SEASONAL DYNAMICS OF EDAPHIC BACTERIAL COMMUNITIES IN THE HYPER-ARID NAMIB DESERT

Alacia Armstrong

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University of the Western Cape

Supervisors: Prof. I.M Tuffin, Dr. J-B Ramond and Prof. D.A Cowan.

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Abstract

The Namib Desert is a hyper-arid, coastal desert with limited bioavailable water and nutrients; characteristics which collectively impose constraints on edaphic microbial communities. Several studies in the Namib Desert have investigated changes in soil microbial communities across space. However, the temporal variation of edaphic bacterial community in response to seasonal microenvironmental variation in the Namib Desert gravel plains has never been investigated *in situ*.

The edaphic bacterial community dynamics were evaluated over short (57 days) and long-term (1 year) sampling intervals using an extensive sampling strategy in combination with community fingerprinting by T-RFLP analyses and microenvironmental characterization. The short-term study was conducted on three distinct locations in the Namib Desert gravel plains. Soil bacterial communities were found to be more similar within habitats than between habitats, with the differences likely shaped by soil pH. These findings are consistent with the concept of habitat filtering.

Investigation of edaphic bacterial communities over 1 year in an 8100 m^2 sampling site revealed seasonal patterns of variation in community structure. Soil moisture, phosphorus, potassium and magnesium were identified as significant abiotic drivers of community temporal dynamics. β diversity was found to increase over time, while the environment remained relatively static. These findings support previous observations that desert communities are likely structured by stochastic and deterministic processes. Taken together, these findings advance understanding of temporal variation of edaphic communities in the Namib Desert.

Declaration

I declare that all work presented in this thesis 'Seasonal dynamics of edaphic bacterial communities in the Namib Desert' is my own, and all sources and work used or quoted in this thesis have been appropriately cited.

Alacia Armstrong

MSc. Candidate

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Table of Contents

List of Figures

List of Tables

Chapter 1: Literature Review

Introduction

Deserts are key biomes as they constitute the largest terrestrial ecosystems covering one-third of the Earth's surface (Figure 1; Collins *et al*., 2008). Moreover, the surface of these regions continues to increase annually, a process known as desertification, at a rate 5.8 billion hectares per annum (p.a; Vernon *et al*., 2006). Desertification is defined as "land degradation in arid, semiarid and dry sub-humid areas, resulting from various factors, including climatic variations and anthropogenic impact" by the United Nations Convention to Combat Desertification (1994). Desertification has adverse impacts on the environment, which include, most notably, the loss of biodiversity, a reduction in plant productivity and decreased carbon sequestration capacity, all of which contribute towards climate change. Currently, it is estimated that 10 - 20% of drylands are already degraded or undergoing desertification (Adeel *et al*., 2005).

Aridity is among the greatest stresses imposed by warm deserts on biotic life, as it influences the bio availability of water in these environments (Pointing & Belnap, 2012). Arid regions can be classified according to an aridity index (AI) that reflects the ratio of precipitation (P) to potential evapotranspiration (PET). Regions for which P/PET< 1, are classed as deserts (Pointing & Belnap, 2012). Using this definition, there are four key areas identified that differ substantially in general topography, climate and vegetation; sub-humid (0.5 - < 0.65), semi-arid (0.2 -<0.5), arid (0.05 - <0.2), and hyper-arid (<0.05; Figure 1).

Temperature is a key factor affecting vegetation and primary productivity in desert environments, as it is closely linked to aridity. Thermal extremes, in both hot and cold deserts, impose stresses in relation to strong seasonal and diurnal cycles (e.g., frequent freeze-thaw cycles). Cold deserts such as the Arctic and Antarctica generally have low average annual temperatures (0 ˚C to < 10 ˚C) with decreased rates of primary productivity in comparison to hot deserts such as the Namib and the Sahara, with average temperatures > 18 ˚C (Peel *et al*., 2007). This study will focus on the hot, hyper-arid Namib Desert.

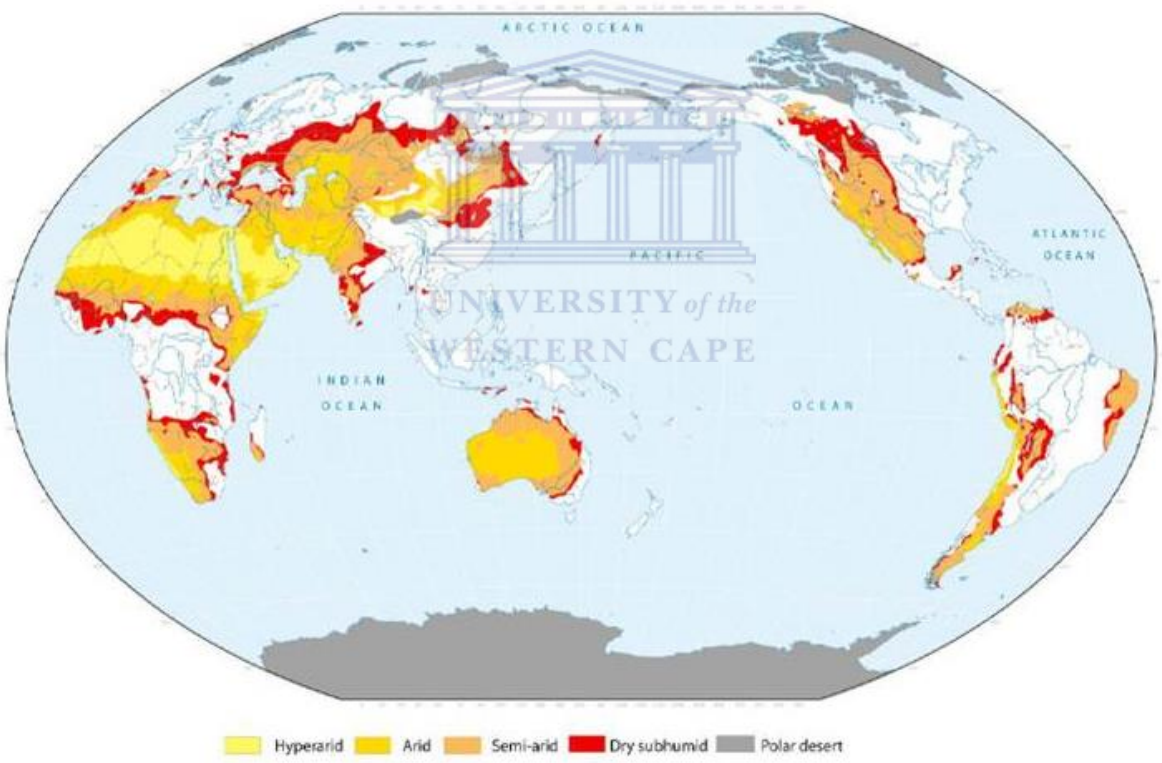

Figure 1: The global distribution of drylands on Earth. Various zones of aridity have been identified according to their aridity index; dry, sub-humid, semiarid, arid and hyper-arid regions (Chan *et al*., 2012).

1. The Namib Desert

1.1. Introduction

The Namib Desert is considered to be the world's most ancient desert, estimated to be 80 million years old (Prestel *et al*., 2008). It extends for over 2000 km from the Carunjamba River in Angola (S14° 16; E12°22) to the Olifants River in South Africa (S31°42; E18°11; Viles, 2005). The desert is located 120-200 km from the Namibian coast, bounded inland by the Great Escarpment to the South (Figure 2). The Namib covers a considerable latitudinal range, while its width is narrow in comparison, allowing rivers to flow from semi-arid regions in the east to hyper-arid coastal regions, feeding into the underground water table in the region (Koris *et al*., 2009).

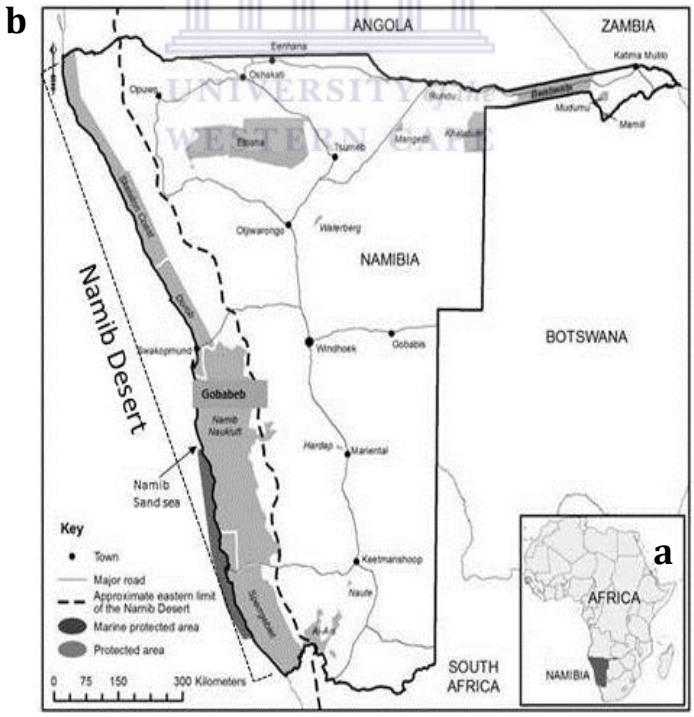

Figure 2: Location map of Namibia in Africa (a) and the Namib Desert in South-Western Africa (b). The Namib Desert stretches 120-200 km along the south-western coast of Namibia.

The regional topography of the Namib is highly variable and can be subdivided into these major areas: the Northern Namib, Central Namib Plains, the Namib Sand Sea, and the Southern Namib, which branches into the East Namib inland, and along the coast. The main landforms in these various regions are sand dunes, inselbergs, savanna grasslands, gravel plains and playas (Figure 3; Eckardt & Drake, 2011).

Figure 3: Photographs of prominent Namib Desert biotopes and landforms. a: Playas (salt pans) in the coastal Namib; b: Gravel plains in the central Namib illustrating bare soil with patches of dry *Stipagrostis sp.* a common perennial grass; c: Dunes with isolated patches of *Stipagrostis sp,*; d: the Kuiseb river flooding in 2011 (Courtesy of Prof. D.A Cowan and the Gobabeb Research and Training Centre).

The vast plains of the Namib are interrupted by dry riverbeds, extensive drainage networks and ephemeral rivers (Hachfield, 2000; Viles, 2005). A series of isolated saline springs are also observed throughout the Namib, the most notable being the gravel plain springs in the central Namib (Figure 3 a).

The ecology of the Namib has been studied extensively, focussing essentially on macro-biodiversity and adaptations to desert-imposed extremes (e.g., water vapour harvesting *Stenocara gracipiles*; Seely, 1979). Due to its distinct convergence of uniquely diverse biotopes, hyper-aridity, unusual water sources and strong climatic gradients, the Namib Desert represents a unique model to study ecosystem drivers in arid environments (Henschel & Seely, 2000; Henschel & Lanchester, 2012). However, studies on microorganisms inhabiting this environment have been severely lacking. Only recently have studies, investigating the adaptive strategies and mechanisms regulating microbial community structures predominantly concentrating on hypolithic (cyano)bacterial communities (Makhalanyane *et al*., 2012**;** Stomeo *et al*., 2013) and bacteriophages and their hosts (Prestel *et al*., 2008), been reported. In a recent study by Stomeo *et al*. (2013), the microbial communities structure of hypoliths (translucent rocks that are principally colonized by cyanobacteria) were compared to open soil communities in a well-established water availability gradient determined by fog and rainfall. The aim of the study was to gain insight into determinist processes that regulate the microbial community assembly. The study found significant structural differences between open soil bacterial community and hypolithic community structures that were most likely differentially influenced by water origin; i.e., rainfall or fog. Water bioavailability is therefore a key element driving microbial

community composition in the Namib Desert (Warren-Rhodes *et al.,* 2013). These studies demonstrate the influence of the Namib deserts' microclimate complexity on shaping microbial community structures in the central Namib and highlight the need for more research focussing on the edaphic microbial communities and the factors that shape them.

1.2. Climate and water availability

The Namib is a coastal desert with a long history of hyper aridity, the onset of which is estimated to have originated 5 million years ago (Ward *et al.,* 1983). The Namib Desert ranges from semi-arid in the highlands towards the central Namib Desert and onto the hyper-arid coast, receiving <20 mm of rainfall p.a. (Eckardt & Drake, 2011). As the Namib Desert is located at the interface of tropical, subtropical and temperate atmospheric and oceanic systems (Stone & Thomas, 2012), its regional weather patterns are influenced by several factors, the strongest of which are the cold Benguela current and the subtropical anticyclonic zone along the south-west coast of Africa. The Namib Desert is unique as it exhibits hyper-aridity yet also abundant and diverse, albeit highly variable, water sources (Schachtschneider & February, 2010). Fog, dew, rainfall and groundwater discharge (coastal springs and pans), constitute important sources of water in this desert. These include ephemeral rivers, aquifers, gravel plain springs, fresh water springs and pans (Schachtschneider & February, 2010; Eckardt *et al*., 2013).

Rainfall occurs mainly during the summer months (January-April), except in the southern regions of the desert which generally receive small amounts of rain during winter (Lancaster, 2002). Extreme rainfall variability (both spatially and temporally; Mattes &

Mason, 1998) with high rates of evaporation (Loutit, 1990), is experienced in the central and northern Namib, with a strong west to east rainfall gradient (Hachfield &, 2000). Notably, there have been long periods (up to 10 years in the coastal Namib) with no rainfall recorded (Shanyengana *et al.,* 2002). These characteristics make the Namib Desert one of the driest regions in the world (van Damme, 1991). Therefore, fog, as in the Atacama Desert (Warren-Rhodes *et al*., 2006), is an essential source of bioavailable water in this region.

Fog is an integral aspect of the Namib Deserts' hydrological cycle (Hamilton & Seely, 1976), although its significance decreases inland from the coast (Lancaster *et al*., 1984). Fog in the Namib Desert most frequently results from the warm air from the Hadley Cell mixing with the cold offshore Benguela Current (Eckardt *et al.*, 2013). It is more reliable than rainfall as a water source due to its decreased variability and high frequency of occurrence (precipitation due to fog is five times higher than precipitation resulting from rainfall; Seely & Henchel, 2000; Viles, 2005). The central Namib especially, is subject to frequent fog events, up to 200 days p.a. along the coast (Seely, 1979).

The Namib Desert has highly variable and generally low precipitation events (in the form of rainfall and/or fog) with high daily fluctuating surface temperatures (ranging from 0 °C to 50 °C; Eckardt *et al*., 2013). Therefore, all forms of life, and notably edaphic microorganisms, require unique adaptation strategies to survive these extreme conditions (Crits-Christoph *et al*., 2013), and to fulfil their crucial roles in biogeochemical cycling (Pointing & Belnap, 2012; Figure 4).

2. The Carbon Cycle in Terrestrial Desert Environments: A Focus on Microbial Communities

2.1. Biogeochemical Cycles in Desert Environments

Biogeochemical cycles are defined as 'the complex interplay between biological, geological and chemical processes, by which materials and energy are exchanged and reused at the Earth's surface' (Hedges, 1992). These cycles are mediated through a series of complex processes involving several microbial communities (Yergeau *et al*., 2007).

Microorganisms are key agents in mediating biogeochemical transformations and constitute reservoirs of several key elements on Earth. The six major elements cycling on Earth include carbon (C), hydrogen (H), nitrogen (N), oxygen (O), sulphur (S), and phosphorus (P; Falkowski *et al*., 2008). Some of these elements (for example, N, P, C and S), may be converted into recalcitrant organic forms and stabilized by interactions with inorganic soil constituents (Stevenson *&* Cole, 1999).

Although cycles of individual elements are complex, certain aspects of their cycling processes are shared, such as immobilization (conversion of inorganic compounds to organic compounds by plants or microorganisms) and mineralization (decomposition or oxidization of chemical compounds into bioavailable forms). Elements can exist in various forms and move between dynamic reservoirs, where the net amount of material exchanged is termed the reservoir flux. The turnover of a reservoir depends on how rapidly a reservoir is created or consumed and is largely determined by the reservoir size (Stevenson & Cole, 1999).

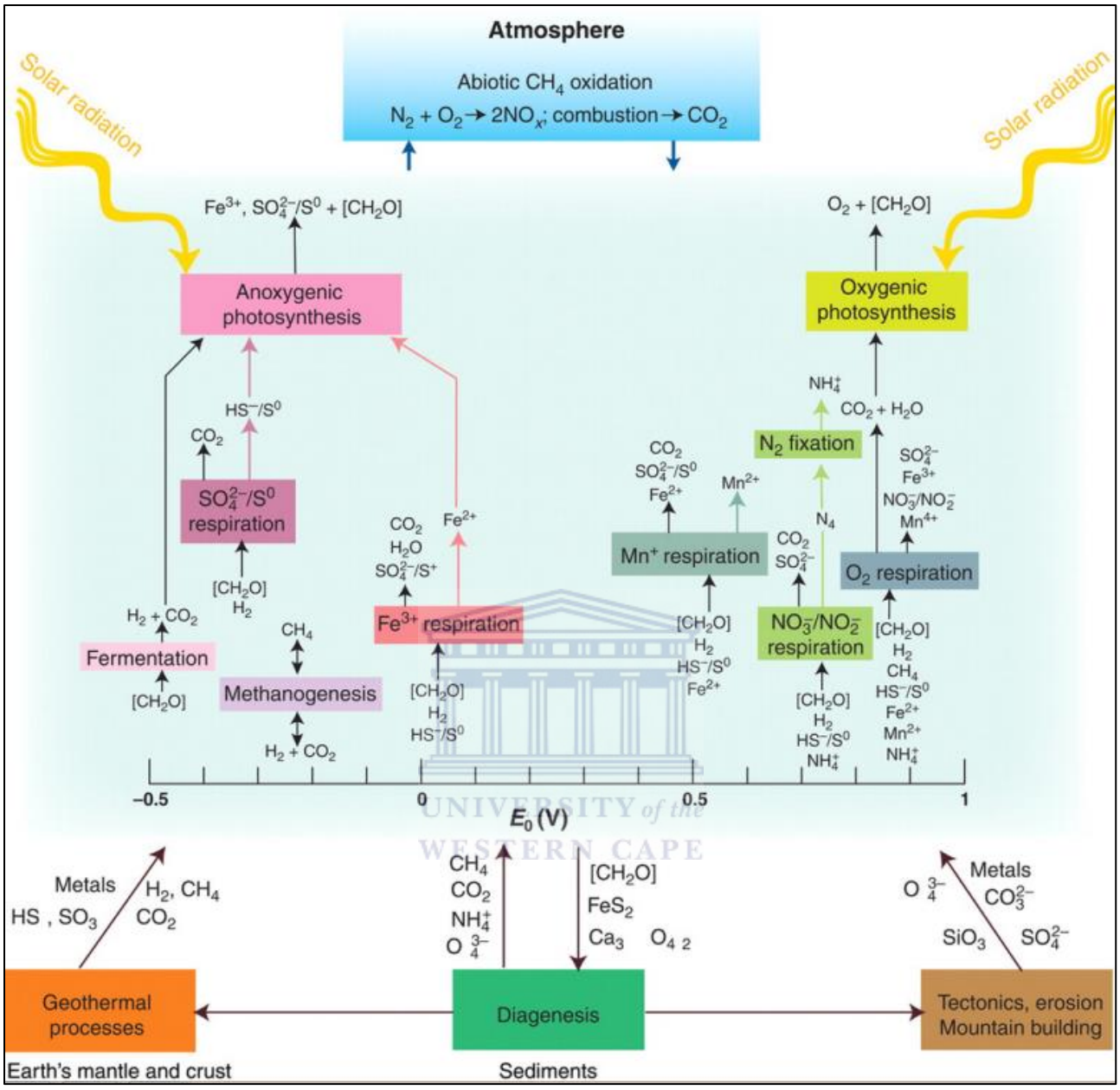

Figure 4: A generalized model of the biosphere showing energy inputs and outputs driven by microbial biochemical processes. Abiotic transformations are represented at the top as atmospheric transformations, and tectonic and geothermal transformations are at the bottom. Biogeochemical processes driven by microbial communities are represented by the biospheric compartments and in sediments (the middle section in blue; Falkowski *et al.*, 2008).

Desert environments are characterized by low levels of net primary productivity (NPP), defined as 'the net photosynthetic accumulation of carbon by plants' (Potter *et al*., 2012) and limited or absent vegetation cover. In arid and semi-arid regions that receive less than 600 mm of precipitation p.a., water bioavailability is the key limiting factor of NPP. Soil microbial communities respond rapidly to pulse water events (Figure 5), resulting in increased C and N mineralization, affecting microbial available substrates, as well as the nutrient immobilization and mineralization balance (Stevenson & Cole, 1999). Edaphic microbial communities thus play essential roles in key desert ecosystem processes such as C, P and N cycling, soil formation and stabilization, water infiltration and nutrient acquisition (Cable & Huxman, 2004; van Der Heijden *et al*., 2008).

The general assumption is that the C and N cycles are coupled in both marine and terrestrial cycles (Brookshire *et al*., 2005. However, this theory does not hold well in arid systems with pulse-patterns of rainfall, which cause cycles to be spatially heterogeneous (Collins *et al*., 2008; Gruber & Galloway, 2008). The frequency and paucity of wet-dry seasonal cycles in arid ecosystems determine the heterogeneous nutrient and vegetal cover as well as microbial mediated N and C turnover in these environments (Figure 5; Austin *et al*., 2004).

The major regulatory elements of N immobilization and mineralization by soil microbial communities in deserts are the ratio of C:N in microbial organic substrates and the efficiency of the communities to utilize N and C as nutrient sources. Of these factors, C:N ratio has been demonstrated to be the most important, as it can vary greatly between

different substrates and therefore largely impacts the balance between N immobilization and mineralization. C:N ratios have also been shown to greatly affect autotrophic respiration (Gruber & Galloway, 2008) and soil quality. N availability is a limiting factor of NPP in natural ecosystems and is therefore intimately linked to the C cycle (Vitousek & Howarth, 1991).

