Resolving Drivers of Microbial Community Structure in The Dry Valleys of Antarctica

A thesis

submitted in fulfilment

of the requirements for the degree

of

Doctor of Philosophy in Biological Sciences

at

The University of Waikato

by

ERIC BOTTOS

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2015

Abstract

Understanding the processes that underlie patterns of microbial distribution is fundamental to the field of microbial ecology, but extremely challenging given the complexity of natural systems. Antarctica's ice-free regions possess unique ecosystems of simple trophic structure, shaped by the harsh environmental conditions that typify the continent. The Dry Valleys comprise the largest of these regions and have one of the simplest food webs on the planet, making them a tractable system to comprehensively define relationships that influence microbial distributions at the landscape scale. The New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) was aimed at identifying factors that control, and can predict, biological distributions in the Dry Valleys. As part of nzTABS, the goal of the research presented in this thesis was to elucidate the factors that influence bacterial community structure in Dry Valley soils.

This study explored how topographic, physicochemical, and spatial variation influence bacterial diversity and community structure across a Dry Valley landscape. Bacterial communities were characterized in 471 soil samples using automated ribosomal intergenic spacer analysis (ARISA). Diversity and community composition were most strongly related to variation in physicochemical soil properties, though significant relationships with topographic and spatial variables were also observed. This identified, for the first time, the influence of environmental variables on bacterial diversity and community composition across the landscape, and presents a structural equation model identifying those relationships.

The phylogenetic diversity of bacterial communities in Dry Valley soils was also examined. High-throughput sequencing of 16S rRNA gene amplicons was used to analyze bacterial communities in 177 soil samples. This work identified significant relationships between the relative abundances of bacterial taxa and both abiotic and biotic variables, though these relationships explained only a small amount of community variation collectively. The relative abundances of several bacterial taxa were, however, significantly coupled to one another, suggesting that interactions between bacterial taxa may influence community compositions.

Lastly, the bacterial composition of aerosols above the Dry Valleys was examined. High-throughput sequencing of 16S rRNA gene amplicons was used to analyze two aerosol samples collected in the Miers Valley, and their compositions were compared to those of previously characterized aerosols and soils from across the continent. Bacteria present in the aerosols were found to be distinct from those of local soils; instead, aerosol compositions were more similar to those of air samples reported from elsewhere on the planet. Importantly, these findings suggest that local redistribution of Dry Valley soil bacteria through atmospheric processes may be largely restricted to periods when high winds mobilize soil particles and associated biota.

This study provides novel insights into the microbial ecology of the Dry Valleys. Despite the relative simplicity of the ecosystem, the factors that influence bacterial distributions within the Dry Valleys appear to be highly complex, and include a combination of abiotic and biotic drivers. Continued research will help to disentangle relationships that influence microbial community compositions in Antarctica's ice-free ecosystems, and will improve understanding of processes that influence microbial community assembly globally.

Statement of Authorship

The work presented in this thesis results from several collaborative efforts. I made substantial contributions to all works presented. Co-authorship forms outlining author contributions to all co-authored works are included here. The full citations for published works and the nature of author contributions are further detailed in the prefaces of relevant chapters.

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Co-Authorship Form

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Student and Academic Services Division
Wahanga Ratonga Matauranga Akonga
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 858 5096
Website: http://www.waikato.ac.nz/sasd/postgraduate/

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Nature of contribution by PhD candidate	Wrote the majority of the chapter, compiled all contributions
Extent of contribution by PhD candidate (%)	70

CO-AUTHORS

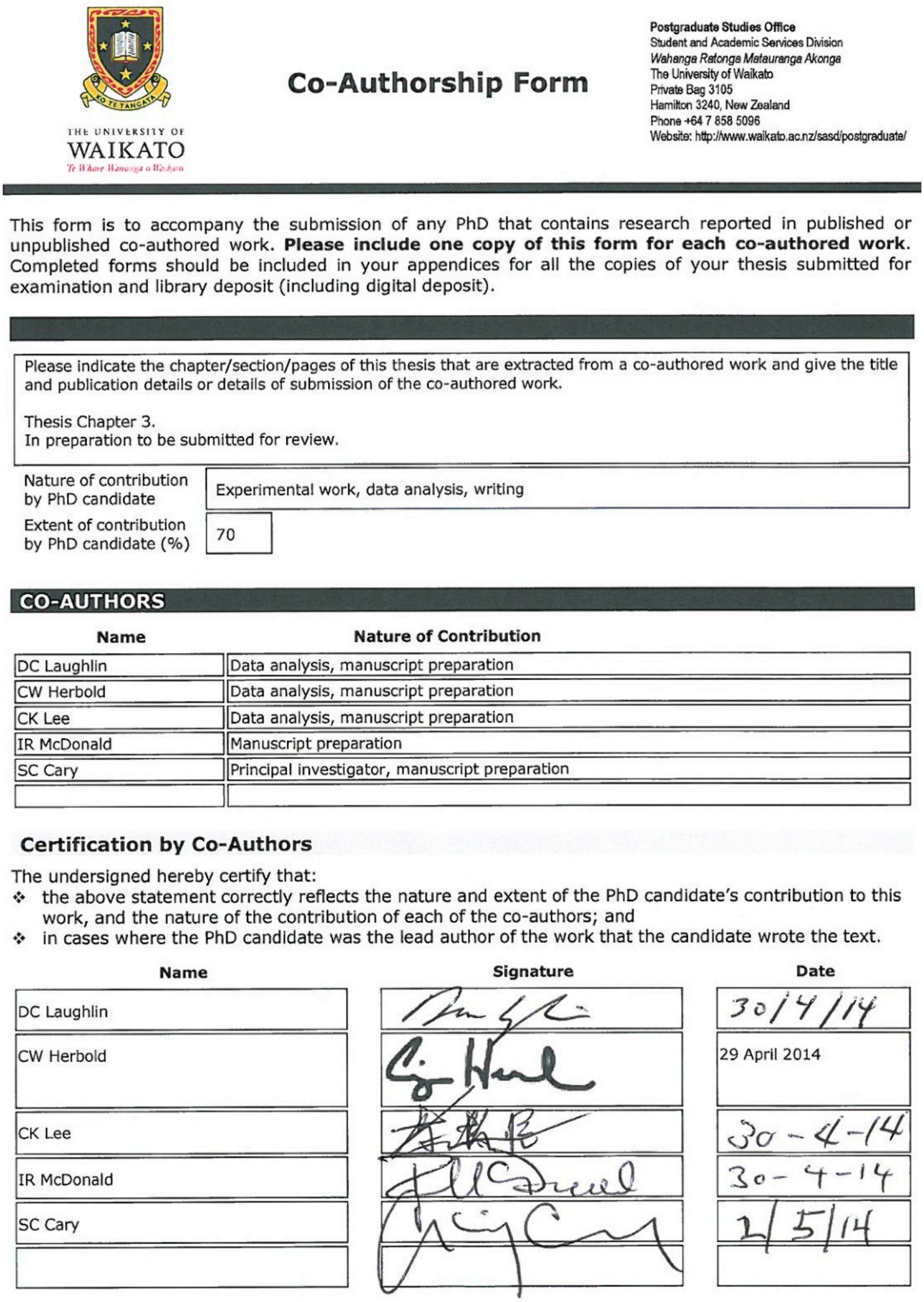
Name	Nature of Contribution
JW Scarrow	Writing (primarily sections on geology), chapter preparation
SDJ Archer	Writing (primarily sections on aquatic systems), chapter preparation
IR McDonald	Preparation of Figure 2.3, chapter preparation
SC Cary	Chapter preparation

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
JW Scarrow	J.W. Scarrow	2/5/14
SDJ Archer		30.04.14
IR McDonald		30-4-14
SC Cary	S. Craig Cary	2/5/14



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Thesis Chapter 3.
In preparation to be submitted for review.

Nature of contribution by PhD candidate	Experimental work, data analysis, writing
Extent of contribution by PhD candidate (%)	70

CO-AUTHORS

Name	Nature of Contribution
DC Laughlin	Data analysis, manuscript preparation
CW Herbold	Data analysis, manuscript preparation
CK Lee	Data analysis, manuscript preparation
IR McDonald	Manuscript preparation
SC Cary	Principal investigator, manuscript preparation

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- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
DC Laughlin		30/4/14
CW Herbold		29 April 2014
CK Lee		30-4-14
IR McDonald		30-4-14
SC Cary		2/5/14

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Thesis Chapter 4.
In preparation to be submitted for review.

Nature of contribution by PhD candidate	Experimental work, data analysis, writing
Extent of contribution by PhD candidate (%)	70

CO-AUTHORS

Name	Nature of Contribution
CK Lee	Data analysis, manuscript preparation
DC Laughlin	Data analysis, manuscript preparation
IR McDonald	Manuscript preparation
SC Cary	Principal investigator, manuscript preparation

Certification by Co-Authors

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- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
CK Lee		30-4-14
DC Laughlin		30/4/14
IR McDonald		30-4-14
SC Cary		2/5/14

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Thesis Chapter 5. Published as Bottos EM, Woo AC, Zawar-Reza P, Pointing SB, Cary SC. (2014). Airborne bacterial populations above desert soils of the McMurdo Dry Valleys. *Microbial Ecology* **67**: 120-128.

Nature of contribution by PhD candidate	Field and lab work, data analysis, wrote manuscript
Extent of contribution by PhD candidate (%)	70

CO-AUTHORS

Name	Nature of Contribution
AC Woo	Data analysis
P Zawar-Reza	Data analysis, manuscript preparation
SB Pointing	Principal investigator, manuscript preparation
SC Cary	Principal investigator, manuscript preparation

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The undersigned hereby certify that:

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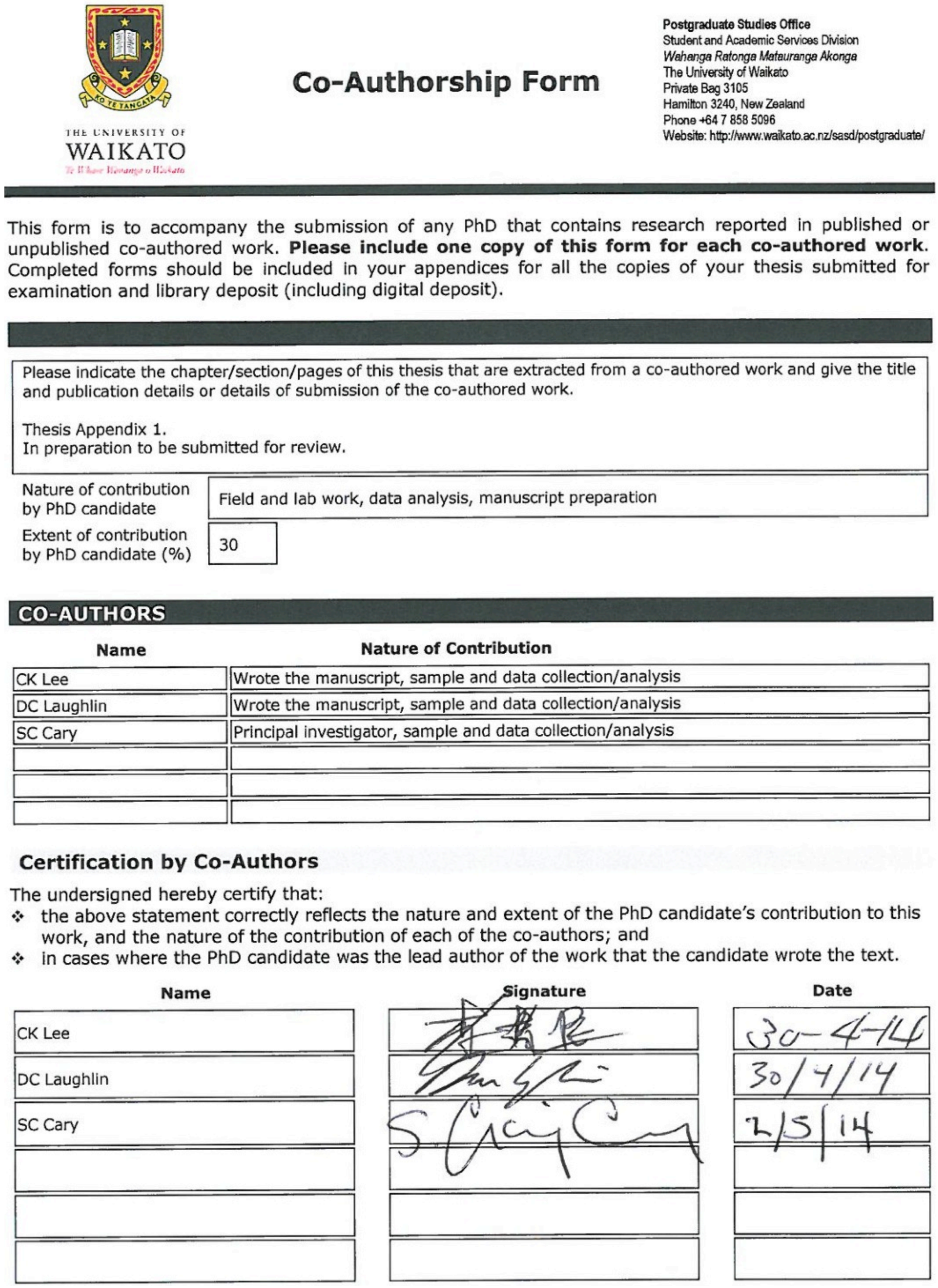
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
P Zawar-Reza		12 May 2014
SB Pointing		6 May 2014
SC Cary	Craig Cary	12/5/2014



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Thesis Appendix 1.
In preparation to be submitted for review.

Nature of contribution by PhD candidate	Field and lab work, data analysis, manuscript preparation
Extent of contribution by PhD candidate (%)	30

CO-AUTHORS

Name	Nature of Contribution
CK Lee	Wrote the manuscript, sample and data collection/analysis
DC Laughlin	Wrote the manuscript, sample and data collection/analysis
SC Cary	Principal investigator, sample and data collection/analysis

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- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
CK Lee		30-4-14
DC Laughlin		30/4/14
SC Cary		2/5/14

Acknowledgements

Firstly, I would like to thank my supervisors Craig Cary, Ian McDonald, and Hugh Morgan for the opportunity to undertake this research, and for their guidance and support over the course of my study. I would also like to thank the many researchers involved in the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS), who made this work possible. Thank you to all those I worked with in Antarctica: your efforts made for three safe, successful, and memorable field seasons. Special thanks to Ashley Sparrow for hosting me at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) facility in Alice Springs, Australia, during the early stages of analysis of the nzTABS dataset, and to Janet Jansson for hosting me at Lawrence Berkeley National Lab in Berkeley, California, USA, to carry out sample processing as part of the Earth Microbiome Project.

I would like to thank the students and staff in the Thermophile Research Unit at the University of Waikato, who I had the privilege of working alongside as colleagues and friends. I am also thankful to the many researchers in the Department of Biological Sciences at the University of Waikato, who were available for advice. The collaborative nature of the department was truly invaluable for fostering this work.

Finally, I would like to thank my family and friends. To the many friends I have made here in New Zealand, thank you for making me feel at home. Thanks especially to you Chelsea, for your love and encouragement. To my family and friends back in Canada, thank you for your support in this endeavor. Most importantly, thank you Dad, Mom, Jenny, and Kara: your love and support have always allowed me to follow my dreams.

Table of Contents

List of Figures

List of Tables

Chapter 1: Understanding Microbial Distributions

Introduction

Understanding the factors that influence biological distributions is central to the field of community ecology, and critical to inform best conservation and environmental management practices. Knowledge of relationships between organisms and their environment allows particular community attributes to be used as indicators of ecosystem health, and enables forecasting of possible community shifts in response to environmental change. These abilities are becoming increasingly important as anthropogenic disturbance to natural systems intensifies (Turner, 2010).

Microorganisms are the most abundant and genetically diverse organisms on Earth (Whitman *et al.*, 1998), yet little is known about the factors that influence their distributions. Ecological theory has largely been shaped by investigations of plant and animal communities and the degree to which such theory is applicable to explaining patterns of microbial distributions is not well established. Indeed, the technical inability to adequately characterize microbial biodiversity and community composition has precluded examination of basic patterns in microbial ecology. With recent advances in the ability to characterize microbial communities, patterns of microbial distributions are being revealed and questions about the processes that determine these patterns are beginning to be addressed.

This chapter aims to orient the reader to the concepts underlying this thesis. It begins with a brief overview of the methods used in microbial ecology, with emphasis on those employed in the studies presented in the following chapters. It then reviews current understanding of the factors that influence microbial community composition and discusses the processes that can shape patterns of microbial distributions. Finally, the studies that makeup each chapter in this thesis are introduced and an outline for the thesis structure is presented.

Methods of Microbial Community Analysis

Investigating the ecology of microorganisms presents a unique challenge, as microbial communities are not readily observed. Traditionally, microbial community studies have relied on growing microorganisms from samples of interest on artificial media, and identifying isolates based on morphology and physiology. While there are many benefits to using culture-dependent techniques that allow for direct observations of the microorganisms present in a community, it is estimated that more than 99 % of microorganisms in most environments cannot be established in pure culture using current methods (Béjà, 2004). Culture-based analyses, therefore, do not accurately represent the true composition of a microbial community. To overcome the limitations associated with culture-based methods, a number of culture-independent genetic techniques have been established, and are now the basis for most microbial community analyses.

Genetic techniques allow researchers to characterize microbial communities based on analyses of total community DNA, as individual populations within a community can be distinguished based on their genetic signatures. Ribosomal operons are attractive genetic targets in studies of microbial communities as they are present in all organisms and contain genes that are highly conserved evolutionarily (Woese and Fox, 1977). These conserved sequences can be exploited using PCR to amplify regions of the operon that can be used to provide a genetic fingerprint of the total community or sequenced directly to identify the taxa present in a community.

Community fingerprinting techniques exploit genetic differences that exist between taxa to discriminate individuals in a community. Automated ribosomal intergenic spacer analysis (ARISA) is a commonly used fingerprinting technique, which discriminates taxa based on the length of the internally transcribed spacer (ITS) between the 16S and 23S ribosomal RNA (rRNA) genes in their ribosomal operons (Fisher and Triplett, 1999) (Figure 1.1). As a non-coding region of the operon, the ITS is hyper variable in both sequence and length between different microbial taxa. ARISA involves PCR amplification of the ITS from a template of total community DNA, using primers specific to conserved regions of the rRNA genes that flank the target. One of the primers in this reaction is labeled with a fluorescent tag, which is incorporated into the PCR products during the reaction. The products are then separated by size on an automated capillary electrophoresis instrument equipped with a fluorescence detector. An electropherogram generated by this process indicates the size of all products in a given sample and a relative measure of the abundance of each sized product. These fingerprints allow for comparisons of relative diversity and community structure between samples. Fingerprinting methods provide a rapid, cost-effective, means of obtaining microbial diversity and community structure information that can be compared between samples; however, they do not provide an appropriate means of phylogenetic assessment of community compositions.

DNA sequencing of the gene encoding the small subunit of the ribosomal operon (16S rRNA gene) is typically employed to obtain information about the phylogenetic identity of microorganisms present in a community. The gene, or a portion of the gene, is PCR amplified from total community DNA and sequencing is carried out on the amplicons produced in the reaction. The advent of high throughput sequencing techniques has allowed microbial communities to be surveyed at unprecedented depths, as hundreds of samples can be sequenced simultaneously on a single instrument and yield information from thousands to hundreds of thousands of amplicons per sample. The cost of sequencing has fallen considerably in recent years, greatly increasing the accessibility of these methods to researchers (Tringe and Hugenholtz, 2008).

Public databases and computer programs that efficiently process the vast amounts of sequence data generated by modern sequencing technologies are necessary to allow meaningful analyses of datasets. Sequencing data can be shared and researched through repositories such as GenBank (Benson *et al.*, 2005), The Ribosomal Database Project (Cole *et al.*, 2005), and Greengenes (DeSantis *et al.*, 2006). Open-source software such as Mothur (Schloss *et al.*, 2009) and QIIME (Caporaso *et al.*, 2010) bring together a suite of applications to process and quality check raw sequence data, query sequences against public databases, and perform community analyses. These platforms are allowing analyses that are commonplace in plant and animal ecology to be applied to questions in microbial ecology.

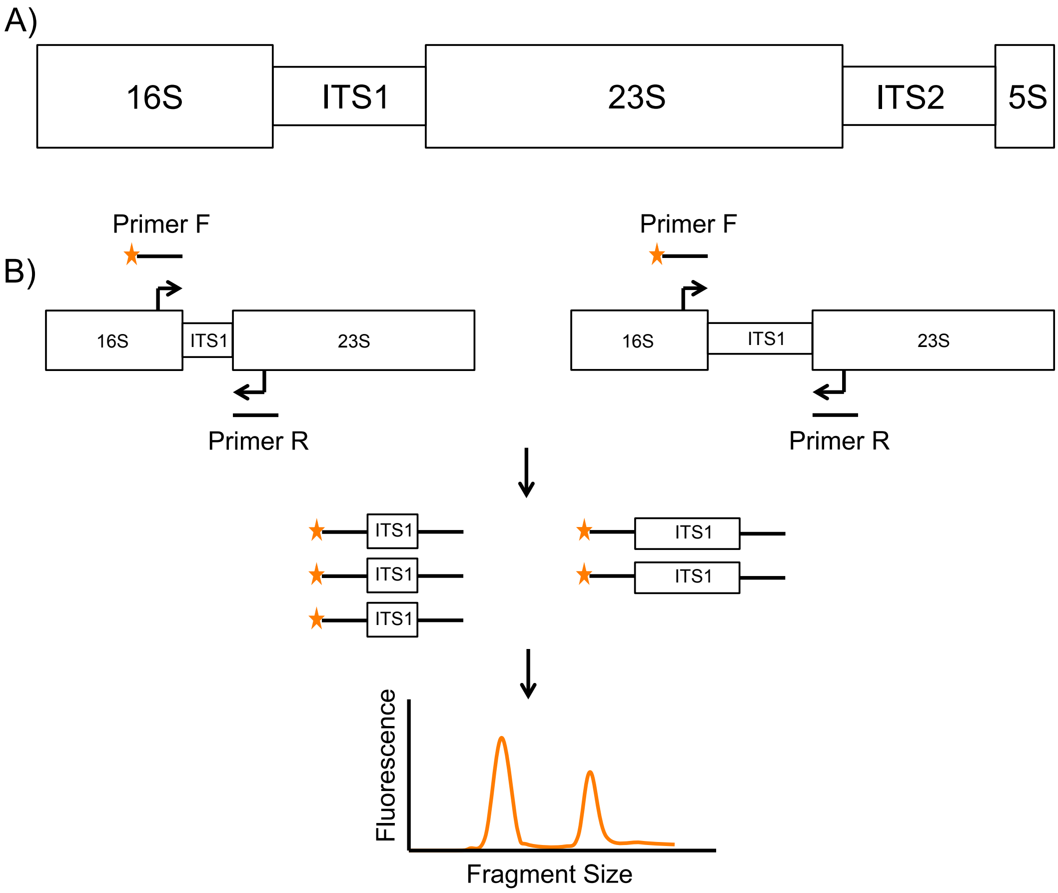

Figure 1.1: The bacterial ribosomal operon and its use as a target in automated ribosomal intergenic spacer analysis (ARISA). The bacterial ribosomal operon (A) contains the 16S, 23S, and 5S ribosomal RNA (rRNA) genes, separated by internally transcribed spacers (ITS). The rRNA genes are highly conserved and the ITS regions vary in sequence and length between different taxa. ARISA (B) exploits these characteristics to yield a community fingerprint, in which different taxa in a community are distinguished based on their different ITS1 lengths. Primers specific to conserved regions of the 16S and 23S rRNA genes are used to amplify ITS1 of all taxa present in a pool of total community DNA. One primer is labeled with a fluorescent tag, which is incorporated into all amplicons generated in the reaction. These amplicons are size separated on an automated capillary electrophoresis instrument equipped with a fluorescence detector, to yield an electropherogram that indicates the size and relative abundance of all amplicons generated.

Patterns and Processes in Microbial Ecology

The principle "everything is everywhere, *but* the environment selects" was introduced by Lourens Baas-Becking in 1934 to explain the nature of microbial distributions (Baas-Becking, 1934; de Wit and Bouvier, 2006). This view suggests that microbial taxa are not bound spatially by dispersal limitation, and patterns of their distribution can, therefore, be explained solely by environmental variability. The small sizes of individuals coupled with the large sizes of populations make the concept of ubiquitous microbial dispersal tenable, and provide the basis for support of Baas-Becking's principle (Fenchel and Finlay, 2004; Finlay, 2002). However, in recent years, investigations of the patterns of microbial distributions have revealed that similar spatial relationships exist for microorganisms and macroorganisms, suggesting that the laws that govern the distributions of microorganisms are not fundamentally different to those that govern distributions of macroorganisms (Green and Bohannan, 2006).

Under the assumption of ubiquitous dispersal, patterns of microbial distributions are expected to arise entirely from selection. Selection refers to deterministic processes that result from differences in fitness between organisms or taxa (Vellend, 2010), and selective pressures may come from abiotic and biotic characteristics of an environment. Abiotic factors are recognized as important drivers of microbial community composition, and their influence is demonstrated in several studies that show shifts in community composition along environmental gradients. Microbial communities have been found to vary in composition with gradients of salinity (Lozupone and Knight, 2007), pH (Griffiths *et al.*, 2011; Rousk *et al.*, 2010), moisture (Pointing *et al.*, 2007; Zhang *et al.*, 2013; Zeglin *et al.*, 2011; Brockett *et al.*, 2012), and temperature (Garcia-Pichel *et al.*, 2013), to name a few. Biotic interactions may additionally influence microbial distributions as the presence of particular organisms in an environment may have positive or negative effects on particular microbial taxa. The importance of these mutualistic interactions are exemplified in plant microbe associations (Marschner *et al.*, 2001; Bonfante and Anca, 2009) and syntrophic relationships between microbial taxa (Stams and Plugge, 2009), while antagonistic relationships include predation by higher taxa (Rosenberg *et al.*, 2009) and competition between microorganisms (Hibbing *et al.*, 2009). In a scenario of ubiquitous microbial dispersal, the taxa best suited to the environmental conditions would inhabit different locations solely as a result of environmental selection, and patterns of microbial distribution could be explained entirely if all abiotic and biotic associations could be understood.

Observed spatial patterns of microbial distributions appear to contradict assumptions of ubiquitous dispersal. Many studies have demonstrated that microbial communities become less similar in composition as the distance between communities increases (Horner-Devine *et al.*, 2004; Bell, 2010; Green *et al.*, 2004; Martiny *et al.*, 2011). These distance-decay relationships are not explicitly at odds with the principle of ubiquitous distribution, as the community dissimilarity observed might simply reflect the decrease in environmental similarity that occurs over the same geographic distance (Nekola and White, 1999). In many cases, however, variation in community composition cannot be adequately explained by environmental variation alone (Finkel *et al.*, 2012; Martiny *et al.*, 2011), and thus the patterns appear to have a true spatial component (Nekola and White, 1999). These observations suggest that, in addition to deterministic processes, stochastic processes are important for shaping patterns of microbial community composition, and must be accounted for in descriptions of microbial distributions.

The importance of stochastic processes in shaping microbial communities has been demonstrated in several systems. Stochastic processes are non-deterministic, or neutral, processes that influence community composition. These include passive dispersal of taxa, genetic differentiation of taxa due to mutation or horizontal gene transfer, and drift in relative taxa abundances that result from random growth and decline of populations (Nemergut *et al.*, 2013). Analyses of a wastewater treatment community found that abundances of common taxa could be explained by neutral models and the authors suggest that stochastic processes may be similarly important to microbial community assembly in any open system (Ofiţeru *et al.*, 2010). This suggestion has been supported experimentally through analyses of community assembly in open sterile microcosms (Langenheder and Székely, 2011). Stochastic processes have also been found to be important in explaining compositions of subsurface microbial communities (Stegen *et al.*, 2012) and desert hypolith communities (Caruso *et al.*, 2011). In all these studies, deterministic factors were also found to influence community composition, and the relative contributions of stochastic and deterministic processes on microbial community composition have been found to vary greatly by environment (Wang *et al.*, 2013).

Given the complexity of natural systems and the abundance of processes that may influence community assembly, simple ecosystems are appealing as a starting point to resolve the drivers of community composition. The number of potential outcomes for a given model of ecosystem interactions grows exponentially as the number of variables is increased (Vellend, 2010). However, it is necessary to account for all potentially important abiotic and biotic variability in order to understand the factors that influence community composition in natural systems. In particular, the degree to which stochastic processes can be investigated is dependent on how well environmental and biological variability can be quantified. As a result, an ecosystem can only be adequately characterized if the variables in the system can be comprehensively surveyed, without yielding a dataset too complex to interpret.

The Current Study

Antarctica is dominated by ice and snow covered landscapes; however, several areas of the continent remain ice-free for part or all of the year. These areas collectively makeup approximately 0.35 % of the continent and provide important terrestrial oases to Antarctica's edaphic (soil associated) communities (Hopkins *et al.*, 2006). At approximately 4500 km^2, the McMurdo Dry Valleys of Southern Victoria Land comprise the largest ice-free area of the continent (Levy, 2013) and are the subject of intense scientific interest.

The Dry Valleys provide a tractable ecosystem to investigate the factors that influence microbial distributions in terrestrial environments. The harsh environmental conditions of Antarctica have shaped microbial dominated ecosystems of simple trophic structure (Cary *et al.*, 2010). Therefore, interactions between trophic levels are greatly reduced in relation to most temperate ecosystems, amplifying the relative importance of abiotic influences on the local biota (Hogg *et al.*, 2006). The isolation of the continent is expected to limit the frequency with which microorganisms are introduced to Antarctica from external sources (Pearce *et al.*, 2009), suggesting the regional pool of microbial taxa may remain reasonably constant. The reduced complexity of Antarctica's terrestrial ecosystems make them ideal environments to study the influences of abiotic factors on the composition and structure of edaphic microbial communities.

Understanding the factors that drive community composition in Antarctic soils is also critical to manage these ecosystems and predict how they will respond to change. The recent increase in human activity on the continent has the potential to disrupt sensitive environments (Cowan *et al.*, 2011), and understanding the probable consequences of such activity will influence policy aimed at mitigating detrimental impacts. Climate change poses additional threats to these ecosystems, as Antarctica's terrestrial environments will see significant alteration of the physical landscape under projected climate scenarios (Anisimov *et al.*, 2001). The biological responses to such change can only be predicted through increased understanding of the relationships between the local biota and the physical environment. Information about the potential responses of biological communities in these systems may additionally provide insight into the shifts that can be expected to occur globally in response to environmental change.

The New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) was established in 2008 as a multidisciplinary, international initiative, aimed at elucidating the factors that influence biological distributions in Antarctic soils (nztabs.ictar.aq). Distinct in its landscape scale approach, nzTABS aims to comprehensively characterize biological communities across the range of environmental variation that exists within the Dry Valley ecosystem. nzTABS initial survey in the 2009 and 2010 austral summers included analyses of geochemical and physicochemical soil attributes and biological characterization of vegetation, insects, all infauna and flora (nematodes, rotifers, tartigrades), and microorganisms in nearly 500 samples collected from across a study area of 220 km^2. As the largest biological survey of terrestrial Antarctic ecosystems undertaken to date, the efforts of the nzTABS programme are greatly advancing understanding of the ecology of the Dry Valleys.

The research presented in this thesis was carried out as part of the nzTABS programme, and describes biological investigations that were completed on the set of Dry Valley soil samples collected in January of 2009 and 2010. This thesis evaluates several aspects of microbial ecology and biogeography in the Dry Valleys of Antarctica, with the goal of providing novel insights into the factors that influence microbial community compositions in Antarctic soils. The chapters of this thesis have been prepared as independent manuscripts and result from several collaborative efforts. I am primary author of all chapters in the body of this thesis and made significant contributions to works presented in all chapters and appendices. Contributions of all authors and citations for works that have been accepted for publication are outlined at the preface of each chapter and appendix. An overview of the chapters of this thesis is outlined below.

Chapter 2 presents a literature review of studies that have examined bacterial community structures in soils across Antarctica. Bacterial community characteristics are discussed in relation to environmental variation for the diverse range of habitats found in the ice-free regions of the continent. As a synthesis of research completed to date, this chapter summarizes current understanding of the patterns of bacterial distributions in Antarctica's terrestrial ecosystems and the environmental influences that shape them. This review has been published as a book chapter with the following citation:

> **Bottos EM**, Scarrow JW, Archer SDJ, McDonald IR, Cary SC. (2014). Bacterial community structures of Antarctic soils. In: *Antarctic Terrestrial Microbiology: Physical and biological properties of Antarctic soils*, Cowan, DA (ed.). Springer-Verlag, Berlin, pp 9-33.

Chapter 3 describes patterns of bacterial diversity and community structure in relation to topographic, physicochemical, and spatial variation across the nzTABS Antarctic Dry Valley study area. ARISA was used to compare bacterial communities in 471 soil samples, and a combination of structural equation modeling (SEM), variance partitioning, and Mantel tests were used to assess relationships between explanatory variables of interest and bacterial community characteristics. As the largest survey of terrestrial microbiology completed in Antarctica to date, this work identifies numerous significant relationships between environmental variation and bacterial community diversity and composition. This chapter has been prepared for submission to the *ISME Journal*.

Chapter 4 describes patterns of distributions of particular phylogenetic groups of bacteria in 177 of the samples from the nzTABS Dry Valley study area. In addition to being part of the nzTABS programme, the work in this chapter results from collaboration with the Earth Microbiome Project (EMP) (Gilbert *et al.*, 2010), which included the nzTABS soil samples in its global sequencing programme. In addition to relationships observed between community composition and environmental variation, this work identifies numerous significant relationships between bacterial taxa, suggesting that both abiotic and biotic interactions may influence microbial distributions in Dry Valley soils. This manuscript is in preparation for submission to the *ISME Journal*.

Chapter 5 describes the bacterial composition of aerosols in the Dry Valleys of Antarctica. This work was undertaken as a pilot study to determine the importance of bacterial inputs to local soils from the air. Aerosols were sampled at two locations in the Miers Valley between December 2009 and January 2010, and high-throughput DNA sequencing of bacterial 16S rRNA genes was used to assess the bacterial composition of the aerosols. Bacteria detected in the aerosols were distinct from previously described Antarctic soil communities, suggesting local redistribution of soil organisms through the air may be restricted to periods in which weather events mobilize soil particulates and associated biota. This work provides novel insights into the dispersal of bacteria across the landscape and suggests that inputs of bioaerosols from atmospheric deposition have little effect on local soil community compositions. In addition to being part of the nzTABS research programme, this study was undertaken in collaboration with Steve Pointing's group at Auckland University of Technology. This work has been published with the following citation:

> **Bottos EM**, Woo AC, Zawar-Reza P, Pointing SB, Cary SC. (2014). Airborne bacterial populations above desert soils of the McMurdo Dry Valleys. *Microbial Ecology* **67**: 120-128.

Finally, Chapter 6 presents general conclusions and future directions of this work.

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Chapter 2: Bacterial Community Structures of Antarctic Soils

Preface

This chapter presents a literature review of studies that have examined bacterial community structures in Antarctic soils. As primary author, I wrote the majority of the chapter and compiled and edited all writings provided by co-authors. Joshua Scarrow earned co-authorship by contributing sections related to landscape geology and the physical environment. Stephen Archer earned co-authorship by contributing a section on the aquatic systems of the Dry Valleys and their biology. Glen Stichbury, GIS analyst at the University of Waikato, prepared Figures 2.1 and 2.2, and my supervisor Ian McDonald prepared Figure 2.3. My supervisors Ian McDonald and Craig Cary reviewed and edited content. This review has been published as a book chapter with the following citation:

> **Bottos EM**, Scarrow JW, Archer SDJ, McDonald IR, Cary SC. (2014). Bacterial community structures of Antarctic soils. In: *Antarctic Terrestrial Microbiology: Physical and Biological Properties of Antarctic Soils*, Cowan DA (ed.). Springer-Verlag, Berlin, pp 9-33.

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Chapter 2
Bacterial Community Structures of Antarctic Soils

Eric M. Bottos, Joshua W. Scarrow, Stephen D. J. Archer,
Ian R. McDonald and S. Craig Cary

Abstract Antarctica's ice-free environments span diverse habitats, ranging from well developed and nutrient rich soils in the coastal areas, to poorly developed and oligotrophic soils in the continent's deserts and high elevation sites. Though most terrestrial environments in Antarctica are typified by harsh environmental conditions, many soils are home to abundant and diverse bacterial communities. These communities are locally adapted, varying both between and within different regions of the continent, and typically reflecting the local physicochemical and biological characteristics of the soils. Environmental conditions are changing rapidly in many areas, due to increased human activity on the continent and the impacts of climate change. This chapter reviews characteristics of bacterial communities in soils across Antarctica in relation to their environment, and discusses the potential responses of bacterial communities to contemporary environmental change. Continued and coordinated efforts to understand bacterial community structure and function in Antarctic soils will be necessary to monitor and predict ecological responses in these changing environments, and to shape management practices that will ensure the protection and preservation of biodiversity in Antarctica's terrestrial ecosystems.

2.1 Introduction

While the majority of continental Antarctica is permanently covered by the Antarctic Ice Sheet, approximately 0.35 % of the continent remains free from ice and snow cover for part or all of the year (Hopkins et al. 2006b). These ice-free areas are largely confined to the perimeter of the continent at coastal sites and regions cut off

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E. M. Bottos · J. W. Scarrow · S. D. J. Archer · I. R. McDonald · S. C. Cary (✉)
The University of Waikato, Hamilton, New Zealand
e-mail: caryc@waikato.ac.nz

D. A. Cowan (ed.), *Antarctic Terrestrial Microbiology*,
DOI: 10.1007/978-3-642-45213-0_2, © Springer-Verlag Berlin Heidelberg 2014

from the Antarctic Ice Sheet, but also include isolated nunataks and mountain peaks that protrude through the extensive ice cover of the Antarctic Plateau. Soils exist in these ice-free regions at various stages of development (Vincent 1988), varying greatly in age and physicochemistry and influenced uniquely by local climate, geography, and biology (Claridge and Campbell 1985). Though exposed soils make up only a small proportion of the continent, they are important terrestrial oases that support unique edaphic (soil associated) communities.

The harsh environmental conditions of continental Antarctica have shaped soil ecosystems of low diversity and simple trophic structure. In most areas of the continent, soil organisms face severe conditions, including low water and nutrient availability, extremely cold temperatures, frequent freeze–thaw cycles, periods of prolonged darkness in winter, and exposure to high levels of ultraviolet radiation in summer (Cary et al. 2010). Conditions across most of the continent are inhospitable to many plant and animal populations that are common in temperate soils. With the exception of the Antarctic Peninsula and surrounding subantarctic islands, vascular plants are absent from Antarctic soils, with vegetation is restricted to cryptogamic species (Bargagli 2008). Mosses and lichens are the only conspicuous vegetation; however, cyanobacteria and, to a lesser extent, algae are typically the dominant phototrophs in Antarctic soils (Vincent 2002). The complete absence of terrestrial vertebrates means heterotrophic organisms are limited to invertebrates, protozoa, fungi, Bacteria, and Archaea. Antarctic soil communities are, therefore, primarily microbial and appear to be structured almost entirely by abiotic factors due, in part, to extremely limited biotic interactions (Hogg et al. 2006).

The bacterial communities of Antarctic soils have been a focus of many studies since the pioneering work of the 1930s (Darling and Siple 1941). Early-cultivation-based studies successfully isolated and described many bacterial strains (Flint and Stout 1960; Johnson et al. 1978); however, these strains probably represent only the small proportion of bacteria that are amenable to culture (Smith et al. 2006). As a result, Antarctic soils were reported to be depauperate in terms of bacterial abundance and diversity (Flint and Stout 1960), with many soils reported to be sterile (Horowitz et al. 1972). The recent application of molecular techniques has greatly improved the resolution of microbial analyses and revealed that bacterial communities are far more abundant (Cowan et al. 2002) and diverse (Tindall 2004; Smith et al. 2006; Barrett et al. 2006b; Niederberger et al. 2008; Cary et al. 2010) than initially understood.

This chapter summarizes our current understanding of bacterial community diversity and how environmental conditions affect these structures, in soils across the continent. It begins with a brief description of soil distributions in Antarctica using the current terrestrial Antarctic Conservation Biogeographic Regions (Terauds et al. 2012), which are adapted to help structure this review. In the subsequent discussion of bacterial communities in Antarctic soils, an effort is made to present the important environmental features of particular regions to preface discussions of bacterial community characteristics. Finally, the importance of exogenous inputs to bacterial community structures and the temporal responses of bacterial communities to change are examined.

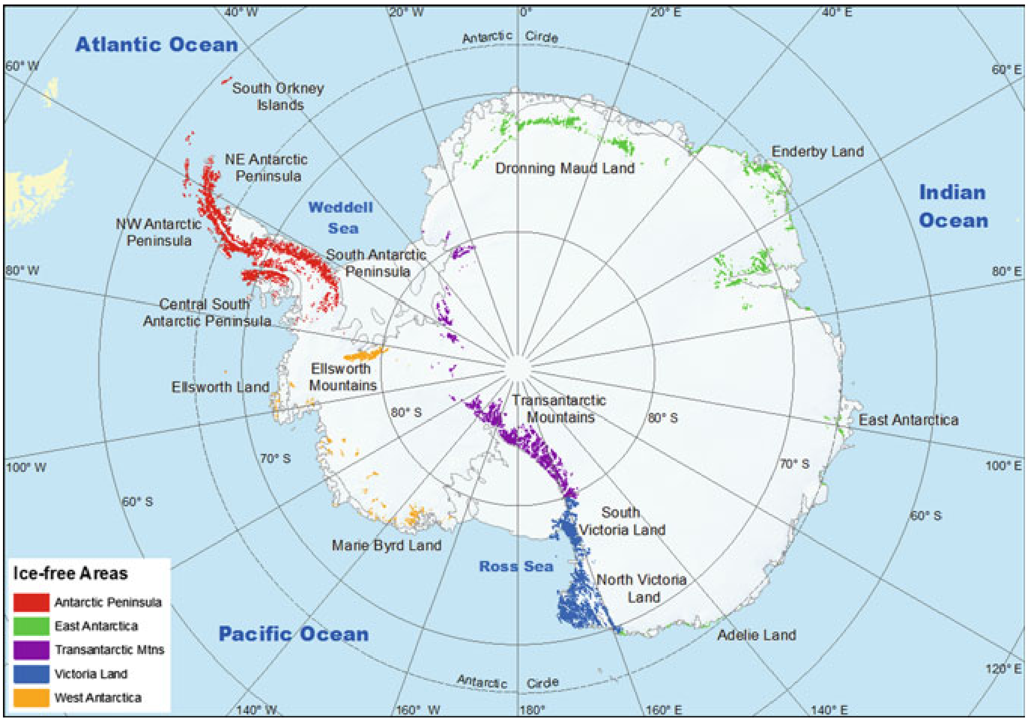


Fig. 2.1 Map of Antarctica indicating the terrestrial Antarctic conservation biogeographic regions (Terauds et al. 2012)

2.2 Continental Distribution of Antarctic Soils

Antarctica's ice-free areas are patchily distributed across the continent. The most recent effort to group these terrestrial areas into manageable conservation bioregions has seen the continent divided into fifteen geographically distinct regions, based on expert opinion and available environmental and biological information (Terauds et al. 2012). As many of these bioregions have not been well characterized microbiologically, it was necessary to adapt the classification system to provide an appropriate means of structuring this review; as a result, the fifteen bioregions have been consolidated here into five regions (Fig. 2.1). Here, the Antarctic Peninsula refers to five biogeographic regions: the South Orkney Islands, North-east Antarctic Peninsula, North-west Antarctic Peninsula, Central South Antarctic Peninsula, and South Antarctic Peninsula. West Antarctica refers to the three biogeographic regions that lie South of the Antarctic Peninsula and West of the Transantarctic Mountains: The Ellsworth Mountains, Ellsworth Land, and Marie Byrd Land. The designation Victoria Land refers to the bioregions South Victoria Land and North Victoria Land. East Antarctica refers to the four biogeographic regions that lie East of the Transantarctic Mountains: Dronning Maud Land, Enderby Land, East Antarctica, and Adelie Land. Finally, the Transantarctic Mountains bioregion is addressed here as its own region.

