65	11.1	6.51	0.15	0.08	[a]Honda et al., 1987

[a] as μg/g wet weight

One of the limitations in interpretation of presence-absence binary data generated from DGGE using diversity indices relates to the inability to differentiate between sites with same or similar number of bands (Gafan, et al., 2005). For example, a site which produces three bands associated with *Pseudomonas* in DGGE will have no difference (in terms of

index) with another site which produces three bands associated with cyanobacteria. Hence, analysis of similarity (ANOSIM - Table 5.4a and 5.4b) was carried out to supplement H'. ANOSIM indicated a significant difference across all sites, and that the dominant bacteria community structure in all the studied locations was significantly different. Notwithstanding this difference between sites, when comparison was made between aggregated site data (Table 5.4b), the highest similarity was observed between seal wallows, penguin rookeries and vegetated fellfield soil, suggesting a general effect of vertebrate guano and vegetation in influencing soil bacterial community structure. Conversely, Jane Col exhibited the most distinct dominant bacteria community structure. This observation was partially in agreement with the genetic distance (Table 5.2), as Jane Col also showed the greatest genetic distance. Sites with animal influence (rookeries 0.203-0.236; wallows 0.217-0.245) recorded lower genetic distances than vegetated fellfield soil (0.276) and barren soil (0.283).

The majority of the retrieved bacterial sequences were from the Bacteroidetes. This class is commonly found in Antarctica and has been reported to possess the ability to degrade a wide range of polymeric substances such as chitin and cellobiose (Aislabie, et al., 2006; Li, et al., 2006). The highest Bacteroidetes proportion was obtained in DGGE profiles from seal wallows and penguin rookeries (77.27%-83.33%). Members of the Bacteroidetes have been found to be prevalent in the intestine and faeces of mammals (Dick & Field, 2004; Flint, Duncan, Scott, & Louis, 2007), and members of Flavobacteriaceae are prominent during the early stages of guano decomposition (Zdanowski, et al., 2004).

The cyanobacterium *Phormidium autumnale* had been isolated previously from Jane Col, Signy Island (Wynn-Williams, 1996). In the current study, sequences showing high homology to this cyanobacterium were detected from all sites except Cemetery Flats 1 and

2. In addition, sequences affiliated with Firmicutes were also present across all sampling sites. A sequence associated with Acidobacteria was only detected at Jane Col. This group of bacteria is commonly extracted from Antarctic soils (Aislabie, et al., 2006) and tends to occur in moist soils with alkaline pH and low EC (Aislabie, et al., 2008), as found here in Jane Col. The low occurrence of cyanobacteria and acidobacteria in this study is generally in agreement with studies conducted elsewhere in Antarctica (Powell, et al., 2003; Aislabie, et al., 2006; Aislabie, et al., 2008), suggesting lower overall abundance of these groups in Antarctic soil.

Although our finding of pH as the most important factor influencing bacterial diversity is in agreement with Fierer & Jackson, (2006), care has to be taken when interpreting these data as no consistent correlations between soil variables and bacterial diversity or bacterial abundance had been reported from previous studies. Based on Yergeau et al. (2007a), the abundance of 16S rRNA genes obtained from the soil of vegetated and fellfield sites from three locations across different latitudes progressing into Antarctica (Falkland Islands, Signy Island, Anchorage Island) was significantly positively correlated with water content ($r = 0.56$), conductivity ($r = 0.54$) and chloride ($r = 0.51$). Stark et al. (2003) found that certain heavy metal (Cd, Cu, Pb, Sn, and Zn) concentrations might provide the strongest explanatory power for the microbial assemblage pattern in near shore sediments.

5.5 Summary

The bacterial communities of all eight studied locations on Signy Island differed significantly, despite those from vegetated soil, seal wallows and penguin rookeries also showing considerable overlap. Although vertebrate influenced sites generally showed

greater dominant bacterial diversity, the genetic distances calculated from retrieved sequences suggested otherwise. Thus, the greatest genetic distance was observed at Jane Col, a site with alkaline pH and the lowest nutrient (carbon and nitrogen) levels. A combination of pH, conductivity, Cu and Pb showed the highest explanatory value for the bacterial community structure across these study locations.

CHAPTER SIX

STUDY 3. HIGH LEVELS OF SPATIAL HETEROGENEITY IN THE BIODIVERSITY OF SOIL PROKARYOTES ON SIGNY ISLAND, ANTARCTICA

6.1 Introductions and objectives

There is a consensus that spatial variation amongst soil organisms is not random but exhibits predictable patterns over different spatial scales, with small scale variation exhibiting greater diversity than large scale variation (Wiens, 1989; Ettema & Wardle, 2002; Fraschetti, Terlizzi, & Benedetti-Cecchi, 2005). Such small scale variation could be more susceptible to local environmental influences such as areas of increased substrate availability (Horner-Devine, Carney, & Bohannan, 2004a). Bailey and Wynn-Williams, (1982) reported that organic content (loss on ignition), total N, and water content showed significant direct correlations with microbial counts from soil at 6 locations on Signy Island, while pH showed an inverse relationship. However, Wynn-Williams, (1990) proposed carbon as the limiting factor for cyanobacterial and algal colonization of frost-sorted soil polygons at Jane Col. In addition, recent culture-independent studies have also shown the direct influence of soil properties such as soil nutrients, moisture and pH on bacterial diversity (Barrett, et al., 2006a; Barrett, et al., 2006b; Aislabie, et al., 2008; Aislabie, et al., 2009) and these parameters also showed close relationship to specific functional genes such as glutamate dehydrogenase and nitrate reductase (Yergeau, et al., 2007b).

In the preliminary study soil microbial biodiversity on Signy Island (Study 2), pH, conductivity, copper and lead content were found to correlate most strongly with soil prokaryote biodiversity. In addition, substantial overlap was observed across sites visibly

affected by seals, penguins and the presence of vegetation. Extending from these knowledge, this study aim to further examine the links between soil prokaryote biodiversity and a range of biological, disturbance and soil chemistry factors across a larger set of 15 disparate locations on Signy Island.

6.2 Materials and Methods

The methodologies were listed in Chapter 3

6.3 Results

6.3.1 Soil chemical properties

Soil chemical properties varied significantly across all locations (one way-MANOVA, Wilks' $\lambda = 0.00001$, $F_{140, 558.77} = 12.870$, $P<0.0001$). The pH values of the soils on Signy Island were generally acidic (pH 4.79 - 6.81) with barren soils being least acidic. Highest mean water, carbon and nitrogen content were recorded from penguin rookeries (Gourlay Peninsula, North Point and Cummings Cove), with the exception of carbon content of soil from Pumphouse. However water, carbon and nitrogen contents in Cummings Cove (less densely populated rookery) were 2-3 times lower than the other rookery locations. Electrical conductivity was higher (130.52 -1088.67 $\mu S\ cm^{-1}$) at sites closer to the sea as opposed to inland locations (27.06 – 60.23 $\mu S\ cm^{-1}$). The highest copper contents were detected from Gourlay Peninsula and North Point, both heavily populated penguin rookeries, whilst the highest zinc and lead contents were found in human impacted barren soil at Signy Station and Pumphouse. Among all 5 measured heavy metals, iron was the

most abundant element (3875.15 – 12202.90 mg kg^{-1}), and nickel the least abundant (0 – 11.89 mg kg^{-1}).

Significant differences in soil properties between soil under different environmental influences (Factorial MANOVA, Wilks' λ = 0.05556, $F_{30,\ 214.95}$ = 12.016, P < 0.0001) suggested that the patterns of soil properties varied when compared across vegetated, barren, shore and vertebrate influenced sites. In addition, the variability in soil parameters was effected by the presence of human disturbance (Factorial MANOVA, Wilks' λ = 0.32227, $F_{10,\ 73}$ = 15.323, P<0.0001). Furthermore, both factors (environmental influences and human disturbance) showed interdependency in explaining the soil chemical variations across all sampling sites, as indicated by significant interactions in measured soil properties between different environmental influences and the presence of human disturbance (Factorial MANOVA, Wilks' λ = 0.11949, $F_{30,\ 214.95}$ = 7.611, P<0.0001) (Table 6.1). For example, seal wallows and penguin rookeries away from human influence had significantly higher water, nitrogen, copper and iron contents, and lower pH, in comparison with wallows associated with human impact. Higher levels of carbon, lead, zinc and nickel were also present in relatively pristine barren locations in comparison with highly impacted but barren soil from at Pumphouse and Signy Station. Finally, higher nickel content was detected in soils obtained under unimpacted than from impacted vegetation, and the impacted shore location showed greater pH and EC in comparison with the unimpacted Cummings Shore.

Overall, comparison across the sites with different external impacts (environmental and human disturbance) suggested significantly higher water, nitrogen and copper contents (with the exception of "impacted barren") at vertebrate influenced locations not subject to

human disturbance, while significantly greater lead and zinc contents were measured from human disturbed barren soils compared with other locations.

Table 6.1 Selected environmental properties and diversity indices (mean ± standard error) of the aggregated locations. Aggregation classes based on subjective assessment of the major environmental influence at each site are given in Table 3.2 (see section 3.1.2). Reference to 'disturbance' indicates historical or current present of human disturbance at a location.

Aggregation	% Water	pH	E.C.	% Carbon	% Nitrogen	Copper (mg kg^{-1})	Lead (mg kg^{-1})	Zinc (mg kg^{-1})	Iron (g kg^{-1})	Nickel (mg kg^{-1})	H'	Distance
Undisturbed Vertebrate	37^{a} ± 3.7	5.14^{c} ± 0.10	$226^{b,c}$ ± 36.3	10.9^{a} ± 1.8	2.0^{a} ± 0.3	100.3^{a} ± 18.7	13.1^{b} ± 0.6	47.1^{b} ± 6.5	9^{b} ±0.6	$5.7^{a,c}$ ± 0.8	2.20^{a} ± 0.11	0.22^{a} ± 0.03
Disturbed Vertebrate	15^{b} ± 1.7	$5.81^{a,b,e}$ ± 0.09	336^{b} ± 70.4	2.2^{a} ± 0.5	0.6^{b} ± 0.1	11.0^{b} ± 3.6	11.9^{b} ± 1.3	23.0^{b} ± 4.0	6^{a} ± 1.1	$5.0^{a,b,c}$ ± 1.6	$2.20^{a,b}$ ± 0.07	0.18^{a} ± 0.01
Undisturbed Barren	12^{b} ± 0.6	$6.23^{b,d}$ ± 0.06	$31^{d,e}$ ± 2.6	0.7^{b} ± 0.1	$<0.5^{i\,b}$	2.7^{b} ± 0.5	11.2^{b} ± 0.7	17.2^{b} ± 1.0	6^{a} ± 0.5	$7.6^{c,d}$ ± 1.0	1.56^{b} ± 0.15	0.33^{b} ± 0.02
Disturbed Barren	16^{b} ± 1.4	$6.19^{b,d}$ ± 0.19	$76^{c,d}$ ± 17.2	22.8^{c} ± 6.9	0.7^{b} ± 0.1	$53.5^{a,b}$ ± 14.7	646.5^{a} ± 205.6	265.8^{a} ± 60.9	$8^{a,b}$ ± 0.3	0.4^{b} ± 0.1	$2.06^{a,b}$ ± 0.25	$0.23^{a,b}$ ± 0.03
Undisturbed Vegetated	16^{b} ± 1.0	$5.17^{a,c}$ ± 0.09	$47^{c,d}$ ± 5.0	$1.7^{a,b}$ ± 0.2	$<0.5^{i\,b}$	5.2^{b} ± 0.7	10.2^{b} ± 0.3	21.5^{b} ± 1.4	$6^{a,b}$ ± 0.5	$8.4^{a,c,d}$ ± 1.0	2.42^{a} ± 0.24	$0.23^{a,b}$ ± 0.01
Disturbed Vegetated	15^{b} ± 1.7	$5.09^{c,e}$ ± 0.28	$60^{c,d}$ ± 13.1	$3.8^{a,b}$ ± 0.8	0.8^{b} ± 0.1	2.9^{b} ± 0.2	8.7^{b} ± 1.1	5.4^{b} ± 1.0	$8^{a,b}$ ± 0.5	$^{j}N.D.^{\,b}$	$1.97^{a,b}$ ± 0.28	0.15^{a} ± 0.05
Undisturbed Shore	22^{b} ± 3.5	$5.89^{a,b,e}$ ± 0.30	$131^{b,c,e}$ ± 14.8	$2.3^{a,b}$ ± 0.5	0.5^{b} ± 0.0	25.6^{b} ± 3.7	10.0^{b} ± 1.0	16.7^{b} ± 2.7	$9^{a,b}$ ± 0.2	$9.7^{a,c,d}$ ± 1.1	2.53^{a} ± 0.14	$0.25^{a,b}$ ± 0.01
Disturbed Shore	14^{b} ± 3.2	6.81^{d} ± 0.05	1089^{a} ± 180.7	$<0.5^{i\,a,b}$	$<0.5^{i\,b}$	14.7^{b} ± 0.9	16.3^{b} ± 0.6	18.5^{b} ± 1.3	10^{b} ± 0.3	$11.9^{c,d}$ ± 1.5	1.34^{b} ± 0.13	$0.28^{a,b}$ ± 0.04

Note: For the ease of calculation, value <0.5 was defined as 0.5 and not detected was defined as 0 in ANOVA

Superscripts ^{a,b,c,d,e} denote significant differences among means. Sites with different letter are significantly different ($P < 0.05$) ^{i}All replicates generated results lower than 0.5%; ^{j} Not Detected; H' = Shannon Diversity Index; Distance = Genetic distance based on Jukes Cantor Model

6.3.2 Bacterial diversity index and genetic distance

Significant variation in Shannon diversity index (H') and genetic distance was observed among sites (one way MANOVA, Wilks' $\lambda = 0.3034$, $F_{28, 148} = 4.310$, $P < 0.0001$) and across all environmental influences (One way MANOVA, Wilks' $\lambda = 0.5622$, $F_{6, 170} = 4.248$, $P = 0.0005$). Although genetic distance showed a significant separation (ANOVA $F_{1, 88} = 5.015$, $P = 0.0276$) between sites with or without history of human disturbance, H' showed a negligible variation (ANOVA $F_{1, 88} = 0.323$, $P = 0.5709$). In general, H' values derived from the presence-absence binary matrix were highest from Gourlay Peninsula (2.68) and Pumphouse (2.64), and lowest from Knob Lake (1.34). When assessed on aggregated data (Table 6.1), significantly higher H' values were obtained from undisturbed shore, vegetated and vertebrate influenced locations, and lower values from undistrubed barren fellfield and human impacted shore.

Despite having low diversity as indicated by H', soils from relatively pristine barren locations were genetically distinct, with a high genetic distance being observed. This was especially apparent for soils from Jane Col and Knob Lake, which harboured the greatest genetic variation. In general, lower H' and greater genetic distance were observed from shore and barren soil locations, while vegetated and vertebrate influenced sites showed higher H' and lower genetic distance.

Table 6.2 Identity of excised and sequenced DGGE bands from BLAST search in GenBank (assessed on December 2008)

Band	Closest relative from GenBank BLAST search	Hit (%)	Phylum/ Sub-phylum	Accession No	Origin
B1	Uncultured cyanobacterium clone A822	99	Cyanobacteria	EU283559	Anderson Lake, USA
B2	Uncultured bacterium; KD8-75	99	Bacteroidetes	AY218692	Penguin Droppings Sediments, Ardley Island
B3	Uncultured bacterium; KD2-72	97	Bacteroidetes	AY218589	Penguin Droppings Sediments, Ardley Island
B4	Uncultured bacterium; KD6-86	99	Bacteroidetes	AY188323	Penguin Droppings Sediments, Ardley Island
B5	Uncultured bacterium clone KD7-36	99	Bacteroidetes	AY218708	Penguin Droppings Sediments, Ardley Island
B6	Uncultured bacterium; UOXD-e10	98	Bacteroidetes	EU869765	Onyx River, Antarctica
B7	Uncultured bacterium; KD1-64	98	Bacteroidetes	AY218564	Penguin Droppings Sediments, Ardley Island
B8	Uncultured Bacteroidetes; AI-1F_E12	99	Bacteroidetes	EF219546	Unvegetated soil environments, Anchorage Island
B9	Uncultured bacterium; KD8-102	99	Bacteroidetes	AY218677	Penguin Droppings Sediments
B10	Uncultured soil bacterium; E08_bac_con	100	Bacteroidetes	EU861850	Dry meadow surface soil
B11	Uncultured bacterium clone FFCH15238	94	Unclassified	EU133921	Soil, undisturbed mixed grass prairie preserve
B12	Antarctic bacterium R-9217	94	Bacteroidetes	AJ441008	Antarctica:Vestfold Hills, Ace Lake
B13	Uncultured bacterium clone KD5-100	100	γ-Proteobacteria	AY218723	Penguin dropping sediment
B14	*Cellulophaga algicola* strain KOPRI_22183	93	Bacteroidetes	EU000229	Marine Sample
B15	Uncultured bacterium; KD3-93	99	Bacteroidetes	AY218622	Penguin Droppings Sediments
B16	Uncultured bacterium; zEL27	97	Bacteroidetes	DQ415821	Frasassi sulfidic cave stream biofilm
B17	*Pedobacter kribbensis* strain PB93	96	Bacteroidetes	EF660752	Soil
B18	*Lewinella nigricans*, strain ATCC 23147T	90	Bacteroidetes	AM295255	Beach Sediment
B19	Uncultured Flavobacteria; GASP-MB2W3_B01	99	Bacteroidetes	EF665452	Forest at the GASP KBS-LTER sampling site
B20	Uncultured bacterium; BFA_075	95	Bacteroidetes	EF444134	Freshwater wetland soils
B21	Uncultured bacterium clone KS-124	93	Bacteroidetes	EU809887	Soil Sample from Kafni Glacier, Himalaya
B 22	Uncultured bacterium; KD3-67	97	Bacteroidetes	AY188307	Penguin Droppings Sediments
B23	Uncultured bacterium; KD3-67	98	Bacteroidetes	AY188307	Penguin Droppings Sediments
B24	Uncultured soil bacterium; TIIA5	98	Bacteroidetes	DQ297951	hydrocarbon contaminated soil
B25	Uncultured bacterium; SRRB35	98	Bacteroidetes	AB240509	Environmental sample

B26	*Ginsengisolibacter* sp. NP11	99	Bacteroidetes	EU196345	Cold Saline Sulfidic Spring, Canadian High Arctic
B 27	Uncultured bacterium clone Tet1mes2B8	94	Bacteroidetes	EU290279	marine sample
B28	Uncultured bacterium clone FCPT767	99	Unclassified	EF516481	grassland soil
B29	Uncultured Firmicutes; GASP-MA1W3_H06	97	Firmicutes	EF662740	Cropland
B30	Uncultured bacterium; KD4-4	97	Bacteroidetes	AY218633	Penguin Droppings Sediments
B31	*Phormidium autumnale* CCALA 697	99	Cyanobacteria	AM778710	Cultures
B32	Uncultured bacterium clone LOXA-g02	97	Actinobacteria	EU869563	Onyx River, Antarctica
B33	Uncultured Clostridiaceae; pCOF_65.7_F11	94	Firmicutes	EU156149	Hot Spring
B34	Uncultured Firmicutes; GASP-MB1S2_E01	91	Firmicutes	EF664607	Forest Soil
B35	Uncultured beta proteobacterium clone A6YF12RM	95	β-Proteobacteria	FJ570320	soil early snow melt site B, Alpes
B36	*Oscillatoria* sp. 327/2	100	Cyanobacteria	FJ461751	Cultures
B37	Uncultured bacterium; ½/3B	99	Bacteroidetes	FJ380137	Antarctica: Cape Hallett
B38	Uncultured Comamonadaceae; BFM20(8)	99	β-Proteobacteria	DQ628936	subglacial water or ice or sediment
B39	Uncultured bacterium clone Elev_16S_1335	85	Acidobacteria	EF019956	trembling aspen rhizosphere
B40	Uncultured bacterium clone MBD7	92	γ-Proteobacteria	EU044946	Canadian Arctic microbial mat: Markham Ice Shelf
B41	Uncultured Gemmatimonadetes; C12_WMSP2	100	Gemmatimonadetes	DQ450683	saturated alpine tundra wet meadow soil

Table 4.3.2, Continued

6.3.3 DGGE profiles and sequences identity

A total of 41 DGGE banding positions were identified and sequenced (Table 6.2) from the 15 locations around Signy Island. The 16S rRNA sequences were clustered into 9 phylum/sub-phylum groups, with Bacteroidetes as the dominant group (63.4% of the total band positions). A majority of the affiliated sequences under Bacteroidetes were sourced elsewhere in Antarctica. This class was found to be more prominent in human disturbed locations (with the exception of Pumphouse) than undisturbed sites, accounting for 76.9% - 92.6% of total detected bands (Table 6.3). The remaining sequences were distributed among Cyanobacteria, Firmicutes, β-Proteobacteria, γ-Proteobacteria, Gemmatimonadetes and Actinobacteria, while 2 sequences remained unclassified. Most of the affiliated sequences from these groups also originated from cold environments such as subglacial sediments, alpine tundra soil and cold saline springs (Table 6.2). Although representing low proportion of the retrieved sequences, both Cyanobacteria and Firmicutes were found in almost all sampling sites, potentially suggesting high adaptability of these groups to different environmental stresses. A trend of greater prevalence of γ-Proteobacteria in animal-influenced locations was observed, while Acidobacteria were only detected in the dry barren soil of Jane Col and Pumphouse (Table 6.3).