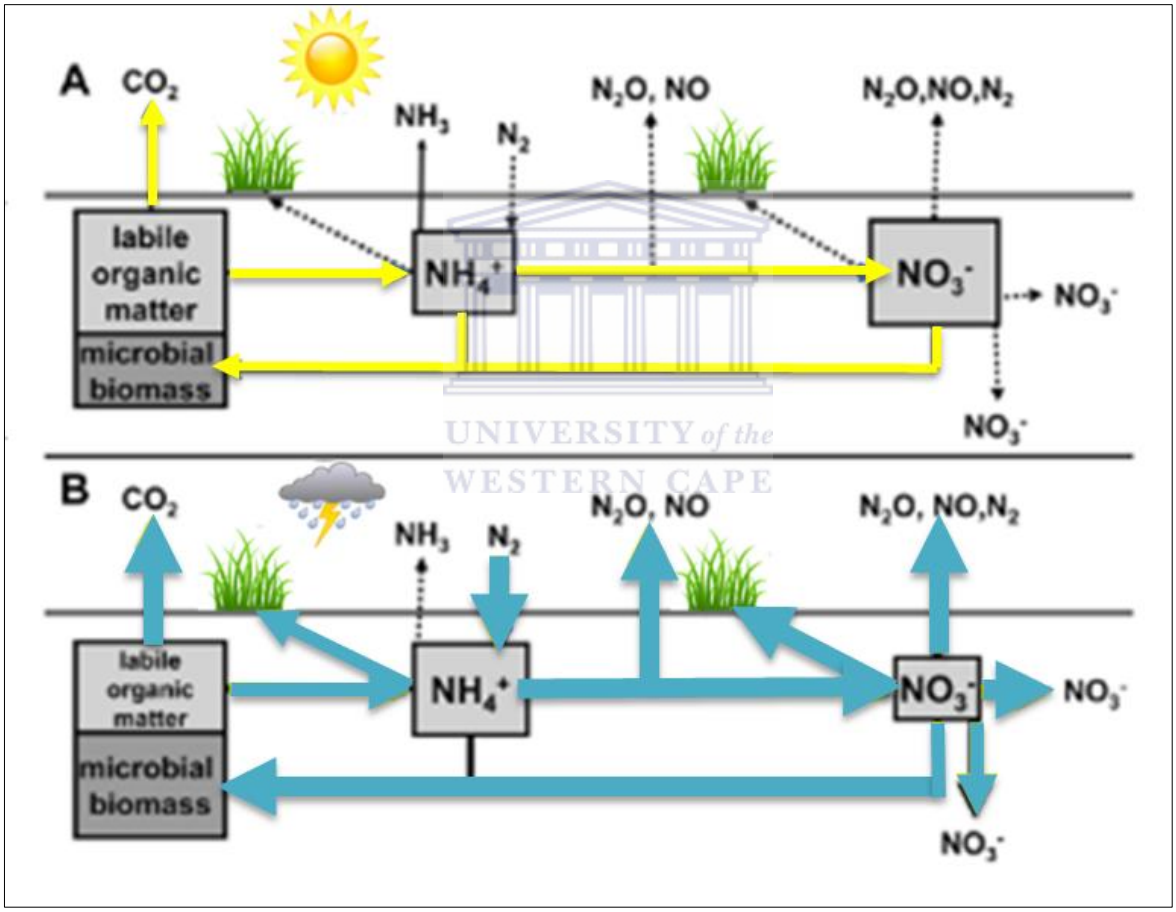

Figure 5: Schematic diagram of the C and N biogeochemical cycles in arid ecosystems under (A) dry conditions (yellow) and, (B) after a pulsed rainfall event (blue). Dotted arrows represent flows that are either low or undetectable, while the width of arrows represents the relative importance of the events under the two different conditions. Adapted from Austin *et al.* (2004).

N and P have been found to be major limiting factors to autotrophic growth in fresh water, marine and terrestrial environments (Elsar *et al*., 2007). In order to understand the mechanisms underlying P limitations, it is essential to understand the conceptual framework of general nutrient limitation which is more pronounced in desert systems as well as the complex interplay between the biogeochemical P and N cycles mediated by microbial communities in these extreme environments.

Biogeochemical models have been successful in predicting the cycling of C and N under stable conditions. However, within the context of global change, major discrepancies in the predictions of future biosphere-atmosphere fluxes and ecosystem feedbacks exists (Bolker *et al*., 1998). The magnitude of the effect of increased microbial activity on the global N cycle is still unclear. What is apparent is the intimate coupling between the processes driving N availability, C fluxes and microbial activity (Billings *et al*., 2002). It is therefore essential to understand the microbial diversity and function in mediating C, N and P cycles, especially in environments with extreme conditions such as deserts.

2.1.1. Phosphorus (P) cycle

P is not cycled through the atmosphere and is thus dominated by geological factors. The loss of P from a system can only be replenished through primary minerals such as apatite, which is essentially mediated by complex microbial communities. P cycles between the biosphere, hydrosphere and lithosphere (Richardson *et al*., 2009).

The lithosphere represents a crucial reservoir of P, and is therefore a major element of soil organic matter (SOM), where its primary source is the weathering of minerals from parent rock material (Vitousek, 2004). The reservoir of P in soil is dynamic and subject to variation in response to water, temperature and C availability (Richardson & Simpson, 2011). In soil, P exists both in inorganic fractions and in organic forms (e.g., orthophosphate; PO). Inorganic P is adsorbed (attached) to the mineral surfaces, whereas organic P is either adsorbed or assimilated to biomass, or linked to SOM. While the bulk of soil P is fixed or absorbed to soil particles, a fraction of P is lost by leaching. Mineralogical transformations in soil cause continual sequestration of P, thereby making soil age a key factor in P limitation (Vitousek, 2004). Precipitated forms of P, such as calcium-phosphates (Ca-P), are the dominant forms of P in desert soils under alkaline conditions. Ca-Ps are effectively solubilized by organic microbial or plant -related cations and anions (Khan *et al*., 2009). They are also effective in chelating metal ions normally linked to complex forms of soil P or by facilitating the adsorption of orthophosphate (PO) or organic P via ligand exchange reactions (Ryan *et al*., 2001).

The immobilization and solubilization, mineralization and redistribution of soil P are dependent on a range of physio-chemical properties including P sorption by colloidal surfaces, and plant and microbial P uptake (Stewart & Tiessen, 1987). These processes are mediated by microbial groups involving *Rhizobium, Enterobacter, Bacillus, Pseudomonas, Azospirillum and Azobacter* (Figure 6; Oberson & Joner, 2005; Khan *et al*., 2006; Richardson & Simpson, 2011). Edaphic microorganisms mediate P availability in soil through a range of mechanisms. Briefly, these include facilitating organic P mobility (either directly or indirectly) via microbial turnover and inducing metabolic processes to solubilize and

mineralize P from limited available forms of inorganic and organic P in soil (Seeling & Zasoski, 1993; Richardson *et al*., 2009).

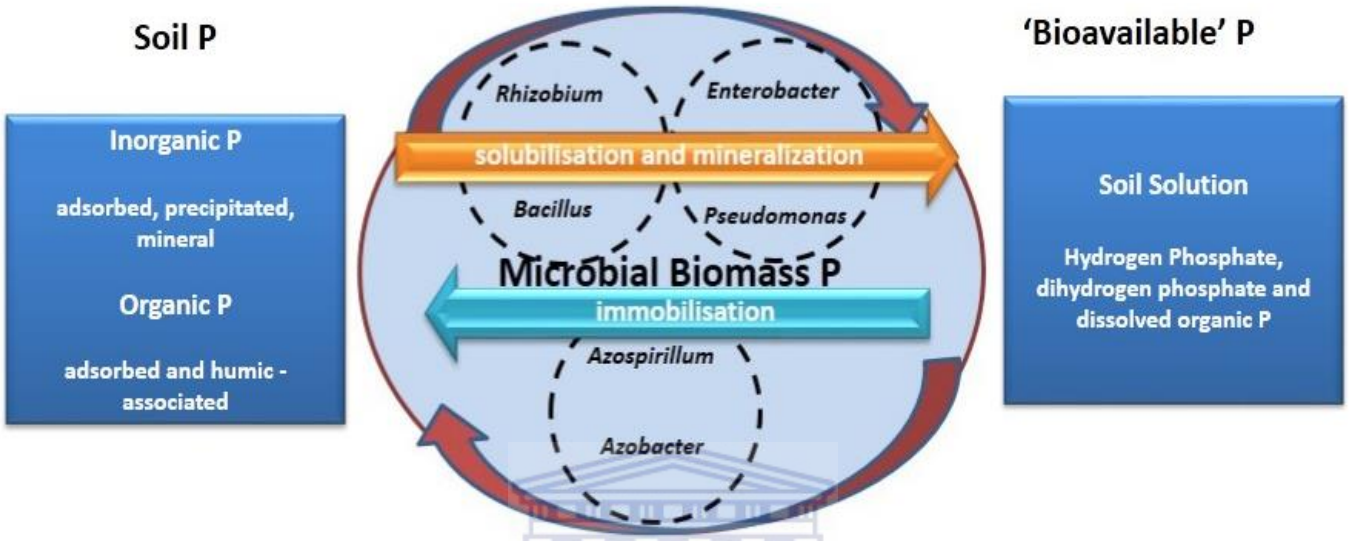

Figure 6: Schematic representation of the P cycle highlighting the importance of microbial communities to P availability in soil. Phosphorus exists as either soil P which consists of inorganic and organic phosphorus, or 'bioavailable' P which forms part of the 'soil solution' and includes hydrogen phosphate, dihydrogen phosphate and dissolved organic phosphorus Adapted from Khan *et al*. (2009).

The rate of P solubilization is determined by microbial activity through the release of metabolites such as organic acids and mechanisms, including organic acid production and proton extrusion (Sagoe *et al*., 1998; Khan *et al*., 2009). Thus, P availability is largely due to the rate of the reactions replenishing the soluble P reservoir (Ryan *et al*., 2001). Microorganisms thus compete for the limited available P; i.e., orthophosphate (PO), with plants from the soil solution. Microbial P pools therefore represent a key reservoir of

immobilized P, temporarily unavailable to plants (Richardson & Simpson, 2011). The uptake of P by edaphic microbial communities and its release and translocation, have major impacts on soil P availability to plants and the biogeochemical cycling of P (Seeling & Zasoki, 1993; Oehl *et al*., 2004).

2.1.2. Nitrogen (N) cycle

N is a key element used by organisms to form complex organic compounds including amino acids, nucleic acids and proteins. N thus drives key metabolic processes associated with growth and energy transfer (Falkowski *et al*., 2008; Butterbach-Bahl & Gundersen, 2011). Atmospheric N is highly inert with a residence time of 1 billion years and is cycled in various forms (both physical and biological processes) throughout all spheres (Figure 7; Gruber & Galloway, 2008; Falkowski *et al*., 2008).

N cycling in terrestrial ecosystems is well studied and involves complex microbial, physiochemical and plant physiological processes (Butter-Bahl *et al*., 2011). N cycling in soil is characterized by numerous N transformations involving both organic (ammonium (NH_4^+)) and inorganic (nitrate (NO_3^-)) N species and the immobilization of N by plants and microorganisms. Atmospheric N exists as dinitrogen (N_2) and is inaccessible to many organisms in this form. N_2 fixation into ammonia (NH_3) initiates the cycle which is catalyzed by nitrogenase (encoded in the *nif* gene; Zehr *et al*., 2003).

Under acidic conditions, NH_3 is readily converted to NH_4. Nitrogen fixation is a biological process involving the oxidation of NH_4 resulting in the production of nitrite (NO_2^-) by ammonia oxidation and NO_2^- into nitrate (NO_3; Seefeldt *et al*., 2009). The first step of NH_4^+ oxidation is catalyzed by the ammonia monooxygenase enzyme, encoded by the *amo*A gene (Figure 7). This gene has been extensively studied as a molecular marker for ammonia oxidizing bacteria (AOB) and archaea in terrestrial ecosystems (Zumft, 1997). Nitrate reductases (encoded by either the *nar* or *nap* genes) catalyzes the reduction of NO_2^- into NO_3^- (Zumft, 1997; Jia & Conrad, 2009). NO_3^- can be reduced further by one of three anaerobic pathways (Offre *et al*., 2013). These include denitrification, the step-wise process of the reduction of soluble NO_3- (through NO_2- and NO to gaseous nitrous oxide (N_2O)) encoded by *nir*S and *nir*K, the formation of NH_4 by dissimilary nitrate reduction (DNRA), or the formation of N_2 by anaerobic ammonium oxidation (annamox; Mulder *et al.,* 2006). DNRA is catalyzed by formate dehydrogenases encoded by the *nrf*A gene (Darwin *et al*., 2006).

N enters the soil and becomes part of the SOM matrix following internal N cycling through plants. Extracellular microbial enzymes depolymerize SOM and the resultant cleavage of macromolecules results in bioavailable N for plants and microorganisms. Biological N fixers have the capacity to convert N_2 to biological N substantially at ecosystem scales. This is most commonly associated with symbiotic N_2 fixation of the bacterium *Rhizobium* with plant root nodules, although free-living N fixers also exist (e.g., *Azospirillum*). N can further be converted into NH_4^+ (ammonification or N mineralization) aerobically or anaerobically (Butterbach-Bahl & Gundersen, 2011).

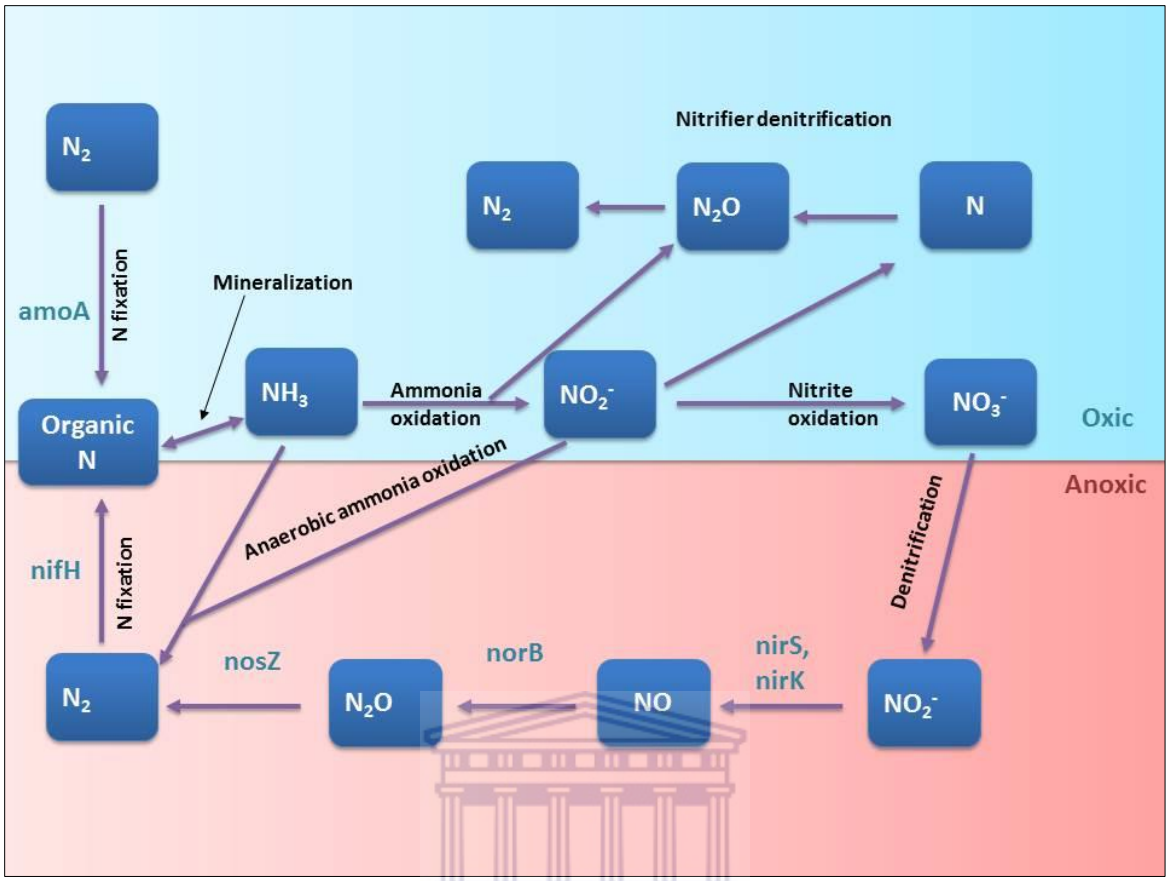

Figure 7. Schematic representation of the microbial mediated N cycle. The major biological transformation pathways and genes encoding enzymes are presented. Oxic (in blue) and anoxic (in red) microbial mediated N processes are shown. Adapted from Offre *et al*. (2013).

N fixation is essential for ecosystem function and sustainability and microbial N fixers (e.g., N fixing cyanobacteria) are therefore key drivers of N biogeochemical cycling Galloway *et al*., 2003. In deserts with limited vegetation, microbial communities i.e., soil biological crusts (SBCs) and/or hypolithic communities, mediate N cycling, in particular diazotrophy; i.e., the processes by which N is fixed into a bioavailable form (Pointing & Belnap, 2012).

Hypoliths have been demonstrated to be key drivers of N fixation in both cold and hot deserts (Cowan *et al*., 2011).

Increased N availability leads to eutrophication of terrestrial and water sources, increased release of greenhouse gas nitrous oxide (N_2O) from soil and soil and water acidification as a result of reactive N (N_r) deposition (Galloway *et al*., 2003; Erisman *et al.,* 2008). Because of these environmental threats, it remains important to understand N dynamics in desert ecosystems as they are more susceptible to accelerated rates of climate changes.

2.1.3. Carbon (C) cycle

Globally, there are three major reservoirs of C: the oceanic pool, the atmospheric pool and the terrestrial pool (Batje, 1996; Kirkby *et al*., 2013). Terrestrial C storage is one of the largest global C pools, twice the size of the atmospheric and biotic pools combined (2500 Pg) and represents an important reservoir in the global cycling of C throughout the different C pools (Rayment & Jarvis, 2000; Kirkby *et al*., 2013). C in soil exists in two forms: soil organic C (SOC) and soil inorganic C (SIC). SOC forms part of SOM, a dynamic, functional component in terrestrial ecosystems. Fluxes in SOM structure and composition have great impacts on ecosystem processes, most importantly on soil C reservoirs and fluxes (Figure 8; Batje, 1996). SIC is especially significant in soils in arid regions (Lal, 2004), where inorganic C is primarily in the form of carbonate (Wang *et al*., 2002). The terrestrial C reservoir is likely to be affected by N deposition, C mineralization, soil management practices, land-use change, water bioavailability and edaphic microbial communities (Davidson & Janssens, 2006).

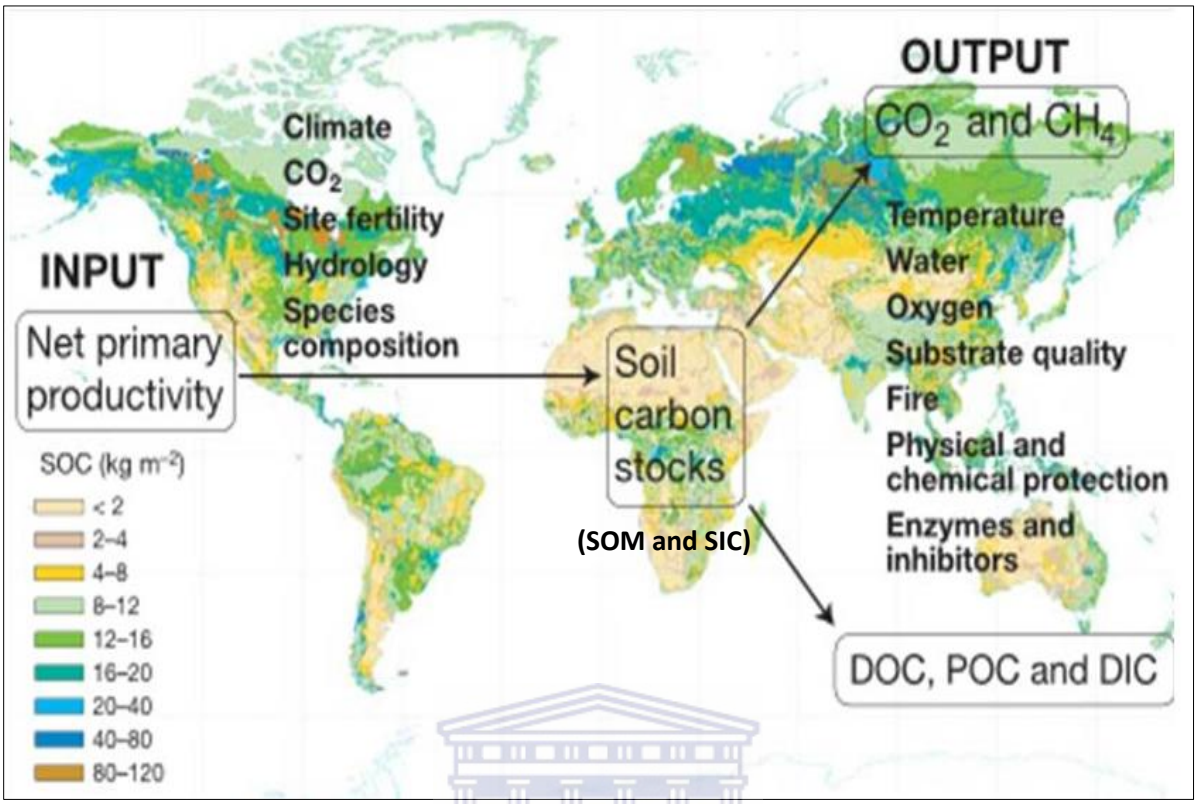

Figure 8: Achematic representing the key parameters influencing major soil C inputs (net primary productivity) and outputs (CO_2 and CH_4 ; Davidson & Janssens, 2006).

The C balance of terrestrial ecosystems is adversely affected by anthropogenic activity such as pollution, deforestation, biomass burning and land-use practices which increase the release of 'greenhouse gases' (e.g., CO_2 and CH_4). As levels of atmospheric CO_2 and CH_4 are predicted to increase globally at a steady rate, it is important to understand the effect of global warming on the activity of soil microbial communities, especially within the context of global biogeochemical cycling (Tabita *et al*., 2007). The cycling of C is essential for NPP in terrestrial ecosystems (Bardgett *et al*., 2008). C enters terrestrial ecosystems through photosynthesis and is emitted from soil though a variety of processes collectively termed as respiration (Trumbore, 2006).

Due to increased global awareness of climate change, there is a growing focus on soil microbial communities, and their active role in global C cycling (Mazzarino *et al*., 1993). However knowledge gaps exist in the microbial mediation of key C sources and the contribution of edaphic cyanobacteria to the regional C cycle in deserts (Pointing *et al*., 2012).