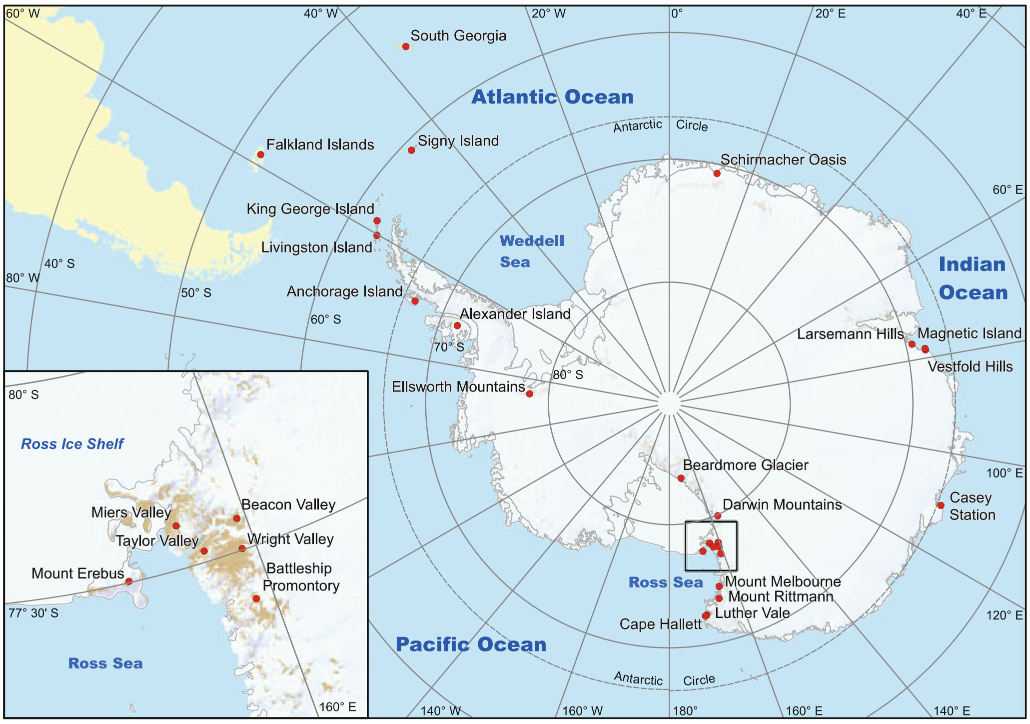


Fig. 2.2 Map of Antarctica indicating ice-free areas referenced in this review

Soils between regions can be quite heterogeneous due to variable influences on soil development. Globally, soil development is dependent on five major factors: time, climate, topography, parent materials, and resident organisms (Jenny 1941). Soil properties pertinent to soil biota such as conductivity, pH, nutrient availability, and carbon content are the result of the particular age and development of a soil from bedrock or an emplaced deposit. Antarctic soils are generally coarse-grained sands due to the dominance of physical weathering processes over chemical processes; thus, clays are a minor component of most soils, and nutrient retention and availability are minimal (Campbell and Claridge 1987).

The largest expanses of ice-free land are found in Victoria Land, the Transantarctic Mountains, and the Antarctic Peninsula, while the areas in East and West Antarctica are substantially smaller. To date, microbiological analyses have been most extensively undertaken on the Peninsula and in Southern Victoria Land, while descriptions of bacterial communities in several ice-free regions of the continent are limited (Fig. 2.2).

2.3 Bacterial Communities of Antarctic Soils

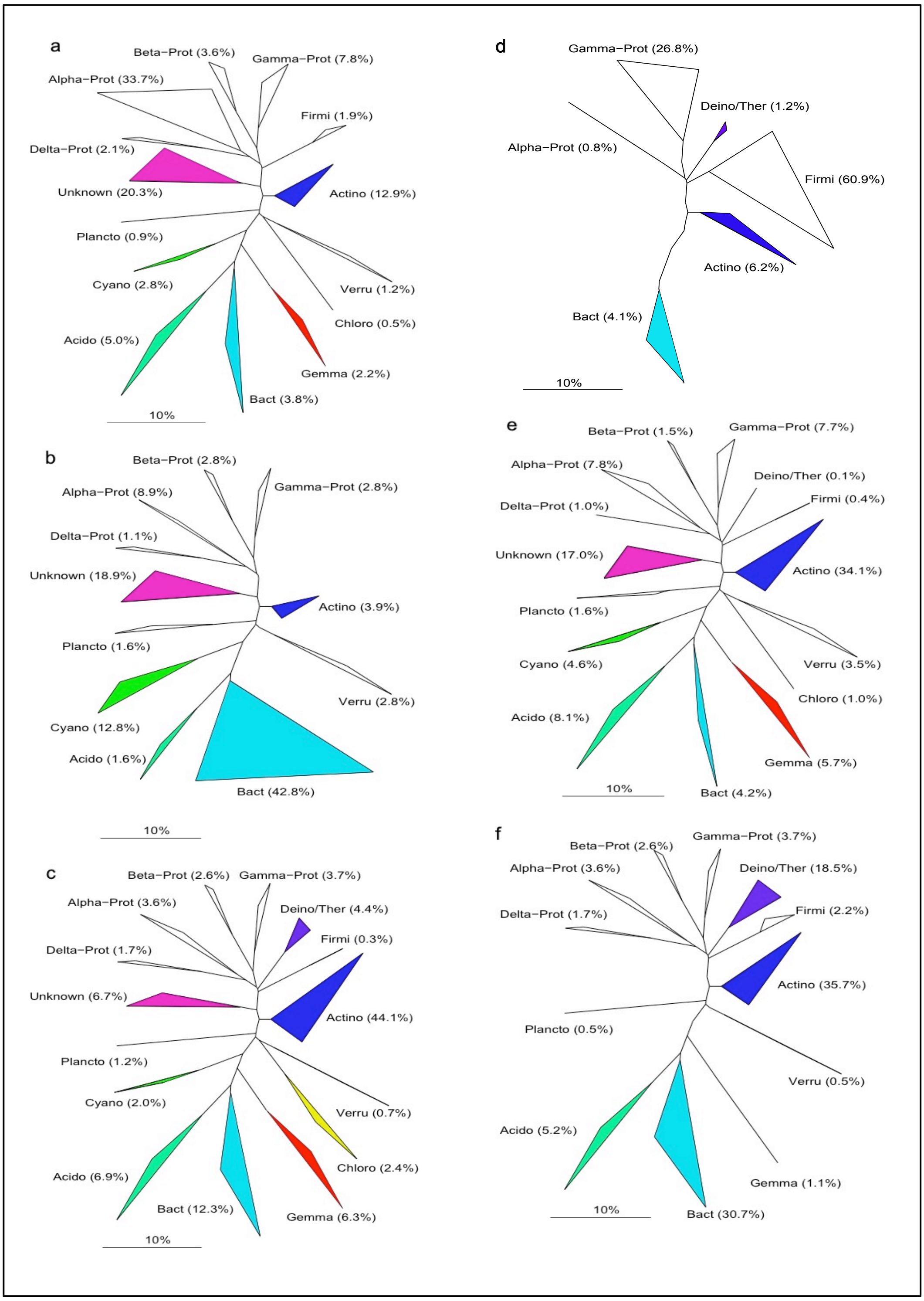
Bacterial community structures of Antarctic soils are highly heterogeneous, though some community characteristics are maintained across many Antarctic soil environments. Several bacterial phyla are frequently observed, including

Actinobacteria, *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Gemmatimonadetes*, *Deinococcus-Thermus*, and *Cyanobacteria*, though relative abundances of these groups differ between soils of different regions of the continent (Fig. 2.3). A high proportion of phylotypes observed in culture-independent analyses of Antarctic soils is from unknown or unclassified bacteria, which may reflect that they are unique to Antarctic soils or that similar environments globally have not been well characterized microbiologically (Vincent 2000). Many studies also report dominant phylotypes (Saul et al. 2005; Aislabie et al. 2006b, 2009), in contrast with more even phylotype representation characteristic of temperate soils, which may reflect the harshness of the Antarctic soil environment. That bacterial communities of Antarctic soils can be considered similar by only a few broad features is, perhaps, not surprising, considering the steep environmental gradients that exist both between and within, regions of the continent. Indeed, bacterial communities across Antarctic soils have been found to vary significantly with geography, climate, soil physicochemical parameters, and local biological influences.

2.3.1 Antarctic Peninsula

Soils of the Antarctic Peninsula have developed under the most amenable climatic conditions on the continent. With mean annual temperatures ranging from 0 to −12 °C and mean annual precipitation of 200–1,000 mm water equivalent (Balks et al. 2013), these ‘coastal oases’ are exceedingly warm and wet in an Antarctic context. The biology of the Peninsula is also significantly different from that of the interior of the continent, as few invertebrate and plant species found here are shared with other regions of Antarctica (Chown and Convey 2007). The greater availability of water in coastal soils allows significant organic matter buildup, with nutrients generally not limiting plant growth (Balks et al. 2013). Some soils contain up to 10 % carbon in subsurface ‘humic horizons’ as a result of leaching and concentration of organic carbon from overlying biological production (Balks et al. 2013). This is even more advanced in the Peninsular region, where extensive moss peat development is a relatively common feature, with soils formed on peat often being strongly acidic (Balks et al. 2013). The facilitation of more chemical and biological processes allows soil development to progress at a greater rate relative to other areas in Antarctica (though soil development is still weak in a global context) and allows the accumulation, recycling and retention of nutrients to occur. Acid-sulfate soils on King George (Simas et al. 2008) and Seymour Islands (Balks et al. 2013) result from acid production from the oxidation of sulfides and represent an extreme soil habitat as a result of moisture-facilitated chemical weathering.

Bacterial communities have been examined along the entire latitudinal range of the Antarctic Peninsula, allowing trends in diversity and composition to be examined. Bacterial diversity and evenness have been found to decrease with increasing latitude along the Peninsula in unvegetated soils (Yergeau et al. 2007b).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Chloro (1.0%)
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Bact (30.7%)

Fig. 2.3 Phylum level diversity of bacterial 16S rRNA gene sequences from Antarctica. **a** Antarctic Peninsula soils, **b** West Antarctic soils, **c** Victoria Land soils, **d** Ornithogenic soils, **e** East Antarctic soils and **f** Transantarctic Mountain soils. Peninsula soil sequences (1216) are from studies of Signy Island, Anchorage Island, Mars Oasis, Fossil Bluff and Coal Nunatak fellfield and vegetated soils (Yergeau et al. 2007b); West Antarctic soil sequences (180) are from a study in the Ellsworth Mountains (Yergeau et al. 2007b); Victoria Land soil sequences, a total of 426 clone sequences and 25976 pyrosequencing reads, were included from the McMurdo Dry Valleys, from studies in the Miers Valley (Lee et al. 2012; Tiao et al. 2012), Beacon Valley, Wright Valley, Battleship Promontory (Lee et al. 2012), Luther Vale (Niederberger et al. 2008), and Bull Pass and Vanda in the Wright Valley (Aislabie et al. 2006b); ornithogenic soil sequences (514) are from Cape Hallett and Cape Bird in the Ross Sea region (Aislabie et al. 2009); East Antarctic soil sequences (1396) were included from the Larsmann Hills (Bajerski and Wagner 2013) and Schirmacher Oasis (Shivaji et al. 2004); and the Transantarctic Mountain soil sequences (361) were from the Darwin Mountains (Aislabie et al. 2013). The trees were constructed using ARB (Ludwig et al. 2004), with DNADIST and Neighbor joining analysis, and the percentage of sequences in each phyla is shown (*in brackets*). Abbreviations for labels are: *Acido* Acidobacteria; *Actino* Actinobacteria; *Bact* Bacteriodetes; *Chloro* Chloroflexi; *Cyano* Cyanobacteria; *Deino/Ther* Deinococcus/Thermus; *Firmi* Firmicutes; *Gemma* Gemmatimonadetes; *Plancto* Planctomycetes; *Prot* Proteobacteria; *Verru* Verrucomicrobia

This geographical trend explained a greater degree of variation than soil characteristics, suggesting increasingly harsh and unstable climatic conditions are responsible for the observed relationships (Yergeau et al. 2007b). Similar trends with latitude were not observed in vegetated soils, suggesting that effects of climatic stress on bacterial communities are mitigated by the stable temperature, water availability, and nutrient availability conferred by vegetation (Yergeau et al. 2007b). Bacterial abundances may be similarly influenced, as bacterial numbers were found to be enriched in vegetated soils compared with unvegetated sites (Yergeau et al. 2007a).

Across much of the Peninsula, soils are dominated by similar phyla, though several trends with geography and physicochemistry have been reported. The phyla *Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Proteobacteria,* and *Verrucomicrobia* are frequently observed, and *Proteobacteria* makes up a substantial proportion of soil sequences in the region, with frequent representation of the *Alpha, Beta, Delta,* and *Gamma Proteobacteria* classes (Yergeau et al. 2007b). Microarray analyses have indicated that *Actinobacteria*, *Epsilonproteobacteria*, and *Verrucomicrobia* decreased in relative abundance with increasing latitude, whereas *Cyanobacteria* representation increased (Yergeau et al. 2009) findings that largely corroborated earlier clone-library analyses (Yergeau et al. 2007b). Additionally, clone-library analyses have revealed a significant decrease in *Acidobacteria* representation with increasing latitude (Yergeau et al. 2007b). *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, and *Alphaproteobacteria* were found to be more abundant relative to other taxa in soils of Fossil Bluff (71°19′S) and Coal Nunatak (72°03′) on Alexander Island than at sites on the Falkland Islands, Signy Island, and Anchorage Island (51°76′S–67°34′S) (Yergeau et al. 2009). Several phyla were significantly influenced by soil physicochemical factors, as the proportion of *Chloroflexi* and *Betaproteobacteria* were negatively correlated with pH, while the proportion of *Firmicutes* and *Verrucomicrobia* were positively correlated with pH (Yergeau et al. 2009). *Chloroflexi* and *Planctomycetes* were positively associated with soil water, organic matter content, and soil nitrogen, and the proportion of *Betaproteobacteria* was positively correlated with soil nitrogen and nitrate (Yergeau et al. 2009).

Soils of Mars Oasis on Alexander Island have been found to be outliers in latitudinal studies of the Peninsula, with higher than expected diversity (Yergeau et al. 2007b). A comparison of soil communities of Mars Oasis, Ares Oasis, and Viking Valley on Alexander Island also found Mars Oasis to have the highest diversity of the three sites (Chong et al. 2011). Soils across the three sites were dominated by *Bacteroidetes*, *Actinobacteria*, and *Acidobacteria*; *Cyanobacteria* were also abundant at Mars Oasis, but below detection limits at the other two locations (Chong et al. 2011). Despite relatively similar representation at the phylum level, few phylotypes were shared between the different soil sites, and variation in community composition was found to be most strongly associated with soil pH and copper content (Chong et al. 2011). Within Mars Oasis, no significant variation in bacterial diversity or community structure was observed in a comparison between two different soils varying in water, carbon, nitrogen, and phosphorous content, suggesting that these parameters have little influence on microbial community structures at this location (Newsham et al. 2010). Newsham et al. (2010) report similar phyla level distributions in soils from Mars Oasis to those found by Chong et al. (2011), though representation of *Proteobacteria* were substantially higher in the former study.

Vegetation has a pronounced influence on bacterial community characteristics in the Antarctic Peninsula. On Livingston Island, *Bacteroidetes* were found to be enriched in moss covered soils compared with the surrounding unvegetated soils, which may be related directly to the plant cover or to the reduced soil pH (Ganzert et al. 2011). On King George Island, rhizosphere soils of the flowering plants *Deschampsia antarctica* and *Colobanthus quitensis* have been found to support similar bacterial communities, and these communities are very different to those reported in unvegetated soils in the region (Teixeira et al. 2010). Rhizosphere soils were dominated by representatives of the phyla *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. The high representation of *Firmicutes* in rhizosphere soils is notable as this group is not found to make up a substantial proportion of the bacterial communities in unvegetated soils of the region (Yergeau et al. 2007b; Teixeira et al. 2010). A high proportion of *Firmicutes* was found to group to the *Clostridia*, suggesting bacterial community structure may be influenced by anaerobic conditions of the rhizosphere.

2.3.2 West Antarctica

Ice-free areas make up a small proportion of West Antarctica, at coastal locations in Ellsworth Land and Marie Byrd Land, and at inland nunataks and mountain ranges that rise above the 500–1500-m-thick ice of the West Antarctic Ice Sheet (Convey and McInnes 2005). Mean annual temperatures range from −12 °C in the vicinity of Russkaya Station in Marie Byrd Land (Abakumov 2010) to approximately −30 °C in the southern Ellsworth Mountains (Dahe et al. 1994). At high altitude sites of West Antarctica, soil is rare and poorly developed (Denton et al. 1992; Convey and McInnes 2005).

Few microbial analyses have been undertaken in the soils of West Antarctica, with the exception of clone-library analyses from soils of the Ellsworth Mountains completed by Yergeau et al. (2007a, b) as part of their latitudinal study of the Antarctic Peninsula. Bacterial community structures were highly dissimilar to those found along the Antarctic Peninsula. Communities were dominated by *Bacteroidetes* of the order *Sphingobacteriales*, which, at 42 % of the sequences observed, more than double the proportion of *Bacteroidetes* observed at any other site in the study (Yergeau et al. 2007b). Additionally, *Cyanobacteria* made up nearly 14 % of sequences in the Ellsworth Mountain clone libraries, which was similar to levels observed in soils of Mars Oasis but substantially higher than all other sample sites along the Peninsula (Yergeau et al. 2007b). Analysis of this data in a continent-wide study of bacterial distributions found the communities of the Ellsworth Mountains were more similar to those on Alexander Island than to those in Victoria Land, despite being located at the similar latitude to the soils of Victoria Land (Chong et al. 2012).

2.3.3 Victoria Land

A diverse range of soil types is observed across Victoria Land. Covering a latitudinal gradient of approximately 8° and positioned between the polar plateau and the coast, soils in Victoria Land are exposed to a wide spectrum of climatic variation, including variable temperature and precipitation regimes (Barrett et al. 2006b). Desert ecosystems dominate the landscape of Southern Victoria Land and the high altitude areas of Northern Victoria Land, while low-elevation coastal soils of Northern Victoria Land see considerable marine and biological influence (Barrett et al. 2006b). This region also contains geothermal soils on Mount Erebus in Southern Victoria Land and Mount Melbourne and Mount Rittman in Northern Victoria Land.

2.3.4 Desert Soils

The McMurdo Dry Valleys of Southern Victoria Land comprise the largest ice-free area on the continent, with approximately 4,500 km^2 of ice-free area (Levy 2013). The mean annual air temperatures ranges from −20 to −35 °C, and mean precipitation ranges from less than 10 to 100 mm (Bockheim and McLeod 2008).

Strong and dry katabatic winds facilitate increased evaporation and sublimation, thus contributing to the aridity of the environment. Considerable salt accumulation in soils high up the valley walls indicates extremely low moisture availability and negligible leaching over long periods of time (Campbell and Claridge 1987). Soil pH is generally alkaline, and carbon and nitrogen contents are typically low except in wetted areas and regions receiving contemporary or legacy organic matter subsidies from nearby high-productivity sites (Elberling et al. 2006; Barrett et al. 2006b).

In the Dry Valleys, any soils of considerable moisture content are patchily distributed and generally concentrated around existing water bodies with ephemeral melt conditions. Soils at the margins of lakes, glacial streams, and ponds are reliant on the short period of the austral summer when liquid water can be sustained (Stanish et al. 2012). Ponds are the most common inland bodies of water and are scattered at all elevations around the Dry Valley system; they are usually small and typically undergo complete freeze/thaw cycles each year relying on snow and ice melt for replenishment (Vincent and James 1996). Lakes are larger inland bodies of water typically with permanent ice cover and a large reservoir of liquid water year round (Howard-Williams and Hawes 2007), and are usually replenished during summer by glacial and snow pack melt water streams (Vincent and James 1996). These streams vary greatly in size and occur with intermittent flow for a few weeks during the summer months (Howard-Williams and Hawes 2007). Within and along the margins of these aquatic systems are microbial (cyanobacterial) mats, which are thought to contribute the most significant primary productivity to the Dry Valleys (de la Torre et al. 2003; Aislabie et al. 2006b).

A large amount of biomass is created by primary production each year within the aquatic systems of the Dry Valleys. This biomass accumulates at the edges of these water bodies where it is freeze-dried and can be blown throughout the valley distributing organisms and providing carbon and nitrogen subsidies to areas of low productivity (Parker et al. 1982; Elberling and Brandt 2003; Moorhead et al. 2003; Nkem et al. 2006; Hopkins et al. 2006a; Barrett et al. 2006b; Wood et al. 2008). The size of these aquatic systems, along with wind intensity and direction, influence the relative contribution of these sources to valley-wide nutrient cycling and productivity (Hopkins et al. 2006a, b). While there is evidence of increased organic carbon with increased proximity to lakes (Elberling and Brandt 2003), ponds (Moorhead et al. 2003), and streams (Aislabie et al. 2006b), similar bacterial and metazoan taxa across geographic latitudes indicate this mat material is also widely dispersed (Barrett et al. 2006a). Stable isotope signatures indicate that carbon and nitrogen in contemporary lake sediments provide the bulk of organic matter to the valleys especially for the contiguous low-elevation areas (Barrett et al. 2006b). Additionally, ‘legacy carbon’ from ancient lake mats may be an important source of organic matter to soils lacking contemporary primary productivity (Burkins et al. 2000, 2001; Hopkins et al. 2006a).

Other conspicuous sources of organic matter in the Dry Valleys include lithic communities, and mummified seal, and penguin carcasses; however, these sources appear to have more localized influences on soils than microbial mats. Lithic communities in McKelvey Valley (Pointing et al. 2009) and the Miers, Marshall, and Garwood Valleys (Khan et al. 2011) were found to be distinct from surrounding soil communities, suggesting substantial dissemination of biomass to the local environment is restricted. Similarly, while the presence of an animal carcass at a site may drastically alter the physicochemical parameters and bacterial community composition of the soil, the effects have been found to be restricted to the immediate vicinity of the augmentation (Tiao et al. 2012).

Bacterial community structures in the Dry Valleys vary considerably from those observed in other regions of the continent (Cary et al. 2010). At the phylum level, there is a reduction in the relative abundance of *Proteobacteria* and an increase in the relative abundance of *Actinobacteria* and *Bacteroidetes* in Dry Valley soils compared with those on the Peninsula (Cary et al. 2010). The frequent representation of *Deinococcus-Thermus* and *Gemmatimonadetes* in Dry Valley soils also distinguishes these soils from those with more temperate influences (Cary et al. 2010). These variations are apparent even at the course scale of phyla representation, indicating the uniqueness of the bacterial communities in this region.

Within the Dry Valleys, bacterial communities may be highly localized. A comparison of bacterial community structures in soils from similar areas in four valleys (Beacon Valley, Upper Wright Valley, Battleship Promontory, and Miers Valley) revealed variable levels of diversity between valleys, and despite very similar representation of taxa at the phylum level, only 2 of 214 species-level phylotypes observed in the study were shared between all four valleys (Lee et al. 2012). This variability in community composition was best explained by variation in salt content, altitude, and copper content of the soils from the four valleys. These findings suggest bacterial populations may be highly regionalized and challenge assumptions that Antarctic soils may be dominated by a small number of cosmopolitan species (Vishniac 1993).

Much of the variation in bacterial community structures in desert soils of Victoria Land may be linked to water availability. In a survey of soils in Luther Vale, Northern Victoria Land, Niederberger et al. (2008) noted the presence of representatives of the *Deinococcus-Thermus* and *Bacteroidetes* in dry (<4 % soil moisture) low-productivity soils but not in high-productivity soils of higher moisture content (9 % soil moisture). Conversely, *Cyanobacteria*, *Verrucomicrobia*, *Betaproteobacteria*, and *Gammaproteobacteria* of the genus *Xanthomonas* were found only in the high moisture content soils (Niederberger et al. 2008). In a survey of soils from the Wright Valley in Southern Victoria Land, *Deinococcus-Thermus* and *Actinobacteria* of the genus *Rubrobacter* were also found to be most prevalent in drier soils (Aislabie et al. 2006b). Water content was also found to have an important influence on community composition of stream sediments along the Onyx River of Wright Valley, with *Bacteroidetes* found to be more abundant in wet sediments and *Acidobacteria* more abundant in dry sediments (Zeglin et al. 2011).

In contrast to the findings in Northern Victoria Land (Niederberger et al. 2008), the distribution of *Cyanobacteria* in the Dry Valleys was not found to be related to soil water content (Wood et al. 2008). Community fingerprinting and sequencing were completed using *Cyanobacteria*-specific PCR primers to analyze distributions in Beacon Valley and Miers Valley soils. Cyanobacterial signatures were below detection or limited to a single phylotype in samples analyzed from Beacon Valley, despite having higher average soil water content than samples collected from Miers Valley, where several phylotypes, grouping to the orders *Chroococcales*, *Nostocales*, and *Oscillatoriales*, were observed (Wood et al. 2008). Variation in cyanobacterial community structures was best explained by differences in elemental composition of soils (Wood et al. 2008).

The similarity of cyanobacterial phylotypes in Dry Valley soils to local lake and hydroterrestrial cyanobacterial mat phylotypes supports suggestions that soils are seeded directly through wind dispersal of mat communities (Aislabie et al. 2006b; Wood et al. 2008). In particular, *Leptolyngbya* spp., which are dominant in microbial mats across the Dry Valleys (Adams et al. 2006), are commonly detected in the surrounding soils (Aislabie et al. 2006b, 2008). Mat samples have been shown to be capable of remaining dormant for many years and returning to activity in the presence of water (Vincent and Howard-Williams 1986), suggesting that windblown mat material not only provides important nutrients to the surrounding soils, but also disseminates potentially active bacterial species throughout the valley floor. The absence of hydrological features (ponds and lakes) in the Beacon Valley compared to Miers Valley was considered an important factor in explaining the differences in cyanobacterial compositions between the two valleys (Wood et al. 2008). Heterotrophic bacteria in microbial mats, which include representatives of the phyla *Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes*, and *Deinococcus-Thermus* (Brambilla et al. 2001; Van Trappen et al. 2002; Rojas et al. 2009; Peeters et al. 2011), may be similarly redistributed by aeolian processes in Antarctic ecosystems. Indeed, proximity to hydrological features was found to be more important than soil moisture for describing variation in both bacterial and cyanobacterial community structures in Miers Valley soils (Niederberger et al. 2012).

Bacterial community structure and biomass have not been found to vary significantly with variation in metazoan communities in Victoria Land soils (Barrett et al. 2006a). Bacterial community diversity and structure were found to be unrelated to nematode abundance, suggesting there is little top–down control on bacterial communities by these predators (Barrett et al. 2006a). These findings support suggestions that biotic interactions have little influence in shaping Dry Valley soil communities (Hogg et al. 2006), though more detailed studies need to be undertaken to assess the importance of biotic influences.

2.3.5 Coastal and Ornithogenic Soils

Low-elevation sites at the northern limit of Victoria Land, such as Edmonson Point (74°S) and Cape Hallett (72°S), can have considerably more developed soils than those found in the Dry Valleys (Hofstee et al. 2006a; Barrett et al. 2006b). Cape Hallett receives annual precipitation of 183 mm of water equivalent per year and has a mean annual temperature of −15.3 °C (Barrett et al. 2006b). Moisture is high in comparison with other Antarctic soils and can reach saturation during summer (Hofstee et al. 2006b). These northern soils support extensive moss beds, which, together with inputs from sea-bird guano and marine salts, maintain soils of high organic carbon and nitrogen content in relation to soils in Southern Victoria Land (Barrett et al. 2006b).

The presence of large populations of penguins at Cape Hallett, and other coastal areas around the continent, has a pronounced effect on soils. Stone-nest building impacts soil structure, while inputs from penguin guano, feathers, eggs, and corpses result in high organic matter content (Aislabie et al. 2009). Uric acid, derived from penguin guano, is the dominant organic matter, comprising approximately 8 % of soil dry weight (Staley and Henvig 1993). The impacts are so profound that the penguin-impacted soils are classified as ornithogenic soils (Hofstee et al. 2006a; Balks et al. 2013), distinct from all other soil types on the continent.

Bacteria in ornithogenic soils face markedly different conditions to those in surrounding mineral soils. Ornithogenic soils of the Ross Sea region contain similar levels of bacterial diversity to mineral soils in the same region, but differ in abundance and community composition (Aislabie et al. 2009). Culture-based methods estimate bacterial abundances at 10^5–10^7 cells/g of dry soil (Ramsay and Stannard 1986), while direct microscopic counts suggest concentrations are closer to 10^{10} cells/g of dry soil (Ramsay and Stannard 1986; Aislabie et al. 2009). *Firmicutes* and *Gammaproteobacteria* of the genus *Psychrobacter* were found to dominate soils colonized by penguins, whereas *Actinobacteria* and *Gammaproteobacteria* of the family *Xanthomonadaceae* were found to dominate soils that had been previously colonized (Aislabie et al. 2009). These differences were attributed to variation in nutrient and salt content of the soil sites, which were higher in colonized soils than in previously colonized soils. The importance of *Psychrobacter* species in ornithogenic soils is supported by culture-based studies of soils from penguin colonies of Magnetic Island in East Antarctica, in which several *Psychrobacter* isolates were found to be halotolerant and capable of utilizing uric acid, or its metabolite allantoin, as their sole carbon and energy source (Bowman et al. 1996). The phylogenetic affiliations of several phylotypes grouping within the *Firmicutes* suggest similar abilities to withstand high salinities and utilize uric acid under anaerobic conditions, which together with the ability of *Firmicutes* to form endospores to withstand environmental stress, would explain their ability to inhabit ornithogenic soils (Aislabie et al. 2009). The dominance of *Actinobacteria* and *Xanthomonas* in previously colonized soils may be the result of a shift in bacterial community structure in response to changing environmental conditions that accompany guano decomposition (Speir and Ross 1984; Zdanowski et al. 2004, 2005) and leaching of ammonium stocks from soils (Aislabie et al. 2009).

2.3.6 Geothermal Soils

Geothermal soils exist on the active volcanoes of Victoria Land: Mount Erebus (3,794 m), Mount Melbourne (2,733 m), and Mount Rittman (2,600 m) (Bargagli et al. 1996). Soil temperatures reach 65 °C near fumarolic vents, and soil moisture is sustained surrounding these sites through snow melt and condensation of steam (Bargagli et al. 1996). Steep physicochemical gradients of temperature and pH exist surrounding fumaroles, as demonstrated on Mount Erebus, where soil temperatures were found to decrease from 65 to 3 °C, and pH decrease from approximately pH 7–pH 4, over a distance of 55 cm from fumaroles (Soo et al. 2009).

The geothermal soils of Antarctica's volcanic sites provide the sole oases for thermophilic bacterial communities on the continent. Soils of Mt. Erebus have been shown to support comparable bacterial abundances to geothermal sites in the rest of the world (Hudson and Daniel 1988). Several thermophilic *Firmicutes* (Hudson and Daniel 1988; Logan et al. 2000; Imperio et al. 2008) and *Cyanobacteria* (Melick et al. 1991; Bargagli et al. 1996) have been isolated from Mount Erebus, Mount Melbourne, and Mount Rittman. Much greater diversity, however, was observed in culture-independent analyses of Mount Erebus, which reported representation of the phyla *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *Cyanobacteria, Deinococcus-Thermus*, OP10, and a large number of unclassified bacteria; notably, *Proteobacteria* were not detected in these analyses (Soo et al. 2009). Total bacterial and cyanobacterial community structures were found to be primarily driven by soil temperature and pH (Soo et al. 2009). Geothermal soils are discussed in more detail in this volume in the chapter entitled 'Microbial ecology of geothermal habitats in Antarctica'.

2.3.7 East Antarctica

With the notable exception of the Prince Charles Mountains, the ice-free areas of East Antarctica are generally restricted to coastal locations, with the Polar Plateau often sloping right to the coast. The East Antarctic coastline occupies lower latitudes (66–72°S) than the majority of West Antarctica and Victoria Land and lies in a more similar climate band to portions of the Antarctic Peninsula (Balks et al. 2013). This region is considerably affected by marine influences: Soils can be in the order of four times wetter than the Dry Valley equivalents, thus leaching of nutrients and salts is common. Soil pH can be as low as 4 in some areas, ranging through to mildly alkaline (Balks et al. 2013). Landscapes in the East Antarctic oases contain a patchwork of dry soils and 'bogs', arising from the patchy distribution of snow accumulation and subsequent melt (Goryachkin et al. 2004). Moss and lichen cover is considerable in places (Balks et al. 2013) although not in the order of peat-generating beds of the Peninsula.

Several studies have described bacterial communities in soils surrounding the stations operated by various international programmes in the region. Bacterial diversity in a soil from Maitri Station, near Lake Zub, in Schirmacher Oasis has been examined using both culture-dependent and clone-library analyses (Shivaji et al. 2004). Representatives of the phyla *Gemmatimonas*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Chlamydiae*, and *Protebacteria* representing the classes *Alpha*, *Beta*, and *Gamma* were observed in the clone library, with the greatest proportion of sequences grouping to the *Gammaproteobacteria*. Several species of *Proteobacteria* and *Actinobacteria* were also successfully cultured.

Bacterial communities from a range of habitats around Casey Station were analyzed using denaturing gradient gel electrophoresis (DGGE) as a community fingerprinting technique (Chong et al. 2009). Sites included soils that are heavily impacted by human disturbance, soils with low levels of human disturbance, and specially protected sites both formerly and actively colonized by Adelie Penguins. Despite the range of sites sampled, bacterial diversity and community structure were not found to be significantly related to environmental variation (Chong et al. 2009). Sequencing of bands from DGGE gels revealed that majority of bacterial signatures from across all sample sites were from bacterial grouping to the phylum *Bacteroidetes*.

An analysis of bacterial communities associated with glacier forefields in the Larsemann Hills has been completed using a suite of culture-dependent and molecular techniques (Bajerski and Wagner 2013). The study revealed the dominance of *Actinobacteria, Acidobacteria, Proteobacteria, Bacteroidetes, Cyanobacteria*, and *Chloroflexi* and several trends in phyla level distributions within the glacial forefield sites (Bajerski and Wagner 2013). *Cyanobacteria*, *Deltaproteobacteria*, and *Gemmatimonadetes* were positively associated with soil moisture and pH, while magnesium, calcium, and potassium were found to influence distributions of *Actinobacteria* (Bajerski and Wagner 2013). *Bacteroidetes* were found to be the most abundant in the vicinity of glaciers, which the authors suggest may be related to low temperature and high water availability at these locations (Bajerski and Wagner 2013). This work provides insight into the process of bacterial succession following glacial retreat and establishes relationships between bacterial community structure and physicochemical properties of soils in the region.

2.3.8 Transantarctic Mountains

The Transantarctic Mountains separate the Polar Plateau from the Ross Sea, with ice flow concentrated in major outlets such as the Beardmore and Shackleton Glaciers (see Figs. 2.1, 2.2). At areas where the Transantarctic Mountains exceed altitudes of 1,500–2,000 m, ice-free regions rise above the Polar Plateau. These areas form a discontinuous chain of isolated island-like soil habitats, often separated by expanses of tens to hundreds of kilometers. The high altitude and proximity to the Polar Plateau lead to the most extreme climatic conditions on the continent. A mean annual temperature of −40 °C and a mean annual water accumulation of only 36 mm per year were estimated from snow pit data at the Beardmore Glacier, Central Transantarctic Mountains (Bockheim 1990). Winds ubiquitously originate from the plateau, and the extreme cold and dryness of this air mass facilitates intense sublimation, exacerbating the influence of precipitation deficiency.

The extreme climate at high altitudes severely depresses soil development, resulting in weakly developed soils even over very long timescales. Soil properties are more homogenous across the Transantarctic Mountains relative to other areas of the continent, as the extreme lack of water precludes many soil development pathways, thereby limiting soil diversity (Claridge and Campbell 1968). With chemical weathering impeded by low water availability, ultraxerous soils are almost exclusively the product of physical processes. Any significant chemical alterations likely indicate an extremely long soil development history. The severe moisture deficit results in dry-frozen permafrost throughout the profile, and ice-cemented soil is generally not observed (Claridge and Campbell 1968). Nutrient levels are low, with organic carbon contents ranging from 0.02 to 0.08 % and organic nitrogen typically below quantifiable levels in relation to inorganic nitrogen content (Claridge and Campbell 2004). In the absence of snow melt and subsequent leaching, atmospheric salts deposited in snow can accumulate to very high concentrations over long timescales (Claridge and Campbell 1968). Salts in far-inland soils are dominated by sulfates and nitrates, relative to chlorides, indicating the influence of the polar air mass rather than marine influence (Campbell and Claridge 1987). A higher sulfate-to-chloride ratio generally correlates with a lower pH; as such, soils on the plateau side of the Transantarctic Mountains are generally less alkaline than those closer to the Ross Sea (Campbell and Claridge 1987).

Recently, cultivation-independent analyses of the bacterial communities in soils of the Darwin Mountains have shown bacterial abundance and composition to be influenced most strongly by soil age and physicochemical properties (Aislabie et al. 2012, 2013; Magalhães et al. 2012). Bacterial community fingerprinting analyses of soils in the Darwin Mountains revealed bacterial diversity was greatest in younger, less developed soils, which had lower salinity and higher C/N ratios than older soils (Magalhães et al. 2012). The authors suggest that salt accumulation in older terrains may constrain bacterial diversity. Additionally, cyanobacterial community fingerprints were examined, and signatures were detected in 57 % of sampling sites, with diversity most strongly correlated with soil pH, C/N ratios, and soil salinity. Interestingly, despite the extremely low water availability in this environment, soil moisture was not found to be a strong determinant of total bacterial or cyanobacterial communities.

In analyses of four drifts in the Darwin Mountains, representing a soil chronosequence ranging from early Holocene (10 ka) to mid-Quaternary (900 ka), phospholipid fatty acids and heterotrophic counts showed microbial biomass to be greatest in less developed soils (Aislabie et al. 2012). Bacterial community structures were found to be influenced by soil development, soil water content, organic carbon content, and pH (Aislabie et al. 2012), and soil diversity was found to be highest in the least developed soil (Aislabie et al. 2013). Soils were dominated by bacteria grouping to the phyla *Deinococcus-Thermus*, *Actinobacteria*, and *Bacteroidetes* (Aislabie et al. 2013).

Analyses of bacterial communities at locations south of the Darwin Mountains are extending our understanding of bacterial biogeography to the southernmost soils of the continent. Fingerprinting techniques have recently been applied to characterize bacterial and cyanobacterial distributions in soils of the Beardmore Glacier region in relation to soils in the Dry Valleys (Sokol et al. in press). Distributions of the overall bacterial community were found to be influenced by gradients in pH and soil moisture, while dispersal limitation from aquatic features appeared to have a more important role than environmental gradients in shaping cyanobacterial distributions specifically (Sokol et al. in press). A combination of bacterial community fingerprinting and high-throughput community sequencing has recently been applied to characterize communities in soils from latitudes 83° to 87°S and preliminary findings indicate significant differences in bacterial diversity and community structures between sites across the region (Scarrow, pers comm).

2.4 Exogenous Factors and Inputs

Increased human activity on the continent has the potential to drastically impact bacterial communities, both directly by introducing novel organisms to the environment and indirectly by causing environmental disturbances (Kennicutt et al. 2010). Visitors to Antarctica are exceptional hosts to foreign bacteria and vectors for the transport of organisms to and within the continent (Cowan et al. 2011). A recent study of soils in ice caves on Mount Erebus has indicated potential fungal contamination from human visitation (Connell and Staudigal 2013). Anthropogenic activities in the region also necessitate some level of physical disturbance to soils and increase the threat of chemical contamination to terrestrial and marine ecosystems alike (Kennicutt et al. 2010). Disturbances of terrestrial systems are greatest surrounding permanent settlements; however, even minor levels of activity in sensitive areas of the continent can leave lasting effects on soil communities.

The impacts of the introduction of foreign bacteria to Antarctic soils by human activity remain largely uncharacterized. The harshness of Antarctic soils make them inhospitable to many potential colonizing bacteria (Cowan et al. 2011); however, soils of lower diversity may be more susceptible to colonization by foreign organisms (Van Elsas et al. 2012). While there is evidence of inputs of foreign organisms to Antarctic environments through natural atmospheric processes (Vincent 2000), these exchanges are thought to be restricted by the geographic isolation of the continent and barriers to foreign air masses produced by the Antarctic Circumpolar Current (Pearce et al. 2009). Traces of the human enteric bacteria *Escherichia coli* have been detected in Dry Valley field camps

(Sjoling and Cowan 2000) and the human skin commensal *Staphylococcus epidermis* has been detected through PCR amplification in soils at frequently visited sites (Ah Tow and Cowan 2005). Both *E. coli* (Boyd and Boyd 1963) and *S. epidermidis* strains (Ah Tow and Cowan 2005) have been shown to lose viability quickly in Antarctic soils, suggesting human-associated bacteria have little impact on endemic bacterial communities. However, in light of current climate change predictions, the suggested immunity of the continent to these types of introductions is in question. Given several recent studies showing the local and regional uniqueness of soil microbial communities (Lee et al. 2012; Chong et al. 2012), the transfer of soil bacteria between regions of the continent may be of paramount immediate concern to the biosecurity of the ecosystem as repercussions of such transfers are not yet fully understood (Hughes et al. 2010; Cowan et al. 2011).

Shifts in bacterial communities in response to hydrocarbon contamination have been reported in several Antarctic soils (Aislabie et al. 1998; Whyte et al. 2002; Saul et al. 2005). Soils in the Ross Sea region contaminated with alkanes and polyaromatic hydrocarbons were found to have higher counts of cultureable heterotrophic bacteria and lower bacterial diversity than uncontaminated control soils (Saul et al. 2005). Contaminated soils were dominated by *Proteobacteria* of the genera *Pseudomonas*, *Sphingomonas*, and *Variovorax*, while *Proteobacteria* were found to make up only a minor component of control soils (Saul et al. 2005). These responses are similar to those observed in hydrocarbon contaminated soils of temperate regions (Aislabie et al. 2006a). The breakdown of hydrocarbons in Antarctic soils is extremely slow as biodegradation is largely restricted to the warm summer months (Aislabie et al. 2006a).

Shifts in bacterial community structure may also be induced through physical disturbance of soils. Soils around permanent bases can be intensely disrupted by building and heavy equipment use (Kennicutt et al. 2010), while environmentally sensitive regions can be impacted by foot traffic (Ayres et al. 2008). Trampling has been shown to impact invertebrate species and reduce CO_2 fluxes in Dry Valley soils (Ayres et al. 2008), though specific affects on bacterial communities have not been investigated. Bacterial community structures may be altered by trampling due to disturbance of soil crusts, as has been demonstrated in other cold deserts (Kuske et al. 2012).

2.5 Temporal Variation in Bacterial Communities

Recent evidence suggests that temporal variation in bacterial community structure may occur rapidly in response to environmental change in Antarctic soils. In a soil warming experiment on the Antarctic Peninsula, soil community compositions were found to shift in response to an increase of 2 °C over the course of just 3 years (Yergeau et al. 2012). *Acidobacteria* representation was found to decrease in soils of higher temperature, while *Alphaproteobacteria* representation increased. Additionally, warming resulted in a significant increase in bacterial abundance in vegetated soils. Taxonomic diversity and evenness were not significantly affected by warming; however, functional gene analyses showed a decrease in diversity and redundancy of functional characteristics of soil communities in response to warming.

An experiment in Miers Valley, in which a mummified seal was transplanted to a previously pristine site, showed that bacterial community structure was also altered considerably in just 3 years (Tiao et al. 2012). The augmentation resulted in a significant decrease in bacterial diversity and shifted the community structure from *Actinobacteria* dominated, to *Proteobacteria* and *Firmicutes* dominated. This response was attributed to physical factors, as temperatures were more stable, and relative humidity was both higher and more stable, in soils under the seal carcass than in the surrounding soils.

