Modelling Microbial Diversity in Antarctic Soils

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Memorandum

Except where acknowledgement is given this thesis is the unaided work of the author. Material presented has never been submitted to Newcastle University or to any other educational establishment for the purposes of obtaining a higher degree.

Abstract

Microorganisms play a crucial role in supporting biodiversity, maintaining marine and terrestrial ecosystems at the crux of the nutrient cycle. They are the most diverse and abundant of all living creatures, yet little is understood about their distribution or their intimate relationship with the environment. Antarctic ecosystems are among the most simple on Earth; with basic trophic structuring and the absence of many taxonomic groups, they are also isolated geographically with small patchy areas of nutrient inputs. In this instance, Antarctica becomes a pristine laboratory to examine the ecological paradigms already applied to macro-organisms, to determine if common biological laws govern the distribution of biology globally. The decline of biodiversity with increasing latitude is one such observation in the distribution of macro-organisms. In this study, soil microbial community samples were retrieved over a latitude of 56 to 72 °S across the Antarctic Peninsula region. This is a region of special interest due to a rapidly warming climate with mean temperatures increasing at several times the rate of mean global warming. Sites were biologically and environmentally profiled and data used in a variety of multivariate analysis in order to identify spatial trends and infer mechanisms that may be driving Antarctic terrestrial food webs; or where this was not possible, the areas where focus was needed to increase the information profile to allow this. Results indicate a lack of linear latitudinal gradient in microbial diversity, but do show a correlation with environmental heterogeneity; analysis of site diversity identified a gradient between warmer wetter areas, and areas synonymous with cold desert environment at 66°S, supported by both phylum composition and indicative soil chemistry. This was confirmed through principal co-ordinates of neighbours' matrices analysis (PCNM), with distinct regions of community composition being identified when viewed with respect to environmental variables. Considering an overview of diversity with respect to environmental variables provided additional structure to test hypotheses about nutrient webs through structural equation modelling (SEM), and inferred that areas of patchy nutrient input exist and by means of ornithogenic guano additions promote higher C and N availability, increasing microbial abundance and richness.

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Common Terms and Abbreviations

AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CA	Correspondence Analysis
CCA	Canonical Correspondence Analysis
CFI	Comparative Fit Index
DOC	Dissolved Organic Carbon
EC	Electrical Conductivity
ELFA	Ester Linked Fatty Acid
LGM	Last Glacial Maximum
MDR	Mean Daily Range
MEM	Moran's Eigenvector Map
MLE	Maximum Likelihood Estimation
MSPA	Multi-Scale Pattern Analysis
NH_4^+	Ammonium
NO_3^-/NO_2^-	Nitrate/Nitrite
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
T-RFLP	Terminal Restriction Fragment Length Polymorphism
PCA	Principle Components Analysis
RDA	Redundancy Analysis
RDP-II	Ribosomal Database Project
PCNM	Principle Components of Neighbours Matrices
RMSEA	Root Mean Square Error of Approximation
SEM	Structural Equation Modelling
VIF	Variable Inflation Factor Analysis

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Thesis Introduction

1.1. The Antarctic Environment

Antarctica is a pristine field laboratory like no other. The only continent to remain uninhabited since the late Cenozoic era approximately 40,000 years ago, human presence on Antarctica was not recorded until 1821 with the landing of seal hunter John Davis at Hughes Bay. With only 0.32% of land recurrently ice free (BAS, 2007), the extreme conditions of the Antarctic environment have restricted colonization to only the most robust of organisms. Excluding the presence of few avifauna and seal colonies, terrestrial food webs are typically of basic trophic structure with nutrient cycles heavily reliant on soil microbes (Wynn-Williams, 1996; Hopkins *et al.*, 2005).

Across the continent, species level diversity is low and many higher level taxonomic groups, such as molluscs, trees and vertebrates, are missing (Convey, 2010; Barnes *et al.*, 2006). Until recently, Antarctic soil was also regarded as biotically depauperate, or species poor (Adams, 2006) but recent estimates of both diversity and abundance have considerably higher values than previously thought. Cowan *et al.* 2002 found total microbial cell counts of between 10^6 and 10^8 cells/g^{-1}, 4 fold previous estimates, but still much less than temperate soils, where microbial numbers are in the region of 2.1 x 10^9 g^{-1} (Trevors, 2010).

Low species diversity is typically associated with lower functional capability and/or output of a community (Tilman *et al.*, 1997) and consequential instability to perturbations and environmental change. As the Antarctic Peninsula is the fastest warming region in the southern hemisphere (Vaughan, 2007; Turner *et al.*, 2011), understanding how climatic and consequential environmental changes impact on these soils and their ecological function, may

have global importance in understanding the effects of global warming on terrestrial communities.

Across much of the Antarctic Peninsula, daily soil temperature fluctuations about 0°C are common and freeze-thaw cycles occur several times daily over the austral summer (Davey *et al.,* 1992). Antarctic soils at higher latitudes may experience several months where soil remains frozen continuously, this means microbial access to water and nutrients is limited. Freeze-thaw cycles cause physical disturbance to soil substrate structure (Schimel and Mikan, 2005) and may damage or destroy some microorganisms, but through these effects, also results in the liberation of nutrients from ruptured organisms when thawing occurs (Skogland *et al.,* 1988; Edwards and Cresser, 1992). The frequency of freeze-thaw cycles have been found to impact Antarctic bacterial nutrient cycle processes, via changes in functional gene density and also by altering fungal community structure (Yergeau *et al.,* 2008).

Recently, a temperature increase of up to 0.54 °C per decade has been reported for the western peninsula (Turner *et al.,* 2013) significantly greater than the estimated rate of global warming at 0.8 °C per century (Hansen *et al.,* 2010). An increase in temperature may lead to what is regarded as more 'hospitable conditions' for soil microbes with increased liquid water availability and the consequential release of minerals and energy sources (Emanuel *et al.,* 1985). It has been suggested that microbial communities exposed to frequently unstable environments, such as those of polar soils, will be more resilient to changes in climate (Waldrop and Firestone, 2006). Recent studies of polar soil communities have been divergent and presented results supporting evidence of both acclimation (Rinnan *et al.,* 2009; Yergeau *et al.,* 2012) and a lack of response (Hartley *et al.,* 2008) in microbial communities or tolerance (Bale, 2002; Bokhorst *et al.,* 2012) to changing temperatures in larger organisms.

Currently, our understanding of terrestrial Antarctic communities is limited due to a lack of sampling coverage with many studies concentrated around a small number of accessible areas (Convey *et al.,* 2012). However, although simple compared to temperate soils, it has broadly been acknowledged that Antarctic ecosystems are more diverse than previously thought (Cary *et al.,* 2010; Chong *et al.,* 2012). Additionally, there are several examples of regional trends in terrestrial organisms, including an observed latitudinal gradient in flora and endemism of 33% - 50% of lichens in continental Antarctica (Øvstedal and Lewis Smith,

2001; Peat *et al.,* 2007), endemism in invertebrate taxa (Adams *et al.,* 2006; Maslen and Convey, 2006; McGaughran *et al.,* 2010; Pugh and Convey, 2008; Caruso *et al.,* 2009; Mcgaughran *et al.,* 2011) and microorganisms (Vyverman *et al.,* 2010; Chong *et al.,* 2012). Why these patterns exist is difficult to ascertain but one mechanism suggested is that species in some areas have been geographically isolated and preserved creating areas of ecological 'refugia'; an example of which has been named the 'Gressitt Line', a boundary where the Antarctic Peninsula meets the continent across which few species are shared (Chown and Convey, 2007). This is further supported by bio-molecular evidence suggesting endemic lineages may be traced back millions of years, some as far as the break up of Gondwana (Allegrucci *et al.,* 2006: Convey and Stevens, 2007; Convey *et al.,* 2008, 2009).

Molecular studies cataloging microbial communities have increased over the last decade, though the majority have been concentrated around the South Orkney and South Shetland Islands (Yergeau *et al.,* 2007; Chong *et al.,* 2009; Ganzert *et al.,* 2011; Hill *et al.,* 2011) or the Dry Valleys (Aislabie *et al.,* 2006; Niederberger *et al.,* 2008; Teixeira *et al.,* 2010) and restricted to less than 10 localised sites. This lack of sampling scope in any single study may be the reason no clear geographical trend has been observed in Antarctic microbial communities, unlike Arctic soils (Chu *et al.,* 2010) which appear to follow the same distribution characteristics as microbial communities globally (Green and Bohannan, 2006; Fierer and Jackson, 2006). A meta-analysis by Chong *et al.* (2012) pooled 16s rRNA genetic data from several previous studies across a range of Antarctic soils, and distinctive soil communities were identified separating those of McMurdo Dry Valleys (latitude 77-78.5° S; longitude 170.13-161.33 W°) and those of the Scotia Arc/ Antarctic Peninsula region (latitude 51-72.03° S; longitude 58-85.60 W°), implying the existence of a broad scale factor such as difference in climate or geological history as a feasible explanation for distinct community distributions. Many Antarctic microorganisms have also displayed consistent dependencies upon environmental conditions such as differences in soil pH, heavy metal content, nutrients and salinity (Yergeau *et al.,* 2007; Aislabie *et al.,* 2008; Niederberger *et al.,* 2008; Chong *et al.,* 2009; Chong *et al.,* 2010; Pointing *et al.,* 2010; Ganzert *et al.,* 2011).

Recent developments in high throughput genetic technologies profiling microbial data emphasize the importance of analogous numerical tools to describe relationships between community and environment (James and McCulloch, 1990). Such tools, though widely available in ecology, instill a level of reticence in microbial ecologists because data often

requires preparation prior to analysis which can be time consuming (Ramette, 2007) and even basic multivariate methods; such as ordination, assume an understanding of the data type and flaws before an appropriate technique may be chosen (Legendre and Legendre, 1998). Exploratory analysis, which attempts to reveal trends or clusters of objects, is often preferred to hypothesis driven techniques (Ramette, 2007) because of the simplicity of conducting such analyses. However, mere identification of gradients or archetypes; typically produced by exploratory techniques, exert limitations on intellectual interpretation and identification of key mechanisms within a system.

Assessing patterns in microbial diversity is cumbersome as most microbial species are thought yet to be discovered; it is crudely (and conservatively) estimated less than 0.01% of bacterial species have been isolated and identified (Schloss and Handlesman, 2006). Furthermore, community profiling methods are often inadequate in sampling scale to capture a true representation of community diversity, due to the high heterogeneity of the terrestrial environment and cosmopolitan distribution of microorganisms throughout**.** Being at the extremity of both latitude and life, the depauperate nature of Antarctic biota is in this case advantageous to scientific studies; Antarctic microbial systems provide the perfect basic canvas to examine the complex relationship between taxa and their distribution patterns. Current evidence suggests that Antarctic ecosystems, in particular those of the Antarctic Peninsula where global warming is accelerated, represent a harbinger of the consequences of climate change. Increasing temperatures represents an improvement in living conditions for many polar organisms (Walther *et al.*, 2003). Preliminary studies have indicated that it will be the dramatic expansion of plant colonies, rather than temperature increase per se, which will have the greatest impact on soil communities (Roberts *et al.*, 2009). However, before the impact of climate change on Antarctic microbiology can be quantified, we must first unravel the trends exhibited in terrestrial diversity and community composition and their underpinning mechanisms (Wynn-Williams, 1996).

The broad aims of this study are:-

- To explore patterns in diversity of Antarctic soil microorganisms using two different microbial community datasets and environmental data.
- To assess the validity of the application of common ecological theory to contemporary microbial community data.
- To identify and utilise appropriate ecological models in order to delineate biological trends in the data.

Chapter 1 introduces various aspects of the Antarctic environment and biology, biogeography and microbial diversity and ecological modelling approaches.

Chapter 2 describes the data used in this study and the method of collection, and introduces the modelling and analysis methods used to form results. Specific methods as applied in each analysis are reported in the relevant results chapters.

Chapter 3 presents the dataset used with simple analyses of differences in soil chemical properties, physical influences, microbial abundance and diversity across all samples. The results helped to form hypotheses tested in subsequent chapters.

Chapter 4 examines the hypothesis that increasing latitude, which also correlates to decreasing temperature in the southern hemisphere, will result in differences in microbial diversity and community composition across the range of sites sampled.

Chapter 5 uses a spatially scaled modelling approach to examine differences and similarities in the structure of environmental and bacterial community data.

Chapter 6 attempts to link microbial fatty acid and bacterial genetic community profiling datasets to gain a broader perspective of environmental dependency; this is performed by characterising sites according to dominant external influences.

Chapter 7 tests a theoretical representation of a microbial energy web to determine the major influences driving community abundance and richness in Antarctic soil.

Chapter 8 presents a final synthesis of the major results from the study.

1.1.1. Continental History

Antarctica once belonged to the super-continent Gondwana, along with most of the present day southern hemisphere land mass. The early paleo-climate was thought to be tropical with no polar ice and supported a diverse range of animal and plant life (Pross *et al.,* 2012). Rifting and stretching began in both Gondwana and Laurasia with the separation of India and Africa from Gondwana at 125 mya BP. Towards the end of the Mesozoic era, a mass extinction of up to 90% of marine and 85% of terrestrial species occurred, probably the result of one or several asteroid collisions with earth - the 'K-T extinction event' (Mcleod *at al.,* 1997). Phototrophic plants suffered due to the atmospheric restrictions of the asteroid ash cloud on solar energy penetration and toxic sulphuriouse emissions from high volcanic activity called continental super-plumes, such as those of the Deccan Traps in central India (Chenet *et al.,* 2007). The presence of the chironomid midges *Belgica antarctica* and *Eretmoptera murphy***,** both still found in Antarctica, may also be dated back to the Mesozoic era around 68.5 Myr (Allegrucci *et al.,* 2006). Various species of mesoorganisms belonging to mite and copepod taxa, which are present now across the continent, can also be traced back to around this time (Bayly *et al.,* 2003; Kellogg and Taylor, 2004; Convey and Stevens, 2007; Convey *et al.,* 2008)

By the beginning of the Eocene epoch (54 mya), soil temperatures were around 15˚C (Robert *et al.,* 1994). Australia remained attached to Antarctica until the late Eocene era around 41 mya (Scher and Martin, 2006); when Antarctica moved towards its contemporary location over the South Pole. Continental isolation was completed with the separation of the Antarctic Peninsula from South America by the opening of the Drake Passage 43-10 mya (Scher and Martin, 2006). Global temperatures began to cool due to the 'Azolla event', where a mass Azolla fern bloom across the 50Mkm^2 Arctic Ocean floor acted as a carbon sink (Speelman *et al.,* 2009). The first Antarctic glaciers are thought to have formed 40-25 mya (Liu *et al.,* 2009) with substantial cooling onset with the establishment of the circumpolar current.

Carbon sequestration dropped CO_2 levels by 80% by the late Oligocene (Miller *et al.,* 2009) resulting in the 'Ice-house' climate we know today.

1.1.2. Modern climate and environment

However considered, environmental conditions of Antarctica are extreme. Even compared to the Arctic, Antarctica is colder at comparative latitudes, due to high altitude (significantly thicker ice cover), land mass allowing thermal release and warming effect of arctic being comprised centrally of ocean. Most climatic parameters are driven by the geographic position of Antarctica as a continent. The low temperatures experienced are maintained by a combination of high latitude and high altitude. Increasing latitude reduces sunlight exposure via low angle of incidence i.e. due to the large angle of radiation over the poles, solar energy is spread over a larger surface area, limiting surface temperatures. This also results in a varying degree of days either without sunlight completely, complete winter darkness, or days without night. Due to the size of the Antarctic continent this is seen to different extremes dependent of latitude. The South Pole experiences a period of 6 months where the sun doesn't rise, albeit there are periods of lengthened twilight which extend the daylight period. This effect diminishes towards the Antarctic coast where only 2 to 4 weeks may be in complete darkness (Australian Antarctic Division, 2013). In addition, being the world's highest continent with an average altitude of 2,300m, Antarctica experiences significant environmental lapse rate (ELR); where temperature drops -1◦C per 100m increase in height. Annual temperatures average between -2 and -8◦C for the Antarctic Peninsula, in summer reaching around 0 ◦C for northerly Peninsula sites and dropping as low as -15◦C in winter at lower latitudes (see Table 1.1). Low level annual precipitation of 50 mm and 200 mm for continent and coastal regions respectively (Ward, 2001) categorize Antarctica as the world's largest desert.

Table 1.1. Summary of mean surface air temperatures at research bases from the north to south

Base Location	Latitude (decimal degrees ◦S)	Longitude (decimal degrees ◦W)	Summer temperature (◦C)	Winter temperature (◦C)	Annual temperature (◦C)
Signy Island[2]	60.7	45.6	-0.85	-3.10	-1.90
Bellingshausen[1]	62.2	58.9	-0.63	-4.82	-2.76
Auturo-Prat[1]	62.5	59.7	-0.03	-4.40	-2.28
O'Higgins[1]	63.3	57.9	-1.75	-6.52	-4.23
Rothera[1]	67.5	68.1	-1.97	-6.30	-3.75
San Martin[1]	68.1	67.1	-2.03	-6.87	-4.04
Mars Oasis[2]	71.9	68.3	-2.70	-13.95	-7.90

Notes

Peninsula [1] temperatures based on 2012 monthly mean adapted from SCAR READER project (BAS,2012). [2] temperatures over three and five years at Signy Island and Mars Oasis respectively, adapted from Dennis *et al.*, 2013 courtesy of Dr Helen Peat, BAS.

High continentiality and low temperatures promote little atmospheric moisture, which is maintained by fluxes in air pressure. A high pressure cell sits over the South Pole and, as air moves towards lower pressure coastal areas, strong katabatic winds carry precipitation away from the continent towards areas of low altitude increasing accumulation in these areas (Knuth, 2007). The Peninsula is strongly influenced by low pressure and despite a low amount of rainfall the precipitation rate exceeds the evaporation rate resulting in relatively high soil moisture (Vaughan *et al.*, 2003). With increasing latitude there is significant decrease in moisture availability and temperature. General Bernard O'Higgins station (~63º 19' S, ~57º 04' W) situated on the north peninsula is on average 20 ◦C warmer annually than Sky Blu station (~74º 58´ S, ~70º 46´ W) , the most southern station of the Peninsula (Jones and Reid, 2001). Geographic and climatic conditions are summarised in Figure 1.1.

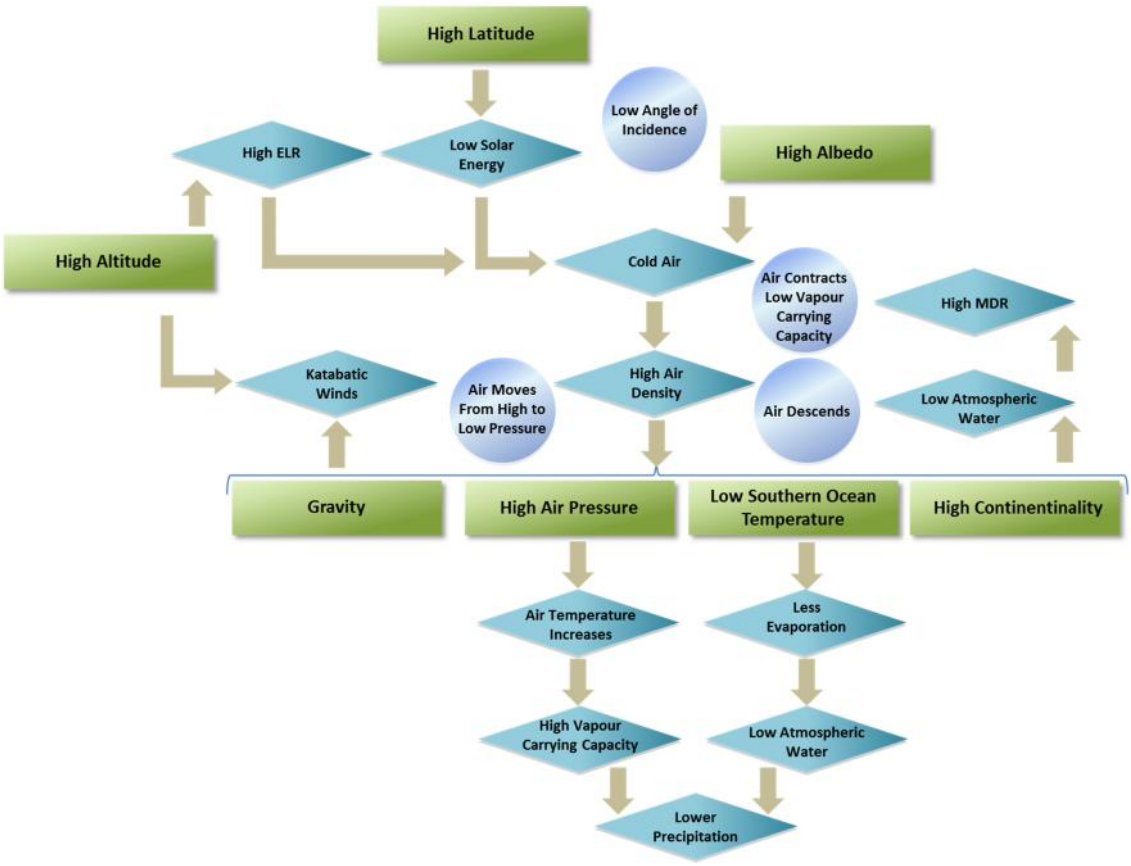

Figure 1.1. Summary of the abiotic influences driving and maintaining the Antarctic climate. Compiled using Vaughan *et al.*, 2003 & BAS, 2012. ELR is environmental lapse rate and MDR is mean daily range.

Three biogeographic zones of Antarctica have been identified: sub-, continental and maritime (Figure 1.2.). Continental Antarctica is generally regarded as the eastern peninsula and the area below the 'Gressitt Line' (Smith, 1984; Convey, 2013). Conditions on the continent are far more severe than other regions; with low precipitation and temperature levels. Continental conditions will not be discussed in detail as none of the sites in this study fell into this category. The sub-Antarctic mainly comprises of the islands above the northern limit of sea-ice. Conditions are significantly wetter than at higher latitudes and temperature range tends to be small across all seasons with average temperatures above zero for most of the year (Pendlebury and Barnes-Keoghan, 2007). South Georgia (54°30′S 37°00′W) is the most northern location sampled in this study. Climatically, it is characteristic of the sub-Antarctic and is considered ecologically rich**.**

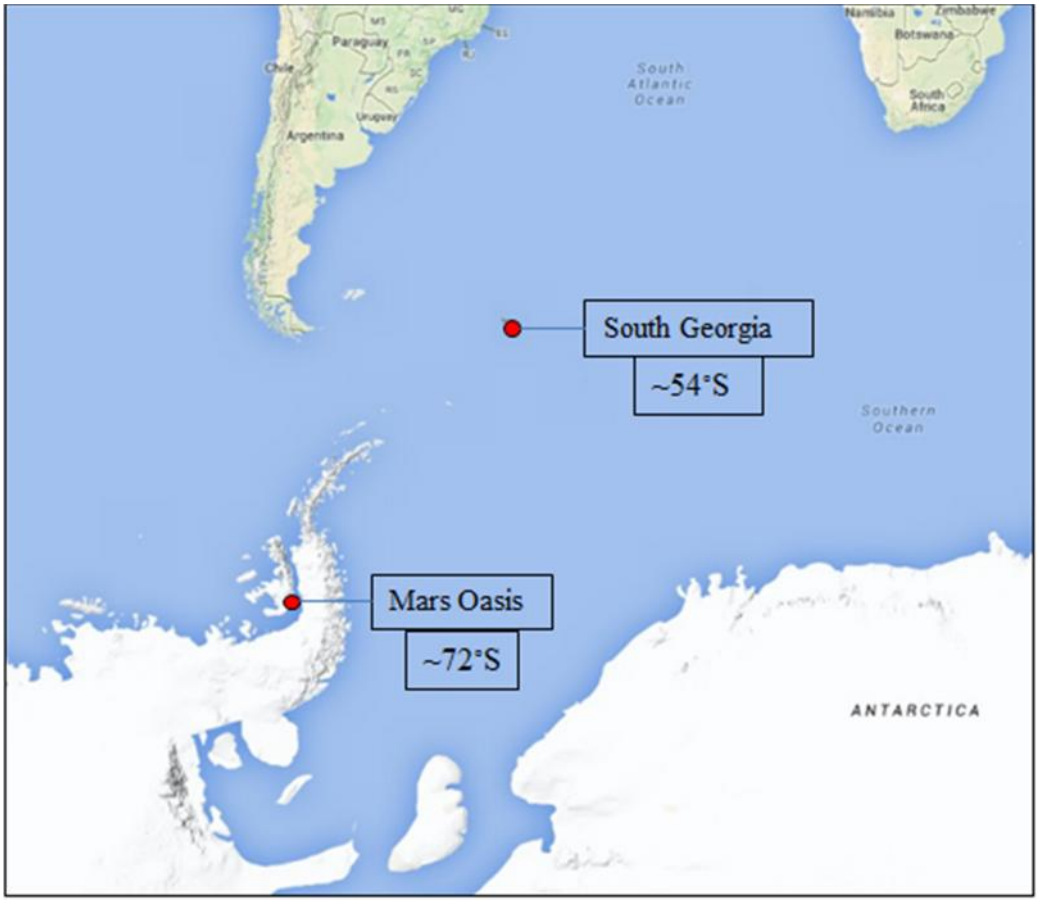

Figure 1.2 Map of the Antarctic Peninsula region.

Showing the extent of the study region from the most northern site, South Georgia, down to the most southern site, Mars Oasis. Image from Google Maps (2014).

The majority of sampling sites in this study may be classed as 'maritime' and span northern to southern Graham Land. Maritime locations are those with coastal association on or around the west coast of peninsula where influence of seasonal changes is strong. Surface temperatures in the maritime region are highly variable and diurnal freeze thaw cycles common throughout summer months (Wynn-Williams, 1996). With only 0.34% of the continent permanently ice free, these exposed soil areas tend to be 'coastal oases' characterised by low altitude and close to areas of sea ice formation (BAS, 2004). Input and disturbance from marine vertebrates may be locally abundant (Convey, 2003). Conditions further south show decreasing nutrient input, transient low level water availability and high UV exposure (Yergeau *et al.*, 2007). Vegetation is largely dominated by cryptogams (lichen, mosses) which become less prevalent toward southern Graham Land. Mesofaunal groups are

largely restricted to the north and southern sites are dominated by microbial producers (Maslen and Convey, 2006). The most southern location in our transect, Mars Oasis, is generally regarded as a 'biological hotspot' with high bacterial and mesofaunal diversity (Convey and Smith, 1997) although there is no consensus on why this is the case.

1.1.3. Impacts of climate change

Climatic observation only began systematically in Antarctica with the International Geophysical year (1957-58). Since then, there have been mixed trends of warming and cooling observed in continental Antarctica, for example, surface temperatures have increased across West Antarctica (Turner *et al,* 2013) but, at Admundsen-Scott base, close to the South Pole, temperatures have cooled over recent decades (Turner *et al.,* 2013). The Antarctica Peninsula however, is the fastest warming part of the Southern Hemisphere; with temperatures increasing 10 times that of mean global warming for winter averages (BAS, 2007), and representing a net change 5 times that of the global average over the last century (Vaughan *et al.*, 2003; Hansen *et al.*, 2010). The Southern Ocean is also experiencing dramatic response to changing climate, warming of around 0.2°C per decade has been observed, (Domingues, 2008). In response to this, in the last 50 years 25000 KM^2 of ice from floating ice shelves has been lost (BAS, 2012), and along the west coast of the Antarctic Peninsula sea ice extent has decreased by 40%, (ASOC, 2008; Turner *et al.*, 2013), although a mild increase in sea ice extent has been observed in other areas (Turner *at al.,* 2013). Also, while 87% of glaciers along the west coast of the Peninsula have retreated over last 50 years (Cook *et al.*, 2005), the loss of ice shelves has increased meltwater run off and glacial acceleration, showing an increase flow rate of 12% between 1992 and 2005 (Pritchard, 2007). This increase in meltwater run off and glacial flow rate, suggests a combined northern Antarctic Peninsula contribution of 0.16 ± 0.06 mm per year to global sea-level rise (Pritchard, 2007).

Inconsistency amongst sea ice boundaries and accumulation of sea ice has begun to affect the continent's largest inhabitants, both directly by change in suitable breeding grounds for some penguin species (Ducklow *et al.,* 2007).and indirectly by affecting food supplies. One example of this is the loss of krill stocks in the southern ocean (Atkinson, 2004) due to a reduction in growth habitat for the krill because of Southern Ocean warming (Hill *et al.,* 2013). Chinstrap and adelie penguins, which reside in the western Antarctic Peninsula

(WAP) and Scotia Arc area, are thought to be among of the most vulnerable species to climate change through the changes in krill stocks (Smith *et al.*, 1999). With krill stocks reducing (as much as 80% since 1970) chinstrap and adelie penguins are showing declining abundance with an average annual reduction in abundance of -4.3% and -2.9% respectively (Trivelpiece *et al.*, 2011).

For many Antarctic flora and fauna climate change represents improved living conditions and signs of transformation in response to these improvements have already begun (Walther *et al.*, 2003). Environmental alleviation is predicted to increase colonisation leading to increased diversity and biomass, a more complex ecosystem structure, and a switch of dominance from abiotic to biotic environmental drivers (Convey, 2010). Increasing temperatures and subsequent increased water availability is thought to be responsible for more than doubling the population size of Antarctica's two flowering plants (*Deschampsia* and *Colonbathus*) in some regions of the Peninsula (Convey, 2006), which is predicted to have already had profound changes on dissolved soil carbon and nitrogen and microbial communities (Roberts *et al.*, 2009). However due to the complexity of these ecosystems and their response to multiple stressors, the continuing effects of global warming may provide other drivers which could oppose the improved conditions provided by global warming, e.g. increased UV exposure (Convey, 2002), or increasing influence of alien species due to the environmental alleviation (Frenot *et al*, 2005).

Antarctic microbial communities respond quickly to what would be regarded as an improvement in environmental conditions, less than 3 years in response to warming (Yergeau *et al.*, 2011). This rapid response to warming is due to Antarctic bacteria being resilient to temperature stress; bacterial communities acclimate their minimum growth temperature (T_{min}) according to the temperature of the soil they inhabit, allowing growth to occur well below 0◦C, (Rinnan *et al.* 2009). Rinnan *et al.* (2009) found T_{min} averaged -10.5◦C across a range of locations on the Antarctic Peninsula making them cold tolerant, furthermore bacteria have been found to be less affected by freeze-thaw stress and more responsive than fungi to increases in temperature, being most active at 15◦C (Tibbles and Harris, 1996; Yergeau and Kowalchuk, 2008). In addition, although Antarctic bacteria can function in low temperatures, for many psychrotolerant organisms, their optimum temperature is above 20°C, so even a small increase in temperature alleviates stress and increases bacterial affinity for available substrates (Nedwell, 1999). Whilst Antarctic bacteria can respond rapidly to increased

temperature, this has been shown to lead to a weakened linkage between the bacterial community taxonomic and functional richness (Yergeau *et al.,* 2011), developing a more generalist microbial community, with functional genes shared by many species

1.1.4. Humans in Antarctica

Human impacts began most notably with whaling and sealing; leading to extensive localised impact at sites of high anthropogenic activity, e.g. South Georgia was occupied on a permanent basis by Norwegian and British sealers from 1786, and the introduction of many non-native species from ships' stores, food waste and animals such as rats, rabbits and sheep (Convey and Lebouvier, 2009). Later during the exploration years on the Antarctic continent microbes were increasingly introduced to the continent through food, waste, ponies etc. e.g. the Shackleton and Scott parties' impact at Cape Royds and Cape Evans (Meyer, 1962). More recently, tourism and intensive science programmes have resulted in the introduction of several invasive alien species and persistent pollutants which has greatly increased the scope for human impact on the Antarctic environment. Numbers of tourists visiting Antarctica are growing exponentially with a fourfold increase in the number of inflatable boat landings between 1995 and 2005 (Frenot *et al.,* 2005), thus areas of human contact are not restricted to a small number of sites nor are the landings consistent in location (Frenot, 2005). The Committee for Environmental Protection of the Antarctic Treaty System has recognised these to be issues of political concern (Chown and Convey, 2007).

The greatest human impact has perhaps been observed on flora, both in the introduction of alien species (e.g. *Elymus repens*), or unexpected concentration of some species at sites of anthropogenic influence (e.g. *Prasiola crispa*), and in the destruction of species (e.g. *Usnea*) and expansion of geographic range in others (e.g. *Poa annua*). Bourzat and Monie (1977) reported that seeds of *Elymus repens*, or 'couch grass', along with several other grass species, had been deliberately sown on the Desolation Islands in the 1970's to provide grazing ground for sheep. *Poa annua*, a cosmopolitan grass regarded as 'a harmful organism and contaminant' (USDA-ARS, 2011) is thought to have been accidentally introduced to King George Island in 1985 (Olech, 1996), where it was first observed growing in the hollows of a foot grid outside of Arctowski Polar station. Since 1985, *Poa annua* populations have expanded considerably across all major sub-Antarctic islands; colonisation of the grass is typically associated with soil disturbance and it commonly thrives on well-trodden ground

(ISSG, 2010). *Prasiola crispa* is an alga formerly most commonly found at sites high only in seal and penguin guano, has become prevalent in recent years in soils enriched with organic waste, in close proximity to Antarctic buildings (Olech, 1996). The sensitive lichen *Usnea* - "old man's beard", has become a common destructive souvenir of tourists visiting Antarctica (Olech, 1996), given its symbiotic role with fungi, microbial diversity is likely to be altered at these localised anthropogenic sites.

Endemecity of Antarctic microbial groups and the true level of human interference across Antarctica are more difficult to determine. Species which appear unique to Antarctica may be the result of lack of comparable data from elsewhere or may be unreliable due to the logistical problems associated with collecting robust data from polar environments (Frenot *et al.,* 2005). Teixeria *et al.* (2010) found bacterial communities proximate to human presence were notably different, with an abundance of proteobacteria and not firmicutes as was the case with all other sites. Various human-associated pathogens have been isolated in proximity to Antarctic stations and *Clostridium perfringens,* a bacterium found predominantly in human faeces was isolated as far as 400m from McMurdo sewage outfall (Smith and McFeters, 1999). Though there is increasing evidence to suggest the transmission of human-mediated disease to Antarctic fauna (Kerry and Riddle, 2009), especially avian species; this is confounded by the fact great numbers tend to migrate to South America and Australia during winter months.

1.1.5 Antarctic soils

Antarctic soil has not been profiled systematically across the continent as only 0.34% of land is ice free (BAS, 2007). Antarctic soil is generally regarded as mineral but varies greatly in moisture and nutrient content between maritime and continental locations (Beyer *et al.,* 2000). Due to a lack of higher trophic organisms, nutrient cycles are heavily reliant on microorganisms, and are constrained by low temperatures, restricting moisture availability and suppressing metabolic activity. Nutrient pools are generally low. Some coastal areas have proportionately large nitrogen input from guano though most is restricted to plant-microbe interactions (Roberts *et al.* 2009*)* or in the case of carbon, to microbial fixation and mineralization of contemporary detritus additions (Hopkins *et al.,* 2006). Soil amino acid turnover is extremely rapid (Jones *et al.,* 2004) and, in conjunction with short peptides, are the main source of available nitrogen to *Deschampsia antarctica* and other vegetation (Hill *et al.,* 2011). Variation in pH depends mostly on colonization status of penguins; pH ranges

from 5.6-8.0 in previously colonized sites and 5.7-7.0 in presently occupied sites (Jones *et al.*, 2004; Aislabie *et al.*, 2009). Fellfield soils which have no history or proximity to ornithogenic soils tend to be neutral 6.9-7.7 (Yergeau *et al.*, 2007a). The basic bacterial processes of nitrogen and carbon cycles in Antarctic soils are summarised in Figure 1.3.

Soil temperature fluctuation is highly seasonal and thermal variation at the surface soil can be wide ranging, especially at fellfield sites where black body absorption is high (Block *et al.*, 2009). Frequent freeze thaw cycles impose perhaps the greatest stress on Antarctic soil communities (Tearle, 1987). Tearle (1987) reported that this stress was linked to the destruction of some organisms, altering soil aggregate structure and the exudation patterns of cryptogams resulting in the release of organic matter to soil microorganisms. Freeze-thaw cycles occur more frequently at lower latitudes across the Antarctic region and may represent a gradient of environmental instability (Yergeau *et al.*, 2007a). Yergeau and Kowalchuk (2008) found that freeze thaw cycles were more influential than vegetation or warming on microbial community functionality.

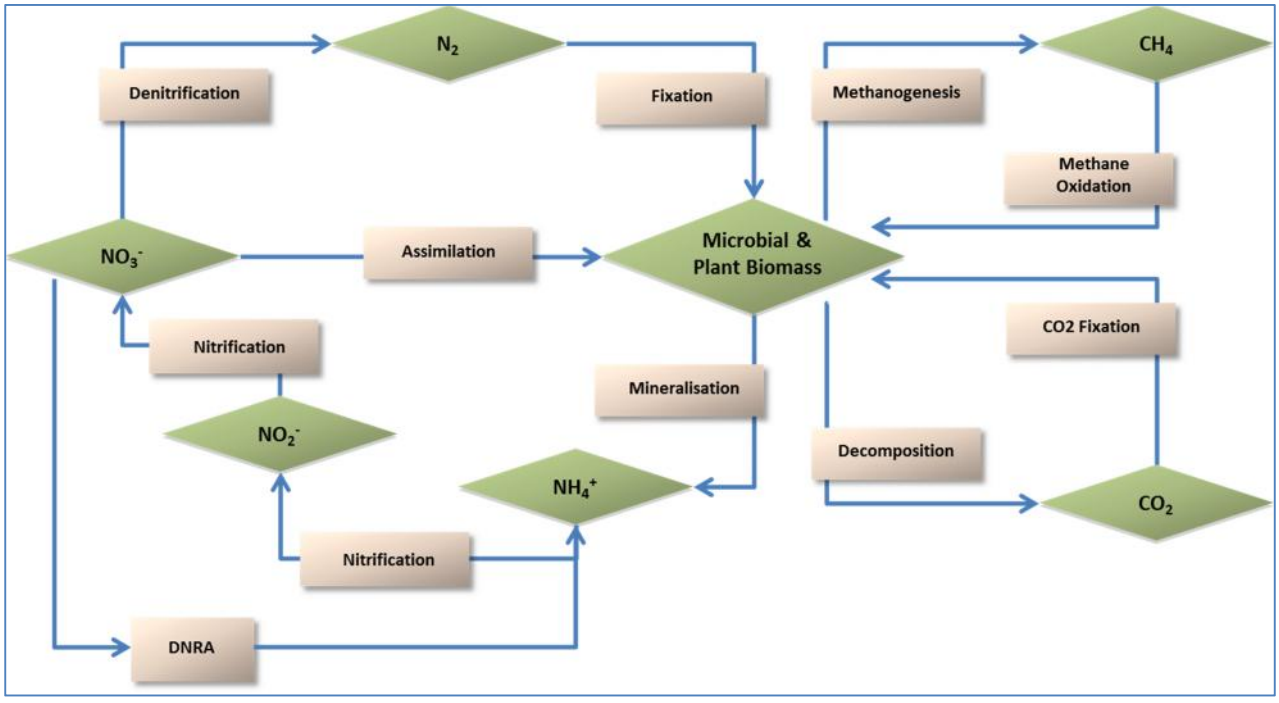

Figure 1.3. Basic bacterial processes of nitrogen and carbon cycles in Antarctic soil.

1.1.6 Carbon cycle

The dynamics of carbon cycling can be difficult to elucidate due to the vast array of viable substrates. Decomposition in Antarctic soils is driven by saprotrophic fungi and bacteria and has been found to be most heavily influenced by temperature. Parsons *et al.* (2004) found that CO_2 flux in the Dry Valleys was associated with changes in temperature and in cellulase abundance, with a decrease in cellulase gene abundance with increasing latitude (Yergeau *et al.,* 2009). Phototrophic primary production (microbial C-fixation) is highest in places of low vegetation and is probably an important source of soil C in these areas (Hopkins *et al.,* 2006; Cary *et al.,* 2010). The methane cycle occurs in environments where hydric stress is extreme (low or high) by inhibiting gas diffusivity and therefore promoting anaerobic respiration which is essential for methanogenesis (Nazaries *et al.,* 2013). Methane oxidation occurs where urea/ammonia concentration are low (Wahlen, 1993), by . Methane oxidation has been poorly described in Antarctic soil. Yergeau *et al.* (2009) found that methane oxidation gene abundance was negatively correlated with nitrate concentration, as nitrate concentration is driven through nitrification of ammonia.

Labile carbon has been found to be the limiting nutrient for respiration in Antarctic soil (Smith, 2005). Microbial communities compete with plants efficiently for nutrients to the point of affecting plant growth when microbial access to labile C is high in nutrient-deficient soils (Schmidt *et al.,* 1997). Arid soils are thought to accumulate uric acid from penguin rookeries, which can be degraded to serve as a source of both N and C (Aislabie *et al.,* 2009). Yoshitake *et al.* (2007) found that mineral soils of the Arctic were both C and N limited, and with addition of both, respiration increased but not biomass, reflecting a nutrient trigger response in dormant organisms and increased metabolic activity in others.

1.1.7 Nitrogen cycle

Nitrogen is thought to be both the most limited nutrient (Sjorgerstein and Wookey, 2005) and the key driver of primary productivity (Hopkins *et al.,* 2006) in high latitude systems. The main sources of nitrogen in Antarctic soil are from N-fixing bacteria and from guano additions (Christie, 1987). Cyanobacteria are important nitrogen fixers in polar environments as they are photosynthetic and are not likely to be inhibited by low substrate availability.

Nostoc, a blue-green alga, has been found to be highly abundant in freshwater lakes and soils and could be the single most important N-fixing organism in Antarctica (Davey, 1983). Cyanobacteria have been found to be metabolically robust to soil hydric pressures, being capable of N-fixation at temperatures as low as -7°C (Davey and Marchant, 1983) until complete cellular freezing occurs, but are more sensitive to light (Smith, 1984) and soil acidity. The pH optimum for N fixation by cyanobacteria is 8.7 but N-fixation has been found to occur at pH 6.6 in a soil sample from Signy Island (Horne, 1972).

Mineralization of N and C from guano is thought to be rapid, 50% mineralization was recorded after 3 weeks in King George Island soils (Myrcha and Tatur, 1991). One of the major components of guano is urea, which contributes significantly to soil basicity, increasing rates of nitrification. However, *Nitrobacter*, the major bacterium responsible for nitrite oxidation, is sensitive to ammonia toxicity so accumulation of nitrite is likely in ornithogenic soils (Mulvaney, 1994). Aislabie *et al.* (2009) suggested levels of ammonium akin to Cape Bird quantities of 734 ppm could have inhibitory effects.

Ammonia oxidation is the first, rate-determining step in nitrification and leads to the conversion of ammonia to the less stable nitrite. Conditions favorable for nitrification include high levels of NH_4^-, high pH and aerated soil but it is thought the second step of nitrification, nitrite oxidation, is restricted by low temperatures (Siciliano *et al.,* 2009). Increasing soil temperature has been found to have dramatic effects. Over a 5 year experiment Chapin *et al.* (1995) discovered large increases in nitrate levels compared to that of ammonia due to microbial immobilization.

Increased concentrations of dissolved soil nitrogen enable nitrogen loss through nitrate assimilation, and can be seen through nitrate leaching in wet soils (Kowalchuk and Stephen, 2001). Increased soil water levels in marine influenced Antarctic soils could promote conditions supportive of anaerobic bacteria (Ramsey, 1983), which is supported by higher relative abundance of anaerobic bacteria when compared to temperate soils (Teixeria *et al.,* 2010). Also, anaerobic conditions and restricted nitrogen availability provide conditions under which dissimilatory nitrate reduction to ammonium (DNRA) can occur, in which non-denitrifying NO_3^- reducing bacteria take a greater role over denitrifiers (Rutting *et al.,* 2011), Christie (1987) found that nitrifying bacteria were more abundant than denitrifying bacteria in Antarctic soil.

Plants are likely to play a key role in the future regulation of nitrogen. Populations of *D. antarctica* are thought to have increased substantially in number and range, and this is continuing (Gerighausen *et al.,* 2003; Peat *et al.,* 2007; Hill, 2011) although, there is also evidence the trend is slowing down dramatically (Parnikoza *et al.,* 2009) . These expansions are predicted to lead to lower soil pH and increased concentrations of dissolved soil nitrogen, encouraging microbial and enzymatic activity (Roberts *et al.,* 2009).

1.2. Antarctic Biology

Life in Antarctica is sparse, concentrated to pockets of suitable environments and dominated by response to seasonal variation (Chown and Convey, 2007). It is thought that the major dynamic defining Antarctic ecosystems is not the harshness of the environment itself, but the stress exerted by the variation in conditions (Yergeau *et al.,* 2008). Fluctuation is characteristic of all Antarctic environments so metabolic flexibility is an essential part of life strategies for most organisms (Wynn-Williams, 1996). To assess the link between environmental heterogeneity and ecosystem response, recent studies of terrestrial Antarctica have been concerned with the biogeography of communities (Adams *et al.,* 2006; Yergeau *et al.,* 2007; Niederberger *et al.,* 2008; Cannone *et al.,* 2008; Chong *et al.,* 2010; Arenz and Blanchette, 2011; Chong *et al.,* 2012), reporting on quantities and (less so) on dynamics of nutrient cycling (Elberling *et al.,* 2006; Barrett *et al.,* 2007; Bokhorst *et al.,* 2007; Hopkins *et al.,* 2008; Roberts *et al.,* 2009) and assessing the impacts of global warming on soil communities (Doran *et al.,* 2002; Tosi *et al.,* 2005; McGeoch *et al.,* 2006; Caldwell *et al.,* 2007; Yergeau and Kowalchuk, 2008; Rinnan *et al.,* 2009).

Recent work on terrestrial invertebrates has challenged historical theory and provide strong evidence that contemporary Antarctic metazoa must have been isolated and preserved in a persistently active state (Convey *et al.,* 2008, 2009; Pugh and Convey, 2008; Chown and Convey, 2012. Climate models have previously implied that, due to successive glacial maxima in the Neocene and late-Pleistocene era, terrestrial Antarctic life would have become all but extinct, and therefore that present diversity must be result of post Holocene colonization of ice free areas. Vyverman *et al.,*(2010) summarize findings from studies of several independent taxa, which report biogeographical trends for microorganisms,

concurrent to the patterns observed for metazoa. Antarctic microbial diversity has not been well documented, with most studies involved in identifying abundant genera in specific habitats, but a transect study by Yergeau *et al.* (2007) linked bacterial distribution to vegetation cover and latitude.

The environmental alleviation predicted to arise from warming, including increased periods of time where the temperature is above 0°C and water and nutrient availability, have had mixed effects on terrestrial biota in simulation experiments. Most studies have found warming to have little impact on microbial and bacterial communities directly unless the frequencies of freeze-thaw cycles are altered (Bokhorst *et al.,* 2007). Nutrient supplementation increases metabolic activity in microbial communities but does not change community composition (Hopkins *et al.,* 2008). The most significant effect already observed due to warming is the increase in plant populations; the consequences of this are perhaps most crucial at present for research.

1.2.1. Flora and fauna

Almost all Antarctic macro-organisms are highly regionalised, dictated by the severity of the environment. Maritime and Peninsula locations contain the majority of avian nesting sites and pinniped wallows. Peat has been found to harbour some of the most hospitable conditions where high numbers of microorganisms have been observed compared to fellfield sites and tundra environments (Steyn and Smith, 1981). Peat bogs and forelands of glaciers are also becoming dominated by *Deschampsia antarctica* monocultures, which provide attractive grounds for pinnipeds (Olech, 2010). Penguins represent the majority of bird biomass and guano contributions are thought to be the greatest source of nitrogen addition to soils (Bokhorst *et al.,* 2007). Despite this, ornithogenic soils have not been found to be biologically more diverse than other soils (Aislabie *et al.,* 2009). Little has been reported on avian species ranges or numbers, aside from sphenisciformes, but microarthropod distribution has been linked to ornithogenic transmission (Krivolutsky *et al.,* 2004.).

Other terrestrial fauna consists of soil micro- and mesoorganisms including nematodes, microarthropods, tardigrades and rotifers. Nematodes are at the top of Antarctic soil food webs, highly abundant with pivotal roles as both consumers and predators, but are also vulnerable to perturbation (Freckman and Virginia, 1997). They have been of special interest

to evolutionary ecologists as they display discrete taxonomic distributions between continental and maritime Antarctica and most species are found nowhere else on earth (Maslen and Convey, 2006). Contrary to previous understanding, some of these nematodes have also been found to be specialist rather than generalist in their microbial prey; with preferences attributed to chemotaxis of microbial produced compounds (Newsham *et al.,* 2004). This is likely to have not only important implications on food web energy flow but also on spatial structures displayed by different trophic levels.

The majority of vegetation is found on the South Orkneys, South Shetland Islands and on the west coast of the Peninsula, seeing a diversity gradient with diminishing species richness with increased latitude (Peat *et al.,* 2007). There are around 350 species in total and are mainly comprised of simple plant species (mosses and liverworts), lichen and fungi, but the largest group in terms of species are the 'sac fungi' *Ascomycota* (Malosso *et al.,* 2003) of which many form symbiotic relations with algae to form lichens. Lichens are reasonably abundant across Antarctica due to their ability to withstand drought and low temperatures. Some species have been found as far as 86◦26'S at an altitude of 1750m, where they utilise fog and condensation as their main source of water (Broady and Weinstein, 1998). Higher plants are strongly correlated with latitude. There are two flowering plants, *Deschampsia antarctica* (Antarctic hair grass) and *Colobanthus quintensis* (Antarctic pearlwort) which extend from South Georgia through the South Orkneys and South Shetland Islands, down the west cost of the Peninsula as far south as Lazarev Bay on Alexander Island (Parnikoza *et al.,* 2011; Convey *et al.,* 2011). *Poa annua*, one of the most widely distributed plant species in the world has also been found in the South Shetland Islands, one of many invasive species accidently introduced to Antarctica (Frenot *et al.,* 2005).

1.2.2. Bacteria

The most common phyla encountered in soil globally are Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Verrucomicrobia, Bacteroidetes, Plancomycetes, Gemmatimonadetes and Firmicutes, with members of these nine phyla making up 92% of soil 16S rRNA and 16S rRNA gene libraries (Janssen, 2006). Antarctic soils are characterised by few dominant bacterial phylotypes in all terrestrial environments (Aislabie *et al.,* 2009) but notably differ from temperate soils by a lack of dominance of Proteobacteria, which account for 39% of soil bacterial communities globally (Janssen, 2006).

Antarctic vegetation has been found to have a buffering effect on the severity of climatic influence on soil communities (Yergeau *et al.,* 2008) by increasing thermal stability and improving nutrient networks. The species of the plant has also been found to influence the composition of microbial communities in rhizosphere soil (Kowlachuk *et al.,* 2002). Due to this, it was thought diversity in respective microbial communities may be lowered by creating a specific environmental niche. However, in a study looking at bacterial diversity associated with the rhizosphere of Antarctica's two vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* in maritime soils, Teixeira *et al.* (2010) found no difference between communities. But contrary to temperate soils, Actinobacteria and Firmicutes phyla represented 70% of the sequences. Actinobacteria are generally considered k-strategists (van Elsas *et al.,* 2006), which tend compete to survive when resources are limited and Firmicutes encompass endospore formers, a primary function of which is to ensure survival through periods of environmental stress.

Bacterial abundance is thought to be ten-fold higher in sites with proximity to sea animals (Smith, 1985). Sites currently occupied by penguins are high in Firmicutes and Gammaprotebacteria (Chong *et al.,* 2012). High abundance of Firmicutes in maritime soils is likely to be due directly to penguin guano additions (Aislabie *et al.,* 2009). Salinity has also been found to be of significance in bacterial communities which are adjacent to penguin rookeries (Aislabie *et al.,* 2009). After guano additions and sea water flooding, high summer temperatures and lack of precipitation mean that bacteria may have to tolerate highly alkaline soils. Actinobacteria and Gammaproteobacteria are abundant in soils formerly occupied by penguins, but are thought to contain considerably lower levels of soil nutrients due to decomposition and leaching (Aislabie *et al.,* 2009).

Yergeau *et al.,* (2007) sampled a latitudinal transect from the Falkland Islands (51◦S) to the Elsworth Mountains (78◦S), high numbers of Cyanobacteria were found in all samples. Conditions of terrestrial Antarctica are highly transient so ecophysical strategies and metabolic flexibility are required for many microorganisms. Cyanobacteria, common in all Antarctic habitats are known as opportunists - with the metabolic and morphological diversity to combat environmental pressures. They have shown a rapid response to moisture availability post desiccation or freezing (Hawes *et al.,* 1992), the ability to form heterocysts containing nitrogenase so they may fix nitrogen under anoxic conditions and a much reduced

temperature optimum compared to that of respective temperate strains, supporting evidence of cold-adaptation (Pandey *et al.*, 2004). Dry Valley soils have been found to be dominated by Cyanobacteria, Acidobacteria, Actinobacteria and Bacteroidetes (Smith *et al.*, 2010). Niederberger *et al.* (2008) found *Deinococcus*/*Thermus* lineage to be linked to areas of dry, low productivity soil whilst Gammaproteobacteria (*Xanthomonas*) were found exclusively in high productivity soil. But rarefaction analyses indicated soils were under-sampled, suggesting a higher biodiversity than previously expected.

1.2.3. Fungi

Fungi encompass a range of functions within soil food webs acting as saprophytes, decomposing dead matter to contribute to nutrient cycles, forming mutualistic associations with plants to aid nutrient uptake and improving soil structure by means of hyphal networking, helping to buffer soil against moisture instabilities. Over 1000 species of fungi have been reported across Antarctica (Bridge *et al.*, 2008) though higher fungi incidence declines below 66◦S (Bridge *et al.*, 2010). Yeasts dominate continental Antarctica due to their ability to withstand desiccation, freezing and high soil ionic content (Vishniac and Klingler, 1986) whilst filamentous species are more abundant across maritime areas. Onofri (2006) discovered a variety of endemic and indigenous species, indicated by high numbers of instances of isolation - *Cryptococcus vishniacii*, *Geomyces pannorum* and *Thelebolus microsporus* have all been frequently recorded from different sites and substrata. Newsham *et al.* (2009) conducted a study at Mars Oasis at sites of varying soil moisture content and found chytrids to be most abundant at high moisture sites and black yeasts and *Tetracladium* among the most common in dry soils.

Fungi are known to be most prolific in acidic soils, with up to a 30 fold increase in fungal growth ratio compared to that of bacteria from neutral pH soils to ~pH4 (Rousk *et al.*, 2010). However, fungal diversity is thought to be driven by water availability in Antarctic soils (Newsham *et al.*, 2009) though fungi are more resilient than bacteria to low temperatures and consequential variation in moisture availability (Pietikainen *et al.*, 2005). Bapiri *et al.* (2010) found that, whilst bacterial growth was inhibited by drying/rewetting events, fungal growth remained constant even at different bacterial:fungal biomass ratios. Freeze-thaw cycles are thought to have less effect on fungi than bacteria but have still been found to be influential on community composition and size (Yergeau *et al.*, 2007a).

Many of the fungal species found in Antarctica are cosmopolitan taxa but have metabolically adapted to cope with the environment (Selbmann, 2005). Some species have melanin-rich hyphae to protect against high UV radiation (Robinson, 2001) or altered cellular lipid composition acting as a cryoprotectant (Weinstein *et al.,* 1997). In fungal reproductive structures, a disaccharide called trehalose has been found in high concentrations and is reported to stabilize membranes during dehydration. Weinstein *et al.* (2000) found increased concentrations of trehalose, up to 75%, in some fungi when incubated at low temperatures. Cryptoendolithic black fungi from the Dry Valleys have developed a much simplified method of reproduction, producing propagules from toruloid pre-existing hyphae rather than by sexual reproduction (Ruisi *et al.,* 2006). Here it is assumed low genomic diversity favours selection of the most adaptive strains to survive environmental hostility.