2.2. Terrestrial Microbial-Mediated C cycle

C is cycled through the environment via a series of fixation, decomposition and respiration processes (Shiveley *et al*., 2001) involving multiple complex microbial communities including bacteria, archaea, fungi (Figure 9; Nielsen *et al*., 2011) and viruses, representing the microbial loop (Kimura *et al*., 2008).

There is a delicate balance between primary productivity (photosynthesis) and organic matter decomposition which must be maintained in order to preserve soil source/sink dynamics (Bardgett *et al*., 2008). Once C has been decomposed by both bacterial, archaeal and fungal- degraders, C is released into the atmosphere in the form of CO_2 and CH_4 gases. Microbial decomposition is responsible for 86% of CO_2 produced worldwide (Shiveley *et al*., 2001).

The functional diversity of microbial communities mediating N cycling in soil (e.g., N fixation and denitrification) has been well studied (Carreiro *et al*., 2000; Coleman & Whitman, 2005). In contrast, the microbial contribution to the terrestrial C cycle is not well understood. This lack of knowledge is due to the complexity of the cycle and the great diversity of microorganisms and functions involved (Nielson *et al.,* 2011).

In general, there are four major microbial groups involved in the C cycle (Figure 9); methanogens (archaea; mediate the formation of CH_4 by converting either bicarbonate (CHO_3-) or CO_2 and other C-compounds including formate), Methanotrophs and Methylotrophs (convert CH_4 into CO_2), primary producers such as cyanobacteria (fix C by converting CO_2 into carbohydrates, and heterotrophic bacteria and fungi (convert organic C into CO_2).

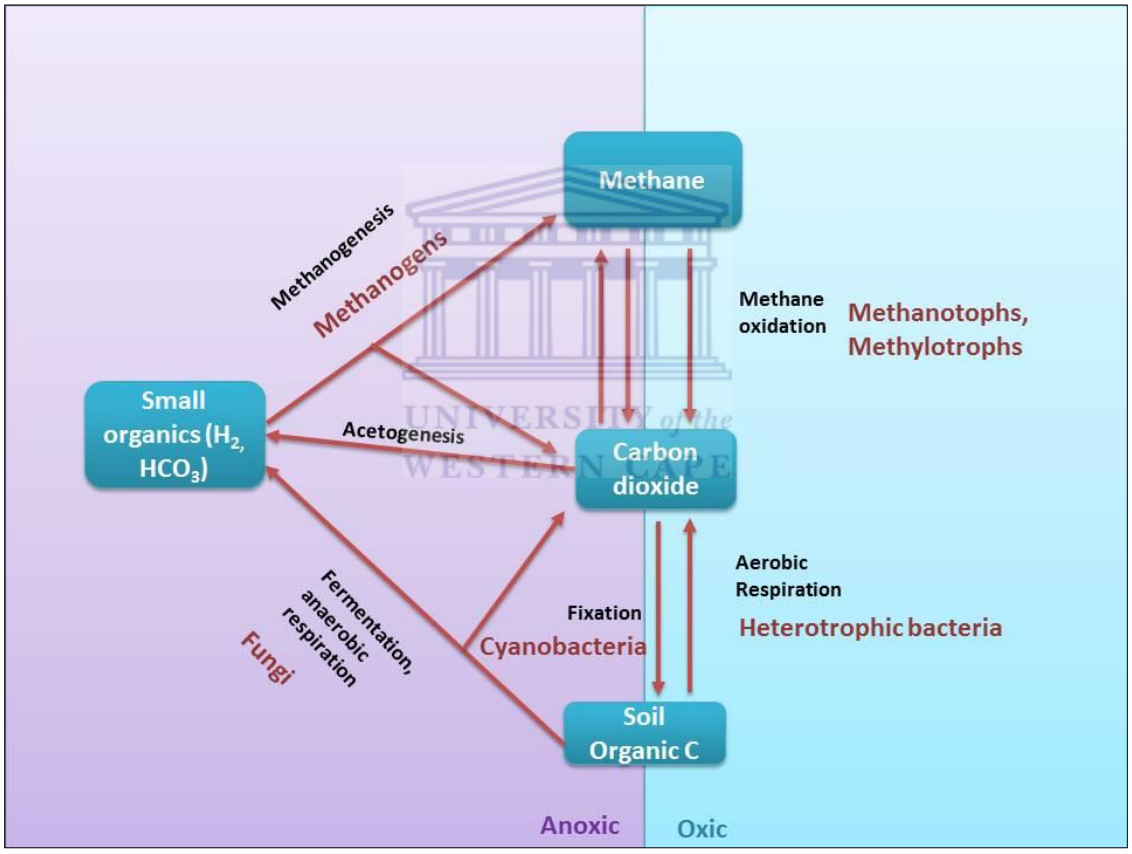

Figure 9: Simplified schematic representation of the major microbial groups and processes mediating C turnover in the global C cycle. A complex mixture of methanogens, fungi, methanotrophs/methylotrophs and heterotrophic bacteria are involved in specific aspects of the microbial-mediated global C cycles. Adapted from Offre *et al.* (2013).

2.2.1. Microbial C fixation: CO_2 → CH_2O

Plants and specialized autotrophic microbial communities (photoautotrophs e.g., cyanobacteria) have evolved the ability to fix and concentrate large amounts of atmospheric CO_2 (Tabita *et al*., 2007). C can also be fixed anaerobically by acetogenic bacteria or archaea (chemolithoautotrophy) via complex microbial pathways (Yamanaka, 2008), or through archaeal methanogenesis (Conrad, 2009). However, CO_2 is removed from the atmosphere and translocated into terrestrial systems principally through photosynthesis, (Paterson *et al*., 2009).

CO_2 fixation is therefore an important step in the C-cycle and has been extensively studied (e.g., Shively *et al*., 1998; Selesi *et al*., 2005; Saini *et al*., 2011). C fixation pathways include the Calvin-Benson-Bassham (CBB) pathway (or Calvin cycle), Arnon-Buchanan cycle or reductive tricarboxylic acid (rTCA) cycle, the Acetyl-CoA pathway or Wood- Ljundahl pathway, the 3-Hydroxypropionate (3HP) bicycle (Fuchs- Holo bicycle). The CBB pathway has been reported in both plants and diverse microbial populations and is the main cycle in terrestrial C-fixation (Saini *et al*., 2011). The CBB cycle is especially important in extreme arid environments where soils contain limited nutrients, and this cycle is well studied in these regions (Bliss & Gold, 1994; Shively *et al*., 1998; Montaya *et al*., 2012). The key enzyme involved in this process is ribulose-1,5-biphosphate carboxylase and is present in plants, algae and cyanobacteria (Shively *et al*., 1998; Montaya *et al*., 2012).

In deserts where vegetation cover is low, organic C accumulation is heavily dependent on C fixation by cyanobacteria that form part of the active biological soil crusts (BSCs; Pointing

& Belnap, 2012) and hypolithon communities (Warren-Rhodes *et al*., 2006). Cyanobacteria are ancient photoautotrophs estimated to be about 3.5 billion years old and are thought to have converted the early reducing atmosphere into an oxidizing environment through photosynthesis (Schopf, 1996). Cyanobacteria have colonized virtually all habitats on earth, possibly as a result of their accumulative physiological adaptations over the period of their existence, and are considered the most dominant phototroph in marine and terrestrial ecosystems (Taton *et al*., 2006). In deserts, cyanobacteria constitute the main members of photosynthetic microbial communities in cryptic niches, the hypolithons (i.e. under quartz rocks; Pointing & Belnap, 2012; Makhalanyane *et al*., 2012). Moreover, in such extreme environments lacking non-vascular vegetation, they constitute key species in enabling the cycling, and especially the fixation, of C and N (Pointing *et al*., 2007; Wong *et al*., 2010).

2.2.2. Microbial C Decomposition: $CH_2O \rightarrow CO_2$; $CH_2O \rightarrow CH_4$

Soil microbial communities are the major decomposers of C sources (either aerobically or anaerobically), as they are among the few organisms in soil that possess the necessary enzymes to degrade recalcitrant plant-associated compounds (Hättenschwiler *et al*., 2005). Bacteria and fungi play key roles in nutrient cycling in soil, especially C and N cycling, and are responsible for 90% of the total SOM decomposition (Swift *et al*., 1979).

The major determinant factors influencing decomposition rates are climate, soil moisture, substrate chemical composition, litter quality, C:N ratios, lignin content, nutrient availability (Bardgett *et al*., 2008; McGuire & Treseder, 2010), the direct activity and the composition of microbial communities and their association with soil animals including

nematodes and earthworms (Hättenschwiler *et al*., 2005; Davidson & Janssens, 2006; Yuste *et al*., 2007; Nielsen *et al*., 2011). Temperature sensitivity of soil C is thought to increase in proportion to the complexity of the substrate (Bosatta & Agren, 1999; Davidson & Janssen, 2006) and is affected by environmental constraints such as drought (arid conditions) which inhibit extracellular enzyme diffusion and the degradation of soluble organic-C substrates (Collins *et al*., 2008).

The decomposition of plant biomass is an essential step in soil organic matter formation, C balance in terrestrial ecosystems and the mineralization of organic nutrients. The major component of biomass is lignocellulose which consists of three types of polymers namely; cellulose, hemicellulose and lignin (Pérez *et al*., 2002). The degradation of lignocellulose requires the co-operation of complex microbial communities and various enzymes (Wongwilaiwalin *et al*., 2010) which play important roles in the cycling of organic carbon. Cellulose is the most abundant source of C in soils, and its enzymatic hydrolysis is an important step in the C cycle (Wilson, 2011).

Microbial degraders of cellulose in soil are central to the flow of energy from plants to other trophic levels, resulting in the release of atmospheric CO_2 (el Zahar Haichar *et al*., 2007). Cellulose is an essential substrate to microorganisms including bacteria (e.g., notably *Cellulomonas* and *Cytophaga*) and fungi (Basidiomycota and Ascomycota; Sukumaran *et al*., 2005). The products of lignocellulosic biodegradation subsequently serve as substrates for other microbial species unable to degrade cellulose-like compounds. This enables rapid cellulose decomposition rates which essentially increases fungal diversity. It

has been suggested that fungi, rather than bacteria, dominate decomposition of C substrates in arid ecosystems, predominantly as a result of their innate ability to degrade both polysaccharides and polyphenols in SOM (Baldrian *et al*., 2011).

The biological production of methane (CH_4), the ultimate step of the C decomposition process, is essential in the global C cycle and is primarily due to archaeal methanogenesis (Conrad, 2009). CH_4 plays essential roles in the atmosphere and is a potent greenhouse gas. The majority of CH_4 produced however, is not released into the atmosphere, but converted to biomass by methanotrophic bacteria (metabolize CH_4 as their only source of C and energy; Shively *et al*., 2001). Three methane sinks exist, the largest being the photochemical oxidation of CH_4 introduced by the reaction of OH radicals. The remaining two sinks are microbial CH_4 oxidation into soil and stratosphere diffusion (Conrad, 1996). H_2 serves as a potential substrate for methanogenic archaea. The production of CH_4 mainly occurs under anaerobic conditions, where the decomposition of SOM or other oxidants including nitrate, sulphate or ferric iron (Lelieveld *et al*., 1998). The CH_4 produced serves as a substrate for either aerobic or anaerobic methane oxidation (Liu & Whitman, 2008) or is emitted to the atmosphere Under anaerobic conditions, methanogenesis represents the final step of organic matter degradation (Conrad, 1999; Watanabe *et al*., 2009) resulting in the production of acetate, CO_2, and H_2 which is released into the atmosphere (Shively *et al*., 2001).

Methanogens use three pathways in the production of CH_4, in which the key enzyme is methyl-coenzyme M reductase gene (MCR): the reduction of CO_2, fermentation of acetate and the disproportionation of methanol and methylamines (Thauer, 1998). During the final stage of Ch_4 production, MCR catalyses the reduction of a methyl-coenzyme M forming Ch_4 (Luton *et al*., 2002; Inagaki *et al*., 2004).

In marine environments, archaea (mainly from the order *Methanosacrinales*) are involved in methanogenesis, linked with sulfate-reducing bacteria (Inagaki *et al*., 2004). Sulfate-reducing bacteria are anaerobes that utilize sulphate as the terminal electron acceptor in the degradation of organic compounds to produce sulphide. The reduction of sulphate accounts for approximately 50 % of organic C mineralization and in anoxic environments, the sulphur cycle is intimately linked with the C and N cycles (Muyzer & Stams, 2008).

2.2.3. Microbial Respiration: $CH_2O \rightarrow CO_2 + H_2O$ + Energy

Respiration represents the flow of C from organic to inorganic pools. The cycling of organic C is mediated via the activity of heterotrophic bacterial communities and viral lysis (Coleman *et al*., 1992; Jahnke & Craven, 1995). Soil respiration (CO_2 emission) produces approximately 80 Pg of CO_2 annually (Li *et al*., 2005) and constitutes the predominant release of C into the atmosphere. The process of soil respiration is divided into an autotrophic (involving plant roots and their associated microorganisms, e.g., mychorrhiza fungi and bacteria), and heterotrophic (resulting from soil C degradation) cycle of C turnover. Heterotrophs convert organic C and related nutrients into carbohydrates, lipids and proteins photosynthesis (Schlesinger & Andrews, 200).

3. Investigating soil microbial communities

Microbial diversity in soil is estimated to be extremely high (thousands of species in 1 g of soil, Hättenschwiler *et al*., 2005) with the majority of microorganisms still being uncharacterized (Fierer *et al*., 2007). Furthermore, a lack of taxonomic knowledge (Kirk *et al*., 2004) and the complexity of soil microbial communities limit the meaningful interpretation of microbial ecology in the context of environmental parameters (Fierer *et al*., 2009). However, the increasing interest in microbial soil ecology, especially in understanding the relationship between microbial community dynamics and climate variations, has resulted in the rapid progression of various molecular techniques that enable these relationships to be probed (Andrén *et al*., 2008; Fierer *et al*., 2009).

3.1. Assessing the sampling strategy

Soil is a dynamic and complex environment, influenced by a combination of physiochemical, biological and environmental factors (e.g., pH, temperature; Bronick & Lal, 2005). The wide-ranging heterogeneity of soil varies across spatial (Ritz *et al*., 2004) and temporal scales (Rayment & Jarvis, 2000) and is inhabited by highly heterogenous microbial communities (Baker *et al*., 2009).

In an attempt to obtain reliable and reproducible samples that lend themselves to comparisons and statistical scrutiny (Gawlik *et al*., 2003), soil sampling strategies should be informed by the dynamic relationship between soil heterogeneity and microbial communities (Baker *et al*., 2009). The spatial scale of a study site determines the degree of sample variation (Ettema & Wardle, 2002) and therefore requires techniques of sufficient

resolution to detect small scale differences (Bending *et al*., 2006). Frequently, differences between samples are assumed to be negligible and hence sample pooling is a regular practise among microbial ecologists. This practise grossly underestimates microbial diversity (Green & Bohannan, 2006) and destroys biological variability required for intra (within)- and inter (between) - sample comparisons (Prosser, 2010).

While it is commonly accepted knowledge that microorganisms in soil possess the ability to detect and respond rapidly to environmental stimuli such as variations in temperature, water and nutrient availability (Fenchel, 2002), there is currently a lack of knowledge regarding the timescale required to observe shifts in microbial communities relative to environmental impacts (Cain et al., 1999, Andrén *et al*., 2008). Significant seasonal shifts in the microbial community structure have been observed in terrestrial ecosystems as a result of microclimatic influences (Schmidt *et al*., 2007), but high-resolution experiments regarding environment-specific conditions relating to community change are lacking. Environments with high temporal variations require the use of detailed spatial-statistical analyses (Cain *et al*., 1999) employing methods that possess the sensitivity to detect major as well as subtle variations (Cain *et al*., 1999; Andrén *et al*., 2008).

While studies have investigated the effect of spatial and temporal variability on microbial community dynamics (e.g., Schmidt *et al*., 2007; Weisskopf *et al*., 2008), the use of small sample sets limits these studies for extrapolation to larger geographical areas and reduces the meaningful interpretation of the datasets (Cao *et al.,* 2002). An example of poor sampling methodology employed is demonstrated in a study by Bell & colleagues (2009).

Microbial community responses to temporal variations of moisture and temperature were investigated in the Chinuanhuan Desert. In this study, 12 3 x 3 m plots were sampled biannually for a period of 3 years. The results from the study are unsubstantiated as it is not possible to statistically assess biological variability between samples that lack a well-replicated sampling design. A sampling design that is truly representative of a study site requires a holistic examination of all testable parameters that may exert influences on the fluctuating biological system. This is highlighted in a study by Baker *et al*. (2009). Soil samples were collected from 40m x 45m plots using a sampling grid and a random number generator in an attempt to obtain unbiased sampling. They employed 2 different experimental designs, with a large number of independent replicates as well as pooled samples for comparisons. Variability introduced from DNA extractions were minimized by repeating the experiment in triplicate and amplifying the pooled DNA template. T-RFLP analysis of the PCR amplicons revealed a decrease in variability of the pooled samples, in comparison to individual samples (of both pH and bacterial community composition across spatial scales), substantiating the need for sample replicates when studying natural environments (Knight *et al*., 2012). The study of edaphic microbial communities thus requires robust sampling strategies, complemented by molecular fingerprinting techniques (Prosser, 2010; Jansson & Prosser, 2012).

3.2. Community Fingerprinting Techniques

Previous culture-based techniques characterized microorganisms on the basis of morphological, physiological and biochemical properties (Nocker *et al*., 2007). The isolation and cultivation of soil microorgansims only access 0.1% - 1.0 % of the total gene

complement and thus the majority of microorganisms in soil remain uncultured. This is largely due to the inability to understand and thus accurately reflect environmental conditions to grow microorganisms (Muyzer & Smalla, 1998) as natural soil environments are complex and highly heterogenous (Daniel, 2005). Culturing methods therefore, inaccurately reflecting true microbial composition within natural habitats (Hugenholtz, 2002; Aguilera *et al*., 2006).

The direct isolation of DNA from soil yields total microbial genomic DNA, termed the metagenome (Rondon *et al*., 1999). Metagenomics circumvent the limitations and biases associated with culturing techniques, and involve the extraction, amplification and analysis of the complete genetic complement of an environment. This allows direct access to a diverse range of novel genes and their products (Schmeisser *et al*., 2007).

Molecular-based techniques are widely used to investigate microbial populations in natural ecosystems (e.g., Makhalanyane *et al.,* 2013). Fingerprinting techniques targeting molecular markers such as the 16S ribosomal RNA (bacteria; Muyser & Smalla, 1998) and 18S rRNA genes (fungi; Fierer *et al*., 2007) enable the assessment of community diversity. The rRNA molecule contains highly conserved domains comprised of functionally important sequence information (Osborn *et al*., 2000). The polymerase chain reaction (PCR) amplification of the 16S rRNA gene using universal primers enables phylogenetic studies of complex soil microbial communities (Nocker *et al*., 2007). This has allowed for the study of microbial communities in a variety of habitats to analyse cross-biome metagenomics (Fierer *et al*., 2012). Despite these numerous advantages, metagenomic

techniques are subject to the limitations of variable DNA extraction and biases introduced by PCR (Blackwood *et al*., 2003).

The increasing awareness of the fundamental importance of microorganisms in ecosystem processes has encouraged the investigation of microbial diversity and stability in relation to environmental and anthropogenic perturbations. Molecular microbial fingerprinting techniques enable the differentiation between sequences without the need for sequencing (von Wintzgerode *et al*., 1997), allow the analysis of complex microbial assemblages in natural environments and are reproducible, affordable and provide rapid results (Brown *et al.,* 2005). These techniques include denaturing gradient gel electrophoresis (DGGE; Muyser & Smalla, 1998), automated ribosomal intergenic spacer analysis (ARISA; Fisher & Triplett, 1999), and terminal restriction fragment length polymorphism (T-RFLP; Liu *et al*., 1997).

DGGE is a popular, if now old-fashioned, technique that uses sequence variation in ribosomal RNA to provide an overview of the community diversity profile (Kirk *et al.,* 2004). This technique allows for the separation of PCR amplicons migrating under a gradient of increasing denaturing strength (denaturants are usually urea or formamide; Figure 10). The DNA fragment is only partially denatured due to primers that incorporate a G-C clamp (30-50) nucleotide sequence consisting of guanines (G) and cytosines (C), allowing for the differential separation of fragments (Muyzer *et al.,* 1993).

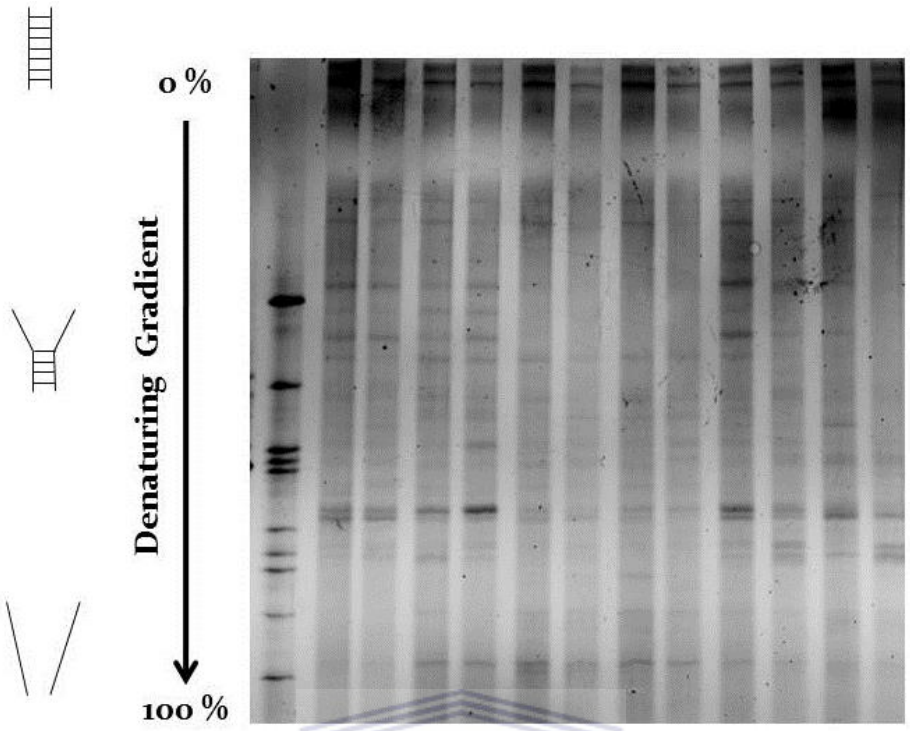

Figure 10: Community fingerprinting profile generated by DGGE analysis. DNA fragments partially separate along a gradient of increasing denaturing strength.