As revealed by CAP (Fig. 6.1) bands 5, 10, 13, 15 and 22 occurred more frequently in soils from vertebrate influenced sites. These consisted of 4 Bacteroidetes and 1 γ-Proteobacteria, all except one of these isolated previously from penguin rookery sediments (the exception being band 10, from dry meadow surface soil). A few members of Bacteroidetes, including bands 14, 18 21, 25 and 27, were most often recovered from soil samples collected near shore, supported by bands 14, 18 and 27 being of marine origin. Bacterial assemblages from barren fellfield soils included highly diverse sequences (band 33, 36, and 41) from

Firmicutes, Cyanobacteria and Gemmatimonadetes, while vegetated sites did not showed any strong correlations with the vector overlays in CAP.

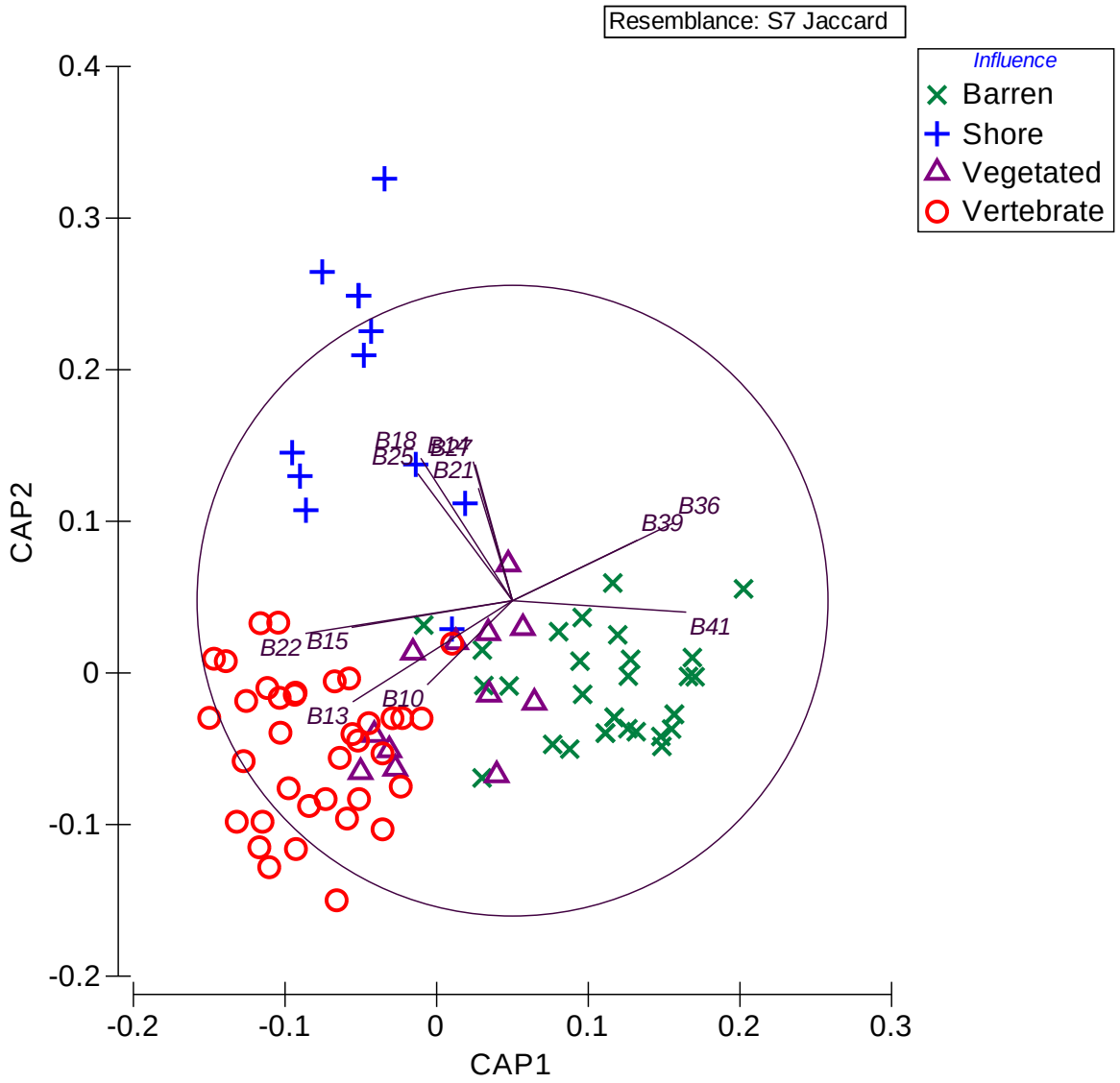

Fig. 6.1 CAP ordination of bacterial assemblages based on different influence (barren, vegetated, vertebrate and shore).

Notes:
[1]CAP1 eigenvalue = 0.8918, CAP2 eigenvalue = 0.8351
[2]Number of orthonormal PCO axes used (*m*) = 16
[3] Explained total variation = 81.11%
[4] Relationship between bands and assemblages was generated based on Pearson correlations, only vector length > 0.35 was shown

Table 6.3 Cumulative number of bands and proportions of affiliated prokaryotic taxonomic groups at each location (n = 6). GP = Gourlay Peninsula; NP = North Point; CCO = Cummings Cove; EF = Elephant Flats; CF1 = Cemetery Flats 1; CF2 = Cemetery Flats 2; DP = Deschampsia Point; BP = Berntsen Point; ST = Skua Terrace; JC = Jane Col; KL = Knob Lake; Ph = Pumphouse; SS = Signy Station; Csh = Cummins Shore; Fsh = Factory Shore. In each pair of values given, the top number (a) represents the sum of affiliated band occurrence from 6 replicates, and the lower (b) represents the percentage from the given group detected from all bands.

Phylum	**GL**	**NP**	**CCO**	**EF**	**CF1**	**CF 2**	**DP**	**BP**	**SK**	**JC**	**KL**	**PH**	**SB**	**Csh**	**Fsh**
Bacteroidetes	[a]69 [b]*77.5%*	40 *85.1%*	33 *78.6%*	51 *79.7%*	48 *84.2%*	50 *92.6%*	56 *72.7%*	40 *76.9%*	18 *66.7%*	32 *55.2%*	12 *57.1%*	53 *59.6%*	27 *84.4%*	61 *77.2%*	21 *87.5%*
Cyanobacteria	8 *9.0%*	2 *4.3%*	5 11.9%	1 *1.6%*	2 *3.5%*	[c]*N. D.*	5 *6.5%*	7 13.5%	4 14.8%	9 *15.5%*	3 *14.3%*	13 *14.6%*	4 *12.5%*	6 *7.6%*	*N. D.*
Firmicutes	7 *7.9%*	4 *8.5%*	4 *9.5%*	4 *6.3%*	3 *5.3%*	1 *1.9%*	3 *3.9%*	4 7.7%	*N. D.*	7 *12.0%*	1 *4.8%*	1 *1.1%*	1 *3.1%*	7 *8.9%*	3 *12.5%*
β-Proteobacteria	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	2 *2.6%*	*N. D.*	*N. D.*	1 *1.7%*	*N. D.*	4 *4.5%*	*N. D.*	2 *2.5%*	*N. D.*
γ-Proteobacteria	5 *5.6%*	1 *2.1%*	*N. D.*	5 *7.8%*	4 *7.0%*	2 *3.7%*	3 *3.9%*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	3 *3.4%*	*N. D.*	*N. D.*	*N. D.*
Gemmatimo-nadetes	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	5 *6.5%*	*N. D.*	5 18.5%	*N. D.*	5 *23.8%*	3 *3.4%*	*N. D.*	*N. D.*	*N. D.*
Acidobacteria	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	6 *10.3%*	*N. D.*	3 *3.4%*	*N. D.*	*N. D.*	*N. D.*
Actinobacteria	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	*N. D.*	5 *5.6%*	*N. D.*	*N. D.*	*N. D.*
Unclassified	*N. D.*	*N. D.*	*N. D.*	3 *4.6%*	*N. D.*	1 *1.9%*	3 *3.9%*	1 1.9%	*N. D.*	3 *5.2%*	*N. D.*	4 *4.5%*	*N. D.*	3 *3.8%*	*N. D.*
Total detected bands	89	47	42	64	57	54	77	52	27	58	21	89	32	79	24

[c] Not Detected

6.3.4 Differences and variations among assemblages

The DGGE binary matrix data were subjected to non-metric multidimensional scaling (NMDS) ordinations under the Jaccard similarity model in order to visualize the multivariate patterns among groups (data not shown). From the NMDS, the variations in bacterial assemblages showed random distribution, although this might be linked with the high stress value in the NMDS plot (Kruskal stress = 0.19). When the data were viewed under constrained CAP ordination, clustering of bacterial assemblages was apparent in relation with both environmental influence (Fig. 6.1) and history of human disturbance (data not shown). Indeed, as suggested in PERMANOVA (Table 6.4), significant spatial effects of both environmental influence ($F_{3, 87}$ = 5.629, P = 0.001) and human disturbance ($F_{1, 89}$ = 6.0034, P = 0.001) were exerted on bacterial assemblages. Furthermore, a significant interaction between environmental influence and human disturbance ($F_{3, 82}$ = 4.984, P = 0.001) was also identified.

In spite of the differences, subsequent pairwise T-tests showed higher similarity between vegetated soil and marine vertebrate influenced sites as compared to suppralitoral soil and barren fellfield locations (Fig. 6.1, Table 6.5). In addition, sites classified as shore contained the most distinct bacterial communities, with low relationship values to the other three assemblages. It was also noteworthy that, despite significant differences between each grouping, high within replicates variation was observed, as low similarity within each environmental influence was achieved (Table 4.3.5; 17.40 % - 27.98%).

A test of homogeneity of dispersion (PERMDISP) was performed to identify any effect of dispersion within assemblages. In brief, this is to test whether the bacterial diversity of one group is significantly more variable than any other. From PERMDISP, sites with or without

influence from human disturbance did not vary in terms of dispersion ($F_{1, 89}$ = 0.3079, P = 0.632). Nevertheless, significant dispersion effects were identified in comparisons between environmental influence assemblages ($F_{3, 87}$ = 7.3987, df = 3, P = 0.001), with the lowest within grouping variation in the vegetated assemblage and the highest in barren soil (Table 6.6).

Table 6.4 PERMANOVA on Jaccard similarities for DGGE derived assemblages of bacterial diversity with different environmental influence on Signy Island. Information given includes degrees of freedom (df), sum of squares (SS) and mean square (MS).

Source	**df**	**SS**	**MS**	**Pseudo-F**	***P* Value**
Environmental Influence (Inf)	3	0.45×10^5	15010	5.6	0.0001
With/ Without history of human disturbance (Dis)	1	0.16×10^5	16006	6.0	0.0001
Inf x Dis	3	0.36×10^5	11194	4.2	0.0001
Residual	82	2.86×10^5	2666.2		
Total	89	3.17×10^5			

Table 6.5 Pairwise similarity between/within groups based on Jaccard similarities (P value for all pairs < 0.01)

	Barren	Shore	Vegetated	Vertebrate
Barren	17.4			
Shore	8.2	23.1		
Vegetated	16.2	11.4	28.0	
Vertebrate	12.9	14.8	21.0	26.0

Table 6.6 Mean and standard error (SE) within grouping variation (distance from centroid) and Pairwise PERMDISP of environmental influences

Group	**Size**	**Mean**	**SE**
Influences			
Barren	30	58.5	1.1
Shore	12	54.4	1.4
Vegetated	12	49.8	2.1
Vertebrate	36	52.0	1.2

Pairwise grouping	**t value**	***P* value**
Barren vs Shore	2.11	0.106
Barren vs Vegetated	3.99	0.004
Barren vs Vertebrate	3.90	0.002
Shore vs Vegetated	1.85	0.063
Shore vs Vertebrate	1.07	0.415
Vegetated vs Vertebrate	0.89	0.450

6.3.5 Correlations of soil properties with bacterial assemblages

DISTLM was carried out to identify correlations between measured soil parameters and the composition of bacterial assemblages (Table 6.7). When considering groups singly in the overall marginal test (all samples regardless of environmental influences or presence of human disturbance), “edaphic” explained nearly 21 % of variation and “metal” up to 18 % of variation in bacteria community patterns. The explanatory value was very similar for “edaphic” (21.97 %) but slightly increased for “metal” (25.82 %) using only data from relatively non-human-impacted sites. Greater dependence on soil properties was observed in the bacterial diversity of the human disturbed soil, where “edaphic” explained 36.41 %

and “metal” 32.27 % of variation. The breakdown of the marginal proportions of explained variation attributed to each single variable from these three conditions is illustrated in Fig. 6.2. Across all samples, pH was the most important soil parameter underlying variation in bacterial community structure. However, conductivity, water, and copper content were also important limiting factors at sites with no human disturbed history, while pH, conductivity, carbon, lead and nickel content were the most important soil factors in human disturbed sites.

Table 6.7 DISTLM marginal test results for the regression of the DGGE derived bacterial community patterns and forth root transformed soil properties. The results were obtained by grouping the measured soil properties to “edaphic factor” and “metal factor”. ‘Prop’ is the proportion of explained variation, ‘res. df’ the residual degrees of freedom, and ‘regr. df’ the regression degrees of freedom.

All Group	**SS(trace)**	**Pseudo-F**	**P**	**Prop**	**res. df**	**regr. df**
edaphic	65785	4.39	0.0001	0.21	84	6
metal	55610	3.57	0.0001	0.18	84	6
Undisturbed Locations	**SS(trace)**	**Pseudo-F**	**P**	**Prop**	**res. df**	**regr. df**
edaphic	39138	2.70	0.0001	0.22	48	6
metal	45987	3.34	0.0001	0.26	48	6
Human-disturbed locations	**SS(trace)**	**Pseudo-F**	**P**	**Prop**	**res. df**	**regr. df**
edaphic	44522	3.44	0.0001	0.36	30	6
metal	39453	2.86	0.0001	0.32	30	6

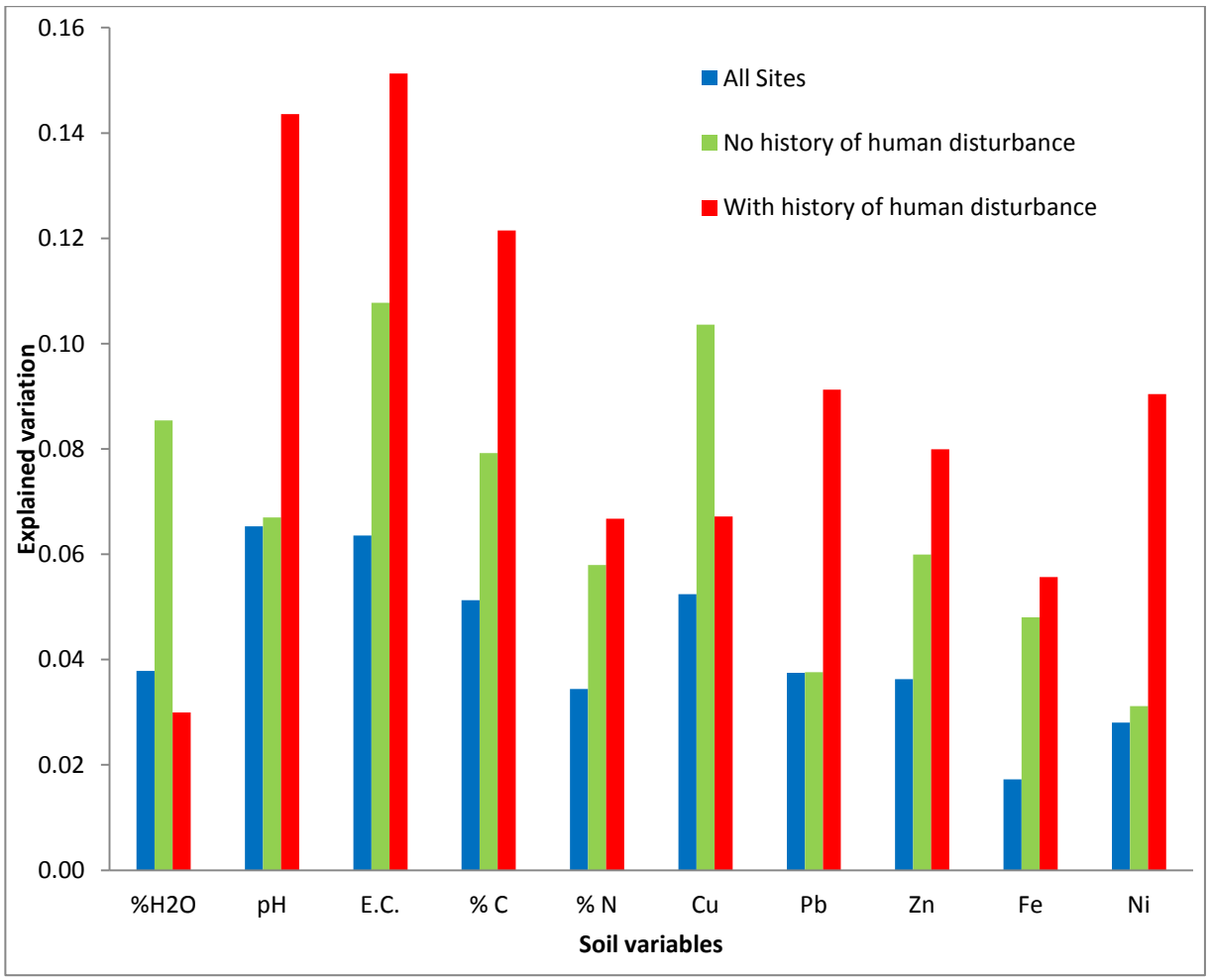

Fig. 6.2 Explanatory value of single variables from all, natural and impacted sites inferred from marginal test of DISTLM

Note: all correlations are statistically significant ($P < 0.05$) except: 'all sites: Fe'; 'No history of human disturbance: Ni'; and 'With history of human disturbance: % water'.

6.4 Discussion

The results showed that prokaryote biodiversity across terrestrial environments on Signy Island displayed high levels of spatial heterogeneity. This is consistent with previous reports from continental Antarctica (Barrett, et al., 2006b; Xiao, Li, You, & Wang, 2007; Aislabie, et al., 2008). On Signy Island, relatively greater within site variation was present in nutrient-poor barren soil in contrast with vegetated and vertebrate influenced soils. Notwithstanding these differences, clustering of both soil parameters and prokaryotic diversity was apparent related to the major classes of environmental influence experienced, and between sites with or without a history of human disturbance. Although with a different strength of influence in human disturbed and undisturbed sites, soil pH gave the highest explanatory value for the measured microbial diversity.

Studies such as that of Yergeau et al. (2007b), which cover a much larger geographical area, have reported significant differences in bacterial diversity between locations separated by hundreds or thousands of kilometers. At a much smaller scale of organization, this study highlight that small-scale variation is also an important factor in understanding patterns of prokaryotic distributions in Antarctica. This is generally consistent with the observations that prokaryote diversity is sensitive to local environmental conditions such as nutrient and water availability (Horner-Devine, et al., 2004a; Barrett, et al., 2006a; Barrett, et al., 2006b) and that soil heterogeneity is apparent over small spatial scales (Wiens, 1989; Ettema & Wardle, 2002; Fraschetti, et al., 2005).

6.4.1 Soil properties and correlations with bacterial community profile

When soil chemical parameters are considered individually, pH was the most important factor in determining prokaryote diversity on Signy Island (Fig. 6.2), as has previously been reported elsewhere (Bååth, 1996; Wardle, 1998; Fierer & Jackson, 2006; Aciego Pietri & Brookes, 2008). In addition, bacteria are known to favour low salinity over high salinity environments (Wichern, Wichern, & Joergensen, 2006), which is consistent with our finding of a high correlation between electrical conductivity and the bacterial community profile (Fig. 4.3.2), along with low diversity (H') in shore samples (Table 6.1). A feature of the soil chemical data obtained was the high variability across sampling locations (Table 6.1), likely driven by their different environmental influences (c.f. Horner-Devine, et al., 2004a). For example, ornithogenic soil and seal wallows were generally enriched in carbon and nitrogen while barren soils had low water content and were nutrient-poor.

Considering the DGGE-inferred community structure, there was higher variation between different nutrient-poor barren soils than present within nutrient rich vegetated and vertebrate influenced locations (Table 6.6). Significant spatial separation was also indicated by the PERMANOVA (Table 6.4) and CAP ordinations (Fig. 6.1), the latter showing clear separation between all four environmental classifications (shore, vertebrate, vegetated and barren), consistent with previous reports of environmental influences being vital factors in determining bacterial diversity (Barrett, et al., 2006a; Barrett, et al., 2006b; Aislabie, et al., 2008; Aislabie, et al., 2009). The overlap detected between prokaryote communities of vegetated and vertebrate-influenced sites (Table 6.5) may reflect the fact that scattered vegetation is typically present in most penguin rookery and seal wallowing areas.