1.3. Biodiversity and Biogeography

Biogeography is the study of the distribution of biodiversity over space and time (Hughes-Martiny *et al.,* 2006), often revealing the mechanisms behind structures of diversity. The biogeography of plant and animal life has been broadly documented for centuries and common ecological trends have been predicted to hold across species globally. In macro-ecology, perhaps the most widely recognized but least understood spatial pattern is the latitudinal gradient in taxonomic diversity. Diversity is thought to increase towards the equator and decrease towards the poles, although the gradient has not always been found to be linear (Kaufman, 1998). There are currently more than 30 hypotheses proposed to explain the latitudinal gradient, but despite this, none is regarded as a single plausible mechanism (Willig *et al.,* 2003; Rohde, 1992). Hillebrand (2004) conducted a global meta-analysis of over 600 latitudinal studies over a range of trophic levels and biomes, and found that the gradient was more pronounced for larger organisms. This is thought to be primarily due to lower dispersal ability and slower life cycles of large organisms. Whether the patterns observed in macroorganisms are also exhibited by microorganisms and whether biotic and abiotic dynamics are the same, has been the focus of many recent studies (Hughes-Martiny *et al.,* 2006; Fierer and Jackson, 2006; Lauber *et al.,* 2009; Chu *et al.,* 2010).

Spatial and temporal variation are the key characteristics of biodiversity globally and are also strongly characteristic of Antarctic environments, if poorly assessed (Chown and Convey, 2007). Spatial variations occur on an infinite hierarchy of scales, but in the simplest sense are made up of global and local structures. Global patterns represent gamma or beta diversity; the trend between communities in different or the same ecosystem, and local variations are those driven by biotic relations between individuals within a community. Global trends may be the result of contemporary abiotic mechanisms, e.g. dispersal, fluctuations in nutrient inputs and environmental parameters or due to historical biogeographic events resulting in isolation of relict populations. Local trends are a result of biological processes such as competition or parasitism.

Contemporary biodiversity is thought to be amidst a sixth mass extinction event (Vie *et al.*, 2008) with 38% of IUCN red list documented species listed as threatened. The 'black box' of the terrestrial environment means the extent to which microbial communities are concurrent with this trend is currently unknown (Hughes Martiny *et al.*, 2006). Species loss, particularly in terrestrial ecosystems is thought to be intrinsically linked to a decline in ecosystem functionality and production (Hector *et al.*, 1999). The importance of microbes in driving all major soil ecological processes is well established; hence the consequence of species loss on these processes is high on the agenda of global research. Examining diversity in spatial and temporal planes requires first the identification of patterns (Gaston and Blackburn, 1999). This is made complicated in microbiology due to a lack of consensus on species definition, inability to sample microbial habitats in adequate resolution and difficulty identifying the boundaries of ecological niches due to the complexity of soil environments (Chu *et al.*, 2010). This aside, ecologists provide a number of interesting tools to deal with microbial data, though most require specialist tailoring to suit the nature of the microbial community datasets.

1.3.1. Microbial diversity: considerations and analysis

Several factors have hampered the study of microbial diversity. In the past, methods for cataloguing microbial communities were time-consuming, expensive and inadequate to describe for large-scale screening efforts. Recent developments in high-throughput technologies, such as pyrosequencing, almost certainly address these issues, if the

considerable confusion on how to define a microbial species is resolved (Tamames *et al.,* 2010). Current approaches are based upon genotypic similarity but these approaches are known to group strains together inappropriately (Hall *et al.,* 2010). OTU (operational taxonomic unit) grouping, based upon 16S rRNA sequence identity of 97 to 98% for bacteria is the most common designation, as this corresponds most closely with previously established species divisions (Griffin *et al.,* 2011), but there has been increasing sway in favour of a more polyphasic approach where functional role is considered alongside genotype (Tamames, *et al.,* 2010; Cai *et al.,* 2009; Gillis *et al.,* 2005).

In addition, the resolution of modern community profiling techniques highlights inadequacies in sampling strategy, the issue of microorganisms being abundantly distributed throughout the soil environment (Schloss and Handlesman, 2006) needs to be considered. Due to this, experimental strategies which attempt to compare microbial communities between transects or across a gradient would need to analyze an extremely large sample in order to capture the true nature of community composition. Furthermore, more cost effective molecular community profiling techniques currently utilized such as DGGE, are not sensitive enough to detect rare species (Woodcock *et al.,* 2006) so many studies do not capture true diversity in a population, only give an indication of the abundance of the most common organisms.

Microbial community profile data is commonly represented by presence/absence or by quantitative matrices of species abundance which are often zero heavy. The majority of biomass tends to belong to several dominant phylotypes, though many species occur at only a few sites but contribute little to overall abundance. Thus, the interpretation of microbial diversity into a meaningful vector of quantity is a continuing challenge for ecologists. Indices classically used for assessing plant and animal communities, though increasing in their popularity in microbial ecology as a ‘quick fix’, offer a distorted or erroneous solution and lack meaningful biological interpretation (Jost, 2007). The ubiquitous nature and vast abundance exhibited by microorganisms means common indices such as Shannon (Shannon, 1948), which do not account for sample size, are likely to represent abundance and richness disproportionally amongst samples. Furthermore, after the financial and time costs of generating high complexity community information, the use of diversity indices has been viewed as ‘sacrificial pseudoreplication’ (Hurlbert, 1984), where all information about species identity and relative functions is lost only to produce a vector of limited biological

value. The pitfalls highlighted previously indicate a less binary more dynamic approach is needed to extract the maximum value from the data we have.

An all-encompassing ecosystem approach has been suggested to best describe patterns and functions in microbial diversity (Ramette *et al.,* 2007). This involves identifying spatial and temporal scales at which populations vary, used in combination with environmental parameters as a means of explaining patterns or functions of the community. The commitment of collecting such a dataset may however be outside of the logistical or financial capabilities of many studies. Multivariate analysis has been applied to microbial data sets although has been largely dominated by exploratory techniques such as Cluster analysis and Principal Component Analysis (PCA) (Ramette, 2007). This is reflective of several factors. First, the complexity of the microbe-environment relationship instills reticence towards conclusions drawn from confirmatory techniques, such as multiple regression analysis, as often these models are driven by the analyst's personal hypotheses, and do not allow 'free' expression of the data. Second, traditionally microbiologists have felt that the dispersal of microorganisms throughout the environment is random and unrelated to the mechanisms driving macro-organism distribution (Finlay, 2002), thus the application of more intensive ecological analyses are not appropriate. O'Donnell *et al.* (2007) highlighted the limited ability of modeling approaches to address spatial heterogeneity in microbial ecology. With the ability to analyse multiple scales within the data and across sampling regimes, recent developments in spatial eigenanalysis techniques may come some of the way to remedy this problem.

The analysis of diversity almost always involves two properties: species richness and species evenness or abundance. Species richness is a relative term that refers to the number of species in a community, and is directly associated with measuring the diversity of species in a given area. Evenness is another dimension of diversity which defines the number of individuals from each species in an area, so that areas can be compared. Together, these terms have been used to describe species diversity patterns on Earth, though potentially they represent separate aspects or mechanisms of community composition. Richness is often attributed to historical events or climatic isolation whilst abundance is much more likely to represent contemporary biotic and/or abiotic conditions (Barrantes and Sandoval, 2008). Diversity for microbial communities can be seen as a species list as a first approximation of site-specific species diversity (Wilson, 1992), but to provide a more detailed picture species

richness is normally used which encompasses the number of each species type into the diversity measurement (Wilson, 1992). However a drawback of using species richness is that this can hide the absolute abundance of each species, and pays no consideration to specialised species or specific functional characteristics (Wilson, 1992). To ensure that these concerns are captured, a measure of species evenness is often used in conjunction with species richness, and provides the balance or 'spread' of species by type and numbers (Whittaker, 1972).

Diversity as a property is commonly examined in three forms, Alpha, Beta and Gamma (Whittaker, 1960). Alpha diversity is considered as the local species diversity at a subunit level (Whittaker, 1972), however it has also been classed as the mean species diversity across a number of subunits (Tuomisto, 2011), and represents the diversity *within* a site specific community (Vane-Wright, 1991). Beta diversity considers the species diversity *among* communities (Wilson, 1992), and provides an estimate of the regional or environmental diversity gradient. There are a number of proposed definitions for beta diversity, but largely there are major types: non-directional variation and directional variation (Anderson *et al.*, 2010). Non-directional variation considers variation between a group of samples within a sampling area. Directional beta diversity looks at the changes in samples over a temporal or spatial gradient, this is also known as 'species'turnover. Gamma diversity is considered as the total diversity and is derived from the Alpha and Beta component independently ($D\alpha + D\beta = D\gamma$) (Jost, 2007).

Often diversity is measured by common diversity indices (Shannon, 1948; Simpson, 1949), and each in their own way provide an estimation that is representative of one or more aspects of species diversity, whether primarily concerned with species richness (e.g. Shannon indices), species evenness (e.g. Brillouin E indices) or with species concentration of dominance (e.g. Simpson indices) (Jost, 2007). Considering distinct communities within species diversity is furthered by the proposed use of similarity matrices (e.g. Sorensen, 1948) with an effective species alpha diversity metric, considering the similarity between sites where abundance weight is unequal (Magurran, 2004).

1.3.2. Trends in Antarctic biodiversity

Patterns in Antarctic diversity are not well documented and terrestrial systems have in the past been investigated in a non-systematic manner (Chown and Convey, 2007). Studies have tended to focus on specific species, regions and scales - mostly due to the logistical inaccessibility of many regions. Nevertheless, species diversity and abundance are enough to expose biogeographical trends at continental scale, given the correct sampling resolution, which would otherwise be clouded by a myriad of trophic interactions (Yergeau, 2011). Variation across a range of spatial and temporal scales has been observed for many organisms and research over the last 10 years has dramatically challenged much of what was previously thought about distributions of Antarctic biota (Chong *et al.,* 2012). Though many taxonomic groups are absent from Antarctic food webs, surveyed areas have revealed biodiversity hotspots, a gradient in species richness and even global dominance of some Antarctic fauna (O'Loughlin *et al.,* 2011).

The most significant finding of terrestrial studies has perhaps been in microfauna. Traditionally it was thought colonization of all life on Antarctica came about post the last glacial maximum approximately 20,000 years ago (Clark *et al.,* 2009). But recent studies present evidence of biological regionalization across terrestrial Antarctica in various metazoan, bacteria and algal taxa (Vyverman *et al,* 2010; Maslen and Convey, 2006; Cromer *et al.,* 2005). Cromer *et al.* (2005) found evidence in sediment cores that some species of rotifer and water fleas (*Cladocera*) have been present in continental Antarctica for at least 130,000 years and chironomid midges have had a sustained presence on the continent for an estimated 49 million years (Allegrucci *et al.,* 2006). Though some regions may have been more hospitable than others, and maintained specific areas of refugia during periods of climatic extremes, the continued presence of many of these populations casts doubt over past estimates of ice coverage (Newman *et al.,* 2009)

The widely-assumed pattern of decreasing diversity with increasing latitude found in macroscopic fauna and flora is not supported by Antarctic nematodes. Indeed soils from Alexander Island have species richness almost 80% greater than northern Marguerite Bay-making colonisation from the north highly unlikely. Likewise colonisation from the south also seems unlikely due to a complete lack of nematode species in Ellsworth Land (Maslen and Convey, 2006), suggesting relict and truly endemic populations. On Alexander Island, Mars Oasis (71◦54'S) has been identified as a hotspot for microbial and mesofaunal diversity

(Lawley *et al.*, 2004; Maslen and Convey, 2006), although soil chemical characteristics have not been found to differ from nearby equally surveyed areas (Yergeau *et al.*, 2007).

Avifauna and pinnipeds encompass the top level in the marine trophic hierarchy although their distribution within the Antarctic region is almost certainly restricted to availability of suitable breeding grounds. Guano additions are comprised of mainly of uric acid, which following decomposition provides an important source of nitrogen to maritime areas. Guano is also responsible for 'zones of phosphatisation', where guano compounds react with rock resulting in areas of extremely phosphate rich soil (Myrcha and Tatur, 1991). Lindeboom (1984) found that decomposition of organic nitrogen in penguin rookery soil is rapid and quantitivley more important than nitrification or denitrication. Volatized ammonia then feeds back to nearby soil, resulting often in ornithocoprophilous plant communities (Mizutani and Wada, 1988). Guano has been found to be strongly influential on the composition of bacterial communities in rhizosphere soil (Teixeira *et al.*, 2010), where Firmicutes dominate, but no difference in bacterial diversity has been observed in ornithogenic soils from that of mineral soils (Aislabie *et al.*, 2009; Chong *et al.*, 2012).

Plant diversity is low in Antarctica, with only two species of higher plant whose colonization range extends only as far as 69°S, in contrast with the Arctic where they reach at least 80°N (Mori *et al.*, 2008). Plant life has been widely catalogued and been found to be linked to latitude in the Antarctic peninsula region (Clarke, 2003) but a matching gradient has not been observed along the Victoria Land coast, 72◦S to 86◦S (Peat *et al.*, 2007). The majority of flora comprises mosses and lichens, but only 6-7% of mosses are thought to be endemic to maritime areas and none to continental Antarctica (Peat *et al.*, 2007).

Yergeau *et al.* (2009) found numbers of bacterial and archaeal taxa, detected using phylochip microarray analyses, significantly decreased with increasing latitude with a more pronounced effect at the southernmost sites. Availability of liquid water has been found to be more important than temperature to biological activity in terrestrial habitats (Sømme 1995; Block 1996; Convey and Lewis Smith 2006), but only when carbon and nitrogen are not limiting in microbial communities (Yergeau *et al.*, 2008; Dennis *et al.*, 2013). Yergeau *et al.* 2007b) found that vegetated soils contained enhanced bacterial diversity and abundance, presumably because environmental conditions become more favourable with plant-mediated soil enrichment. There is also a contrasting opinion that diversity is likely to be lower, due to

substrate enrichment favouring only a specific niche of microbial utilizers (Yergeau *et al*., 2007b). Whether environmental stability is more important to the maintenance of diversity than environmental severity is a matter of interest.

1.3.3. Functional diversity

In the harsh environment of the Antarctic, abiotic selective pressure is particularly high, forcing methods of adaptation in many organisms (Rogers, 2007). The manner in which organisms respond can be best understood by quantifying functional response to their environment, which is key in predicting the survival of populations in times of change. Functional diversity has been defined as 'the number, type and distribution of functions performed by organisms within an ecosystem' (Diaz and Cabido, 2001) and involves ecological and evolutionary processes such as nutrient cycling and gene flow (Schleuter *et al.*, 2010). It is the functional diversity (species traits), not the species richness, of ecosystems that provides the greatest stability, and is most efficient and resistant to fluctuations in environmental conditions (Cleland, 2012).

In the study of community assembly, the taxon has been regarded as the unit of diversity although there is growing scepticism about the relevance of linnaen classification in microbiology (Green *et al.*, 2008). Furthermore, reporting taxonomic diversity gives only a comparison of genotypes present and no information about phenotypic expression or the importance/redundancy of co-occurring species (Schleuter *et al.*, 2010). Variation in phenotypic expression can be a reflection of response to substrates and environmental conditions, so changes observed between communities allow us to understand the key mechanisms behind selection pressures in that environment. The principal behind functional diversity is to bridge the gap between ecosystem processes and individual organisms to give a measure of community output and stability.

Quantifying functional diversity is still a problem to ecologists. It has been suggested that an approach which considers functional richness, evenness and divergence may be most suitable. Functional richness is thought to be orthogonal to both functional evenness and species richness (Mason *et al.*, 2005). Low functional richness implies either some of the community resources remain unused or community resources are too limited to support a wide breadth of functions, the result of which is reduced buffering against environmental fluctuation (Tilman *et al.*, 1997). Low functional evenness indicates under-utilisation of

some parts of the niche space (Mason *et al.*, 2005). Four key criteria have been described by Petchey and Gaston (2006) to measure functional diversity: consideration of appropriate traits to be measured, traits to be weighted according to importance, consideration to be given to maximizing statistical value from trait type and the measure to be able to explain and predict variation in ecosystem processes.

Functional traits in microorganisms have been best represented in literature by the expression of certain genes (Gilbert *et al.*, 2010). 'Hard traits' like these are particularly useful because they account for the organism's ability to function and the organism's measurable contribution to that ecosystem process. The choice of trait is in essence, a compromise between which traits best describe the function or process of interest and what is possible to measure and the weighting of traits should be done according to the biological question at hand. In systems where a variety of parameters are available, gradient analysis may be used to identify those which have maximum explanatory power (Petchey and Gaston, 2006).

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2

Project Data and Thesis Methods

2.1. Project Data

The data that are fundamental to this project have been brought together through two means: site collected physical observations and laboratory generated data post external testing of samples. The statistical and modeling analysis of these data elements begin to describe the diversity and variation at the sites and within the species range. The point of data provision into this project forms the basis of this thesis, and from that point the later introduced modelling and statistical methodologies begin to consider total diversity and variation across latitude. Understanding the site characteristics and the diversity between the two data elements underpins the later analysis between sites and across the project regions. This chapter introduces the raw data and provides context for its use. Later chapters consider the diversity across all sites (beta diversity) and bacterial composition (alpha diversity) and, through statistical and hierarchical methods reflect on the conclusions that can be drawn from this for total diversity (gamma diversity) (Whittaker, 1972).

This was a collaborative project funded by NERC- AFI7/05
"Microbial Diversity in Antarctic Soils"

Project leader: David Hopkins
Co-investigators: Tony O'Donnell, Steven Rushton, Kevin Newsham
Post-Doctoral Research Assistant: Paul Dennis
PhD Candidate: Victoria Ord (Chester)

The project was devised by Hopkins, O'Donnell, Rushton and Newsham. Samples were collected across the Antarctic Peninsula region by Hopkins and Dennis in the 2007/2008 austral summer. Chemical analysis of the samples was directed by Hopkins and conducted by Dennis, and Ord with help in these analyses from Dr Armando Laudicina and Dr Benhua Sun, and the molecular community analysis was conducted by Hopkins and Dennis. Data from these analyses was provided to Ord as a series of excel data tables. The data was compiled into a database by Ord and all statistical and modeling analysis conducted by Ord under the supervision of Rushton.

2.1.1. Soil collection Sites

During the 2007-2008 austral summer, a total of 68 soil samples from 25 locations, location descriptions in Table 2.1, with further site descriptions from field notes provided in Appendix 1. Our sampling locations spanned a 2000km gradient across the Antarctic Peninsula, from South Georgia (54◦S, 38◦W), Figure 2.1, to Mars Oasis (72◦S, 68◦W), Figure 2.2. Locations were chosen largely based upon opportunistic access at the time of sampling, as appropriate to the nature of work in an extreme environment. Locations ranged between research bases, mainland Antarctic Peninsula and sea-isolated islands on both the east and west coast of the Peninsula and were varied widely in geology, climate and presence of higher taxonomic groups i.e. higher plants, seal colonies etc.

Table 2.1. Site locations represented by the flag icons in Figure 2.1

Icon	Location	Icon	Location
a	Alexander Island	n	Port Lockroy
b	Greenwich Island	o	Livingston Island
c	Seymour Island	p	Jenny Island
d	Trinity Island	q	Cape Evenson
e	Alectoria	r	Nelson Island
f	King George Island	s	Gand Island
g	Deception Island	t	Antarctic Peninsula
h	Wiencke Island	u	Detaille Island
i	Rothera	v	Lagoon Island
j	South Georgia	w	Berthelot Island
k	James Ross Island	x	Mars Oasis
l	Blaiklock Island	y	Signy Island
m	Rothschild Island		

Figure 2.1. Map of site locations.

Created using latitude and longitude, populated into Excel fusion tables; an experimental data visualization web application to gather, visualize, and share larger data tables, which links as a KML file to view in Google Earth to create a map (Google, 2013).

Figure 2.2.A

Figure 2.2.B

Figure 2.2.A. South Georgia (54.2500˚S), our most northern sampling location, has the highest number of tourist visits in excess of 14,000 annually (Frenot *et al.*, 2005) (Source: www.wanderingalbatross.org).

Figure 2.2.B. Mars Oasis (71.9800˚S), one of the most southern sites sampled, has low contemporary input of soil nutrients and low available soil moisture, with temperatures reaching as low as -41˚C in winter (Dennis *et al.*, 2013) (Photograph: Ord, 2008)

2.1.2. Physical Observation Data

Due to the variation in observable characteristics, additional site-specific information was estimated and recorded for each location by professor David Hopkins and Dr Paul Dennis. Characteristics included: birds (seabirds and penguins), seals, higher plants, rock density, mosses, lichens, algae, and human activity. These were all graded 0-10 corresponding to the level of influence each parameter was thought to have at each soil site sampled. Latitude, Longitude and Altitude were also recorded. Bulk soil samples from the 68 sites were retrieved and stored at -20◦C. Samples for microbial community analyses were snap frozen in field then stored at -80◦C. The uppermost 5 cm of soil was collected in 50 ml tubes, immersed in a mixture of dry ice and ethanol (ca. -80ºC) in the field, and then transferred to -80ºC freezers. Maintenance at this temperature was to stop RNA degradation and enable active microbial communities to be investigated using RNA-based methods at a later date. Table 2.2. provides the data descriptions for the physical observation data.

Table 2.2. Physical Observation Data

Data Element	Description	Source
Site	Specific location of sampling	GPS measured/map
Tub	Sample identifier specific by site	Number reference only
Moss	Estimated relative value 1-10	Observation
Latitude	GPS coordinates	GPS measured
Longitude	GPS coordinates	GPS measured
Altitude	Metres above sea level	GPS measured
Lichen	Estimated relative value 1-10	Observation
Rocky	Estimated relative value 1-10	Observation
Humans	Estimated relative value 1-10	Observation
Plants	Estimated relative value 1-10	Observation
Seals	Estimated relative value 1-10	Observation
Birds	Estimated relative value 1-10	Observation

Notes

Physical observation data recorded at time of soil sampling at site and provided as a collated data table for this project. The table shows data element nomenclature, data description and data source. Review of data is provided in chapter 3.

Two additional environmental variables were created using the BAS Antarctic Peninsula and Weddell Sea Sheet 13A (BAS, 2007). Distance to west coast or 'Disttowest' is estimation in kilometers of the distance of the sampling site from the nearest west coast peninsula shoreline. This encompasses the variation in climate caused by ocean climate, which may be significant as the majority of our soils were sampled along the western side of the Antarctic Peninsula. The west coast is subject to the brunt of the circumpolar current which flows west to east, and is much less glaciated/isolated (Vaughan, 2003).

Oceanicity is a representation of 'Island effect', the % land mass within 50km and 250 km of the site was measured and a PCA was performed, the first axis extracted (95% variance explained) and used for further analysis. Island sites often are exposed to different weather fronts, may have different geological and glacial history and are more likely to be home to pinniped wallows and avian colonies.

2.1.3. Major Soil Characteristics Data

Major characteristics of the collected soil samples were measured by Dennis, Laudicina and Ord at the Scottish Crop Research Institute (SCRI) Dundee, in July/August 2008, for each soil sample across the range of sites. Analyses were directed by Hopkins and Dennis, and were provided to this project as a series of excel spread sheets. Table 2.3 shows the major soil characteristics data descriptions for the characteristics chosen for statistical analysis in this thesis.

Table 2.3. Major soil characteristics

Data Element	Description	Unit of measurement	Source
P	Phosphate concentration, 3 replicates per site	µg g^{-1} dry soil	Total Water Extractable Phosphate
Amino	Full amino acid profiles, 3 replicates per site	Pmolg^{-1} dry soil	Water extractable amino acids
EC	Electrical conductivity, 2 replicates per site	µS	Soil in water extracts
PH	Acidity, 2 replicates	pH	Soil in water extracts
% C by weight	Organic C	Mg C g-1 soil	Elemental analysis following desiccation, treatment with HCl to remove inorganic carbon.
% N by weight	Total N	Mg N g^{-1} soil	Elemental analysis
NH_4^-	Ammonium, 3 replicates per site	Mg N kg^{-1} dry soil	N dissolved in KCL
NO_3^-	l Nitrate, 3 replicates per site	Mg N kg^{-1} dry soil	N dissolved in KCL
DOC	Dissolved Organic Carbon, 3 replicates per site	Mg C kg^{-1} dry soil	C dissolved in KCL
C:N	Ratio of organic carbon to total nitrogen, 3 replicates	Ratio	Organic C and total N
H_2O	% Water Holding Capacity, 3 replicates	%	% moisture content at field moisture

Notes
Data was measured and recorded at the the Scottish Crop Research Institute, and provided as a collated data table into this project. The table shows the selected data element nomenclature, data description and data source. A review of this data is provided in chapter 3.

2.1.4. Microbial Community Data; Fatty Acid Analysis

Fatty acid analysis uses reagents to break the ester links in cell membranes releasing lipids which are used as chemotaxic markers of microbial communities and are a means of calculating microbial biomass. Phospholipid fatty acid (PLFA) and whole cell fatty acid analysis such as by the Microbial Identification System (MIDI) are perhaps the most commonly used methods for fatty acid analysis, but are time consuming (Schutter and Dick, 2000). For this reason, and to provide whole cell fatty acid analysis which PFLA does not, Ester Linked Fatty Acid (ELFA) analysis was the approach chosen here for data acquisition.. Fatty acids may be taken collectively to represent a particular subset of the microbial community (Table 2.4). ELFAs are designated as the number of carbon atoms followed by a colon, then the number of double bonds and their position from the aliphatic (ω) end. The prefixes "*i*" and "*a*" refer to iso and ante-iso methyl branching. Cyclopropyl groups are denoted by "cy". "10Me" refers to a methyl group on the tenth carbon from the carboxylic end of the fatty acid. ELFAs with fewer than 14 carbon atoms and greater than 19 carbon atoms were excluded as these are thought to originate from non-microbial sources (Zelles *et al.*, 1995).

Table 2.4. Summary of ELFAs used to estimate the biomass of different microbial groups

Microbial Biomass Component	ELFA
Total microbial	14-19 carbon atoms
Total bacteria	14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω9c, 16:1ω7c, 16:0, br17:0, 10Me16:0, i17:0, a17:0, cy17:0ω7, 17:0, br18:0, 10Me17:0, 18:1ω7c, 18:0, 10Me18:0, 19:1, cy19:0ω9
Gram positive bacteria	I15:0, a15:0, br16:0, i16:0, br17:0, 10Me16:0, i17:0, a17:0, br18:0, 10Me17:0, 19:1
Gram negative bacteria	16:1ω9c, 16:1ω7c, cy17:0ω7, cy19:0ω9, cy19:0ω7
Fungi	16:1ω5c, 18:2ω6, 18:1ω9
Actinomycetes	10Me17:0, 10Me18:0

VA-Mycorrihizal fungi, C-responsive organisms	16:1ω5
AM-fungi	16:1ω5c
Sulphate reducing bacteria	10Me16:0
Methanotrophs	16:0, 16:1, 16:1ω8c, 18:1ω8c

Notes
Compiled using Lechevalier and Lechevalier, 1988; Frostegard *et al.,* 1993; Zelles, 1999; Olsson, 1999.

ELFA analysis was carried out by Dr Paul Dennis at the Scottish Crop Research Institute (SCRI) with collaboration from project staff and SCRI staff, on the 68 samples from the 68 site locations. ELFA data were provided as a collated dataset into this project for subsequent statistical modeling and analysis. The initial data were provided as a spreadsheet of abundance, absolute concentration, for each fatty acid for each of the sampling sites, with 3 replicates per site. These data were used to produce a community dataset based upon known associations between fatty acids and components of microbial biomass to provide more specific microbial community composition data. Table 2.5 provides the data elements and definitions

Table 2.5. Microbial community data elements

Data Element	Description	Source (Fatty acids used)
Total microbial biomass	All fatty acids representing microbial biomass	14:0, i15:0, a15:0,15:0, br 16:0, i16:0, 16:1 7c, 16:1 9c, 16:1, 11c16:0, br 17:0, 10Me 16:0, i 17:1 , i 17:1 , i17:0, a17:0, 17:1 n-8, 17:0 9, cy17:0, br 18:0, 10Me 17:0, 18:2, 9c12c, 18:1 9c, 18:1 n-7c, 18:1 n-5, 18:1 n-5 , 18:0, 10Me 18:0, 19:1, 19:0 9cy, 19cy n-7, 19:0
Total bacterial biomass	All fatty acids representing bacterial biomass	14:0, i15:0, a15:0, 15:0, i16:0, 16:1 7c, 16:1 9c, 16:0, br 17:0 , 10Me 16:0, i17:0,

		a17:0, 17:0 9cy, 17:0, br 18:0, 10Me 17:0 18:1 n-7c, 18:0, 10Me 18:0 19:1, 19:0 9cy
Gram positive bacterial biomass	All fatty acids representing Gram Positive bacterial biomass	br 16:0, i15:0, a15:0, i16:0 br 17:0 , i17:0, a17:0, br 18:0 19:1
Gram negative bacterial biomass	All fatty acids representing Gram Negative bacterial biomass	19cy n-7, 16:1 7c, 16:1 9c, 17:0 9cy, 19:0 9cy
Actinomycetes biomass	All fatty acids representing Actinomycetes bacterial biomass	10Me 16:0, 10Me 17:0, 10Me 18:0
Fungi	All fatty acids representing fungal biomass	18:2 9c12c, 18:1 9c

Notes

Microbial community data elements following ELFA analysis as provided by Dr Paul Dennis. The table shows data element nomenclature, data description and data source. Review of data is provided in Chapter 3.

2.1.5. Microbial Species Taxonomic Diversity Data; Pyrosequencing Analysis

The species taxonomic diversity data used in this project were provided by Dr Paul Dennis and analysed at the University of Liverpool, the data definitions are shown in Table 2.6. These data were provided following 454 pyrosequencing of 16s rRNA gene fragments amplified from 49 soil samples. The full 68 samples were not sequenced due to time and budgetary constraints.

Pyrosequencing is a technique for DNA sequencing based upon the 'sequencing by synthesis principle' introduced by Mostafa Ronaghi and Pål Nyrén at the Royal Institute of Technology in Stockholm in 1996 (Nyrén, 2007). The sequencing by synthesis methodology works by creating a primer DNA bead incubation mix, layered with enzyme beads from the subject sample, incubated with substrates APS and luciferin, and DNA polymerase, ATP sulfurylase, luciferase and apyrase. A dNTP is added to the reaction in sequence and incorporated into the DNA strand if complementary to the base in the template strand. After each addition, an eqimolar quantity to the incorporated nucleotide of pyrophosphate is released. In the presence of APS, pyrophosphate is converted to ATP by ATP sulfurase. The ATP fuels the conversion of luciferin to oxyluciferin by luciferase which also results in a proportional release of light to the quantity of ATP. A charge coupled device camera is used to detect the light and produce a pyrogram where peak height represents the number of nucleotides added to the strand. Apyrase degrades unused dNTPs and ATP, halting light omission before the next dNTP is added (Elahi and Ronaghi, 2004)

454 pyrosequencing is specifically an array-based pyrosequencing approach developed by 454 life sciences (454 Life Sciences, 2000), following licencing of the pyrosequencing technology. The abundance of all sequences within each sample was recorded for each cluster and forms the data provided by Dennis for subsequent statistical modeling and analysis within this project. Data were provided as a series of excel workbooks for each level of taxonomic classification. Advised at the provision of the data was that sequences were matched using the ribosomal database project (RDP-II) (Cole et al., 2003), however no specific packages or pipelines were defined. Organism matches were identified for c. 2100 sequences, with the remaining c. 29400 sequences being unclassified and/or unknown, and that CD-HIT software (Weizhong and Godzik, 2006) was used to cluster sequences at 95% similarity threshold to provide sufficient confidence sequences over this threshold are

considered to be of the same operational taxonomic unit (OTU) (Weizhong and Godzik, 2006). No further detail was provided into the source of, or subsequent investigation of, the c. 29400 unclassified and/or unknown sequences.

Table 2.6. Species taxonomic diversity data elements

Data Element	Description	Source
Tub	Site Specific Sample Identifier	At Site Collection
Phylum	Bacterial phylum abundance for 49 sites	Sequence clusters
Class	Bacterial class abundance for 49 sites	Sequence clusters
Order	Bacterial order abundance for 49 sites	Sequence clusters
Family	Bacterial family abundance for 49 sites	Sequence clusters
Genera	Bacterial genera abundance for 49 sites	Sequence clusters
Species	Bacterial species abundance for 49 sites	Sequence clusters

Notes

Species taxonomic diversity data elements following 454-pyrosequencing analysis as provided by Dennis. The Table shows data element nomenclature, data description and data source. Review of data is provided in Chapter 3.

2.2. Thesis Methodologies

This section introduces the statistical and modelling techniques used in this project, which are later expanded on in the analysis chapters in which they were used. The fundamentals of the methodologies are discussed, as are the key issues and recommendations for their application. Specific tailoring (a key aspect when applying any modelling technique) to the datasets discussed in Section 1 of this chapter is covered in the appropriate analysis chapters and summarised in Table 2.7.

Table 2.7. Summary of each analysis chapter's aims and the modelling methodologies applied.

Chapter	Topic	Methodologies Applied
4	Examines the hypothesis that increasing latitude will result in differences in microbial diversity	Principal Coordinates of Neighbours Matrices, Principal Coordinates Analysis, Generalized Linear Modelling
5	Uses a spatially scaled modelling approach to examine differences and similarities in the structure of environmental and bacterial community data	Multi-scale Pattern Analysis and Canonical Multi-scale Pattern Analysis
6	Links both 454 and ELFA community profiling datasets to gain a broader perspective of environmental dependency and species profiling	Redundancy Analysis and Canonical Correspondence Analysis
7	Tests a theoretical representation of a microbial energy web to determine the major influences driving community abundance and richness	Structural Equation Modelling

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Several statistical and modelling software packages were used in the analyses of the project dataset including R, PRIMER 6, M-Plus and Excel. R is a language and platform for

statistical computing and graphics devised by Ihaka and Gentleman at the University of Auckland, New Zealand in the mid 2000's. R was used for the majority of the analyses in this thesis due to the transparency and flexibility of the programming environment and the wide range of packages available. The chief analyses conducted in R were Principal Coordinates of Neighbours Matrices (PCNM), Generalized Linear Modelling (GLM), Multi-scale Pattern Analysis (MSPA), Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA); more detail on the specific libraries, authors and functions associated with these analyses are provided in the corresponding thesis chapters. In addition to chief analyses, many simple data mining and exploration procedures were conducted prior to advanced modelling, which were encompassed by core R functions and did not require the downloading of additional libraries.

Plymouth Routines In Multivariate Ecological Research (PRIMER with PERMANOVA+ addition) was created collaboratively by Anderson, and Gorley and Clarke, creators of the original version of the program. PRIMER is a collection of statistical techniques which focus on clustering or constraining data, partitioning variation and measuring and testing relationships between variables. Chief analyses conducted in PRIMER were Principal Coordinates Analysis (PCO) with testing of homogeneity of group dispersions (PERMDISP); these were analyses belonging to chapter 4 only. Both of these functions are present within the PERMANOVA+ addition and default settings were used in their application.

M-Plus by Munthen and Munthen is a package dedicated to conducting different simple and complex forms of Structural Equation Modelling (SEM), including options for introducing time-series data. A simple version of SEM was utilized in chapter 7, including a selected range of available SEM model fit diagnostics.

Data were initially presented as Microsoft Excel data tables. As most of the analyses were conducted in R, many of these tables were simplified by taking an average of data replicates per sample and converting the excel files into CSV files, a format requirement of R. Excel was also used to create the Heat Maps present in chapter 6 using the heat map graphical function.

2.2.1. Ordination

Ordination can best be described as a means of ranking a group of objects along an environmental gradient by graphical representation, where the data are displayed in such a way that the axes seek to explain the major patterns of variation in the data. The x axis is the major principal axis i.e. the axis which explains the dominant trend in the data, and the y axis (the principal axis) is determined by the next most influential trend - this is orthogonal to the trend portrayed in the x axis. Before selecting the correct technique to be used consideration needs to be given to two factors: whether the relationships exhibited by the data are linear or unimodal, and whether the gradient of sites may be described by an extenuating factor or 'constrained'.

Unconstrained ordination is considered a purely exploratory tool, the most commonly used example of which is PCA, currently used in almost half of all statistical analysis of microbial communities (Ramette, 2007). PCA is an eigenanalysis-based technique which uses Euclidean distance to measure the difference between sites based upon their correlation or covariance co-efficient. Due to the tendency of ecological data to exhibit a large number of zero's, Euclidean distance wrongly assumes likeness between sites of conjoint absence (of species or biophysical variables). Ecological data often displays unimodal species response curves (Whittaker, 1967) rather than the monotonic or linear relationships assumed by PCA. The resulting ordination assumes a 'horseshoe' or 'arch' effect where what should be the opposite ends of a gradient are folded inward, portraying sites with no species as having common ecological similarity (Gauch, 1982). RDA is the constrained form of PCA in which the distribution of species amongst sites is sought to be explained by one or more environmental variables, and also suffers from the same caveat. PCO is a technique, which when using Euclidean distance as a measure of similarity or dissimilarity between data, yields the same results as a PCA. However, PCO is commonly used with similarity measures such as Bray-Curtis (Bray and Curtis, 1957) to identity similar samples on the basis of species occurring in both, but not the absence of a species in both samples as a sign of similarity

To combat the problems related to the use of Euclidean distance in ordination, Legendre and Gallagher (2001) proposed that performing a transformation on the species data prior to analysis would be beneficial. Of the transformations proposed, Hellinger and Chord proximity were found to be the best transformations to be used on ecological data prior to

PCA or RDA. Hellinger, Chi-square and Chord metric are shown in Box 2.1. By considering incidence of mutual presence rather than absence, these methods provide a more reliable measure of association between sampling units and their respective descriptor variables, conserving the true geographic distance across an ecological gradient. Another practical consideration for using these transformations is that they do not give high weight to rare species, as with Chi-square distance; the algorithm used in Correspondence Analysis (CA) and the constrained form of CA, CCA. This is especially important in soil environments where sampling is blind and double absence of a species is more likely to indicate lack of information rather than exhaustive sampling. CA and CCA are suited to model unimodal relationships, although still potentially suffer the same pitfalls with zero inflation, and it is now generally preferred that PCA/RDA with appropriate transformation are used whatever the relationship dynamics (Zuur *et al.*, 2007).

$$D_{Hellinger}(x_1,x_2) = \sqrt{\sum_{j=1}^{p}\left[\sqrt{\frac{y_{1j}}{y_{1+}}} - \sqrt{\frac{y_{2j}}{y_{2+}}}\right]^2}$$

$$D_{\chi^2 metric}(x_1,x_2) = \sqrt{\sum_{j=1}^{p}\frac{1}{y_{+j}}\left(\frac{y_{1j}}{y_{1+}} - \frac{y_{2j}}{y_{2+}}\right)^2}$$

$$D_{chord}(x_1,x_2) = \sqrt{\sum_{j=1}^{p}\left(\frac{y_{1j}}{\sqrt{\sum_{j=1}^{p} y_{1j}^2}} - \frac{y_{2j}}{\sqrt{\sum_{j=1}^{p} y_{2j}^2}}\right)^2}$$

Box 2.1. Equations for Hellinger, Chi-square and Chord metric between sites x1 and x2 across p species (Legendre and Gallagher 2001).

Both RDA and CCA were chosen as modelling approaches in Chapter 6 analyses. These ordinations were performed in R using the library 'vegan'(Oksanen *et al.*, 2011), and the functions CAPSCALE and CCA respectively. Prior to analyses, all microbial community data were transformed using the 'Hellinger' metric. This was achieved using the R library 'vegan'and the function DECOSTAND.

PRIMER software was used to perform PCO for analyses in Chapter 4 using the function PCO after applying a Bray-Curtis transformation on the data using the function Transform. The option to overlay the environmental parameters was also selected. PRIMER was selected for the PCO analyses instead of R, due to the ability to perform the test for homogeneity of group dispersions on the data,

which was not easily accessible in R at that time. For more detail on the methods used, see sections 4.2.and 6.2.

2.2.2. Spatial eigenvector analyses

Principal coordinates of neighbours matrices (PCNM) and Moran's eigenvectors maps (MEM) (Borcard and Legendre, 2002) have been introduced as a family of spatial predictors with the capacity of identifying different spatial structures present in multivariate community data. Structures may be classified as either global or local by means of permutation testing. Global structures are thought to represent abiotic influences and gradient effects, and local structures represent contagious biotic processes demonstrating spatial autocorrelation. The advantage of identifying such structures in the soil microbial community is that it may be possible to recognize key ecological dynamics behind observed patterns in diversity and composition. In ecology, PCNM and MEMs are used to dissect the spatial patterns of the ecological variability into separate scales. These variables can be used as spatial predictors in multiple regressions as they are un-correlated (Bocard and Legendre, 2002). When environmental data are available they can also be used as spatial predictors in constrained ordinations (Borcard *et al.* 2004), however when used this way can be subject to high type I errors (Jombart *et al.*, 2009)

Multiscale Pattern Analysis (MSPA) has been introduced as an extension of MEM which can be used with quantitative and qualitative variables and does not use the forward selection method of vector selection associated with high errors (Jombart *et al.,* 2009). MSPA be used for both canonical and partial canonical analyses by performing a multivariate regression of data onto a set of explanatory variables, as done in constrained ordinations (Jombart *et al.*, 2009). Canonical MSPA serves to extract the environmental component of the multi-scale spatial patterns of species, thereby focusing on spatial dependence of species on environment. Partial canonical MSPA can be used to study multi-scale spatial patterns in species after removing the environmental effect from the data.

PCNM was performed using R programming software and the key functions taken from the libraries 'spacemakeR' (Anon, 2011) and 'sedarjombart' (Jombart *et al.,* 2010). PCNM was performed using the function PCNM. MSPA and canonical MSPA were also conducted in R, using the libraries 'ade4' (Chessel *et al.,* 2011), 'spdep' (Bivand, 2011), 'adegenet' (Jombart, 2011), 'spacemakeR' (anon, 2010) and 'sederJombart' (Jombart *et al.,* 2010), using the

function MSPA. For more detail on the methods used, see sections 4.2 and 5.2.

2.2.3. Multiple regression: Generalised linear modelling (GLM)

Multiple regression seeks to model the relationship between a dependent variable and several independent variables and to explore the forms of these relationships. In restricted circumstances, regression analysis may also be used to infer causal relationships between the independent and dependent variables. The main considerations in the application of regression techniques are concerned with several assumptions about the data, which have varying importance depending on the method of choice.

Though most regression techniques are reasonably robust against the violation of data assumptions (Fitzmaurice *et al.*, 2004), it is expected that variables will be normally distributed, as non-normal variables such as those with outliers or heavy skew, distort relationships and significance tests. Several visualisations of the data both prior to and post analyses (such as box-plots and QQ-plots) can be informative to identify problems. Outlier removal reduces the occurrence of type I and type II errors (Osbourne, 2001). There is however no standard protocol for outlier removal, outliers are identified and removed based purely upon knowledge about the data under scrutiny. When outlier removal cannot be ecologically justified, data transformations (e.g. square root, log) can be used to improve normality.

Other assumptions include homogeneity of variance or homoscedasticity, in which the dependant variable exhibits similar amounts of variance across the range of values as does the independent variable. This can be assessed by plotting residuals against fitted values in a spread level plot, this is a significance test of the homogeneity of variance with the data. Where heterogeneity is observed, a transformation of the response variables can help stabilize the variance (Zuur *et al.*, 2010)

Collinearity is where two or more independent variables exhibit a high degree of correlation, which means they represent the same source of variation. Collinearity is a particular problem in regression when explanatory variables are highly correlated (collinear). Variable Inflation Factor analysis (VIF) can be used to offset one explanatory variable against all the others in a linear regression in order to assess which encompass the same source of variation. A VIF value of 4 or more (Montgomery and Peck, 1992) indicates a highly collinear variable which

may need to be removed from the model.

GLMs were performed using R statistical software and the function GLM, a function present in the core program. Prior to GLM analyses, VIF was performed in the 'car' package in R (Fox 2010) using the function VIF. For more detail on the method used, see section 4.2

2.2.4. Structural Equation Modelling (SEM)

SEM is an extension of multiple regression and represents both an exploratory and confirmatory tool to investigate hypothesised cause-effect relationships in a composite statistically dependant model (Shipley, 2000). Relationships between parameters can be measured and correspond to the magnitude of dependence between pairs, represented by path-coefficients. The aim is to produce a model where a series of dependencies is simultaneously expressed in order to find the most parsimonious but best-fitting model. The key difference between SEM and other regression analyses is the ability to use latent variables. These are hypothetical constructs which cannot be directly observed but may be indicated using a subset of measured variables within the model.

There are two general types of variables used in SEM, exogenous and endogenous. These are variables which are explained by one of more of the other variables in the model. The path co-efficients to endogenous variables are estimated by multiple regression on a correlation matrix of the variables, meaning that there is a limitation of SEM to deal better with linear relationships. This is not always the case in ecology, but certain data transformations (e.g. log) can help transform the data to linearity

Many of the caveats of multiple regression also apply to SEM. Construct reliability is the first assumption, that independent variables are measured without error. This is confounded by construct validity, which is concerned with how well the measured variable represents the construct it seeks to measure (Bollen, 1989). Multicollinearity between variables can cause unreliable parameter estimates, but is easily accounted for in the model by interaction terms between latent variables. In order to cope with latent variable estimation and experimental power a sample size minimum of 100 sites is recommended, and optimally over 200 (Lei, 2007). In Chapter 7, SEM analysis uses 49 and 68 samples for 454 bacterial and ELFA community data respectively, the concerns with sample size are discussed in that section. A

general rule of thumb is to have a minimum of five times the number of observations than parameters (Klem, 1995).

SEM was performed in M-plus using the 'type=general command' and outputs selected were 'standardized', 'sampstat' and 'modindices'. Modindices is a function to return modification indices which can be used to suggest relationships between variables which may improve model fit but are not currently being implemented in the model. This information assisted in model building as relationships were omitted or introduced with different model runs. Standardized and sampstat provide standardized model estimates to remove the effects of parameter scaling for easy comparison; the default provides only unstandardized estimates, and covariance between variables respectively. Models which best represented the hypothetical relationships between variables were tested until the best fit combination of parameter relationships were found. For more detail on the method used see section 7.2.

2.2.5. Model selection

Finding a model with the best set of explanatory variables is a key aim in most multivariate analyses, especially in regression-based techniques (Zurr *et al.*, 2006). By finding the best subset, noise and error can be minimised and model predictions are likely to be more accurate. Akaike Information Critieria (AIC) and the adjusted coefficient of determination (adjusted R^2) are commonly used such statistical criteria. For every possible combination of explanatory variables, these criteria provide a means of assessing the goodness of fit for the most parsimonious model (i.e. including the least parameters) with the lowest AIC and highest R^2 values (Box 2.2).

Several indices are available that may be used to assess the true model fit, power and comparative model fit for any structural equation model. Goodness of fit was assessed here using chi-squared tests, where a significant statistic indicates that the model is not supported by the data. For true model fit, both the Root Mean Square Error of Approximation (RMSEA) and the Comparative Fit Index (CFI) are not heavily influenced by sample size or non-normality but concentrate on testing an overall hypothesis .i.e the variance explained by a latitudinal gradient. Using MLE (Maximum likelihood estimation), Hu and Bentler (1999) proposed that a cut-off of 0.08 for standardised RMSEA together with 0.95 for CFI was the most appropriate approach. Information criteria such as Akaike's information criterion (AIC)

and Bayesian information criterion (BIC) may be used to compare two similar models for goodness of fit. Models with lowest AIC and highest BIC values are regarded as the best-fitting models. Also, adjusted BIC may be used to account for sample size and number of parameters estimated, putting more importance on parsimony.

$$\text{Adjusted } R^2 = 1 - \frac{SS_{residual}/(n-(p-1))}{SS_{total}/(n-1)} \qquad AIC = n\log(SS_{residual}) + 2(p+1) - n\log(n)$$

Box 2.2. Adjusted R2 and AIC (Zuur *et al.*, 2006)

2.3 References

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3

Data Exploration: Site Variation and Species Diversity

3.1. Introduction

Over the last decade there has been substantial addition to our knowledge of soils and their associated micro-biota from the Antarctic Peninsula region (Yergeau *et al.*, 2007, 2009; Yergeau and Kowalchuk, 2008; Aislabie *et al.*, 2010; Convey *et al.*, 2012; Chong *et al.*, 2012). However most of these studies present either a detailed description of several sites in close proximity or are concentrated around the same areas, popular for their logistic ease. The following analyses present the main results created from a sampling effort in the austral summer 2007/2008 where 68 soil samples were collected from 24 locations from South Georgia (54.2833° S, 36.5000° W) to Mars Oasis, South East Alexander Island (71.5244° S, 68.1500° W) and were chemically and biologically profiled later in 2008-2009. Prior to the following analysis chapters, this chapter serves as a presentation of the key trends in the data which help form a solid basis for further exploration. The methodology and results will be discussed for each section independently as most are a simple graphical representation and a discussion will follow to help amalgamate the major findings with the current literature.

3.2. Variation in environmental factors between sampling locations

3.2.1. Site locations and data acquisition

Sites locations, data acquisition and modelling strategies are described in Chapter 2.

3.2.2. Site influences

Site influence data was documented in the field on a scale of 1-10 according to 'by-eye' estimation by the field scientist (see Section 2.1.1. for more information). The variation of the site influence data was considered in both a site-specific view and also a global trend analysis. The variation of the site physical characteristics was captured in isolation, but has been presented so reflection can be made across the sites. Data are presented with sites orientated by latitude. Figure 3.1 shows the site-observed influences with sites orientated north (top) to south (bottom) based upon averaged site influence scores to account for the differing number of sites sampled at each location. Figure 3.2 shows a 100% stacked bar graph where the percentage each category contributes to the total for each location is displayed and Figure 3.3 shows the global trend of site characteristics moving north to south in a clockwise fashion on a stacked line radar graph so contribution of influence across all of the locations sampled can be compared.

Figure 3.1 does not show any major trend with latitude but, generally, external influences were stronger at sites further south, as seen by the cumulative influences at each site, peaking at Jenny Island. As expected, seal and bird presence appeared to coincide in locations. Rocky, moss and lichen retained a presence throughout most sites. Human and plant influence did not show a clear pattern with latitude. Both the stacked graph (Figure 3.2) and the global analysis (Figure 3.3) identified birds and rocky as the two most influential characteristics across all sites. As these data are purely observational and also subjective based upon the field scientist's opinion at the time of the visit, the validity of trends observed cannot be assessed objectively.

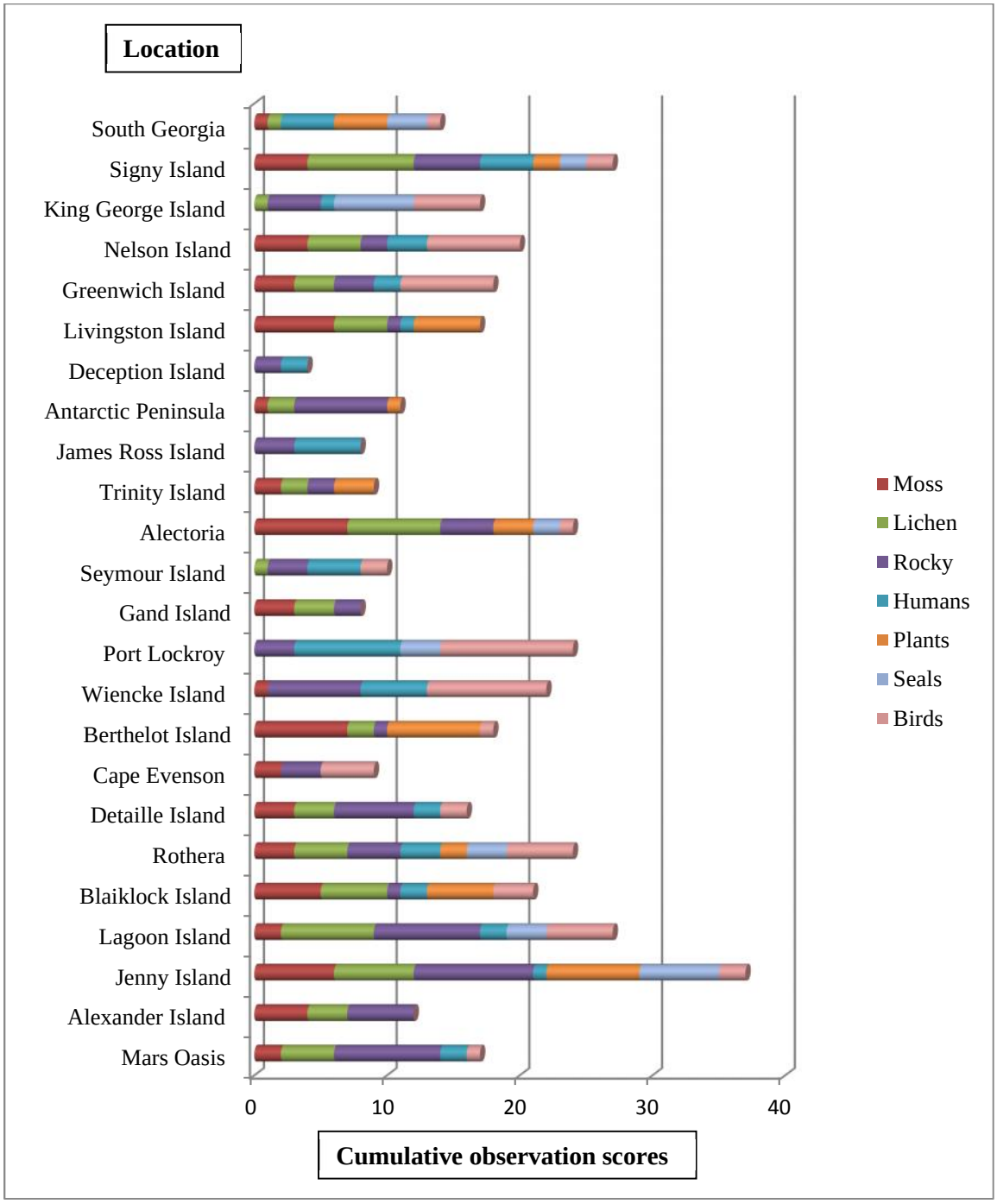

Figure 3.1. Absolute site influences data were collated using Microsoft Excel 2010; the observation values for the physical characteristics were averaged to provide a relative equivalent scale regardless of the number of sites sampled at each location. This was sorted by latitude, north to south, to support comparative data presentation. The derived table was used to present the data in a simple excel bar chart of actual numbers.