These studies have important implications for understanding how bacterial community structures may respond to climate change. A continent-wide increase in temperature of 0.12 °C per decade has been observed over the 50 year period from 1957 to 2006 (Steig et al. 2009). Increases in temperature are predicted to continue to drive decreases in snow and ice cover, increases in water and nutrient availability, and increases in vascular plant and bryophyte populations, which have already been reported along the Scotia Arc and Antarctic Peninsula (Convey and Smith 2006). Bacterial communities will likely show rapid and significant responses in structure, activity and diversity to the changes predicted by current climate change scenarios. These may well be the most immediate biological responses to environmental change, making bacterial communities important as early-warning indicators of ecological shifts. What is also clear is that these extreme terrestrial ecosystems harbor unique, locally adapted, microbial communities that may be threatened by climate change. Under the Antarctic Treaty, we have a responsibility to protect this diversity and archive what currently exists for future generations. To this end, New Zealand is pioneering a new Antarctic genetic Archive (AGAr) to capture and preserve Antarctic genetic resources.

2.6 Conclusions

The terrestrial ecosystems of Antarctica are influenced by some of the harshest conditions on Earth, resulting in communities that are structured predominantly by abiotic influences. The application of both culture-dependent and molecular techniques has greatly improved understanding of bacterial communities in Antarctic soils, and the degree to which local environmental factors shape community structure. Bacterial communities have been found to be highly heterogeneous between different regions of the continent and within regions, varying primarily with climate and soil physicochemical conditions. However, the current paucity of data from several ice-free areas, coupled with the varied methodologies employed in studies from different locations, severely limits biogeographic interpretations of available datasets.

Increased understanding of the environmental factors shaping bacterial community structure and function is necessary to inform management of terrestrial Antarctic ecosystems and to predict how these regions may respond to environmental change. Microorganisms may well show the most rapid and significant immediate biological responses to climate change, making them important as early-warning indicators of ecological shifts. What is also clear is that these extreme terrestrial ecosystems harbor highly adapted unique microbial communities that may be threatened by climate change, and under the Antarctic Treaty, we have a responsibility to not only protect this diversity but to archive what currently exists for future generations. An immediate response is required by the research community in order to establish bacterial community structure data from across terrestrial Antarctica, as a means of understanding bacterial biogeography and to provide a baseline from which to measure community responses to climate change. To do this, appropriate universal metrics must be established to measure biological change over various timescales, and a concerted effort must be made to bank the current genetic biodiversity, as a resource to monitor change.

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Chapter 3: Abiotic Factors Influence Patterns of Bacterial Diversity and Community Composition in the Dry Valleys of Antarctica

Preface

This chapter describes the patterns of bacterial diversity and community composition in relation to abiotic factors in soils of the Antarctic Dry Valleys. This work was undertaken as part of the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS). As primary author, I was involved in fieldwork and lab analyses, completed the data analyses, and wrote the manuscript. Daniel Laughlin earned co-authorship by assisting with statistical analyses and testing and revising the *a priori* structural equation model. Craig Herbold and Charlie Lee earned co-authorship by implementing computer script for the analyses of the community fingerprinting data. All authors reviewed and edited manuscript content.

Sample collection and environmental surveys were completed as part of nzTABS, and result from the efforts of the entire nzTABS team. Preparation of the maps presented in Figure 3.2 was completed by Glen Stichbury, GIS analyst at the University of Waikato.

This chapter has been prepared for submission to the *ISME Journal*.

Abiotic factors influence patterns of bacterial diversity and community composition in the Dry Valleys of Antarctica

Running title: Bacterial communities in Antarctic soils

Eric M. Bottos[1,2], Daniel C. Laughlin[1], Craig W. Herbold[1,2], Charles K. Lee[1,2], Ian R. McDonald[1,2], Stephen C. Cary[1,2*]

[1]Department of Biological Sciences, The University of Waikato, Private Bag 3105, Hamilton, New Zealand
[2]The International Centre for Terrestrial Antarctic Research, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

*Corresponding Author

Abstract

Bacteria are fundamentally important to many soil processes, however the factors that govern bacterial distributions in soils remain unclear. The Dry Valleys of Antarctica are an ecosystem of simple trophic structure, where the lack of top-down biotic controls allows abiotic influences on bacterial communities to be readily identified. The goal of this study was to evaluate the degree to which aspects of topographic, physicochemical, and spatial variation explain patterns of bacterial taxon richness and community compositions across the Dry Valleys of Southern Victoria Land. Using automated ribosomal intergenic spacer analysis (ARISA), bacterial communities were analyzed in 471 Dry Valley soil samples collected from across a 220 square kilometer study area. Richness was most strongly influenced by physicochemical soil properties, particularly soil conductivity, though significant trends with several topographic and spatial variables were also observed. Structural equation modeling (SEM) supported a final model in which the variation in community composition was best explained by physicochemical variables, particularly soil water content, and where the effects of topographic variation on community composition were largely mediated through their influence on physicochemical variables. Community dissimilarity increased with distance between samples, and though most of this variation was explained by topographic and physicochemical variation, a small but significant relationship remained after controlling for this environmental variation. As the largest survey of terrestrial bacterial diversity and community composition completed in Antarctica to date, this work provides fundamental knowledge of the Dry Valleys ecosystem, and has implications for global understanding of the environmental factors that influence bacterial distributions.

Introduction

Many fundamental questions in microbial ecology relate to the distribution of free-living microorganisms in the environment. Microorganisms are the most abundant and genetically diverse organisms on the planet, and their activity is vital for many aspects of biogeochemical cycling and ecosystem function (Whitman *et al*., 1998); as such, understanding the factors influencing microbial distributions is paramount to understanding all natural systems. Microbial community compositions are influenced by a combination of environmental filtering and neutral processes, though the relative influence of particular factors can vary greatly between habitats (Stegen *et al*., 2012; Lindström and Langenheder, 2012; Langenheder and Székely, 2011). While understanding of microbial biogeography has been advanced in recent years, the principles that govern microbial distributions remain poorly understood (Nemergut *et al*., 2011).

Soil environments are particularly difficult to characterize microbiologically due to their complexity. In soils, bacteria are influenced by a multitude of abiotic and biotic interactions (Horner-Devine *et al*., 2007), and the nature of these interactions can change rapidly over small spatial and temporal scales (Ettema and Wardle, 2002; Ramette and Tiedje, 2007). Across landscapes, pH has been found to be among the most important abiotic factors influencing bacterial diversity and community structure (Chu *et al*., 2010; Fierer and Jackson, 2006; Griffiths *et al*., 2011; Lauber *et al*., 2009; Rousk *et al*., 2010), though salinity (Lozupone and Knight, 2007), nutrient content (Andrew *et al*., 2012; Cruz-Martínez *et al*., 2012; Fierer and Jackson, 2006), and soil moisture (Cruz-Martínez *et al*., 2012; Fierer and Jackson, 2006) have also been found to significantly influence bacterial communities. The structure and function of many soil bacterial communities are also intimately linked to those of plant and animal communities (Miki *et al*., 2010; Sylvain and Wall, 2011), complicating efforts to characterize the importance of abiotic controls on bacterial distributions. This complexity is reduced in desert ecosystems, where abiotic factors appear to be more important than biotic factors in shaping microbial community compositions (Fierer *et al*., 2012).

Antarctic Dry Valley soils are a simplified and isolated cold desert ecosystem, where biotic interactions are thought to be minimal (Hogg *et al*., 2006) and the environmental factors affecting life can be more easily identified than in temperate ecosystems (Adams *et al.*, 2006). Organisms in these soils face extreme conditions, including exposure to cold temperatures, lack of water and nutrient availability, high salinity, high exposure to ultraviolet radiation in the austral summers, and periods of prolonged darkness during austral winters (Vincent, 1988). This harsh environment has shaped an ecosystem of relatively low biocomplexity in comparison to most terrestrial ecosystems (Adams *et al.*, 2006; Yergeau *et al.*, 2007). The lack of vascular plants means primary production in these soils is limited to mosses, lichens, algae, and cyanobacteria, while heterotrophic prokaryotes, fungi, protozoa, and a small number of invertebrate animals comprise the system's heterotrophs (Adams *et al.*, 2006). Few terrestrial ecosystems on Earth are characterized by such a simple food web. This environment, therefore, offers access to a biological system that is primarily microbial and that appears to be largely structured by abiotic drivers (Hogg *et al.*, 2006), making it an ideal place to resolve the important relationships between bacteria and their environment.

The visible uniformity of the Dry Valley landscape masks a highly heterogeneous system, as current research reveals patchy distributions of macro- and micro- organisms throughout the environment (Cary *et al.*, 2011). Microbial community structures are highly variable, with assemblages reported to vary significantly in samples from different soil types, and in samples of the same soil type but with differing physicochemical properties and geography (Aislabie *et al.*, 2006; Barrett *et al.*, 2006; Lee *et al.*, 2012; Niederberger *et al.*, 2008; Wood *et al.*, 2008). These variations in bacterial communities between sites have not been found to correlate strongly with physicochemical characteristics of soils or with metazoan diversity (Barrett *et al.*, 2006). While these studies have been valuable in demonstrating the heterogeneity of soils in the Dry Valleys, a comprehensive survey, of sufficient geographic scope and sampling effort to elucidate the factors that dictate microbial community structures across the landscape, has not been undertaken.

The goal of the present study is to describe how bacterial diversity and community structure in Dry Valley soils are related to topographic, physicochemical, and spatial variation in the landscape. To this end, we tested the following hypotheses:

1. Bacterial taxon richness varies significantly with environmental gradients, particularly physicochemical variation in soil properties found to be important in other ecosystems;
2. Bacterial community composition varies significantly with topographic and physicochemical gradients in the landscape, with the effects of topographic variation on bacterial community structures mediated through physicochemical variation in soil properties as illustrated in the *a priori* model presented in Figure 3.1;
3. Bacterial community similarity decreases with distance between samples in a distance decay relationship, but this spatial variation in bacterial community composition is explained by topographic and physicochemical heterogeneity in the landscape rather than dispersal limitation.

Using automated ribosomal intergenic spacer analysis (ARISA), we generated bacterial community genetic fingerprints from 471 samples from across a 220 km^2 landscape in the Dry Valleys of Southern Victoria Land to assess bacterial taxon richness and community compositions in relation to environmental variation. Structural equation modeling (SEM) was applied to relate community structure to environmental variation, as this approach is well suited to handle models with indirect relationships among variables (Kline, 2005). SEM has been shown to be highly useful in multivariate analyses in plant ecology (Laughlin and Abella, 2007; Scherber *et al*., 2010), but is seldom applied to similar problems in microbial ecology. Here, this technique is used to test our second hypothesis and adjust our initial *a priori* model. As the largest survey of terrestrial bacterial diversity and community structure completed in Antarctica to date, this work identifies numerous significant effects of environmental variation on bacterial diversity, and direct and indirect effects of abiotic variables on bacterial community structures across space in the Dry Valley landscape.

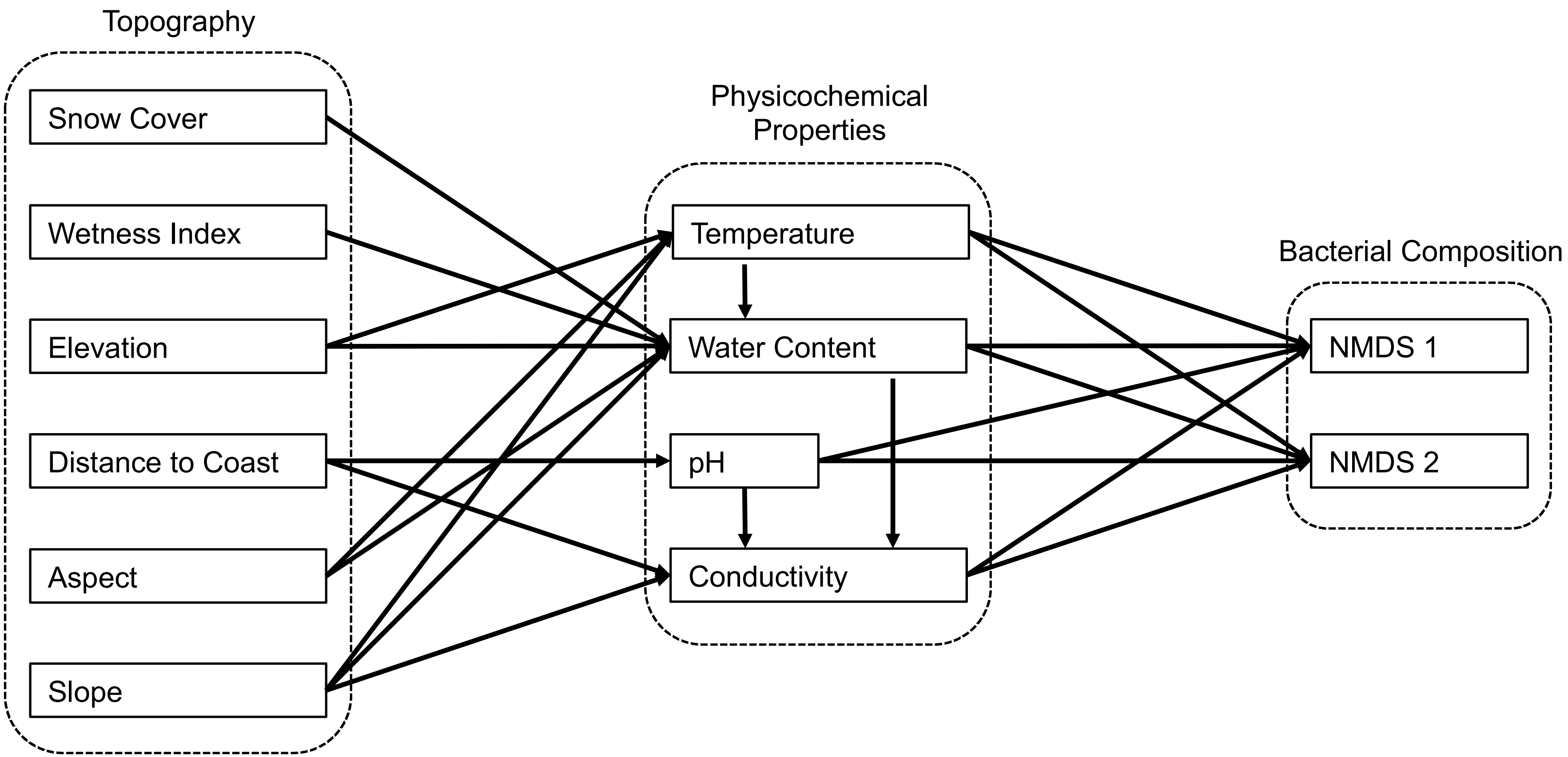

Figure 3.1: Initial *a priori* model of environmental factors that influence bacterial community compositions in Dry Valley soils.

Materials and Methods

Site Selection, Site Surveys, and Sample Collection

A study area of 220 km^2, encompassing the Miers, Marshall and Garwood Valleys in the southern end of the Dry Valleys (Figure 3.2a), was divided using 4 primary attributes obtained by remote sensing into spatially distinct regions, termed ‘tiles’. Each tile was defined by its elevation (200 m increments from sea level to 1400 m), slope (less than or greater than 20 degrees), aspect (North-, South-, East-, or West- facing, or zero), and overriding geology (aeolian, alluvial, gneiss, granite, lacustrine, mafic, marble, moraine type 1 (M1), moraine type 2 (M2), moraine type 3 (M3), schist, scree, or complex). Tile boundaries were drawn where the combination of these environmental attributes changed. Environmental assessments were completed within each tile in November 2008, to confirm the reliability of the remote sensing data and to identify microhabitats in each tile that varied from the general tile attributes (See Appendix 1 for further details on tile delineation). This process ultimately divided the landscape into 545 tiles (Figure 3.2b).

During the 2008-2009 and 2009-2010 austral summers, biological surveying and soil sampling were completed at 471 sites that had been strategically selected within tiles and their microhabitats to capture the environmental heterogeneity of the landscape (Figure 3.2c). Soil samples for microbiological and physicochemical analyses were collected aseptically from the top 10 cm of soil below the desert pavement and stored in sterile Whirl-Packs (Nasco International, Fort Atkinson, WI, USA). At the earliest opportunity, samples for total gravimetric water content determination were transported to McMurdo Station for processing, and samples for microbiological and physicochemical analyses were frozen at -20 °C to be transported to New Zealand for processing.

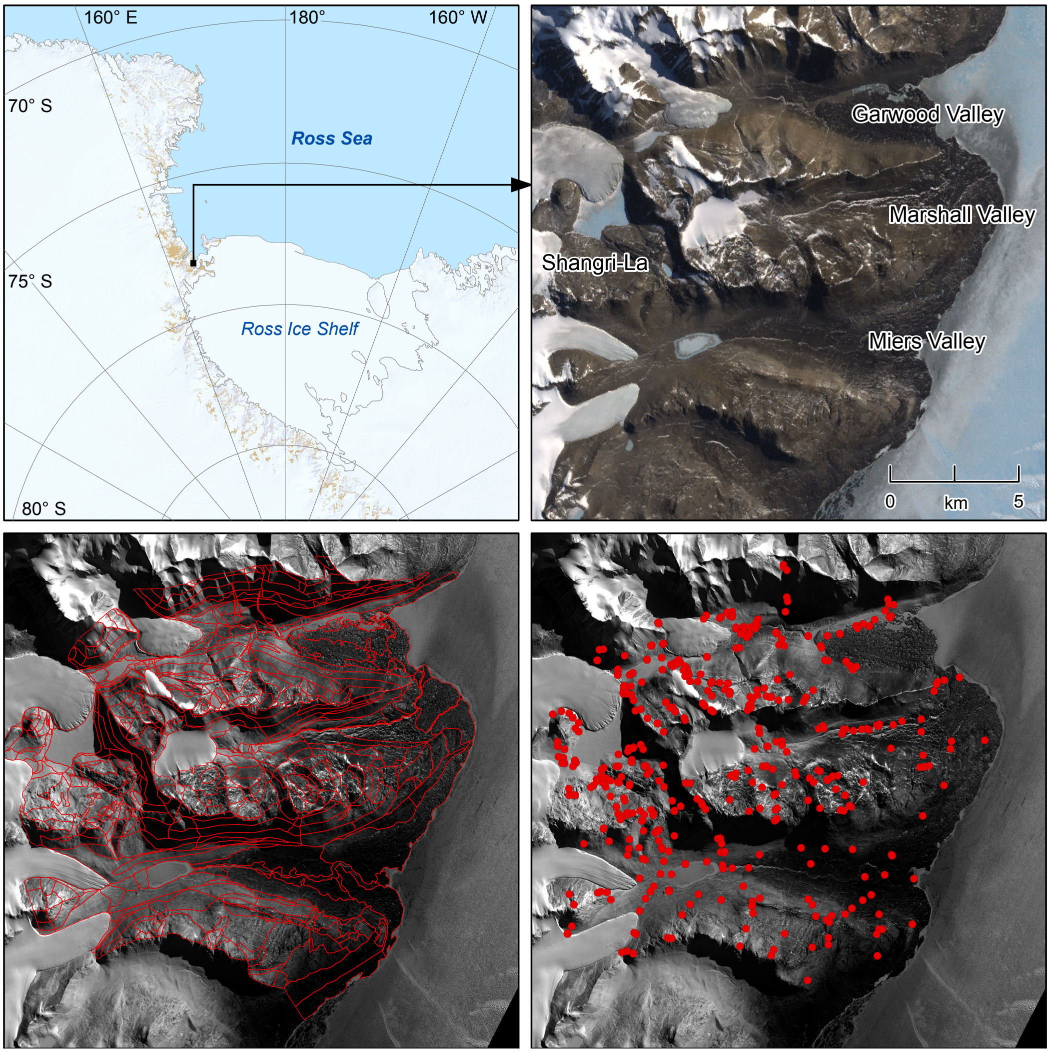

Figure 3.2: A map of the Ross Sea Region of Antarctica (A), indicating the location of the study area (B), tile boundaries (C) and sample sites (D).

Determination and Treatment of Environmental Variables

Six topographic variables, six physicochemical variables, and two spatial variables were examined in relation to bacterial diversity and/or community composition. Topographic variables included elevation (m), aspect (degrees from North), slope (degrees), distance to the coast (m), an index of yearly snow cover, and an index of soil wetness. Elevation, aspect, and slope of sample sites were determined using LIDAR derived digital elevation models (Wilson and Csathó, 2007). Distance to the coast was defined as the Euclidean distance of the sample site to the nearest coastline. The index of yearly snow cover and index of wetness were derived as previously described (Stichbury *et al.*, 2011), and provide relative estimates of snow cover and liquid water availability for sites based on Geographic Information Systems (GIS) and remote sensing data. Physicochemical variables analyzed included soil pH, electrical conductivity (mS/cm), water content (w/w %), carbon content (w/w %), nitrogen content (w/w %), and average summer temperature (°C). Soil pH and electrical conductivity were determined for 2 ml of soil slurried in 10 ml of deionized water (Lee *et al.*, 2012) using a Thermo Scientific Orion 4-Star Plus pH/Conductivity Meter (Thermo Scientific, Auckland, NZ). Water content was determined from the mass loss of soil following incubation at 105 °C for 48 hours (Barrett *et al.*, 2004). Organic carbon and total nitrogen content was determined from 300 mg of dried acidified soil using a CE Elantech Flash EA 1112 Elemental Analyzer (Lakewood, NJ) as previously described (Barrett *et al.*, 2009). Average summer temperature was predicted based on land surface temperature data from Landsat 7 ETM+ using band 6 (at 60 m resolution) and validated by field measurements (Lee *et al.*, Appendix 1). Longitude and latitude were included as spatial variables.

No topographic variable data was transformed prior to analysis. Soil conductivity, water content, carbon content, and nitrogen content were log(x+1) transformed prior to analysis, while pH and average summer temperature were not transformed. Longitude and latitude were transformed to X and Y coordinates (m), respectively. For the purpose of variance partitioning and spatial analyses, environmental variables were normalized and environmental distance matrices based on Euclidean distances between samples were generated. Coast distance was removed as a variable in these instances to ensure that no degree of spatial variation was explicitly built into the topography and environmental distance datasets.

DNA Extraction and Preparation

DNA was extracted from soils as described by Barrett and colleagues (2006), with modifications to facilitate high-throughput sample processing. Briefly, 0.7 g of soil was added to a microcentrifuge tube containing 0.5 g of both 0.1 mm and 2.5 mm silica-zirconia beads (BioSpec Products, Bartlesville, OK, USA). To each sample, 270 µL phosphate buffer (100 mM NaH_2PO_4) and 270 µL SDS lysis buffer (100 mM NaCl, 500 mM Tris pH 8.0, and 10% SDS) were added, and samples were bead-beaten for 10 minutes on a Vortex Genie 2 with a 24-tube vortex adapter (Mo Bio Laboratories Inc., Carlsbad, CA, USA). To each sample, 180 µL CTAB extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.4% BME) was added and samples were shaken at 300 rpm and 60 °C for 30 minutes. Samples were centrifuged at 16,000 x g for 3 minutes, prior to the addition of 350 µL chloroform:isoamyl alcohol (24:1) and 35 µL 10 M ammonium acetate. Samples were vortexed to mix and centrifuged at 16,000 x g for 5 minutes. The aqueous phase of each sample was transferred to a 96 well lysis block and processed using an X-tractor Gene liquid handling robot (Corbett Life Sciences, Concorde, NSW, Australia), using the DX Universal liquid sample DNA Extraction Protocol (CorProtocol No. 14104 Version 02). Samples were eluted in 80 µL TE pH 8.5 (10 mM Tris-HCl, 0.5 mM EDTA). Negative controls, consisting of bead tubes with no sample added, were processed as described above and included in each lane of the lysis block to assess potential contamination of extracts.

DNA extracts were quantified using Quant-iT Picogreen dsDNA reagent (Invitrogen, Auckland, New Zealand) on a FLUOstar optima fluorescence plate reader (BMG Laboratories, Offenburg, Germany). Briefly, 100 µL of picogreen solution (picogreen diluted 1:200 in TE) was added to each well of a black 96 well plate, containing 95 µL TE and 5 µL sample or standard containing 0 to 25 ng/µL lambda dsDNA (Invitrogen). Samples were excited at 485 nm and emission was measured at 520 nm. All extracts with DNA concentrations exceeding 2 ng/µL, were adjusted to 2 ng/µL in TE.

ARISA of Community DNA

PCR targeting the intergenic spacer between the 16S and 23S rRNA genes of the bacterial ribosomal operon was completed for each extraction and extraction negative controls. Each 25 µL reaction contained 1X PCR buffer, 3 mM $MgCl_2$, 1 U Platinum *Taq* DNA Polymerase (Invitrogen), 0.25 µM primers ITSF (5'-GTCGTAACAAGGTAGCCGTA-3') (Integrated DNA Technologies, Auckland, New Zealand) and ITSReub (5'-HEX-GCCAAGGCATCCACC-3') (Applied Biosystems, Auckland, New Zealand) (Cardinale *et al.*, 2004), 0.2 mM dNTPs (Invitrogen), and 5 µL of template DNA. Thermal cycling was completed on a Bio-Rad DNA Engine Peltier Thermal Cycler 200 (Bio-Rad, Hercules, CA, USA) using conditions described by Cardinale and colleagues (2004).

Amplicons were diluted 1:20 in de-ionized water. A mixture containing 2 µL of diluted amplicon, 0.13 µL of Liz-1200 internal size standard (Applied Biosystems), and 7.87 µL of HiDi formamide (Applied Biosystems) was heat denatured at 95 °C for 5 minutes and cooled to 4 °C for 2 minutes, before being resolved on an ABI 3130 Genetic Analyzer (Applied Biosystems) at the University of Waikato DNA Sequencing Facility.

All peaks between 100 and 1200 base pairs in length, that made up greater than 0.3 % of the entire signal over 10 rfu in each electropherogram were accepted as true peaks. The total number of true peaks was taken as a measure of bacterial taxon richness for each sample. Peaks within one base pair of one another in a pairwise comparison between fingerprints were binned together, and a Bray Curtis dissimilarity matrix using proportional peak abundance data was generated based on each pairwise comparison of the electropherograms.

Data Analyses

Bivariate relationships between taxa richness and environmental variables were assessed using linear models, and significant relationships were identified using Pearson's product-moment rank correlation. Non-linear associations were identified by examining residual plots, and appropriate non-linear models were then fit based on these analyses. The amount of variation in taxa richness explained by topographic variation, physicochemical variation, and spatial variation was assessed using multiple linear regression.

Bray Curtis community dissimilarities were examined in a two-dimensional ordination using non-metric multidimensional scaling (NMDS). Ordinations were completed in R using the 'vegan' library (Oksanen *et al*., 2011). The best solution was accepted after 1000 iterations. Pearson's product-moment rank correlation between the matrix of Bray Curtis dissimilarities and a matrix of the Euclidean distances between the points in the final NMDS ordination was calculated in order to assess the amount of variation captured in the final two dimensional solution (McCune and Grace, 2002). The relationships between the NMDS results and the continuous environmental variables were assessed using bivariate analyses and structural equation modeling (SEM).

The *a priori* model of the factors hypothesized to influence microbial community structure was tested using the program Mplus (v3.12). The model was evaluated using maximum likelihood estimators and a chi-squared goodness of fit index. Modification indices and sound theoretical justification were used to assess the need for adjustments to the structure of the *a priori* model to obtain a good fitting model consistent with the data. Total effects and indirect effects of the final model were assessed between variables.

Spatial variation in community structure was assessed using Mantel tests and related to environmental distance using partial Mantel tests. Both tests were completed with 9999 permutations. Additionally, variance partitioning based on redundancy analysis (RDA) was used to assess the amount of variation in the community structure that was explained by topographic variation, physicochemical variation, and spatial variation.

Results

Patterns of Bacterial Richness

Bacterial richness varied significantly with several physicochemical, topographic, and spatial variables (Figure 3.3). Richness was most strongly positively correlated with soil water content and most strongly negatively correlated with soil conductivity. A non-linear association between bacterial taxon richness and pH revealed richness was highest around pH 8, and decreased with increasing acidity and alkalinity. Together, the physicochemical, topographic, and spatial variables explained 38 % of the variation in richness, with space only accounting for 1 % of this variation independent of physicochemical and topographic heterogeneity (Figure 3.4).

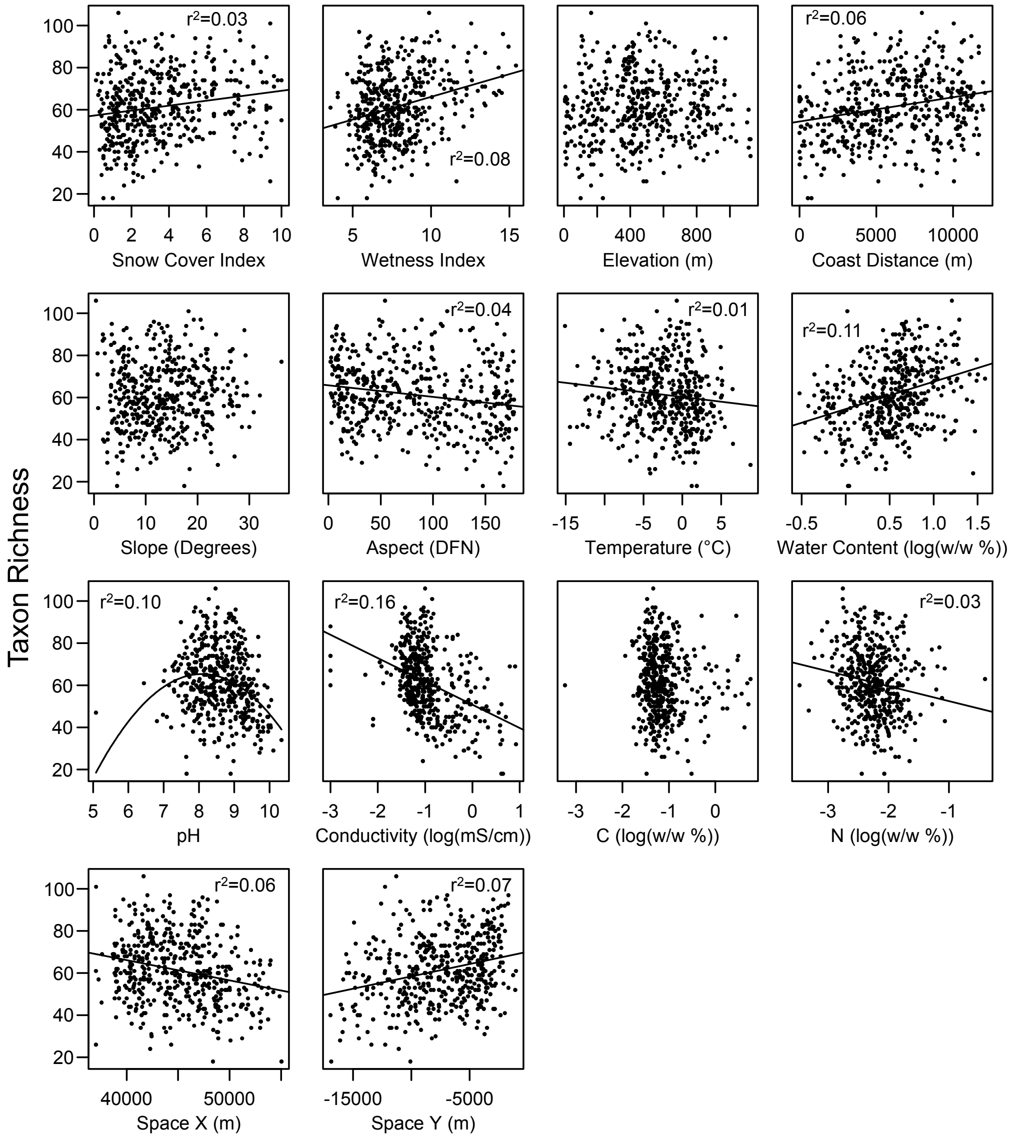

Figure 3.3: Bivariate relationships between environmental variables and bacterial taxon richness. Trend lines and r-squared values are included for significant relationships (P<0.05).

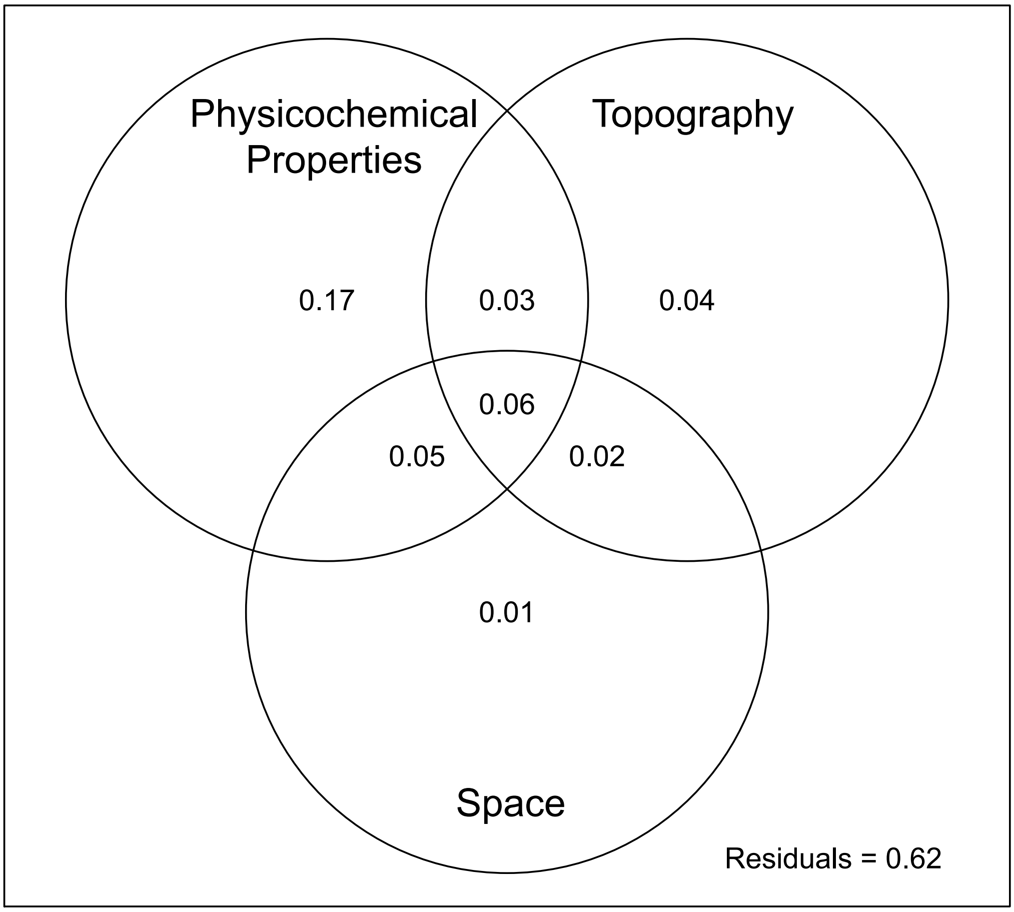

Figure 3.4: Venn diagram indicating the amount of variation in bacterial taxon richness explained by three groups of environmental variables: physicochemical soil properties, topography, and space. Coast distance was removed as a topographic variable for the purpose of variance partitioning to ensure that no degree of spatial variation was explicitly built into the topography dataset. An additional variable of pH^2 was added to the physicochemical soil properties to account for the non-linear relationship between richness and pH.

Patterns of Community Structure

A two-dimensional NMDS ordination, representing similarities of bacterial community compositions between sites, was reached with a stress of 0.25, and environmental gradients were assessed within the ordination (Figure 3.5). Comparison of the Euclidean distances in the two–dimensional NMDS configuration and the Bray Curtis distances revealed that the final two-dimensional solution captured 66 % of the variation in the community composition data. All the continuous environmental variables assessed showed significant bivariate relationships with one or both of the NMDS axes (P<0.01) (Table 3.1). NMDS axis 1 was found to vary significantly with snow cover, wetness, elevation, coast distance, aspect, temperature, soil water content, pH, and conductivity, but not slope. NMDS axis 2 varied significantly with snow cover, elevation, coast distance, aspect, temperature, and conductivity, but not wetness, soil water content, or pH.

The initial *a priori* model was not consistent with the data (X^2 = 242.9, d.f. = 28, P < 0.0001), and so the model was revised to find a stable final solution reflective of the relationships between the variables. Paths to soil water content from elevation, aspect, and snow cover were removed, as were paths to temperature from slope, paths to pH from coast distance, and paths to conductivity from pH and soil water content. Modification indices supported the addition of paths from snow cover and coast distance to temperature, paths from aspect and soil water content to pH, and paths from aspect to conductivity. Direct paths from wetness index and snow cover to community structure were also added. Finally, the variation in community structure captured in NMDS axis 2 was not explained by a unique set of variables to those explaining NMDS axis 1, so axis 2 was removed to simplify the final model.

The final model did not differ significantly from the data (X^2 = 27.6, d.f. = 21, P = 0.15) and explained 43 % of the variation observed in NMDS axis 1 (Figure 3.6). In addition, the model explained 45 % of the variation in temperature, 12 % of the variation in soil water content, 11 % of the variation in conductivity, and 5 % of the variation in pH. Soil water content had the strongest direct effect and total effect on axis 1 (Table 3.2). The effects of the topographic variables on community composition were largely mediated through soil property variables, with the exception of wetness index and snow cover, which had both indirect and direct effects on community composition.

Spatial Patterns of Community Structure

The average Bray Curtis community dissimilarity was found to increase with geographic distance between samples (Mantel $r = 0.103$, $P < 0.0001$), as did environmental distance (Mantel $r = 0.170$, $P < 0.0001$) (Figure 3.7). Partial correlations revealed a significant, but weak, correlation between community dissimilarity and geographic distance after accounting for environmental relationships (Mantel $r = 0.058$, $P < 0.01$). This correlation became non-significant if distance from the coast was included as a variable in generating the environmental distances (Mantel $r = 0.013$, $P = 0.244$). Variance partitioning of the community dissimilarity data also indicated that a small amount of the spatial variation in the data could not be explained by topographic and physicochemical distance (Figure 3.8).

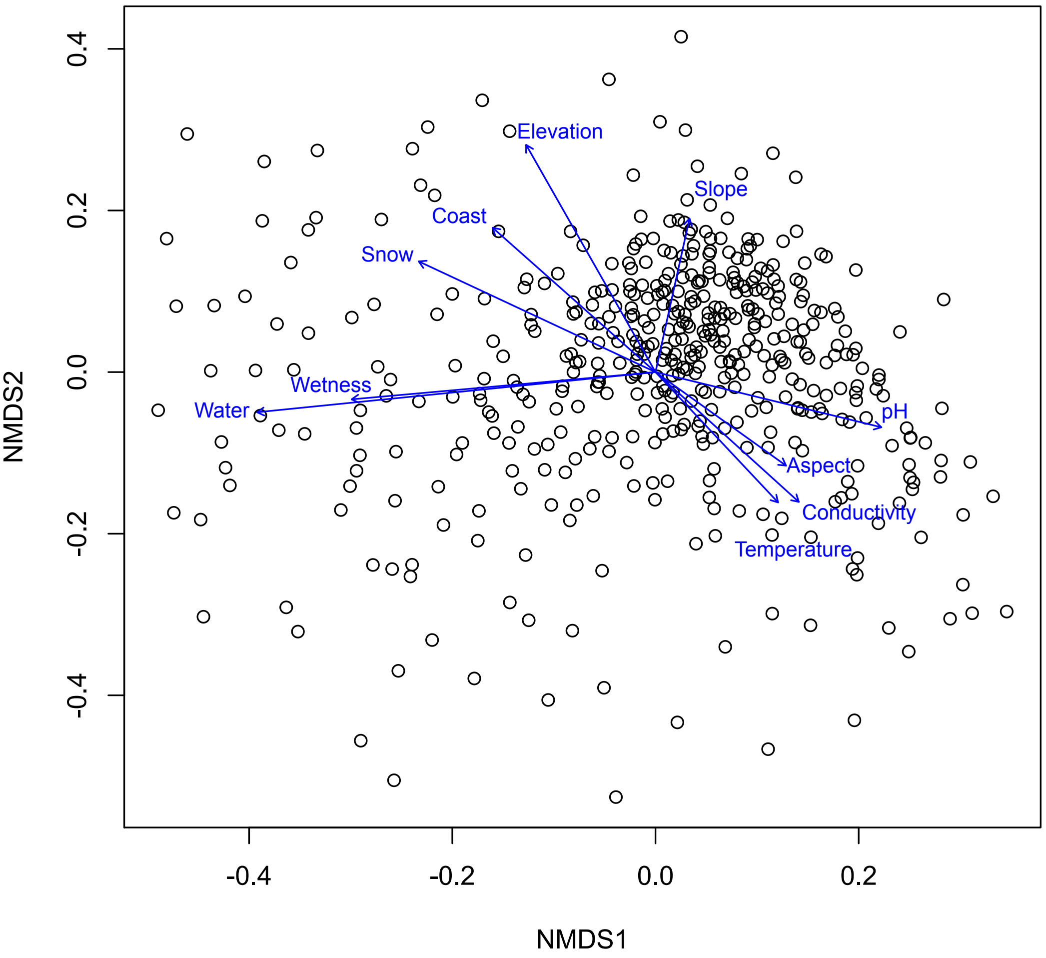

Figure 3.5: Non-metric multidimensional scaling (NMDS) ordination of bacterial community compositions in Dry Valley soils, with overlaid vectors indicating the strength and direction of environmental gradients.

Table 3.1: Bivariate correlation coefficients (r) of environmental variables with axes one and two of the non-metric multidimensional scaling (NMDS) ordination of bacterial community compositions. Significant ($P < 0.05$) correlations are in bold.

Variable Group	Variable	NMDS Axis 1	NMDS Axis 2
Topography	Snow Index	**-0.31**	**0.16**
	Wetness Index	**-0.38**	-0.04
	Elevation	**-0.18**	**0.35**
	Coast Distance	**-0.22**	**0.21**
	Slope	0.05	**0.24**
	Aspect	**0.17**	**-0.14**
	Temperature	**0.17**	**-0.20**
Soil Properties	Water Content	**-0.50**	-0.06
	pH	**0.29**	-0.08
	Conductivity	**0.19**	**-0.19**

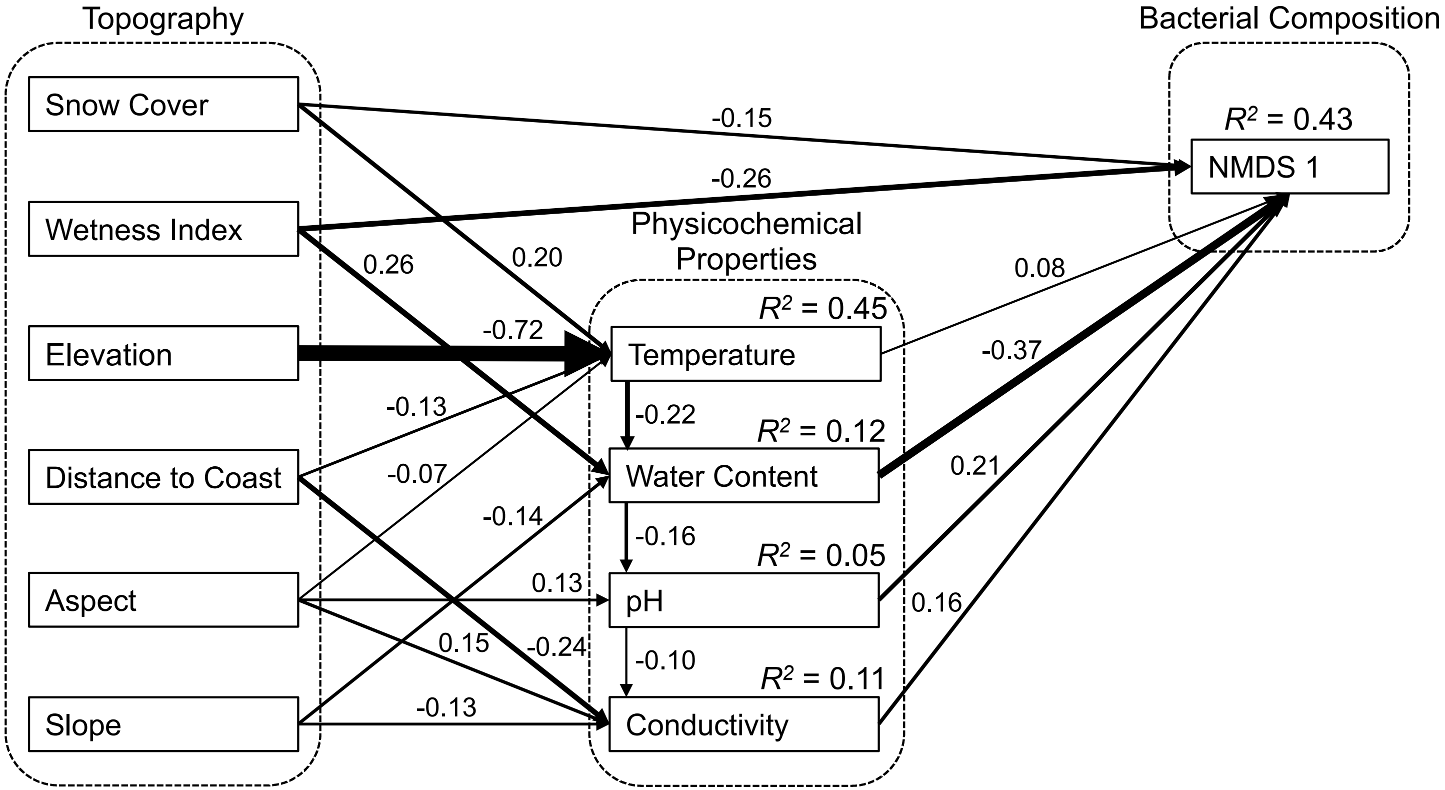

Figure 3.6: Final structural equation model (SEM) results ($X^2 = 27.6$, d.f. = 21, $P = 0.15$) with standardized coefficients. All pathways were significant.