6.4.2 Prokaryote diversity

Bacterial diversity as inferred from DGGE is most appropriately regarded as "dominant bacterial diversity", due to the preferential amplification by PCR of the abundant and the more active members of the community (Pearce, et al., 2003), and care is required in interpretation of DGGE data (Muyzer & Smalla, 1998). Nevertheless, this method is routinely used in assessment of soil bacterial diversity (Muyzer, 1999; Nakatsu, 2007), and shows relatively consistent sequence identity from bands with identical melting positions using nested PCR approach (e.g. Dar, Kuenen, & Muyzer, 2005; Foti, et al., 2008). Thus, within these limitations, DGGE profiles exhibit a "snapshot" of the dominant bacteria community, and showed high reproducibility in this study.

As reported from a smaller range of sites on Signy Island (Chong, et al., 2009a), bacterial diversity obtained from excised DGGE bands was dominated by the Bacteroidetes, a group frequently reported to be prevalent in Antarctic soils (Bowman, McCammon, Gibson, Robertson, & Nichols, 2003; Saul, et al., 2005; Shravage, Dayananda, Patole, & Shouche, 2007; Aislabie, et al., 2008; Chong, et al., 2009b). It may also be noteworthy that a higher proportional occurrence of Bacteriodetes was detected in human disturbed sites as opposed to undisturbed locations (Table 6.3), which might be attributed to the ability of these bacteria to degrade a wide range of polymers such as starch, gelatin, casein, xylan and Tween 80 (Saul, et al., 2005). Cyanobacteria such as *Oscillatoria* spp. and *Phormidium autumnale*, detected in this study, have been recorded previously from Signy Island (Davey & Rothery, 1993). Additionally, a small number of sequences belonging to Firmicutes, Proteobacteria, Acidobacteria, Actinobacteria and Gemmatimonadetes were also detected here. However, as most of these sequences provide matches with 'uncultured' sequences in Genbank, no functional interpretation can be proposed. These phyla have again been

reported in previous studies of terrestrial environments in Antarctica (Smith, et al., 2006; Xiao, et al., 2007; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Chong, et al., 2009b).

6.4.3 External influences on soil properties

The presence of elevated heavy metal contents in soils in Antarctica has been widely associated with anthropogenic contamination (Claridge, et al., 1995; Sheppard, et al., 2000; Powell, et al., 2003; Stark, et al., 2003; Santos, et al., 2005; Chong, et al., 2009b). However, there are also natural sources of heavy metals in soils, such as from the weathering of rock and from sea spray (Malandrino, et al., 2009). Previous studies by Chong et al. (2009a) and Nygård et al. (2001) have also identified native vertebrate contributions to the metal content in soil, especially in areas of high seal and penguin density due to bioaccumulation of metal via the food chain, as seen in comparison of metal levels (copper and zinc) at sites with vertebrate influence and elsewhere. Similarly, high iron levels have been reported at sites with visible vegetation (Bargagli, Brown, & Nelli, 1995; Yergeau, et al., 2007a).

In the current study, extremely high lead and zinc contents were measured in soils at Pumphouse, the abandoned coal-powered engine room used for pumping fresh water from the adjacent lake in the 1920s. The lead level was up to 10 times higher (1205.32 mg kg^{-1}) than that reported from coal in England (1.7 – 2.8 mg kg^{-1}) and Scotland (5.6 – 137 mg kg^{-1}) (Farmer, Eades, & Graham, 1999). In comparison with other Antarctic studies, a similarly high level of lead and greater level of zinc were reported by Sheppard et al. (2000) from soil around Scott Base, Victoria Land. The enriched levels in Pumphouse soil most likely relate to the previous burning of coal, and indicate the low mobility of these metal ions (Claridge, et al., 1995) and their longevity in the Antarctic terrestrial environment (Tin, et

al., 2009). Elevated levels of iron and zinc around Signy Station itself are likely sourced from both the metal elements of the station structure and the burning of fuel (oil, and previously coal) to power the station.

6.4.4 Human influences superimposed on prokaryote assemblages

In contrast with Chong et al.'s (2009b) study at Casey Station (East Antarctic coastline), no apparent elevation of bacterial diversity (H', Tables 6.1) or obvious segregation was exhibited in relatively pristine locations as distinct from human disturbed sites on Signy Island, although this might simply indicate an artefact of the application of the Shannon diversity index to diversity data inferred from the DGGE absence/presence binary matrix (see also Hartmann & Widmer, 2006; Chong, et al., 2009a). Nevertheless, increased genetic distance (Tables 6.1) was observed in relatively unimpacted sites in contrast with most human-disturbed locations (excluding impacted and unimpacted shore), possibly indicative of greater genetic diversity in undisturbed locations. Bacterial communities obtained from anthropogenically impacted locations generally included taxa from a more restricted phylogenetic cluster. Notwithstanding this, high bacterial diversity was detected from one impacted location, Pumphouse, which also had the greatest level of metal contamination. This observation is perhaps comparable with the bacteria diversity found at Wilkes Tips, a relict dumpsite on Windmill Island (Chong, et al., 2009b), and may relate to the recovery of bacterial diversity over decades (50-80 y) after the original contamination (Gillan, Danis, Pernet, Joly, & Dubois, 2005).

Human impact occurs in many forms in Antarctica (Bargagli, 2005, 2008; Tejedo, et al., 2009; Tin, et al., 2009), including hydrocarbon contamination, heavy metal contamination,

sewage waste and physical disturbance. Intentional or unintentional human alterations of environments in Antarctica can lead to changes in soil properties and directly contribute to shifts in bacterial diversity (Delille, 2000; Delille & Delille, 2000; Aislabie, Fraser, Duncan, & Farrell, 2001; Delille, Coulon, & Pelletier, 2007; Tin, et al., 2009). For instance, decreased bacterial diversity (Saul, et al., 2005) and decreased bacterial abundance (Delille, 2000- 1 year after contamination; Delille, et al., 2007- 2-4 months after contamination) has been reported on hydrocarbon polluted sites in Antarctica. The discrimination of bacterial community patterns between undisturbed and human impacted locations identified by PERMANOVA (Table 6.4) might suggest that human disturbance forms a new selective pressure in natural Antarctic environments, facilitating microbes that can tolerate changes in the soil environments such as increases in heavy metal and hydrocarbon content. This is consistent with recent reports of greater occurrence of hydrocarbon degraders in oil-polluted Antarctic soil than in control locations (Delille, 2000; Aislabie, et al., 2001; Saul, et al., 2005).

In comparisons between sites with and without a history of human disturbance, the soil factors which showed the highest explanatory value varied. Metal contents explained a greater proportion of variation in sites with no history of disturbance (Table 6.7), which, perhaps, indicates that soil bacteria in natural Antarctic habitats show higher sensitivity to changes in metal levels. Furthermore, a greater proportion of variation in soil bacterial diversity was explained by differences in soil properties at human disturbed locations than at undisturbed sites. Overall, this supports the suggestion of Barrett et al. (2006a) that, while Antarctic organisms are well-adapted to extreme environmental conditions, they may be highly sensitive or intolerant to change.

6.5 Summary

In a Study 2, soil bacterial diversity at environmentally distinct locations on Signy Island was examined using denaturing gradient gel electrophoresis (DGGE) profiling, and a range of chemical variables in soils was determined in order to describe variations between them. The dominant bacterial communities of all locations were found to be significantly different, although higher levels of similarity were observed between locations with similar physico-chemical characteristics, such as at penguin rookeries, seal wallows and vegetated soils. Extending from the study, soil prokaryote biodiversity on Signy Island was reanalyzed by comparing 15 distinct locations in order to elucidate any interaction between four general habitat types on Signy Island (South Orkney Islands, maritime Antarctic) and any influence of previous human impacts at these sites. Specific sites were selected to represent the range of different soil environments present and to cover a range of environmental factors present in the maritime Antarctic which are known to influence bacterial community composition in soils elsewhere. A diverse prokaryote community is described, again with the majority of excised and sequenced bands belonging to the Bacteroidetes. Although DGGE profiling identified significant differences in prokaryotic biodiversity between all sampling sites, aggregations of banding patterns were also apparent across the different soil environments examined. Correlations between specific DGGE profiles and 10 selected soil parameters suggested that much of this variation could be explained by differences in the levels of environmental disturbance and soil pH. In particular, a greater proportion of variation in soil bacterial diversity was explained by differences in soil properties at human disturbed locations than at undisturbed locations, with higher explanatory values by edaphic factors in the former and soil metal content in the later. In general, the data indicate that small-

scale variation is an important factor in understanding patterns of prokaryotic distributions in soil habitats in the maritime Antarctic environment.

CHAPTER SEVEN

STUDY 4. ASSESSMENT OF SOIL BACTERIAL COMMUNITIES ON ALEXANDER ISLAND (IN THE MARITIME AND CONTINENTAL ANTARCTIC TRANSITIONAL ZONE)

7.1 Introduction and objectives

Alexander Island, western Antarctic Peninsula, hosts important ice-free terrestrial habitats in the southern maritime Antarctic (Smith, 1988a; Convey & Smith, 1997). It is situated close to the maritime/continental Antarctic biogeographical boundary, which is particularly characterised by an increase in overall levels of water stress relative to the large majority of the maritime Antarctic (Smith, 1984; Smith, 1988a; Maslen & Convey, 2006), and provides a natural laboratory to study the terrestrial ecosystem at the boundary between these two important ecological zones. The soils on Alexander Island are generally arid, nutrient poor and exhibit lower mean temperatures than typical maritime Antarctic soils found at more northern locations (Lawley, Ripley, Bridge, & Convey, 2004; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Engelen, et al., 2008). Precipitation levels and soil water content found on Alexander Island are, however, higher and less extreme than those reported for the relatively well-studied and arid Dry Valleys of continental Antarctica (Fell, Scorzetti, Connell, & Craig, 2006). Recent environmental change in the Antarctic Peninsula region may potentially threaten these environments, as consequences including increases in UV radiation levels, temperature rise and changes in liquid water availability (Convey, 2003) are likely to affect fundamental ecosystem processes. The transition zone studied might

therefore provide an indicator of the trajectory of future changes to continental Antarctic soils as a result of environmental change.

To date, only a very limited understanding of the microbial diversity of this unique habitat can be inferred for the prokaryotic (Hughes & Lawley, 2003; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Newsham, Pearce, & Bridge, 2010) and eukaryotic elements of the community (Wynn-Williams, 1996; Convey & Smith, 1997; Hughes & Lawley, 2003; Malosso, Waite, English, Hopkins, & O'Donnell, 2006; Maslen & Convey, 2006; Bridge & Newsham, 2009). Using a principal coordinate analysis based upon the Steinhaus similarity of clone library data, Yergeau et al. (2007a) showed that the soil bacterial community compositions of several locations on Alexander Island were relatively distinct from samples collected from other sub- and maritime Antarctic environments (separated over large distances of hundreds to thousands of kilometres).

The distributions of Antarctic soil organisms are generally driven by differences in space, climate, chemistry and environments (Holdgate, 1977; Chown & Convey, 2007), a feature also reported in the invertebrate fauna of maritime Antarctic habitats (Usher & Booth, 1986). However, very few investigations have attempted to examine bacterial distribution and community structure patterns within Antarctica. Newsham et al. (2010) reported that soil bacterial diversity at two sites on Alexander Island was insensitive to differences in soil water and nutrient content, in contrast with the influence of water on fungal community composition (Bridge & Newsham, 2009). In Studies 1,2 and 3, it has been shown that the bacterial diversity from varying environments on the Windmill Islands, coastal continental Antarctica (Chong, et al., 2009b) and Signy Island, maritime Antarctica (Chong, et al., 2009a; Chong, et al., 2010) was sensitive to the distance between sampling sites, and

environmental factors such as animal, human and vegetation influences, and specific soil chemical parameters.

The primary aim of the current study was to build on this background by extending the geographical range and examining the soil bacterial communities from three sites located in the maritime/continental Antarctic transitional zone, on south-east Alexander Island. The new sites were generally arid relative to the bulk of the maritime Antarctic, physically close (~4 km apart), with the same underlying geology and geomorphology, and influenced by the same macroclimate. In addition, and to provide a more robust analysis, three complementary but independent molecular methods specifically to compare the variation in bacterial assemblage patterns (T-RFLP and DGGE) and taxonomic relationships (cloning) between locations. This study also sought to determine whether the environmental factors that determined bacterial community structure elsewhere in the Antarctic might be also applicable here and would therefore be of wider significance in polar terrestrial habitats.

7.2 Materials and Methods

The methodologies were listed in Chapter 3

7.3 Results

7.3.1 Soil properties

The studied soils were generally nutrient poor (<0.65% total carbon and <0.5% total nitrogen), dry (2.60 – 5.41% water content) and slightly alkaline. The overall soil properties

were significantly different between Viking Valley, Mars Oasis and Ares Oasis (Table 7.1; PERMANOVA pseudo-F = 17.394, *P*=0.001). Among the most notable differences were higher zinc content at Ares Oasis and iron content at Mars Oasis. In addition, up to ten times higher salinity was recorded at Mars Oasis than at Ares Oasis or Viking Valley.

7.3.2 Taxonomic identity and composition of clone libraries

Three 16S rRNA gene clone libraries of nearly full length sequences (~1500bp) were generated to compare the taxonomic composition obtained from the soil samples collected. From 288 selected clones, 18% were found to be faulty (incorrect insert size or unamplifiable vector), and the remaining clones generated 105 unique RFLP patterns that were subsequently sequenced. A total of 83 OTUs or phylotypes (Table 7.2 & 7.3) covering 11 bacterial classes (Table 7.3) were then inferred by grouping of sequences with > 97% homology and removal of plastid and chimeric sequences. The overall similarity of the retrieved ribotypes to the reported sequences available in the GenBank database was ~96% (accessed on 7 October 2010). Considering each location separately, 30, 37 and 25 OTUs were detected from Viking Valley, Mars Oasis and Ares Oasis, respectively (Table 7.4, Fig 7.1). The sample coverage of the three libraries was 79-88% and the rarefaction curve is presented in Fig 7.2.

Comparing the bacterial richness of Mars Oasis inferred from our data and that reported by Newsham et al.(2010), very similar Shannon diversity index values were obtained (~3.3-3.6), even though both studies used different phylogenetic cut-offs in defining OTUs. Further, a direct comparison of the detected phyla and orders in both studies showed 69% and 73% overlap, respectively. These provide support to our estimates of bacterial richness.

Table 7.1 GPS locations and selected surface soil variables (mean ± standard deviations) for the three studied locations on Alexander Island (n = 6 for each location) and in comparison with other locations from the maritime and continental Antarctic. Water content is expressed as percentage of soil dry mass and E.C. (electrical conductivity) as μS cm^{-1}. Elemental values are expressed as mg kg^{-1}. N.D. – not detected, N. R. – not recorded.

Locations	GPS	% Water	pH	E.C.	% Carbon	% Nitrogen	Copper	Lead	Zinc	Iron	Nickel
Alexander Island, Southern Antarctic Peninsula (This Study)											
Mars Oasis (MO)	71° 52.677'S 68° 14.915'W	5.41[a]	7.75[a]	291.07[a]	<0.5-0.55	<0.5	9.63[a]	11.71[a,b]	16.44[a]	3574.9[a]	0.89
		± 2.08	± 0.03	± 157.71			± 0.79	± 2.59	± 3.98	± 191.95	± 0.52
Ares Oasis (AO)	71° 50.329'S 68° 13.487'W	2.60[b]	7.54[b]	39.08	<0.5-0.65	<0.5	4.63[b]	16.04[a]	18.77[b]	2560.14[b]	N. D.
		± 0.75	± 0.16	± 11.14[b]			± 0.62	± 2.92	± 0.30	± 281.16	
Viking Valley (VV)	71° 49.989'S 68° 20.632'W	4.57[a,b]	7.035[c]	27.1[b]	<0.5	<0.5	8.06[c]	8.6[b]	16.84[a]	2155.22[b]	N. D.
		± 0.53	± 0.11	± 1.66			± 1.07	± 1.77	± 0.89	± 660.06	
Superscripts [a,b,c] denote significant differences among means. Sites with different letter are significantly different (P < 0.05)											
Other studies on Alexander Island (Lawley et al. 2004; Yergeau et al. 2007a; Newsham et al. 2010)											
Fossil Bluff	71°19'S 68°18'W	6	7.7	85	0.16[j]	0.02	N.R.	N.R.	N.R.	1.5	N.R.
Mars Oasis	71°52'42''S, 68°15'00''W	5.6-10	7.9	N.R.	0.3-1.9	0.03-0.2	N.R.	N.R.	N.R.	N.R.	N.R.
Coal Nunatak	72°07'S, 68°32'W	12.36	7. 67	N.R.	1.5[i]	0.05[i]	16	23	61	1.7	6.9
Signy Island, Maritime Antarctica (Chong et al. 2010)											
Deschampsia Point	60° 40.837'S 45° 37.973'W	16.22	5.17	47.05	1.65	<0.5	5.23	10.21	21.46	6648.29	8.42
Skua Terrace	60° 40.950'S 45° 37.918'W	12.26	6.1	27.73	0.81	<0.5	4.15	11.27	18.65	8271.9	8.5
Jane Col	60° 41.861'S 45° 37.760'W	13.06	6.3	27.05	0.57	<0.5	2.81	10.98	18.34	5691.87	8.85
Berntsen Point	60° 42.442'S 45° 35.547'W	14.64	5.09	60.23	3.8	0.76	2.88	8.7	5.35	7624.42	N.D.
Adelaide Island, Maritime Antarctic (Lawley et al. 2004)											
Rothera Point	67°34'S, 68°08'W	24.74	5.47	N.R.	2.3[i]	0.31[i]	220	17	1100	1.8	4.5

Ellsworth Land (Lawley et al. 2004)											
Sky Hi Nunataks	74°50'S, 71°36'W	10.51	5.42	N.R.	0.42[a]	0.05[a]	5.6	10.6	38.4	2.9	10.8
Continental Antarctica, Dry Valleys and Transantarctic Mountains (Lawley et al. 2004; Aislabie et al. 2006; Wood et. al. 2008; Chong et al. 2009a)											
Browning Peninsula	66º28'S 110º32'E	8.99	6.22	24.62	<0.50	N.D.	3.25	5.07	7.12	1304.6	N.R.
Mitchell Peninsula	66º18'S 110º32'E	1.65	5.28	14.28	<0.50	<0.50	2.37	6.2	4.65	734.68	N.R.
Red Shed	66º16'S 110º31'E	5.84	7.58	21.34	<0.50	<0.50	3.62	8.69	16.61	2348.2	N.R.
Marble Point	77°25'S 163°41'E	2.4	9.6	650	0.28	0.02	N.R.	N.R.	N.R.	N.R.	N.R.
Bull Pass	77°31'S 161°52'E	0.2	7.6	5580	0.03	0.01	N.R.	N.R.	N.R.	N.R.	N.R.
Near Lake Vanda	77°31°S 161°40'E	0.2	9.1	90	0.06	0.00	N.R.	N.R.	N.R.	N.R.	N.R.
Mt Fleming	77°33'S 160°17'E	1.8	7.1	3560	0.03	0.08	N.R.	N.R.	N.R.	N.R.	N.R.
Beacon Valley	77°48'S, 160°48'E	3.1	N.R.	N.R.	0.1	0.1	67.7	3.9	28.5	14237.7	11.6
Miers Valley –North	78°60'S, 164°00'E	1.1	N.R.	N.R.	0.7	0.04	11.3	2.1	24.9	12893.7	35.3
Miers Valley –South		0.8	N.R.	N.R.	0.3	0.1	10.8	1.7	25.2	13967.8	38.8
LaGorce Mountain	86°30'S, 147°W	12.4	6.8	N.R.	0.04[i]	0.01[i]	12	9.8	39	1.6	10
Regional Comparison	*Range*										
Transition Zone	Min	2.6	5.4	27.1	<0.5	<0.5	4.6	8.6	16.4	1.5	N.D.
	Max	12.4	7.9	297.1	1.9	0.05	16	23	61	2560	10.8
Maritime Antarctica	Min	12.2	5.1	27.1	0.6	<0.5	2.8	8.7	5.4	1.8	N.D.
	Max	24.7	6.3	60.2	2.3	0.8	220	17	1100	7624	8.9
Continental Antarctica	Min	0.2	5.28	21.3	<0.5	N.D.	2.4	1.7	4.7	1.6	10
	Max	12.4	9.6	5580	0.7	0.08	67.7	9.8	39	13968	38.8

[a] measured in mg kg^{-1}
[b] % organic carbon

Table 7.2 Similarity of 16S rRNA gene sequences from clone libraries obtained in the current study compared to the GenBank database. OTUs were defined as clones with unique restriction patterns after digestion with restriction enzyme *MSP-1*. Clones with distinct RFLP patterns but showing 97% sequence homology were further grouped together.