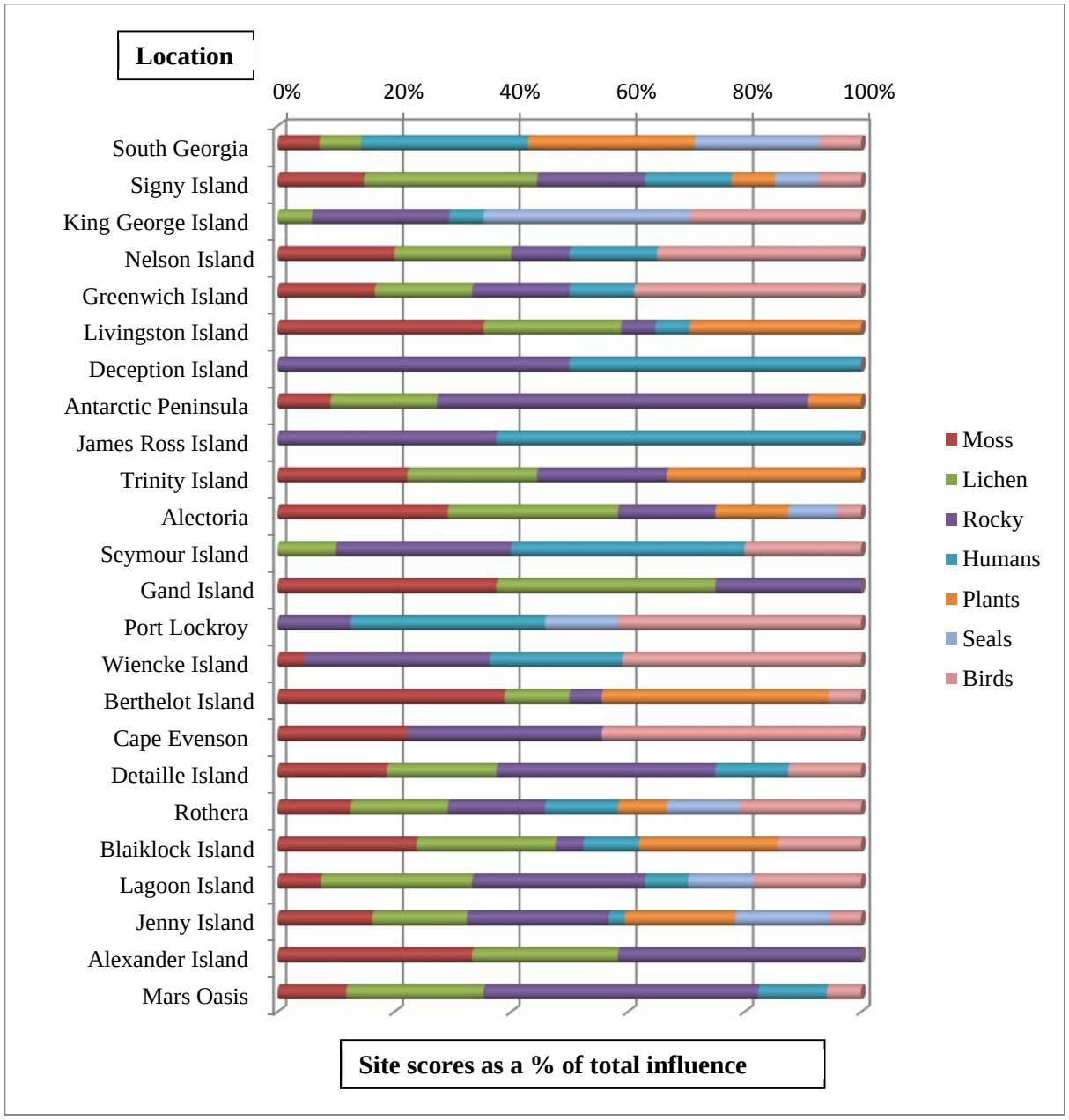

Figure 3.2. Percentage site influences data were collated using Microsoft Excel 2010; the observation values for the physical characteristics were averaged to provide a relative equivalent scale regardless of number of sites per location. This was sorted by latitude, north to south, to support comparative data presentation. The data was presented north to south by site using a 100% stacked bar chart to show the percentage representation of each influence per location.

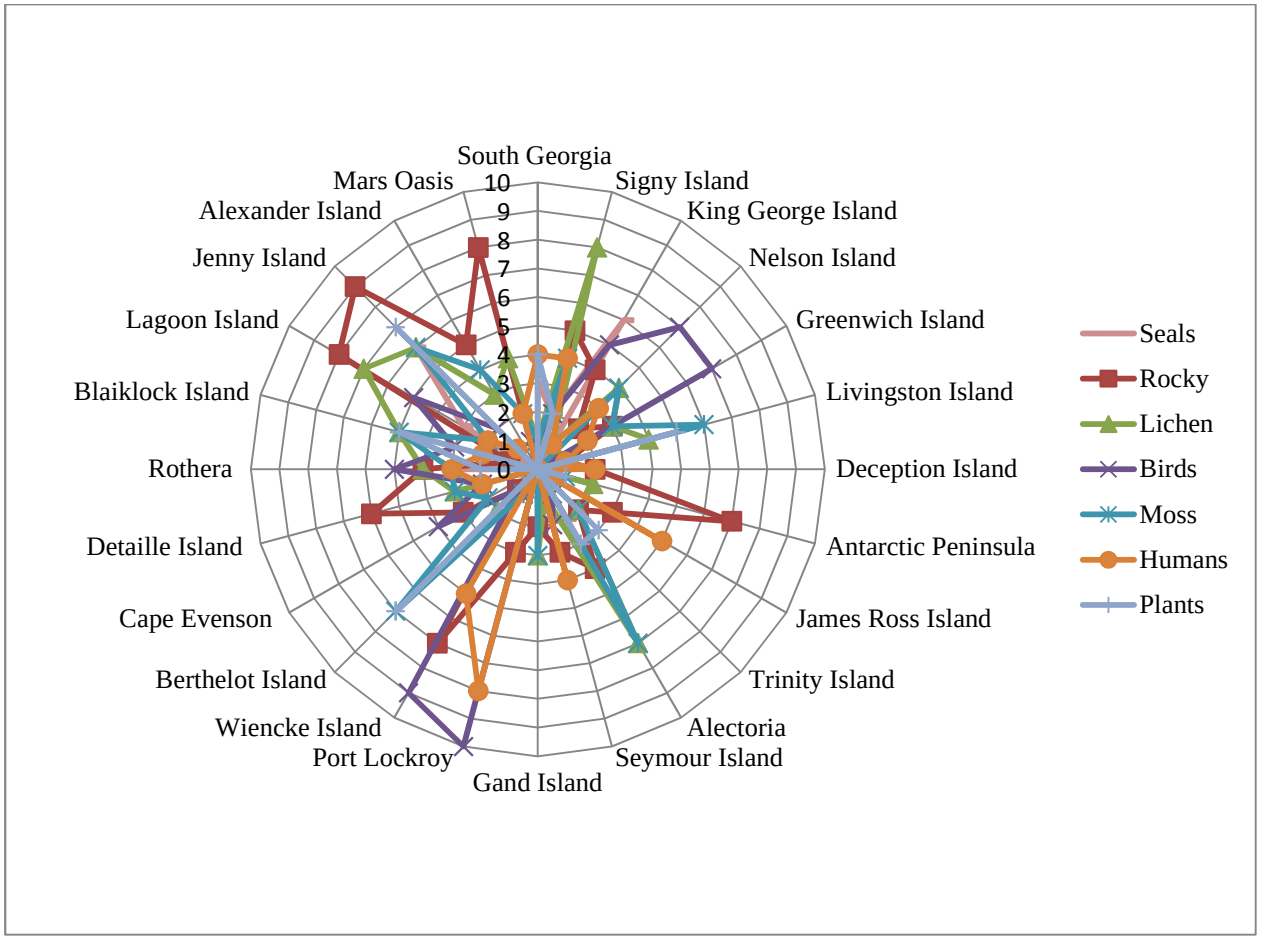

Figure 3.3. Global spread of location influences averaged site data, collated in Microsoft Excel 2010 and presented by location north to south in a clockwise direction; physical characteristics sorted from major to minor observations, layered deepest to shallowest on an excel radar graph.

3.3. Soil chemical properties

Measured soil chemical properties (see Section 2.1.2) were compared across regions using a principal coordinates analysis (PCO) using the software package PRIMER 6 & PERMANOA+ (Clarke & Gorley, 2006). PCO was performed based upon normalized chemical data across all locations and using Euclidean distance to measure similarity between sites, so that sites of similar soil chemistry were grouped together. An individual chemical property was then overlaid using the bubble graph function to show how that specific property differed between locations. Table 3.1 lists the abbreviations for location names used in the PCO.

Table 3.1. List of abbreviations used in the PCO

Abbreviation	Location	Abbreviation	Location	Abbreviation	Location
Alt.	Alectoria	Grn Is.	Greenwich Island	Rothera	Rothera
Alx Is.	Alexander Island	JR Is.	James Ross Island	Rsthcld Is.	Rothschild Island
Ant Pn.	Antarctic Pn.	Jn Is.	Jenny Island	Sym Is.	Seymour Island
Brth Is.	Berthlot Island	KG Is.	King George Island	Signy Is.	Signy Island
Blk Is.	Blaiklock Island	Lg Is.	Lagoon Island	SG	South Georgia
Cape Ev.	Cape Evenson	Lvg Is.	Livingston Island	Trn Is.	Trinity Island
Dcpt Is.	Deception Island	MO	Mars Oasis	Wiencke Is.	Wiencke Island
Det Is.	Detaille Island	Nls Is.	Nelson Island		
Gand Is.	Gand Island	PL	Port Lockroy		

The PCO, used to group sites according to the similarity of their soil chemistry, had only limited success in representing the trend across different locations, with the first two axes explaining only 55% the variation (all graphs). According to the first axis, Seymour Island, James Ross Island and most of the other locations sampled are similar in soil chemical properties but are distinct from Cape Evenson, Detaille Island and Gand Island. By examining, the individual chemical trends dissolved organic carbon (Figure 3.4), organic carbon (Figure 3.5.) and total nitrogen (Figure 3.6.), all show a trend correlated with the first axis where quantities of these increase linearly towards Cape Evenson (far right PCO). By referring back to Figure 3.1 and the field notes in Appendix 3, these locations lack plant and seal influence but have rocky soils, Conversely, phosphate (Figure 3.7) and pH (Figure 3.8) decrease across the primary gradient (PCO1).

The second axis (PCO2) separates Port Lockroy from the other locations, with Berthelot Island being placed at the opposite end of the gradient. According to the bubble plot overlays, Port Lockroy soils are basic (Figure 3.8) and have high nitrate quantities (Figure 3.9), Port Lockroy is also well known for high ornithgenic inputs as shown in Figure 3.1 and Appendix 3. Berthelot Island soil is high in moisture content as seen in Figure 3.11 but generally low in other substrates, major influences observed close to the area of soil collection were plant and moss presence (see Figure 3.1).

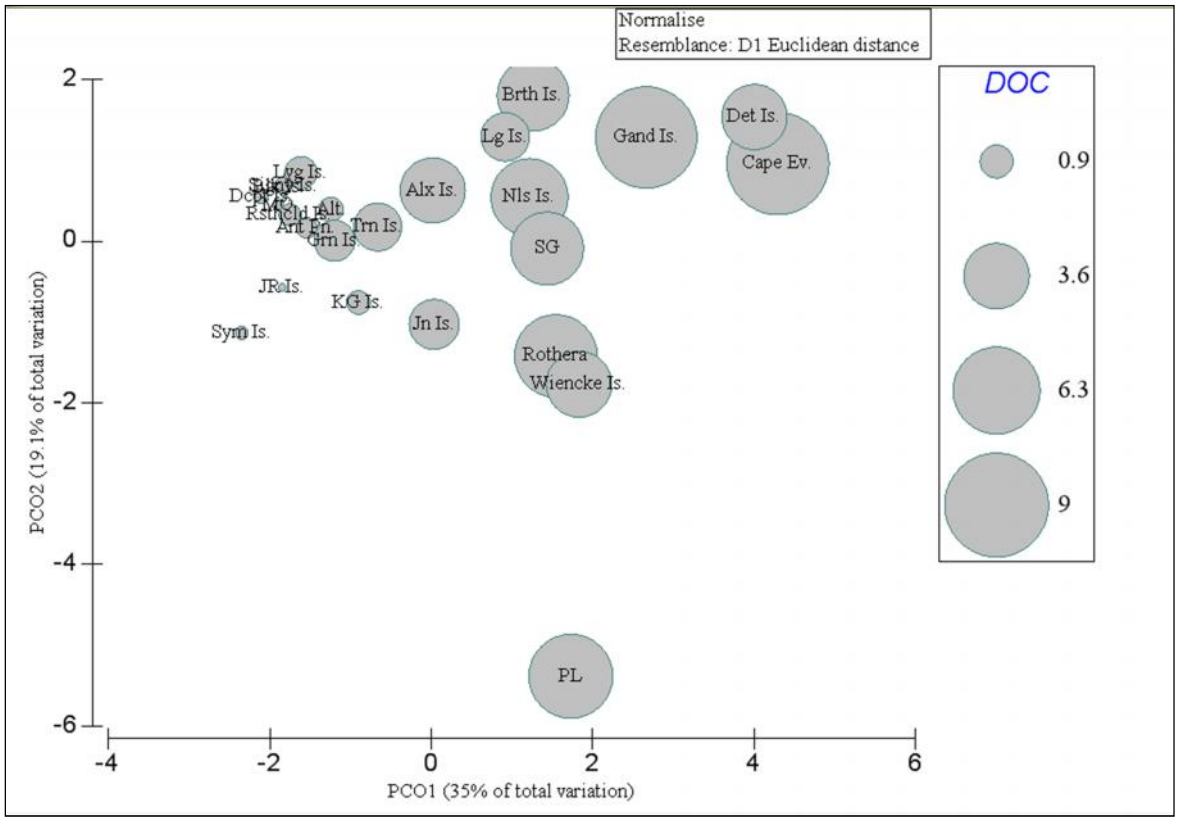

Figure 3.4 PCO of DOC, PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of dissolved organic carbon (DOC). Scale for each bubble graph is representative of the individual data range for each variable.

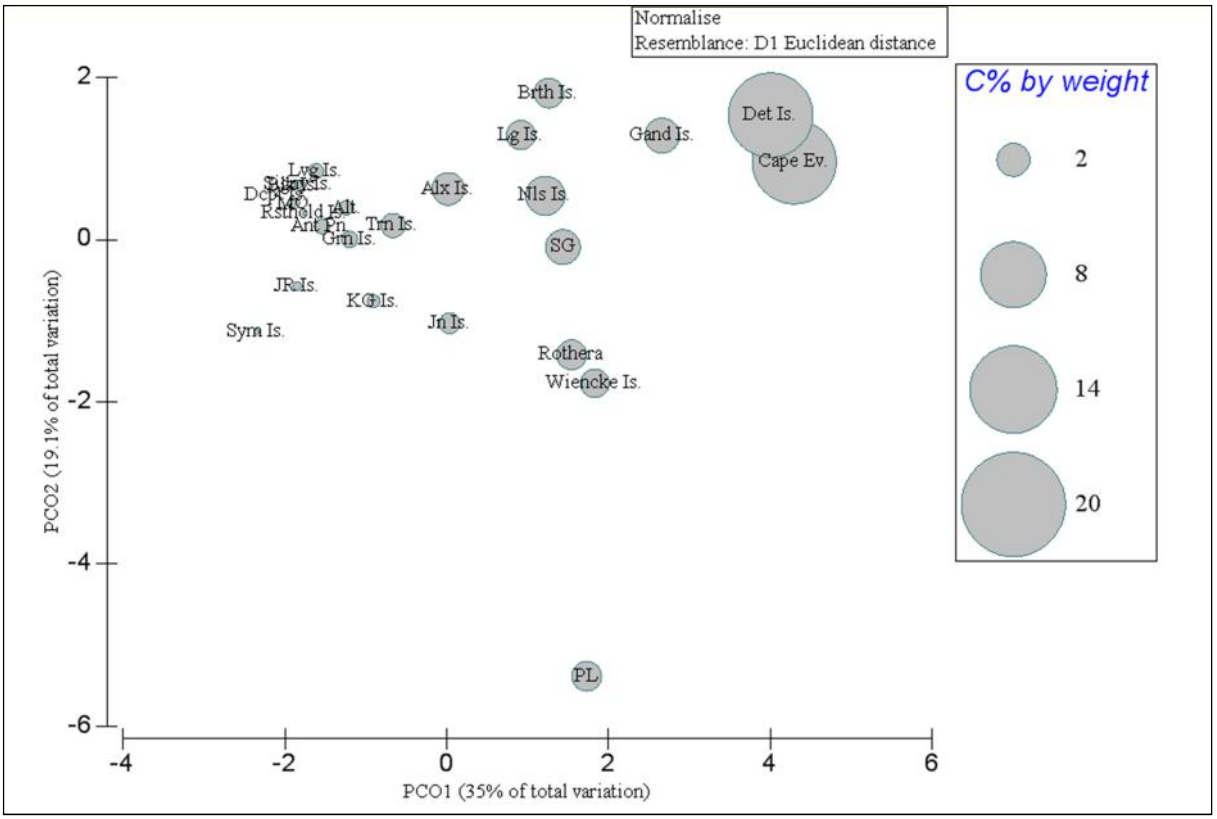

Figure 3.5. PCO of organic C. PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of the total % carbon (% C by weight), respectively. Scale for each bubble graph is representative of the individual data range for each variable.

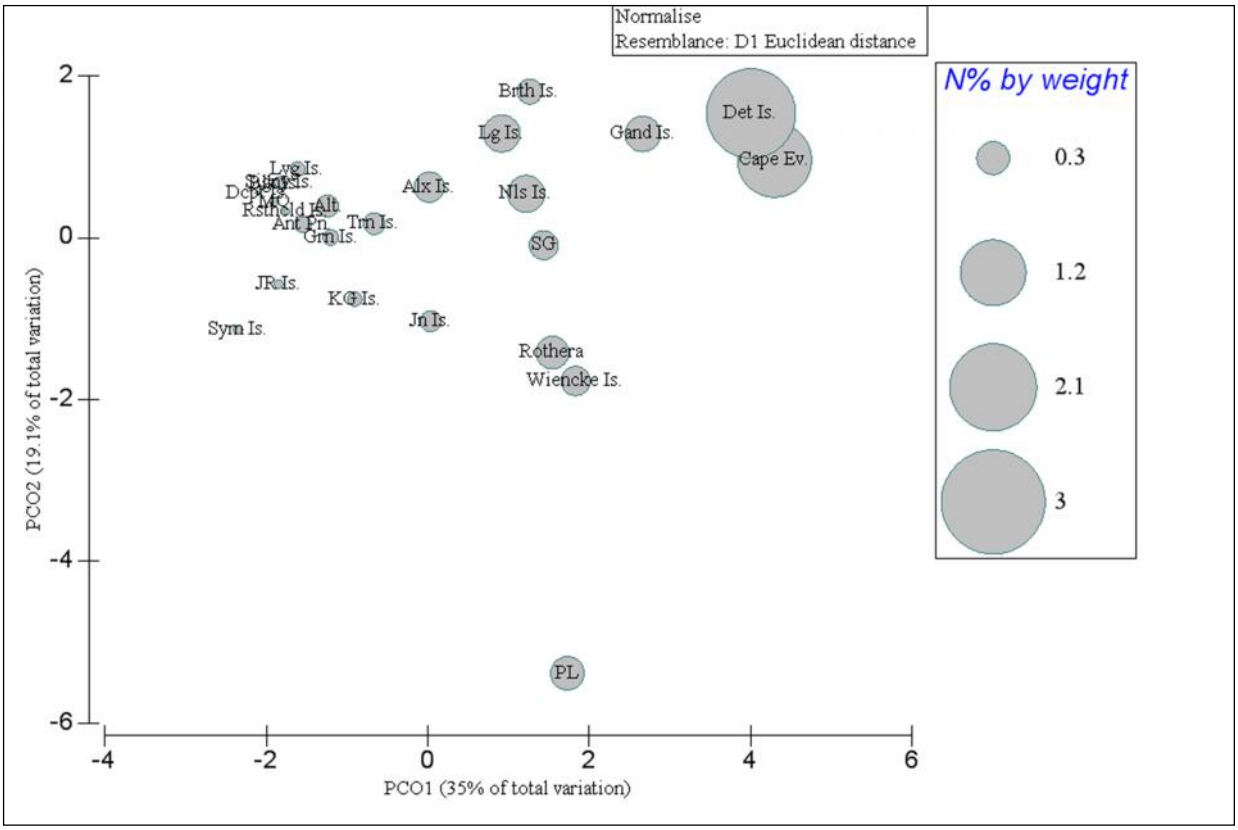

Figure 3.6 PCO of total N. PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of total % nitrogen (% N by weight). Scale for each bubble graph is representative of the individual data range for each variable.

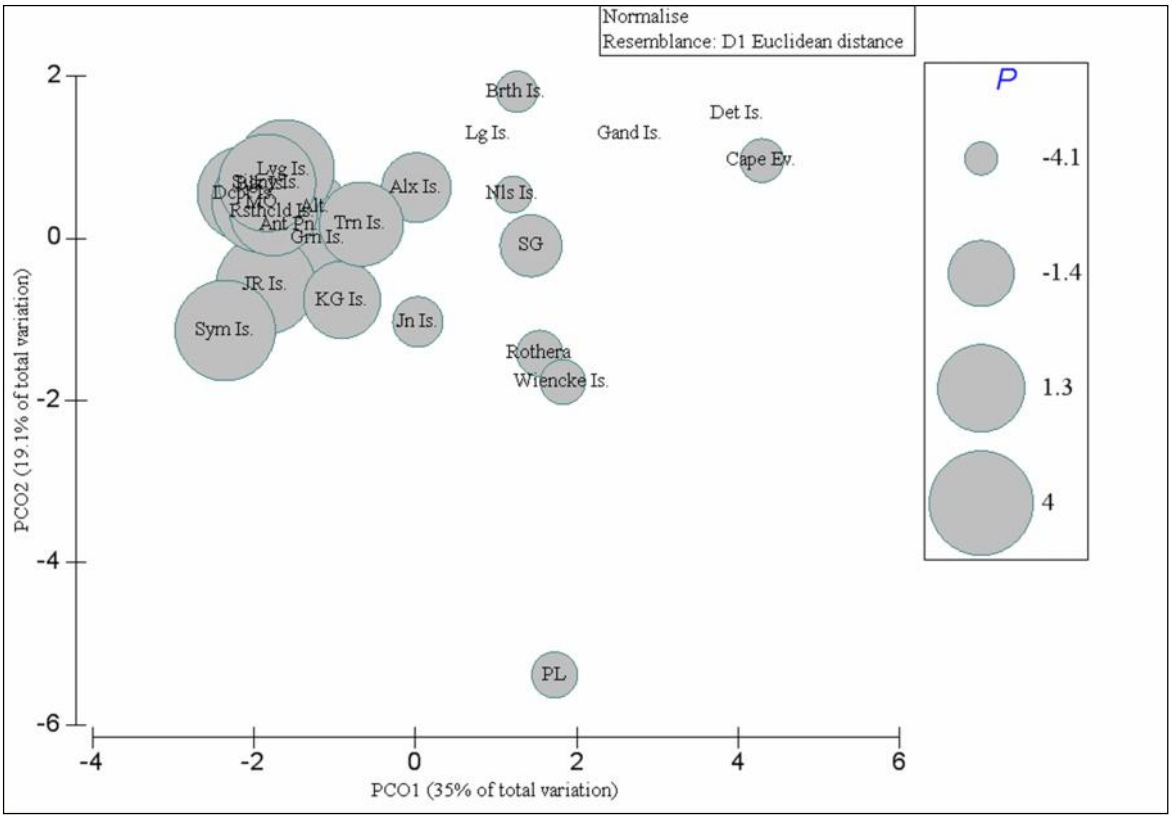

Figure 3.7. PCO of Total P. PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of total phosphate (PO_4^{3-}), . Scale for each bubble graph is representative of the individual data range for each variable.

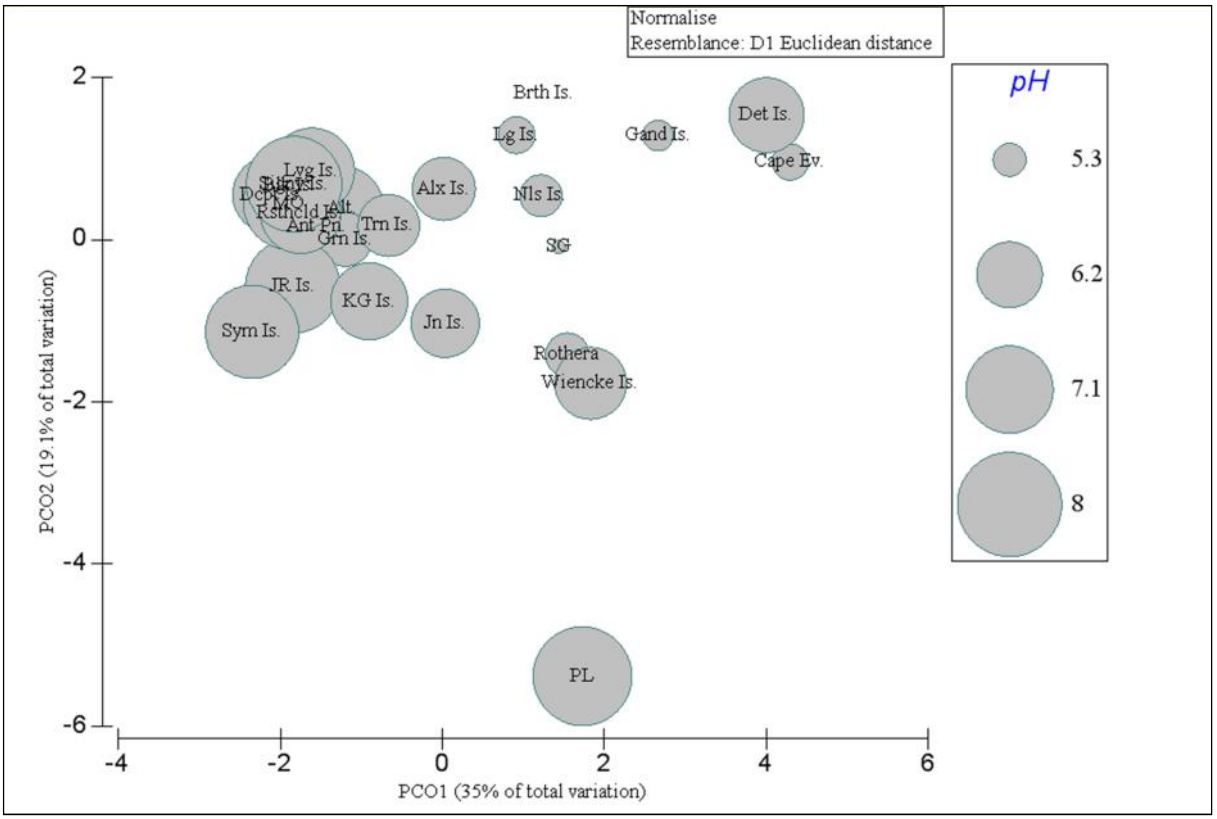

Figure 3.8. PCO of pH: PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of pH. Scale for each bubble graph is representative of the individual data range for each variable.

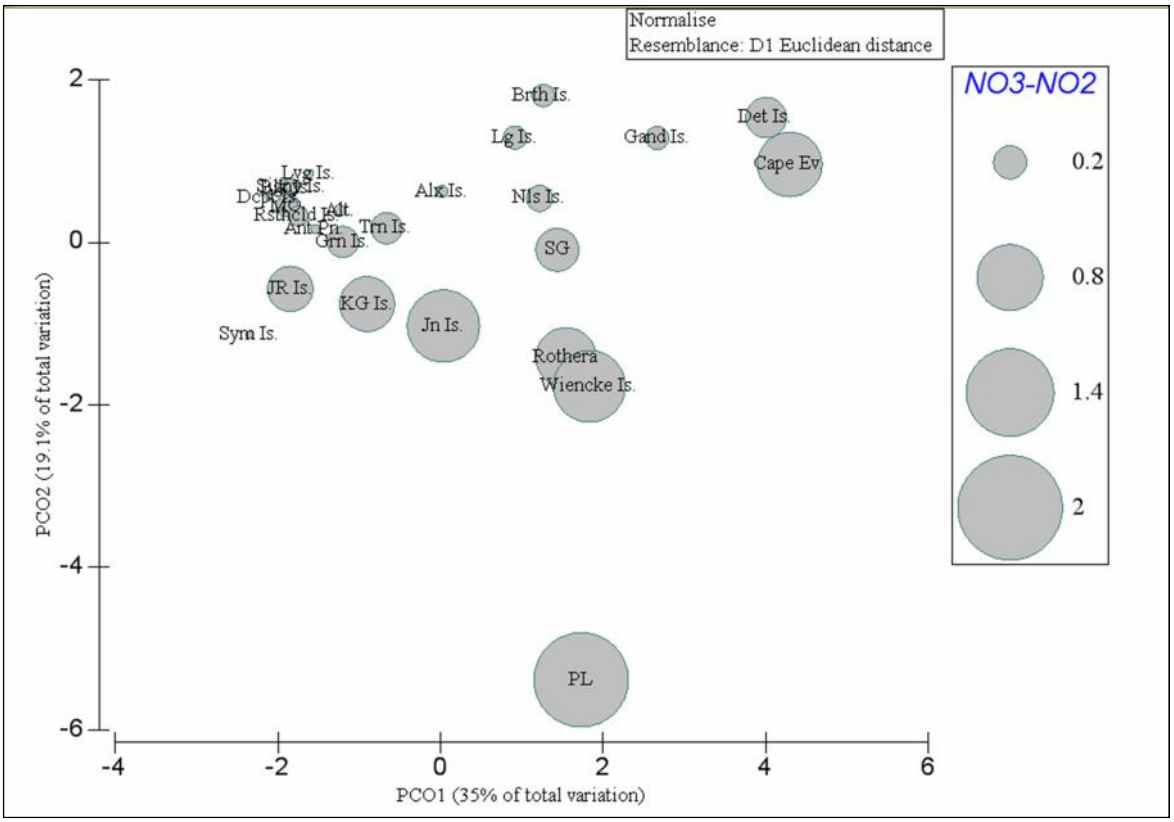

Figure 3.9. PCO of Nitrate/Nitrite. PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of nitrate/nitrite (NO_2^-, NO^-_3). Scale for each bubble graph is representative of the individual data range for each variable.

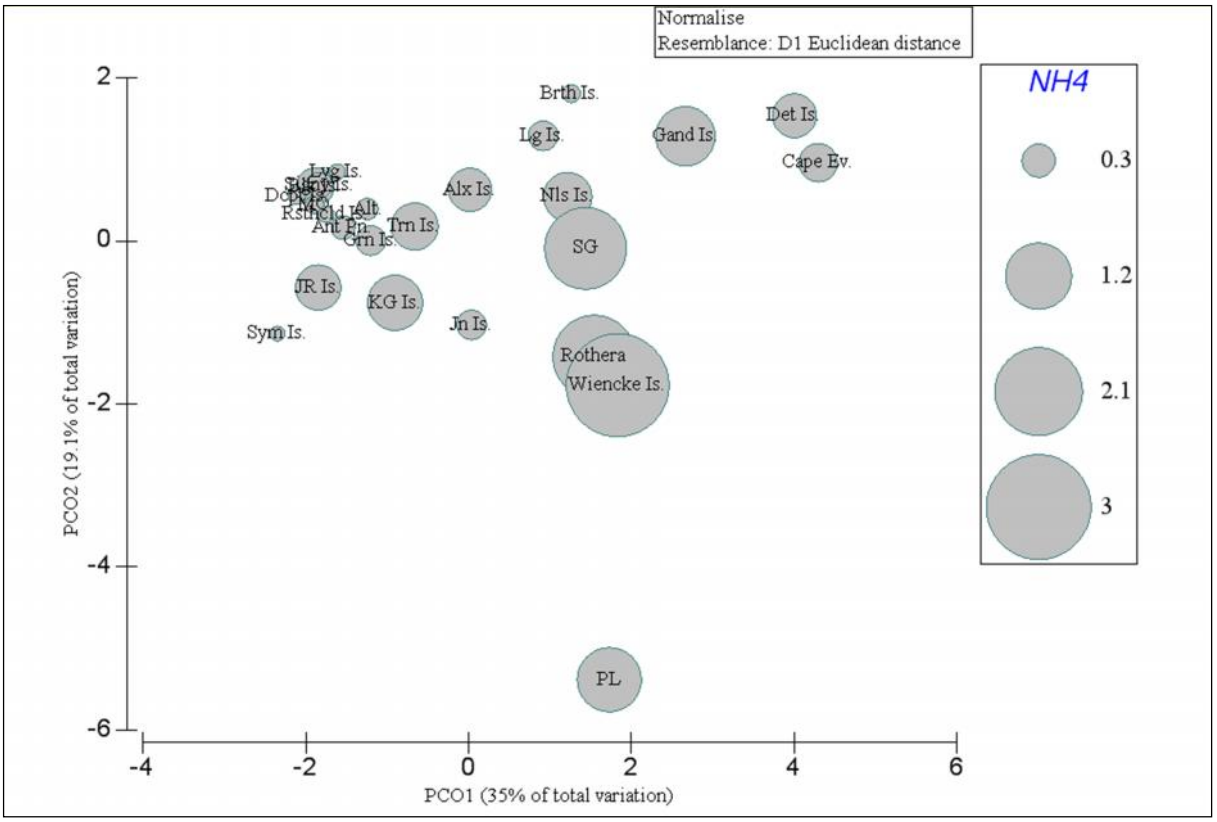

Figure 3.10. PCO of Ammonium (Bottom). PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of ammonium (NH_4^+), . Scale for each bubble graph is representative of the individual data range for each variable.

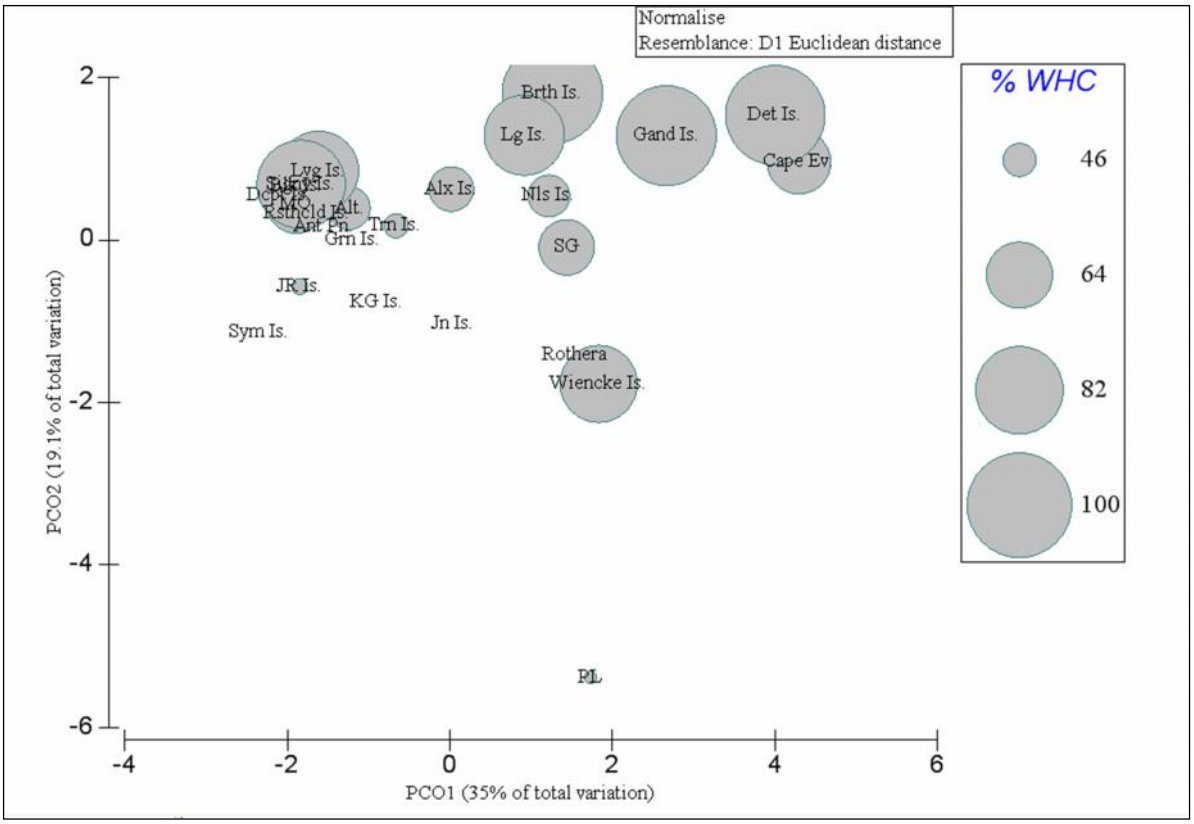

Figure 3.11. PCO of %WHC: PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of % soil at water holding capacity (%WHC). Scale for each bubble graph is representative of the individual data range for each variable.

3.4. Abundance

Microbial abundance was explored through presentation of simple bar charts of abundance plotted against latitude and sorted from North (South Georgia) to south (Mars Oasis). First, the ELFA data was partitioned according to microbial representative (see Table 2.4), and then total microbial biomass in n mol g^{-1} dry soil was calculated for all sites (Figure 3.12). For the pyrosequencing data, relative abundance was calculated using total classified sequences for each bacterial phylum, over all sites (Figure 3.13).

Microbial biomass abundance (ELFA) increased with latitude, peaking around Port Lockroy and Cape Evenson (Figure 3.12). There was also a proportionate increase in the abundance of Gram Positive bacteria in these areas. Abundance varied at other most latitudes but consistently declined at the sites furthest south, Rothschild Island and Mars Oasis. Fungal fatty acids were present in all locations and, in most, accounted for a high fraction of the biomass there.

From the bacterial pyrosequencing data, the most abundant bacteria were Proteobacteria and Bacteriodetes, which maintained strong proportional presence throughout most samples and do not appear to vary with latitude. Cyanobacteria were dominant in some soil from South Georgia and Berthelot Island but showed low abundance elsewhere. Gemmatimonadetes peaked in abundance around James Ross Island. Sites with the highest abundance represent 'mid' latitude sites in our gradient, around between Port Lockroy and Wienke Island, which is consistent with the microbial community data.

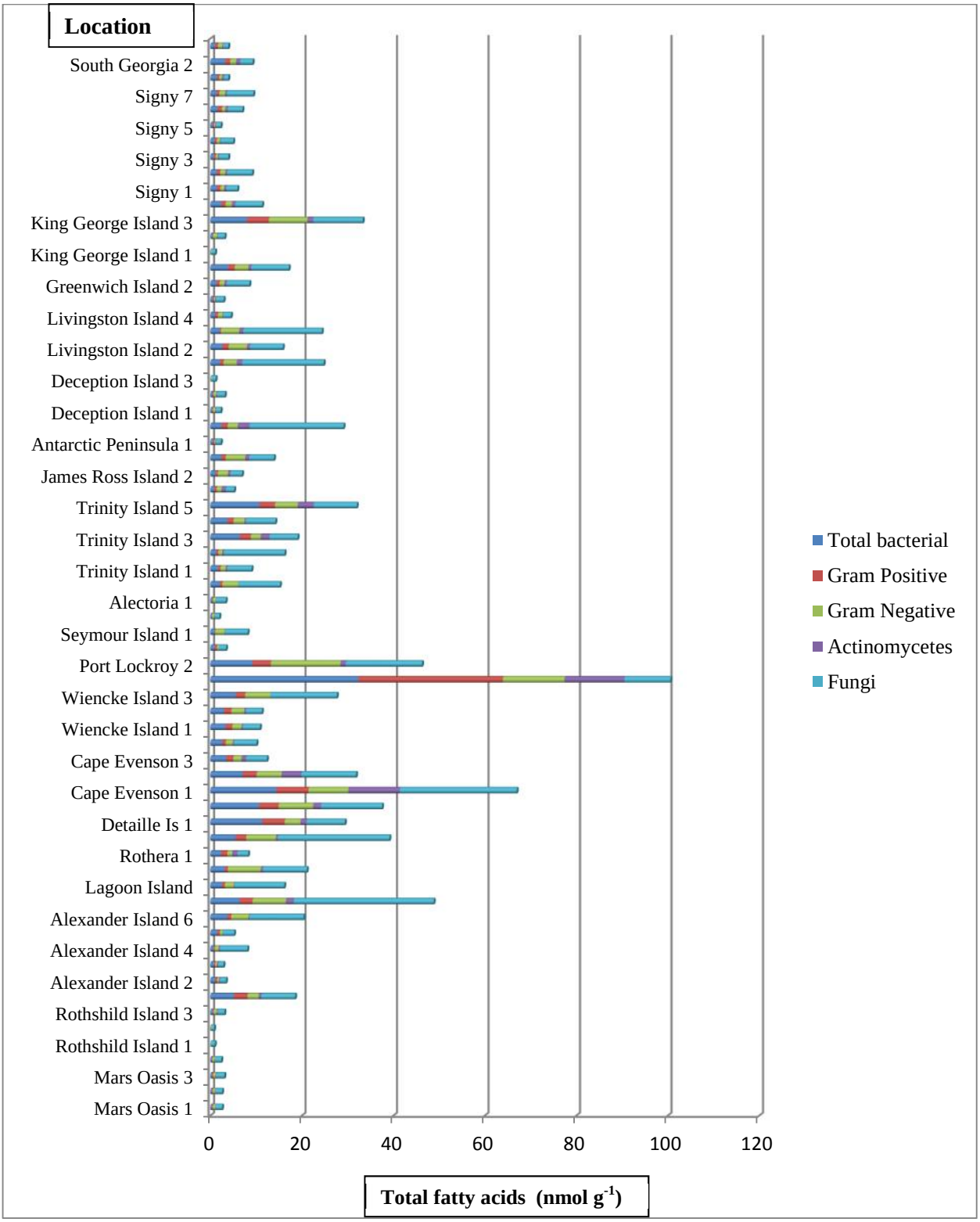

Figure 3.12. Abundance variation of the ELFA data was collated in Microsoft excel with the bar chart function and sorted by latitude, north to south. Individual sites are presented and no average was taken by site to present the rawest form of alpha diversity. No scaling was applied.

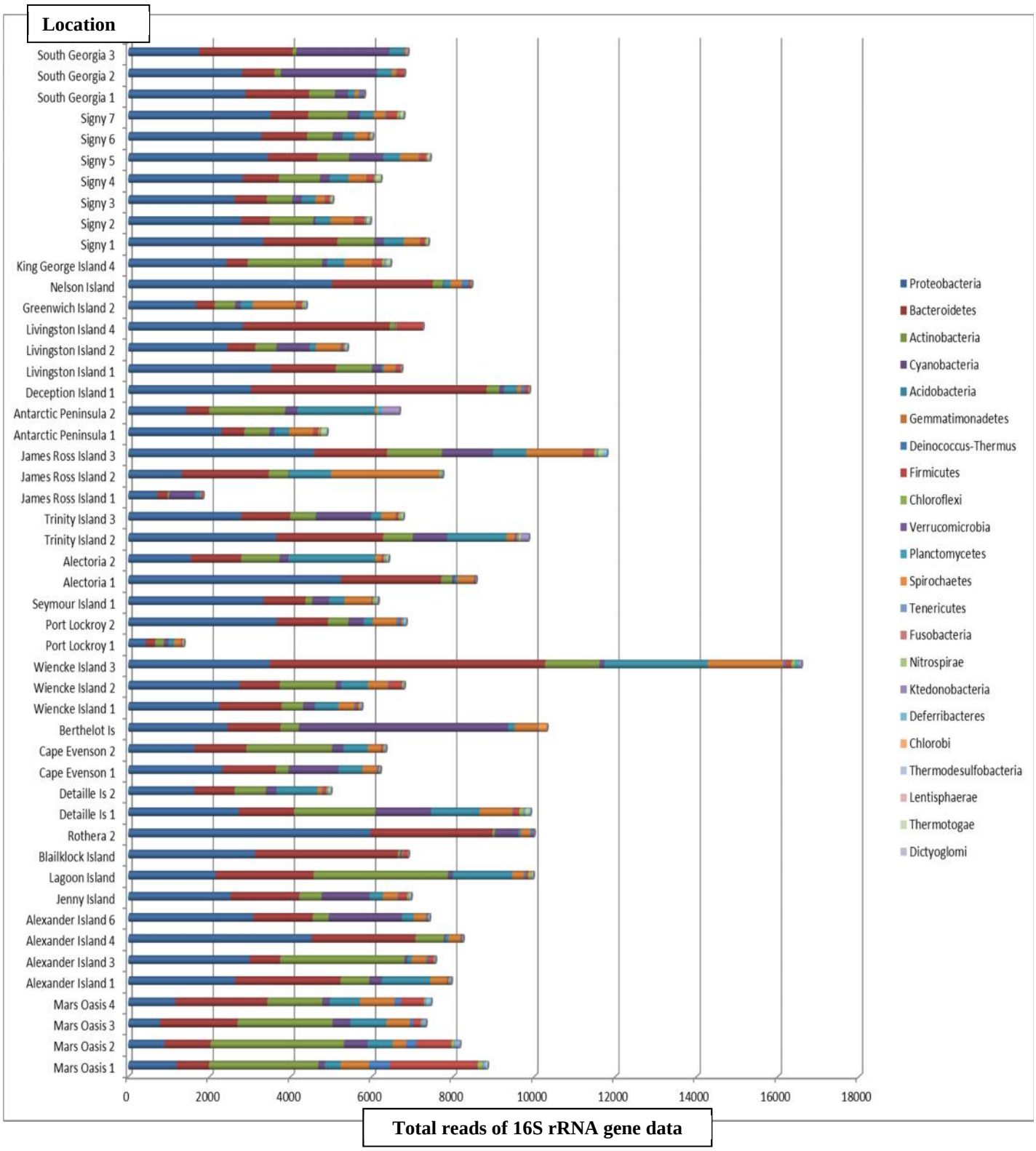

Figure 3.13. Relative sequence abundance of the 16S rRNA bacterial 454 data was collated in Microsoft excel with the bar chart function and sorted by latitude, north to south. Individual sites are presented and no average was taken by site to present the rawest form of alpha diversity. No scaling was applied.

3.5. Alpha Diversity

Alpha diversity is considered as the local species diversity at a subunit level (Whittaker, 1972), or at one site in a collection of comparable locations. It has also been classed as the mean species diversity across a number of subunits (Tuomisto, 2011) and represents the diversity *within* a site specific community (Vane-Wright, 1991). The Simpson index (*D*) equation (Box 3.1), provides a means of presenting the alpha diversity as a function of species concentration and is particularly suited when there is a known range of possible species but data are sensitive to a potentially small sample size, and there are recordable observations for each species per site, allowing a 0 return against a species range when not present at a given site (Jost, 2007). The Shannon diversity Index (H') is the most common representation of microbial diversity, and focuses on species richness (Box 3.1).

$$D = (1-(\text{SUM } (ni*(ni-1)) / (N*(N-1))))*100$$

$$H' = -\Sigma i \text{ pi ln(pi)}, (i = 1,2,3,...S), 0 \leq H' \leq \infty$$

Box 3.1. Diversity equations (Wilson, 1992)
Top: Simpson Diversity D: N = total number of individuals; ni = number of ith species at site. The Simpson index is actually a measurement of dominance and assesses the probability that two randomly selected individuals from a community will belong to the same species.
Bottom: Shannon Diversity H: where pi is the proportion of the total community abundance represented by the ith species and ln (pi) is the natural log of pi.

Simpson diversity was selected as the alpha diversity used in this project, to address any potential issues with sample sizes and a focus on species concentration, however for comparison the Shannon diversity index has also been provided. Diversity (D) is represented as the percentage likelihood that any two 'individuals' extracted from the same community sample would not be of the same species. The higher the value, the more diverse a community is considered (Jost, 2007).

Total microbial diversity using the ELFA community data and 454- bacterial taxonomic data were each used to calculate Simpson and Shannon index values. Prior to analyses, community datasets were standardized to account for bacterial library comparison and also to

amend the un-even numbers of samples between datasets. Figure 3.14 shows the Shannon and Simpson Diversity indices across the study sites. the higher the index number, the more diverse the site was estimated to be. Index calculations were implemented using PRIMER 4 & PERMANOVA software (Clarke & Gorley, 2006), and are provided in Appendix 2 as data tables for reference. Both indices are mapped against latitude, to allow comparison of trend across the latitudinal gradient, although it should be noted the values of each index are independent from each other.

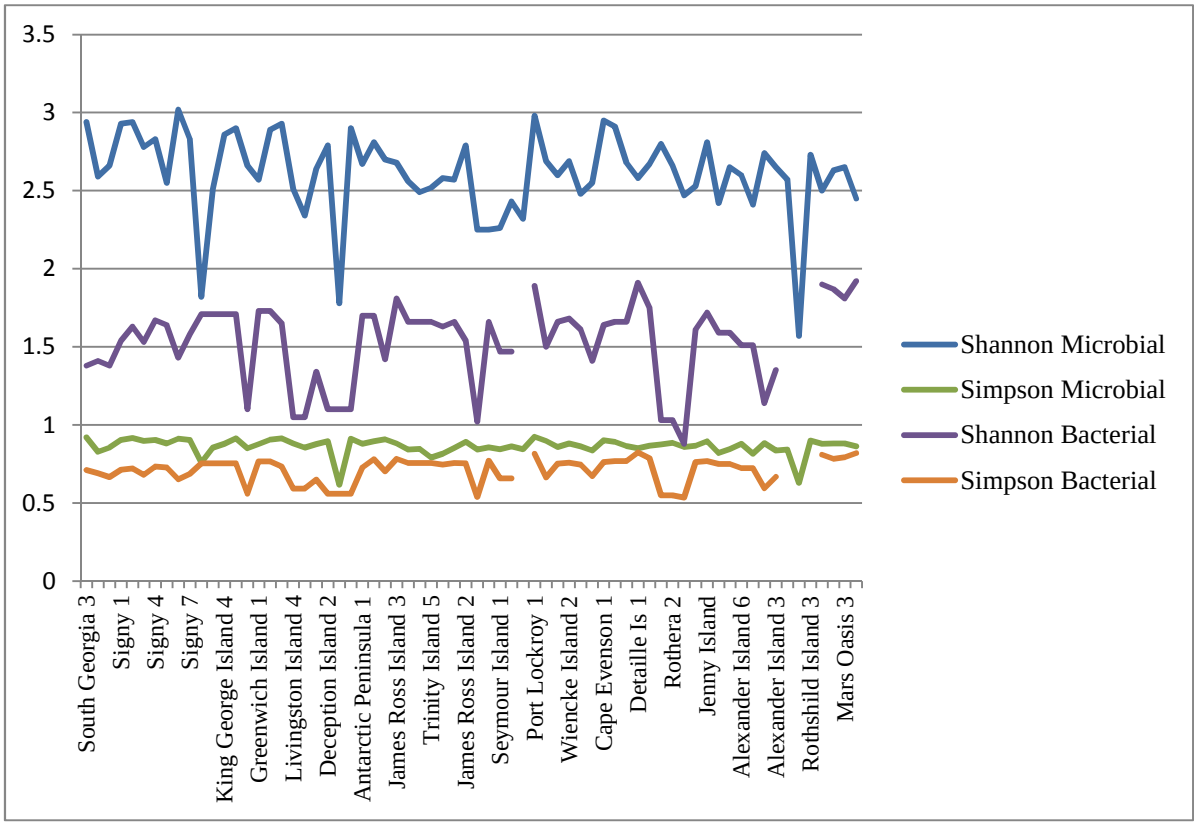

Figure 3.14. Shannon and Simpson indices plotted against sites across the latitudinal gradient, with increasing latitude from left to right. Gaps in bacterial data are due to a fewer number of sites sequenced (see Section 2.1.3). Simpsons index has been scaled by division by 100 so that the two indices can be presented simultaneously. Appendix 2 shows the corresponding data tables.

According to Shannon's diversity index, diversity generally declined with latitude, but there was much fluctuation across the range of locations, with peaks apparent around Signy Island

and Port Lockroy for all data. However, latitude was not found to have a strong correlation either the ELFA microbial data (R^2 = 0.34) or the 454-bacterial data (R^2 =0.27). Bacterial diversity was also high at the most southern site, Mars Oasis. The Simpson index shows no clear linear trend with latitude but flucuated more for bacterial data, whereas the ELFA microbial data appeared more linear. There was a noticable drop for both indices around Deception Island and again at Blaiklock Island.

Shannon's diversity index highlighted Port Lockroy (2.98) and Signy Island (3.02) as the most diverse for the microbial community data and Mars Oasis (1.92) and Detaille Island (1.91) as the most diverse for the bacterial community data, this makes *H′* for Antarctic soils comparable to values for temperate soils, which range between 2.3 and 3.7 (Fierer and Jackson, 2005). Rothschild Island and Blaiklock Island were least diverse for microbial (1.57) and bacterial data respectively (0.88).

Simpson's Index identified Port Lockroy and South Georgia to be the most diverse locations (92.5% and 91.95% respectively) for the microbial data and Detaille Island (82.41%) and Mars Oasis (82.02%) were the most diverse for the bacterial data. The least diverse were Deception Island (61.76%) and Rothschild Island (62.98%) for the microbial community data and Blaiklock Island (53.4%) for the bacterial data.

3.6. Discussion

3.6.1. General Conclusions

Measuring external influences and their subsequent addition or change to ecosystems is not straightforward (Convey, 1996) and often relies on a secondary source to act as a bio-indicator (Kimberling *et al.,* 2001). For example, Smith (1994) identified Antarctic plants as bioindicators of regional warming, as the expansion of plant ranges were strongly correlated to increasing temperatures. Being able to monitor effects through such a medium requires a timescale which would not be attainable in studies where sampling is delivered from a single opportunity. Examination of our observed site influence parameters led to the conclusion that they are of limited value when examined in isolation. All sites will likely have some exposure

to all the influences measured, so analyses must be focused around when the influence is significantly larger than in other areas. Thus, the data obtained, alone, offer limited insight into diversity gradients.

Most of our analyses used three replicates and then were pooled if multiple soils were sampled from the same area. Soils are known to be chemically distinct in different areas (Convey, 2010) and the PCA was successful in separating locations based upon the properties measured. We observed examples of similar gradients in several chemical properties, the most notable being a gradient in total carbon, total nitrogen and dissolved organic carbon, with an inverse gradient in ph (Figure 3.4. to Figure 3.11). High quantities of these substrates have been linked to avi-faunal presence and seal colonies, which are influential on Detaille Island, Cape Evenson, Port Lockroy and Gand Island. Most sites from these locations were found to be diverse according to Shannon and Simpson indices. These analyses are consistent with previous studies which have identified a general trend of decreasing total C and total N for sites moving inland or with decreased bird and seal abundance, and an inverse trend with soil pH along the same gradients (Barratt *et al.*, 2006). Barratt et al. (2006) observed that, whilst diversity remained consistent with soil chemical gradients, community composition changed, proposing that broad-scale trends in soil chemistry are important factors influencing community profile.

This is supported in our data in that no clear trend of diversity with latitude was apparent with either of the indices used, although there was confluence between 'diverse' and 'low-diversity' locations between Simpson and Shannon indices (Figure 3.14). Diversity indices should be independent of one another (Jost, 2007), so with two types of community data producing similar high diversity index trends; this gives a level of confidence in the diversity trends that are being displayed across the latitudinal gradient. Our analyses show that the bacterial diversity, in terms of phylum numbers, is high, with the top five phylotypes being Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria and Acidobacteria (Figure 3.14.). It has been recognised in similar analyses (Pointing *et al.*, 2010), that this can be misleading due to the abundance in soils of the closely related acidobacterial and actinobacterial phylotypes as found here. This is evident when the lower Simpson diversity indices are viewed for the community data (Figure 3.14). The high relative abundance of the phyla Actinobacteria, Bacteroidetes, and Cyanobacteria is supportive of previous studies of bacterial communities in desert soils, both hot and cold (Lee *et al.*, 2012*)*, and supports a

hypothesis that, given the environmental dissimilarities in temperature ranges and plant abundance, factors such as pH and moisture content may be more significant in structuring microbial communities (Fierer, 2012).

3.6.2. Specific Observations

The aim of this chapter was to identify trends that may lead to model assumptions or potential hypothesis as to the drivers in total diversity. However, several points of interest were raised which may fall outside of the scope for analysis, but are interesting to note. Pie charts were created using 454-bacterial species data, to compare most abundant species for each site (See Appendix 2 for most common bacterial species at each sampling location).

Figure 3.15 shows the top 10 species for the 'Antarctic Peninsula' (Site 1- 63.70°S- See Appendix 1 for detailed site information), representing 64% of the sequences at this site, and therefore can be considered a representative scale. *Dechloromonas* sp. *PC1*, a member of the phylum Proteobacteria and dominant in the phyla diversity returns, shows clear dominance in the community profile, whilst 'Rocky' shows clear dominance in the percentage site influence profiles (Figure 3.1 and Figure 3.2).

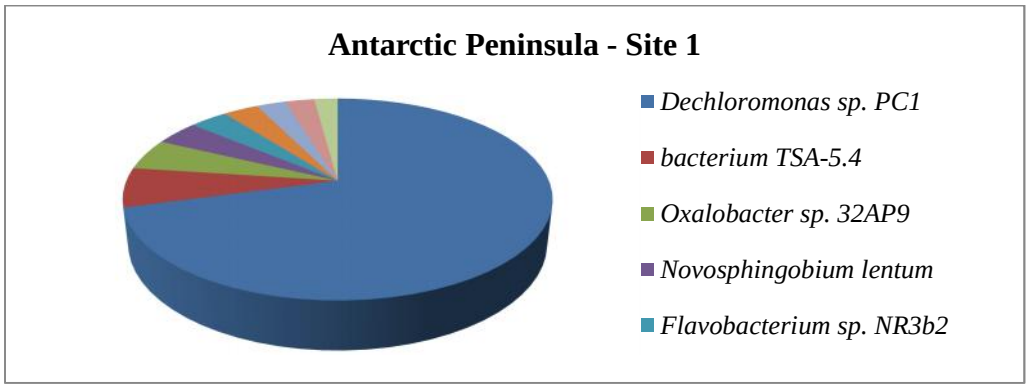

Figure 3.15. Antarctic Peninsula 1 Species Diversity site 454 data were collated in Microsoft excel and sorted by species abundance (high to low), the top 10 species (or nearest significant value) were isolated, and presented in an excel pie chart.