Limitations of DGGE include insufficient discriminatory sensitivity, and the possibility that organisms may contain multiple copies of 16S rRNA genes with accumulated mutations, leading to overestimation of community diversity (Kirk *et al.,* 2004). To increase the integrity of the diversity profiles obtained by DGGE, results can be qualitatively analysed in conjunction with other molecular techniques such as T-RFLP and ARISA (Boon *et al*., 2002; Kirk *et al.,* 2004).

ARISA is a method that exploits the variability of the length of the intergenic spacer (ITS) region between the 16S and 23S genes. This region has been found to contain various tRNAs that can be used to discriminate between bacterial species (Fisher and Triplett,

1999) and fungal species (Ranjard *et al*., 2001). As with T-RFLP, ARISA negates the use of culture-dependent methods and involves the use of fluorescently labelled PCR primers. However, a drawback of this method is the inability to measure OTU abundance (Ramette, 2009).

T-RFLP analysis is a high-throughput fingerprinting method that is frequently employed to detect and monitor changes in microbial community composition and structure. The gene of interest (e., 16S rRNA) is targeted PCR amplification, with one or both primers fluorescently tagged (e.g., 6' carboxyfluorescein). The products are digested with either one or a combination of restriction enzymes and the relative abundance of fluorescently labelled T-RFs is determined by a DNA sequencer. An electrophorogram is produced which represents the profile of a community i.e., DNA fragments at varying lengths reflecting the composition and presence of dominant community members. This method distinguishes between sequences based on the presence or absence of specific restriction sites (Blackwood *et al*., 2003; Kirk *et al*., 2004).

T-RFLP has been demonstrated to be effective at detecting variations between microbial populations in an assortment of environments (Tiedtjie *et al*., 1999; Schütte *et al*., 2008) including desert soils (Stomeo *et al*., 2013). Functional gene diversity can also be studied by T-RFLP, by targeting genes encoding for N (*nif*H) and C fixation (*cbb*L) and methane oxidation (*pmo*A), for example. Microbial community profiling techniques do not yield phylogenetic information to directly compare communities. T-RFLP partially overcomes this limitation through the use of clone libraries, from which sequences may be assigned to individual T-RF peaks, providing taxonomic information specific to the environmental

sample under study (Kirk *et al*., 2004). There are also various software tools available to allow for the assignment of T-RFs using online databases (Kent *et al*., 2003).

3.3. Next-generation sequencing

Recent advances in molecular biology have resulted in the emergence of next generation sequencing (NGS) as a powerful tool in microbial ecology, resulting in a higher degree of resolution with which community diversity can be studied in complex environments (Chu *et al*., 2010; Roh *et al*., 2010). 454 sequencing (Figure 11) is a high throughput sequencing platform provided by Roche/454 Life sciences for use in metagenomics (Petrosino *et al*., 2009).

Pyrosequencing has been successful in increasing the resolution at which ecologists are now able to study patterns and drivers of microbial biogeography (Lauber *et al*., 2009). For example, Fierer *et al.* (2012) recently undertook a comparative study investigating the functional diversity of edaphic microbial communities in hot and cold deserts, forests, grasslands and tundra. Evidence suggested microbial communities in desert environments contained a higher abundance of stress-response genes related to osmoregulation and dormancy. These studies highlight the importance of employing pyrosequencing in microbial ecology studies, with new insight into desert-adaptation strategies of microbial communities. Improvements of NGS technologies are constantly being developed, providing more robust tools to analyse microbial communities and their phylogenetic and functional relationships. For example, employing "barcoding" technology to pyrosequencing (Hamady *et al*., 2008), which involves multiplex sequencing of a barcode

sequence; i.e., a sample-specific identifier is attached to the DNA to be sequenced. The appropriate barcodes are detected and phylogenetically assigned to the sequence reads (Berry *et al*., 2011).

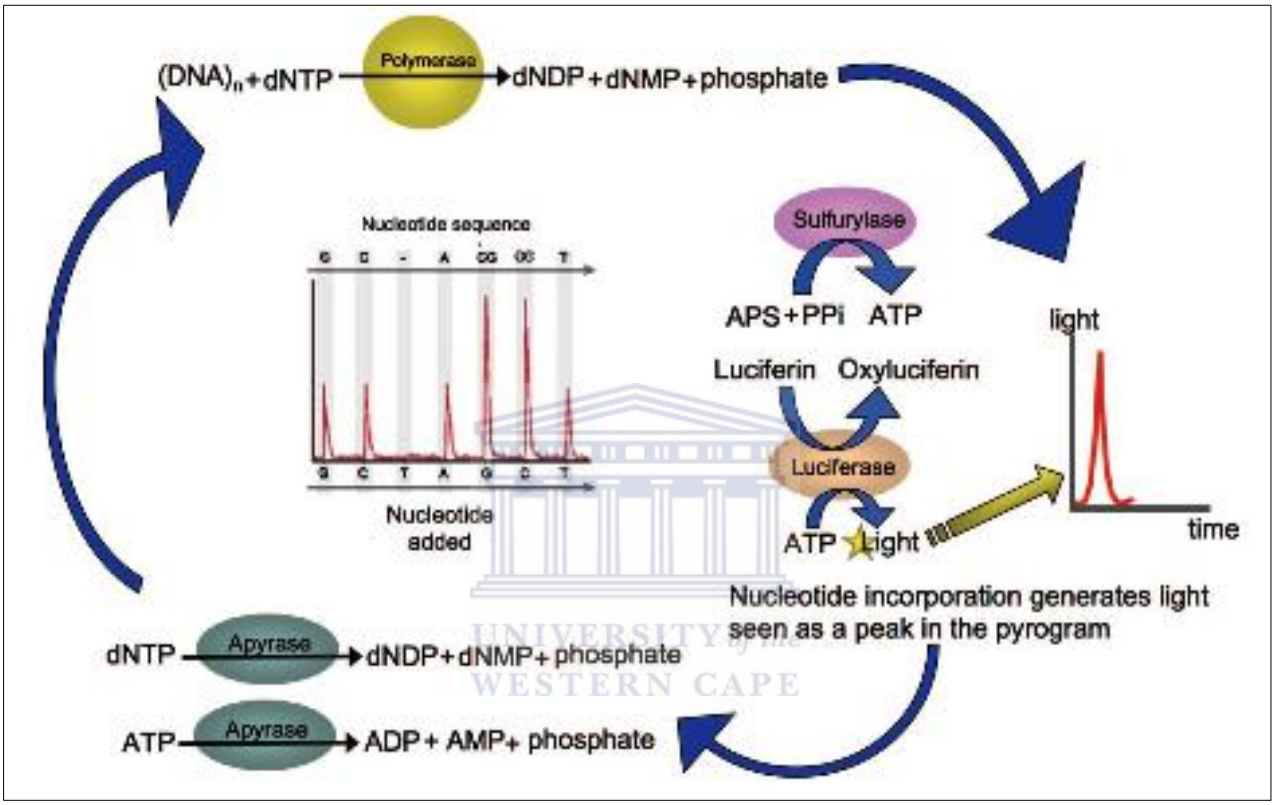

Figure 11: A generalized outline depicting the pyrosequencing chemistry. The biochemical reactions and enzymes depicted are involved in generating light signals. Each peak in a pyrogram represents a pulse of light detected in the instrument (Petrosino *et al.*, 2009).

While NGS technologies have improved sequencing outputs, read length and accuracy, challenges have been reported in the total sequencing output in relation to the cost and labour. However, these technologies have revolutionized the ability to study microbial ecology at very high resolution, especially when used in parallel to software platforms that

analyse the vast sequencing output, such as MOTHUR and QIIME (Caporaso *et al*., 2010; Shokralla *et al*., 2012).

4. Research objectives

Deserts are low energy systems and constitute the most extensive terrestrial ecosystems on Earth (Laity, 2009). In arid regions, high temperatures in association with limited and sporadic rainfall impose constraints on vegetation and edaphic microbial communities. The increase of global levels of atmospheric CO_2 is predicted to increase drought events and episodic floods in such environments (Adeel *et al.,* 2005). These pulse-precipitation events may potentially increase temporal vegetation cover, stimulating the bioavailability of nutrients in generally oligotrophic soils (Austin *et al*., 2004). This increases edaphic microbial community activity and, notably, their involvement in biogeochemical cycles (Hättenschwiler *et al*., 2005).

Bacterial communities have demonstrated rapid response to environmental events (Fenchel, 2000), however the temporality of their response (e.g., changes in composition and/or function) is not well studied in deserts. Furthermore, investigating the impact of seasonal vegetation on edaphic microbial communities in such regions will shed light on their role in ecological processes and the dynamics of nutrient turnover in this extreme environment.

The central Namib Desert received 165 mm of rainfall in 2011, recorded as the wettest year in the last 49 years (Eckardt *et al*., 2013). This extreme event transformed regions of the central Namib into primary successional grasslands (Figure 12). This observation

stimulated us to consider the role of seasonal nutrient availability in shaping the edaphic bacterial communities in this hyper-arid and depauperate environment.

Within this framework, the specific objectives were:

i. To investigate the variability of microenvironmental variables and edaphic bacterial communities in a 8100 m^2 vegetation-covered site (Site A), in comparison to two 100 m^2 vegetation-free sites (Sites B and C), by designing a representative sampling strategy and assessing short-term dynamics across spatial and temporal scales.

ii. To examine whether or not edaphic bacterial communities in the vegetation-covered site exhibited seasonal patterns of change over a one year period,

iii. And finally, to evaluate the role of the microenvironment in shaping temporal patterns of variation in the edaphic communities in the central Namib Desert gravel plains.

This study utilizes a holistic approach which combines the use of T-RFLP for microbial community fingerprinting, fine-scale soil physio-chemical characterization and a robust sampling strategy.

Figure 12: Photographs of the gravel plains in the central Namib Desert before an extreme rainfall event in April 2010 (a), and after the 165 mm rainfall event in April 2011 (Courtesy of Prof. D.A Cowan)

Chapter 2: Materials and Methods

2.1. Study site and sampling strategy

Sampling was conducted 2 km east of the Gobabeb Training and Research Centre in the central Namib Desert from 01 May 2012 to 28 April 2013. The study area was divided into 3 sites (Figure 13) consisting of Site A (S23° 33.302', E15° 3.288) Site B (S23°33.235, E15°03.232) and Site C (S23°33.332, E15°03.343).

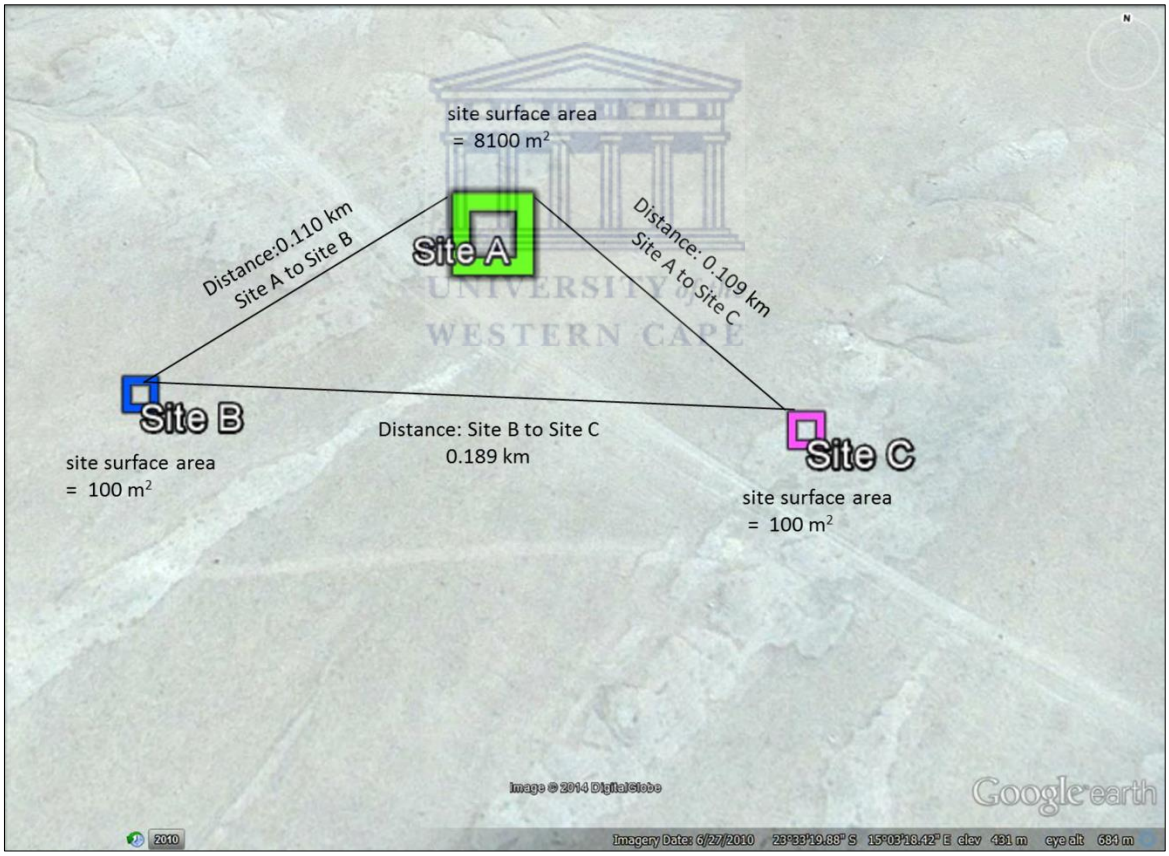

Figure 13: Location map of the study area in the Namib Desert gravel plains. The study area was divided into three sites (A, B and C). The distance between sites and their surface areas are depicted (Source: Google Earth; 6/27/2010).

The study area was selected where the region was generally consistent in terms of geology aspects (south-facing), slope (est. 5°) and the presence or absence of vegetation cover during May 2012 (Figures 14 and 15). Site A represented a vegetation-covered study site and extended over a total surface area of 8100 m^2, divided into 81 (10 x 10 m) plots (Figure 14).

Figure 14: Photograph depicting Site A in the Namib Desert gravel plains. In May 2012, seasonal *Stipagrostis* sp., a common perennial grass in the region, was growing on the study site.

Sites B and C, which were immediately adjacent to the Site A, were selected for the absence of *Stipagrostis* sp (representing vegetation free sampling sites) .These sites were divided into 4 (10 x 10 m) plots covering a total surface area of 100 m^2 (Figure 15).

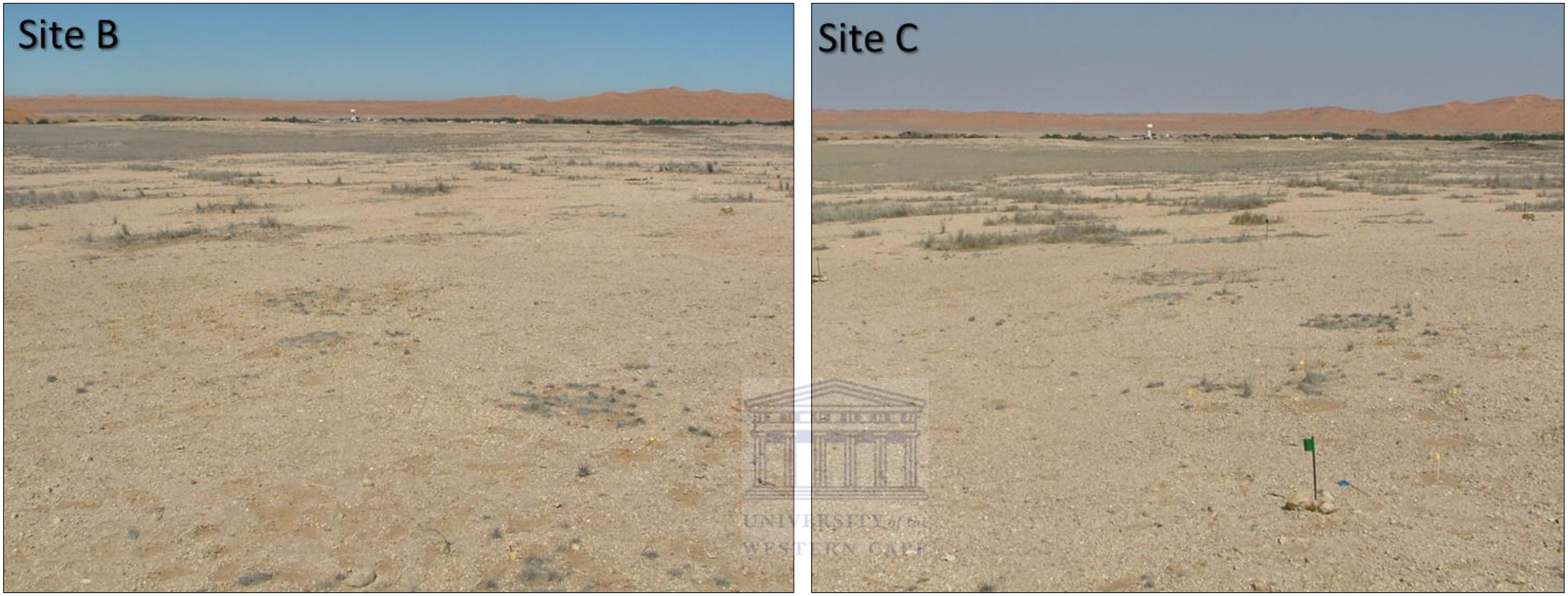

Figure 15: Photographs of the 100 m² Sites B and C study areas in the Namib Desert gravel plains in May 2012. These sites represented vegetation-free sites (*Stipagrostis* sp. was largely absent on the study sites) in this study and were located approximately 0.189 km apart.

Soil samples were collected between 8 and 10 AM on days 0, 4, 12, 28, 42, 57, 88, 118, 138, 178, 198, 238, 268, 298, 328 and 355 (from 01 May 2012 to 28 April 2013). On each sampling day, surface soil (0-3 cm) samples were collected using a 1 m^2 grid divided into 16 quadrats (Figure 16). These samples were pooled and homogenized into a single sample. This strategy was repeated for randomly selected 8 plots Site A and on 3 plots for Sites B and C each (n = 14 total per sampling day). This approach allowed us to assess bacterial community variability at different time periods. A random sampling strategy was employed which minimized biases; ensuring samples to be representative of the environment (Quinn & Keough, 2002; Baker *et al*., 2009).

Figure 16: Equipment used in the sampling of desert surface soil in this study. 1 m^2 wired sampling grid (divided in 16 individual 25 x 25 cm quadrats) and a trowel is depicted.

Soil samples were homogenized and separated into 2 g aliquots for FDA analysis and DNA extraction and 7 g for storage in RNAlater™ (Sigma-Aldrich, USA). Soil for molecular analyses was stored at -20 °C, while the remaining soil (~ 70 g) was stored at 4°C for physio-chemistry characterization.

2.2. Temperature and relative humidity data

Air temperature and relative humidity was obtained from the Gobabeb Land Surface Temperature (LST) weather station, established by the Karlsrughe Institute of Technology (KIT) in the central Namib Desert gravel plains (23°33'S, 15°03'E). The stations instruments were mounted at varying heights (e.g., 2m and 25m) measuring air temperature, relative humidity and wind speed at 1 minute intervals (Göttsche *et al*., 2013). In chapter 4, air temperature and relative humidity (measured at 2 m) was represented as monthly averages to observe seasonal trends (Figure 26).

2.3. Soil physio-chemical characterisation

Soil chemistry analyses were conducted at the Soil Science Laboratory of the University of Pretoria, South Africa, according to standard quality control procedures (SSSA, 1996). All solutions and reagents used in the chemistry analyses were supplied by Merck Chemicals, South Africa. Soil samples were sieved (2 mm) prior to analysis, as recommended.

2.3.1. Assays for inorganic N (ammonium and nitrate)

A method for determining exchangeable ammonium (NH_4^+) and nitrate (NO_3^-) by steam distillation described by Bremmer & Keeney (1966) was used with minor modifications.

Briefly, 5 g of soil was mixed with 2M potassium chloride (KCl; 10 ml/g of soil) solution and shaken for 30 minutes at 220 rpm. Samples were allowed to settle for 1 minute and the supernatant was filtered through a 110 mm Whatman no. 2V filter paper and stored at 4 °C overnight. The extractant was processed where ammonia (NH_3) is volatised from a weak alkaline solution. The addition of 0.2 g magnesium oxide (MgO) powder liberates ammonium (NH_4) and residual nitrate (expressed as mg N g^{-1}) and is determined by the reduction to nitrite (NO_2) via the addition of 0.2 g of Devarda alloy powder (Keeney & Nielson, 1982).

2.3.2. Total organic C

The Walkley-Black method (Walkley, 1935) was used to determine organic C content of soils, with minor modifications. To 2 g of soil, 10 ml of 1M potassium dichromate ($K_2Cr_2O_7$) solution was mixed by swirling. Ten ml sulfuric acid (96%, H_2SO_4) solution was added and the mixture was cooled at room temperature for 30 min. Deionized water (150 ml) and concentrated (10 ml, 96%) orthophosphoric acid (H_3PO_4) were added and the mixture was cooled as before. One ml (2.5 mM) phenylalanine colour indicator was added and the mixture was titrated with iron (II) ammonium sulphate [$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$] solution, until the endpoint of the reaction was reached i.e. when the solution changed from purple to green.

2.3.3. Total organic P

Determination of total organic P was performed using the P Bray method described by Bray & Kurtz (1945), with minor modifications. To 4 g of soil, 50 ml P Bray-1 Solution was added and the bottles shaken for exactly 1 min. After shaking, the solution was filtered through a 110 mm Whatman no. 2V filter paper. Phosphorus concentrations

were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES**;** Spectro genesis, Germany).