Ribotypes	Source[a]	Closest match in GenBank, (% homology)	Match	Accession Number	Sequence origin
UMAB-cl-1	VV	Uncultured bacterium clone SedCS35, (98%)	1417/1446	FJ849354	Arctic stream sediment, Alaska, USA
UMAB-cl-2	VV	Uncultured bacterium clone AK1DE1_04E, (96%)	1449/1495	GQ396974	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-3	VV	Uncultured soil bacterium clone UF8, (98%)	1442/1471	DQ297978	hydrocarbon contaminated soil
UMAB-cl-4	VV	Uncultured bacterium clone Pia-s-46, (93%)	1407/1503	EF632938	freshwater sediment
UMAB-cl-5	VV	Uncultured Acidobacterium sp. clone MBT1, (97%)	1453/1488	FJ538114	paddy field soil
UMAB-cl-6	VV	Uncultured Acidobacteria bacterium clone 354D, (99%)	1446/1453	AY571790	Southern Victoria Land, Ross Island, Antarctica
UMAB-cl-7	VV	Uncultured bacterium clone 1S10, (92%)	1361/1478	GU359062	rhizosphere soil of peanut
UMAB-cl-8	VV	Uncultured bacterium, clone: Hados.Water.Eubac.3, (90%)	1343/1506	AB355044	surface water
UMAB-cl-9	VV,MO	Uncultured soil bacterium clone M60_Pitesti , (99%)	1448/1484	DQ378273	oil-polluted soil
UMAB-cl-10	VV	Uncultured bacterium clone FCPP630, (97%)	1448/1491	EF516042	grassland soil
UMAB-cl-11	VV	Uncultured bacterium clone ANTLV1_H10, (99%)	1404/1412	DQ521501	McMurdo Dry Valleys, Antarctica
UMAB-cl-12	VV	Uncultured bacterium clone p7d07ok, (94%)	1418/1541	FJ478800	undisturbed tall grass prairie, top 5 cm
UMAB-cl-13	VV, AO	Uncultured endolithic bacterium; NB10-WNS, (94%)	1437/1513	AB374386	central Alps, canton of Grisons
UMAB-cl-14	VV	Uncultured bacterium clone 3y-7, (95%)	1444/1512	FJ444699	cotton rhizosphere
UMAB-cl-15	VV	Uncultured bacterium clone V201-118, (95%)	1405/1488	HQ114133	biofilms in a full-scale vermifilter
UMAB-cl-16	VV	*Lysobacter ginsengisoli*, strain: Gsoil 357, (97%)	1454/1496	AB245363	type strain
UMAB-cl-17	VV, AO	Uncultured bacterium clone p8e22ok, (97%)	1459/1495	FJ479370	undisturbed tall grass prairie, top 5 cm
UMAB-cl-18	ALL	Uncultured organism clone DLE084 (99%)	1429/1436	EF127611	Antarctic ice sample
UMAB-cl-19	VV	Uncultured bacterium clone AK4DE1_09D, (99%)	1438/1451	GQ397053	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-20	VV	Uncultured bacterium isolate AL clone P5, (98%)	1358/1372	EU117171	arctic tundra soil
UMAB-cl-21	VV	Uncultured bacterium clone AK1AB1_04H, (94%)	1431/1515	GQ396821	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-22	VV, AO	Uncultured soil bacterium clone A05_bac_con, (98%)	1458/1487	EU861820	control dry meadow surface soil
UMAB-cl-23	VV	Uncultured bacterium clone AK1AB2_06H , (91%)	1405/1528	GQ396865	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-24	VV	Uncultured bacterium clone AK4AB2_08E, (95%)	1468/1530	GQ396942	unvegetated, perhumid, recently-deglaciated soils

UMAB-cl-25	VV	Uncultured bacterium clone AK4AB1_10E, (97%)	1469/1509	GQ396910	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-26	VV	Uncultured bacterium clone KC-2, (96%)	1419/1467	EU421849	soil sample under a glacier from Lahaul-Spiti Valley
UMAB-cl-27	VV,MO	Uncultured soil bacterium clone M35_Pitesti, (98%)	1459/1479	DQ378253	oil-polluted soil
UMAB-cl-28	VV	*Modestobacter* sp. CNJ794 PL04, (93%)	1394/1496	DQ448774	marine sediment
UMAB-cl-29	VV	Uncultured bacterium clone HF_NC_4, (92%)	1404/1511	FJ625343	boreal pine forest soil
UMAB-cl-30	VV, MO	Uncultured bacterium clone 1-9F, (96%)	1448/1505	EU289467	endophytes or symbionts enriched from stem bark
UMAB-cl-31	MO	*Phormidium pristleyi* ANT.PROGRESS2.6, (98%)	1408/1432	AY493585	Antarctica
UMAB-cl-32	MO	Uncultured bacterium clone AK4AB2_01H, (99%)	1509/1515	GQ396922	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-33	MO	Uncultured bacterium clone AK4AB1_05A, (99%)	1439/1451	GQ396895	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-34	MO	*Microcoleus vaginatus* CJI-U2-KK1, (99%)	1444/1449	EF654062	culture
UMAB-cl-35	MO	*Crinalium magnum* SAG 34.87, (93%)	1337/1433	AB115965	Not reported
UMAB-cl-36	MO	Uncultured soil bacterium clone bac2nit44, (93%)	1398/1501	EU861947	nitrogen amended dry meadow surface soil
UMAB-cl-37	MO	Uncultured alpha proteobacterium, Dolo_14, (97%)	1419/1448	AB257639	surface of dolomite rock in the Alps
UMAB-cl-38	MO	Uncultured Acidobacterium sp. clone sw-xj281, (97%)	1480/1525	GQ302592	cold spring sediment
UMAB-cl-39	MO	Uncultured bacterium clone Bas-7-33, (95%)	1420/1490	GQ495390	Hnausahraun lava flow
UMAB-cl-40	MO	Uncultured bacterium clone AK4AB1_12E, (96%)	1444/1491	GQ396918	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-41	MO	Uncultivated soil bacterium clone S023, (98%)	1501/1519	AF013550	volcanic cinder field
UMAB-cl-42	MO	Uncultured bacterium clone Ld1-14 , (94%)	1408/1489	GQ246409	North Yellow Sea sediments
UMAB-cl-43	MO	Uncultured bacterium clone JMYB12-53, (98%)	1470/1497	FJ810580	coal tar waste-contaminated groundwater
UMAB-cl-44	MO	Uncultured cyanobacterium clone R8-B31, (98%)	1414/1430	DQ181689	Lake Rauer, Rauer Islands, East Antarctica
UMAB-cl-45	MO	*Leptolyngbya* sp. ANT.LH52.1, (98%)	1409/1431	AY493584	Antarctica
UMAB-cl-46	MO, AO	Uncultured actinobacterium, (99%)	1444/1448	DQ366004	Victoria Land, Antarctica
UMAB-cl-47	MO	Uncultured bacterium clone JSC8-B11, (98%)	1471/1495	DQ532226	Johnson Space Center
UMAB-cl-48	MO	Uncultured soil bacterium clone A05_bac_con, (95%)	1415/1488	EU861820	control dry meadow surface soil
UMAB-cl-49	MO	Uncultured bacterium clone AK4AB1_12B, (98%)	1458/1487	GQ396917	unvegetated, perhumid, recently-deglaciated soil
UMAB-cl-50	MO	*Coleodesmium* sp. ANT.LH52B.5, (99%)	1426/1432	AY493596	Antarctica
UMAB-cl-51	MO	*Flexibacteraceae* bacterium VUG-A141a, (97%)	430/1472	EU155016	Victoria Upper Glacier, Antarctica
UMAB-cl-52	MO	Uncultured bacterium clone AK4DE1_08B, (96%)	1413/1463	GQ397048	unvegetated, perhumid, recently-deglaciated soil
UMAB-cl-53	MO	*Nostoc* sp. KVJF16, (99%)	1441/1452	EU022729	Norway

UMAB-cl-54	MO	Uncultured bacterium clone p7e09ok, (93%)	1393/1489	FJ479147	undisturbed tall grass prairie, top 5 cm
UMAB-cl-55	MO	*Nostoc sp. 'Pannaria aff. leproloma cyanobiont'* 2 Ch, (98%)	1431/1450	EF174228	Not Reported
UMAB-cl-56	MO	Uncultured candidate division TM7 bacterium; MAFB-C4-28, (90%)	1366/1516	AY435496	propane-sparged ground water
UMAB-cl-57	MO	Uncultured bacterium clone p7d07ok, (88%)	1374/1546	FJ478800	undisturbed tall grass prairie, top 5 cm
UMAB-cl-58	MO	Uncultured bacterium clone p35m05ok, (97%)	1462/1506	FJ478794	undisturbed tall grass prairie, top 5 cm
UMAB-cl-59	MO	Uncultured bacterium partial 16S rRNA gene, clone FA01A05, (95%)	1478/1492	FM872485	floor dust
UMAB-cl-60	MO	Uncultured bacterium clone AK1AB2_10F, (95%)	1411/1483	GQ396878	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-61	MO	Uncultured bacterium clone AK1AB2_06H, (92%)	1421/1544	GQ396865	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-62	MO	*Leptolyngbya sp*. 0BB19S12, (98%)	1423/1440	AJ639895	Italy:Emilia-Romagna, Imola, Bubano Basin
UMAB-cl-63	MO	Uncultured bacterium clone p25g20ok, (94%)	1418/1497	FJ479461	undisturbed tall grass prairie, top 5 cm
UMAB-cl-64	AO	Uncultured Bacteroidetes bacterium; OS-C131, (97%)	1433/1475	EF612369	abandoned semiarid lead-zinc mine tailing site
UMAB-cl-65	AO	Uncultured soil bacterium clone 2_H11, (97%)	1449/1485	EU589307	rice paddy field soil
UMAB-cl-66	AO	Uncultured bacterium clone p35b10ok, (95%)	1445/1508	FJ479298	undisturbed tall grass prairie, top 5cm
UMAB-cl-67	AO	Uncultured endolithic bacterium; 31B-WNS, (88%)	1322/1500	AB374381	central Alps, canton of Grisons
UMAB-cl-68	AO	Uncultured actinobacterium clone E1B-B6-114, (95%)	1409/1482	EF016809	hyperarid Atacama Desert soils
UMAB-cl-69	AO	Uncultured bacterium clone AK4DE1_06C , (91%)	1377/1501	GQ397039	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-70	AO	Uncultured bacterium clone 342B, (95%)	1329/1396	FJ542866	Southern Victoria Land, Ross Island, Antarctica
UMAB-cl-71	AO	*Sporocytophaga sp*. 4v, (90%)	1351/1489	FJ372724	hot spring
UMAB-cl-72	AO	Uncultured bacterium clone AK1AB1_11D, (95%)	1421/1491	GQ396844	unvegetated, perhumid, recently-deglaciated soils
UMAB-cl-73	AO	Uncultured *Rubrobacter sp*. clone 354H, (99%)	1440/1450	AY571811	Southern Victoria Land, Ross Island, Antarctica
UMAB-cl-74	AO	Bacteroidetes bacterium P3, (96%)	1424/1480	DQ351728	La Gorce Mountains, Antarctica
UMAB-cl-75	AO	Uncultured bacterium clone p6i06ok, (96%)	1454/1505	FJ478731	undisturbed tall grass prairie, top 5 cm
UMAB-cl-76	AO	Uncultured Acidobacterium sp. clone sw-xj91, (96%)	1425/1482	GQ302572	cold spring
UMAB-cl-77	AO	Uncultured bacterium clone glb352c, (95%)	1415/1474	EU978840	glacier ice
UMAB-cl-78	AO	Uncultured bacterium clone ADB-26, (97%)	1439/1493	HM366486	urban aerosols
UMAB-cl-79	AO	Uncultured bacterium; BF0001B033, (92%)	1345/1461	AM697007	indoor dust
UMAB-cl-80	AO	Uncultured CFB group bacterium; SM1G04, (94%)	1400/1480	AF445698	Environmental sample
UMAB-cl-81	AO	Uncultured bacterium clone B02_SB3A, (98%)	1368/1393	FJ592635	non-fumarole soil (elev. 5235 m) collected at Socompa

					Volcano, Andes
UMAB-cl-82	AO	*Nocardioides sp*. JS614, (95%)	1434/1496	CP000509	Not Reported
UMAB-cl-83	AO	Uncultured bacterium clone FCPN412, (91%)	1323/1453	EF516361	grassland soil
UMAB-cl-84	VV	Uncultured bacterium clone Bas-7-64, (98%)	1500/1525	GQ495421	Hnausahraun lava flow

[a] Sources of which the ribotypes were isolated.

Table 7.3 TRF lengths and representative OTUs. The dominant TRF lengths (±1bp) which occurred in >72% of the T-RFLP community profiles were marked in bold. T-RF lengths 97, 102, 127, 466, 467 were not retrieved by clone libraries and hence unable to assigned the putative identities. The taxonomic classification was associated based on Naïve Bayesian Classifier algorithm from RDP 10 classifier with confident threshold set at 80%.

T-RF	Representative	Phylum	Class	Order	Family	Genus
None[a]	UMAB-cl-8	Cyanobacteria	Cyanobacteria		Chloroplast	Chlorophyta
	UMAB-cl-23	TM7				
	UMAB-cl-83	Unclassified				TM7 *incertae sedis*
59	UMAB-cl-69	OP10				
60	UMAB-cl-66	Actinobacteria	Actinobacteria	Solirubrobacterales		
	UMAB-cl-58, 75	Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter
61	UMAB-cl-10	Proteobacteria	Deltaproteobacteria	Myxococcales		
	UMAB-cl-17	Actinobacteria	Actinobacteria	Solirubrobacterales		
72	UMAB-cl-36	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
77	UMAB-cl-81	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Segetibacter
80	UMAB-cl-78	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
86	UMAB-cl-48	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
	UMAB-cl-9, 22, 40	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Ferruginibacter
87	UMAB-cl-2	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Terrimonas
	UMAB-cl-39	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
90	UMAB-cl-3, 18, 64	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Flavisolibacter
91	UMAB-cl-54	Actinobacteria	Actinobacteria	Actinomycetales		
93	UMAB-cl-42	Actinobacteria	Actinobacteria			
104	UMAB-cl-56	TM7				TM7 *incertae sedis*
118	UMAB-cl-4, 21, 25	Proteobacteria	Betaproteobacteria			
	UMAB-cl-31	Cyanobacteria	Cyanobacteria			
120	UMAB-cl-80	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Spirosoma
125	UMAB-cl-67	Unclassified				

130	UMAB-cl-68	Actinobacteria	Actinobacteria	Actinomycetales		
134	UMAB-cl-47, 73	Actinobacteria	Actinobacteria	Rubrobacterales	Rubrobacteraceae	Rubrobacter
135	UMAB-cl-63	Acidobacteria	Acidobacteria Gp7			Gp7
136	UMAB-cl-30	Actinobacteria	Actinobacteria	Solirubrobacterales		
	UMAB-cl-79	Actinobacteria	Actinobacteria	Actinomycetales		
137	UMAB-cl-71	Bacteroidetes	Sphingobacteria	Sphingobacteriales		
138	UMAB-cl-28	Actinobacteria	Actinobacteria	Actinomycetales		
	UMAB-cl-7	Acidobacteria	Acidobacteria Gp4			Gp4
139	UMAB-cl-41	Acidobacteria	Acidobacteria Gp4			Gp4
140	UMAB-cl-38	Acidobacteria	Acidobacteria Gp4			Gp4
142	UMAB-cl-12, 76	Acidobacteria	Acidobacteria Gp4			Gp4
147	UMAB-cl-11, 19, 37	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
148	UMAB-cl-55	Cyanobacteria	Cyanobacteria		Family I	GpI
149	UMAB-cl-26	Acidobacteria	Acidobacteria Gp3			Gp3
150	UMAB-cl-53	Cyanobacteria	Cyanobacteria		Family I	GpI
	UMAB-cl-27, 84	Acidobacteria	Acidobacteria Gp4			Gp4
151	UMAB-cl-35	Cyanobacteria	Cyanobacteria		Family I	GpI
156	UMAB-cl-14	Proteobacteria	Deltaproteobacteria	Myxococcales		
167	UMAB-cl-65	Actinobacteria	Actinobacteria	Acidimicrobidae	Acidimicrobidae *incertae sedis*	Ilumatobacter
	UMAB-cl-74	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Hymenobacter
181	UMAB-cl-20	Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Iamia
203	UMAB-cl-1	Bacteroidetes				
208	UMAB-cl-24, 32	Acidobacteria	Acidobacteria Gp6			Gp6
210	UMAB-cl-46	Actinobacteria	Actinobacteria	Rubrobacterales	Rubrobacteraceae	Rubrobacter
277	UMAB-cl-82	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides
281	UMAB-cl-5	Acidobacteria	Acidobacteria Gp4			Gp4
	UMAB-cl-29	Unclassified				

290	UMAB-cl-6, 59	Acidobacteria	Acidobacteria Gp6			Gp6
308	UMAB-cl-57	Acidobacteria	Acidobacteria Gp4			Gp4
377	UMAB-cl-51	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Hymenobacter
416	UMAB-cl-77	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Hymenobacter
431	UMAB-cl-16	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
441	UMAB-cl-60	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter
443	UMAB-cl-72	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	
476	UMAB-cl-61	TM7				TM7 *incertae sedis*
478	UMAB-cl-49	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	Haliscomenobacter
483	UMAB-cl-43	Proteobacteria	Betaproteobacteria			
485	UMAB-cl-13	Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter
	UMAB-cl-62	Cyanobacteria	Cyanobacteria		Family IV	GpIV
488	UMAB-cl-33	Cyanobacteria	Cyanobacteria		Family I	GpI
489	UMAB-cl-44, 45	Cyanobacteria	Cyanobacteria		Family IV	GpIV
490	UMAB-cl-50	Cyanobacteria	Cyanobacteria		Family I	GpI
495	UMAB-cl-70	TM7				TM7 *incertae sedis*
497	UMAB-cl-34	Cyanobacteria	Cyanobacteria		Family XIII	GpXIII
	UMAB-cl-52	Cyanobacteria	Cyanobacteria		Family IV	GpIV
499	UMAB-cl-15	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	

[a] RE cutting site <50bp or >500bp

Each clone library included representatives of between 6 and 10 phyla or subphyla. Little overlap in OTUs were observed between locations; for example, only one OTU (UMAB-cl-18) was present at all three study sites, three OTUs were shared between Mars Oasis and Viking Valley (UMAB-cl-9, 27, 30) and Viking Valley and Ares Oasis (UMAB-cl-13, 17, 22), and one between Mars Oasis and Ares Oasis (UMAB-cl-46). In general, all three sites were well represented by Actinobacteria (16% - 40%) and Bacteroidetes (20% - 40%). As most of the sequences showed highest affinity with uncultured representatives in GenBank, the lack of taxonomic information is compensated by assigning the phylotypes' taxonomic hierarchy based on the Naïve Bayesian Classifier algorithm using the classifier option from Ribosomal Database Project (RDP) 10 (Table 7.3). According to this tool (confidence threshold 80%), the Actinobacteria phylotypes were grouped within the Orders Acidimicrobiales, Actinomycetales, Rubrobacterales, and Solirubrobacterales. In contrast, the detected Bacteroidetes were exclusively from the single Order Sphingobacteriales, covering the genera *Ferruginibacter, Flavisolibacter, Hymenobacter, Terrimonas, Spirosoma, Segetibacter, Haliscomenobacter* and *Pedobacter*. The Proteobacteria obtained (α, β, γ, δ sub-classes) mainly comprised clones from Viking Valley, with the α-Proteobacteria closely related to the genus *Sphingomonas* and the δ-Proteobacteria to *Lysobacter*. Additionally, Acidobacteria were prevalent in both Viking Valley (27%) and Mars Oasis (19%), although only one phylotype was retrieved from Ares Oasis. Representatives of Cyanobacteria were rare (i.e. below the detection limit) at Ares Oasis and Viking Valley but constituted the highest percentage (30%) of the total phylotypes obtained from Mars Oasis. Clones belonging to the candidate division OP10 were only found at Ares Oasis.

Table 7.4 Distributions of 16S rRNA gene phylotypes in clone libraries. For each library, 96 clones were picked and screened using RFLP analysis. Subsequent phylotype assignment was carried out by grouping sequences showing > 97% sequence homology.