The dominance of 'Rocky' as a characteristic, is repeated in the Mars Oasis sample, as is the abundance of *Dechloromonas sp. PC1* in Mars Oasis sites 1, 2 and 4. Site 4 is shown in Figure 3.16. where *Dechloromonas sp. PC1* represents 74% of the total sequence abundance. *Dechloromonas sp. PC1* is a perchlorate reducing bacterium that is also capable of nitrate reduction. Perchlorate salts have been used for over 50 years in many man-made flammable substances, such as rocket fuel, and have become a contamination concern in the United States (Nozawa-Inoue *et al.*, 2005). Although it appears here that the rocky presence at both sites may be coincidental, the conspicuous presence of *Dechloromonas* at these sites- and many others (See Appendix 2), may actually suggest a link to human contamination,

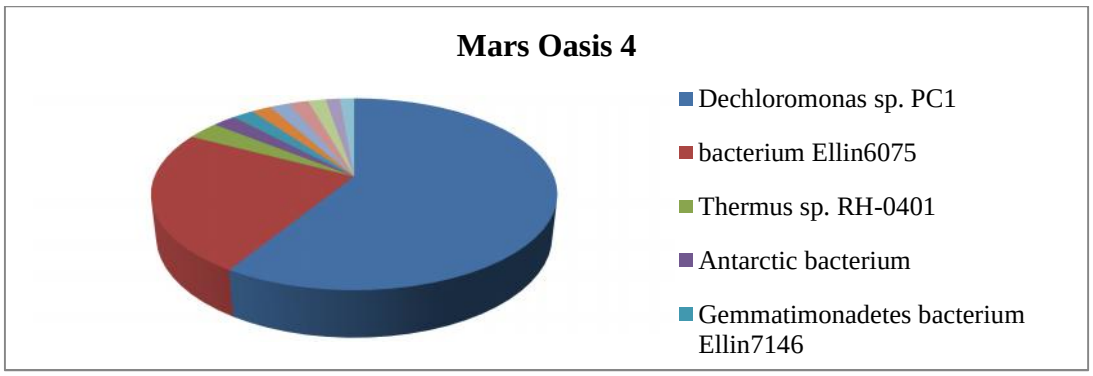

Figure 3.16. Mars Oasis 4 Species Diversity site 454 data were collated in Microsoft excel and sorted by species abundance (high to low), the top 10 species (or nearest significant value) were isolated, and presented in an excel pie chart.

Reviewing the core data underpinning this project has provided initial insight into the potential areas of interest for this study, and contributes to forming the hypothesis to be tested in later chapters. First, a simple linear relationship between latitude and diversity was not observed, similar studies have had positive results for bacteria (Yergeau *et al.*, 2007b but not for fungi along the Peninsula (Dennis *et al.*, 2012). This does not exclude the existence of a biodiversity gradient but requires a different approach to investigate this, non-linear latitudinal gradients of diversity have been observed previously (Stevens *et al.*, 2003) Consistencies of high diversity and biomass were observed in areas where seal and avian influence are high, which has been attributed to higher substrate levels where these levels would otherwise be limited (Cowan *et al.*, 2002; Aislabie *et al.*, 2009). The Principal coordinates analyses of chemical data revealed a gradient of sites where consistent patterns in

soil substrates were observed, such as increased nitrogen and carbon in the same areas. The relationship between the direct effect of ornithgenic inputs on soil chemistry, therefore must also be examined in following analyses.

3.7. References

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4

Environmental Heterogeneity Accounts for Microbial Biogeography in Antarctic Soils*

Abstract

Macro-organism distribution is represented in ecology by predictable paradigms, an example of which is the decline of taxonomic diversity with increasing latitude. Recent studies of the Antarctic Peninsula have addressed this pattern and some have suggested it also applies to soil microorganisms, largely due to the relationship between increasing latitude and decreasing temperature and the consequential effects on soil substrate availability. In addition, reviews of Antarctic microalgae, terrestrial metazoan and microorganism distribution provide proxy biological evidence to indicate that an area of cross-continental regionalization exists, which does not intuitively represent selective adaptation to environmental constraints, but rather evolutionary genesis by continental accretion. In this study, microbial community profile data were analyzed from soils retrieved over 53-72◦S latitude (2000km gradient) in the most comprehensive terrestrial survey of the Antarctic Peninsula yet attempted. We applied multivariate modelling techniques to examine the structural and spatial heterogeneity of microbial communities. Concurrent with other recent studies across the continent we find evidence of microbial regionalisation but a lack of evidence to suggest either a link to historic provincialism or a direct effect of latitude. Instead, disparities in community composition are accounted for by a gradient of increasing environmental heterogeneity with increasing latitude, characterized by ecologically distinct regions of the Antarctic Peninsula.

Keywords: Microbial community; Biogeography; Latitudinal gradient

*complementary analyses for this Chapter are presented in Appendix A4

4.1. Introduction

The universal distribution of soil microorganisms has until recently been shrouded by the 'black box' of the terrestrial environment (Tiedje *et al.*, 1999). Now that advanced community profiling techniques have provided the perfect platform, the direct influence that microorganisms and their distribution have on ecosystem processes can be better estimated. Antarctica is a 'pristine' field laboratory like no other and, with soil of basal trophic structuring, provides the perfect canvas to examine the relationship between taxa and their distribution throughout the environment. Antarctic soil conditions are characterized by low moisture, low temperature and nutrient cycles being heavily reliant on soil microbes (Hopkins *et al.*, 2006). But, and of further universal importance, the Antarctic Peninsula is also the fastest warming region in the southern hemisphere (Turner *et al.*, 2013; Vaughan, 2007; Pritchard and Vaughan 2007; Vaughan *et al.*, 2003). There is intensified interest in climate change scenarios, with particular regard to polar regions, where the effect is likely to be more dramatic. Along much of the Antarctic Peninsula, daily soil temperature fluctuations about 0°C are common. This is significant as 0°C is thought to loosely define the threshold beyond which soil microbes are metabolically active. With a temperature increase of 3.7 ± 1.6◦C in the last century, several times the average rate of global warming (Vaughan *et al.*, 2003), profound changes in future microbial community composition, nutrient cycling and energy flow are likely to occur. However, the impact climate change may have on Antarctic microbiology cannot be predicted without first unraveling the factors underlying terrestrial diversity and community composition and their underpinning mechanisms (Wynn-Williams, 1996; Vincent and Pienitz, 1996).

Microorganisms are thought to account for the majority of the world's biodiversity and yet little is known about trends in their distribution at local and global scales (Green and Bohannan, 2006). In macro-ecology, perhaps the most widely recognized but least understood spatial pattern is the latitudinal gradient in taxonomic diversity. Diversity is thought to increase towards the equator and decrease towards the poles, although the gradient has not always been found to be linear (Kaufman, 1998). There are currently more than 30 hypotheses proposing explanations for the latitudinal gradient, but despite this, none are regarded as a singularly plausible mechanism (Willig *et al.*, 2003; Rohde, 1992). Several hypotheses relate directly to geographical characteristics of the tropics such as the Mid-domain effect, where species ranges are thought to overlap more towards the centre of their

domain than the edges, forcing a peak in species richness around the equator (Colwell and Lees, 2000). Also, the species-energy hypothesis suggests that increased solar energy and water availability leads to increased primary production, where more species can be energetically supported in an area (Currie, 1991). Other hypotheses are concerned with historical perturbation (Brown and Lomolino 1998), evolutionary rates (Cardillo *et al.,* 2005) and biotic population interactions (Pianka 1966).

The idea of a structured diversity gradient in microbial systems may also seem unfeasible because of their cosmopolitan distribution and vast dispersal capability (Bardgett *et al.,* 2005), however, the past decade has generated contentious evidence that soil microbial communities display predictable spatial structures, many of which are analogous to patterns displayed by macroorganisms (Green *et al.,* 2004; Vyverman *et al.,* 2010; Chong *et al.,* 2012). Studies from microscale to continental planes have found edaphic parameters, predominantly pH, to be the fundamental drivers of diversity in microbial soil communities (Lozupone and Knight, 2007; Fierer and Jackson, 2006) even when a latitudinal effect is observed (Staddon *et al.,* 1998). Recent studies present evidence of terrestrial biological regionalization across the Antarctic Peninsula in various metazoan, bacteria and algal taxa (Convey *et al.,* 2008, 2009;; McGaughran *et al.,* 2010; Maslen and Convey, 2006), though none of these studies examined spatial distributions in conjunction with environmental parameters.

Although space is considered to be the most influential dynamic on environmental heterogeneity and community structure (Legendre and Fortin, 1989; Tilman and Karieva, 1997; Jombart *et al.,* 2008), decomposing spatial trends can be a complex process. Spatial variation is multidimensional and can be attributed to evolutionary legacy or numerous environmental drivers acting simultaneously upon population dynamics. Microbial community structures are thought to be due to one of, or a combination of, spatial factors: geological heritage, where areas of relict populations have been isolated in tectonic congregation (Pugh and Convey, 2008; McGaughran *et al.,* 2010), induced spatial dependence, where species distributions are shaped by environmental gradients (Fierer and Jackson, 2006) and spatial autocorrelation, brought about by biotic assemblage interactions (Legendre and Legendre, 1998; Dray *et al.,* 2006). Identification of different spatial scales at which ecological trends are exhibited allows insight into the global and local processes inducing spatial dependence between community and environment (Jombart *et al.,* 2009).

O'Donnell *et al.* (2007) highlighted the limited ability of modelling approaches to address spatial heterogeneity in microbial ecology. The availability of high throughput technologies for detailing community profiles requires matching initiative in statistical analyses. Principal coordinates of neighbours matrices (PCNM) and Moran's eigenvectors maps (MEM's) (Borcard and Legendre, 2002) have been introduced as a family of spatial predictors with the capacity of identifying different spatial structures present in multivariate community data. Structures may be classified as either global or local by means of permutation testing. Global structures are thought to represent abiotic influences and gradient effects, and local structures contagious biotic processes, where interactions between neighbouring individuals demonstrate spatial autocorrelation. The advantage of identifying such structures in the soil microbial community is that it may be possible to recognize key ecological dynamics behind observed patterns in diversity and composition.

Beta diversity can be represented as species turnover (Whittaker, 1972), or the measurement of change in community structure from one sampling unit to another, along a spatial, temporal or environmental gradient (Anderson *et al.*, 2011). Species turnover can be estimated at multiple scales using PCNM (Peres-Neto, 2006) and is the fundamental approach applied for considering beta diversity in this chapter. Here a PCNM-based modeling strategy to investigate spatial patterns of microbial species turnover present in fatty acid community data (ELFA) is proposed. In Addition, an attempt is made to relate spatial structures to presence and magnitude of environmental drivers to better understand microbial diversity patterns, and the reasons behind these patterns, in terrestrial Antarctica.

4.2. Methods

4.2.1. Site locations and data acquisition

Sites locations, data acquisition and modelling methodologies are described in Chapter 2.

This section discusses species turnover, community composition and environmental variables, as summarised in Table 4.1.

Table 4.1. Key modelling terminology

Terminology	Description	Source
Species turnover	The changing profile of the ELFA fatty acid data over the latitudinal gradient	ELFA data, see Chapter 2.1.3 Table 2.4
Community composition	The changing profile of the grouped fatty acid data into indicative microbial communities	ELFA data, see Chapter 2.1.3 Table 2.5
Environmental variables	The environmental and chemical properties either observed or later provided from lab testing	Observation on site, and Lab provided data, see Chapter 2.1.2, and Table 2.2 / 2.3.

4.2.2. Statistical analysis

Following methodology developed by Stephane Dray *et al.* (2006), raw ELFA community data were incorporated into a 'relative neighbour' connection network - a straight line graph connecting nearest points in a point set, ensuring all sites had at least one neighbour (see Figure 4.1). This was preferable over a more distributed network, such as Delaunay triangulation (Delaunay, 1934), for several reasons. First, unlike macro-organisms we assume movement of microorganisms to be dynamic, although largely limited to aerial dispersal and due to the nature of this, to also favour local rather than regional migration. Second, due to the relatively small number of sites sampled over a large area, a more intricately connected network increases the chance of masking large scale trends, which was our primary interest. The relative neighbour network was created as a function using site latitudes and longitudes, in the 'spacemakeR' package in R (Anon, 2011)

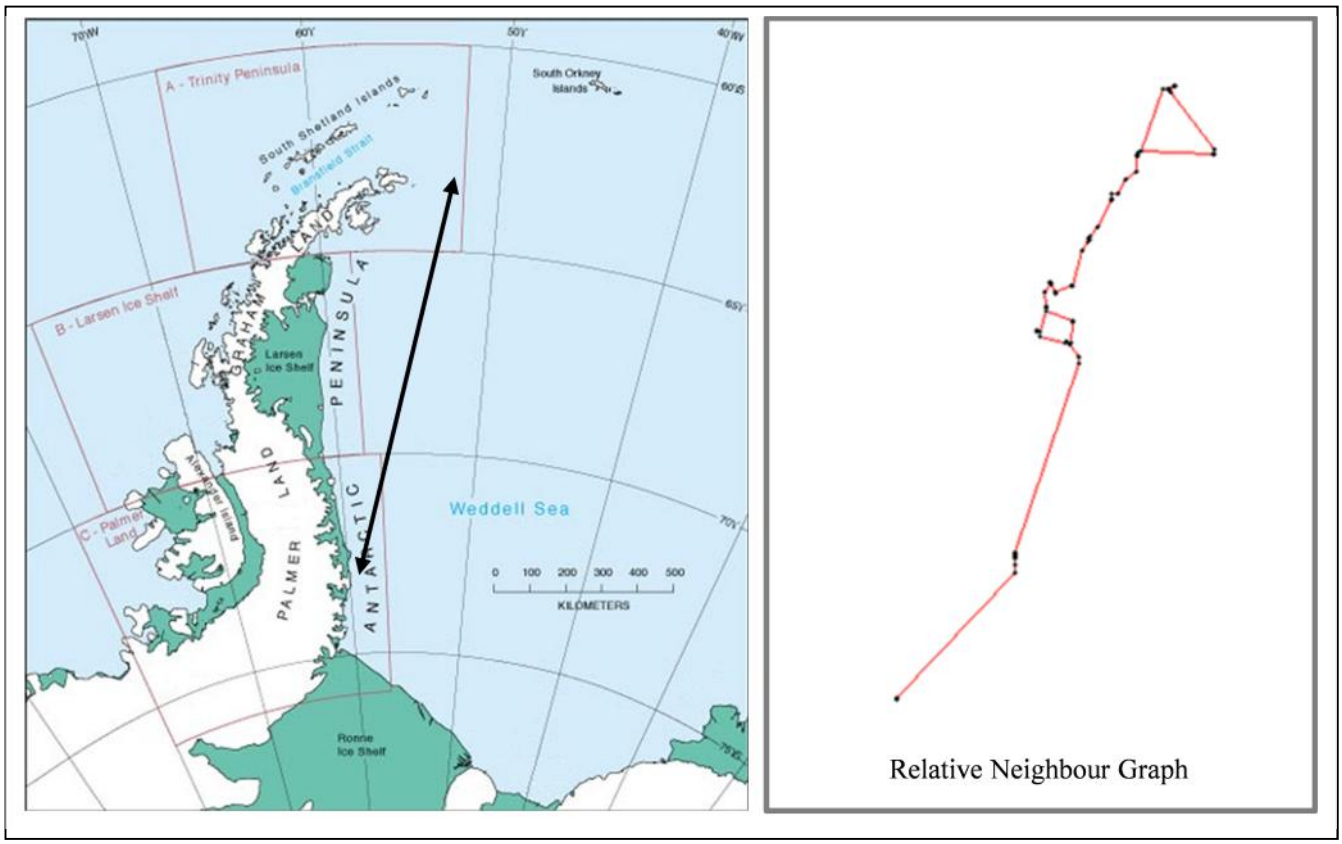

Figure 4.1. The Antarctic Peninsula (left): Latitudinal gradient over which sites were sampled (Ferringo *et al.*, 2005). (Right) The relative neighbour connection network chosen to represent potential site connectivity in the calculation of Moran's I

The relative neighbour graph was used to identify sites of geographic and ecological proximity. This is achieved by using the geographic connectivity between sites as a means of spatial weighting in the calculation of Moran's *I* (Moran, 1950) for changes in fatty acid composition in the ELFA data. Moran's I values range from -1, indicating perfect dispersal to +1, indicating perfect correlation. A zero value is indicative of a random spatial pattern. These identified the level of spatial autocorrelation between site fatty acid compositions and thus the nature of the gradient in species turnover along the latitudinal transect. PCNM eigenfunctions were calculated for all our Peninsula locations in the 'spacemakeR' package in R (Anon, 2010). The Akaike Information Criterion (AIC) was used as a means of model

evaluation, also following recommendations of Burnham and Anderson (2002) to accept a threshold value of ≤ 2 for AIC_i - AIC_{min} to suggest substantial evidence for an adequate model fit. Monte Carlo permutation analysis was applied to test for the global and local significance of AIC identified spatial structures, using the R package 'sedarjombart' (Jombart *et al.*, 2010). We retained the best fitting spatial model (vector 1) as an indicator of major trend in species turnover to test for evidence of spatial structuring in the species turnover gradient.

The sites were partitioned and a dummy variable created according to the spatial trend exhibited in PCNM vector 1 map to further explore how ecological conditions may be driving spatial community structure. Multivariate dispersion as proposed by Anderson *et al.* (2006) is a technique which weights an order of magnitude change in abundance the same as a change in species composition. This was important for the ELFA data which does not define individual microbial species but rather abundance of fatty acids. First, Bray-Curtis was chosen as a measure of ecological dissimilarity, then Euclidean distance based upon the dissimilarity algorithm was preserved by use of principal coordinate analysis (PCO), so that distance of an individual unit to the group centroid could be calculated. A p-value was then obtained by permuting least square residuals. The availability of this multivariate test, termed PERMDISP, for homogeneity amongst group diversity also allows for the option to superimpose biological diversity with environmental heterogeneity and test robustly for differences in structure. PRIMER 6 and PERMANOVA + software (Clarke and Gorley, 2006) was used to assess group dispersal post north-south separation.

Prior to PERMDISP, we normalized the environmental variables to adjust for different measurement scales and used Euclidean distance as a measure between sites to form a resemblance matrix. PERMDISP was performed using distance to group centroids and P-values obtained via 9999 permutations as recommended by Anderson (2006). PCO was used for visual representation of group dispersal for environmental factors.

To identify more specific relationships between community composition and environmental drivers we also pooled fatty acid abundances according to their taxonomic representatives (e.g. 10Me 16:0 is indicative of Actinomycetes; see Chapter 2.1.3, Table 2.4). These pooled abundances were used as broad-scale indicators of key microbial community groups to partition more effectively the specific environmental influences on key group dispersal. These groupings were used as response variables in Generalized Linear Models (GLM) and

in PERMDISP to distinguish environmental drivers specific to each microbial fraction of the community.

Variable inflation factor (VIF) analysis was used in selection of appropriate environmental variables to minimise collinearity, performed in the 'car' package in R (Fox 2010) via the methodology of Zuur *et al.* (2007). All variables used in this analysis had a VIF value of < 4, beneath adequate threshold values recommended by Montgomery and Peck (1992). In the GLM, full models were initially fitted including all VIF selected variables and model fit inspected. Parameters, representative of environmental variables, were removed if doing so improved model fit until the minimum adequate model was found. In this way, the GLM models contained the strongest set of environmental predictors for each of the microbial responses variables.

4.3. Results

4.3.1. Species turnover gradient

Of the PCNM eigenfunctions calculated, 6 models were chosen under AIC selection as significant but the best fitting of these, Vector 1, we retained as the dominant spatial structure occurring in ELFA species turnover. The map of vector 1 (Figure 4.2.) indicates ecological disparity in community composition representing two, possibly three biologically distinct provinces: the identification of regional groupings are concurrent with concepts for bio-geographic zones (Convey, 2010), and identified within the MEM is a regional divide between sites characterised by warmer and wetter conditions in the northern Antarctic Peninsula, north of 66°S and east of 64°W, and sites along the west coast of the in the Southern Antarctic Peninsula, south of 66°S. A third possible divide is partially evident where the sites become more characteristic of continental Antarctica (Mars Oasis), however with such limited data points for the southern sites this is not clear in the representation. Figure 4.2. shows the major trends across vector 1 for the main grouping of sites, extreme north and south sites are not depicted as they have only one nearest neighbour on which to base a correlation.

A Monte Carlo permutation test (9999 permutations) for the presence of global and local structuring was performed on all retained PCNMs as a measure of the overall trend in the ELFA data and also on vector 1 alone as significance test of the dominant structure. Neither of these tests revealed statistically significant global or local structures present in the data. This being the case, there is still some observable evidence of structuring in vector 1, indicated by clear delineations in species turnover. It may simply be that for this to become statistically significant more locations would need to be sampled in order to increase the robustness of the connectivity network.

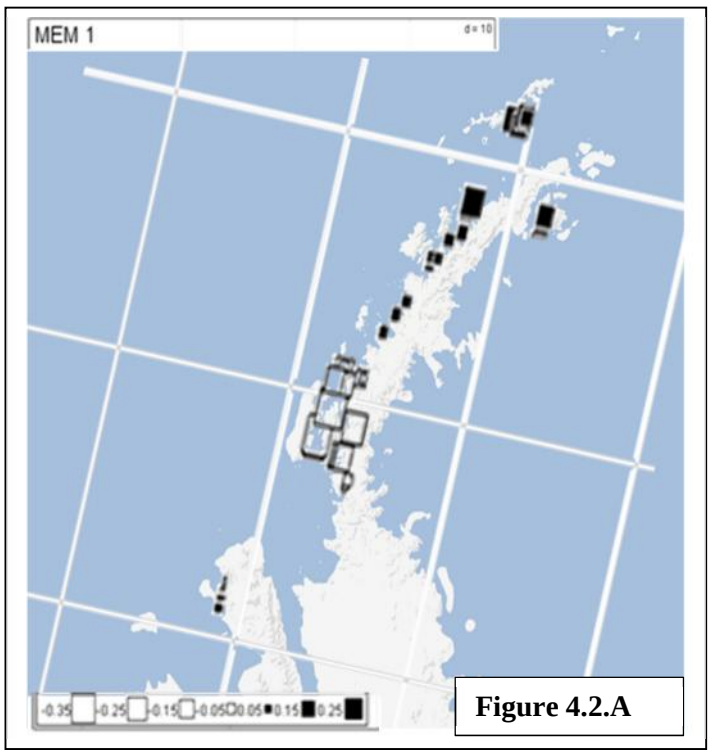

Figure 4.2.A

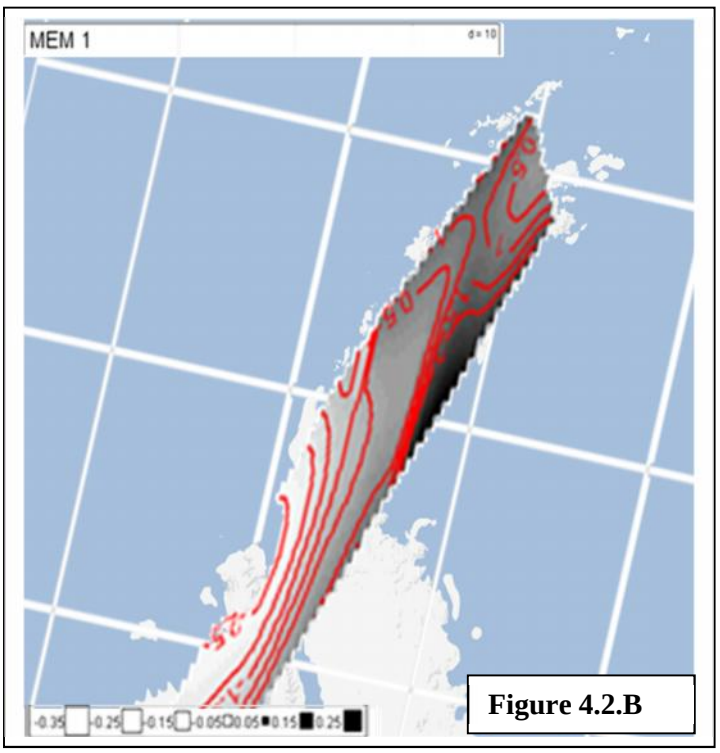

Figure 4.2.B

Figure 4.2.A An S-graph representation, where change in population similarity is indicated by a change in colour of squares from white to black. The bigger the square the more 'different' a site is from other colours (i.e. ecologically more diverse) and the more weight that site gives to the overall trend. **Figure 4.2.B** MEM Contour map indicating changes in beta diversity (Species Turnover), proximity of contour lines indicates the strength of the gradient of ecological change. Both figures are the MEM 1 output as a representative overlay onto a map of the Antarctic Peninsula (Google, 2013), which is provided to give regional context only. Extreme north and south sites were excluded from the plot due to their having only one 'proximity' neighbour.

4.3.2. Multivariate dispersion

The PERMDISP function tests for homogeneity of multivariate dispersion (similarity in variance between sites) and was used to test across the identified regions for the level of ecological heterogeneity in species turnover, community composition and environmental variability The *p*-values (Table 4.2.) did not support the existence of heterogeneity in species turnover with latitude, although the results indicated there may be an increasing level of heterogeneity in community composition with decreasing latitude. A transect spanning a greater distance may have permitted more robust statistical analysis to substantiate this.

Multivariate heterogeneity in community composition (p=0.0338) was observed between the north and south Antarctic Peninsula. This variance is based upon differences in key microbial groupings (the higher level indicative nature of fatty acids) rather than species turnover (the changing profile of the individual fatty acids themselves), The results of the environmental PERMDISP suggested a significant dispersion between regions overall (f=25.969, p=0.0023) and pair-wise comparisons (Table 4.2.) show evidence of strong environmental heterogeneity between the south Peninsula and north Peninsula groups and also increasing heterogeneity with decreasing latitude.

Table 4.2. PERMDISP P - values for environmental PERMDISP comparison by region pairs.

	Species turnover	**Community composition**	**Environmental variables**
Region	*P*(perm)	*P*(perm)	*P*(perm)
South Peninsula to 'Continental'	0.1149	0.0741	0.5716
North Peninsula to 'Continental'	0.0738	0.1666	**0.0048****
North to South Peninsula	0.0788	**0.0338***	**0.0001*****

Notes

Species turnover analysed based upon ELFA profile data of raw matrix form, Community composition by ELFA indicator groupings and Environmental variables were all environmental variables as selected by VIF. Significant results are shown in bold, according to level of confidence: '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$, '.' $p < 0.1$, ' ' $p < 1$.

4.3.3. Environmental heterogeneity

To visualize the scope of environmental dispersion across regions and between sites within those regions, we used a Principal Coordinates Analysis (PCO) measuring site environmental variable dispersion to the regional modelled centroids that described the mean environmental dispersion by region (Figure 4.3). 36.1% of environmental variation was captured by the first two axes. This is a low figure, suggesting a more complex suite of environmental parameters is required than may be portrayed on two axes. Group numbers were assigned to sites, based upon the trend observed in the PCNM analysis (Figure 4.2.) with groups 1 to 3 representing continental, south and north peninsula regions respectively. The PCO revealed group 3, the most northern group, displayed the greatest dispersion amongst sites, and therefore was most heterogeneous group in terms of microbial soil community profiles. Group 1, the most southern group, displayed the least dispersion amongst sites, indicating a relationship of heterogeneity and change in latitude. Environmental variables were overlaid onto the ordination with Pearson correlation used to indicate the strength of relationship the environmental variables have with the axes. The PCO indicated soil conditions or availability of key nutrients appear to be governing this trend; soil moisture, carbon (dissolved organic carbon and phosphorus) driving the main source of variation (PCO1) and nitrogen inputs (nitrate, nitrite) and birds driving axes 2.

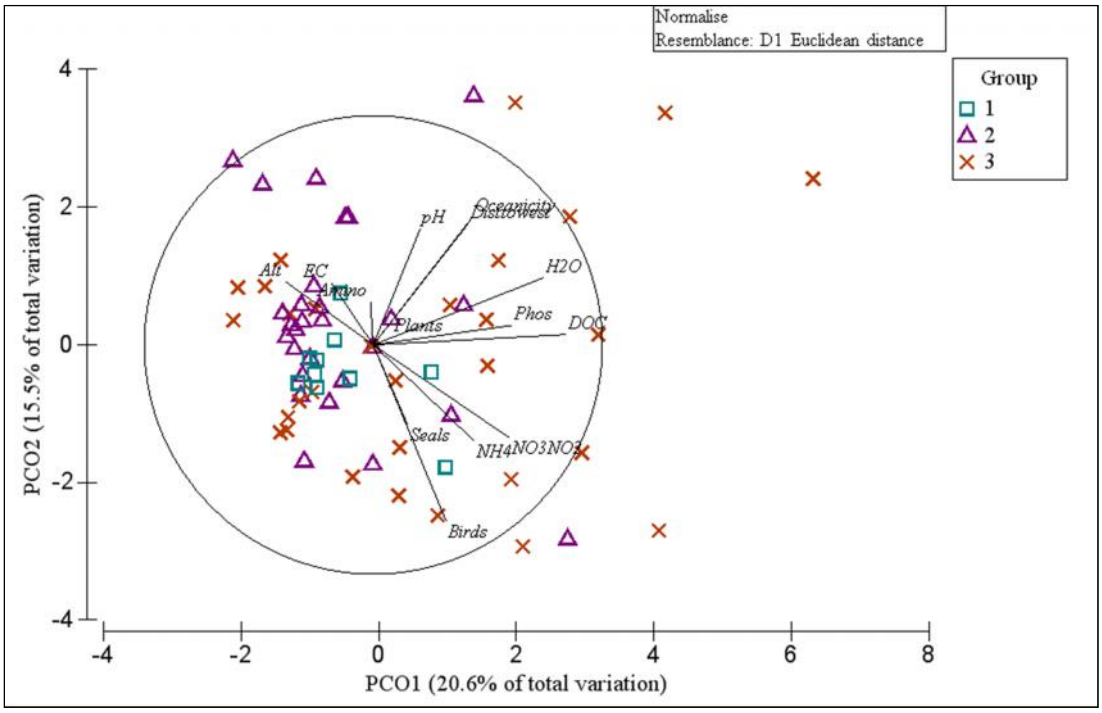

Figure 4.3. Principal coordinates analysis of group dispersal based upon environmental variables as partitioned by PCNM. Group 1 corresponds to continental region, group 2 the South Peninsula region and group 3 to the North Peninsula region. Pearson correlations of environmental variables to axes are superimposed and displayed as grey lines with their respective labels.

General linear models, used to represent each separate community component (Table 4.3.) showed a dominance of nitrate and nitrite (NO_3^-/NO_2^-) over all microbial components. The environmental variable oceanicity (see Table 4.3.), a measure of how exposed each site was to coastal influences, was also closely related to ELFA community structure but not significantly to fungi. DOC was linked to Gram positive bacteria including Actinomycetes, which are key decomposers in alkaline soils. The fungal community was significantly correlated to soil amino acids. Distotwest, birds, seals and plants were not found to be related to any of the microbial community directly.

Parameter	Unit	Total Bacteria	Gram +ve Bacteria	Gram -ve Bacteria	Actinomycetes	Fungi
Altitude	(M)	-	-	**0.04** *	-	0.10.
EC	(µS)	-	-	-	-	0.13
PO_4^{3-}		-	-	-	-	-
pH		-	-	-	-	-
DOC	(mgC/Kg soil)		**0.01** *	-	**0** ***	-
NO-3NO-2	(mgN/Kg soil)	**0** ***	**0** ***	**0** ***	**0** ***	-
H2O	(%WHC)	**0** ***	-	0.06	-	0.07.
C:N		-	-	-	-	-
Amino	(pmol AAg-1 soil)	-	-	0.1	-	**0.01** *
NH_4	(mgN/Kg soil)	-	0.09.	-	**0.01** **	-
Birds		-	-		-	-
Seals		-		-	-	-
Plants		-	-	-	-	-
Dist to West Coast	(km)	-	-	-	-	-
Oceanicity		**0.01** **	**0.04** *	**0.03** *	**0.01** **	0.06

Table 4.3. P-values for GLM best fitting models where microbial components were predicted by all environmental parameters. AIC values were used to select for best fitting models. Significant results are shown in bold, according to level of confidence: . '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$, '.' $p < 0.1$, ' ' $p < 1$.

4.4. Discussion

The PCNM analysis provides visual evidence supporting the existence of spatial structure (Figure 4.2), with a boundary between ecologically distinct north and south Peninsula soil microbial communities around 66°S. This is close to the Antarctic circle and is a divide consistent with previous studies (Lewis Smith, 1984). Around this latitude, there is a reduction in ice-free areas, the number of islands and also in flora (Peat *et al.*, 2007) Although the spatial structure permutation test on this boundary was non-significant, this was contrasted by the significant results from multivariate dispersion tests, where a difference in

dispersion was observed between north and south Peninsula sites, when sites were partitioned according to the spatial trend (see Table 4.2.).

Multivariate dispersion is used to test for homogeneity, or heterogeneity, between areas or groups of samples, (Anderson, 2006) and has been used to describe beta-diversity for a range of organisms (Soininen *et al.,* 2007). Here, there was no significant trend in microbial beta-diversity along the gradient of peninsula, but there were marked difference between north and south Peninsula sites for both community composition and environmental variables. This suggests the lack of a latitudinal effect, but supports that change in microbial community may be related to changing environmental conditions, or environmental heterogenity. Recent studies on soil organisms distribution in Antarctica, have inferred that examples of biological heritage may be indication of relict lineage, the enduring or native existence at a particular site (Pugh and Convey, 2008; McGaughran *et al.,* 2010; Vyverman *et al.,* 2010). Results here suggest environmental heterogeneity may also be related to disparities in microbial distribution across the Peninsula region,

The Principal Coordinates Analysis (Figure 4.3) which was used to visualise the dispersion of the environmental variables, indicated that DOC, nitrate, ammonium and soil moisture were likely to be the main influences over environmental heterogeneity. Large amounts of carbon and nitrogen are contributed to Antarctic soil via guano inputs from avi fauna and seals, however, in other areas these substrates are limited (Smith, 2005; Roberts *et al.,* 2009; Aislabie *et al.,* 2009). General linear models (Table 4.3.) confirmed these variables as significant predictors of fatty acids related to bacteria and actinomycetes, but not fungi. Similarly, the ratio of total organic carbon to nitrogen was not significantly related to fungi, this is contrary to findings from other studies (Dennis *et al.,* 2012; Yergeau *et al.,* 2007b) but it should be noted GLM only accounts for linear and not unimodal relationships between variables.

Unlike similar studies of soil transects, pH did not play a major role governing microbial diversity in our dataset. The most convincing evidence currently presented (Baker *et al.,* 2009; Fierer and Jackson, 2006; Lauber *et al.,* 2009; Chong *et al.,* 2012), found pH to be the dominant driver of diversity from local to global scales ($1m^2$ to continental transects), but unlike Antarctic soil, none of the environments sampled were thought to be limited by key soil nutrients like carbon or nitrogen. In Antarctic soils, variation in pH depends mostly on

colonization status of penguins - pH ranges vary greatly from 5.6-8.0 in currently and previously colonized sites (Jones *et al.*, 2004; Aislabie *et al.*, 2009), while fellfield soils which have no history or proximity to ornithogenic soils tend to be pH neutral (Yergeau *et al.*, 2007a). In soils from this transect, pH ranged from 4.44 to 7.98, though we found no evidence to suggest pH was correlated to ornithogenic presence (R^2= 0.02, P=<0.01).

The ratio of carbon to nitrogen was found to have little influence on all ELFA community components (Table 4.3.), consistent with the findings of Yergeau *et al.* (2009). Cleveland and Liptzin (2007) recently suggested a ratio to broadly represent 'normal soil' of 60:7:1 for C:N:P., but Dennis *et al.*, (2012) found Antarctic soils have a lower ratio of organic carbon to nitrogen (mean=6.3:1). Unusual stoichiometry of these key soil nutrients is characteristic of Antarctic soils (Barrett *et al.*, 2007). Commonly, Antarctic soil is low in C and high in N and P, particularly in proximity to ornithogenic guano deposits. Although guano can be a rich source of nutrients, it also contains high levels of ammonium-N. Aislabie *et al.* (2009) suggested that levels akin to those found at Cape Bird, 734 (mg kg^{-1}), could have inhibitory effects on microbial communities. *Nitrobacter* species, thought to be the key nitrite oxidising bacterial genus in Antarctic soils (Mulvaney, 1994)., are particularly sensitive to ammonia toxicity (Anthonisen *et al.*, 1976), so accumulation of nitrite is likely in ornithogenic soils Indeed, high levels of NO_{-3}/NO_{-2} were detected in ornithogenic sites, the highest concentration found at a Port Lockroy (307mg kg^{-1}), being similar to that found at penguin rookeries of Cape Hallet (Aislabie *et al.*, 2008). There was no evidence to infer toxic effects from the high NO_3^-/NO_2^-. GLM analysis showed a positive relationship with all bacterial FAs but not those of fungal origin. Fungal distribution could be most strongly correlated with amino acid content, turnover of which is extremely rapid in Antarctic soils (Jones *et al.*, 2004), whereas Gram positive bacteria generally, and Actinomycetes particularly, were linked to DOC. In other high latitude systems, niche partitioning of carbon sources has been observed between fungal and bacterial soil populations. Ley and Schmidt (2002) observed partitioning of phenolic compounds and amino acids between bacteria and fungi which also varied seasonally. Additionally it is thought that fungi outcompete bacteria at low temperatures (Selbmann *et al.* 2005).

The oceanicity variable was used to represent the differences in climatic exposure of sites located on an island compared to those of a more inland/continental location. Islands in the main may be subject to more severe climatic fluctuations, including increased salinity from

sea spray, flooding and nasal and guano ornithogenic additions (Aislabie *et al.,* 2009), fluctuating soil hydrology due to increased precipitation and soil evaporation rates (Convey, 2006) and more frequent freeze-thaw cycles due to air pressure and temperature instability. These factors combined represent a gradient in environmental stress and GLM results indicate (Table 4.3.) that bacteria are more vulnerable to these stressors than fungi as can be seen by significant relationships with the oceanicity variable. Many of the fungal species found in Antarctica are cosmopolitan species which have metabolically adapted to cope with the environment. Some species have melanin-rich hyphae or cell walls, such as 'black yeast' to protect against high UV radiation (Robinson, 2001) or increased levels of the cryoprotectant disaccharide trehalose such as with *Mortierella elongata,* a psychrotrophic fungus, which stabilizes membranes during dehydration (Weinstein *et al.,* 2000). Fungal diversity has been linked to water availability in Antarctic soils (Newsham *et al.,* 2009), however, because fungi are more functionally resilient than bacteria to low temperatures they are also less affected by the consequential variation in moisture availability (Pietikainen *et al.,* 2005). Bapiri *et al. (*2010) found that, whilst bacterial growth was inhibited by drying/rewetting events, fungal growth remained constant even at different bacterial:fungal biomass ratios.

The heterogeneity of the Antarctic environment- in this case a boundary between the north and south Peninsula, is not unexpected, spatial autocorrelation of edaphic variables is common in areas where nutrient additions are patchy. Increasing environmental heterogeneity exerts strong selection pressures on microbial communities and has been observed as the driving force behind bacterial diversity in other Antarctic habitats (Villaescusa *et al.,* 2010). Unlike many terrestrial habitats, the variation in the Antarctic environment can be at least partially predicted. Major nutrient pools are associated with ornithogenic inputs and soil hydric properties become more restricting but more stable with increasing distance south. Clarifying whether the environment and microbial communities vary at similar spatial scales could be vital in proving the origin of diversity gradients and hotspots observed in Antarctic biota.

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5

Decomposing Spatial Scales of Bacterial Ecology in Antarctic Soils*

Abstract

Bacteria are present in all environments on Earth but their distribution varies immensely across spatial and temporal scales. The spatial distribution of soil bacteria is thought to be at least partially driven by ecological processes, but at which scale the environment is most influential over these processes is unknown. In addition, the availability of high throughput genetic data to characterise bacterial communities allows the link between taxonomic rank and spatial distribution to be better understood. Spatial structuring at each level permits inferences about distribution mechanisms behind microbial dispersal. Antarctic soils host the most basic terrestrial food webs and, over a latitudinal gradient encompassing 19˚ latitude, essential relationships between bacterial communities and the influence of space and environment were addressed. Using Multi-scale Pattern Analysis (MSPA), spatial structures represented in both the environment and bacterial communities were analysed at each taxonomic rank. We found that, overall, bacterial communities shared only weak spatial structures with the environment at all taxonomic levels, but were more predictable by the environment as rank decreased. The spatial structure associated with the principal gradient in environmental dependence was related to the C:N ratio, and had the strongest influence on the spatial distribution of Proteobacteria (R^2=0.19, p=0.001), *Deinococcus* (R^2=0.18, p=0.001) and Planctomycetes (R^2=0.18, p =0.001).

Keywords: Antarctic bacteria; Biogeography; Spatial Scale; Multi-scale Pattern Analysis

*complementary analyses for this Chapter are presented in Appendix 4

5.1 Introduction

Bacteria are commonly described as 'ubiquitous' and 'cosmopolitan' but have also been observed to exhibit predictable spatial trends across the full spectrum of taxonomic resolution (Pointing *et al.,* 2010). In studies where sampling design is arbitrary and based upon logistic opportunity, as is common in Antarctic science, reliance on the marriage of community structures to the most relevant abiotic drivers may not be uniform at every taxonomic resolution. Structures observed at one taxonomic level may be an artefact of scale and not representative of true system dynamics (Weins, 1989). In soil communities, reoccurring spatial trends have been observed (Ettema & Wardle, 2002) and signify either spatial autocorrelation, brought about by biotic assemblage interactions (Legendre and Legendre, 1998; Dray *et al.,* 2006), the influence of contemporary environmental variation or geological heritage where populations have remained geographically isolated over long time periods *in situ* in areas of refuge (Hughes-Martiny *et al.,* 2006). Relict populations have recently been identified across many taxa in Antarctic soils and may be dated back as far as 68 million years with some chironomid species (Allegrucci *et al.,* 2006, 2012). The maintenance of these, which have survived through the Last Glacial Maximum and many preceding glacial cycles, have been largely attributed to an overestimation (Newman *et al.,* 2009) or high variability in ice cover (Convey *et al.,* 2008). How the environment influenced the survival of these communities can at best be only estimated, unlike the influence of contemporary dynamics shaping the future of Antarctic life.

What happens to future bacterial communities is of special interest because of the pivotal role they play in nutrient cycling in the terrestrial environment. It is globally estimated that microbial respiration of carbon from soil, accounts for CO_2 release an order of magnitude higher than anthropogenic emissions (Luo and Zhou, 2006). Some Antarctic microorganisms are subject to increasing environmental alleviation by a changing climate. The Antarctic Peninsula is warming at a rate of $3.7 \pm 1.6^{\circ}C$ $century^{-1}$, several times the mean rate of global warming (Vaughan *et al.,* 2003), and is expected to experience an increase in soil moisture and nutrient availability. In a field manipulation study by Dennis *et al*. (2013), the combination of warming and of carbon and nitrogen addition resulted in increased activity and biomass of microorganisms, particularly at sites where conditions were more severe. How microbial response to warming temperatures will impact on Antarctic ecosystems is

unknown, but there is a concern that the potential thawing of permafrost and higher metabolic activity will facilitate the release of stored carbon compounds and efflux of CO_2 into the atmosphere (Pearce, 2008).

The main environmental driver of bacterial diversity in soil is thought to be pH (Fierer and Jackson, 2006) though few studies have found pH to be influential in Antarctic soil (Yergeau *et al.,* 2007; Aislabie *et al.,* 2008; Aislabie *et al.,* 2009; Chong *et al.,* 2009; Newsham *et al.,* 2009). It is likely that the severity of the environment, compared to that of more temperate soils, brings about more basic constraints on diversity from low soil moisture, C and N content and tolerance to freeze-thaw cycle stress (Yergeau *et al.,* 2008). However, the Antarctic Peninsula is a region of high heterogeneity in which isolated areas of oligotrophy may be in reasonable proximity to penguin colonies, where soil nutrient composition is rich (Aislabie *et al.,* 2009). Areas of nutrient abundance are largely associated with the western maritime Peninsula, where sites are exposed to less severe climatic fluctuation, making grounds more hospitable to pinniped and avian colonisation.

Spatial trends exhibited by Antarctic microorganisms have not been explored in great detail as studies have in the main been restricted to specific areas of logistic accessibility (Chown and Convey, 2007). Yergeau *et al. (*2007a) found increasing latitude correlated with decreasing bacterial diversity in some soils, but in a study encompassing only a few locations, some of which were outside the maritime Antarctic area. Otherwise, to our knowledge, there are few studies currently which have addressed bacterial biogeography over a large scale in Antarctica (Chong *et al.*, 2013). Here, we have applied Multiscale Pattern Analysis (MSPA) and canonical MSPA to address bacterial biogeography across 49 sites on the Antarctic Peninsula. MSPA is an eigenanalysis technique which identifies the variation in species/environment at multiple spatial scales. The canonical form determines where variation in community is related to that of the environment at multiple scales. That is, a large scale gradient effect - such as a latitudinal relationship with diversity - will be identified simultaneously as a localised biotic effect, such as a hotspot for soil amino acids. This should indicate the major scales at which bacterial communities interact with environmental parameters. Identification of different spatial scales at which ecological trends are exhibited, allows insight into the global and local processes inducing spatial dependence between community and environment (Jombart *et al.,* 2009).

At lower taxonomic rank, such as species level, we would expect to observe a dominance of fine-scale structures, where spatial autocorrelation comes about through utilization of microhabitat resources and functional redundancy promotes weakness in spatial structures. The presence of a global pattern at high resolution may be indicative of historical refugia. At high taxonomic rank we might expect a gradient effect by large or medium structures, as the environment selects for groups of organisms who function according to specific environmental conditions. Understanding the nature of the link between scales at which the environment and bacteria interact will be embedded in the ability to predict patterns and processes across scale (Wheatley and Johnson, 2009), which could prove invaluable in a rapidly changing Antarctic climate.

5.2 Methods

5.2.1. Site locations and data acquisition

Sites locations, data acquisition and modelling methodologies are described in Chapter 2.

5.2.2. Statistical analysis

Variable inflation factor (VIF) analysis was used in the selection of appropriate environmental variables to minimise collinearity. VIF was performed in the 'car' package in R (Fox *et al.,* 2010) via the methodology of Zuur *et al*. (2006). All variables used in analysis had a VIF value of less than 4, indicating low collinearity and beneath adequate threshold values recommended by Montgomery and Peck (1992). Environmental variables included in analysis were soil chemical data: DOC, organic C:N ratio, ammonia, nitrate, soil at % of its water holding capacity, total P, pH, EC, amino acid content and presence of plants, seals and birds. Soil chemical data were scaled by logarithmic transformation prior to statistical analyses as recommended by Palmer (1993).

Following Jombart *et al.* (2009) we performed MSPA and canonical MSPA on community and environmental data in the software package R (R Development Core Team, 2008). Libraries 'ade4' (Chessel *et al.*, 2011), 'spdep' (Bivand, 2011), 'adegenet' (Jombart, 2011),

'spacemakeR' (anon, 2010) and 'sederJombart' (Jombart *et al.*, 2010) were used. Only the taxonomically classified sequence data was used for clarity amongst taxonomic groups.

The latitude and longitude of each site were used to create a network representing how sites interact. In this case, a relative neighbour connection network was chosen which assumed each location had at least one neighbour (see Section 4.2.2 and Figure 4.1). This was preferable over a more heavily connected network for several reasons. Data were sampled over a relatively large scale, with in some cases many km between sites. Therefore we had to assume any autocorrelation observed between communities at these sites reflects only large-scale processes, not biotic interactions between individuals. Furthermore, the mechanisms identified in community ecology which are responsible for species distributions such as distance-decay relationships, can only be revealed if sites furthest away from each other are identified in the model. If a more intricate connection network was used, where a higher degree of connectivity was assumed between sites, the significance of sampling along a latitudinal gradient may have been masked.

The relative neighbour graph was used to identify sites of geographic and ecological proximity. This is achieved by using the geographic connectivity between sites as a means of spatial weighting in the calculation of Moran's *I* (Moran, 1950) for changes in bacterial community data. Moran's I values range from -1, indicating perfect dispersal to +1, indicating perfect correlation. A zero value is indicative of a random spatial pattern. Moran's I values were then used to identify the level of spatial autocorrelation between site bacterial community composition and/or environmental characteristics along the latitudinal gradient of the Peninsula.

The following analytical procedure to perform the calculations for MSPA was used. First, an initial raw community matrix (X) was centred and scaled and qualitative variables replaced by dummy vectors to produce the community data matrix (Y). For canonical MSPA, the community data, Y, was linearly regressed onto environmental covariates (C), the predicted vector $\hat{Y}$ was used to replace Y in the MSPA calculation (Figure 5.1. equation i) and, for partial MSPA, $\hat{Y}$ was replaced by $Y-\hat{Y}$ to remove the environmental variation. The weighted site network was then used to obtain n-1 orthogonal Moran's Eigenvector Maps (MEM's) which range from U_1 (smallest) to U_{49} (largest) as described by Dray *et al*. (2006) and the

variability of the community data was decomposed via linear regressions of the spatial maps (U_{01}-U_{49}) to produce a set of predicted values (Figure 5.1. equation ii).

i) $\hat{Y}= C(C^TC)-^1C^TY$

ii) $S= ((Y^TU)*(Y^TU))/(n^2)$

iii) $Z= S-(1_q1_{n-1}{}^T)/(n-1)$

Box 5.1. Linear regression calculations:

i) The calculation of the predicted vector $\hat{Y}$, used in canonical MSPA, where C^T was the transposed matrix of environmental covariates.

ii) The regression of the predicted vector Y onto MEMs (U) where '*' denotes the Hadamard product and n was equal to the number of MEMs to produce matrix S

iii) The centring of S, to produce Z before MSPA using 1q, a dimensional vector whose components are all one.

_Notes

'The Hadamard product' refers to a binary operation where two matrices are used to form a third matrix of equal dimensions, it is unlike 'the matrix product' as the result is commutative.

The matrix S was centred by subtracting R^2 coefficients to produce matrix Z (Box 5.1. equation iii). MSPA then functioned as a PCA of Z, where rows were weighted by D (diagonal matrix of row weights). Bi-plots were used to display the principal and second axis in accordance with scree-plot analysis (a plot ranking all variation trends in an ordination diagram) of eigenvalue decrease, in order to determine whether more than the first two axes were needed to be included in the analyses.

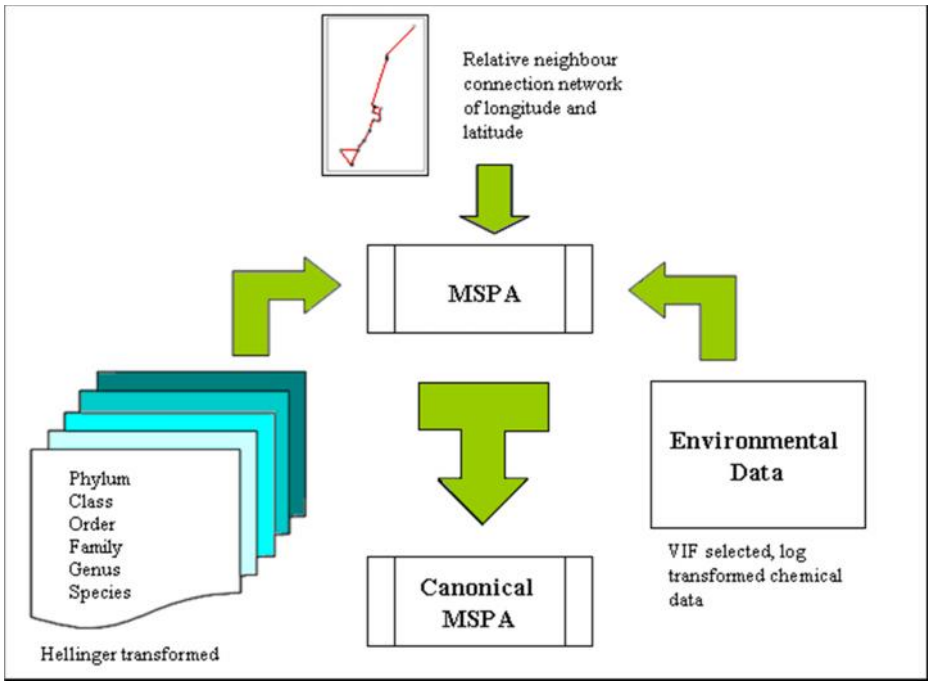


Figure 5.1. The MSPA approach to modelling multiple taxonomic scale data

Community data from each taxonomic rank, phylum to species, were used in standard MSPAs to look at spatial scales relating to bacterial distributions, canonical MSPAs to compare spatial trends between taxonomic levels which could be related to environmental predictors and also partial MSPAs, where the variation shared between the environment and community is removed and only structures present in biological data are preserved (See Figure. 5.2). An MSPA of environmental variables alone was also produced for comparison. MEMs U_1 to U_{49} were displayed on a bi-plot and, where MEMs were closest to circle of radius one or furthest from 'zero' on the axis the stronger the loading onto that axis. Variables indicating strongest spatial pattern are displayed further from the origin.

5.3. Results

Overall, the pyrosequencing data, which encompass both bacterial taxon diversity and relative abundance, showed little spatial association with the environmental variables across the range of sites sampled in this study. MEMs constructed to represent co-varying autocorrelation between community and environment displayed weak to moderate correlation with individual taxa across all taxonomic levels based upon environmental conditions ($R^{2\ range}$ 0- 0.46, $R^{2\ mean}$=0.02), but correlations were highest at genus level (see Table 5.1). Furthermore, the maintenance of predictable spatial structures (MEMs) was more prevalent at lower taxonomic rank as shown by the re-occurrence of MEM_06 throughout MSPAs, which is the most dominant spatial structure observed at phylum level but decreases in significance as taxonomic resolution decreases (Figures 5.3 and 5.4).

	MSPA Best R^2	Canonical MSPA Best R^2	Partial MSPA Best R^2
Phylum	0.31	0.22	0.36
Class	0.24	0.25	0.32
Order	0.35	0.26	0.35
Family	0.38	0.27	0.42
Genus	0.43	0.42	0.46
Species	0.38	0.32	0.46

Table 5.1. Best fit R^2 values for community only MSPA with spatial constructs (MEM U_0*i*) and canonical MSPA with spatial constructs (MEM U_0*i*). All values are significant to a threshold of P <0.01.

For community MSPA (not canonical), U_12 was the dominant MEM at all levels, and is associated with *Lentisphaerae* (R^2=0.31), *Verrucomicrobia* (R^2=0.22) and *Planctomycetes* (R^2=0.20). Species and genus data are difficult to interpret but there are considerably more MEMs contributing to species distribution and many of them are intermediate to fine-scale (Fig. 5.4), reflecting high heterogeneity in species distribution patterns. Using phylum data

only (Figure. 5.2.) two broad-intermediate scale MEMs were most influential U_10 and U_12 as they are furthest from the axes centre,, neither of which correlated strongly with the environmental variables. The spread of phyla across the bi-plot is restricted but clustered around the axes, indicating the presence of one or more gradients of phylotypes ranging from Lentisphaerae and the sister phylum Verrucomicroba at one end and many thermophilic bacteria at the other. It is likely that MEM U_12 represents the latitudinal transect which is intrinsic in the relative neighbour connection network thus could reflect a gradient in temperature, soil moisture content or plant prescence, for examples, all of which may be related to latitude (Smith *et al.*, 2009; Peat *et al.*, 2007).

