Microbial ecology of hot and cold desert edaphic communities

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Abstract

This thesis presents significant advances into the microbial ecology of hypolithic communities in two hyperarid deserts.

Deserts account for one fifth of the Earths total surface area. These zones differ substantially in terms of climate, geomorphology, hydrology and vegetation. Desert biomes are, however, generally depauperate with particularly with respect to macroorganisms. Hypoliths, photosynthetic microbial assemblages associated with quartz rocks, are widely distributed in hot and cold desert environs where they may represent a large fraction of the standing biomass and mediate key ecosystem processes, including nutrient cycling. However, important questions regarding their (i) development (ii) community structure and assembly patterns and (iii) functional structure remain unaddressed. Here, molecular tools (T-RFLP, clone libraries and pyrosequencing) and multivariate data analyses were used to address these questions.

This study presents evidence of species recruitment in the development of hypolithic communities in the Namib Desert. Hypolithic bacterial communities were compared at a fine scale (10 m radius). Multivariate analysis of T-RFLP-derived data showed that hypolithic and open soil communities were structurally distinct. Applying the ecological concept of ‘indicator species’, 6 and 9 indicator lineages were identified for hypoliths and soil, respectively. Hypolithic communities were dominated by cyanobacteria affiliated to Pleurocapsales, whereas actinobacteria were prevalent in the open soil. These results are consistent with the concept of species sorting and suggest that the underside of the quartz rocks provide conditions suitable for the development of discrete and demonstrably different microbial assemblages. However, strong evidence for neutral assembly processes was found, as almost 90% of the taxa present in the hypoliths were also detected in the open soil. All together, these results suggest that hypolithons do not develop independently from microbial communities found in the surrounding soil, but selectively recruit from local populations.

The bacterial community structure and assembly patterns in hypolithons from Miers Valley (Antarctica) were investigated. Previous studies in this valley have identified three morphologically distinct hypolithic community types: cyanobacteria dominated (Type I), fungus dominated (Type II) and moss dominated (Type III). The bacterial composition of surface soils and hypolithic communities were shown to be clearly and robustly distinct, using T-RFLP analysis. Moreover, the bacterial assemblages were similar in Type II and Type III hypolithons and clearly distinct from those found in Type I. Using 16S ribosomal RNA gene (rRNA) 454 pyrosequencing, *Proteobacteria* were shown to be the most important bacterial component of all three types of hypolithic communities. As expected, *Cyanobacteria* dominated Type I hypolithons, whereas *Actinobacteria* dominated Types II and III hypolithons. Using a probabilistic dissimilarity metric and random sampling, deterministic processes were demonstrated to be relatively more important in shaping the structure of the bacterial community found in Type II and Type III hypolithons. Taken together, these results suggest that hypolithic development favors a sequential pathway with Type II hypolithons serving as an intermediate development state between Type I and Type III.

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In a more in depth analysis of the diversity patterns of key nutrient cycling genes in Antarctic Miers Valley edaphic communities, genes coding for carbon fixation (green- and red-like *cbb*L), nitrogen fixation (*nif*H), nitrification (*amo*A) and denitrification (*nir*K and *nir*S), were targeted. Multivariate analysis (PERMANOVA) showed that hypolithic and open soil communities were functionally distinct. Type I hypoliths were functionally more diverse than soils, suggesting higher potential for enzymatic activities. Taxonomic structure (derived from 16S rRNA data) showed congruence with functional traits (genes involved in C and N cycling). Redundancy analysis suggested that chemical variables (S, F, and NO_3) were important structuring forces in the different communities. Taken together, the results suggest that stochastic processes such as dispersion cannot override the influence of environmental factors on functional diversity patterns.

Declaration

I declare that this thesis which details the “Microbial ecology of hot and cold desert edaphic communities” is my own work. Although aspects of this thesis have been published in peer reviewed journals, none of the work within this thesis has been submitted for any degree or examination at any university. Appropriate citations and complete references have been included to cite all work and sources I have used or quoted.

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Dedication

This thesis is dedicated to the memory of my aunt Nketile Mothibedi, my friend Dr. Zama Mtshali, auntie Anna Le-Roux and Fungai Manhanga. You are greatly missed.

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To my dearest Andiswa, this journey has come to an end, but ours is only just beginning. You love and support has helped me through this period.

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Table of Abbreviations

Abbreviation	Definition
Alpha	α
ANOVA	analysis of variance
ARDRA	amplified ribosomal DNA restriction analysis
ATP	adenosine triphosphate
Bases	b
Base pairs	bp
Beta	β
Bp	base pairs
BSA	bovine serum albumin
°C	degrees Celsius
Ca	calcium
$CaCl_2$	calcium chloride
CCA	canonical correspondence analysis
Cfu	colony forming units
Chloride	Cl
Cm	centimeter
Cs	caesium
df	degree of freedom
Degrees Celsius	ºC
DGGE	denaturing gradient gel electrophoresis
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
et al	et alia
EtBr	ethidium bromide
EtOH	ethanol
F	Fluoride
Gamma	γ
g/L	grams per litre
H’	Shannon diversity index

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HCl	hydrochloric acid
h	hours
IPTG	isopropyl β-D-thiogalactosidase
KCl	potassium chloride
Km	kilometer
km^2	square kilometer
l	litre
LB	Luria Bertani medium
LB-amp	Luria Bertani medium containing ampicillin
LBA	long branch attraction
Li	lithium
m	metre
M	molar
MDS	multi-dimensional scaling
nMDS	non multi-dimensional scaling
mg	milligram
MgCl	magnesium chloride
$MgSO_4$	magnesium sulphate
min	minute
ml	milliliter
mm	millimetre
µg	microgram
µl	microlitre
µm	micrometre
µM	micromolar
mM	millimolar
$MnCl_2$	manganese chloride
NaCl	sodium chloride
NaH_2PO_4	sodium phosphate
ng	nanogram
NGS	Next Generation Sequencing
OTU	operational taxonomic unit
PCA	principal component analysis
PCR	polymerase chain reaction

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ppm	parts per million
RCF	relative centrifugal force
RDA	redundancy analysis
rDNA	ribosomal deoxyribonucleic acid
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
rRNA	ribosomal oxyribonucleic acid
s	second
S	Sulfur
SD	standard deviation
sp.	Species
SSU	small subunit
TAE	tris-acetic acid EDTA
TE	tris EDTA
Tris	tris (hydroxymethyl)-aminomethane
T-RFs	terminal restriction fragments
T-RFLP	terminal restriction fragment length polymorphism
OTU	operational taxonomic unit
UV	ultraviolet
v/v	volume per volume
WGA	whole genome amplification
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactosidase

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CHAPTER 1: Literature Review

1.1 Deserts

With the growing awareness of global climate change, Desert research has undergone a resurgence of interest. Desertification, the degradation of land towards greater aridity, has increased as a result of changes in temperature and anthropogenic activities (Le Houérou, 1996). Increased desertification is likely to affect over 15% of the global population, with obvious consequences in losses of plant productivity, water supplies and a loss of livelihoods often in impoverished areas (UNEP, 2007). An estimated 69% of agriculturally used arid lands are either degraded or experiencing desertification, costing an estimated US$ 42 billion annually (Pointing and Belnap, 2012). The serious implications of this problem underlie the importance of conservation, rehabilitation and sustainably managing land and water resources in regions that are vulnerable to continued degradation (UNEP, 2007, Durant *et al*., 2012).

Deserts, also known as arid/dry lands, are a key biome and account for one fifth of the Earths total surface area (approximately 33.7×10^6 km^2) (Laity, 2009) (Figure 1). Deserts are widely defined as areas where the ratio precipitation to potential evaporation (P/ETP) is less than 1 based on direct meteorological observations (UNEP, 1992). There are four key zones of aridity using the UNEP definition (P/ETP), namely: sub-humid (0.5 - < 0.65), semi-arid (0.2 -<0.5), arid (0.05 - <0.2), and hyperarid (<0.05). These zones differ widely in terms of climate, geomorphology, hydrology and vegetation (Tooth, 2012, Thomas, 2011, Pointing and Belnap, 2012). Annual temperature is also a key delineator of deserts. Hot deserts (such as the Atacama, Sahara, and Namib) display average temperatures above 18 °C, while cold deserts (Arctic and Antarctic) have average temperatures of either close to zero or marginally above (typically < 10 °C) (Peel *et al.*, 2007).

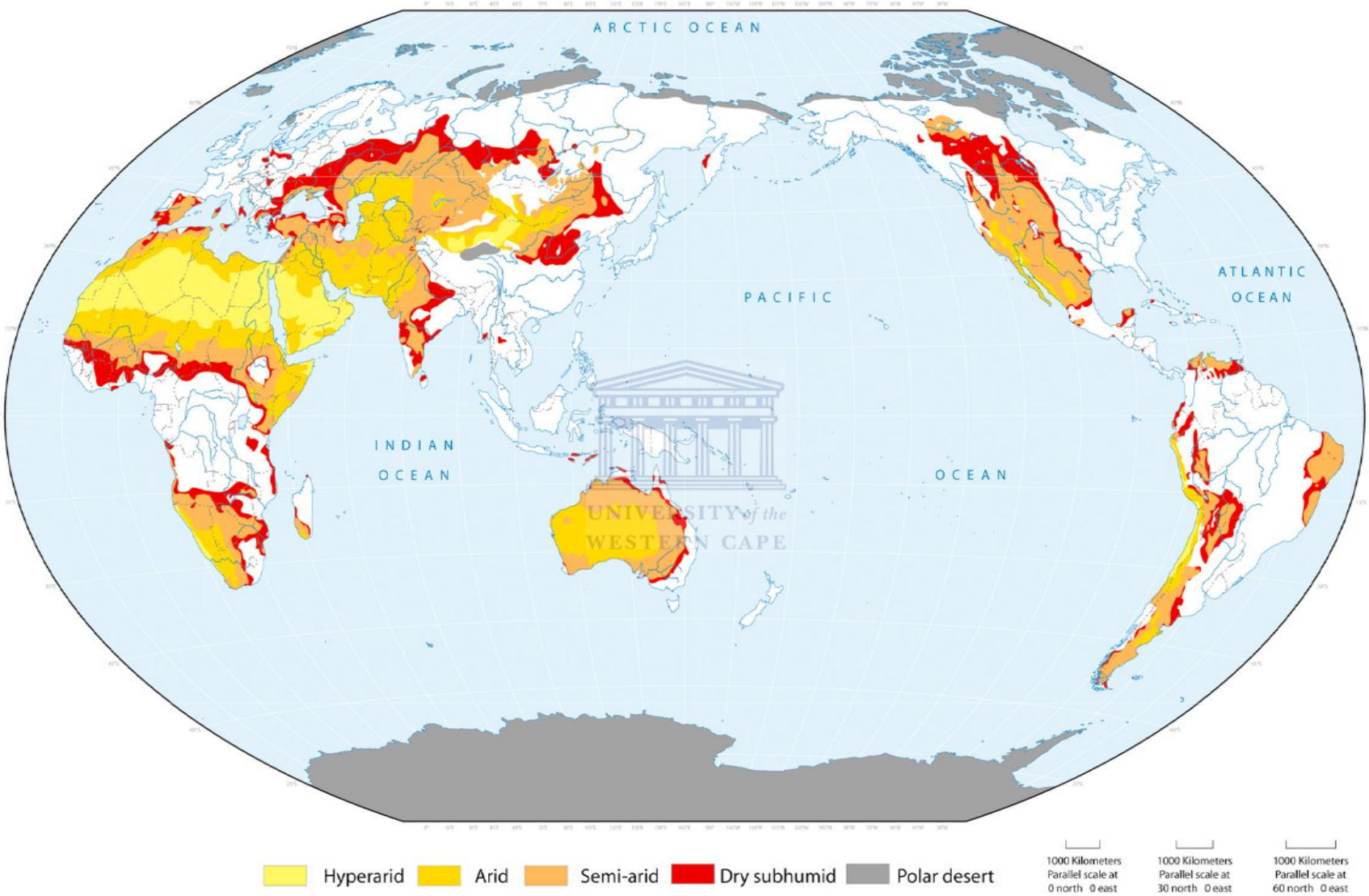

Figure 1: Global distribution of arid regions (Chan *et al.*, 2012).

1.1.1 Namib Desert

The Namib Desert is estimated to be upwards of 80 million years old and stretches for more than 2,800 kilometers along the southwest coast of Africa, from Luanda (8°45'S) in Angola to St. Helena Bay (32°45'S) in South Africa (Prestel *et al.*, 2008). From the Atlantic coast eastward, the Namib gradually ascends in elevation, reaching up to 200 km inland to the foot of the Great Escarpment. The desert geology consists of sand seas near the coast, with gravel plains and scattered mountain outcrops occurring further inland. The sand dunes, some of which are 300 metres high and span 32 kilometres long, are the second largest in the world after the Badain Jaran Desert dunes in China (Figure 2).


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Figure 2 Namib Desert study area near Gobabeb Desert Research Station, Namibia (Photo credit: Prof. D.A. Cowan)

The majority of this desert can be classified as arid (Eckardt *et al.*, 2012) with certain regions demonstrating hyperaridity (Eckardt *et al.*, 2012) for at least the past 5 million years (Ward *et al.*, 1983). Temperatures along the coast are stable and generally range between 9-20 ºC annually, while the central Namib is characterized by wide fluctuations, with minimum temperatures of around 0 ºC being recorded and maxima of above 50 ºC (von Willert *et al.*, 1992, Makhalanyane *et al.*, 2012).

Fog events, that originate offshore from the collision of the cold Benguela Current and warm air from the Hadley Cell, are common in the zone extending from the coast to approximately 60 km inland (Eckardt *et al.*, 2012) and are thought to be the dominant source of biologically available water in the region (Budel *et al.*, 2009)**.** Coastal fogs occur, on average, around 65 days annually at Swakopmund, resulting in fog precipitation of approximately 34 mm annually (Lancaster *et al.*, 1984). Rainfall is reported to show a gradual increase from the coast inland, with average annual rainfall values of around 18 mm. At the Gobabeb Desert Training and Research Station (55 km inland), mean annual rainfall is around 21 mm compared with Ganab (over 100km inland) is 55 mm (Lancaster *et al.*, 1984, Henschel *et al.*, 1998). Taken together, the data reveals fog as a more predictable source of water (Shanyengana *et al.*, 2002), reinforcing a previous observation of the importance of events in this ecosystem (Olivier, 1995).

1.1.2 Antarctic Dry Valleys

Continental Antarctica consists of roughly 0.3% of ice free regions of which only a tiny fraction is composed of terrestrial biotopes (Convey and Stevens, 2007, Cary *et al.*, 2010). The McMurdo Dry Valleys of Eastern Antarctica encompass a considerable portion of the ice-free regions of the continent, and are glacially carved valleys separated from the Polar Plateau by the Transantarctic Mountains (Bockheim and McLeod, 2008). The surface topologies are by no means homogenous and vary greatly in terms of height (ranging from sea level to over 2,000 m) (Doran *et al.*, 2003).

The annual surface temperatures in the Antarctic Dry Valleys are characterized by large scale fluctuations, ranging from around -53 °C in winter to above 9 °C in summer (Doran *et al.*, 2002). These fluctuations limit the amount of atmospheric water, and consequently result in very low mean atmospheric humidity values (Bargagli, 2004, Connolley and King, 2006). The annual precipitations is extremely low, measured at an average of 3.6 kg/m^2 at coastal sites over East Antarctica, and less than 0.5 kg/m^2 on the inland plateau (Connolley and King, 2006). Snow is the principal source of precipitation over most of Antarctica, although summer rains may fall on the western parts of the Antarctic Peninsula (Bargagli, 2004). The mean annual precipitation is estimated at around 15 g/cm^2, although this value is well below the rate of evaporation and sublimation (Cowan and Ah Tow, 2004). Because of the absence of vascular plants and macrofauna, the Dry Valleys have long been considered to represent habitats that are essentially hostile to life (Claridge and Campbell, 1977, Smith *et al.*, 1992, Vishniac, 1993).

1.2 Desert microbial communities

Microorganism, the so called “unseen majority” (van der Heijden *et al.*, 2008), are highly diverse and mediate key ecosystem processes, including nutrient acquirement (Smith and Read, 1997, Sprent and Parsons, 2000, Prosser *et al.*, 2007), nitrogen cycling (Tiedje, 1988, Kowalchuk and Stephen, 2001), carbon cycling (Högberg *et al.*, 2001) and soil formation (Rillig and Mummey, 2006). It is estimated that a gram of soil harbours thousands of bacterial, archaeal, and eukaryotic taxa (Claire Horner-Devine *et al.*, 2003, Curtis *et al.*, 2002, Leake *et al.*, 2004). This taxonomic diversity is mirrored by the diversity of the microorganisms’ protein-encoded functions, what makes microbes important targets in the search for novel pharmaceuticals and other compounds of industrial importance.

Soil associated microbial communities, along with invertebrate populations, may represent major biotic components in Antarctic desert ecosystems (Cary *et al.*, 2010, Pointing and Belnap, 2012, Convey and Stevens, 2007).

Although temperature and water availability are thought to be critical for the composition and survival of desert microbial communities, other factors may also be important. These include radiation (UVA and UVB radiation and photosynthetically active radiation (PAR)), the physical stability of soil surfaces and the geochemical properties of local soil (Cowan *et al.*, 2010b, Cowan *et al.*, 2011a).

An estimated 50% of the deserts surfaces are covered with pebbles or rocks which represent the desert 'pavement' (Laity, 2009). These pebbles or rocks (often quartz or marble) have been reported in all major desert surfaces on Earth (Bahl *et al.*, 2011) but are particularly important components of hyperarid desert pavements. The undersides of these pebbles are often colonized by microbial communities known as hypoliths (Golubic *et al.*, 1981), where the overlying mineral substrate provides protection from incident UV radiation and excessive photosynthetically active radiation (Schlesinger *et al.*, 2003, Cowan *et al.*, 2010a, Wong *et al.*, 2010), thermal buffering in hot (Warren-Rhodes *et al.*, 2006) and cold deserts (Broady, 1981), protection from freeze–thaw events (Cockell and Stokes, 2006), physical stability (Wong *et al.*, 2010), and enhanced moisture availability compared with the surrounding soil (Warren-Rhodes *et al.*, 2006). These communities are dominated by cyanobacteria and are thought to represent a significant input source of carbon (Burkins *et al.*, 2001b) and nitrogen (Cowan *et al.*, 2011) into depauperate desert soils. Interestingly, several studies have suggested an interesting divergence in cyanobacteriarial compositions. Hypoliths from warmer deserts appear to be comprised of largely coccoid Pleurocapsalean cyanobacteria of the genus *Chroococcidiopsis* (e.g., Warren-Rhodes *et al*., 2006; 2007; Bahl *et al*., 2011). Hypoliths in extreme cold and polar deserts appear to support a higher abundance of filamentous oscillatorian cyanobacterial morphotypes and to support lower cyanobacterial richness (e.g., Cockell and Stokes, 2006; Pointing *et al*., 2009; Wong *et al*., 2010). The *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* appear to be ubiquitous in all hypoliths (Pointing *et al*., 2007; 2009; Wong *et al*., 2010; Makhalanyane *et al.,* 2012), and these may represent keystone taxa essential to community assembly in hypolithic communities.

Hypoliths can be envisioned as “ecosystems engineers” (*sensu* Jones *et al*., 1994), as they play critical roles in the structuring of desert communities. Hypolithic biomass (and associated EPS) may contribute to soil stability around colonized rocks (Pointing *et al.*, 2007, Warren-Rhodes *et al.*, 2007). Microbial EPS can also promote soil fertility, as the components of the matrix create a mosaic of polyfunctional binding sites that keep excess heavy metals from the cell surface while concentrating growth-promoting nutrients on the sheath (Gadd, 1990). Hypoliths also collect and absorb dew, and the quantity collected correlates with EPS abundance (Gorbushina, 2007). Since hypolithons have a relatively simple trophic structure, they are also a good model system to study community assembly processes (Chan *et al.*, 2012).

1.2.1 Community assembly theory in hypoliths

Community theory seeks to explain and predict observable phenomena, such as temporal and spatial patterns of diversity (Prosser *et al.*, 2007). There are many conceptual and theoretical approaches to community theory (Table 1). However, only four different processes create and shape diversity in local assemblages: dispersal, diversification, selection and ecological drift (Vellend, 2010, Costello *et al.*, 2012).

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Dispersal, or the spatial movement of organisms, is a fundamental process by which diversity accumulates in local microbial communities. The hypothesis that “everything is everywhere, but the environment selects” (Baas-Becking, 1934) had a powerful impact on thinking about community assembly (Martiny *et al.*, 2006), but a more recent appreciation of the limitations of microbial dispersal suggests that this conceptualization was an oversimplification (Martiny *et al.*, 2006). Assuming that microbial dispersal is restricted, hypoliths may be viewed as “islands”, and as sinks for available colonizers.

Table 1: Framework to disentangle metacommunity paradigms in experimental and observational studies (Logue *et al.*, 2011)

Paradigm	Criteria used in experimental studies	Criteria used in observational studies
Species-sorting (SS)	Habitat patches are environmentally heterogeneous. Dispersal is high enough to enable species to fill niches within habitat patches because of niche diversification. Studies lacking information on dispersal rates or frequencies cannot distinguish between SS or ME.	
Mass-effects (ME)	Habitat patches are environmentally heterogeneous. Dispersal is high enough to override local dynamics (i.e. spatial dynamics are considered explicitly). Studies lacking information on dispersal rates or frequencies cannot distinguish between SS or ME.	
Patch-dynamic (PD)	Habitat patches are environmentally homogeneous. Species differ in their ability to disperse. Along a colonisation–competition trade-off, successful colonisers outcompete poor competitors and vice versa. Experimentally, this requires active mobility or diffusive dispersal based on differing passive mobility rates. Testing PD is counteracted by researcher-mediated bulk dispersal (e.g. via pipetting).	Observationally, differing dispersal abilities among species are considered relevant a priori (although few observational studies have measured dispersal rates). The main criterion here is that habitat patches are environmentally homogeneous. Moreover, dispersal has to be low enough to restrict mobility of the most competitive species.
Neutral-model (NM)	Species do not differ in their fitness or niche (i.e. species composition within habitat patches is not driven by differences in competitiveness or mobility).	

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A second process that operates in microbial communities is local diversification. Unlike in most plant and animal communities, for microbes this process can take place over short ecological time scales. Large microbial population sizes, high growth rates and strong selective regimes result in rapid microbial adaptation via mutation or recombination. Recombination via horizontal gene transfer may be especially common among members of hypolithic assemblages. Hypolithic cyanobacteria form extensive biofilms and biofilms are uniquely suited for HGT, as they sustain high bacterial density and metabolic activity, even in the harshest environments (Sørensen *et al.*, 2005)

Two other processes are important in shaping the structure of microbial communities: selection (environmental conditions and interspecific interactions) and ecological drift or demographic stochasticity (Vellend, 2010). As a result of ecological drift, low-abundance species are more likely to proceed toward local extinction and become lost from the system, unless they have (or can gain) a competitive advantage, access a different niche or become replenished by dispersal from outside the community (Costello *et al.,* 2012).

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Metacommunity theory integrates the four processes described above and provides a useful framework for considering community assembly in hypoliths. Metacommunity theory views the world as a collection of patches (spatially distinct areas of suitable habitat surrounded by a matrix of unsuitable habitat) and is especially helpful for understanding the relative importance of dispersal and environmental selection in shaping hypolithic communities, an issue that has received little attention in desert microbial communities (Logue *et al*., 2011).

1.3 Methods in Microbial Ecology

The first record of a human observation of a bacterial cell dates from 1663, when Antonie van Leeuwenhoek observed bacteria through a homemade microscope. During the following 320 years, microscopy and pure cultures dominated microbiological studies.

In 1985, an experimental advance radically changed the way that the microbial world is visualised. Building on the pioneering work of Carl Woese, which showed that rRNA genes can be used as evolutionary chronometers (Woese, 1987), Pace and colleagues created a new branch of microbial ecology (Lane *et al.*, 1985a, Stahl *et al.*, 1985). They used direct analysis of 5S and 16S rRNA gene sequences in the environment to describe the diversity of microorganisms without culturing (Stahl *et al.*, 1985, Pace *et al.*, 1986). The development of PCR technology and the design of primers that could be used to amplify almost the entire 16S rRNA gene (Giovannoni *et al.*, 1990) accelerated the discovery of diverse taxa as habitats across the Earth were surveyed by the new technique (Barns *et al.*, 1994, Giovannoni *et al.*, 1990). The application of PCR technology provided a view of microbial diversity that was not distorted by the culturing bias and revealed that the uncultured majority is highly diverse and contains members that diverge deeply from the readily culturable minority.

DNA extracted from a microbial community can be analysed in several ways: (i) by using fingerprinting methods, (ii) by cloning and sequencing phylogenetically informative genes, such as those for 16S ribosomal RNA; (iii) by using high-throughput sequence analysis of phylogenetically informative short sequence adaptors (tags); or (iv) by analysing all the genes (“metagenome”) studied, either by random cloning into large or small insert libraries and sequencing those, or by clone-free methods such as pyrosequencing.

Although there are many variants of these technologies; only those that have been used in this study are described below.

1.3.1 Molecular fingerprinting techniques

Molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (Muyzer *et al.*, 1993), terminal restriction fragment length polymorphism (T-RFLP) (Liu *et al.*, 1997), amplified ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999), and single-strand conformation polymorphism (SSCP) (Schwieger and Tebbe, 1998), have proven useful for time-efficient sample processing and comparative analysis of microbial com-munity structure (e.g. Bent and Forney 2008).

1.3.1.1 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is by far the most popular technique in microbial ecology (based on the number of citations). The method itself is relatively simple, and is based on electrophoretic separation of amplicons through a polyacrylamide gel. The primers for DGGE incorporate a 30 – 50 nucleotide sequence made up of guanines (G) and cytosines (C) on the 5' end. This G-C clamp prevents the DNA from completely denaturing when subjected to electrophoresis (in a linear gradient of DNA denaturant, typically a combination of urea and formamide) and separation occurs due to differences in the melting temperature (Muyzer *et al.*, 1993).

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A major reason for the initial popularity of the technique is that amplicons can be excised from the gel, re-amplified using primers without a GC clamp and then sequenced (Sanyika *et al.*, 2012, Diez *et al.*, 2001) or blotted onto nylon membranes and hybridized to molecular probes specific for different taxonomic groups (Straub and Buchholz-Cleven, 1998).

DGGE has been used, for example, to explore differences among samples or environments (Khan *et al.*, 2011, Makhalanyane, 2009, Sanyika *et al.*, 2012, Stomeo *et al.*, 2012, Babalola *et al.*, 2009) or to assess shifts in microbial community structure after environmental disturbance or change (Bourne *et al.*, 2007, Ramond *et al.*, 2012). DGGE analysis has also been used in an investigation which showed that evaporate

rocks harbour communities predominantly made up of cyanobacteria, along with heterotrophic bacteria and archaea (de los Ríos *et al.*, 2010).

1.3.1.2 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Discrimination in this technique is based on the differences in the length of the intergenic space (ITS) region located between the 16S and 23S rRNA genes (Fisher and Triplett, 1999), as it encodes different tRNAs depending on the bacterial species. In ARISA PCR amplicons are separated via capillary electrophoresis. A drawback of this technique is that an OTU may represent more than one taxon, leading to underestimation of species diversity (Crosby and Criddle, 2003).

ARISA has been used to assess the community profiles of different valleys in Antarctica (Lee *et al.*, 2012a) and in a study investigating sources of edaphic cyanobacterial communities (Wood *et al.*, 2008b). As for DGGE, ARISA may be used in order to investigate the variations in time or depth (Boer *et al.*, 2009).

1.3.1.3 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP analysis is one of the most frequently used fingerprinting techniques. The method is similar to classical RFLP but at least one of the primers used is labeled with a fluorescent dye (e.g., 6' carboxyfluorescein (6-FAM)). The resulting mixture of gene amplicons is then digested with one or more restriction enzymes that have four base-pair recognition sites and the sizes and relative abundances of the fluorescently labeled T- RFs are determined using an automated DNA sequencer. Since the differences in the sizes of T-RFs reflects differences in the sequences of the genes (i.e., sequence polymorphisms), phylogenetically distinct populations of organisms can be resolved. Thus, the pattern of T-RFs is a composite of DNA fragments with unique lengths that reflects the composition of the numerically dominant populations in the community.

An obvious weakness of T-RFLP analysis is the lack of directly obtainable phylogenetic data. Unlike DGGE, bands cannot be excised and sequenced in order to assign phylogeny. However, there are online tools which facilitate putative assignments of T-

RFLP (e.g., https://secure.limnology.wisc.edu/trflp/) (Kent *et al.*, 2003) by making use of *in silico* digestions of database sequences. These are generally useful up to phylum level, but it is difficult to assign all TRFs. Many studies have used other sequence based approaches, such as clone libraries, in order to assign phylogeny (Makhalanyane *et al.,* 2012).

These techniques are primarily used to amplify small subunit (16S or 18S) rRNA genes from total community DNA (Taylor *et al.*, 2012, He *et al.*, 2012, Pointing *et al.*, 2009). However, T-RFLP has also been used for the analysis of functional genes such as those encoding for nitrogen fixation (Rosch and Bothe, 2005, Tan *et al.*, 2003) methane oxidation (Horz *et al.*, 2001, Mohanty *et al.*, 2006) or chitin degradation (Peter *et al.*, 2011).

1.3.2 Sequencing Technologies

Sanger Sequencing chemistry was originally described in 1977 and revolutionized studies in the biological sciences (Sanger *et al.*, 1977). The ability to generate amplicons though the introduction of Polymerase Chain Reaction (PCR) has allowed for selective amplification of various genes which has consequently led to improvements in Sanger Sequencing (Mullis *et al.*, 1986). However, in microbial ecology, the 16S rRNA gene has been the most widely used for exploring phylogeny.

1.3.2.1 Clone library analysis in microbial ecology

Construction of clone libraries has been routinely used in microbial ecology for over two decades (Schloss *et al.*, 2004). The method involves extraction of DNA from environmental sample material and the subsequent amplification, by specific primers, of the genes of interest. The amplicons are then purified and ligated to a cloning vector. Further, the ligation mixture is transformed to competent *E. coli* cells and successful transformants screened. Unique clones are then usually sequenced using Sanger sequencing.

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The limitations of clone libraries are now well documented (Curtis *et al.*, 2002, Bent and Forney, 2008); however the method remains useful for inferring community structure of the largely untapped genetic reservoir of soil microbial communities (Daniel, 2005). For example, clone libraries were constructed in order to infer hypolithic community structure in the Atacama (Lacap *et al.*, 2010). In another instance clone libraries were constructed in order to explore the diversity of bacteria in Antarctic Dry Valleys using 16S rRNA gene libraries (Pointing *et al.*, 2009).

1.3.2.2 Next Generation Sequencing

Next Generation Sequencing (NGS) has emerged as an alternative to the more traditional approaches. These techniques have a greater capacity and produce higher volumes of data (Table 2). With the aid of these technologies, microbial ecologists are now able to investigate a range of new questions unhindered by the limitations imposed screening clones (Tringe and Hugenholtz, 2008).

High throughput pyrosequencing technology was developed in 2005 (Margulies *et al.*, 2005). This technology has made it possible to obtain 100-times more sequences for the same cost as traditional Sanger sequencing technology. The first commercially available NGS platform, the Roche GS20 (454 Life Sciences), generated reads averaging 100 bp and could produce 20 Megabases (Mb) of data in a single run (Tringe and Hugenholtz, 2008). One of the first studies using this platform allowed Sogin *et al.* (2006) to generate a total of 118,000 16S rRNA gene pyrosequencing tags. Since then, the use of NGS has increased dramatically, with rapid improvements in sequencing chemistries and signal detection, facilitating more rapid, accurate and cheaper generation of very large volumes of sequence data (Desai and Jere, 2012).

There are currently 6 commercially available sequencing platforms (Table 2), with more under development (Glenn, 2011, Radford *et al.*, 2012). These platforms have often targeted for different applications. For instance, whereas the Illumina has been favored for de novo sequencing of BACS, plasmids and microbial genomes the HeliScope has primarily been used for re-sequencing and transcript counting (Glenn, 2011). Microbial

ecology studies have primarily utilized the 454 and Illumina platforms (Caporaso *et al.*, 2012, Lee *et al.*, 2012b). The high read length (up to 1000 nucleotides) of the 454 sequencing giving more reliable phylogenetic data, while the Illumina platform allows massively parallel sequencing at high accuracy (Caporaso *et al.*, 2012, Liu *et al.*, 2012).

Table 2: The platforms and the detailed information for the NGS technologies

Platform-Instrument	Amplification	Read length (bp)	Yield Mbp per run	Sequencing method
Roche/GS-FLX Titanium	Emulsion PCR	400 to 650	50–650Mbp/run	Pyrosequencing
Illumina/HiSeq 2000, HiScan	Bridge PCR (Cluster PCR)	100 to 150	200 Gbp/run	Reversible terminators
ABI/SOLiD 5500xl	Emulsion PCR	50 – 100	>100 Gbp/run	Sequencing-by-ligation (octamers)
Helicos/Heliscope	None	35 (25 - 55)	21- 37 Gbp/run	True single-molecule sequencing (tSMS)
Life Technologies/ Ion Torrent	Emulsion PCR	50 - 250	200 - 400	Synthesis (H+ detection)
Pacific Biosciences/ PacBio	None	850 -1500	5 -10	Synthesis

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NGS applications in microbial ecology studies have been enhanced by incorporating unique sequence tags into the amplification primers. This “barcoding” technology allows for different samples to be multiplexed in a single sequencing run (Hamady and Knight, 2009, Parameswaran *et al.*, 2007). Multiplexing involves amplification using the different “tag” primers, pooling the amplicons and sequencing. Later, the sequence reads are computationally assigned to different samples. Manufacturers now offer kits for multiplexing. There are also online resources (http://www.grenoble.prabi.fr/trac/OBITools), which allow for tag design subjected to user defined lexical constraints (Coissac *et al.*, 2012). Pyrosequencing technology has been used, for example, to study the biogeography of high-alpine bacteria (King *et al*., 2010) or microbial community variation in human body (Costello *et al.*, 2009).

1.3.2.3 Shortcomings of molecular techniques

All phylogenetic methods are subject to a number of disadvantages. For example, the 16S rRNA gene cannot be used to discriminate between deep branching, closely related taxonomic groups (Janda and Abbott, 2007, Vos *et al.*, 2012). An additional limitation is that PCR may often result in selective amplification of 16S rRNA gene sequences and has been reported to miss almost half the microbial diversity in some samples (Hong *et al.*, 2009). Another drawback with the use of 16S rRNA gene-based phylogeny from mixed-culture DNA is the development of chimeric sequences, which are hybrid products between multiple parent sequences. However, chimeric sequences can be removed by using chimera detection tools (e.g. Chimera Check, UCHIME, Black Box Chimera check) (Ashelford *et al.*, 2006, Gontcharova *et al.*, 2010, Cole *et al.*, 2003, Edgar *et al.*, 2011). An inherent weakness in fingerprinting techniques is that overly-abundant taxa tend to be disproportionately represented (Bent and Forney, 2008), making it difficult to relate banding patterns to changes in particular species or lineages. Current NGS platforms are also prone to various drawbacks such as very short read lengths, and higher error rates (Hamady and Knight, 2009, Schloss *et al.*, 2011).

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1.3.3 Data Analysis in microbial ecology

Once information is obtained using either molecular fingerprinting or sequencing based analysis there are two principal options available for data analysis. Firstly, the data are usually converted to tables where the columns may represent samples and the rows indicate either a taxonomic group or a gene function (these are called data matrices), and the fields contain abundance or presence/absence data (Thomas *et al.*, 2012). Secondly, the data are used to produce phylogenetic trees. These are typically graphical representations of sequence data which consists of branches and nodes. The branches represent a genetic lineage through time while the nodes arise for every new lineage included in the tree. Because these data contain multiple variables (e.g. species, OTUs or genes), the application of multivariate statistics is named multivariate analysis.

There is a wealth of multivariate analyses methods (Table 3) (Ramette, 2007); however for the purposes of this thesis discussions will be limited to the following:

(i) Quantification of microbial diversity, rank abundance and Venn diagrams
(ii) Cluster and ordination analysis
(iii) Assessing why microbial groups differ in space and/or time
(iv) Assessing differences between and within groups

1.3.3.1 Quantification of microbial diversity, rank abundance curves and Venn diagrams

A frequent point of departure for studies focused on microbial ecology is the examination of species diversity. Species enumeration is important for understanding community structure. While definitions of species are unambiguous for macroorganisms, this is not the same for microorganisms. Species are most commonly defined through the biological species concept promoted by Mayr (1942). This is a genetic definition that envisages a species as a group of interbreeding individuals that is isolated from other such groups by barriers to recombination. However, prokaryotes (and some eukaryotes) reproduce asexually, and frequently acquire new genetic information through horizontal gene transfer (HGT). Consequently, the concept of a microbial species is arduous, and microbial ecologists have preferred the use of operational taxonomic units (OTUs) or defined phylotypes.

Beyond the core definition of a species, community diversity has been measured in three main ways. First, diversity within a given community (α-diversity) is usually characterized using the total number of species (species richness), the relative abundance of the species (species evenness) or a combination of the two dimensions (Table 4) (Lozupone and Knight, 2008). Diversity between different communities in an environment (β-diversity) is often characterized using the number of species shared between two communities. Secondly, the analysis can be either qualitative (measuring only the presence-absence data) or quantitative (also taking into account relative abundance). Thirdly, all defined OTUs or phylotyes can be treated as being equally related to one another (taxon diversity), whether the phylogenetic distance between

Table 3: Usage (%) of multivariate analysis in different fields (Ramette, 2007)

	Exploratory analysis				Hypothesis-driven analysis						
Keywords	Cluster	PCA	MDS	PCoA	CCA	RDA	Manova	Mantel	ANOSIM	CVA	Total number
Bacter*	48.5	38	4.5	0.4	3.2	1.8	1.3	0.4	0.9	1.1	1141
Microb*	45.8	40.2	3.9	1.1	2.2	2.2	1.1	1.7	0.6	1.1	179
Plant*	40.2	28.5	4.6	1.7	15.5	3.7	1.9	2.3	0.6	0.9	3335
Fung*	54	27.2	2.8	1.1	8.5	2.8	0.9	1.1	0.2	1.4	563
Fish	30.1	33.7	9.8	0.3	13.5	2.7	3.6	2.9	2.3	1.2	1464
Bird*	41	20.5	5.4	0.7	21.2	3.5	2.1	4.2	0.5	0.9	429
Insect*	54.3	13.7	6.1	0.8	11.5	4.4	3.5	3	1.1	1.7	637

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* Asterisks were placed at the end of each keyword to accommodate for variations.

each pair of the OTUs is considered (Phylogenetic diversity), or whether the ecological roles are taken into account (Functional diversity). Diversity indices have been used to study, for example, the drivers of bacterial communities in soil (Ge *et al.*, 2008) or the temporal variation in microbial communities in water (Gilbert *et al.*, 2010).

Table 4: Categories of diversity measurements

	Measurement of diversity within a single community (α-diversity)	**Measurement of diversity Between communities (β-diversity)**
Only presence/ absence of taxa considered	Qualitative alpha-diversity (Richness) Species-based: Chao 1 ACE Rarefaction Divergence-based: Phylogenetic diversity (PD)	Qualitative beta-diversity Species-based: Sorensen index Jaccard index Divergence-based: Unweighted UNIFRAC Taxonomic similarity (DS)
Additionally accounts for the number of times that each taxon was observed	Quantitative alpha-diversity (Richness and/or Evenness) Species-based: Shannon index Simpson index Divergence-based: Θ	Quantitative beta-diversity Species-based: Bray-Curtis index Morisita-Horn index Divergence-based: Weighted UNIFRAC F_{ST} DPCoA

Whittaker plots, commonly known as rank abundance curves, depict the relative abundance of species. The curve is a two dimensional chart showing the taxon rank number (in order of abundance) on the horizontal axis and their abundance on the vertical axis. A rank abundance is a graphic means of observing species richness and species evenness. Species richness can be viewed as the number of different species on the chart (i.e., how many species were ranked), whereas species evenness is derived from the gradient of the line that fits the graph. Low evenness is indicated by a steep gradient as the high-ranking species have a much higher abundance than the low ranked species. A gentle gradient indicates high evenness, as the abundance of different species is similar. Rank abundance curves were used to show, for example, that dormancy contributes to the conservation of microbial diversity in lake ecosystems

(Jones and Lennon, 2010). Rank abundance has also been used to demonstrate that bacteria are confined to single assemblages and that abundant bacteria are more widely distributed (Nemergut *et al.*, 2011).

A simple and visual manner of depicting the number of unique and shared taxa across localities is by means of a Venn diagram (Figure 4). These diagrams are based on the presence or absence of defined OTUs, where the circles (or squares in other cases) are used to denote the different microbial communities. Shared OTUs are denoted by overlaps in the circles (core taxa). It is thought that commonly occurring organisms that appear in all assemblages associates with a particular habitat are likely to be critical for the function of that particular community (Shade and Handelsman, 2012). Venn diagrams have been used to show that, for example, no OTUs are shared between fresh and marine water samples in an Atlantic rain forest, Brazil (Silveira *et al.*, 2011). These diagrams have also been used to investigate the impact of long term organic and inorganic amendments on the actinobacterial community in soils (Piao *et al.*, 2008).

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1.3.3.2 Cluster and ordination analysis

The objective of cluster analysis is to group objects into separate categories based on their dissimilarities. Cluster analysis is therefore generally recommended for use when distinct discontinuities, instead of continuous differences, are expected between communities. Many clustering methods have been used in microbial ecology studies (reviewed in Ramette 2007). For example, hierarchical clustering has revealed the existence of a characteristic brackish bacterial community in the central Baltic Sea (Herlemann *et al.*, 2011), while k-means clustering has shown that, under aerobic conditions, strains with relatively enhanced tolerance to As(III) predominated over the most As(V)-tolerant strains across a soil arsenic gradient (Valverde *et al.*, 2011).

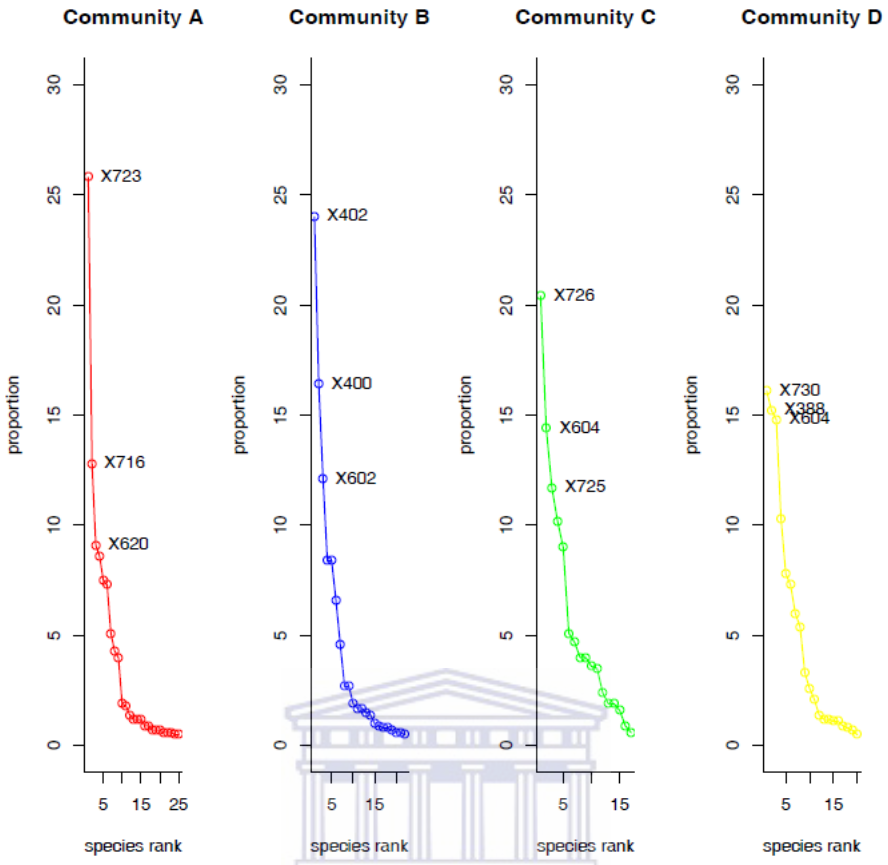

Figure 3: Rank-abundance plots of the T-RFLP profiles which may be used to visualize the distribution of OTUs in various communities. The y axis shows the relative abundance of each TRF, whereas the x axis is the ordinal rank of the TRFs from most abundant (1) to least abundant (n).

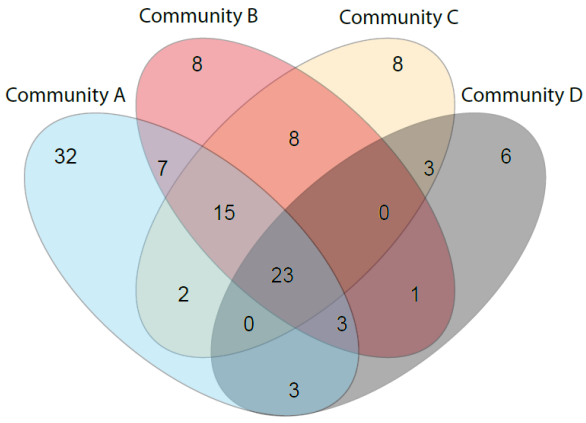

Figure 4: Venn diagram plot of T-RFLP profiles which may be used to visualize "core" group of taxa.

While cluster analysis looks for discontinuities in a dataset, ordination extracts the main trends in the form of continuous axes. It is therefore especially well adapted to analyze data from communities structured in gradients. Among ordination methods non-metric multidimensional (nMDS) is one of the most widely used (Figure 5). The nMDS algorithm ranks distances between objects, and uses these ranks to chart the objects nonlinearly onto a simplified, two-dimensional ordination space to preserve their ranked differences, and not the original distances (Ramette, 2007). As a result, on an nMDS plot, the proximity between objects (microbial communities) corresponds to their similarity, but the ordination distances do not correspond to the original distances among objects. Because nMDS uses an iterative procedure we can inspect the stress and R^2 (goodness-of-fit) values, which describe the quality of the ordination. A plot with a stress value below 0.2 is considered a reliable representation of the data (Clarke, 1993). nMDS has been used, for example, to study the global patterns of bacterial beta-diversity in seafloor and seawater ecosystems (Zinger *et al.,* 2011), or to study soil bacterial community structure at the continental scale (Lauber *et al.*, 2009b).

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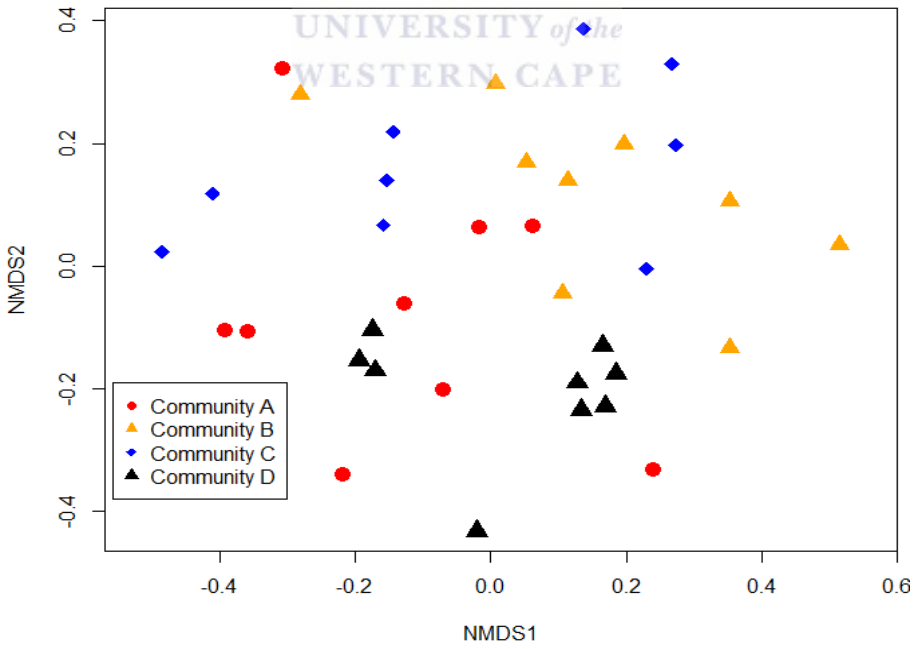

Figure 5: nMDS plot allowing community structure patterns to be visualized easily on a 2D plot.

1.3.3.3 Assessing differences between and within groups

Due to the necessity imposed by microbial ecology studies (that is, experimental design and replication) (Knight *et al.*, 2012, Prosser, 2010) it has become increasingly important to test whether the variation between and/or within groups of samples is significantly different. To this effect, methods such as permutational analysis of variance (PERMANOVA) (Anderson, 2001) and analysis of similarities (ANOSIM) (Clarke, 1993) are regularly implemented to assess the differences between groups, while permutational dispersion (PERMADISP) has been used to assess differences within groups.

In permutational ANOVA, the test statistic is a multivariate analog to Fisher's F ratio. However, the F ratio is not distributed like Fisher's F ratio (ANOVA) since (i) the distribution of the individual species may follow an irregular pattern (that is, species are not normally distributed) and (ii) similarity is not calculated from a single experimental unit. Therefore traditional probability values (P values) are not used. A randomly generated F value is calculated by randomly shuffling the similarity matrix several times without regard to treatment. The P value is calculated by comparing the value of F obtained with the actual labeling experimental treatments to the distribution created by permutation of the labels (Chase, 2007). PERMANOVA has been used, for instance, to experimentally test the bacterial distance decay relationship (Bell, 2010).

ANOSIM is a randomization-based method of multivariate analysis used to compare the variation in species abundance and composition among sampling units (β-diversity) in terms of some grouping factor or experimental treatment level (Clarke, 1993). Consequently two types of ranks are compared, and the resulting R test statistic measures whether separation of community structure is found (R = 1), or whether no separation occurs (R = 0). R values >0.75 are commonly interpreted as well separated, R>0.5 as separated but overlapping, and R<0.25 as barely separable (Clarke, 1993). As in PERMANOVA, a permutational approach is used to test the statistical significance of R. ANOSIM has been used, for instance, to investigate environmental influences on bacterial diversity of soils on Signy Island, maritime Antarctica (Chong *et al.*, 2009).

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Permutational dispersion uses the similarity matrix of all samples to calculate the spatial median value of community composition (centroid) and the distance of each experimental community to that centroid (Anderson *et al.*, 2006). Calculating distance-to-centroid (dispersion) values for each group of samples gives the F ratio. A P value is then obtained by comparing the actual F ratio to 999 randomly generated (group randomly assigned to each community in the similarity matrix) F ratios (Chase, 2007). Permutation dispersion has been used, for example, to assess the intra-habitat bacterial diversity associated with cold-water corals (Schöttner *et al.*, 2009), or to study the changes in biodiversity produced by nitrogen deposition (Zhang *et al.*, 2011).

1.3.3.4 Assessing why microbial communities differ in space and/or time

The need to understand the factors leading to differences in microbial communities in different places and times remains a key issue in microbial ecology (Anderson *et al.*, 2011). Collection of metadata has become a critical requirement for microbial ecology. These data are crucial in examinations and explanations of observed patterns. Network analysis, vector fitting, mantel tests and redundancy analysis (RDA) are just some of the methods suitable for such examinations. RDA is a constrained ordination method combining regression and principal component analysis (PCA) (Legendre and Gallagher, 2001). The method seeks, in successive order, a series of linear combinations of the variables (environmental data) that best explain the variation of the response matrix (species abundance data). RDA is, therefore, a hypothesis-driven technique where the matrix of exploratory variables conditions the “weights”, the orthogonality and the direction of the ordination axes (Borcard *et al.*, 2011). More important, a null hypothesis of absence of linear relationship between the response and explanatory matrices can be tested in RDA; this is not the case in PCA. Redundancy analysis has been used, for example, to study the biogeography of soil archaea and bacteria along a steep precipitation gradient (Angel *et al.*, 2010) and the changes in coral-associated microbial communities during a bleaching event (Bourne *et al.*, 2007).

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1.3.4 Phylogenetic analysis in microbial ecology

Molecular phylogenies are pivotal in evaluating the ecology of microbes. Once the data are generated, either through conventional or next generation sequencing, the next step is sequence validation (removal of chimeric sequences etc.). The sequences are then aligned using multiple sequence alignments. The choice of the type of algorithm varies and is normally predetermined by the type of final analysis to be carried out. After the output alignment has been attained, the branches and nodes of the resultant tree may be calculated using either distance or character based methods.

Neighbour joining trees utilize distance based methods calculating pairwise distances between two sequences in a sequence alignment. In this type of phylogeny it is possible to employ a number of different substitution models, depending on the nucleotide frequency and the rates of transitions and transversions which apply to the given data set. Neighbour joining phylogeny is computationally efficient and fast but does, however, make it prone to increased occurrence of random errors.

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Maximum Likelihood (ML) infers phylogeny based on a character dataset. ML implements a parametric statistical method, where the probability of proposed substitution models and evolutionary histories that would give rise to a data set being studied, are evaluated (Felsenstein, 1981, Yang, 1997). By this means, the algorithm searches for the tree topology with the highest likelihood or probability given an evolutionary model. A major advantage of the ML algorithm is its statistical power, as a number of different evolutionary and substitution models can be applied and tested. A drawback is its extensive time and computational demand (Yang and Rannala, 2010).

After obtaining a phylogenetic tree it can be imported into, for example, Unifrac (Lozupone and Knight, 2005) to facilitate rapid identification of patterns in large and complex datasets. These visualizations include 3D views of any combination of the first 10 principal coordinates, and parallel coordinates plots that plot the position of each sample along each of the first 10 principal coordinates, showing which coordinates discriminate among groups of samples. Moreover, parallelization of the resampling

techniques, such as jackknifing, makes it more feasible to test whether particular clusters are robust to the sampling effort.