Site	Viking Valley (VV)	Mars Oasis (MO)	Ares Oasis (AO)
[a]*Coverage*	79%	88%	88%
Phylum/ Class			
Bacteroidetes	6 (20%)	8 (22%)	10 (40%)
Acidobacteria	8 (27%)	7 (19%)	1 (4%)
Actinobacteria	5 (17%)	6 (16%)	10 (40%)
α-proteobacteria	2 (7%)	1 (3%)	0
β-proteobacteria	3 (10%)	1 (3%)	0
γ-proteobacteria	1 (3%)	0	0
δ-proteobacteria	3 (10%)	0	0
Cyanobacteria	0	11 (30%)	0
TM7	1 (3%)	2 (5%)	1 (4%)
OP10	0	0	1 (4%)
Gemmatimonadetes	0	1 (3%)	0
Unclassified	1 (3%)	0	2 (8%)
Total taxa	30	37	25

[a] Sample coverage was estimated using formula $C = 1 - (n_1/N)$ derived from Good's Coverage (1953), where n_1 is the number of clones which occurred only once in the library and N is the total number of sequences in the library

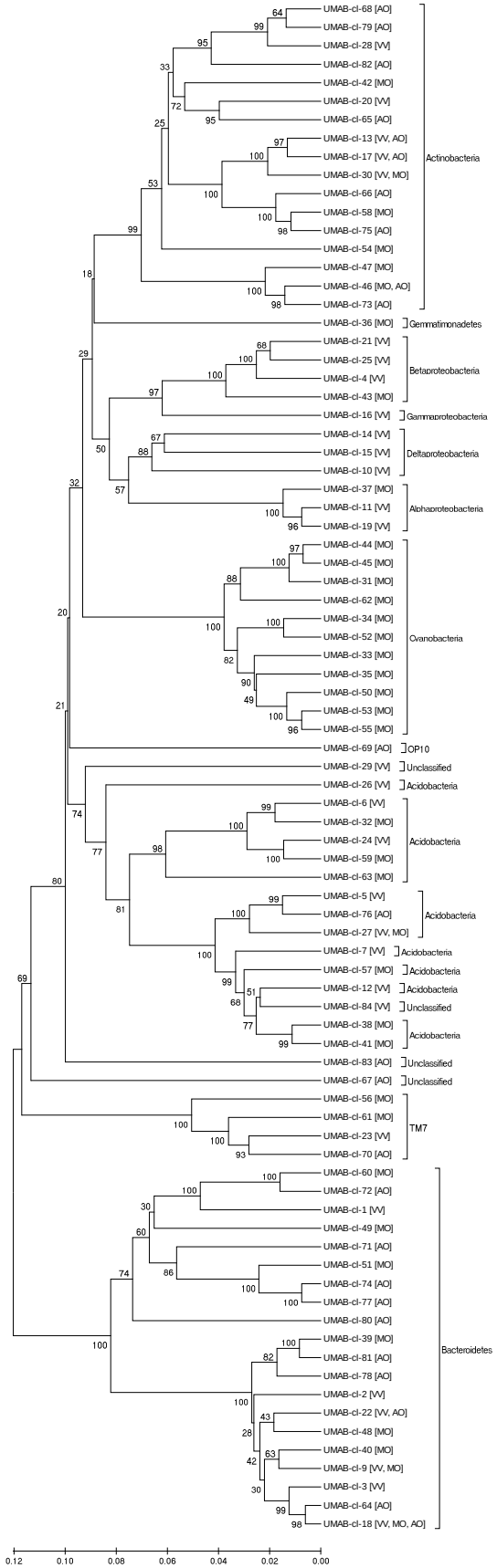

Fig 7.1 UPGMA tree showing the phylogenetic relationship of 16S rRNA gene clones retrieved from Viking Valley (VV), Mars Oasis (MO) and Ares Oasis (AO). Numbers on the branch indicate bootstrap values from 500 re-sampling replicates. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. Parentheses next to the phylotypes represent the location where the phylotype was detected.

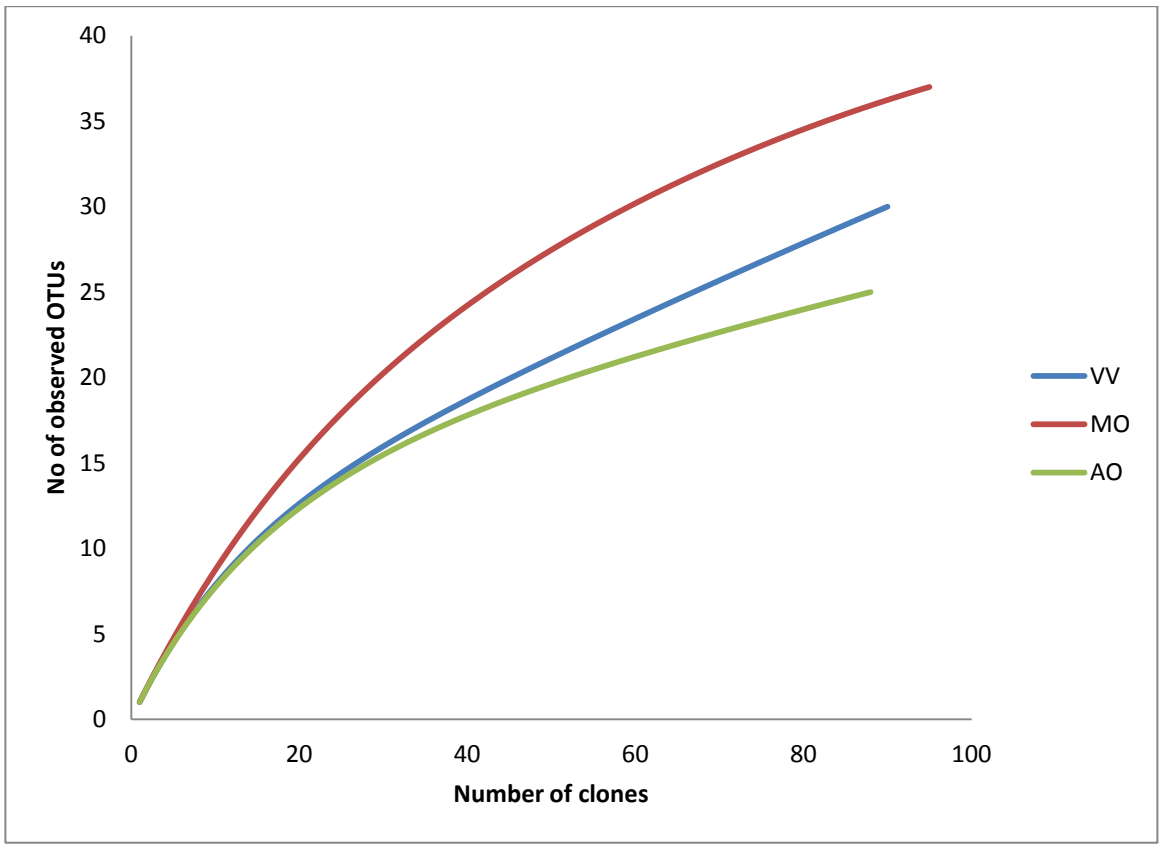

Fig 7.2 Rarefaction curve obtained from each clone library. The OTUs was defined based on RFLP and 97% sequence homology cutoff.

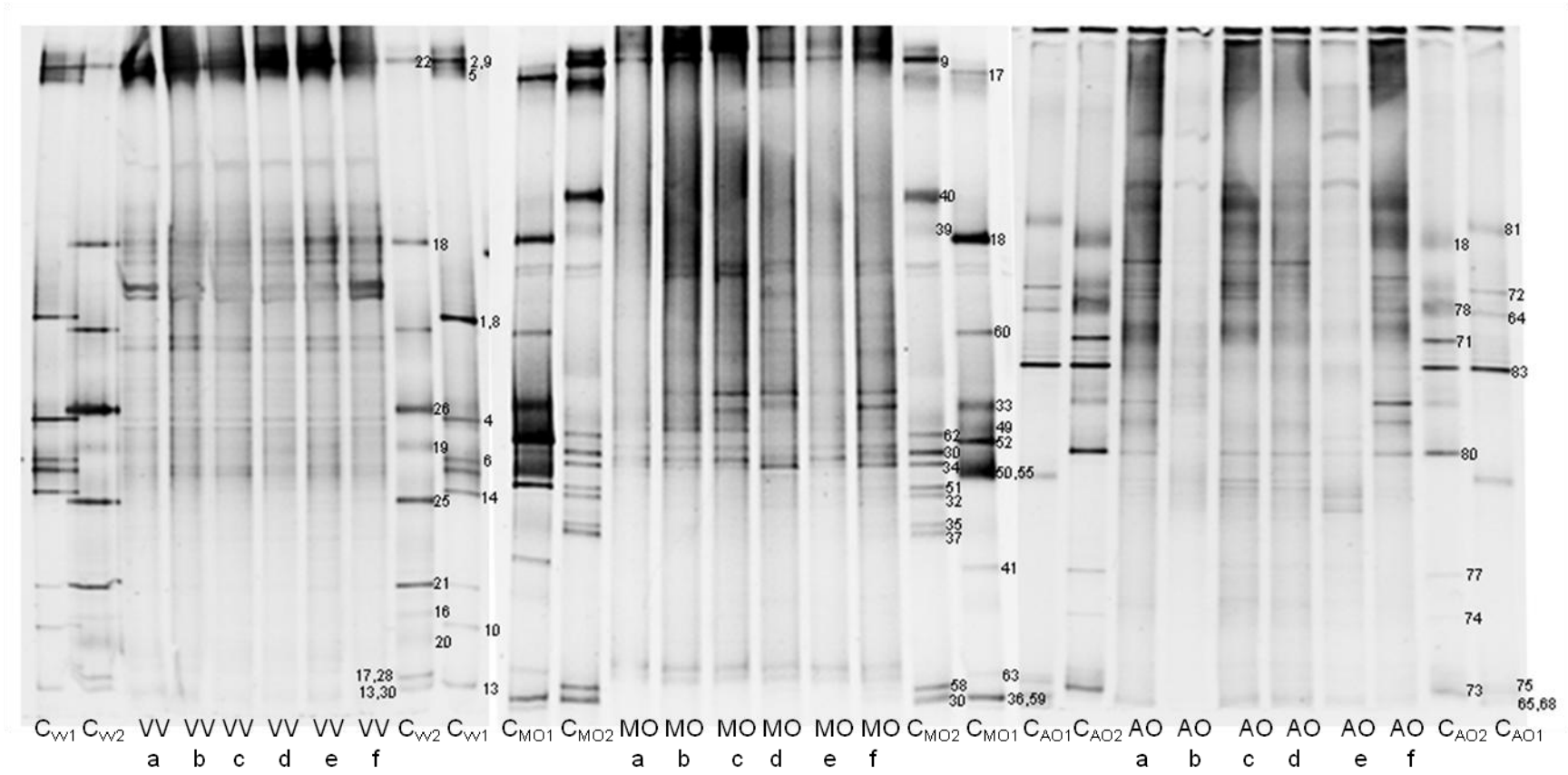

Fig. 7.3 DGGE banding patterns of Viking Valley (VV), Mars Oasis (MO) and Ares Oasis (AO). Lane C_x were pooled clones from clone libraries and the numbers indicate the identity of the bands as reported in Table 7.2. Banding positions produced by chimeric sequences were not labeled.

7.3.3 T-RFLP and DGGE community structure analyses

A total of 143 T-RF lengths were obtained from the T-RFLP fingerprinting analysis and an average of 32.28 T-RFs were detected from the three studied locations. Approximately 41% of the T-RFs were site specific, with most originating from Ares Oasis (30 site specific T-RFs) followed by Mars Oasis (18) and Viking Valley (10). The remaining T-RFs showed overlap between sites and 13 dominant T-RFs (occurrence >72%) were identified. A parallel T-RFLP analysis for OTUs obtained from clone libraries was carried out to corroborate the taxonomic identity of the corresponding T-RFs (Table 7.3). From the 55 T-RF lengths obtained from the clone libraries, approximately 54% (±1bp) matched with that of the dominant T-RF in the T-RFLP community profiles. The assignment of the putative T-RFs identity showed that the main T-RFs detected represented the phylum Bacteroidetes (genera *Ferruginibacter*, *Terrimona*s, and *Flavisolibacte*r), Actinobacteria (order Actinomycetes and genus *Conexibacter*), Cyanobacteria (genera *GpI* and *GpIV*), Gemmatimonadetes (genus *Gemmatimonas*), α-proteobacteria (genus *Sphingomonas*) and β-proteobacteria. As with the clone libraries, most of the putative cyanobacteria T-RFs were prevalent at Mars Oasis (T-RFs 148, 150, 151, 490).

The DGGE profiles are shown in Fig. 7.2. A similar approach as T-RFLP was applied for DGGE, where the GC-clamped PCR products of the combined OTUs from clone libraries were loaded on the side of the community profile to corroborate the taxonomic identity. Based on Fig. 7.2, a total of 49 banding positions were scored and ~43% of the bands were site specific. Amongst these, 10 were found only in Ares Oasis, 7 only in Mars Oasis and 4 only in Viking Valley. The dominant DGGE bands (intense bands with high occurrence) in Viking Valley were affiliated with clones UMAB-cl-2, 9, 22, 18 (Bacteroidetes), Mars Oasis with clones UMAB-cl-9 (Bacteroidetes) and UMAB-cl-30 (Actinobacteria) and Ares

Oasis with clone UMAB-cl-80 (Actinobacteria). The DGGE and T-RFLP assemblage patterns were transformed into absence/presence binary matrices for further analysis to reduce potential variation caused by methodological limitations (Enwall & Hallin, 2009). In order to measure the extent of similarity between binary data generated from T-RFLP and DGGE analysis, the resemblance matrices from both methods were matched using Spearman rank correlation. These two methods were correlated ($\rho = 0.363$; $P = 0.008$).

7.3.4 Soil bacterial assemblage patterns and taxonomic composition

The soil bacterial assemblage patterns inferred from DGGE and T-RFLP analyses were ordinated using Non-metric Multidimensional Scaling (NMDS) (Fig. 7.3a,b). A clear clustering between the three sampling sites was apparent in the DGGE-derived NMDS. In comparison, although distinct clustering of T-RFLP-derived soil bacterial patterns was again clear for samples from Viking Valley and Mars Oasis, the replicates from Ares Oasis were highly variable. Despite these differences, significant spatial clustering between the three locations was evident in both DGGE and T-RFLP based PERMANOVA analyses (pseudo $F_{DGGE} = 12.5$; pseudo $F_{T\text{-}RFLP} = 2.2$; both $P < 0.01$).

The differences between clone libraries were assessed using normalized weighted UniFrac analysis. The results showed that all three sampling sites on Alexander Island harboured significantly different phylogenetic composition ($P = 0.001$). This pattern was in good agreement with data obtained using T-RFLP and DGGE analysis.

Fig 7.4 NMDS of (a) DGGE and (b) T-RFLP derived bacterial assemblage patterns

7.3.5 Correlations between measured soil variables and bacterial assemblage patterns

The DISTLM procedure was used to identify correlations between measured soil parameters and the composition of bacterial assemblages inferred from T-RFLP and DGGE. The correlation of soil parameters with clone libraries was not carried out due to the lack of replication in clone libraries. When the soil parameters were considered singly, each variable contributed significantly with a different proportion of explained variation to the bacterial assemblage patterns recorded by T-RFLP (~7% to 11%; pseudo-F = 1.3-1.9, all $P < 0.05$ with the exception of Zn, $P = 0.152$) and DGGE analysis (~14 to 34%; pseudo-F = 2.7-7.2, all $P < 0.05$). Using the AIC to find the simplest combination of soil parameters which best explained the bacterial assemblage patterns, copper and pH were identified as the best fit model, explaining 20.4% of variation in T-RFLP data and 56.1% of variation in DGGE data.

7.4 Discussion

7.4.1 Soil chemistry and bacterial community composition on Alexander Island compared with other regions

Comparing soils from Signy Island, Adelaide Island and the Windmill Islands (Table 7.1), from more northern latitudes in the maritime and continental Antarctic, the soils from Alexander Island were generally drier, less acidic and exhibited lower carbon contents. Conversely, soil environments in the interior of continental Antarctica (77-86 ºS) were more alkaline and showed higher levels of desiccation. Although it is difficult to compare the trace metal contents reported here with previous studies due to different extraction methods, higher copper content was generally observed in Alexander Island compared with other maritime Antarctic soils. In addition, lower zinc and iron contents were recorded from Alexander Island in contrast with most other locations in continental Antarctica.

The soil bacterial community of Alexander Island exhibits some overlap with those of other cold and extreme environments. For example, 33% of the retrieved ribotypes from clone libraries (Table 7.2) showed highest homology with sequences in GenBank from unvegetated, perhumid and recently deglaciated soil at Juneau, Alaska (Sattin, et al., 2009), and elsewhere in Antarctica. Further, the prevalent orders of Actinobacteria such as Actinomycetales and Rubrobacterales from this study were in agreement with the predominant bacteria detected from hyperarid Atacama desert soils (Connon, Lester, Shafaat, Obenhuber, & Ponce, 2007). Additionally, when compared with other studies in Antarctica, the bacterial taxonomic composition in Alexander Island soils (Table 7.2) showed high phylum level resemblance with those of mineral soil and dry desert soils from Victoria Land (Aislabie, et al., 2006; Smith, et al., 2006; Aislabie, et al., 2008;

Niederberger, et al., 2008; Pointing, et al., 2010) in continental Antarctica. For instance, the prevalence of Actinobacteria, Acidobacteria and Bacteroidetes were generally reported in these studies. Coincidently, these three groups have also been reported to be prevalent in elevated cold non-fumarole soils on Socompa Volcano, Andes mountains (Costello, et al., 2009).

Representatives from these phyla are known to possess the ability to thrive in extreme environments. For example, members of the genus *Rubrobacter* (Actinobacteria) have been reported to show high resistance to radiation (Ferreira, et al., 1999), a feature that may be advantageous for bacteria during the austral summer months when Alexander Island soils experience high levels of solar radiation. Others, such as *Actinomycetes* and *Nocardioides*, are able to become anhydrobiotic to survive prolonged periods of desiccation (Potts, 1994). were found to contain high affinity ATP-binding cassette (ABC), which provides competitiveness during nutrient uptake in nutrient-poor environments (Ward, et al., 2009). In addition, the former contain genes that encode putative addiction modules that might be able to switch on rapidly to prevent DNA and protein synthesis in unfavourable conditions (Ward, et al., 2009). Although it is still unclear which factors underlie the prevalence of Bacteroidetes in cold arid soils, culturable members of genera including *Pedobacter* (Margesin, Sproer, Schumann, & Schinner, 2003) and *Hymenobacter* (Oren, 2006) have been isolated from cryoconites on glacier ice and from soils and sandstone of the continental Antarctic. As the Bacteroidetes sequences obtained in the current study affiliated with a number of Bacteroidetes genera with different hydrolytic abilities (e.g. *Pedobacter* degrade casein and gelatin; *Segetibacter* hydrolyze aesculin, urea and arginine) (Margesin, et al., 2003; Mulder & Deinema, 2006; Oren, 2006; Xie & Yokota, 2006; An, Lee, Im, Liu, & Lee, 2007; Yoon & Im, 2007), it is possible that the Bacteroidetes obtained

might be opportunists which mainly rely on external nutrient inputs such as from melt snow or windblown material of decaying lichens and mosses from nearby vegetated areas.

Several less abundant phylotypes associated with the α, β, γ and δ subgroups of Proteobacteria, as well as candidate divisions TM7 and OP10, were also recovered. Although they were not the dominant phylotypes, their presence has been noted elsewhere in Antarctica (Hughes & Lawley, 2003; Aislabie, et al., 2006; Smith, et al., 2006; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Aislabie, et al., 2008; Pearce, Hughes, Lachlan-Cope, Harangozo, & Jones, 2010; Pointing, et al., 2010).

7.4.2 Chemistry and bacterial assemblage patterns in the study sites

Despite the overall proximity and similarity in geological and environmental features between the three study locations on Alexander Island, significant variation in some trace chemical and physical variables was detected, particularly in conductivity. The higher conductivity observed in Mars Oasis soils was likely related to the formation and presence of salt crusts on soils in this area (Hughes & Lawley, 2003; Andr é& Hall, 2005).

The variation in the underlying soil chemical parameters may have driven the observed differences in the bacterial community compositions of the three study sites. From PERMANOVA of DGGE and T-RFLP data, it is apparent that Mars Oasis, Viking Valley and Ares Oasis harboured different bacterial community structures. Additionally, the retrieved clones from the clone library analysis suggested that the three sites harbour different phylogenetic composition (normalized weighted UniFrac analysis).

Using DISTLM analysis, this study showed that the variation in bacterial assemblage patterns in the study sites was largely linked with differences in soil pH and copper content. The significant correlation between pH and soil bacterial communities is in agreement with the previous studies (Studies 2 and 3) on Signy Island, northern maritime Antarctic (Chong, et al., 2009a; Chong, et al., 2010), and various other studies on polar terrestrial environments, including northern Victoria Land, continental Antarctica (Aislabie, et al., 2008) and the Arctic (Fierer, Bradford, & Jackson, 2007; Männistö, Tiirola, & Häggblom, 2007; Chu, et al., 2010). Furthermore, when assessing DGGE derived bacterial assemblage patterns in soil samples collected at several locations between 51 °S and 78 °S, Yergeau et al. (2007a) also reported a significant relationship between the diversity of the bacterial community and soil pH. It is becoming clear that soil pH plays a pivotal role in the ecology of bacteria in polar soils. The availability of trace metals, including copper, is often highly correlated to soil pH (Salam & Helmke, 1998; Kunito, Saeki, Oyaizu, & Matsumoto, 1999), possibly explaining the high correlation between soil copper content and the soil bacterial assemblage patterns reported here.

In conclusion, despite differences in the geographical location and underlying soil parameters, the soil bacterial community composition on Alexander Island was generally similar to reports from other Antarctic environments, and particularly to those of arid soils in the continental Antarctic. This may suggest a strong relationship between the overall environmental conditions and the distribution of soil bacteria. Nevertheless, when assessed at a local scale, it was also found that the bacterial assemblage patterns were highly sensitive to variation in soil pH and copper content.