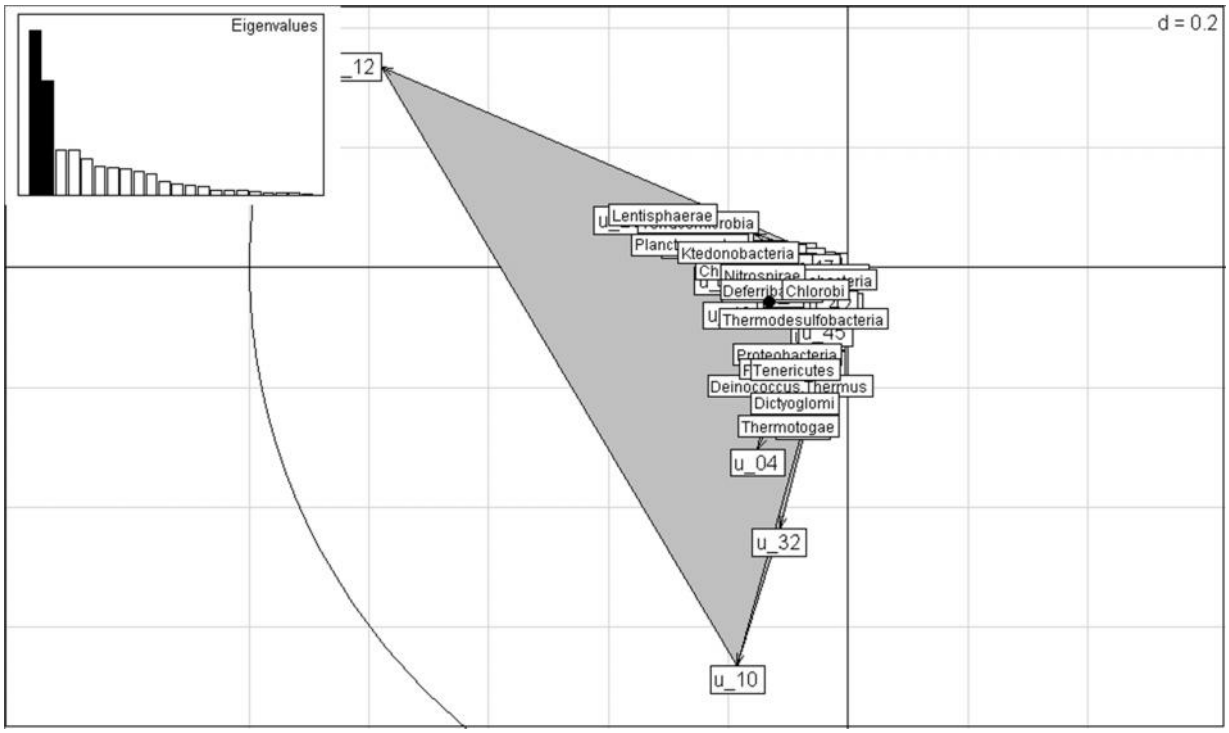


Figure 5.2. MSPA of phylum data with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla names superimposed to indicate associations with spatial structures. The grid size of the plot is notated with'd' and is equivalent to the coefficient of multiple determination (R^2).

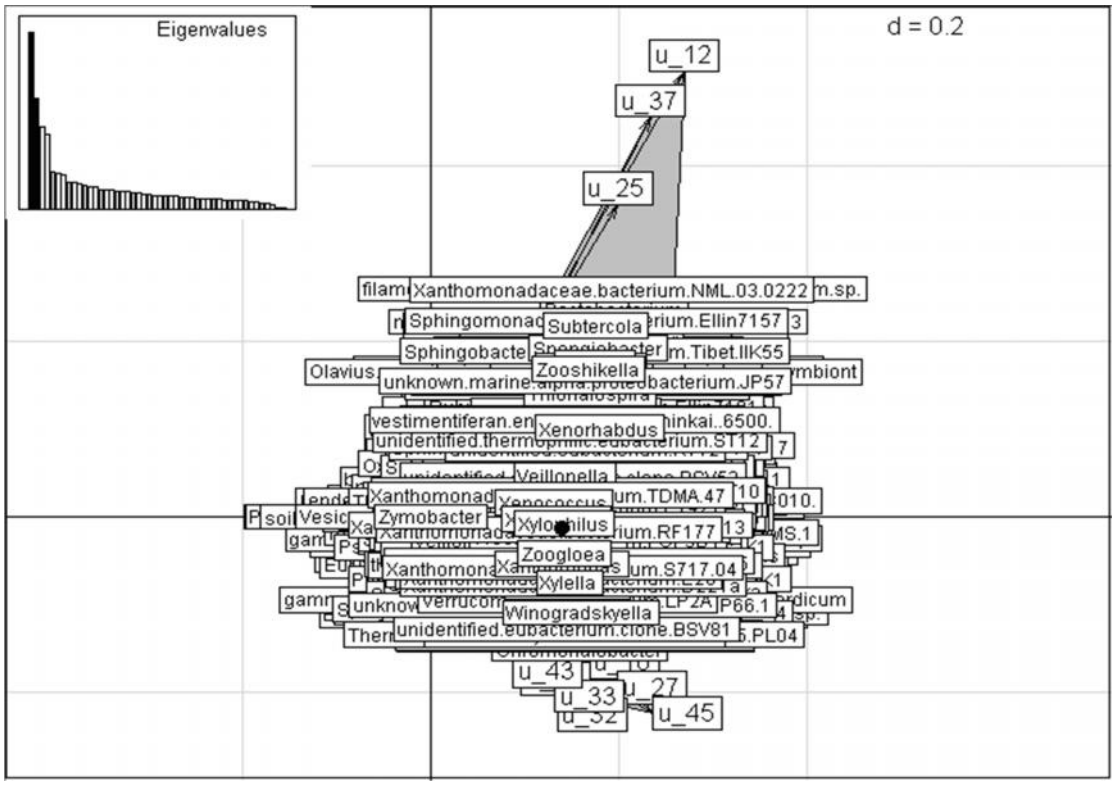


Figure 5.3. MSPA of genus data with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and species names are superimposed to indicate associations with spatial structures. The grid size of the plot is notated with'd' and is equivalent to the coefficient of multiple determination (R^2).

Several large scale structures were present in an MSPA of environmental variables only. Axis one was dominated by U_03, a broad scale structure, which was best linked to quantity of DOC and axis two was related to spatial structures U_07 and U_08 and driven by seals and birds (Figure. 5.4).

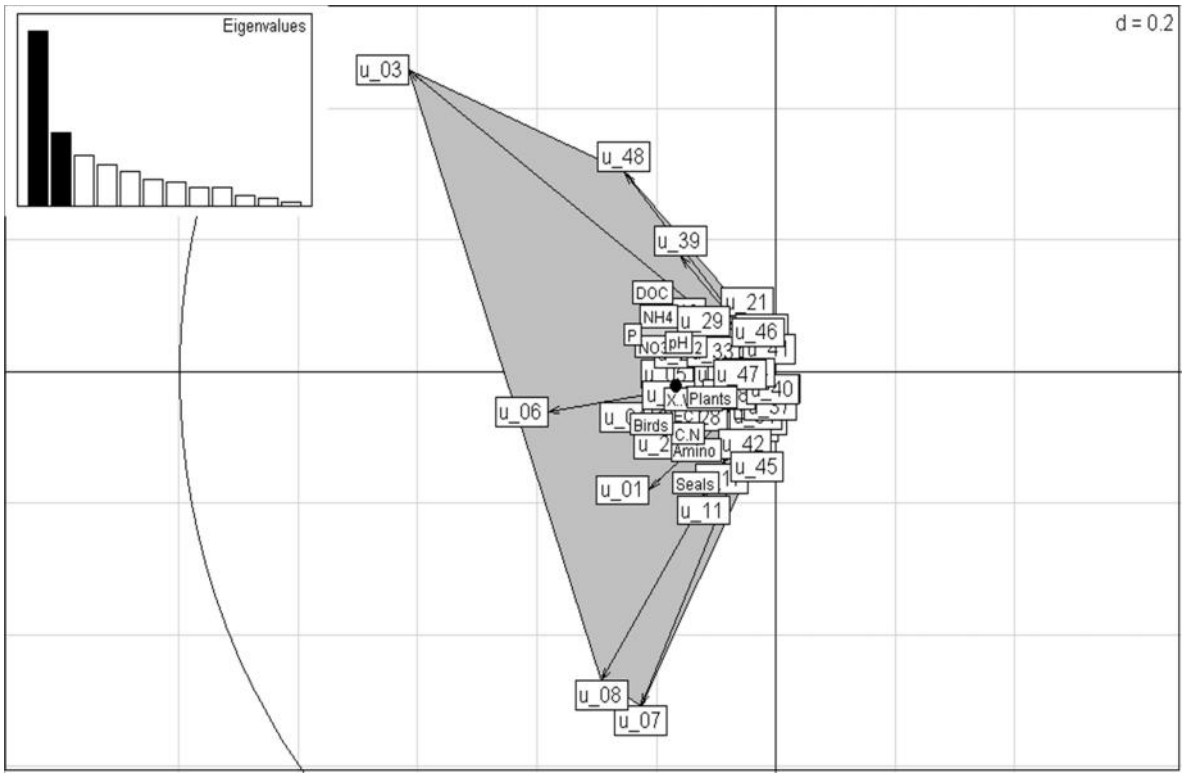


Figure 5.4. MSPA of environmental variables with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with'd' and is equivalent to the coefficient of multiple determination (R^2).

Canonical MSPAs (Figures 5.5 and 5.6) showed some spatial structures which were common to both bacterial community and the environment, for example with the phylum data MEMs U_04, U_06 and U_10 were present in constrained and unconstrained MSPA (Figure. 5.5), all of which represent large-scale spatial structures, indicating that spatial trends in the bacterial community data are only observable over a large distance. Furthermore, all canonical bi-plots indicated the presence of a single dominant spatial structure, U_06, between community and environment at all taxonomic levels, the scree-plots in the main suggesting the retention of only one dominant spatial structure.

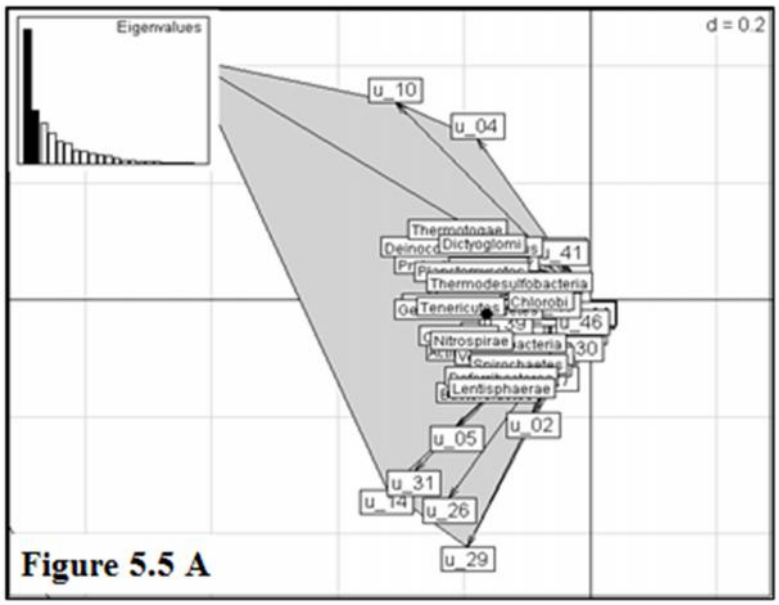


Figure 5.5 A

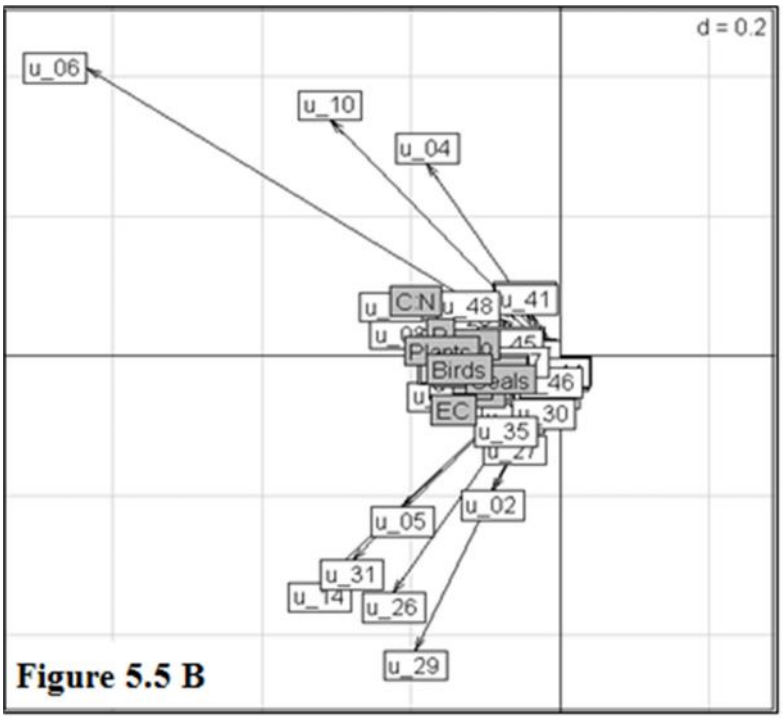
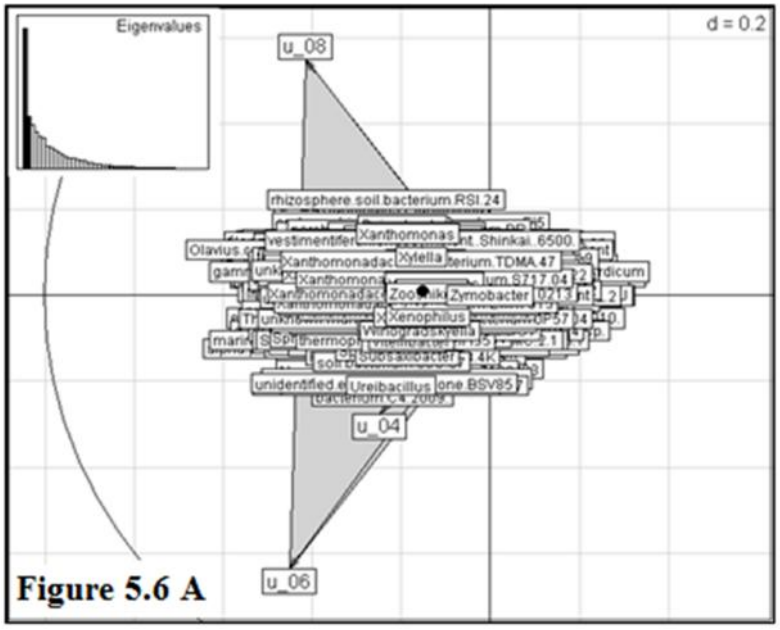


Figure 5.5 B

Figure 5.5. (**A)** Canonical MSPA of phylum constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with'd' and is equivalent to the coefficient of multiple determination (R^2). **Figure 5.5.** (**B**) reveals the environmental variables which are present but obscured in the full canonical MSPA graph above.



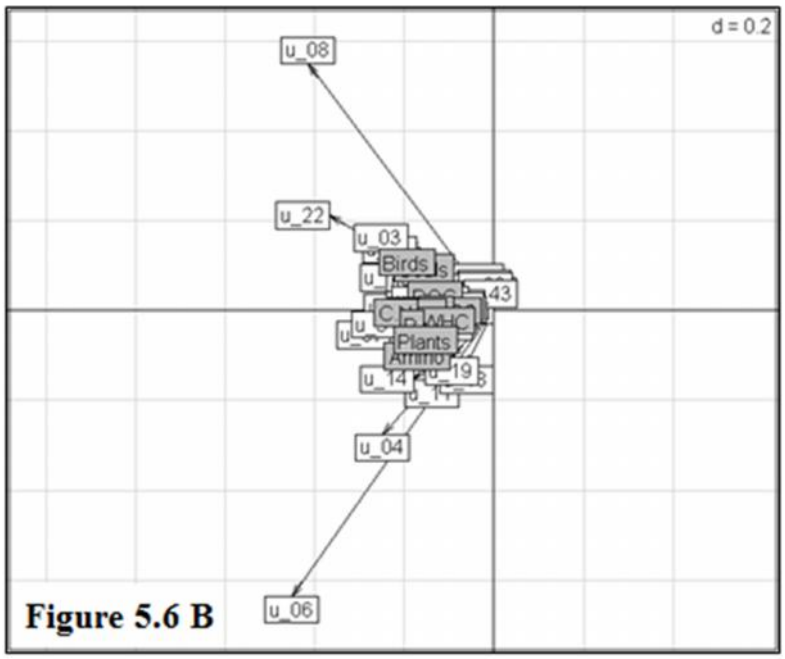


Figure 5.6. (**A**) Canonical MSPA of genus constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with'd' and is equivalent to the coefficient of multiple determination (R^2). **Figure 5.6.** (**B**) reveals the environmental variables which are present but obscured in the full canonical MSPA graph above.

MEM U_06 represents a large-scale rather than fine-scale structure, indicating that the bacterial community data only exhibit trends over a large distance and do not reflect smaller interactions amongsub-communities. MEM U_06 was most strongly associated with Proteobacteria (R^2=0.19), *Deinococcus* (R^2=0.18) and Planctomycetes (R^2=0.18) and, of all environmental parameters examined, was most strongly influenced by C:N (R^2=0.13). The influence of U_06 decreased with increasing taxonomic resolution and it became associated with the second axis by species level, where U_08, associated with bird presence, became more dominant. Overall, there was not a large amount of variation between the spatial vectors , MEM_U_01 to MEM_U_49, seen across different taxonomic groupings which could be related to the environment. The partial MSPA (Figure. 5.7) where environmental influence was removed from the MSPA plot was almost identical to the pure community MSPA plots (Figures. 5.2 and 5.3), indicating our environmental variables had little influence over bacterial distributions.

Figure 5.7. Partial MSPA of phylum data with environment removed with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla names superimposed to indicate associations with spatial structures. The grid size of the plot is notated with 'd' and is equivalent to the coefficient of multiple determination (R^2).

5.4. Discussion

Space is a theme commonly addressed in microbial ecology and several hypotheses exist concerning the biogeography of microorganisms, one such notion is the expression of ubiquitous microbial presence across all environments (Findlay, 2002). The results here indicate Antarctic bacterial populations can be weakly to moderately predicted by spatial influences with varying success amongst taxonomic groups, for example we might expect to see high numbers of Lentisphaerae and Verrucomicroba at similar locations but not Thermotogae (Figure 5.2.), but would have less success predicting bacterial species distributions. Also, we would expect to observe a trend in microbial diversity over a large scale, such as a latitudinal gradient than we would over several meters, as shown by the dominance of MEMs U_01-12. This is important as it reiterates bacterial distribution is non-random throughout terrestrial environments (Fierer and Jackson, 2006; Lauber *et al.,* 2009), though in this case, predictability is poor based upon the environmental variables used. Some recent studies in Antarctica have presented evidence of biological regionalization in terrestrial environments for various metazoan, bacteria and algal taxa (Vyverman *et al*, 2010; McGaughran *et al.,* 2009; Maslen and Convey, 2006), which have been attributed to areas of glacial refugia rather than environmental dependence.

The presence of dominant MEM U_12 in all community-only MSPAs, which is unrelated to the environmental constraints, reveals a linear gradient with the thermophile Thermotogae (Huber and Hannig, 2006) and desiccation- and UV-resistant Deinococcus (Hirsch *et al.,* 2004) at one end, and more abundantly distributed soil bacteria at the other such as Verrucomicrobia and the sister phylum Lentispherae (Cho *et al.,* 2004). Verrucomicrobia are associated with soil and fresh water environments, and with faeces (Bisset *et al.,* 2005), and Lentispherae with terrestrial gut microbiota from mammals, birds, fish as well as in coral microbiomes and marine sediment (Cho *et al.,* 2004). Deinococcus is well known for being robust to environment stress, in particular having high tolerance to radiation and able to withstand a wide temperature range (Ferrerira *et al.,* 1997). Thermotogae and Dictyoglomi, both present at the same end of the gradient, also tend to be prefer warmer temperatures. This tentatively suggests that less specially adapted microorganisms (such as Verrucomicrobia) which are present in most environments, can tolerate less extreme environments than metabolically more robust taxa (such as Deinoccocus) and therefore do not flourish in these environments.

The canonical biplots (Figure 5.5 and Figure 5.6), where the environmental variables are used to attempt to predict the distribution of bacteria, also indicate the presence of a single dominant spatial structure, U_06, at all taxonomic levels. This MEM had at best a weak correlation to C:N (R^2=0.13), which could either infer a significant level of environmental dependence unrelated to the variables we used or highlight a flaw in using linear relationships to represent environmental heterogeneity, which for many variables is likely to be unimodal even post-transformation. The association between U_06 and Proteobacteria (R^2=0.19), Deinococcus (R^2=0.18) and Planctomycetes (R^2=0.18) is not obvious. These phyla encapsulate a wide range of functional abilities and the importance of the C:N ratio is common for many microorganisms. It is thought the same ecological process may display different patterns at different scales though it may be of more importance in some than others (Wheatley and Johnson, 2009). This is best demonstrated by the significance of MEM U_06, which decreases with increasing taxonomic resolution, and becomes associated with the second axis by species level, where U_08, associated with bird presence, becomes more dominant.

In all MSPAs, higher taxonomic levels display the most predictable spatial trends with only a few important structures. As diversity at higher levels is thought to be more revealing to broad-scale environmental pressures and changes (James *et al.*, 1995) where environmental conditions select for specific functions expressed by the community, the maintenance of diversity of higher taxonomic groups is more important to ecosystem function due to the high amount of functional redundancy at species level in most soils (Vane-Wright *et al.*, 1990). However, in this analysis genus level community data were found to exhibit the strongest relationship with spatial scale. This could be important considering the trend in changing emphasis in microbiology from reporting species taxonomy to considering phenotypic and functional traits (Fenchel and Findlay, 2006), and the analysis of species abundance may not be the most appropriate method of analysis in microbial community ecology.

The application of MSPA highlights that different spatial trends can be observed across taxonomic groupings, some of which cannot clearly be linked to an environmental parameter and indicate that another mechanism is responsible for species distributions. Furthermore, different information can be gathered by the examination of spatial distributions on multiple taxonomic levels, which is useful given the format of high-throughput microbial datasets

produced by many modern sequencing methods. In a well-designed experiment, methods such as MSPA could be used to model mechanisms of species distributions by comparing spatial dependencies across taxonomic groups. Examination of spatial relationships with the environment or spatial structures exhibited only in the community may give an indication of whether environmental dependency or historical provenance governs the broad-scale trends observed.

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6

Ecological Profiles of Antarctic Soils*

Abstract

Environmental conditions on the Antarctic Peninsula are significantly different from those of the continental mainland and are characterised by areas of patchy heterogeneity. Heterogeneity is an ecological phenomenon which acknowledges that all ecological structures are included in the total diversity at a broad range of environmental scale. This has recently been identified as a driver of Antarctic biodiversity. Heterogeneity can be observed at small spatial scales but is more commonly associated with climatic and biotic influences, reflected in specialized soil environments, such as ornithogenic soils with high guano input or gelisols, high in permafrost. Different soil types tend to be geographically predictable and to varying degree, as are the microbial communities associated with them. In the following analyses, a classification of microbial communities from different locations across the Antarctic Peninsula is explored to help gain a greater understanding of the influences on diversity. Over 49 locations, two separate microbial community datasets were analysed simultaneously and site characteristics known to influence nearby biota were investigated to compare their individual influence with both soil properties and the associated microbial diversity. We present evidence to suggest the dependence of some bacterial species on specific habitats and that those habitats also exhibit varying soil chemical profiles.

Keywords: Environmental Heterogeneity; Ecology; Microbial Diversity;

*complementary analyses for this Chapter are presented in Appendix 5

6.1. Introduction

Life in terrestrial Antarctic ecosystems is constrained by low temperatures, impeding energy availability and utilisation for many organisms. However, environmental conditions across the maritime Antarctic Peninsula region are significantly different to those of continental Antarctica (Beyer *et al.,* 2000), where there are many examples of discontinuous physiochemical gradients. The maritime region covers around 15◦ latitude, which by its nature represents a pseudo gradient in temperature (see Chapter 1, Table 1.1). Signy Island (60◦43'S), one of the South Orkney Islands is at the most northerly end of the project's maritime Antarctic area, and represents a rich and diverse ecosystem (Smith, 1988) with frequent freeze-thaw cycles representing a major environmental stressor on soil biota (Wynn-Williams, 1996; Yergeau and Kowalchuk, 2008). Conditions further south show decreasing nutrient input, transient low level water availability and high UV exposure (Yergeau *et al.,* 2007), which select for oligotrophic or specially adapted communities. As the Antarctic Peninsula is warming at a rate of 3.7 ± 1.6 ◦C per century (Vaughan *et al.,* 2003), ten times the mean of global warming (BAS, 2007). Conditions observed at northern sites are likely to be reflected at higher latitudes in future years.

In order to assess how the Antarctic Peninsula's rapidly changing climate will impact on microbial communities, first we must better understand the way microorganisms are distributed throughout the environment. This requires the determination of not only spatial trends, but more importantly the mechanisms behind spatial dependencies (Hughes-Martiny *et al.,* 2006). The primary limitation on Antarctic microbes is thought to be the availability of soil carbon and nitrogen, which in some areas is conserved by a high degree of internal cycling (Smith, 2005; Hopkins *et al.,* 2006). However, there are several 'higher order' inputs to influences in the soil food web hierarchy which have been observed to influence both soil chemical composition (Aislabie *et al.,* 2009; Zhu *et al.,* 2011) and microbial community composition (Aislabie *et al.,* 2004; Texeira *et al.,* 2010) directly. As higher level influences on soil communities are highly regionalised in the Antarctic Peninsula area, this has led to a characterisation of different soil types. For example soils currently or previously colonised by avifauna have been termed 'ornithogenic soils'. They have been generically and consistently described by conditions including higher concentrations of organic matter, phosphorus,

electrical conductivity and variable, but often basic, pH (Barrett *et al.*, 2007; Aislabie *et al.*, 2009).

A number of other inputs could also lead to specific soil characteristics. Human presence for instance, has already had notable impact on Antarctic environments with the introduction of several invasive alien species, such as the cosmopolitan grass *Poa annua* (Frenot *et al.,* 2005) and persistent pollutants (Bargagli, 2000; Chown and Convey 2007). *Prasiola crispa* is a foliose alga most commonly found near penguin colonies, (Karsten, 2005), which has also become prevalent in recent years in soils enriched with organic waste in close proximity to Antarctic buildings (Olech, 1996). There also is evidence to suggest the bacterial communities' found close to human presence are notably different from other soil communities (Teixeria *et al.,* 2010; Cowan *et al.,* 2011). One prominent impact of the warming Antarctic Peninsula climate is the expansion of plant and shift of seal colonies (Turner *et al.,* 2013; Weimerskirch *et al.,* 2003), both of which are likely to lead to significant changes in the terrestrial environment. Freshwater lakes adjacent to seal colonies are becoming increasingly eutrophic, (Laybourn-Parry, 2006) and fragile vegetation has been irreversibly damaged in places such as Signy Island (Smith, 1988), though the effects on microbial communities are unknown. Also, numbers of Antarctica's two species of higher plants *Deschampsia antarctica* (Antarctic hair grass) and *Colonbanthus quintensis* (Antarctic pearlwort) have more than doubled (Lewis Smith, 1994) in some regions due to increasing temperatures and associated increased water availability (Convey, 2006). This is predicted to have already had profound effect on dissolved soil carbon and nitrogen and microbial communities (Roberts *et al.,* 2009). We investigate here whether higher inputs such as these can lead to unique soil chemical or microbial profiles.

The composition of soil communities is often assessed by fatty acid analysis, where reagents are used to sever the ester links in cell membranes, releasing lipids. As these lipids are a component of living cell membranes which are rapidly broken down upon cell death, they can be used as chemotaxanomic markers of metabolically active microbial communities (Zelles, 1999). Several studies have linked individual fatty acids to microbial components and to a lesser degree to individual functional group (Lechevalier and Lechevalier, 1988; Frostegard *et al.,* 1993; Zelles, 1999; Olsson, 1999). As some species provide crucial ecosystem functions, their relative importance may alter with varying environmental conditions (Collins and Benning, 1996). The aim of this study is to examine and characterise

different Antarctic habitats in terms of abiotic conditions, which may lead to distinctive fatty acid soil profiles and also to relate fatty acids to bacterial taxa.

Nannipieri *et al.* (2003), suggests that most microorganisms are functionally redundant therefore the emphasis of community studies should focus on how taxa and functions vary over space, rather than demonstrating that microorganisms are present or absent from a particular geographic area (Hughes-Martiny *et al.*, 2006). As Antarctic soils are relatively low diversity communities, functional redundancy may be low and soil biotic processes highly vulnerable to any changes in substrate availability (Yergeau *et al.*, 2012). By exploring the relationship between fatty acids and bacterial taxa, we may also gain better understanding of which functions may be most influenced by habitat type in Antarctic terrestrial environments.

6.2. Methods

6.2.1. Site locations and data acquisition

Sites locations, data acquisition and modelling methodologies are described in Chapter 2.

6.2.2. Data selection

Pyrosequencing data for 49 sites were used to represent bacterial phylum and class. ELFA data for the same 49 sites were selected so, both community datasets were comparable in the analysis (see Section 2.2 for more information about community datasets).

In order to create an comparison of environment 'types', the types needed to first be identified. Various site influences were estimated through field observations (see Section 2.1, Table 2.2, for more information on collection, and Appendix 1) and were used in this instance to classify 'different' environments. It is probable that some of these characteristics, for example bird and seal presence, will be correlated due to a location's proximity to the coast and subsequent selection for nesting/feeding grounds, but this does not mean that an area strongly influenced by seals (and less so by birds) will have the same soil properties as an area strongly influenced by birds (and less so by seals).

Environment characteristics were represented by some measured soil properties (see Section 2.1, Table 2.3, for more detail on collection and measurement). These included pH, total water, extractable phosphate (PO_4^{3-}), electrical conductivity (EC), total nitrate with nitrite ($NO_3^-NO_2^-$), ratio of total organic carbon to total nitrogen (C:N), total amino acids; as summarised in a PCA and axis 1 extracted and termed 'Amino', total ammonium (NH_4^+), dissolved organic carbon (DOC) and percentage soil saturation of soil water holding capacity (%WHC). Latitude was also included amongst the environmental variables. These variables were then linked to environment types by heat maps, a simple data visualisation tool where two-dimensional tables of numbers are presented as shades of colours (Gehlenborg and Wong, 2012), in order to differentiate between soil profiles.

6.2.3. Data Modelling

Table 6.1. Summary of data and modelling approaches used for comparison

Comparison	**Data Used**	**Method and Presentation**
Individual fatty acids with bacterial phylum and class	ELFA and Pyrosequence data	Db-RDA
Individual fatty acids with site characteristics	ELFA and Site influences	CCA
Site characteristics and environmental data	site influences estimated in-field, latitude and soil chemical properties	Heat maps

Constrained ordination was used to make associations between fatty acids and bacterial phyla/classes. Both sets of community data were Hellinger-transformed prior to analyses to better represent high zero abundance (Legendre and Gallacher, 2001). Redundancy analysis (db-RDA) was used with bacterial taxa data constrained by ELFA data with species-weighted scaling (scaling=2) to maximise correlations between biological relationships and reduce influence of site skewing. Hellinger-transformed ELFA data were constrained by habitat characteristics. Canonical correspondence analysis (CCA) was used in this case to maximise the differences between rare occurrences or site characteristics, which is typical of the Chi-

square metric (Legendre and Gallacher, 2001), because it was important to maximise the variance to help separate site 'types'. All ordinations were performed in the software package R (R Development Core Team, 2008) using library 'vegan' (Oksanen *et al.*, 2011), an overview of the modelling approaches is listed in Table 6.1.

Heat maps are frequently used in molecular biology to represent gene expression in microarrays through coloured indication of positive and negative returns (Shannon, 2003). Here they were utilized in order to visualise site influences against chemical analysis results and latitude (environmental characteristics), so that it could easily be discerned when 2 parameters have a correspondingly high or low value. Sites were classified under low, medium and high degrees of influence, where these corresponded to recorded values of 0-4, 5-7 and 8-10 respectively (See Section 2.1 for data definitions). For environmental conditions, an average was taken of all values across sites and used to define the category 'medium', a 33% percentile above average was used as a threshold for 'high' and 33% below average for 'low'. These values were then used to create a heat map for each site influence, so that it was clear where relationships existed between variables. Heat maps were constructed in Excel (2010) using a spreadsheet of the data with a low, medium and high pre-defined. Heat maps were derived from the bubble graph function.

6.3. Results

6.3.1. Constrained ordination

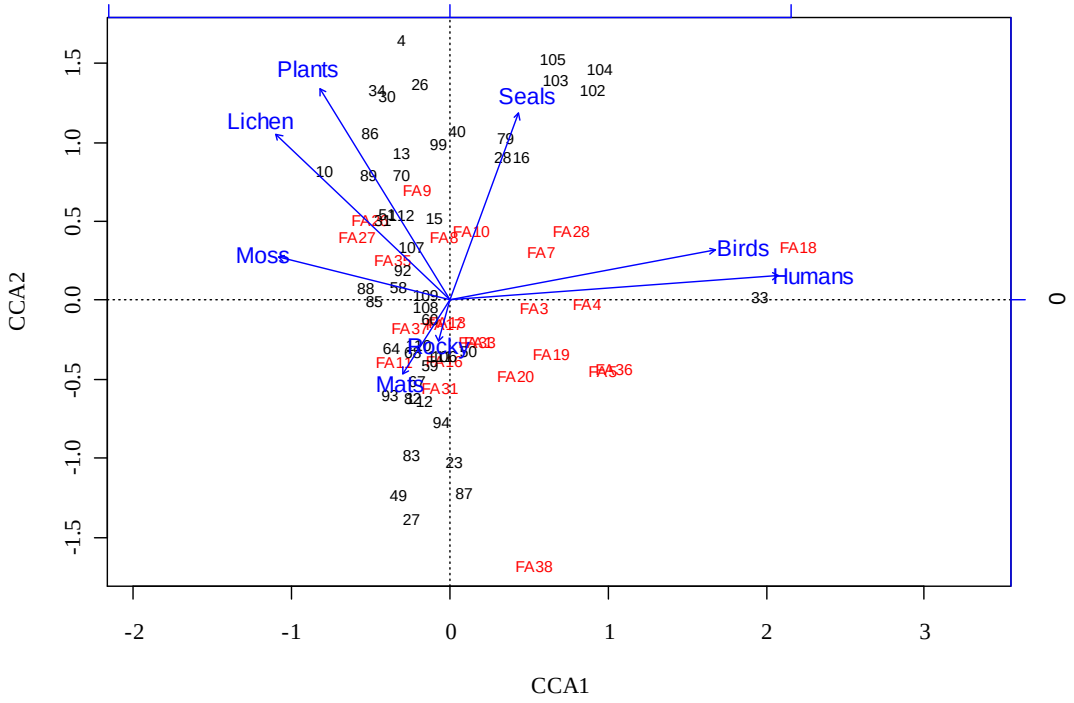

Figure 6.1. Canonical correspondence analysis of ELFA data constrained by site characteristics. Length and direction of blue arrows correspond to influence over community data, arrows close together show correlation between variables. Fatty acids are annotated in red and are partitioned according to their correlation with site characteristics.

The CCA (Figure. 6.1) explained 47% of the variation, with axis 1 accounting for 27% and axis 2 for 8% of the constrained inertia. These are low scores and indicate, first, that ELFA individual fatty acids can only moderately be predicted by site characteristics and, second, that the variation shared between ELFA and site characteristics cannot be well explained by only two dimensions. Axis one had a strong correlation with birds and humans and shows they are unlikely to be in the same area as Moss. Axis 2 was correlated with seals and plants. The RDA of bacterial taxon (Figure. 6.2) constrained by ELFA accounted for 54% and 23% of the community variation for Phylum and Class, respectively, with low axis scores for all those retained (Axis 1= 20%, 11%, Axis 2= 10%, 6%).

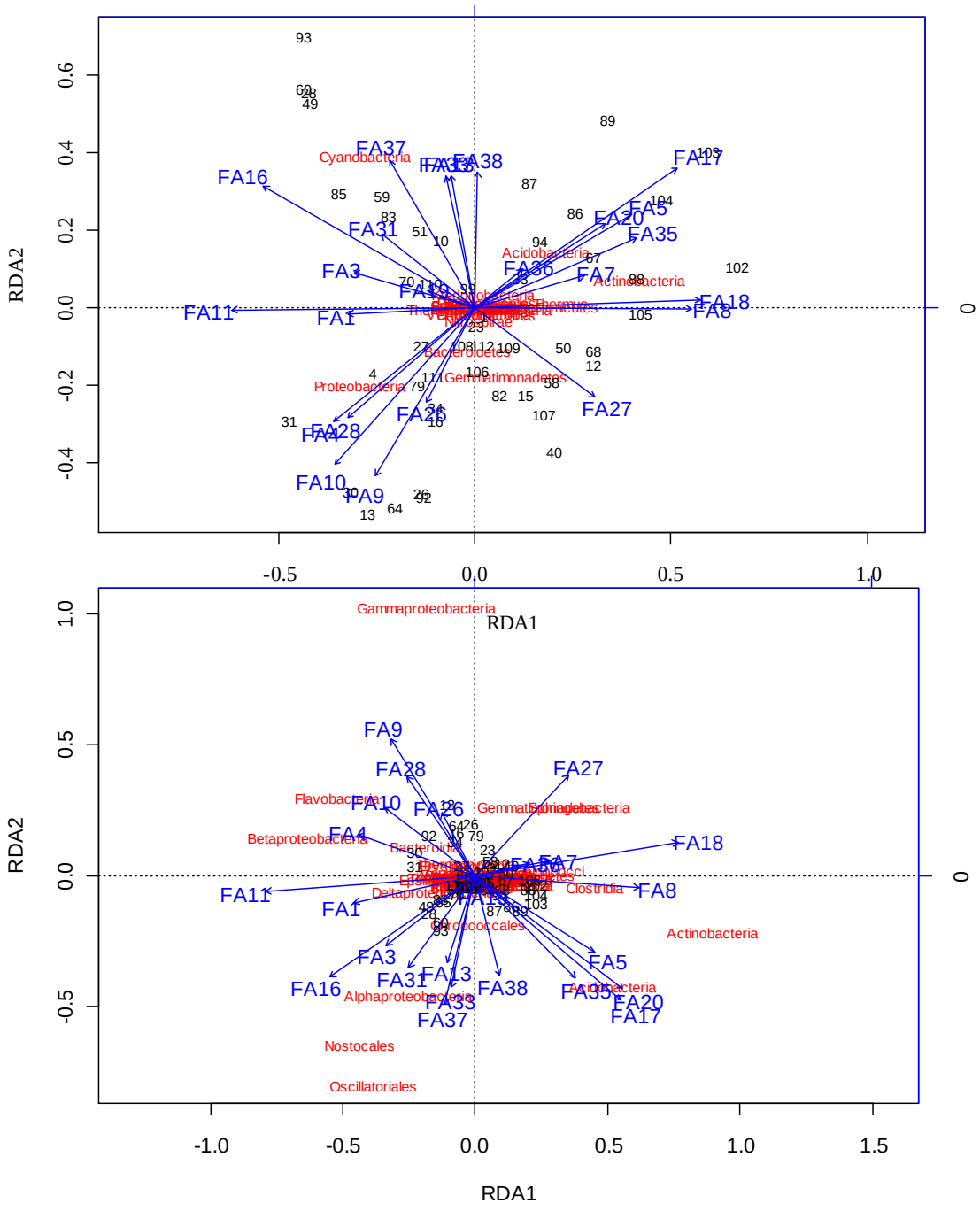

Figure 6.2. Distance-based redundancy analysis (db-RDA) of 454 bacterial data constrained by microbial fatty acids for bacterial Phylum (Top) and by bacterial Class (Bottom). Length and direction of blue arrows correspond to influence over community data, arrows close together show correlation between variables. Fatty acids are notated in red and are partitioned according to their correlation with site characteristics.

6.3.2. Heat map: Birds with constrained ordination analysis

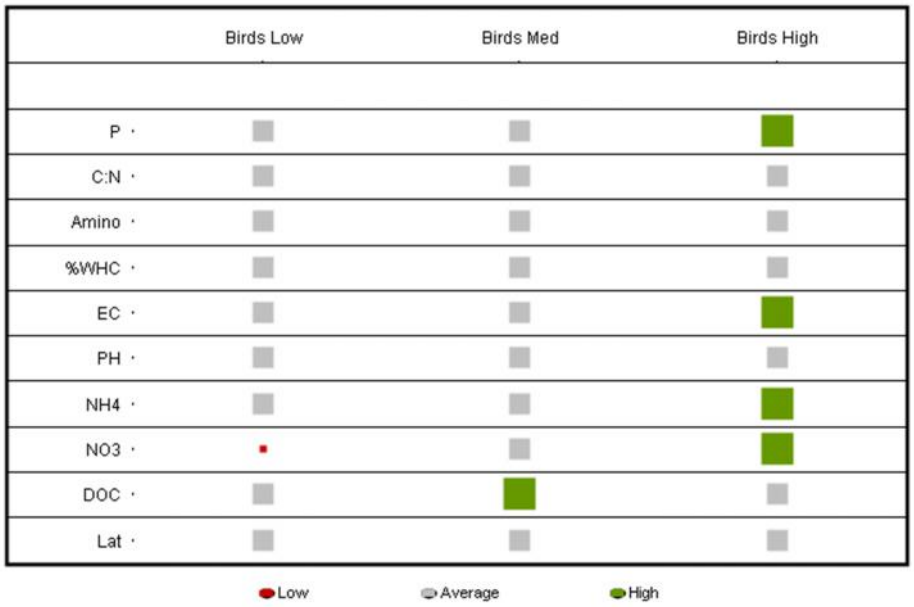

Figure 6.3. Heat map of Bird influence; Colour and size of square corresponds to correlation between site characteristic grade and environmental parameter; where squares are coloured green correlation is high, grey is average and red is low.

The heat map used to describe ornithogenic soil (Figure 6.3), shows soil nutrient conditions concurrent with those found in other studies, with high N (nitrate and ammonium N), high P and high EC in proximity to a high abundance of avifauna. The lack of nitrate associated with low bird influence implies birds are a major driver of nitrate-N in Antarctic soils. All other environmental parameters appeared reasonably unaffected by birds, although high DOC was linked to moderate bird influence.

The CCA between site characteristics and ELFA data showed a correlation between birds and FA18 (17:1ω8) and to a lesser degree to FA07 (i16:0) and FA28 (18:1ω7c), which represent total microbial, Gram positive bacterial and total bacterial fatty acids, respectively. According to bacterial RDAs, FA07 and FA18 could both be derived from Actinobacteria, which were the third most abundant phylum in our bacterial sequence data, and would suggest a prevalence of Actinobacteria in ornithogenic soils. 14-methyl-pentadecanoic acid (i16:0) has been observed in polar psychrophilic actinobacteria previously (Mannisto *et al.*, 2000) but heptadecenoic acid (17:1ω8) has been found in cold-tolerant fungi (Singh *et al.*,

2013). FA28 looks to be related to Proteobacteria and in several recent studies has been linked to new species of Proteobacteria, *Pseudorhodobacter antarcticus sp. nov*. and *Puniceibacterium antarcticum gen. nov.*, (Chen *et al.*, 2013; Chang *et al.*, 2014), isolated from Antarctic marine sediment and seawater.

6.3.3. Heat map: Seals with constrained ordinationanalysis

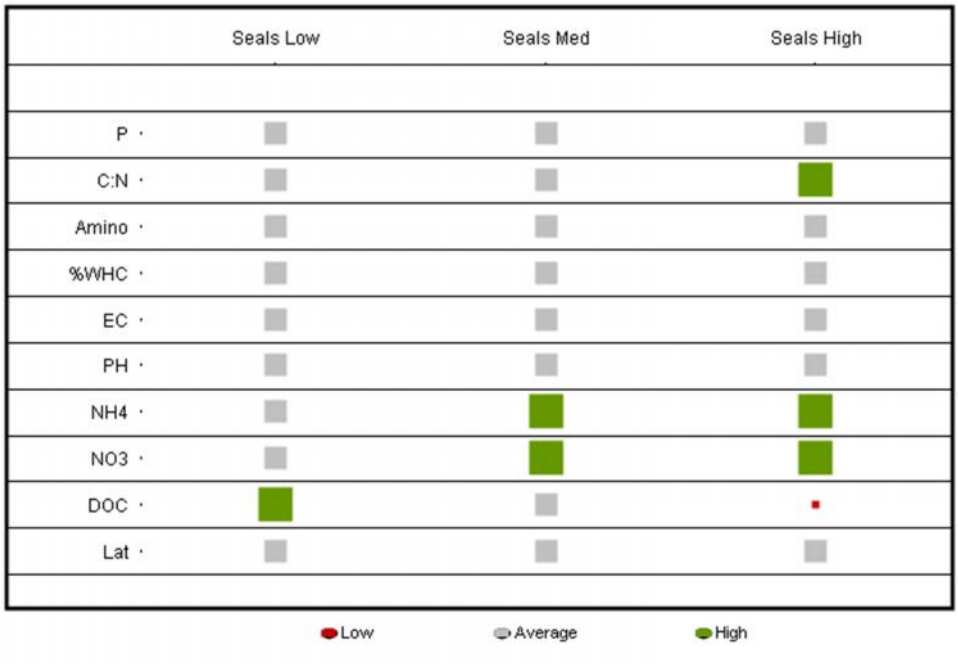

Figure 6.4. Heat map of seal influence; colour and size of square corresponds to correlation between site characteristic grade and environmental parameter; where squares are coloured green correlation is high, grey is average and red is low.

As seals are also directly contributing guano to the soil, as with birds, a positive relationship with both nitrate-N and ammonium-N was again observed (Figure 6.4). However, a positive relationship with total carbon in the form of increasing C:N was confounded by a negative relationship with dissolved organic carbon (DOC), indicating that a complex cycle of carbon depletion and addition may be related to seal presence. FA10 (16:1ω11c) was correlated to seals on the CCA, this being a fatty acid about which little is known, although it has been

detected in organisms related to Gram negative bacteria (Zelles, 1999). According to the class RDA (Figure 6.2), Flavobacteria may have an association with 16:1ω11c, although Flavobacteria contain high proportion of 16:1ω7c normally (McCammon and Bowman, 2000).

6.3.4. Heat map: Plants with constrained ordination analysis

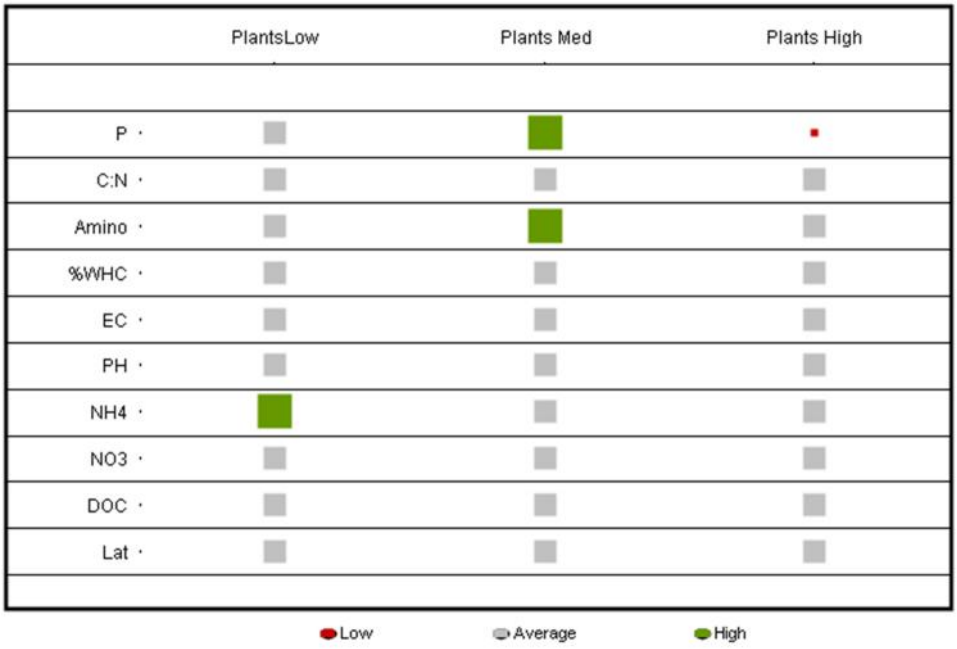

Figure 6.5. Heat map of plant influence; colour and size of square corresponds to correlation between site characteristic grade and environmental parameter; where squares are coloured green correlation is high, grey is average and red is low.

The heat map for plants (Figure 6.5) did not indicate a high correlation of plants with any of the environmental parameters, but did suggest areas of high guano (high NH_4^+, high P) are not related or are even negatively related. This is similar to the trend shown where lichen (Appendix 6) is particularly low in areas of high ammonium. Lichen was the only parameter in the heat map analysis which showed a correlation with latitude, with increased lichen at lower latitudes. Moderate plant presence was associated with high amino acid abundance. FA09 was most closely linked to plants and represents 16:1ω9c, Gram negative bacteria. Moss (Appendix 6) behaved differently and was abundant in areas of high ammonium-N, DOC and soils with high moisture. Moss and lichen were correlated with fatty acids 26

(18:2ω9c12c) and 27 (18:1ω9c), which both represent fungi. Also, 18:1ω9c has been found in high concentration in the soils of mixed species forests (Xing *et al.*, 2010) FA35 (19:1) which is linked to Gram positive bacteria and bacteria which have high tolerance for polluted soil (Bååth *et al.*, 1992), according to the RDA (Figures 6.2 and 6.3) is strongly correlated to abundance of Acidobacteria. 19:1 is not commonly found in Acidobacteria (Thrash and Coates, 1991).

6.3.5. Heat map: humans with constrained ordinationananalysis

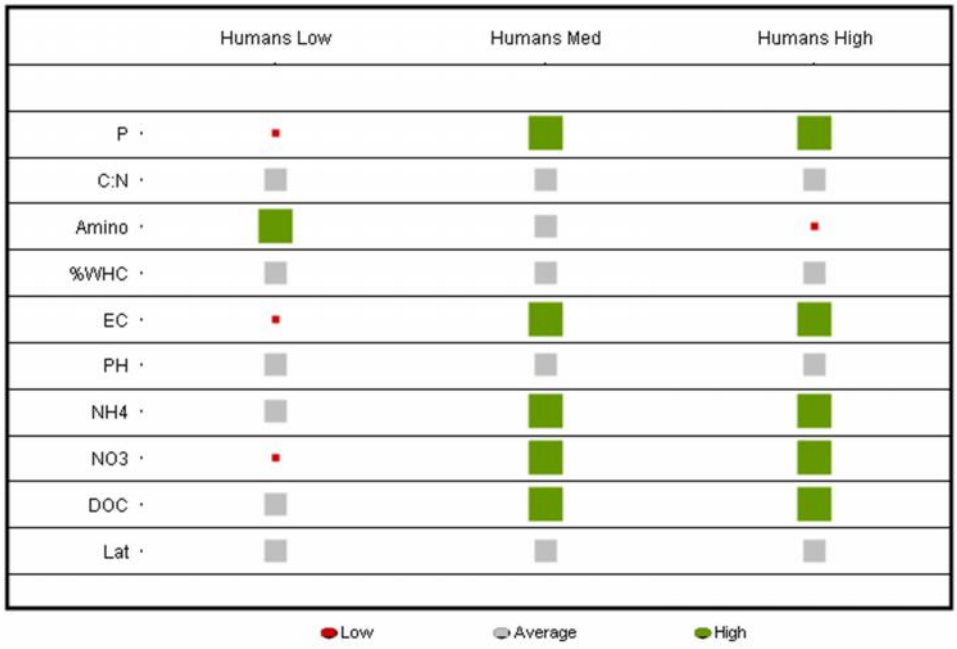

Figure 6.6. Heat map of human influence; colour and size of square corresponds to correlation between site characteristic grade and environmental parameter; where squares are coloured green correlation is high, grey is average and red is low.

The human heat map (Figure 6.6.) showed a high abundance of PO_4^{3-}, NH_4^+, NO_3^-, DOC and high EC and a negative relationship with amino acid in areas of high and average human influence, which suggests a correlation between human site locations in areas of marine animal and bird colonised areas. FAs 03 (i15:0) and 04 (a15:0) had the strongest correlation with human influence (Figure 6.1) and both relate bacteria, usually Gram positive bacteria (See Chapter 2, Table 2.4). a15:0 also showed a strong correlation to Betaproteobacteria,

though there is little to support this in the literature. A mild correlation was observed between i15:0 and Alphaproteobacteria, which is supported by Kwon *et al.,* (2005), who found i15:0 to be one of the most dominant fatty acids present in *Kordiimonas gwangyangensis gen. nov., sp. nov.,*a marine sediment bacterium.

6.4. Discussion

The significance of ornithogenic inputs to microbial communities is well documented, and avi-guano is thought to be one of the primary sources of nitrogen to Antarctic microorganisms (Christie, 1987). Due to the high compositional amounts of urea which add to soil basicity (Bundy, 2009), nitrification rates are increased in these areas (Myrcha and Tatur, 1991). The relationships expressed in the heat map (Figure 6.3) support previous findings that ornithogenic soils are characterized by high conductivity and have high levels of nitrogen and phosphorus (Breuning-Madsen, 2009). The dominance of Actinobacteria at ornithogenic sites has been found in other studies (Sanyika *et al.*, 2012) and these bacteria are thought to be the most important decomposers of complex material (Pankratov *et al.*, 2006). The possible link between FA28 (18:1ω7c) and Gammaproteobacteria, the largest class excluding Firmicutes for metabolic abilities such as temperature adaptation (Williams *et al.*, 2006), could indicate increased bacterial diversity at these sites. This is generally supported by diversity analyses in previous chapters (See Figure 3.14) where, for example, high Shannon and Simpson indices were observed in Port Lockroy, a site with high ornithogenic inputs.

Soils from seal colonies are less frequently examined. Recent studies (Chong *et al.,* 2009) have found that N and C contributions were increased in seal colony soils though these values were as much as 45 times less than ornithogenic inputs (Zhu *et al.*, 2011). Results here suggest a positive relationship between high abundance of seals and these soil nutrients, however the sites sampled which were particularly high in seals were, in this case, high in avifauna also. Gram negative bacterial representative FA10 (16:1ω11c), correlated to high seal sites, has also been observed in abundance in sites heavily contaminated by smelter and metals (Kelly *et al.*, 2003) so is likely to be associated with metabolically robust microbial communities. According to the RDA (Figure 6.2), Flavobacteria may have an association

with FA10 (16:1ω11c), however high concentration of 16:1ω7c is normally observed for Flavobacteria (McCammon and Bowman, 2000).A study by Pearce *et al.* (2008) which investigated the diversity of Heywood Lake, a lake frequented by elephant and fur seals on Signy Island, also found common occurrence of flavobacterial genera, which they attributed to a specialist role in organic matter decomposition. Species of Flavobacteria have also been observed in Antarctic sea water (Nohi, *et al.,* 2005) and Saline lake (Shi *et al.,* 2012) samples.

The best-vegetated areas in Antarctica are found on the South Orkney Islands, South Shetland Islands and along the west coast of the Antarctic Peninsula. Antarctic vegetation has been found to have a buffering effect on the severity of climatic influence on soil communities (Yergeau *et al.,* 2008) by increasing thermal stability and improving nutrient networks. However, sites high in plants could not be directly linked to environmental nutrient alleviation, but instead suggested they may be restricted by the high quantities of P and N deposited in guano, which is not unusual since N toxicity commonly occurs in areas of high application (Barrett *et al.,* 2007). Also, microbial communities compete with plants efficiently for nutrients, to the point of affecting plant growth when microbial access to labile C is high in heterogeneous soils (Schmidt *et al.,* 1997).