2.3.4. Cation exchange capacity (CEC) and Elements

Determination of CEC was performed using a modified method described by USDA (1972) which uses ammonium acetate (NH_4OAc) and potassium chloride (KCl) solutions as extractants. Four g of soil was mixed with 0.2M ammonium acetate (10 ml/g of soil) and shaken for 60 min. The samples were centrifuged (8800 rpm for 10 min) and the supernatant was filtered through a 110 mm Whatman no. 2V filter paper into a flask. The weight of the remaining soil and container was determined and 50 ml (2 M) potassium chloride solution was added prior to shaking at for 60 min. The samples were centrifuged at 8800 rpm for 10 min and stored at 4°C overnight. Prior to titration, 0.2 g magnesium oxide powder was added to the ammonium acetate and potassium chloride extractant solution. The CEC value was calculated as the difference between the values of the two extractant solutions, as determined by titration with 0.25 M iron (II) ammonium sulphate. Fifteen ml aliquots of the ammonium sulphate solution were used to determine the concentration of magnesium (Mg), sodium (Na), calcium (Ca) and potassium (K) by ICP-OES. These variables have previously been shown to be important factors shaping edaphic microbial communities (O'Donnell *et al*., 2007).

2.3.5. Moisture Content (MC)

The moisture content of soils was determined according to the standard method by weighing 2 g of soil into glass beakers and incubation in an oven at 100 °C for 24 hours. The moisture content was calculated according to: $MC\% = W1 - W2x100$, where: W1= weight of container and moist soil (g) and W2 = weight of dried soil and container (g).

2.3.6. Particle size distribution

The particle size distributions of soil samples were determined according to the method by USDA (1972). Ten ml of Calgon dispersing solution was added to 30 g of soil. Deionized water was added to the soil up to 150 ml and shaken for 5 min at maximum speed. In order to separate the various sand fractions, the samples were passed through differently sized sieves (0.5 mm for coarse sand, 0.1 mm for fine sand and 0.05 mm for very fine sand), i.e., clay, silt and sand fractions. After each sieving, the remaining soil fraction was transferred to a beaker and dried at 105 °C to constant mass and weighed. The silt and clay fractions were suspended in 1L deionized water, shook for 30 s and incubated at room temperature for 6 hours. The percentage for the different soil fractions were calculated according to the formula: $sand\% = \frac{Ax100}{M}$; $clay\% = \frac{Ex50}{M}$; $silt\% = 100 - (\%sand - \%clay)$, where: A = mass for sand fraction, E= mass of clay fraction and M = mass of soil.

2.3.7. pH.

The slurry technique, as described by Eckert & Simms (1995), was used to measure pH by mixing 2 g of soil with 5 ml of deionized water and allowing it to settle for 30 min. A Crison Bench pH meter (Crison Instruments, Barcelona, Spain) was used to measure the soil pH.

2.4. Molecular Techniques

2.4.1. Metagenomic DNA Extraction

Metagenomic DNA was extracted from 0.25 g of soil using the Powersoil® DNA Isolation Kit (MOBIO, West Carlsbad, CA, USA) according to the manufacturer's instructions.

2.4.2. Polymerase Chain Reaction (PCR)

To amplify the 16S rRNA gene in metagenomic DNA, the primer pair E9f/U1510r was used as described in Table 3. Standard 50 µl PCR reactions contained 25 ng of metagenomic DNA as template, 200 µM dNTPs, 0.5 µM of each primer, 0.600 U of DreamTaq® DNA polymerase 1X DreamTaq® buffer (Fermentas, USA).

Table 1: Primer combinations and PCR parameters used in this study.

Primer Set	Sequence (5' to 3')	Amplification Cycle	Specificity	Reference
E9f	GAGTTTGATCCTGGCTCAG	95 °C/5 min 30x (95 °C/30 s - 52°C/30 s -72 °C/8 min)	16S rRNA gene (Bacteria)	Reysenbach & Pace, 1995
U1510r	GGTTACCTTGTTACGACTT			Marchesi *et al.*, 1998

2.4.3. Analytical Procedures

2.4.3.1. Agarose Gel Electrophoresis

Agarose gels (1%-2%) were prepared by dissolving agarose in 1X TAE buffer and adding 0.5 µg/ml GelRed mixed with standard loading dye to aid visualization. DNA fragments and PCR amplicons were separated by electrophoresis at 100 V in 1 X TAE buffer. Gels were visualized using ultraviolet (UV) light illumination and photographed with the Molecular Imager® Gel Doc™ XR+ digital imaging system (Bio-Rad, South Africa).

2.4.3.2. DNA Quantification

The DNA concentrations (OD_{260} nm x 50 ng/µl) and purity (OD_{260} nm/ OD_{280} nm) were measured using the Nanodrop® ND-100 UV-Vis Spectrophotometer (Nanodrop Technologies, USA). The ratio of OD_{260} nm/ OD_{280} nm is acceptable in the range of 1.8–2 (Wilfinger *et al.*, 1997).

2.5. DNA Purification

PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit according to the manufacturer's instructions (Macherey-Nagel, Duren, Germany).

2.6. Terminal - Restriction Fragment Length Polymorphism (T-RFLP)

Bacterial 16S rRNA genes were amplified using the universal primers as described in Section 2.1. The forward primer was fluorescently FAM-labelled (6-carboxyfluorescein) at the 5' end. PCR reactions were carried out in duplicate, in a Bio-RAD T100 Thermal Cycler (BioRad, USA). PCR amplicons were purified using the NucleoSpin Gel Extraction

Kit as described in section 2.5. Amplicon concentrations were normalized to 200 ng and digested with *Hae*III restriction enzyme (Fermentas, USA) at 37 °C overnight. The digested fragments were purified and eluted in 20 µl of the elution buffer. Purified products were sequenced at the Stellenbosch University's Central Analytical Facility (CAF: http://academic.sun.ac.za/saf/). Fluorescently labelled T-RF fragments were subjected to capillarity electrophoresis in a ABI3130XL sequencer (Applied Biosystems, USA) co-injected with the molecular size ladder GeneScan Rox 1.1 (sizes: 47, 51, 55, 82, 85, 93, 99, 126, 136, 292, 317, 362, 439, 557, 692. 695, 946). T-RFLP profiles from resultant ABI files were then analysed using Peak Scanner™ (version 1.0, Applied Biosystems, available online: https://products.appliedbiosystems).

2.7. Multivariate Statistical Analysis

Multivariate analyses of T-RFLP and abiotic data were performed as described by Makhalanyane *et al*. (2013). Software programs Primer 6 (version 6.1.5.81; Primer E Ltd, Plymyth, UK) and R statistical package 2.15.1 using the vegan, gpolts and labdsv packages (www.r-project.org), were used to analyse the multivariate data sets. Specifically, multivariate analyses of data were performed on square-root transformed T-RFLP data (reflecting OTU abundance), and on normalized data for environmental variables.

T- RFLP data and environmental variables sets were used to calculate Bray-Curtis dissimilarity matrices (Bray & Curtis, 1957) and Euclidean distance similarity matrices, respectively. This allowed T-RFLP profiles and environmental data to be visualized

using non-metric multidimensional scaling (NMDS) plots. A single point on an NMDS ordination represents a complex bacterial fingerprint (for each sampling day), consisting of numerous T-RFs (where one T-RF represents one OTU). The NMDS algorithm ranks distances between objects (here, samples representing bacterial communities) and plots them based on their ranking, nonlinearly onto a dimensional ordination space (Ramette, 2009). This method of ordination is popular because it provides a simplified representation of community relationships whilst preserving the rank order of sample dissimilarity and distances (Clarke, 1993). Therefore, sample-relatedness can be determined by their position in space; i.e., the more dissimilar samples are, the further apart they will be positioned (and vice versa). The quality of the ordination is indicated by a stress value, where the closer the value is to zero, the more aligned the rank orders are: Stress <0.05 constitutes an excellent representation with no possibility of misinterpretation; Stress <0.1 represents a good ordination with a low risk of false interpretation; Stress <0.2 is a usable ordination, with the potential for misinterpretation; Stress >0.2 represents an ordination that is close to random and therefore unreliable (Clarke, 1993).

ANOSIM was used to test for inter-variation (between group variations) between *a priori* defined groups (Clarke, 1993). For example in chapter 3; ANOSIM was used to test the significance of Site A, B and C communities and in chapter 4 ANOSIM tested seasonal (summer 2012, winter 2012, autumn 2012, autumn 2013 and spring 2012 communities. ANOSIM provides a p-value to test for significance of grouping ($P < 0.05$) and yields an R value (i.e., $R > 0.75$, groups are well separated, $R > 0.5$, groups overlap

but clearly different, R<0.25, groups are barely distinguishable (Clarke, 1993; Clarke & Gorley, 2001).

Intra-variability (within-group variation) of bacterial communities was assessed using the function betadispers (vegan package in R; Oksanen *et al*., 2011). The function implements PERMDISP2 procedure for the analysis of multivariate homogeneity of group dispersions (variances; Anderson *et al*., 2001). In chapter 3, dispersion analysis was used to test within-group variation among Site A, B and C bacterial communities and environmental variables. The F ratio is obtained by calculating the distance-to-centroid (dispersion) values for each group of samples. The P value is subsequently obtained by comparing the actual F ratio to 999 randomly generated F ratios, and $P \leq 0.05$ is considered significant (Chase, 2007).

The non-parametric Kruskal-Wallis one-way analysis of variance (Kruskal & Wallis, 1952) was used to test differences in environmental variables among sites (chapter 3) and seasons (chapter 4). Wilcoxon-Mann-Whitney *post hoc* tests for pairwise comparisons were used after ensuring that an overall Kruskal-Wallis test was significant ($P < 0.05$). Tests were Bonferroni-corrected for multiple errors (Gotteli & Ellison, 2012). This correction however, is often judged to be rather conservative as it leads to significance for fewer pairwise comparisons (Legendre & Legendre, 1998; Ellison & Gotelli, 2004).

A Venn diagram provides a simple and visual representation of the number of unique and shared taxa across groups. These illustrations are based on a presence/absence data set in which the shared OTUs are calculated. The circles are used to represent the

different communities and the shared OTUs are represented by overlaps in the circles (Shade & Handelsman, 2012). In chapter 4 a Venn diagram was used to depict comparisons of T-RFLP-derived OTUs in the bacterial communities among seasons.

Spearman's rank order correlations is a non-parametric version of the Pearson correlation test and is therefore less restrictive as a Spearman's test does not require the data to have a linear relationship (Spearman, 1906). In chapter 3, Spearman's rank order correlations were used to for correlations among environmental variables.

Mantel tests (Mantel, 1967) were conducted in R (vegan package) to examine the correlations between (i) Euclidean distances of environmental variables vs. Euclidean distance of time (sampling days; chapters 3 and 4), (ii) Bray-Curtis dissimilarity distances vs. Euclidean distance of time (chapters 3 and 4) and (iii) averaged Bray-Curtis dissimilarity distances (β diversity) vs. averaged Euclidean distances of soil moisture, P, K^+ and Mg^{2+} (chapter 4).

Redundancy analysis (RDA; Legendre & Legendre, 1998) was selected to test the effect of abiotic data in explaining bacterial community variation in R (vegan package). RDA is a constrained ordination method (Legendre & Gallagher, 2001) related to principal component analysis (PCA). The ordination sequentially seeks the combination of environmental variables that best explain the variation of the biotic matrix (in this study, T-RFLP data). The impact of the environmental variables on the matrix with biological data is displayed as arrows, where direction of the arrow indicates the direction of maximum change of that variable, and the length of the arrow is

proportional to the rate of change. The amount of variation explained by each axis are depicted with scores (eigenvalues) on the axes (Legendre and Anderson, 1999). The significance of the RDA models and of the selected variables was determined by 999 Monte Carlo permutations at $P < 0.05$ for each group. Only the significant variables ($P \leq 0.05$) were selected from all the environmental variables tested (C, P, NO_3^-, NH_4^+, Mg^{2+}, Ca^{2+}, Na^+, K^+, CEC, MC and pH) and fitted to the ordination as arrows.

In chapter 3, due to the large amount of soil required for soil structure analysis, only four replicates per site were measured and this variable was therefore not included in the redundancy analysis.

2.8. Buffers, solutions and media

Luria-Bertai agar (LB; Sambrook & Russell, 2001)

Yeast extract 10g

Tryptone 5g

NaCl 10g

Agar 15g

The pH was adjusted to 7 before autoclaving and the medium was supplemented with 100 mg/ml of ampicillin.

TAE Buffer

50X TAE (pH8) stock

Tris-HCl 242.2 g

Glacial acetic acid 57.1 ml

0.5M EDTA 100 ml

The solution was made up to 1L with deionized water.

Phosphate Buffered Saline (PBS)

NaCl 80 g

KCl 2.0 g

Na_2HPO_4 14.4 g

KH_2PO_4 2.4 g

The pH adjusted to 7.4 before autoclaving, made up to 1L with deionized water.

Tris (tris-hydroxyaminomethane) Buffer

Tris-hydroxyaminomethane 60.54 g

The pH was adjusted to 8.6 before autoclaving and made up to 1L with deionized water.

P Bray Solution

NH_4F 600 ml (0.25 mg/L)

32% HCl 50 ml

The solution was made up to 1L with deionized water.

Boric acid-indicator solution

Bromocresol green 0.5 g

Methyl red 0.1 g

p-nitrophenol 0.1 g

Dissolved in 100 ml 95% ethanol and made up to 1L with deionized water. The pH was adjusted to 4.6.

Calgon dispersing solution

Sodium hexametaphosphate [$(NaPO_3)6$] 35.7 g

Sodium carbonate (Na_2CO_3) 7.94 g

The solution was made up to 1L with deionized water.

Chapter 3: Short-term bacterial community dynamics and microenvironmental variability of Namib Desert gravel plain soils

3.1 Introduction

Research has highlighted the importance of edaphic microbial communities in maintaining ecosystem functioning by acting as key drivers of the essential C and N biogeochemical cycles (Yergeau *et al*., 2007; Pointing & Belnap, 2012). Therefore, investigating soil microbial community dynamics (i.e., their patterns of structural change over time) has been the focus of considerable research (Butler *et al*., 2003; Stickland *et al*., 2009; Lauber *et al*., 2013). Studies have demonstrated links between temporal variability of edaphic microbial communities and seasonal changes in soil moisture, temperature and vegetation cover (Waldrop & Firestone, 2006; Koch *et al*., 2007; Horz *et al*., 2004; Buckley & Schmidt, 2001). Local environmental factors (e.g., pH and soil physiochemical characteristics), spatiality, resource factors and soil structure have also been reported to influence edaphic community structure (diversity and composition; Zhou *et al*., 2002; Fierer & Jackson, 2006; Rasche *et al.,* 2010; Fierer *et al*., 2012). However, *in situ* research focussing on identifying the drivers of spatial variation and temporal community dynamics across habitat types in deserts remains scant.

Desert terrestrial environments are typically characterised by low levels of bioavailable water and nutrients, diurnal and seasonal temperature extremes, and high levels of ultraviolet (UV) radiation (Pointing & Belnap, 2012). Namib Desert soil ecosystems are among the most extreme environments on Earth, with low (< 25 mm) and variable rainfall and high daily fluctuating temperatures (0 °C to 50 °C; Eckardt *et al*., 2013).

Despite such challenges, microbial communities proliferate and constitute key process drivers in deserts, functioning as sites of primary productivity (Pointing *et al*., 2009; Tracy *et al*., 2010; Caruso *et al.,* 2011; Makhalanyane *et al*., 2012; Stomeo *et al*., 2013). We know that desert microbial communities exhibit seasonal (Lipson, 2007; Bell e*t al*., 2009) and annual (*d*e Bruyn *et al*., 2011) patterns of change, and generally respond rapidly to moisture events (Garcia-Pichel & Pringault, 2001; Fierer & Schimel, 2002). However, the lack of replication in temporal studies (Knight *et al*., 2012) decreases their discriminatory power to differentiate between "real" temporal changes in communities or differences reflecting soil spatial heterogeneity (Lauber *et al*., 2013),

It has been suggested that external environmental drivers (e.g. pH) rather than biological factors (e.g. competition) are the key determinants shaping edaphic microbial community structures in desert environments (Fierer *et al*., 2012). Caruso *et al.* (2011), however, demonstrated that both stochastic and deterministic processes interact to structure desert microbial communities at a global scale. These contradicting studies highlight the need to resolve the temporal variability of edaphic communities in natural environments using robust sampling strategies (i.e., highly replicated; Schmidt *et al*., 2007; Prosser *et al*., 2010; Knight *et al*., 2012; Lauber *et al*., 2013; Jansson & Prosser, 2013), as their discriminatory power is dependent on the analytical methods and sampling design employed (Frostegard *et al*., 2011; Lombard *et al*., 2011).

The aim of this study was therefore to investigate and compare the variability of bacterial communities and soil physiochemistry among different sites in the Namib

Desert gravel plains using (i) short-term sampling intervals, (ii) fine-scale soil physiochemical analyses and (iii) molecular fingerprinting through T-RFLP analyses. A total of 83 surface (0-3 cm) soil samples from three distinct locations were collected in the Namib Desert gravel plains over 57 days using a randomized sampling design.

3.2. Results

3.2.1. Metagenomic DNA extraction

An important initial step in investigating the structure of environmental microbial communities is the extraction of the soil metagenome. This is particularly challenging as the complex soil matrix contains PCR-inhibiting substances such as humic and fulvic acids which co-extract with DNA (Schneegurt *et al.*, 2003). In low biomass environments, the extraction of high quality DNA of sufficient yields may be another limiting factor.

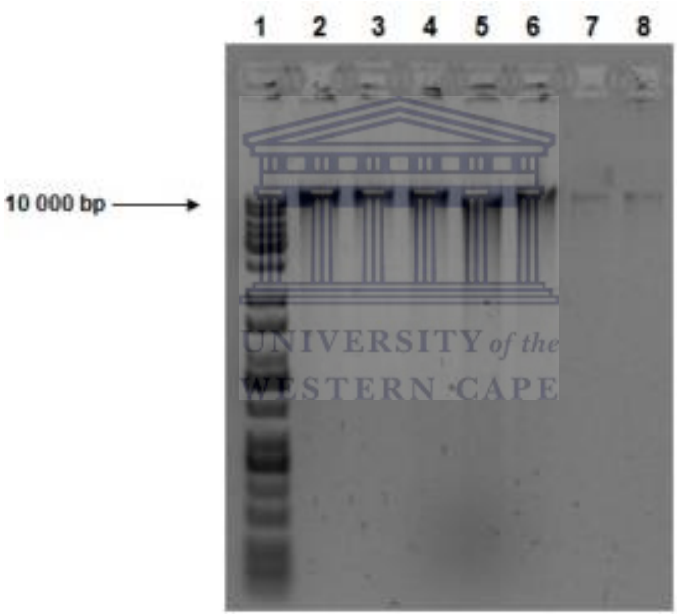

Figure 17: Desert soil total metagenomic DNA extraction. Lane 1: DNA molecular weight marker (Kappa), Lanes 2-8, soil metagenomic DNA.

In this study all soil samples metagenomic DNA (n = 127) were successfully extracted with the MoBio Powersoil® DNA Isolation Kit (Chapter 2, section 2.3.1). High molecular weight (~10 000 bp) DNA was consistently recovered with an $A_{260/280}$ ratio between 1.6 and 1.9 (Figure 15).

3.2.2. PCR amplification of bacterial 16S rRNA genes

In order to obtain reproducible community fingerprints with T-RFLP, PCR-amplifications must have high specificity, and PCR optimization is typically required to improve amplification efficiency and specificity prior to downstream analysis. The additions of glycerol, magnesium chloride, or formamide, as well as DNA template dilutions, were tested (data not shown). However, some control site soil samples remained recalcitrant to amplification (Figure 18 a, Lanes 2-3,5,7), or yielded multiple bands (Figure 18 a, Lanes 4,6,8,9). It was noted that the addition of 4% dimethyl sulfoxide (DMSO) and 0.4 mM bovine serum albumin (BSA) yielded highly specific amplifications (Figure 16 b), as previously reported (Frackman *et al*., 1998). Amplifications of the 16S rRNA gene using universal bacterial primers (E9f/U1510r; Table 1, Chapter 2) were successful for 82 samples, with the expected product size of 1,500 bp (Figure 16). The negative control yielded no visible amplification (Lane 5). 16S rRNA gene amplification products were therefore suitable for T-RFLP analysis.

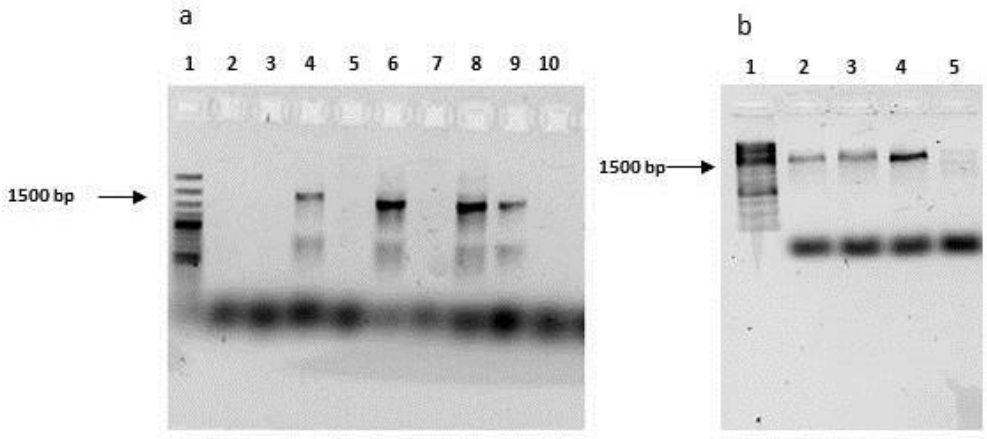

Figure 18: 16S rRNA gene PCR amplification from metagenomic DNA before (a) and after (b) optimization. a: Lane 1: DNA molecular marker (Kappa), Lanes 2-9, 16S rRNA gene amplification result, Lane 10, negative control. b: Lane 1: DNA molecular marker (Kappa),Lanes 2-4 16S rRNA gene amplification result, Lane 5, negative control.

3.2.3. Assessing the sampling strategy

A total of 83 soil samples were collected from the study area over 57 days, this included 56 soil samples from Site A and 18 samples from Sites A and B, respectively (Chapter 2, section 2.1). In assessing the distribution of samples randomly collected on study site A (n = 7 x 8), it was observed that of the site was sampled over the 2 month period, 11% (n = 6) was sampled at least twice, while 44 % 56% (n= 31) was never sampled (n = 36%; Figure 19). Sites A and B covered a smaller area (100 m^2 each) and therefore the plots on the sites were sampled more frequently (sites sampled at least twice; n = 100 %, data not shown).