7.5 Summary

Despite an increasing number of site specific Antarctic soil bacterial diversity assessments, an understanding of soil bacterial community composition in the arid soil environments of the maritime/continental Antarctic transitional zone remains lacking. To date, most existing microbiological studies of this type have focused on either the wetter environments of more northern latitudes on the Antarctic Peninsula/Scotia arc, or the exceptionally arid deserts of the Dry Valleys of continental Antarctica. In this study, soil bacterial diversity and the physicochemical parameters which might influence it, from three relatively arid locations on Alexander Island were assessed. The study sites generally contained levels of pH, hydration and metal content different from previous reports of maritime or continental Antarctic soil habitats. Surprisingly, each site harboured significantly different soil chemistry and bacterial assemblages, although, the bacterial community taxonomic composition was similar to those of other cold and arid environments. Significantly, three complementary molecular methods (DGGE, T-RFLP, cloning) selected to address different elements of diversity, gave consistent results across the techniques. It is further showed the bacterial community differences between the sites were best explained by the soil pH and copper content. Using these data, this study suggests that the soil pH might play an important role in structuring bacterial assemblage patterns across polar soils.

CHAPTER EIGHT

STUDY 5. GEOGRAPHICAL PROXIMITY HAS LESS INFLUENCE ON SOIL BACTERIAL COMMUNITY PATTERNS AND TAXONOMIC COMPOSITIONS COMPARED TO ALTITUDE AND SOIL PH

8.1 Introduction and objectives

There has been a long history of interest in the relationship between the community diversity of soil bacteria and environmental gradients. This is perhaps especially true for the Antarctic terrestrial environments, where major biogeochemical functions within ecosystems are achieved almost exclusively by microorganisms (Wall, 2005; Hopkins, et al., 2008), as the harsh conditions severely restrict the occurrence of larger plants and animals. Prior to the advent of molecular ecology methods, the study of soil bacterial diversity was mainly dependent on culturing approaches and morphological characterization (e.g. Williams, 1969; Baker, 1970; Smith & Tearle, 1985; Bolter, 1992). Despite providing valuable information on morphology and factors such as enzymatic characteristics, the community patterns and diversity inferred from culture dependent methods were inevitably underestimated and inaccurate, being skewed towards the few easily cultivated species (Torsvik & Ovreas, 2002; Coleman & Whitman, 2005).

More recently there has been a surge in culture-independent DNA-based analyses which, in most cases, show that the bacterial community in Antarctica is generally more diverse than previously thought (e.g. Cowan, et al., 2002; de la Torre, Goebel, Friedmann, & Pace, 2003; Aislabie, et al., 2008; Niederberger, et al., 2008; Yergeau, et al., 2009). Additionally, such data coupled with statistical inferences had provided some insight into fundamental

questions relating to patterns in and the control of bacterial spatial distribution. For example, it has been shown that the distribution of soil bacteria in Antarctica is not random as both small (<1 km) and high (>1000 km) spatial scale clustering has been reported (Yergeau, et al., 2007b; Niederberger, et al., 2008; Chong, et al., 2010).

The soil diversity and communities inferences from 16S rRNA gene based molecular methods might be highly dependent on the diversity measures applied (i.e. species-based or divergence-based – see section 2.3.4). Nevertheless, qualitative species-based measure (DGGE and T-RFLP) in Studies 1, 2, 3 and 4 has been shown to be able to distinguish bacterial community patterns in relation to factors including geographical proximity and anthropogenic, animal or vegetation influences. In this study, the soil bacterial communities of nine sites from three environmentally distinct regions off the west coast of the Antarctic Peninsula were assessed using both species-based and divergence-based measures. The primary hypotheses were (a) that community diversity patterns would be expressed differentially using the different diversity measures, (b) that the soil bacterial communities would exhibit predictable patterns in relation to the geographical sampling region, (c) that the studied regions would be taxonomically distinct, and (d) that bacteria distribution would be influenced by heterogeneity in soil parameters.

8.2 Materials and Methods

The methodologies were listed in Chapter 3

8.3 Results

8.3.1 Soil chemical properties

The soil chemical properties across all sites varied significantly (one-way MANOVA, Wilk's $\lambda = 0.00001$, $F_{56, 215.33} = 31.297$, $p<0.001$) (Table 8.1). Soil samples from Ryder Bay (AI, LI, KB and RP) were relatively nutrient rich as compared to Reptile Ridge (AC and IC) and Alexander Island (MO, VV and AO). The highest carbon and nitrogen contents were detected from LI and KB, where the sampling sites were situated close to an elephant seal wallow (*Mirounga leonina*) and blue eyed shag *(Phalacrocorax atriceps bransfieldensis*) colony, respectively. Vertebrate faeces/guano deposits may also have contributed to the high water holding capacity, with high water and heavy metal content in soils at these two sites. In general, the soil pH at sites in Ryder Bay, with the exception of RP, was acidic. Located close to Rothera Research Station, the soil from RP was slightly alkaline and characterized by elevated Zn, Pb and Cu content, which might be a reflection of anthropogenic impact.

Mineral soils from Reptile Ridge and Alexander Island were nutrient poor and exhibited lower heavy metal content than those of Ryder Bay. Contrasting soil pH values were obtained from Reptile Ridge and Alexander Island, with strongly acidic values recorded in the former and slightly alkaline pH in the latter. When all measured soil chemical properties were considered together (except %C, %N and Ni – these three parameters were excluded as they showed undetectable or detectable but unmeasurable signals), the Euclidean distance based principle coordinates analysis (PCO) showed no clear patterns in relation to the three sampling regions. Nevertheless, two main clusters were formed, demarcating the two highest nutrient sites (LI and KB) and the remaining samples (Fig

8.1a). Closer inspection of the latter cluster showed further partitioning (Fig 8.1b). Within the seven sites contained in this cluster, the soil chemical properties of Alexander Island were similar to those of RP while the soils of IC were more similar to AI from Ryder Bay than AC from the same origin, Reptile Ridge.

Table 8.1 Soil chemical properties of the 9 studied sites

	%Water	pH	EC µS/cm	% Carbon	% Nitrogen	Cu mg/kg	Pb mg/kg	Ni mg/kg	Fe mg/kg	Zn mg/kg
Iron Col (IC)	8.01 ±1.10	2.93 ±0.15	80.60 ±12.47	<0.5	<0.5	6.62 ±0.73	4.00 ±0.71	1.37 ±0.39	7447.47 ±1619.66	10.15 ±1.19
Ammo Col (AC)	13.17 ±6.30	4.57 ±0.25	13.57 ±0.85	<0.5 - 0.83	<0.5	8.10 ±1.20	8.22 ±0.79	1.43 ±0.69	399.86 ±53.06	6.25 ±0.23
Kilingbeck Island (KB)	35.79 ±2.52	4.80 ±0.18	507.83 ±146.91	4.86	1.40	20.36 ±4.98	27.95 ±4.34	8.12 ±1.02	17613.88 ±4649.80	727.21 ±37.62
Lagoon Island (LI)	28.50 ±3.70	4.78 ±0.71	233.32 ±114.98	4.41	0.81	66.87 ±14.31	17.82 ±2.36	3.96 ±0.95	22945.70 ±3379.83	530.61 ±169.24
Anchorage Island (AI)	5.89 ±0.59	6.41 ±0.48	43.07 ±15.41	<0.5 - 0.97	<0.5	6.59 ±2.06	5.79 ±0.91	1.58 ±0.68	5871.03 ±1711.57	7.71 ±2.32
Rothera Point (RP)	8.10 ±1.32	8.17 ±0.10	50.70 ±6.92	0.87	<0.5	81.99 ±108.74	21.34 ±6.23	N.D.	2458.70 ±352.17	136.28 ±54.44
Vikings Valley (VV)	4.57 ±0.53	7.04 ±0.11	27.11 ±1.66	<0.5	<0.5	8.06 ±1.07	8.60 ±1.77	N.D.	2155.22 ±660.06	16.84 ±0.89
Ares Oasis (AO)	2.60 ±0.75	7.54 ±0.16	37.73 ±11.14	<0.5 - 0.65	<0.5	4.63 ±0.62	16.04 ±2.92	N.D.	2560.14 ±281.16	18.77 ±0.30
Mars Oasis (MO	5.41 ±2.08	7.75 ±0.03	291.07 ±157.71	<0.5 - 0.55	<0.5	9.63 ±0.79	11.71 ±2.59	0.89 ±0.52	3574.90 ±191.95	16.44 ±1.47

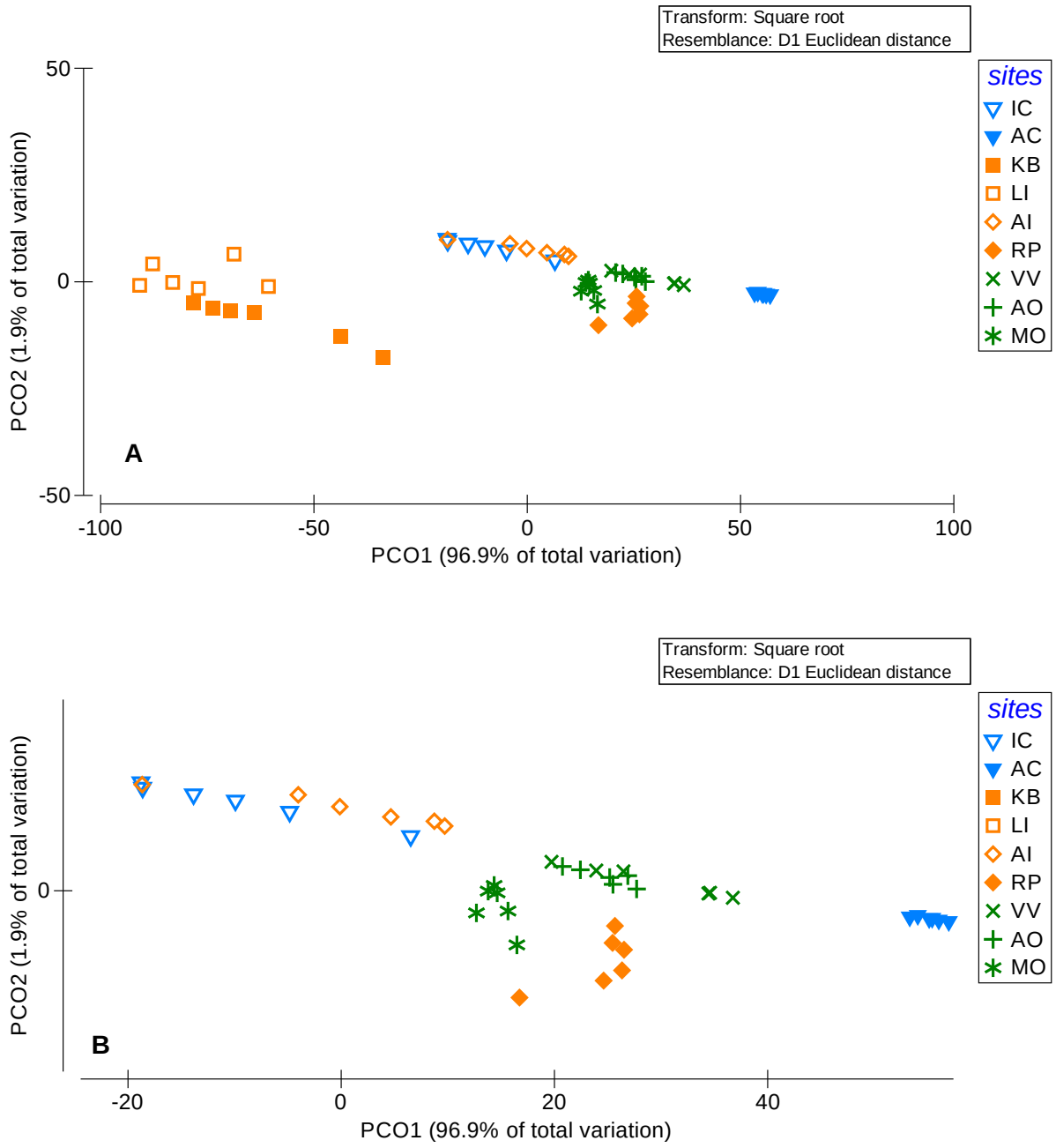

Fig 8.1 PCO ordination of soil chemical parameters based on Euclidean distance: (A) all sites, (B) without KB and LI.

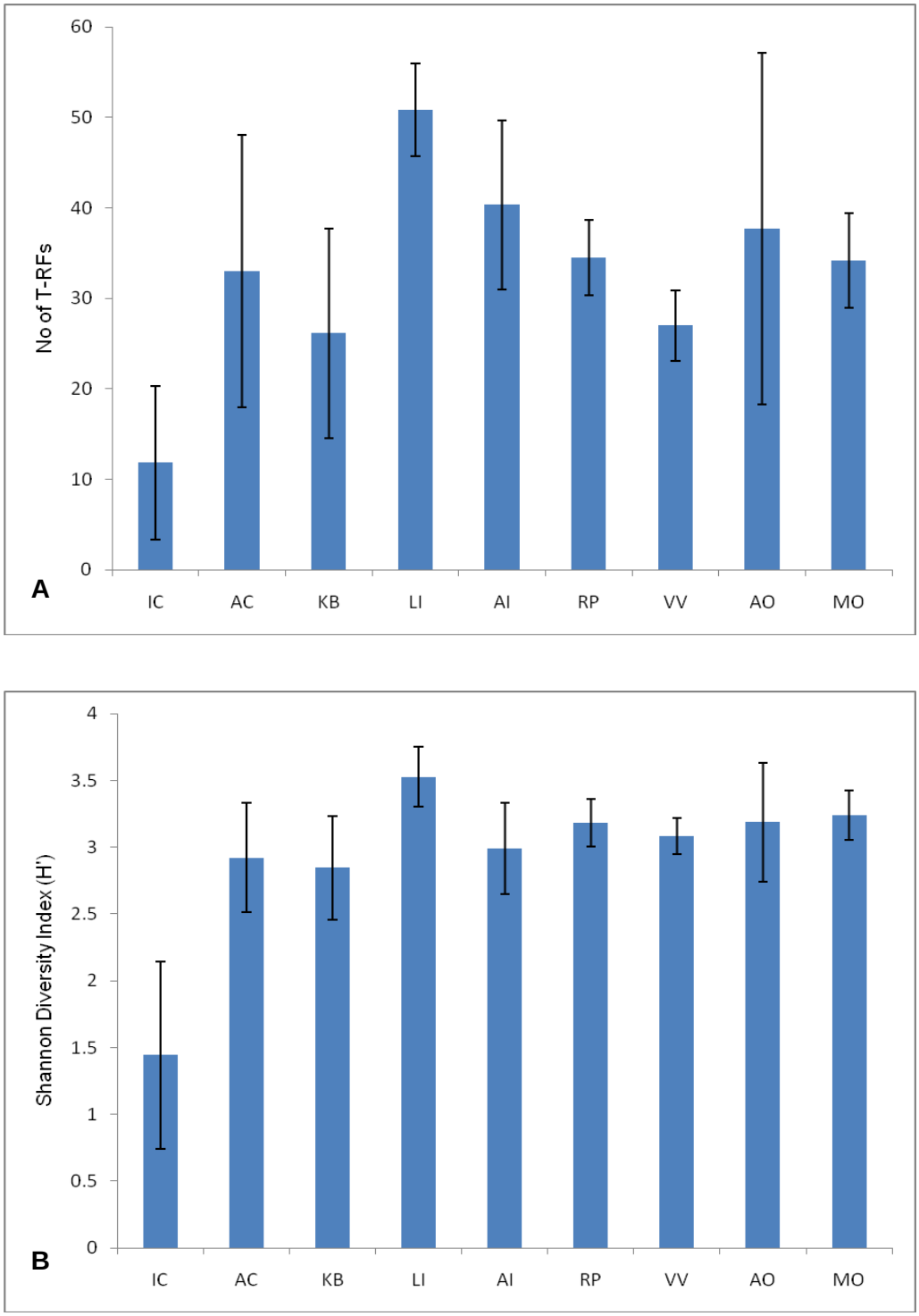

Fig 8.2 Species based α-diversity indices from T-RFLP analysis. (A) qualitative, (B) semi-quantitative

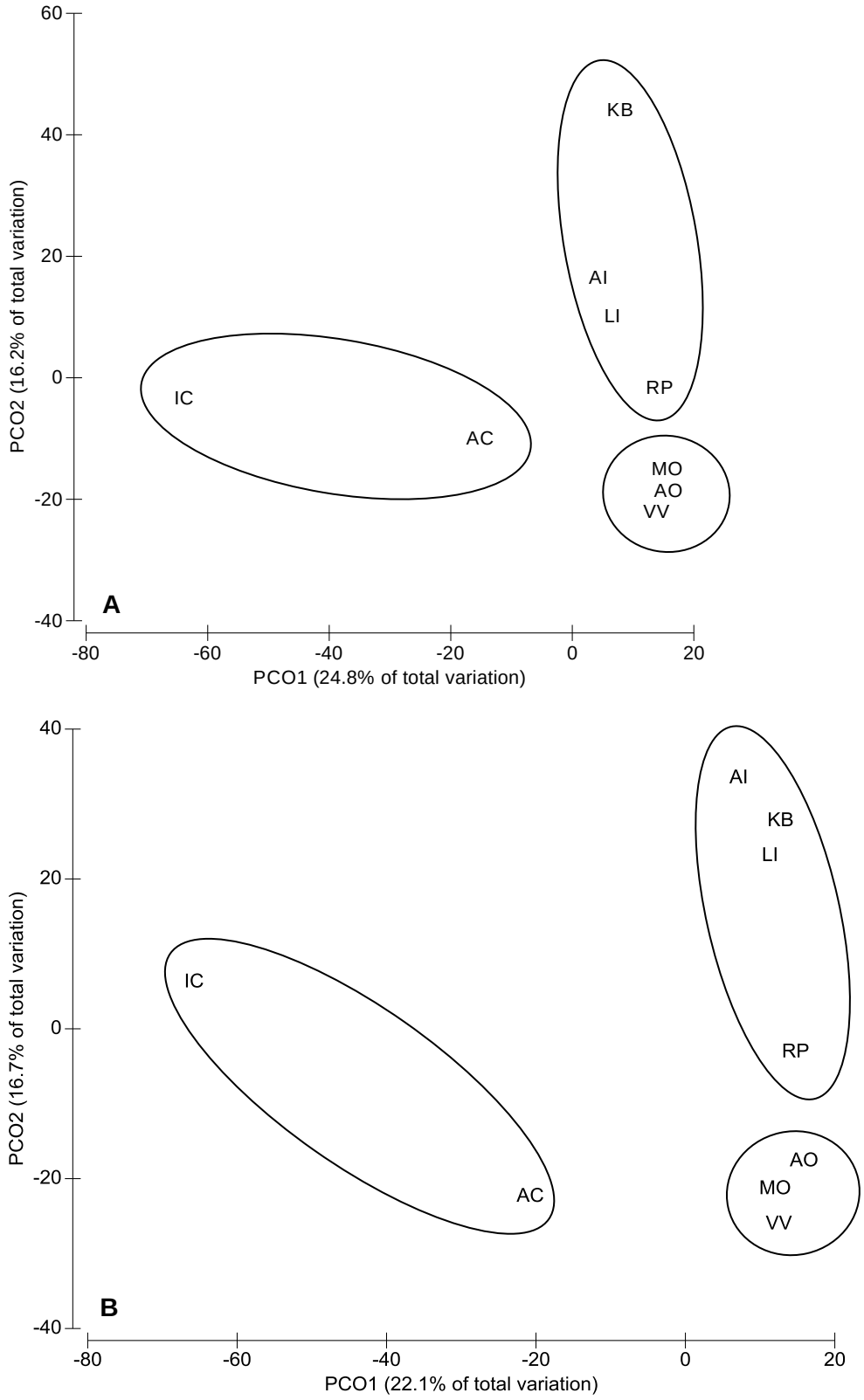

Fig 8.3 PCO ordination based on PERMANOVA pairwise similarity for (A) qualitative, (B) semi-quantitative

8.3.2 Spatial variability of qualitative and semi-quantitative measures derived from species-based T-RFLP analysis

A total of 211 T-RF lengths were obtained from the T-RFLP fingerprinting analysis. Fragment lengths were highly variable, with AC, KB and AO showing highest variability within the replicate samples. Overall, IC showed lowest mean T-RFs while LI contained highest mean T-RFs (i.e. highest species richness) (Fig. 8.2a). The variation between sites was less apparent when the data were analysed using the Shannon diversity index which incorporates both T-RF richness and evenness (Fig. 8.2b). Nonetheless, IC again exhibited lowest diversity and LI highest diversity.