1.3.5 Next Generation Sequence Analysis

NGS platforms have completely revolutionized the field of microbial ecology (Metzker, 2009, Harismendy *et al.*, 2009, Edwards *et al.*, 2006). However, the massive volumes of data generated from NGS runs pose large bioinformatic and computational challenges. For instance, between 1 and 6 billion reads may be generated from 454 GS FLX or Illumina HiSeq runs. The reads produced, although somewhat smaller in terms of length to those from Sanger sequencing, have been shown to be sufficient for accurate community profiling (Liu *et al.*, 2007). However, there currently exists, a chasm between the knowledge of the molecular ecologists generating the data and the required bioinformatic skills required to process it.

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The type of analysis to be carried out would be similar to analysis done on Sanger sequence output, although computers with higher processing capabilities would be required. Typical workflows involve checking the raw data for quality and trimming the sequences (removing primers, pyrosequencing noise and chimeric sequences). Alignments are then generated using a number of available platforms (e.g. Ribosomal Database Project pyrosequencing pipeline, or Greengenes) (Cole *et al.*, 2009, DeSantis *et al.*, 2006) and OTUs defined.

There are a number of routines for analysis of amplicon data generated from pyrosequencing reads. MOTHUR (Schloss *et al.*, 2009) and QIIME (Caporaso *et al.*, 2010) are among the most popular. Both software packages offer a relatively simple, easy to use command based application allowing the user to analyses NGS data from start (checking sequence quality) to finish (OTU based approaches and phylogenetic analysis). The programs allow for microbial community analysis and produce visualizations such as networks of co-occurring species, histograms of within- or between-sample diversity or Venn diagrams.

1.4 Research objectives

Desert environments are ‘epicenters’ for ecosystem change and are primary sites for global climate change. Extreme conditions (e.g. temperatures, low water availability) represent severe ecological constraints which limit the diversity of higher plant and animal life. Lithic microbial communities, especially hypolithic communities, are widely dispersed in arid environments. It is assumed that the hypolithic ‘microenvironment’ attenuates some of the extreme conditions, making it more feasible for microbial communities to survive (Pointing *et al.*, 2007, Cowan *et al.*, 2010b). In these depauperate desert environments, key ecosystem functions are likely to be driven by microbial communities (Whitman *et al.*, 1998). Although such processes are now better understood in the context of other biomes, key issues relating to hypolithic communities in hyperarid environments, such as the kinetics and pathways of assembly, remain unanswered. This thesis aims to address important questions regarding hypolithic development, community structure and assembly patterns, and functional structure.

The specific research objectives are:

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i) To investigate the processes driving community assembly in hypolithons at a microscale in the hyperarid Namib Desert.
ii) To assess the community assembly patterns in hypoliths in Antarctic cold desert hypolithons.
iii) To explore the diversity patterns of key nutrient cycling genes in Antarctic soil communities.

CHAPTER 2: Materials and Methods

2.1 General chemicals and enzymes

Agarose	Lonza
Ammonium persulphate (APS)	Merck
Ampicillin	Roche
Bacteriological agar	Merck
Bovine serum albumin	Roche
Bromophenol blue	Sigma
DNase	Roche
Ethylene diamine tetra acetic acid (EDTA)	Merck
Ethanol	BDH
Ethidium bromide	Sigma
Glacial acetic acid	Merck
Glucose	BDH
Glycine	BDH
Hydrochloric acid (HCl)	Merck
Phenylmethylsulphonyl fluoride (PMSF)	Roche
Restriction enzymes	Fermentas
Sodium dodecyl sulphate (SDS)	Promega
Sodium chloride	Merck
Sodium hydroxide	Merck
T4 Ligase	Fermentas
Tris (Tris[hydroxymethyl] aminoethane)	BDH
Tryptone	Merck
Yeast extract	Merck

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2.2 Buffers, stock solutions, and media

EDTA (0.5 M, pH 8) stock solution*
EDTA salts 186.1 g
NaOH pellets approx. 20 g
Deionized water added up to 800 ml

Tris-HCl (pH8) *
Tris salts 121.1 g
Concentrated HCl 42 ml
Deionized water added up to 800 ml

TAE buffer*
50X TAE (pH8) stock
Tris-HCl 242.2g
Glacial acetic acid 57.1ml
0.5M EDTA 100ml
Deionized water added up to 1L

SOB agar (LB) *
Tryptone 20.0 g
Yeast extract 0.5 g
NaCl 0.50 g
250 mM KCl 1.87 g
Agar 15.0 g

TAE buffer*
50X TAE (pH8) stock
Tris-HCl 242.2g
Glacial acetic acid 57.1ml
0.5M EDTA 100ml
Deionized water added up to 1L

The pH was adjusted to 7 before autoclaving and the medium was supplemented with 100 mg/ml of ampicillin and 100 mM $MgCl_2$ after cooling to less than 55 ºC.

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Ethidium Bromide (EtBr) staining solution
10 mg/ml for pre-staining
Ethidium Bromide powder 1g
Deionized water added up to 100ml
0.3µg/ml for post-staining
10mg/ml Ethidium Bromide 3µl
Deionized water 100ml

SOC Media
Tryptone 20.0 g
Yeast extract 5.0 g
NaCl 0.5 g
250 mM KCl 10.0 ml

The pH was adjusted to 7 before autoclaving; the medium was cooled to approximately 50ºC and the following filter sterilized and added aseptically, 5 ml of 2 M $MgCl_2$ and 20 ml of 1M glucose; the media was made up to 1L.

TE buffer (pH8) *
1M Tris-HCl 1ml
0.5M EDTA 200µl
Deionized water added up to 100ml

* Autoclaving of solutions was carried out at 120 ºC for 20 min unless otherwise stated.

Detailed protocols can be found on Sambrook & Russell (2001): all values in g/L unless otherwise specified.

2.3 Sample Collection and chemical analysis

2.3.1 Namib Desert

Sampling was conducted close to the Gobabeb Training and Research Centre, Namib Desert in April 2010 by Thulani Makhalanyane and Prof. S.B. Pointing. Samples were collected within a 10 m radius site (S 23°32.031', E 015°01.813'). At each of the 5 discrete sampling points, one hypolith and 6 soil samples, at 0 - 10, 10 - 20 and 20 - 30 cm below the hypolith (hereafter, sub-lithic) and at 0 - 10, 10 - 20 and 20 - 30 cm one meter from the hypolith (hereafter, open soil) were aseptically collected (Figure 6).

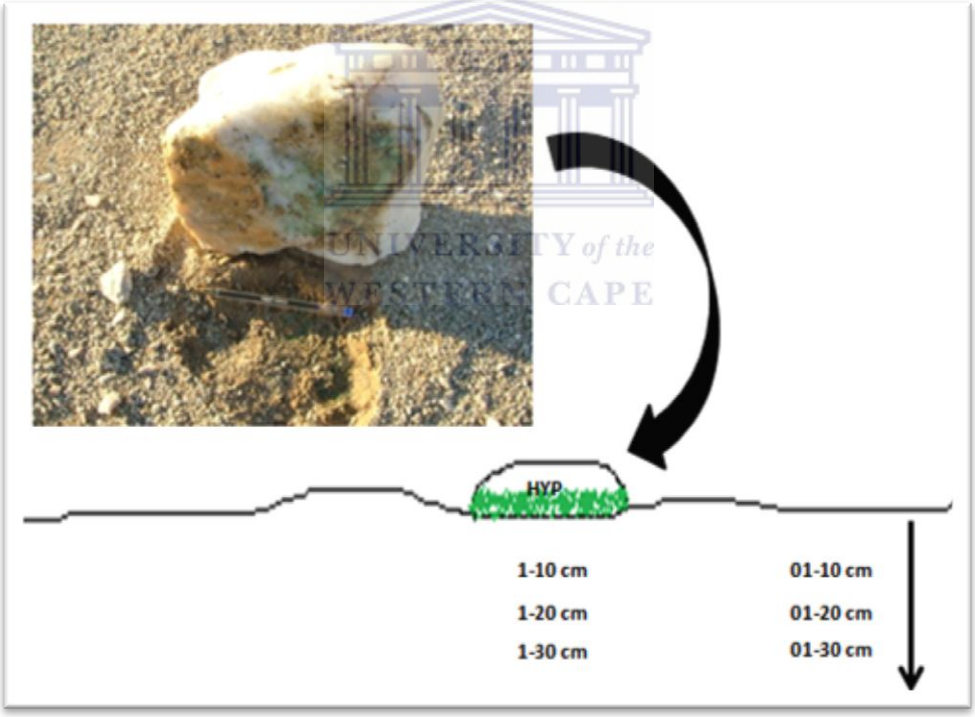

Figure 6: Diagrammatic depiction of the sampling layout. Photos insert showing a hypolithic rock in the Namib Desert.

Hypolithic biomass was recovered by scraping adherent material from the rock sub-surface. Samples (5 hypolithic, 15 sub-lithic and 15 open soils), were transported to the laboratory, homogenized with a sterile spatula, transferred into 2 ml tubes and frozen at

-80°C until further analysis. Soil chemical analysis were determined at the Central Analytical Facility (SANAS Accredited Testing Laboratory, Somerset West, South Africa), according to standard quality control procedures (SSSA, 1996) (Table 5).

Table 5 Physico-chemical properties from the environment where Namibian samples were collected.

Soil Type	Sand
pH (KCl)	7.0
% C	0.09
% N	0.016
Na^+ (mg/kg)	160.93
K^+(mg/kg)	164.21
Ca^+ (mg/kg)	2793.44
Mg^+ (mg/kg)	93.22

*Values are presented as means of five samples.

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In situ micro-environmental data [relative humidity [(% RH) and temperature (ºC)] were recorded in Namib Desert samples using Thermochron/Hygrochron iButtons (model DS1921, Embedded Data Systems, USA). iButtons were positioned beneath hypolithic quartz rocks at the soil surface. Measurements were recorded automatically every 5 min over a 6-month period at different depths of (i.e. 0 - 10, 10 - 20, and 20 - 30 cm).

2.3.2 Miers Dry Valley

Samples were collected from the Miers Valley (S78°05.558', E163°48.557') during the austral summer of 2010, collected as part of a study focused on the distribution of lithic and hypolithic communities (Figure 7) by Prof. D.A. Cowan (Cowan *et al.*, 2010b). Hypolith and open soil samples were collected aseptically in sterile falcon tubes at 0 - 10 cm. In total 36 samples were collected. Samples (9 from each hypolith Type I, II, III, and from open soil) were transported to the laboratory on dry-ice and stored at -80 °C until further analysis. Soil chemical analyses were determined as described in section 2.1.1.

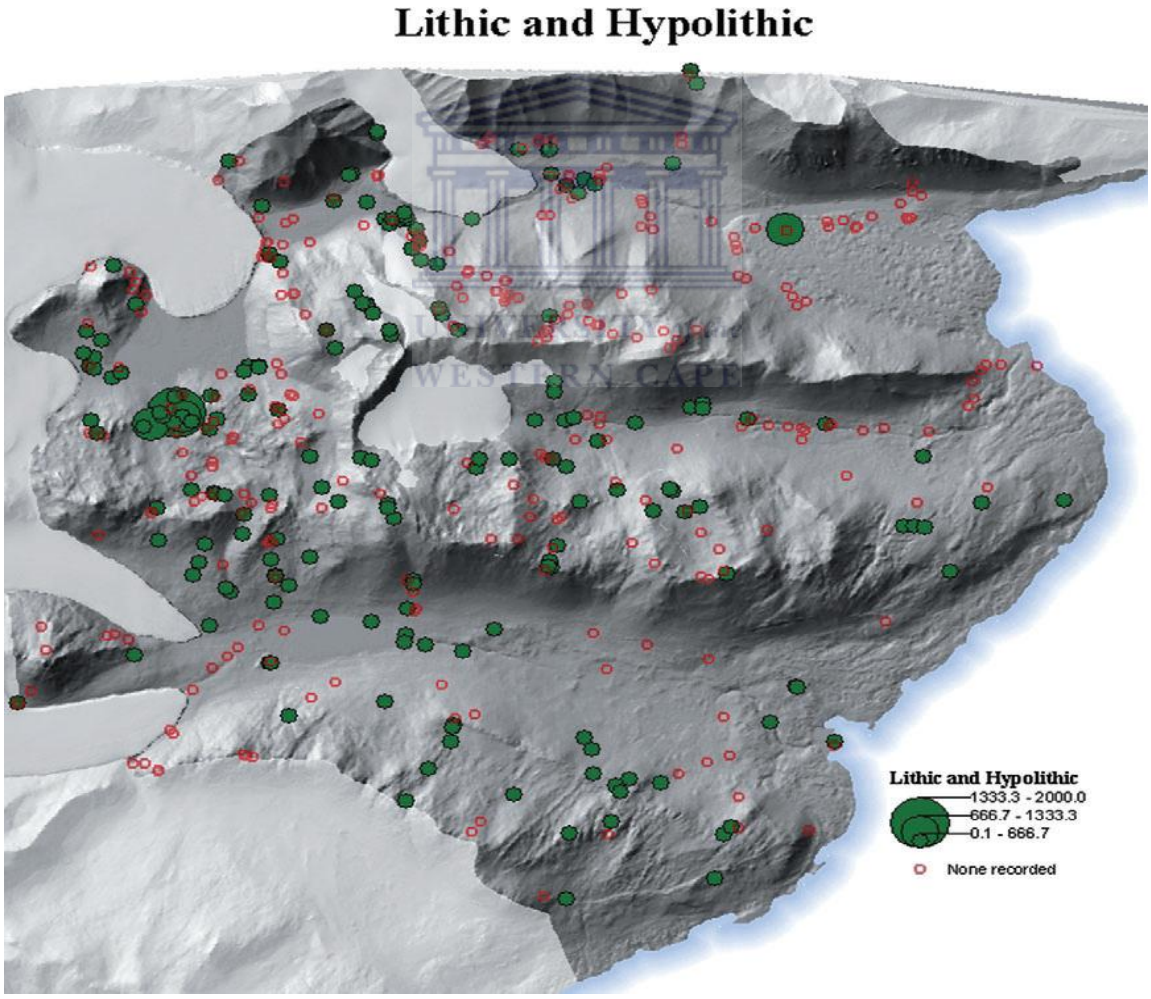

Figure 7: Survey data of hypolith distribution across the Miers, Marshall and Garwood Valleys, Eastern Antarctica

2.4 DNA extraction

Metagenomic DNA was extracted from hypolith and soil samples using the PowerSoil® DNA Isolation Kit (MoBio, West Carlsbad, CA, USA) as specified by the manufacturer with minor modifications. To a 2 ml PowerBead Tube, 0.5 g of sample material was added and gently mixed by vortexing. 60 µl of Solution C1 was added to the mixture, inverted several times before vortexing. The PowerBead Tubes were then horizontally secured using the MoBio Vortex Adapter tube and centrifuged at maximum speed for 15 min. Centrifugation at 10 000 x g was applied for 30 sec at room temperature. The resultant supernatant was carefully transferred to a new 2 ml Collection Tube. 250 µl of Solution C2 was added to the tube then vortexed for 5 sec and incubated at 4^{o}C for 5 min. Centrifugation at 10 000 x g was applied for 1 min at room temperature. 750 µl of the supernatant was transferred into a new 2 ml Collection Tube. 1200 µl of solution C4 was added to the supernatant and vortexed for 5 sec. 675 µl of the resultant supernatant was loaded onto the Spin Filter and centrifuged at 10 000 x g for 1 min. The eluent was discarded and an additional 675 µl of supernatant was loaded to the Spin Filter and centrifuged further at 10 000 x g for 1 min. The column was then washed with Solution C5. To elute DNA, 50 µl of Solution C6 was applied to the column and collected in a new tube.

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2.5 Analytical Techniques

2.5.1 Agarose gel electrophoresis

DNA was separated by agarose gel electrophoresis. Total genomic DNA fragments and PCR amplicons were separated in 1% - 2.5% agarose gels, prepared in 0.5 X TAE buffer (Sambrook and Russell, 2001) (Ethidium bromide (0.5 µg/ml), was added to the agarose gels during preparation for the staining and visualization. Samples were prepared by mixing with 6 X concentrated loading buffer (20% (v/v) glycerol and 5 mg/ml bromophenol). Electrophoresis was performed in 0.5 X TAE buffer at 100 V. DNA bands were sized according to their migration in the gel as compared to DNA molecular weight markers (e.g., DNA cut with *Pst*I restriction enzyme). Gels were visualized via

ultraviolet (UV) light illumination and photographed with a digital imaging system (AlphaImager 2000, Alpha Innotech, San Leandro, CA).

2.5.2 Spectrophotometry

The DNA concentrations (calculated as OD260 nm x 50 ng/µl) and purity (ratio OD260 nm/ OD280 nm) were measured using the NanoDrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA). The solvent used for DNA elution was the reference sample for the determination of the DNA spectrophotometric parameters.

2.5.3 Picogreen assay

The Picogreen assay was conducted as outlined in the Roche Amplicon Library Preparation Method Manual for the GS Junior Titanium Series. Briefly, the DNA standard (100 ng/ul) provided with the Picogreen Assay Kit was thawed and serially diluted to 8 tubes in order to set up a standard. The assay was conducted by transferring 99 ul of 1X TE Buffer and 1 ul of each sample to be quantified into an Eppendorff tube. To the mixture, 100 ul of a 1 in 200 dilution of Picogreen reagent was added to each Ependorff tube. The solution was mixed by pipetting up and down at least 4 times. Quantification was not carried out if the R^2 value of the standard curve was not above 0.98.

2.6 PCR clean-up and Gel Extraction

PCR products were cleaned either directly or after separation on 0.8% TAE agarose gels by electrophoresis as described above (section 2.5.1) using the NucleoSpin® Gel and PCR Clean-up kit. PCR clean-up was done through first amending the DNA binding conditions by adjusting the volume of the reactions to 100 ul with water. To 1 volume of the sample, 2 volumes of Buffer NTI were added. 700 ul of the solution was then applied to the NucleoSpin® Gel and PCR Clean-up Column and centrifuged for 30 sec at 11 000 x g. After discarding the flow-through the silica membrane was washed with 700 ul of Buffer NT3 before centrifugation at 11 000 x g for 30 sec. The silica membrane

was then dried by centrifugation for 1 min at 11 000 x g to remove Buffer NT3. DNA was eluted by placing the column into a new column, adding 15 ul of Buffer NE, incubating at room temperature for 1 min and applying centrifugation for 1 min at 11°000 x g. For gel purification a sterile scalpel was used to excise DNA fragments from the gel, adding 200 ul of Buffer NTI and incubating for 10 min at 50 °C. Thereafter the same routine was applied as for PCR product purification.

2.7 Polymerase chain reaction (PCR)

PCR amplifications were carried out in 50 ul reaction volumes in a Thermal cycler (model 2700 or 2720, Applied Biosystems, CA, USA). The reaction mixture contained 1X PCR Buffer (with $MgCl_2$), 0.2 mM of dNTPs mix, 1/500 Tween-20 and varying amounts of template, primer-pairs and *Taq* polymerase. Autoclaved MQ water was used in making all of the PCR reagents. All primers used in this project, and associated PCR profiles, are listed in Table 6.

2.8 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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Computation analysis preceded T-RFLP analysis in order to select the enzyme which would yield the highest polymorphism in the terminal restriction fragments (T-RFs) and highest specificity to target sequences. Functional gene sequences were obtained from the FunGene (Functional Gene pipeline and repository) (http://fungene.cme.msu.edu//index.spr). Sequences for *cbb*L genes (Green-like and Red-like) were downloaded from the NCBI database (www.ncbi.nlm.nih.gov/). *In silico* digestions were performed using the program TRiFLE (Junier *et al.*, 2008). After determining restriction enzyme and primer sets for T-RFLP (Table 6), PCR was carried out using fluorescently labelled primers. Fluorescein label (FAM) tetrahydrochloro 6-carboxyfluorescein) was added to the 5' end of the forward primers. After triplicate PCR reactions for each sample, amplicons were purified using the NucleoSpin Gel Extraction Kit as described in section 2.6. Amplicon concentrations were normalized and then digested as described in section 2.9.5. The digested fragments were then purified and eluted in 15 ul of deionized water. Restriction fragments were quality checked on agarose gels. Purified products were the sent for GeneScan service at the Stellenbosch

University's Central Analytical Facility (http://academic.sun.ac.za/saf/). Sizing of fluorescently labelled fragments was done by capillary electrophoresis in an ABI3130XL (Applied Biosystems, USA) co-injecting with GeneScan Rox-labeled GS600 (which sizes fragments between 35 bp to 600 bp) or Rox 1.1 (35 bp to 1200 bp) (Table 2.3). T-RFLP profiles from resultant ABI files were then analysed using Peak Scanner™ (version 1.0) (Applied Biosystems, available online (https://products.appliedbiosystems.com). T-RFs shorter than 30 bp were omitted from further analysis.

True peaks and fragments of similar size were identified and binned using the software R (http://www.r-project.org) and Perl (http://www.perl.org) as previously described (Abdo *et al.*, 2006). Each T-RF is assumed to correspond to an Operational Taxonomic Unit (OTU) and the relative abundance of each T-RF was determined by the relative peak area. Further statistical operations were performed on the resultant set of T-RF/abundance data matrix as described in section 2.12.

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Table 6: Primer combinations, cycling conditions and specificity of primers used in this study.

Specificity	Primer Set	Sequence (5' to 3')	Amplicon size (approx.)	Amplification Cycle	Reference
16S rRNA gene	341F 908R	CCTACGGGAGGCAGCAG CCGTCAATTCCTTTRAGTTT	585 bp	Initial denaturation 94ºC for 6 min, 30 cycles of 94ºC for 40 sec, 55ºC for 45 sec ,72ºC for 1 min, Final elongation step of 72ºC for 10 min	(Ishii and Fukui, 2001) (Lane *et al.*, 1985b)
*nif*H gene	PolF PolR	TGC GAY CCS AAR GCB GAC TC ATS GCC ATC ATY TCR CCG GA	360 bp	Initial denaturation 94ºC for 6 min, 30 cycles of 94ºC for 40 sec, 55ºC for 45 sec ,72ºC for 1 min, Final elongation step of 72ºC for 10 min	(Poly *et al.*, 2001) (Poly *et al.*, 2001)
Green-like *cbb*L First rate limiting step of photosynthesis	595F 1387R	GACTTCACCAAAGACGACGA TCGAACTTGATTTCTTTCCA	890 bp	Initial denaturing: 94ºC for 5 min 30 cycles of: 94ºC for 1 min, 60ºC for 1 min, 72ºC for 1 min Final elongation step of 72ºC for 10 min	(Elsaied and Naganuma, 2001a)

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Specificity	Primer Set	Sequence (5' to 3')	Amplicon size (approx.)	Amplification Cycle	Reference
Red-like *cbb*L First rate limiting step of photosynthesis	*cbbL*R1F cbbLR1R	AAG GAY GAC GAG AAC ATC TCG GTC GGS GTG TAG TTG AA	800 bp	Initial denaturing: 94 ºC for 2 min 30 cycles of: 94 ºC for 1 min, 58 ºC for 1 min, 72 ºC for 1 min Final elongation step of 72 ºC for 10 min	(Selesi *et al.*, 2005) (Selesi *et al.*, 2005)
*amo*A	amoA1F amoA-2R	GGGGHTTYTACT GGTGGT CCCCTCKGSAAAGCCTTCTTC	470 bp	Initial denaturing: 94 ºC for 5 min 30 cycles of: 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min Final elongation step of 72 ºC for 10 min	(Rotthauwe *et al.*, 1997) (Rotthauwe *et al.*, 1997)
*nir*K Nitrite reduction	nirK1F nirK5R	GGMATGGTKCCSTGGCA GCCTCGATCAGRTTRTGG	514 bp	Initial denaturing: 94ºC for 5 min, 30 cycles of: 94 ºC for 1 min, 53ºC for 1 min, 72ºC for 1 min Final elongation step of 72ºC for 10 min	(Braker *et al.*, 1998) (Braker *et al.*, 1998)

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Specificity	Primer Set	Sequence (5' to 3')	Amplicon size (approx.)	Amplification Cycle	Reference
*nir*S Nitrite reduction	nirS1F nirS6R	CCTAYTGGCCGCCRCART CGTTGAACTTRCCGGT	890 bp	Initial denaturing: 95ºC for 6 min, 30 cycles of: 94ºC for 1 min, 58 ºC for 1 min, 72 ºC for 1 min Final elongation step of 72 ºC for 10 min	(Braker *et al.*, 1998) (Braker *et al.*, 1998)
*nar*G Nitrite oxidation	narG1960f narG2650r	TAYGTSGGSCARGARAA TTYTCRTACCABGTBGC	650 bp	Initial denaturing: 95ºC for 10 min, 38 cycles of: 94ºC for 1 min, 59ºC to 52ºC for 1 min (decreasing 0.5ºC/cycle,72ºC for 1 min) Final elongation step of 72ºC for 10 min	(Philippot *et al.*, 2002) (Philippot *et al.*, 2002)
*nos*Z Nitrous oxide reduction	nosZ 752F nosZ 1773	ACCGAYGGSACCTAYGAYGG ATRTCGATCARYTGNTCRTT	800	Initial denaturing: 94ºC for 5 min 35 cycles of: 94ºC for 30 s, 57ºC for 1 min, 72ºC for 1 min Final elongation step of 72ºC for 10 min	(Hunter *et al.*, 2006) (Scala and Kerkhof, 1999)

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Specificity	Primer Set	Sequence (5' to 3')	Amplicon size (approx.)	Amplification Cycle	Reference
*nap*A	napA v66 napA v67	TAYTTYYTNHSNAARATHATGTAYGG DATNGGRTGCATYTCNGCCATRTT	707	Initial denaturing: 94 ºC for 5 min 35 cycles of: 94 ºC for 30 s, 49 ºC for 40 s, 72 ºC for 30 s Final elongation step of 72 ºC for 10 min	(Flanagan *et al.*, 1999) (Flanagan *et al.*, 1999)
c*nor*B	cnorB2F cnorB-6R	GACAAGNNNTACTGGTGGT TGNCCRTGNGCNGCNGT	578	Attempted: Initial denaturing: 94 ºC for 5 min 35 cycles of: 94 ºC for 30 s, 55 ºC for 40 s, 72 ºC for 30 s Final elongation step of 72 ºC for 10 min	(Braker and Tiedje, 2003) (Braker and Tiedje, 2003)
q*nor*B Nitric oxide reduction	q-norB2F q-norB5R	GGNCAYCARGGNTAYGA ACCCANAGRTGNCANACCCACCA	262 bp	Attempted: Initial denaturing: 94 ºC for 5 min 35 cycles of: 94 ºC for 30 s, 55 ºC for 40 s, 72 ºC for 30 s Final elongation step of 72 ºC for 10 min	(Braker and Tiedje, 2003) (Braker and Tiedje, 2003)

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Specificity	Primer Set	Sequence (5' to 3')	Amplicon size (approx.)	Amplification Cycle	Reference
*nrf*A nitrate ammonification	nrfA 2F nrfA 2R	CACGACAGCAAGACTGCCG CCGGCACTTTCGAGCCC	520	Attempted: initial denaturing: 94 ºC for 5 min 30 to 35 cycles of: 94 ºC for 10 to 45 s, 53 ºC to 62 ºC for 40 s to 1 min, 72 ºC for 40 s to 1 min Final elongation step of 72 ºC for 10 min	(Mohan *et al.*, 2004) (Smith *et al.*, 2007)
M13 For M13 Rev	Cloning Vector specific	CCCAGTCACGACGTTGTAAAACG AGCGGATAACAATTTCACACAGG		Initial denaturing: 94 ºC for 10 min 30 cycles of: 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min Final elongation step of 72 ºC for 10 min	Cloning vector pGEM® T Easy

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2.9 Cloning of 16S rRNA genes

2.9.1 Preparation of Electrocompetent cells

Electrocompetent DH5α *E. coli* cells were prepared by inoculating a single freshly streaked colony in 20 ml SOB media and cultured overnight at 37ºC with agitation at 250 rpm. 2 ml of the overnight culture was inoculated into a 250 ml sterile flask and cells were grown at 37ºC with shaking (250 rpm) for 3.5 - 4 h to an OD_{600} nm of 0.6. Cells were kept on ice and harvested by centrifugation at 4000 x *g* for 10 min at 4ºC. The cells were then re-suspended in a volume of ice-cold sterile water equal to the original culture volume, and then harvested as before. The supernatant was discarded and the cells were re-suspended in ice-cold sterile 10% glycerol and then centrifuged for 15 min at 4000 x *g*. After decanting the supernatant, cells were re-suspended in ice-cold sterile 15% glycerol, 2% sorbitol using a volume of 2 ml per L initial culture. Cells were harvested by centrifugation at 4000 x *g* for 10 min at 4ºC and then re-suspended in a volume of ice-cold sterile 15% glycerol, 2% sorbitol equal to that of the cell pellet. 50 µl volumes of cells were then aliquoted into clean 0.5 ml Eppendorf tubes. Liquid nitrogen was used to snap freeze the cells which were then stored at -80ºC until further use.

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2.9.2 Ligation of PCR products using Promega pGEM-T™ Easy Vector System

Ligations were carried out using p-GEM™ T Easy Vector System according to manufacturer's instructions. Ligations were carried out in 10 µl volumes. To each tube 5 µl of Rapid ligation buffer was mixed with 1 µl of pGEM-T™ Easy vector. To each reaction 3 µl of PCR product was added to the mixture with 1 µl of ligase being added.

2.9.3 Transformation of competent *E. coli* DH5α strain

The electro-competent DH5α *E.coli* cells were transformed with purified DNA. An Eppendorf tube containing 50 µl of electrocompetent cells was removed from -80ºC storage and allowed to thaw on ice. 2µl of the ligation mixture (section 2.10.2) was added to the thawed cells and gently mixed. The mixture was incubated on ice for approximately 1 min then pipetted into a pre-chilled 0.1 cm sterile electroporation cuvette (Bio-Rad). Electroporation was performed under the following conditions: 1.8 kV, 25 µF, 200 Ω on the BioRad Gene Pulser machine. Immediately following electroporation, 1 ml SOB broth was added to the cuvette; the cells were then transferred to a 15 ml Falcon tube and incubated at 37°C for 1 h with agitation. 100 µl of cells were plated onto LB-agar plates supplemented with ampicillin (100 µg/ml), IPTG (20 µg/ml), and X-Gal (30 µg/ml). Recombinant transformants were selected by blue/white colour selection based on insertional inactivation of the *lac*Z gene for transformations done using pGEM T-Easy™.

2.9.4 Screening of clones

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2.9.4.1 Colony PCR

Colonies were picked from overnight culture plates using sterile toothpicks and swirled in 50 µl TE buffer. 2 µl was directly used as a template for PCR. PCR reaction consisted of 5 µl of 10X Buffer, 4 µl of 25 mM $MgCl_2$, 5 µl of 5 µm of each primer M13 (Table 6), 5 µl of 1 mM dNTPs, 0.5 µl of *Taq* DreamTaq polymerase (Fermentas, Burlington, Canada) and 2 µl of the re-suspended colony in a final volume of 50 µl. Amplification was performed as described in section 2.7. The products were separated by agarose gel electrophoresis (section 2.5.1) and cleaned using the method described in section 2.6 before sequencing.

2.9.5 Amplified Ribosomal DNA restriction analysis (ARDRA)

ARDRA analysis was performed in 96-well plates in volumes of 10-30 ul. Restriction digests were carried out as stipulated by the manufacturer. Appropriate amounts of 10 X buffer for a specific enzyme, and 5-10U of the enzyme per ng of amplicon DNA were added. Reactions were typically incubated overnight to allow for complete digestion in a water bath at 37 °C, unless otherwise stated. The digestion products were analysed by gel electrophoresis in 2% (w/v) agarose gels as described in Section 2.5.1.

2.9.6 Plasmid extraction

Plasmid extraction was performed with the QIAGEN Plasmid Mini Kit (QIAGEN GmbH, Hilden, Germany), using manufacturer's instructions with minor modifications. A single bacterial colony was streaked onto a selective plate and inoculated into a starter culture of 5 ml LB medium containing 100 mg/ml ampicillin. Following overnight incubation at 37 ºC with vigorous shaking (at approximately 300 rpm), cells were harvested by centrifugation at 6000 x g for 15 min at 4 ºC. The bacterial pellet was resuspended in 0.3 ml of Buffer P1. To the suspension 0.3 ml of Buffer P2 was added, mixed thoroughly by inverting the sealed tube 4-6 times, and then incubated at room temperature (15 -25 ºC) for 5 min. 0.3 ml of Buffer P3 was added and vigorously mixed by inverting 4 – 6 times, then incubated on ice for 5 min. Centrifugation was then applied at maximum speed (18 000 x g) before transferring the supernatant to an equilibrated column and allowing the column to empty by gravity flow. The column was washed twice with 2 ml of Buffer QC, before eluting DNA with 0.8 ml of Buffer QF. The eluted DNA was precipitated by adding 0.7 volumes of isopropanol, and then centrifuged immediately at 10 000 x g for 30 min. After decanting the supernatant, the DNA pellet was washed with 1 ml of 70% ethanol at 10 000 x g for 10 min. Once the supernatant was decanted, the pellet was air-dried for 5 -10 min, before dissolving in 10 mM Tris-HCl.

2.10 Sequence Analysis of phylogenetic marker genes

Inferences for phylogenetic and functional affiliations were determined by BLAST searches on the NCBI GenBank nucleotide database (http://blast.ncbi.nlm.nih.gov/) and phylotypes were delineated by 97 % sequence similarity as determined by MOTHUR (version 1.21) (Schloss *et al.*, 2009). Sequences retrieved from this study together with respective sister and outgroup sequences (obtained from the NCBI database) were aligned using ClustalW in BioEdit version 7.0.5.3, (Hall, 1999), followed by manual inspection and editing. The alignments were tested against 88 prescript models of evolution using a free java program jModeltest (version 0.1.1; (Posada, 2003)). The criterion described by the most appropriate evolutionary model were input for maximum likelihood analysis using Genetic Algorithm for Rapid Likelihood Inference (GARLI) (version 0.95) (Zwickl, 2006). The resultant phylogenetic trees were visualized using FigTree (version 1.3.1) (tree.bio.ed.ac.uk/software/figtree). The robustness of furcated branches were supported by both bootstrap values (1000 replicates) determined using PAUP* 4.0b10 (Posada, 2003) and Bayesian posterior probabilities calculated using Mr Bayes (mrbayes.csit.fsu.edu, version 3.04). Values (in percentage) were shown in all branch nodes supported by more than 50% of the trees.

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2.11 16S rRNA gene Amplicon Pyrosequencing

In order to reduce the number of samples, equal amounts of DNA from each of the 9 samples (section 2.3.2) were pooled; generation 3 type specific samples plus an open soil control (Table 7). Multiplex identifiers were added to the primers with a 4 base tag for each sample. The V3 16S rRNA genes were amplified using the primers 341F (5'-CCTACGGGAGGCAGCAG -3') and 805R (5'- CCGTCAATTCCTTTRAGTTT -3'), containing the 454 FLX adaptors, with the sample-specific multiple identifier (Table 7).

Table 7: The Adaptor and Tag sequences used for pyrosequencing on the Roche 454 Platform.

Sample Name	Adaptor (5' to 3')	Tag Sequence
Hypolith Type I	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATA
Hypolith Type II	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACAG
Hypolith Type III	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TTCA
Type IV (Soil)	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCG

A PCR mixture contained each primer at 0.5 µM, 0.2 mM of each dNTP, 0.02 nM of Phusion High-Fidelity DNA Polymerase DNA Polymerase and 1 nM Phusion HF buffer (Finnzymes, Espoo, Finland). PCRs were conducted in triplicate and pooled as previously described (section 2.6) and quantified using the Picogreen assay kit (Molecular Probes, Invitrogen, Carlsbad, CA, USA). Samples were then submitted to the GATC Biotech (Konstanz, Germany) for pyrosequencing.

Pyrosequencing data were analysed using MOTHUR (version 1.27.0) following a previously established pipeline developed by Schloss and colleagues (http://www.mothur.org/wiki/Download_mothur) (Schloss *et al.*, 2009). Briefly, the Fasta, quality and flow data were extracted using the sffinfo command. In order to remove sequences of low quality MOTHUR uses the shhh.flows command which is an implementation of the PyroNoise component of the AmpliconNoise suite of programs. The dataset was then simplified by obtaining the unique sequences using the unique.seqs command. An alignment was then generated using the align.seqs command by aligning the data to the SILVA reference alignment (http://www.arb-silva.de/download/arb-files/). In order to make sure that there was no overlap between the sequences, the screen.seqs command was used. Sequences that did not match the reference alignment were removed. Identification of chimeras was through the chimera.slayer application. The taxonomic affiliations of the OTUs were determined using the naïve Bayesian rRNA classifier (Wang *et al.*, 2007) and a confidence threshold of 80 %. The sequence data are available at the NCBI Sequence Read Archive under the accession number SRA058593.

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2.12 Multivariate Statistical Analysis

In order to perform multivariate analysis, various software programs were used including Arlequin (version 3.1) (Excoffier and Scheider, 2005), Primer 6 (version 6.1.5.81 (Primer E Ltd, Plymyth, UK), and R statistical package 2.15.1 using vegan, BiodiversityR, picante (Kembel *et al.*, 2010), gplots, labdsv packages (www.r-project.org).

2.12.1 Clone Library analysis

Arlequin v3.0 (Excoffier and Scheider, 2005) and Unifrac (Lozupone *et al.,* 2006) were used to assess the phylogenetic differences between communities using the FST and P tests, respectively. Phylogenetic OTUs at a similarity level of 97 % were determined using CD-HIT http://weizhonglab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=h-cd-hit-est). Diversity estimates (Chao1) were calculated using an online tool (http://www.aslo.org/lomethods/free/2004/0114a.html) (Kemp and Aller, 2004). *In silico* predictions of terminal restriction fragments (T-RFs) from clone libraries were performed using TRFCUT (Ricke *et al.,* 2005). Sequence data have been submitted to NCBI GenBank database (accession numbers JN714842 - JN714926). All other analyses were conducted using R (http://www.R-project.org).

2.12.2 T-RFLP analysis

Chapter 3

T-RFLP data reflecting relative OTU abundance were Hellinger-transformed (Legendre and Gallagher, 2001) and used to calculate Bray-Curtis dissimilarity matrices (Bray and Curtis, 1957), which were further visualized using non-metric multidimensional scaling (NMDS). To account for the unequal number of samples (5 hypoliths vs. 15 of each soil type); a resampling procedure was done; taking 5 samples of each habitat type to achieve 100 randomly generated nMDS plots. Permutational multivariate analysis of variance (PERMANOVA), function adonis (vegan package for R), was performed to test for significant differences between sample groups (hypoliths, sub-lithic soil and open soil). MRT analysis (De'Ath, 2002) was used to determine correlations between

bacterial community composition and habitat parameters (e.g. type and depth) (mvpart package for R). Indicator species analysis (IndVal index) (Dufrene and Legendre, 1997), which combines relative abundance and relative frequency of occurrence, was used to identify the species that were statistically significant indicators of the habitat type (labdsv package for R).

Chapter 4 and 5

Multivariate analysis including diversity indices, species richness and nMDS and RDA were used to analyse the microbial diversity. Similarity matrices of community compositions based on T-RFLP and 454 pyrosequencing data (Chapter 4) were calculated using the presence/absence based Jacard index and relative abundance based Horn index. Nonmetric multidimensional scaling (NMDS) analysis was performed on the similarity matrices to visualize community structure. Similarity matrices were also compared using Mantel's matrix randomization test (Mantel, 1967) (Chapter 5) with Pearson's correlation and 999 permutations. Data analyses were performed using predefined R functions and packages described above.

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2.12.3 Pyrosequencing analysis

To estimate whether a defined bacterial community (subsidiary community) represented a subsample of another community (original community) we used a random sampling procedure (Besemer et al 2012). This procedure can be regarded as conservative as the subsidiary community was reduced to OTUs which occurred also in the original community, therefore increasing the chance that the subsidiary community resembles the original community.

CHAPTER 3: Evidence of species recruitment and development of hot desert hypolithic communities

3.1 Introduction

The Namib Desert in South West Africa is considered to be the world's most ancient desert and has substantially varied ecotopes including gravel plains, dunes, inselbergs, escarpments, and playas (Eckardt and Drake, 2010). This desert spans a longitudinal distance of over 200 km, stretching from the western coastline to the eastern mountains along the Tropic of Capricorn (Figure 8). The Namib has been classified as an arid zone with some regions demonstrating hyperarid characteristics (Eckardt *et al.*, 2012). The desert surface is subject to wide temperature fluctuations (from 0 °C to as high as 50 °C) with a general increase from the coast inland. Rainfall patterns within this desert are scant and erratic, with long periods of aridity (Eckardt *et al.*, 2012).


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Figure 8:**Landscape topology in the central Namib Desert (Courtesy of Prof D.A. Cowan)**

The undersides of rocks in climatically extreme deserts, such as the Namib, act as a refuge for microorganisms (defined as "hypoliths") and their community (the "hypolithon") (Pointing and Belnap, 2012, Chan *et al.*, 2012). The overlying rock creates a favourable sub-lithic microhabitat where microorganisms benefit from greater physical

stability, desiccation buffering, increased water availability and protection from UV fluxes (Cowan *et al.*, 2010b, Pointing *et al.*, 2009). As they are typically dominated by primary producers (Cockell and Stokes, 2004, Wood *et al.*, 2008a) hypolithic communities are thought to be significant contributors to regional carbon and nitrogen inputs (Burkins *et al.*, 2001a, Cowan *et al.*, 2011b).

Previous studies have suggested that hypolithons develop independently from surrounding soil communities (Warren-Rhodes *et al.*, 2006, Pointing *et al.*, 2007, Tracy *et al.*, 2010, Davila *et al.*, 2008). However, data on the mechanisms of community assembly leading to site-to-site variations (β-diversity) in community composition in deserts remain scant. Recently, Caruso *et al.,* (2011) reported that deterministic and stochastic processes interact in the assembly of hypolithons on a global scale. However, the drivers of bacterial beta-diversity are known to depend both on spatial (Martiny *et al.*, 2011) and temporal scales (Lindström and Langenheder, 2012, Langenheder *et al.*, 2012). For example, dispersal limitation was found to drive Nitrosomondales beta-diversity at the scale of an individual marsh (Martiny *et al.*, 2011). In direct contrast, the environment was the most important factor in explaining differences between these communities across regional and continental scales (Martiny *et al.*, 2011). These differences highlight the need to identify the patterns and mechanisms that shape bacterial community composition in different habitat types and at different spatial scales.

Here, the ecological concept of "indicator species**"** (Dufrene and Legendre, 1997) is applied to investigate the process behind hypolithic community assembly at a microscale (10 m radius), and strong evidence is presented that in the Namib Desert recruitment from soil sources supports hypolithic community assembly. It is predicted that should deterministic processes be significant, hypoliths and surrounding soil should demonstrate greatly dissimilar bacterial communities (specialists). If the effect of the environment is limited, both hypolith and surrounding soil should contain similar bacterial communities (generalists).

3.2 Results and discussion

The comparative bacterial composition of hypolithic and nearby soil samples at a site in the hyperarid Namib Desert was assessed using T-RFLP analysis and clone libraries (Chapter 2 section 2.8). A total of 98 T-RFs were obtained, ranging from 23 to 44 OTU's for the individual samples. When averaged for the different sample types, hypoliths and surrounding soil contained similar numbers of OTUs, with values of 22.0 [±4.7 (SD)], 25.5[±7.2 (SD)] and 30.3 [±6.7 (SD)] for hypoliths, open soil and sub-lithic soil, respectively. A comparison in OTU composition (β-diversity) revealed that 5 OTUs were unique to the hypoliths, 10 were unique to the open soil and 29 were unique to sub-lithic soil (Figure. 9). In total, 38 OTUs (38 % overlap) were shared between hypolith and soil samples.

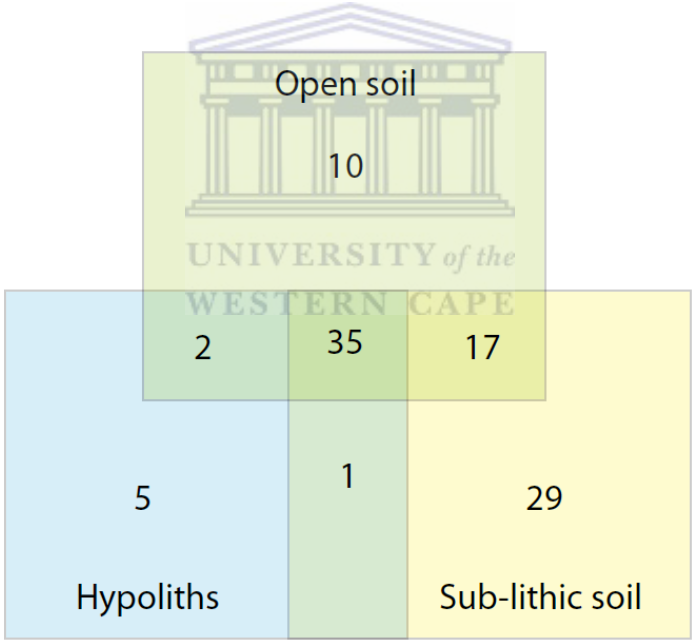

Figure 9: **Venn diagram comparing the distribution of bacterial T-RFLP fragments between hypolith and soil samples.**

When bacterial community patterns were visualized by NMDS of Bray Curtis similarities, communities grouped separately according to their habitat (Figure 10). Similar results were obtained after accounting for the unequal number of samples by applying a random resampling procedure (Appendix A). When habitat type, depth and the

interaction between both factors were assessed in an *adonis* model (PERMANOVA analysis), habitat was found to have a significant effect ($F_{2,28}$=4.82, P=0.001). Each group was clearly distinct (hypoliths vs. sub-lithic soil R^2=0.26, P=0.001; hypoliths vs. open soil R^2=0.30, P=0.001; sub-lithic vs. open soil, R^2=0.08, P=0.02); that is, the overlying quartz rocks not only influenced the hypolithon but also the soil bacterial community below the rock. Although differences between hypolithic and soil bacterial community structure have been reported in polar deserts (Pointing *et al.*, 2009, Khan *et al.*, 2011), similar observations have not been reported for hot desert communities. In contrast to previous studies of microbial communities (Zhou *et al.*, 2002, Ge *et al.*, 2008) no spatial variation on vertical axes was observed, although these studies were performed on a broader scale and bacterial community patterns are known to depend on both spatial and resource factors (Zhou *et al.*, 2002, Martiny *et al.*, 2011).

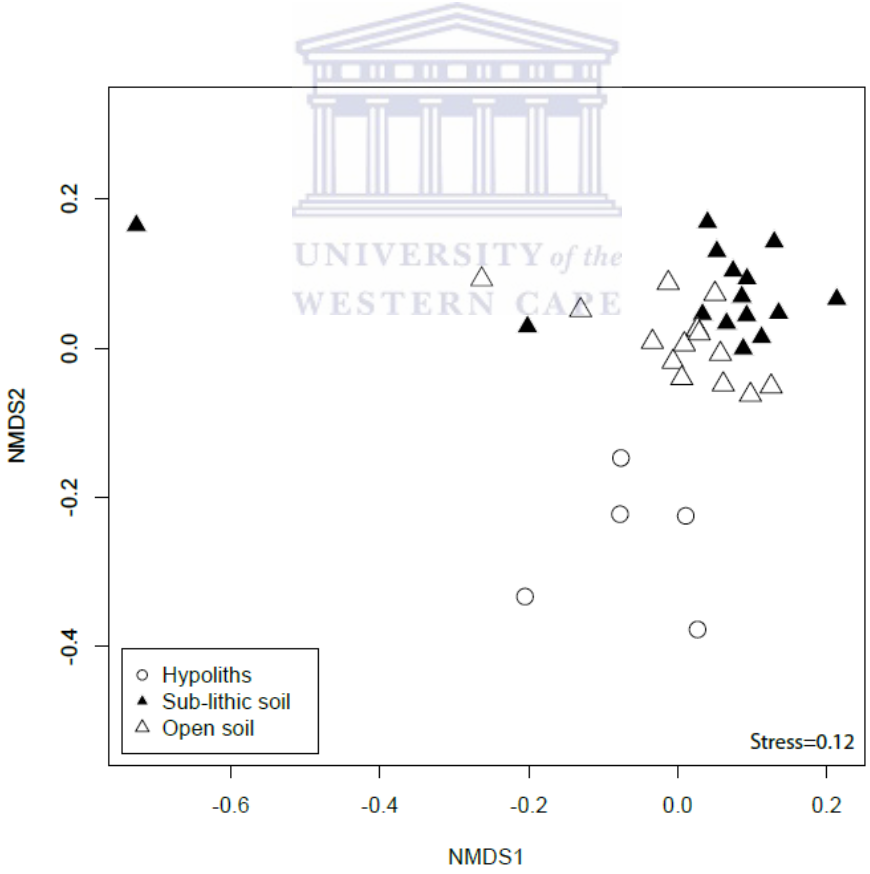

Figure 10: nMDS ordination plot (Bray-Curtis distance matrix) of T-RFLP profiles for soil- and hypolith-derived samples. The quality of the ordination is indicated by a low-stress value.

In order to relate OTU abundance and habitat type, a multivariate regression tree (MRT) analysis was performed. Habitat type alone explained 10 % of the variation observed. Indicator OTUs identified using the IndVal indexes were mainly responsible for the topology of the tree (Figure .11a) suggesting that these specialist lineages represented ecological indicators of the prevailing environmental. Overall, 6 and 9 OTUs were found to be statistically significant indicators of the hypoliths and surrounding soil, respectively ($P < 0.05$) (Figure 11b).

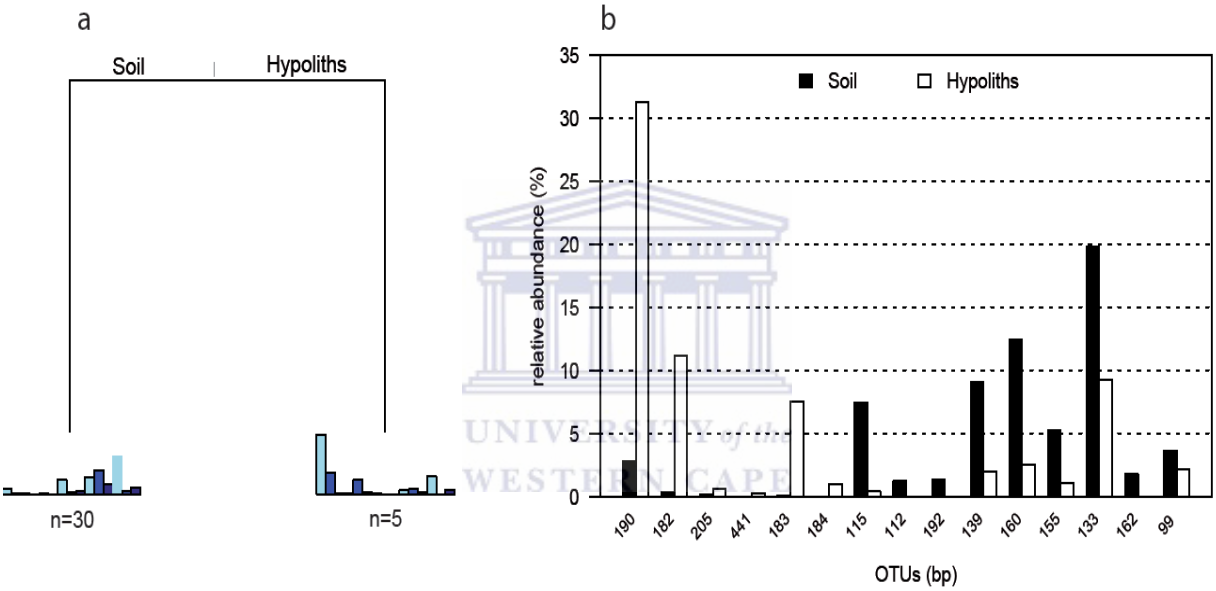

Figure 11: Multivariate regression tree (MRT) analysis (A). The model explained 10% of the variance in the whole data set. The bar plot under each leaf [magnified in (B)] shows the contribution of the different indicator species. The number of samples included in the analysis is shown under the bar plots.

Clone libraries yielded a total of 85 unique, non-chimeric sequences, of which 33 and 52 clones were sequenced from hypolith and soil, respectively (Appendix B). Phylogenetic analysis of the clone libraries was consistent with multivariate analysis of the T-RFLP profiles. Both F_{ST} and P tests were significant (Appendix C), indicating a lower genetic diversity within each community than for two communities combined and that the different communities harboured distinct phylogenetic lineages (Martin, 2002).

Rarefaction curves and Chao 1 estimates indicated that sampling had approached an asymptote only for hypoliths (Figure 12, Appendix D). In spite of the relatively low number of clones sampled, this is not unexpected since previous studies have shown low phylogenetic diversity in hot desert ecosystems (Wong *et al.*, 2010). The majority of the clones displayed homology to sequences retrieved from hot hyperarid deserts (Appendix B). Nonetheless, only 6 OTUs showed identity values higher than 97 %, indicating that the majority of sequences might represent novel taxa.