Figure 19: Schematic diagram of the sampling strategy employed in this study. The days sampled over 57 days and the respective plots are colour-coded, while white plots were never sampled. The 10 x 10m plots were identified by numbers between 1-81, which allowed the selection of individual plots to be sampled using a random number generator.

3.2.4 The variability of environmental parameters

The three study sites (Figure 14 and 15, Chapter 2) separated in relation to their chemical composition on the NMDS ordination is presented in Figure 20. The clustering of environmental variables from Sites B and C suggests that these sites are more similar in their chemical composition, than Site A, as confirmed by ANOSIM pairwise comparisons (ANOSIM, A vs. B, R = 0.56, P = 0.001; A vs. C, R = 0.54, P = 0.001; B vs. C = R = 0, P = 0.9).

Significant differences in soil chemistry intra-variability was observed (Figure 20) and confirmed by betadispersion analyses (betadispers, P = 0.035), with Site A showing the greatest variability.

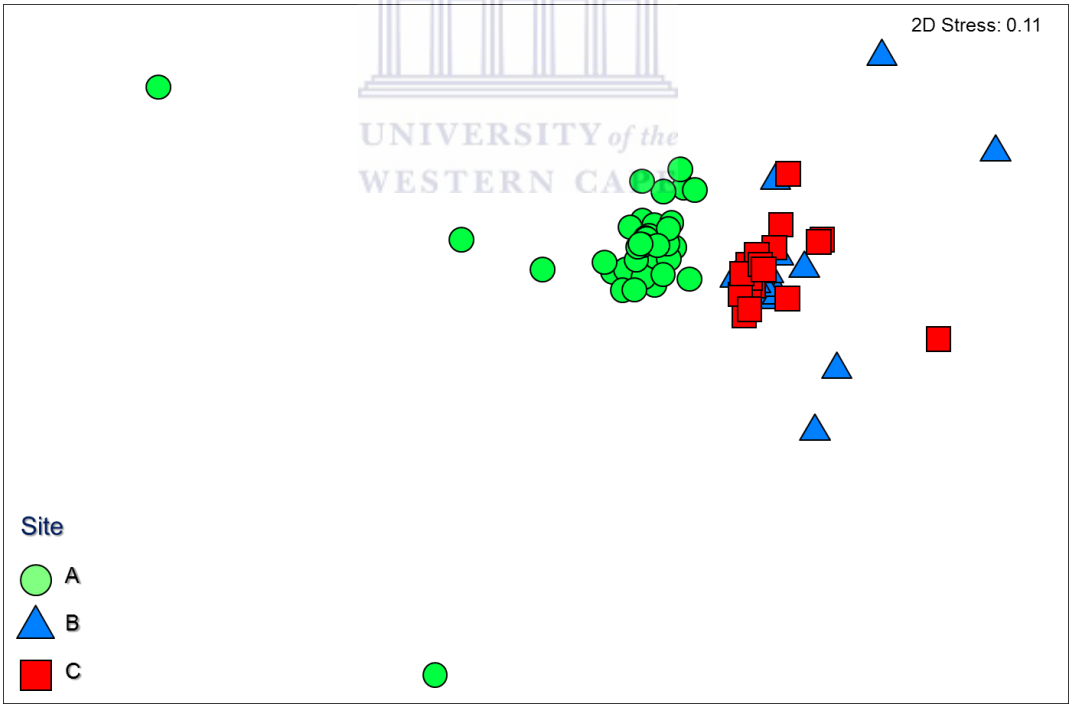

Figure 20:2-D NMDS plot (Euclidean distance) of normalised soil chemistry variables (soil moisture content, C, P NO_3^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Na^+, pH) for soil samples over 57 days. Site-specific grouping of bacterial communities is displayed. Site A represented the vegetation-covered study site, while Sites B and C were vegetation-free.

Soil pH, C, P, K+, NH_4^+, Mg^{2+}, Moisture content and Ca^{2+} were significantly higher in Site A (Kruskal-Wallis test, $P < 0.02$) than in soils from Sites B and C. Contrastingly, levels of soil NO_3^- and Na^+ were significantly higher in Sites B and C (Kruskal-Wallis test, $P < 0.005$) compared to Site A. Indeed, pH was found to be significantly higher in Site A compared to Sites B and C (pairwise Wilcoxon-Mann-Whitney tests, Sites A vs. B, $P = 0.001$; Sites A vs. C, $P = 0.001$; Sites B vs. C, $P = 1$), substantiating earlier observations that Sites B and C share a similar habitat type. Soil pH was found to be significantly correlated to the variables P, NH_4^+, NO_3^-, Na, K, Mg, Ca (Spearman's $\rho > 0.6$, $P < 0.05$) in all three sites.

Environmental variables were not correlated with time (Mantel, $r = 0.03$, $P = 0.001$), suggesting that the composition of environmental variables remained generally static over the 57 day sampling period.

Analyses of grain size distribution suggests that Sites B and C displayed similar soil structures by only representing sand and silt fractions (Figure 21) while Site A exclusively contained clay (4%). Based on soil chemistry and structure analyses, Site A represents a significantly different edaphic environment compared to soils from Sites B and C, which appeared to be more similar.

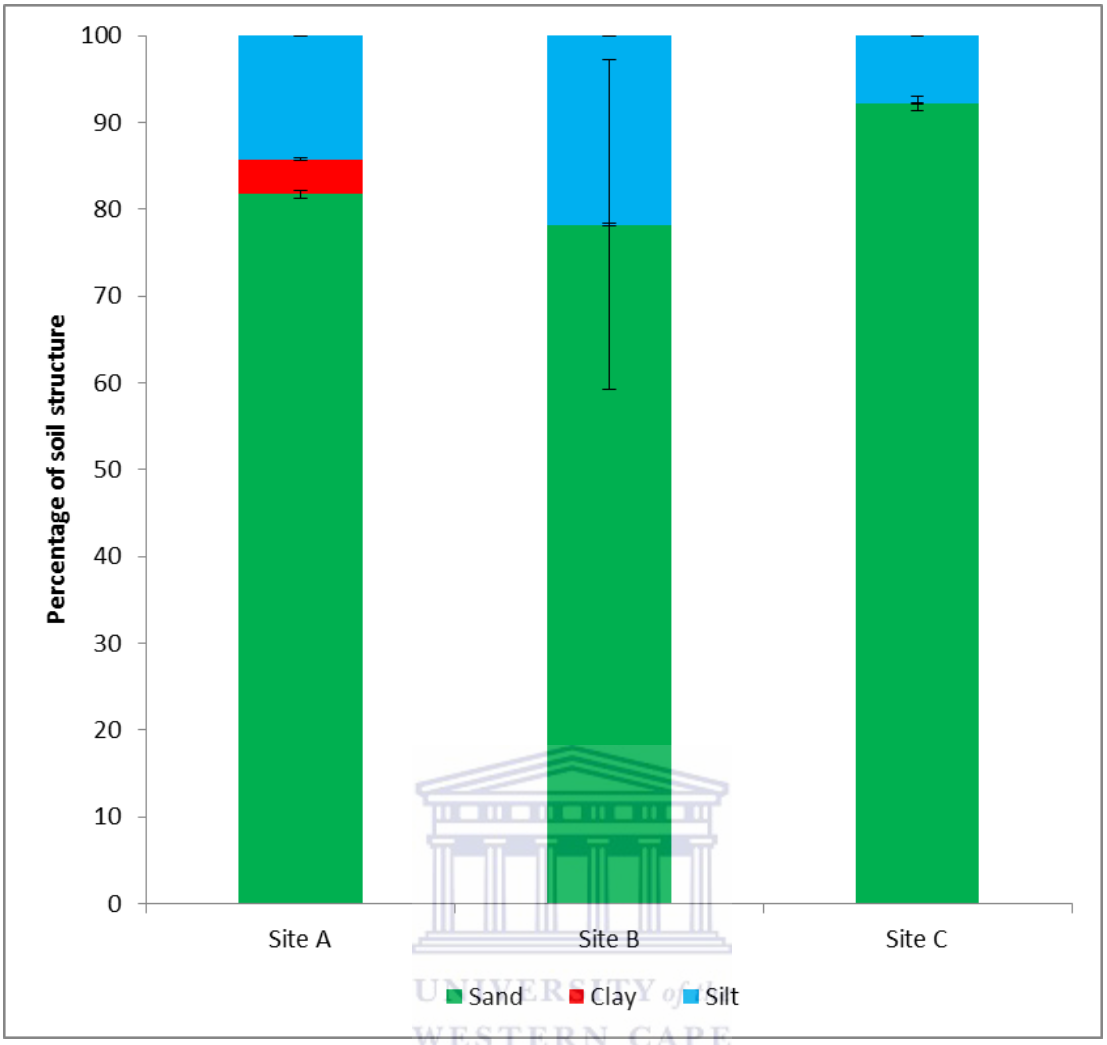

Figure 21: Histogram depicting percentage soil structure (sand/silt/clay) analysis for sites. Data was generated using 4 replicates per site (n = 4 x 3), to obtain a 'global' representation of the soil structure specific to individual sites. Error bars depict standard deviation.

3.2.5 Bacterial community patterns

A NMDS ordination displaying bacterial community structure (Figure 3) revealed community overlap between Sites B and C, while bacterial communities from Site A separate from Sites B and C bacterial communities. This was supported by ANOSIM pairwise comparisons (A vs. C: R = 0.54, P = 0.001; A vs. B: R = 0.56, P = 0.001; B vs. C: R = 0, P = 0.9). Furthermore, bacterial communities displayed no significant differences in intra-variability over time (betadispers, P = 0.1).

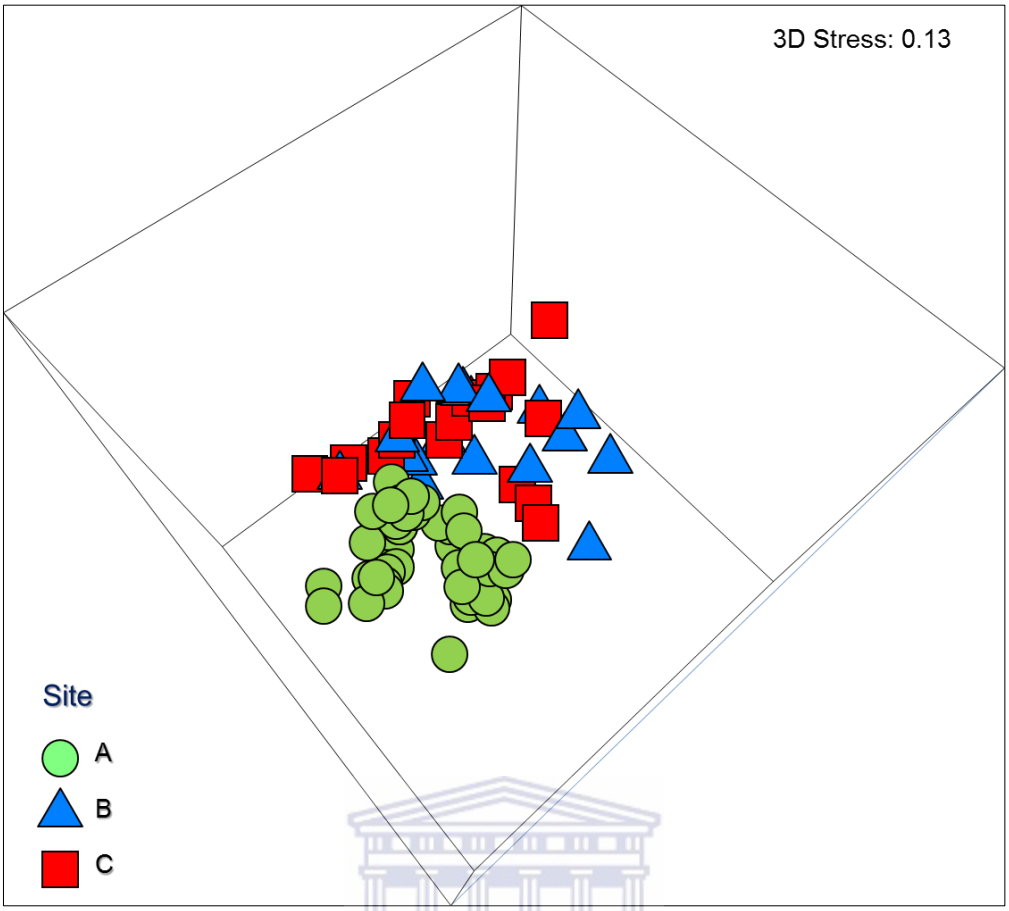

Figure 22: NMDS plot (Bray-Curtis dissimilarity) of relative abundances comparing bacterial community T-RFLP profiles between sites. T-RFLP data was square-root transformed prior to analyses.

To better observe the temporal variation, communities in their respective sites were studied separately (Figure 23), and found to display different temporal dynamics. For example, shifts in bacterial community composition between D0 and D4 were larger in Sites A and B, than in Site C. Furthermore, larger shifts between D4 and D12 were observed in Sites A and C, compared to Site B.

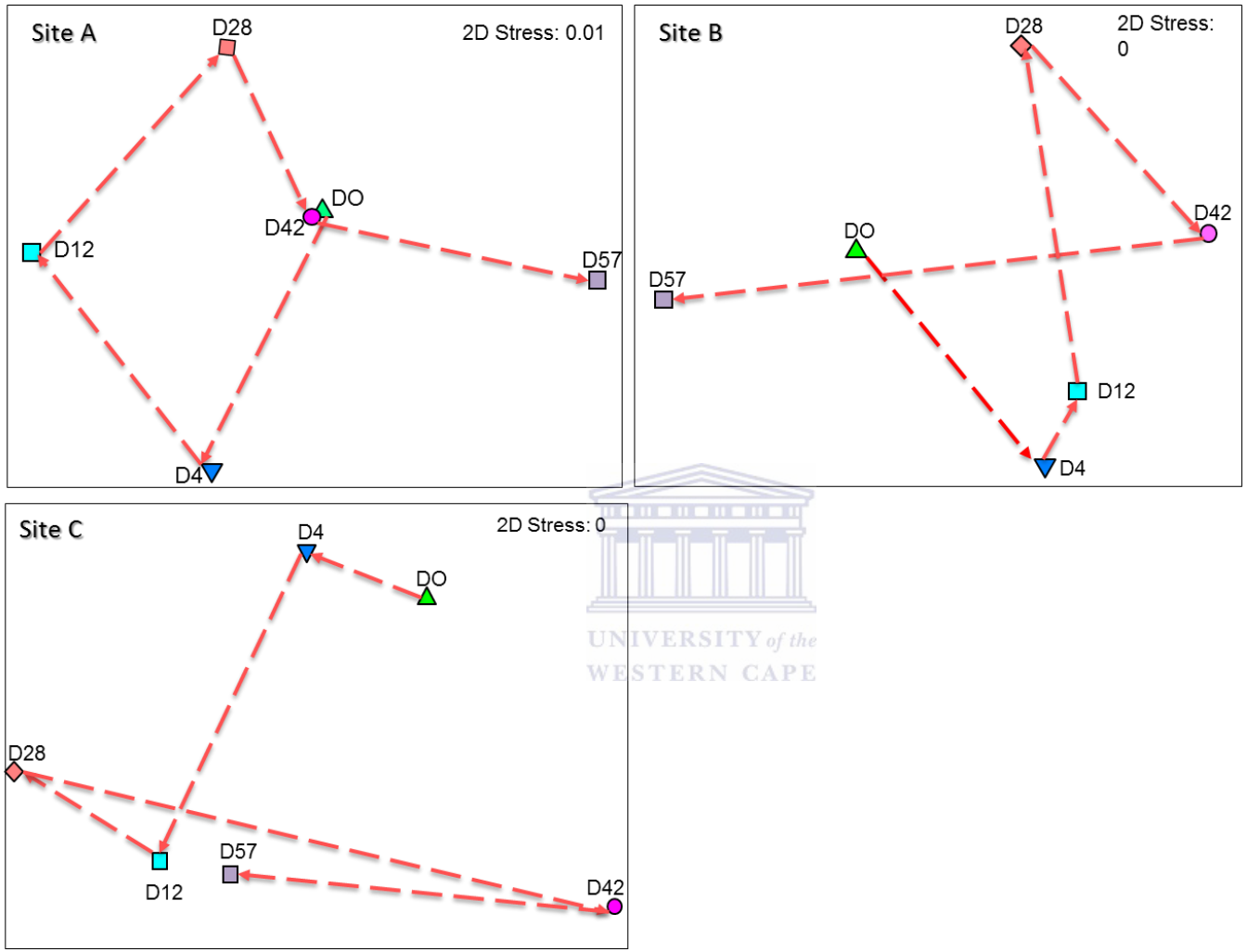

Figure 23: Changes in bacterial community composition at each sampling point. NMDS showing Bray-Curtis dissimilarity of T-RF abundance averaged across replicates (so that one point represents a complex bacterial fingerprint of each sampling day). Connecting lines are trajectories displaying temporal dynamics of bacterial community composition. T-RFLP data was square-root transformed prior to analyses.

3.2.6 The role of the environment in shaping edaphic bacterial communities

The effect of environmental variables (C, P, NO_3^-, NH_4^+, MC, K^+, Ca^{2+}, Mg^{2+}, Na^+, pH) on the bacterial community structure among Sites A, B and C was assessed using canonical RDA analysis (Figure 24). Grouping of samples in the ordination plot along axis 1 demonstrated overlap between Site B and C communities, whereas Site A communities were separate and likely correlated with pH. Soil pH was the only variable found to play a role in the observed variation between sites, however only 11% of the measured variation could be explained (RDA analyses, P = 0.04).

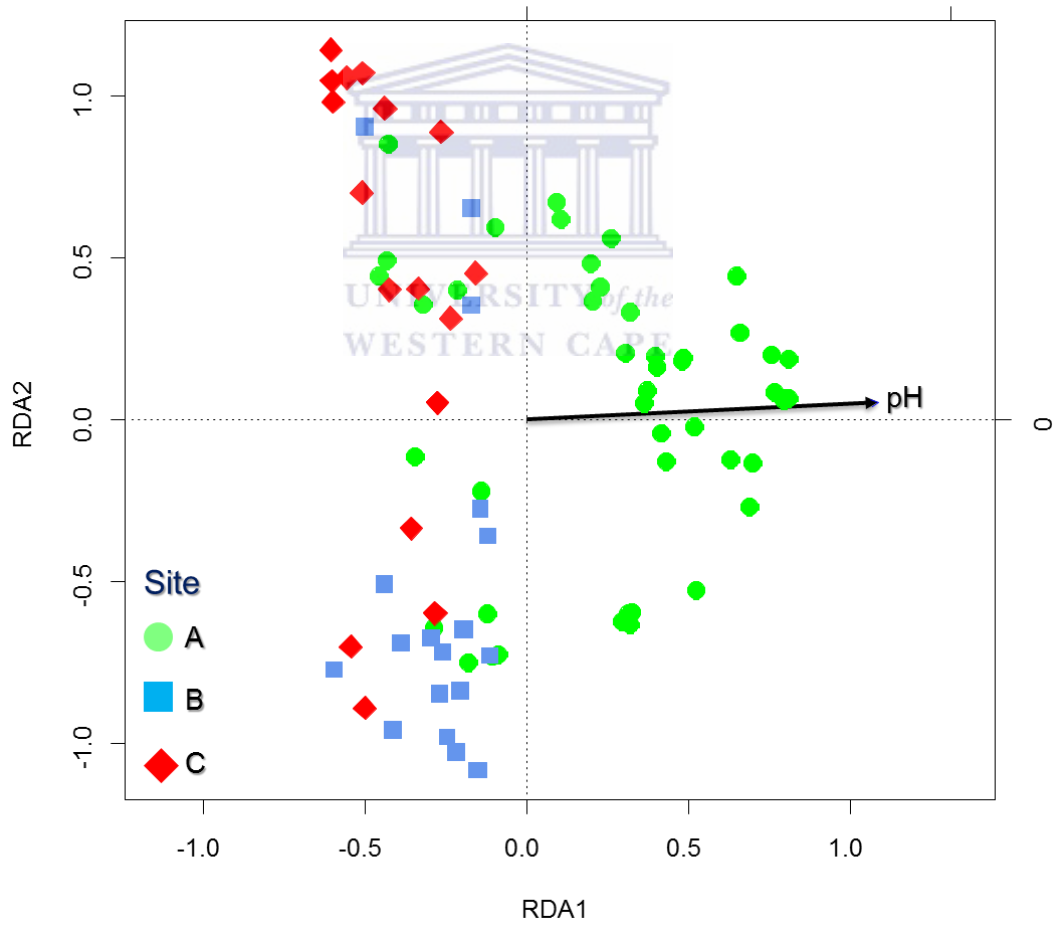

Figure 24: Redundancy analysis (RDA) biplot of bacterial abundance and microenvironmental parameters. Only the environmental variable that significantly explained variation of bacterial community structures was fitted to the ordination (arrows; P = 0.04).

3.3 Discussion

Different community dynamics were observed for site-specific bacterial communities, however none of the measured environmental variables were significant in explaining the observed temporal variation. These results suggest that the observed temporal changes in bacterial community composition could be driven by stochastic rather than deterministic factors in the Namib Desert gravel plain soils. The Neutral Theory (Hubbel, 2001), in which the structure of communities with equal fitness is driven by stochastic drift (Rosindell *et al*., 2012), resulting in random patterns of species co-occurrence (Bell, 2005), could explain the observed intra-site variability. Alternatively, environmental factors not specifically targeted in this study could account for the variation observed. For example, the quantity and quality of organic carbon accessible through root exudation and/or plant litter inputs have been linked to temporal changes in specific taxonomic groups (Sherman & Steinber, 2012). Furthermore, temporal variations in soil moisture and temperature have been demonstrated to influence the soil microbial community composition in deserts (Bell *et al*., 2009).

Due to the extreme environmental characteristics and the limited bioavailability of water of the region, it was not surprising to observe that environmental variables remained relatively static over time (Eckardt *et al*., 2013). Furthermore, the rate of C turnover in arid environments has been estimated to be in the order of decades (Warren-Rhodes *et al*., 2006) and low rainfall could limit microbial nutrient availability, resulting in decreased microbial decomposition rates (Austin *et al*., 2004). The availability of other environmental

variables, for example soil phosphorus, is dependent on soil moisture, temperature and nutrient availability (Richardson & Simpson, 2011) and therefore long-term monitoring of the soil physiochemical characteristics of this environment is warranted.