There are contrasting views on whether plant species alter microbial community composition (Kowlachuk *et al.,* 2002; Teixeira *et al.,* 2010) but differences in abundant bacterial species ratios have been observed. In the rhizosphere of Antarctica's two vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis*, Teixeira *et al.* (2010) found, contrary to temperate soils, Actinobacteria and Firmicutes represented 70% of sequences obtained. Fatty acid 16:1ω9c, Gram negative bacteria are most associated with plants and may be linked to Gammaproteobacteria also. There are around 350 species of plants in total and are mainly comprised of lower plants such as mosses, and liverworts (Malosso *et al.,* 2003) of which, many form symbiotic relations within lichen. This is indicated by the correlation of fungal fatty acids 18:2ω9c12c and 18:1ω9c with lichen and moss in the CCA. Areas high in moss and lichen may also be abundant in Acidobacteria (see Figure 6.1), these bacteria are common throughout soils and represent a range of functions. Acidobacteria are widely thought to dominate in resource limited environments (Smit *et al.,* 2001; Fierer *et al.,* 2007). The ratio of %Proteobacteria : (%Proteobacteria + Acidobacteria) is thought to be indicative of nutritional status of soils, and is low in ogliotrophic soil (Smit *et al.,* 2001), this study found a mean ratio of 0.83, closer to the ratio of a high input agricultural system as defined

by Smit (2001), however it is recognised that this ratio indication is based on only a few studies.

How human presence affects the Antarctic environment is perhaps the most controversial factor investigated here. The detrimental recent human impact on the Antarctic environment has been highlighted by the Committee for Environmental Protection of the Antarctic Treaty System as a matter of political concern (Chown and Convey 2007). However, the analyses presented here indicated a high level of correlation between key soil nutrients associated with ornithogenic soil, and therefore we have to assume a level of sampling error may be associated with the proximity of human influence to ornithogenic sites. As heat maps are not inclusive of interaction terms between site characteristics and are merely exploratory, it is likely that the data used in this study will have some overlap of site characteristics. For example, sites highest in human influence are primarily research bases which are often located in close proximity to penguin or seal colonies due to the nature of the research being conducted. Despite any overlap between environmental influences, the level of impact indicated by even only moderate human influence may warrant some concern. Fatty acids i15:0 and a15:0 showed a correlation with human presence (Figure 6.1). Previously, i15:0 and a15:0 have been chosen to represent Gram positive bacteria (Federle, 1986; Zelles, 1997) but here showed a correlation with Gram negative bacteria, and in particular the Betaproteobacteria and Alphaproteobacteria. Proteobacteria are regarded as classic examples of R-selected organisms with high growth rates when nutrient availability is limited (Smit *et al.*, 2001).

In conclusion, the simplicity of the Antarctic environment aids in the identification of key relationships between higher tropic influences and additions, as well as those of soil chemistry and microbiology. Ornithogenic soils display the most predictable chemical characteristics due to N and C substrates from guano (Aislabie *et al*, 2009), and now growing consistency of predictable associated microbiota (Bownam *et al*, 1996) but it is clear there is potential for better interpreting N dynamics within Antarctic soils, particularly if community data are combined with functional gene analysis. The true nature of human influence is unclear, although the data suggest that human presence may significantly influence environmental conditions or that presence is too highly correlated with other factors, a limitation of data collection. Plant environments were crudely described in this study, though plants are known to have a buffering effect maintaining diversity (Yergeau *et al*, 2007).

Teixeira *et al*, (2010) failed to identify difference between microbial communities of different plant species. This relationship is reiterated by the association of plant influence with high microbial abundance.

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Terrestrial Antarctic Ecosystems: Ornithogenic Influence on Microbial Food Webs*

Abstract

Antarctic soil ecosystems operate under closed conditions. Energetic inputs are limited and nutrient cycles are reliant almost solely upon efficient microbial function. Guano inputs from avifauna and seal colonies are thought to provide the chief nitrogen input to soils, but the effect of such additions on microbial distribution and biomass are yet to be understood. The extent to which microbial diversity can be predicted by edaphic parameters is of global interest and the simple trophic structure provided by pristine Antarctic environments provides an opportunity for a greater resolution within these relationships. Using environmental and community data, we created a conceptual structural equation model to test hypotheses about energy flow through terrestrial Antarctic food webs. Fatty acid analysis was used to investigate soil microbial communities and soil bacterial diversity was analysed in greater depth using high throughput bacterial community data. We found substantial evidence to support ornithogenically-driven energy pathways where guano addition contributed substantially to the maintenance of the soil N-cycle. The implications and use of causal modelling techniques to represent ecosystem dynamics are also discussed.

Keywords: structural equation modelling, food webs, ornithogenic soils

*Complementary analyses for this Chapter are presented in Appendix 6

7.1. Introduction

Antarctic soil food webs are probably the simplest on earth. The absence of many taxonomic groups has magnified the role of microorganisms as the key facilitators of ecosystem processes (Wynn-Williams, 1996). Soil geochemical cycles are heavily reliant on microbial participation. Biological fixation alone is estimated to produce 69% of available soil nitrogen (Bezdicek and Kennedy, 1998). As nitrogen is thought to be both the most limiting nutrient (Marion and Everett, 1989) and the key driver of primary productivity (Hopkins *et al.,* 2006), the ways in which N substrates are distributed throughout Antarctic soil represent a major dynamic of Antarctic ecosystem functionality.

The main sources of nitrogen in Antarctic soil are from N-fixing bacteria and from guano additions, mainly from seabirds or penguin colonies (Christie, 1987). Soils currently or previously colonised by avifauna have been termed 'ornithogenic soils' and are characterised by higher concentrations of organic matter and phosphorus, high electrical conductivity and variable, but often basic pH (Barrett *et al.,* 2007; Aislabie *et al.,* 2009). High microbial biomass has been observed in ornithogenic soils but not increased diversity (Aislabie *et al.,* 2009).. This may suggest a high degree of dormancy in areas of low nutrient availability where microbes do not have access to adequate levels of substrates (De Nobili *et al,* 2001). Though ornithogenic inputs are considered a fundamental source of carbon and nitrogen to soil communities, it is also thought that high levels of ammonia-N, present in guano, may be toxic in driers soils where less leaching can occur (Barrett *et al.,* 2006). Whether the energetic benefit of an increased N-substrate pool to community biomass outweighs the risk of toxicity, is still unknown.

In addition to nitrogen, labile carbon is also known to be limiting on microbial mineralisation (Smith, 2005). Dynamics of carbon cycling are more difficult to elucidate due to the vast array of viable substrates but a degree of C substrate priming has been observed (Malosso *et al.,* 2003). Substrate priming has been defined as an increase in the mineralisation of soil organic C following the addition of a new input of organic carbon (Bingeman *et al.,* 1953; Kuzyakov *et al.,* 2000) Priming in Antarctic soils has been observed with substrates containing N (Hopkins *et al.,* 2008), which suggests C mineralization may be N-limited in some areas. Decomposition in Antarctic soils is driven by a number of controls to which decomposers are fundamental, namely saprotrophic fungi and bacteria (Robinson, 2002).

Robinson (2002) cites that mass loss is sensitive other factors, which have been confirmed in recent studies as temperature (Parsons *et al.,* 2004; Yergeau *et al.,* 2009) and nitrogen (Bokhorst *et al.,* 2007) which all influence decomposition rates. Microbial primary production is high where vegetation is limited and is probably an important source of soil carbon in these areas (Hopkins *et al.,* 2006). Other carbon inputs include amino acids and, in ornithogenic soil, uric acid, which serves as a source of both C and N. In conjunction with short peptides, these are the main source of available nitrogen to *Deschampsia antarctica* and other vegetation (Hill *et al.,* 2011). Methane oxidation, though poorly described in Antarctic soil, generally occurs in environments where hydric stress is extreme and urea/ammonia concentrations are low (Yergeau *et al.,* 2009). Yergeau et al. (2009) found that methane oxidation gene abundance was negatively correlated with nitrate quantity.

In addition to C and N compounds, other abiotic parameters are known to influence microbial community structure and functioning. pH is thought to govern soil bacterial diversity in temperate soils (Fierer and Jackson, 2006; Baker *et al.,* 2009), though such a relationship has not been observed with any consistency in Antarctic soils (Chong *et al*, 2012). Fungal diversity has been most strongly correlated to water availability rather than substrates directly (Newsham *et al.,* 2009) though fungi are more resilient than bacteria to low temperatures and consequential soil hydric fluctuation (Pietikainen *et al.,* 2005). Soil temperatures are highly seasonal and thermal variation of surface soil can be significant, especially at fellfield sites where black body absorption is high (Block *et al.,* 2009). Freeze-thaw cycles occur frequently at lower latitudes across the Antarctic region and may represent a gradient of environmental instability (Yeargeau *et al.,* 2007a). Yergeau and Kowalchuk (2008) found that freeze thaw cycles were more influential than vegetation or warming on microbial community functionality. Several climate change scenarios have demonstrated a lack of response to small increases in temperature (Dennis *et al.,* 2013; Yergeau and Kowalchuk, 2008), which is thought to be due to nutrient limitation on the microbial communities' ability to respond to temperature increase.

The type and amount of available organic substrate strongly influences the abundance of microbial groups in soil communities. This is especially true in Antarctic soil systems where many geochemical processes in terrestrial organisms are energetically constrained (Yergeau *et al.,* 2012). Furthermore, understanding the mechanics of community functionality becomes particularly important in an environment where nutrient pools are low and contemporary

nutrient additions patchy. The routes in which energy flows through microbial food webs may be essential to predicting the way in which environmental and anthropogenically-induced changes will affect future communities. The carbon-nitrogen relationship in soil is multifaceted with interactions occurring between different compound forms. In order to build a better understanding of how carbon and nitrogen dynamics impact upon microbial communities, other soil substrates and characteristics need to be considered in unison. It has been well established that guano inputs contribute significantly to soil N (Aislabie *et al.*, 2009), but few studies have attempted to address how these contributions affect energy flow between other soil substrates and microbial communities. The aim of this study is to investigate the effect of ornithogenic inputs on Antarctic soil food webs, inclusive of substrate interactions, to create a model which best represents the most significant pathways which energy flows through to microbial communities from ornithogenic sources.

7.1.1. Model hypotheses:

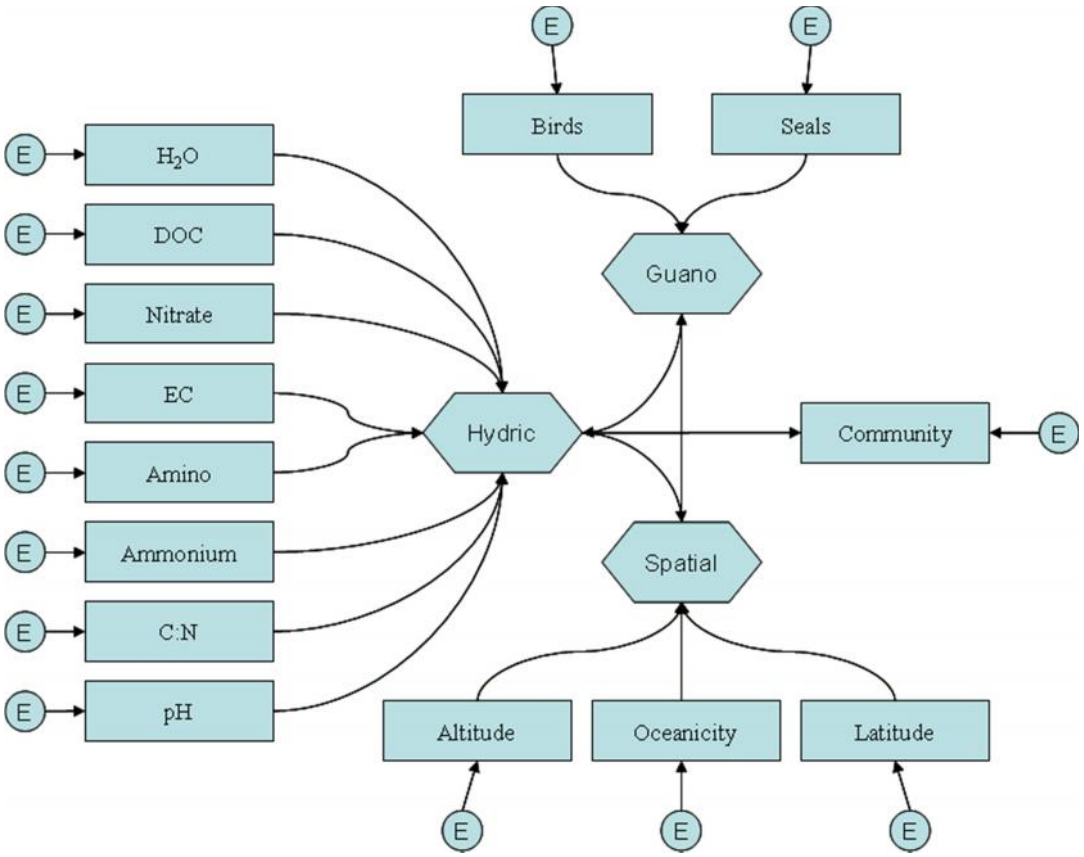

Figure 7.1. Full SEM of hypothesised environmental influences governing microbial abundance and distribution. Measured variables, shown as rectangles, are regressed onto latent variables and have associated error terms. 'Community' in this case represents ELFA microbial biomass, ELFA microbial richness 454-bacterial relative abundance or 454-bacterial richness

The conceptual model created was a generalisation of the main geochemical interactions expected within a soil system and the major abiotic stressors of polar environments (see Figure 7.1.), and was designed as a representation of the perceived energy web. To enable this, three conceptual latent variables were hypothesised (Table 7.1). *Please refer to Chapter 2 for data nomenclature and descriptions.*

Table 7.1. Latent variables created for SEM

Latent Variable	Description	Justification
Hydric	A latent variable 'Hydric' was created to indirectly represent the cumulative effect of hydro-climatic constraints present in Antarctic soils, which include highly regionalised variance in precipitation levels, flooding and aridity and freeze-thaw cycles.	Fluctuations in soil moisture all affect various nutrient availability, soil pH and EC, which formed the measured predictors of 'hydric'. This is in agreement with Grace *et al.* (2001) who found soil hydric influences could be amalgamated in this way to predict plant biomass.
Spatial	A second latent variable 'spatial' was used to represent the broad scale variation in climate across the peninsula by combined influence of latitude, altitude and oceanicity.	Previously, we found no significant effect of latitude on microbial diversity but we did observe a relationship with environmental heterogeneity with further distance south (Chapter 3). Oceanicity was also found to be influential over bacterial diversity; this is likely to be due to the fluctuation in climate experienced by more coastal locations.
Guano	The last latent variable 'guano' was the cumulative effect of estimated levels of guano additions from both seals and bird colonies.	Seal and avian influence were combined as they often occupy the same maritime locations in close proximity to one another. In addition, guano from both is thought to enhance soil C and N quantities to varying degrees (Vincent, 1988).

Notes
Data elements described in Chapter 2.

We hypothesised and tested the following relationships between latent variables:-

i) Hydric will be dependent on Guano due to deposition of large amounts of organic matter associated with bird and seal droppings.

ii) Guano will be more dependent than Hydric on Spatial, due to limited availability of suitable nesting grounds near coastal areas.

iii) Hydric to be the most important driver of variation at community level, for all community data.

In essence, the structural equation model created was a representation of a top-down, bottom-up scenario where geologically-isolated areas of guano additions would provide nutrient-rich soil areas, stimulating more diverse and abundant microbial communities in those areas.

7.2. Methods

7.2.1. Site locations, data acquisition and modeling methodology

Sites locations, data acquisition and modelling methodologies are described in Chapter 2.

7.2.2. Data treatment.

Soil chemical measurements were found to exhibit right-tailed skew so were log (normal) transformed, as was altitude, to maximise normality. Variables were then standardised according to the PRIMER 'normalise' function to account for differences in scale. Outliers observed were mainly found to be related to sites 33 and 79, which was soil sampled in close proximity to a large penguin and seal colony at Port Lockroy on Wienke Island. Based upon the likely unique and influential location and that the patterns in environmental data reflected patterns found in ELFAs, we decided against removing the outliers. Instead we down-weighted sites 33 and 79 by standardising against all Wienke Island sites, so we would still maximise trends in variable relationships. Based upon variance inflation factor analysis (VIF), longitude was omitted from analyses as it was found to be highly collinear with latitude (R=0.89), we considered latitude to be more likely to be influential over other environmental structures present.

7.2.3. Assessing SEM model fit:

All models were built using the software package M-PLUS 4.2 (Muthen and Muthen, 2006). An initial model was run according to the measurement model (Figure 7.1), for which model iterations could not be completed, therefore indicating a failure in the model. When

parameters were reduced to a simple model, all individual parameters could be incorporated into a working SEM, so the problem was deduced to be related to the number of samples and/or the number of parameters to be estimated. In order to cope with latent variable estimation and experimental power, a sample size minimum of 100 sites is recommended, optimally over 200. For our taxonomic diversity and species community data 49 and 69 sites were available respectively, so our original measurement model was likely to be over-parametised and unable to cope with the small number of sites. Following recommendations by Marsh *et al.* (1998) our small sample size coupled with a low number of indicators (<4) per latent variable meant that the latent variables 'guano' and 'spatial' would be estimated incorrectly, and so were replaced using measured variables for each. This reduced the hypothesis testing of statements i. to iii. (Section 7.1.1.) in their ability to test between latent variables but still enabled the testing of a more simple hierarchical SEM model representing guano influence on soil chemistry and soil chemistry influence on community data.

To minimise model over-parameterisation, whilst still assessing model fit, we used a bottom-up model building strategy where abundance and richness were first retained as the key parameter for all models. Then, factors related to richness and abundance were eliminated, based upon their lack of statistical significance, in a step-wise fashion until only the significant factors were remaining. We then hypothesised a new model from this in order to predict the likely pathways in which these factors could be linked to the environment.

Species Richness or Abundance was first selected for both microbial ELFA and 454-bacterial community datasets separately, to be used as the response variable in the final models. This resulted in 4 path models, these were microbial abundance, microbial richness, bacterial relative abundance and bacterial richness. All soil chemical variables were then used as predictors of community and the initial models were run to explore the relationship between these predictors and each community dataset. The least significant, non-significant variables were then deselected for the next step in the modelling procedure, until a basic model with significant pathways and reasonable fit statistics was found. Birds and Seals were then added to the initial models as predictors of soil chemistry and the models were re-run with variables selected and de-selected to improve model fit statistics (Table 7.2 shows model fit statistics for best fitting models; see Section 7.3.1 for an explanation of how models were chosen).

Several indices are available that may be used to assess the true model fit, power and comparative model fit for any structural equation model. Goodness of fit was assessed using chi-squared tests, where a significant statistic indicates that the model is not supported by the data. For true model fit, both the Root Mean Square Error of Approximation (RMSEA) and the Comparative Fit Index (CFI) are not heavily influenced by sample size or non-normality but concentrate on testing overall hypotheses. Using MLE (Maximum likelihood estimation), Hu and Bentler (1999) proposed that a cut-off of 0.08 for standardised RMSEA together with 0.95 for CFI was the most appropriate approach. Information criteria such as Akaike's information criterion (AIC) and Bayesian information criterion (BIC) may be used to compare multiple similar models for goodness of fit. Models with lowest AIC/BIC are regarded as best-fitting models. Also, adjusted BIC may be used to account for sample size and number of parameters estimated, putting more importance on parsimony.

7.3. Results

7.3.1. SEM model fit:

Final parsimonious models were chosen for each community criterion via addition and deletion of measured variables to attain best overall model fit, removing statistically weak variables (less covariance) until a robust statistical prediction was formed. Simultaneous alterations of interactions between variables, as suggested by modification indices, were also tested in situations where interactions between variables were possible, but unknown. AIC and BIC were used to select between best-fitting models where other criteria could not be used to separate them. Fit statistics shown (Table 7.2.) are a summary of the full model diagnostics (Appendix 6). The chi-squared statistic was non-significant in all final models, indicating no significant difference between predicted covariance structure and that observed in the data. RMSEA were well below the recommended threshold and CFI was 1.00 in all cases, indicating good model fit.

Table 7.2. Summary of model fit statistics for final SEM models

	Chi-square (value, p-value)	**RMSEA** (estimate, probability RMSEA <= .05)	**CFI**
ELFA Biomass	5.752 0.4516	0.000 0.559	1.00
ELFA Richness	0.655 0.8837	0.000 0.907	1.00
454-Bacterial Relative Abundance	0.000 0.9998	0.000 1.000	1.00
454-Bacterial Richness	2.014 0.8472	0.000 0.878	1.00

Notes

For ELFA Biomass, ELFA Richness, 454-Bacterial Relative Abundance and 454-Bacterial Richness SEM models, see Figures 7.2., 7.3., 7.4. and 7.5. respectively.

7.3.2. ELFA SEM model implications:

For ELFA total microbial biomass (Figure 7.2.) and richness (Figure 7.3), the best-fitting models indicated a hierarchy of substrate transfer from ornithogenic origin. Bird presence was associated with higher nitrate levels, though a positive significant pathway to ammonia was not observed. In both models a positive relationship between EC and nitrate was observed, concurrent with a number of investigations where EC has been used to linearly predict quantities of nitrate with reasonable success (Miyamoto *et al.*, 2010).

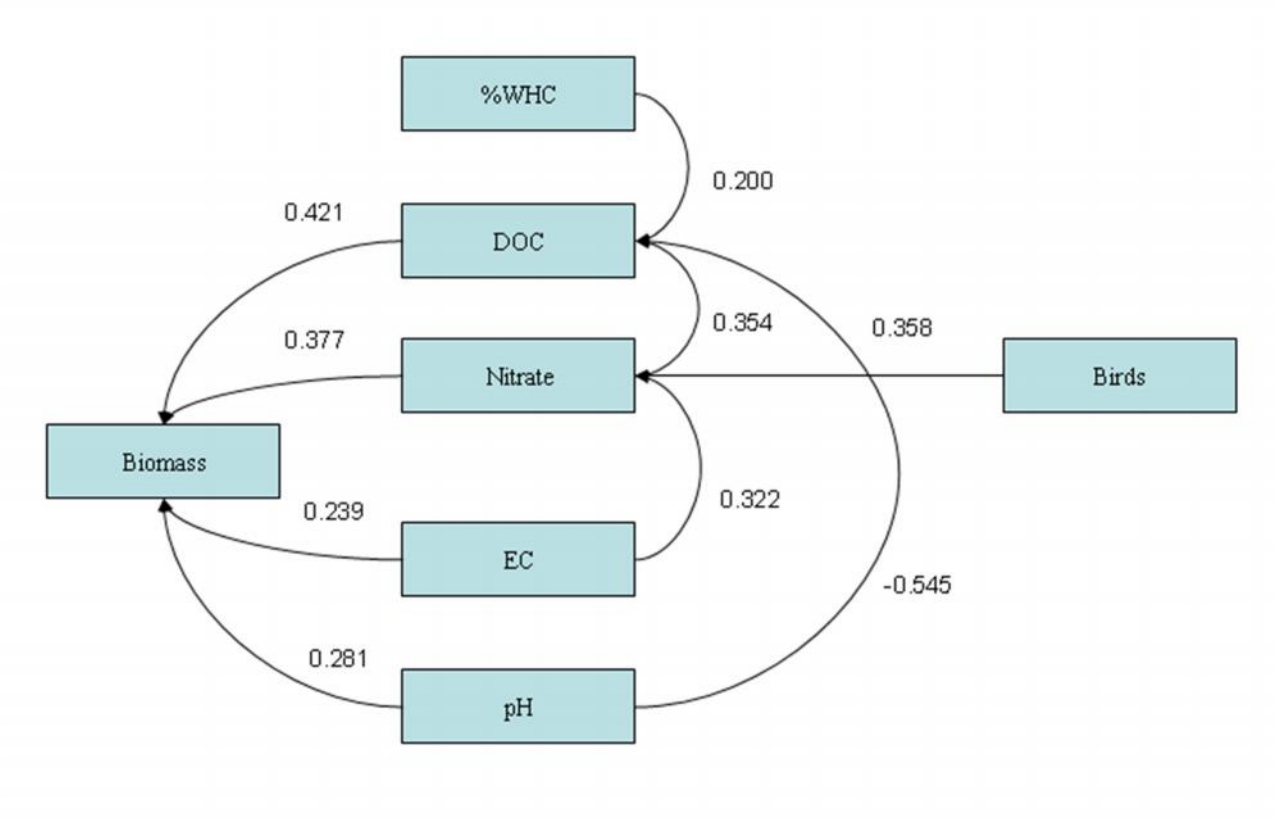

Figure 7.2. Best fit model representing system driving ELFA total microbial biomass. Figures shown are corresponding standardised path coefficients, which indicate the strength of a relationship between a pair of variables and also if the relationship is positive or negative. A value close to 1.0 indicates a strong correlation and a value close to 0.0 no correlation. Only significant terms are shown.

Figure 7.2. shows that DOC was most influential over ELFA biomass with a path coefficient of 0.421 along with, to a lesser degree, nitrate (0.377), pH (0.281) and EC (0.239) respectively. These values indicate a moderate relationship between ELFA biomass and key soil substrates, concurrent with C and N limitation in Antarctic soils. Furthermore, increased nitrate was also associated with increased dissolved organic carbon (DOC) which could imply several things: (i) in areas where DOC exists in highest quantities conditions are favourable for nitrification (not denitrification), thus leading to DOC and nitrate accumulation in soil, (ii) N additions to soil have a facilitating role in C availability, or (iii) that birds guano increases both soil C and N. All of these assumptions consider DOC as a substrate only, though it is known to also be a biproduct of microbial metabolism which confounds the role of DOC in this context. DOC also appears to be more abundant in moist, basic soils, conditions which are likely to be characteristic of soils occupied by bird colonies.

A weak correlation was observed where ELFA biomass was increased in areas where pH was not extremely acidic – these are pH neutral soils close to but not containing active colonies.

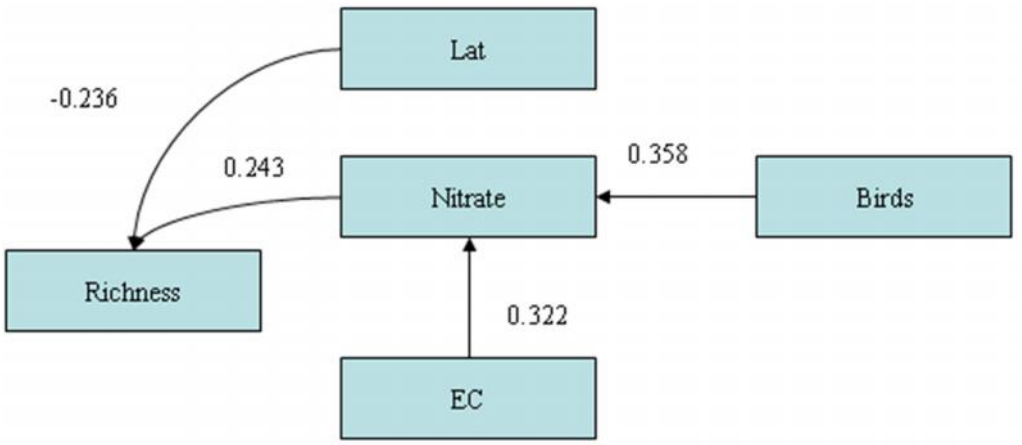

Figure 7.3. Best fit model representing system driving ELFA microbial richness. Figures shown are corresponding standardised path coefficients, , which indicate the strength of a relationship between a pair of variables and also if the relationship is positive or negative. A value close to 1.0 indicates a strong correlation and a value close to 0.0 no correlation. Only significant terms are shown.

Latitude independently was found to weakly influence ELFA richness, with increased richness at lower latitudes. This is an interesting observation and contrary to findings in previous chapters. It should be noted that SEM is not used to indicate a relationship between a single pair of variables but suggests here, that in models predicting microbial community richness, latitude should be included to improve overall model accuracy. Nevertheless, this result was not unexpected as lower latitude sites have a greater capacity for species influx via aoelian, avian or human distribution (Frenot et al., 2005; Turner *et al.*, 2013). Neither of the other spatial parameters; altitude and oceanicity had a significant relationship with the remaining parameters.

7.3.3. Bacterial 454 SEM model implications:

Like the ELFA models, bacterial richness and relative abundance both include a relationship of birds with ammonium and nitrate (Figure 7.4 and Figure 7.5). However the relationship between nitrate and bacteria is negative, inferring high bacterial relative abundance and richness in areas of low nitrate or vice-versa. By establishing birds are responsible for significant input of ammonia, on which nitrate levels are dependant via oxidation, this would suggest nitrate is being lost rapidly from the system in some areas. As these areas are higher in dominant bacterial taxa, lower nitrate could be a response to high rates of denitrification and/or ogliotrophic bacterial use of nitrate. Leaching of nitrate is also a plausible explanation, although a significant correlation in the SEM between nitrate and soil moisture would have better supported this.

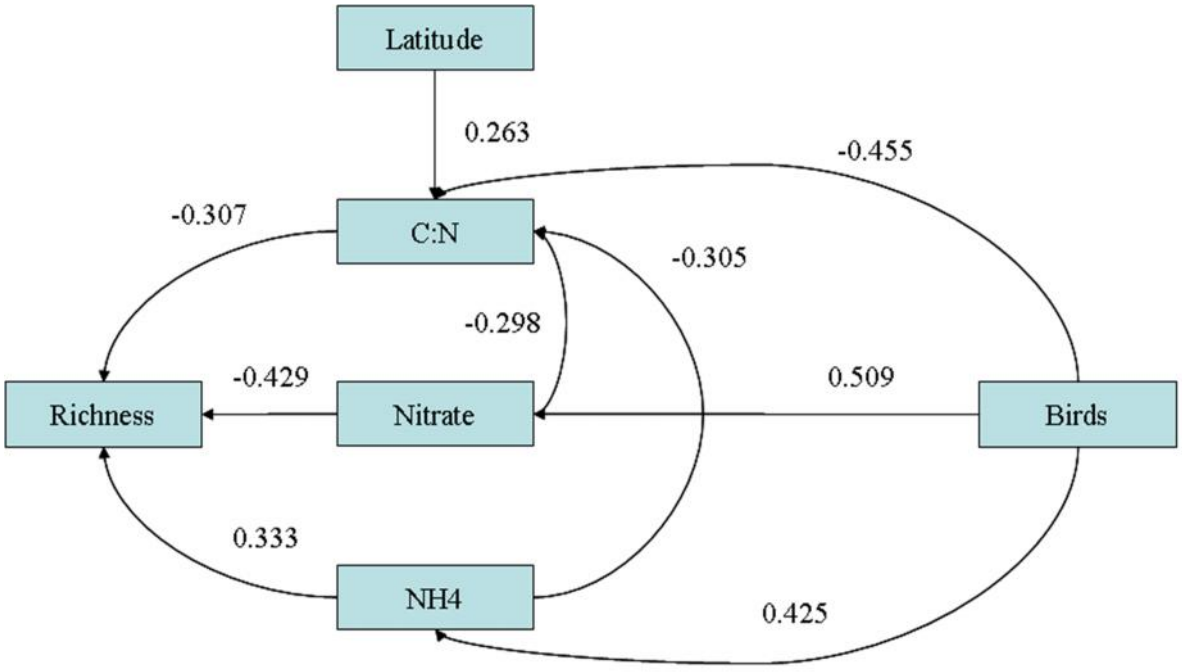

Figure 7.4. Best fit model representing system model driving 454- bacterial richness. Figures shown are corresponding standardised path coefficients, which indicate the strength of a relationship between a pair of variables and also if the relationship is positive or negative. A value close to 1.0 indicates a strong correlation and a value close to 0.0 no correlation. Only significant terms are shown.

The ratio of organic C to total N increases with increasing distance south, but negatively affects bacterial richness. This is logical because inputs from avifauna will become less frequent as habitat conditions become less hospitable for colonisation thus lowering ammonium and nitrate quantities in the soil. Also due to increasing environmental isolation and decreasing temperatures, other contemporary sources of nutrient input and internal C cycling in soil will become restricted, thus total quantities of C and N are likely to be much lower in more southern soils and communities may be both C and N limited.

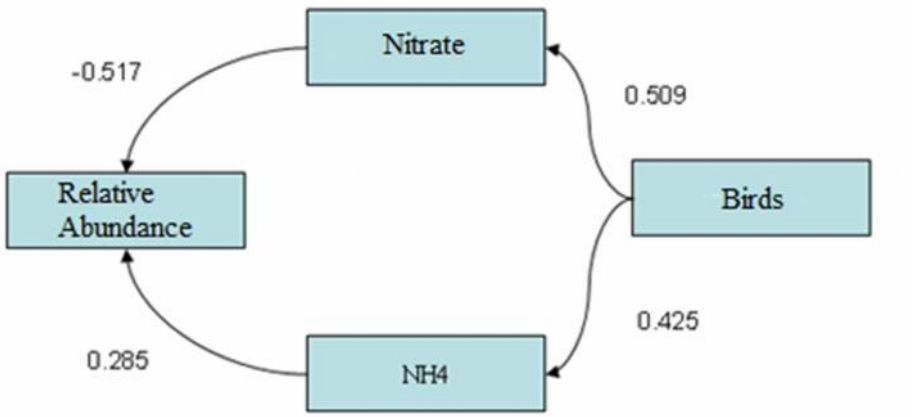

Figure 7.5. Best fit model representing system driving 454- bacterial relative abundance. Figures shown are corresponding standardised path coefficients, , which indicate the strength of a relationship between a pair of variables and also if the relationship is positive or negative. A value close to 1.0 indicates a strong correlation and a value close to 0.0 no correlation. Only significant terms are shown.

7.4. Discussion

7.4.1. Application of SEM for Antarctic food-web analysis

There are several limitations to using a structural equation modeling approach which mean results must be interpreted with caution. Certainly any assumption of process driven activity must be conservative, the path models are formulated from statistical covariance and imply a

predictive relationship, so formulation of any underlying process driver would be purely speculative. Also, the initial path model is created based upon our limited knowledge of how a soil food web functions and can only encompass the variation observed in the variables used. Although, based upon similar multivariate models of soil energy webs, abiotic parameters found to be the most influential such as pH, carbon and nitrogen have been sucessfully included in the final models (Laughlin *et al.*, 2007; Brahim *et al.*, 2011). As Antarctic ecosystems have limited exposure to external nutrient inputs and very simple food web hierarchy, they represent a far more predictable system than other terrestrial environments. Even if it is impossible to perfectly represent the complex interactions between microbial communities and their energy sources, Antarctic ecosystems offer the best opportunities to further our limited understanding.

There were several inadequacies associated with our data set. Due to the nature of sampling in an extreme environment, it was not logistically possible to reach the minimum recommended 200 sites (Kline, 2010), which meant we had to reduce the complexity of the hypotheses which could be represented to avoid over-parameterization. Furthermore, the small number of sites also meant we could not use a split data set to validate our model results, so it must be stressed that the models should be considered only as a means of hypothesis testing in a crude form and results interpreted as such, rather than as proof of causal relationships or underlying process, to form the basis for more comprehensive future investigation.

7.4.2. Model implications

Overall, despite having to simplify the original path models hypothesised, SEM did provide evidence to support some of the hypotheses (see Section 7.1.1.). A relationship between guano additions and soil chemical properties was observed, most consistently for nitrate, where bird presence was positively correlated with nitrate levels in all models, this is compliant with current literature on ornithogenic soils (Aislabie *et al.*, 2009; Cocks *et al.*, 1999). Furthermore, soil hydric parameters such as nitrate, ammonium and DOC were most strongly correlated to variation in the community data, but pH which was expected to have a significant role in all models, showed only a weak positive correlation to microbial biomass and no significant pathways to either bacterial richness or relative abundance. This is contrary to many studies which have found pH to be a dominant influence in temperate

(Fierer and Jackson, 2006; Nicol *et al.,* 2008; Lauber *et al.,* 2009) and polar soils (Chu *et al.,* 2010; Yergeau *et al.,* 2007a)

For final models, the only spatial parameter retained in any of the models was latitude. No significant relationship was found between latitude and avian presence in any of the final SEM models. Avian distribution within polar regions is almost certainly restricted to availability of suitable breeding grounds with coastal access and with increasing latitude, a lack of ice free areas is problematic for some smaller seabirds (Laidre *et al.,* 2008). However, this is not the case for penguins, the distribution of which is often dictated by sea-ice dynamics (Croxall *et al.,* 2002). Though most of the locations sampled in this study were coastal, more than half the sites were not estimated to have substantial ornithogenic input. Therefore this study emphasises the importance of the role of avifauna at the top level of the terrestrial trophic hierarchy in the Antarctic Peninsula region. The effect of guano additions represent a key pathway through which N, and to a lesser degree C flow down to and are responsible for the maintenance of microbial communities.

Labile carbon has been found to be the limiting nutrient for respiration in Antarctic soil (Smith, 2005). We found here that DOC was influential over microbial communities as a whole, correlated with increased microbial biomass. The exact nature of DOC dynamics is difficult to elucidate due to a dual role as both a substrate and bi-product of microbial metabolism, but DOC fluxes are primarily hydrologically driven (Neff and Asner, 2001). Frequent freeze-thaw cycles across much of the Antarctic Peninsula exert the greatest stress on soil organisms themselves (Yergeau and Kowalchuk, 2008), though it is the moisture content of the soil which determines the distribution and availability of soil substrates. As many of our soils were well below optimum field moisture holding capacity (60-80%), with an average of 59%, much of the soil nutrient pool may be unavailable, though DOC is likely to be conserved. The strong association between microbial communities and DOC may represent an ogligotrophic response to the most readily available form of carbon (Hua *et al.,* 1999). pH had a minor positive correlation with microbial biomass and a negative correlation with DOC. Since our range of soils were pH 4.4 to 7.8, higher biomass was most likely associated with pH neutral soils, this is supported by trends in the raw data, and is congruous with a lack of a monotonic relationship between pH and abundance presented in earlier chapters. DOC quantities were negatively related to pH, and the relationship between parameters was most strongly illustrated by highest quantities of DOC found in areas of low

pH. Interestingly, these are mostly areas which have a moderate rather than a strong influence of ornithogenic input.

Organic carbon and total nitrogen were represented in the models in the form C:N and a significant pathway was found only to bacterial richness, on which high C:N negatively affected richness. Though richness gives no indication about the metabolic output of the community, a lower diversity of species in a community is usually congruent with a lower functional capability. This would be in accordance with a system where C mineralization is N-limited, as other recent studies of Antarctic soils have also implied (Malosso *et al.,* 2003; Hopkins *et al.,* 2008; Dennis *et al.,* 2012 Yergeau *et al.* (2007b) found that C:N, as well as freeze-thaw cycle frequency and pH, were also related to the expression of N-cycle related genes.

A direct correlation between nitrate-N and birds was significant in all models, and between ammonium-N and birds in bacterial communities only. The model suggests high richness and bacterial relative abundance (and therefore low eveness) are associated with areas of high ammonium. As ammonia oxidisers are rare or absent in some Antarctic soils (Vishniac, 1993), this could lead to an accumulation of ammonium. High microbial biomass and richness were associated with high nitrate, but in these areas bacterial relative abundance and richness were low. This seems somewhat paradoxical as bacteria are a major contributor to the soil nitrate pool, although ammonium oxidisers are a highly specialized group of bacteria, comprised of only a few genera (Kowalchuk and Stephen, 2001). A possible explanation for this could be that, in the areas where bacterial abundance is higher, denitrification rates could be rapid. Yergeau *et al.* (2007b) also found that nitrate and nitrite reductase genes (denitrifying genes) were most associated with areas where quantities of nitrite were low. Electrical conductivity in soils is known to correlate with nitrate concentration (Miyamoto *et al.,* 2010) and is demonstrated in other studies (Aislabie *et al.,* 2009) and here, by a positive path between them. High EC, as well as nitrate, becomes characteristic of ornithogenic soils in this way.

Several parameters had little or no influence in the final food web models. Amino acids provide both a source of C and N to microbial communities and constitute a large proportion of organic N in Antarctic soils, however turnover is thought to be extremely rapid (Jones *et al.,* 2004). The lack of any relationship between amino acids and microbial community in the

SEM analysis, in this instance, may highlight a problem with the final models, reflecting the inadequacy of measuring soil characteristics at only one time point to truly represent highly changeable ecosystem dynamics. Also it may be that by looking only at cumulative amino acid quantity in soil, we have masked the influence of one or several highly influential amino acids which are better indicators of microbial utilization. A lack of influence of P was also found, which is unexpected in the context of the model as high soil P is associated with ornithogenic soils and has been found to inhibit biomass, respiration and N-mineralisation (Tserchko *et al.*, 2003).

7.4.3. Conclusions

Though the value of this modelling approach lies in understanding Antarctic food web dynamics by allowing us to test hypotheses about key system mechanisms and better revealing the interactions between substrates, SEM may not be the most appropriate technique. In the context of polar studies, our dataset may be regarded as sophisticated, but the complexity of such a modelling approach may require a level of data complexity which would be very costly to collate. Given the constraints of data collection in such extreme environments, the future value of approaches such as SEM, will be in experiments with highly specific hypotheses. However, the repetition with which an ornithogenic food web hierarchy is presented in all our models cannot be ignored. These models have strong implications about energy flow and form a hypothesis for further study.

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8

Synthesis

Antarctic ecosystems are driven by abiotic processes (Hogg *et al.*, 2006). In this study we present evidence through the modelling of our data that penguin and seal guano presence - by the means of guano additions - increase carbon and nitrogen substrate distribution in Antarctic soil (Chapter 7), which creates areas of chemical heterogeneity in different areas of the Antarctic Peninsula (Chapter 4). Microbial communities are more diverse in these locations (Chapter 3) and specific microbial and bacterial taxa can be linked to specific environmental conditions (Chapter 6). There is no evidence of decreasing diversity with increasing latitude, but there is evidence to suggest a north/south divide in community composition around 66°S. Spatial structuring of bacterial communities is best demonstrated at genus level with the presence of a broad-scale gradient, but can only weakly be linked to a dependency on the environment, inferring there may be other mechanisms behind bacterial distribution (Chapter 5).

There is a recent school of thought that, contrary to classical ecology, the diversity of microorganisms can be predicted by latitude, with studies finding decreased diversity at higher latitudes (Hughes-Martiny *et al.*, 2006; Fierer and Jackson, 2006; Lauber *et al.*, 2009; Chu *et al.*, 2010). Examination of Shannon and Simpson indices both for each site and across all sites we found no clear evidence of this for Antarctic soils. We also modelled a species turnover gradient (beta diversity) where links between sites implicitly tested for a gradient by measuring autocorrelation along the Peninsula and found no evidence of a linear relationship with latitude and diversity. Latitudinal effects are observed over smaller ranges in temperate soil, so over 68 sampling points of microbial communities at 25 sites and over a 2000km transect, we must reject the hypothesis that there is a linear relationship present for the Antarctic Peninsula soils. We did observe that bacterial abundance was low at the most southern site sampled, Mars Oasis, but this contrasted with high richness.

This does not mean latitude is not an important factor in the governance of biological communities. If we accept decreasing latitude is not the mechanism behind microbial distribution but is instead linked to decreasing temperatures, it's probable that latitude is intrinsically linked to the level of functional dormancy within a community, where soil substrates remain unavailable due to low soil moisture availability, irrespective of the level of nutrient supply. This is best demonstrated in the soils of the McMurdo Dry Valleys, where organic matter content is high though turnover is restricted by low soil temperatures (Parsons *et al.,* 2004; Hopkins *et al*., 2006).

The change in microbial community composition at 66°S was attributed to a change in the soil environment as other abiotic conditions change between north and south Peninsula areas (Lewis-Smith, 1984). This boundary marks the Antarctic circle, where there is a lack of ice-free areas and few islands (Peat *et al.,* 2007) , avifauna have been observed at this latitude in abundance, after which distributions become more scarce (Ainley and Jacobs, 1981). As this study has found, avian inputs to soil can be an important driver. We note that the influence of latitude over ornithogenic presence potentially does affect microbial communities, but a relationship between bird distribution and latitude has not been reflected in this study. This may be due to a lack of detailed observational data or a lack of sites south of 66°S to substantiate this.

The quality of the soil environment is, however, a fundamental driver here and in other studies of microbial diversity (Fierer *et al.,* 2012; Pointing *et al.,* 2010) and abundance (Aislabie *et al.,* 2009). High nutrient quality in Antarctic Peninsula soils is related to organic matter input from ornithogenic sources which can be considerable yet patchy in distribution. Ornithogenic soils are characterized by high concentrations of nitrogen and phosphorus, high electrical conductivity and variable, but often basic pH (Barrett *et al.,* 2007; Aislabie *et al.,* 2009). In these areas, microorganisms also have the ability to function optimally when temperature allows. This has been observed in the laboratory where C-substrate priming has occurred with substrates containing N (Malosso *et al.,* 2003; Dennis *et al.,* 2013), which suggests C mineralization may be N-limited for many soils. In northern locations such as South Georgia, where mean summer temperatures are higher than those on the Antarctic Peninsula (BAS, 2012), mineralization of N and C from guano is very rapid, and around 50% volatization was recorded at 3 weeks in King George Island soils (Myrcha and Tatur, 1991).

In temperate soils, a correlation as great as 0.9 has been reported to describe the relationship between bacteria and organic matter distribution (Ranjard *et al.,* 2000) though here the highest correlations we found were 0.52 and 0.43 between nitrate and bacterial abundance and richness respectively in the Structural Equation Models.

The relationship between soil nutrients and microbial diversity is an example of spatial dependence, of which, we found supporting evidence in Chapters 4,5,6 and 7. Spatial dependence is one of many hypotheses used to describe how organisms are distributed throughout their environment. Though abiotic parameters have already been identified as drivers of diversity, another such hypothesis is that of historical provenance. Examples of biological regionalization in various metazoan, bacteria and algal taxa have recently been observed across terrestrial Antarctica (Allegrucci *et al.,* 2006; Maslen and Convey, 2006; McGaughran *et al.,* 2009; Vyverman *et al,* 2010). These communities have been attributed to areas of historical refugia, where some regions may have maintained a more hospitable eco-climate than others during periods of climatic extremes, such as glacial maxima (Newman *et al.,* 2009). These two hypotheses are not mutually exclusive but, to our knowledge, no studies have attempted to incorporate both into a mechanistic model of Antarctic communities. Results here do not discourage the existence of microbial provinces. Indeed Multiscale pattern analysis identified a gradient in the bacterial community data which was unexplained, but the results generally support a gradient which is more likely to be related to the heterogeneity of the environment, and the chemical profile that the environmental variance imposes (Fierer *et al.,* 2012).

Distance is considered to be the most influential dynamic on environmental heterogeneity and community structure (Legendre and Fortin, 1989; Tilman and Karieva, 1997; Jombart *et al.,* 2009). Antarctic ecosystems are typically extremely heterogeneous (Hopkins *et al.,* 2006; Chown and Convey, 2007). This makes community response to environmental changes difficult to predict, though prediction is at the forefront of Antarctic research due to the rapidly changing climate. Though perhaps coarsely modelled, we found it may be possible to catalogue major characteristics of the few higher trophic influences and their corresponding soil profiles.. Already ornithogenic, fellfield, mineral and soils specific to higher plant species have been chemically and biologically profiled (Yergeau *et al.,* 2007b; Aislabie *et al.,* 2009; Teixeria *et al.,* 2010). The simplistic environment which allows the disentanglement between such influences should be utilized in future studies to maximize prediction potential. This is

in agreement with current motivations adopted at the 31st Antarctic Treaty Consultative Meeting (ATCM) where the implementation and development of existing environmentally bespoke domains (Morgan *et al.*, 2007) was encouraged as part of a larger drive towards future conservation (Terauds *et al.*, 2012).

Distance also needs to be better incorporated into experimental design. Too few studies employ random spatial design in extreme sampling environments, which is likely to hamper the validity or power of results (O'Donnell *et al.*, 2007), especially as environmental conditions are of such importance. MSPA was employed here to distinguish between global (often abiotic) processes and those at smaller scales. This would have been better employed if not restricted to the consideration of broad scale (2000km) variation, with many km between separate microbial communities, which have the prospect for many varying scales of ecological interactions but may be too coarse for revealing biological interactions. An experimental design incorporating incremental increases in scale, in conjunction with microbial community components may give the best distinction between biotic and abiotic processes which are scale dependant.

A Structural Equation Modeling (SEM) approach has been used before successfully with soil chemical parameters in ecological studies (Grace, 2006; Unger *et al.*, 2012) and here provides some simple but ecologically vital models to represent key nutrient relationships in Antarctic Peninsula soils. The major caveat in our modeling approach was a lack of sampling locations to be able to fully utilize the modeling power of SEM, as we had to remove many variables before we were able to run a basic model which was not over-parametised. Also, the ability to include functional gene abundance into a model with some additional process measurements could yield a level of understanding about nutrient flux which has not been possible before, as seen in a recent paper by Petersen *et al.* (2012) for Alaskan soils.

Top-down control in ecological terminology is vague, but often taken to mean 'predation'. However, in the context of Antarctic ecosystems it must instead represent 'limitation', a term usually reserved for bottom-up analysis, and used to describe the limitations imposed by the environment. In a wider context, limitation needs also to be seen as the top-down boundary conditions superimposed by the environment and/or spatial scale. Antarctic environments are not without predation, indeed nematodes which are the top microbial predator are highly abundant throughout maritime soils (10^6 individuals $m^{-2)}$ (Maslen, 1981). However, the

importance of biotic processes such as predation on terrestrial ecosystems is conflicted by spatial dependence exhibited by these predators, whose abundance has been attributed to soil moisture and temperature (Maslen, 1981). Oksanen's synthesis (Oksanen *et al.*,1981) argued that effective top-down forces are only likely to occur in habitats with high primary productivity, since low productivity habitats do not have functional upper trophic levels. We suggest here that Antarctic soil food webs represent an exception, where a lack of classic predators are replaced by a climate which limits the distribution of avifauna and other external influences and therefore guano imput and the consequential limitation on organic matter.

The approach taken in this project has been to design modelling and analysis techniques to interpret and to give insight into the diversity of microbiology from a series of provided datasets, and has used a variety of statistical techniques to inform on diversity gradients and spatial trends. Whilst the modelling approach has identified diversity gradients (Chapter 3 and Chapter 4), and has been able to relate these at different scales to environmental conditions (Chapters 5 and 6), perhaps the most important outcome of applying this modelling approach has been to identify its inherent restrictions and the potential pitfalls in retrospective analysis of data collected without thought to the analysis techniques available to make good use of this data. Seen throughout the analysis and discussions is evidence that variation and diversity between sites, correlation to latitude, environmental conditions and locations cannot be easily separated by the use of standard indices. The permutations for influence on diversity are complex, as is the ecosystem that is influencing community structures. The approach to understand this therefore needs to be sensitive to these complexities and must allow for variations that cannot be quantified by simple laboratory testing or mathematical equations. As has been shown in this project, no one model or 'sum', can adequately describe the observations, it is only by utilising a range of techniques that this can be understood.

The recommendation following this project would be to consider the techniques that have been applied, construct a meta-framework of the data needs and data outputs. Starting from a top-down hypothesis for a process model that describes the systems, designing the modelling approach and the suite of models that would do this, the raw data requirements will be derived, and the potential impacts of error understood. This approach would then define the

data collection and laboratory test, to provide the greatest efficiency in moving the question of ‘what drives microbial diversity?’ forward.

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Appendix 1: Site Field Note Descriptions

Site Descriptions

These descriptions were recorded in field at the time of sample collection and provide supplementary information about individual sites within locations. Locations are listed alphabetically.

Alectoria

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site Description
13	Alectoria	Alectoria	25	64.16	59.14	NNW	Small island rock out crop NW or James Ross Is close to peninsula coast. Mostly composed of heavily fractured gneiss and schist intruded with quartzite. Abundant mosses and lichens. Soil was course grain with fractured rock
86	Alectoria	Alectoria	50	64.16	59.14	NNW	Small island rock out crop NW or James Ross Is close to peninsula coast. Mostly composed of heavily fractured gneiss and schist intruded with quartzite. Abundant mosses and lichens. Soil was finer texture and wet from snow melt.

Alexander Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
1	Alexander Island	NW Peninsula	62	69.37	72.02	NE	Sample taken from below large snow patch where there was a 2-3 cm deep peat bed. Soil collected from between moss patches and where soil had been washed down on top of moss.
11	Alexander Island	NW Peninsula	62	69.37	72.03	NW	Soil from between rocks beneath snow patch with abundant algal/cyano-bacterial mat in vicinity.
12	Alexander Island	NW Peninsula	70	69.62	71.99	NNW	Un-vegetated site on W facing side of headland. Few mosses and lichens in the vicinity. Frost-shattered rock fragments.
22	Alexander Island	Mount Holt	?	69.54	71.89	?	?
26	Alexander Island	Mount Holt	501	69.54	71.89	N	Soil surrounded by large rocks and sparse cryptogams on top of some cliffs. In an area that had previously been covered with snow. In the videos I use the wrong name it is not Rothschild Island although the large bags are labelled Rothschild as well.
75	Alexander Island	NW Peninsula	64	69.53	72.00	SW	Some mosses in vicinity. Sample taken from un-vegetated area damp because of snow melt. Frost shattered rock fragments at surface. Finer texture beneath.
85	Alexander Island	NW Peninsula	66	69.39	72.02	Flat	Soil from un-vegetated area of fell field site. Relatively fine textured soil. Mosses and cyanobacteria/algae in vicinity

Antarctic Peninsula

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
82	Antarctic Peninsula	Pass	1617	63.70	57.93	E	Apparently organic rich soil outcrop in amongst many rock about 5 m down from the summit where Andrew Fleming was taking his GPS measurement. No vegetation, no lichen although there was sparse coverage in the area.
89	Antarctic Peninsula	Pass	1612	63.72	57.95	S	Nearly at the top of the mountain. Soil was found in the cracks between rocks where a few lichens could be found

Berthelot Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
93	Berthelot Island	Berthelot Island	52	65.50	64.29	Flat	Plateau with abundant mosses and *D. antarctica* in the vicinity. Soil un-vegetated collected from hollows between rocks.

Blaiklock Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
30	Blaiklock Island	Blaiklock Island	7	67.69	64.29	Flat	Sample taken from un-vegetated area above raised beach. Some grass, pearlwort, mosses and lichens in vicinity.

Cape Evenson

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
59	Cape Evenson	Antarctic Peninsula	58.8	66.33	65.75	N/A	Rocky soil collected top of cliff. Skua's in area. No vegetation (extreme sparse moss) but not on sampled soil. Raised from melt of glacier behind so not elevated in moisture as a result.
67	Cape Evenson	Antarctic Peninsula	30.8	66.33	65.83	W	Soil collected from a shelf on a cliff. Skua's in area. Some vegetation (moss) but not on sampled soil. X 1.1 is just top 3 cm X 1.2 is all depths
76	Cape Evenson	Antarctic Peninsula	12	66.33	65.83	N/A	Soil collected from base of cliffs. The sediments from above are likely to have been washed down and accumulated at the base. The soil was oak coloured and had a sandy silt texture. The soil was structured in large (30 x 50) blocks that were probably 5

Deception Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
16	Deception Island	Whaler's Bay	91	63.14	60.59	N/A	Volcanic soil with rocks < 1cm or equal. Washed down volcanic ejector mid way up the hill from site 1. Next to a yellow boulder possibly rich in Sulphur. No vegetation.
18	Deception Island	Whaler's Bay	105	63.12	60.66	N/A	Soil surrounded by large rocks and sparse cryptogams on top of some cliffs. In an area that had previously been covered with snow. In the videos I use the wrong name it is not Rothschild Island although the large bags are labelled Rothschild as well.
98	Deception Island	Whaler's Bay	14	63.13	60.77	SW	Volcanic soil ashy texture. Next to yellow rock covering the summit of the hill. No vegetation.

Detaille Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
87	Detaille Island	Detaille Island	26	66.89	66.86	Flat	Soil sampled between shattered rocks on highest point of the island. Some sparse lichen, moss and algae growing epilithically.
94	Detaille Island	Detaille Island	21	67.01	66.78	Flat	As with Detaille 1, but from a more sheltered site where some wind-blown feathers have collected.

Gand Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
71	Gand Island	Gand Island	25	64.51	62.98	WSW	Ice and stone chute above beach and headland. The "soil" had been washed down in meltwater and overlay ice. Some moss and lichen in vicinity.