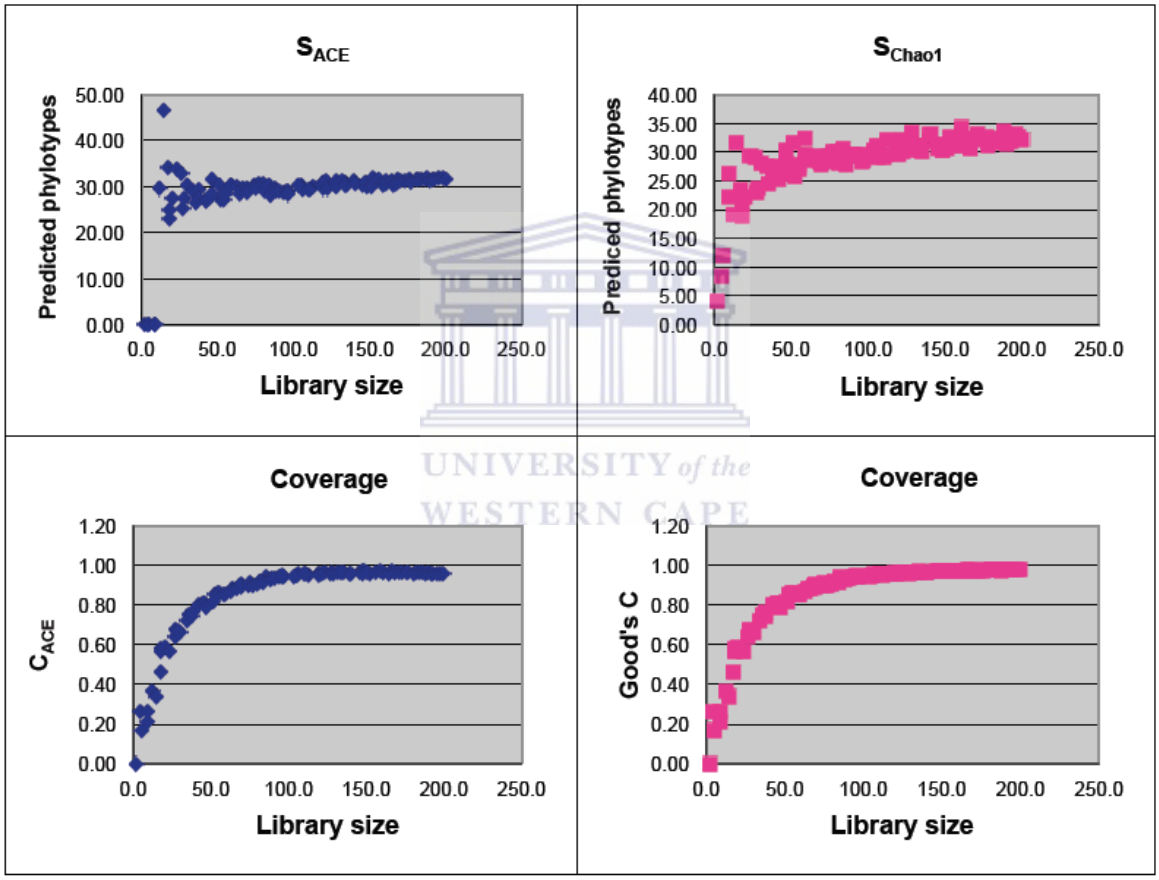

Figure 12: **Hypolithic clone library coverage (Good's and CACE) and estimator (Schao1 and SACE) plots.**

Soil samples were dominated by the phyla Actinobacteria (49%) and Proteobacteria (21%). Acidobacteria, Cyanobacteria, Bacteroidetes and Chloroflexi phylotypes were detected in lower numbers (Figure 13 a, b). Members of these phyla are generally

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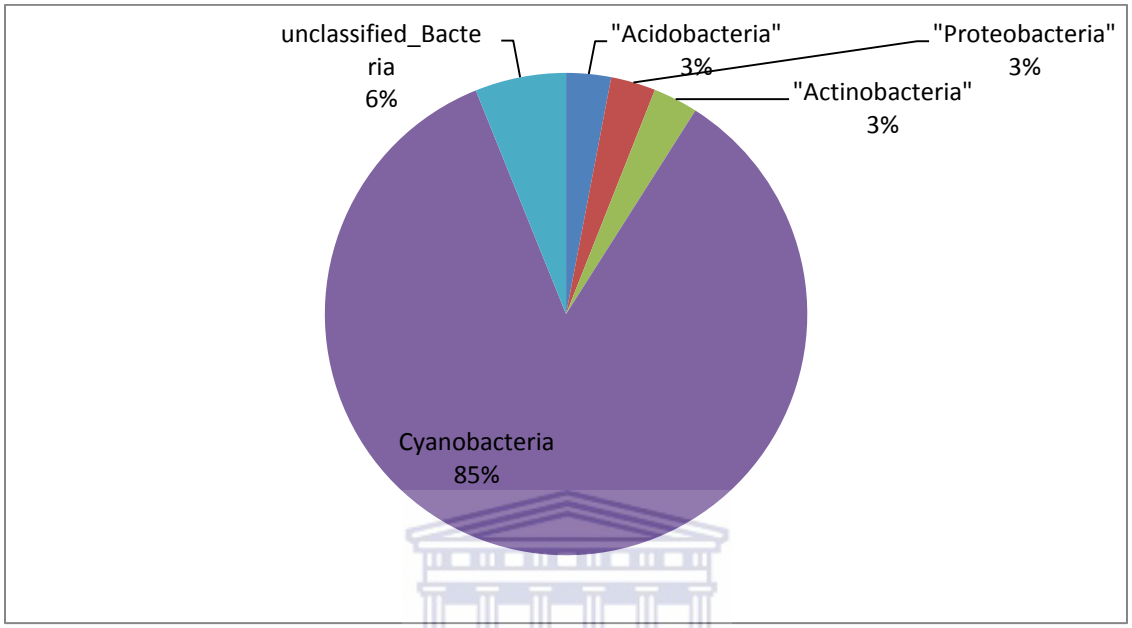
among the most common inhabitants of soils (Fierer and Jackson, 2006, Jones *et al.*, 2009, Lauber *et al.*, 2009a). Clones derived from hypoliths were mostly affiliated to the phylum Cyanobacteria (85%), dominated by *Chroococcidiopsis* lineages (order Pleurocapsales), although members of the orders Oscillatoriales, Stigonematales, and Chroococcales were also observed. *Chroococcidiopsis* has been identified as one of the common primary producers occurring in both hot and cold deserts (Tracy *et al.*, 2010, Caruso *et al.*, 2011, Lacap *et al.*, 2011, Bahl *et al.*, 2011). Other phyla represented in the hypolithic clone library included Acidobacteria (2.9%), Proteobacteria (2.9%), Actinobacteria (2.9 %) and unclassified bacteria (3%) (Figure 13 a, b). A total of 60 (out of 98) T-RFLP-defined OTUs were matched to 16S rRNA gene sequences resulting in an overall assignment of 61 %.

Hypolithic and surrounding soil indicator species were identified as Cyanobacteria and actinobacteria, respectively. If indicator lineages play a pivotal ecological role within the habitat (Auguet *et al.*, 2010), these results support the view that Cyanobacteria are among the most important functional groups in hypoliths (Cowan *et al.*, 2011b). Cyanobacteria are ubiquitous in most terrestrial habitats, and have central ecological roles in energy transduction, nitrogen fixation and as pioneer species (Whitton and Potts, 2000).

Only 5 OTUs were exclusive to hypolithic samples and the most abundant OTUs were present in both soil and hypolithic samples. This is consistent with neutral theory predictions (Hubbell, 2001) that assume species are ecologically equivalent. Thus, the compositions of local communities are regulated only by chance without considering deterministic factors (intra-specific competition or niche differentiation). Although these assumptions are still controversial, there is empirical evidence that both deterministic and stochastic processes shape the structure of microbial communities (Langenheder and Szekely, 2011, Caruso *et al.*, 2011, Ofiteru *et al.*, 2010, Dumbrell *et al.*, 2010). Notably, a global-scale study of hypolithic communities found that neutral models failed to show evidence of deterministic processes when Cyanobacteria and heterotrophic

bacteria were analyzed separately, whereas species co-occurrence was non-random when both groups were analyzed together (Caruso *et al.*, 2011).

a



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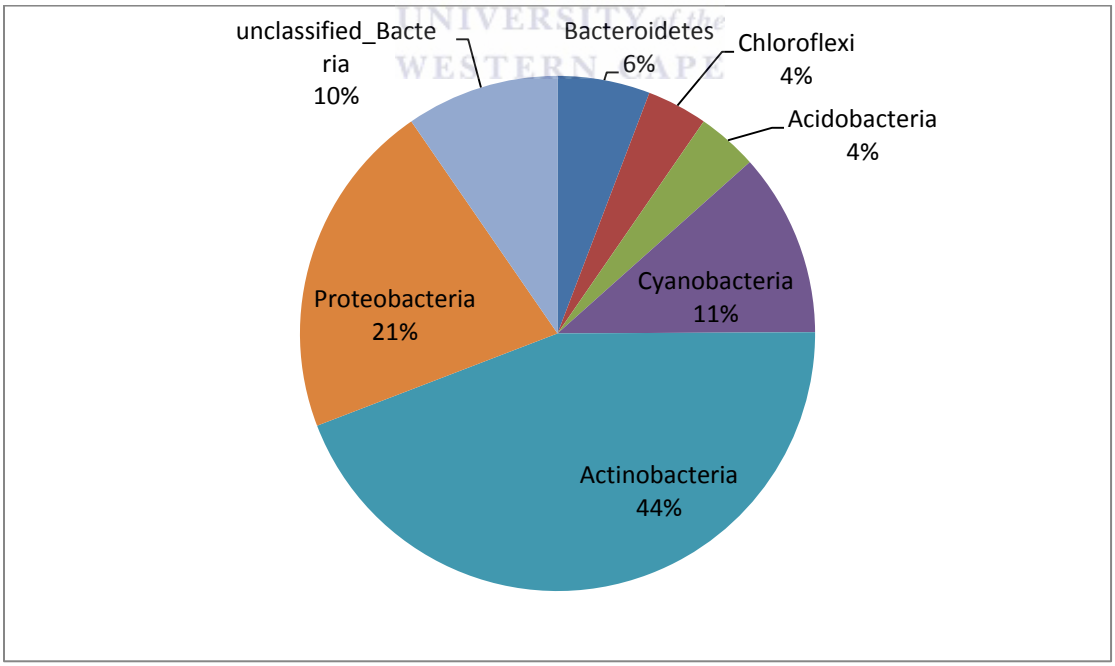

Figure 13: Clone libraries distribution for both (a) hypoliths and (b) soil communities.

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Caruso and co-workers identified demographic stochasticity as a major factor influencing community assembly, and here we present evidence that stochasticity also plays a pivotal role in local community assembly. Since 88 % of the OTUs observed in hypolithic community samples were also found in soil it is most likely that a great proportion of taxa that “seeded” hypolithons were recruited from the surrounding soil. It is also possible that a common source (e.g., bio-aerosols) seeded both soil and hypolithic communities. In any case, under the assumptions of neutral theory it might be expected that taxa composition and abundance should be approximately the same in hypoliths and in soil (Ostman *et al.*, 2010, Sloan *et al.*, 2006). As has been observed previously in rock pools seeded by rainfall water (Langenheder and Szekely, 2011) or lakes seeded by soils (Crump *et al.*, 2012), we found that most abundant taxa in the soil were also present in hypoliths albeit in lower abundance (Appendix F). Nevertheless, this was not always the case as demonstrated by the presence of indicator species (Figure 11). Consequently, the neutral theory failed to explain all the variation found in the bacterial community structure. In fact, Cyanobacteria and actinobacteria were over-represented in hypoliths and surrounding soil, respectively, suggesting that deterministic processes (habitat filtering) are also important.

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Three non-exclusive reasons for the relatively weak deterministic effect are proposed. Firstly, it could reflect a limitation of the technique (i.e., T-RFLP), as it is well known that fingerprinting methods only target the most abundant taxa (Bent and Forney, 2008). Secondly, critical deterministic elements of local environmental conditions in hypoliths and surrounding soil at the Namib study site may not differ significantly (temperature and % RH values are shown in Figure 15 a, b). Finally, high dispersal rates (source-sink dynamics) (Cottenie, 2005) could buffer the effect of selection by continued homogenization of the communities involved. Indeed, there was a high degree of overlap between the soil and hypolithic communities (Figure 14).

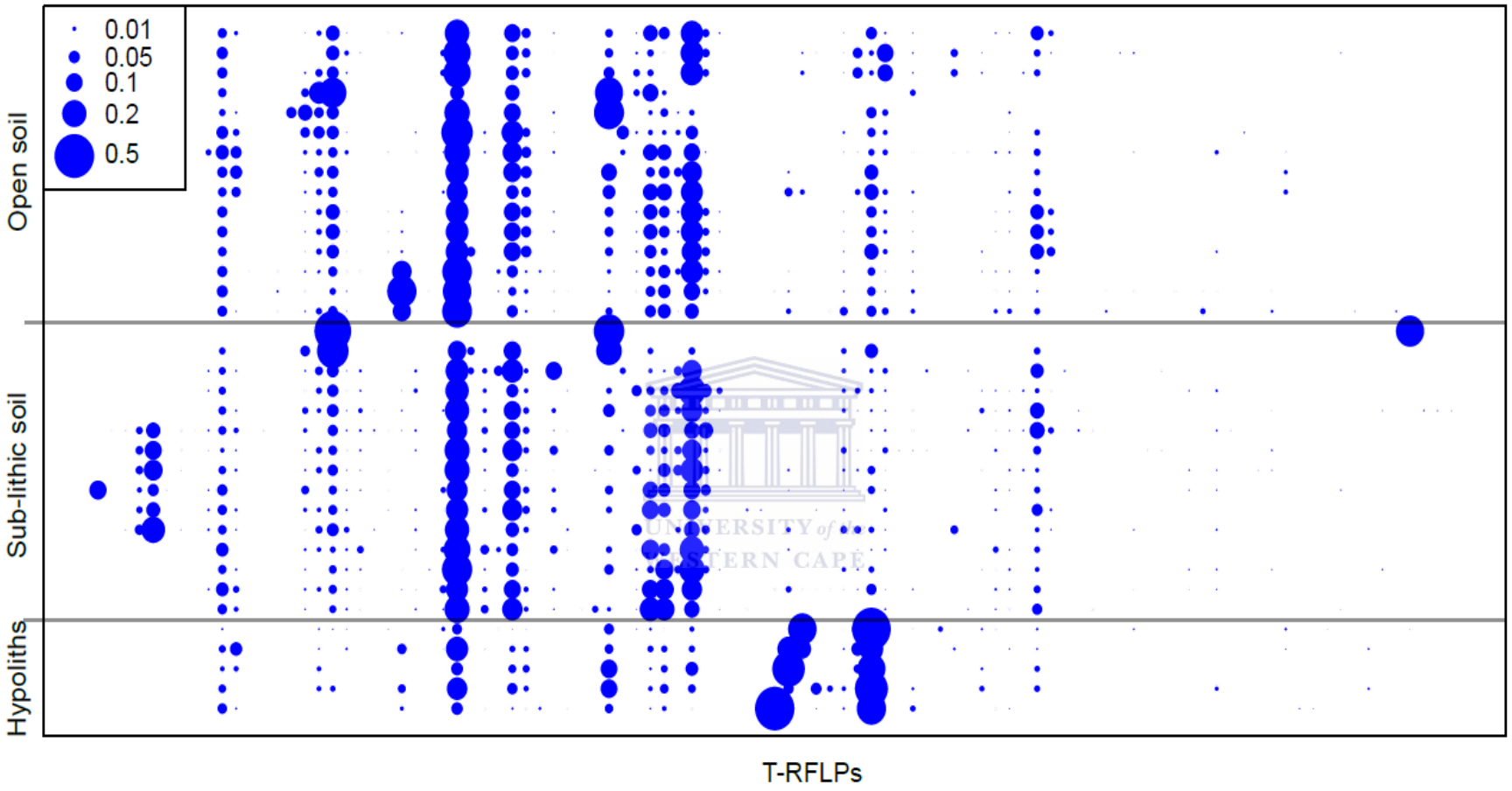
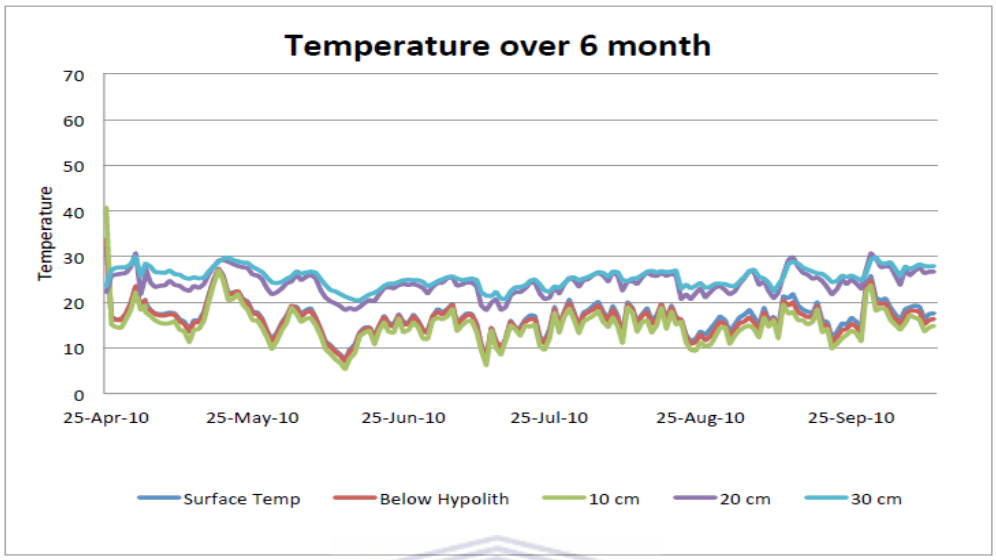


Figure 14: Bubble plot. Each bubble represents one T-RF (columns) and is sized according to its relative abundance in the sample (rows).

a



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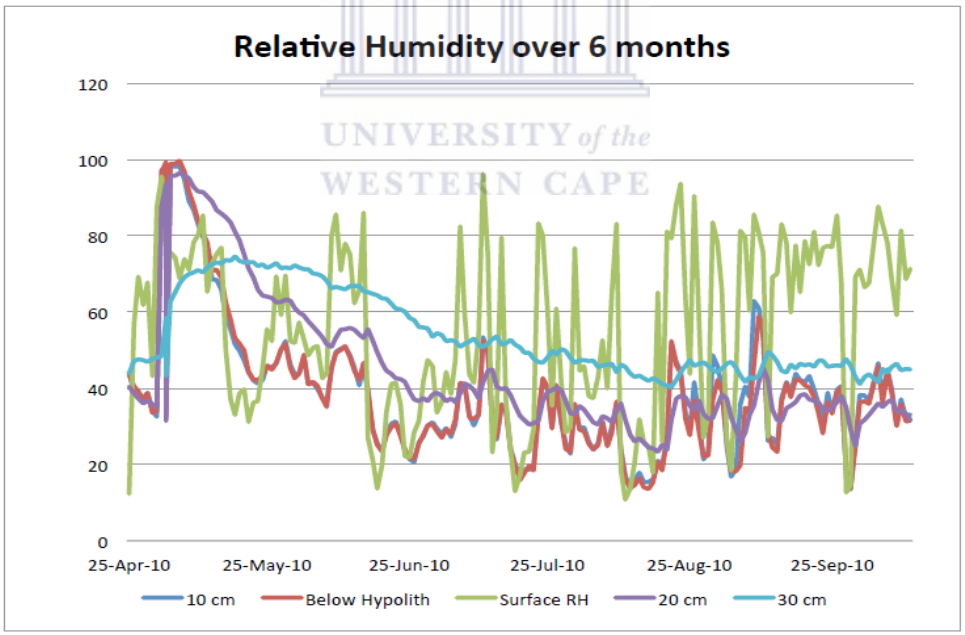

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Figure 15: Temperature (a) and %RH (b) over a 6 month period at the sampling location. Data was acquired at 5 min intervals.

It is important to note, however, that non-neutral processes such as intra-species interactions, invariance under assemblage or the complexity of ecological interactions and the 'melting' of competitive hierarchies can generate neutral patterns (Alonso *et al.*, 2006). Clearly, more focused research is required in order to explain the differences in microbial community structure between hypoliths and soil.

Metacommunity studies typically relate assembly processes to the entire community and do not take into account different categories of species. However, it has been shown for aquatic bacteria that habitat specialists and generalists have different population dynamics (Shade *et al.*, 2010). Co-occurrence patterns were also found for soil microbial communities (Barberan *et al.*, 2011). More important, habitat generalist and specialist have been shown to differ in their respective contributions to ecosystem functioning (Gravel *et al.*, 2011).

In conclusion, the presence of generalist lineages is strongly suggestive that Namib hypolithic bacterial communities did not develop independently from the surrounding soil. This is in contrast to some hyperarid Antarctic hypoliths where Cyanobacteria-dominated hypolithon occurs in soils where cyanobacterial signatures were undetectable by sequence analysis of environmental clone libraries (Pointing *et al.*, 2009). Similarly in the hyperarid Atacama Desert hypoliths occur in soils devoid of recoverable Cyanobacteria, although other reservoirs of Cyanobacteria exist in this desert within deliquescent minerals (Davila *et al.*, 2008, de los Rios *et al.*, 2004, Wierzchos *et al.*, 2012). The significant fog-moisture input to this Namib study site may be a factor affecting microbial diversity in soil reservoirs, and the extent to which aridity affects this will be a fruitful area for future work. In this study empirical evidence is provided that cyanobacteria are indicator species (specialists) for hypoliths, suggesting that both habitat filtering and stochastic processes shaped the assembly of hypolithic bacterial communities in the Namib. Since specialist assemblages seem to be more productive (Gravel *et al.*, 2011) and more susceptible to extinction than generalists when habitat conditions are altered (Tilman *et al.*, 1994a), these results have implications for habitat conservation in drylands that support hypoliths. This study

suggests that future investigations of hypoliths could exploit findings that Cyanobacteria are indicator taxa and focus more closely on this component to infer ecological patterns.

CHAPTER 4: Species assembly patterns in Antarctic hypolithic communities

4.1 Introduction

Antarctica is regarded as one of the most “extreme” environments on Earth (Cary *et al.*, 2010, Cowan and Ah Tow, 2004, Convey and Stevens, 2007). The McMurdo Dry Valleys of Eastern Antarctica are characterized by very low levels of precipitation, episodic katabatic winds, high salt content and extremely low temperatures (Cary *et al.*, 2010, Cowan and Ah Tow, 2004). While it has frequently been assumed that these would result in both low cell numbers and species diversity, recent data have supported the view that species diversity is higher than initially thought in a range of Antarctic biotopes (Cowan *et al.*, 2002, Babalola *et al.*, 2009, Smith *et al.*, 2006, Aislabie *et al.*, 2006). Evidence of potentially novel microbial species has been found in various niches ranging from permafrost to the ice-free arid terrestrial zones (Yergeau *et al.*, 2007b, Stomeo *et al.*, 2012) (Figure 16).

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Figure 16: Desert surface pavement rich in quartz rocks colonized by hypoliths, Miers Valley, Eastern Antarctica (Picture courtesy of Prof. Don Cowan)

Hypolithic communities, microbial assemblages which colonize the underside of rocks, are widely distributed in hot (Schlesinger *et al.*, 2003, Warren-Rhodes *et al.*, 2006, Warren-Rhodes *et al.*, 2007, Makhalanyane *et al.*, 2012) and cold deserts (Smith *et al.*, 2006, Cockell and Stokes, 2004, Wood *et al.*, 2008, Pointing *et al.*, 2009, Wong *et al.*, 2010, Khan *et al.*, 2011). The hypolithic "lifestyle" is proposed to circumvent environmental stress since the rock provides attenuation from excessive UV, photosynthetically active radiation (PAR), freeze thaw events and enhanced water availability (Cowan *et al.*, 2010a, Cary *et al.*, 2010). Hypoliths in extreme environments may be the dominant sites of primary productivity (Tracy *et al.*, 2010), N input (Cowan *et al.*, 2011) and the basis for the survival of whole ecosystems (Thomas, 2005). Therefore, understanding their species composition (alpha-diversity), how they vary across sites (beta-diversity) and the factors and processes that control them is of particular relevance in desert microbiology research.

Bacterial community composition in the most common hypolithic morphotype (Type I) have been shown to differ from that of soil communities (Pointing *et al.*, 2009, Khan *et al.*, 2011, Makhalanyane *et al.*, 2012), and to be dominated by Cyanobacteria (reviewed in Chan et al., 2012 and Pointing and Belnap, 2012). *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* are ubiquitous to all hypoliths (Pointing *et al.*, 2007, Pointing *et al.*, 2009, Wong *et al.*, 2010), whereas *Bacterioidetes*, *Acidomicrobia*, *Verrucomicrobia*, *Archaea,* fungi or mosses represent a small component (Khan *et al.*, 2011). However, recently, two other hypolithic morphotypes have been described in the Miers Valley, Antarctica (Cowan *et al.*, 2010a). Type II hypolithons are dominated by fungal mycelia and Type III hypolithons are bryophyte based communities. It has been suggested that these three different types of hypolithons may represent sequential development stages (Cowan *et al.*, 2010a). Since Antarctic soils contain relatively low levels of organic carbon (Cary *et al.*, 2010) the obvious hypothesis is that photoautotrophic hypolithons (Type I) are the primary stage in succession. The increased eutrophication of the hypolithic microenvironment resulting from Cyanobacterial colonization could serve as a trigger for heterotrophic fungal colonization (Type II) (Cowan *et al.*, 2010a). It is suggested that the development of the

Type III community may be controlled more by growth kinetics than by nutrient status, on the basis that very slow growth rates of Antarctic mosses (Clarke *et al.*, 2012) is probably much less than the growth rate of Cyanobacteria. Several possible alternatives for the structural link between the three types, and the development pathways have been proposed (Cowan et. al., 2011a, Figure 17).

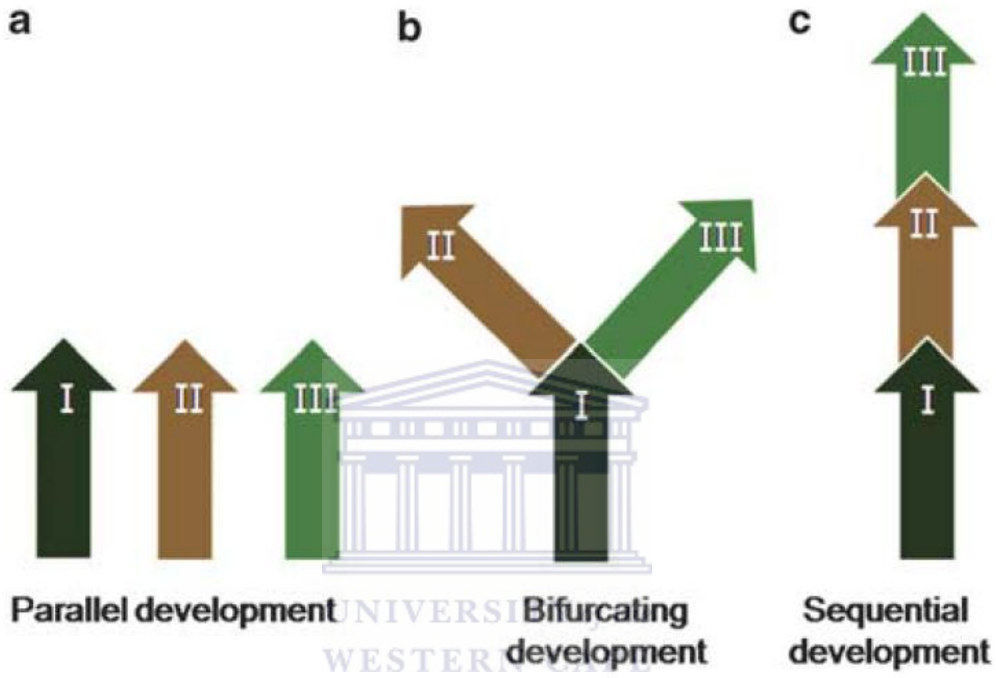

Figure 17: Hypothetical pathways for the development of hypolithic communities (Cowan *et al.,* 2010a).

Increasing evidence indicates that local (e.g., environmental conditions and species interactions) and regional (e.g., demographic stochasticity and dispersal) habitat factors are important in shaping the landscape distribution patterns of Type I hypolithons (Pointing *et al.*, 2009, Caruso *et al.*, 2011). Most importantly, as found in other systems (e.g., Barberán and Casamayor, 2010; Drakare and Liess, 2010; Ofiteru *et al*., 2010; Langenheder and Szekely, 2011; Ofiteru et al 2010) different parts of the hypolithic community may assemble via different mechanisms (Caruso *et al.*, 2011). Thus, while the heterotrophic component was influenced significantly by environmental factors, the cyanobacterial component was shaped by high levels of demographic stochasticity

(Caruso *et al.*, 2011). In contrast, very little is known about the bacterial composition and drivers that determine the structure of Type II and Type III hypolithic communities. In this study, bacterial beta-diversity patterns for the different Types of hypolithic morphotype communities were assessed. Since biomass accumulation (productivity) is often highest in the earlier stages of succession (Fierer *et al.*, 2010) and stochastic processes predominate in higher-productivity systems (Chase, 2010), we hypothesize that stochasticity will be stronger in cyanobacterial- relative to fungal- and moss-dominated hypolithons.

4.2 Results and Discussion

Here, representative quantities of hypolith and open soil samples (Table 8) were assessed. This study investigates the bacterial community structure of hypolithic morphotypes, with the aim of demonstrating discreteness from open soil. Further, we aim to demonstrate community assembly patterns with the view to elucidate possible implications for community succession.

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A total of 117 T-RFLP derived OTUs were detected of which 23 (19.6 %) were shared between the four different habitats and 52 (44.4 %) were unique to the respective habitats (Figure 18a). The number of T-RFLPs per sample (α-diversity) ranged from 19 to 33. Overall, hypolithons contained higher bacterial OTU numbers than soil and Type II hypolithons appears to harbor a higher level of bacterial diversity (pairwise Wilcoxon-Mann-Whitney tests, $P<0.05$) (Appendix E). At a regional level (γ-diversity) 85, 65 and 57 OTUs were observed in Type I, Type II and Type III hypolithons, respectively. Meanwhile, only 39 OTUs were observed in open soil (ca. 54% reduction respective to Type I hypolithons). This means that (within-habitats) compositional differences were reduced in the order Type I => Type II => Type III => open soil. Furthermore, the frequency of occurrence of OTUs differed between communities. OTU occurrences in Type I hypolithons was considerably less abundant than those in Type III hypolithons and open soil (Table 9).

Table 8: Soil samples collected from Miers Valley, Antarctica. Physicochemical characteristics of hypolith and open soil samples is shown below.

	Sample	F^- mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	% N	% C	% S	pH
Hypolith Type I	sample1	0.33	8.65	3.45	1.13	0.07	0.56	0.28	7.98
	sample2	0.35	7.63	3.11	1.23	0.01	0.68	0.29	8.21
	sample3	0.56	9.17	8.88	1.32	0.03	1.45	0.3	8.43
	sample4	0.48	10.12	22.55	24.4	0.06	1.71	0.29	8.55
	Sample5	0.75	9.98	17.84	11.75	0.04	1.29	0.25	8.12
	Sample6	1.05	6.71	7.25	4.91	0.03	1.41	0.22	8.1
	sample7	1	76.52	32.1	4.66	0.16	2.86	0.33	7.98
	sample8	0.75	15.6	28.18	21.26	0.03	1.02	0.3	7.89
	sample9	0.64	11.63	10	26.26	0.08	1.58	0.24	7.54
Hypolith Type II	sample10	0.25	4.3	1.72	7.89	0.01	0.75	0.31	8.34
	sample11	0.44	2.71	3.9	5.98	0.11	0.68	0.25	7.88
	sample12	0.7	4.61	2.88	2.35	0.09	0.81	0.29	8.23
	sample13	0.35	12.44	10.95	0.45	0.03	1.08	0.29	7.81
	sample14	0.32	4.09	3.25	5.67	0.01	0.78	0.21	8.6
	sample15	0.21	21.85	5.65	4.87	0.02	0.6	0.2	8.25
	sample16	0.59	18.68	13.22	15.94	0.04	1.14	0.23	8.23
	sample17	0.27	3.54	2.25	7.18	0.02	0.7	0.17	8.12
	sample18	0.94	23.2	23	6.87	0.02	0.87	0.3	7.89

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Hypolith Type III	sample19	0.5	18.1	9.9	4.89	0.06	1.04	0.21	7.66
	sample20	0.51	3.97	10.82	9.81	0.03	0.78	0.34	7.76
	sample21	0.26	4.6	2.88	1.56	0.01	0.66	0.31	7.89
	sample22	0.27	3.97	1.94	0.29	0.02	0.9	0.35	8.1
	sample23	0.54	8.17	4.9	1.73	0.03	0.78	0.17	7.89
	sample24	0.49	4.61	4.7	7.02	0.05	1.24	0.21	8.34
	sample25	0.49	6.93	6.86	0.72	0.03	0.98	0.22	8.1
	sample26	0.71	22	16.28	7.46	0.09	1.92	0.23	8.34
	sample27	0.31	50.55	27.91	1.67	0.03	0.98	0.21	8.23
	Sample28	0.12	2.36	1.36	0.88	0.04	0.62	0.25	8.48
Open soil	Sample29	0.15	1.63	2.34	0.75	0.07	0.68	0.45	8.31
	Sample30	0.13	1.5	4.23	0.69	0.04	0.43	0.38	8.23
	Sample31	0.1	3.11	2.44	0.46	0.02	0.36	0.27	8.36
	Sample32	0.09	1.25	3.23	0.77	0.06	0.48	0.23	8.25
	Sample33	0.1	1.01	0.27	0.69	0.05	0.77	0.58	8.45
	Sample34	0.17	1.9	1.45	0.52	0.03	0.57	0.34	8.35
	Sample35	0.18	2.24	1.02	0.28	0.02	0.59	0.35	8.43
	Sample36	0.16	2.8	1.34	0.36	0.05	0.56	0.27	8.53

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Table 9: Values of several diversity metrics, frequency of occurrences and multivariate dispersions, including results of permutation tests to compare all four bacterial communities or microenvironmental data.

Diversity metrics	**Type I**	**Type II**	**Type III**	**OS**	***F ratio***	***P value***
$\overline{\alpha}$	24.11^{ab}	26.66^{a}	26.33^{ab}	22.77^{b}	3.37	0.05
γ	85	65	57	39	-	-
β_{Add} $(\gamma - \overline{\alpha})$	60.88^{a}	38.33^{b}	30.66^{c}	16.22^{d}	342.5	0.001
Frequency of occurrences	2.5±2.2^{a}	3.7±3.0^{ab}	4.2±3.0^{b}	5.3±3.4^{b}	22.84	0.001
Multivariate measures	$\bar{d}_{cen}$ **Type I**[§]	$\bar{d}_{cen}$ **Type II**	$\bar{d}_{cen}$ **Type III**	$\bar{d}_{cen}$ **OS**	***F ratio***	***P value***
Jaccard	0.48^{a}	0.35^{b}	0.33^{b}	0.22^{c}	31.78	0.001
Raup-Crick	0.32^{a}	0.09^{b}	0.14^{b}	0.02^{b}	9.14	0.001
Euclidean[†]	3.0^{a}	2.1^{ab}	2.0^{ab}	1.3^{b}	3.22	0.05

[†] Microenvironmental data. [§] Indicates that the magnitude of the dispersion within a habitat differs between habitats. Different letters indicate statistically significant differences in means (for the diversity metrics), in frequency of occurrences or in dispersions (for the multivariate measures) between bacterial communities or microenvironmental data ($P < 0.05$).

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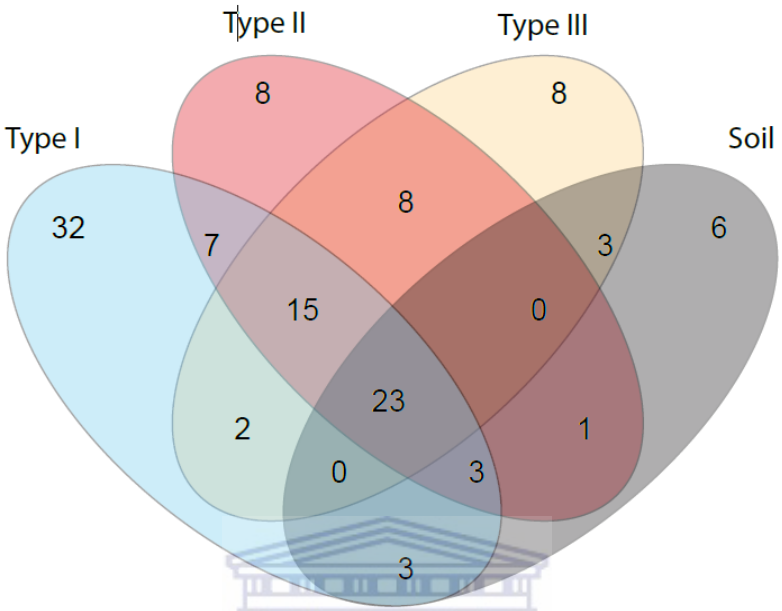
Pyrosequencing analysis of partial 16S rRNA gene PCR amplicons, generated from pooled DNA from each habitat type (n=9), supported findings from bacterial T-RFLP analysis. The sequence data are available at the NCBI Sequence Read Archive under the accession number SRA058593. A total of 569 OTUs (cutoff defined at a 97% sequence similarity level) were found, of which 314 were singletons (Table 10). 156 OTUs occurred only in Type I hypolithons, 77 OTUs only in Type II hypolithons and 146 OTUs only in Type III hypolithons. 129 OTUs were unique to the soil and only 6 were shared by all four bacterial communities (Figure 18b). The observation that a large number of the OTUs were unique to the habitat type could be explained in part by the fact that rarefaction curves did not reach a plateau (Appendix F). Thus, it is possible that bacteria found in a given habitat may be present in the rest of the habitats, albeit below the detection threshold. However, although it is likely that average bacterial diversity was not complete and methodological differences preclude exhaustive comparisons, these values are higher than previously found in Antarctic hypolithons (Khan *et al.*, 2011) and Dry Valley soils (Lee *et al.*, 2012a) and suggest that bacterial biodiversity in the Miers Valley desert pavement may have been underestimated.

a



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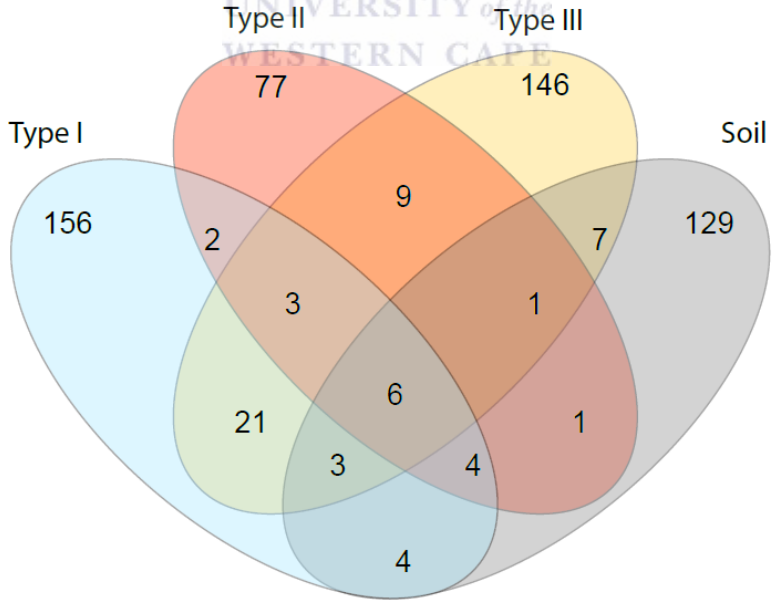

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Figure 18: **Venn diagram from (a) T-RFLP and (b) Pyrosequencing analysis for bacterial OTUs at 97 % sequence similarity cutoff found in hypoliths and soil.**

After the number of OTUs was resampled to the smallest effort (n=103 OTUs) richness was found to be lower in open soil samples than in hypolithons (Table 10), confirming the trend shown by the T-RFLP analysis. The rank-abundance distributions displayed a strong dominance of a few OTUs and a long tail of rare OTUs (not shown) what is a common feature among bacterial communities (Fuhrman, 2009).

A total of 13 phyla were detected of which all were detected in hypolithons and 9 were found in the open soil (Appendix E). Those phyla contributing most to the observed diversity were present in hypolithic and open soil bacterial communities; although in some cases the distribution of their relative abundance indicated a preference for one of the four habitats (Table 10, Appendix E). As in previous reports cyanobacterial phylotypes were found in both open soil and hypolithic Miers Valley samples (Wood *et al.*, 2008b) but dominated in Type I hypolithons (Cowan *et al.*, 2010a, Khan *et al.*, 2011). *Actinobacteria* were more abundant in Type III hypolithons and open soil samples, whereas *Proteobacteria* (mainly *Alphaproteobacteria*) were prevelant in Type I and II hypolithons. In total, four (out of 6) of the most cosmopolitan OTUs were classified to the genus level. Those genera were *Methylobacterium* (29 OTUs), *Novosphingobium* (375 OTUs), *Roseomonas* (11 OTUs) and *Sphingomonas* (370 OTUs) (Figure 19, Table 10). Interestingly, the most abundant OTUs in hypoliths were found in lower numbers in open soil and *vice versa*. This is in contrast with what the composition of hypolithic communities from the Namib Desert (Makhalanyane *et al.*, 2012) and suggests a stronger deterministic effect in Antarctic hypolithons.

Table 10: Distribution and composition of OTUs (97% sequence similarity cutoff)

Habitat	Reads	OTUs	Rarefied OTUs	Singletons	Ratio of rare
Type I	1518	199	96.6 ± 5.3[a]	95	48 %
Type II	369	103	103	58	16 %
Type III	1419	196	97.7 ± 5.4	87	44 %
OS	1461	155	77.7 ± 4.7	74	48 %

[a] Standard deviation

When microenvironmental conditions (elemental chemical analysis) were incorporated in an nMDS ordination plot, hypolithic and soil bacterial communities were found to be shaped by disparate environmental factors (PERMANOVA: $F_{3,35}$=4.08, P=0.001; Figure 19, Table 8). However, no differences were found among the three different types of hypolithons (P>0.05 for all comparisons). A post-hoc test using Wilcoxon-Mann-Whitney showed that soil samples presented lower values than hypolithons (Appendix H), suggesting that nutritional constraints in this depauperate environment are higher for soil bacterial communities than hypolithic communities. This is entirely consistent with expectations the different levels of primary productivity in the two habitat types, as predicted by the different photoautotrophic compositions.

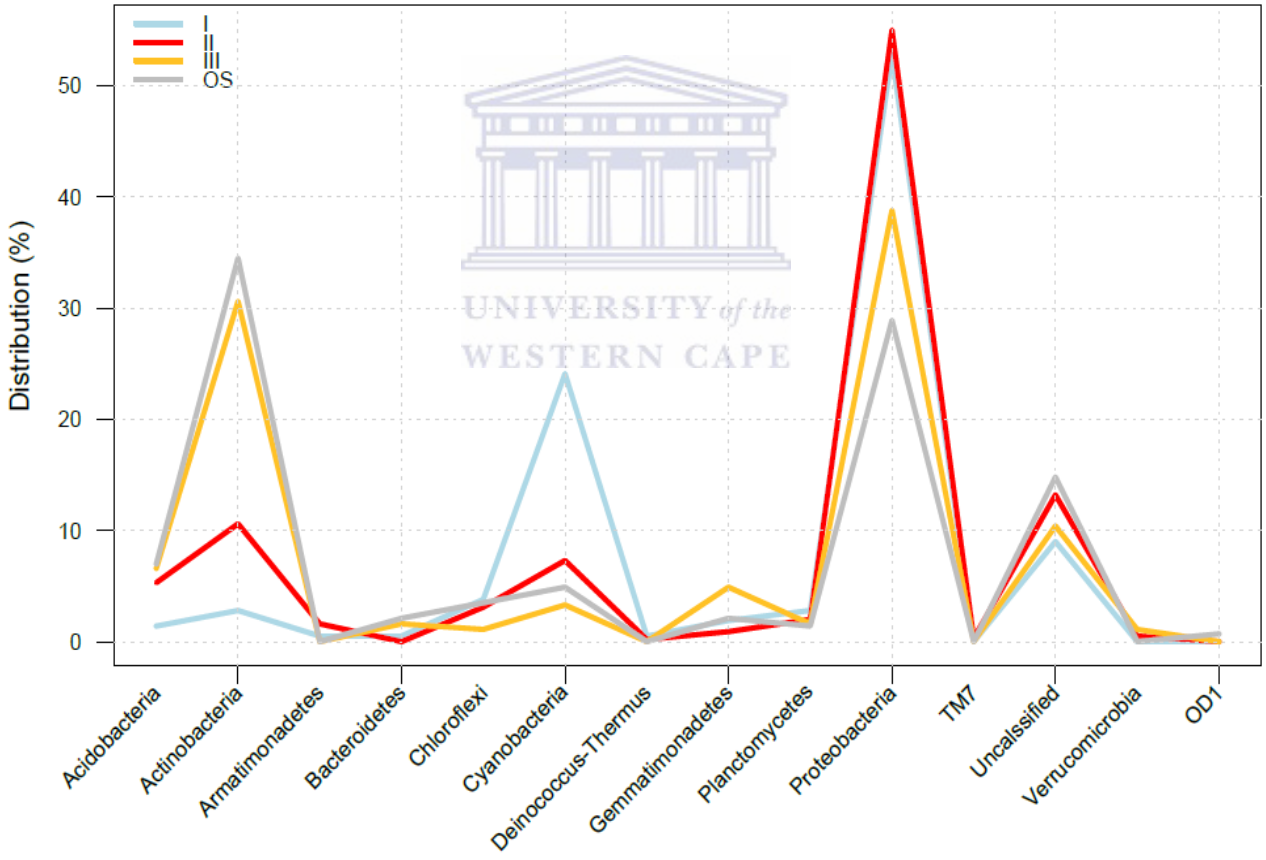

Figure 19: Line graph depicting taxonomic classification of bacterial reads retrieved from different hypolith and soil samples at phylum level using RDP classifier with a confidence threshold of 80%.

Table 11. Phylogenetic assignment and sequence distribution of selected OTUs (97%)

OTU ID	Number of sequences				Taxonomy	Taxonomic level and confidence
	Type I	Type II	Type III	OS		
69	83	0	0	0	*Amaricoccus*	Genus/100%
139	90	0	7	0	*Sphingopyxis*	Genus/100%
148	28	30	1	0	Sphingomonadaceae	Family/100%
163	0	5	81	0	Rhizobiales	Order/100%
165	0	4	112	0	Phyllobacteriaceae	Family/100%
210	6	3	19	1	*Methylobacterium*	Genus/97%
211	40	1	26	3	Caulobacteraceae	Family/100%
212	107	16	240	12	*Novosphingobium*	Genus/81%
213	2	2	4	3	*Roseomonas*	Genus/100%
214	189	61	119	1	*Sphingomonas*	Genus/96%
243	1	13	0	0	Actinobacteria	Phylum/100
281	0	0	0	150	*Patulibacter*	Genus/100%
316	1	0	0	156	Acidimicrobiales	Order/100%
329	1	13	0	118	*Patulibacter*	Genus/98%
440	2	8	33	8	Acidobacteria	Phylum/100%
541	0	0	0	202	*Kistimonas*	Genus/100%

The four most abundant OTUs in each habitat type are included, with the six OTUs found in all four habitats underlined. Taxonomic assignments are the finest level that passed the RDP Classifier's (80% confidence threshold).

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T-RFLP bacterial community analysis using Jaccard's dissimilarity index showed significant differences in community structure between hypoliths and soil samples (PERMANOVA: $F_{3,35}$=6.84, P<0.001; Figure 20). Despite similar environmental conditions, two different hypolithic clusters were detected. The first cluster was formed by Type I hypolithons while a second cluster composed of Type II and III hypolithons. These results were confirmed using a randomization test (Table 12), which demonstrated surprisingly that the bacterial composition appears to be very similar in, the two very different types of eukaryotic hypolithons (Type II, III). Type I hypolithic communities were considerably more variable in their within-OTUs composition than were Type II and III (Table 10). Soil samples showed a very high level of compositionally consistency. Similar results were found using Bray-Curtis and Morisita-Horn dissimilarity metrics (Appendix H, Appendix I).

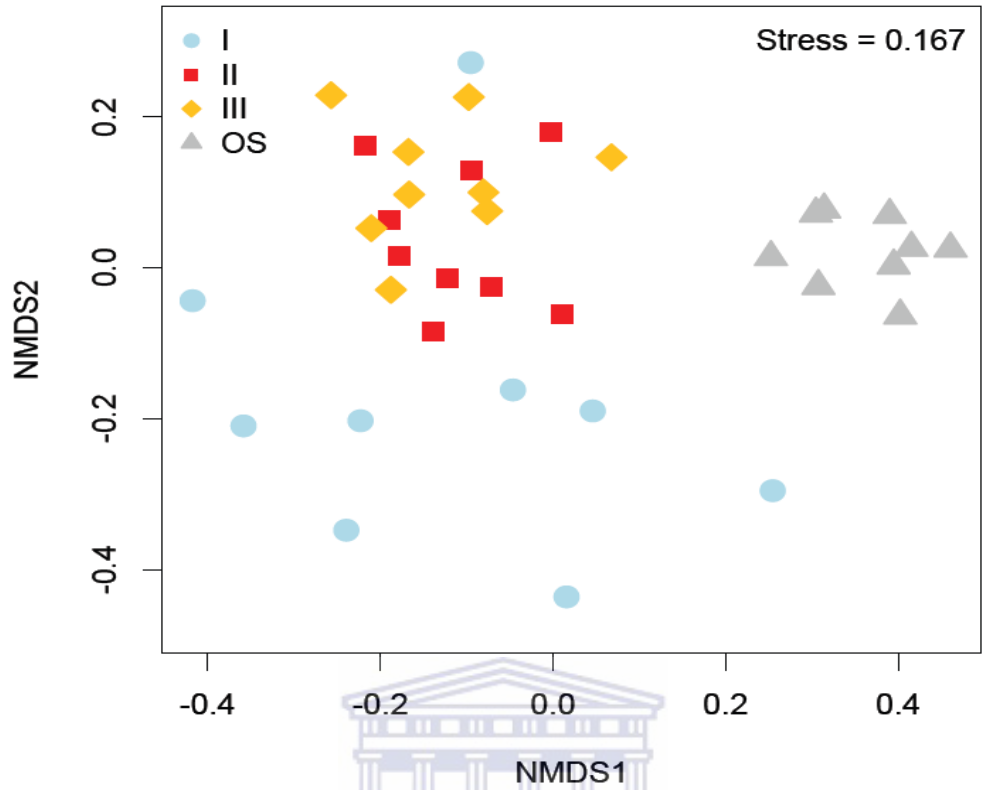

Figure 20: **nMDS ordination plot (Jaccard dissimilarity matrix) of T-RFLP profiles hypolith Type I, Type II, Type III and Open soil (OS) derived samples. The quality of the ordination is indicated by the low-stress value.**

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Table 12: **Permutational multivariate analysis of variance**. **A significant PERMANOVA indicates that the multivariate composition of the community differs between habitats.**

	Biotic data (T-RFLP)		Abiotic data
	Jaccard	**Raup-Crick**	**Euclidean**
Type I – Type II	1.96[a] (<0.01)[b]	1.85	1.24
Type I – Type III	3.23 (<0.001)	6.01 (<0.05)	0.76
Type I – Open soil	8.71 (<0.001)	19.08 (<0.001)	6.24 (<0.001)
Type II – Type III	1.37	0.38	0.39
Type II – Open soil	16.25 (<0.001)	152.61 (<0.001)	8.59 (<0.001)
Type III – Open soil	19.50 (<0.001)	160.88 (<0.001)	5.85 (<0.001)

[a] *F ratio*; [b] *P value*

To investigate how abiotic factors affected T-RFLP patterns we performed redundancy analysis (Figure 21). We found that sulphur, nitrate and fluoride were the most important factors explaining variability of T-RFLP patterns (P<0.001).