Environmental heterogeneity has been shown to play a pivotal role in shaping bacterial community composition (Green & Bohannan, 2006; Ranjard *et al*., 2013). Similarly, in this study significant differences between bacterial communities from distinct soil types (i.e., which encompasses soil texture and chemical properties) were found (Chau *et al*., 2011). While numerous ecological determinants potentially shaping desert edaphic bacterial community structures exists (e.g. water source; Stomeo *et al*., 2013), this discussion will focus on vegetation (*Stipagrotis* sp. present exclusively on Site A), soil structure (different in Site A vs. Sites B and C) and pH (identified as a significant factor).

Research into the links between above and belowground communities confirms the view that bacterial communities will vary depending on the presence or absence of vegetation (Bardgett *et al*., 2008; Berg & Smalla, 2009). In oligotrophic desert soils, plants provide nutrient rich habitats representing islands of fertility (Herman *et al*., 1995; Schelsinger *et al*., 1998; Aguilera *et al*., 1999), selectively influencing the edaphic microbial community (Acosta-Martínez *et al*., 2008; el Zahar Haichar *et al*., 2008). The above-ground *Stipagrostis* sp. associated with Site A (absent from Sites B and C) could therefore directly influence the observed differences in bacterial community structure. For example, the existence of fungal-associated communities in the presence of plants may influence bacterial community composition (abundance and diversity) through resource competition (Boer *et*

al., 2005). Studies have shown the importance of plant and soil microbial community interactions through litter and root exudates, particularly in low nutrient environments (Knelman *et al*., 2012). While extremely low (characteristic of desert soils), Site A presented the highest percentage of carbon. Higher resource availability may favour fast-growing community members, increasing their heterogeneity and abundance (Royer-Tardif *et al*., 2010) and ultimately influencing their response to environmental change (de Vries & Shade, 2013).

Differences in soil structure (as determined by % clay/sand/silt composition) has been demonstrated to correlate with soil environmental parameters (e.g., organic matter, moisture content) and the edaphic microbial community assemblages in arid regions (Pasternak *et al*., 2013). Soil clay has been found to provide protection of edaphic microbial communities against predation (Chau *et al*., 2011), and to influence the turnover of organic carbon, potentially affecting microbial community dynamics (Sagger *et al*., 1999). For example, the adsorption of minerals to clay particles has been proposed to protect proteins and nucleic acids against proteolysis, and thermal and pH denaturation (Nannipieri *et al*., 1990, 2002). It has also been suggested that plant species and soil type cooperatively shape microbial community structure in soils (Berg & Smalla, 2009). Thus, it is likely that clay and/or vegetation play a role in structuring desert soil microbial communities. However, experimentation is required to define the potential role of soil clay and *Stipagrotis* sp. in shaping bacterial communities in Namib Desert gravel plain soils.

Shifts in community structure may be related to local environmental variables (Van der Gught *et al*., 2007). Similarly, this study presents evidence that significant difference in soil

pH structures bacterial communities over relatively short spatial scales in the Namib Desert gravel plains. Soil pH has been proven to be a significant determinant of bacterial community composition at local (Rousk *et al*., 2010), regional (Chu *et al*., 2010; Griffiths *et al*., 2011) and continental scales (Fierer & Jackson *et al.,* 2006; Lauber *et al*., 2009). The relationship between edaphic bacterial community composition and soil pH has been well established across an array of biomes, soil types, and spatial scales, independent of the molecular techniques employed (e.g. DNA fingerprinting; Fierer & Jackson, 2006; clone libraries; Lauber *et al*., 2008 and pyrosequencing; Lauber *et al*., 2009; Rousk *et al*., 2010). However, the specific mechanisms governing the observed patterns of bacterial community structure cannot be identified without further experimentation. Since soil pH was correlated with the majority of measured environmental variables in this study, it is likely a combination of factors shaping bacterial communities, and not soil pH alone. For example, nutrient availability, soil moisture, salinity and cationic metal solubility are often related to soil pH (Brady *et al*., 2010), and all these factors could independently drive the observed changes in community structure. Alternatively, bacterial communities have been shown to survive in a narrow yet optimal pH range (Madigan *et al*., 1997) and therefore minor deviations of *in situ* soil pH could lead to a population being rapidly outcompeted by unconstrained members (Rousk *et al*., 2010). The response of edaphic bacterial communities to changes in soil pH has been demonstrated as shifts in the relative abundance of community members across pH gradients (Jones *et al*., 2009).

Although soil pH was the only statistically significant variable identified in explaining the observed differences of bacterial communities between sites, a large amount of variation remained unexplained (89 %). While a standard suite of soil characteristics were measured

in this study, it is possible that other variables could be important in explaining bacterial community variability. For example, cation exchange capacity (CEC) is an important environmental variable driving microbial community variation in natural habitats (Seghers *et al*., 2003), yet CEC is rarely measured in microbial ecology studies. It is recognised that due to the inherent low levels of nutrients and minerals in desert environments (Pointing & Belnap, 2012) the use of highly sensitive techniques could therefore increase the resolution of such studies. For example, GeoChip analyses have been employed to investigate edaphic microbial communities in various ecosystems, for example targeting specific microbial-related N, C, S and P biogeochemical cycles (He *et al*., 2010). Moreover, the impact of soil chemistry temporal variability may rather induce variations in active community members, detected using RNA-based approaches (Buckley & Schmidt, 2003) and not DNA-based approaches as used here.

Dispersal mechanisms have been suggested to play a significant role in shaping bacterial communities, and even more so in edaphic environments due to their heterogeneity (Ritz *et al*., 2004). However, dispersal limitation is significantly influenced by the sampling area size (Ranjard *et al*., 2013), and therefore care should be taken when interpreting results over varying spatial scales. Due to the close proximity of the sites (> 200 metres apart), it is therefore unlikely that dispersal limitation could have been a dominant factor influencing the observed community variation. If dispersal is not limited, the community composition could be dominated by environmental selection as predicted by niche-based theories (Leibold & McPeek, 2006). Similarly, bacterial communities in different soil types were

significantly different and likely shaped by variation in soil structure and physiochemical composition in this study.

Conclusion

Taken together, these results suggest that stochasticity and habitat filtering through soil pH play an important role in shaping edaphic communities in Namib Desert gravel plain soils. Secondly, the data suggests that soils with similar environmental characteristics support similar bacterial communities. Previous studies in the Namib Desert have suggested strong evidence for environmental filtering (Makhalanyane *et al*., 2012; Stomeo *et al*., 2012), however this is the first evidence for soil pH as a determinant for structuring bacterial communities across spatial scales in the region.

The study of bacterial community dynamics in oligotrophic environments such as the Namib Desert would ideally require a temporal evolution longer than two months as seasonality has been demonstrated to be an important driver of microbial community assembly in arid regions (Cregger *et al*., 2012; Pasternak *et al*., 2013). Consequently, in the next chapter, the temporal variations of Site A bacterial communities and soil physiochemistry over a 1 year period is presented.

Chapter 4: Temporal variability of edaphic bacterial communities in the Namib Desert gravel plains

4.1. Introduction

Understanding community distribution and abundance across both spatial and temporal scales is essential in microbial ecology research, specifically the factors that shape them. The turnover of microbial communities in space and time (β diversity) have been shown to vary over environmental gradients such as productivity (Chase & Leibold, 2002; Chase & Ryberg, 2004; Chase, 2010), drought (Pointing *et al.,* 2007) and salinity (Chrits-Christoph *et al*., 2013; Stomeo *et al*., 2013). In general, β diversity can be shaped by local environmental factors such as nutrient availability and/or species interactions (Langenheder *et al*., 2012), in addition to regional factors such as ecological drift (Ricklefs, 2003; Langenheder & Szekely, 2011).

The few studies that have investigated temporal changes in soil microbial communities have shown that community composition can vary across different time scales. For example, temporal variation over days (Zhang *et al*., 2011), seasons (Shade *et al*., 2013; Lipson, 2007) and years (De bruyn *et al*., 2011) have been reported. We know that desert microbial communities can exhibit seasonal trends of variability (Bell *et al*., 2008; Cregger *et al*., 2012) and are influenced by water availability (Pointing *et al*., 2007), temperature (Cregger *et al*., 2012) and the geochemical properties of local soil (Cowan *et al*., 2011). However, research into the role of seasonal soil microenvironmental conditions in shaping temporal dynamics of desert edaphic communities is lacking.

Water availability, resulting from both rainfall (Warren-Rhodes *et al*., 2006; Pointing *et al*., 2007) and fog (Azua-Bustos *et al*., 2011), is thought to be among the most important factors affecting microbial communities in desert terrestrial environments (Warren-Rhodes *et al*., 2006; Pointing *et al*., 2007; Cary *et al*., 2010; Pointing & Belnap, 2012), The Namib Desert is arid, with scarce and highly variable rainfall events (< 25 mm p.a.), experiencing extended periods of drought (Eckardt *et al*., 2013). Fog events are a frequent occurrence, (65 days p.a. on average), resulting in approximately 34 mm of annual precipitation (Lancaster *et al*., 1984) and are thus thought to be a dominant and more predictable source of bioavailable water in the region (Shanyengana *et al*., 2002). The Namib Desert is an extreme environment, characterised by low nutrient levels, yet high salt content (Stomeo *et al*., 2013) and limited water (Eckardt *et al*., 2013). Previous studies investigating microbial communities in the region have been restricted in resolution as single time-point investigations (Makhalanyane *et al*., 2012; Stomeo *et al*., 2013). The major aim of this study was therefore to assess whether or not edaphic desert communities demonstrated seasonal patterns and to what extent the local environmental factors would drive temporal dynamics. Specifically, we investigated the temporal changes of the soil bacterial community in the Namib Desert gravel plains over 1 year, using T-RFLP fingerprinting and fine scale soil characterization.

4.2. Results

4.2.1. Assessing the sampling strategy over 1 year

A total of 127 soil samples were collected from the experimental site over 12 months (Chapter 2, section 2.1). 86 % (n = 70) of the site was sampled at least once, 63% (n = 51) was sampled at least twice, while only 14 % (n = 11%) was never sampled (Figure 25).

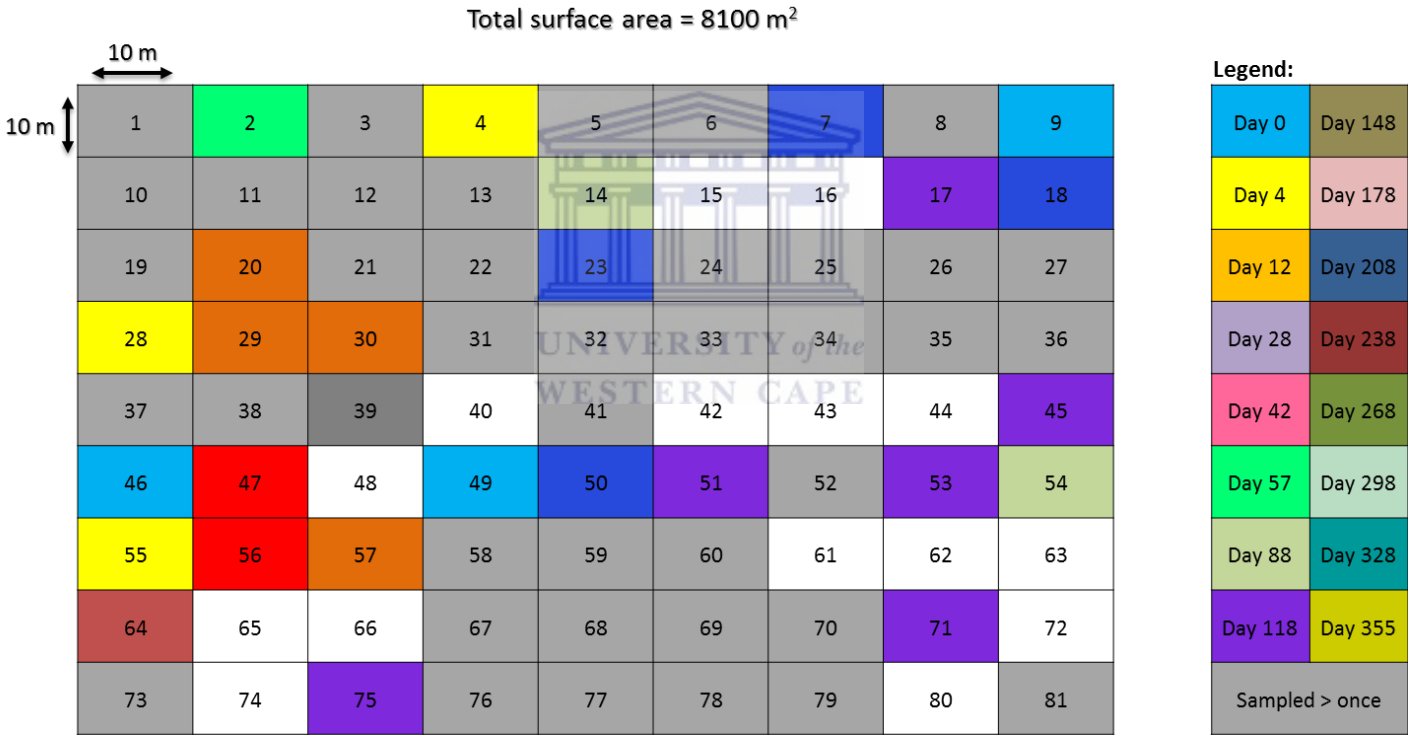

Figure 25: Schematic diagram of the sampling strategy employed in this study. The days sampled over 1 year and the respective plots are colour-coded, while white plots were never sampled. The 10 x 10m plots were identified by numbers between 1-81, which allowed the selection of individual plots to be sampled using a random number generator.

4.2.2. Environmental characterisation

Air temperature and relative humidity data was collected by the Gobabeb LST weather station established by KIT, from January 2012 to April 2013 (Figure 26). Months were grouped according to the classic seasons of the Southern hemisphere, i.e., autumn (1 March to 31 May), winter (1 June to 31 August), spring (1 September to 1 November) and summer (1 December to 28 February). Air temperature and relative humidity demonstrated significant differences between seasons, with summer being the hottest (~ 25 °C) and wettest (~60 % RH) season, while winter (°C) was the coldest (~16 °C) and driest (~40% RH; Kruskal-Wallis, $P < 0.05$) season.

Microenvironmental variables (C, P, NO_3^-, NH_4^+, Mg^{2+}, Ca^{2+}, Na, K, CEC, MC and pH) were measured to characterize each soil sample (n = 127; Appendix A). Sample concentrations were low, as expected in a desert environment (Appendix A). Some of the variables demonstrated significant differences between seasons (Wilcoxon-Mann-Whitney, $P < 0.05$). For example, soil P, Mg^{2+}, NH_4^+ and NO_3^- were significantly higher in autumn 2013, while K^+, C, CEC and soil moisture were significantly higher in autumn 2012. The majority of variables were lowest in winter 2012 (Wilcoxon-Mann-Whitney, $P < 0.05$).

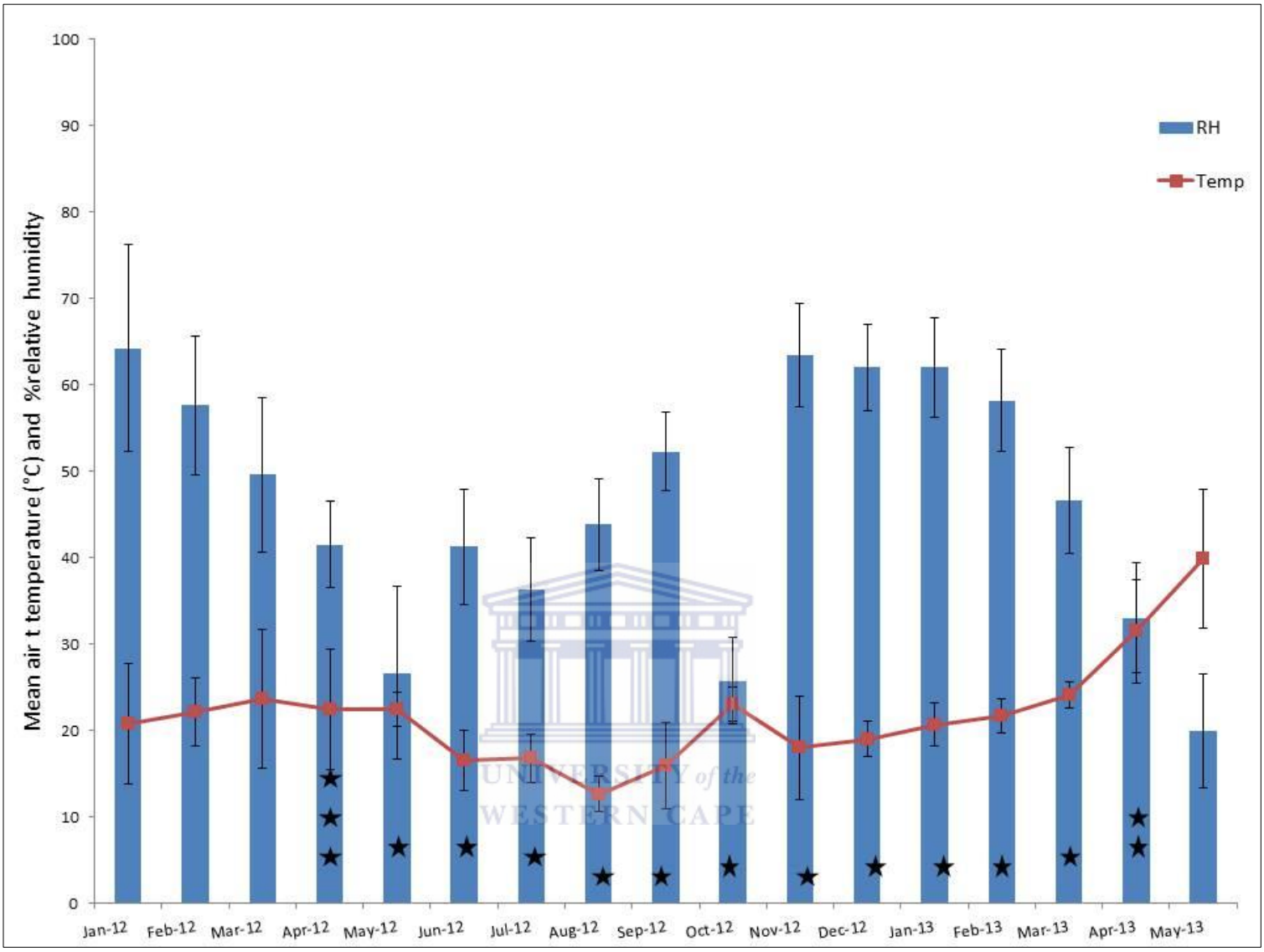

Figure 26: Mean air relative humidity and temperature from January 2012 to May 2014. Data was collected at a height of 2m near the Gobabeb Research and Training Centre by KIT. Stars indicate time points when soil was collected for soil chemistry and bacterial community analyses across a 1 year sampling period.

4.2.3 Temporal variation of bacterial communities

Bacterial community composition was assessed using T-RFLP analysis. A total of 214 T-RFs were obtained from 127 processed samples, of which 110 OTUs (51.4 %) were unique to respective seasons (Figure 27), with Autumn 2013 and summer 2012 containing the highest number of unique OTUs (30 and 49, respectively), while winter 2012 contained the lowest (7). The remaining 104 OTUs (48.6 %) were shared among at least two seasons. A total of 15 OTUs (7 %) were shared among all seasons.

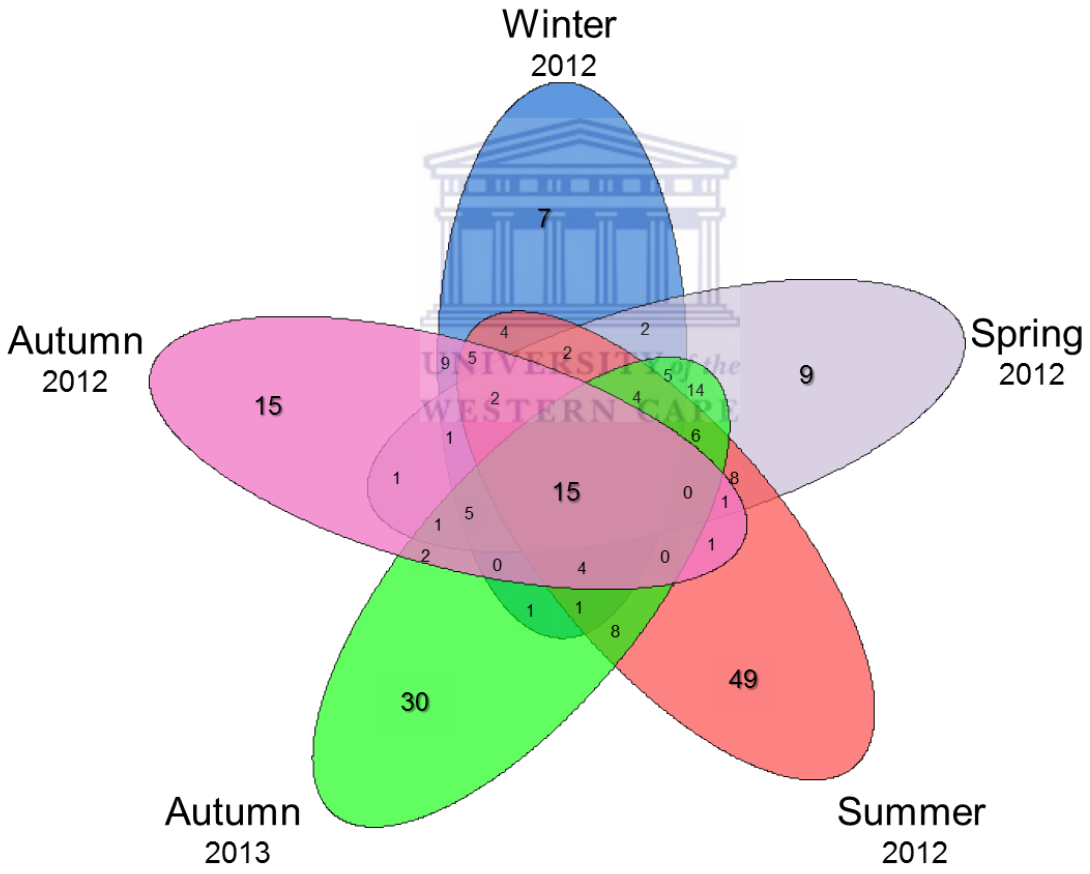

Figure 27: Venn diagram comparing the distribution of T-RFs in the bacterial community among seasons (summer, autumn, winter and spring). Autumn 2012 and autumn 2013 were separated as they represented different temporal points over the sampling period.