For a between sites comparison, the qualitative bacterial membership and semi-quantitative composition were elucidated using PERMANOVA. Analyses of both absence/presence and relative T-RF abundance data showed that the soil bacterial community membership ($F_{8,45}$ = 5.847, P = 0.001) and composition ($F_{8,45}$ = 6.215, P = 0.001) varied significantly across all samples. The pairwise PERMANOVA relationships of both measures were illustrated using PCO ordination (Fig. 8.3a and 8.3b). Visualization of the PCO plots suggested that the bacterial membership and composition from same local region (i.e. Ryder Bay, Reptile Ridge or Alexander Island) were more similar. For instance, a clear clustering was observed for samples collected from Alexander Island. The samples from Ryder Bay were generally more similar among each other than to those from Reptile Ridge. Concurrently, the bacterial community structure and composition within Ryder Bay were not consistent: higher resemblances in community membership were detected between AI, LI and RP while AI, KB and LI were similar in terms of the bacterial composition. RP being barren and free of vegetation showed some similarity with Alexander Island. From the PCO plot, samples from Reptile Ridge were clearly distinct from those from Alexander Island and the

Ryder Bay islands. Furthermore, IC contained the most distinct bacterial community compared to all other samples.

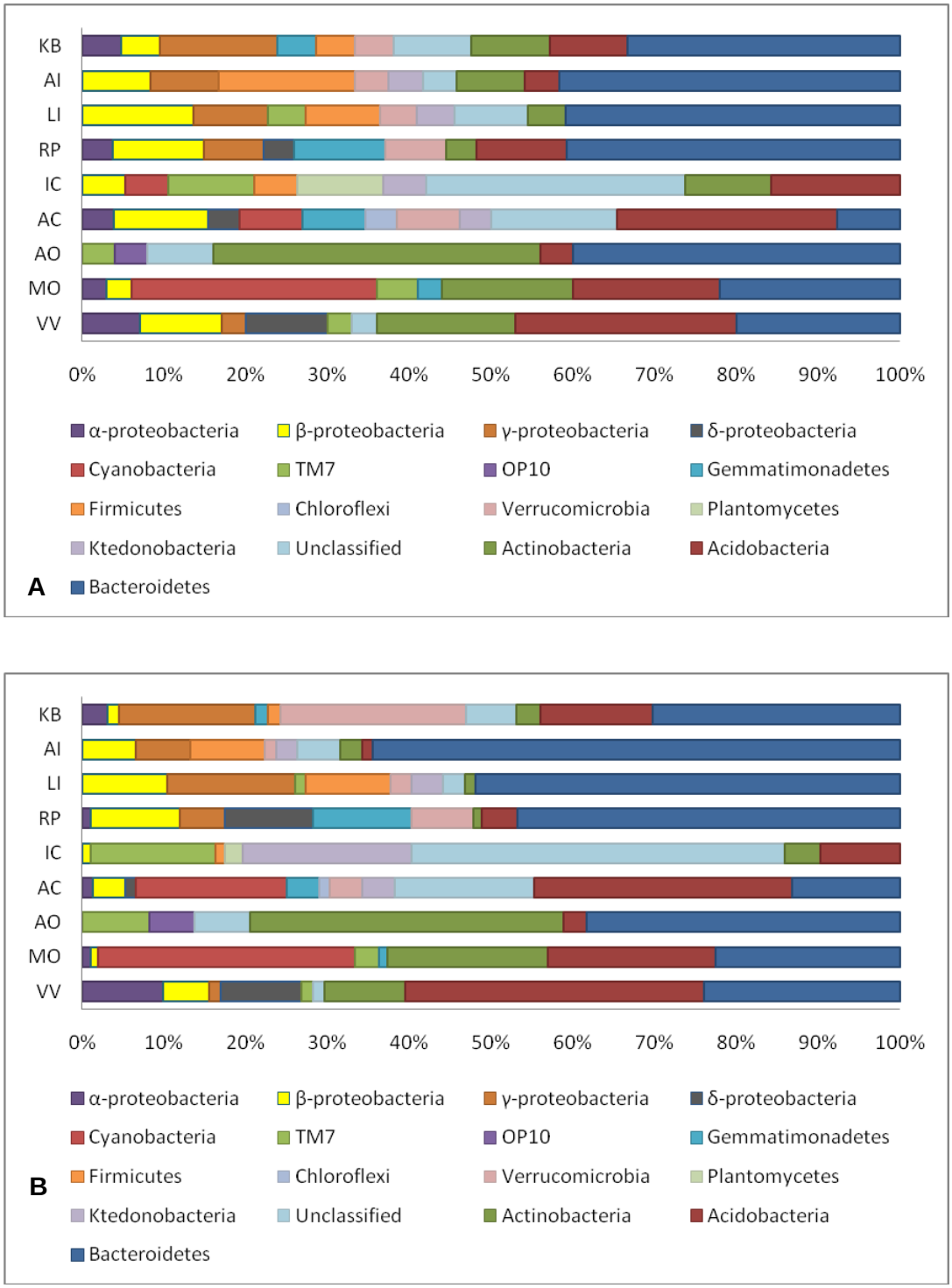

Fig 8.4 Phylum/class level 16S rRNA gene composition derived using (A) number of unique phylotype, (B) relative cumulative number of occurrence based on RFLP and 97% gene homology grouping

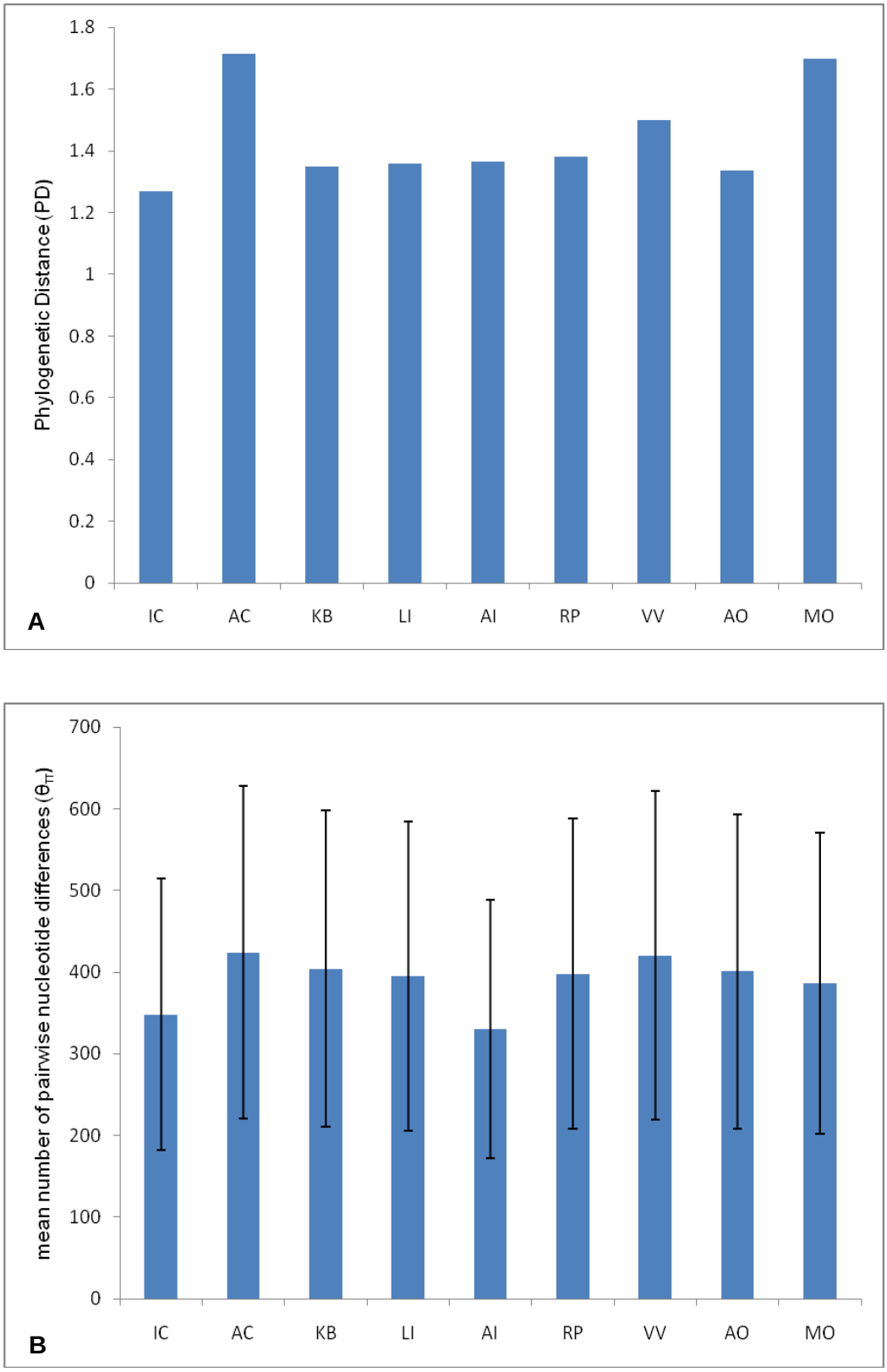

Fig 8.5 Divergent based α-diversity indices from cloning. (A) qualitative, (B) quantitative

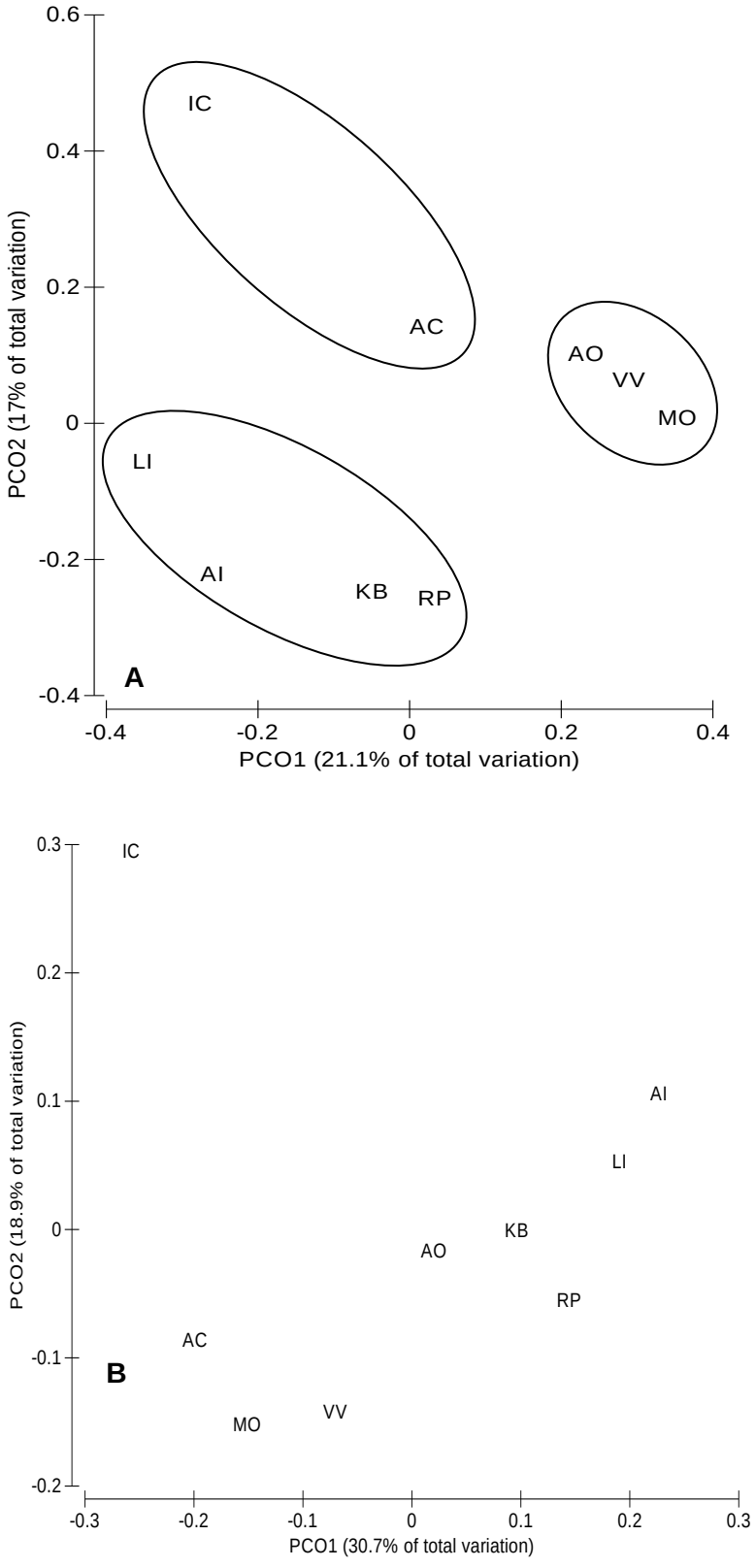

Fig 8.6 PCO ordination based on: (A) pairwise unweighted UniFrac distance (qualitative), (B) pairwise weighted UniFrac distance (semi-quantitative)

8.3.3 Spatial variability of qualitative and semi-quantitative measures derived from divergence-based clone libraries analysis

Calculated based on the 200 obtained ribotypes (Fig 8.3a,b) spanning 17 different taxa (including 'unclassified' and the class Ktedonobacteria whose taxa are *insertae sedis*), the PD showed that the overall phylogenetic richness of Alexander Island, Reptile Ridge and Ryder Bay were similar (Fig 8.5a). In addition, when we compared the phylogenetic composition with θ_π, no discernable differences were observed (Fig 8.5b).

In order to measure the β-diversity, the sequence data were analysed using both unweighted and weighted UniFrac algorithms (Fig 8.6a,b). From the PCO, the distribution patterns of unweighted UniFrac data were analogous to that of pairwise PERMANOVA derived from both absence/presence and relative abundance of T-RFs. From Fig 8.6a, greater similarity between Alexander Island and Ryder Bay was apparent than between either of them and Reptile Ridge. Although AC showed some similarity with Alexander Island (AO, VV and MO), IC generated the most distinct pattern of phylogenetic diversity. Partitioning of bacterial assemblages with reference to local region was more subtle in the PCO produced using the weighted UniFrac algorithm (Fig 8.6b). Here, while IC remained as the most distinct location, AO was more similar to Ryder Bay samples than to other Alexander Island sites.

Although variations were seen between data obtained from T-RFLP profiling (species-based) and clone libraries (divergence based), Spearman rank correlation showed that the pairwise similarity/distance matrices of the qualitative β-diversity measures ($\rho = 0.521$, $P = 0.013$) and quantitative β-diversity measures ($\rho = 0.812$, $P = 0.01$) derived from the two methods were comparable.

Table 8.2 Net relatedness index (NRI) and nearest taxa index (NTI) for the studied sites. [a] Index not weighted with abundance [b] Index weighted with abundance. Number in bold: *P < 0.05;* Number in Italic: P <0.10

	Phylogeny membership[a]		**Phylogenetic composition[b]**	
	NRI	**NTI**	**NRI**	**NTI**
AC	-0.5607	-0.0375	*-1.3235*	0.028
AI	0.3493	0.7238	0.5492	*1.2778*
AO	*1.2308*	**1.5978**	0.4236	*1.2976*
IC	-0.5395	0.6017	1.1252	**1.7101**
KB	-0.2256	0.5550	-0.3776	-0.0271
LI	0.0296	0.5193	0.7508	*1.5512*
MO	**2.9370**	**1.9120**	**3.5069**	**3.0818**
RO	0.2372	*1.4621*	0.3517	*1.4960*
VV	**2.2796**	**1.7922**	**1.6345**	**1.6913**

8.3.4 Taxonomic distinctness of the retrieved ribotypes

Taking advantage of the high taxonomic resolution of the nearly full length 16S rRNA sequences obtained from the clone libraries, the taxonomic distinctness of the ribotypes retrieved from each studied location was compared using the NRI and NTI indices (Table 8.2). Significant terminal and/or overall phylogenetic clumping were detected in all three sites from Alexander Island when considering the phylogenetic diversity of the retrieved ribotype groups. As this measure disregards the relative abundance information, this suggested that the bacterial community structures in Alexander Island were mainly composed of genotypically related bacterial species. When the same test was repeated by taking into account the ribotype abundances (the occurrence frequency of each ribotype), MO and VV in Alexander Island remained significantly phylogenetically clustered while IC showed significant terminal clumping. Four other sites including AO, RO, LI and AI also showed non-significant suggestion of terminal clumping ($P < 0.08$). Together these results suggest that the clone libraries of the three regions were generally dominated by a

high proportion of dominant ribotypes or a high prevalence of related ribotypes. For example, IC was dominated by UMAB-186 and UMAB-190 (94% sequence homology to each other), which contributed 40% of the detected clones. Likewise, LI was dominated by ribotypes from Chitinophagaceae, accounting for ~29% of the retrieved clones.

Overall, the most common ribotypes detected across all locations derived from Bacteriodetes, these being especially conspicuous in Ryder Bay. Soils from Reptile Ridge generated the highest percentage of unclassified taxa (Fig 8.4a,b).

Table 8.3 Correlation of altitude, geological distance and soil chemical parameters to the bacterial assemblage patterns derived from different measures. Note: x = not significant; * = P <0.05; ** = P <0.01

	Parameters								
Bacterial assemblage patterns	**Altitude**	**Distance**	**%H_2O**	**pH**	**EC**	**Cu**	**PB**	**Fe**	**Zn**
Species based									
PERMANOVA of T-RF occurrence (qualitative)	*	x	x	**	x	x	x	x	x
PERMANOVA of T-RFs relative abundance (semi-quantitative)	*	x	x	**	x	x	x	x	x
Divergent based									
unweighted UniFrac (qualitative)	x	*	x	*	x	x	x	x	x
weighted UniFrac (semi-quantitative)	*	x	x	x	x	x	x	x	x
Taxonomic Distinctness									
unweighted NRI	x	**	*	*	x	x	x	x	x
Unweighted NTI	x	**	**	*	x	x	x	x	X
weighted NRI	x	x	x	x	x	x	x	x	X
weighted NTI	x	x	x	x	x	x	x	x	X

8.3.5 Correlations of bacterial assemblage patterns and taxonomic distinctness with elevation, separation distance and measured soil chemical parameters

Environmental and soil chemical factors which showed the strongest influence on taxonomic distinctness and bacterial assemblage patterns inferred using different measures are compared in Table 8.3. Both bacterial community structure and composition derived from T-RFLP analysis showed significant correlations with soil pH and altitude. In contrast, the divergence-based unweighted UniFrac distance was significantly correlated with pH and geological distance while the weighted UniFrac distance was significantly correlated with altitude. Additionally, the variations in taxonomic distinctness (NRI and NTI values) of the ribotype groups were significantly correlated with pH, altitude and water content while no correlation was established between the abundance weighted NRI and NTI and any of the environmental and soil variables.

8.4 Discussion

In this study, several complementary measures were applied to elucidate the bacterial richness (α-diversity) and bacterial assemblage overlap (β-diversity) across three distinct regions near to the Antarctic Peninsula. Both species-based and divergence-based α-diversity did not show any distinguishable patterns in relation to the sampling region, suggesting that the bacterial species richness (number of T-RFLP peaks and H') and phylogenetic richness (PD and θ_π) were not influenced by the environmental differences between them. The comparison of PD and θ_π with the existing studies in Antarctica and elsewhere is problematic, as the former is strongly influenced by the phylogenetic tree topology specific to the different studies and the methods used in phylogenetic tree

construction (Lozupone & Knight, 2008). The latter measure has been more widely used in population genetic studies and is relatively less common in microbial ecology studies (Lozupone & Knight, 2008).

Nonetheless, the finding that soil bacterial richness from disparate environments was similar is consistent with other Antarctic studies. For example, Niederberger et al. (2008) found similar Shannon diversity index values (~3.3 to 4) in comparison between high and low productivity soils in Luther Valley, Northern Victoria Land. In addition, Yergeau et al. (2007b) showed that the bacterial richness estimated using the Chao1 estimator did not show a clear latitudinal pattern. Interestingly however, the Shannon diversity index inferred from clone library analyses of various Antarctic soil bacterial studies (H' ranging from 1 to 4) (Saul, et al., 2005; Aislabie, et al., 2006; Smith, et al., 2006; Niederberger, et al., 2008; Pointing, et al., 2010) exhibited lower values than those obtained from tropical and temperature regions (H' ranging from 3.8 to 7) (Dunbar, et al., 2000; Hartmann & Widmer, 2006; Kim, et al., 2007). In line with this, using Phylochip microarray analysis, Yergeau et al. (2009) found lower bacterial diversity in samples collected from a latitudinal transect between 51°S and 78°S than reported in previous PhyloChip studies from temperate soil environments. This perhaps suggested that although bacterial richness can be a good indicator with which to compare soil bacterial communities from climatically disparate zones, it is however less effective in stratifying environmental gradients within Antarctica itself.