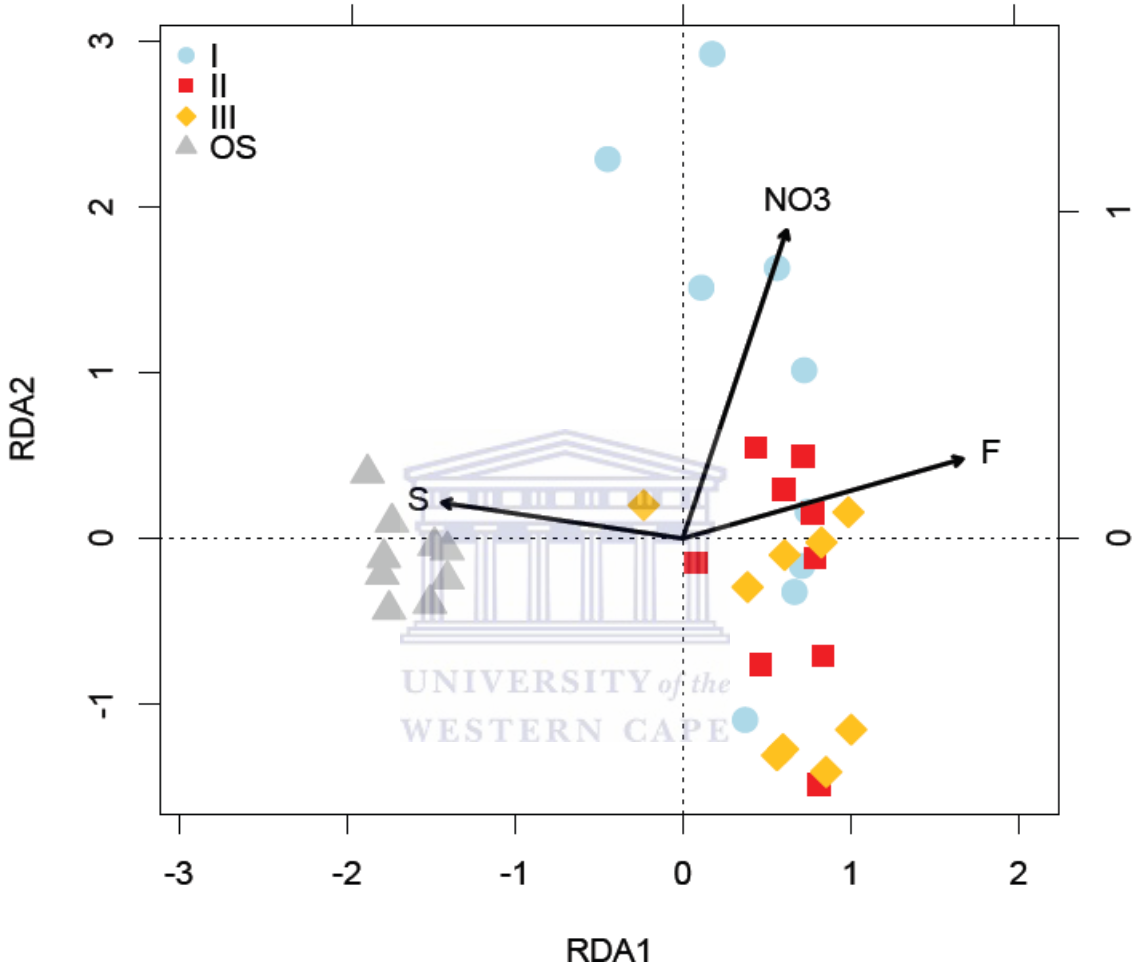

Figure 21: **Redundancy analysis (RDA) biplot of bacterial diversity and microenvironmental parameters. T-RFLP analysis profiles for hypolithic and soil samples are depicted (n=36). Only the environmental variables that significantly explained variability in microbial community structure are fitted to the ordination (arrows).**

There are various possible interpretations of these results. First, it is possible that if differences in environmental parameters are higher among hypolithons, over dispersion in Type I hypolithons can arise because of environmental heterogeneity. However, environmental heterogeneity, measured by permutational analysis, showed no differences between the three types of hypolithons (Table 9). Substrate-related and/or

the environmental variables that were not recorded might also influence the structure of bacterial communities. Using RDA analysis, only 18 % of the total variation in community composition could be explained by the measured variables (Figure 21), indicating that other factors may be dominant. For example, it has been shown that Type I cyanobacterial hypolithons colonized rocks with approximately 50 % lower PAR transmittance than fungal or moss hypolithons (Cowan *et al.*, 2010b). Salinity and porosity have been also found to be important variables determining community structure below quartz rocks (Pointing *et al.*, 2009). Species can also alter the abiotic conditions of their environment (Jones *et al.*, 1994). For instance, changing from a bacterial to a fungal-dominated community over the course of succession may lead to fundamental differences in nutrient availability (Cherif and Loreau, 2007). Overall, bacterial communities were more similar within habitats than among habitat's which is consistent with the concept of habitat filtering (Van der Gucht *et al.*, 2007).

Other ecosystem features (e.g., productivity) or species traits (e.g., body size or dispersal and competitive abilities) may be important in explaining bacterial community assembly. Recent work has demonstrated that ecological determinism increases with organism size (Farjalla *et al.*, 2012) and that higher beta-diversity at higher productivity resulted from a stronger role for stochastic relative to deterministic assembly processes with increasing productivity (Chase, 2010). Interestingly, hypolithons dominated by cyanobacteria have been shown to be as important in sequestering carbon (productivity) as plants, lichens and bryophytes on Devon Island, in the high Arctic (Cockell and Stokes, 2004) and the dominant sites of N input in Antarctic desert soils (Cowan *et al.*, 2011). Also, species can show negative co-variances when trade-offs between competitive and dispersal abilities create patch dynamics (Cottenie, 2005). Similar investigations focused on microorganisms are scant, but it is acknowledged that dormancy (reviewed by Lennon and Jones, 2011) may affect rates of dispersal to new locations. Alternatively, production of antibiotics may mediate competition between bacterial populations (Cordero *et al*., 2012). Nevertheless, given the small spatial scale of our study, the patterns we observed are more likely to be caused by the effects of differences in productivity and/or competition.

It is noted that most dissimilarity metrics (e.g., Jaccard's dissimilarity index) are biased by the number of species (Anderson *et al.*, 2011). To circumvent this bias, we took advantage of a probabilistic dissimilarity metric (Chase *et al.*, 2011), which measures the deviation of pairwise comparisons of community dissimilarity from the null expectation under random assembly. This approach allowed us to compare the beta-diversity of the different habitat types independent of differences in alpha-diversity and provides some indications of the possible underlying mechanisms of community assembly (Chase *et al.*, 2011, Anderson *et al.*, 2011). Interestingly, this approach confirmed that the bacterial community composition was clearly different between hypolithons and open soil (PERMANOVA: $P<0.001$ all pairwise combinations; Figure 22) and between Types I and Type II/Type III hypolithons. Moreover, the values of this metric were larger among Type I samples (that is, less deviant from the null expectation) relative to samples from the other three communities (Table 10), supporting the view that deterministic processes are relatively more important in explaining variations in Types II/Type III hypolithons and soil communities. Since cyanobacteria dominated Type I communities, this is consistent with conclusions by Caruso *et al.* (2011), who found that stochasticity is an important driver for the autotrophic component of hypolithic communities. Furthermore, simulated hypolithic communities from random sampling of the soil community demonstrate that stochastic dispersal was unlikely to shape the observed community structure of the hypolithons (Figure 23). In contrast, we found by random sampling that Type II hypolithons could be originated from Type I, whereas Type III could be created from Type II but not from Type I.

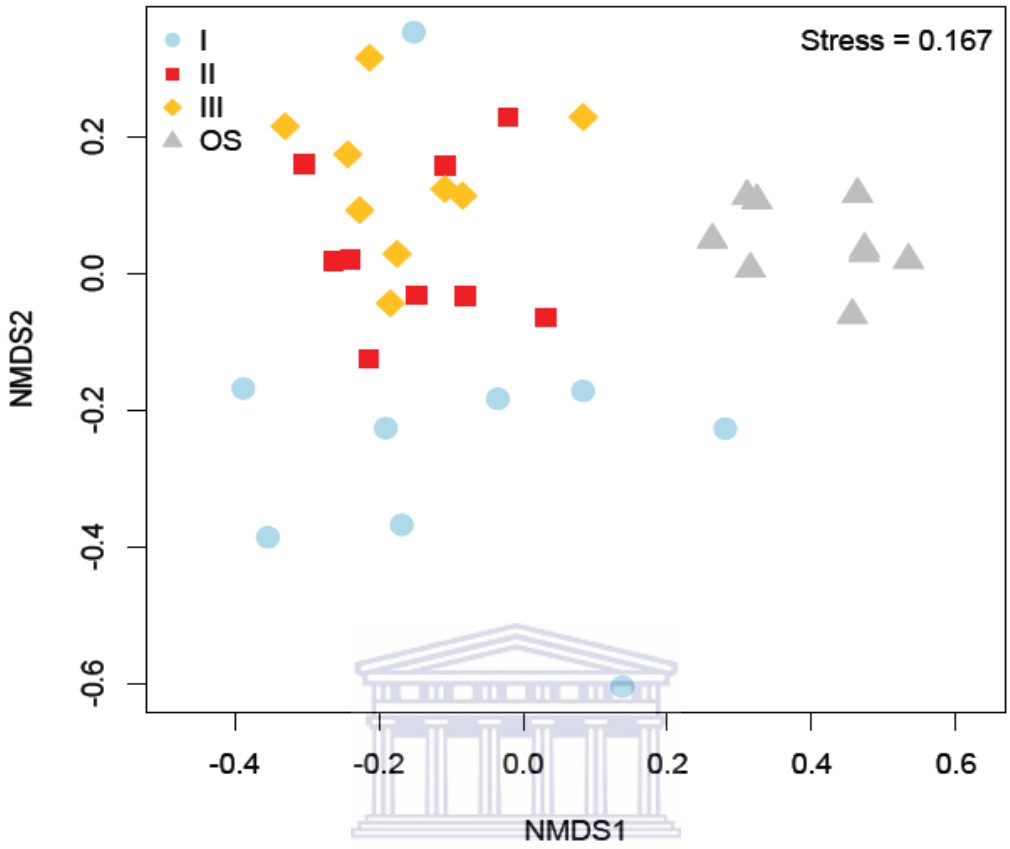

Figure 22: nMDS ordination plot of T-RFLP profiles hypolith Type I, Type II, Type III and Open soil (OS) derived samples. The quality of the ordination is indicated by the low-stress value. Points represent the composition of a community in multidimensional space, and the distance between any two points represents the difference between those two communities according to a modified Raup-Crick dissimilarity metric.

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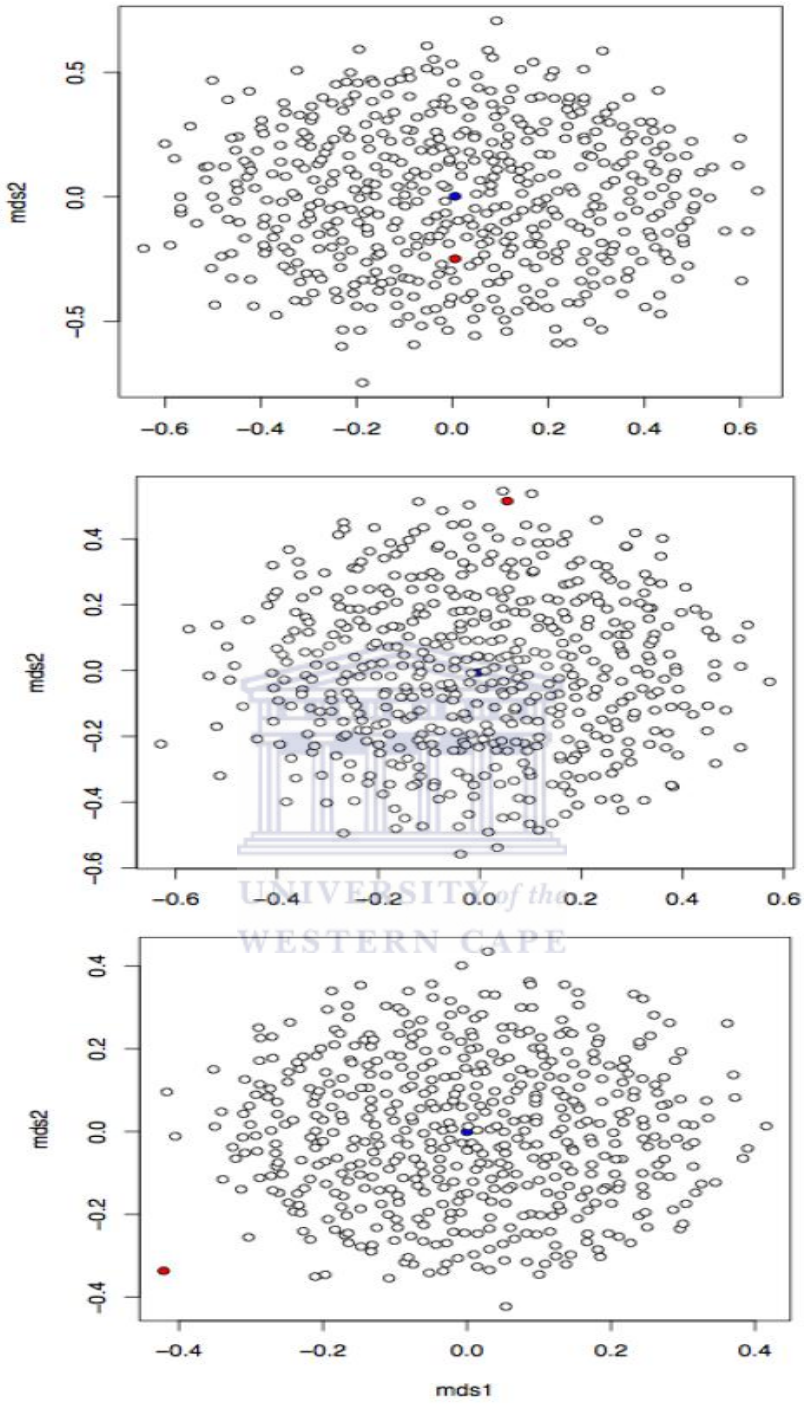

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Figure 23: **nMDS analysis depiction of a random subsampling procedure to estimate the probability that hypolithons represented random samples of their respective original communities. A total of 1000 random subsamples were assembled for each sample pair. White circles represent the random subsamples while the filled blue and red represents the original and subsidiary community, respectively. (a) Type I and Type II (b) Type II and Type III (c) Type I and Type III.**

Here evidence is presented that Type II and Type III hypolithons contain similar communities that contrast clearly from those of Type I. Moreover we found that Type I and Type II/Type III bacterial communities differ in the way they assemble.

In summary we have demonstrated that hypolithons and open soils display demonstrably different bacterial community compositions. In addition, we found that beta-diversity is higher in Type I hypolithons respective to Types II and III suggesting that deterministic processes in the bacterial component of hypolithons might increase in the order Type I => Type II => Type III. If we assume that the effects of stochastic variation may become less important as communities develop over time (Fierer *et al.*, 2010), these findings may validate the sequential development model proposed by Cowan *et al.* (2010), which suggests Type II hypolithons as an intermediate development state between Type I and III. However, it is important to note that our analysis represents a snapshot in the development of these communities, with a focus on the most abundant taxa, and diversity may not have reached its maximum yet (Fierer *et al.*, 2010). Long term monitoring programs of hypolithic bacterial composition involving larger sample numbers and ultra-deep sequencing may help us to resolve how the relative importance of deterministic/stochastic processes changes over time and the consequent reasons for such.

CHAPTER 5: Functional patterns of key nutrient cycling genes in Miers Valley, Antarctica

5.1 Introduction

The potential for biological diversity to affect ecosystem functionality has been widely recognized (Loreau *et al.*, 2001, Kinzig *et al.*, 2002). It has been suggested that a sustained decrease in biodiversity, affecting ecosystem functionality, could have adverse effects on humanity in many ways (Loreau *et al.*, 2002, Hooper *et al.*, 2005, Cardinale *et al.*, 2012). Understanding how functional diversity influences conservation is pivotal, especially in the areas which are most at risk from factors such as climate change. Desert biomes, such as the hyperarid Miers Valley (Figure 24), are potentially susceptible to ecosystem losses which could ultimately result in changes leading to a reduction in biodiversity and function (Sala *et al.*, 2000). As deserts have a relatively simple trophic structure, typically with a low diversity of macroorganisms, microorganisms are likely to drive the key processes of inputting carbon and nitrogen into the system (Cary *et al.*, 2010)**.**

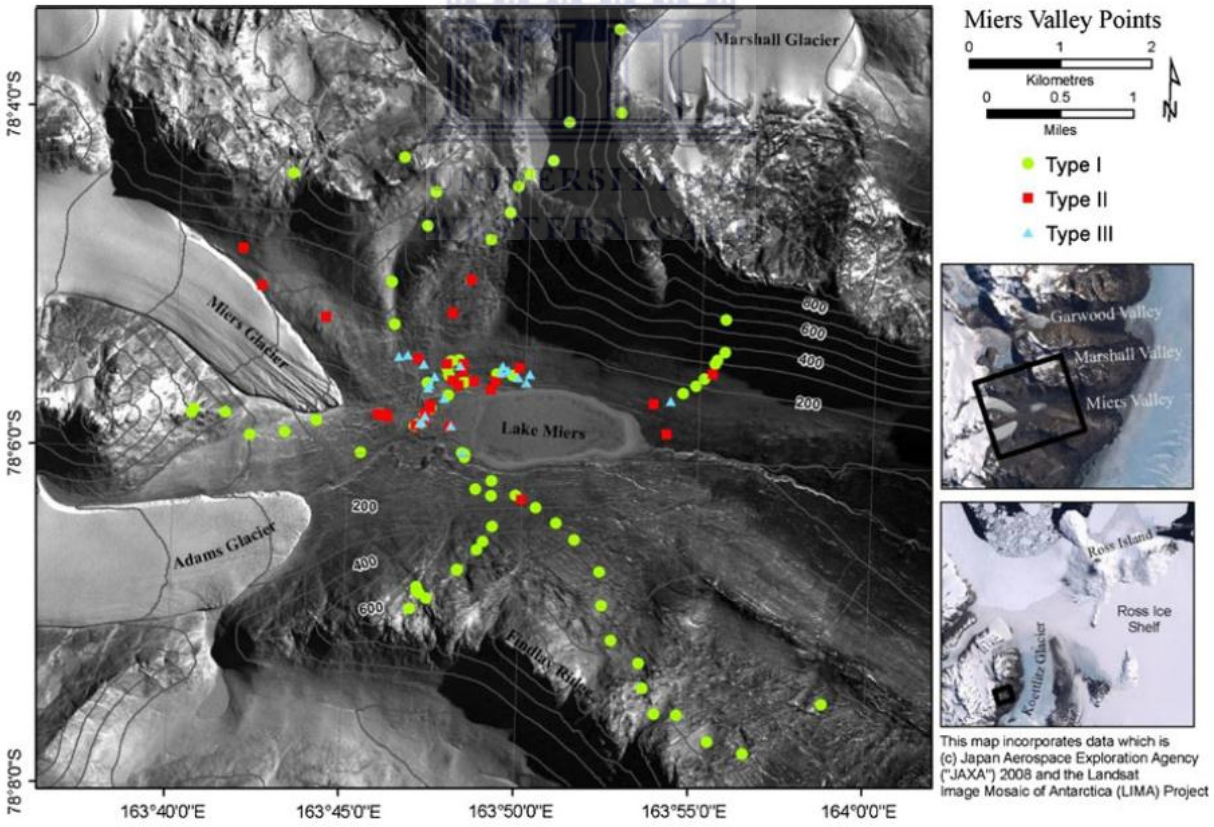

Figure 24: GIS satellite map of the upper Miers Valley (in relation to the immediate coastal region and Ross Island (insets) showing the distribution of different hypolithon types (Cowan *et al.*, 2010b)

Carbon fixation (Anderson and Domsch, 2007) and mineralization (Gregorich *et al.*, 2006) are important processes in soil communities. Ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) catalyzes the first rate limiting step of photosynthesis (Ellis, 1979), of which the large subunits of the predominant form (form I; Spiridonova et al 2004, Selesi et al 2005) are encoded by *cbb*L genes (Kusaid and Bowien 1997; otherwise named *rbc*L in older nomenclature or when referring to eukaryotic organisms; Tabita 1988). Two variants of the gene are present in organisms; green-like and red-like *cbb*L. Green-like *cbb*L gene forms are present in plants, green algae, cyanobacteria, and representatives of α-, β- and γ-proteobacteria (Selesi *et al.*, 2005, Watson and Tabita, 2006, Elsaied and Naganuma, 2001b). The red-like type includes non-green algae and α- and β-Proteobacteria (Selesi *et al.*, 2005). A preliminary investigation employing clone library analysis found that hypolithic communities were dominated by taxa closely related to cyanobacteria and proteobacteria (Makhalanyane, 2009).

The nitrogen cycle has been extensively studied in soil**.** The cycle is initiated by fixation of nitrogen gas into ammonia (NH_3) (Howard and Rees, 1996); catalyzed by nitrogenase, subunits of which are the nitrogen fixation by the nif gene (Zehr *et al.*, 2003). Ammonia is readily converted to ammonium (NH_4^+ ionized ammonia), under acidic pH conditions (Howard and Rees, 1996). The collective process of nitrification is an energy producing reaction involving the aerobic oxidation of ionised ammonia into nitrite (NO_2) (by ammonia oxidation) and nitrate into nitrate (NO_3) by nitrite oxidation (Bothe *et al.*, 2006). The first step of ammonia oxidation is catalysed by a monooxygenase (Hollocher *et al.*, 1981), the first subunit of which is encoded by the *amo*A gene (McTavish *et al.*, 1993). The reduction of nitrate to nitrite is catalysed by nitrate reductase proteins that are either membrane bound (encoded by the *nar* operon; Warnecke-Eberz and Friedrich 1993) or are located within the periplasm (encoded by the *nap* gene; Siddiqui et al. 1993). Once nitrate is formed, it can be reduced further by one of three anaerobic pathways: i) the multistep reduction of nitrite to form dinitrogen gas, termed denitrification (Zumft, 1997), ii) the formation of ammonium ions by dissimilatory nitrate reduction (DNRA) (Knowles, 1982) or iii) the coupling of ammonium oxidation to the reduction of nitrite to form dinitrogen gas by anaerobic ammonium oxidation (anammox) (Mulder *et al.*, 2006, Van

de Graaf *et al.*, 1995). In denitrification, nitrite is reduced to nitric oxide (NO) using either of two nitrite reductase proteins NirS and NirK (encoded by the nitrite respiration genes, *nir*S and *nir*K), nitric oxide reductase (encoded by the nitric oxide respiration gene, *nor*) and nitrous oxide is reduced to dinitrogen gas using nitrous oxide reductase (encoded by the nitrous oxide respiration gene, *nos*Z; Zumft 1997). DNRA is catalysed by formate dehydrogenases, encoded by the *nrf*A gene (Darwin *et al.*, 2006), and the anammox pathways are partially catalyzed by hydroxylamine oxidoreductase encoded by the *hao* gene (Schalk *et al.*, 2000, Strous *et al.*, 2006).

A major question in community ecology is how community structures change in space and/or time. This is because structure has been shown to significantly affect ecosystem processes (e.g., Tilman 1997). Here, structure refers to the composition and diversity of biological communities, and function relates to the processes that the communities drive. Both are directly determined by the environmental context. In addition, functions can be directly or indirectly affected by altered community structure.

Previous studies targeting the 16S rRNA gene have shown that hypolithic and soil bacterial communities from Antarctica vary in taxonomic diversity (structure and composition) (see Chapter 4). Here, the hypolithic and soil bacterial community patterns are investigated in the context of functional guilds (N and C cycling). The hypothesis is that habitat type, and specifically environmental conditions, would be a major driver of change of bacterial functional diversity. Accordingly, we expect to find: (i) spatial variation of functional diversity in soil and hypoliths and (ii) systematic relationships between taxonomic and functional structures.

5.2 Results and discussion

Here, T-RFLP analysis is used to assess the functional community structure of hypolith and soil bacterial guilds implicated in C and N fixation. While genes associated with photosynthesis, nitrogen fixation, nitrification and denitrification have previously been identified in Antarctic environs (Cameron *et al.*, 2012, Yergeau *et al.*, 2007a, Jung *et al.*,

2011), this is the first study which has comprehensively assessed the patterns of occurrence of these key functional genes in hypolith and open soil communities.

5.2.1 Spatial variability of functional diversity

A total of 57 distinct green-like *cbb*L OTUs, ranging in size from 29 to 780 bp were identified. Eight (14 %) were shared between all four habitats, with 9 (16 %) unique to Type I hypolithons, 3 (5 %) to Type II, 6 (10 %) to Type III and 5 (9 %) unique to open soil.

A survey of red-like genes showed a total of 115 T-RFS, substantially higher than the green-like *cbb*L genes. The T-RFs ranged in size from 31 to 785 bp. From the total number of OTUs, 20 (17 %) were shared between all samples. A fairly high proportion of OTUs, 22 %, were exclusive to Type I hypoliths, whereas 7 %, 9 %, and 3 % of OTUs were distinctive to Type II, Type III and open soil, respectively.

Bacterial *amo*A gave a total of 49 T-RF that ranged from 40 to 479 bp. Twenty four (49%) were shared between habitats, with 2 (4%), 2 (4%) and 1 (2%) been unique to Type I, Type II and Type III, respectively. No T-RFs were found to be exclusive to open soil samples.

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*nif*H T-RFs ranged in size from 24 to 358 bp. From a total of 70 T-RFs, 10(14.3%) were shared between all sample types. Type I hypoliths had the highest proportion of exclusive OTUs (30 %), with 14 %, 9 % and 4 % being limited to Type II, III and open soil, respectively.

*nir*K T-RFs had a total of 20 T-RFs, ranging in size from 69 to 464 bp. From the total OTUs (20), 3 (15%) were shared between hypolith and open soil. A remarkably high proportion of OTUs (30%) were exclusive to open soil samples while 10% and 5% were distinct to Type I and Type II hypoliths, respectively. No T-RFs were exclusive to Type III hypoliths.

*nir*S gave a total of 11 T-RFs, ranging in size from 30 to 850 bp. From a total of 11 T-RFLPs, 27% were shared between all samples and 18 % were exclusive to open soil. No T-RFs were unique to Hypoliths.

Red-like *cbb*L presented the highest numbers of T-RFs per sample, followed by *amo*A, Green-like *cbb*L, *nir*K and *nir*S (Figure 25). Only *nir*K from Type III hypolithons yielded significantly higher OTU numbers than those from open soil (Figure 26).

When functional genes were displayed by habitat type, hypoliths showed higher T-RF number than open soils (Figure 27). However, only the difference between Type I and open soil was statistically significant (P<0.05). It might be concluded that the potential enzymatic capacity is higher in Type I hypolithons than in soil.

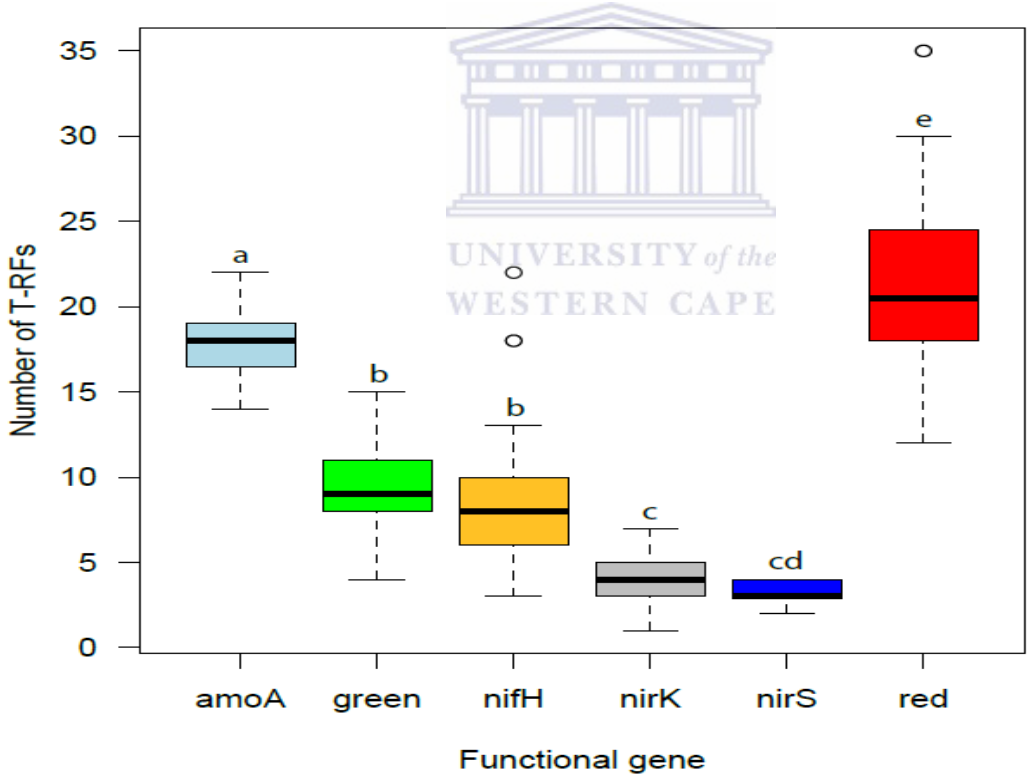

Figure 25: The number of T-RFs obtained for each functional gene. Different letters denote that the differences were statistically significant.

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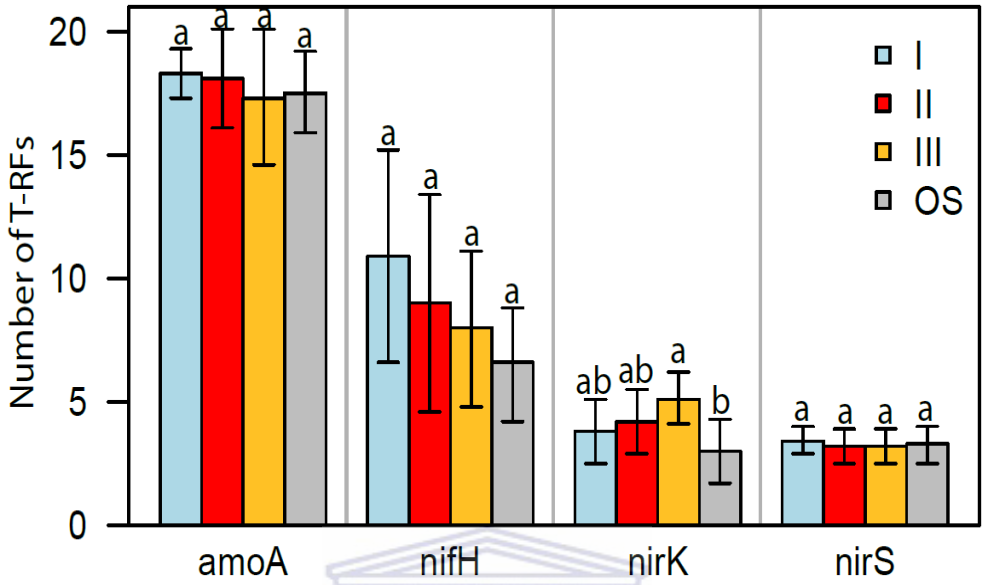

Figure 26: **The total number of T-RFs displayed nitrogen cycling genes. Different letters denote that the differences were statistically significant.**

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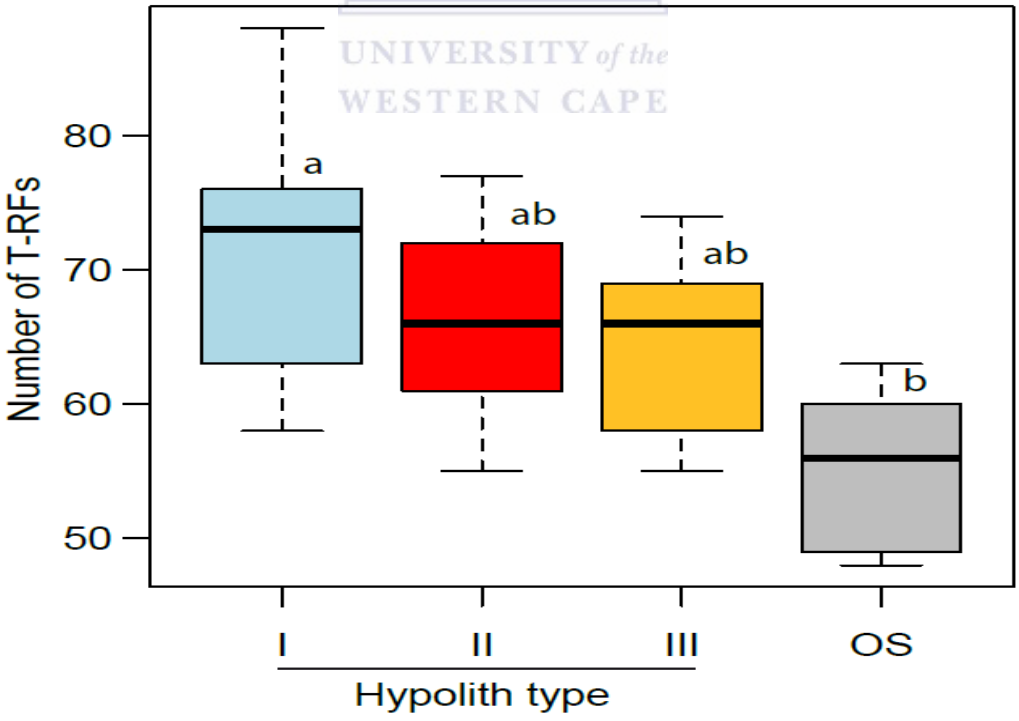

Figure 27: Functional genes displayed by habitat type. Different letters denote that the differences were statistically significant.

5.2.2 Functional profile of bacterial communities

T-RFLP data gathered from the six different functional genes were combined to create functional community profiles for each sample. Patterns of differences among assemblages from the different habitats were difficult to examine due to the stress of nonmetric multidimensional scaling plots being high (>0.20, Figure 27) (Clarke, 1993). Three-dimensional nMDS plots were examined in each case and were found to display similar patterns to the two-dimensional plots, although the stress using three dimensions was in each case reduced to below 0.20. Consequently, only the two-dimensional plots are shown, but should be interpreted with some caution. nMDS plots showed that hypolithic samples clustered separately from open soil samples (Figure 28). Permutational multivariate analysis of variance (PERMANOVA) revealed that all four different habitat types harbored distinct community profiles ($P < 0.05$ for all 6 combinations). In addition, the differences in NMDS plots suggested that there might also be important differences in dispersion (variability) between the different assemblages, with greater spread observed for Type I hypolithons than for open soil.

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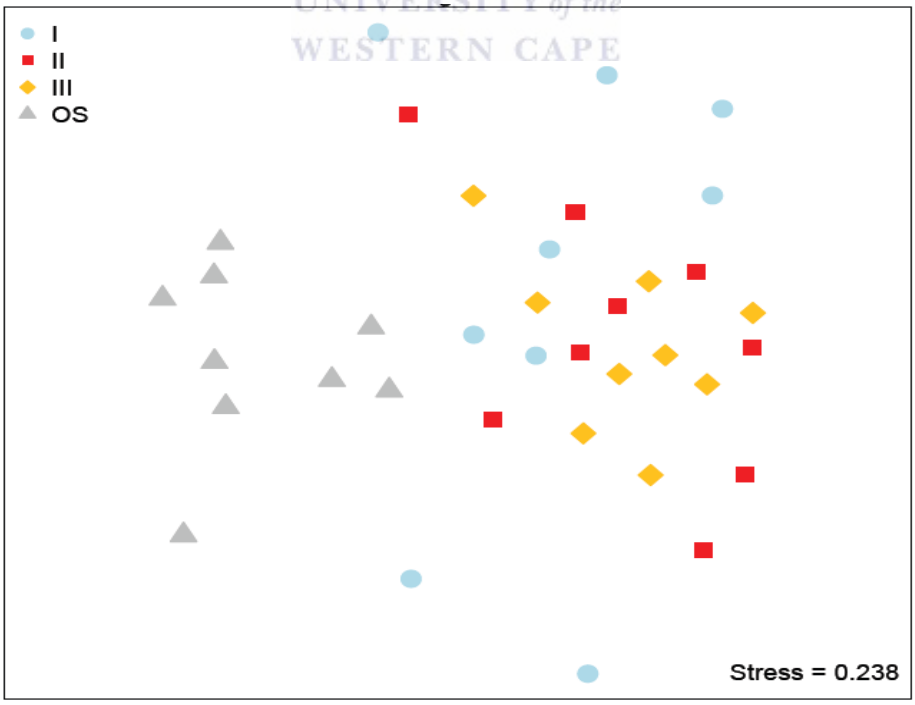

Figure 28: **nMDS ordination plot of combined functional gene T-RFLP profiles for soil- and hypolith derived samples.**

This pattern was supported by differences in the value of multivariate dispersion (Anderson 2006), which was 0.44 and 0.37 for Type I and open soil, respectively ($P < 0.05$). Similar results were obtained using qualitative (Jaccard) and quantitative (Bray-Curtis) similarity metrics. When both taxonomic and functional data matrices were compared, Jaccard similarity showed that on average there was 33.5 % similarity in taxa composition across samples, and 34.7 % similarity in functional composition, indicating that many encoded functions are unique to the different phylotypes detected by the T-RFLP analysis.

5.2.3 Functional and taxonomic structure

An analysis of functional and taxonomic structure showed that genes coding for nitrogen and carbon cycling related enzymes were linked to the structure of bacterial communities in the different Antarctic habitats (Mantel r=0.51, P=0.001). The same environmental variables were also influential in accounting for variation in both taxonomic and functional structure (S, F, and NO_3) (Figure 29). These data suggest that there is a significant correlation between taxonomic and functional structure, and both are responsive to local environmental conditions. The existence of community related patterns suggests non-random distributions of populations following the species sorting community assembly model (Leibold *et al.*, 2004, Van der Gucht *et al.*, 2007), where, community structure in environmental patches is shaped by the environment and/or interspecies interactions (Cottenie, 2005, Langenheder and Szekely, 2011). The results presented here indicate that stochastic processes such as dispersion cannot override the influences of environmental factors.

The connection between taxonomy and functional structure that was observed is in agreement with results from stream macro invertebrate communities (Heino *et al.*, 2007) and controlled experiments with assembled communities, where bacterial diversity (as a component of community structure) has been found to be an important element of ecosystem functioning (Bell, 2005, Peter *et al.*, 2011, Langenheder and Szekely, 2011). In contrast, algal-associated (Burke *et al.*, 2011) and nascent stream corridors (Frossard

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et al., 2011) bacterial communities have shown a disconnect between taxonomy and functional genes, and taxonomy and potential enzyme activities (ecosystem functioning), respectively.

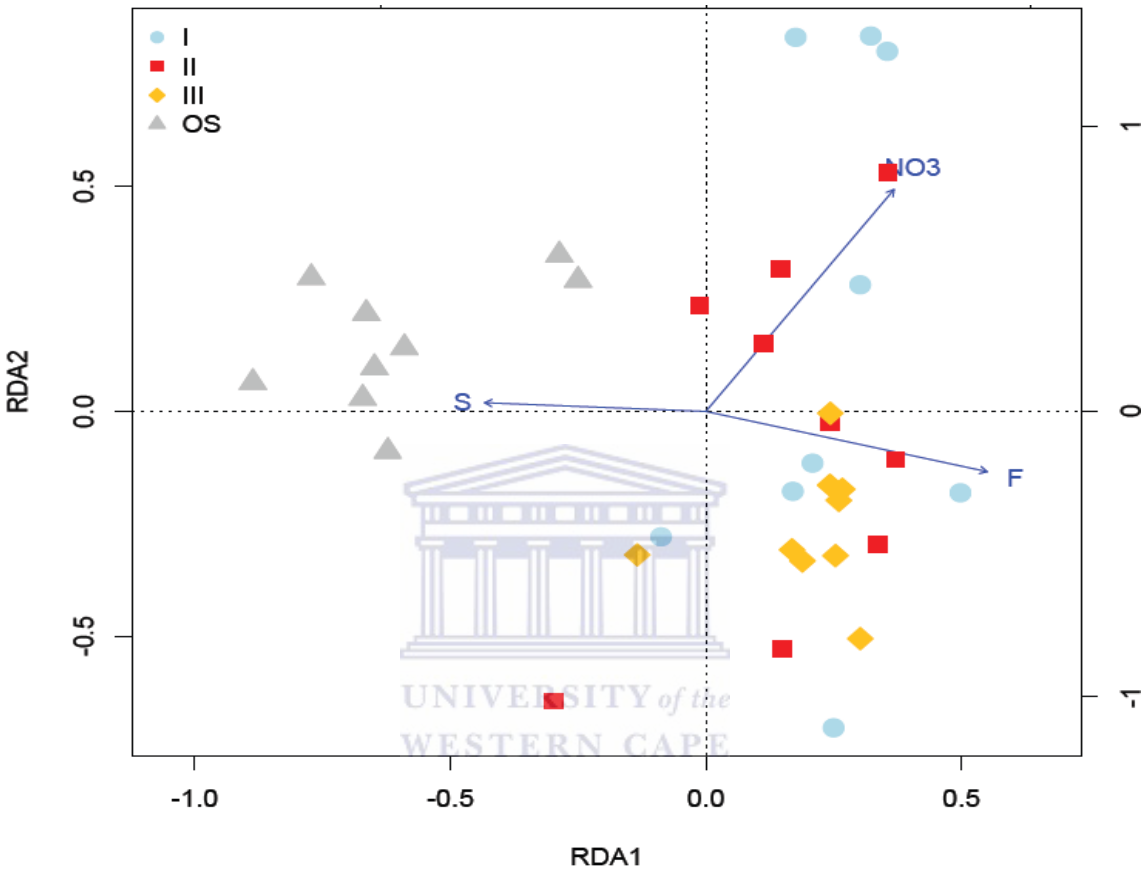

Figure 29: Redundancy analysis (RDA) biplot of functional diversity and microenvironmental parameters. T-RFLP analysis profiles for hypolithic and soil samples are depicted (*n*=36). Only the environmental variables that significantly explained variability in microbial community structure are fitted to the ordination (arrows).

One reason for the congruency between taxonomic and functional structure might be methodological, because T-RFLP tends to detect mainly dominant taxa, resulting in a simplified fingerprint (Bent and Forney, 2008, Verbruggen *et al.*, 2012). This explanation would imply that functional patterns are primarily driven by dominant taxa that are best detected by T-RFLP. In fact, a widespread tenet in microbial ecology is that the dominant microorganisms in a sample are those that play the most important functional role under normal conditions. However, the patterns we observed do not translate necessarily into

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function, as dominant taxa are not always the most active (Lennon and Jones, 2011). For example, dormant bacteria have been estimated to account for an average of ca. 50 % of all bacterial OTUs in soils (Lennon and Jones, 2011).

A link between taxonomic and functional structure is often implied (Selesi *et al.,* 2009). However, it is understood that DNA-based techniques provide no direct evidence for community function. The use of molecular techniques based on environmental RNA, e.g. metatranscriptomic analysis, would however provide strong evidence of functional capacity and could contribute to an understanding of whether the patterns observed are important for ecosystem functioning.

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CHAPTER 6: Thesis Summary and Synthesis

6.1 Thesis Summary

The principal aim of this thesis was to develop a broader understanding of taxonomic and functional bacterial diversity in extreme hot and cold hyperarid deserts. A combination of culture independent tools, ecological theory and multivariate statistical analysis was used to elucidate the microbial ecology in these environments. The Namib and Miers Valley, Antarctica were used to represent hot and cold hyperarid deserts, respectively.

Chapter 1 reviewed the current literature on desert biome research, methods of data acquisition used in this thesis, and current theoretical and multivariate approaches for data analysis. Here, emphasis was placed on desert biomes and the significance of the hypolithic niche. Furthermore, the chapter presented community theory in hypoliths which led to the development of a hypothesis which formed the basis for subsequent results chapters (Chapter 3-5). The second chapter provided detailed materials and methodology followed in the course of the study.

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The third chapter explored the development of hypolith communities in the Namib Desert. Here the ecological concept of “indicator species” was applied to investigate the processes leading to hypolithic community assembly at a microscale in a hot hyperarid desert. In the event of deterministic processes being significant, the hypothesis was that hypoliths and surrounding soil would demonstrate greatly dissimilar bacterial communities (specialists). Alternatively, should environmental effects be limited, both hypoliths and surrounding soil should contain similar bacterial communities (generalists). Empirical evidence is presented (for the first time for a hot desert) that hypolithic colonization in the Namib does not develop independently from microbial communities found in the surrounding soil, but these communities selectively recruit from local populations. This result is in contrast, for instance, to some hyperarid Antarctic desert hypoliths were cyanobacteria-dominated hypolithon occurs in soils where cyanobacterial signatures were undetectable by sequence analysis of environmental clone libraries (Pointing *et al.*, 2009).

The forth chapter expands on previous investigations which have established Antarctic hypolithons as three morphological types (Khan *et al.*, 2011, Cowan *et al.*, 2010a). This chapter sought to explore the bacterial community structure and assembly patterns in hypolithons from Miers Valley, Eastern Antarctica. The principal aim of the study was to assess the bacterial beta-diversity patterns in the different types of hypolithic communities. In addition, the study sought to investigate the effect of community assembly processes in hypolithic communities. Productivity is known to be highest at nascent stages of succession (Fierer *et al.*, 2010), and stochastic processes are known to predominate in higher-productivity systems (Chase, 2010). The hypothesis was that stochasticity would be greater in cyanobacterial dominated communities, followed by fungal and moss-dominated communities, respectively. Here, T-RFLP and pyrosequencing analysis of the 16S rRNA genes in all three hypolith morphotypes were combined with multivariate analysis of microenvironmental conditions. The results are strongly suggestive that hypolithons and open soils display different community compositions. Additionally, β-diversity was found to be higher in Type I hypolithons relative to Type II and III, respectively, suggesting that deterministic processes in the bacterial component of the hypolithons may increase in the order Type I => Type II => Type III. Taken together, the results present empirical evidence suggesting that the different morphological types of hypolithons may represent different successional stages of development. The conclusion of the chapter contradicts two other possible hypotheses on hypolithic community development (Cowan *et al.*, 2010a).

The fifth chapter assesses the presence and diversity of key functional genes implicated in C and N cycling in Antarctic environments. As a continuation of the previous chapter, the functional guilds implicated in C and N fixation were assessed in both hypolith and soil bacterial communities using T-RFLP analysis. The hypothesis was that habitat type together with environmental conditions would be a major driver of change in bacterial functional diversity. As a consequence, a spatial variation of functional diversity in hypoliths and soil would be found. Furthermore, there would be a systematic relationship between results obtained from the taxonomic and functional elements. Spatial variability analysis showed that red-like *cbb*L genes displayed the highest number

of T-RFs per sample, followed by *amo*A, green-like *cbb*L, *nir*K and *nir*S. The *nir*K gene from Type III hypolithons yielded a significantly higher OTU number than open soil. When functional genes were displayed according to habitat type, hypoliths showed higher T-RF numbers than open soils, with differences between Type I and open soil being statistically significant ($P<0.05$). These results suggest that functional gene potential is substantially higher in Type I hypoliths. The functional profile of bacterial communities was further assessed by combining the six different functional genes to create functional community profiles for each sample. PERMANOVA revealed that all four different habitats harboured distinct community profiles ($P < 0.05$ for all 6 combinations). nMDS plots showed important differences in variability between the different assemblages, with the greatest spread observed for Type I hypolithon. A remarkable finding from this chapter was the demonstrated statistically significant link between genes coding for nitrogen and carbon cycling related enzymes and the structure of bacterial communities in the different Antarctic habitats.

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6.2 Synthesis of the findings

This thesis represents a detailed assessment of desert bacterial edaphic communities in hot and cold deserts (Namib Desert and Miers Valley, Antarctica). Culture independent approaches (T-RFLP, clone library sequence analysis, and 454 pyrosequencing of the 16S rRNA gene) were combined with computational analysis (multivariate statistical analysis, *in silico* analysis, phylogenetic analysis) and theoretical approaches to investigate the microbial ecology of hyperarid deserts.

The finding that Namib Desert hypolithic communities recruit selectively from the open soil environment has several implications. First, this provides evidence that cyanobacteria are indicator species for hypoliths (specialists). Previous investigations have found that specialist assemblages have higher productivity levels (Gravel *et al.*, 2011). Productivity is of particular importance in Desert biomes where the species pool is lower than that of other biomes. It has been found that a large species pool is essential for the sustenance of ecosystem function and assembly (Balvanera *et al.*, 2006). In depauperate environments, specialists assemblages are more susceptible to extinction

than generalists (Tilman *et al.*, 1994). A loss in specialist lineages has a direct net negative effect on levels of biodiversity and productivity (Cardinale *et al.*, 2012, Duffy, 2008).

This study has, for the first time, presented evidence for community succession in Antarctic Miers Valley edaphic communities. In view of the virtual absence of 'vegetation` in Antarctic communities, preliminary confirmation of early stages of succession is especially noteworthy. The possible validation of the model proposed by Cowan *et al*., (2010) is based on the assumption that the effects of stochastic variation may become less important as communities develop over time. This study brings into focus the productivity of climax communities in discrete microbial assemblages. Although the study was conducted by sampling at only a single time point, it is possible to speculate on the processes underlying developmental pathways which follow the initial establishment of microbial communities. One of the factors relating to community development which is essentially unknown is the kinetics of processes. The growth rates of Antarctic moss communities (mean 0.6 – 1.3 mm y^{-1}) (Clarke *et al.*, 2012) are far higher than those of lichen communities (mean <0.1 mm y^{-1}) (Sancho *et al.*, 2007). With the limited light availability of hypolithic communities, it is possible that growth and decay rates are even lower than for surface mosses and lichens. This would make it technically difficult to estimate growth rates and relate these to successional pathways in hypoliths.

Genes coding for nitrogen and carbon cycling were combined to create functional community profiles for each sample. A strong correlation linking function and taxonomy was found (Chapter 5). It was also observed that the same environmental variables were influential in accounting for variation in both taxonomy and functional structure (S, F, and NO_3). Taken together, these results strongly suggest non-random distributions of populations following the species sorting community assembly model (Leibold *et al.*, 2004).

6.3 Limitations of the study and future work

Soil communities are influenced by numerous environmental variables, making studies focusing on soil microbial communities difficult. Soil, as an environment, contains high levels of genetically variable taxa involved in a wide range of intricate processes. These add complexity to any experiment where several numbers of measured variables could lead to observed changes to members of the community. Although a wide suite of abiotic parameters was measured in this study, there remain many unmeasured microclimatic and physical variables in the environment that may be linked to microbial community structure and function. Measurement of relevant environmental variables and continual monitoring over a temporal scale is likely to provide more useful information required for the interpretation of biodiversity patterns, and relating these to the environment. It is noted, however, that the very low functional rates in such communities make the acquisition of data very challenging.

The limitations of this study are mostly associated with the methodological constraints, as reviewed in Chapter 1. Here, molecular based approaches such as PCR, T-RFLP, clone libraries, and NGS were used. All these methods have inherent biases which may lead to a skewed picture of biodiversity. T-RFLP, for instance, is known to detect mainly dominant taxa, resulting in a fingerprint is inevitably over simplified. Throughout the thesis, care has been taken to objectively highlight these limitations. Where possible, steps such as the introduction of replicates, and removal of chimeric sequences were taken in order to increase the robustness of the results. These techniques, however, remain useful for assessing diversity patterns and remain the most viable tools in microbial ecology (e.g. Besemer *et al.,* 2012, Fierer and Jackson, 2006, Knight *et al.,* 2012).

The theoretical approaches (ecological concepts) and models which have been used in this study are designed principally for macroecology. While microbial communities are significantly different from those dominated by higher eukaryotes, similar biogeographic trends have been shown (Martiny *et al.,* 2006). Ecological theories must be applied with

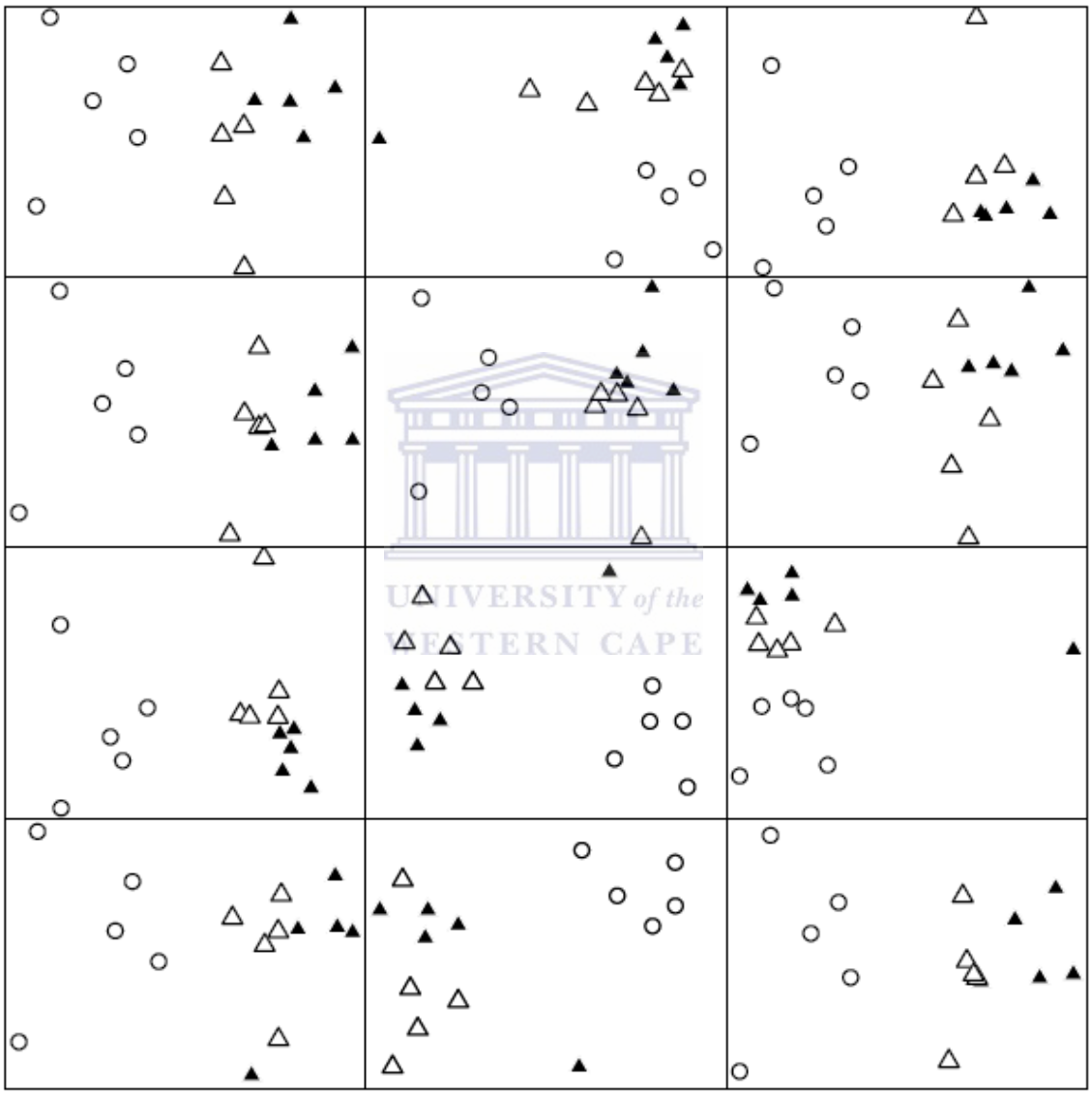
requisite modifications and with some caution to retain validity in microbial ecology (Horner-Devine *et al.*, 2003, Prosser *et al.*, 2007).

The findings made in this thesis could be further validated by combining other polyphasic approaches. Future studies should integrate the use of metatranscriptomic and metaproteomic approaches (Jansson *et al.*, 2011). The use of these approaches is expected to "permit us to 'see' the who, what, when, where, why and how of microbial communities" (Knight *et al*., 2012).