Greenwich Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
3	Greenwich Island	North beach	16	62.64	59.95	S	Soil collected at the foot of a rocky outcrop. Surface covered with shattered small stones (presumably from out crop). Mosses and lichens in the vicinity and also, guano, feathers and bones (Petrel nests on the outcrop). Soil collected from and unvegetated.
15	Greenwich Island	North beach	13	62.67	59.78	N	

James Ross Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
28	James Ross Island	Cape Lachman	104	63.83	57.94	N/A	Volcanic soil, dry, very sparse lichen (none on sample). More rocky than the other sites. On N facing slope 4 m below Col plateaux
40	James Ross Island	Cape Lachman	85	64.04	57.84	S	E side of Col on high ground very sparse lichen but none on sample area. Appears dry to 3 cm depth. Mod shelter from rock outcrop on N side, open to South Hi
99	James Ross Island	Cape Lachman	81	64.02	57.79	NW	Volcanic with very sparse lichen cover (none on sample). Close to Helio landing spot, just to N. West of Col of site 2

Jenny Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
51	Jenny Island	Jenny Island	12	67.97	68.60	NE	Raised beach pebbles and some larger rocks that had fallen from higher cliffs. Several locations where fur seals lie. Both D. *antarctica* and *Colobanthus* in the vicinity, as well as lichens and mosses.

King George Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
14	King George Island	Round Point	7	61.95	58.63	Flat	Tombolo beach formed since the 1950s aerial photo. Beach now joins Round Pt with what was once a small island that contains a significant penguin colony. The land surface is un-vegetated, but there are abundant Fur and Weddell seals and penguins, guano, feathers.
37	King George Island	Brazilian Base	64	62.13	58.41	N/A	About 50 m from site 1 down a hill and then up again. Very sparse lichen and moss. Sample taken from vegetation free area. Not very rocky. Dry on surface but reasonably moist 1cm down.
43	King George Island	Round point	65	62.16	58.47	ENE	Edge of penguin colony on "island" at end of beach. Lots of chinstraps all around, also some fur seals. Lots of guano and feathers in and near the soils.
68	King George Island	Brazilian Base	12	62.13	58.66	W	On a hill about 500 m behind the Brazilian base. Modern lichen very sparse moss. Sample taken from vegetation free area. Quite rocky.

Lagoon Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
88	Lagoon Island	Lagoon Island	20	67.76	68.46	SSE	Soil collected on a small peak beyond the beach. The area was very rocky and cryptogams were common although these were sparse on the soil collected. There were many skuas, fur seals and some elephant seals on the beach. There were also Adelie penguins nearby.

Livingston Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal Degrees	Aspect	Site description
34	Livingston Island	Byers Peninsula	64	62.73	61.19	SW	About 1km from sea over beach raised beach and then eroded cliff to a plateau with very sparse lichen and moss cover (not in sample area). Snowing at time of sampling. DH des - Plateau above raised beach at centre of Byer's Peninsula coastline. Abundant mosses
70	Livingston Island	Byers Peninsula	75	62.70	61.23	WSW	About 40 m from site 3 towards mid lands. Very wet thixotropic soil similar to Wynn Knolls, Signy. D. Antarctica still green surrounded by moss. Moss removed to obtain rhizosphere soil and bulk soil taken from just outside the moss are so vegetation free. Lichen present.
80	Livingston Island	Byers Peninsula	78	62.70	61.22	Flat	About 15 m from sample site 1 to left when facing the sea. Still on plateau but completely different soil type. Apparently frost sorted such that finer materials are nearer the surface. The consistency was like peanut butter. DH des - Fine textured soil.
92	Livingston Island	Byers Peninsula	64	62.74	61.20	SSW	Further up from plateaux about 120 m to left of site 2 when facing sea. Bleached D. Antarctica. Relatively moist when compared with site 2 with larger rocks. DH. Des - Sample collected in wet area inland from plateau, where snow and melt-water have accumulated.

Mars Oasis

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal Degrees	Aspect	Site description
102	Mars Oasis	Near Melon Hut	15	71.98	68.38	N/A	Surface soil collected from level ground consisting of dry crushed rock and fines. Large area of ground with no evidence of recent snowmelt. No vegetation or cyano-bacterial mat cover.
103	Mars Oasis	Near Melon Hut	15	71.98	68.38	N/A	As above
104	Mars Oasis	Near Melon Hut	15	71.98	68.38	N/A	As above
105	Mars Oasis	Near Melon Hut	15	71.98	68.38	N/A	As above

Nelson Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
64	Nelson Island	Edward point	26	62.63	59.66	Flat	Flat land on top of the headland 50 m from lighthouse. Many skuas, some nests at site and albatross nearby. Fell-field soil with abundant mosses and lichens. Soil samples taken from un-vegetated part of the polygons.

Port Lockroy

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
33	Port Lockroy	Port Lockroy		64.82	63.48	Flat	Gritty soils not immediately beneath penguins
79	Port Lockroy	Port Lockroy		64.82	63.48	Flat	Ornithogenic soil close to the PLR hut - lots of Gentoo guano and feathers.

Rothera

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
24	Rothera	ASPA	35	67.58	68.35	Flat	Boulder field at the highest point within the Rothera ASPA. Abundant lichens and mosses in the vicinity, also some D. ant. Soil collected from between rocks.
31	Rothera	East Beach	6	67.60	68.34	Flat	Sample collected by Mark Gorin (with gloves). No other information

Rothschild Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
32	Rothschild Island	Overton Peak	469	69.81	72.19	N/A	Sample collected by Mark Gorin (with gloves). No other information
62	Rothschild Island	Overton Peak	414	69.72	72.28	N/A	As above.
96	Rothschild Island	Overton Peak	405	69.81	72.06	N/A	As above.

Seymour Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
4	Seymour Island	Marambio	160	64.28	56.77	N/A	Windblown sand sandy soils containing large number of fossils and volcanic ejecta collected on headland between two melt water gullies. No vegetation in the vicinity. Samples collected about 0.5 km to the N of the airstrip at the Argentinean base.
9	Seymour Island	Marambio	190	64.28	56.77	N/A	As above.

Signy Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
106	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	Thixotropic soil very wet and frozen at 10 cm depth. Covered with frost shattered angular rocks and lichen some moss also in surrounding area. Some Skuas but no seals, the highest point around Jane Col area.
107	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	As above
108	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	As above
109	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	As above
110	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	As above
111	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	As above
112	Signy Island	Wynn Knolls	199	60.72	45.85	N/A	.As above

South Georgia

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
27	South Georgia	Bird Island	422	54.24	38.05	N/A	Just 10 m down from site 4. In an area of tussock but with large mainly vegetation free areas. Soil sampled from a mainly vegetation free area (just some tiny green plants < 1 mm).
49	South Georgia	Bird Island	430	54.23	38.05	N/A	Site followed cliffs around towards the base. This was completely vegetation free with very sparse lichen coverage only.
60	South Georgia	Bird Island	480	54.03	38.31	N/A	Upland Meadows. Soil sampled next to a cliff falling to the sea. The area was vegetation free but may have been a blow out. Sparse lichen and moss.

Trinity Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
8	Trinity Island	Spert Island	93	64.02	60.98	N/A	Vegetation free soil surounded by rock and some snow. Soil quite moist and contained more clay and silt than site 10.
10	Trinity Island	Spert Island	92	64.02	61.18	N/A	Vegetation free sandy soil next to a cliff. Very low MC.
83	Trinity Island	Spert Island	92	64.03	60.96	NW	Vegetation free soil down from knoll in a rocky area where melt water is running down to the pond.
84	Trinity Island	Spert Island	103	64.03	61.20	N/A	Melt water pond 10 m2 1m deep in middle, 10-20cm around the sample. The sediments had some orange pigment on the surface. When the sediments were disturbed, bubbles with a methane like smell came to the surface
91	Trinity Island	Spert Island	106	64.02	60.98	NE	D. Antarctica on a vegetated (lichen and moss) knoll up from but very close to site 8. Took rhizosphere and bulk soil.

Wiencke Island

Site	Location I	Location II	Altitude (m)	Latitude Decimal degrees	Longitude Decimal degrees	Aspect	Site description
23	Wiencke Island	Near Port Lockroy	2	64.89	63.76	Flat	Pebbly plateau above beach. Mosses in vicinity and some feathers and guano, but not a colony site.
50	Wiencke Island	Near Yelcho	6	65.06	63.59	WNW	Vegetation free orthinogenic soil. On rock outcrop near rocky beach frequented by birds. At base of glacier so lots of sediment deposited and enriched with guano. 20 m from site 50.
58	Wiencke Island	Near Yelcho	5.9	65.07	63.60	WNW	Vegetation free orthinogenic soil. On rock outcrop near rocky beach frequented by birds. At base of glacier so lots of sediment deposited and enriched with guano. 20 m N from site 50. Base of glacier. Area of soil that was browner than the rest.

Appendix 2: Chapter 3 Supporting Analysis

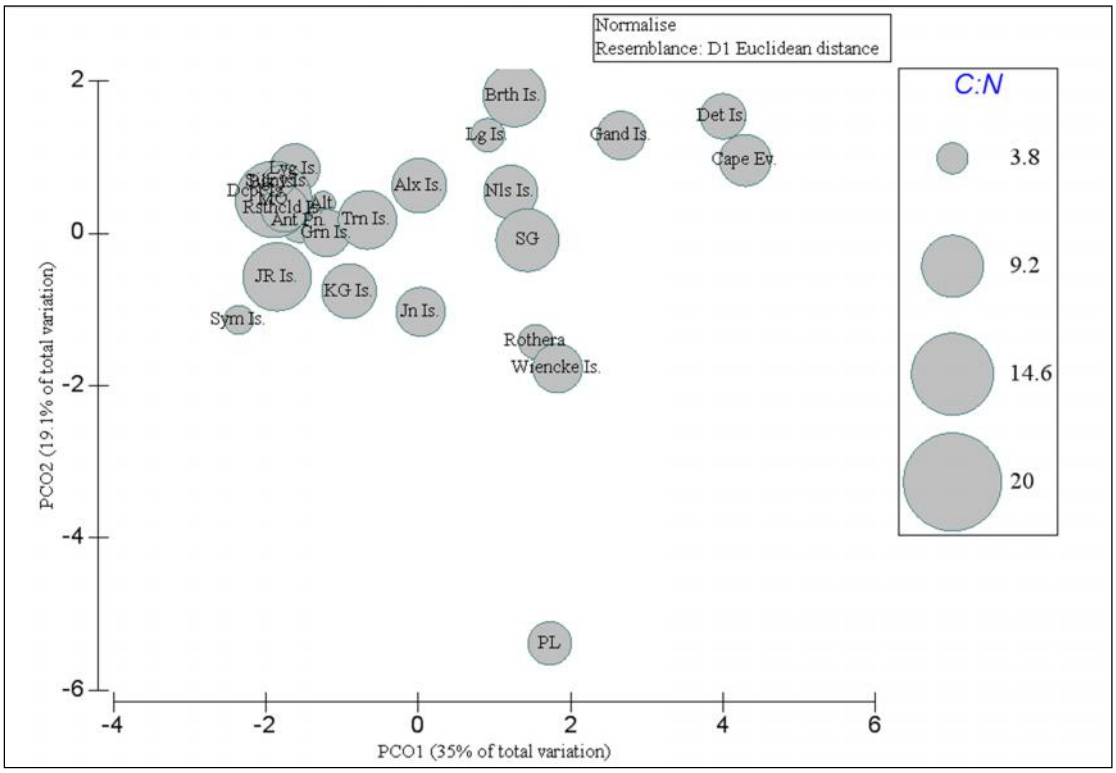
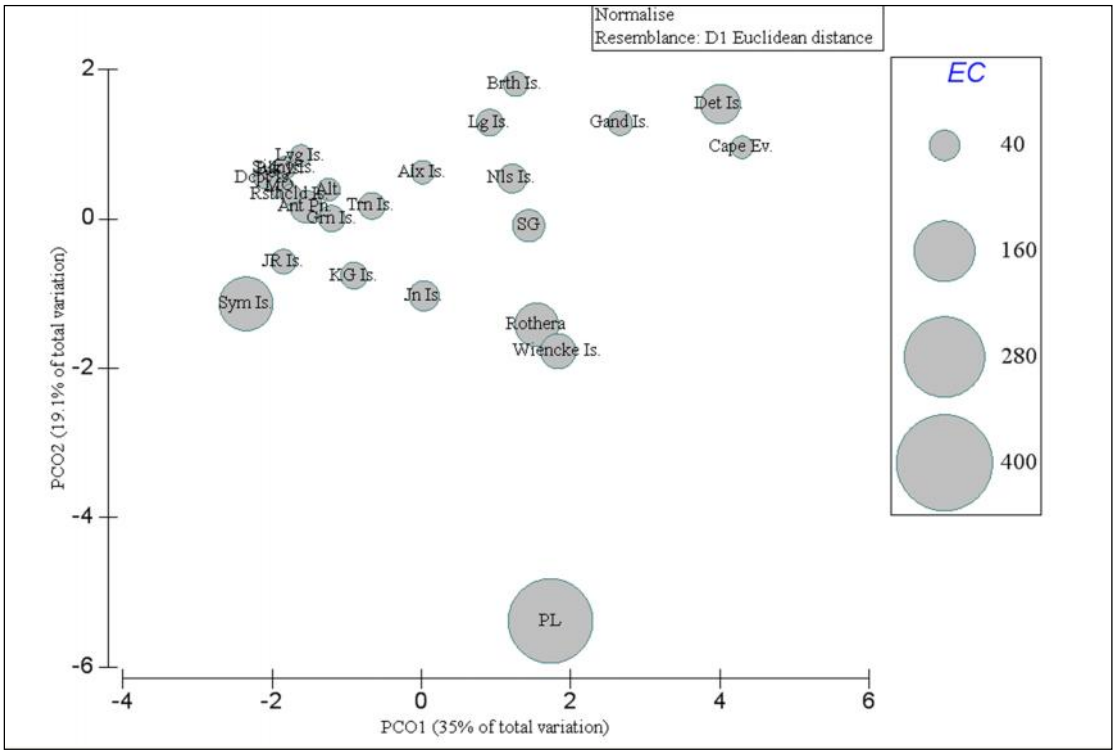


Figure A2.1. PCO of C:N: PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of C:N ratio. Scale for each bubble graph is representative of the individual data range for each variable.



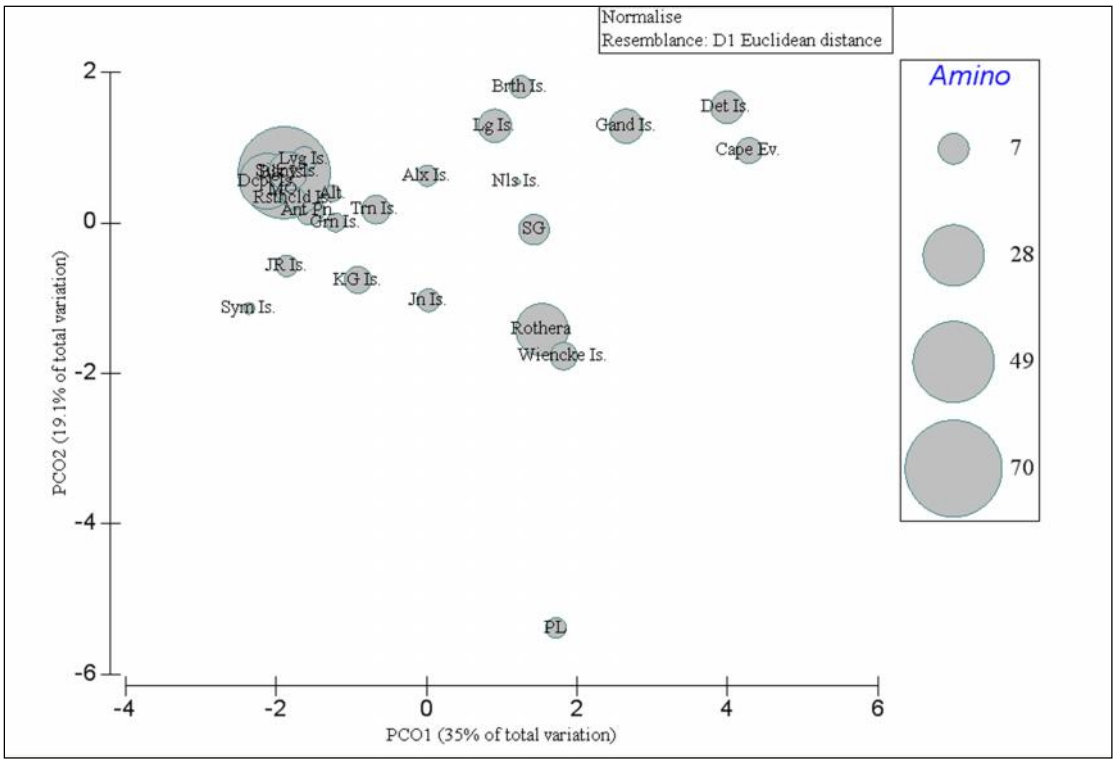


Figure A2.2. PCO of % EC (top) and **Figure A2.3.** PCO of amino acid profile (bottom): PCO analysis to group sites of similar chemical profile together with an overlay bubble graph of Electrical Conductivity and amino acid profile (Amino) for figures respectively. Scale for each bubble graph is representative of the individual data range for each variable.

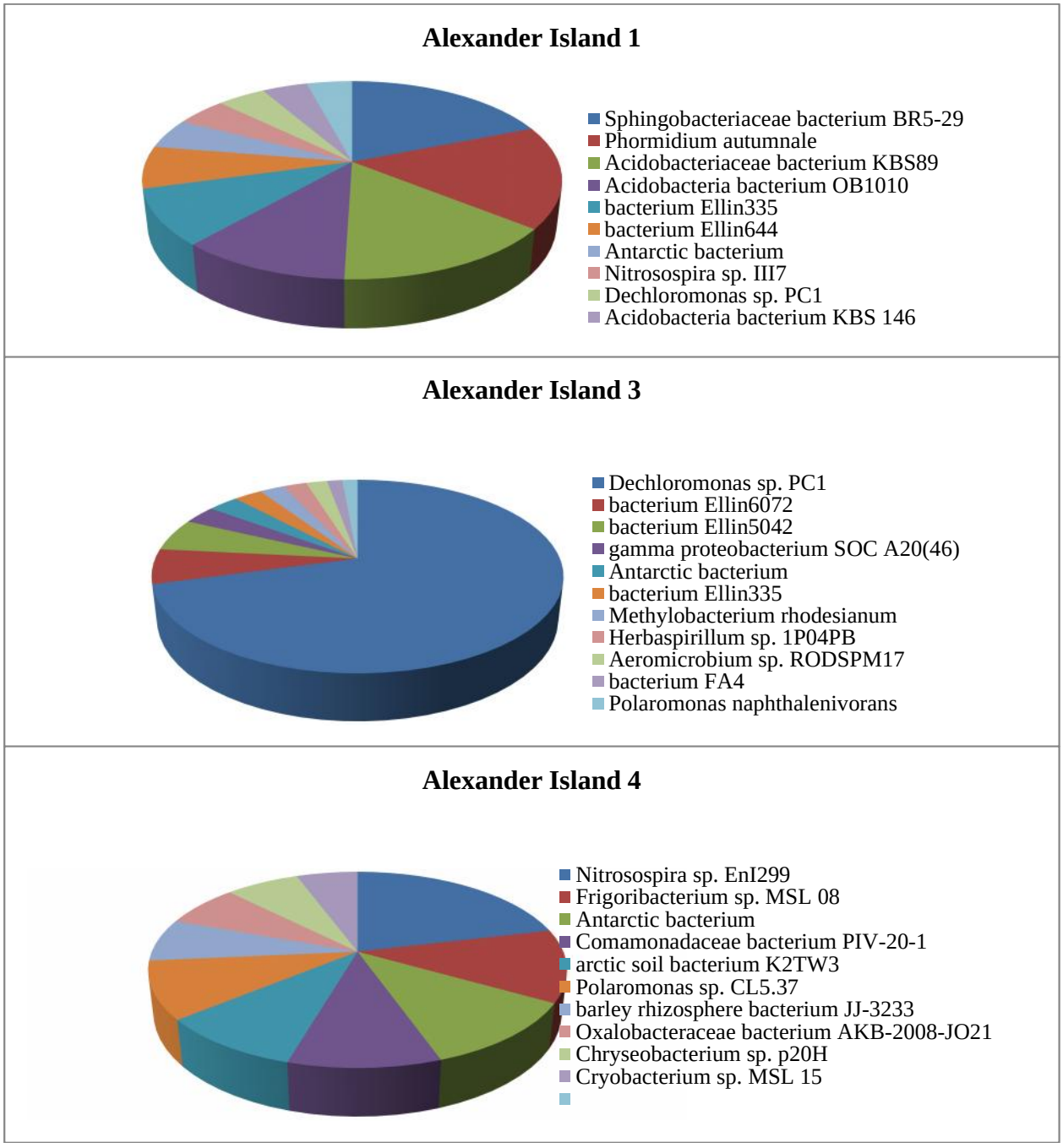
Location	Shannon	Simpson	Location	Shannon	Simpson
Alectoria 1	2.25	84.26	Livingston Island 3	2.34	85.48
Alectoria 2	2.25	85.56	Livingston Island 4	2.51	88.21
Alexander Island 1	2.42	81.92	Mars Oasis 1	2.50	87.83
Alexander Island 2	2.65	84.37	Mars Oasis 2	2.63	88.11
Alexander Island 3	2.65	83.61	Mars Oasis 3	2.65	88.13
Alexander Island 4	2.74	88.36	Mars Oasis 4	2.45	86.35
Alexander Island 5	2.41	81.54	Nelson Island	2.66	84.94
Alexander Island 6	2.60	87.84	Port Lockroy 1	2.98	92.50
Antarctic Peninsula 1	2.67	87.96	Port Lockroy 2	2.69	89.66
Antarctic Peninsula 2	2.81	89.64	Rothera 1	2.80	87.58
Berthelot Is	2.55	83.57	Rothera 2	2.66	88.60
Blailklock Island	2.47	85.87	Rothshild Island 1	1.57	62.98
Cape Evenson 1	2.95	90.14	Rothshild Island 2	2.57	84.16
Cape Evenson 2	2.91	89.10	Rothshild Island 3	2.73	90.06
Cape Evenson 3	2.68	86.53	Seymour Island 1	2.26	84.35
Deception Island 1	2.90	91.11	Seymour Island 2	2.43	86.28
Deception Island 2	2.79	89.58	Signy 1	2.93	90.31
Deception Island 3	1.78	61.76	Signy 2	2.94	91.52
Detaille Is 1	2.58	85.07	Signy 3	2.78	89.84
Detaille Is 2	2.67	86.69	Signy 4	2.83	90.45
Gand Is 1	2.32	84.44	Signy 5	2.55	88.05
Greenwich Island 1	2.57	87.61	Signy 6	3.02	91.14
Greenwich Island 2	2.89	90.64	Signy 7	2.83	90.37
James Ross Island 1	2.70	90.82	South Georgia 1	2.66	85.47
James Ross Island 2	2.79	89.18	South Georgia 2	2.59	82.87
James Ross Island 3	2.68	88.12	South Georgia 3	2.94	91.95
Jenny Island	2.81	89.51	Trinity Island 1	2.56	84.29
King George Island 1	1.82	75.91	Trinity Island 2	2.49	84.53
King George Island 2	2.51	85.44	Trinity Island 3	2.58	81.55
King George Island 3	2.90	91.47	Trinity Island 4	2.57	85.30
King George Island 4	2.86	87.90	Trinity Island 5	2.52	79.15
Lagoon Island	2.53	86.59	Wiencke Island 1	2.60	85.82
Livingston Island 1	2.64	87.72	Wiencke Island 2	2.69	88.13
Livingston Island 2	2.93	91.36	Wiencke Island 3	2.48	86.32

Table A2.1. Shannon-Weiner and Simpson's ELFA diversity calculated using indices (Fig. 3.14) for microbial alpha diversity using ELFA data at each site. Sites order alphabetically and from left to right.

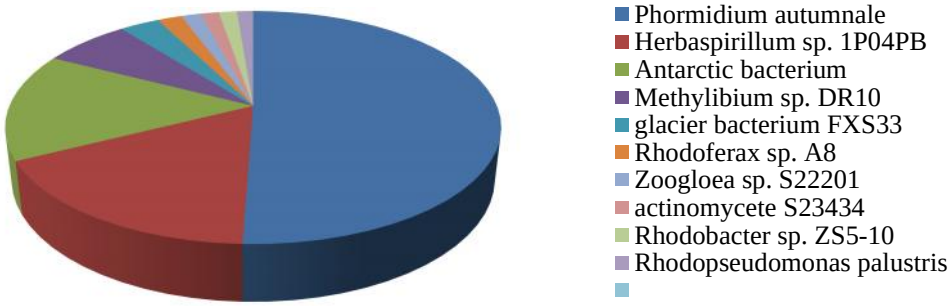
Location	Shannon	Simpson	Location	Shannon	Simpson
Alectoria 1	1.02	53.91			
Alectoria 2	1.66	76.98	Mars Oasis 1	1.90	80.89
Alexander Island 1	1.59	74.94	Mars Oasis 2	1.87	78.22
Alexander Island 3	1.35	66.70	Mars Oasis 3	1.81	79.35
Alexander Island 4	1.14	59.50	Mars Oasis 4	1.92	82.02
Alexander Island 6	1.51	72.39	Nelson Island	1.10	55.94
Antarctic Peninsula 1	1.70	72.79	Port Lockroy 1	1.89	81.63
Antarctic Peninsula 2	1.70	78.07	Port Lockroy 2	1.50	66.37
Berthelot Is	1.41	67.21	Rothera 2	1.03	54.97
Blailklock Island	0.88	53.40	Seymour Island 1	1.47	65.87
Cape Evenson 1	1.64	76.14	Signy 1	1.54	71.31
Cape Evenson 2	1.66	76.92	Signy 2	1.63	72.22
Deception Island 1	1.10	55.86	Signy 3	1.53	67.96
Detaille Is 1	1.91	82.41	Signy 4	1.67	73.45
Detaille Is 2	1.75	78.59	Signy 5	1.64	72.82
Greenwich Island 2	1.73	76.55	Signy 6	1.43	65.11
James Ross Island 1	1.42	70.21	Signy 7	1.58	68.69
James Ross Island 2	1.54	75.40	South Georgia 1	1.38	66.51
James Ross Island 3	1.81	78.22	South Georgia 2	1.41	69.12
Jenny Island	1.72	76.93	South Georgia 3	1.38	71.02
King George Island 4	1.71	75.46	Trinity Island 2	1.66	75.67
Lagoon Island	1.61	76.24	Trinity Island 3	1.63	74.62
Livingston Island 1	1.34	65.00	Wiencke Island 1	1.66	75.13
Livingston Island 2	1.65	73.41	Wiencke Island 2	1.68	75.80
Livingston Island 4	1.05	59.22	Wiencke Island 3	1.61	74.57

Table A2.2. Shannon-Weiner and Simpson's 454 diversity calculated using indices (Box 3.1) for bacterial alpha diversity using pyrosequncing data at each site. Sites order alphabetically and from left to right.

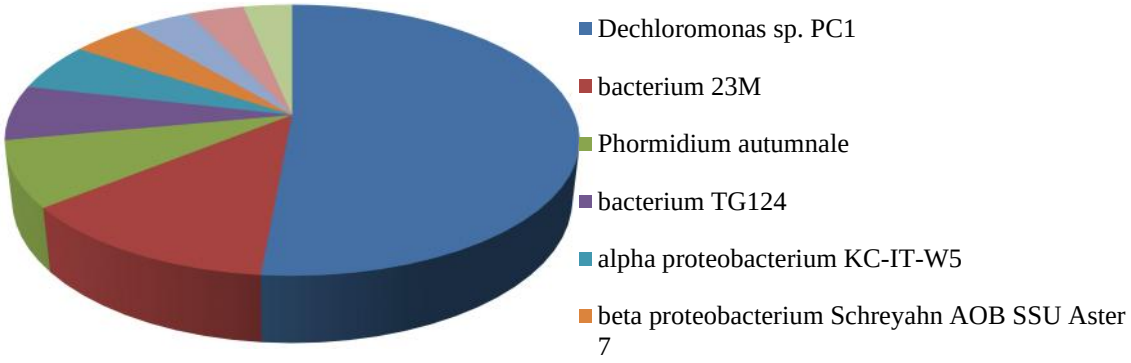
Figure A2.4. All Site Top Ten Species: Species Diversity 454 data was collated in Microsoft excel and sorted by species abundance (high to Low), the top ten species (or nearest significant value) was isolated, and presented in a simple excel pie chart.



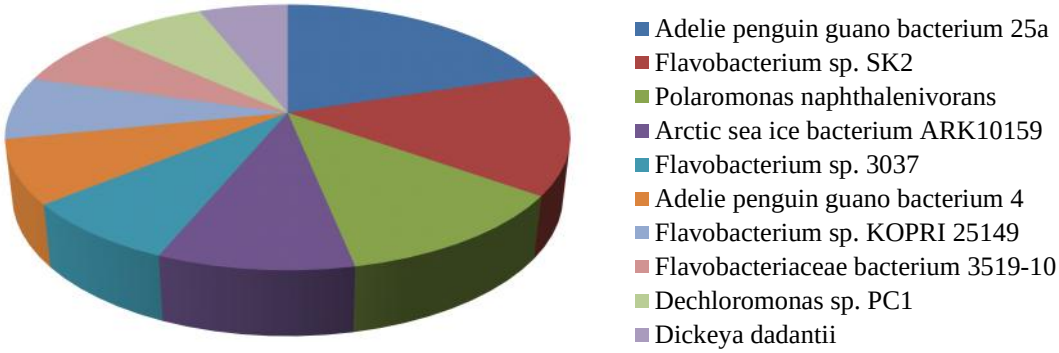
Alexander Island 6



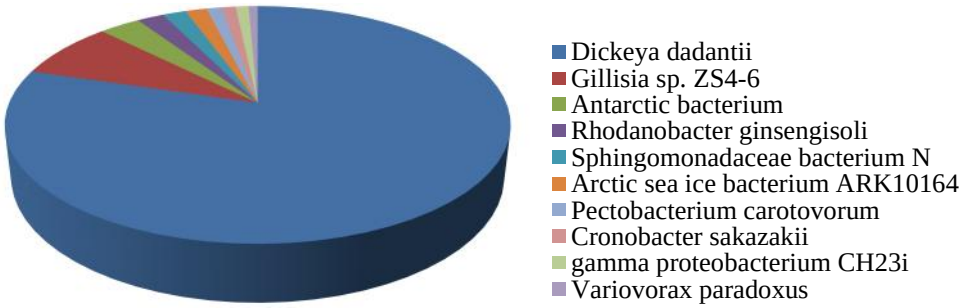
Signy 4



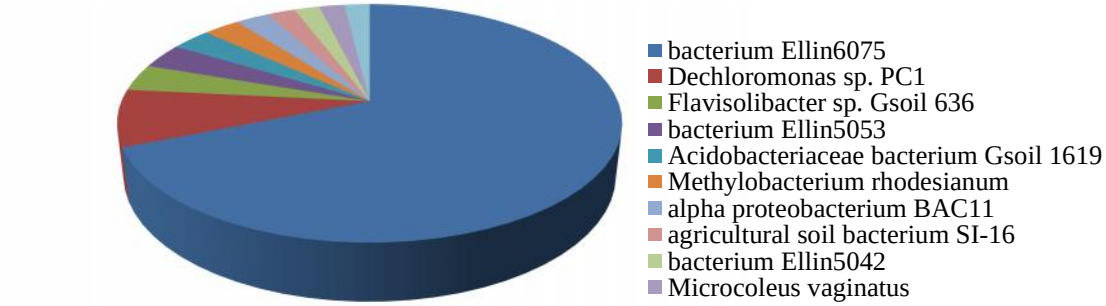
Wiencke Island 3



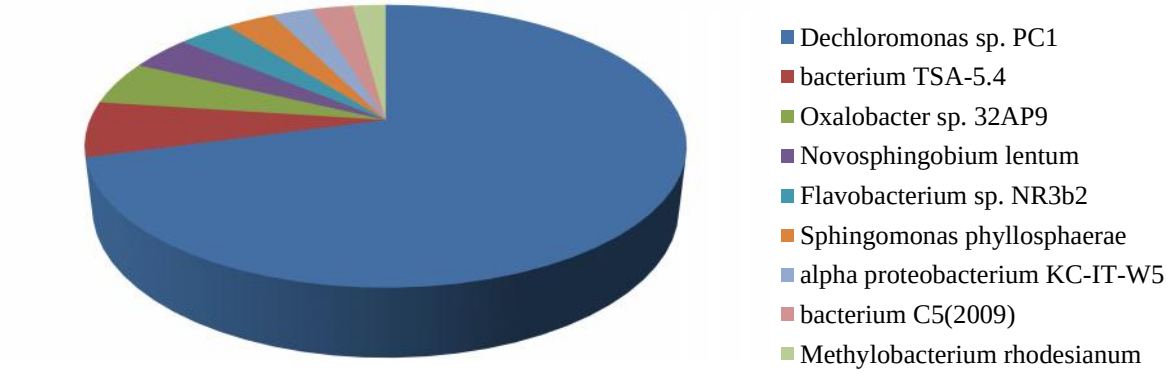
Alectoria 1



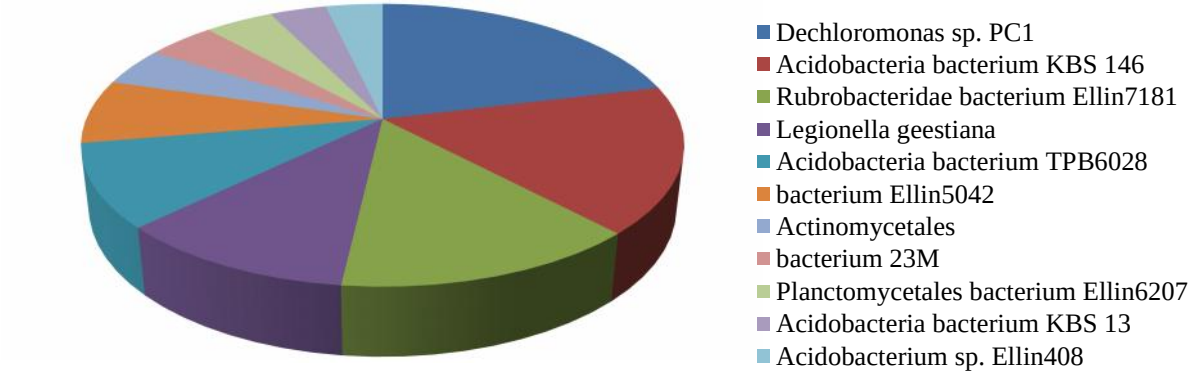
Alectoria 2



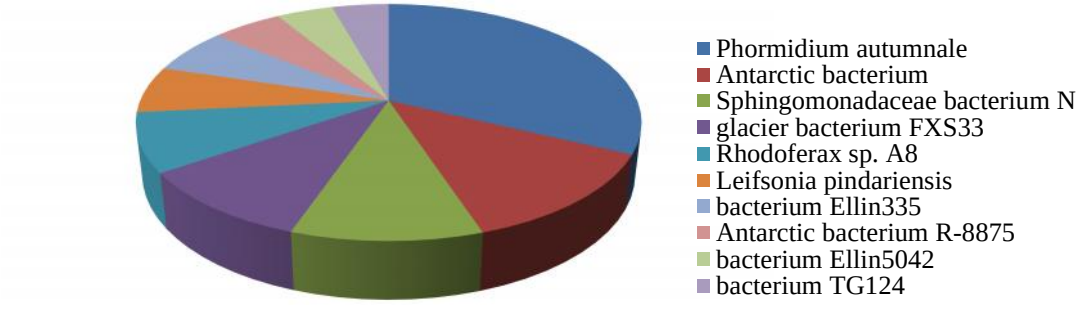
Antarctic Peninsula 1



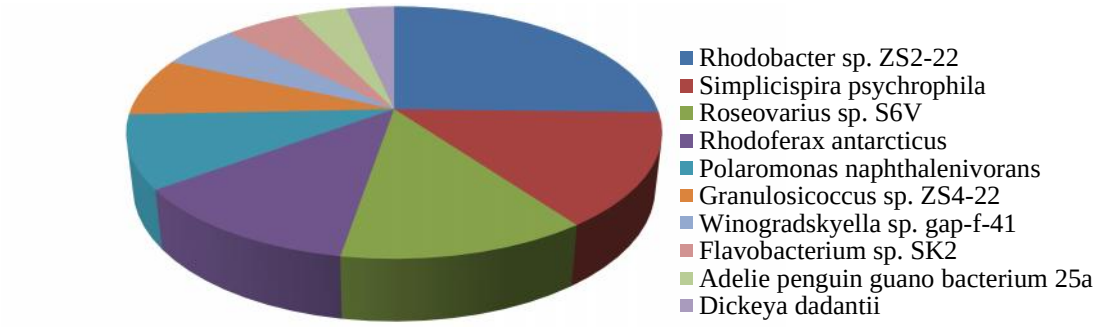
Antarctic Peninsula 2



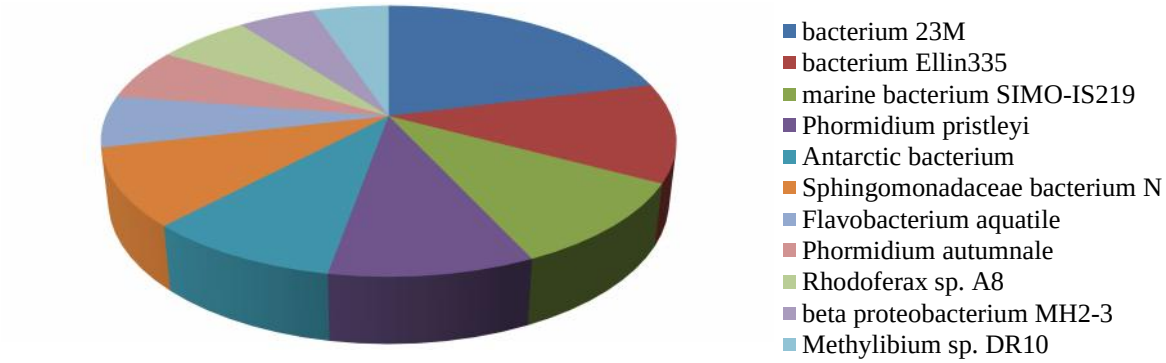
Berthelot Is



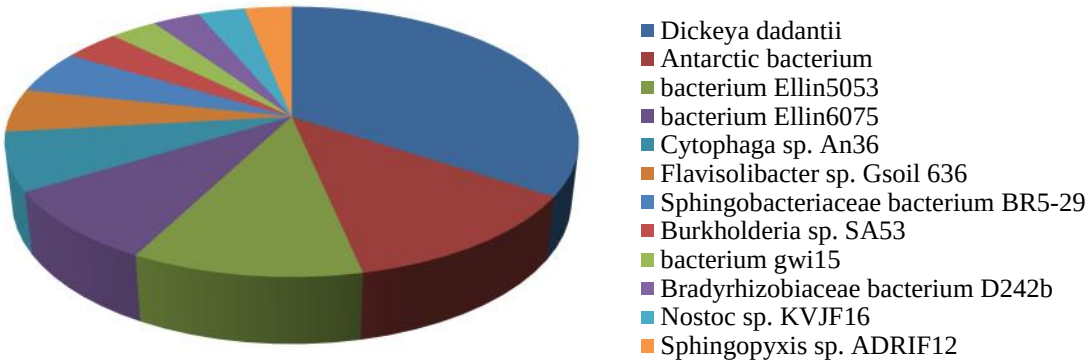
Blailklock Island



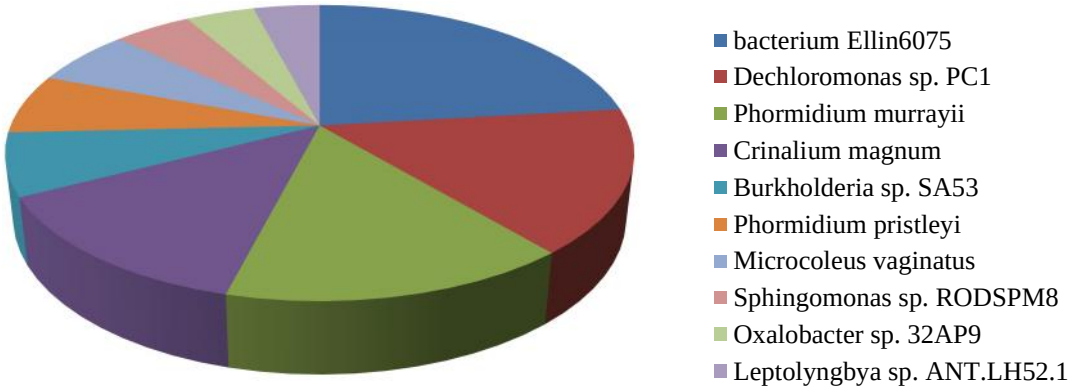
Cape Evenson 1



Cape Evenson 2



Detaille Is 1



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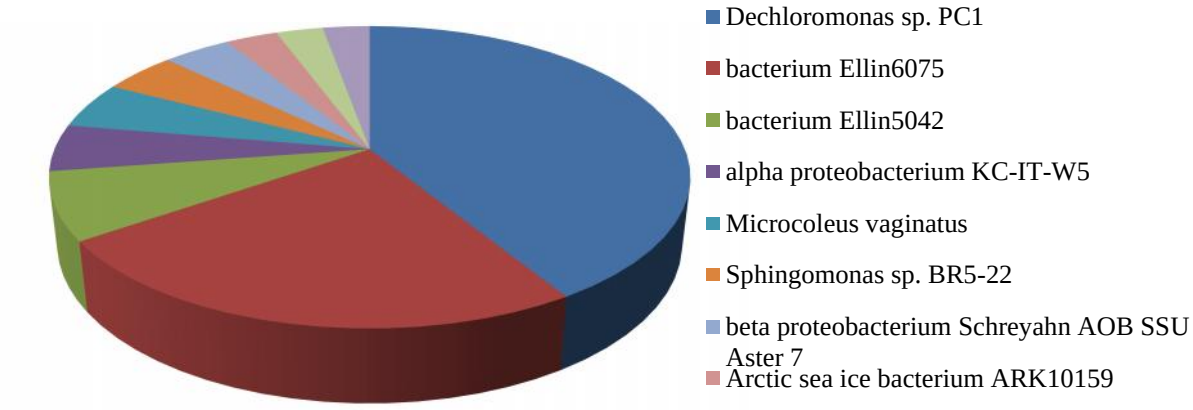
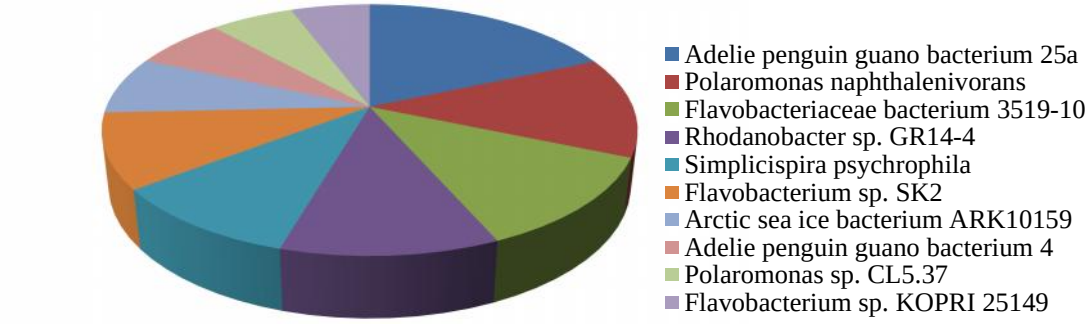
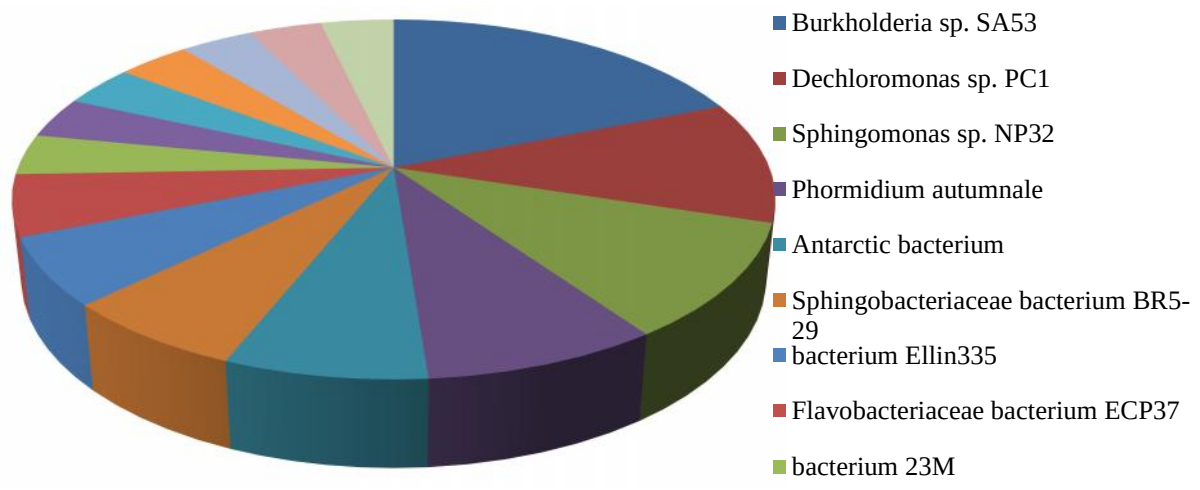