Table 3.2: Standardized direct effects, indirect effects, and total effects of environmental factors on NMDS axis one.

Factor	Direct Effects	Indirect Effects	Total Effects
Aspect		0.039	0.039
Slope		0.035	0.035
Elevation		-0.121	-0.121
Distance to Coast		-0.061	-0.061
Snow	-0.154	0.034	-0.120
Wetness	-0.258	-0.104	-0.361
Temperature	0.080	0.088	0.168
Water Content	-0.370	-0.032	-0.402
pH	0.213	-0.016	0.196
Conductivity	0.163		0.163

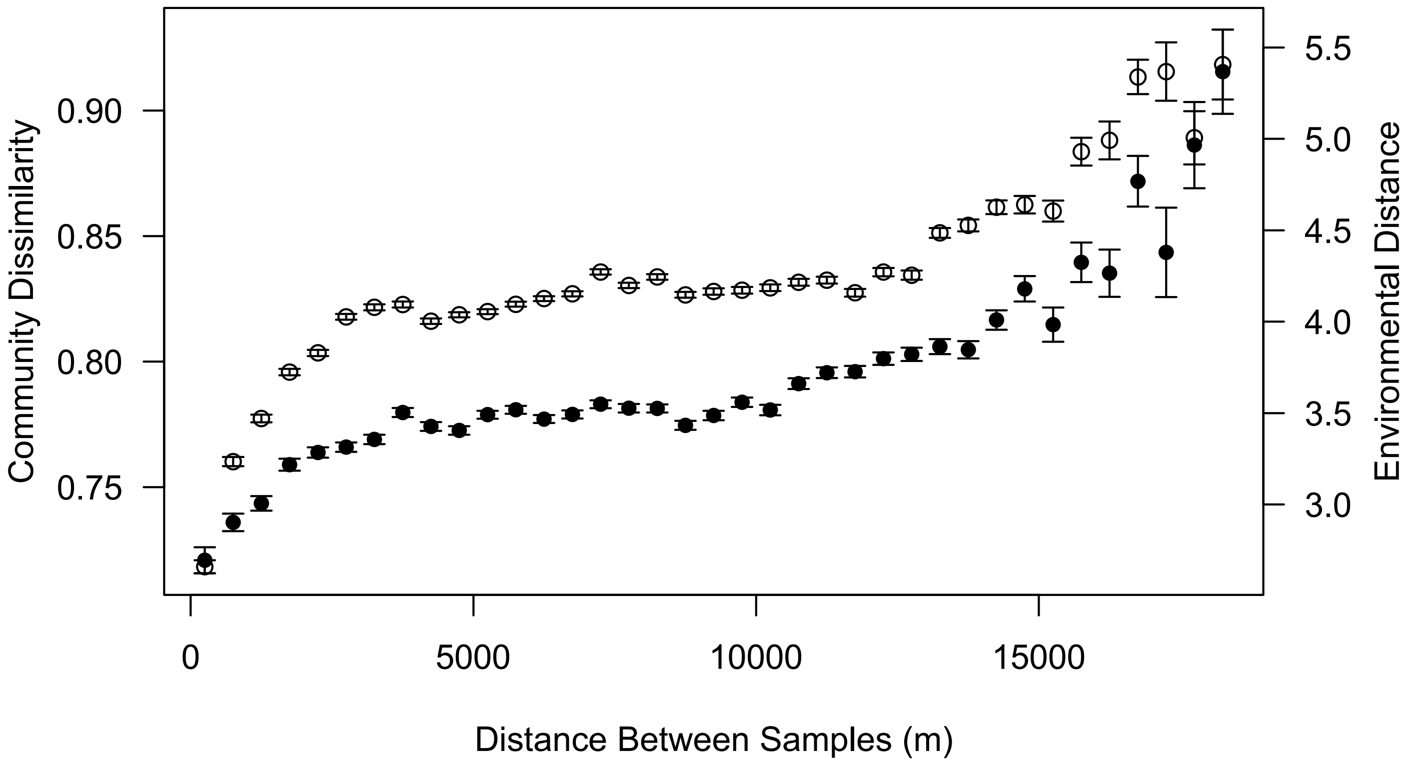

Figure 3.7: The relationship of community dissimilarity (filled circles) and environmental distance (open circles) with space. Mean variance in community dissimilarity and environmental distance were calculated between samples at 500 m intervals. Error bars show standard error. Coast distance was not included as a variable in calculating environmental distance to ensure that no degree of spatial variation was explicitly built into the environmental dataset.

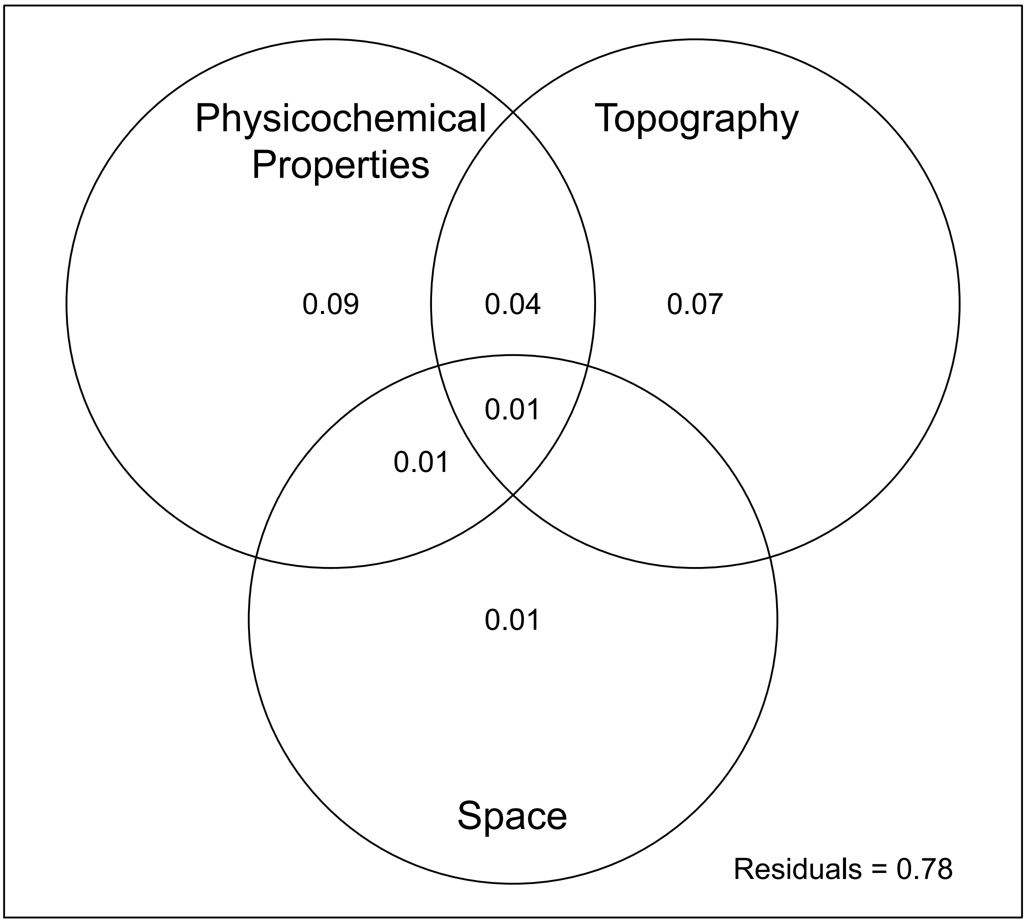

Figure 3.8: Venn diagram indicating the amount of variation in bacterial community structure explained by three groups of environmental variables: physicochemical soil properties, topography, and space. All physicochemical and topographical variables in the final model were included in the variance partitioning analysis except for coast distance, which was removed to ensure that no degree of spatial variation was explicitly built into the topography dataset.

Discussion

In the field of microbial ecology, recent emphasis has been placed on understanding the factors that control bacterial distributions (Martiny *et al.*, 2006). Previous studies of Dry Valley soils have revealed unexpectedly diverse bacterial communities, which appear to be shaped largely by abiotic variation, as opposed to biotic interaction (Cary *et al.*, 2010; Hogg *et al.*, 2006; Lee *et al.*, 2012). Here, we demonstrate that significant differences in bacterial diversity and community composition follow variation in topography and physicochemical properties across the Dry Valley landscape.

Patterns of Bacterial Richness

Bacterial richness was measured as the number of peaks in ARISA profiles from each sample. Despite the resolution limitations inherent to DNA fingerprinting techniques, ARISA has been accepted as a useful method for comparing taxon richness between samples (Brown and Fuhrman, 2005; Fuhrman *et al.*, 2008; Kovacs *et al.*, 2010) and remains an important rapid, cost-effective, technique for large scale analyses of microbial communities. Applying this methodology to the current study, we detected significant variation in bacterial taxon richness with environmental gradients, and found physicochemical variables to be stronger predictors of bacterial richness than topographic variables.

Richness was most strongly negatively associated with conductivity. The importance of salinity in shaping bacterial diversity and community structure has been reported previously (Lozupone and Knight, 2007), and similar negative trends in bacterial diversity with salinity have been reported in Antarctic environments (Chong *et al.*, 2010; Magalhães *et al.*, 2012; Zeglin *et al.*, 2011). High salt concentrations are typical of Antarctic soils (Claridge and Campbell, 1977; Vincent, 1988), and in the current study conductivity values averaged approximately 0.32 mS/cm and ranged greatly from 0 to 8.3 mS/cm in soils across the landscape. Salinity presents a strong selective pressure, as few bacteria are capable of growth over large ranges of salt concentrations (Oren, 2006). As such, one would expect the presence of non-halotolerant taxa to be greatly reduced in saline soils.

Soil water content was the second strongest predictor of bacterial richness and the strongest positive association. While soil water is expected to have an important influence on bacterial diversity in desert ecosystems, some recent studies have found the importance of water to be ancillary to other factors in shaping bacterial diversity in arid landscapes (Andrew *et al.*, 2012), including the Darwin Mountains of Antarctica (Magalhães *et al.*, 2012). Wetness index was found to be the most important topographic factor explaining bacterial richness.

Soil pH was found to be a less important predictor of bacterial richness in Dry Valley soils compared to temperate (Fierer and Jackson, 2006; Griffiths *et al.*, 2011) and Arctic ecosystems (Chu *et al.*, 2010). These studies have described patterns of bacterial diversity in soils ranging from pH 3 to pH 9, and found richness to be highest in soils near neutral pH. Soils in the current study nearly all ranged from neutral to alkaline with greater than 95 % of the values ranging from pH 7 to 10. The reduced importance of pH in shaping bacterial richness in Dry Valley soils could, therefore, be because (1) the general response in richness as pH increases from neutral is unequal to the response observed as pH decreases from neutral, (2) the smaller range of pH values observed over the landscape in the present study compared to previous reports provided an insufficient range to strongly affect richness, or (3) the harsh environmental constraints of the Dry Valley ecosystem compared to other ecosystems makes pH a secondary factor influencing richness in these soils.

Spatial trends in bacterial richness were not expected; however, significant relationships were observed with both latitude and longitude. Richness was found to increase moving North in latitude and West in longitude (with increasing distance from the coast). As expected, these spatial trends were almost entirely explained by topographic and physicochemical variation in the landscape.

Patterns of Community Structure

As predicted in the *a priori* model, the effects of most of the topographic variables on community structure were mediated through physicochemical variation. Elevation had the largest indirect effect on community composition, due to its strong effect on temperature. Variation in bacterial communities with elevation has been described previously in other systems (Bryant *et al.*, 2008), and trends in cyanobacterial distributions have been related to elevation in the Dry Valleys (Smith *et al*., 2006; Wood *et al*., 2008). Aspect, slope, and coast distance had more modest effects on physicochemical variables, with aspect found to affect temperature, pH, and conductivity, while slope was found to affect water content and conductivity, and coast distance was found to affect temperature and conductivity.

Direct effects on community composition were supported for two topographic variables: snow cover and wetness index. In the *a priori* model, snow cover was hypothesized to impact community structure indirectly through influencing soil moisture; however, this path was not supported in the final model. It is possible that the moisture in snow rarely becomes bioavailable, as it is often removed by sublimation and strong winds rather than melting (Gooseff *et al*., 2003). As such, the direct impact of snow cover on community structure could be the result of the change imparted to the physical environment by shielding the soils from sunlight and ultraviolet radiation. The influence of wetness index on community composition could not be explained entirely through measures of soil moisture. As it is difficult to assess the influence of water in a soil solely by an instantaneous measure at the time of sampling, a direct path from wetness index to community composition provides a meaningful indication of potential availability of water in soils over time.

Water content and wetness index had the strongest direct and total effects on bacterial community composition. Soil moisture has been identified as the strongest predictor of bacterial community compositions in recent analyses of other terrestrial systems, including soils of the Tibetan Plateau (Zhang *et al*., 2013), lithic communities across hot and cold hyper-arid landscapes in deserts of China (Pointing *et al*., 2007), and in stream sediments of the Onyx River in the Dry Valleys and Rio Salado in New Mexico (Zeglin *et al*., 2011). Water is understood to be a limiting variable in the Dry Valleys (Barrett *et al*., 2007; Kennedy, 1993) and the presence of particular bacterial taxa have been related to water availability in Antarctic soils (Aislabie *et al*., 2006; Niederberger *et al*., 2008); however, the current work is the first evidence that water is the most important variable in explaining bacterial community compositions in soils across the landscape as a whole.

The total effects of pH, conductivity, and temperature on community composition were found to be roughly similar. Soil pH had a strong positive direct effect on community composition, but the total effects of pH were tempered slightly by a negative indirect effect mediated through conductivity. The strength of the direct and total effect of pH is not surprising, considering that pH has been found to be the most important factor shaping microbial community compositions in other soils (Chu *et al.*, 2010; Fierer and Jackson, 2006; Griffiths *et al.*, 2011; Lauber *et al.*, 2009; Rousk *et al.*, 2010). Conductivity, despite being the strongest predictor of bacterial diversity across the landscape, had the second smallest direct effect and smallest total effect on community composition of all the physicochemical variables analyzed. Temperature had the weakest direct effect on community composition, but a relatively strong indirect effect mediated through water content contributed to a substantial total effect. While cold-adapted bacteria may be selected for by the Dry Valley landscape as a whole, the community compositions within the landscape appear to show little differentiation based directly on variation in mean summer temperature. Mean summer temperatures across the landscape were predicted to range from -15 to 9 °C, entirely within the psychrotrophic range (Scherer and Neuhaus, 2006), providing little capacity to select different populations between sites based on different temperature optima; it is, perhaps, not surprising then that temperature has at least an equally important role in shaping community composition by influencing water availability.

Spatial Patterns of Community Structure

A significant distance decay relationship was observed, in which community dissimilarity increased with increasing distance between samples; however, once accounting for environmental variation, the correlation, while still significant, was quite weak. Spatial variation that cannot be explained by environmental variability has been used as an indicator of dispersal limitation for bacterial populations (Martiny *et al.*, 2011), consistent with neutral models of larger organisms (Bell, 2001; Condit *et al.*, 2002). Dry Valley landscapes are understood to be linked through aeolian and hydrological processes (Barrett *et al.*, 2007), increasing the likelihood of dispersion of biomass over the landscape. Recently, however, cyanobacterial taxa have been shown to vary spatially over short distances (< 3 km) in the Dry Valleys and Transantarctic Mountains, independent of the environmental gradients found to influence the composition of bacterial communities as a whole (Sokol *et al*., 2013). The small amount of unexplained spatial variation observed here may, therefore, reflect the presence of a subset of bacterial taxa whose distributions are better explained by dispersal limitation than environmental filtering. Alternatively, this variation may be the result of unmeasured environmental variation or legacy effects of landscape history, not accounted for in this study. One must be careful not to overstate the importance of weak trends in environment-space relationships (Griffiths *et al*., 2011), particularly as they are known to vary with the resolution of the taxonomic data (Horner-Devine *et al*., 2004). Based on the data presented here, however, we cannot rule out the possibility that some bacterial populations show dispersal limitation across the landscape, which may have an additional effect on shaping bacterial communities to that of taxa sorting due to environmental variation.

Conclusions

At the outset of this work, three hypotheses were proposed to explain variation in bacterial diversity and community composition across the Dry Valleys of Antarctica. We confirmed our first hypothesis that bacterial taxon richness varied with environmental gradients, and found soil conductivity and water content to be the most important explanatory variables. Our second hypothesis presented an *a priori* model indicating paths through which environmental gradients were expected to influence bacterial community composition. Based on empirical data, this *a priori* model was rejected in favor of a model that supported numerous significant direct and indirect effects of abiotic variables on bacterial community compositions that were not present in the initial model. Our third hypothesis, that a significant distance decay relationship exists in soils across the study area, was confirmed; however, the cause of the relationship remains inconclusive, as the observed variation could not be explained entirely by environmental heterogeneity. This work provides fundamental knowledge of the environmental factors influencing bacterial communities in Dry Valley soils. Such knowledge will be important locally and regionally, for shaping management strategies for Antarctica’s ice free areas and predicting ecosystem responses to climate change scenarios of increased temperature, water availability, and environmental variability (Wall, 2007), as well as globally, as we seek to describe patterns in microbial ecology across the planet.

Acknowledgements

This research was supported through an IPY award from the Foundation for Research, Science and Technology of New Zealand (UOWX0710 and UOWX0715) and the National Science Foundation (NSF OPP ANT-0944556 and ANT-0944560) through awards to SCC. DCL, CKL, IRM, and SCC were supported by the New Zealand Marsden Fund (UOW1201, UOW1003, and UOW0802). Logistics support for the field seasons was provided by Antarctica New Zealand. We especially thank the many researchers who were involved in the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) for their time and commitment to this work.

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Chapter 4: Patterns of Bacterial Taxa Distributions in Soils of the Antarctic Dry Valleys

Preface

This chapter describes the phylogenetic diversity of bacterial communities in soils of the Antarctic Dry Valleys, and examines ecological drivers of taxa distribution. This work was undertaken as part of the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) and the Earth Microbiome Project (EMP), and was supported as a Community Sequencing Program (CSP) funded by the United States Department of Energy (DOE). As primary author of this chapter, I was involved in fieldwork and lab analyses, completed the data analyses, and wrote the chapter. Charlie Lee earned co-authorship by assisting with, and advising, the processing of sequencing data. Daniel Laughlin earned co-authorship by advising statistical analyses. All authors reviewed and edited chapter content.

Sample collection, environmental surveys, and biological surveys completed as part of nzTABS, were the result of efforts from the entire nzTABS team. Nematode, rotifer, and tardigrade enumeration was completed by nzTABS members Dianna Wall (Colorado State University, USA) and Byron Adams (Brigham Young University, USA) and their lab groups. Preparation of samples for inductively coupled mass spectroscopy (ICPMS) was carried out by technicians Roanna Richards-Babbage and Robin Hacker-Cary of the University of Waikato Department of Biological Sciences, and ICPMS was carried out by technician Steve Cameron of the University of Waikato Department of Chemistry.

DNA sequencing was completed as part of the EMP and through a CSP funded by the United States DOE. I was involved in writing the successful CSP proposal, along with Charlie Lee, Craig Herbold, Janet Jansson, and S. Craig Cary. DNA extractions were completed primarily by staff at Lawrence Berkeley National Lab (LBNL), Berkeley, California, USA. I spent time in LBNL validating DNA extraction protocols and assisted with DNA extractions. DNA sequencing was completed by staff at the Joint Genome Institute (JGI).

This chapter is being prepared for submission to the *ISME Journal*.

Patterns of bacterial taxa distributions in soils of the Antarctic Dry Valleys

Eric M Bottos[1,2], Charles K Lee[1,2], Daniel C Laughlin[1], Ian R McDonald[1,2], S. Craig Cary[1,2*]

Department of Biological Sciences, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

The International Centre for Terrestrial Antarctic Research, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

*Corresponding Author

Abstract

The Dry Valleys of Antarctica are a microbially dominated ecosystem of simple trophic structure, where environmental drivers of microbial distributions are expected to be readily discernable in the absence of extensive trophic level interactions. While environmental variation is known to influence bacterial community composition in Dry Valley soils, the degree to which abiotic, biotic, and spatial variation can predict patterns of taxa distribution across the landscape is not well established. Here, high-throughput 16S rRNA gene sequencing was used to characterize bacterial communities in 177 Dry Valley soil samples, and community composition was examined with respect to global patterns of bacterial distributions and in relation to extensive environmental metadata associated with the samples. Soil libraries were dominated by *Actinobacteria* (mean 28.3 %, SD 13.2 %), *Acidobacteria* (mean 22.5 %, SD 10.8 %), *Bacteroidetes* (mean 16.6 %, SD 7.7 %), and *Proteobacteria* (mean 14.0 %, SD 10.0 %), and community composition was found to reflect the arid, alkaline, and oligotrophic nature of the Dry Valleys landscape. Within the system, several significant associations between bacterial community composition and abiotic and biotic variation in the environment were observed; however, these variables explained only a small amount of the total variation in community composition, and the distributions of individual taxa were only weakly associated with environmental gradients. The abundances of many bacterial taxa were significantly coupled to one another, suggesting that, even in a trophically simple system subjected to harsh abiotic stressors, interactions between bacterial taxa may influence bacterial community assembly.

Introduction

Recent surveys of bacterial communities globally have greatly increased understanding of the patterns of bacterial distributions, and the processes that shape community composition. In soils, bacterial community compositions appear to be structured predominantly by soil pH (Fierer & Jackson, 2006; Griffiths *et al.*, 2011; Lauber *et al.*, 2009; Chu *et al.*, 2010), and the relative abundance of particular taxa show relationships to numerous physicochemical gradients, including pH (Lauber *et al.*, 2009; Jones *et al.*, 2009), soil moisture (DeBruyn *et al.*, 2011), and nutrient content (Fierer *et al.*, 2007). While these trends appear to be important both within and across terrestrial biomes, there are few ecosystems where the importance of these abiotic factors on bacterial distributions can be examined in the absence of potentially confounding co-variation in distributions of higher taxa. The Dry Valleys of Antarctica are a microbially dominated ecosystem of simple trophic structure, where the influence of environmental heterogeneity on bacterial distributions can be examined, and where the full extent of biological interactions in the ecosystem can be accounted for, both within and between trophic levels (Cary *et al.*, 2010).

The composition of bacterial communities in Antarctica's Dry Valley soils are thought to be unique, shaped by the harsh environmental conditions that typify the ecosystem (Cary *et al.*, 2010). This perception of community uniqueness is supported by analyses of bacterial communities that show phyla and class level representation of taxa are atypical of soils from temperate locations, hot desert soils, and other regions of Antarctica (Cary *et al.*, 2010). Bacterial communities across Dry Valley soils are also highly heterogeneous, which may reflect the steep physicochemical gradients and unique glacial histories that exist in the landscape (Lee *et al.*, 2012). While abiotic variation is expected to be the dominant driver of bacterial community composition in Dry Valley soils (Hogg *et al.*, 2006; Adams *et al.*, 2006; Cary *et al.*, 2010), the distributions of bacterial taxa have not been adequately linked to measured environmental variation.

Biotic interactions have been assumed to be largely unimportant in shaping community compositions in Antarctic soils (Hogg *et al.*, 2006); however, there is increasing evidence that taxa distributions in the Dry Valleys may be influenced by species interactions, as well as environmental filtering, particularly for microbial taxa. Patterns of taxa co-occurrence have been observed between bacteria and diatoms in Dry Valley stream communities (Stanish *et al*., 2013) and, most recently, cyanobacterial diversity has been linked to the diversity of fungi and multicellular taxa in Dry Valley soils (Lee *et al*., Appendix 1). It has been suggested that the initial colonization of harsh environmental niches by keystone species may alter local environmental conditions, facilitating the subsequent establishment of other taxa (Chan *et al*., 2013). Conversely, the recent detection of antibiotic resistance genes in functional gene analyses of soils from McKelvey Valley has been cited as evidence of potential competitive interaction between bacterial taxa in Dry Valley soils (Chan *et al*., 2013). These findings suggest that patterns of taxa co-occurrence should be considered along with abiotic variation to elucidate drivers of community composition in Dry Valley soils.

To date, studies of Antarctic soils have largely relied on small sample numbers to infer patterns of bacterial distributions. With the advent of high-throughput sequencing, it is now feasible to characterize the phylogenetic diversity of bacterial communities in large numbers of samples (Tringe and Hugenholtz, 2008); consequently, surveys of bacterial diversity can move beyond simple point assessments of community composition to include analyses of the variation in community composition that occurs across samples. Elucidating the patterns of this variation in relation to environmental variability is necessary to understand the processes that influence community composition and function, and ultimately to predict ecosystem responses to environmental change. In this study, we analyzed the bacterial community composition in 177 Dry Valley soil samples from across a 220 km^2 study area using high-throughput sequencing, and describe patterns of bacterial distributions across the landscape. As the largest and most comprehensive phylogenetic survey of bacterial communities completed in Antarctic soils to date, this work provides novel insight into how bacterial community compositions of Dry Valley soils are related to global patterns of bacterial distributions and how the distributions of bacterial taxa within the Dry Valleys are related to the local environmental conditions.

Materials and Methods

Sample Collection and Site Surveys

Samples were collected as part of the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) programme (nztabs.ictar.aq) during the 2008-2009 and 2009-2010 austral summers, as described previously (Lee *et al.*, Appendix 1). From the 490 samples collected by nzTABS, 192 were selected for high-throughput 16S rRNA gene amplicon sequencing, as part of the Earth Microbiome Project (Gilbert *et al.*, 2010). All samples were accompanied by extensive environmental and biological metadata, collected as part of nzTABS (Lee *et al.*, Appendix 1). Samples were selected for sequencing to cover the broad range of abiotic conditions that exist across the nzTABS study site and the range of bacterial community compositions known to exist in these soils based on previous bacterial fingerprinting analyses (Bottos *et al.*, Chapter 3) (Figure 4.1).

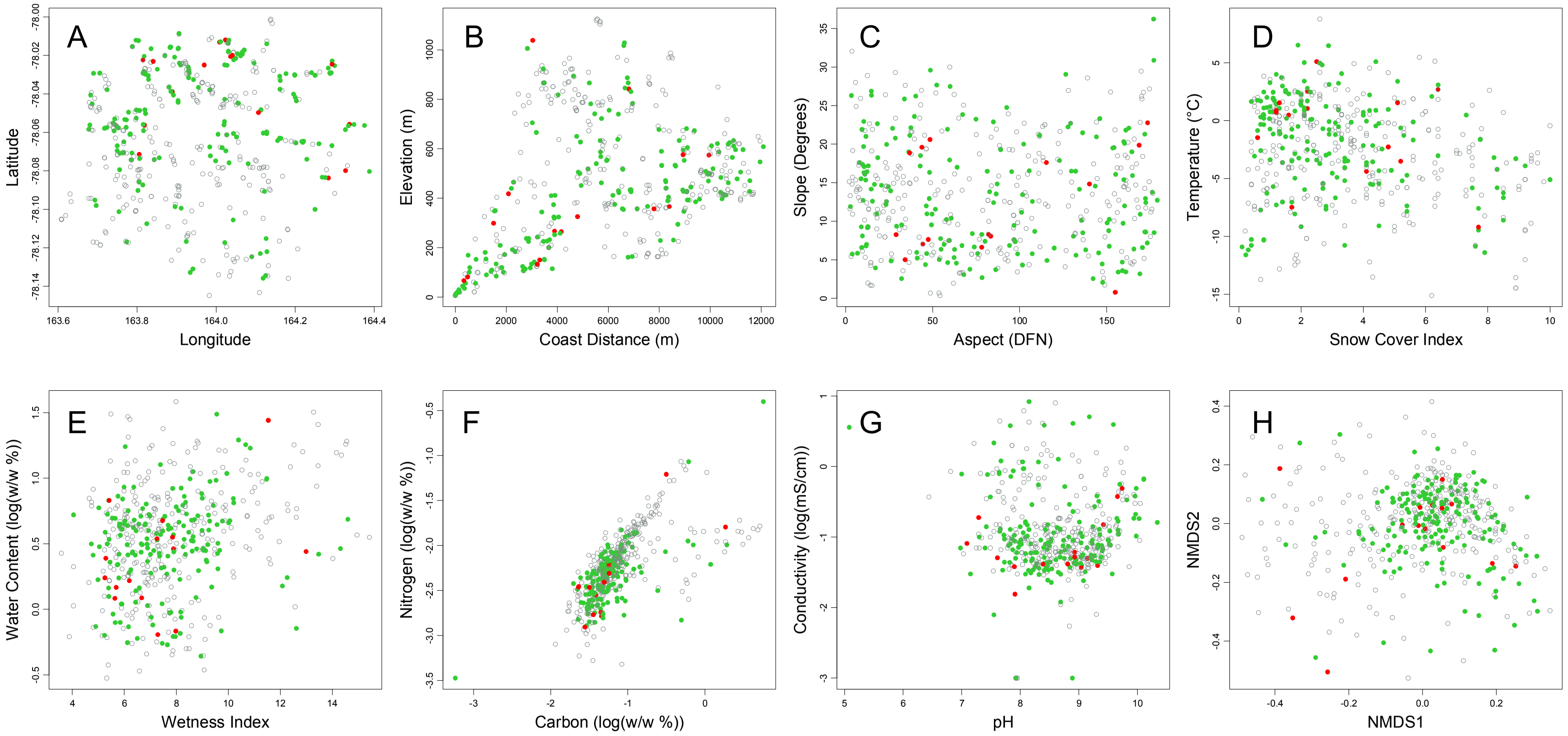

Figure 4.1: Samples selected for sequencing from the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) were chosen to cover the range of spatial (A) and environmental (B-G) variation in the Dry Valleys ecosystem, as well as the variation in bacterial community compositions across samples, as assessed previously by community fingerprinting (Bottos *et al.*, Chapter 3) (H). Filled circles represent sequenced samples and unfilled circles represent unsequenced samples; samples sequenced at sufficient depth to be included in analyses are filled green, while samples dropped due to insufficient reads are filled red.

Sample, Sequence, and Data Processing

Sample processing, sequencing and core amplicon data analysis were performed by the Earth Microbiome Project (www.earthmicrobiome.org) and all amplicon and metadata has been made public through the data portal (www.microbiome.me/emp). Briefly, DNA was extracted from 0.1-0.25 g of soil using the PowerSoil-htp 96 Well DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). PCR targeting the V4 region of the 16S rRNA gene was completed in triplicate for each sample as described previously (Caporaso *et al.*, 2011). Triplicate PCRs were pooled and paired-end sequencing of amplicons was completed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) (Caporaso *et al.*, 2012). As part of the JGI amplicon processing pipeline, all reads were trimmed to 165 base pairs and paired reads were assembled using the software FLASH (Magoč & Salzberg, 2011). The assembled reads were trimmed from both the 5' and 3' ends using a 20 base pair sliding window and a quality threshold of 30.

The trimmed assembled reads were processed in Mothur (Schloss *et al.*, 2009). Chimeras were removed using Mothur's implementation of the program Perseus (Quince *et al.*, 2011). Sequences were aligned (DeSantis *et al.*, 2006a) against the Silva database (Quast *et al.*, 2012) between positions 11894 and 25319, and sequences that did not align over this region were removed from further analysis. Reads were then preclustered (Huse *et al.*, 2010) to recognize sequences with less than two mismatches across the alignment as the same. Taxonomic assignment of sequences was completed in Mothur against the May 2013 release of the Greengenes database (DeSantis *et al.*, 2006b), using the command *classify.seqs*, with default settings and a bootstrap cutoff of 80 %. Any reads not grouping to the kingdom *Bacteria* were removed. Samples were clustered into operational taxonomic units (OTUs) at 97 % sequence identity using Cd-hit-est (Huang *et al.*, 2010). The processed dataset was resampled to 10,000 sequences per sample to normalize the number of observations per sample. Samples with less than 10,000 sequences were dropped from further analysis, yielding a final dataset consisting of 177 samples. Representative sequences from abundant OTUs were compared to those in the NCBI database using the BLASTn algorithm (Altschul *et al.*, 1990).

Determination and Treatment of Variables

Data collected previously as part of the nzTABS programme was used to assess patterns of bacterial community composition in relation to abiotic, biotic, and spatial variation in the landscape. Abiotic variables included from the nzTABS dataset were elevation (m), aspect (degrees from North), slope (degrees), distance to the coast (m), an index of yearly snow cover (Stichbury *et al.*, 2011), an index of soil wetness (Stichbury *et al.*, 2011), average summer temperature (°C), pH, conductivity (mS/cm), water content (w/w %), carbon content (w/w %), and nitrogen content (w/w %): these will be referred to as 'abiotic variables'. Abundance measures of multicellular taxa included from the nzTABS dataset included mosses, lichens, mites, springtails, three genera of nematode (*Eudorylaimus*, *Scottnema*, *Plectus*), rotifers, and tardigrades: these will be referred to as 'biotic variables'. Mosses and lichens were reported as percent coverage in a 20 m^2 transect at the sample site, mites and springtails were reported as the number sighted during a 10 minute survey of the sample site, and nematodes, rotifers, and tardigrades were reported as the number of total live and dead organisms per 100 grams of soil. Latitude and longitude were included from the nzTABS dataset as 'spatial variables'. Abiotic, biotic, and spatial variables were determined as described previously (Bottos *et al.*, Chapter 3; Lee *et al.*, Appendix 1). In addition to the characterizations completed as part of nzTABS, analyses of soil elemental composition were completed on 147 of the 192 sequenced samples by inductively coupled plasma mass spectrometry (ICPMS), as previously described (Lee *et al.*, 2012). These 'geochemical variables' included the following elements: B, Na, Mg, Al, P, S, K, Ca, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cs, Ba, Tl, Pb, U.

Prior to analysis, conductivity, water content, carbon content, and nitrogen content data were all log(x+1) transformed to address skewness; all other abiotic variables remained untransformed. Moss and lichen coverage estimates were transformed to presence/absence data, while all other biotic variables were log(x+1) transformed. Longitude and latitude were transformed to X and Y coordinates (in meters), respectively. Geochemical variables were filtered to replace any negative values with zero, and all concentrations exceeding the maximum range of standard curves used to quantify element concentrations were replaced with the maximum concentration observed across samples for that particular element. Following data filtering, all geochemical variables were log(x+1) transformed.

Data Analyses

The abundances of bacterial taxa were log(x+1) transformed prior to analyses of community composition. Bray Curtis dissimilarities were calculated between samples at the phyla, class and OTU levels of taxa definition in the PRIMER 6 package (PRIMER-E Ltd, Ivybridge, UK). Biota Environmental Stepwise (BEST) analysis was performed in PRIMER 6 to analyze the relationships between bacterial community composition and abiotic, biotic, and geochemical variables. BEST analysis selects the subset of environmental variables that maximize the rank matrix correlation between the environmental and community dissimilarity matrices. The abiotic, biotic, and geochemical datasets were analyzed against bacterial community dissimilarity matrices independently. In BEST analyses involving abiotic data and geochemical data, all variables were normalized prior to analysis. In all analyses, the BIOENV procedure was run using default settings and with 99 permutations.

Pearson's correlation coefficients between bacterial taxa abundances and the explanatory variables identified by BEST analysis as most important for explaining bacterial community composition were calculated in R (R Core Team, 2014). Variance partitioning based on redundancy analysis (RDA) was used to determine the relative influence of abiotic, biotic, and spatial variation on bacterial community composition, and was completed in R using the vegan package (Oksanen *et al.*, 2013). Correlations between relative bacterial taxa abundances were completed using SparCC (Sparse Correlations for Compositional data) with default settings and 100 permutations (Friedman and Alm, 2012).

Results

Community Composition

A total of 52 bacterial phyla or candidate phyla included in the Greengenes taxonomy (McDonald *et al*., 2012) were represented in the soil community libraries, and 17 of these phyla comprised greater than 1 % of sequences in any sample (Figure 4.2). Libraries were typically dominated by *Actinobacteria* (mean 28.3 %, SD 13.2 %), *Acidobacteria* (mean 22.5 %, SD 10.8 %), *Bacteroidetes* (mean 16.6 %, SD 7.7 %), and *Proteobacteria* (mean 14.0 %, SD 10.0 %). *Actinobacteria* were the most abundant phylum in 50 % of samples, while *Acidobacteria*, *Bacteroidetes*, and *Proteobacteria* were most abundant in 31 %, 12 %, and 6 % of samples, respectively. Together, these four phyla accounted for 81 % of sequences observed across all samples. The most abundant phyla were represented by several classes, with varying relative abundance (Figure 4.3).

The phylum *Actinobacteria* was dominated by sequences grouping to the class *Thermoleophilia* but also included sequences grouping to the classes *Actinobacteria*, *Acidomicrobiia*, *Rubrobacteria*. *Thermoleophilia* comprised 54 % of sequences grouping to the phylum, with 88 % of these sequences grouping to the order *Solirubrobacterales* and 12 % grouping to the order *Gaiellales*. The class *Actinobacteria* made up 22 % of sequences in the phylum, and 99% of these sequences grouped to the order *Actinomycetales*. *Acidomicrobiia* (order *Acidomicrobiales*) and *Rubrobacteria* (order *Rubrobacterales*) comprised 11 % and 6 % of sequences grouping to the phylum, respectively. Sequences grouping to other classes in the *Actinobacteria* made up 4 % of the phylum, while 2 % of the sequences could not be further classified.

The class *Chloracidobacteria* accounted for 90 % of the sequences assigned to the phylum *Acidobacteria*. These sequences grouped almost exclusively to the order RB41, with 82 % of these sequences grouping to the family Ellin6075. Sequences grouping to the classes *Solibacteres*, Acidobacteria-6, and iii1-8 had nearly equal representation, comprising 4 %, 3 %, and 3 % of *Acidobacteria* sequences, respectively. Sequences grouping to other classes and sequences that could not be classified beyond the phylum level made up less than 1 % of the *Acidobacteria*.

Sequences grouping to the class *Saprospirae* were the most abundant representatives within the *Bacteroidetes*, comprising 68 % of the phylum. All sequences classified within the *Saprospirae* grouped to the order *Saprospirales*, and 97 % of these sequences grouped to the family *Chitinophagaceae*. Sequences grouping to the class *Cytophagia* made up 17 % of the phylum, and the classes *Sphingobacteriia* and *Flavobacteriia* each made up 4 % of the phylum. Other classes comprised less than 1 % of the sequences grouping within the *Bacteroidetes*, while 5 % of the sequences remained unclassified beyond the phylum level.

The phylum *Proteobacteria* included sequences grouping to the classes *Alpha*-, *Beta*-, *Gamma*-, *Delta*-, and *Epsilon-proteobacteria*. *Gamma*- and *Alpha-proteobacteria* were present in all samples and were the most dominant classes, comprising 39 % and 31 % of the phylum, respectively. *Beta*- and *Delta-proteobacteria*, were also present in all samples, but typically at lower sequence abundance, representing 18 % and 8 % of the sequences in the phylum, respectively. *Epsilonproteobacteria* were present in 8 % of the samples and comprised a total of 1 % of sequences grouping to the *Proteobacteria*. Sequences grouping to other classes and sequences that could not be classified beyond the phylum level each made up 1 % of the phylum.

Other phyla were detected across all samples, typically at lower abundance than the four dominant phyla. The phyla *Verrucomicrobia*, *Cyanobacteria*, and *Planctomycetes* were present in all samples, comprising 5 %, 2 %, and 1 % of all sequences observed, respectively. Sequences grouping to the phyla *Gemmatimonadetes* and *Chloroflexi* were present in all but one sample and each comprised 3 % of all sequences observed. An additional 13 phyla were observed in greater than 50 % of samples; however, at 1 % of the total sequences observed, the phylum *Chlorobi* was the most abundantly represented of these phyla. Collectively, all other phyla comprised less than 3 % of the total sequences observed and 1 % of sequences could not be grouped to a phylum.

Less than 1 % of OTUs were observed in greater than half of the samples; however, many of the most abundant OTUs were observed across the majority of samples (Figure 4.4). The most abundant OTUs were closely related to bacteria previously reported from environmental samples (Supplementary Table 4.1). The closest matches in the NCBI database to many OTUs were bacteria previously observed in Antarctic soils, including those from Southern and Northern Victoria Land, East Antarctica, and the Antarctic Peninsula. Other common sources of close OTU matches were arid ecosystems, Arctic soils, glacial forefields, and volcanic soils.

Relating Community Composition to Environmental Variation

Bacterial community composition was poorly described by the measured explanatory variables. Variance partitioning revealed that the combination of abiotic, biotic, and spatial variation collectively explained approximately 7 % of the variation in bacterial community composition at the OTU level and less at the class and phylum level (Figure 4.5). At the OTU level, abiotic and biotic variation alone explained 1 and 2 % of the community variation, respectively, while approximately 3 % of the community variation was explained by both abiotic and biotic variation. Spatial variation did not explain any variation independent of that explained by biotic and abiotic variables.

BEST analysis revealed weak, but significant ($p<0.05$), relationships between community composition and both abiotic (Table 4.1) and biotic variables (Table 4.2). At all taxonomic levels analyzed (phylum, class, and OTU), community composition was most strongly correlated with conductivity as a single abiotic variable, and with conductivity, pH, and soil water, as a combination of abiotic variables. Community composition was most strongly correlated with the nematode *Eudorylaimus* abundance as a single biotic variable, and with *Eudorylaimus*, springtail, and moss abundance as a combination of biotic variables. The distributions of individual taxa were also only weakly associated with abiotic variables and biotic variables (Table 4.3), though several significant associations were observed. Soil geochemistry was not significantly related to bacterial community composition.