Here we have linked the diversity and relative abundance of major soil microbial taxa and functional genes with broad scale gradients in biotic and abiotic characteristics. The results reported in this thesis represent significant advancements to the study of microbial ecology in desert biomes. Key issues relating to (i) the development of microbial communities in hyperarid desert communities, (ii) the bacterial community structure and assembly patterns in hypolithons from the Miers Valley and, (iii) the functional patterns of key nutrient cycling genes have been examined. These findings have stimulated questions on the productivity of niche microbial communities in desert biomes, a possible avenue for fruitful future research.

APPENDICES

Appendix A



nMDS generated from random “resampling”. This was applied in order demonstrate that the effect of sample size does not influence the overall structure of hypolithic and soil bacterial communities. Code indicators: Circles (hypolithons), filled triangles (sub-lithic soil) and empty triangles (open soil). 12 randomizations are depicted.

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Appendix B: BLASTN results against the NCBI database. E values were zero and omitted from the table.

Seq. Identification	Accession number	Closest Homologos in GenBank	%	Origin -country	Isolation source	Source clone library
NamSP1	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP2	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP3	AF493850.1	Uncultured bacterium clone 11	99	Southern Mojave Desert	Surface of desert rocks	Hypolith
NamSP4	FJ805942.1	Uncultured Chroococcidiopsis sp. clone A4_1 16S	97	Botswana: Kalahari	Desert quartz	Hypolith
NamSP5	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP6	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Atacama Desert	Quartz	Hypolith
NamSP7	FN813975.1	Uncultured bacterium 16S rRNA gene, clone 26B1-D3	91		*Lactuca sativa* (phyllosphere)	Hypolith
NamSP8	FJ230783.1	Uncultured bacterium clone agateC2	92	Australia	Hypolithic soil	Hypolith
NamSP9	AY615380.1	Uncultured bacterium clone AP18	96	Atacama Desert	Rock	Hypolith
NamSP10	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP11	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP12	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP13	FJ230828.1	Uncultured bacterium clone Prehnite44	98	Australia	Hypolithic slime	Hypolith
NamSP14	FJ230827.1	Uncultured bacterium clone	95	Australia	Hypolithic slime	Hypolith
NamSP15	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	hypolithic slime	Hypolith
NamSP16	FJ891051.1	Uncultured cyanobacterium clone AY6_21	99	Atacama Desert	Rock	Hypolith
NamSP17	HM241076.1	Uncultured bacterium clone 211	98	Global hypolith study	Quartz hypoliths from desert	Hypolith
NamSP18	FR849426.1	Uncultured bacterium clone B16S-XJcc-2-29	95	Xinjiang Province	Soil	Hypolith
NamSP19	JF295649.1	Uncultured bacterium clone Ovdat61c11	100	Xinjiang Province	Soil	Hypolith
NamSP20	HM241001.1	Uncultured bacterium clone 136	96	Global hypolith study	Quartz hypoliths from desert	Hypolith
NamSP2	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith

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1						
NamSP22	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP23	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP24	FJ230783.1	Uncultured bacterium clone agateC2	96	Australia	Hypolithic slime	Hypolith
NamSP25	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP26	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	95	China	Uncultured Chloroflexi bacterium	Hypolith
NamSP27	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP28	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP29	FJ230783.2	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP30	FJ230783.2	Uncultured bacterium clone agateC2	95	Australia	Hypolithic slime	Hypolith
NamSP31	HM241076.1	Uncultured bacterium clone 211	97	Australia	Hypolithic slime	Hypolith
NamSP32	JF173381.1	Uncultured bacterium clone ncd1994h09c1	96	china	Soil	Hypolith
NamSP33	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Yungay, Atacama Desert	Quartz	Hypolith
NamSP34	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Soil	Soil
NamSP35	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma, Kessler Farm	Undisturbed tall grass prairie, top 5 cm	Soil
NamSP36	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP37	FJ592827.1	Uncultured bacterium clone G01_SB3A	97	Atacama	Socompa Volcano, Andes	Soil
NamSP38	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP39	FR687056.1	Uncultured bacterium clone d21h4b13	95	China	Paddy soil	Soil
NamSP40	GU219537.1	Uncultured bacterium clone Obs1-15	94	Iceland	Bsidian outcrop, Valafell	Soil

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NamSP41	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Soil
NamSP42	HM240933.1	Uncultured bacterium clone 068	95	Global hypolith study		Soil
NamSP43	AJ555203.1	Uncultured Actinobacterium	98	Lower Austria, Marchfeld	Agricultural soil	Soil
NamSP44	EF540530.1	Uncultured soil bacterium clone P21_J20 16S	97	Estonia	Semi-coke	Soil
NamSP45	JN037870.1	Uncultured Actinobacterium clone UHAS5.5	99	India	Saline-alkaline soil	Soil
NamSP46	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP47	DQ906857.1	Uncultured bacterium clone 10D-4	95	Oman	Subsurface soil	Soil
NamSP48	JF834545.1	Kocuria sp. PM0532155	99	India	Environmental sample	Soil
NamSP49	AB248528.2	Arthrobacter sp. LC7 gene for 16S rRNA,	99	Niigata (Japan)	Soil	Soil
NamSP50	FN550146.1	Micrococcaceae bacterium isolate MI-BOA	98	Marion Island	Soil	Soil
NamSP51	GQ425963.1	Uncultured bacterium clone Adulam-209	99	Adulam (Israel)	Soil	Soil
NamSP52	EF016806.1	Uncultured actinobacterium clone E1B-B3-11	95	Atacama Desert	Soil	Soil
NamSP53	FR667915.1	*Azospirillum brasilense* 16S rRNA gene, strain Gr59	99	Greece	Soil	Soil
NamSP55	GU552232.1	Uncultured actinobacterium clone D-16S-130	99	Atacama Desert	Desert soil	Soil
NamSP57	JF295718.1	Uncultured bacterium clone Lehavim48d08			Soil	Soil
NamSP58	AB205958.1	Uncultured bacterium clone OS-27	97	Niigata (Japan)	Activated sludge	Soil
NamSP59	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP60	AJ535735.1	Uncultured actinobacterium clone CF2	99	Marchfeld (Austria)	Soil	Soil
NamSP61	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP6	FR667915.1	*Azospirillum brasilense* strain Gr59	99	Greece		Soil

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2						
NamSP63	HM565047.1	Uncultured Actinomycetales bacterium clone N-35	98	China	Concrete	Soil
NamSP66	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP67	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP68	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP69	DQ336134.1	Frankia sp. strain BCU110345	96	Argentina	Soil	Soil
NamSP70	FR849478.1	Uncultured bacterium clone B16S-XJrs-3-8	99	Xinjiang Province		Soil
NamSP73	JN684205.1	Uncultured bacterium clone H144	100	China	Environmental sample	Soil
NamSP74	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Environmental sample	Soil
NamSP75	FJ230801.1	Uncultured bacterium clone QuartzC15	92	Australia	Hypolithic slime	Soil
NamSP76	EU440648.1	Actinomycetales bacterium clone Plot17-A07	99			Soil
NamSP77	FR849480.1	Uncultured bacterium clone B16S-XJrs-3-61	99	China	Desert	Soil
NamSP78	HQ910327.1	Uncultured bacterium clone P-8_B22	97	Utah (USA)	Desert soil	Soil
NamSP79	FR852514.1	Uncultured bacterium clone W3-199	98	China	Red soil	Soil
NamSP80	JF295697.1	Uncultured bacterium clone Lehavim48g01	93	Israel	Soil	Soil
NamSP81	EU029450.1	Uncultured Bacteroidetes bacterium clone T4174	95	Israel	Environmental sample	Soil
NamSP82	JF707601.1	Uncultured Chloroflexi bacterium clone HKTK7-4	93	India	Desert soil	Soil
NamSP83	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP84	HM240929.1	Uncultured bacterium clone 064	98	Global hypolith study	Quartz hypoliths from desert	Soil
NamSP85	GQ425235.1	Uncultured bacterium clone Ovdat-4	99	Ovdat (Israel)	Soil	Soil

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NamSP86	HM584296.1	Acinetobacter sp. CJ-S-MA3	99	Korea	Environmental sample	Soil
NamSP87	EF651023.1	Uncultured beta proteobacterium clone AUVE_03A05	98	Australia	Cropland	Soil
NamSP88	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma (USA)	Tall grass prairie, top 5cm	Soil
NamSP89	AB622776.1	Uncultured bacterium clone: IMCUGWBC9-1	99	China	High arsenic aquifer	Soil
NamSP91	FJ790550.1	Uncultured bacterium clone VB29	99	Tibet	Soil	Soil
NamSP92	FM209314.1	Uncultured bacterium 16S rRNA gene, clone 230	96	Israel:Negev desert	Soil	Soil
NamSP93	JF295619.1	Uncultured bacterium clone Ovdat61h02	92	Israel	Soil	Soil
NamSP94	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP96	JF706662.1	Uncultured actinobacterium clone w3-15	99	Atacama Desert	Hypolith	Soil
NamSP98	JF706662.1	Uncultured actinobacterium clone w3-15	98	Atacama Desert	hypolith	Soil
NamSP99	FM209314.1	Uncultured bacterium clone 230	91	Negev desert (Israel)	Desert	Soil

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APPENDIX C:

Phylogenetic diversity (PD) data from clone libraries generated from hypolith and open soil

Library	Number screened	ARDRA defined phylotypes	No. of OTU 80 % sequence similarity	No. of OTU 90 % sequence similarity	No. of OTU 97 % sequence similarity	Chao1 richness	F_{st} statistic
Hypolith	200	33	6	9	28	96.9 %	0.132
Open soil	200	52	16	31	41	49.3 %	0.236

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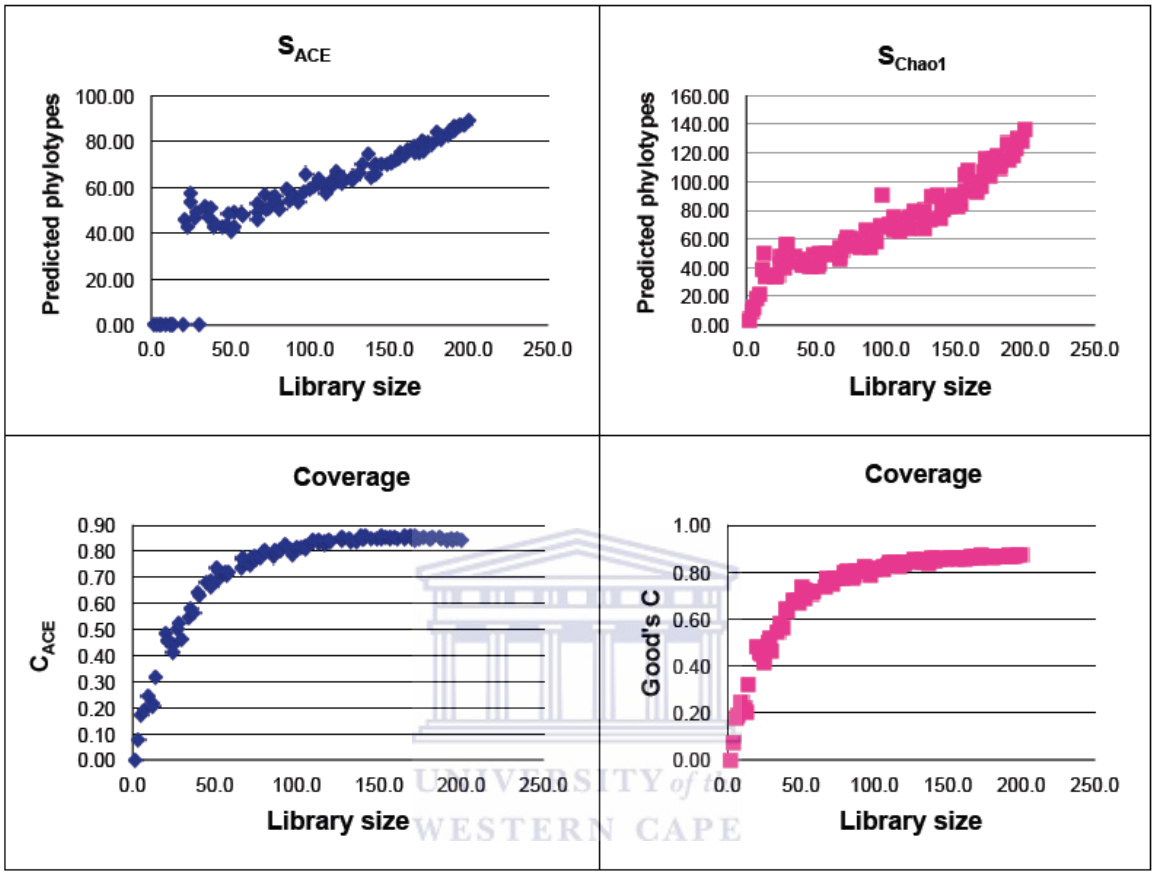
Appendix D:



Open soil clone library coverage (Good's and CACE) and estimator (Schao1 and SACE) plots.

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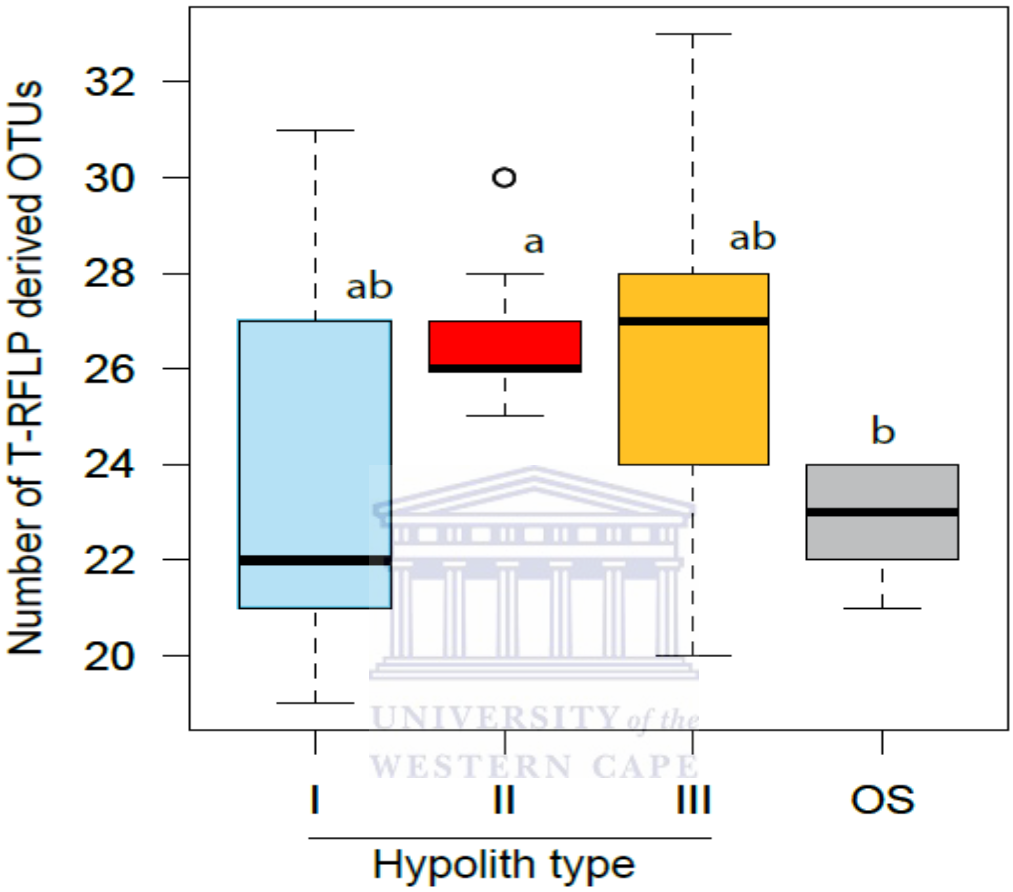
Appendix E



Box plots showing the richness in pooled hypolithons and soil samples.

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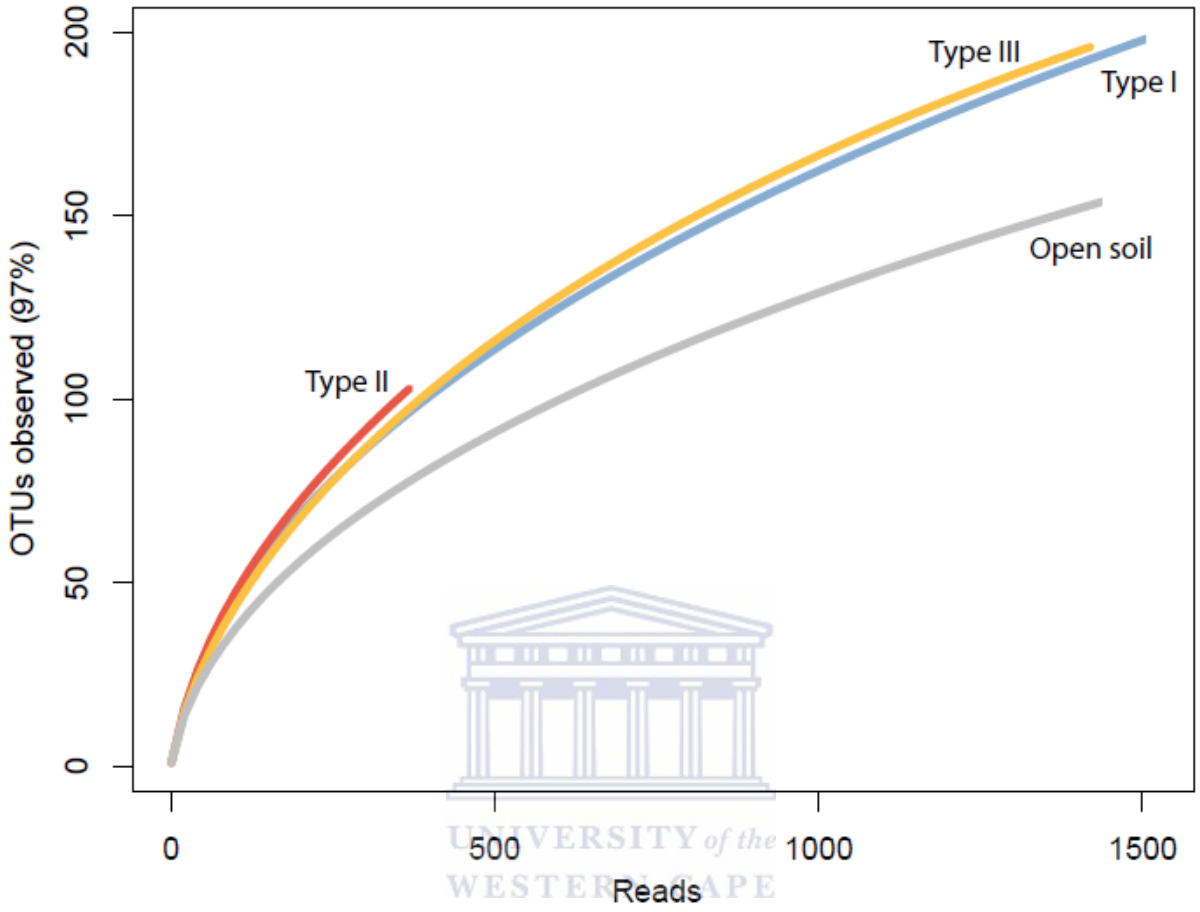
Appendix F



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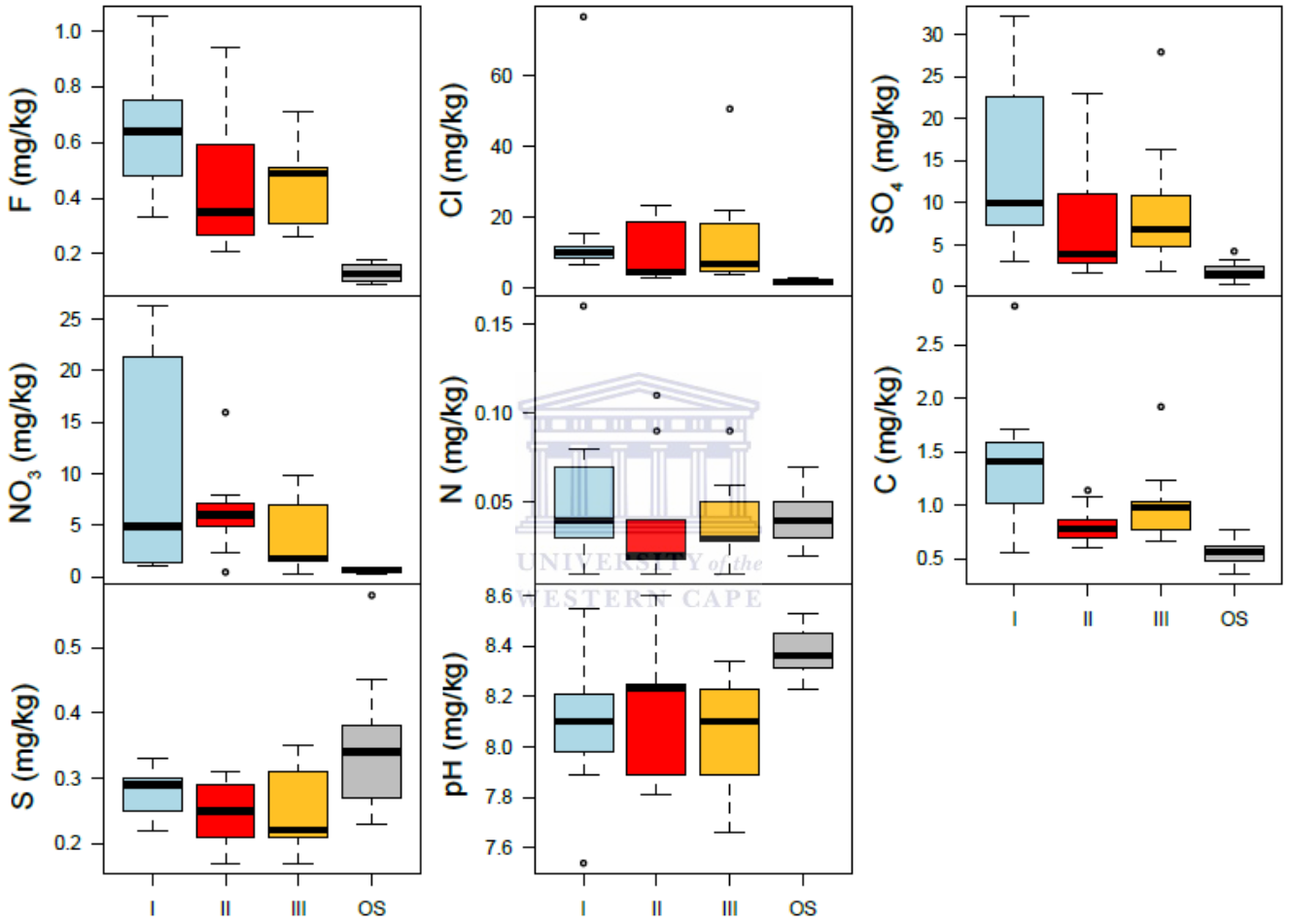
The effect of the sequencing effort on the estimation of the number of OTUs for hypoliths (Type I,II,III) and soil.

Appendix G



Box plots showing post-hoc test using Wilcoxon-Mann-Whitney on microenvironmental data suggesting that nutritional constraints in this depauperate environment are even higher for soil bacterial communities.

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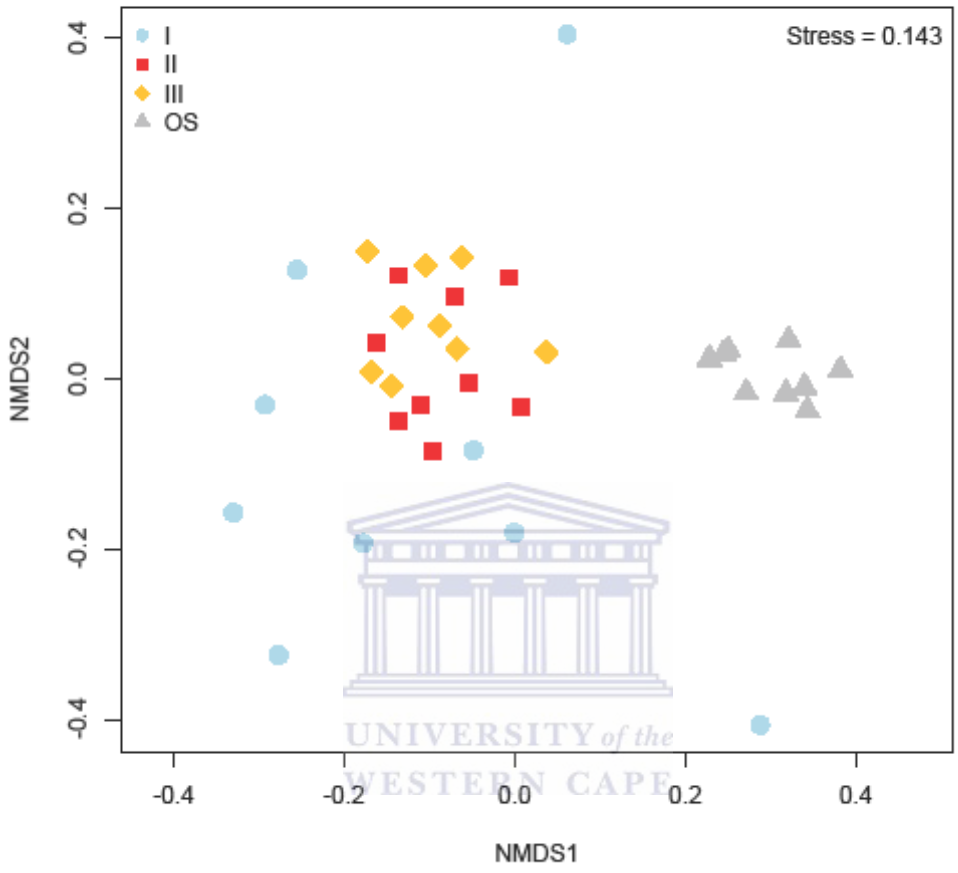
Appendix H



nMDS ordination plot (Bray-Curtis dissimilarity matrix) of T-RFLP profiles hypolith Type I, Type II, Type III and Open soil (OS) derived samples. The quality of the ordination is indicated by the low-stress value.

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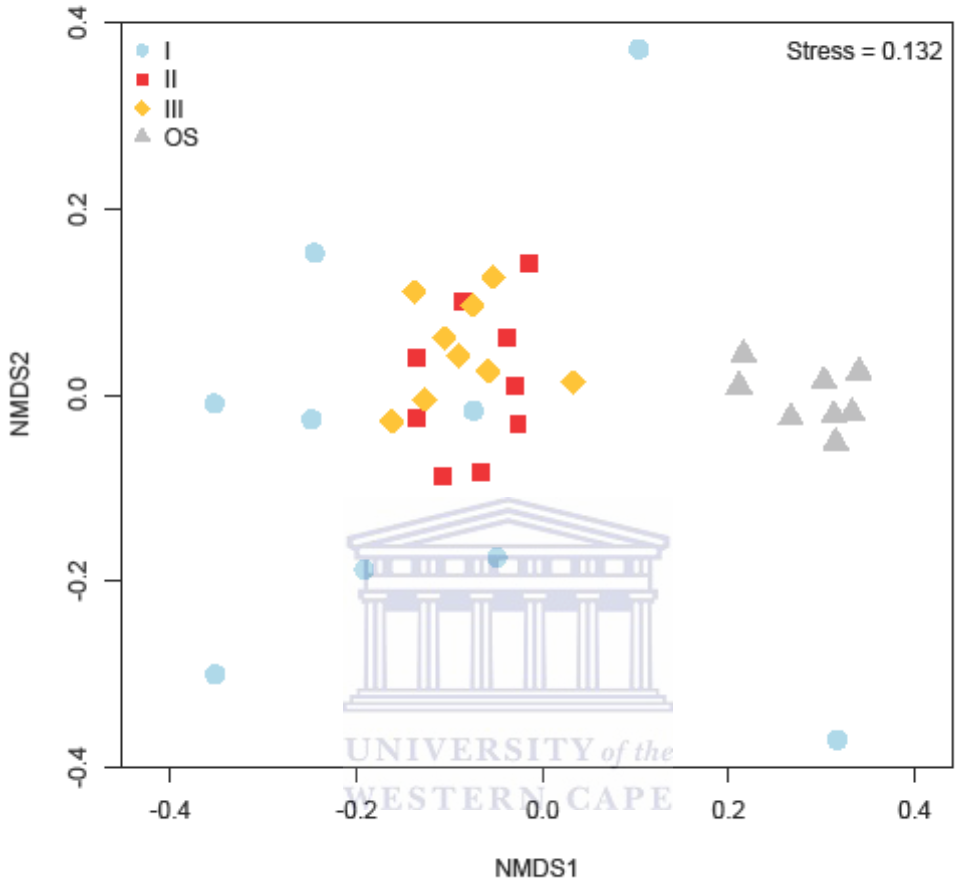
Appendix I



nMDS ordination plot (Morisita-Horn dissimilarity metrics) of T-RFLP profiles hypolith Type I, Type II, Type III and Open soil (OS) derived samples. The quality of the ordination is indicated by the low-stress value.

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Research Outputs since 2010 (PhD registration)

International travel

1. The University of Hong Kong, September 2010 - December 2010. Research Visit. Hong Kong
2. NASA-AMES-IMBM. April 2010 and May 2012 Spaceward Bound Research Trip. Gobabeb Desert Research Station, Namibia.

Publications

3. Cowan, D.A., Sohm, J.A., **Makhalanyane, T.P.,** Capone, D.G., Green, T.G.A., Cary, S.C., and Tuffin, I.M. (2011) Hypolithic communities: important nitrogen sources in Antarctic desert soils. *Environ Microbiol Rep* **3**: 581-586 [Impact factor=3.2]
4. Cowan, D.A., Khan, N., **Makhalanyane, T.P.,** Valverde, A. (2011) Antarctic hypolithic communities - Model systems for a cryptic astrobiological lifestyle. *62nd International Astronautical Congress 2011* **1**: 308-312.
5. Stomeo, F., **Makhalanyane T.P.,** Valverde, A., Pointing, S.B., Stevens, M., Cary S.C., Tuffin, I.M., and D.A Cowan. Abiotic drivers influence the microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. (2012) FEMS Microbiology Ecology, 82, 326 – 340 [Impact factor=3.456]
6. **Makhalanyane, T.P.,** Valverde, A., Lacap, D.C., Pointing, S.B., Tuffin, M., and Cowan, D.A. (2012). Evidence of species recruitment and development of hot desert hypolithic communities. *Environ Microbiol Rep*. In press **doi: 10.1111/1758-2229.12003** [Impact factor=3.2]
7. **Makhalanyane, T.P.,** Valverde, A., Burkland, N-K., Cary, C.S., Tuffin, M., and Cowan, D.A. (2012). Bacterial community structure and assembly patterns in hypolithons from Miers Valley, Antarctica. *Under Review*
8. **Makhalanyane, T.P.,** Valverde, A., Cary, C.S., Tuffin, M., and Cowan, D.A. (2012). Functional patterns of key nutrient cycling genes in Miers Valley, Antarctica. I*n preparation*

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Conference outputs

9. **Makhalanyane, T.P.,** Cary, S.C., Pointing, S.B., Tuffin I.M., Cowan D.A. 2010. The Diversity of Key Anabolic Genes in Antarctic hypolithons**.** International Symposium for Microbial Ecology -13, Seattle, USA. **Oral** *
10. .Cowan D.A., Khan, N., **Makhalanyane, T.P**., Sohm, J., Capone, D., Cary. S.C., Tuffin, IM 2010. International Symposium for Microbial Ecology -13**.** Hypolithic communities in Antarctic Dry Valley soils. **Invited keynote**
11. **Makhalanyane, T.P,** Cary, S.C., Pointing, S.B., Tuffin I.M., Cowan D.A**.** The Diversity of Key Anabolic Genes in Antarctic hypolithons. Extremophiles 2010. **Poster**
12. **Makhalanyane, T.P,** Cary, S.C., Pointing, S.B., Tuffin I.M., Cowan D.A. Cryptic microbial communities. International Astronautical Conference. 2011. **Poster***
13. Cowan, D.A., Khan, N., **Makhalanyane, T.P**, Valverde, A. Antarctic hypolithic communities – model systems for a cryptic astrobiological lifestyle. International Astronautical Conference. 2011. **Invited keynote**
14. Makhalanyane, T.P, Pointing, S, Stomeo, F, Lacap-Bugler, D.C, Tuffin I.M, Cowan D.A. Microbial niche differentiation determines community composition in a hot hyperarid desert. SASM. 2011. Cape Town 2011 **Poster**
15. Cowan, D.A., Khan, N., **Makhalanyane, T.P.**, Cary, S.C, Pointing, S.B. Tuffin I.M. Hypolithic communities in the Antarctic Dry Valleys; Biology and Genomics, Alpine and Polar Biology. 2011. Ljubljana. **Invited keynote**
16. .**Makhalanyane, T.P,** Pointing, S, Lacap-Bugler, D.C, Tuffin I.M, Cowan D.A. Microbial niche differentiation determines community composition in a hot hyperarid desert – **SASBMB.** 2012. Drakensburg 2011. **Poster**
17. Tuffin, I.M Stomeo, F., **Makhalanyane, T.P.**, Valverde, A., McKay, C., Warren-Rhodes, K., Lacap, D., Pointing, S.B., and Cowan, D.A. Evidence of species recruitment and development of hypolithic communities along an aridity gradient in the Namib Desert. Extremophiles 2012. Seville, Spain**. Oral**
18. Cowan, D.A., **Makhalanyane, T.P.**, Cary, S.C., and Tuffin, I.M. Understanding complexity and processes in Antarctic niche habitats. Extremophiles 2012 Seville, Spain. **Invited keynote** *Presenting author

Technical Reports

19. Ramond, J.B., **Makhalanyane, T.P.,** Cowan. D.A**. NRF Blue Sky Project:** Annual Progress Reports 2012: Culturing the Uncultured.

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Hypolithic communities: important nitrogen sources in Antarctic desert soils

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Summary

Hypolithic microbial communities (i.e. cryptic microbial assemblages found on the undersides of translucent rocks) are major contributors of carbon input into the oligotrophic hyper-arid desert mineral soils of the Eastern Antarctic Dry Valleys. Here we demonstrate, for the first time, that hypolithic microbial communities possess both the genetic capacity for nitrogen fixation (i.e. the presence of *nifH* genes) and the ability to catalyse acetylene reduction, an accepted proxy for dinitrogen fixation. An estimate of the total contribution of these communities suggests that hypolithic communities are important contributors to fixed nitrogen budgets in Antarctic desert soils.

Introduction

The soil surface environment in the Dry Valley deserts of Eastern Antarctica is too extreme to support macroscopic microbial communities (Wynn-Williams, 1990), although these desert soils contain a high diversity of microbial phylotypes (Smith *et al.*, 2006; Cary *et al.*, 2010). Hypoliths, prokaryote-dominated biological communities found under translucent stones (see Fig. 1A and B), are 'refuge' habitats which extend the range of life into some of the most extreme desert environments on Earth (Cowan, 2009; Pointing *et al.*, 2009). The overlying quartz or marble allows the transmission of incident light while providing protection from the deleterious effects of extreme desiccation, physical disturbance and high UVa/b fluxes (Cockell and Stokes, 2004).

Hypolithic communities are typically dominated by photoautotrophs including cyanobacteria and green algae (Cockell and Stokes, 2004; Wood *et al.*, 2008), but moss-dominated hypolithons have recently been identified in Antarctic desert soils (Cowan *et al.*, 2010). These communities are now thought to make an important contribution to the carbon input budget in depauperate habitats such as polar deserts (Burkins *et al.*, 2001).

However, little is known of the nitrogen budgets in Antarctic desert soils. Organic nitrogen inputs may be derived from legacy biomass or from lake-derived cyanobacterial biomass distributed within the Dry Valleys by aeolian action (Moorhead *et al.*, 1999). Here we demonstrate, for the first time, that hypolithic communities both possess the genetic capacity for N_2 fixation and exhibit significant nitrogenase activity, making them an important, and hitherto disregarded source of fixed nitrogen in Antarctic deserts.

Results and discussion

Using PCR amplification of metagenomic DNA extracts with *nifH* primers PolF and PolR (widely used universal primers for identification of nitrogenase gene clusters) (Poly *et al.*, 2001), we have demonstrated that all hypolith samples investigated ($n = 6$) contain multiple *nifH* phylotypes, which belong to a wide range of known nitrogen fixers (Fig. 2). All of the cyanobacterial sequences belong to the order *Nostocales*, a group of filamentous cyanobacteria that form specialized cells (heterocysts) where nitrogen fixation is localized. 16S rRNA gene sequences from this order, along with *Oscillatoriales* and *Chroococcales*, can be found in soil environments throughout the Dry Valleys (Wood *et al.*, 2008) and globally in cold environments (Yergeau *et al.*, 2007; Jungblut and Neilan, 2010). However, dry soils in Miers Valley do not show nitrogenase activity (J.A. Sohm and D.G. Capone, unpubl. data). The other sequences were identified as members of the proteobacteria, but it is impossible to predict their capacity to fix nitrogen in hypolithic communities. However, we note that certain proteobacteria (e.g. *Azotobacter vinelandii*) are nitrogen fixing symbionts of plants (Chen *et al.*, 2003).

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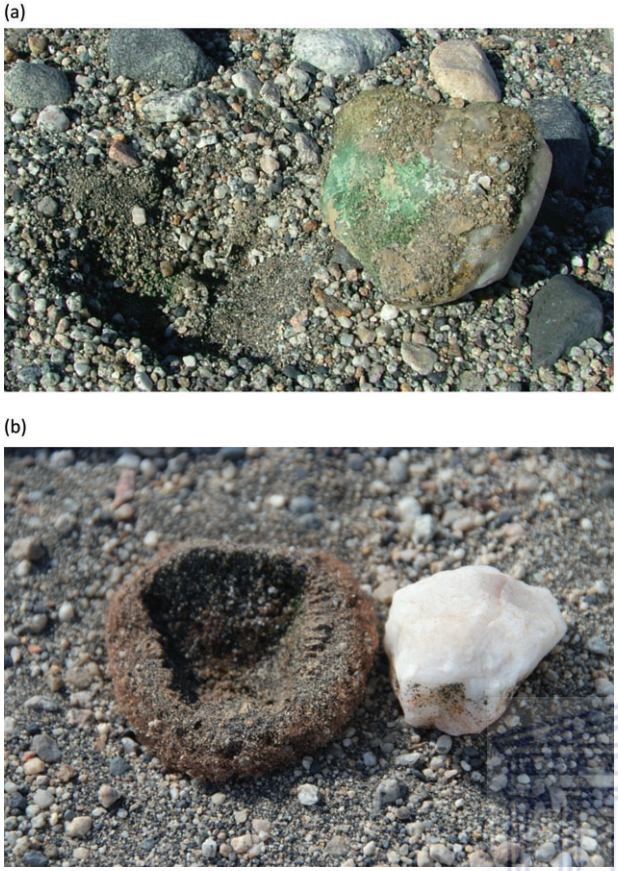

Fig. 1. (A) Cyanobacterial hypolithon; (B) moss-dominated hypolithon.

Nitrogenase activity, determined using the acetylene reduction assay (Capone, 1993), was found in six of the 12 hypolith community samples tested, and ranged over an order of magnitude, 0.02–0.174 nmol N g^{-1} h^{-1} (Fig. 3). Hypoliths selected for this experiment were independent community samples, were not chosen for size or potential stage of development, or for consistency of geochemical factors such as the translucence of the overlying rock. Substantial variability in rates is therefore to be expected.

Acetylene reduction assays were also performed on open soil samples (controls) from 14 different locations in the Miers Valley. No activity was detected in any of these samples.

When acetylene reduction data were recalculated on the basis of total organic carbon content, all values fell within a fourfold range and gave a mean value of 0.73 (SD 0.34) nmol N (mg C)$^{-1}$ h^{-1}. Because these are the first published rates for hypolithic communities, it is difficult to find appropriate examples for comparison. Desert soil crusts are also cyanobacteria-dominated biological communities typical of dry habitats, making them a valid comparative system. Although the range of N fixation rates from the different habitats spans around five orders of magnitude, our surface area normalized acetylene reduction rates (see below) fall within this range (Table 1). Surprisingly, they are also within the range of acetylene reduction found in the wet climate of a Hawaiian rainforest (Matzek and Vitousek, 2003). In comparison, endolithic communities (cyanobacterial- or chlorophyte-dominated communities found within the interstices of granular translucent rocks) both in hot deserts and on the Antarctic continent rarely demonstrate detectable nitrogenase activity (Friedmann and Kibler, 1980).

It was not possible to carry out extensive experiments on the environmental controls on nitrogenase activity in hypoliths because of low sample mass and the desire to minimize environmental disturbances. However, one hypolith was sufficiently large to subdivide into six subsamples in order to assess the effects of light and temperature (sample 5 in Fig. 3). Two samples were incubated under the standard conditions, while the other four were incubated at ambient temperature (ranging from 0°C to +6°C over the course of the 8 h experimental period). Of these four, two were exposed to an ambient sunlight regime, and two were covered in foil to completely exclude light. Nitrogenase activity was not significantly different between any of the treatments ($P > 0.22$), indicating that in the short term, activity is not highly sensitive to the range of temperature or light tested. Assuming that temperature is a major driver of metabolic activity, we note that temperature difference between *in vitro* and *in vivo* incubation conditions was only approximately 10°C, where the maximum difference in activity predicted by Arrhenius behaviour would be twofold.

Over the following austral summer we carried out a second trial on the effects of water on nitrogenase activity. Water availability is known to be very important to nitrogen fixation in cryptic microbial communities (see, for example, Zaady *et al*., 1998; Boison *et al*., 2004). In two experimental samples, nitrogenase activity was not detectable in prior to water addition, but increased to levels equivalent to previous values in samples where 0.5 ml of water was added (0.113 and 0.085 nmol N g^{-1} h^{-1}). While the sample size is small, these results suggest that hypolithic nitrogenase activity is rapidly responsive to water addition. We suggest that this response may be mediated by the state of desiccation of the samples, which might account for the consistently higher acetylene reduction rates in the 2008 experiments and their failure to show a response to water addition.

Using these data on nitrogen fixation in hypoliths together with other data collected as part of an international landscape-scale biocomplexity survey we

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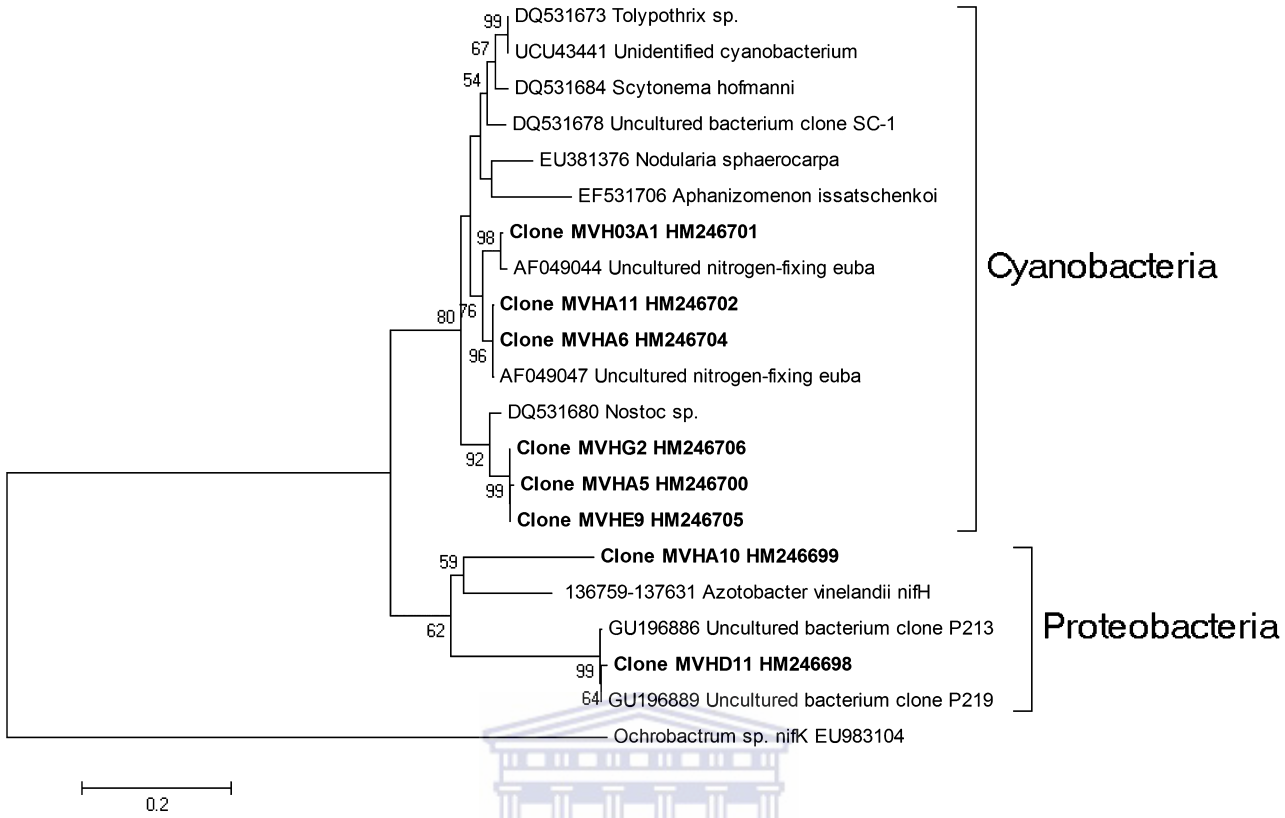

Fig. 2. Phylogenetic tree of *nifH* sequences obtained from hypolith metagenomic DNA. DNA was extracted from six hypolithon samples as described by Miller and colleagues (1999) and used as template for PCR. DGGE analysis of all six samples (data not shown) showed similar 16S rRNA gene amplicon fingerprints, and DNA samples were pooled for further analysis. Primers PolF and PolR (5′-TGCGAY CCS AAR GCB GAC TC-3′ and 5′-ATS GCC ATC ATY TCR CCG GA-3′ respectively) were used to amplify a 360 bp *nifH* fragment. Amplification was carried out as described by Poly and colleagues (2001). PCR products were eluted from agarose gels using a NucleoSpin® Extract II kit. Eluted PCR products were ligated into the pGEM®-T Easy vector (Promega) and transformed into competent *Escherichia coli* Gene Hogs™ (Invitrogen). Plasmids from *nifH* libraries were isolated by use of a Zippy™ Plasmid Miniprep Kit (Zymo Research Cooperation). Sequencing of representative plasmid DNA was carried out by the University of Stellenbosch Sequencing Service using a Hitachi 3730xl DNA Analyser (Applied Biosystems) and the Big Dye Terminator v3.1 system. Chromas® was used for editing sequences. Edited sequences were aligned using Bio-Edit with MEGA 4 (Tamura *et al.*, 2007) being used to construct phylogenetic trees, based on the Maximum Composite Likelihood method and substitution model using Neighbour-Joining. The test of phylogeny was used based on 1000 bootstraps of replication and a pairwise deletion of gaps. DNA sequences were identified by BLAST homology searches against the NCBI non-redundant database. The sequences determined in this study are available at GenBank under Accession No. HM 246698–HM 246706.

attempted to estimate the potential hypolith-derived contributions to the regional nitrogen budget of a 220 km^2 area of three McMurdo Dry Valleys. Using a mean hypolith dry weight value of 29.4 $\pm$ 41.5 g ($n = 31$), we calculate that an 'average' hypolith has the capacity to fix approximately 2.2 $\pm$ 1.6 nmol N h^{-1}. The survey dataset (Fig. 4) showed that 38% of all transects exhibited some colonization by hypolithic communities, and that mean colonization area was 25 cm^2 per transect (i.e. equivalent to an average surface area colonization of 0.024%). Using this value, we calculate that the total hypolith footprint in the three-valley region is approximately 5200 m^2.

Using field measurement data, we estimate the average surface dimensions (i.e. coverage) of a hypolith to be 22.5 $\pm$ 8.4 cm^2 ($n = 43$). Combining the acetylene reduction data with the survey data in order to estimate a value for regional hypolithic N fixation capacity, we obtain an 'instantaneous' value of 5.1 mmol N h^{-1}. The total annual photosynthetically active period for McMurdo Station (some 40 km N-E of the Miers Valley) has been calculated at approximately 2690 h (Frederick and Liao, 2005). We note that this is likely to be a minimum value, since there is evidence that metabolic activity in cold-adapted organisms extends well below freezing point (Bakermans *et al.*, 2003). We

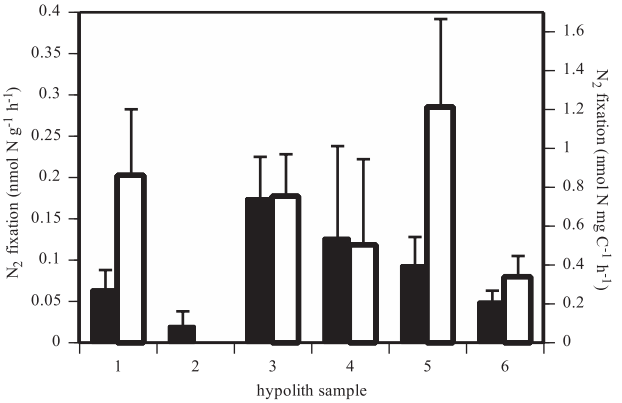

Fig. 3. Specific nitrogen fixation rates calculated from acetylene reduction data. Twelve samples were assayed, with only six showing activity and reported in this figure. No organic carbon measurement was obtained for sample 2, and therefore no normalized carbon rate is reported. Nitrogenase activity was measured using the acetylene (C_2H_2) reduction method, as described by Capone (1993). Multiple hypolithic samples were fragmented and placed into duplicate or triplicate 27 ml serum vials. Vials were sealed immediately, injected with 2 ml of C_2H_2 and incubated at approximately 15°C and 0.4% incident light, in order to mimic *in situ* austral summer conditions. The increase in ethylene (C_2H_4) was monitored in the field over a 24 h period using a gas chromatograph fitted with a flame ionization detector. The rate of increase of C_2H_4 was converted to a N_2 fixation rate by dividing by a C_2H_4:N_2 ratio of 3:1, then multiplying by 2 nitrogen atoms per N_2. Data were expressed as nmol N g^{-1} h^{-1}. Specific rates [nmol N (g C)$^{-1}$ h^{-1}] were calculated by including organic carbon content values. Following measurement of N fixation rates, hypolith samples were retained for organic carbon analysis. Fractions (2–6 g) were ground in a Retsch MM 2000 ball mill to homogenize and reduce the particle size for efficient removal of soil carbonates. An acid digestion method (Midwood and Boutton, 1998) was used to remove soil carbonates. The acid washed soils were dried at 60°C to constant weight, then re-ground in the bead mill. Samples were then weighed into combustible foil sample packages for subsequent analysis. Per cent organic carbon and nitrogen of 0.250 ± 0.002 g samples was determined using a TruSpec Carbon/Nitrogen determinator (LECO Corp., St Joseph, MI, USA) at the Stable Isotope Laboratory, University of Waikato, Hamilton, NZ.

therefore calculate that the minimum annual N input from hypolithic communities to the three valley system investigated is approximately 14 200 mmol N (0.38 kg N).

This result is surprising, given that it has been widely accepted that Dry Valley lake and stream systems are the principal sources of local N input. While the availability of experimental data is very limited, the annual contribution from Lake Hoare in Taylor Valley was estimated to be 0.37 kg N year^{-1} (Moorhead *et al.*, 1999). Since not all of the Antarctic Dry Valleys contain lakes, we suggest that hypolithic communities may be the only significant organic nitrogen contributor to lake-free valleys, and are probably important contributors of fixed nitrogen even in lake-containing valleys.

In conclusion, we have demonstrated that cryptic microbial communities play a much more important role in nutrient cycling in Antarctic desert soils than previously suspected. These results also offer intriguing opportunities for more detailed analysis of the functional behaviour and adaptive strategies of organisms in these highly specialized niches.

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Table 1. Nitrogen fixation rates from other studies of cryptic microbial communities.

Location	Sample type	Acetylene reduction (nmol N cm^{-2} h^{-1})	Study
Miers Valley, Antarctica	Hypolith	0.026–0.23	This study
Gurbantunggut Desert, China	Soil crust	0.028–0.65	Wu *et al.* (2009)
Great Basin Desert, Utah	Soil crust	< 0.033–8.7	Belnap (1996)
Chihuahuan Desert, New Mexico	Soil crust	0.00067	Hartley and Schlesinger (2002)
Negev Desert, Israel	Soil crust	34–61	Zaady *et al.* (1998)
Antarctica (various)	Endolith	Activity in one of 29 samples	Friedmann and Kibler (1980)
Hot deserts (various)	Endolith	No activity	Friedmann and Kibler (1980)
Tropical montane forest, Hawaii	Bryophyte	0.033–0.19	Matzek and Vitousek (2003)
	Lichen	0.10–2.0	Matzek and Vitousek (2003)
	Wood decay	0.054–0.63	Matzek and Vitousek (2003)

Where rates were reported in terms of C_2H_4 production, a conversion factor of 3:1 was used to convert values to N_2 equivalents, multiplied by 2 to yield molar N production.