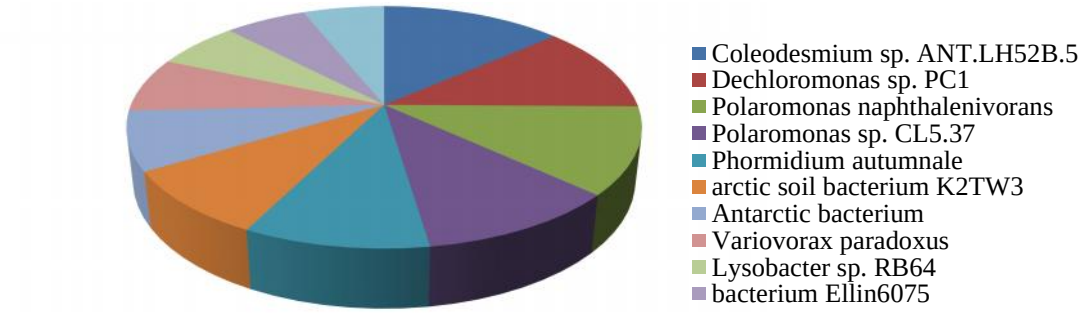
Deception Island 1



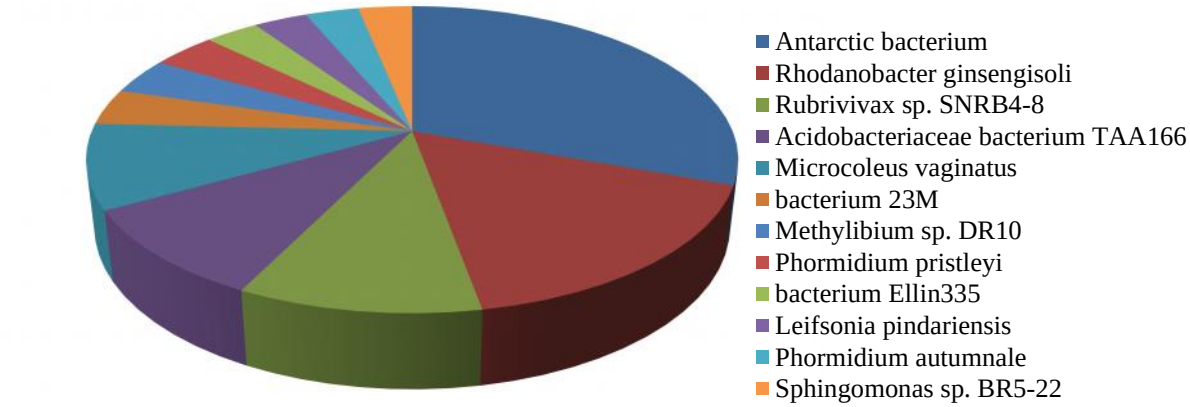
Greenwich Island 2



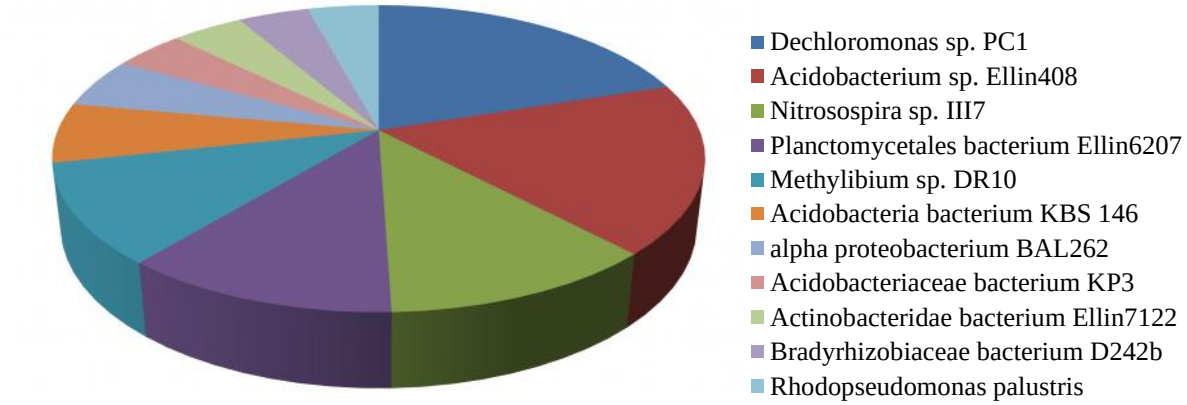
Jenny Island



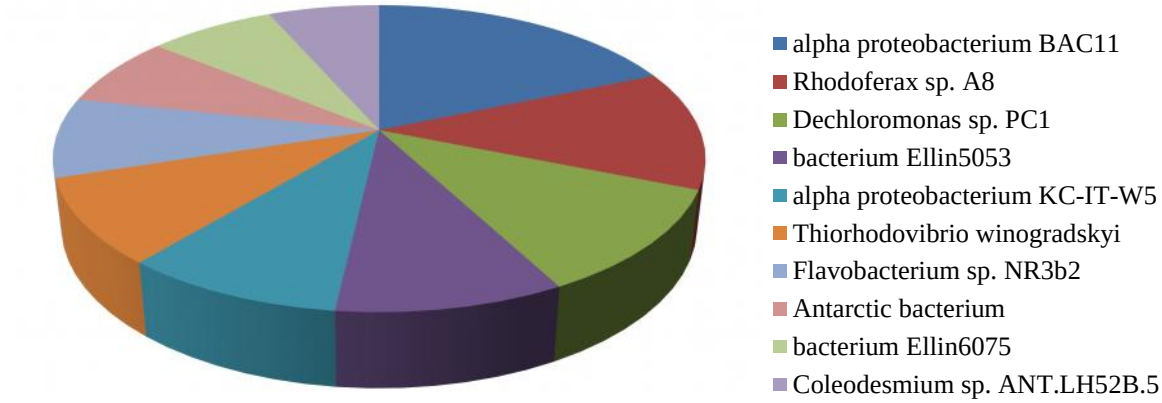
James Ross Island 1



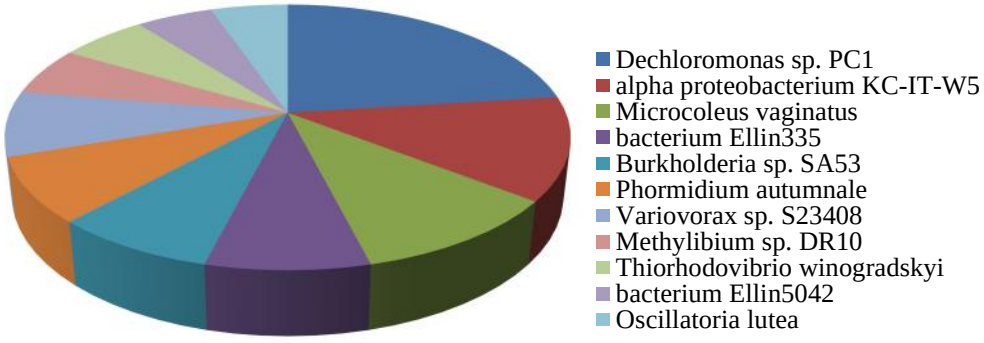
James Ross Island 2



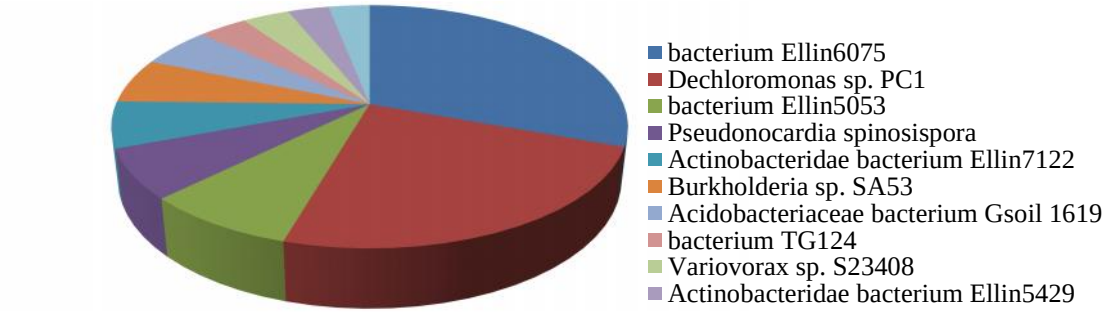
James Ross Island 3



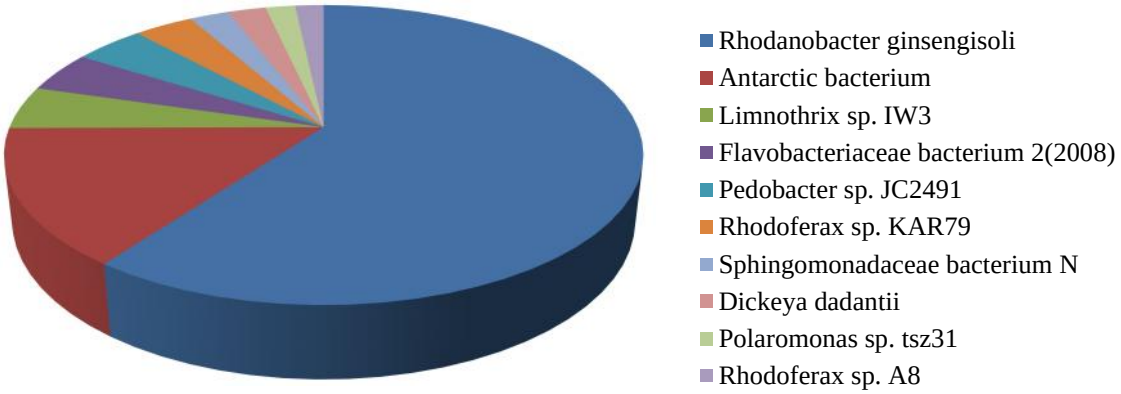
King George Island 4



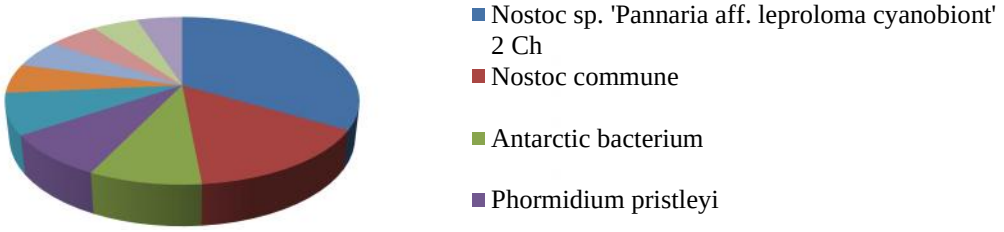
Lagoon Island



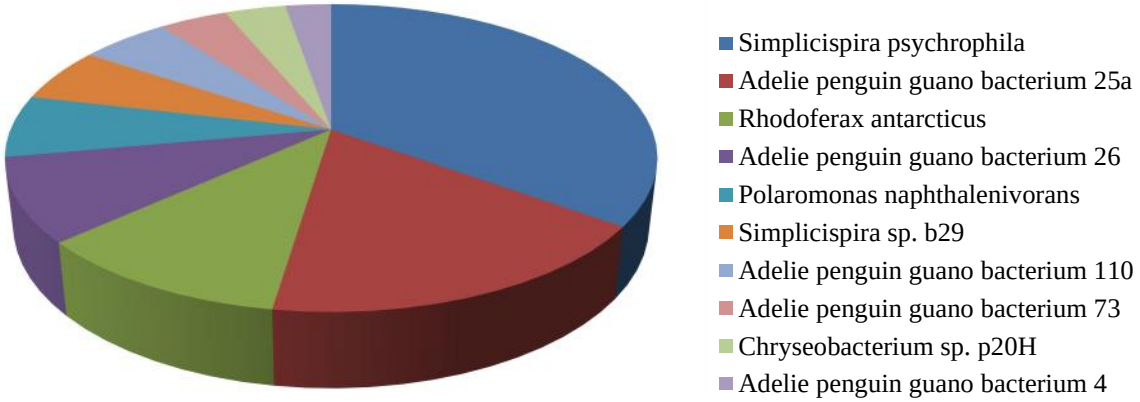
Livingston Island 1



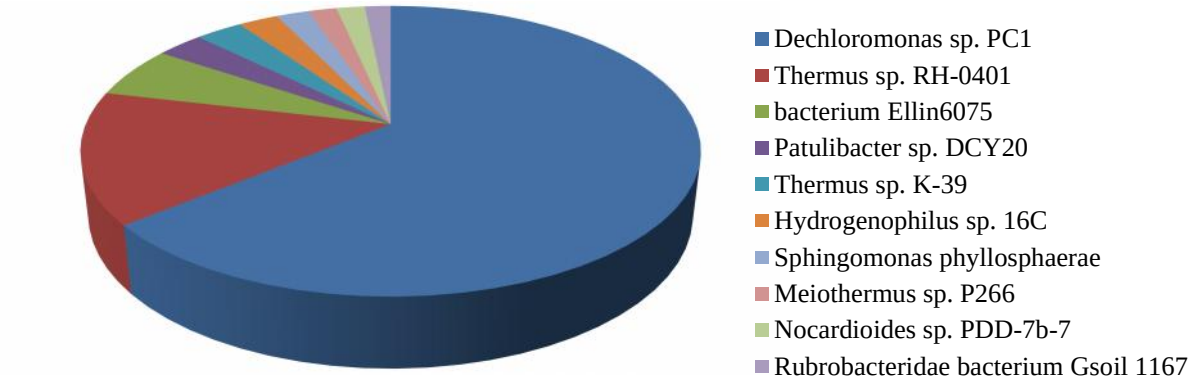
Livingston Island 2



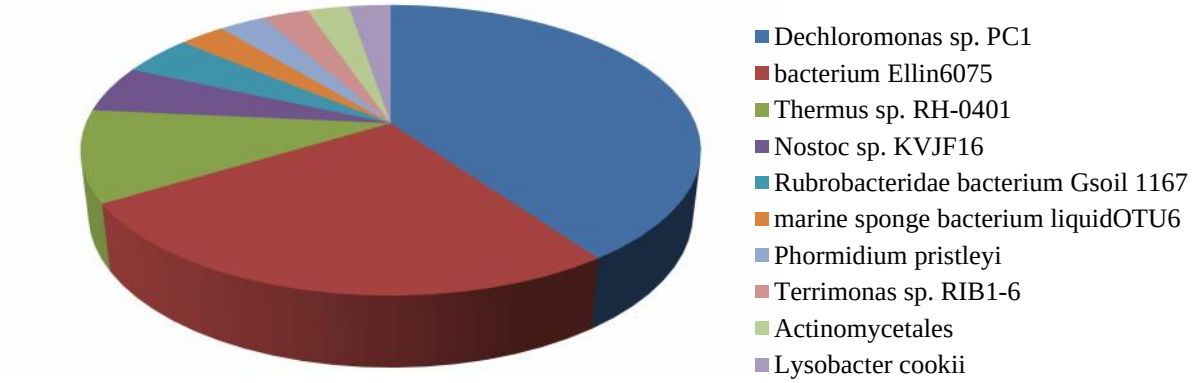
Livingston Island 4



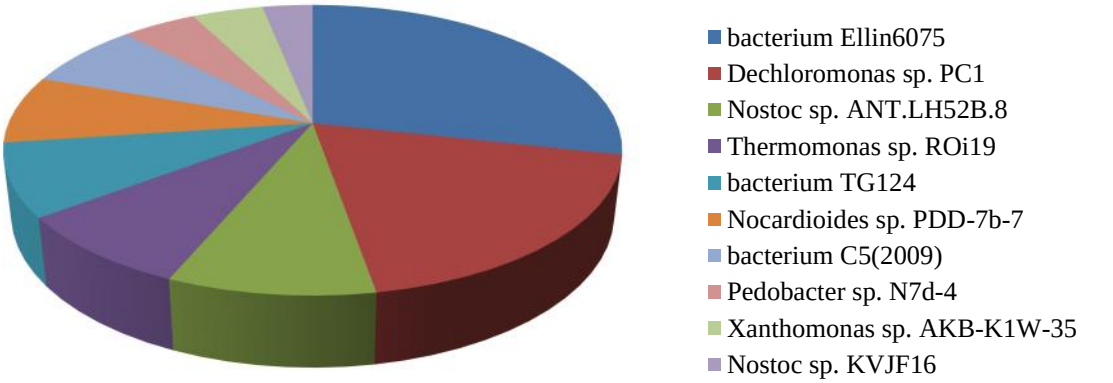
Mars Oasis 1



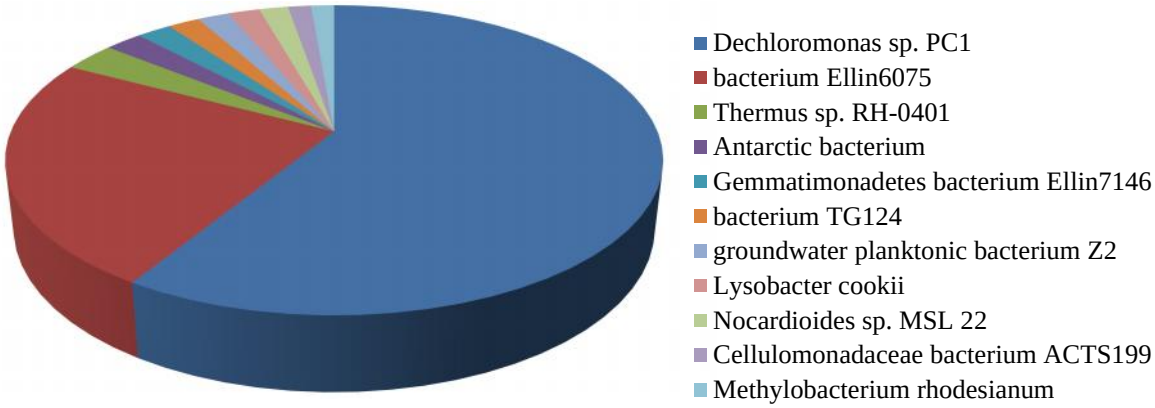
Mars Oasis 2



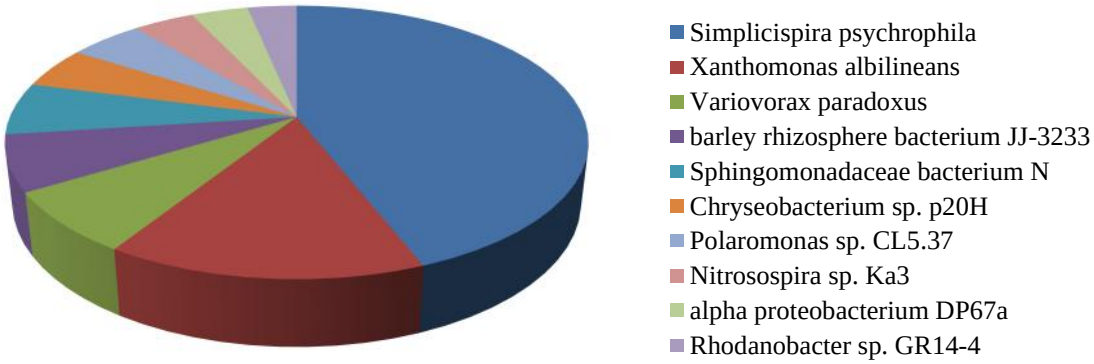
Mars Oasis 3



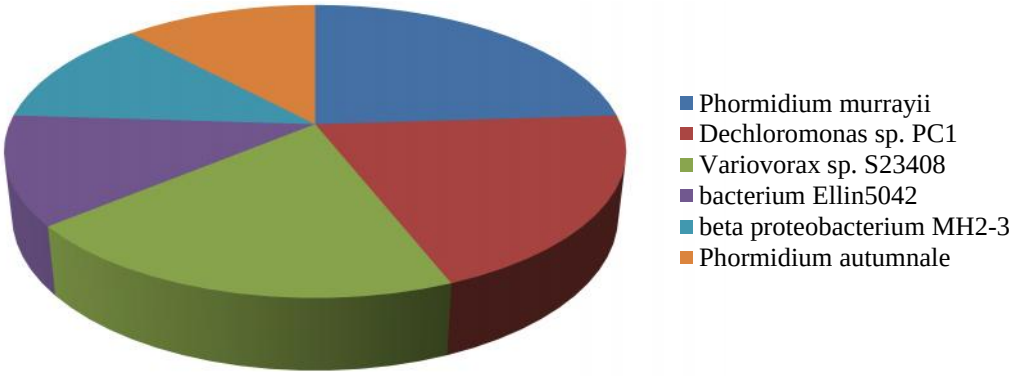
Mars Oasis 4



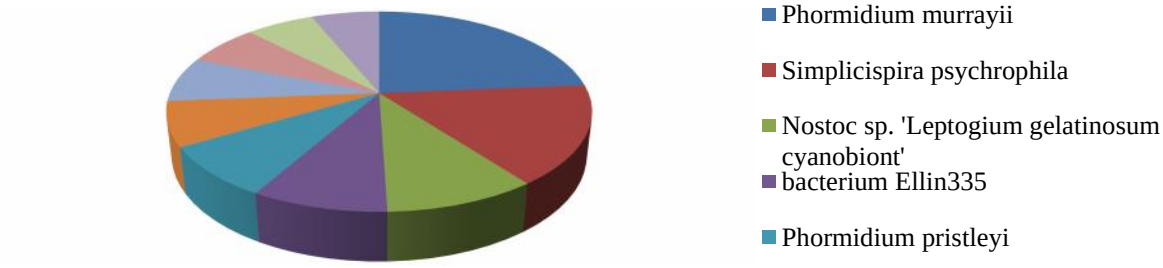
Nelson Island



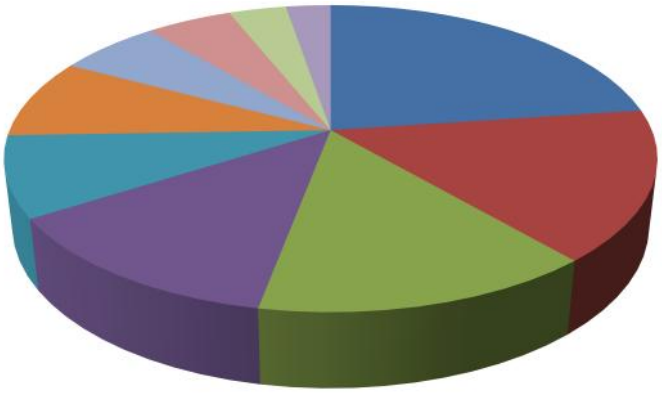
Port Lockroy 1



Port Lockroy 2

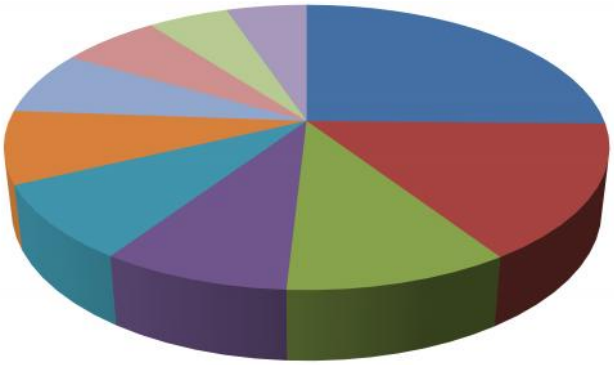


Rothera 2



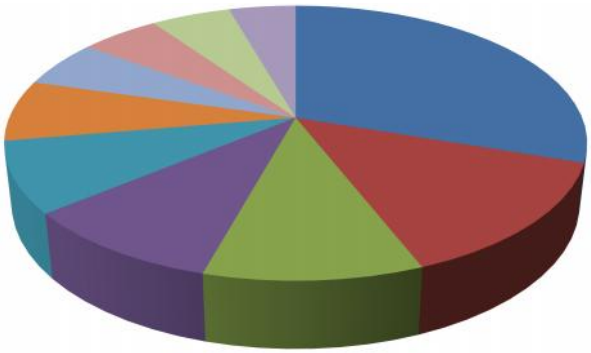
- glacier bacterium FXS33
- Polaromonas sp. Asd M3-1
- Polaromonas sp. CL6.24
- bacterium HTCC4111
- Sphingomonadaceae bacterium N
- Antarctic bacterium
- glacier bacterium FXS1
- Polaromonas sp. tsz31
- glacier ice bacterium sp. glbI11
- Flavobacterium segetis

Seymour Island 1



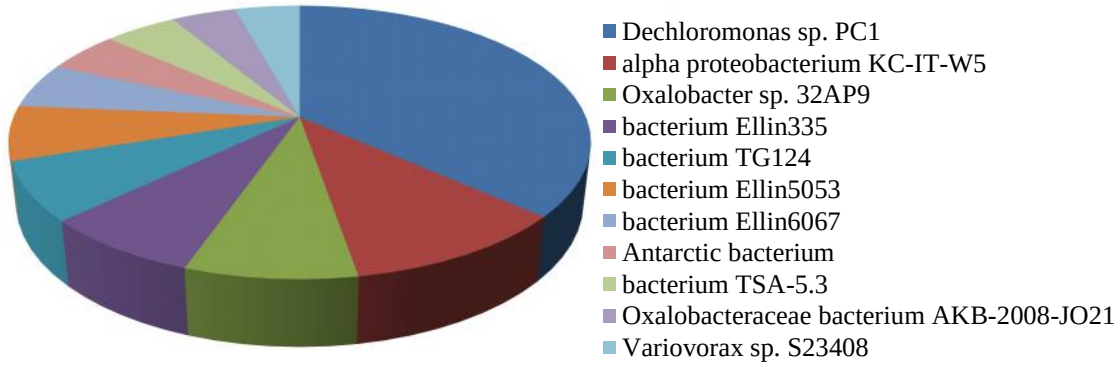
- Rhodoferax sp. A8
- Sphingomonadaceae bacterium N
- Cytophaga sp. An36
- Antarctic bacterium
- Sphingobacteriaceae bacterium BR5-29
- bacterium Ellin335
- Antarctic bacterium R-8875
- Methylibium sp. DR10
- bacterium Ellin5042
- Leptothrix sp. PW10C

Signy 1

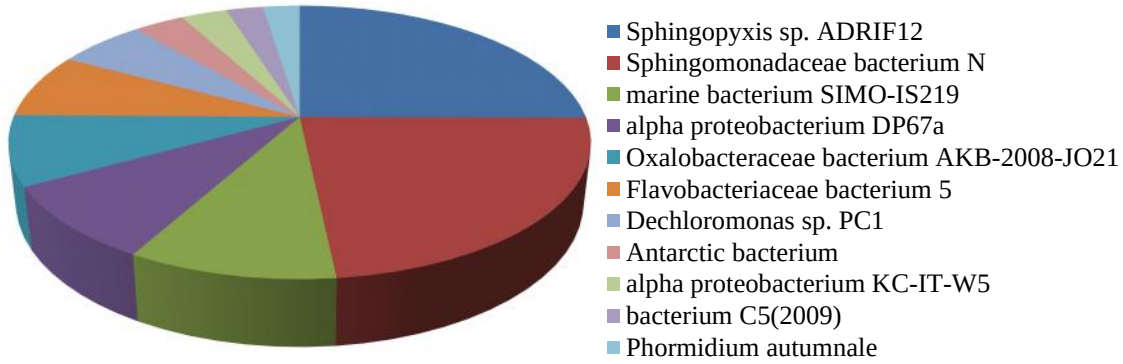


- Sphingopyxis sp. ADRIF12
- alpha proteobacterium DP67a
- marine bacterium SIMO-IS219
- bacterium Ellin335
- bacterium Ellin6075
- bacterium TG124
- bacterium 16M
- Microcoleus vaginatus
- Phormidium autumnale
- Dechloromonas sp. PC1

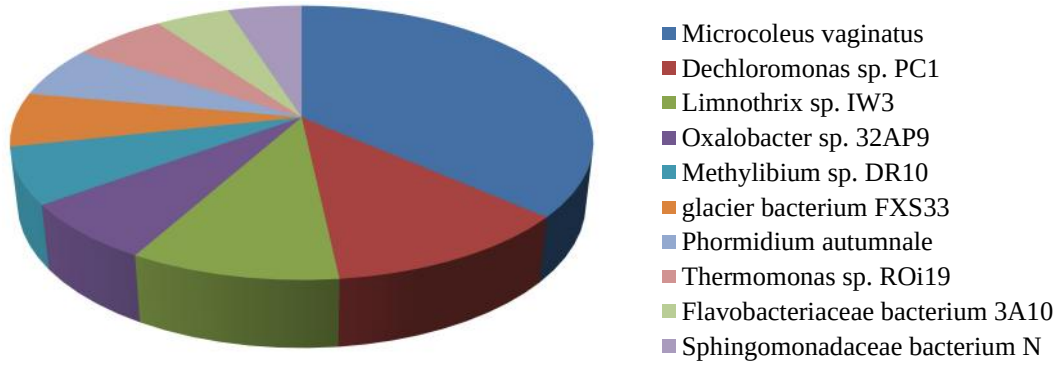
Signy 2



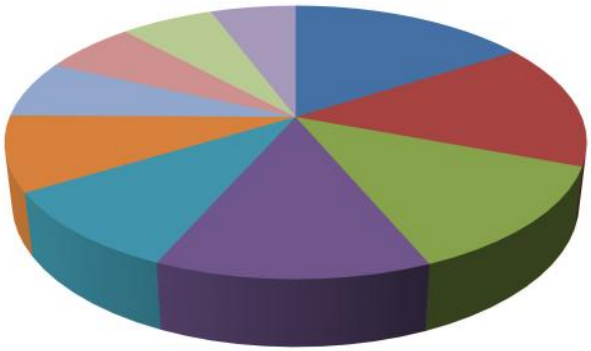
Signy 3



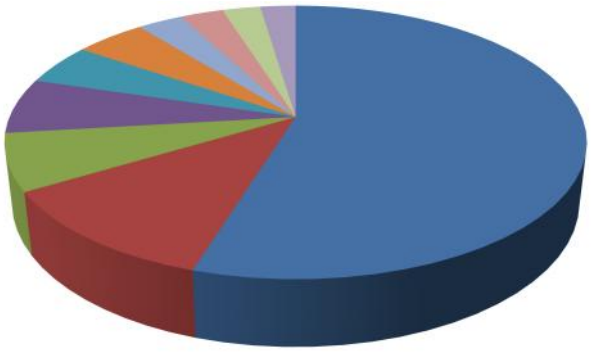
Signy 5



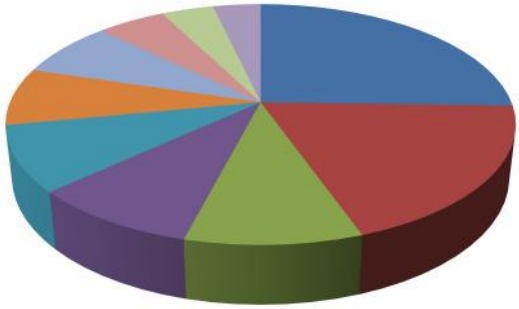
Signy 6



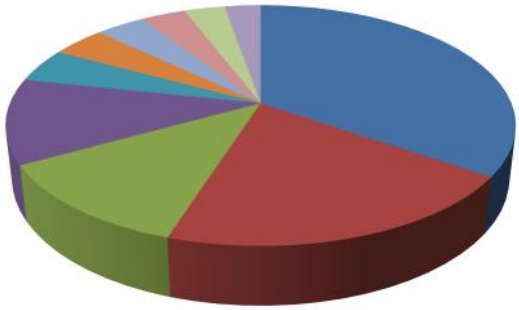
Signy 7



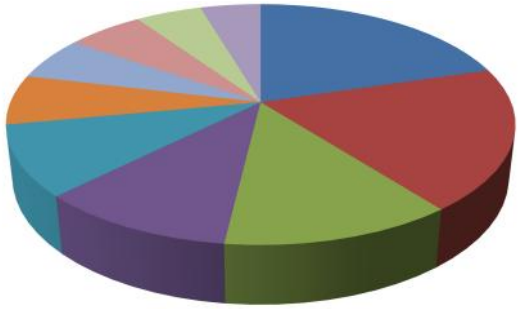
South Georgia 1



South Georgia 2

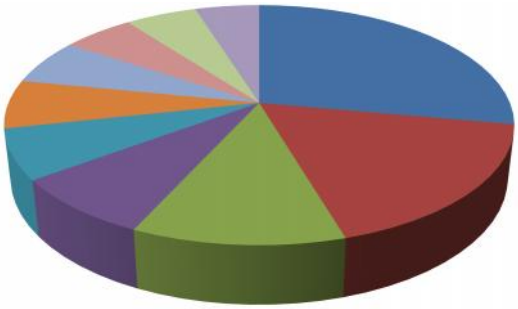


South Georgia 3



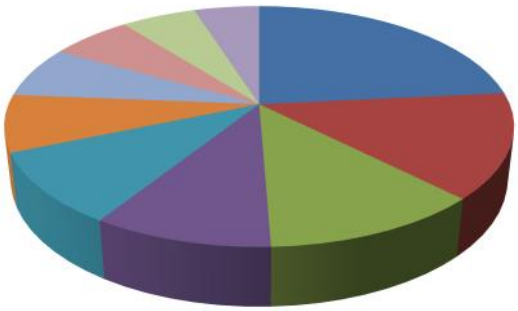
- Cloning vector pPRV111B
- Limnothrix sp. IW3
- Algoriphagus boritolerans
- Antarctic bacterium
- Gillisia sp. ZS4-6
- Loktanella salsilacus
- Leptolyngbya antarctica
- Phormidium autumnale
- Dechloromonas sp. PC1
- Algoriphagus sp. ZS3-3

Trinity Island 2



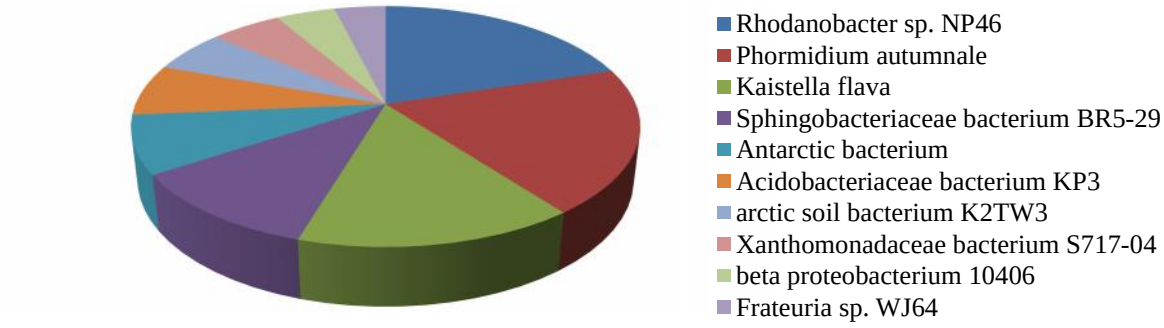
- Limnothrix sp. IW3
- Phormidium autumnale
- bacterium Ellin644
- Acidobacteriaceae bacterium KBS89
- Antarctic bacterium
- Adelie penguin guano bacterium 25a
- Polaromonas naphthalenivorans
- Flavobacterium sp. SK2
- Rhodanobacter sp. GR14-4
- Arctic sea ice bacterium ARK10159

Trinity Island 3

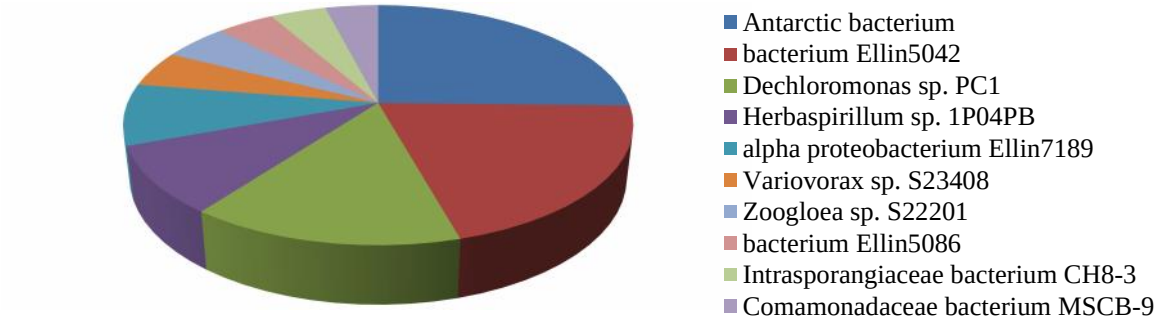


- Antarctic bacterium
- Flavobacteriales bacterium HD-O-03-10
- Limnothrix sp. IW3
- Chryseobacterium sp. IMMIB L-1519
- Sphingomonadaceae bacterium N
- glacier bacterium FXS33
- beta proteobacterium MH2-3
- Rhodoferax sp. A8
- Antarctic bacterium R-8875
- Flavobacterium sp. NR3b2

Wiencke Island 1



Wiencke Island 2



Appendix 3: Chapter 4 Connectivity Maps

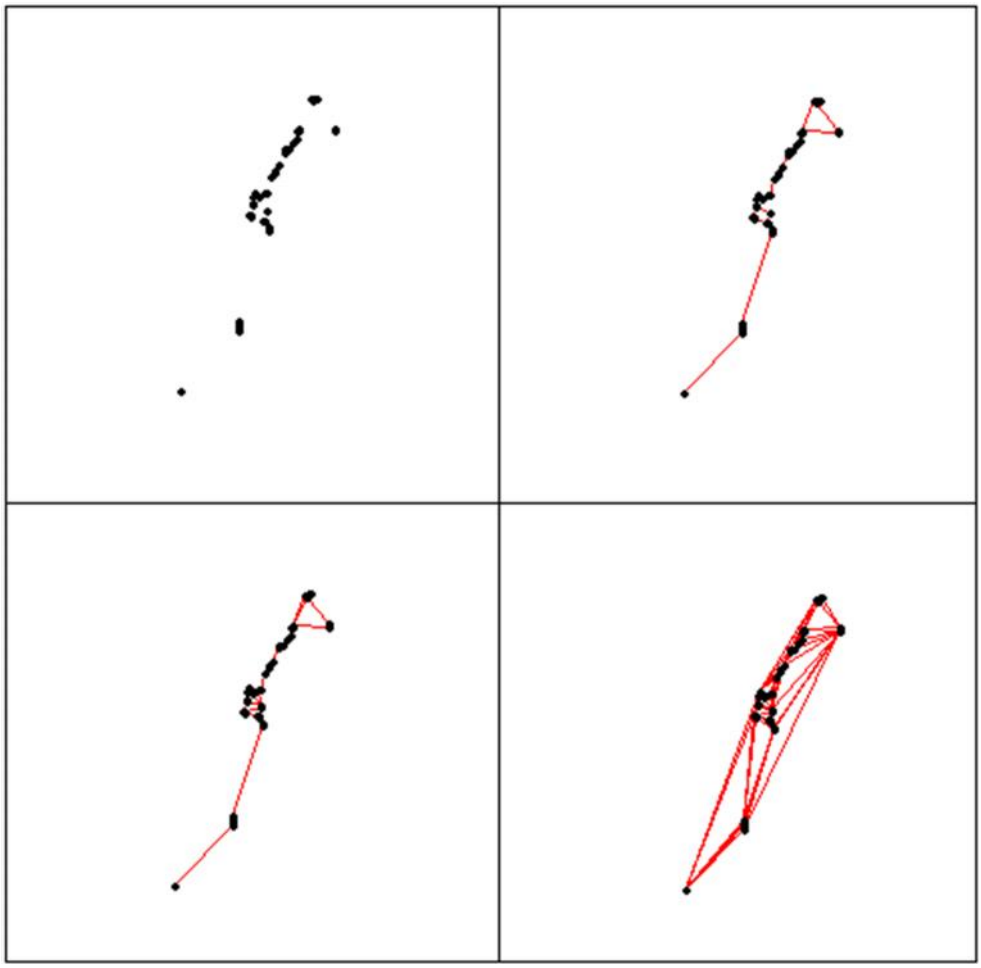


Figure A3.1. Connection networks constructed using latitudes and longitudes (from top left), Delaunay triangulation, Gabriel graph, Relative Neighbour graph, Sphere of Influence graph.

Appendix 4: Chapter 5 Supporting Analysis

	u_02	u_03	u_04	u_05	u_06	u_07	u_08	u_09	u_10	u_11	u_12	u_14	u_15	u_16	u_18	u_19	u_20	u_21	u_22	u_23	u_24
Proteobacteria				0.06					0.16	0.06									0.05		
Bacteroidetes											0.07	0.07									
Actinobacteria											0.12										
Cyanobacteria							0.08														
Acidobacteria			0.05		0.07													0.05			
Gemmatimonadetes	0.06			0.06								0.06			0.05						
Deinococcus.Thermus			0.16					0.15	0.10									0.06			
Firmicutes			0.11						0.12				0.06					0.05			
Chloroflexi											0.13	0.21				0.05					
Verrucomicrobia							0.10				0.22						0.12				
Planctomycetes			0.07		0.05						0.20										
Spirochaetes			0.05			0.08														0.05	
Tenericutes				0.07					0.20												
Fusobacteria						0.07							0.08		0.06				0.06		0.12
Nitrospirae					0.07		0.08	0.09			0.07						0.09				
Ktedonobacteria										0.05	0.08										
Deferribacteres																					
Chlorobi						0.07				0.07		0.06				0.06					
Thermodesulfobacteria								0.09				0.10	0.06								
Lentisphaerae		0.05									0.31			0.06			0.06				
Thermotogae			0.11	0.06				0.07	0.17									0.15			
Dictyoglomi									0.12											0.05	

	u_25	u_27	u_28	u_29	u_30	u_31	u_32	u_33	u_34	u_35	u_36	u_37	u_38	u_39	u_40	u_41	u_43	u_44	u_45	u_46	u_47	u_48
Proteobacteria																			0.10	0.11		
Bacteroidetes		0.05																0.07		0.15		
Actinobacteria		0.06		0.07						0.11												
Cyanobacteria									0.07	0.12										0.11		
Acidobacteria	0.13									0.06										0.10		
Gemmatimonadetes			0.07										0.09	0.07								
Deinococcus.Thermus																						
Firmicutes							0.09															
Chloroflexi													0.05		0.09							
Verrucomicrobia									0.07				0.13	0.05								
Planctomycetes	0.18																			0.08		0.05
Spirochaetes		0.20																				
Tenericutes				0.06		0.08					0.06											
Fusobacteria			0.06			0.06								0.06							0.11	
Nitrospirae						0.06						0.05	0.06									
Ktedonobacteria	0.18					0.06						0.10	0.10									
Deferribacteres							0.07						0.16			0.07	0.13					
Chlorobi					0.10			0.08							0.05				0.10	0.06		
Thermodesulfobacteria								0.07								0.06						0.07
Lentisphaerae	0.09																					
Thermotogae							0.12															
Dictyoglomi							0.24										0.10					

Table A4.1. Determination coefficients between species predicted by MEMs. Only values over 0.05 are shown.

---

	u_01	u_02	u_03	u_04	u_05	u_06	u_07	u_08	u_09	u_10	u_12	u_13	u_14	u_16	u_17	u_18	u_19	u_20	u_26	u_29	u_31	u_35	u_39	u_41	u_45
Cyanobacteria					0.06								0.06												
Deferribacteres					0.08					0.05										0.12					
Ktedonobacteria						0.08		0.14						0.07											
Proteobacteria						0.19		0.10																	
Firmicutes				0.08						0.22															
Thermodesulfobacteria			0.06			0.07				0.05														0.11	
Tenericutes						0.13				0.06										0.10					
Planctomycetes	0.12					0.18																			
Bacteroidetes													0.17		0.06					0.12					
Spirochaetes					0.07									0.05					0.13	0.11					
Thermotogae				0.12		0.12				0.16															
Fusobacteria					0.07		0.09															0.13	0.12		
Actinobacteria						0.09							0.11			0.07			0.15						
Deinococcus.Thermus				0.06		0.18			0.06	0.06							0.06								
Acidobacteria						0.18	0.05						0.12				0.08								
Chlorobi		0.06		0.09			0.05								0.09			0.10							0.10
Chloroflexi	0.08		0.06		0.09	0.11							0.18												
Gemmatimonadetes					0.19	0.07				0.12		0.05	0.08												
Dictyoglomi				0.12		0.10		0.06		0.11	`							0.06							
Verrucomicrobia		0.12	0.10		0.07			0.11							0.06						0.10				
Nitrospirae					0.09	0.08		0.07	0.06				0.06					0.07			0.15				
Lentisphaerae	0.10	0.14							0.07										0.12	0.10	0.13				

Table A4.2. Coefficients between species by the env. variables and MEMs. Only values over 0.05 are shown.

	u_01	u_02	u_03	u_04	u_05	u_06	u_07	u_08	u_10	u_11	u_13	u_14	u_16	u_17	u_21	u_22	u_23	u_35	u_39	u_45	u_48
DOC			0.17												0.05						0.07
NO3.NO2			0.05									0.07									0.16
NH4			0.12			0.08													0.12		
pH		0.07	0.07		0.09	0.05					0.05										
EC	0.10				0.08		0.05														
X..WHC							0.07						0.07								
Amino				0.08		0.05	0.12			0.09		0.06									
C.N						0.11		0.09	0.05							0.10					
P			0.16	0.10		0.09															
Plants						0.08										0.06	0.05	0.07			
Seals							0.14	0.12						0.08						0.05	
Birds	0.14		0.09					0.15													

Table A4.3. Determination coefficients between environmental variables and the MEMs. Only values over 0.05 are shown.

Figure A4.1. Canonical MSPA of class constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with 'd' and is equivalent to the coefficient of multiple determination (R^2).

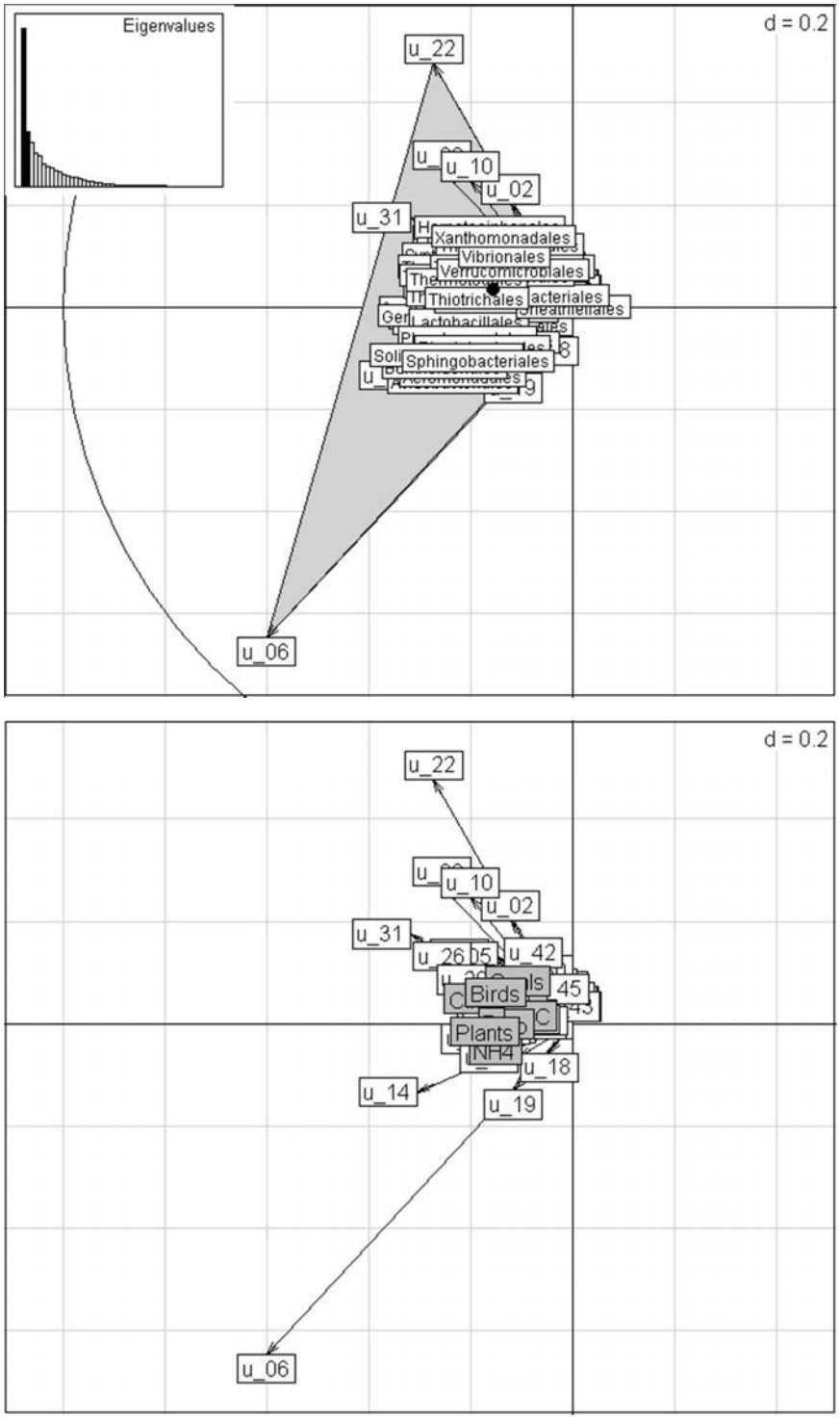


Figure A4.2. Canonical MSPA of order constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influences are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with 'd' and is equivalent to the coefficient of multiple determination (R^2).

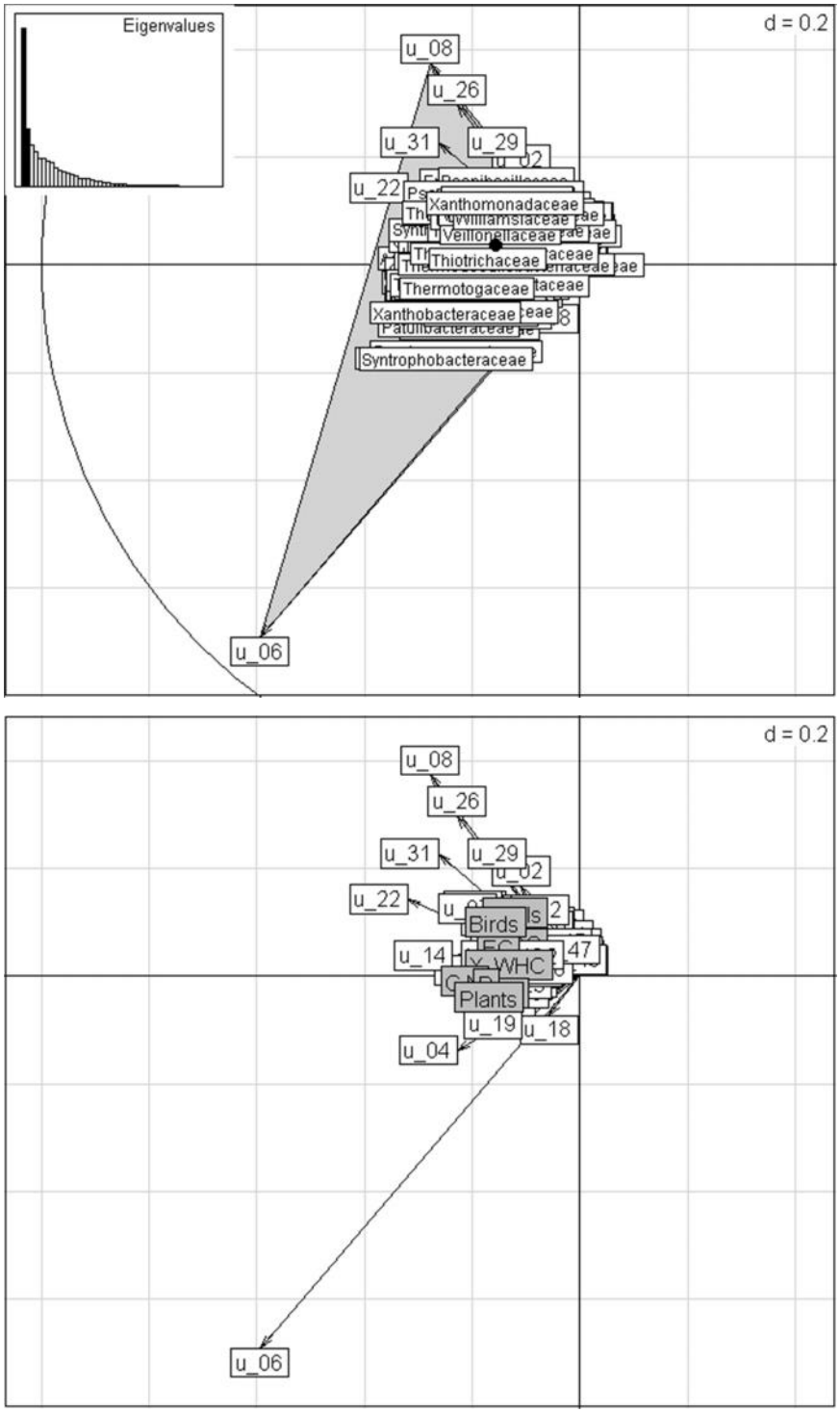


Figure A4.3. Canonical MSPA of family constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with 'd' and is equivalent to the coefficient of multiple determination (R^2).

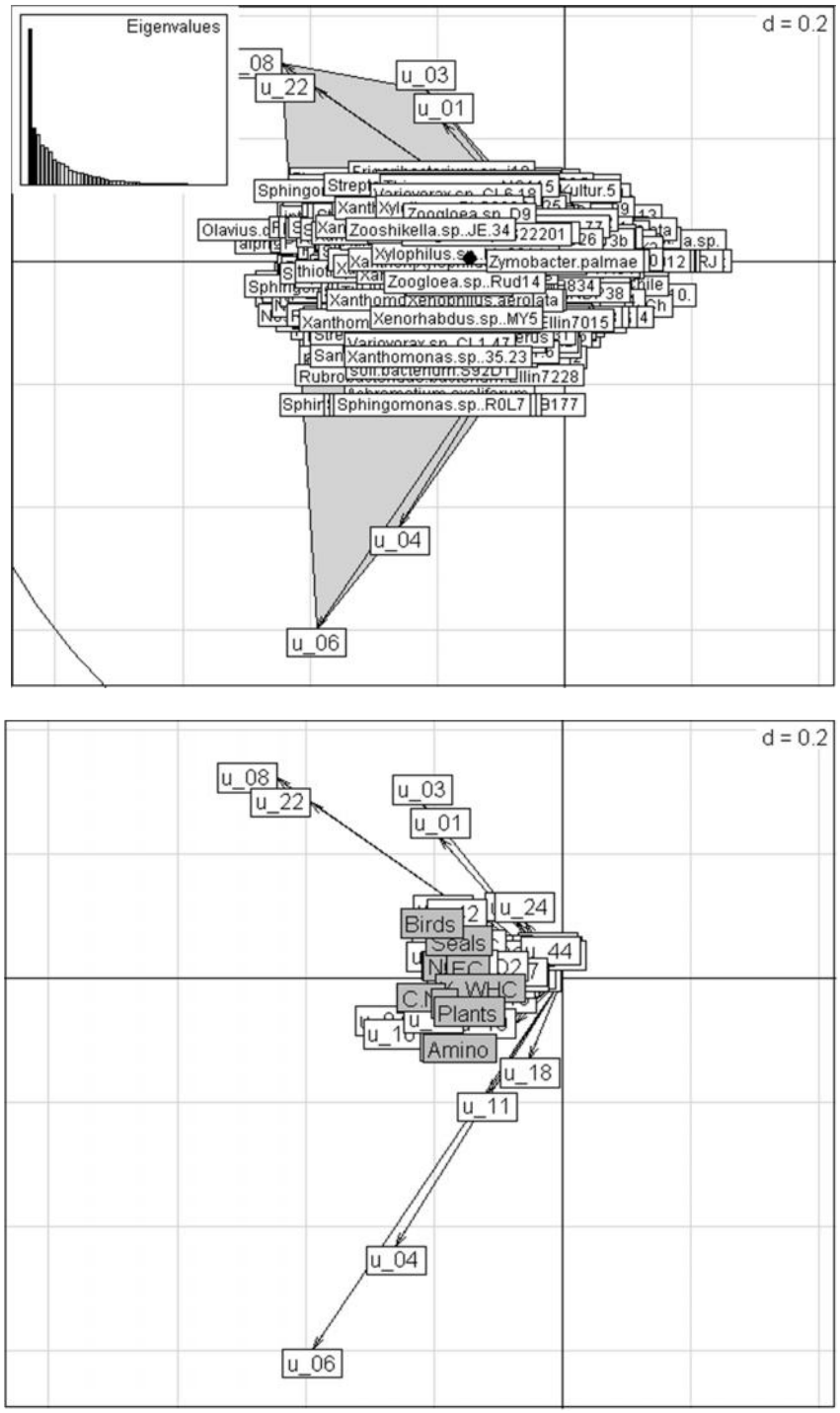


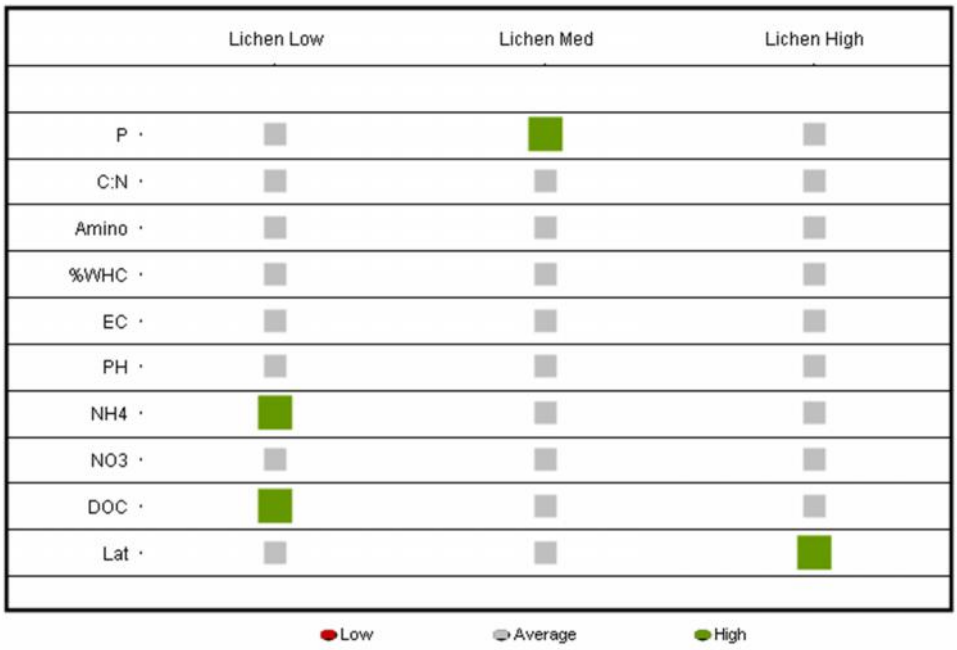
Figure A4.4. Canonical MSPA of family constrained by environment with scree plot of axes eigenvalues. MEM vectors (U_0*i*) exhibiting the strongest spatial influence are further from the origin and phyla/environmental parameters superimposed to indicate associations with spatial structures. The grid size of the plot is notated with 'd' and is equivalent to the coefficient of multiple determination (R^2).

PHYLUM	Mean Abundance
Proteobacteria	2677.306122
Bacteroidetes	1682.918367
Actinobacteria	950.377551
Cyanobacteria	586.9795918
Acidobacteria	557.8673469
Gemmatimonadetes	465.8163265
Deinococcus-Thermus	42.20408163
Firmicutes	181.8877551
Chloroflexi	27.37755102
Verrucomicrobia	3.387755102
Planctomycetes	12.6122449
Spirochaetes	7.06122449
Tenericutes	9.56122449
Fusobacteria	0.948979592
Nitrospirae	20.04081633
Ktedonobacteria	16.30612245
Deferribacteres	11.66326531
Chlorobi	0.091836735
Thermodesulfobacteria	0.102040816
Lentisphaerae	0.12244898
Thermotogae	0.571428571
Dictyoglomi	0.040816327

CLASS	Mean abundance
Sphingobacteria	1120.83
Betaproteobacteria	1001.37
Actinobacteria	863.15
Alphaproteobacteria	775.85
Gammaproteobacteria	687.19
Acidobacteria	422.61
Oscillatoriales	306.71
Flavobacteria	301.90
Gemmatimonadetes	277.99
Nostocales	204.39
Clostridia	152.80
Deltaproteobacteria	97.63
Chroococcales	45.66
Deinococci	40.65
Bacteroidia	38.97
Nitrospira	19.94
Bacilli	15.37
Deferribacteres	11.66
Dehalococcoidetes	11.56
Planctomycetacia	9.73
Mollicutes	9.50
Spirochaetes	7.06
Ktedonobacteria	5.79
Epsilonproteobacteria	5.20
Thermolithobacteria	4.21
Erysipelotrichi	3.65
Thermomicrobia	3.35
Caldilineae	3.18
Holophagae	3.04
Verrucomicrobiae	1.83
Chloroflexi	1.56
Stigonematales	1.19
Spartobacteria	1.14
Pleurocapsales	0.97
Fusobacteria	0.91
Thermotogae	0.57
Opitutae	0.28
Prochlorales	0.16
Gloeobacteria	0.12
Thermodesulfobacteria	0.10
Zetaproteobacteria	0.08
Chlorobia	0.07
Dictyoglomia	0.04

Table A4.4. Mean abundances of phyla and class present in bacterial community

Appendix 5: Chapter 6 Supporting Heat Maps



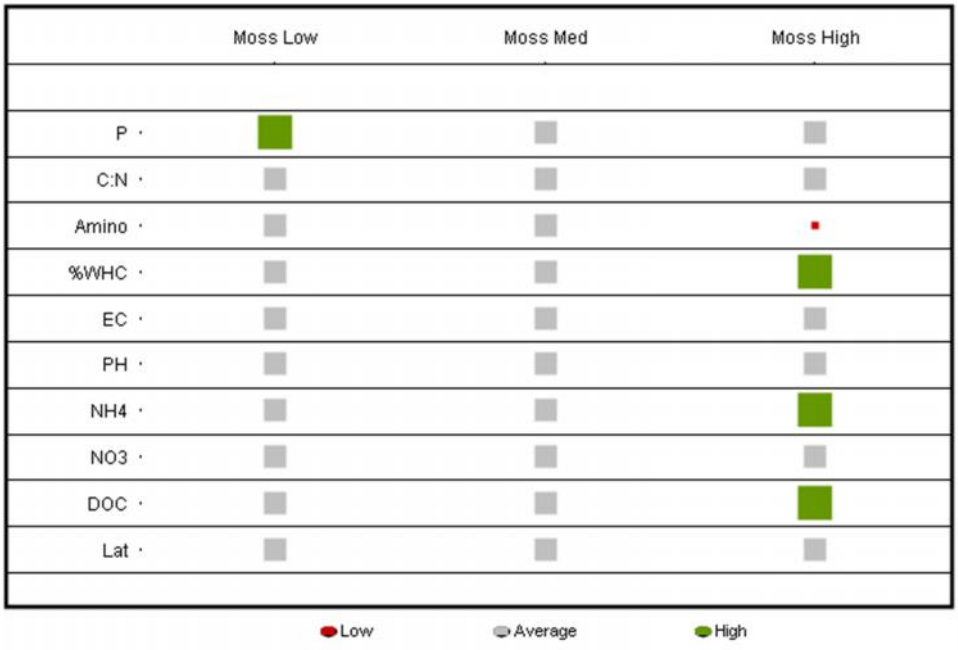


Figure A5.1. Heat map of Lichen (Top) and **Figure A5.2.** Heat map of Moss influence (Bottom). Colour and size of square corresponds to correlation between site characteristic grade and environmental parameter; where squares are green correlation is high, grey is average and red is low.

Appendix 6: Chapter 7 Full Fit Diagnostics

Chi-Square Test of Model Fit

Value	5.752
Degrees of Freedom	6
P-Value	0.4516

CFI/TLI

CFI	1.000
TLI	1.005

Loglikelihood

H0 Value	-886.703
H1 Value	-883.827

Information Criteria

Number of Free Parameters	12
Akaike (AIC)	1797.407
Bayesian (BIC)	1824.216
Sample-Size Adjusted BIC (n* = (n + 2) / 24)	1786.422

RMSEA (Root Mean Square Error Of Approximation)

Estimate	0.000
90 Percent C.I.	0.000 0.153
Probability RMSEA <= .05	0.559

R-SQUARE

Observed Variable	R-Square
DOC	0.452
NO3	0.353
BIO	0.561

QUALITY OF NUMERICAL RESULTS

Condition Number for the Information Matrix 0.107E-06
(ratio of smallest to largest eigenvalue)

Table A6.1. Full fit diagnostics for ELFA Richness

Chi-Square Test of Model Fit

Value	0.655
Degrees of Freedom	3
P-Value	0.8837

CFI/TLI

CFI	1.000
TLI	1.169

Loglikelihood

H0 Value	-527.859
H1 Value	-527.532

Information Criteria

Number of Free Parameters	6
Akaike (AIC)	1067.719
Bayesian (BIC)	1081.123
Sample-Size Adjusted BIC (n* = (n + 2) / 24)	1062.226

RMSEA (Root Mean Square Error Of Approximation)

Estimate	0.000
90 Percent C.I.	0.000 0.096
Probability RMSEA <= .05	0.907

R-SQUARE

Observed Variable	R-Square
NO3	0.353
RICH	0.122

QUALITY OF NUMERICAL RESULTS

Condition Number for the Information Matrix 0.895E-07
(ratio of smallest to largest eigenvalue)

Table A6.2. Full fit diagnostics for ELFA Abundance

Chi-Square Test of Model Fit

Value	0.000
Degrees of Freedom	2
P-Value	0.9998

CFI/TLI

CFI	1.000
TLI	1.172

Loglikelihood

H0 Value	-31.021
H1 Value	-31.021

Information Criteria

Number of Free Parameters	7
Akaike (AIC)	76.043
Bayesian (BIC)	89.286
Sample-Size Adjusted BIC (n* = (n + 2) / 24)	67.319

RMSEA (Root Mean Square Error Of Approximation)

Estimate	0.000
90 Percent C.I.	0.000 0.000
Probability RMSEA <= .05	1.000

R-SQUARE

Observed Variable	R-Square
NO3	0.259
NH4	0.181
ABUN	0.285

QUALITY OF NUMERICAL RESULTS

Condition Number for the Information Matrix 0.168E-06
(ratio of smallest to largest eigenvalue)

Table A6.3. Full fit diagnostics for 454 Abundance

Chi-Square Test of Model Fit

Value	2.014
Degrees of Freedom	5
P-Value	0.8472

CFI/TLI

CFI	1.000
TLI	1.188

Loglikelihood

H0 Value	-255.324
H1 Value	-254.317

Information Criteria

Number of Free Parameters	13
Akaike (AIC)	536.647
Bayesian (BIC)	561.241
Sample-Size Adjusted BIC (n* = (n + 2) / 24)	520.446

RMSEA (Root Mean Square Error Of Approximation)

Estimate	0.000
90 Percent C.I.	0.000 0.111
Probability RMSEA <= .05	0.878

R-SQUARE

Observed Variable	R-Square
NO3	0.259
NH4	0.181
RICH	0.326
CN	0.241

QUALITY OF NUMERICAL RESULTS

Condition Number for the Information Matrix 0.136E-06
(ratio of smallest to largest eigenvalue)

Table A6.4. Full fit diagnostics for 454 Richness

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