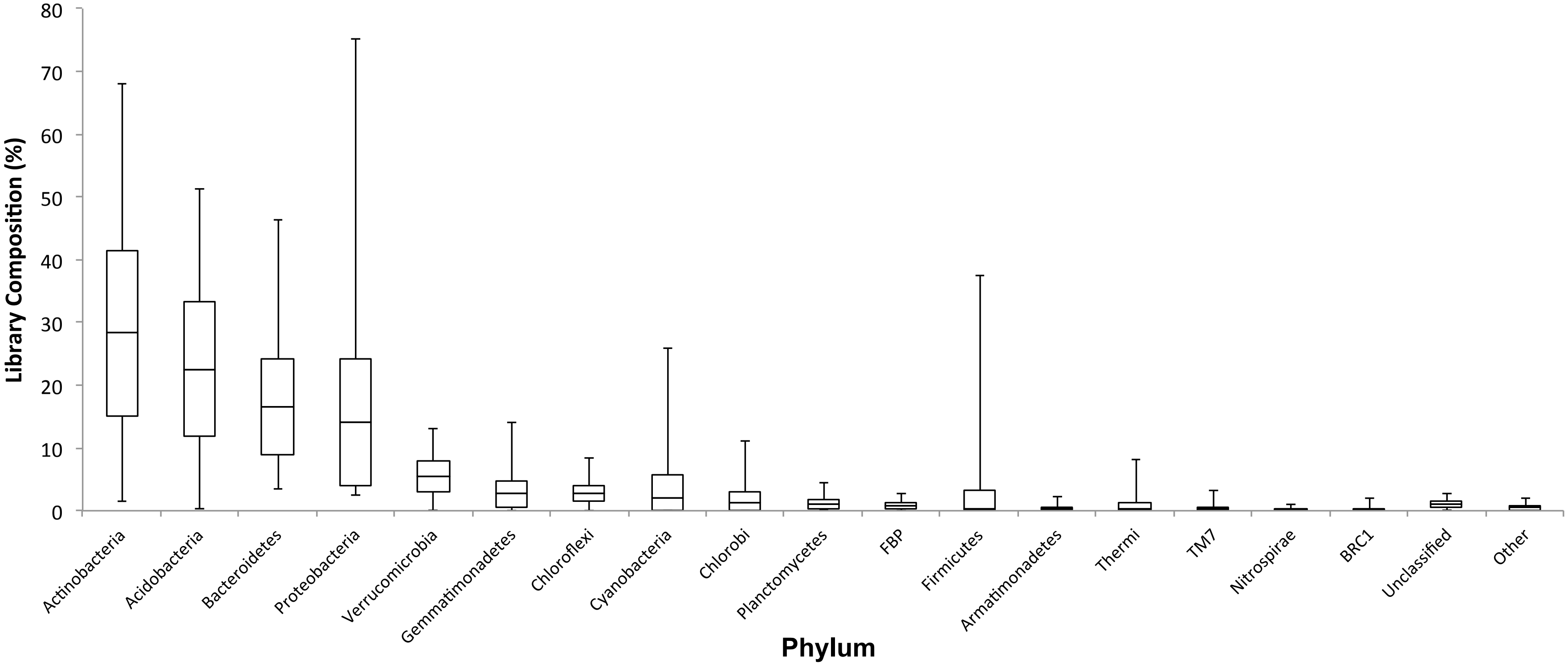

Figure 4.2: Average phylum level representation of sequences observed in 177 Antarctic Dry Valley soil samples. Horizontal lines within each box indicate mean representation of each phylum, the vertical extent of the boxes indicate one standard deviation of the mean, and the extent of the vertical bars indicate the minimum and maximum representation of each phylum. All phyla comprising more than 1 % of sequences in any sample are shown.

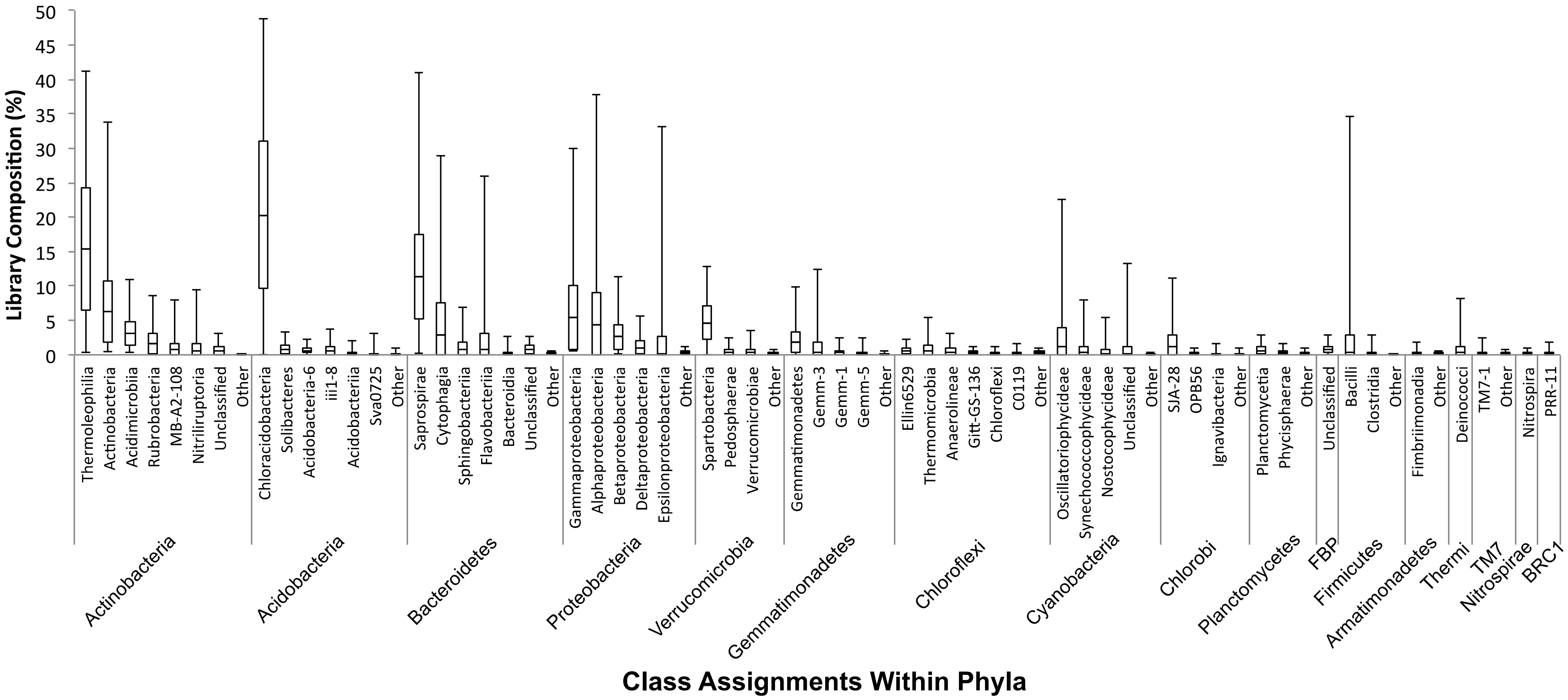

Figure 4.3: Average class level representation of sequences observed in 177 Antarctic Dry Valley soil samples. Horizontal lines within each box indicate mean representation of each class, the vertical extent of the boxes indicate one standard deviation of the mean, and the extent of the vertical bars indicate the minimum and maximum representation of each class. All classes comprising more than 1 % of sequences in any sample are shown.

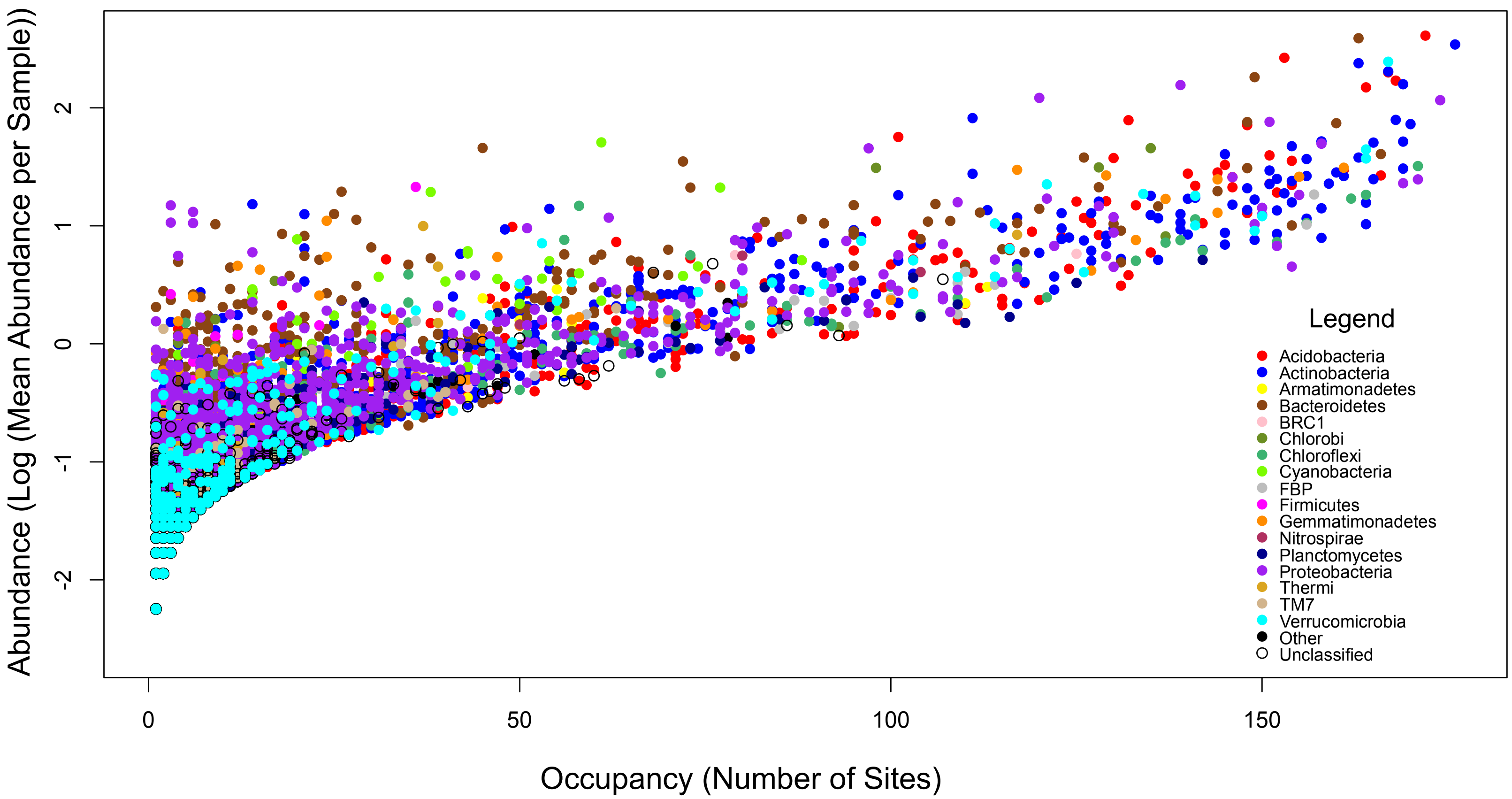

Figure 4.4: Relationship between average OTU abundance and occupancy (number of samples in which an OTU was observed).

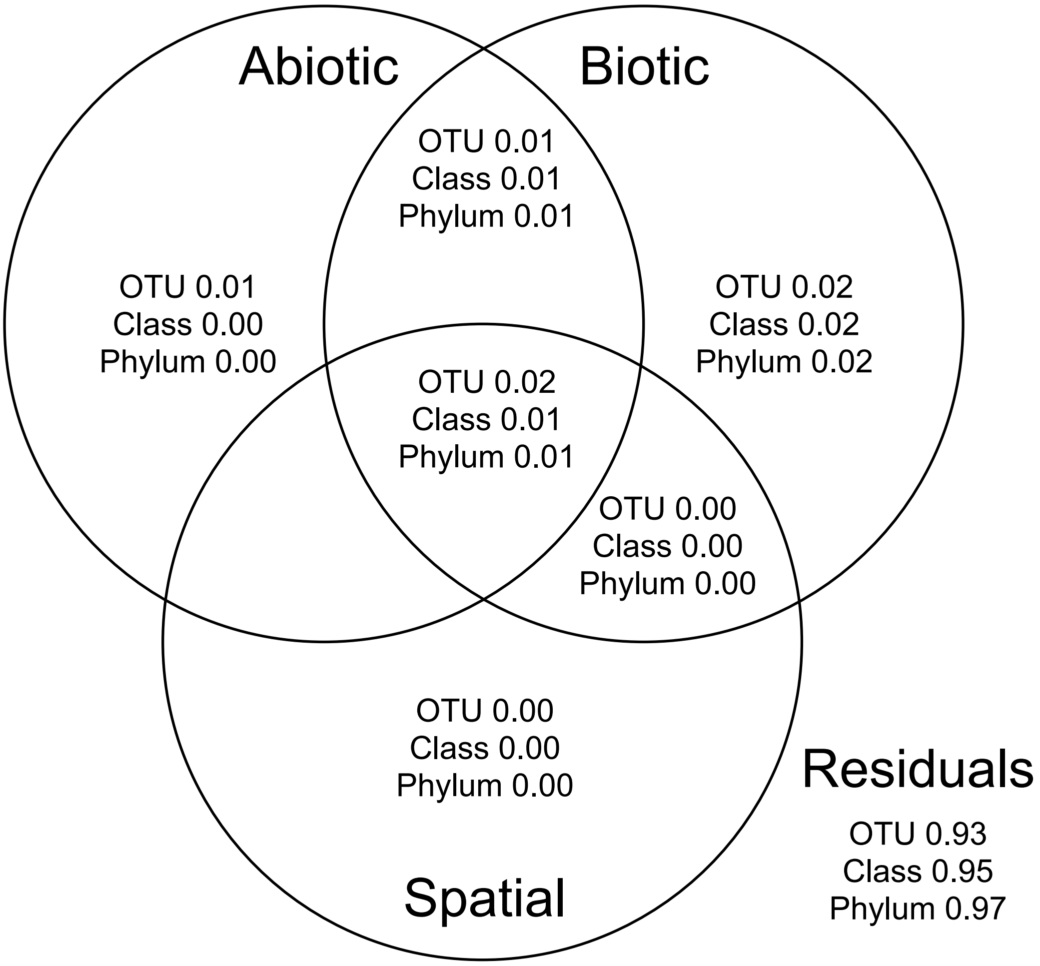

Figure 4.5: Venn diagram indicating the variation in bacterial community composition at the OTU, class, and phylum levels explained by abiotic, biotic, and spatial variation.

Patterns of Bacterial Taxa Co-occurrence

Pairwise comparisons of the representation of the dominant bacterial groups revealed the abundance of several phyla were significantly (two-sided pseudo p-values < 0.05, based on 100 bootstraps) correlated with each other (Table 4.4). The relationships between the four dominant phyla were negative or neutral. *Actinobacteria* abundance was negatively correlated with both *Bacteroidetes* and *Proteobacteria* abundance, while *Acidobacteria* abundance was negatively correlated with *Proteobacteria* abundance. Strong associations involving less abundant phyla included negative correlations between *Acidobacteria* and *Firmicutes*, between *Thermi* and *Chlorobi*, and between *Actinobacteria* and both *Cyanobacteria* and *Nitrospirae*. The strongest positive correlations were observed between *Gemmatimonadetes* and *Chloroflexi*, between *Acidobacteria* and both *Verrucomicrobia* and FBP, and between *Actinobacteria* and both *Chloroflexi* and FBP.

Table 4.1: Spearman rank correlations of abiotic variables found to explain the greatest variation in bacterial community composition at the phyla, class, and OTU levels. Analyses were completed using the BIO-ENV procedure in PRIMER 6, with 177 samples and a total of 12 abiotic variables*. The highest correlations at all taxonomic levels were from the combination of conductivity, soil water content, and pH.

	Phyla	Class	$OTU_{0.03}$
Conductivity, Water, and pH	0.161	0.195	0.223
Conductivity (alone)	0.121	0.168	0.216
Water (alone)	0.063	0.068	0.082
pH (alone)	0.063	0.068	0.082

*Other variables included in analyses: elevation, slope, aspect, coast distance, wetness index, snow cover index, average summer temperature, soil carbon content, soil nitrogen content.

Table 4.2: Spearman rank correlations of biotic variables found to explain the greatest variation in bacterial community composition at the phyla, class, and OTU levels. Analyses were completed using the BIO-ENV procedure in PRIMER 6, with 177 samples and a total of 9 variables*. The highest correlations at all taxonomic levels were from the combination of Eudorylaimus, Springtails, and Moss.

	Phyla	Class	$OTU_{0.03}$
Moss, Springtails, Eudorylaimus	0.208	0.215	0.237
Springtails, Eudorylaimus	0.201	0.211	0.231
Eudorylaimus (alone)	0.182	0.192	0.210
Springtails (alone)	-0.018	-0.014	-0.001
Moss (alone)	-0.030	-0.024	-0.029

*Other variables included in analyses: lichens, mites, Scottnema, Plectus, rotifers, tardigrades.

Table 4.3 (next page): Pearson's correlation coefficients indicating the strength of associations between taxa relative abundance and abiotic and biotic variables in 177 soil samples. Significant correlations ($p < 0.05$) are indicated in bold, with positive correlations highlighted red and negative correlations highlighted blue.

	Conductivity	Water	pH	Eudorylaimus	Springtails	Moss
Actinobacteria	**0.17**	**-0.19**	0.02	-0.05	**-0.18**	-0.09
Thermoleophilia	0.05	**-0.17**	0.04	0.01	**-0.16**	-0.06
Actinobacteria	**0.23**	**-0.17**	0.03	-0.13	-0.12	-0.12
Acidimicrobiia	0.11	-0.07	0.05	-0.03	-0.04	-0.07
Rubrobacteria	**0.20**	-0.13	-0.04	-0.06	**-0.20**	-0.07
MB.A2.108	-0.11	-0.02	**0.15**	0.12	-0.10	0.02
Nitriliruptoria	**0.27**	-0.12	0.05	**-0.22**	**-0.23**	-0.04
Acidobacteria	**-0.22**	0.02	0.09	**0.24**	0.03	-0.01
Chloracidobacteria	**-0.18**	0.00	0.04	**0.23**	0.05	-0.04
Solibacteres	**-0.33**	**0.19**	0.06	**0.29**	0.11	-0.05
Acidobacteria.6	**-0.20**	0.07	0.11	**0.18**	0.04	0.03
iii1.8	**-0.33**	0.10	0.04	**0.26**	0.12	-0.02
Acidobacteriia	0.11	-0.11	0.12	-0.07	-0.08	0.02
Sva0725	-0.08	-0.02	0.12	-0.02	0.02	-0.01
Bacteroidetes	-0.13	0.11	**-0.17**	-0.01	0.13	-0.13
Saprospirae	**-0.31**	0.10	0.01	**0.25**	0.13	-0.07
Cytophagia	0.12	0.03	**-0.16**	**-0.17**	0.02	-0.12
Sphingobacteriia	0.02	0.07	0.06	-0.09	0.09	-0.03
Flavobacteriia	0.11	-0.01	0.01	**-0.16**	0.04	0.03
Bacteroidia	-0.01	-0.12	0.13	0.01	0.03	0.12
Proteobacteria	0.05	0.01	-0.02	-0.09	0.07	0.06
Gammaproteobacteria	0.13	-0.02	-0.09	**-0.16**	0.02	0.01
Alphaproteobacteria	0.05	-0.07	0.04	-0.12	0.05	0.04
Betaproteobacteria	-0.04	**0.21**	0.06	0.12	0.11	-0.03
Deltaproteobacteria	**-0.19**	0.12	-0.03	0.08	**0.19**	0.05
Epsilonproteobacteria	-0.03	**-0.16**	0.06	0.08	-0.02	0.10
Verrucomicrobia	**-0.20**	0.03	**0.19**	**0.21**	0.02	0.02
Spartobacteria	**-0.15**	-0.04	0.11	**0.21**	-0.02	0.03
Pedosphaerae	**-0.29**	0.12	0.09	0.11	**0.18**	0.00
Verrucomicrobiae	0.02	**0.19**	-0.08	-0.06	0.14	-0.02
Gemmatimonadetes	**0.16**	0.02	-0.05	0.05	0.00	-0.05
Gemm-3	**0.33**	**-0.16**	-0.08	**-0.23**	-0.11	-0.07
Gemm-1	**-0.19**	**0.15**	**0.23**	**0.21**	0.11	0.09
Gemm-5	0.12	0.05	0.04	-0.03	0.04	0.05
Chloroflexi	0.10	-0.03	0.05	0.04	0.05	-0.05
Ellin6529	**-0.18**	-0.07	**0.16**	**0.17**	-0.01	0.01
Thermomicrobia	**0.35**	-0.11	0.02	**-0.15**	**-0.16**	0.00
Anaerolineae	-0.05	0.09	0.09	0.03	**0.17**	0.05
Gitt.GS.136	**-0.15**	0.02	0.09	**0.18**	0.03	0.03
Chloroflexi	**-0.26**	0.03	0.11	**0.20**	0.09	0.00
C0119	**-0.26**	-0.07	0.09	**0.26**	0.12	0.02
Cyanobacteria	-0.04	**0.18**	-0.04	0.07	**0.16**	0.09
Oscillatoriophycideae	0.01	0.14	-0.04	0.07	0.07	0.13
	-0.13	**0.15**	-0.08	0.12	**0.22**	0.02
Nostocophycideae	-0.11	**0.20**	-0.09	0.14	**0.15**	0.07
Chlorobi	**-0.27**	**0.18**	0.02	**0.27**	**0.17**	0.03
SJA-28	**-0.29**	**0.23**	-0.03	**0.29**	0.13	0.02
OPB56	-0.06	-0.02	0.06	0.07	0.08	0.06
Ignavibacteria	0.03	0.11	-0.01	0.06	0.11	-0.07
Planctomycetes	0.04	-0.01	**0.16**	-0.01	0.09	0.10
Planctomycetia	0.05	-0.03	**0.15**	-0.05	0.06	0.10
Phycisphaerae	-0.10	-0.01	0.13	**0.16**	0.08	-0.02
FBP	0.08	-0.13	0.02	0.01	-0.09	-0.12
Firmicutes	0.07	0.03	-0.03	-0.09	0.08	-0.02
Bacilli	0.08	0.04	-0.02	-0.07	0.12	-0.02
Clostridia	0.05	-0.01	-0.12	-0.05	0.12	-0.02
Armatimonadetes	-0.13	-0.03	0.06	**0.19**	0.14	-0.02
Thermi	**0.28**	-0.10	0.02	**-0.28**	-0.14	-0.08
TM7	**-0.18**	0.09	0.07	0.12	**0.18**	0.05
Nitrospirae	-0.10	**0.17**	-0.04	0.09	**0.15**	0.04
BRC1	0.05	-0.12	0.10	-0.05	-0.09	0.02

Table 4.4: SparCC correlation coefficients indicating the strength of relationships between the abundances of phyla in 177 Dry Valley soil libraries. Significant correlations (two-sided pseudo p-values < 0.05, based on 100 bootstraps) are indicated in bold, with positive correlations highlighted red and negative correlations highlighted blue.

	Actinobacteria	Acidobacteria	Bacteroidetes	Proteobacteria	Verrucomicrobia	Gemmatimonadetes	Chloroflexi	Cyanobacteria	Chlorobi	Planctomycetes	FBP	Firmicutes	Armatimonadetes	Thermi	TM7	Nitrospirae
Acidobacteria	0.16															
Bacteroidetes	**-0.30**	-0.08														
Proteobacteria	**-0.26**	**-0.37**	-0.04													
Verrucomicrobia	0.08	**0.48**	**-0.37**	**-0.23**												
Gemmatimonadetes	**0.19**	-0.15	**-0.17**	**-0.15**	0.08											
Chloroflexi	**0.45**	-0.02	-0.13	**-0.34**	**0.17**	**0.62**										
Cyanobacteria	**-0.58**	-0.13	**0.16**	**0.28**	**-0.16**	**-0.17**	**-0.38**									
Chlorobi	**-0.37**	**0.33**	0.06	**-0.20**	**0.32**	0.03	-0.04	0.07								
Planctomycetes	**-0.18**	**-0.25**	**-0.37**	0.11	**0.26**	**0.19**	**0.15**	0.11	0.01							
FBP	**0.45**	**0.55**	**0.16**	**-0.24**	**0.17**	0.11	**0.28**	**-0.16**	**-0.18**	**-0.20**						
Firmicutes	**-0.22**	**-0.57**	**0.15**	**0.30**	**-0.34**	**0.16**	**0.12**	0.10	**-0.22**	**0.15**	**-0.30**					
Armatimonadetes	-0.14	**0.34**	**-0.16**	**-0.20**	**0.35**	**0.17**	0.08	-0.02	0.13	0.13	**0.24**	-0.07				
Thermi	**0.31**	**-0.30**	0.12	0.05	**-0.35**	**0.21**	**0.24**	0.03	**-0.57**	0.01	**0.26**	**0.18**	-0.11			
TM7	**-0.31**	0.02	0.01	-0.08	**0.19**	0.11	-0.02	**0.13**	**0.35**	**0.20**	**-0.25**	0.03	0.11	**-0.21**		
Nitrospirae	**-0.51**	-0.07	0.07	0.03	0.07	0.00	**-0.24**	**0.32**	**0.37**	**0.18**	**-0.31**	0.04	0.01	**-0.25**	**0.31**	
BRC1	**0.28**	-0.01	0.08	**-0.16**	0.00	**0.18**	**0.30**	**-0.28**	-0.12	0.08	**0.21**	0.02	**-0.13**	**0.24**	0.07	**-0.22**

Discussion

Community Composition

The bacterial community compositions reported here support the idea that Dry Valley soils are fundamentally different from those found in other soil habitats globally, including other terrestrial regions of Antarctica (Cary *et al*., 2010). The dominant representation of the phyla *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Proteobacteria* in sample libraries is consistent with findings in previous culture independent surveys of Dry Valley soils, as is the poor representation of *Proteobacteria* in relation to the other three dominant phyla (Cary *et al*., 2010). Globally, soils tend to be dominated by *Proteobacteria*, particularly *Alphaproteobacteria* (Janssen, 2006; Lauber *et al*., 2009), and this holds true for many soil communities that have been characterized on the Antarctic Peninsula (Yergeau *et al*., 2007). The reduced relative abundance of *Proteobacteria* in Dry Valley soils is therefore a notable characteristic of these communities, and may be reflective of the unique abiotic conditions of the Dry Valley landscape. Indeed, the community compositions observed here are largely consistent with the global patterns of taxa representation that would be predicted given the hyper-arid, alkaline, and oligotrophic nature of the Dry Valley soil environment (Lauber *et al*., 2009), and the closest matches of dominant OTUs were predominantly to bacteria described from other extreme environments (see Supplementary Table 4.1).

In temperate soils, *Actinobacteria* account for approximately 13 % of bacterial communities on average (Janssen, 2006), and tend to be more abundant in soils of high pH and low soil moisture (Lauber *et al*., 2009). In the present study, *Actinobacteria* comprised nearly 30 % of samples on average. In arid and hyper-arid environments *Actinobacteria* can be highly dominant, as demonstrated recently in analyses of soils from the Atacama Desert, where they accounted for 72 to 88 % of community libraries (Crits-Christoph *et al*., 2013). *Actinobacteria* are often abundant in dry soil crusts and their relative abundance can drop drastically and rapidly in response to wetting events (Angel and Conrad, 2013; Placella *et al*., 2012). In the Dry Valleys, *Actinobacteria* relative abundance was found to drop drastically within three years in response to physical changes to the soil environment, including increased humidity, stabilized temperature, and reduced ultraviolet irradiance, imparted by the presence of a mummified seal carcass (Tiao *et al.*, 2012). The majority of sequences observed in the current study grouped to the order *Solirubrobacterales*, which are found in diverse soil environments and are an important component of biological soil crusts in arid environments (Reddy and Garcia-Pichel, 2009).

Acidobacteria are among the most abundant bacterial phyla in soils globally (Lauber *et al.*, 2009; Janssen, 2006) and tend to be most abundant in acidic soils (Jones *et al.*, 2009). In the current study, however, acidobacterial sequences grouped predominantly to *Acidobacteria* subdivision 4 (Joseph *et al.*, 2003), which tend to increase in relative abundance with increasing soil pH (Jones *et al.*, 2009). While this group remains poorly understood, its members are thought to be well adapted to oligotrophic conditions (Eichorst *et al.*, 2011). The high abundance of the class *Chloracidobacteria* reported here is consistent with recent descriptions of bacterial community composition in soils from the Garwood Valley (Fierer *et al.*, 2012), and indicates that this class of bacteria are ubiquitously distributed in soils across the region.

Bacteroidetes are also abundant in soils globally, and their high relative abundance in Dry Valley soils is consistent with their tendency to be most abundant in neutral and alkaline soils (Lauber *et al.*, 2009). Here, *Bacteroidetes* were found to be abundant in all soils analyzed, and were dominated by sequences grouping to the family *Chitinophagaceae* and, to a lesser extent, several families within the class *Cytophagia*. Interestingly, *Bacteroidetes* are largely copiotrophic in temperate soils, which would suggest they are best adapted to nutrient rich environments (Fierer *et al.*, 2007); however, many of the closest matches to the dominant *Bacteroidetes* OTUs reported here were also from oligotrophic environments.

Though *Proteobacteria* were underrepresented here in comparison with more temperate locations, they still comprised a substantial proportion of all Dry Valley samples analyzed. *Gammaproteobacteria* were the dominant class, which is consistent with recent reports of high *Gammaproteobacteria* representation within an otherwise poorly represented proteobacterial phylum in arid soils of the Atacama Desert (Neilson *et al.*, 2012). *Alphaproteobacteria*, which typically dominate temperate soils (Janssen, 2006; Lauber *et al.*, 2009), were the second most abundant class in the phylum. Representatives of the order

Sphingomonadales accounted for 53 % of the *Alphaproteobacteria*, which is consistent with trends that show increased relative abundance of this order in soils of high pH, despite the opposite trend being observed for the class *Alphaproteobacteria* as a whole (Lauber *et al*., 2009).

Other phyla in the Dry Valley soil libraries were represented at similar relative abundances to those observed in temperate soils (Janssen, 2006; Lauber *et al*., 2009) or were only minor constituents of the libraries. While many of these phyla were represented across the majority of sites, the number of sites in which a given OTU was present (occupancy) was strongly related to its abundance, and the occupancy of OTUs from all phyla varied greatly (see Figure 4.4). The vast majority of OTUs from all phyla were observed in very few samples, supporting the understanding that Dry Valley soil communities are highly heterogeneous (Lee *et al*., 2012), even over relatively small spatial scales.

Relating Community Composition to Environmental Variation

Variation in community composition was best explained by variation in soil conductivity as a single abiotic variable, and by soil conductivity, water content, and pH in combination. This is consistent with findings of community fingerprinting analyses of 471 Dry Valley soils samples, which also revealed water, pH, and conductivity to be the most important factors in shaping community compositions across the study area (Bottos *et al*., Chapter 3). However, the degree of variation in community composition explained by abiotic variation in the present study was lower than that observed based on community fingerprinting analyses.

Discrepancies between the findings of the sequencing analyses presented here and previous ARISA fingerprinting analyses of these samples may be expected for several reasons. Firstly, the methods of DNA extraction and the targets of PCR amplification differ between the studies, so biases inherent to these procedures may explain variability between the findings (Suzuki & Giovannoni, 1996; Martin-Laurent *et al*., 2001). Secondly, the phylogenetic level at which ARISA differentiates taxa is difficult to define, as the length of the intergenic spacers targeted by ARISA do not vary consistently with 16S rRNA gene sequence identity (Brown and Fuhrman, 2005). Finally, while the samples analyzed in the current study are representative of the entire sample set analyzed by fingerprinting, the current study includes less than half of the samples analyzed by fingerprinting. Despite efforts to capture the full extent of the environmental heterogeneity in this study, samples with high soil moisture and wetness index were not well represented in the sequenced dataset (see Figure 4.1e), so variation in community composition related to gradients of soil moisture may not be as well reflected here as they were in analyses of the entire sample set. With increased sample sequencing, particularly of samples with high soil moisture content, the strength of the relationships between community composition and abiotic variation may be expected to converge with those found in analyses based on community fingerprinting.

Soil geochemistry was also a poor predictor of bacterial community composition at the phyla, class and OTU level. Soil elemental compositions may result from weathering of parent material, atmospheric deposition, and leaching from sedimentary rock (Campbell and Claridge, 1987). Elemental composition of soils has been linked to bacterial distributions in comparisons between communities from geologically disparate locations in the Dry Valleys, but has not been found to be an important determinant within valleys (Wood *et al.*, 2008; Lee *et al.*, 2012). In these instances of inter-valley comparison, it is difficult to determine whether the distributions observed are driven by soil geochemistry or other variables that distinguish the environments. That no association between soil geochemistry and bacterial community composition was observed here suggests that underlying geology of soils is not an important determinant of bacterial community composition, at least across the range of soil types represented in the current study area.

Despite the weak link between abiotic variation and community composition overall, the distributions of some taxa showed significant relationships with abiotic gradients (see Table 4.3). *Actinobacteria* were found predominantly in soils of low soil moisture and high conductivity, which suggests their dominance in samples arise from their ability to tolerate the arid and highly saline soils that dominate the landscape. Only *Thermi* and classes within the *Gemmatimonadetes* and *Chloroflexi* also showed positive associations with conductivity, and only *Epsilonproteobacteria* and a single class within the *Gemmatimonadetes* (Gemm-3) also showed negative associations with soil moisture. Conversely, *Cyanobacteria* were most prevalent in soils of high soil moisture, where they may be particularly important as primary producers (Vincent, 2002). These environmental associations, though significant, were weak, suggesting that measured abiotic variation is a poor determinant of taxa distributions alone.

Weak significant relationships between bacterial community composition and the distributions of multicellular taxa were also observed (see Table 4.3). The distribution of the nematode *Eudorylaimus* was most strongly associated with bacterial community composition as a single biotic variable, which may reflect its negative association with soil conductivity rather than a true biotic interaction. Indeed, the significant relationships observed between *Eudorylaimus* and bacterial taxa in analyses of pairwise correlations strongly reflect the shared associations with soil conductivity. The positive associations observed between springtails and cyanobacteria may reflect an association between springtails and high productivity niches within the system (Hogg *et al.*, 2006).

Patterns of Taxa Co-occurrence

The abundance of several bacterial taxa were significantly coupled to one another, suggesting similar abiotic drivers may influence multiple taxa or that interactions between bacterial groups may have an important role in shaping community composition. Functional assessment of Dry Valley microbial communities in McKelvey Valley completed by Chan and colleagues (2013) may shed light on the distributions of taxa and patterns of taxa co-occurrence observed here. The abundance of stress response pathways for cold shock, osmotic stress, radiation/desiccation stress, and nutrient limitation found in *Actinobacteria* in communities of McKelvey Valley may explain the dominance of this phylum across Dry Valley soils. *Actinobacteria* only showed positive correlations with phyla of relatively low representation, but these phyla, which include the *Chloroflexi,* and *Thermi*, were also found to have stress response pathways to cope with desiccation and nutrient limitation in McKelvey Valley soils. Additionally, *Chloroflexi* had a strong positive association with *Gemmatimonadetes* (particularly class 3), which was found to have stress response pathways for desiccation in McKelvey Valley soils. The positive relationships between these taxa may be related to their shared ability to tolerate environmental stresses, particularly desiccation stress; however, the strong negative associations with other abundant and ecologically important taxa may indicate these groups are outcompeted in particular niches.

Actinobacteria were most strongly negatively associated with *Cyanobacteria* and *Nitrospirae*. As the dominant autotrophs in Dry Valley soils, *Cyanobacteria* are important keystone taxa, capable of fixing carbon and nitrogen (Vincent, 2002). These nutritional amendments to otherwise oligotrophic soils, may favor secondary colonization by more copiotrophic taxa and taxa integral to nitrogen cycling. Indeed, *Bacteroidetes*, which were negatively associated with *Actinobacteria*, were positively associated with *Cyanobacteria*. The co-occurrence of *Bacteroidetes* with *Cyanobacteria* has been noted previously in Dry Valley stream communities (Stanish *et al*., 2013), and the findings here suggest this relationship is also important in soils across the landscape. *Bacteroidetes* are known to degrade a diverse range of complex carbohydrates, including exocellular polysaccharides produced by algae and cyanobacteria (Thomas *et al*., 2011). Additionally, *Bacteroidetes* may be ecologically important in Dry Valley soils as denitrifiers (Chan *et al*., 2013), and so their co-occurrence with nitrogen fixing *Cyanobaceria* may be functionally important to nitrogen cycling in the system. The strong positive associations between *Cyanobacteria* and *Nitrospirae* (likely important nitrifiers in the system) may also be important to nitrogen cycling, while the positive association between *Cyanobacteria* and *Proteobacteria*, particularly the *Alpha- Beta-* and *Delta- Proteobacteria*, may be important for both nitrogen and carbon carbohydrate cycling (Chan *et al*., 2013).

Acidobacteria were strongly positively correlated with *Verrucomicrobia*, and co-occurrence of OTUs from these phyla has been reported previously in soils from North America, South America, and Antarctica (Barberán *et al*., 2012). While the ecological role of *Verrucomicrobia* remains unclear, they, like *Acidobacteria*, are thought to be particularly important in oligotrophic soils (Bergmann *et al*., 2011). In McKelvey Valley soils, *Acidobacteria* and *Verrucomicrobia* were both found to have stress response pathways to cope with nutrient limitation, but appeared less capable than *Actinobacteria* and its associated taxa of coping with radiation/desiccation stress (Chan *et al*., 2013).

Conclusions

Here, patterns of bacterial distributions were evaluated across a Dry Valley landscape. Though distinctive, the community compositions of Dry Valley soils do not represent a departure from the trends in taxa distribution that are observed globally in response to abiotic variation. Indeed, the most abundant taxa in the system would be predicted to be present in Dry Valley soils based on trends in their distribution globally in relation to pH (Lauber *et al*., 2009; Jones *et al*., 2009). In addition, the dominant OTUs observed here were most closely related to bacteria found in other cold, arid, and oligotrophic environments. Within the system, patterns of community structure (evaluated at the phylum, class, and OTU level) and individual taxa distributions were only weakly associated with abiotic variation and variation in distributions of multicellular taxa; however, strong and significant relationships existed between the relative abundances of specific bacterial taxa. These patterns of taxa co-occurrence suggest that the distributions of keystone taxa are influenced by abiotic conditions, which in turn influence the distributions of other taxa. Increased understanding of the functional relationships between taxa may, therefore, reveal processes important to soil colonization, which in turn have important implications for ecosystem structure and function.

Acknowledgements

This research was supported through an IPY award from the Foundation for Research, Science and Technology of New Zealand (UOWX0710 and UOWX0715), through the National Science Foundation (NSF OPP ANT-0944556 and ANT-0944560), and through a Community Sequencing Program funded by the US Department of Energy. DCL, CKL, IRM, and SCC were supported by the New Zealand Marsden Fund (UOW1201, UOW1003, and UOW0802). Logistics support for the field seasons was provided by Antarctica New Zealand. We especially thank the many researchers who were involved in the New Zealand Terrestrial Antarctic Biocomplexity Survey for their time and commitment to this work.

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Supplementary Information

Supplementary Table 4.1: Analysis of the 10 most abundant OTUs detected in Dry Valley soil communities, including their RDP assignments and closest matches in the NCBI database. Only published entries are reported, except where unpublished entries alone were the closest match.