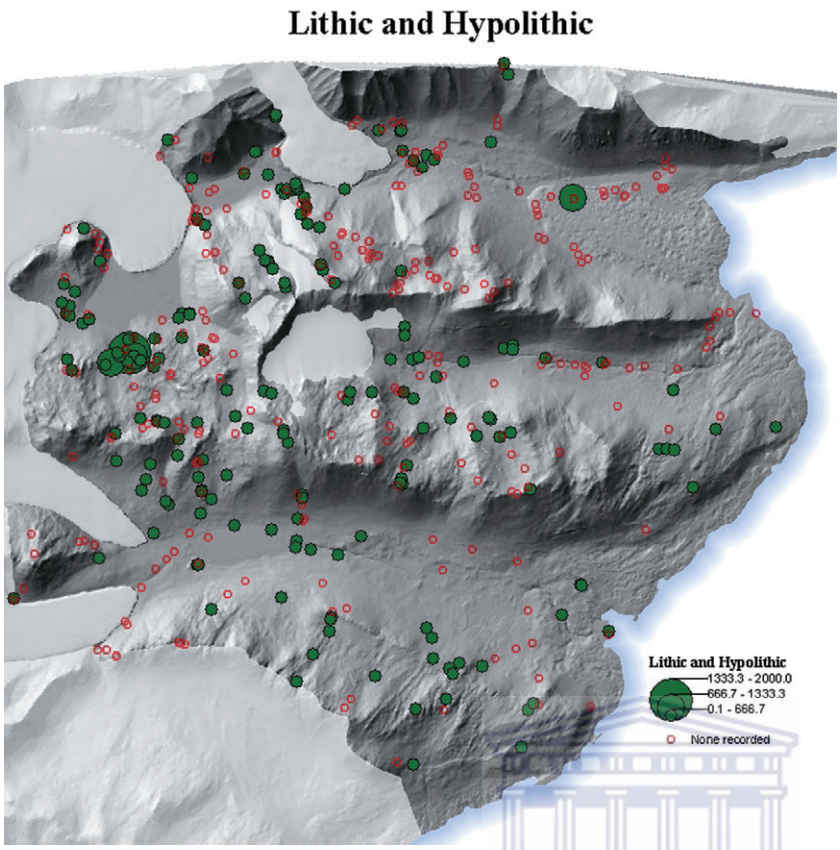

Fig. 4. Survey data of hypolith distribution across the Miers, Marshall and Garwood Valleys, Eastern Antarctica. Hypolith distribution data were recorded as part of a regional survey of surface biology, performed under the auspices of the Waikato University (NZ) FRST project ('Understanding, valuing and protecting Antarctica's unique terrestrial ecosystems: Predicting biocomplexity in Dry Valley ecosystems'). The presence and coverage of hypoliths was recorded in 20 m × 2 m transects at 466 sites, distributed across the Miers, Marshall and Garwood Valleys of the McMurdo Dry Valleys region. Coverage was estimated in units of 10 cm × 10 cm from each transect.

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ANTARCTIC HYPOLITHIC COMMUNITIES – MODEL SYSTEMS FOR A CRYPTIC ASTROBIOLOGICAL LIFESTYLE

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ABSTRACT

Microbial communities found on the underside of translucent quartz pebbles are termed "hypoliths". Such communities are common in both hot and cold deserts (where suitable mineral substrates are present), and represent specialised refuge habitats in otherwise biologically depauperate systems. In the Eastern Antarctic McMurdo Dry Valley cold deserts, hypoliths represent the dominant soil communities. The Dry Valley soil microenvironment is thought to represent one of the most extreme habitats on Earth. Extreme elements of the soil surface microenvironment include extreme cold (mean annual temperature approx. -20°C), low water availability and water activity, very low atmospheric humidity, extreme oligotrophy, high seasonal incident UV radiation, long periods of low or zero PAR and physical instability.Extensive studies of the hypolithic habitat suggest that the presence of an overlying mineral substrate mediates some of the critical microenvironmental factors limiting biological survival. The translucent quartz pebbles effectively allow the passage of light to a depth (typically 1 – 4 cm) where minimum conditions for survival of phototrophic bacteria (principally cyanobacteria) are satisfied – as compared to the soil surface where conditions are too extreme for cyanobacterial existence. Cyanobacterial phototrophy effectively supports the development and maintenance of complex biological communities, supporting a wide range of bacteria and lower eukaryotes. These isolated communities constitute simple and sustainable trophic structures capable of supporting populations of lower eukaryotes (grazers) and showing evidence of elemental cycling. Astrobiological interest in hypoliths derives from the fact that such biological communities are cryptic (i.e., the underlying biological structures are undetectable by direct sensing) and because hypoliths represent a mechanism for supporting simple biological communities under conditions which are otherwise inimical to life.

INTRODUCTION

The coastal Miers Valley (78°06'S, 163°44'-164°12'E) in Eastern Antarctica is one of a number of ice-free valleys that together constitute the McMurdo Dry Valley region. The Miers Valley, located at the southern end of the region, is a typical glacially carved valley, approximately 25 km long and 1.2 - 2.5 km wide. The 20 m deep, permanently ice-covered Lake Miers situated on the valley floor is fed during the summer months by the melt-streams from two glaciers situated at the western end of the valley: the Miers Glacier on the northern side and the Adams Glacier on the southern side of the valley (Fig. 1) [1, 2].

While no long-term temperature data exist for the Miers Valley, the mean annual air temperature of the coastal Dry Valleys is approximately -18ºC [3]. During summer, maximum temperatures are around 0ºC, with soil temperatures exceeding 14ºC for short periods of time. Surface soil temperatures during winter fall to as low as -40ºC. Snow is the sole form of precipitation, most of which sublimes quickly in the desiccating atmosphere. The region receives four months of continuous sunlight during summer and four months of complete darkness during winter, with each phase followed by transition twilight periods.

The extreme environmental conditions experienced in the Miers Valley are thought to pose a challenge to the establishment and development of widespread biological communities. However, discrete hypolithic communities are present in the Miers Valley, confined to the undersides of translucent quartz pebbles that occur in the desert pavement found in this Dry Valley (Fig. 1) [4, 5]. The

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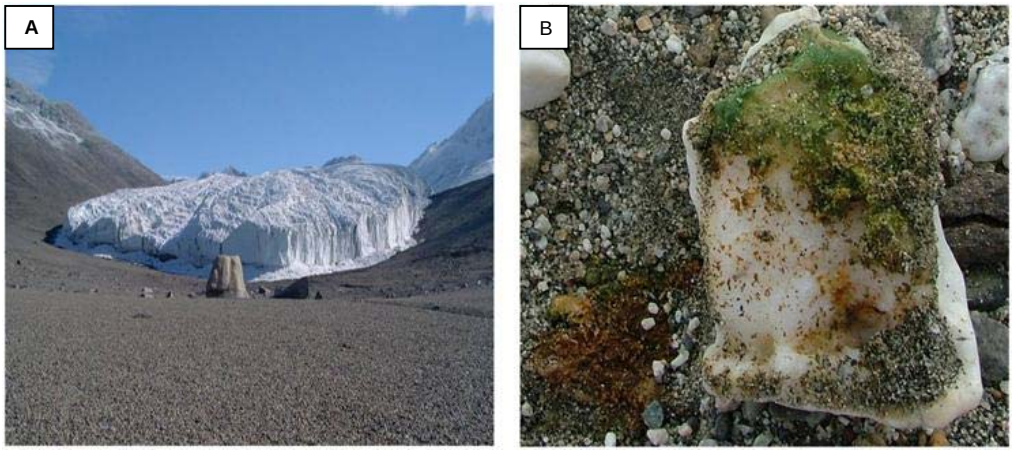

Fig. 1: (A) Typical "pavement structure" the Miers Valley with (B) hypolithon associations on the underside of translucent quartz rocks characteristically embedded in the mineralized soil.

determination of temperature and humidity regimes in hypolithic and control environments may indicate whether these physical parameters are major drivers in the establishment of hypolithic communities. Hypolithic communities in the Miers Valley are dominated by photosynthetic organisms that develop exclusively beneath translucent quartz rocks [3]. Recent studies have demonstrated that hypolithic communities may be very important elements of regional carbon loads [6] and may play a key role in N inputs in Antarctic terrestrial systems [7]

MATERIAL AND METHODS

Temperature and Relative humidity (R_H)

In situ temperature and R_H of quartz rocks, and soil were obtained using the Thermochron/Hygrochron iButtons (model DS1921G, Embedded Data Systems).

Incident and transmitted light through colonised quartz rocks were measured using a LI-COR quantum sensor (LI-190SA) mounted on the levelling fixture (LI-COR 2003S).

RESULTS AND DISCUSSION

Temperature measurements

Temperature measurements were automatically recorded by microsensors at 4-hour intervals from February to December (2006).

During the dark winter season, extremely cold but comparable temperatures were detected in both hypolithic rocks and on the soil surface. Differences between hypolithic and soil surface temperatures became greater with increasing hours of sunlight (Fig. 2). Although there was no significant difference in average annual soil temperatures, there was a significant difference in temperature variance over the same period ($P<0.05$).

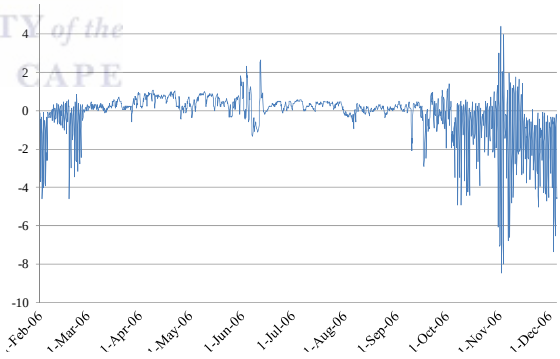

Fig. 2: *In situ* temperature differences between hypolithic and open soil 11 month period. January to December 2006.

The greatest fluctuations in temperature (over short periods of time) took place from October to February (the austral summer period), the period of maximum solar impact. As expected, the soil surface experienced much wider thermal fluctuations (up to 18ºC) than the hypolithic environment, which was buffered from the direct effect of solar irradiation by the presence of the overlying rock.

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Daily temperatures reached maximum values in the mid-afternoon and late evening when the south-facing sampling site in the Miers Valley, which lies on a 7° slope, was subjected to more direct sunlight. The temperature decreased to a minimum in the early hours of the morning when the sun was lower on the horizon and the south-facing slopes of the Miers Valley were shadowed. Sunlight and the angle of the incident irradiation are therefore important in determining the temperature regime of such environments. The temperature regimes in hypolithic, non-translucent rock and sub-soil environments are comparable with no significant difference. This indicates that the buffering of temperature fluctuations experienced under hypolithic rocks is similar to the buffering effect under non-translucent rocks and at soil 3 cm in depth.

Interestingly, and in contrast to previous work by Schlesinger *et al*. (2003) in hot deserts, we did not demonstrate the presence of a 'greenhouse' effect in the hypolithic microenvironment.

Relative Humidity (RH)

Relative humidity can be viewed as an indicator of potential water availability to biological communities. Taking into consideration the absence of rain in the Miers Valley, the high RH values recorded are, at first sight, surprisingly high.

As expected, RH values followed an inverse pattern to the observed temperature changes. RH increased over the winter season and decreases rapidly during the summer, being consistently higher in the hypolithic environment than in the soil surface ($P<0.05$) throughout the annual cycle. As for temperature, the soil surface experienced wider RH fluctuations (up to 80%) during the austral summer than did the hypolithic habitat (Figure 3).

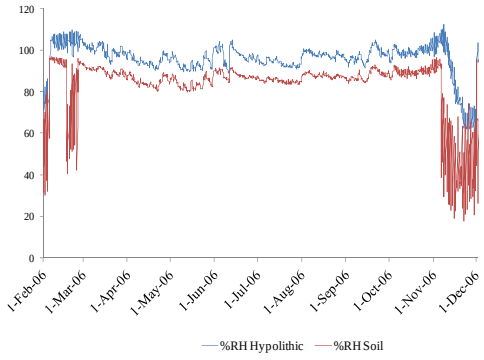

Fig. 3: Average *in situ* %RH profiles over 11 months (Miers Valley, Eastern Antarctica, February to December 2006).

Fluctuations in RH showed a similar trend to that of the temperature regime. Thus, the difference in RH remained stable over the winter months, with less than 10% RH difference between hypolithic environments and the soil surface, and increased during the austral summer becoming statistically significant in early November ($P<0.05$). As a result, hypolithic communities experience higher atmospheric humidities during the austral summer. Given that this period is the 'window of metabolic activity', this may be a significant driver of biological activity and community development.

Statistical analysis showed that the highest RH values occurs beneath non-translucent rocks, while the lowest occurs on the soil surface (Table 1). However, the 'hypolithic' zome of a non-translucent rock is incapable of supporting a phototropic community. There was a significant difference in mean among hypolithic and non-translucent rock and among hypolithic and the soil surface (both, $P<0.05$). Furthermore, a significant difference in fluctuation values was observed between all sites ($P<0.05$). This was particularly evident for the soil surface, where RH fluctuated almost five times as much as the hypolithic environment (Table 1).

Although diurnal fluctuations in RH are evident for all the environments tested, these changes are most prominent at the soil surface. Therefore, in terms of greater RH and lower fluctuation values, the hypolithic environment offers a more suitable environment for biological survival and function than the soil surface. However, these factors were similar in the sub-soil environment suggesting that RH alone may not be the limiting factor in the development of terrestrial biological communities in the Miers Valley, but that the combination of PAR (transmitted through the translucent rock), the higher RH of the hypolithic environment and the greater stability of the RH level may together drive community development.

Table 1: Analysis of temperature over a 7 day period in hypolithic and control environments.

	HP	**SbS**	**NTR**	**SS**
Average	67.15	68.98	100	55.97
StdDev[a]	6.02	5.61	4.87	13.28
Var(P)[b]	36.28	31.41	23.71	176.38

[a] StdDev = Standard deviation
[b] Var(P) = Variance
HP, hypolithic; SbS, sub-soil environment; NTR, Non-translucent rock environment; SS, soil surface environment

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Light transmittance

The hypolithic communities in the Miers Valley are exclusively found under translucent quartz rocks, supporting the concept that light is a critical driver of community, but that other conditions must be satisfied for such communities to survive. [8, 9].

Our results showed that transmission of incident light through hypolithic quartz rocks was low (average 2.3% of incident PAR) (Figure 4) and that the overlying mineral substantially collimates shorter wavelength radiation.

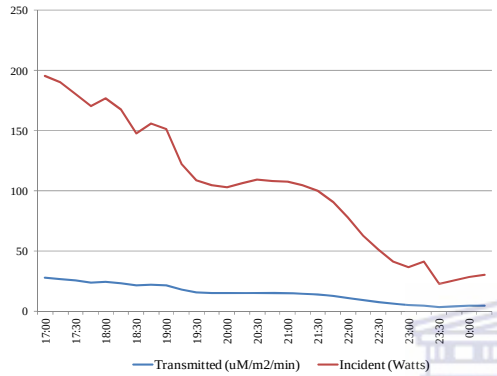

Fig.4: Spectral scan of transmitted and incident light.

The effect of radiation on biological systems depends on the wavelength, the type of radiation and the ability of the microorganisms to adapt [ref]. $UV_{A/B}$ radiation (280 nm to 400 nm) is particularly harmful to cellular processes. Therefore, a reduction in the transmittance of short wavelength UV-radiation in hypolithic environments is advantageous to the survival and stability photosynthetic communities. Furthermore, prolonged high light intensities experienced on the soil surface during the Antarctic austral summer may lead to a continuous and possibly damaging state of photosystem saturation in cyanobacteria, leading to reduce net photosynthetic activity [10]. Proteins involved in photosynthesis can also be sensitive to high irradiation levels. For example, the D1 protein found in photosystem II (PSII) reaction centres is particularly sensitive to high levels of irradiation. When damaged or denatured, D1 polypeptide is removed from the PSII reaction centre and replaced with functioning D1 protein [11]. At high light intensities the rate of damage to the photosystem reaction centres exceeds the rate of repair decreasing photosynthesis performance [12, 13]. Therefore, hypolithic environments, with lower light intensities, would provide a protected and more suitable environment for photosynthesis during the austral summer.

Considered together, these results suggest that a combination of abiotic factors play a major role in the development of hypolithic communities. While the light availability is low, it clearly exceeds the minimum flux required for photosynthesis [10]. Additionally, the physical stability of the hypolithic microenvironment (as compared to the surface environment), together with the increased water availability, may be critical factors in reducing thermodynamic stress and facilitating the development and survival of stable microbial communities.

ACKNOWLEDGEMENTS

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Evidence of species recruitment and development of hot desert hypolithic communities

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Summary

Hypoliths, photosynthetic microbial assemblages found underneath translucent rocks, are widely distributed within the western region of the Namib Desert and other similar environments. Terminal restriction fragment length polymorphism (T-RFLP) analysis was used to assess the bacterial community structure of hypoliths and surrounding soil (below and adjacent to the hypolithic rock) at a fine scale (10 m radius). Multivariate analysis of T-RFs showed that hypolithic and soil communities were structurally distinct. T-RFLP-derived operational taxonomic units were linked to 16S rRNA gene clone libraries. Applying the ecological concept of 'indicator species', six and nine indicator lineages were identified for hypoliths and soil, respectively. Hypolithic communities were dominated by cyanobacteria affiliated to Pleurocapsales, whereas actinobacteria were prevalent in the soil. These results are consistent with the concept of species sorting and suggest that the bottom of the quartz rocks provides conditions suitable for the development of discrete and demonstrably different microbial assemblages. However, we found strong evidence for neutral assembly processes, as almost 90% of the taxa present in the hypoliths were also detected in the soil. These results suggest that hypolithons do not develop independently from microbial communities found in the surrounding soil, but selectively recruit from local populations.

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Introduction

The Namib Desert in South West Africa is considered to be the world's most ancient desert and has substantially varied ecotopes including gravel plains, dunes, inselbergs, escarpments and playas (Eckardt and Drake, 2011). This desert spans a longitudinal distance of over 200 km, stretching from the western coastline to the eastern mountains along the Tropic of Capricorn. The Namib has been classified as an arid zone with some regions demonstrating hyperarid characteristics (Eckardt *et al*., 2012). The desert surface is subject to wide temperature fluctuations (from 0°C to as high as 50°C) with a general increase from the coast inland. Rainfall patterns within this desert are scant and erratic, with long periods of aridity (Eckardt *et al*., 2012).

The undersides of rocks in climatically extreme deserts, such as the Namib, act as a refuge for microorganisms (defined as 'hypoliths') and their community (the 'hypolithon') (Chan *et al*., 2012; Pointing and Belnap, 2012). The overlying rock creates a favourable sub-lithic microhabitat where microorganisms benefit from greater physical stability, desiccation buffering, increased water availability and protection from UV fluxes (Pointing *et al*., 2009; Cowan *et al*., 2010). As they are typically dominated by primary producers (Cockell and Stokes, 2004; Wood *et al*., 2008) hypolithic communities are thought to be significant contributors to regional carbon and nitrogen inputs (Burkins *et al*., 2001; Cowan *et al*., 2011).

Previous studies have suggested that hypolithons develop independently from surrounding soil communities (Warren-Rhodes *et al*., 2006; Pointing *et al*., 2007; Davila *et al*., 2008; Tracy *et al*., 2010). However, data on the mechanisms of community assembly leading to site-to-site variations (beta diversity) in community composition in deserts remain scant. Recently, Caruso and colleagues (2011) reported that deterministic and stochastic processes interact in the assembly of hypolithons on a global scale. However, the drivers of bacterial beta diversity are known to depend on both spatial (Martiny *et al*., 2011) and temporal scales (Langenheder *et al*., 2012; Lindström and Langenheder, 2012). For example, dispersal limitation was found to drive Nitrosomondales beta diversity at the

scale of an individual marsh (Martiny *et al.*, 2011). In direct contrast, the environment was the most important factor in explaining differences between these communities across regional and continental scales (Martiny *et al.*, 2011). These differences highlight the need to identify the patterns and mechanisms that shape bacterial community composition in different habitat types and at different spatial scales.

Here, we apply the ecological concept of 'indicator species' (Dufrene and Legendre, 1997) to interrogate the process behind hypolithic community assembly at a microscale (10 m radius), and present strong evidence that in the Namib Desert recruitment from soil sources supports hypolithic community assembly. We predict that if deterministic processes are strong, hypoliths and surrounding soil should demonstrate greatly dissimilar bacterial communities (specialists). If the effect of the environment is limited, both hypolith and surrounding soil should contain similar bacterial communities (generalists).

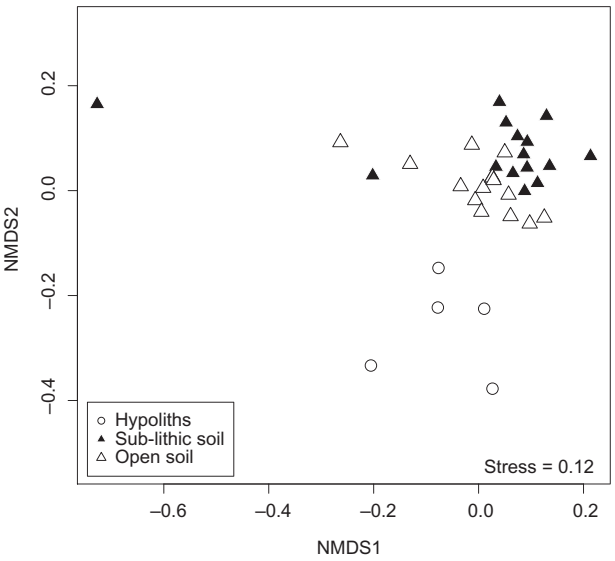

Fig. 1. NMDS ordination plot (Bray–Curtis distance matrix) of T-RFLP profiles for soil- and hypolith-derived samples. The quality of the ordination is indicated by a low-stress value.

Results and discussion

The comparative bacterial composition of hypolithic and nearby soil samples at a desert site in the hyperarid Namib Desert was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis and clone libraries (see *Supporting information* for materials and methods). A total of 98 T-RFs were obtained, ranging from 23 to 44 operational taxonomic units (OTUs) for the individual samples. When averaged for the different sample types, hypoliths and surrounding soil contained similar numbers of OTUs, with values of 22.0 [± 4.7 (SD)], 25.5[± 7.2 (SD)] and 30.3 [± 6.7 (SD)] for hypoliths, open soil and sub-lithic soil, respectively. Shifts in OTU composition (beta diversity) revealed that five OTUs were unique to the hypoliths, 10 were unique to the open soil and 29 were unique to sub-lithic soil (Fig. S1). In total, 38 OTUs (38% overlap) were shared between hypolith and soil samples.

When bacterial community patterns were visualized by non-metric multidimensional scaling (NMDS) of Bray–Curtis similarities, communities grouped separately according to their habitat (Fig. 1). Similar results were obtained after accounting for the unequal number of samples by applying a random resampling procedure (Fig. S2). When habitat type, depth and the interaction between both factors were assessed in an *adonis* model (PERMANOVA analysis), habitat was found to have a significant effect ($F_{2,28} = 4.82$, $P = 0.001$). Each group was clearly distinct (hypoliths vs. sub-lithic soil $R^2 = 0.26$, $P = 0.001$; hypoliths vs. open soil $R^2 = 0.30$, $P = 0.001$; sub-lithic vs. open soil, $R^2 = 0.08$, $P = 0.02$); that is, the overlying quartz rocks not only influenced the hypolithon but also the soil bacterial community below the rock. Although differences between hypolithic and soil bacterial community structure have been reported in polar deserts (Pointing *et al.*, 2009; Khan *et al.*, 2011), similar observations have not been reported for hot desert communities. In contrast to previous studies of microbial communities (Zhou *et al.*, 2002; Ge *et al.*, 2008), no spatial variation on vertical axes was observed, although these studies were performed on a broader scale and bacterial community patterns are known to depend on both spatial and resource factors (Zhou *et al.*, 2002; Martiny *et al.*, 2011).

In order to relate OTU abundance and habitat type, a multivariate regression tree (MRT) analysis was performed. Habitat type alone explained 10% of the variation observed. Indicator OTUs identified using the IndVal indexes were mainly responsible for the topology of the tree (Fig. 2a) suggesting that these specialist lineages represented ecological indicators of the prevailing environmental. Overall, six and nine OTUs were found to be statistically significant indicators of the hypoliths and surrounding soil respectively ($P < 0.05$) (Fig. 2b).

Clone libraries yielded a total of 85 unique, non-chimeric sequences, of which 33 and 52 clones were sequenced from hypolith and soil, respectively (Table S1). Phylogenetic analysis of the clone libraries was consistent with multivariate analysis of the T-RFLP profiles. Both F_{ST} and *P*-tests were significant (not shown), indicating a lower genetic diversity within each community than for two communities combined and that the different communities harboured distinct phylogenetic lineages (Martin, 2002). Rarefaction curves and Chao 1 estimates indicated that sampling had approached an asymptote only for hypoliths (Fig. S3). In spite of the relatively low number of clones

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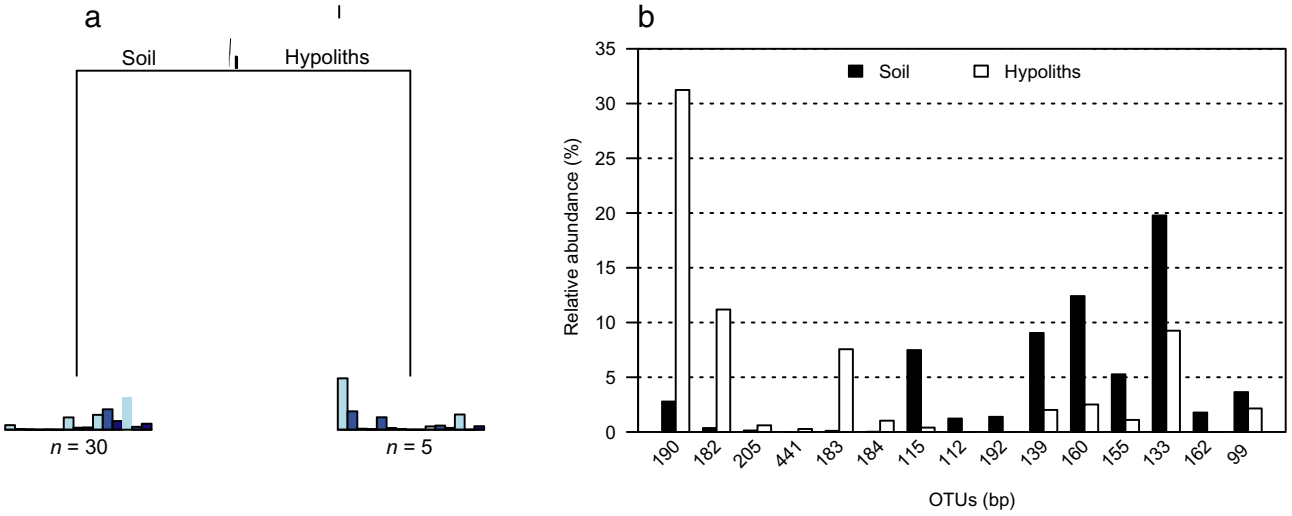

Fig. 2. Multivariate regression tree (MRT) analysis (a). The model explained 10% of the variance in the whole data set. Bar plot under each leaf (magnified in b) shows the contribution of the different indicator species. The number of samples included in the analysis is shown under bar plots.

sampled, this is not unexpected since previous studies have shown low phylogenetic diversity in hot desert ecosystems (Wong *et al*., 2010). The majority of the clones displayed homology to sequences retrieved from hot hyperarid deserts (Table S1). Nonetheless, only six OTUs showed identity values higher than 97%, indicating that the majority of sequences might represent novel taxa.

Soil samples were dominated by the phyla Actinobacteria (49%) and Proteobacteria (21%). Acidobacteria, Cyanobacteria, Bacteroidetes and Chloroflexi phylotypes were detected in lower numbers (Figs S4 and S5). Members of these phyla are generally among the most common inhabitants of soils (Fierer and Jackson, 2006; Jones *et al*., 2009; Lauber *et al*., 2009). Clones derived from hypoliths were affiliated to the phylum Cyanobacteria (85%) dominated by *Chroococcidiopsis* lineages (order Pleurocapsales), although members of the orders Oscillatoriales, Stigonematales and Chroococcales were also observed. *Chroococcidiopsis* has been identified as one of the common primary producers occurring in both hot and cold deserts (Tracy *et al*., 2010; Bahl *et al*., 2011; Caruso *et al*., 2011; Lacap *et al*., 2011). Other phyla represented in the hypolithic clone library included Acidobacteria (2.9%), Proteobacteria (2.9%), Actinobacteria (2.9%) and unclassified bacteria (3%). A total of 60 (out of 98) T-RFLP-defined OTUs were matched to 16S rRNA gene sequences resulting in an overall assignment of 61%.

We found that hypolithic and surrounding soil indicator species were identified as cyanobacteria and actinobacteria, respectively. If indicator lineages play a pivotal ecological role within the habitat (Auguet *et al*., 2010), these results support the view that cyanobacteria are among the most important functional groups in hypoliths (Cowan *et al*., 2011). Cyanobacteria are ubiquitous in most terrestrial habitats and have central ecological roles in energy transduction, nitrogen fixation and as pioneer species (Whitton and Potts, 2000).

Only five OTUs were exclusive to hypolithic samples and the most abundant OTUs were present in both soil and hypolithic samples. This is somehow consistent with neutral theory predictions (Hubbell, 2001) that assume species are ecologically equivalent. Thus, the compositions of local communities are regulated only by chance without considering deterministic factors (intra-specific competition or niche differentiation). Although these assumptions are still controversial, there is empirical evidence that both deterministic and stochastic processes shape the structure of microbial communities (Dumbrell *et al*., 2010; Ofiteru *et al*., 2010; Caruso *et al*., 2011; Langenheder and Szekely, 2011). Notably, a global-scale study of hypolithic communities found that neutral models failed to show evidence of deterministic processes when cyanobacteria and heterotrophic bacteria were analysed separately, whereas species co-occurrence was non-random when both groups were analysed together (Caruso *et al*., 2011). The global study of Caruso and colleagues identified demographic stochasticity as a major factor influencing community assembly, and here we present evidence that stochasticity also plays a pivotal role in local community assembly. Since 88% of the OTUs observed in hypolithic community samples were also found in soil it is most likely that a great proportion of taxa that 'seeded' hypolithons were recruited from the surrounding soil. It is also possible that a common source (e.g. aeolian transport) seeded both soil and hypolithic communities. In any case, under the assumptions of

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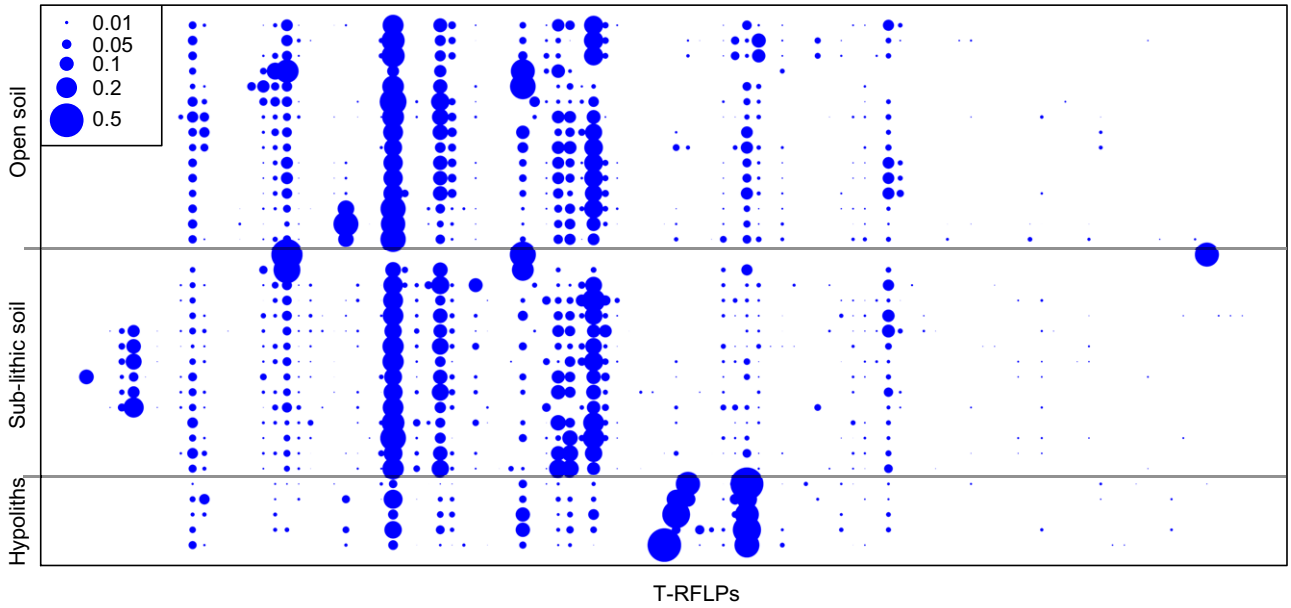

Fig. 3. Bubble plot. Each bubble represents one T-RF (columns) and is sized according to its relative abundance in the sample (rows).

neutral theory it might be expected that taxa composition and abundance should be approximately the same in hypoliths and in soil (Sloan *et al*., 2006; Ostman *et al*., 2010). As has been observed previously in rock pools seeded by rainfall water (Langenheder and Szekely, 2011) or lakes seeded by soils (Crump *et al*., 2012), we found that most abundant taxa in the soil were also present in hypoliths albeit in lower abundance (Fig. 3). Nevertheless, this was not always the case as demonstrated by the presence of indicator species (Fig. 2b). Consequently, the neutral theory failed to explain all the variation found in the bacterial community structure. In fact, cyanobacteria and actinobacteria were overrepresented in hypoliths and surrounding soil, respectively, suggesting that deterministic processes (habitat filtering) are also important.

We suggest three non-exclusive reasons for the relatively weak deterministic effect. First, it could reflect a limitation of the technique (i.e. T-RFLP), as it is well known that fingerprinting methods only target the most abundant taxa (Bent and Forney, 2008). Second, critical deterministic elements of local environmental conditions in hypoliths and surrounding soil at the Namib study site may not differ significantly (temperature and % relative humidity values are shown in Fig. S6). Finally, high dispersal rates (source-sink dynamics) (Cottenie, 2005) could buffer the effect of selection by continued homogenization of the communities involved. Indeed, there was a high degree of overlap between the soil and hypolithic communities (Figs 3 and S1). It is important to note, however, that non-neutral processes such as intra-species interactions, invariance under assemblage or the complexity of ecological interactions and the 'melting' of competitive hierarchies can generate neutral patterns (Alonso *et al*., 2006). Clearly, more focused research is required in order to explain the differences in microbial community structure between hypoliths and soil.

Metacommunity studies typically relate assembly processes to the entire community and do not take into account different categories of species. However, it has been shown for aquatic bacteria that habitat specialists and generalists have different population dynamics (Shade *et al*., 2010). Co-occurrence patterns were also found for soil microbial communities (Barberan *et al*., 2011). More important, habitat generalist and specialist have been shown to differ in their respective contributions to ecosystem functioning (Gravel *et al*., 2011).

In conclusion, the presence of generalist lineages indicates that Namib hypolithic bacterial communities did not develop independently from the surrounding soil. This is in contrast to some hyperarid Antarctic hypoliths where cyanobacteria-dominated hypolithon occurs in soils where cyanobacterial signatures were undetectable by sequence analysis of environmental clone libraries (Pointing *et al*., 2009). Similarly, in the hyperarid Atacama Desert hypoliths occur in soils devoid of recoverable cyanobacteria, although other reservoirs of cyanobacteria exist in this desert within deliquescent minerals (Davila *et al*., 2008). The significant fog moisture input to our Namib study site may be a factor affecting microbial diversity in soil reservoirs, and the extent to which aridity affects this will be a fruitful area for future work. In our study we provide empirical evidence that cyanobacteria are indicator species (specialists) for hypoliths,

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suggesting that both habitat filtering and stochastic processes shaped the assembly of hypolithic bacterial communities in the Namib. Since specialist assemblages seem to be more productive (Gravel *et al*., 2011) and more susceptible to extinction than generalists when habitat conditions are altered (Tilman *et al*., 1994), these results have implications for habitat conservation in drylands that support hypoliths. Our study suggests that future investigations of hypoliths could exploit our finding that cyanobacteria are indicator taxa and focus more closely on this component to infer ecological patterns.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Supplementary materials and methods.

Fig. S1. Venn diagram comparing the distribution of bacterial T-RFLP fragments between hypolith and soil samples.

Fig. S2. NMDS generated from random 'resampling'. This was applied in order to demonstrate that the effect of sample size does not influence the overall structure of hypolithic and soil bacterial communities. Code indicators: circles (hypolithons), filled triangles (sub-lithic soil) and empty triangles (open soil). Twelve randomizations are depicted.

Fig. S3. Hypolithic (A) and soil (B) clone library coverage (Good's and CACE) and estimator (Schao1 and SACE) plots.

Fig. S4. Clone libraries distribution for both hypoliths and soil communities.

Fig. S5. Maximum likelihood tree of eubacteria (A) and cyanobacteria (B). Phylotypes recovered during this study are shown in bold type. NCBI GenBank accession tree topologies are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1000 replications (second number). Code indicators: triangles (hypolithons), squares (soil).

Fig. S6. Temperature (A) and % relative humidity (RH) (B) over a 6 month period at the sampling location. Data were acquired at 5 min intervals.

Table S1. BLASTN results against the NCBI database.

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Supplementary Material for

Evidence of species recruitment and development of hot desert hypolithic communities

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This pdf contains:

Materials and methods

Materials and Methods

Field site, sample collection, and soil chemical analysis

The study was conducted close to the Gobabeb Training and Research Centre. Samples were collected within a 10 m radius site (S 23°32.031', E 015°01.813'). At each of the 5 discrete sampling points, one hypolith and 6 soil samples, at 0 - 10, 10 - 20 and 20 - 30 cm below the hypolith (hereafter, sub-lithic) and at 0 - 10, 10 - 20 and 20 - 30 cm one meter from the hypolith (hereafter, open soil) (see figure below), were aseptically collected. Hypolithic biomass was recovered by scraping adherent material from the rock sub-surface. Samples (5 hypolithic, 15 sub-lithic and 15 open soil), were transported to the laboratory, homogenized with a sterile spatula, transferred into 2 ml tubes and frozen at -80°C until further use.

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The annual mean rainfall at Gobabeb (from 1962 to 2010) was 25 mm (Eckardt *et al.*, 2012), and fog events, which are common in a zone from the coast to ca. 60 km inland (Eckardt *et al.*, 2012), are thought to be the dominant source of bioavailable water in the region (Budel et al., 2009).

Rocks were generally small (40-80 mm) and thin (20-60 mm), and transmission values across the visible spectrum ranged from 0.4 to 14%.

The physico-chemical properties of soil from which sampling was conducted were as follows:

Soil Type	Sand
pH (KCl)	7.0
% C	0.09
% N	0.016
Na^+ (mg/kg)	160.93
K^+(mg/kg)	164.21
Ca^+ (mg/kg)	2793.44
Mg^+ (mg/kg)	93.22

Values are presented as means of five samples.

Field measurements of micro climatic data

In situ micro-environmental data [relative humidity [(%RH) and temperature (ºC)] were recorded, using Thermochron/Hygrochron iButtons (model DS1923, Embedded Data Systems). iButtons were positioned beneath hypolithic quartz rocks at the soil surface. Measurements were recorded automatically every 5 min over a 6-month period at different depths of (i.e. 0 - 10, 10 - 20, and 20 - 30 cm) (see supplementary figure 6).

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DNA extraction

Metagenomic DNA was extracted from 0.5 g aliquots of hypolith and soil samples using the PowerSoil™ DNA Isolation Kit (MoBio, West Carlsbad, CA, USA) and following manufacturer's instructions. Concentrations of DNA yield were determined using a Nanodrop ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

T-RFLP analysis

Terminal restriction fragment length polymorphism (T-RFLP) analysis was conducted using 16S rRNA gene primers 341F-FAM (5′-CCTACGGGAGGCAGCAG-3′; tetrahydrochloro 6-carboxyfluorescein) (Ishii and Fukui, 2001) and 908 R (5'-CCGTCAATTCCTTTRAGTTT -3' (Lane et al., 1985). PCR reactions were carried out in a Thermo Hybrid (Ashford, GB) in a standard 50 µl reaction containing 1 X PCR buffer [(10 X being 200 mM Tris pH 8.8, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1 % (w/v) Triton X-100)], 0.2 mM each dNTP, 0.5 µM of each primer, 0.2 U of Dream*Taq* polymerase (Fermentas, USA) and 10 ng of template DNA. Thermal cycling conditions were as follow; 5 min denaturation at 94 ºC followed by 30 cycles with denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and elongation at 72 ºC for 105 s with a final elongation at 72 ºC for 10 min. Products were purified using the NucleoSpin Kit™ (Clonetech, Japan) and digested using HaeIII (Fermentas, USA). After a second purification, electrophoretic separation of restriction fragments was conducted using an ABI3130XL (Applied Biosystems, USA). T-RFLP profiles were analyzed using Peak Scanner 1.0 (Applied Biosystems, available online (https://products.appliedbiosystems.com). True peaks and fragments of similar size were identified and binned using the software R and Perl (Abdo *et al.,* 2006).

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Statistical analyses

T-RFLP data reflecting relative OTU abundance were Hellinger-transformed (Legendre and Gallagher, 2001) and used to calculate Bray-Curtis dissimilarity matrices, which were further visualized using non-metric multidimensional scaling (NMDS). To account for unequal number of samples (5 hypoliths vs. 15 each soil type) we also performed a resampling procedure, taking 5 samples of each habitat type to achieve 100 randomly generated nMDS plots. Permutational multivariate analysis of variance (PERMANOVA), function *adonis* (vegan package for R), was performed to test for significant differences between sample groups (hypoliths, sub-lithic soil and open soil). MRT analysis (De'Ath, 2002) was used to determine correlations between bacterial community composition and habitat parameters (e.g. type and depth) (mvpart package for R). Indicator species analysis (IndVal index) (Dufrene and Legendre, 1997), which combines relative abundance and relative frequency of occurrence, was used to identify the species that were statistically significant indicators of the habitat type (labdsv package for R).

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16S rRNA gene Clone library construction

Two clone libraries were constructed using primers 341F, without FAM-labeling, and 908R (conditions as above) for pooled hypolith and surrounding soil, respectively. Purified PCR amplicons were ligated to the pGEM-T Easy Vector System® (Promega Corporation, Madison, WI, USA), and transformed into Gene Hoggs® cells. The resulting plasmid vectors were isolated and purified using the Qiagen Miniprep kit (Qiagen, Valencia, USA) following the manufacturer's instructions. For each library, 200 clones were screened using ARDRA (RsaI and AluI, Fermentas) and the de-replicated clones were sequenced at the University of Stellenbosch Sequencing Facility (South Africa). Chimeric sequences were checked using the Chimera slayer implementation in Mothur (Schloss *et al.,* 2009).

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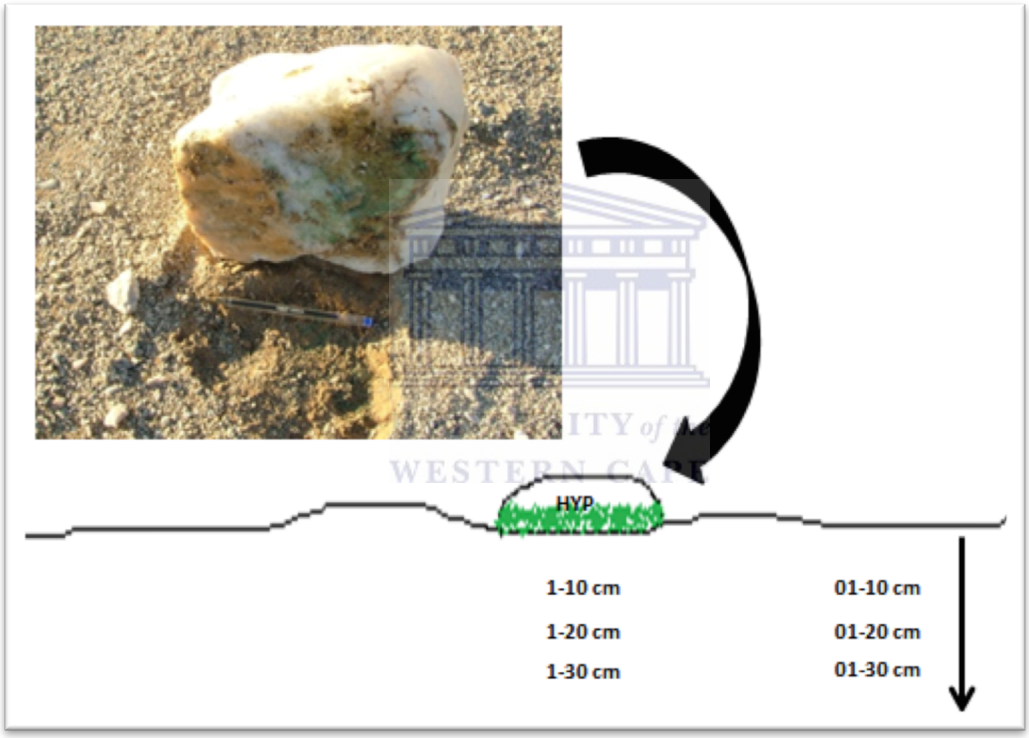
Phylogenetic analysis

Phylogenetic affiliations of representative OTUs were determined using the Classifier tool (Wang *et al.,* 2007) at a confidence interval of 80 % (Ribosomal Database Project II, http://rdp.cme.msu.edu) (Cole *et al.,* 2009). Nucleotide sequences were aligned with references from GenBank database using ClustalX v.1.8.1 (Thompson *et al.,* 1997). Maximum Likelihood trees were constructed using Paup*4.0b10 (Posada, 2003) and GARLI (Genetic Algorithm for Rapid Likelihood Inference) (Swofford, 2003) as described previously (Lacap *et al.,* 2011). Arlequin v3.0 (Excoffier and Scheider, 2005) and Unifrac (Lozupone et al., 2006) were used to assess the phylogenetic differences between communities using the F_{ST} and P tests, respectively. Phylogenetic OTUs at a similarity level of 97 % were determined using CD-HIT (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=h-cd-hit-est). Diversity estimates

(Chao1) were calculated using an online tool (http://www.aslo.org/lomethods/free/2004/0114a.html) (Kemp and Aller, 2004). *In silico* predictions of terminal restriction fragments (T-RFs) were performed using TRF-CUT (Ricke et al., 2005). Sequence data have been submitted to NCBI GenBank database (accession numbers JN714842 - JN714926). All other analyses were conducted using R (http://www.R-project.org).