In contrast, all β-diversity assessments (species-based or divergence-based), with the exception of weighted UniFrac ordination, indicated spatial partitioning in relation to the sampling region. Strong clustering was observed between the soil samples collected from Alexander Island, and the samples from Ryder Bay were more similar to each other than to

those from Reptile Ridge and Alexander Island. This shows that soil bacterial assemblage patterns and phylogenetic community membership within the same region share greater overlap than between different regions, and highlights the influences of the local scale environmental gradients in determining bacterial species distributions (cf.Cannone, Wagner, Hubberten, & Guglielmin, 2008; Chong, et al., 2009a; Chong, et al., 2010). The occurrence of the moderate scale regional segregation is consistent with a number of hypotheses. Wiens et al. (2010) proposed that species tend to retain their niches (set of biotic and abiotic conditions where the species can persist) and related ecological traits over time (niche conservatism). The effects of niche conservatism can be broad and complex but, using a narrower interpretation, niche conservation can result in selective accumulation of species with high affinity to a specific set of environmental conditions. This hypothesis shares some similarity with the phylogenetic relatedness/taxonomic distinctness theory (Webb, et al., 2002; Horner-Devine & Bohannan, 2006; Vamosi, et al., 2009) where genotypically related species with favourable traits are usually preferentially selected in stressful environments (habitat filtering), leading to higher occurrence of taxonomically similar members (phylogenetic clumping). In contrast, under favourable conditions such as high nutrient and water availability, competition can play an important role in filtering genotypically similar species and thus promote phylogenetic overdispersion (i.e. communities containing taxonomically disparate members). Alternately, the bacterial community clustering might be simply a reflection of geographical proximity, as interpreted by Barrett et al. (2006b) using PCR-DGGE strategy. This is consistent with the aerobiological study of Hughes et al. (2004), who reported predominantly local microorganisms in air samples collected from Rothera Station, Adelaide Island, Antarctica.

In order to understand whether the β-diversity patterns were influenced by physical factors such as distance and altitude or soil chemical parameters, Mantel-type tests and multivariate regression were carried out. A complex relationship between the environmental parameters and bacterial community patterns was found. Soil pH and altitude were significantly correlated with bacterial assemblage patterns derived from both qualitative and semi-quantitative species-based matrices. The qualitative divergence-based analysis showed significant correlation with geographical proximity and soil pH, while the semi-quantitative divergence-based analysis gave a significant correlation with altitude.

Such biotic-abiotic correlations are inconsistent both with the previous studies and other reports from Antarctica. For instance, metal ions have been shown to contribute high explanatory value to soil bacterial assemblage patterns observed on Signy Island and Alexander Island (Chong, et al., 2009a; Chong, et al., 2010) while Pointing et al. (2010) advocated the importance of salinity in community structuring of the Antarctic Dry Valleys soil. In the current study, none of these soil chemical factors exhibited a discernable relationship with bacterial assemblage patterns. This perhaps suggested that the influence of the environmental variability to the soil bacterial community is not consistent but sensitive to the spatial scale and type of ecosystem of the study (Barrett, et al., 2006a; Chown & Convey, 2007). Notwithstanding this, pH and altitude clearly had important influence, as the relationship between these two parameters and bacterial assemblage patterns was apparent in three of the four measures applied. Although the use of a single parameter to explain multiple scale bacterial heterogeneity might be an over-simplification, the results presented here support previous suggestion in Study 4 that the high dependence on soil pH by bacterial assemblage patterns is a general feature in polar soils.

Taxonomic distinctness in each location was analysed to test whether habitat filtering or competition can be detected. Using both unweighted and weighted NRI and NTI indices, it is apparent that most of the locations displayed positive or significant positive phylogenetic clumping. More importantly, when taking consideration of the occurrence of each individual taxon, most of the clone libraries were dominated by a high proportion of prevalent ribotypes. This is consistent with several previous studies that have reported bacterial diversity in Antarctic terrestrial ecosystems to exhibit high levels of dominant taxa (de la Torre, et al., 2003; Shravage, et al., 2007; Aislabie, et al., 2008). Nevertheless, the results from this study further indicate that these dominant ribotypes might be genotypically related (high NTI index). Using Spearman rank correlation, the co-occurrence of closely related species were showed to follows trends in geographical proximity, increasing pH and decreasing water content. In contrast, the abundance weighted taxonomic distinctness cannot be explained by any of the tested parameters.

Relationships between geographical proximity and bacterial community patterns were not detected using species-based measures but were apparent in unweighted UniFrac and taxonomic distinctness. This can be explained in the context of the limitations of the species-based measures. As mentioned by Martin (2002), the species-based measures will not be able to detect the similarity between two communities that contain no species in common, but where each species present in one community has a closely related representative in the other. In the light of this, the findings can be interpreted as indicating that locations with relatively closer proximity may not share higher identical bacterial species but instead more closely related ones. Despite this limitation, species-based measures (particularly T-RFLP analysis) is still important to use as it increases the

robustness of the interpretation, being less susceptible to sampling effort and thus providing a more accurate estimation of the relative abundance data.

8.5 Summary

Overall, this study showed that the distribution of soil bacterial community was largely dependent on the differences in habitat (i.e. soil pH and altitude). Nevertheless, between less disparate environments, site with relatively closer proximity might share higher proportion of closely related bacteria lineage. In addition, phylogenetic clumping of dominant ribotypes appears to be a typical feature in these regions, highlighting the importance of niche conservatism and habitat filtering in structuring Antarctic soil bacterial communities. Conversely, phylogenetic overdispersion resulting from competition was not common in the studied region. This finding is in agreement with previous observations of the reduced importance of competition in communities of other higher organisms in the Antarctic terrestrial environment (Convey, 1996; Hogg, et al., 2006). By intergrating different assessment methods, it is also apparent that the bacterial community structures from the three studied regions are complex and can exhibit different features depending on the different diversity measures used, suggesting that the unit of diversity (i.e. species or individual) reacts inconsistently to environmental variation.

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CHAPTER NINE

GENERAL DISCUSSION AND CONCLUSION

9.1 General discussion

The major aim of this thesis was to understand the relationship between soil bacterial community structure and environmental heterogeneity in Antarctica. To this end, investigation was started by comparing bacterial community patterns and soil chemical parameters inferred from eight sites around Windmill Island, continental Antarctica using DGGE profiling method (Study 1). Subsequently, one-way (Study 2) and nested (Study 3) multivariate designs were used to examine the occurrence of spatial clustering on Signy Island, maritime Antarctica. Further, the soil bacterial community patterns of three dry and arid sites on Alexander Island, southern maritime Antarctica were assessed using three molecular methods (i.e. DGGE, T-RFLP, PCR-cloning) (Study 4). Lastly, different diversity measures (i.e. divergence-based versus species-based; qualitative versus semi-quantitative) were applied to investigate soil bacterial communities from three ecologically different regions in the Antarctic Peninsula (Study 5). Each study is presented as a self-contained chapter (Chapter 4-8) with its own discussion on relevant topic. This last chapter summarises the main findings of the preceding chapters, and provides a general review of the results in a broader context.

9.2 Soil bacterial spatial patterns in Antarctica

Soil bacteria are not randomly nor uniformly distributed in the soil environments. Rather, they usually display predictable spatial patterning in relation to the variation in soil chemical and physical parameters (Ettema & Wardle, 2002; Grundmann, 2004). Previous reports on temperate environments had shown that the spatial structuring in soil bacterial communities can occur on scales ranging from micrometers to meters (Nunan, et al., 2002; Franklin & Mills, 2003).

The results from the studies described in this thesis generally supported that spatial structuring was also a general feature in Antarctic soil. From a latitudinal sampling across 51°S to 78°S, Yergeau et al. (2007b) detected community structuring on a scale of thousands of kilometers in relation to the variation in latitude, location and vegetation cover.

In Studies 2 and 3, sampling sites were on smaller spatial scales (i.e. between hundreds of meters to several of kilometers). It was apparent that soil bacterial communities in various locations on Signy Island were significantly different from each other. Further, the soil bacterial assemblage patterns could be aggregated according to subjective assignment of factors such as human disturbance and major external environmental influences including presence of vegetation (grasses and/or mosses), type of vertebrate organisms (seals and/or penguins), and proximity to the sea.

Such spatial patterning was not restricted to milder and more variable environments in sub- or maritime Antarctica. In Study 4, spatial clustering was detected in locations which are relatively close (~4 km apart) and environmentally-similar to each other (cold and arid) on the maritime/continental Antarctic transition zone. Although high xeric stress of the soil habitats in the Dry Valleys of continental Antarctic supported lower bacterial diversity in

comparison to endolith or hypolith environments (Pointing, et al., 2010), discernable spatial structuring in soil bacterial community was also detected (Barrett, et al., 2006b; Niederberger, et al., 2008). Together, these might suggested that the soil bacterial community patterns in all regions in Antarctica are highly site specific

Extending from the array of evidences of spatial patterning in Antarctica, the key question to be addressed is to identify the main factor(s) contributing to the bacterial spatial patterns. Barrett et al. (2006b) suggested that geographical proximity might be the major factor in determining the bacterial community patterns. This is supported by aerobiology studies that the bacterial diversity in the air mass was generally regional specific. For example, Hughes et al. (2004) found that the air samples from Rothera was predominantly similar to those found in the local soil community while Pearce et al. (2010) showed that a portion of airborne bacteria in Halley Research Station, Brunt Ice Shelf was derived from the local human population.

In Study 1, the community overlap (β-diversity) between eight sites around Casey Station was compared. It was found that sites with closer proximity to each other showed higher similarity in bacterial assemblage patterns (i.e. Red Shed and Thala Valley; Wilkes tips and ASPA 136) compared to distantly located sites (Browning Peninsula). However, it was also apparent that the bacterial assemblage patterns were influenced by the local environmental variations. For instance, the oil spill site, having received the highest anthropogenic impact, contained the most distinct bacterial community patterns. Furthermore, it has been shown that external environmental influences can have profound impact on the local soil parameters (Studies 2 and 3). For example, higher nutrient and water content was recorded from vegetated and animal impacted sites while elevated heavy metals content detected in several sites on Signy Island might have originated from anthropogenic contamination and

bioaccumulation of metal in seal faeces and penguin guano. These variations in soil chemical parameters might form selective pressure which preferentially supports the growth of bacteria with suitable trait. In Study 3, It was shown that the variation in soil bacterial community structures from human impacted locations was more dependent (higher explanatory value) on the differences in soil chemical parameters than the undisturbed locations. This might further imply that although Antarctic soil bacteria are well adapted to the cold and harsh environments, they are susceptible to environmental alteration (Barrett, et al., 2006b).

Indeed, regardless of the statistics applied, the bacterial community patterns were well correlated with the soil chemical parameters such as soil pH, salinity and metal content (Studies 2 and 3). In contrast to environmentally heterogeneous locations, Study 4 showed that relatively modest fluctuations in soil pH (between 7.04 to 7.75) and copper level (between 4.63 to 9.63 mg/kg) can result in significant differences in soil community structure in geologically and climatically similar sites. It is noteworthy that the high correlation of soil pH value and bacterial community structure may be a general attribute of polar terrestrial environments as consistent findings have been reported from both Arctic and Antarctic soil systems (Fierer & Jackson, 2006; Yergeau, et al., 2007a; Aislabie, et al., 2008; Chu, et al., 2010).

The importance of both geographical proximity and the underlying environmental differences in structuring soil bacterial community composition was tested in Study 5 using samples collected from three ecologically distinct regions in the Antarctic Peninsula. The results suggested that environmental variation has overriding effect on geographical proximity. As an example, bacterial community compositions of Ryder Bay were more similar to Alexander Island (which is located ~800 km apart) than to Reptile Ridge (~6 km

apart). Using multiple linear regressions, it was shown that the differences in soil bacterial communities between Reptile Ridge and the other two regions were most likely due to the higher altitude and lower pH value. The higher dependence of bacterial community compositions on environmental difference than on geological distance is in agreement with Horner-Devine et al. (2004b) who studied the taxa-area relationship of bacteria in salt marsh sediments.

9.3 Bacterial diversity, richness and taxonomic composition in Antarctic soil

In contrast to community overlap (β-diversity), the patterns of bacterial species richness (α-diversity) in relation to the environmental variations is less obvious. Although most of the sites did not show significant difference in DGGE derived H' (Studies 1, 2 and 5), a tendency of decrease in bacterial richness was observed in sites subjected to active human activities and low nutrient input (Studies 1, 2 and 3). In addition, when correlating the H' with the measured soil parameters (Study 1), no statistically significant relationship was established. Again, using both qualitative (number of T-RFs) and semi-quantitative (T-RFs peak height) approaches, only slight fluctuation in soil bacteria richness was detected from three ecologically distinct regions in the Antarctic Peninsula (Study 5).

Results from the studies conducted appeared to indicate that the bacterial species richness was insensitive to the environmental variation (i.e. differences in physical and chemical parameters) in Antarctica. Nevertheless, it is important to point out that the species richness was only a measure of the amount of distinct "species" (dependent on the assessment methods, the definition of species can be different – see section 2.3.4), it does not take into account the genetic distance of the "species" in the calculation. For example, Gourlay

Peninsula recorded the highest H' value in Study 2 but most of the retrieved sequences was affiliated to the same phylum of Bacteroidetes. Conversely, although Jane Col showed the lowest H' value, the retrieved sequences showed the highest genetic/nucleotide distance. Likewise, contrasting H' value and genetic distance was also reported by Study 3.

This perhaps highlighted the importance of having both species-based and divergence-based indices in complementary assessment as the two indices are of different definition and are capable of revealing different elements of diversity.

By comparing the nucleotide distances of DGGE bands (Studies 2 and 3), it was shown that sites in Signy Island which have the presence of vegetation, vertebrate influence and anthropogenic input habour bacterial communities with lower genetic distance compared to barren and nutriently depauparated sites. The former sites were also found to possess relatively more homogenous bacteria assemblage patterns. Sites with high animal influences such as Gourlay Peninsula, North Point and Elephant Flats registered lower multivariate dispersion indices than Jane Col, the barren and high altitude location on Signy Island (Study 2, Table 5.2; Study 3; Table 6.6). Such findings were also seen in Study 1 whereby the three replicates from oil spill site near Casey Station, Windmill Island were shown to cluster closely in the NMDS plot (Fig.4.2).These patterns are likely to be driven by soil heterogeneity as the soil chemistry and cycling processes in barren and dry landscapes are usually highly variable and more sensitive to the underlying geophysical processes (e.g. cryoturbation) and variation in landscape (e.g. proximity to melt snow and topographic depression leading to accumulation of water) (Barrett, et al., 2004).

The terrestrial soil ecosystem in Antarctica was found to be generally composed of a high proportion of closely related bacteria species and genotypically related dominant members

(Studies 4 and 5). The occurrence of closely related species is consistent with the hypothesis of habitat filtering and niche conservatism (Webb, et al., 2002; Horner-Devine & Bohannan, 2006; Wiens, et al., 2010). This suggests that each soil habitat in Antarctica offered specific niche that only species sharing certain important traits are able to persist. Phylogenetic clumping was also apparent in less extreme habitats (less dry and less nutrient limiting) that received nutrient input from adjacent animal populations (i.e. Study 5; Anchorage Island, Kilingbeck Island and Lagoon Island on Ryder Bay). One possible factor is that although high in nutrient content, the elemental distribution of C, N and P in these ecosystems were far from the optimum ecological stoichiometry (Redfield, 1958 -C:N:P 106:16:1; Cleveland & Liptzin, 2007 -C:N:P 186:13:1). According to Barrett, (2006a) although nitrogen and phosphorus can be in high supply in some Antarctic terrestrial ecosystems, carbon is usually limiting.

It is expected that Antarctica would be the most likely region on Earth to display bacterial endemism due to its geographical isolation from other continents (Vincent, 2000). However, using the lowest taxonomic resolution (i.e. phylum-level), the dominant phyla detected in the studies here (Studies 1, 2, 3, 4, and 5) and other Antarctic habitats (de la Torre, et al., 2003; e.g. Aislabie, et al., 2006; Smith, et al., 2006; Shravage, et al., 2007; Yergeau, et al., 2007a; Yergeau, et al., 2007b; Aislabie, et al., 2008; Pointing, et al., 2010) resemble those of tropical or temperate soils (Janssen, 2006; Youssef & Elshahed, 2008). This perhaps reflects that phylum-level comparison was not able to reveal true endemism. Indeed, when comparing the sequences retrieved in the studies reported in this thesis with other 16S rRNA sequences in GenBank database using a finer taxonomic resolution (i.e. species-level), most of the sequences were found to be <97% homology to the closest representative elsewhere. This is in agreement with other studies (see section 2.2) and

might be indicative that the Antarctic soil environments encompassed a fraction of unique and novel gene pools distinct from non-Antarctic soil environments.

9.4 Limitations and technical considerations

Field work in Antarctica is highly dependent on the weather and logistic availability. Thus, the soil samplings were mainly restricted to those areas which were easily accessible (around stations). Nevertheless, distantly located sites (relative to the stations) were also sampled (Browning Peninsula and Alexander Island) when there were sampling opportunities.

Systematic spatial sampling (i.e. sampling within strictly standardized dimensions in every studied site) was not possible because several sites were composed of mainly scree and pebbles with little soil. Soils, when present, was sometimes restricted to small areas (e.g. < 1 m^2). Systematic sampling was also difficult to carry out in habitat with high animal traffic (i.e. penguin rookeries, shag colonies and seal wallows) as the setting of grid systems (to ensure the sampling dimension) would be time consuming and might disturb the animals.

Nonetheless, in all studies, six replicates were collected from each sampling site (with the exception of Study 1 on Windmill Island where n=3), and these were found to be adequate in providing statistically significant data.

For all the molecular approach applied in the studies here, DNA was used as the starting material. The DNA extraction was carried out in the earliest opportunity usually within 24 hours of sample collection, to prevent DNA degradation and community alteration (Schneegurt, Dore, & Kulpa, 2003). Although DNA is relatively stable, easy to handle and

provides better yield than RNA, the bacterial community inferred from DNA-based assessment might be an overestimation as the legacy DNA from dead and inactive cells can be co-amplified during PCR amplification (Tow & Cowan, 2005). RNA would be more indicative of the metabolically active members in the soil communities. It is however important to note that despite the advantages, RNA extraction is sensitive to contamination and storage time. Such limitations are being addressed by the application of newly available commercial kits such as Soil Preservation Solution (MoBio, USA) that prolong the storage time for soil RNA and thus facilitate the transfer of samples from the field to the laboratory. Another limitation is that most labs in Antarctic stations are inadequately equipped for RNA studies.

As described in the preceding chapters, three 16S rRNA-based molecular methods including DGGE, T-RFLP and clone library analyses were used. The DGGE and T-RFLP were able to provide robust comparison of the species-based (see section 2.3.4) spatial differences between sites as these profiling methods were relatively high throughput and less constrained by the use of multiple replicates compared to cloning. However, both DGGE and T-RFLP contributed little information with regards to the genetic variation within a community. Although excision and sequencing of DGGE bands are common practice, the taxonomic resolution is low (~400 – 500 bp) in comparison to cloning (~1500 bp). On the contrary, although clone libraries provide better estimation of genetic diversity in soil communities, assessment of multiple replicates for each sampling site is not practical.

The data obtained across the five studies were not re-analysed together using standardize statistical analyses because of several limitations. Firstly, the assignment of DGGE banding positions across multiple gels from Studies 1, 2, 3 and 4 was problematic and potentially suffered from high gel to gel variations as the samples were from highly distinct locations

and the DGGE profiles were obtained at different time periods. Secondly, the sequences retrieved from DGGE profiles (Windmill Island and Signy Island – Studies 1, 2 and 3) and clone libraries (Antarctic Peninsula – Studies 4 and 5) can be very different. As shown in Study 4, the DGGE banding profiles obtained from the pooled clones from a specific site had only 50% overlap with the environmental DGGE banding profiles from the same site. This is likely to be due to the use of different PCR strategy in the two methods (direct versus nested PCR).

Diversity in microbial ecology by definition can be referred to the number of cell, and the number of different taxa. In this thesis, the measured diversity is mainly referred to the latter due to the limitations of the molecular tools applied, and partly due to the complexity of the number of the bacterial exist in any given soil environments (e.g. one gram of soil might contained up to 10^{11} microorganism (Torsvik, et al. 2002)). However, relatively new method such as quantitative PCR (qPCR) has been developed to allow quantification of cell number. It is therefore recommended that the bacterial abundance to be measured in the future work.

9.5 Conclusion and future directions

In conclusion, despite the complexity of the Antarctic soil ecosystem and the limitations in the methodologies, several general features were apparent:

1. Spatial structuring is common in Antarctic soil environments and can occur at different spatial scales (i.e. between a few hundreds of meters –Studies 2 and 3; between a few kilometers –Studies 1 and 4; and a few hundreds of kilometers – Study 5).
2. Soil bacterial communities were largely dependent on the differences in the environmental characteristics. Nevertheless, between less disparate environments, sites with relatively closer proximity might share higher degree of community overlap.
3. High correlation was observed between soil pH and Antarctic soil bacterial communities.
4. Habitat filtering and niche conservatism could be important features of Antarctic soil systems as many of the soil bacterial communities were found to harbour a few numerically-dominant species and a high proportion of closely related species.

Based on these evidences, several studies can be carried out to extend the knowledge generated from these studies. Firstly, field experiments can be designed to explore how soil pH impacts the underlying soil bacterial community structure. Secondly, the occurrence of phylogenetic clumping across Antarctic soil habitats can be further tested through advanced sequencing methods such as pyrosequencing. Thirdly, soil sampling can be carried out at different time intervals to understand the relationship between the spatial and temporal patterns of Antarctic soil environments. Together with the results

presented in this thesis, these studies can deepen our understanding on the ecological function of soil system in Antarctica and provide a foundation to predict the impact of environmental perturbation due to climate change to the soil bacterial communities in Antarctica.