OTU	Mean Abundance (±SD)	Occupancy (Number of Samples)	RDP Classification* and Assignment Confidence	Closest Match in NCBI	Source of NCBI Match	Accession **	Identity	Reference
00004	4.08 (±2.81)	172	p_Acidobacteria(100%) c_Chloracidobacteria(100%) o_RB41(100%) f_Ellin6075(100%)	Isolate Ellin6075	Soil, Australia	AY234727	100%	(Joseph *et al.*, 2003)
				Uncultured	Soil, near Moab, USA	eg. JQ402009	100%	(Johnson *et al.*, 2012)
				Uncultured	Soil, Schirmacher Oasis, Antarctica	eg. JX555937	100%	(Teo and Wong, 2014)
				Uncultured	Epilithic biofilm, Ireland	JQ627523	100%	(Ragon *et al.*, 2012)
				Uncultured	Gold mine tailings	AY274121	100%	(Nemergut *et al.*, 2004)
				Uncultured	Arctic soil, Alert, Canada	JQ307037	100%	(Lynch *et al.*, 2012a)
				Uncultured	Arid Shrublands, Moab, USA	JN795635	100%	(Kuske *et al.*, 2012)
				Uncultured	Concrete, Chernobyl, Ukraine	JN020220	100%	(Ragon *et al.*, 2011)
				Uncultured	Soil, Alexander Isl., Antarctica	FN811211	100%	(Chong *et al.*, 2012)
				Uncultured	Skin microbiome	HM332819	100%	(Kong *et al.*, 2012)
				Uncultured	Soil, Atacama Desert, Chile	AY615378	100%	(Warren-Rhodes *et al.*, 2006)
				Uncultured	Ice core, Antarctica	EF127621	100%	(Bidle *et al.*, 2007)
				Uncultured	Soil, Dry Valleys, Antarctica	FJ490263	100%	(Pointing *et al.*, 2009)
				Uncultured	Hnausahraun lava flow, Iceland	GQ495286	100%	(Kelly *et al.*, 2011)
				Uncultured	Mine soils, USA	EU141937	100%	(Rastogi *et al.*, 2010)
00018	3.88 (±3.57)	163	p_Bacteroidetes(100%) c_Saprospirae(100%) o_Saprospirales(100%) f_Chitinophagaceae(100%)	Uncultured	Soil, Darwin Mtns, Antarctica	KC442662	100%	(Aislabie *et al.*, 2013)
				Uncultured	'FACE' soil sample	JQ373129	100%	(Dunbar *et al.*, 2012)
				Uncultured	Soil, Schirmacher Oasis, Antarctica	JX555951	100%	(Teo and Wong, 2014)

				Uncultured	Soil, Atacama Desert, Chile	JX098578	100%	(Lynch *et al.*, 2012b)
				Uncultured	Permafrost, Alaska, USA	JF829180	100%	(Coolen *et al.*, 2011)
				Uncultured	Tundra soil, Alert, Canada	JF508314	100%	(Bartram *et al.*, 2011)
				Uncultured	Soil, Livingston Isl., Antarctica	HQ153331	100%	(Ganzert *et al.*, 2011)
				Uncultured	Soil, Alexander Isl., Antarctica	FN811206	100%	(Chong *et al.*, 2012)
				Uncultured	Hnausahraun lava flow, Iceland	GQ495413	100%	(Kelly *et al.*, 2011)
				Uncultured	Soil, Roopkund Glacier, India	GQ366423	100%	(Pradhan *et al.*, 2010)
				Uncultured	Freshwater, Russia	FJ694289	100%	(Crump *et al.*, 2009)
				Uncultured	Soil, Socompa Volcano, Andes	FJ592618	100%	(Costello *et al.*, 2009)
				Uncultured	Agricultural soil, USA	EU353360	100%	(Morales *et al.*, 2009)
				Uncultured	Sediment, Wright Valley, Antarctica	EU869765	100%	(Zeglin *et al.*, 2011)
				Uncultured	Mixed grass prairie soil, USA	EU133650	100%	(Elshahed *et al.*, 2008)
				Uncultured	Grassland soil, USA	EF663729	100%	(Jangid *et al.*, 2011)
				Uncultured	Gold mine tailings	AY274132	100%	(Nemergut *et al.*, 2004)
00001	3.4 (±3.38)	176	p_Actinobacteria(100%) c_Thermophilia(100%) o_Solirubrobacterales(100%)	Uncultured	Soil, near Moab, USA	JQ401021	100%	(Johnson *et al.*, 2012)
				Uncultured	‘FACE’ soil sample	JQ374804	100%	(Dunbar *et al.*, 2012)
				Uncultured	Soil, Moab, USA	JN795864	100%	(Kuske *et al.*, 2012)
				Uncultured	Semiarid soil, Israel	JF295925	100%	(Bachar *et al.*, 2012)
				Uncultured	Skin microbiome	HM270230	100%	(Kong *et al.*, 2012)
				Uncultured	Semiarid soil, Israel	GQ425686	100%	(Bachar *et al.*, 2010)
				Uncultured	Soil, Dry Valleys, Antarctica	FJ895056	100%	(Pointing *et al.*, 2009)
				Uncultured	Soil, Socompa Volcano, Andes	FJ592846	100%	(Costello *et al.*, 2009)
				Uncultured	Hydrocarbon contaminated soil	FM209078	100%	(Militon *et al.*, 2010)
				Uncultured	Soil, Luther Vale, Antarctica	EF465022	100%	(Niederberger *et al.*, 2008)
				Uncultured	Endolith associated soils, Alps, Switzerland	AB257684	100%	(Horath and Bachofen, 2009)

				Uncultured	Human microbiome	EF702110	100%	(Frank *et al*., 2007)
				Uncultured	Human microbiome	DQ188325	100%	(Harris *et al*., 2007)
00041	2.65 (±3.09)	153	p_Acidobacteria(100%) c_Chloracidobacteria(100%) o_RB41(100) f_Ellin6075(100%)	Uncultured	Hypolith, Miers Valley, Antarctica	HQ197608	100%	(Khan *et al*., 2011)
				Uncultured	Soil, Socompa Volcano, Andes	eg. FJ592791	100%	(Costello *et al*., 2009)
				Uncultured	Sediment, Wright Valley, Antarctica	EU869693	100%	(Zeglin *et al*., 2011)
00012	2.46 (±1.78)	167	p_Verrucomicrobia(100%) c_Spartobacteria(100%) o_Chthoniobacterales(100%) f_Chthoniobacteraceae(100%) g_DA101(97)	Uncultured	'FACE' soil sample	JQ367764	100%	(Dunbar *et al*., 2012)
				Uncultured	Loamy sand, La Jolla, USA	HQ118794	100%	(Williamson *et al*., 2011)
				Uncultured	Ice wedge, Axel Heiberg Isl., Canada	JN220079	100%	(Wilhelm *et al*., 2012)
				Uncultured	Arid Soils, Israel	GQ425504	100%	(Bachar *et al*., 2010)
				Uncultured	Hnausahraun lava flow, Iceland	GQ495401	100%	(Kelly *et al*., 2011)
				Uncultured	Soil, Socompa Volcano, Andes	eg. FJ592671	100%	(Costello *et al*., 2009)
				Uncultured	Sediment, Wright Valley, Antarctica	EU869793	100%	(Zeglin *et al*., 2011)
00006	2.38 (±3.51)	163	p_Actinobacteria(100%) c_Actinobacteria(100%) o_Actinomycetales(100%)	Uncultured	Hypolith, Miers Valley, Antarctica	eg. HQ197638	100%	(Khan *et al*., 2011)
				Uncultured	Snow with Saharan dust layer, Mont Blanc, France	HM104608	100%	(Chuvochina *et al*., 2011)
				Uncultured	Soil, Miers Valley, Antarctica	eg. EU931049	100%	(Babalola *et al*., 2009)
				Uncultured	Soil, Luther Vale, Antarctica	EF464909	100%	(Niederberger *et al*., 2008)
				Uncultured	Hydrocarbon contaminated soil, Ross Island, Antarctica	AY571815	100%	(Saul *et al*., 2005)
00007	2.03 (±2.29)	167	p_Actinobacteria(100%) c_Thermophilia(100%) o_Solirubrobacterales(100%)	Uncultured	Mummified seal associated soil, Miers Valley, Antarctica	EU335887	100%	Unpublished
				Uncultured	Ice core, Antarctica	EF127610	99%	(Bidle *et al*., 2007)
				Uncultured	Arid soil, Israel	GQ425307	98%	(Bachar *et al*., 2010)
				Uncultured	Soils, near Moab, USA	JQ400273	98%	(Johnson *et al*., 2012)
				Uncultured	'FACE' soil sample	eg. JQ369469	97%	(Dunbar *et al*., 2012)

				Uncultured	Rhizosphere soil, India	JQ793391	96%	(Yousuf *et al.*, 2012)
				Uncultured	Arid soils, Israel	GQ425486	96%	(Bachar *et al.*, 2010)
00005	2.00 (±1.95)	167	p_Acidobacteria(100%) c_Chloracidobacteria(100%) o_RB41(100) f_Ellin6075(100%)	Uncultured	Hyaloclastite deposit, Domadalshraun, Iceland	GU219707	99%	(Kelly *et al.*, 2010)
				Uncultured	Nitrogen amended dry meadow soil, USA	EU861884	99%	(Nemergut *et al.*, 2008)
				Uncultured	Sediment, Wright Valley, Antarctica	EU869708	99%	(Zeglin *et al.*, 2011)
				Uncultured	Soil, Larsemann Hills, Antarctica	JX172932	98%	(Bajerski and Wagner, 2013)
				Uncultured	Hypolith, Miers Valley, Antarctica	HQ197608	98%	(Khan *et al.*, 2011)
00098	1.82 (±1.85)	149	p_Bacteroidetes(100%) c_Saprospirae(100%) o_Saprospirales(100%) f_Chitinophagaceae(100%)	Uncultured	Oligotrophic soil, Devon Isl., Canada	EU440385	100%	Unpublished
				Uncultured	Soil, Alexander Isl., Antarctica	FN811248	99%	(Chong *et al.*, 2012)
				Uncultured	Sediment, Wright Valley, Antarctica	EU869569	99%	(Zeglin *et al.*, 2011)
				Uncultured	Soil, Miers Valley, Antarctica	DQ062912	99%	(Smith *et al.*, 2006)
				Uncultured	Snow on glacier, Tibet, China	EU527107	99%	(Liu *et al.*, 2009)
				Uncultured	Semiarid soil, Arizona, USA	EF612369	99%	(Mendez *et al.*, 2008)
00010	1.70 (±1.30)	168	p_Acidobacteria(100%) c_Chloracidobacteria(100%) o_RB41(100); f_Ellin6075(100%)	Uncultured	Tundra soil, Alert, Canada	JF508341	100%	(Bartram *et al.*, 2011)
				Uncultured	Paddy soil, China	FR687171	100%	(Liu *et al.*, 2011)
				Uncultured	Toilet biofilm, Germany	FN401287	100%	(Egert *et al.*, 2010)
				Uncultured	Soil, Socompa Volcano, Andes	FJ592776	100%	(Costello *et al.*, 2009)
				Uncultured	Soil, Luther Vale, Antarctica	EF464984	100%	(Niederberger *et al.*, 2008)
				Uncultured	Soil, Fossil Bluff, Antarctica	EF220302	100%	(Yergeau *et al.*, 2007)
				Uncultured	Puca glacier forefield soil, Peru	DQ514189	100%	(Nemergut *et al.*, 2007)
				Uncultured	Hydrocarbon contaminated soil, Ross Isl., Antarctica	AY571792	100%	(Saul *et al.*, 2005)

* *p*=phylum, *c*=class, *o*=order, *f*=family, *g*=genus

***eg*. indicates the accession number represents one of several entries from the same study

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Chapter 5: Airborne Bacterial Populations Above Desert Soils of the McMurdo Dry Valleys, Antarctica

Preface

This chapter describes the bacterial composition of aerosols in the Dry Valleys of Antarctica. This work was undertaken as a pilot study to determine the importance of bacterial inputs to local soils from the air. As primary author, I completed the sampling, was involved in the lab work, completed the data analyses, and wrote the manuscript. DNA extractions were completed by Stephen Pointing's lab group at the University of Hong Kong. Sequencing preparation and sequencing were completed with the assistance of technician Sarah Kelly and M.Sc. student Chelsea Vickers at the University of Waikato. Anthony Woo earned co-authorship by checking sample sequences for contaminating sequences. Peyman Zawar-Reza earned co-authorship by modeling back trajectories of air masses. All authors reviewed and edited manuscript content. This work has been published with the following citation:

> **Bottos EM**, Woo AC, Zawar-Reza P, Pointing SB, Cary SC. (2014). Airborne bacterial populations above desert soils of the McMurdo Dry Valleys. *Microbial Ecology* **67**: 120-128.

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Microb Ecol (2014) 67:120–128
DOI 10.1007/s00248-013-0296-y

ENVIRONMENTAL MICROBIOLOGY

Airborne Bacterial Populations Above Desert Soils of the McMurdo Dry Valleys, Antarctica

Eric M. Bottos · Anthony C. Woo · Peyman Zawar-Reza · Stephen B. Pointing · Stephen C. Cary

Received: 21 April 2013 / Accepted: 17 September 2013 / Published online: 12 October 2013

Abstract Bacteria are assumed to disperse widely via aerosolized transport due to their small size and resilience. The question of microbial endemicity in isolated populations is directly related to the level of airborne exogenous inputs, yet this has proven hard to identify. The ice-free terrestrial ecosystem of Antarctica, a geographically and climatically isolated continent, was used to interrogate microbial bio-aerosols in relation to the surrounding ecology and climate. High-throughput sequencing of bacterial ribosomal RNA (rRNA) genes was combined with analyses of climate patterns during an austral summer. In general terms, the aerosols were dominated by Firmicutes, whereas surrounding soils supported Actinobacteria-dominated communities. The most abundant taxa were also common to aerosols from other continents, suggesting that a distinct bio-aerosol community is widely dispersed. No evidence for significant marine input to bio-aerosols was found at this maritime valley site, instead local influence was largely from nearby volcanic sources. Back trajectory analysis revealed transport of incoming regional air masses across the Antarctic Plateau, and this is envisaged as a strong selective force. It is postulated that local soil microbial dispersal occurs largely via stochastic mobilization of mineral soil particulates.

Introduction

The aerosphere is important as a conduit for global transport of bacteria and possibly as an ecological niche in itself. Bacteria can be aerosolized from virtually any surface and typically reach atmospheric concentrations in excess of 1×10^4 cells/m^3 above land and marine surfaces [1–3]. Aerosolized bacteria maintain viability during their atmospheric residence and may exist in the air as spores, vegetative cells, or metabolically active and dividing cells [4]. Bacterial residence times can be several days, facilitating transport over great distances, before their removal from the atmosphere by precipitation or direct deposition onto surfaces [5]. Understanding the extent to which an environment may be impacted by the input of foreign organisms, and identifying the sources of these inputs, is important for understanding ecosystem dynamics and for monitoring ecological change.

Little is known about the contribution of bioaerosol transport to the microbial ecology of isolated systems such as the Antarctic continent. Antarctica is considered to be largely decoupled from systems of global atmospheric transport, due to its geographic isolation and barriers to the mixing of air masses caused by the Antarctic Circumpolar Current [6]. Aerosols over Antarctica are predicted to have longer residence

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Electronic supplementary material The online version of this article (doi:10.1007/s00248-013-0296-y) contains supplementary material, which is available to authorized users.

E. M. Bottos · P. Zawar-Reza · S. B. Pointing (✉) · S. C. Cary (✉)
International Centre for Terrestrial Antarctic Research, The University of Waikato, Private Bag 3105, Hamilton, New Zealand
e-mail: steve.pointing@aut.ac.nz
e-mail: caryc@waikato.ac.nz

E. M. Bottos · S. C. Cary
Department of Biological Sciences, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

A. C. Woo
Sorbonne Paris Cité, Faculté de Médecine, Université Paris Descartes, Paris Descartes, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France

P. Zawar-Reza
Department of Geography, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

S. B. Pointing
Institute for Applied Ecology New Zealand, School of Applied Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand

times in the atmosphere than those over most temperate environments [5], suggesting that long-range transport of bacteria over the continent is likely. Few analyses of airborne bacteria over Antarctica have been undertaken, with contemporary reports restricted to analyses of microorganisms in the air above Halley V Research Station on the Brunt Ice Shelf [7] and Rothera Point on the Antarctic Peninsula [8]. Aeolian transport is reported to be an important means of local redistribution of organic material and biomass in deserts globally [9] and including the cold polar deserts of Antarctica's ice-free ecosystems [10–14]; however, there have been no descriptions of bacterial diversity in these aerosols.

The McMurdo Dry Valleys in Victoria Land, Antarctica, are the largest ice-free area on the continent [15, 16], and deposition of organisms into Dry Valley soils has the potential to profoundly impact ecosystem structure and function [17, 18]. It has recently been suggested that the low bacterial numbers present in Dry Valley soils may enhance the ability of invasive colonists compared to those in soil communities of higher biomass [19]. Many of the same selective pressures faced by organisms in the Dry Valleys including desiccation, high UV radiation, low temperature, and oligotrophic conditions [16] are faced by organisms during atmospheric transport [6]. As such, many bacteria entering Antarctic soils from the atmosphere may be well adapted to survive in their new environment [20]. The likelihood of colonization may be increased by the effects of climate change, as increased temperature and water availability may favor growth of foreign organisms in Antarctic soils [6, 21]. Atmospheric processes may, therefore, have a particularly important role in shaping microbial communities in Dry Valley soils.

In this study, the diversity of airborne bacteria was assessed on the floor and ridge of Miers Valley, a location with extensive landscape-scale ecological research data on the surface ecology [18]. The objective was to set up a baseline dataset for airborne inputs of bacteria and assess how they may influence Dry Valley soils, since soil biodiversity is known to vary with elevation in this system [16, 22].

Materials and Methods

Air Sampling

Aerosol samples were collected by filtration onto 0.2-μm-pore-size polycarbonate filters by impaction using solar-powered pumps (SKC, 224-PCXR8, Eighty Four, PA, USA) mounted 1 m above the ground in each location. This height was chosen since the majority of local dust mobilization has been shown to occur at this near-ground interface (Cary et al., unpublished data). Filters inserted into cassette apparatus but not exposed to air flow were used as controls. All filters and cassettes were UV-sterilized and rinsed with 70 % alcohol before use. Sampling apparatus was deployed 1 m above the ground surrounded with a 2 mm gauze baffle on the Miers Valley Floor (78°05′.78S, 163°47′.25E, approx. 270 m) and Miers Valley Ridge (78°06′.83S, 163°47′.18E, approx. 550 m). Samples were collected from a continuous filtration period December 11, 2009 to January 25, 2010 (55 days), with an estimated sample volume of 75,000 l for each location. Filters were stored at −20 °C during transit from Antarctica and until processed.

DNA Extraction, Amplification, and Sequencing

Total DNA was extracted directly from the filters using the DNeasy Plant Mini Kit (Qiagen, CA, USA), after first washing with kit lysis buffer for 10 min. The remaining steps of the extraction were carried out according to the manufacturers instructions. Recovered DNA was quantified using NanoDrop™ (Thermo Scientific, Waltham, MA, USA).

For each sample, PCR targeting the V5–V7 region of the 16S rRNA gene was completed in duplicate. Each 30 μl reaction contained 1× PrimeSTAR buffer, 0.2 mM dNTPs, 0.75 U PrimeSTAR HS DNA Polymerase (Takara Holdings, Kyoto, Japan), 0.4 μM of primers Tx9 (5′-GGATTAGAWACCCBGGTAGTC-3′) and 1391R (5′-GACGGGCRGTGWGTRCA-3′) [23], and 5 μl of template DNA. Thermal cycling conditions consisted of 94 °C for 3 min; 30 cycles of 94 °C for 20 s, 52 °C for 20 s, and 72 °C for 45 s; and 72 °C for 3 min. All thermal cycling was completed on a Bio-Rad DNA Engine Peltier Thermal Cycler 200 (Bio-Rad, Hercules, CA, USA). Duplicate reactions were pooled, and amplicons were size-selected from agarose gels using a MO BIO Gel Extraction Kit (MO BIO Laboratories, Carlsbad, CA, USA). Gel extracted products were cleaned using an Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA) and quantified using a Qubit dsDNA HS Assay Kit and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA).

To prepare the amplicons for sequencing, a second round of PCR was completed in triplicate. PCR reactions were prepared as outlined above, but using 10 ng of purified amplicon as the template and primers MIDX-Tx9F (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID-GGATTAGAWACCCBGGTAGTC-3′) and BacB_1391R (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-GACGGGCRGTGWGTRCA-3′), adapted for one-way reads with unique MID identifiers for each sample. Thermal cycling conditions were as outlined above, but reduced to 13 cycles. Triplicate products were pooled, gel-extracted, cleaned, and quantified as outlined above, before quantification of amplifiable molecules using a KAPA Library Quantification Kit for Roche 454 Titanium/Universal (Kapa Biosystems, Woburn, MA, USA) on a Corbett Rotor-Gene 6000 real-time thermal cycler (Life Technologies). Amplicons were sequenced using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTitrePlate Kit, and GS Junior System (Roche 454 Life Sciences, Branford, CT, USA) at The University of Waikato DNA Sequencing Facility.

Analysis of Sequencing Data

Raw sequence data was analyzed using AmpliconNoise v1.25 with default parameters [24]. Chimeric sequences were removed using Perseus [24]. Sequences were processed in Mothur v1.24.1 [25]. Pairwise distances were calculated using ESPRIT [26] and sequences grouped at 97 % identity into operational taxonomic units ($OTU_{0.03}$) using average neighbour clustering. Unique sequences had to be observed a minimum of three times in order for them to be retained for further analyses. Sequences were assigned to taxonomic groups using the Classifier function of the Ribosomal Database Project (RDP) Release 10 [27] with a confidence threshold of 80 %, and sequences were compared to those in the NCBI database using the BLASTn algorithm [28].

Phylogenetic analyses were carried out in MEGA v5.05 [29]. Sequences were aligned over 266 homologous positions of the 16S rRNA gene using Clustal W. A neighbour-joining tree was constructed based on the alignment using default parameters, except rates among sites were adjusted to be gamma distributed, rather than uniform. The phylogenetic tree was tested based on bootstrap analysis with 1,000 replications.

In addition to the NCBI database, Miers Valley aerosol sequences were compared to those from previously published datasets using the BLASTn algorithm. The first dataset included 31 bacterial clone sequences reported in air samples over Halley Station, West Antarctica [7] (GenBank accession numbers FN431986–FN432029). The second dataset included all bacterial sequences observed in a Miers Valley soil sample that was used as a control site in a recent study [17]. The third dataset included 1,320 sequences from ice-free areas of Antarctica [16]: Anchorage Island, Coal Nunatak, Fossil Bluff, and Mars Oasis [30] (GenBank accession numbers EF219488–EF220012; EF220190–220371; 220735–220903), Miers Valley and Bratina Island [31] (GenBank accession numbers DQ062859–DQ062918), Luther Vale [32] (GenBank accession numbers EF464767–EF465090), Ross Island [33] (GenBank accession numbers AY571818–AY571845), and Marble Point and Wright Valley [34] (GenBank accession numbers DQ365987–DQ366018). Pyrosequencing data was also screened for possible user contamination, using commonly accepted indicators of human microflora and pathogens as previously described [35–39]. The pyrosequencing datasets generated in this study are deposited in the European Nucleotide Archive, under accession number ERP002362 (http://www.ebi.ac.uk/ena/data/view/ERP002362).

Meteorological Data and Back Trajectory Analyses

Back trajectories were generated with the internet-based version of HYSPLIT, to establish the source regions of air masses advecting over Miers Valley during the sampling period [40]. Seven-day back trajectories released at 500 m above ground level (AGL) were obtained using the GDAS database for every other day; to allow for adequate vertical mixing in the atmosphere during transport, the isentropic option for calculating the back trajectories was selected. Although the resolution of GDAS is not adequate enough to show transport within the Dry Valleys system, it should still elucidate the long-range transport source regions accurately.

Results

From the Miers Floor sample, a total of 22,271 reads matching the barcode were obtained, and 12,113 of these (574 unique sequences) were retained following quality checks. From the Miers Ridge sample, 22,101 reads matching the barcode were obtained, and 11,467 of these (600 unique sequences) were retained. Following removal of sequences that occurred less than three times between the two air sample libraries, a total of 202 $OTUs_{0.03}$ were retained for analyses (Supplementary Table 1, Supplementary Figure 1). Control filters yielded no recoverable DNA thus confirming the fidelity of our aerosol sampling setup. The majority of the total sequences observed grouped into $OTUs_{0.03}$ common to both libraries. The 73 $OTUs_{0.03}$ shared between the two libraries accounted for 95.3 % and 93.6 % of the sequences observed in the Miers Floor and Miers Ridge samples, respectively (Supplementary Table 2). A total of 64 $OTUs_{0.03}$ were observed only in the Miers Floor sample, and 65 $OTUs_{0.03}$ were observed only in the Miers Ridge sample, accounting for 4.7 % and 6.4 % of the sequences in these libraries, respectively.

We recorded only ten $OTUs_{0.03}$ (five in both samples, one only in the Miers Floor sample, and four only in the Miers Ridge sample) in the entire dataset for both locations that displayed 91–99 % sequence similarity to human skin (*Dermacoccus*, *Flavobacteria*, *Lactobacillus*, *Micrococcus*, *Propionibacteria*, *Staphylococcus*) or fecal (*Escherichia*) microflora; no pathogenic taxa were detected in the libraries. These taxa indicative of human contamination comprised less than 1 % of the sequences in the two libraries, and since most were phylogenetically also very closely related to common soil bacteria, we conclude negligible human contamination to our sequence dataset.

In both samples, sequences grouping to the Firmicutes dominated libraries, and sequences grouping to the Actinobacteria, Bacteroidetes, and Cyanobacteria constituted minor proportions of the libraries (Fig. 1). The highest diversity was observed in the Proteobacteria with a total of 73

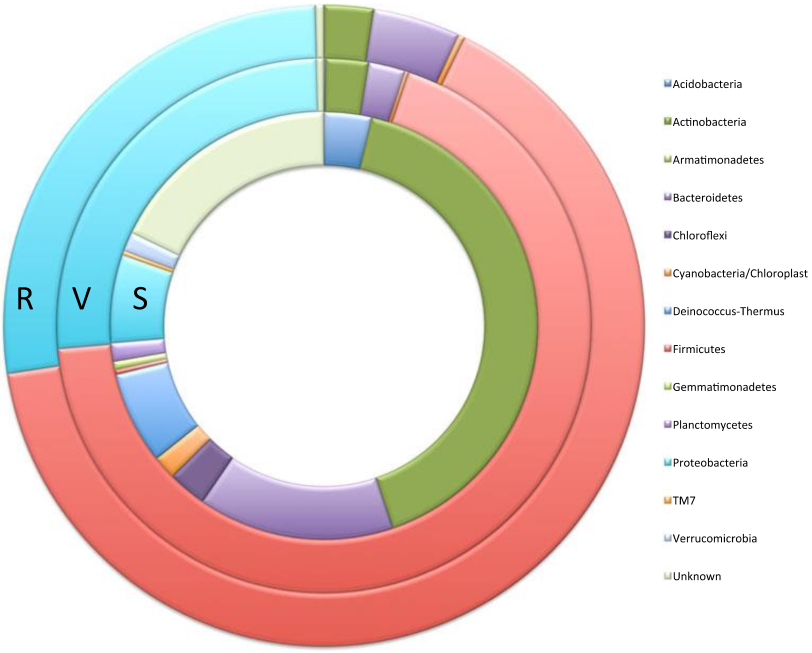

Fig. 1 Phylum level distributions of sequences observed in the Miers ridge (*R*) and Miers valley floor (*V*) bacterial aerosol libraries (this study), plus surrounding soils (*S*) [17]. Phylum assignments were made using the Classifier function of the Ribosomal Database Project (RDP) Release 10 [26] with a confidence threshold of 80 %

OTUs$_{0.03}$ found to group to the phylum, representing the classes Alpha-, Beta-, and Gamma-proteobacteria. The Firmicutes were represented by 56 OTUs$_{0.03}$, which predominantly grouped to the classes Bacilli and Clostridia. The Actinobacteria were represented by 21 OTUs$_{0.03}$, the Bacteroidetes by 13 OTUs$_{0.03}$, and the Cyanobacteria by 4 OTUs$_{0.03}$. A single OTU$_{0.03}$ grouping to the Acidobacteria was observed in low abundance in the Miers Floor library, but could not be confidently grouped beyond the phylum level. Thirty four OTUs$_{0.03}$ could not be confidently grouped to a phylum.

The phylogenetic compositions of the libraries were influenced heavily by five OTUs$_{0.03}$, which dominated both samples (Table 1). These sequences were found to cluster well with cultured representatives of the taxonomic groups to which they were assigned by RDP Classifier (Fig. 2). Many of the dominant sequences were found to group closely with known thermophilic genera. Sequences of OTU$_{0.03}$ 159 grouped within the Firmicutes to the genus *Thermaerobacter* and shared 99 % sequence identity with its closest BLAST match, *Thermaerobacter subterraneus*, an isolate from a hydrothermal system [41]. Sequences of OTU$_{0.03}$ 238 were found to share 100 % sequence identity with isolate *Geobacillus tepidamans*, which has been recovered from highly disparate thermal environments, including geothermal systems and food processing facilities [42]. The most abundant phylotype found to group to the Proteobacteria, OTU$_{0.03}$ 9, also had a closest cultured match to the thermophilic bacteria *Lysobacter thermophiles* YIM 7785^{T}, which was isolated from a geothermal soil in China [43]; however, this sequence has also been reported in numerous culture independent analyses from diverse environments including air from a high elevation site in Colorado, USA [44] and glacial ice in the Antarctic Dry Valleys [45].

The two dominant sequences grouping to the family Paenibacillaceae, OTUs$_{0.03}$ 166 and 176 (Table 1), had closest BLAST matches of 99 % sequence identity to an uncultured

Table 1 Taxonomic assignments of the most abundant OTUs$_{0.03}$ observed in the Miers Floor and Miers Ridge bacterial aerosol libraries

OTU	Miers Floor (%)	Miers Ridge (%)	RDP assignment	Confidence (%)
9	15.7	15.1	Xanthomonadaceae[f]	100
159	24.6	6.7	*Thermaerobacter*[g]	100
166	7.3	11.3	Paenibacillaceae[f]	81–100
176	23.5	14.3	Paenibacillaceae[f]	87–100
238	8.2	24.4	Bacillaceae[f]	100

Assignments were made using the Classifier function of the Ribosomal Database Project (RDP) Release 10 [26] with a confidence threshold of 80 %

[f] Sequences grouped to family

[g] Sequences grouped to genus

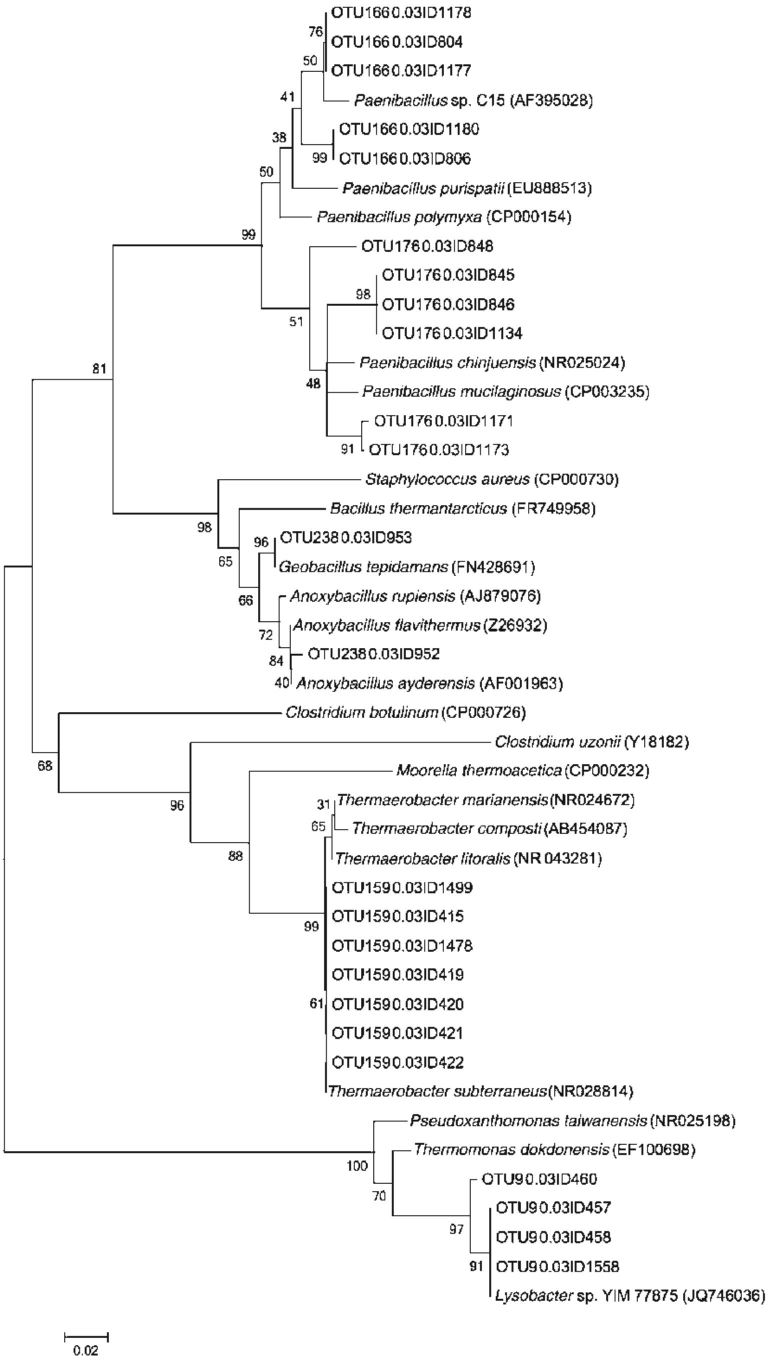

Fig. 2 Neighbour-joining tree illustrating the phylogenetic relationships of sequences from the five most abundant OTUs$_{0.03}$ observed in the Miers Floor and Miers Ridge bacterial aerosol libraries. The tree is based on a Clustal W alignment of 16S rRNA gene sequences over 266 homologous positions. Bootstrap support is shown at each node as a percentage, based on 1,000 resampled datasets. The scale indicates the number of substitutions per nucleotide position

bacteria associated with an insect host [46] and to an uncultured bacteria associated with a mud volcano in the Mediterranean [47], respectively. Their closest cultured matches were with 98 % sequence identity to isolate *Paenibacillus* sp. C15, from Lake Vostok accretion ice and with 98 % sequence identity to *Paenibacillus chinjuensis*, an isolate from a soil in Korea.

Few sequences in the Miers Valley aerosol libraries were also present in the Halley Station aerosols, Miers Valley soil, or Antarctic ice-free areas databases. Sequences from 5 $\mathrm{OTUs}_{0.03}$ observed in the Miers aerosol libraries matched 7 of the 31 clones reported in the Halley Station aerosols with >97 % 16S rRNA gene sequence identity, and these $\mathrm{OTUs}_{0.03}$ accounted for 3.2 % and 0.5 % of the Miers Floor and Miers Ridge samples, respectively. Sequences from 13 $\mathrm{OTUs}_{0.03}$ observed in the aerosol libraries matched sequences in the Miers soil library with >97 % 16S rRNA gene sequence identity, but these sequences made up <3 % of either aerosol library. Sequences from 34 $\mathrm{OTUs}_{0.03}$ were found to be present in either the Miers soil library or soils from other ice-free areas in Antarctica, and these $\mathrm{OTUs}_{0.03}$ accounted for 6.6 % and 5.3 % of the Miers Floor and Miers Ridge samples, respectively.

Seven-day back trajectories indicated that the air masses influencing the sample sites came predominantly from the plateau, with few events immediately influenced by the marine environment (Fig. 3). An Automatic Weather Station (AWS) installed in the valley floor shows that the typical surface flow was easterly (up-valley) for December and January (data is not available between December 12 to January 18 due to equipment failure). As is typical for this season, strong katabatic storms did not occur and wind speeds diurnally oscillated between 7 to 0.5 m/s in response to the solar insolation.

Discussion

The soils and rocks of Antarctica's Dry Valleys support unique microbial communities that are able to tolerate the harsh environmental conditions [16]. This study demonstrated that the aerosol immediately above this edaphic niche supports a bacterial diversity that includes a very low frequency of OTUs related to local soil colonists and instead is dominated by OTUs that suggest that the extensive local active volcanology, together with strong selective pressure from regional transport above the Antarctic plateau, may be the dominant factors determining airborne bacterial dispersal.

The lack of correlation between aerosolized ‘communities’ and local edaphic communities in our study is an enigma. It is recognized that dust transport is a major mechanism for microbial dispersal in deserts worldwide [9], and studies of microbial signatures in Antarctic soils indicate this may also be the case in the Dry Valleys, at least on a local scale [11]. Yet during a large part of the Austral summer, our evidence suggests that local edaphic bacteria form a negligible part of the dust-free airborne biota. We propose that this may be due to microbes associated with mineral soil (dust) particles that were frozen solid to the surface and/or too heavy to have been collected by our pumping apparatus, and therefore dust-associated microbes were under-represented in our study. We may therefore have detected only bacteria being transported as very fine particulates. Soil microbial communities also exhibit relatively low biomass [11, 48] and may be dispersed during occasional stochastic events rather than in a continuous manner, and this may contribute to relatively stochastic demography between such desert locations [49, 50].

At the phylum level, the signatures observed in the Miers Valley aerosols were largely similar to those reported in aerosols over other terrestrial environments. Studies of aerosols from Texas [35], Colorado [51], Hong Kong [39], and the Canadian High Arctic [52] report similar representation of major phyla. This evidence supports suggestions that bacteria from these groups may have characteristics that enhance their ability to be aerosolized and survive harsh conditions in the atmosphere [51]. That the same bacteria that are important globally in aerosols are important in Antarctic aerosols suggests there may be common controls to bacteria during high atmospheric transport, and this may be independent of geographic location.

At the $\mathrm{OTU}_{0.03}$ level, there was also evidence that Miers bio-aerosols were similar to those outside Antarctica. The most abundant $\mathrm{OTU}_{0.03}$ grouping to the Proteobacteria was also observed in air from a high elevation site in Colorado, USA [44]. Several sequences of lower abundance in the Miers library were also recorded from aerosols over tropical terrestrial and marine environments [39], thus suggesting that some taxa are globally distributed in the atmosphere.

Aerosols over Miers Valley showed much higher diversity than those reported from other parts of the continent. This result is at least partially attributable to the use of high-throughput sequencing in the current study (thus resolution power in our study was several orders of magnitude greater than in earlier studies), but may also in part reflect landscape differences between the two study sites influencing aerosol compositions. Pearce and colleagues [7] proposed that the 31 clones and 12 partial sequences reported in aerosols over Halley Station represented a baseline of biodiversity for aerosols over the continent, due to limited impact from local biological sources. Our observation of seven of the taxa described in the Halley Station libraries among the 202 OTUs seen in aerosols over Miers Valley is in agreement with this concept and may indicate that there are some bacterial taxa that can be predictably observed in aerosols over the entire continent. The difference in bacterial assemblages observed between the two studies demonstrates a degree of temporal and/or spatial variation in aerosol compositions over Antarctica.

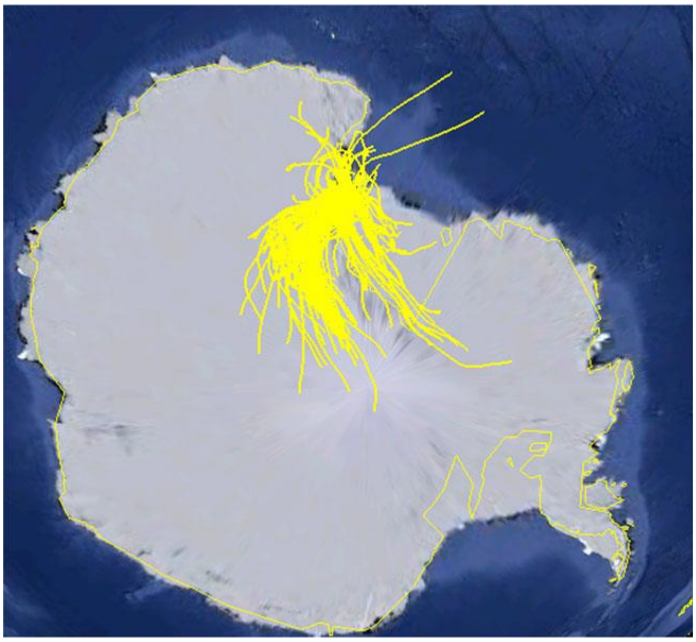

Fig. 3 Hysplit [39] back trajectories showing the origin of air masses influencing the sample site over the sampling period (*yellow highlight*)

There was little evidence that local soils, lakes, glaciers, or the Ross Sea had a marked influence on bacterial aerosol compositions. The air sample libraries were found to be highly dissimilar to the reference soil sample library at both the phylum and $OTU_{0.03}$ level. In stark contrast to the Miers Valley aerosols, work by Tiao and colleagues [17] indicated that the Miers soils were dominated by sequences grouping to Actinobacteria (37.3 %) and Bacteroidetes (12.6 %), while those grouping to Firmicutes (<1 %) and Proteobacteria (Alphaproteobacteria 1.6 %, Betaproteobacteria <1 %, and Gammaproteobacteria<1 %) made up small proportions of the library. All of the phyla observed in the air samples were observed in the soils, but many in the soil samples including the *Deinococcus–Thermus*, Chloroflexi, Planctomycetes, and Verrucomicrobia were not detected in either air sample. Sequences from only 13 $OTUs_{0.03}$ in the Miers aerosol libraries were also observed in the soil library, and none of the dominant signatures in the aerosol libraries were represented. This indicates strong selective pressure in Dry Valleys soils towards community assembly that greatly enhances abundance of 'rare' phylotypes from the incoming airborne (rather than dust-borne) inoculum. We further postulate that dispersal is a largely regional process and occurs via stochastic dust storm events, whilst a more passive low-level but continuous aerosol input from air movements harbours a largely non-invasive aerosolized microbial input. It is also possible that the level of biomass in Dry Valley soils surrounding the sample sites was too low to have a detectable influence on the composition of bacterial aerosols, although cyanobacteria that form high biomass microbial mats around lakes and hypoliths in quartz-bearing soils were undetected and so this appears unlikely.

Of the bacteria detected, none were confidently considered to be of marine origin from the nearby Ross Sea. This is consistent with the analysis of back trajectories for wind, which showed weather arriving predominantly from the interior of the continent. Pearce and colleagues [7] also noted limited marine influence on aerosols at Halley Station in peninsular Antarctica, with only three bacterial sequences of marine origin and no discernable variation in bacterial aerosol compositions when the distance to open water was reduced from 400 to 15 km during seasonal break up of the sea-ice. Hughes and colleagues [8] also reported that only three of the sequences detected in aerosols over Rothera Point were of marine origin, despite close proximity to the open ocean and the marine influence on much of the air arriving at the station. Based on the climatic trends over the sampling period, it is, perhaps, not surprising that little marine influence was observed in the Miers Valley aerosols.

The substantial number of sequences in both libraries that appear to be from thermophilic bacteria was unexpected, but may be explained by influences from the many areas of geothermal activity in the Ross Sea region. Potential sources of thermophilic organisms include Mount Erebus 100 km northeast of the Miers Valley, and Mount Melbourne and Mount Rittman approximately 400 and 500 km north of the Miers Valley, respectively. These sites host diverse and abundant thermophilic microbial communities [53–55]. Bacteria may be aerosolized from these sites in steam that rises from the fumaroles, similar to the mechanism of dispersal described in other geothermal systems [56, 57]. Considering the close proximity of the Dry Valleys to these ecosystems, relative to other significant stocks of microbial biomass, it is reasonable to suggest that these volcanoes, particularly Mt. Erebus, may have an important influence on the composition of bacterial aerosols in the region.

We postulate that at least some aerosolized bacteria arrived via inter-continental dispersal. Indeed, Vincent [58] cites numerous lines of evidence for the frequent transfer of biological material to Antarctica by atmospheric processes. Bacteria have been shown to survive long-distance transport in desert dust blown between Africa and the Caribbean [59]. Many of the bacteria observed in the Miers aerosols may be long lived in the atmosphere, particularly the spore-forming taxa grouping to the phylum Firmicutes. The high proportion of Firmicutes observed in these libraries could be the result of selection for spore-forming bacteria during long-range transport, as the *Paenibacillus*, *Geobacillus/Anoxybacillus*, and *Thermaerobacter* found to dominate the Miers aerosols can all be considered to be cosmopolitan in distribution [42, 60] and may be highly mobile as spores.

We conclude, however, that the majority of the bacteria observed in the aerosols originated in Antarctica, rather than from off the continent. Antarctica has been hypothesized to have one of the lowest rates of microbial exchange with other continents and marine environments, with an estimated 90 % of the aerosols deposited in Antarctic environments predicted to have originated from on the continent [5]. The dust that arrives in Antarctica is predicted to be predominantly from Australia and Patagonia, with the Ross Sea region most heavily influenced by the Australian fraction [61], and there is little reason to expect these aerosols to be dominated by thermophiles. That all of the dominant members are thermophilic suggests that there is a common nearby source, as a mechanism for the selection in favour of thermophilic bacteria in the cold atmosphere is difficult to conceive.

Acknowledgments The authors are extremely grateful to Antarctica New Zealand for logistics and field support in Antarctica.

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Supplementary Information

Supplementary Table 5.1: Summary of Shannon's diversity (H'), Pielou's evenness (J'), Good's coverage (Coverage) and terminal slopes of rarefaction curves (Rarefaction) for aerosol libraries from Miers Floor and Miers Ridge, analyzed with OTUs grouped as unique sequences (Unique) and OTUs grouped at 97 % identity (0.03).

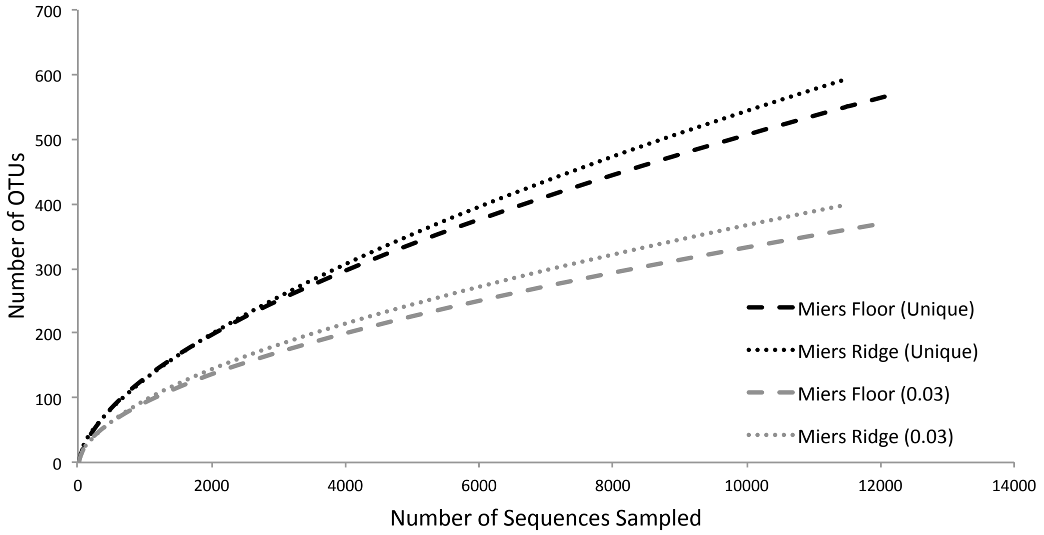
	H'	J'	Good's Coverage	Rarefaction
Miers Floor$_{\text{(Unique)}}$	3.07	0.48	0.97	0.028
Miers Ridge$_{\text{(Unique)}}$	3.25	0.51	0.97	0.033
Miers Floor$_{(0.03)}$	2.74	0.46	0.98	0.018
Miers Ridge$_{(0.03)}$	2.87	0.47	0.98	0.021

Supplementary Table 5.2: Classification of OTUs shared between the Miers Floor and Miers Ridge libraries. All assignments made with greater than 80 % confidence are shown and reported in brackets.