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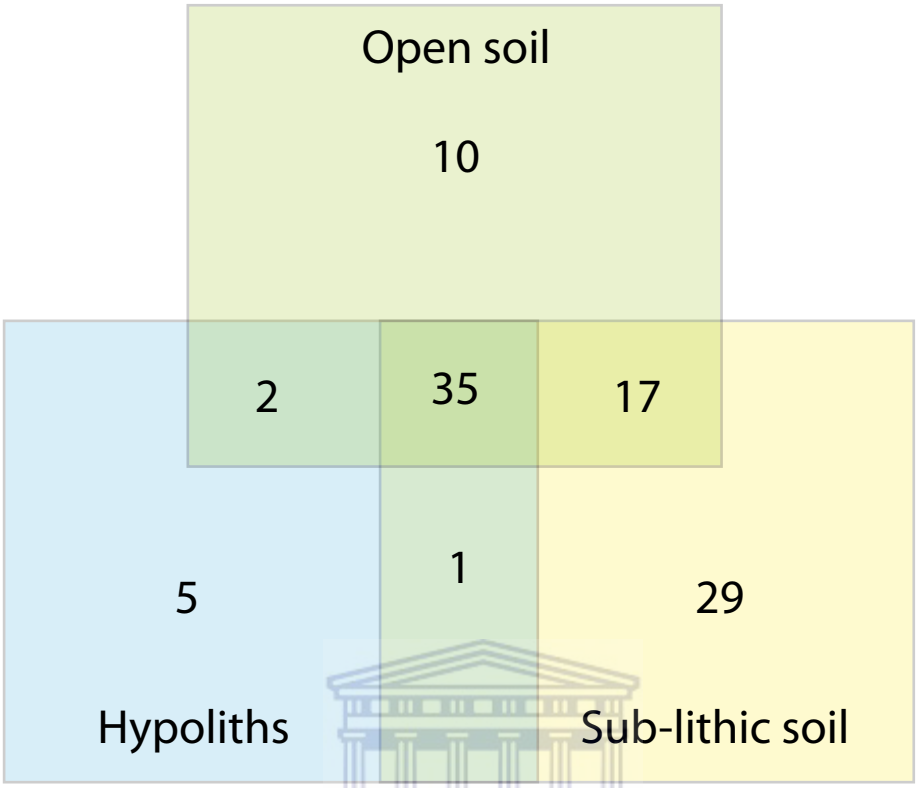
S1



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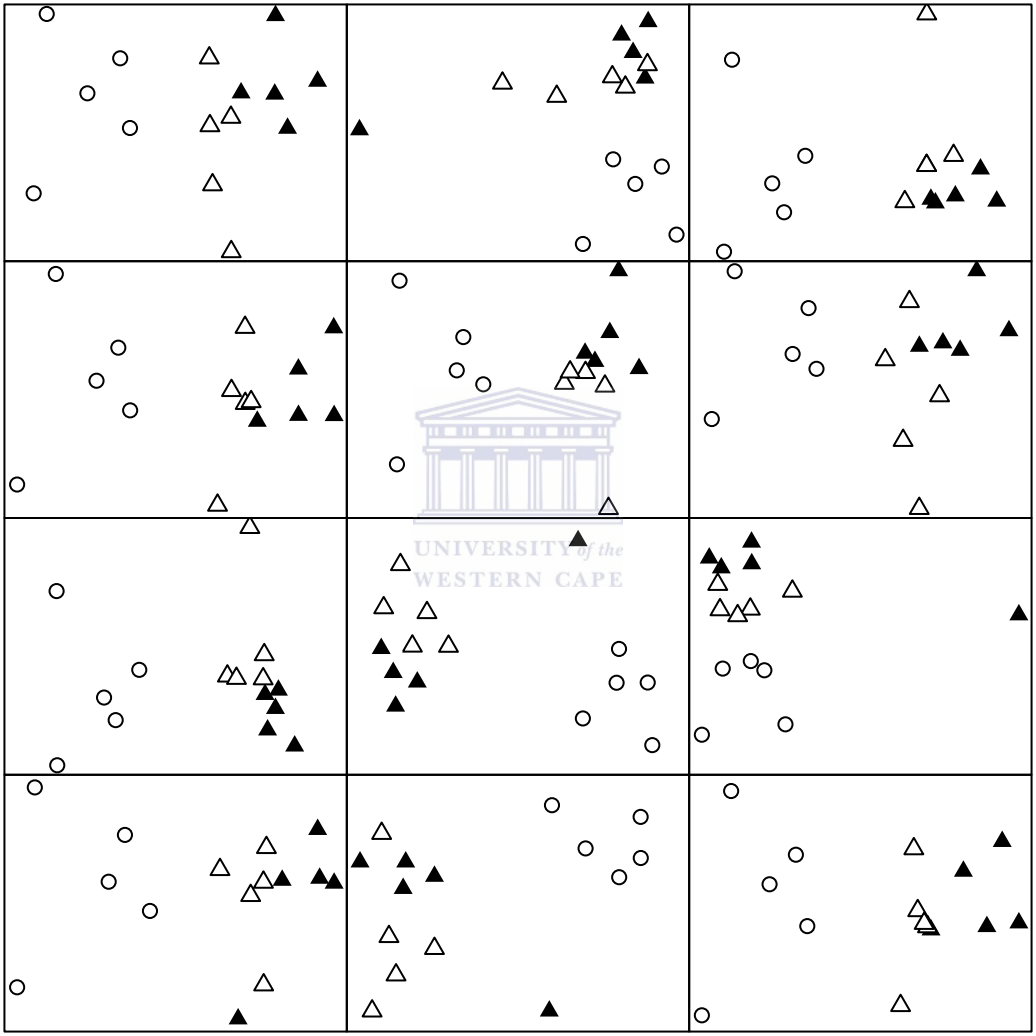
S2



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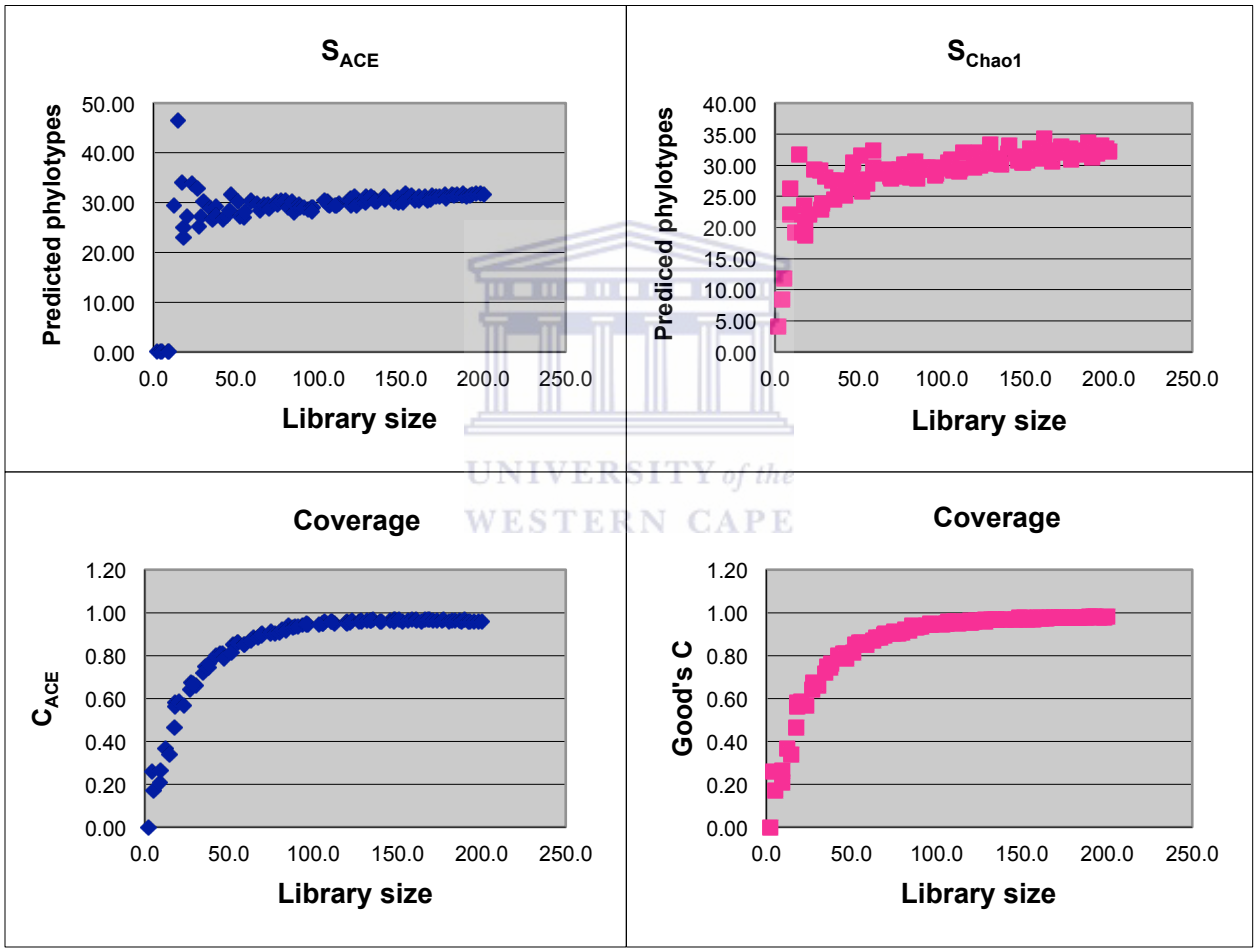
S3

A

Number of clones in library	200
Number of phylotypes observed	30
Predicted value of S_{ACE}	31.64731
Predicted value of S_{Chao1}	32.39386
Observed phylotypes / predicted S_{ACE}	0.956043
Observed phylotypes / predicted S_{Chao1}	0.924231



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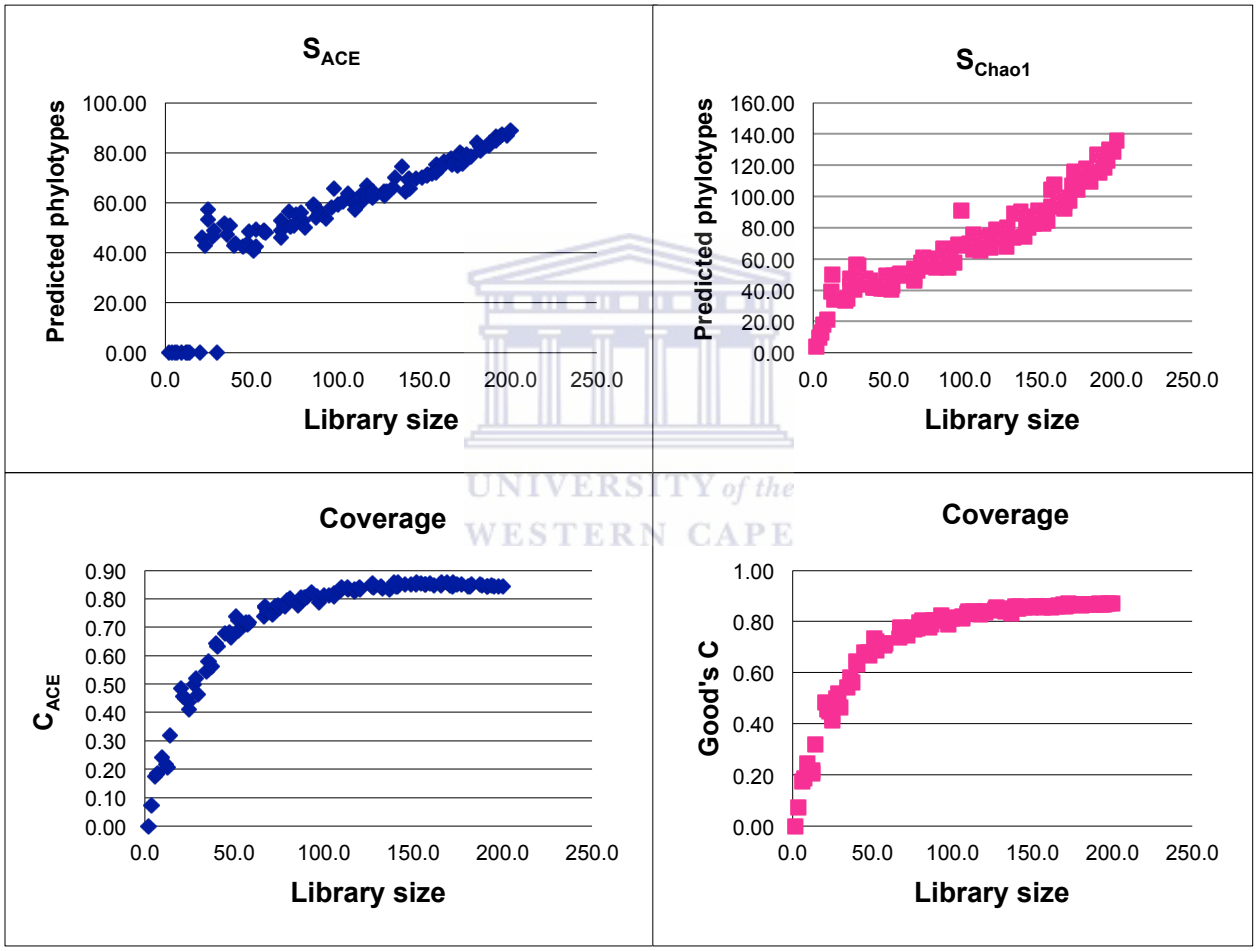
B

Number of clones in library	200
Number of phylotypes observed	54
Predicted value of S_{ACE}	86.87694
Predicted value of S_{Chao1}	127.2837
Observed phylotypes / predicted S_{ACE}	0.668097
Observed phylotypes / predicted S_{Chao1}	0.493066



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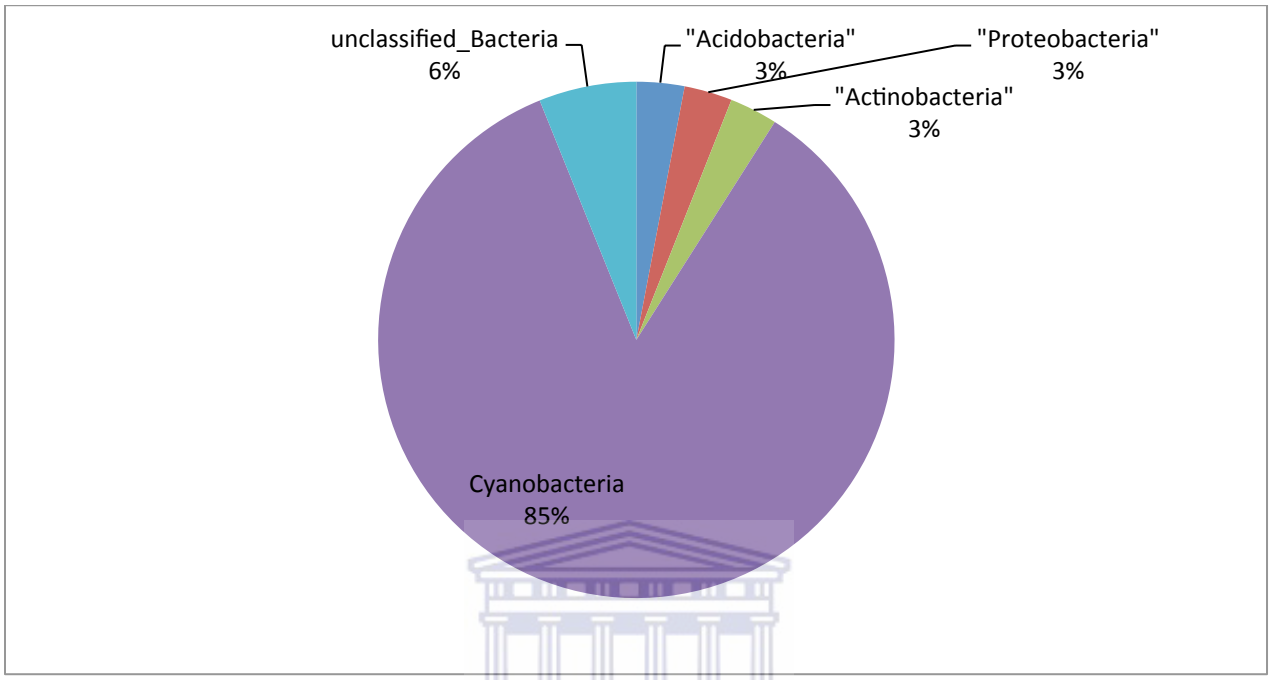
S5

A



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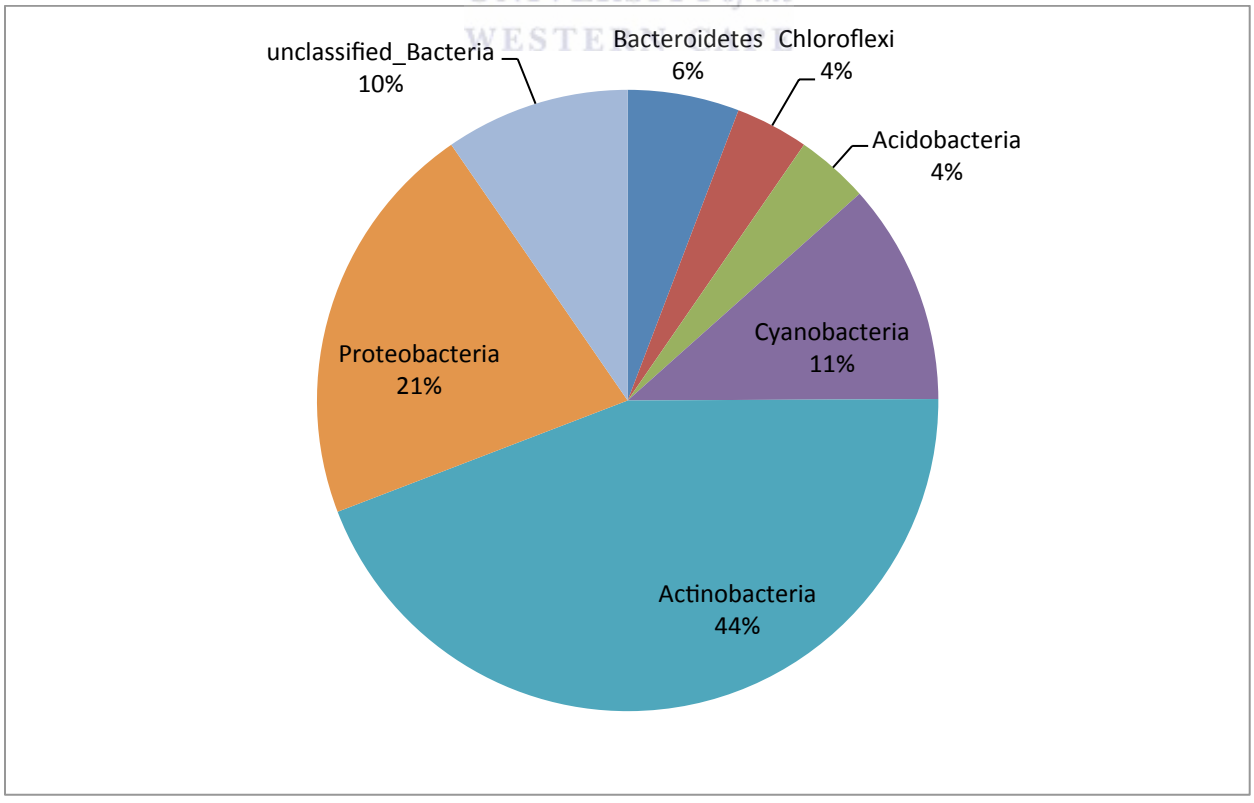
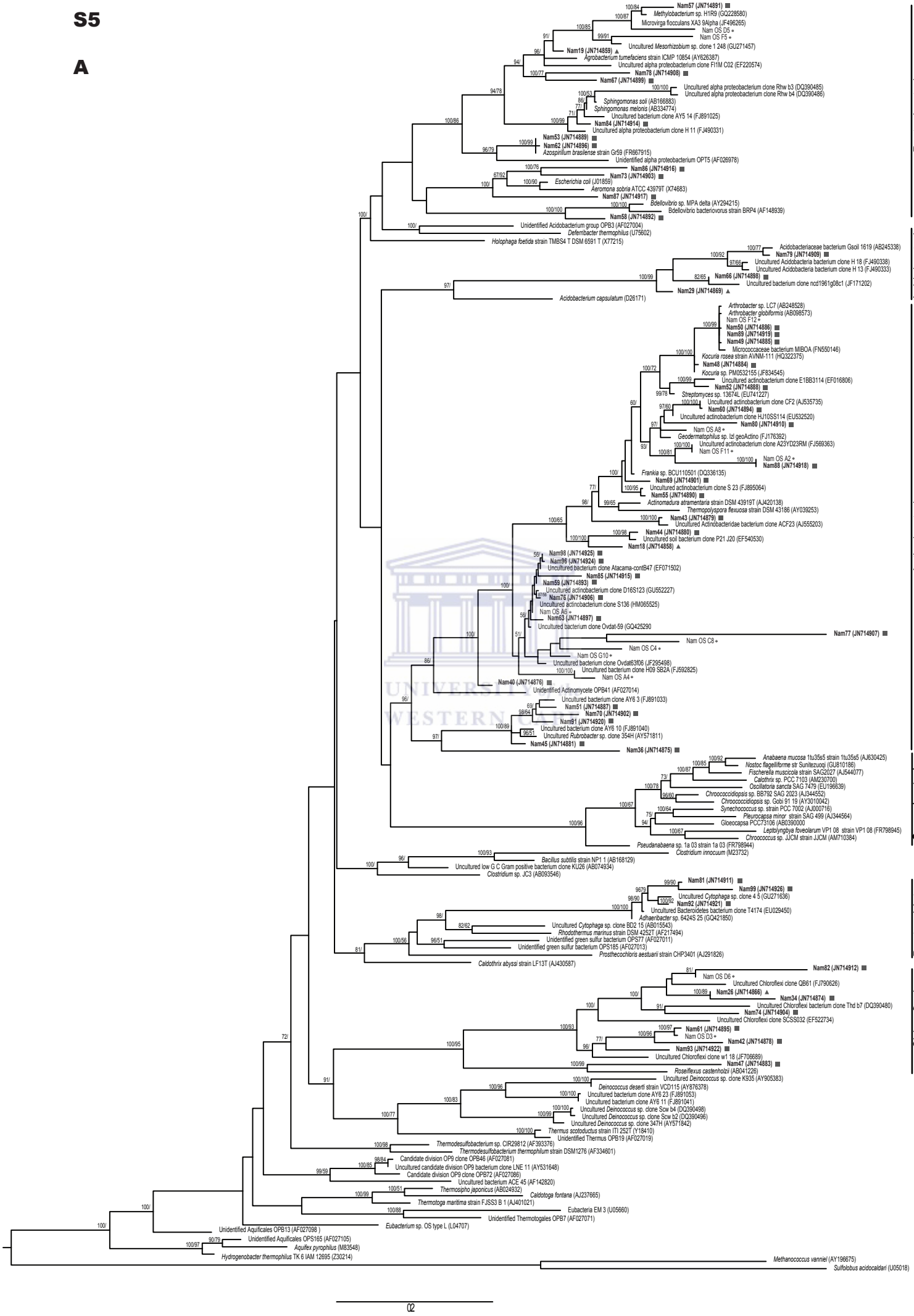
B



S5

A


Proteobacteria
Acidobacteria
Actinobacteria
Cyanobacteria
Bacteroidetes
Chloroflexi
Nam57 (JN714891)
Methylobacterium sp. H1R9 (GQ228580)
Microvirga flocculans XA3 9Alpha (JF496265)
Nam OS D5 *
Nam OS F5 *
Nam19 (JN714859) ▲
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Uncultured alpha proteobacterium clone FI1M C02 (EF220574)
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Uncultured alpha proteobacterium clone Rhw b4 (DQ390486)
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Sphingomonas melonis (AB334774)
Uncultured bacterium clone AY5 14 (FJ891025)
Nam84 (JN714914)
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Holophaga foetida strain TMBS4 T DSM 6591 T (X77215)
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Pseudanabaena sp. 1a 03 strain 1a 03 (FR798944)
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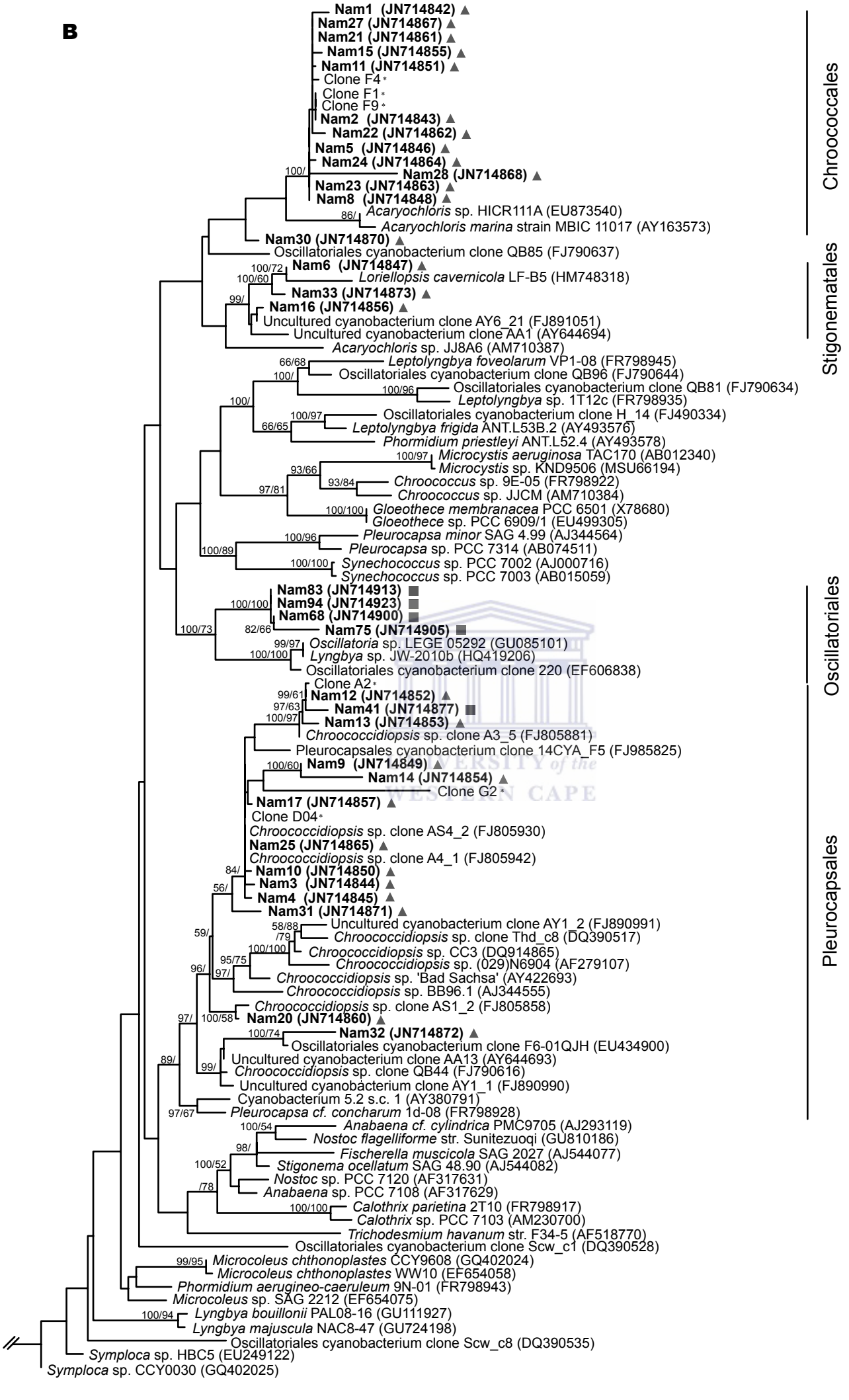
02



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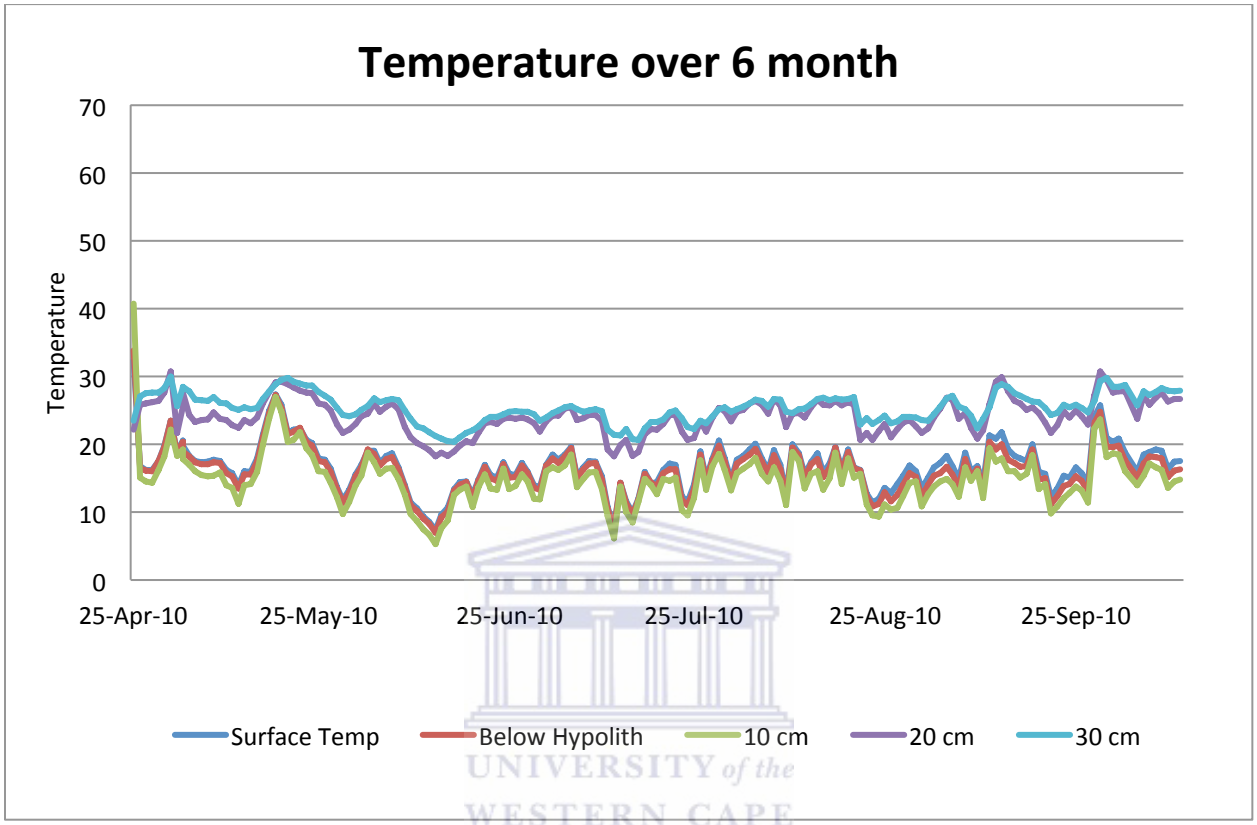
S7

A



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B

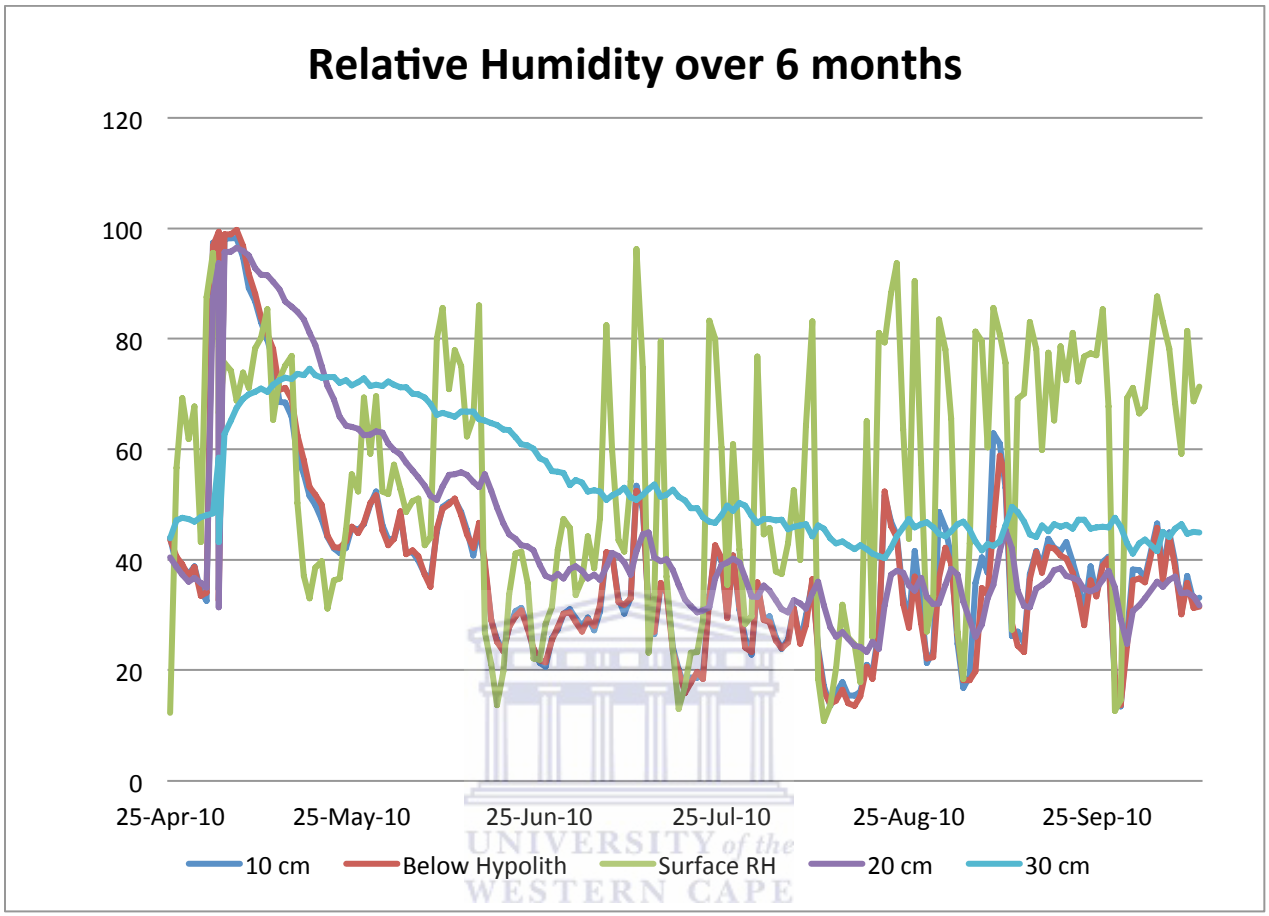

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Table S1

Seq. Identification	Accession number	Closest Homologous in GenBank	%	Origin -country	Isolation source	Source clone library
NamSP1	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP2	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP3	AF493850.1	Uncultured bacterium clone 11	99	Southern Mojave Desert	Surface of desert rocks	Hypolith
NamSP4	FJ805942.1	Uncultured Chroococcidiopsis sp. clone A4_1 16S	97	Botswana: Kalahari	Desert quartz	Hypolith
NamSP5	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP6	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Atacama Desert	Quartz	Hypolith
NamSP7	FN813975.1	Uncultured bacterium 16S rRNA gene, clone 26B1-D3	91		*Lactuca sativa* (phyllosphere)	Hypolith
NamSP8	FJ230783.1	Uncultured bacterium clone agateC2	92	Australia	Hypolithic soil	Hypolith
NamSP9	AY615380.1	Uncultured bacterium clone AP18	96	Atacama Desert	Rock	Hypolith
NamSP10	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP11	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP12	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP13	FJ230828.1	Uncultured bacterium clone Prehnite44	98	Australia	Hypolithic slime	Hypolith
NamSP14	FJ230827.1	Uncultured bacterium clone	95	Australia	Hypolithic slime	Hypolith
NamSP15	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	hypolithic slime	Hypolith
NamSP16	FJ891051.1	Uncultured cyanobacterium clone AY6_21	99	Atacama Desert	Rock	Hypolith
NamSP17	HM241076.1	Uncultured bacterium clone 211	98	Global hypolith study	Hypoliths from desert	Hypolith
NamSP18	FR849426.1	Uncultured bacterium clone B16S-XJcc-2-29	95	Xinjiang Province	Soil	Hypolith
NamSP19	JF295649.1	Uncultured bacterium clone Ovdat61c11	100	Xinjiang Province	Soil	Hypolith
NamSP20	HM241001.1	Uncultured bacterium clone 136	96	Global hypolith study	Hypoliths from desert	Hypolith
NamSP21	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
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NamSP24	FJ230783.1	Uncultured bacterium clone agateC2	96	Australia	Hypolithic slime	Hypolith

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NamSP25	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP26	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	95	China	Uncultured Chloroflexi	Hypolith
NamSP27	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP28	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP29	FJ230783.2	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP30	FJ230783.2	Uncultured bacterium clone agateC2	95	Australia	Hypolithic slime	Hypolith
NamSP31	HM241076.1	Uncultured bacterium clone 211	97	Australia	Hypolithic slime	Hypolith
NamSP32	JF173381.1	Uncultured bacterium clone ncd1994h09c1	96	china	Soil	Hypolith
NamSP33	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Yungay, Atacama Desert	Quartz	Hypolith
NamSP34	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Soil	Soil
NamSP35	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma, Kessler Farm	Undisturbed tall grass prairie, top 5 cm	Soil
NamSP36	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP37	FJ592827.1	Uncultured bacterium clone G01_SB3A	97	Atacama	Socompa Volcano, Andes	Soil
NamSP38	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP39	FR687056.1	Uncultured bacterium clone d21h4b13	95	China	Paddy soil	Soil
NamSP40	GU219537.1	Uncultured bacterium clone Obs1-15	94	Iceland	Bsidian outcrop, Valafell	Soil
NamSP41	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Soil
NamSP42	HM240933.1	Uncultured bacterium clone 068	95	Global hypolith study		Soil
NamSP43	AJ555203.1	Uncultured Actinobacterium	98	Lower Austria, Marchfeld	Agricultural soil	Soil
NamSP44	EF540530.1	Uncultured soil bacterium clone P21_J20 16S	97	Estonia	Semi-coke	Soil
NamSP45	JN037870.1	Uncultured Actinobacterium clone UHAS5.5	99	India	Saline-alkaline soil	Soil
NamSP46	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP47	DQ906857.1	Uncultured bacterium clone 10D-4	95	Oman	Subsurface soil	Soil
NamSP48	JF834545.1	Kocuria sp. PM0532155	99	India	Environmental sample	Soil
NamSP49	AB248528.2	Arthrobacter sp. LC7 gene for 16S rRNA,	99	Niigata (Japan)	Soil	Soil
NamSP50	FN550146.1	Micrococcaceae bacterium isolate MI-BOA	98	Marion Island	Soil	Soil
NamSP51	GQ425963.1	Uncultured bacterium clone Adulam-209	99	Adulam (Israel)	Soil	Soil
NamSP52	EF016806.1	Uncultured actinobacterium clone E1B-B3-11	95	Atacama Desert	Soil	Soil

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NamSP53	FR667915.1	*Azospirillum brasilense* 16S rRNA gene, strain Gr59	99	Greece	Soil	Soil
NamSP55	GU552232.1	Uncultured actinobacterium clone D-16S-130	99	Atacama Desert	Desert soil	Soil
NamSP57	JF295718.1	Uncultured bacterium clone Lehavim48d08			Soil	Soil
NamSP58	AB205958.1	Uncultured bacterium clone OS-27	97	Niigata (Japan)	Activated sludge	Soil
NamSP59	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP60	AJ535735.1	Uncultured actinobacterium clone CF2	99	Marchfeld (Austria)	Soil	Soil
NamSP61	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP62	FR667915.1	*Azospirillum brasilense* strain Gr59	99	Greece		Soil
NamSP63	HM565047.1	Uncultured Actinomycetales bacterium clone N-35	98	China	Concrete	Soil
NamSP66	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP67	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP68	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP69	DQ336134.1	Frankia sp. strain BCU110345	96	Argentina	Soil	Soil
NamSP70	FR849478.1	Uncultured bacterium clone B16S-XJrs-3-8	99	Xinjiang Province		Soil
NamSP73	JN684205.1	Uncultured bacterium clone H144	100	China	Environmental sample	Soil
NamSP74	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Environmental sample	Soil
NamSP75	FJ230801.1	Uncultured bacterium clone QuartzC15	92	Australia	Hypolithic slime	Soil
NamSP76	EU440648.1	Actinomycetales bacterium clone Plot17-A07	99			Soil
NamSP77	FR849480.1	Uncultured bacterium clone B16S-XJrs-3-61	99	China	Desert	Soil
NamSP78	HQ910327.1	Uncultured bacterium clone P-8_B22	97	Utah (USA)	Desert soil	Soil
NamSP79	FR852514.1	Uncultured bacterium clone W3-199	98	China	Red soil	Soil
NamSP80	JF295697.1	Uncultured bacterium clone Lehavim48g01	93	Israel	Soil	Soil
NamSP81	EU029450.1	Uncultured Bacteroidetes bacterium clone T4174	95	Israel	Environmental sample	Soil
NamSP82	JF707601.1	Uncultured Chloroflexi bacterium clone HKTK7-4	93	India	Desert soil	Soil
NamSP83	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP84	HM240929.1	Uncultured bacterium clone 064	98	Global hypolith study	Hypoliths from desert	Soil
NamSP85	GQ425235.1	Uncultured bacterium clone Ovdat-4	99	Ovdat (Israel)	Soil	Soil
NamSP86	HM584296.1	Acinetobacter sp. CJ-S-MA3	99	Korea	Environmental sample	Soil
NamSP87	EF651023.1	Uncultured beta proteobacterium clone AUVE_03A05	98	Australia	Cropland	Soil

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NamSP88	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma (USA)	Tall grass prairie, top 5cm	Soil
NamSP89	AB622776.1	Uncultured bacterium clone: IMCUGWBC9-1	99	China	High arsenic aquifer	Soil
NamSP91	FJ790550.1	Uncultured bacterium clone VB29	99	Tibet	Soil	Soil
NamSP92	FM209314.1	Uncultured bacterium 16S rRNA gene, clone 230	96	Israel:Negev desert	Soil	Soil
NamSP93	JF295619.1	Uncultured bacterium clone Ovdat61h02	92	Israel	Soil	Soil
NamSP94	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP96	JF706662.1	Uncultured actinobacterium clone w3-15	99	Atacama Desert	Hypolith	Soil
NamSP98	JF706662.1	Uncultured actinobacterium clone w3-15	98	Atacama Desert	hypolith	Soil
NamSP99	FM209314.1	Uncultured bacterium clone 230	91	Negev desert (Israel)	Desert	Soil

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RESEARCH ARTICLE

Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley

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Antarctica; Dry Valleys; active layer; microbial diversity; permafrost; water vapour.

Abstract

The McMurdo Dry Valleys collectively comprise the most extensive ice-free region in Antarctica and are considered one of the coldest arid environments on Earth. In low-altitude maritime-associated valleys, mineral soil profiles show distinct horizontal structuring, with a surface arid zone overlying a moist and biologically active zone generated by seasonally melted permafrost. In this study, long-term microenvironmental monitoring data show that temperature and soil humidity regimes vary in the soil horizons of north- and south-facing slopes within the Miers Valley, a maritime valley in the McMurdo Dry Valleys. We found that soil bacterial communities varied from the north to the south. The microbial assemblages at the surface and shallow subsurface depths displayed higher metabolic activity and diversity compared to the permafrost soil interface. Multivariate analysis indicated that K, C, Ca and moisture influenced the distribution and structure of microbial populations. Furthermore, because of the large % RH gradient between the frozen subsurface and the soil surface we propose that water transported to the surface as water vapour is available to microbial populations, either as a result of condensation processes or by direct adsorption from the vapour phase.

Introduction

The Antarctic continent is widely acknowledged as harbouring some of the most extreme climatic conditions on Earth (Hansom & Gordon, 1998). While the bulk of the continent is ice-covered, 0.4% of the terrestrial surface is essentially ice-free, of which the McMurdo Dry Valleys in South Victoria Land comprise the largest coherent region. The extensive mineral soils of the Dry Valleys are characterized by multiple environmental characteristics considered to be biologically extreme, including large seasonal and diurnal variations in temperature, low precipitation and atmospheric humidity (leading to extremely low surface soil water content), low nutrient availability, high levels of salinity and solar radiation (UVB) and strong katabatic winds (Boyd *et al.*, 1966; Cameron *et al.*, 1970; Claridge & Campbell, 1977; Smith *et al.*, 1992; Vishniac, 1993). Microorganisms surviving these extreme conditions are thought to have adopted a wide range of different physiological and adaptive strategies in response to these impacts (Zeglin *et al.*, 2009; Casanueva *et al.*, 2010).

While earlier culture-dependent microbiological surveys of Dry Valley soils suggested that both the biomass levels and microbial diversity were, at best, low (Horowitz *et al.*, 1972; Friedmann, 1993), more recent phylogenetic surveys have indicated otherwise. ATP titres suggested that cell numbers in mineral soils in Miers Valley (a low-altitude coastal valley in the southern McMurdo region) were in the order of 10^6–10^8 per gram (Cowan *et al.*, 2002; Cowan & Casanueva, 2007). Phylogenetic analyses from a wide range of different sites have shown that at least 14 different phyla are present, including psychrophilic and psychrotolerant heterotrophs of the *Actinobacteria*, *Acidobacteria*, *Proteobacteria* and *Bacteroidetes* groups (Elberling *et al.*, 2006; Smith *et al.*, 2006; Aislabie

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et al., 2008) and numerous genera of the photoautotrophic *Cyanobacteria* (Jungblut *et al.*, 2005; Wood *et al.*, 2008). Dry Valley soils have been shown to support relatively low levels of eukaryotic microorganisms (Cowan, 2009; Pointing *et al.*, 2009), and so bacteria are thought to represent the major biotic component in these systems.

While biomass levels, bacterial diversity and community structure in Antarctic habitats are all influenced by multiple environmental parameters (Pointing *et al.*, 2009; Yergeau *et al.*, 2009; Zeglin *et al.*, 2009; Newsham *et al.*, 2010), temperature and water availability are thought to be the most important drivers (Noy-Meir, 1973; Kennedy, 1993; Parsons *et al.*, 2004; Warren-Rhodes *et al.*, 2006; Zeglin *et al.*, 2009; Cary *et al.*, 2010). In Antarctic Dry Valley soils, water availability can vary in both time and space (Noy-Meir, 1973; Hopkins *et al.*, 2006a) as a result of temperature fluctuations and the presence of the permafrost layer. Permafrost, described as permanently frozen sediment (Gilichinsky *et al.*, 2007), is ubiquitous in all but high altitude valleys in the McMurdo region and is present as ground ice or buried ice, ice-cemented permafrost and dry-frozen permafrost (Bockheim, 2008). The Dry Valley permafrost is generally dry and overlain by the active layer, defined as the layer of ground subjected to seasonally freezing and thawing cycles (Adlam, 2010). The active layer extends from the surface to a depth of approximately 10–25 cm (Gilichinsky *et al.*, 2007) depending on the seasonal cycle, aspect, slope (Guglielmin *et al.*, 2008) and location (Campbell *et al.*, 1998) and shows a climate-dependent variation (Campbell & Claridge, 1987). The upper few centimetres of this horizon is susceptible to mobilization by storms and katabatic winds (Gilichinsky *et al.*, 2007) but is partially stabilized by the desert pavement.

Because of the predominance of sublimation processes water is present in the permafrost zone but never in liquid form (Gilichinsky *et al.*, 2007). Nevertheless, permafrost contains diverse populations of both aerobic (gram-positive and gram-negative) and anaerobic bacteria (methanogens, sulphate reducers, etc.) (Gilichinsky *et al.*, 1995; Ponder *et al.*, 2004). These communities are thought to have retained viability for very long periods (from hundreds to millions of years) (Kastovska *et al.*, 2005) and are believed to be at least as old as the permafrost and thus may represent the oldest microorganisms discovered on Earth (Gilichinsky *et al.*, 2007). Observations that microbial activity extends to well below 0 °C (Shi *et al.*, 1997; Vorobyova *et al.*, 1997; Rivkina *et al.*, 2000) suggest that permafrost communities may retain low but significant levels of metabolic activity (Willerslev *et al.*, 2004; Steven *et al.*, 2006; Gilichinsky *et al.*, 2007).

In the active zone, liquid water is present on a seasonal basis. Moist mineral soils are exposed to temperatures above 0 °C for approximately 3 months of each annual cycle, and it is assumed that the conditions necessary for metabolic activity are met. Little is known of either the microbial populations of this zone or of their metabolic activity. Furthermore, moisture movement through the active layer is affected by soil particle size which regulates soil porosity and hydraulic properties (Sauer & Logsdon, 2002). It has been recently shown that water content generally increases with active layer depth and is regulated by temperature and atmospheric relative humidity (RH) and less influenced by latitude (Seybold *et al.*, 2010).

Occasional light snow falls temporarily wet the upper few centimetres of the Dry Valley soils (Hopkins *et al.*, 2006b), but surface liquid water is very rapidly lost because of evaporative and ablation processes driven by the very low atmospheric humidity. Furthermore, in the Dry Valleys sublimation rates exceed precipitation, thus limiting the amount of water able to penetrate the ground (Fountain *et al.*, 2009). Typically, gravimetric analyses of water content in surface soils show extremely low moisture content values (Campbell *et al.*, 1994; Bockheim, 1995; Bockheim & Tarnocai, 1998; Campbell *et al.*, 1998). Recently, Newsham *et al.* (2010) reported that soil water content had only a minor effect on the bacterial community composition on maritime Antarctic soils. This conclusion is in stark contrast to hot desert soils where water availability is the dominant driver of both macro- and microbiological systems (McKay *et al.*, 2003; Warren-Rhodes *et al.*, 2006; Pointing *et al.*, 2007; Warren-Rhodes *et al.*, 2007). Other important variables influencing the diversity and composition of soil bacterial communities include soil pH (Fierer & Jackson, 2006; Männistö *et al.*, 2007; Soo *et al.*, 2009; Yergeau *et al.*, 2009; Zeglin *et al.*, 2009), salinity (Zeglin *et al.*, 2009), latitude (Yergeau *et al.*, 2009), UV impact (for surface microbial community development) (Tosi *et al.*, 2005), soluble salts, K and C (Pointing *et al.*, 2009). An altitude-related effect on development of lithic communities in close association with soil has also been observed (Cowan *et al.*, 2010).

Fine-scale geographic positioning could also affect microbial distribution and diversity. It has been reported that this aspect influences the conditions for microbial development (Wynn-Williams, 1990). Because of higher solar gain, north-facing slopes generally have a larger active layer, a longer seasonal period of thaw and fewer freeze–thaw cycles (Legget *et al.*, 1961; Rieger, 1974). Thus, the north facing slopes are typically less extreme than the south-facing slopes (Keys, 1980) and are characterized by environmental conditions that are biotically more favourable (Wise & Gressit, 1965; Wynn-Williams, 1990).

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Together, these findings suggest that microbial diversity and function in Antarctic Dry Valley soils is dictated by a wide range of abiotic factors, almost certainly in combination. To understand the abiotic 'drivers' of the biotic system, one valuable approach has been to select model communities where it is possible to isolate one or more of these drivers (Cary *et al.*, 2010). A study of soils in shallow-depth transects offers one such system, where clearly defined horizons are subject to quantifiable and distinct regimes of temperature, water availability and chemical properties. Here we have used a range of molecular techniques to analyze the microbial diversity of soil samples collected from shallow-soil-depth transects in a coastal Dry Valley. The use of samples from two sites of different aspect (south and north facing slope) potentially allows us to observe the effects of fine-scale variables. Furthermore, to gain insight into the abiotic drivers influencing and controlling community diversity, structure and composition, microbial profiles have been linked to medium-term microenvironmental variables and soil chemical properties.

Materials and methods

Sample collection

Soil samples (approximately 50 g) were collected from shallow trenches excavated from a north- and a south-facing-site on the lower slopes of Miers Valley during 2009 austral summer (see Table 1 for details of the sampling points). Single soil core samples were recovered aseptically from the surface (0–2 cm) and at depths of 2–5, 5–10, 10–15 and 15–20 cm (south-facing) and from the surface (0–2 cm) and at depths of 2–5, 5–10, 10–15, 15–20, 20–25 and 25–30 cm (north-facing). The permafrost interface level was recorded at approximately 20 and 30 cm, respectively. All samples were stored at < 0 °C in the field and during transport and at −80 °C in the laboratory prior to further analysis.

In situ temperature and humidity measurements

At the surface *in situ* air and soil temperatures (T, °C) were measured using a JENWAY 230 temperature meter. Air and soil relative humidity (% RH) values were measured with a Digitron 2020R meter. Temperature and RH were logged using Thermochron/Hygrochron iButtons (model DS1921G, Embedded Data Systems) embedded at each depth at 10-min intervals for 12 days during November/December 2009 (from 28 November 2009 to 11 December 2009). In addition, soil surface (0–2 cm) temperature and % RH data were logged at 4-h intervals over a 10-month period during 2008. We note, for clarification, that this period does not include the two warmest months of the McMurdo Dry Valley calendar and that mean and maximum temperature and % RH values will be underestimated. Kruskal–Wallis followed by a Wilcoxon–Mann–Whitney *post hoc* test was used to assess T and % RH differences between samples.

ATP assays

ATP measurements were obtained within 90 min of sampling using the commercially available luminometric assay system designed to operate with the SystemSURE Y2 K portable monitor (Celsis Instruments, Cambridge, UK). Data are expressed as relative luminosity units (RLU). All assays were performed in duplicate with appropriate controls.

Soil physicochemical analysis

Soil physicochemical variables including moisture content, pH, organic carbon, total nitrogen, phosphorous, potassium, exchangeable cation and base saturation determinations were performed at BemLab (SANAS Accredited Testing Laboratory, Somerset West, South Africa), according to standard quality control procedures (SSSA, 1996).

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Metagenomic DNA extraction and amplification

Metagenomic DNA was extracted in triplicate from soil samples using the PowerSoil™ DNA Isolation Kit (MoBio, West Carlsbad, CA) according to the manufacturer's instructions. Triplicates were pooled and used as template for PCR amplification. General bacterial primers E9F (5′-GAGTTTGATCCTGGCTCAG-3′; Hansen *et al.*, 1998) and U1510R (5′-GGTTACCTTGTTACGACTT-3′; Reysenbach & Pace, 1995) were used for amplification of 16S rRNA genes. All polymerase chain reactions (PCR) were carried out in a Perkin Elmer Thermocycler (Gene Amp PCR system 6700) in a 50 μL reaction volume containing 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer, 0.2 U of Dream-*Taq*™ polymerase (Fermentas) and 10 ng of template. Thermal cycling conditions were 4 min denaturation at 94 °C; 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 105 s; final elongation step at 72 °C for 10 min. All PCR reactions were carried out in triplicate and DNA concentrations were measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Montchanin, DE).

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Table 1. Samples description, soil T (°C),% RH, ATP values, and diversity indices obtained from the T-RFLP analysis

Sample	S-0	S-5	S-10	S-15	S-20	N-0	N-5	N-10	N-15	N-20	N-25	N30
Slope*,†	South	South	South	South	South	North	North	North	North	North	North	North
Depth (cm)	0–1	2–5	5–10	10–15	15–20	0–1	2–5	5–10	10–15	15–20	20–25	25–30
Zone	Surface	Active layer	Active layer	Active layer	Permafrost boundary	Surface	Active layer	Active layer	Active layer	Active layer	Active layer	Permafrost boundary
ATP‡ (RLU × 10^3)	33 ± 4	7 ± 1	4 ± 0	4 ± 1	2 ± 1	15 ± 4	8 ± 1	5 ± 1	4 ± 0	3 ± 1	1 ± 0	ND
DNA‡ (ng g^{-1})	2033 ± 29	2016 ± 6	1586 ± 30	1523 ± 21	1420 ± 20	1760 ± 56	1753 ± 45	1747 ± 40	1607 ± 179	1660 ± 80	1577 ± 23	1490 ± 30
Min T (°C)§	−4.6	−1.7	−0.4	0.1	−0.2	−3.1	−0.7	−0.2	−0.9	−2.7	−4.2	−4.9
Max T (°C)§	25.4	14.2	10.2	7.9	11.6	25.5	20.4	24.3	23.0	20.4	19.6	26.5
Mean T (°C)§	6.9	5.2	3.8	2.6	1.6	7.9	3.9	2.3	1.31	−0.2	−1.5	−2.1
Min% RH§	23.5	91.6	92.8	85.7	97.3	26.1	76.4	92.4	93.4	92.4	89.0	88.9
Max% RH§	87.5	111.9	109.7	108.7	108.0	86.6	106.9	110.5	107.8	113.8	106.4	108.0
Mean% RH§	50	105.1	105.2	105.0	105.2	39.6	101.7	106.4	104.9	108.6	102.4	104.3
Pielou's Eveness (J′)	0.9	0.9	0.7	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8
Shannon's Index (H′)	1.8	2.5	1.7	3.1	3	1.7	2.8	2.2	1.9	1.9	1.1	1.9
Simpson Index (1 − λ′)	0.8	0.9	0.7	0.9	0.9	0.7	0.9	0.9	0.8	0.8	0.6	0.8

*South facing slope GPS coordinates: S 78° 05.590′, E 163° 48.270′ (slope angle approximately 5°, altitude 177 m).

†North-facing slope GPS coordinates: S 78° 06.144′, E 163° 48.468′ (slope angle approximately 5°, altitude 177 m).

‡Mean (±SD).

§T (°C) minimum, maximum and mean values for South- and North-facing slope samples data logged at 10-min. intervals over a 12-day period. Measurements (South and North T) = 1747; Measurements (South and North % RH) = 1570. ND = Not determined.

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Denaturing gradient gel electrophoresis (DGGE)

Amplicons obtained with the 16S rRNA gene primer set (E9F-U1510R) were used as template for a nested PCR for subsequent DGGE. Template DNA (1 μL) was PCR-amplified using 341F-GC (5′-CCTACGGGAGGCAGCAG-3′, with a GC clamp, CGCCCGCCGCGCGCGGCGG GCGGG GCGGGGGCACGGGGGG, added to the 5′ end) and 534R (5′- ATTACCGCGGCTGCTG-3′) (Muyzer *et al.*, 1993) in a 50-μL reaction containing 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer and 0.2 U of Dream*Taq*™ polymerase (Fermentas). Thermal cycling conditions for DGGE amplification were 4 min at 94 °C; 20 cycles of 94 °C for 45 s, 65 °C for 45 s and 72 °C for 60 s; 20 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s; final elongation step at 72 °C for 10 min. DGGE was performed essentially as described by Muyzer *et al.* (1993) using the DCode DGGE system (Biorad) at 100 V for 16 h at 60 °C in 1× TAE buffer. After EtBr (0.5 μg mL^{-1}) staining, gels were visualized with an AlphaImager 3400 imaging system. DGGE profiles were analyzed using GelCompar® II, version 5.0 (Applied Maths).

Terminal restriction fragment length polymorphism (T-RFLP) analysis

For T-RFLP analysis, PCRs were conducted using the primer set E9F-FAM (5′-labelled with tetrahydrochloro-6-carboxyfluorescein) and U1510R. PCR products were purified with an Illustra GFX™ PCR DNA and gel Band Purification kit (GE Healthcare, UK) and similar amounts of DNA (200 ng) digested with HaeIII (Fermentas). The digested products were purified as above and separated by capillary electrophoresis using an ABI3130XL (Applied Biosystems). ROX 1.1 (Slabert *et al.*, 2010) was used as a size standard. T-RFLP profiles were analyzed using Peak Scanner 1.0 (Applied Biosystems, https://products.appliedbiosystems.com) and the web-based programme T-REX (Culman *et al.*, 2009) (trex.biohpc.org). T-REX software uses the methodology described by Abdo *et al.* (2006) and Smith *et al.* (2005) to identify and align true peaks, respectively. We used one standard deviation in peak area as the limit to identify true peaks and 1 bp as the cluster threshold for the alignment. All T-RFLP analyses were performed in triplicate. An OTU was considered present if it appeared in at least two of the three PCR replicates. Fingerprint profiles were standardized by dividing each individual peak area by the total area of peaks in a sample profile. The Phylogenetic Assignment Tool (PAT) (Kent *et al.*, 2003) (https://secure.limnology.wisc.edu/trflp/) was used to putatively assign T-RFLP peaks. Programme options were set to allow a sizing error of 1 bp only for the smallest T-RFs and up to 4 bp with the longest T-RFs.

Statistical analysis and diversity index calculations of T-RFLP data were undertaken with PRIMER 6 (PRIMER-E Ltd, Ivy-bridge, UK). Analysis of similarities (ANOSIM) was performed to test for significant differences between *a posteriori* sample groupings. BEST analysis (Clarke & Gorley, 2006) was used to rank the influence of abiotic variables on the community structure and to select the combination of variables that better explain biotic patterns. The significance of the correlation between DGGE and T-RFLP matrices was calculated using Mantel test with 999 matrix permutations. Mantel test and other statistical analyses were performed using R (http://www.R-project.org).