OTU$_{(0.03)}$	Floor (%)	Ridge (%)	RDP Assignment
1	0.35	0.15	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Sphingomonadales(100%); Sphingomonadaceae(100%); Sphingomonas(100%)
6	0.06	0.07	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Micrococcineae(100%); Microbacteriaceae(100%); Microbacterium(100%)
9	14.34	14.72	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Xanthomonadales(100%); Xanthomonadaceae(100%)
11	0.05	0.01	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Neisseriales(99%); Neisseriaceae(99%)
12	0.03	0.03	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Neisseriales(88%); Neisseriaceae(88%)
13	0.02	0.03	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(97%); Neisseriales(95%); Neisseriaceae(95%)
26	0.60	0.12	Bacteria(100%); Proteobacteria(99%); Gammaproteobacteria(99%); Xanthomonadales(99%); Xanthomonadaceae(99%)
32	0.02	0.01	Bacteria(100%); Proteobacteria(86%); Gammaproteobacteria(86%); Xanthomonadales(86%); Xanthomonadaceae(86%)
35	0.02	0.01	Bacteria(100%); Proteobacteria(80%); Gammaproteobacteria(80%); Xanthomonadales(80%); Xanthomonadaceae(80%)
75	0.78	0.03	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Burkholderiales(100%); Alcaligenaceae(100%)
82	0.06	0.42	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Burkholderiales(100%); Burkholderiaceae(100%); Ralstonia(100%)
91	1.59	0.03	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Burkholderiales(100%); Comamonadaceae(100%); Diaphorobacter(89%)
105	0.75	0.19	Bacteria(100%); Proteobacteria(100%); Betaproteobacteria(100%); Burkholderiales(100%); Oxalobacteraceae(97%)
121	0.20	0.55	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Caulobacterales(100%); Caulobacteraceae(100%); Brevundimonas(100%)
136	0.02	0.01	Bacteria(100%)
140	0.96	0.46	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Rhizobiales(100%); Methylobacteriaceae(100%); Methylobacterium(100%)
149	0.10	0.13	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Rhizobiales(100%); Bradyrhizobiaceae(100%); Bosea(100%)
150	0.02	0.03	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Rhizobiales(100%); Bradyrhizobiaceae(100%)
157	0.17	0.02	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Rhodobacterales(100%); Rhodobacteraceae(100%); Paracoccus(87%)
158	0.13	0.17	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(99%); Staphylococcaceae(98%); Staphylococcus(97%)
159	24.05	6.49	Bacteria(100%); Firmicutes(100%); Clostridia(100%); Clostridiales(100%); Incertae_Sedis_XVII(100%); Thermaerobacter(100%)
160	0.05	0.19	Bacteria(100%); Firmicutes(100%); Bacilli(96%); Bacillales(96%)
166	7.14	11.02	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(100%); Paenibacillaceae(100%); Paenibacillus(90%)
173	0.11	0.01	Bacteria(100%); Firmicutes(100%); Bacilli(98%); Bacillales(98%); Paenibacillaceae(84%)
176	22.96	13.98	Bacteria(100%); Firmicutes(100%); Bacilli(96%); Bacillales(96%); Paenibacillaceae(95%); Paenibacillus(86%)
179	0.01	0.02	Bacteria(100%)
180	0.27	0.01	Bacteria(100%); Firmicutes(100%); Clostridia(92%); Clostridiales(92%); Incertae_Sedis_XVII(89%); Thermaerobacter(89%)
187	0.03	0.15	Bacteria(100%); Proteobacteria(81%)
202	0.01	0.58	Bacteria(100%); Firmicutes(99%); Bacilli(91%); Bacillales(89%)
216	1.01	3.66	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(100%); Bacillaceae(100%); Geobacillus(100%)
229	0.35	0.02	Bacteria(100%); Firmicutes(96%); Clostridia(89%); Clostridiales(89%); Incertae_Sedis_XVII(89%); Thermaerobacter(89%)
237	0.01	0.24	Bacteria(100%); Firmicutes(96%)
238	8.03	23.77	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(100%); Bacillaceae(100%)

257	0.02	0.09	Bacteria(100%); Bacteroidetes(100%); Flavobacteria(100%); Flavobacteriales(100%); Flavobacteriaceae(100%)
297	0.34	0.47	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Caulobacterales(100%); Caulobacteraceae(100%)
301	0.55	0.05	Bacteria(100%)
305	0.05	0.03	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Lactobacillales(99%); Streptococcaceae(98%); Streptococcus(98%)
308	0.30	0.17	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(100%); Paenibacillaceae(100%); Paenibacillus(100%)
314	0.01	0.02	Bacteria(100%); Firmicutes(99%)
342	0.02	0.02	Bacteria(100%); Firmicutes(100%); Bacilli(98%); Bacillales(98%); Bacillaceae(98%)
348	0.01	0.02	Bacteria(100%); Firmicutes(99%)
350	0.17	1.33	Bacteria(100%); Firmicutes(100%); Bacilli(100%); Bacillales(100%); Paenibacillaceae(99%); Brevibacillus(99%)
363	0.01	0.02	Bacteria(100%); Firmicutes(97%); Clostridia(97%); Clostridiales(97%); Incertae_Sedis_XVII(97%); Thermaerobacter(97%)
371	0.20	0.06	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Sphingomonadales(100%); Sphingomonadaceae(100%); Sphingomonas(100%)
393	0.03	0.14	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(100%); Caulobacterales(100%); Caulobacteraceae(100%); Brevundimonas(100%)
408	0.75	0.04	Bacteria(100%); Bacteroidetes(100%); Flavobacteria(100%); Flavobacteriales(100%); Flavobacteriaceae(100%); Cloacibacterium(95%)
410	0.03	0.02	Bacteria(100%); Bacteroidetes(100%); Flavobacteria(100%); Flavobacteriales(100%); Flavobacteriaceae(100%); Chryseobacterium(100%)
454	0.16	0.17	Bacteria(100%); Firmicutes(99%)
485	0.10	0.06	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Pseudomonadales(100%); Pseudomonadaceae(100%); Pseudomonas(96%)
490	0.08	0.35	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Pseudomonadales(99%); Pseudomonadaceae(99%); Pseudomonas(87%)
499	0.91	0.23	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Enterobacteriales(100%); Enterobacteriaceae(100%); Escherichia/Shigella(97%)
501	0.01	0.02	Bacteria(100%)
503	0.02	0.02	Bacteria(100%)
504	0.02	0.08	Bacteria(100%)
508	0.08	4.07	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Enterobacteriales(100%); Enterobacteriaceae(100%); Klebsiella(91%)
512	0.03	0.01	Bacteria(100%); Proteobacteria(99%); Gammaproteobacteria(95%)
517	0.01	0.06	Bacteria(100%)
533	0.34	0.24	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Pseudomonadales(100%); Moraxellaceae(100%); Enhydrobacter(99%)
555	0.12	0.03	Bacteria(100%); Proteobacteria(100%); Alphaproteobacteria(99%); Rhizobiales(99%); Methylobacteriaceae(99%); Methylobacterium(99%)
565	0.02	0.02	Bacteria(100%)
570	0.07	0.01	Bacteria(100%); Firmicutes(95%); Clostridia(95%); Clostridiales(95%); Incertae_Sedis_XVII(95%); Thermaerobacter(95%)
571	0.97	3.88	Bacteria(100%); Bacteroidetes(100%); Flavobacteria(100%); Flavobacteriales(100%); Flavobacteriaceae(100%); Chryseobacterium(100%)
578	0.02	0.02	Bacteria(100%)
591	0.75	0.12	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Enterobacteriales(98%); Enterobacteriaceae(98%); Serratia(82%)
609	0.04	0.02	Bacteria(100%)
611	1.38	1.49	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Propionibacterineae(100%); Propionibacteriaceae(100%); Propionibacterium(100%)
613	0.02	0.02	Bacteria(100%); Firmicutes(89%); Clostridia(89%); Clostridiales(89%); Incertae_Sedis_XVII(89%); Thermaerobacter(89%)
635	0.31	0.20	Bacteria(100%); Proteobacteria(100%); Gammaproteobacteria(100%); Pseudomonadales(100%); Moraxellaceae(100%); Acinetobacter(100%)
638	0.03	0.01	Bacteria(100%); Actinobacteria(99%); Actinobacteria(99%); Actinobacteridae(99%); Actinomycetales(97%)
640	0.41	0.10	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Corynebacterineae(83%); Corynebacteriaceae(83%); Corynebacterium(83%)

652	0.28	0.08	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Micrococcineae(99%); Micrococcaceae(99%); Micrococcus(99%)
658	0.02	0.10	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Micrococcineae(98%); Micrococcaceae(95%); Kocuria(89%)
664	0.03	0.01	Bacteria(100%); Actinobacteria(100%); Actinobacteria(100%); Actinobacteridae(100%); Actinomycetales(100%); Micrococcineae(100%); Intrasporangiaceae(100%); Janibacter(100%)



Supplementary Figure 5.1: Rarefaction curves for aerosol libraries from Miers Floor and Miers Ridge, analyzed with OTUs grouped as unique sequences (Unique) and OTUs grouped at 97 % identity (0.03).

Chapter 6: Conclusion

Thesis Summary

To date, there has been a lack of systemic studies of sufficient scope and scale to elucidate patterns of microbial distributions in Antarctica's terrestrial environments. The goal of New Zealand's Terrestrial Antarctic Biocomplexity Survey (nzTABS) is to elucidate the factors that determine, and can predict, the distributions of biota in the Dry Valleys of Antarctica (nztabs.ictar.aq). The comprehensive landscape scale approach employed by nzTABS, represents a substantial advance beyond simple point assessments of community composition, providing the power to relate biological distributions to the environmental variation that exists across the ecosystem. Completed as part of the nzTABS research programme, the work presented in this thesis provides novel insights into several important aspects of microbial ecology in the Dry Valleys ecosystem.

Bacterial community fingerprinting analyses revealed diversity and community composition were significantly related to environmental variation across a 220 km^2 study area, comprising the Miers, Marshall, and Garwood valleys (Chapter 2). Bacterial diversity was significantly correlated with several physicochemical, topographic, and spatial variables, but was most strongly influenced by conductivity, soil water content, and pH. Variation in community composition was also better explained by physicochemical variation than topographic or spatial variation in the system. A structural equation model relating community composition to environmental variation indicated soil water content had the strongest direct relationship with community composition, while the effects of topographic variation were largely mediated through physicochemical variables. Spatial variation observed in both bacterial diversity and community composition was also largely explained by the physicochemical and topographic variation in the landscape, though a small but significant relationship between community composition and geographic distance between samples remained after controlling for environmental dissimilarity. This may indicate that some level of dispersal limitation or legacy effects of landscape history are important for explaining variation in bacterial community compositions in Dry Valley soils. This work identifies the primary abiotic influences on bacterial community diversity and composition across a Dry Valley landscape.

High-throughput sequencing allowed phylogenetic analysis of bacterial communities to be completed on 177 nzTABS samples (Chapter 3). Phylum level assessments of community composition showed Dry Valley soil communities were distinct from those found elsewhere on the planet and elsewhere on the continent; however, the compositions were found to be consistent with global trends in taxa composition given the alkaline and oligotrophic nature of Dry Valley soils. Many of the dominant OTUs (defined at 97% sequence identity) in the libraries were most similar to bacteria found in Antarctic soils or other extreme environments, including arid ecosystems, Arctic environments, glacial forefields, and volcanic soils. The environmental variables found to be most important in explaining community composition within the system were consistent with those implicated in analyses of the fingerprinting data; however, relationships between environmental variation and community composition, analyzed at the phylum, class, and OTU levels, were weaker than observed in analyses of the fingerprinting data. In addition to the relationships observed between community composition and environmental variation, the distributions of several taxa were significantly coupled to one another, suggesting that bacterial distributions may be influenced by a combination of abiotic factors and biotic associations in Dry Valley soils. This work adds to growing evidence that readily measurable environmental parameters can only partially explain patterns of bacterial distributions (Hanson *et al.*, 2012), even in the extreme Dry Valleys ecosystem (Van Horn *et al.*, 2013).

The inconsistencies observed in the degree of community variation explained by the fingerprinting and the sequencing data may result from inherent biases of the two methodologies. ARISA provides a means of rapidly and cost effectively comparing community characteristics between samples, making it a particularly valuable tool for analyzing large sample numbers; however, ARISA is limited in its resolution and sensitivity in comparison to next generation sequencing methodologies. This is apparent when the diversity metrics are compared between samples analyzed by ARISA (average 61 OTUs per sample) and those analyzed by next generation sequencing (average 434 OTUs per sample at 97% sequence similarity). ARISA, therefore, may be biased towards detecting more dominant community members in comparison to next generation sequencing techniques. This distinction may be particularly important in analyses of Antarctic soil communities, as genetic signatures from dead or dormant taxa may be preserved in the cold dry soils long after those taxa were active at a particular site (Cary *et al*., 2010). Deep sequencing may, therefore, be more likely than ARISA to detect such legacy signatures, which may partially explain the lower degree of association observed between the community composition and the environmental variation in the next generation sequencing dataset. The inability to attribute phylogenetic information to the fingerprinting profiles provided by ARISA is a major drawback of the method, and makes direct comparisons between the fingerprinting and sequencing datasets difficult. However, the weak relationships between community composition and environmental variation observed using these two independent lines of analysis provide strength to the conclusion that abiotic factors are only partially responsible for structuring microbial communities within the Dry Valleys landscape.

The bacterial compositions of bio-aerosols over the Dry Valleys were analyzed, in order to evaluate the importance of atmospheric dispersion of bacterial taxa to local soil community compositions. The compositions of bio-aerosols were distinct from those observed in Dry Valley soils and other local environments; instead, the major phyla observed were similar to those observed in aerosols over other environments. This suggests that bacterial inputs to Dry Valley soils by atmospheric deposition may be predominantly restricted to widely dispersed taxa, capable of long-range transport in the harsh environmental conditions of the aerosphere. Importantly, these findings also suggest that dispersal of bacterial taxa from Dry Valley soils through the air may be largely restricted to periods when soil particles are mobilized by high winds. Geographic barriers that limit the transport of particulate matter within the Dry Valley landscape may, therefore, influence the distributions of bacterial taxa within the system. As a result, many soil communities may be more isolated from one another than previously recognized within the Dry Valley ecosystem, which may partially explain the community heterogeneity observed in the nzTABS study area (Chapter 4) and that reported previously between valleys (Lee *et al.*, 2012).

In addition to the bacterial community assessments presented in this thesis, the efforts of the nzTABS programme have culminated in an ecosystem model that identifies relationships between environmental and biological variation in the system (Appendix 1). Diversity of cyanobacteria, fungi, and multicellular taxa were all influenced by abiotic factors, but biotic relationships between these groups were also important to explain their distributions. These groups also showed significant spatial variation, independent of abiotic and biotic relationships, which suggest that dispersal limitation and/or legacy effects of landscape history may influence patterns of biotic distribution within the system. As well as improving understanding of the ecology of the Dry Valleys, this study provides an experimental framework for comprehensively quantifying drivers of biotic distributions on a landscape scale through an integrated multidisciplinary approach.

Collectively, the work presented in this thesis reveals that the microbial ecology of the Antarctic Dry Valleys, an extreme environment characterized by simple trophic structure, is still highly complex. Abiotic controls, long believed to be the sole drivers of community composition in Dry Valley soils (Hogg *et al.*, 2006; Barrett *et al.*, 2006; Adams *et al.*, 2006), only partially explain the patterns of biotic distributions observed in the ecosystem. Biotic interactions both within and between trophic levels appear to be additionally important in shaping community compositions, and spatial distributions of taxa may be partially related to dispersal limitation and/or landscape history, as well as neutral effects. While this work has revealed patterns of biological distributions at an unprecedented scale, understanding the processes that influence these patterns must be the subject of future research.

Future Directions

The nzTABS programme continues to expand its scope, having extended its survey from the initial study area (Miers, Marshall, and Garwood) to include Hidden Valley, Victoria Valley, Wright Valley and Taylor Valley over the past five years (Figure 6.1). Many of the analyses presented in this thesis have been used to shape the sampling strategies for these newly surveyed regions. Additionally, due in large part to this thesis work, the methodologies and analytical pipelines used for microbial community fingerprinting of these samples are now established for high-throughput processing at the University of Waikato. This concerted effort to maintain consistency in sampling strategy and analytical methodology as the survey expands ensures that the data generated from newly sampled areas will be directly comparable to that presented in this thesis. The new data collected will be useful to validate and modify the models of biological distributions presented in Chapter 3 and Appendix 1, moving towards ecosystem models that describe the entire Dry Valley region.

The sequencing completed to date on the 177 soil samples presented in Chapter 4 will also be expanded to maximize the environmental and spatial variation covered by the growing nzTABS sample set. An additional 288 nzTABS samples will be sequenced as part of the Earth Microbiome Project (EMP) (Gilbert *et al.*, 2010) and a Community Sequencing Project (CSP) award from the United States Department of Energy. Analysis of these additional samples will be useful to address inconsistencies observed between the findings of the bacterial community fingerprinting described in Chapter 3 and the sequencing dataset presented in Chapter 4. Furthermore, the inclusion of Hidden Valley, Wright Valley, Victoria Valley, and Taylor Valley samples in the sequencing effort will reveal trends in the biogeography of different taxa, and permit analyses aimed at elucidating the possible drivers of the inter-valley heterogeneity known to exist in the Dry Valleys (Lee *et al.*, 2012).

Additionally, metagenomic sequencing of 20 nzTABS samples will be completed as part of the EMP and CSP, and will contribute enormously to understanding the structure and function of Dry Valley soil communities. Metagenomic approaches seek to analyze the genomes of all microorganisms present in a community through shotgun sequencing of total community DNA (Handelsman, 2004). The assembly and annotation of individual genomes allows the taxa present in the community to be identified, and functional genes to be linked to these taxa. Assigning functional capacity to the bacterial taxa in Dry Valleys soils will help to explain how particular groups are suited to different environmental niches and what ecological roles they play in those niches. This will be particularly useful to clarify functional associations between taxa, which may explain the patterns of bacterial taxa co-occurrence described in Chapter 4.

The research presented in Chapter 5 represents one of only three studies using molecular methods, and the only study using high-throughput sequencing technology, to characterize the composition of bio-aerosols on the Antarctic continent. The findings that bacterial taxa in the Dry Valley aerosols were distinct from those in local soil communities raises questions about the local and regional compositions of bio-aerosols over the continent and their relationship to those observed globally. The degree to which bio-aerosol compositions vary both spatially and temporally across the continent should be investigated to determine the importance of the Antarctic atmosphere as a conduit for microbial transport both within and between ecosystems. Studying these trends in relation to terrestrial ecosystem compositions will help to resolve the sources of bio-aerosols on the continent and the importance of atmospheric processes to maintaining connectivity between terrestrial sites, and serve to identify the importance of geographic and climatic boundaries in shaping microbial distributions. Continent-wide expansion of aerosol surveys would be feasible with similar equipment to that employed in the study presented in Chapter 5, setup near research stations across the continent. Given the uniqueness of Antarctica's terrestrial bacterial communities globally (Cary *et al.*, 2010) and the highly disparate bacterial community compositions observed across Antarctica's terrestrial ecosystems (Lee *et al.*, 2012; Yergeau *et al.*, 2007; Cary *et al.*, 2010), increased understanding of the link between terrestrial environments through atmospheric processes will be important to assess the influence of stochastic processes on bacterial distributions.

Continued research of microbial communities in the Dry Valleys of Antarctica will increase understanding of the processes that underlie patterns of microbial distributions. This work is particularly timely, as increased human visitation (Kennicutt *et al.*, 2010) and climate change pose immediate threats to Antarctic ecosystems (Steig *et al.*, 2009). A more complete understanding of the ecological relationships that exist within these microbially dominated ecosystems is necessary to inform best management practices for Antarctica's ice free regions, and will be useful to help disentangle the complexity of ecosystem relationships, as we move toward a better understanding of the ecology of microorganisms globally.

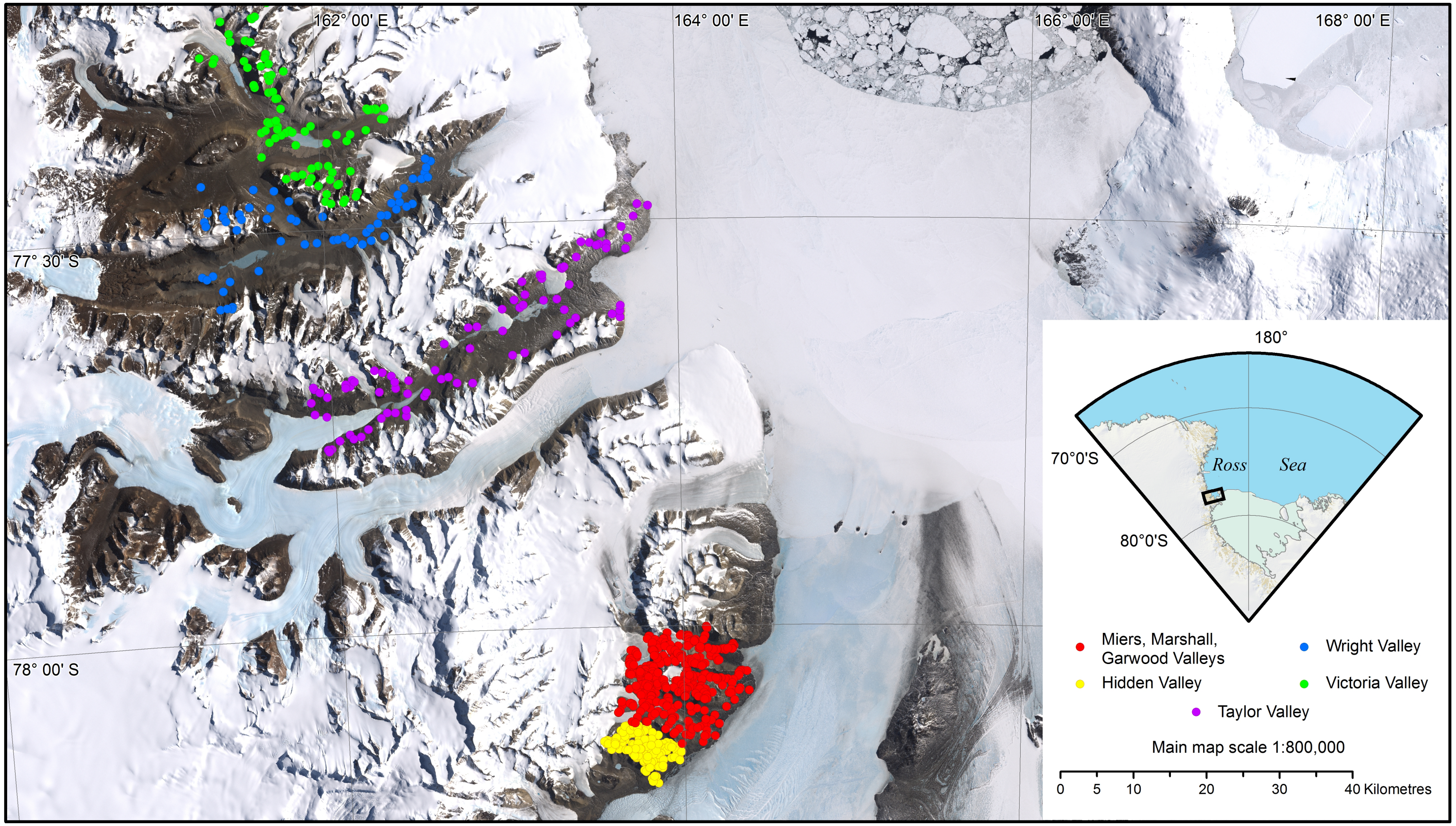


Figure 6.1: Map of the Dry Valleys indicating the sampling sites of the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS) visited from 2008 to 2014.

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Appendix 1: Complex Drivers of Biodiversity in a Simple Yet Extreme Terrestrial Ecosystem.

Preface

This chapter describes a landscape scale model of biology in soils of the Dry Valleys of Antarctica. I earned co-authorship on this paper through my involvement in fieldwork, laboratory analyses, data analyses, and manuscript preparation. In the field, I collected samples, completed biological surveys, and completed sample processing and analyses. In the laboratory, I conducted all microbial analyses, including DNA extraction and quantification, and cyanobacterial and fungal community fingerprinting. I was involved in creating the in-house fingerprinting pipeline and completed all analyses of the fingerprinting data used in the model. I was responsible for managing and curating the entire nzTABS dataset used for model construction and was involved in developing the *a priori* structural equation model. I wrote the supplementary materials for the sections of the analyses I completed, including *DNA Extraction and Characterization* and *Molecular Analysis of Microbial Communities Using ARISA*.

Full author contributions are as follows: SC Cary, TGA Green, BC Storey, AD Sparrow, and L Brabyn. conceived the study; SC Cary, TGA Green, BC Storey, AD Sparrow, ID Hogg, JC Banks, and L Brabyn designed the study and prepared funding proposals; SC Cary, BC Storey, AD Sparrow, I Jones, G Stichbury, L Brabyn, K Joy, EM Bottos, and CK Lee planned the study and field expeditions; SC Cary, AD Sparrow, ID Hogg, M Katurji, P Zawar-Reza, G Stichbury, JC Banks, DA Cowan, IR McDonald, SB Pointing, DW Hopkins, DH Wall, BJ Adams., UN Nielsen, L Brabyn, JE Barrett, K Joy, EM Bottos., DC Laughlin, and CK Lee contributed to sample collection and analysis; SC Cary, ID Hogg, M Katurji, P Zawar-Reza, G Stichbury, L Brabyn, EM Bottos, and CK Lee contributed to data analysis; SC Cary, T Caruso, EM Bottos, DC Laughlin, and

CK Lee contributed to ecological modeling; DC Laughlin and CK Lee wrote the manuscript with inputs from all authors.

This manuscript is in preparation for submission to the journal *Nature Communications*.

Complex drivers of biodiversity in a simple yet extreme terrestrial ecosystem

Charles K. Lee[1,2†] Daniel C. Laughlin[1†], Eric M. Bottos[1,2], Tancredi Caruso[3], Kurt Joy[4], John E. Barrett[5], Lars Brabyn[6], Uffe N. Nielsen[7‡], Byron J. Adams[8], Diana H. Wall[7], David W. Hopkins[9], Stephen B. Pointing[10], Ian R. McDonald[1,2], Donald A. Cowan[11], Jonathan C. Banks[1§], Glen A. Stichbury[1,13], Irfon Jones[4], Peyman Zawar-Reza[13], Marwan Katurji[13], Ian D. Hogg[1,2], Ashley D. Sparrow[14], Bryan C. Storey[4], T. G. Allan Green[1,2,15], S. Craig Cary[1,2,16*]

[1]Department of Biological Sciences, University of Waikato, Hamilton 3216, New Zealand.
[2]International Centre for Terrestrial Antarctic Research, University of Waikato, Hamilton 3216, New Zealand.
[3]School of Biological Sciences, Queen's University Belfast. Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK.
[4]Gateway Antarctica, University of Canterbury, Christchurch 8140, New Zealand.
[5]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24060 USA.
[6]Geography Programme, University of Waikato, Hamilton 3216, New Zealand.
[7]Department of Biology & Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA.
[8]Department of Biology and Evolutionary Ecology Laboratories, Brigham Young University, Provo, UT 84602, USA.
[9]School of Life Sciences, Heriot-Watt University, Edinburgh EH14 4AS, Scotland, UK.
[10]Institute for Applied Ecology New Zealand, School of Applied Sciences, Auckland University of Technology, Auckland 1142, New Zealand.
[11]Department of Genetics, Centre for Microbial Ecology and Genomics, University of Pretoria, Pretoria, South Africa.
[12]Environmental Research Institute, University of Waikato, Hamilton, New Zealand.
[13]Centre for Atmospheric Research, University of Canterbury, Christchurch 8140, New Zealand.
[14]CSIRO Ecosystem Sciences, Alice Springs, NT, Australia.

[15]Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, Spain.
[16]College of Earth and Ocean Sciences, University of Delaware, Newark, DE 19716 USA.
[*]Correspondence author
[†]These authors contributed equally to this work.
[‡]Current address: Hawkesbury Institute for the Environment and School of Science and Health, University of Western Sydney, Penrith NSW 2751, Australia.
[§]Current address: Envirotech, The Cawthron Institute, Nelson, New Zealand.

CKL: cklee@waikato.ac.nz; DCL: d.laughlin@waikato.ac.nz; EMB: e_bottos@hotmail.com; TC: t.caruso@qub.ac.uk; KJ: Kurt.Joy@pg.canterbury.ac.nz; JEB: jebarre@vt.edu; LB: larsb@waikato.ac.nz; UNN: U.Nielsen@uws.edu.au; BJA: byron_adams@byu.edu; DHW: Diana.Wall@colostate.edu; DWH: David.Hopkins@hw.ac.uk; SBP: steve.pointing@aut.ac.nz; IRM: irmcdon@waikato.ac.nz; DAC: don.cowan@up.ac.za; JCB: jonathan.banks@cawthron.org.nz; GS: g.stichbury@gmail.com; IJ: irfon.jones@gmail.com; PZR: peyman.zawar-reza@canterbury.ac.nz; MK: marwan.katurji@canterbury.ac.nz; IDH: hogg@waikato.ac.nz; ADS: Ashley.Sparrow@csiro.au; BCS: bryan.storey@canterbury.ac.nz; TGAG: greentga@waikato.ac.nz; SCC: caryc@waikato.ac.nz

Correspondence

Prof. S. Craig Cary (caryc@waikato.ac.nz). Private Bag 3105, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand 3240. Tel: +64 7 838 4593. Fax: +64 7 838 4324

Abstract

Differentiating the relative importance of environmental and biological drivers on biodiversity is a longstanding challenge in ecology and a seemingly intractable task due to the trophic complexity of most ecosystems. The ultraoligotrophic ecosystem of the Antarctic Dry Valleys hosts one of the simplest food webs known to science, yet supports unexpectedly diverse and spatially heterogeneous microbial communities. Consequently, the Dry Valleys present a uniquely amendable system for resolving drivers of biological diversity; and they have been the subject for an interdisciplinary ecosystem-level investigation of unprecedented spatial resolution and taxa comprehensiveness. Our structural equation model of the Dry Valley ecosystem explained significant variation in biological diversity. The primary drivers of ecosystem organization appeared to be landscape-scale (e.g., climate, topography) and fine-scale (e.g., soil pH, water availability, total nitrogen) abiotic factors. We detected significant spatial patterns linked to population- and landscape-level biological processes such as dispersal limitation. Important positive couplings among the biota highlighted the foundational importance of cyanobacteria as key primary producers. The complex network of relationships among factors driving biodiversity indicates that biotic and spatial processes cannot be ignored even in the simplest and most extreme ecosystem.

Introduction

Understanding how ecosystems self-organize at landscape scales has long been a formidable challenge in ecology (Weiher and Keddy, 1995), and the trophic complexity of most ecosystems obscures the relative contributions of biotic and abiotic factors regulating biological diversity (Loreau *et al*., 2001; Lamb *et al*. 2010; de Vries *et al*., 2012). Given the significant effects of biodiversity on ecosystem function (Cardinale *et al*., 2012), a critical task is to resolve the relative importance of three sets of ecological factors that drive community structure: abiotic environmental filtering, dispersal limitation in space, and biotic interactions, e.g., competition, mutualism, and trophic relationships (Weiher and Keddy, 1995; Borcard and Legendre, 2002). Thorough and spatially explicit descriptions of ecosystem composition and trophic structure are required to elucidate the relative contributions of these factors, but the complexity of most ecosystems creates enormous logistical obstacles for such efforts.

Microbial ecologists have made numerous attempts to resolve broad abiotic drivers of microbial diversity by comparing microbial communities across intra- and inter-continental scales, although such studies generally have not considered biotic or trophic interactions between microbes and more complex organisms (Lauber *et al*., 2009; Nemergut *et al*., 2011; Fierer *et al*., 2012). To date, attempts to examine ecosystems at multiple trophic levels and landscape scales remain scarce (Nielsen *et al*., 2010; Bru *et al*., 2011; Caruso *et al*., 2011; de Vries *et al*., 2012; Zimmerman and Vitousek, 2012), and the mechanisms driving biological diversity patterns remain unclear (Lamb *et al*., 2010; Hanson *et al*., 2012; de Vries *et al*., 2012). Arguably, the scarcity of such attempts can be attributed to the convolution of biotic and abiotic interactions in all but the simplest of ecosystems, which exacerbates the challenge since multiple biotic components can interact with each other while responding to abiotic factors, making it difficult to identify specific (and potentially subtle) effects of abiotic drivers on any particular biota (Lamb *et al*., 2010; de Vries *et al*., 2012).

Located between the Polar Plateau and the Ross Sea (Figure A1.1), the McMurdo Dry Valleys (hereinafter the Dry Valleys) are the largest contiguous ice-free area on the Antarctic continent and hosts an extraordinarily simple ecosystem, with a simple trophic structure and limited evidence for significant biotic interactions (Adams *et al*., 2006; Barrett *et al*., 2006; Hogg *et al*., 2006;

Cary *et al*., 2010) (see Supplementary Figure A1.1 in Supplementary Data). The Dry Valleys are subject to some of the most extreme conditions of any terrestrial habitat on Earth (Cary *et al.*, 2010), which severely constrain the range of biota present (Adams *et al*., 2006; Wall, 2007). Vascular plants and vertebrates are entirely absent, and soils are predominantly ultraoligotrophic, hyperarid, and often hypersaline (Wall, 2007; Cary *et al*., 2010). Consequently, abiotic factors are widely regarded as the primary force shaping the ecology of Dry Valley soils (Poage *et al*., 2008; Pointing *et al*., 2009; Cary *et al*., 2010; Lee *et al*., 2012). These unique characteristics make the Dry Valleys a model system for resolving the role of abiotic factors in shaping community structure.

Initiated during the International Polar Year 2007-2008, the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS, http://nztabs.ictar.aq) draws an unprecedented range of international expertise to profile the biology, geochemistry, geology, and climate of the Dry Valleys, and constitutes the most spatially and biologically comprehensive landscape-scale survey ever undertaken. Here we describe the application of structural equation modeling (SEM) to the broad suite of geological, geographical, geochemical, hydrological, and biological variables captured by nzTABS. Our objective was to test the hypothesis that environmental filters will exert an overwhelming influence on biodiversity in this extreme terrestrial ecosystem. Surprisingly, the final SEM revealed explicit and quantitative effects of spatial processes and biotic interactions on biodiversity in addition to those of environmental filters. These findings highlight the importance of biologically mediated and spatially driven processes in ecosystem organization even in one of the most physically and chemically extreme environments.

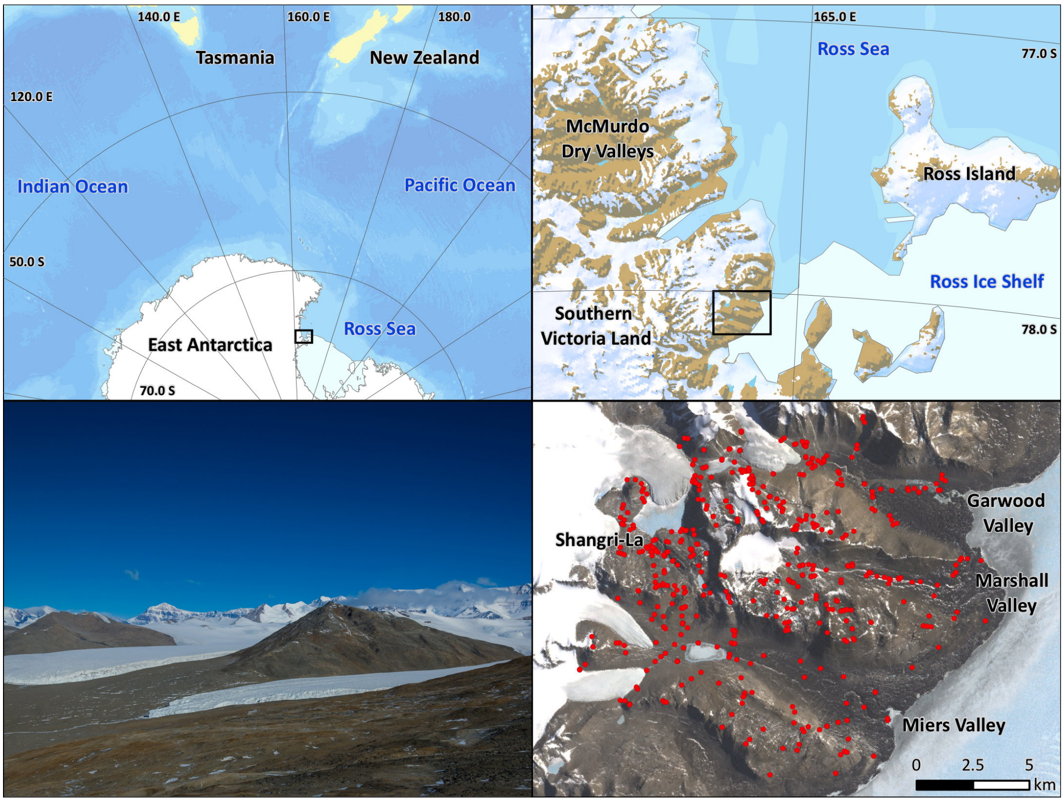

Figure A1.1: Maps of a) Victoria Land, b) McMurdo Dry Valleys and Ross Island, c) nzTABS study area [sampling sites denoted by red circles], d) View of Miers Valley toward the Royal Society Range.

Methods

Study Area

Approximately 0.4% of Antarctica is permanently ice-free, and the main ice-free areas are the Antarctic Peninsula, the McMurdo Dry Valleys, and various mountains and nunataks along the Transantarctic Mountains (Cary *et al.*, 2010). Of these, the McMurdo Dry Valleys contain the largest contiguously ice-free areas (~3,800 km^2) and have been the focus of terrestrial biology research on the continent for the past 50 years (Cary *et al.*, 2010; Levy, 2012). The McMurdo Dry Valleys are situated in southern Victoria Land along the western coast of McMurdo Sound (between 160-164°E and 76-78°S), and contain markedly complex surface geology and topography that result in highly heterogeneous physicochemical conditions in soils across the landscape. The study area comprises 220 km^2 of largely ice-free terrain that includes Garwood, Marshall, and Miers Valleys as well as Shangri-La, an area west of Marshall Valley and enclosed by Joyce Glacier, Mt. Pams, and Mt. Lama (Figure A1.1).

In addition to the extreme cold (mean annual air temperature of ~-20°C), the McMurdo Dry Valleys are characterized by strong winds, extreme aridity (precipitation of <10 cm/yr water equivalent), and lack of significant solar input for much of the year (Cary *et al.*, 2010). Despite the extreme selective pressure, the Dry Valleys appear to sustain a functional but simple ecosystem comprised of prokaryotes, invertebrate fauna, and non-vascular flora. Cyanobacteria (both aquatic and edaphic) appear to be the main primary producers, although significant photosynthetic activity occurs in lithic communities (i.e., endoliths, hypoliths, and chasmoendoliths) as well as mosses and lichens (Adams *et al.*, 2006).

The invertebrate fauna consists of the microarthropods Collembola (i.e., springtails) and Acari (i.e., mites), as well as a range of microinvertebrates including nematodes, tardigrades, and rotifers. Nematodes are the dominant invertebrate taxon across much of the landscape, and their distribution and abundance primarily correlate with the presence of liquid water, pH, salinity, and inorganic carbon (Wall and Virginia, 1999; Virginia and Wall, 1999). Taxonomic diversity for nematodes is low (five species), but abundances can be as high as hot desert soils (Wall and Virginia, 1999; Adams *et al.*, 2006). Rotifers (four species) and tardigrades (eight species) are present but more restricted to ephemerally wetted areas (Wall and Virginia, 1999; Adams *et al.*, 2006). A single species of Collembola (*Gomphiocephalus hodgsoni*) represents the largest (albeit only <1.4 mm in length) terrestrial animal in the Dry Valleys, whereas two species of Acari (*Stereotydeus mollis* and *Nanorchestes antarcticus*) are known within the region. The microarthropods share similar distributional patterns and are more commonly found in soils under rocks on stable and sunny slopes close to water sources. Soil microbial communities are composed of predominantly heterotrophic bacteria (archaeal abundance and diversity appear to be very limited) and fungi, and constitute by far the largest biomass in the ecosystem (Cary *et al.*, 2010).

Tile Delineation

Using a digital elevation model (DEM) based on LIDAR data for the area (Csatho *et al.*, 2005; Wilson and Csatho, 2007), slope (in degrees), elevation (in meters above sea level), and aspect (N, S, E, and W) were generated as the primary inputs for the nzTABS GIS model. The GIS model also included geological (i.e., major bedrock lithologies) and geomorphological (i.e., fluvial, aeolian and glacial) datasets from published sources (Blank *et al.*, 1963; Cook, 1997; Cox *et al.*, 2012), augmented by analyses of ALOS, LandSat, and MODIS satellite imagery, aerial photographs, and subsequent field mapping (Table A1.1). Using the GIS model, the study area (excluding areas covered by ice, snow, and water) was divided into more than 600 geographically and geologically distinct sectors (hereinafter "tiles", minimum 1.5 km^2). Tile boundaries were delineated where the combination of topographic and geologic attributes changed (Table A1.1 & see Supplementary Figure A1.2 in Supplementary Data). Majority filtering was used to smooth spatial variability and avoid the creation of large numbers of small tiles unsuitable for sampling. On-the-ground assessments were carried out in November 2008 to confirm the reliability of delineations, and 554 tiles were chosen for sampling to encompass the entire range of geographical and geological heterogeneity (Figure A1.1c).

Table A1.1: Landscape-scale variables captured by nzTABS.

Category	**Variables**
Remote Sensing and GIS (Satellite and LIDAR)	Elevation* Slope* Aspect* Snow/Ice/Water Presence* Distance to the Coast Soil Surface Temperature Wetness Index (Stichbury *et al.* 2011)
Geology	Bedrock Geology* (Cox *et al.* 2012) Glacial Geomorphology*
Biology	Lichen and Moss (Abundance and Size) Endolith and Hypolith (Abundance) Cyanobacterial Mat (Abundance and Size) Invertebrates (Abundance and Taxonomy) ATP Level Bacterial Diversity (ARISA) Cyanobacterial Diversity (ARISA) Fungal Diversity (ARISA)
Geochemistry	pH Conductivity Water Activity (A_w) Total Soil Moisture Content Total Soil C & N

* Variables used for tile delineation

Tile Sampling

Sampling of soils and biological communities was carried out over two successive austral summers (January 2009 and January 2010). Within each tile, a sampling site was chosen based on feasibility (a safety consideration) as well as sampling route planning. The sampling site must be located inside its corresponding tile and be representative of the geographic and geologic attributes for the tile. At each sampling location (GPS coordinates and elevation recorded), the top 10 cm of soil was collected aseptically using a trowel from multiple spots within a 1 m^2 area for the following subsamples (Figure A1.2): bulk soil (~400 g) with large pebbles (> 2 cm diameter) removed aseptically and homogenized in a sterile 42 oz. Whirl-Pak; soil (~20 g) for moisture content measurement, subsampled from homogenized bulk soil into a sterile 15 mL centrifuge tube sealed with Parafilm; soil (~300 g) for microinvertebrate count, stored in a sterile 18 oz. Whirl-Pak (pebbles not removed to minimize disturbance).

A microarthropod survey (i.e., springtails and mites) was carried out by examining the underside of small (5-10 cm dia.), flat (< 2 cm thickness), and preferably dark rocks within a 20 m radius of the soil sampling location for 10 minutes (Stevens and Hogg, 2002). The number and types of microarthropods observed were recorded, and the organisms were collected using an aspirator and preserved in a vial containing 100% ethanol for later analyses (Stevens and Hogg, 2002). A survey of vegetation (i.e., lichens, mosses, algae, and cyanobacterial mats) and lithic communities (i.e., hypoliths and endoliths) was carried out along a transect (20 m long and 2 m wide, 40 m^2) adjacent to the soil sampling location. Vegetation presence was recorded quantitatively in 100 cm^2 units for each taxon, and the numbers of observed lithic communities were recorded.

Soil samples were subsequently aliquoted and analyzed for total ATP, pH, conductivity, water activity (A_W), total moisture content, microinvertebrate (i.e., nematodes, tardigrades and rotifers) diversity and abundance, and organic carbon and total nitrogen content (Figure A1.2 and see Supplementary Methods), as well as used for bulk DNA extraction (Supplementary Methods).