16S rRNA gene clone library construction

16S rRNA gene fragments, PCR-amplified with primers E9F/U1510R from sample S20 (south-facing slope sample collected at a depth of 20 cm) and N30 (north-facing slope sample collected at a depth of 30 cm), were used for clone library construction (96 clones for each clone library). The PCR products were purified with an Illustra GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare), ligated into pGEM®-T Easy vector System (Promega) and transformed into competent *Escherichia coli* GeneHogs® (Invitrogen). Transformants were selected by blue/white screening. The presence of correctly sized inserts was confirmed by colony PCR and de-replicated by Amplified Ribosomal DNA Restriction Analysis (ARDRA) with restriction enzyme HaeIII (Fermentas). Clones with unique ARDRA patterns were considered as phylotypes and purified with a PeqGOLD Plasmid Miniprep Kit I (Biotechnologie GmbH) according to the manufacturer's instructions and sequenced with a Hitachi 3730xl DNA Analyzer (Applied Biosystems). Chromatograms were edited using Chromas (Technelysium), checked for chimeras using CCODE (Gonzalez *et al.*, 2005) and screened with the GenBank database through a standard BLASTN search (Altschul *et al.*, 1990). Phylotypes were further delineated according to sequence similarities of $\leq$ 97%. CLUSTALW alignments were carried out using Bio Edit (Hall, 1999). Phylogenetic trees were constructed in MEGA4 (Tamura *et al.*, 2007).

A virtual HaeIII digest of sequences obtained from the clone libraries was carried out to confirm the phylogenetic identities of individual peaks (T-DistinctiEnz in silico T-RFLP tool; http://www.bioinformatics.org/~docreza/cgi-bin/restriction/t_DistinctEnz.pl).

Sequences obtained in this study were submitted to GenBank under accession numbers HQ616027–

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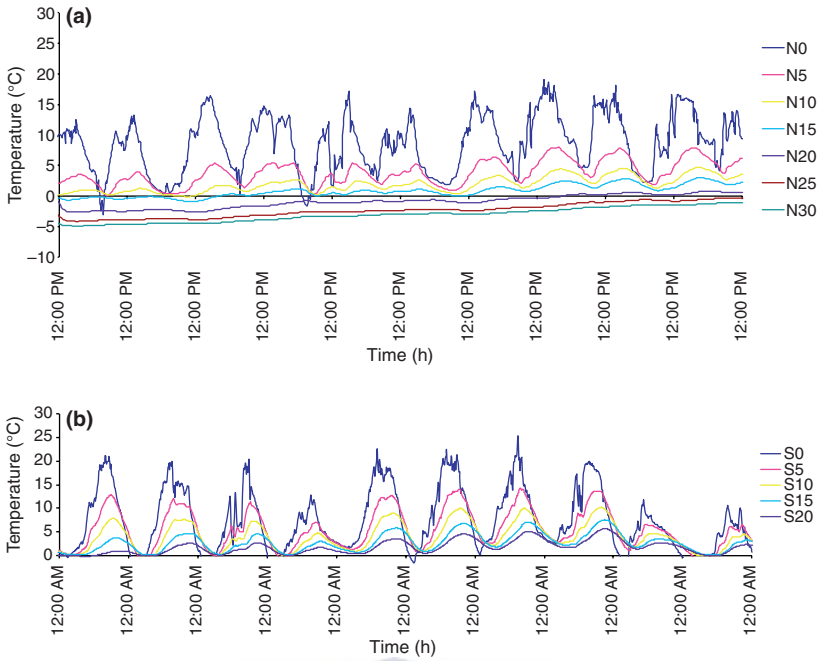

Fig. 1. Temperature (°C) logged at 10-min intervals over 12 days in samples collected from the south (a) and north (b) facing side of the McMurdo Dry Valley during the 2009 expedition. Samples have been collected from the surface and at 5, 10, 15 and 20 cm in depth.

HQ616073. Chao1 estimates were calculated using the web interface provided by Kemp & Aller (2004) (http://www.aslo.org/lomethods/free/2004/0114a.html).

Results

Data sets were acquired from shallow vertical transects at two sites on the lower slopes of the upper Miers Valley. Analysis performed included temperature, humidity, microbial biomass, total DNA, mineral soil physicochemical properties and phylogenetic fingerprinting.

In situ temperature and humidity measurements

Data from the 12-day *in situ* monitoring showed strong diurnal fluctuations in both temperature and % RH for the surface samples with increasing buffering with depth (Figs 1 and 2). Differences in temperature and % RH between the surface and the deepest soil samples from both south- and north-facing aspects were significant ($P < 0.05$). Mean temperature values for south (S)- and north (N)-facing samples ranged from 1.6 (S20) to 6.9 °C (S0) and from −0.2 (N20) to 7.9 °C (N0), respectively. % RH values ranged from 50.0 (S0) to 105.2 (S20) and from 39.6 (N0) to 108.6 (N20), respectively (Table 1). Over the 10-month *in situ* monitoring period (Fig. 3), the minimum temperature and % RH recorded were −41.1 °C and 69.6% and the maximum were 6.7 °C and 106.1%, respectively, while the mean annual temperature was −24.5 °C and the mean annual % RH was 91.8.

Microbial biomass

ATP titres decreased dramatically with depth in both facing slopes [3.3×10^4–2×10^3 RLU (S0-S20) and 1.5×10^4–1×10^3 RLU (N0-N25)] (Table 1). Using the conversion values of Cowan & Casanueva (2007), we estimate the surface cell titres to be 2.3×10^9 cells g^{-1} and 1×10^9 cells g^{-1} in the S- slope and N- slope sites, respectively. Calculated biomass levels for the deepest soil samples were 1.4×10^8 and 7×10^7 cells g^{-1} for south and north, respectively. DNA concentrations decreased significantly from surface samples (2033 ng g^{-1} in sample S0 and 1760 ng g^{-1} in sample N0) to samples collected at depth (1420 ng g^{-1} in sample S20 and 1490 ng g^{-1} in sample N30) from both sides (Table 1).

Community structure

DGGE analysis showed that surface and shallow subsurface soil samples from both sites (except for sample S5) clustered together (71% similarity). Intermediate depth samples from both sites formed a second coherent cluster (81.3% similarity) together with the south permafrost interface sample. The two deepest samples, recovered from the north permafrost interface, clustered together with a 93.1% similarity (Fig. 4).

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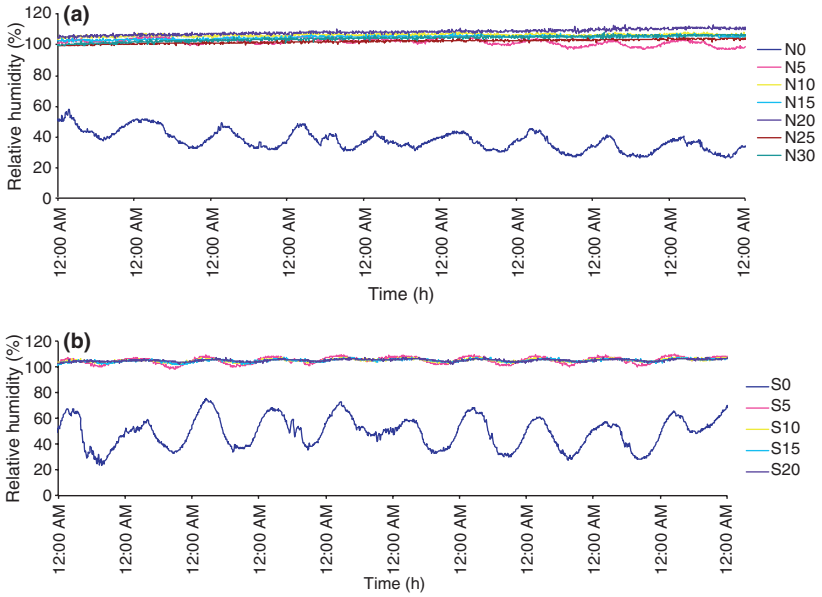

Fig. 2. Relative humidity (%) logged at 10-min intervals over 12 days in samples collected from the south (a) and north (b) facing side of the McMurdo Dry Valley during the 2009 expedition. Samples have been collected from the surface and at 0, 5, 10, 15, 20, 25 and 30 cm in depth.

A cluster analysis of T-RFLP data showed a similar trend to DGGE. This was confirmed by a significant correlation between the two matrices (Mantel R 0.4, $P < 0.05$). However, after ANOSIM analysis no differences were found between samples collected at different depths and samples collected from the two sites (ANOSIM $P > 0.05$). As T-RFLP can be linked to the phylogenetic information (clone libraries) all the data presented here refer to this molecular tool. A total of 76 T-RFs were found, of which 17% were unique to the north and 54% unique to the south (29% overlap). The highest number of OTUs was found in sample S20 (28) and the lowest in sample N25 (4). A few of the TRFs were dominant (e.g., TRFs 77, 92, 96, 121) occurring in over 50% of the samples. OTU richness and number of occurrence of each OTU are shown in Supporting Information, Fig. S1.

Using the Phylogenetic Assignment Tool (PAT) (Kent *et al.*, 2003) putative identities were assigned to a relatively high percentage (70%) of the T-RFLP peaks (Fig. S2). Samples were dominated by *Alphaproteobacteria* (72 bp), *Betaproteobacteria* (77, 91, 199, 205 bp), *Gammaproteobacteria* (210 bp), *Deltaproteobacteria* (234 bp), *Actinobacteria* (94, 121, 183 bp), *Firmicutes* (327 bp), *Bacteroidetes* (254, 259 bp), *Acidobacteria* (267 bp), *Spirochetes* (79 bp), *Cyanobacteria* (127 bp), *Chlorobi* (165 bp), *Planctomycetes* (182 bp) and *Deinococcus/Thermus* (63 bp). Furthermore, a total of 11 TR-Fs could be matched with 16S rRNA gene sequences and their respective taxonomic group (Table S2).

The possible relationships between abiotic parameters and microbial community structures were assessed by BEST analysis (Clarke & Gorley, 2006). C and K were the environmental variables that most strongly influenced the structure of microbial populations ($pw = 0.275$ and $pw = 0.255$, respectively), followed by the combination of K + Ca, K + C and K + C + Ca + moisture ($pw = 0.259$, 0.253 and 0.246, respectively). Including the 12-day mean % RH and temperature values of all the samples (data from Table 1) in the analysis did not alter these patterns. However, as most abiotic factors were highly correlated [except moisture and C (Table 2)], it may be difficult to determine their role in shaping the structure of the microbial communities analyzed.

To elucidate the phylogenetic diversity of the deepest samples (S20 and N30) two 16S rRNA gene clone libraries were generated, as surface soils microbial diversity has been extensively characterized (De la Torre *et al.*, 2003; Smith *et al.*, 2006; Yergeau *et al.*, 2007; Aislabie *et al.*, 2008; Niederberger *et al.*, 2008; Khan *et al.*, 2011). A total of 69 phylotypes were inferred from ARDRA analysis, 39 from sample S20 and 30 from sample N30. Sequence analysis identified 46 OTUs (at 97% identity). *Proteobacteria* and *Actinobacteria* were dominant in the south permafrost interface while *Bacteroidetes*, *Acidobacteria*, *Firmicutes* and *Actinobacteria* were the predominant phyla in sample N30 (Fig. S3). Moreover, the clone library constructed from sample S20 showed higher OTU richness and revealed the presence of other bacterial phyla that were not detected in sample N30 (i.e. *Gemmatinomonadates*, *Verrucomicrobia*,

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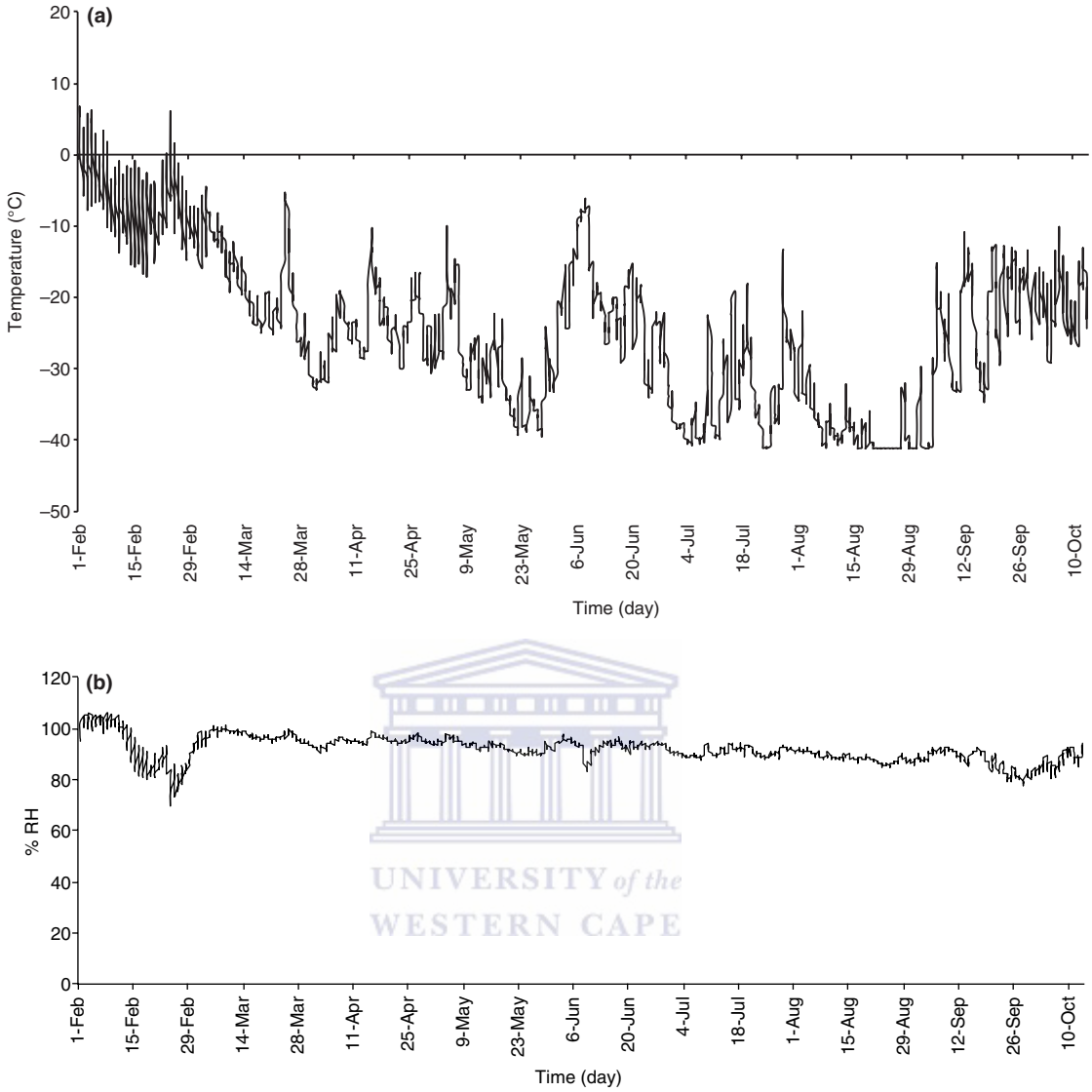

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Fig. 3. Soil surface T (°C) (a) and % RH (b) conditions logged at 4-h intervals over a 10-month period during the 2008 expedition in the McMurdo Dry Valley (n = 2044).

Chlorobi and *Cyanobacteria*) (Fig. S3). This result was in agreement with the Chao1 index, which predicted that only 25% and 52% of the total diversity in samples S20 and N30 was observed, respectively.

The majority of these sequences showed close affinity to uncultivated bacteria (Fig. S4) previously reported from Antarctica (Dry Valleys, Alexander Island) and other cold and/or dry environments (Table S2). Virtual digests of the 16S rRNA gene clone libraries showed 11 OTUs corresponding to those of the dominant T-RFs (±4 bp) in the T-RFLP profiles (Table S2). Although too numerous to list, examples include T-RF 199 affiliated to *Gammaproteobacteria*, T-RF 234 to *Actinobacteria* and T-RF 210 to two different *Gammaproteobacteria* sequences.

Discussion

Recent studies on a wide range of different Antarctic desert soils have demonstrated an unexpectedly rich microbial diversity (Cowan *et al.*, 2002; Aislabie *et al.*, 2006; Smith *et al.*, 2006; Niederberger *et al.*, 2008; Pointing *et al.*, 2009). However, despite extensive phylogenetic analysis, comparatively little is known of the gross functionality of these 'communities', the key functional members and the influence the microenvironmental drivers pose on their development. Here we aimed to establish whether abiotic factors influence microbial profiles in permanent cold soil horizons. Although the number of samples analyzed in this study prevents extrapolation of

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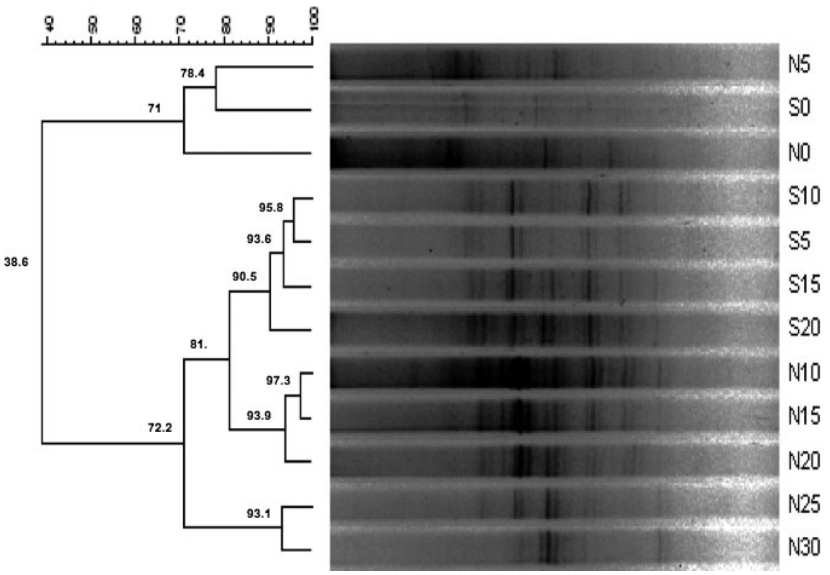

Fig. 4. Cluster analysis (UPGMA algorithm) from DGGE profiles of soil bacterial communities (North- and South-facing slopes of McMurdo Dry Valley).

the results to the wider Antarctic environs, the data does provide evidence that bacterial communities in the Dry Valleys are not homogenous and their development is abiotically driven.

Salinity (Zeglin *et al.*, 2009), pH (Männistö *et al.*, 2007), UV (Tosi *et al.*, 2005), latitude (Yergeau *et al.*, 2009), altitude (Cowan *et al.*, 2010), K, C, soluble salts (Pointing *et al.*, 2009) and other factors have been shown to influence microbial diversity, and temperature and moisture are considered to be the most critical variables (Kennedy, 1993; Noy-Meir, 1973; Parsons *et al.*, 2004; Barrett *et al.*, 2007).

It is widely accepted that water availability is a critical 'driver' of microbial activity in cold desert systems (Barrett *et al.*, 2007). It is also assumed that Antarctic desert soil communities are largely, if not completely, dependent on periodic precipitation in the form of light and intermittent snow falls (Claridge & Campbell, 1977; Hopkins *et al.*, 2006b; Fountain *et al.*, 2009). However, our study and others (e.g. Newsham *et al.*, 2010) have failed to demonstrate a significant relationship between soil water content and biological parameters. Antarctic soil surface microbial communities are in close proximity to a potential source of water in the form of a permafrost horizon (Friedmann, 1993; Bockheim, 1997; Hopkins *et al.*, 2006b). At least 25% of Antarctic desert regions contain permafrost in the form of ground ice soil (Bockheim, 1995). In the maritime Dry Valleys, the permafrost interface during the austral summer season is typically within 30 cm of the soil surface and is commonly overlaid by a moist active layer, where liquid water is entrained upward from the melting interface by capillary action (Bockheim, 2002; Barrett *et al.*, 2007). Active-layer thickness and depth to ice-cemented permafrost are seasonally controlled and related to local climate, solar gain (a product of aspect and slope), proximity to glaciers and albedo of surface rocks (Bockheim, 2002). The active zone represents an intermediate and dynamic continuum between the permanently cemented 'permafrost' and the surface.

Seybold *et al.* (2010) noted an increase in water content with depth. In this study, although soil moisture content did not show a linear increase along the vertical gradient, it did show a clearly increasing trend with depth. A possible explanation for the lack of a linear change in water content with depth would be, as suggested by Seybold *et al.* (2010), that soil water content is influenced both by capillary action (upward entrainment) and recharge from intermittent precipitation events (downward entrainment). However, we did record a very large % RH gradient between surface and depth, suggesting upward water vapour mobility as a strong thermodynamic driver. This gradient occurs within the top 5 cm and stabilizes with depth. We suggest that the transport of water vapour from melted permafrost to atmosphere provides a mechanism for a continuous supply of water to soil microbial populations either as the result of condensation processes or by direct adsorption from the vapour phase (e.g., via hydroscopic exopolymer secretions layers) (Potts, 1994; Azúa-Bustos *et al.*, 2011).

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Table 2. Pearson correlation matrix of physicochemical soil parameters

Variables	pH	Moisture	C	N	Na†	K†	Ca†	Mg†	TValue‡	Na§	K§	Ca§	Mg§	P
Moisture	0.29													
C	−0.01	0.56												
N	−0.59*	−0.32	−0.27											
Na†	0.70*	0.28	0.32	−0.56										
K†	−0.13	−0.2	−0.49	0.34	−0.39									
Ca†	0.69*	0.17	0.25	−0.62*	0.93***	−0.51								
Mg†	−0.34	−0.26	−0.51	0.44	−0.45	0.91***	−0.62*							
TValue‡	0.70*	0.17	0.23	−0.62*	0.95***	−0.46	1.00***	−0.58						
Na§	0.52	0.4	0.36	−0.27	0.65*	0.02	0.35	0.06	0.39					
K§	−0.54	−0.2	−0.4	0.63*	−0.81**	0.66*	−0.95***	0.79**	−0.93***	−0.17				
Ca§	0.52	0.19	0.4	−0.62*	0.75**	−0.68*	0.92***	−0.82***	0.89***	0.08	−0.99***			
Mg§	−0.55	−0.23	−0.43	0.63*	−0.78**	0.67*	−0.92***	0.83***	−0.90***	−0.13	0.99***	−1.00***		
P	−0.29	0.22	0.18	0.36	−0.48	0.42	−0.58*	0.39	−0.57	−0.02	0.59*	−0.57	0.55	
K	−0.14	−0.19	−0.5	0.35	−0.4	1.00***	−0.53	0.92***	−0.48	0.04	0.68*	−0.70*	0.70*	0.43

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.
†Exchangeable cations.
‡Expressed as the sum of exchangeable cations.
§Base saturation.

Biomass levels (as indicated by ATP titres and DNA yields) consistently decreased with depth in shallow vertical soil transects. This observation reinforces the argument for the importance of metabolic capacity (expected to be highest at the surface because of the presence of active phototrophy and because of the higher surface temperatures) and indicates that soil water content is not the most critical factor driving the development and activity of microbial communities in cold desert soils. Although functional microbial ecosystems are thought to exist in permafrost (Vorobyova *et al.*, 1997; Steven *et al.*, 2006) and given that metabolically active microorganisms have been found below −10 °C (Gilichinsky *et al.*, 1995; Shi *et al.*, 1997; Rivkina *et al.*, 2000) most permafrost microorganisms are considered to be in a state of dormancy (Friedmann *et al.*, 1994; Gilichinsky *et al.*, 2007). It is highly likely that viable microorganisms in the intermediate active zone, with consistently low ambient temperatures, exhibit only limited metabolic activity.

Soil organic carbon levels are not inconsistent with this view where organic C levels generally decreased with depth (Table S1). Carbon is usually a limiting factor in the Dry Valley soils (Barrett *et al.*, 2005; Steven *et al.*, 2006; Pointing *et al.*, 2009); however, the origins of organic carbon both in surface soils and at depth are complex. While surface photoautotrophic processes are the obvious renewable source of fixed carbon (Wood *et al.*, 2008), in Dry Valleys harbouring lake systems (such as the Miers Valley) surface soil carbon stocks may be supplemented by the aeolian transport of lacustrine cyanobacterial biomass (Parker *et al.*, 1982; Moorhead *et al.*, 2003). Subsurface carbon is influenced to an unknown extent by transport from the surface and by microbial mineralization processes and may be further complicated by a background of legacy carbon (Moorhead *et al.*, 1999; Burkins *et al.*, 2000). Recent Dry Valley estimates of soil organic C turnover (20–150 years) (Burkins *et al.*, 2000; Elberling *et al.*, 2006) suggest a small contribution of past organic matter to current C cycling. The data presented here and those from other studies (Hopkins *et al.*, 2006a, b; Elberling *et al.*, 2006; Wood *et al.*, 2008) indicate that current soil food webs, marine detritus and endolithic microorganisms subsidize Dry Valley soil organic matter. Surface phototrophic activity and/or the deposition of lacustrine biomass might quantitatively be the more significant processes, resulting in higher organic carbon levels in surface samples.

Bacterial diversity was assessed using three culture-independent techniques: DGGE, T-RFLP and 16S rRNA gene clone libraries. Sediment depth explained changes in community structure, highlighting that surface and shallow subsurface samples cluster separately from the permafrost and active layer samples (Fig. 4). Sample S20

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showed a higher level of diversity, both in clone library and T-RFLP analyses, than sample N30. Whether this is attributed to a north/south influence can only be determined through surveying of additional sampling points.

Furthermore, members of the *Proteobacteria*, *Actinobacteria* and *Firmicutes* were found to be the dominant phyla in all horizons. In contrast the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Gammaproteobacteria* appeared to be more abundant at the permafrost interface. These phyla are typically all well represented in Antarctica (e.g., Aislabie *et al.*, 2006; Smith *et al.*, 2006; Khan, 2008) and other soils (Fierer & Jackson, 2006) and have been shown to contain members well adapted to harsh environmental conditions (Niederberger *et al.*, 2008).

Signatures for photoautotrophic cyanobacteria were recovered from subsurface soil samples. Cyanobacteria usually comprise a relatively small or undetectable fraction of Dry Valley open soil (Cowan, 2009; Pointing *et al.*, 2009; Wood *et al.*, 2008) and permafrost (Gilichinsky *et al.*, 2007). While it is reasonably assumed that these signals do not represent metabolically active organisms, it is unclear whether they represent legacy signals (Cary *et al.*, 2010; Moorhead *et al.*, 1999; Burkins *et al.*, 2000) or the result of soil turnover processes. Furthermore, cyanobacteria are often dominant members of Antarctic hypolithic communities where nitrogen fixation takes place (Cowan *et al.* 2011). Therefore, our data suggest cyanobacteria are cosmopolitan taxa in Antarctic soils and strengthen the view that cyanobacteria are important players in soil ecosystem functioning, either as an active biological component or as a nutrient source (Wood *et al.*, 2008).

A significant portion of the 16S rRNA gene sequences affiliated closely with other uncultured bacteria from cold environments, showing little if any evidence of high-altitude particulate transport and depositional processes, which would be expected to contribute phylotypes with tropical and temperate affiliations (Vincent, 2000) and in general supports the concept of a endogenous origin of Antarctic terrestrial microbial populations. Indeed, this may be an interesting line of enquiry given that hypolithic cyanobacteria from Dry Valley soils have been shown to be genetically isolated from other arid soil populations as before the last glacial maximum (Bahl *et al.*, 2011).

Some of the divisions (i.e. *Spirochaete*, *Planctomycetes*, *Deinococcus/Thermus*) identified by T-RFLP analysis were not detected using clone library analysis or did not show the same abundance (i.e. *Acidobacteria*). Indeed, the Chao1 indexes calculated from both clone libraries revealed the presence of a much richer microbial diversity than estimated, highlighting the importance of employing polyphasic approaches to more accurately evaluate the microbial diversity in environmental samples.

In direct contrast to the results of this study, early culture-dependent analyses indicated that the surface soils of the Antarctic Dry Valleys contained fewer microorganisms than the underlying layers (Cameron *et al.*, 1970; Horowitz *et al.*, 1972) and that microbial diversity was higher in subsurface samples (Friedmann, 1982; Meyer *et al.*, 1988; Nienow & Friedmann, 1993). At least two possible explanations have been suggested: the greater stability and longevity of both vegetative and resting cells at depth, probably as a result of the stable low-temperature environment and the tendency for culture-dependent studies to recover only the fast-growing spore-formers. However, given the possibility of long-term preservation of DNA in Antarctic soils (Ah Tow & Cowan, 2005) and permafrost (Willerslev *et al.*, 2004; Gilichinsky *et al.*, 2007; Johnson *et al.*, 2007) and that culture-independent techniques do not discriminate between extracellular DNA or DNA from live or dead cells, the inference of functionality from such analyses is inappropriate. This is, to an extent, reflected in our data as the decrease in metabolic activity with depth is not directly proportional to the decrease in recoverable DNA (Table 1). The phylogenetic signatures obtained, therefore, reflect both present and historical microbial existence. A transcript-based analysis would more accurately reflect the metabolically active diversity; however, the technical challenges of extracting usable amounts of mRNA from Antarctic desert soils have, so far, prevented the parallel analysis of the functional microbial fraction.

As Antarctica is characterized by an extremely delicate biological equilibrium, the relative simplicity of trophic structures will allow for the examination of future perturbations (i.e. climate changes) and their impact on microbial diversity and ecosystem sustainability.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. OTU Richness (left) and T-RFLP fragments occurrences (right).
Fig. S2. (a) Putative phylogenetic assignment of the T-RFLP peaks using the Phylogenetic Assignment Tool. (b) Relative peak height of the T-RFs.
Fig. S3. Distribution of phyla detected in 16S rRNA gene libraries from soil at the permafrost boundary of Miers Valley.
Fig. S4. 16S rRNA gene neighbor joining tree (Not necessary. This reference is provided in Supporting Information).
Table S1. Soil chemical analysis of the samples.
Table S2. 16S rRNA gene sequences from clone libraries obtained in this study.

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Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley

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Supplementary Material

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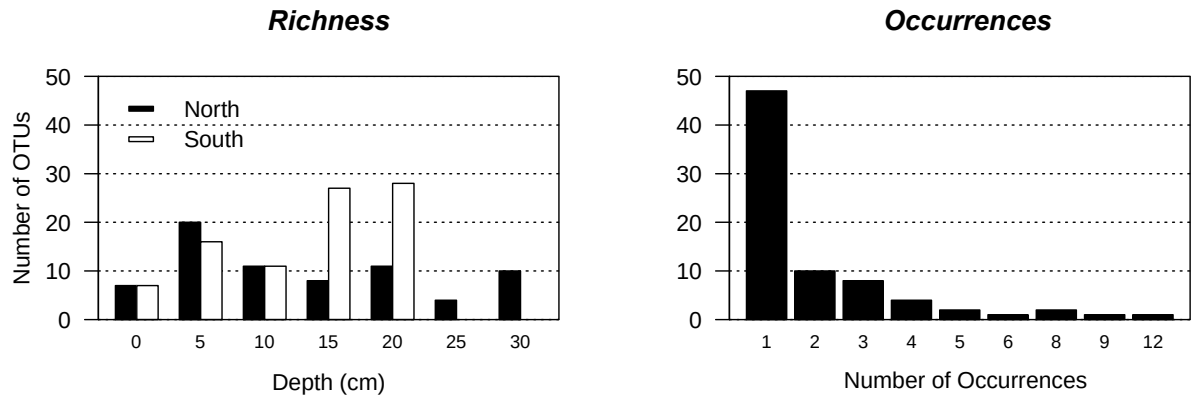

Figure S1. OTU Richness (left) and T-RFLP fragments occurrences (right).

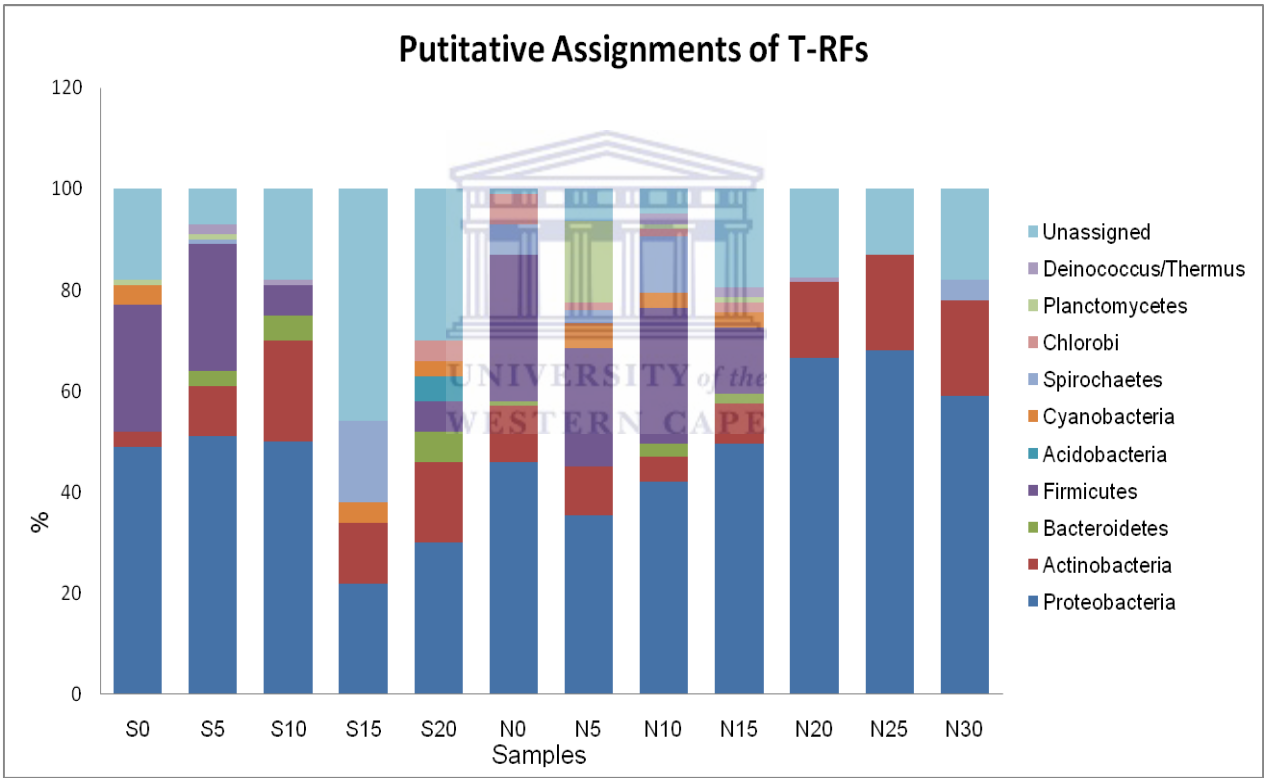

Figure S2a. Putative phylogenetic assignment of the T-RFLP peaks using the Phylogenetic Assignment Tool. (PAT: https://secure.limnology.wisc.edu/trflp/)

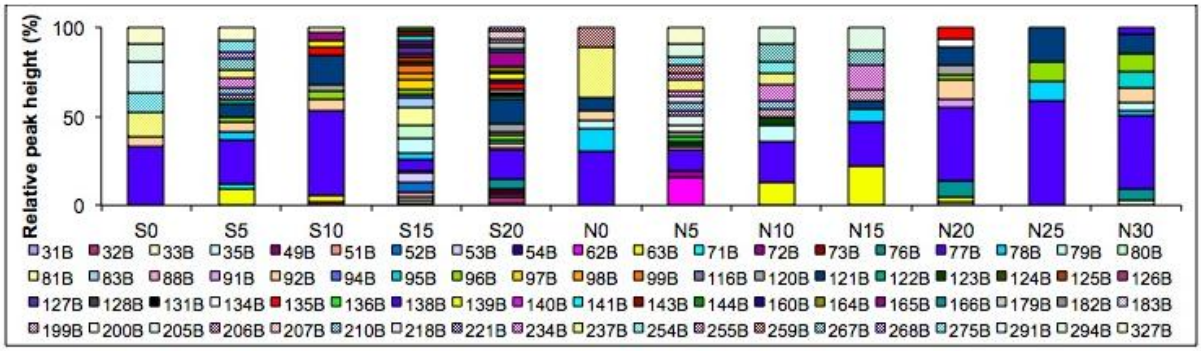

Figure S2b. Relative peak height of the T-RFs.

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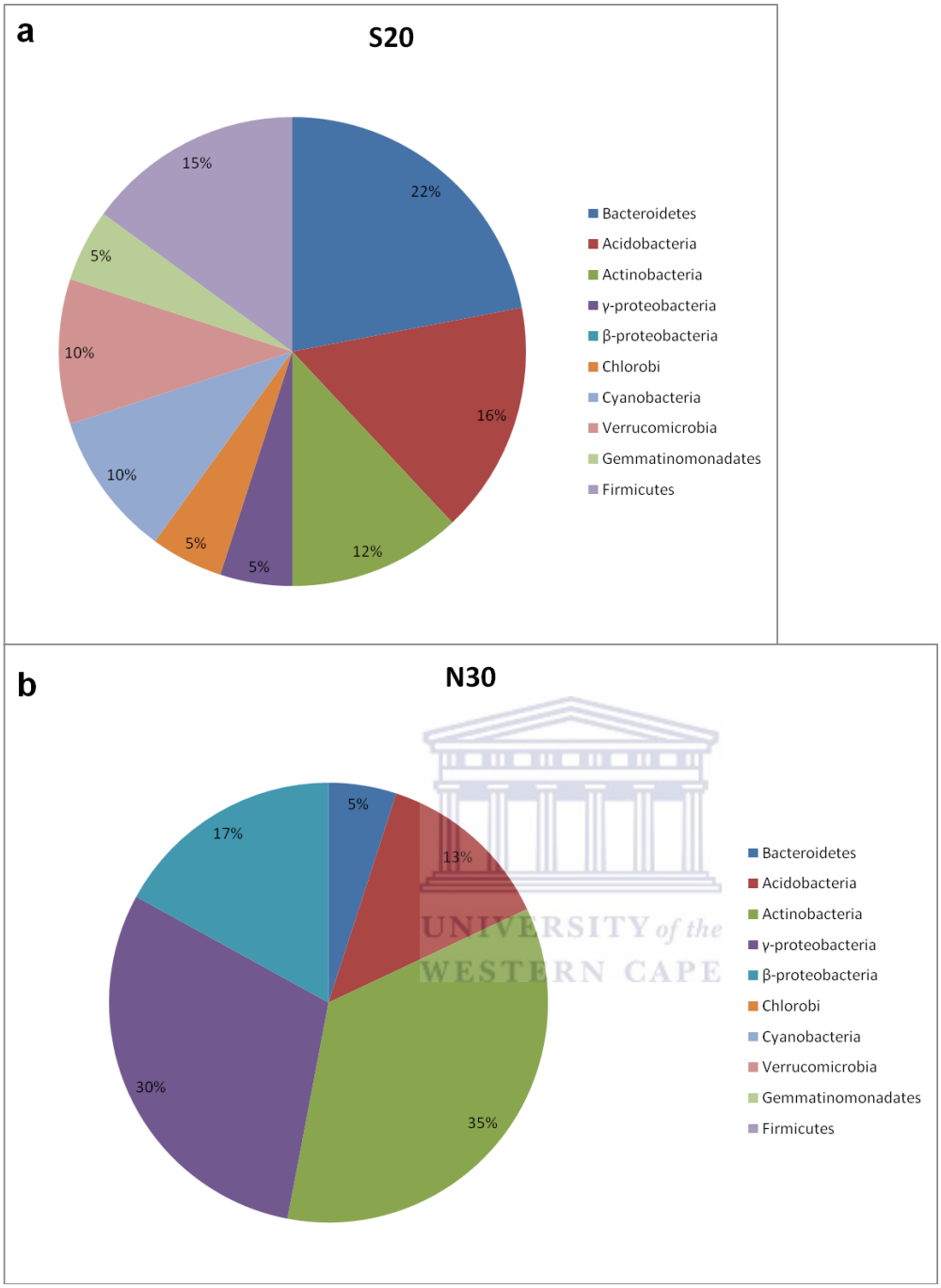

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Figure S3. Distribution of phyla detected in 16S rRNA gene libraries from soil at the permafrost boundary of Miers Valley. (a) south slope (sample S20); (b) north slope (sample N30).

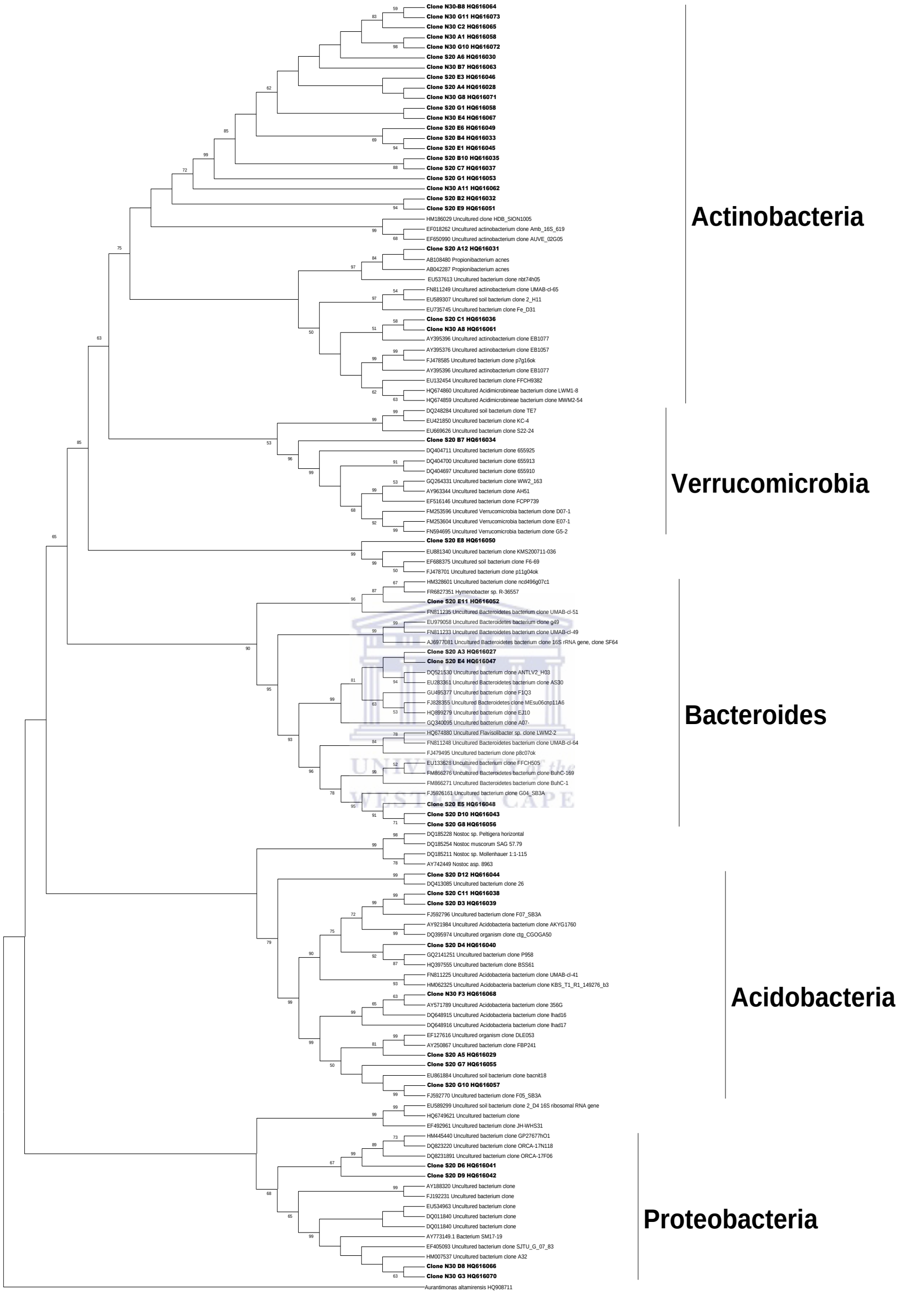

Figure S4. 16S rRNA neighbor joining tree (Saitou & Nei, 1987). Phylogenetic distances were computed using the Maximum Composite Likelihood method (Tamura, et al., 2004). Bootstrap values (Felsestein, 1985) higher than 50% are shown. Phylogenetic analyses were conducted in MEGA4 (Tamura, *et al.*, 2007).

Table S1. Soil chemical analysis of the samples.

Sample	pH	Moisture %	C %	N %	Na[a] cmol/kg	K[a] cmol/kg	Ca[a] cmol/kg	Mg[a] cmol/kg	T-Value[c] cmol/kg	Na[b] %	K[b] %	Ca[b] %	Mg[b] %	P mg/kg	K mg/kg
S0	7.1	2.7	0.3	0.028	0.18	0.4	4.6	0.61	5.78	3.07	6.9	79.47	10.57	2	156
S5	7.5	5.1	0.32	0.023	0.13	0.38	2.47	0.54	3.53	3.66	10.9	70	15.44	35	150
S10	7.7	3.3	0.2	0.028	0.17	0.53	3.12	0.8	4.63	3.72	11.45	67.53	17.29	37	207
S15	7.8	2.9	0.16	0.027	0.19	0.53	2.89	0.82	4.43	4.34	12.04	65.13	18.49	40	209
S20	7.9	4.4	0.16	0.026	0.17	0.45	2.6	0.7	3.92	4.29	11.6	66.31	17.8	2	178
N0	8.2	3.7	0.33	0.024	0.58	0.35	10.37	0.47	11.77	4.93	2.96	88.09	4.01	2	136
N5	8.2	6.9	0.3	0.009	0.52	0.46	8.96	0.63	10.57	4.92	4.37	84.76	5.95	2	181
N10	8	2.1	0.25	0.019	0.32	0.43	7.58	0.51	8.82	3.58	4.82	85.85	5.75	2	166
N15	8.1	7.1	0.34	0.022	0.28	0.4	6.86	0.46	7.99	3.48	4.96	85.86	5.7	28	155
N20	8.1	3	0.2	0.015	0.27	0.39	6.5	0.46	7.62	3.52	5.1	85.32	6.06	1	152
N25	7.9	6.4	0.69	0.012	0.37	0.38	6.34	0.48	7.57	4.85	5.01	83.82	6.32	28	148
N30	8.2	3.8	0.23	ND	0.4	0.39	8.86	0.54	10.19	3.9	3.8	86.96	5.34	2	151

[a] Exchangeable cations. [b] Base saturation. [c] Expressed as the sum of exchangeable cations.

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Table S2. 16S rRNA gene sequences from clone libraries obtained in this study. The % of similarities with the closest homologues in Genbank, accession numbers and the T-RFs lengths of the representatives OTUs are shown.

Ribotypes (T-RF bp)	**Accession no.**	**Closest sequence in GenBank**	**%**	**Sequence origin**
S20-A3	HQ616027	HM439883: Uncultured bacterium (Bacteroidetes)	99	Greenland ice sheet
S20-A4	HQ616028	FN811225: Uncultured Acidobacteria	99	Alexander Island, Antarctica
S20-A5	HQ616029	EU869708: Uncultured bacterium (Acidobacteria)	97	Hot/cold deserts
S-20-A6 (127)	HQ616030	AY742449: *Nostoc* sp. 8963 (Cyanobacteria)	99	New Zealand (from *Gunnera* species)
S20-A12 (183)	HQ616031	HQ848535: Uncultured Actinomycetales (Actinobacteria)	97	Fluvial lake (Canada)
S20-B2	HQ616032	EU869788: Uncultured bacterium (Actinobacteria)	89	Hot/cold deserts
S20-B4	HQ616033	EU715871: Uncultured bacterium (Verrucomicrobia)	95	Environmental soil samples
S20-B7	HQ616034	HM119286: Uncultured bacterium (Verrucomicrobia)	98	Cold volcanic desert
S20-B10	HQ616035	DQ514128: Uncultured Gemmatimonadetes bacterium	99	Deglaciated soil
S20-C1	HQ616036	GU552173: Uncultured actinobacterium	98	Atacama Desert
S20-C7	HQ616037	HQ213781: Uncultured Gemmatimonadaceae	100	Arctic soil
S20-C11 (267)	HQ616038	EU297244: Uncultured Firmicutes bacterium	99	Environmental soil samples
S20-D3	HQ616039	EU297244: Uncultured Firmicutes bacterium	98	Atacama Desert
S20-D4	HQ616040	DQ444079: Uncultured bacterium (Firmicutes)	99	Songhuajiang River sediments
S20-D6 (199)	HQ616041	DQ823229: Uncultured bacterium (γ-proteobacteria)	98	Oregon Caves
S20-D9	HQ616042	EU869631: Uncultured bacterium (Firmicutes)	92	Hot/cold deserts
S20-D10 (259)	HQ616043	EU297865: Uncultured bacterium (Bacteroidetes)	99	Environmental soil samples
S20-D12	HQ616044	EU883148: Uncultured bacterium (Chlorobi)	97	Devon island, Canada
S20-E1	HQ616045	EU715871: Uncultured bacterium (Verrucomicrobia)	92	Environmental soil samples
S20-E3	HQ616046	HQ197646: Uncultured bacterium clone (Acidobacteria)	99	Dry Valleys, Antarctica
S20-E4	HQ616047	HM439883: Uncultured bacterium (Bacteroidetes)	99	Greenland ice sheet
S20-E5	HQ616048	EF651325: Uncultured Flavobacteria (Bacteroidetes)	98	Environmental soil samples
S20-E6	HQ616049	EF516048: Uncultured bacterium (Verrucomicrobia)	97	Environmental soil samples
S20-E8 (182)	HQ616050	EF688375: Uncultured soil bacterium	97	Environmental soil samples
S20-E9	HQ616051	FM865646: Uncultured *Nocardia* sp. (Actinobacteria)	87	Tibetan Plateau

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Ribotypes (T-RF bp)	**Accession no.**	**Closest sequence in GenBank**	**%**	**Sequence origin**
S20-A3	HQ616027	HM439883: Uncultured bacterium (Bacteroidetes)	99	Greenland ice sheet
S20-A4	HQ616028	FN811225: Uncultured Acidobacteria	99	Alexander Island, Antarctica
S20-A5	HQ616029	EU869708: Uncultured bacterium (Acidobacteria)	97	Hot/cold deserts
S-20-A6 (127)	HQ616030	AY742449: *Nostoc* sp. 8963 (Cyanobacteria)	99	New Zealand (from *Gunnera* species)
S20-A12 (183)	HQ616031	HQ848535: Uncultured Actinomycetales (Actinobacteria)	97	Fluvial lake (Canada)
S20-B2	HQ616032	EU869788: Uncultured bacterium (Actinobacteria)	89	Hot/cold deserts
S20-B4	HQ616033	EU715871: Uncultured bacterium (Verrucomicrobia)	95	Environmental soil samples
S20-B7	HQ616034	HM119286: Uncultured bacterium (Verrucomicrobia)	98	Cold volcanic desert
S20-E11	HQ616052	JF043382: Uncultured bacterium (Bacteroidetes)	95	Environmental soil samples
S20-G1	HQ616053	GQ306052: Uncultured bacterium (Bacteroidetes)	97	Periglacial soils
N30-F6	HQ616069	FM165205: Uncultured Thiothrix (γ-proteobacteria)	86	Environmental soil samples
S20-G2 (165)	HQ616054	AJ229196: Unidentified eubacterium (Chlorobi)	87	Environmental soil samples
S20-G7	HQ616055	GU219801: Uncultured bacterium (Firmicutes)	99	Icelandic volcanic glasses
S20-G8	HQ616056	FN811206: Uncultured Bacteroidetes	99	Alexander Island, Antarctica
S20-G10	HQ616057	AY234727: Bacterium Ellin6075 (Firmicutes)	98	Environmental soil samples
N30-A1 (210)	HQ616058	GU598692: Uncultured bacterium (γ-proteobacteria)	99	Environmental soil samples
N30-A2	HQ616059	HM748714: Bacterium Ellin7504(Acidobacteria)	94	Environmental soil samples
N30-A3 (234)	HQ616060	HM748735: Bacterium Ellin7525(Actinobacteria)	94	Environmental soil samples
N30-A8	HQ616061	HQ265246: Uncultured bacterium (Actinobacteria)	96	Tibetan hot springs
N30-A11	HQ616062	HQ330617: Uncultured bacterium (Actinobacteria)	81	Lake Wivenhoe, Australia
N30-B7	HQ616063	HQ224931: Uncultured actinobacterium	89	Environmental soil samples
N30-B8	HQ616064	HQ327150: *Polaromonas* sp. (β-roteobacteria)	100	Tibetan Plateau
N30-C2	HQ616065	HM583567: *Polaromonas* sp.(β-proteobacteria)	98	Glacier cryoconite
N30-D8 (199)	HQ616066	FJ823386: *Escherichia coli* strain TCP-1	99	Environmental soil samples
N30-E4	HQ616067	AB267477: *Flavisolibacter ginsengisoli* (Bacteroidetes)	96	Environmental soil samples
N30-F3	HQ616068	JF394379: Uncultured bacterium (β-proteobacteria)	98	Arctic soil
N30-G3	HQ616070	HM582742: Uncultured bacterium (γ-proteobacteria)	100	Ethiopian soda lake
N30-G8	HQ616071	AM887754: Acidobacteria bacterium (Acidobacteria)	91	Wetlands, Russia

Ribotypes (T-RF bp)	**Accession no.**	**Closest sequence in GenBank**	**%**	**Sequence origin**
S20-A3	HQ616027	HM439883: Uncultured bacterium (Bacteroidetes)	99	Greenland ice sheet
S20-A4	HQ616028	FN811225: Uncultured Acidobacteria	99	Alexander Island, Antarctica
S20-A5	HQ616029	EU869708: Uncultured bacterium (Acidobacteria)	97	Hot/cold deserts
S-20-A6 (127)	HQ616030	AY742449: *Nostoc* sp. 8963 (Cyanobacteria)	99	New Zealand (from *Gunnera* species)
S20-A12 (183)	HQ616031	HQ848535: Uncultured Actinomycetales (Actinobacteria)	97	Fluvial lake (Canada)
S20-B2	HQ616032	EU869788: Uncultured bacterium (Actinobacteria)	89	Hot/cold deserts
S20-B4	HQ616033	EU715871: Uncultured bacterium (Verrucomicrobia)	95	Environmental soil samples
S20-B7	HQ616034	HM119286: Uncultured bacterium (Verrucomicrobia)	98	Cold volcanic desert
N30-G10 (210)	HQ616072	FJ826377: Uncultured bacterium (γ-proteobacteria)	99	Yellow sea
N30-G11	HQ616073	GQ200828: *Massilia* sp. (β-proteobacteria)	95	India

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