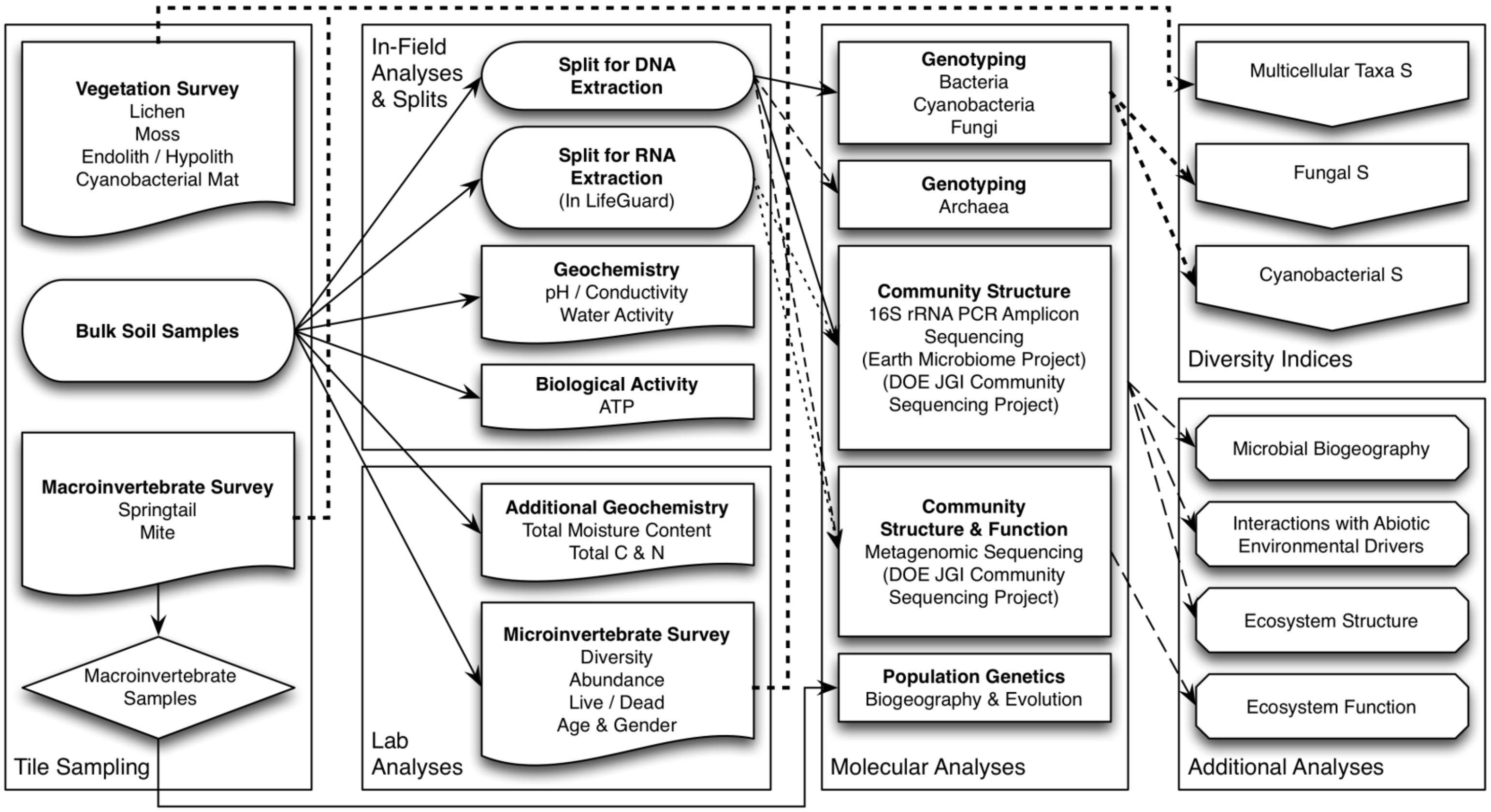

Figure A1.2: Flowgram for nzTABS sample analysis.

DNA-based Analysis of Microbial Communities

After quantification and quality check (Supplementary Methods), extracted DNA samples were used for molecular fingerprinting of bacterial (total and cyanobacteria-only) and fungal communities based on automated ribosomal intergenic spacer analysis (ARISA) (Gardes and Bruns, 1993; Sequerra *et al*., 1997; Wood *et al*., 2008). Briefly, the intergenic spacer between the 16S and 23S rRNA genes of the bacterial/cyanobacterial ribosomal operon and the intergenic spacer between the 18S and 23S rRNA genes of the fungal ribosomal operon was amplified using PCR for each sample (Supplementary Methods).

ARISA fragment length profiles were analyzed using an in-house pipeline that examines all peaks between 100 and 1200 base pairs for cyanobacterial electropherograms and 100 and 1400 base pairs for fungal electropherograms. Peaks in these size ranges that made up greater than 0.3 % of all peaks over 30 relative fluorescence units in each electropherogram were accepted as true peaks. The total number of true peaks was taken as a measure of taxon richness for each sample. Peaks within one base pair of one another were binned for the purpose of comparing electropherograms between samples.

Environmental Metadata

A number of key environmental attributes were derived from satellite imagery and the DEM, including surface soil temperature, a topographically derived 'wetness index', and distance to the coast. Soil surface temperatures were obtained from Landsat 7 ETM+ using band 6 (at 60 m resolution), which captured the up-welling thermal infrared spectrum (in the 10.4-12.5 μm band). Landsat 7-derived temperature data corresponding to locations of forty-five on-the-ground temperature loggers (DS1921G iButtons, Maxim Integrated, San Jose, CA) were compared with records from the iButtons, and significant positive correlations between the two data sets were found (Brabyn *et al*., 2014).

Wetness index, which produces a relative index of liquid water availability in summer, was calculated using a GIS-based model using variables that influence the volume and distribution of water. Remote sensing images from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor collected over several years were used to calculate an average index of snow cover, which was then combined with other water sources such as glaciers and lakes. This resulted in a probable water source model representing the highly heterogeneous distribution of water sources in the Dry Valleys (Stichbury *et al.*, 2011). The water source model was used to weight a hydrological flow accumulation model (Tarboton, 2010) that used slope derived from LIDAR elevation data captured for most parts of the Dry Valleys (Wilson and Csatho, 2007). These data were then used to calculate a Compound Topographic Index (CTI), a steady-state wetness index based on both slope and upstream contributing area (Moore *et al.*, 1993). CTI takes the form:

$$CTI = ln\left(\frac{A_s}{\tan\beta}\right)$$

where A_s is the upslope contributing area in m^2 per unit width orthogonal to the flow direction, and β is the slope angle in radians (Gessler *et al.*, 1995). The resultant model is a relative index of potential water availability, given the availability of melt water sources and topographical features.

Distance to the coast value was calculated as the Euclidean distance (in meters) from the sampling point to the closest point on coastline, which in turn was defined by cells with zero elevation in the DEM. Specifically, the shortest distance was determined by the perpendicular from the coastline to the sampling point. After quality control, data for 490 samples were included in the analysis.

Data Analysis

A broad suite of geological, geographical, geochemical, hydrological, and biological variables (Table A1.1) were collected and evaluated to derive the most parsimonious set of predictors of biodiversity in our study area. Biodiversity is represented by the richness of key autotrophic and heterotrophic groups and the presence of known taxa. Specifically, species richness of cyanobacteria and fungi was estimated using the number of ribosomal intergenic spacer length-polymorphic fragments observed from community fingerprinting analyses. These intergenic spacers exhibit length polymorphism across species and even at the intra-species level, and the length profiles of PCR fragments are therefore indicative of the diversity and abundance of microbial communities. Taxon richness for multicellular taxa was represented by the number of the following supraspecific taxa present in a sample: nematodes, rotifers, tardigrades, springtails, mites, cyanobacterial mats, mosses, lichens, and hypolithic consortia.

To verify that inferences made from diversity patterns apply similarly to community composition, diversity was correlated with community composition in all three groups of organisms (i.e., cyanobacteria, fungi, and multicellular taxa) based on an analysis of *nestedness*. Nestedness occurs when species-poor communities are generally subsets of species-rich communities, and when rare species tend to only occur in species-rich communities. The nestedness of each of the three community matrices was evaluated by calculating their respective 'temperatures', which determine whether species-poor communities are subsets of species-rich ones. The 'temperatures' were calculated using the 'nestedtemp' function (Rodríguez-Gironés and Santamaría, 2006) in the 'vegan' library of R (Oksanen *et al.*, 2013), and their significance was assessed via permutation using the 'oecosimu' function. The relationship between diversity and non-metric multidimensional scaling (NMS) ordinations1 of community composition (obtained using the 'metaMDS' function in 'vegan') (Oksanen *et al.*, 2013) was quantified using correlation analysis.

Cyanobacterial diversity, rather than total bacterial diversity, was included in our analysis for the following reasons: 1) including both would effectively be 'double-counting' since total bacterial diversity includes cyanobacteria as well; 2) cyanobacteria are arguably the most critical group of bacteria, given their large proportional input to primary production in this extreme environment; and 3) cyanobacterial diversity was significantly and positively correlated with total bacterial diversity ($r = 0.31$, $P < 0.0001$), so knowing the diversity of one group provides reasonable estimates about the diversity of the other.

Biological communities closer in space are likely to be more similar in species diversity and community composition. Historical population- and landscape-level processes that are relatively independent of environmental conditions can also be a driver of Antarctic diversity (Huiskes *et al.*, 2006, Chown and Convey, 2007; Caruso *et al.*, 2011). Thus, environmentally independent spatial variables were computed to account for spatial patterns linked to intrinsic population- and landscape-level processes, such as dispersal limitation or source-sink dynamics (Borcard and Legendre, 2002). Competition- and predation-related direct biotic interactions were not explicitly considered due to limited evidence for such interactions among Dry Valley biota (Adams *et al.*, 2006; Hogg *et al.*, 2006).

To represent spatial patterns driven by intrinsic population and community-level processes (e.g., limited dispersal), environmentally independent spatial variables were obtained as follows. First, optimal (in terms of describing spatial autocorrelation) combinations of Principal Coordinates of Neighbor Matrices (PCNM) were calculated (Borcard and Legendre, 2002). To explicitly model spatial patterns that are independent of environmental gradients, the PCNMs were regressed against all environmental variables to allow extraction of the residuals (aka 'spatial residuals'). The 'spatial residuals' were then used in a linear regression model to predict the three biotic diversity variables, and their predicted values were derived. This was followed by a principal components analysis (PCA) on these predicted values, allowing spatial patterns to be summarized in the multivariate distribution of the three biotic diversity variables. The first two components ('s1' and 's2') accounted for 90% of the environmentally independent spatial patterns. Finally, the net effect of spatial variation (s1+s2) was captured through the use of a composite variable (diamond shape) (Grace and Bollen, 2008).

Structural equation modeling (SEM) with composite latent variables was used to determine the relative importance of abiotic conditions, biotic interactions, and spatial patterns due to population- and community-level processes. Based upon previous work (Barrett *et al.*, 2006; Elberling *et al.*, 2006; Huiskes *et al.*, 2006; Chown and Convey, 2007; Wall, 2007), an *a priori* SEM of biodiversity was built, in which topographic properties and surface temperature (summer average) are mediated through the effects of soil properties and indirectly influence the diversity of cyanobacteria (which positively correlates with total bacterial diversity as described above), fungi, and multicellular taxa (see Supplementary Figure A1.3 in Supplementary Data). To identify variables to be included in the *a priori* model, the entire set of predictors was evaluated to determine which variables were most likely to be important for reasons of parsimony, thereby eliminating soil age, geology, soil C, and conductivity.

To derive a final model with good fit to the data from the *a priori* SEM, non-significant pathways were removed, and theoretically justifiable pathways were added that were deemed to be important through inspecting the residual covariance matrices and modification indices. The relationship between biotic diversity and geology was analyzed using ANOVA, and the final SEM was found to explain more variation than geology alone. The ‘total effects’ of each variable on each biotic variable were calculated (see Supplementary Table A1.1 in Supplementary Data), which provides an order to which factors are most important by taking into account both direct and indirect effects (total effects = direct + indirect effects; indirect effects = sum of the products along each pathway). Finally, composite variables were used to estimate the net effects of three constructs (abiotic environmental filters, spatial processes, and biotic interactions) on each of three biotic response variables (cyanobacterial, fungal, and multicellular taxon diversity) (Grace and Bollen, 2008).

Results

Our data indicated that diversity was related to community composition in each of the three groups of organisms (i.e., multicellular taxa, cyanobacteria, and fungi) examined. Multicellular taxa communities were significantly nested (observed temperature = 17.5, $P = 0.01$), and the diversity of multicellular taxa was strongly correlated ($r = -0.99$, $P < 0.001$) with the first NMS axis of multicellular community composition. Nematodes were by far the most frequently observed organism, occurring in 80% of the sampled tiles, followed by hypoliths (40%), rotifers (32%), lichens (25%), tardigrades (23%), springtails (19%), cyanobacterial mats (15%), mites (12%), and mosses (11%). Cyanobacterial communities were significantly nested (observed temperature = 2.0, $P = 0.01$), and cyanobacterial diversity was correlated with both NMS axes (axis 1: $r = -0.75$, axis 2: $r = -0.66$, $P < 0.001$) derived from the cyanobacterial community matrix. Fungal communities were also significantly nested (observed temperature = 2.6, $P = 0.01$), and fungal diversity was correlated with both NMS axes (axis 1: $r = 0.71$, axis 2: $r = -0.71$, $P < 0.001$) derived from the fungal community matrix.

The initial *a priori* SEM (Supplementary Figure A1.3) did not fit the data well ($P < 0.0001$). Consequently, we added theoretically plausible pathways (e.g., direct pathways from abiotic variables to biotic variables) and removed non-significant pathways to obtain a model with an implied covariance structure that matched the observed data. The final model (Figure A1.3a) fit the data well ($\chi^2=$

45, df = 35, *P* = 0.12) and represents the most parsimonious model possible. Each pathway in the model is significant (the standardized path coefficients can be interpreted as partial correlation coefficients). The model explains between 30-40% of the variance in each biotic diversity variable. Importantly, soil properties do not clearly mediate the effects of topography and climate on biotic diversity, and topographic and climate variables have many important direct pathways to biota. Attempts to trim the model by removing select pathways substantially reduced the goodness-of-fit of the model, so each pathway is important. This model is particularly valuable and robust because it explicitly accounts for spatial patterns that do not depend on environmental variables but instead emerge from population- and community-level processes (e.g. dispersal and biotic interactions). The 'total effects' of each variable on each biotic variable indicate that elevation, slope, aspect, distance to coast, and wetness index all have significant total effects on the three components of diversity (Supplementary Table A1.1).

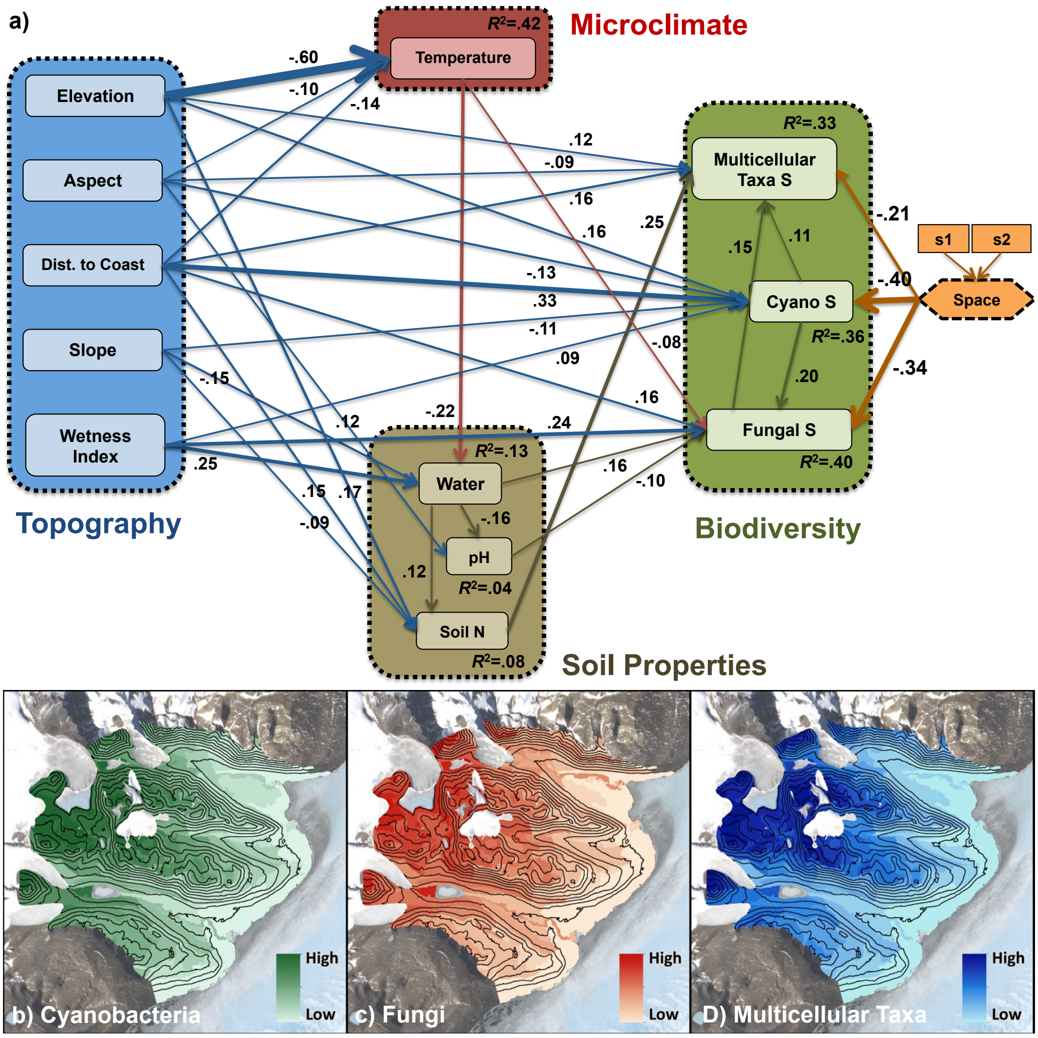

Figure A1.3 (previous page): a) Final structural equation model (χ^2= 45, df = 35, P value = 0.11) with standardized path coefficients (all paths significant, P value < 0.05). "S" represents the diversity and composition of multicellular taxa and microbial assemblages. "Space" represents environmentally independent spatial variables. Cyanobacterial richness was positively correlated with elevation, distance to the coast, and the wetness index; negatively correlated with aspect (degrees from north) and slope; and strongly related to spatial covariates. Fungal richness was positively correlated with distance from the coast, soil water content, and cyanobacteria richness; negatively correlated with pH and temperature; and strongly related to spatial covariates. Diversity of multicellular taxa was positively correlated with cyanobacterial richness, fungal richness, soil nitrogen, distance to the coast, and elevation; negatively correlated with aspect; and less strongly related to spatial covariates. Higher surface temperatures were associated with lower soil water content. b-d) Predictions of cyanobacterial, fungal, and multicellular taxon diversity across the landscape.

It is also important to note that the positive pathways among the diversities of the groups of organisms were essential to the model; removing these pathways yielded very poor model-fit indices. The chosen directions of these pathways were guided by both empirical data and theory. There were eight possible combinations of directed paths among three variables, and after arriving at the final model (Figure A1.3a), we tested all eight combinations to evaluate the sensitivity of the directions of these three pathways. Four of these eight models yielded poor fitting models ($P < 0.05$), and each of these poor models included a pathway from multicellular taxa to fungi, which is strong evidence against that particular pathway. However, the other four models were indistinguishable from a model-fitting perspective (all $P > 0.05$), and so we relied on theory to determine the direction of these pathways. Ecological theory supports pathways from cyanobacterial diversity to fungal diversity and from cyanobacterial diversity to multicellular taxa diversity, given the foundational contribution of these autotrophic single-celled organisms to this extreme ecosystem. Importantly, the positive covariance among the biota is not simply due to similar responses to abiotic conditions because each group responds individually to the sets of abiotic variables in the model. This implies that processes other than abiotic filtering drive the positive covariance among the three groups of biota.

Overall, environmental filtering imposed the strongest net effects on biotic diversity (Table A1.2). Spatial processes were the second most important set of diversity drivers, with nearly the same magnitude of effect as environmental filtering for cyanobacteria (Table A1.2). Biotic interactions were significant in determining fungal and multicellular taxon diversity, and their impact on multicellular taxa was comparable to that of spatial processes (Table A1.2). Finally, the SEM was used to generate spatially explicit prediction of biodiversity across the study area (Figure A1.3b-d) to demonstrate its potential as a tool for understanding the spatial heterogeneity of soil biota in the Dry Valleys for both scientific investigation and environmental management.

Table A1.2: Net effects of abiotic environmental filters, spatial processes, and biotic interactions on cyanobacterial, fungal, and multicellular taxon diversity. Effects were calculated using composite variables within the SEM and represent the absolute standardized path coefficients (ranging from 0 to 1).

	Abiotic	**Spatial**	**Biotic**
Cyanobacteria	0.45	0.40	0
Fungi	0.42	0.34	0.20
Multicellular Taxa	0.39	0.21	0.21

Discussion

The empirical nzTABS SEM is particularly valuable and robust because it demonstrates clear partitioning of variance according to abiotic environmental filters, spatial processes, and biotic interactions (Figure A1.3a and Table A1.2). It therefore provides unique insight into the relative importance of each factor to ecosystem organization at the landscape-scale. The prominence of abiotic drivers (in particular total soil N, soil wetness index, elevation, and distance to the coast) corroborates earlier suggestions that the species richness of Antarctic terrestrial vegetation south of 72ºS (Green *et al.*, 2011) and the structure of Dry Valley invertebrate communities (Janetschek, 1970; Poage *et al.*, 2008) are determined by local conditions. This supports the view that abiotic factors are the most important ecological filter in extreme environments (Weiher and Keddy, 1995).

Spatial processes that were independent of environmental filters impose the second strongest constraint on biodiversity (Table A1.2). Spatial patterns can be related to both biological processes (e.g., dispersal limitation) and legacy effects (e.g., historic distribution of glaciers). Such effects are important in establishing and maintaining ice-free refugia for terrestrial biota (McGaughran *et al.*, 2008), and the importance of the spatial variable in the SEM thus potentially supports a role for legacies linked to glacial geomorphology in shaping distributions of biota in the Dry Valleys (Janetschek, 1970; Convey *et al.*, 2009).

Cyanobacteria provide the energetic foundation of Dry Valley food webs and do not appear to be influenced by biotic interactions with fungi and multicellular taxa (Table A1.2). Fungal diversity was highest where cyanobacterial diversity was high, and multicellular taxon diversity was highest where both cyanobacterial and fungal diversity was high (Figure A1.3b-d), highlighting the fundamental importance of autotrophic cyanobacteria as the primary producers in this extreme ecosystem. Therefore, understanding drivers of microbial diversity is central to predicting higher trophic level responses to abiotic factors, since more diverse microbial communities support more diverse biota at higher trophic levels (Loreau *et al.*, 2001; van der Heijden *et al.*, 2008). We are confident that the positive covariance among the biota is not a result of similar responses to measured abiotic conditions because the model explicitly allowed each group to respond to a unique combination of abiotic variables. The biotic relationships are essential to the fit of the model, and their directions were supported by both theory and empirical tests.

Distance to the coast is a simple variable that includes a composite of nonlinear factors that may influence biodiversity. As expected, distance to the coast was negatively and nonlinearly correlated with soil conductivity ($r = -0.24$, $P < 0.0001$), but conductivity itself was not related to biodiversity. Distance to the coast was positively correlated with log(elevation) ($r = 0.50$, $P < 0.0001$), and negatively correlated with surface temperature ($r = -0.31$, $P < 0.0001$). However, the variance and effects of these factors were accounted for in the model, so the effects of distance to the coast are likely representing some other unmeasured (or unknown) environmental gradient influencing biodiversity.

The empirical SEM (Figure A1.3a) incorporated all measured factors, including physicochemical soil properties that cannot be obtained through remote sensing. Therefore, an additional SEM was derived that uses only unstandardized coefficients associated with factors that are obtainable through remote sensing and GIS (e.g., wetness index, temperature, elevation, aspect, distance to the coast, and slope). This “predictive” SEM can be used to make spatially explicit predictions of biodiversity across the entire Dry Valley landscape. Ongoing data collection can test the generality and power of the “predictive” SEM across a full range of environments throughout the Dry Valley ecosystem. Ultimately, the model will be used to guide future research, underpin future applications in predicting biotic response to environmental change, and support the development of best management practices for this unique ecosystem protected by the Antarctic Treaty System (http://www.ats.aq).

We sought to determine the relative importance of abiotic drivers and spatial processes to microbial and multicellular taxon diversity in an extremely simple ecosystem where biotic interactions were expected to be minimal. Indeed we found that abiotic factors such as soil temperature and topography had significant direct effects on diversity as well as indirect effects mediated through physicochemical soil properties (Figure A1.3a). However, contrary to our expectations, we also detected positive covariance among the biota as well as significant relationships between diversity and environmentally independent spatial effects. Therefore, despite our attempt to study abiotic influences in isolation, we found that the biotic and spatial drivers of diversity cannot be ignored even in the simplest and most extreme of terrestrial ecosystems. Our findings highlight the fundamental importance of incorporating biotic effects and spatial constraints when forecasting community responses to changing environmental conditions. This has direct relevance to more complex terrestrial ecosystems where biotic interactions play a markedly greater role in shaping community structure and ecosystem functioning.

Acknowledgements

This research was supported by Foundation for Research, Science and Technology of New Zealand (UOWX0710 and UOWX0715). C.K.L., D.C.L., I.R.M., and S.C.C. were supported by the New Zealand Marsden Fund (UOW1003, UOW1201, and UOW0802). D.H.W. and B.J.A. were supported by the National Science Foundation McMurdo LTER (NSF OPP ANT-1115245). J.E.B. and S.C.C. were supported by the National Science Foundation (NSF OPP ANT-0944556 and ANT-0944560). D.W.H. was supported by the UK Natural Environment Research Council, the Royal Society of London, and the Carnegie Trust for the Universities of Scotland. We thank C. M. Beard, M. J. Bentley, N. Carson, C. Colesie, Y. Cook, N. J. Demetras, R. Gademann, J. R. Quilez, A. de los Rios, P. Ross, U. Ruprecht, L. Sancho, R. Seppelt, T. Smith, M. I. Stevens, and G. Tiao (in alphabetical order) for their participation in nzTABS field expeditions. We also thank Antarctica New Zealand for logistics support. We acknowledge C.W. Herbold of the University of Waikato for his assistance with genotyping data analysis.

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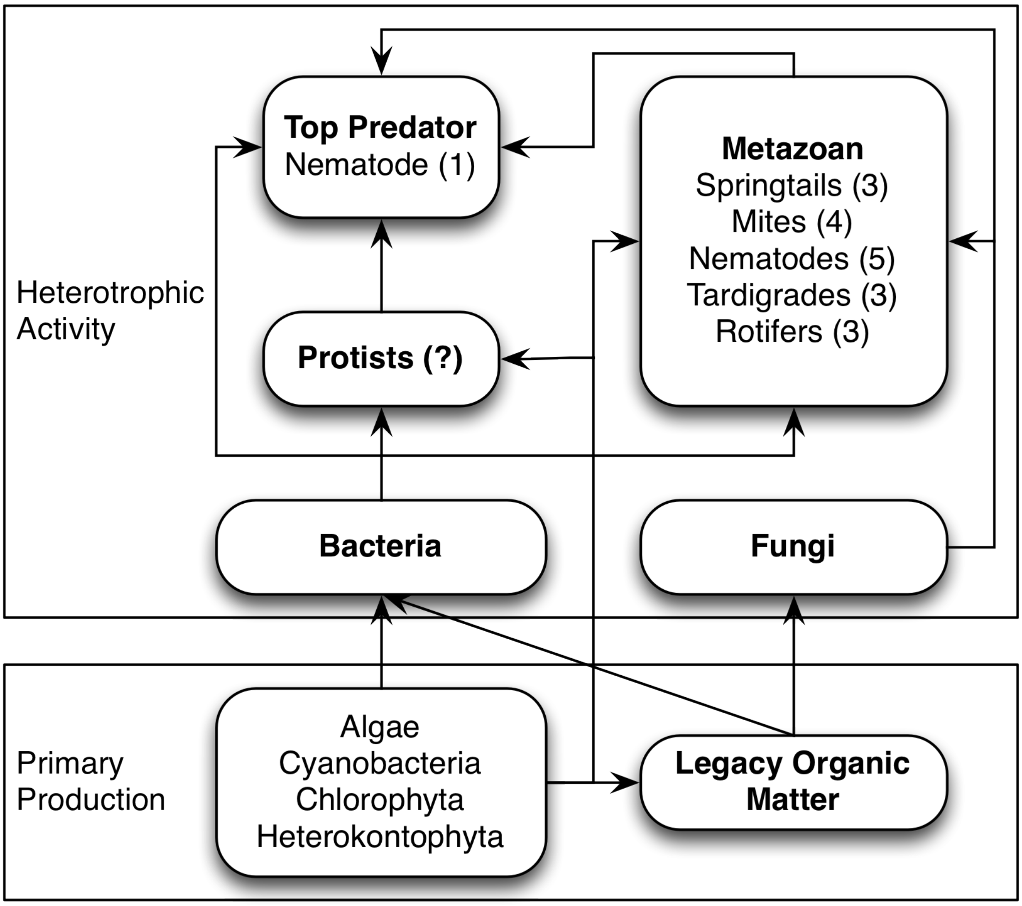
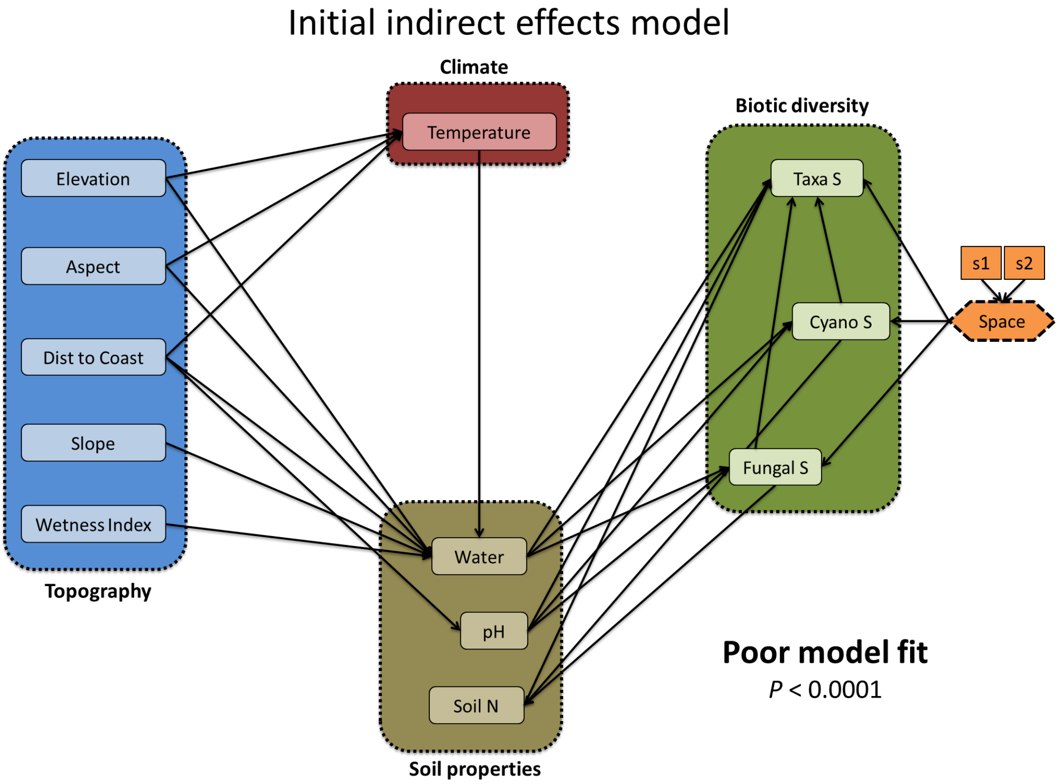
Supplementary Data

Supplementary Figure A1.1: Trophic layers of the Antarctic Dry Valley ecosystem (adapted from Cary *et al.*, 2010). The number of species known for each taxon is denoted in parentheses. Cyanobacteria are the dominant primary producer, and cyanobacterial detritus associated with historic lacustrine features (i.e., legacy organic matter) remains an important input for heterotrophic microorganisms (Adams *et al.*, 2006; Barrett *et al.*, 2006). The top predators in the Dry Valley soil ecosystem are *Eudorylaimus antarcticus*, an omnivorous nematode, and *Scottnema lindsayae*, a bacterivorous nematode.



Supplementary Figure A1.2: Example of tile delineation. Tile boundaries were defined by where change occurred in one of the following topographical or geological properties: elevation, slope (average), aspect (average), and bedrock geology. For example, tile #193B is separated from #179 by elevation (600-800 m vs. 800+ m); from #4011 by geology (granite vs. aeolian), elevation (600-800 m vs. 800+ m), and aspect (E vs. N); from #165 by geology (granite vs. M1), aspect (E vs. flat), and slope (20+° vs. 0°); and from #144 by geology (granite vs. marble) and slope (20+° vs. 0-20°).

Supplementary Figure A1.3: Initial *a priori* graph-theoretic model. Topography and climate were hypothesized to indirectly affect biodiversity through the mediating effects of soil properties in this *a priori* model.



Supplementary Table A1.1: Total and indirect standardized coefficients (all are significant, P < 0.05).

Response	Predictor	Total Effects	Indirect Effects	Direct Effects
Taxon S	Elevation	0.20	0.08	0.12
	Aspect	-0.11	-0.02	-0.09
	Distance to the Coast	0.27	0.11	0.16
	Slope	-0.05	-0.05	
	Wetness Index	0.06	0.06	
	Temperature	-0.02	-0.02	
	Soil Moisture	0.06	0.06	
	pH	-0.02	-0.02	
	Soil N	0.25		0.25
	Cyanobacterial S	0.14	0.03	0.11
	Fungal S	0.15		0.15
	Spatial Variable	-0.21		-0.21
Cyano S	Elevation	0.16		0.16
	Aspect	-0.13		-0.13
	Distance to the Coast	0.33		0.33
	Slope	-0.11		-0.11
	Wetness Index	0.09		0.09
	Spatial Variable	0.40		0.40
Fungal S	Elevation	0.10	0.10	
	Aspect	-0.03	-0.03	
	Distance to the Coast	0.24	0.08	0.16
	Slope	-0.05	-0.05	
	Wetness Index	0.30	0.06	0.24
	Temperature	-0.12	-0.04	-0.08
	Soil Moisture	0.18	0.02	0.16
	pH	-0.10		-0.10
	Cyanobacterial S	0.20		0.20
	Spatial Variable	-0.34		-0.34

Supplementary Methods

Analysis of Soil Samples (Field)

In the field laboratory, bulk soil samples were further aliquoted and processed for additional analyses:

1. DNA-based analyses (~80 g soil): aliquoted from homogenized bulk soil sample into a sterile 50 mL centrifuge tube. Analyses were carried out at the University of Waikato.
2. Soil total ATP analysis (100 mg soil x 2): aliquoted from homogenized bulk soil sample into sterile ATP assay tubes.
3. Soil geochemistry (~80 g soil): aliquoted from homogenized bulk soil sample through a 2 mm sieve into a 4 oz. Whirl-Pak. Analyses were carried out at Virginia Tech, Blacksburg, VA, USA.
4. Soil pH and conductivity (2 mL volume): aliquoted from homogenized bulk soil sample through a 2 mm sieve into a 15 mL centrifuge tube.
5. Soil water activity (A_w) (~15 g): aliquoted from homogenized bulk soil sample through a 2 mm sieve into an A_w measurement cup.

Total soil ATP was measured in duplicates (triplicates where the duplicates did not match) using a 3M Clean-Trace Beverage Test Kit (Acorn Scientific, Auckland, NZ) with a modified protocol. In short, 100 µL of Extractant Buffer was added to 100 mg of soil and allowed to incubate for 60 seconds. 75 µL of ATP Assay Solution was then added to the sample, which was immediately read using a 3M Clean-Trace NG Luminometer (Acorn Scientific). Total soil ATP levels were recorded as relative fluorescence units, and pure ATP solutions were used to check for signs of inhibition in samples with low readings. Soil pH and conductivity were measured using the slurry method (Lee *et al.*, 2012). In brief, 10 mL of deionised water was added to a soil aliquot (2 mL) and mixed thoroughly. The pH and conductivity of the resulting slurry was measured using a Thermo Scientific Orion 4-Star Plus pH/Conductivity Meter (Thermo Scientific, Auckland, NZ). Soil water activity (A_w) was measured using an AquaLab Pawkit Water Activity Meter (Decagon Devices Inc., Pullman, WA).

Analysis of Soil Samples (Laboratory)

Soil samples for moisture content and microinvertebrate analyses were transported to McMurdo Station within 72 hours of collection and analyzed at the Crary Laboratory. Soil total moisture content was determined gravimetrically by the mass loss of soil heated to 105°C for 48 hours and recorded as percentage moisture content (Barrett *et al.*, 2004). Microinvertebrates (i.e., nematodes, tardigrades and rotifers) were extracted from soils using a modified sugar-centrifugation technique (Freckman and Virginia, 1997) and identified and enumerated using bright-field microscopy (Olympus CK40 Inverted Microscope, Olympus America Inc., Center Valley, PA). Population abundances were recorded as numbers of individuals per kg soil, corrected to oven-dry weight equivalent. Demographic information for nematode populations (i.e., gender, juvenile/adult, alive/dead) was also recorded but not used for the construction of the SEM. Observed protozoan (i.e., flagellates, amoebae, and ciliates) abundances were recorded, but the data were not included in the SEM since reliable characterizations of protozoan abundance and diversity greatly exceeded our logistical capability (Bamforth *et al.*, 2005).

Soil samples for DNA-based analyses were transported to Scott Base within 72 hours of collection, where they were stored at -20°C. They were then shipped to University of Waikato under refrigerated conditions and stored at -80°C until analyzed. DNA was extracted from soils and analyzed as described below. A subsample of soil was air-dried and ground in a ball mill to a fine homogenous powder for geochemical analyses. Organic carbon and total nitrogen (inorganic and organic) content was determined from a 300 mg acidified aliquot of this dried, homogenized material with a CE Elantech Flash EA 1112 Elemental Analyzer (Lakewood, NJ) at the Virginia Tech Ecosystem Analysis Laboratory (Barrett *et al.*, 2009).

DNA Extraction and Characterization

For each sample, 0.7 g of soil was added to a microcentrifuge tube containing 0.5 g each of 0.1 mm and 2.5 mm silica-zirconia beads (BioSpec Products, Bartlesville, OK, USA). 270 µL phosphate buffer (100 mM NaH_2PO_4) and 270 µL SDS lysis buffer (100 mM NaCl, 500 mM Tris pH 8.0, and 10%

SDS) were added, and samples were bead-beaten for 10 minutes on a Vortex Genie 2 with a 24-tube vortex adapter (Mo Bio Laboratories Inc., Carlsbad, CA, USA). 180 µL CTAB extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.4% BME) was then added, and samples were shaken at 300 rpm and 60 °C for 30 minutes. Samples were centrifuged at 16,000 g for 3 minutes, prior to the addition of 350 µL chloroform:isoamyl alcohol (24:1) and 35 µL 10 M ammonium acetate. Samples were vortexed and centrifuged at 16,000 g for 5 minutes. A 200 µL aliquot of the aqueous phase of each sample was transferred to a 96 well lysis block and processed using an X-tractor Gene liquid handling robot (Corbett Life Sciences, Concorde, NSW, Australia), using the DX Universal liquid sample DNA Extraction Protocol (CorProtocol No. 14104 Version 02). Samples were eluted in 80 µL TE pH 8.5 (10 mM Tris-HCl, 0.5 mM EDTA). Negative controls, consisting of bead tubes with no sample added, were processed as described above and included in each lane of the lysis block to assess potential contamination of extracts.

For samples yielding DNA concentrations less than 1.8 ng/µL, extractions were repeated manually, without processing on the X-tractor Gene, to increase yields. The lysis steps were completed as outlined above, and the method after the chloroform step was modified as follows. The final ammonium acetate concentration of the lysate was brought to 2.5 M, 300 µL chloroform:isoamyl alcohol (24:1) was added, samples were vortexed, and centrifuged at 16,000 g for 5 minutes. The entire aqueous phase was transferred to a new tube and the chloroform step repeated with an equal volume of chloroform:isoamyl alcohol (24:1). The aqueous phase was transferred to a new tube and DNA was precipitated with addition of 0.54 volumes of isopropanol followed by centrifugation at 16,000 g for 20 min. Pellets were washed by adding 1 mL 70 % ethanol, centrifuged at 16,000 g for 5 min, and the supernatant discarded. Dried pellets were resuspended in 30 µL TE pH 8.5.

DNA extracts were quantified using Quant-iT Picogreen dsDNA reagent (Invitrogen, Auckland, New Zealand) on a FLUOstar optima fluorescence plate reader (BMG Laboratories, Offenburg, Germany). Briefly, 100 µL of picogreen solution (picogreen diluted 1:200 in TE) was added to each well of a black 96 well plate, containing 95 µL TE and 5 µL sample or standard containing 0 to 25 ng/µL lambda dsDNA (Invitrogen). Samples were excited at 485 nm and emission was measured at 520 nm. All extracts with DNA concentrations exceeding 2.2 ng/µL, were adjusted to between 1.8 and 2.2 ng/µL in TE to ensure consistent template concentrations in community analyses. The high-throughput DNA extraction method yielded >1.8 ng/µL for 386 samples while the manual adaptation of the method yielded >1.8 ng/µL for a further 87 samples. For the remaining 17 samples, DNA yields were <1.8 ng/µL but were sufficient for PCR amplification. DNA was below limits of detection in all negative controls.

Molecular Analysis of Microbial Communities Using ARISA

PCR targeting the intergenic spacer between the 16S and 23S rRNA genes of the cyanobacterial ribosomal operon and the intergenic spacer between the 18S and 23S rRNA genes of the fungal ribosomal operon were completed for each extraction. Primer pairs for cyanobacterial amplifications were CY-ARISA-F (Wood *et al.*, 2008) (5'-PET-GYC AYR CCC GAA GTC RTT AC-3') (Applied Biosystems) and 23S30R (Wood *et al.*, 2008) (5'-CHT CGC CTC TGT GTG CCW AGG T-3') (Integrated DNA Technologies) and for fungal amplifications were ITS1F (Gardes and Bruns, 1993) (5'-FAM-CTT GGT CAT TTA GAG GAA GTA A-3') and 3126T (Sequerra *et al.*, 1997) (5'-ATA TGC TTA AGT TCA GCG GGT-3') (Integrated DNA Technologies). Each 25 µL reaction contained 1X PCR buffer, 3 mM $MgCl_2$, 1 unit of Platinum Taq DNA Polymerase (Invitrogen), 0.25 µM primers, 0.2 mM dNTPs (Invitrogen), and 5 µL of template DNA. Thermal cycling was completed on a Bio-Rad DNA Engine Peltier Thermal Cycler 200 (Bio-Rad, Hercules, CA, USA). Thermocycling conditions for cyanobacterial ARISA consisted of 94 °C for 2 min; 35 cycles of 94 °C for 20 s, 55 °C for 15 s, and 72 °C for 1 min 30 s; and 72 °C for 3 min. Thermocycling conditions for fungal ARISA consisted of 94 °C for 3 min; 35 cycles of 94 °C for 20 s, 52 °C for 20 s, and 72 °C for 1 min 15 s; and 72 °C for 5 min. Amplicons were diluted 1:20 in de-ionized water. A mixture containing 2 µL of diluted amplicon, 0.13 µL of Liz-1200 internal size standard (Applied Biosystems), and 7.87 µL of HiDi formamide (Applied Biosystems) was heat denatured at 95 °C for 5 minutes and cooled to 4 °C for 2 minutes, before being resolved on an ABI 3130 Genetic Analyzer (Applied Biosystems) at the University of Waikato DNA Sequencing Facility.

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