A NMDS ordination plot displaying the bacterial community structure over 1 year (Figure 28) revealed community separation according to seasons. The observed seasonal trends were significant (ANOSIM: R = 0.43, P = 0.001) and pairwise comparisons demonstrated significant differences in most groups (P = 0.001; Table 2).

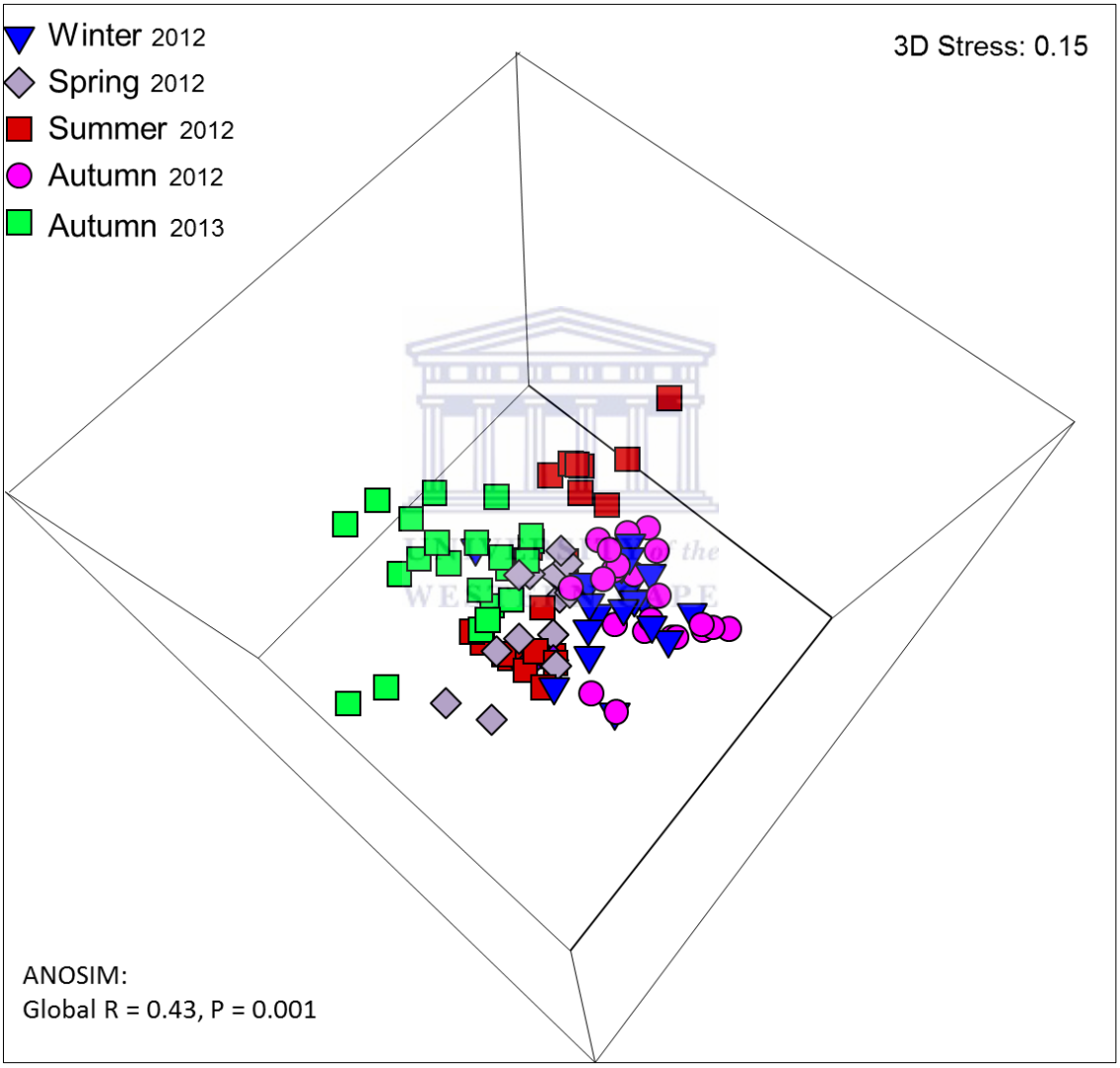

Figure 28: Seasonal patterns of bacterial community structure variation. NMDS ordination plot (Bray-Curtis similarity) of T-RFLP profiles for all soil samples (n = 127) based on the abundance of T-RFs. T-RFLP data was square-root transformed prior to analyses.

Table 2: ANOSIM pairwise comparisons of seasonal bacterial communities. Significant groupings (P = 0.001) are indicated in bold.

Groups	R-value	P-value
Autumn 2012, Winter 2012	0.034	1.24
Autumn 2012, Spring 2012	**0.561**	**0.001**
Autumn 2012, Summer 2012	**0.482**	**0.001**
Autumn 2012, Autumn 2013	**0.727**	**0.001**
Winter 2012, Spring 2012	**0.574**	**0.001**
Winter 2012, Summer 2012	**0.373**	**0.001**
Winter 2012, Autumn 2013	**0.661**	**0.001**
Spring 2012, Summer 2012	0.044	1.28
Spring 2012, Autumn 2013	**0.377**	**0.001**
Summer 2012, Autumn 2013	**0.415**	**0.001**

4.2.4 The role of the environment in shaping the temporal variability of edaphic bacterial communities

To assess the influence of abiotic factors in shaping bacterial communities composition, redundancy analysis (RDA) was performed. Soil moisture, K^+, Mg^{2+} and P were found to be significant in explaining the variability of the bacterial communities over 1 year (RDA, P = 0.001; Figure 29). Grouping of samples in the RDA plot was similar to the NMDS ordination (Figure 28), which showed seasonal differences between groups. Communities in autumn 2012 and winter 2012 were primarily influenced by soil moisture and K^+, while summer 2012 and spring 2012 communities were primarily influenced by P, whereas Mg^{2+} was associated with autumn 2013 communities. Overall, only 22% of the total variation in community composition could be explained by the environmental variables that were measured.

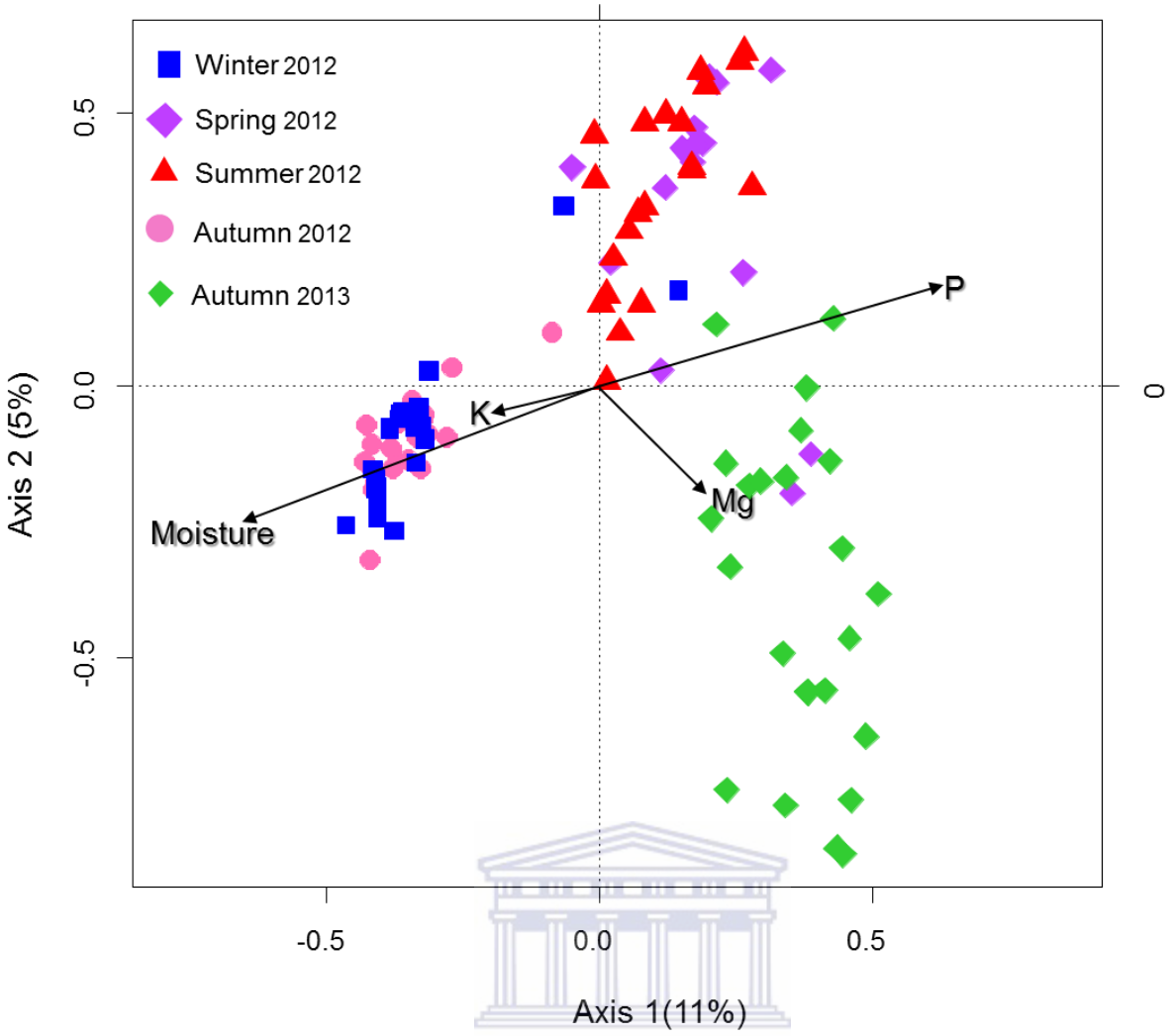

Figure 29: Redundancy analysis (RDA) biplot of bacterial abundance and microenvironmental parameters. Only the environmental variables that significantly explained variation of bacterial community structures were fitted to the ordination (arrows; P = 0.001).

Mantel correlations were conducted to investigate whether or not β diversity and environmental variables changed over time. Furthermore, mantel correlations tested changes in β diversity in relation to environmental distance (Figure 30). β diversity was found to be correlated with time (mantel r = 0.5; P = 0.001). However, environmental variables displayed no changes in relation with time (mantel r < 0.05; P = 0.07). Furthermore, changes in β diversity was not related to environmental distance of soil K^+, P, Mg^{2+} and moisture content (mantel r < 0.1; P > 0.1; Figure 30).

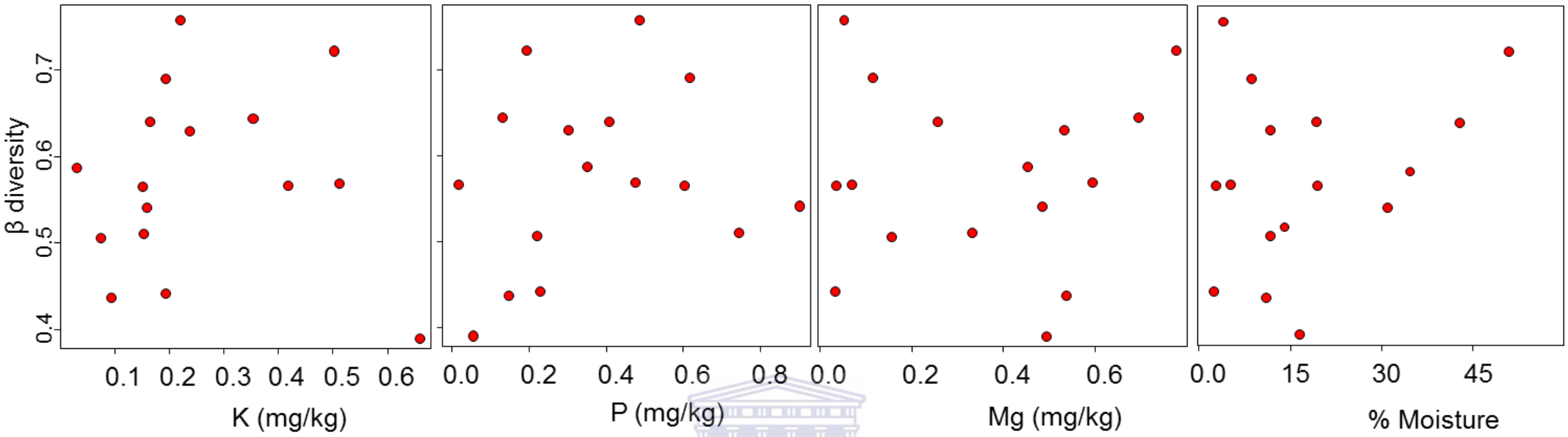

Figure 30: Changes in β-diversity in relation to total soil potassium (K^+) phosphorus (P), magnesium (Mg^{2+}) and % moisture content. For each point, β-diversity was calculated as average Bray-Curtis dissimilarity of pairwise comparisons of the total bacterial community. Values for P, Mg and K were calculated as the average concentration values obtained for all replicates per day.

4.3. Discussion

Previous studies investigating the temporal variability of microbial communities in marine (Eiler *et al*., 2011), terrestrial (Boer *et al*., 2009) and desert environments (Bell *et al*., 2009) have demonstrated strong evidence of seasonality. However, such investigations have been restricted by the use of wide-sampling intervals and often short investigation periods. To overcome these limitations, we employed a replicated sampling regime over varying timeframes, to study the effect of seasonality and soil environmental conditions on the desert edaphic bacterial community over 1 year.

We found soil water content to be important in explaining the observed temporal patterns of community composition. Previously, soil water and temperature have been linked to changes in microbial community composition over time (Shen *et al*., 2008; Tourna *et al*., 2008). Furthermore, seasonality in deserts is reflected by variations in temperature and water availability , being proven regulating factors of edaphic microbial communities (Stres *et al*., 2008; Tabuchi *et al*., 2008; Cleveland *et al*., 2007; de Vries *et al*., 2013). The importance of water availability in shaping desert microbial communities has been well established (Bell *et al*., 2009; Pasternak *et al*., 2012; Stomeo *et al*., 2013). Pointing and colleagues (2007) similarly found water availability, rather than temperature, to be the key determinant structuring arid desert communities. It is important to note that water availability is a function of interacting temperature, rainfall and relative humidity, underscoring the importance of climate for edaphic communities (Pointing *et al.,* 2007).

Furthermore, seasonal changes in soil climate have been correlated with short to medium term variations in nutrient availability (Krave *et al*., 2002; Bell *et al*., 2009; Cookson *et al*.,

2006). Soil nutrients (P, Mg^{2+}, NH_4^+, NO_3^-) were found to be highest during autumn 2013, suggesting increased soil nutrient levels could be related to changes in bacterial community composition. Indeed edaphic bacterial community variability was found to be significantly shaped by K^+, P and Mg^{2+} (in addition to soil moisture). P is often a limiting nutrient for microbial communities in terrestrial environments and has been demonstrated to increase (predominantly organic P) as a result of drying and rapid rewetting of the soil (Belnap, 2011). This was positively correlated with an increase of microbial P biomass (Turner & Haygarth, 2001). These findings suggest soil microbial biomass is a potential source of newly available P, possibly resulting from cell lysis and osmotic shock (Turner *et al.,* 2002). While the role of microbial communities in mineral weathering has been well studied (reviewed in Uroz *et al*., 2009), no information regarding the mineralizing capabilities of bacterial communities in this environment is available. Therefore, research into the geology and phosphorus-solubilizing activity of microbial communities in this region is needed.

In deserts, modest levels of soluble salts are considered important regulators of communities, because water activity (which determines the availability of biological water availability) is reduced in the presence of soluble salts (Cowan, 2009). Previously, Stomeo *et al.* (2013) demonstrated soil salinity (sodium content) to be an important factor shaping microbial communities in the Namib Desert. Similarly, we found K^+ and Mg^{2+} (soil salinity) to be important factors. K^+ and Mg^{2+} have been shown to affect cell physiology by stimulating enzyme reactions (as cofactors) in synthesizing cell materials and are recognized as essential cations for sustaining life (Simard *et al*., 1992). K^+ plays an important role in regulating cell membrane permeability and osmotic adjustment of cells to the environment (Hu & Schmidhalter, 2005). Mg^{2+} has been implicated in playing a role in

haloadaptation of microorganisms, an important mechanism in extreme environment such as the Namib Desert, characterised by the presence of soluble salts (Mulet *et al*., 1999). Recently, a study by Crits-Christoph *et al*. (2013) demonstrated a significant correlation between soil salinity and water availability. Furthermore, relative humidity and soil salinity were found to be the dominant factors shaping microbial communities in the hot and hyper-arid Atacama Desert.

Fog occurs frequently along the coastal region of the Namib Desert, including the site used for this study. The bioavailability of water from fog has been previously shown to be an important factor shaping microbial communities in this environment (Stomeo *et al*., 2013). Fog is a reliable source of bioavailable water (Henschel & Seely, 2008), which can potentially stimulate the solubilisation of P by microbial communities and/or the lysis of microbial cells discharging available P into the system. The analysis of fog chemistry near the Gobabeb Desert Research and Training Centre has previously identified high levels of K^+ and Mg^{2+} present in the fog composition (Eckardt & Schemenauer, 1998; Shanyengana *et al*., 2002). However, further investigations are required to elucidate the role of water availability and soluble salts in shaping the temporal patterns of bacterial community variation identified in this study, and whether such environmental changes are reflected in the functional attributes of these communities.

In contrast to previous studies in arid environments, C and N were not significant in explaining the seasonal variation in edaphic communities. Bell *et al.* (2009) showed

increased rates of nitrogen mineralization and organic matter turnover (as a proxy for soil organic C) to be significantly higher during summer months, in relation to increased precipitation from rainfall and increased microbial biomass. Soil moisture and temperature were found to be the dominant factors regulating mineralization rates in this system. Appel (1998) and Cui & Caldwell (1997) have similarly observed increased microbial mineralization activity in relation to increased temperature and moisture.

There are several possible reasons we did not observe similar trends. Firstly, the potential utilization of labile C sources (which do not require microbial-mediated decomposition) by edaphic communities was not measured in this study. Recently a study in a cold hyper-arid desert (Antarctica) suggested that microbial communities were efficient utilizers of available and easily accessible C sources (Dennis *et al*., 2013). We could therefore have missed an important regulating component of the labile soil C cycle and its potential influence on the edaphic communities. Secondly, as a result of limited water availability (required for biological decomposition of organic matter) and the fact that C in arid environments has a long residence time (estimated to be in the order of decades; Ewing *et al*., 2008), the rate of C turnover in this environment could therefore require a temporal resolution of decades. Thirdly, xeric conditions in deserts have been shown to limit the diffusivity of substrates and enzymes, resulting in decreased rates of C, N and P mineralization in soils (Nadeau *et al.,* 2007). Further investigation into the functional microbial community responses in relation to the potential bioavailability of soil nutrients (P, N and C), is an aspect that warrants future research in the Namib Desert.

Environmental variables were poor predictors of temporal β diversity patterns, suggesting that temporal changes in soil conditions were not linked to changes in community diversity, i.e., community dissimilarity increased over time, while the environment remained relatively stable. Furthermore, while the community composition was influenced by soil moisture, K^+, P and Mg^{2+}, none of these variables were correlated to temporal changes in β diversity. Although these results are in agreement with Lauber *et al*., (2013), several studies have demonstrated a significant relationship between environmental variables and β diversity (Verleyen *et al.* 2009; Martiny *et al*., 2011; Zinger *et al*., 2011; Lindström & Langenheder, 2012; Langenheder *et al*., 2012; Andrew *et al*., 2012). However, studies have presented contradicting results. For example, Andrew *et al*. (2012), found beta diversity to be correlated to soil carbon, although carbon was not significant in explaining patterns of variation in the community. Furthermore, Zinger *et al*. (2011) showed that different microbial communities may exhibit contrasting diversity patterns (e.g., among archaeal, fungal and bacterial communities) which may be related to diverse environmental variables (e.g., plant species composition, soil pH and spatial distance). These differences highlight the need for more studies targeting a comprehensive list of biological and environmental drivers of β diversity over time, as β diversity remains useful for understanding overall community dynamics (Green & Bohannen, 2006; Lozupone *et al*., 2008; Gilbert *et al*., 2012).

We can think of two non-exclusive explanations for not observing a pattern between β diversity and environmental variables. Firstly, β diversity has been shown to vary across spatial scales (Martiny *et al*., 2011). The observed temporal variations may therefore be due to spatial heterogeneity (not measured in this study), particularly as a result of patchy vegetation cover present on the study site at the inception of this work (Figure 14). The

presence of vegetation has been shown to influence bacterial community dissimilarity, potentially through plant-mediated modification of soil properties (Yergeau *et al*., 2007; Zinger *et al*., 2011). Secondly, we may have missed an important abiotic or biotic factor that strongly influences bacterial community dissimilarity in this region (e.g., other microbial groups such as fungi that interact with bacterial communities).

As observed in studies by Makhalanyane *et al*. (2012) and Stomeo *et al*. (2013), a large part of variation (78 %) could not be explained by the measured variables, suggesting that variations in edaphic bacterial communities in this region are caused by yet unknown deterministic drivers and/or stochastic events. Indeed, several studies have alluded to an importance of both stochastic and deterministic processes in structuring desert edaphic communities (Stomeo *et al*., 2013; Makhalanyane *et al*., 2013; Caruso *et al*., 2013). For example, environmental parameters such as substrate availability and niche differentiation (a consequence of environmental heterogeneity), and biological factors e.g., viral lysis and competition, may be important in shaping bacterial community dynamics in this region (Sander & Kalff, 1993; Epstein, 1997; Fuhrman, 1999; Cregger *et al*., 2012). However, competition has been proven to be an unlikely dominant factor affecting edaphic microbial community structures in deserts, due to the low abundance of identified genes associated with antibiotic resistance and microbe-microbe interaction (Fierer *et al*., 2012). Future research into such biological relationships could be tested by laboratory experiments. Under the neutral theory, stochastic processes e.g., demographic stochasticity, dispersal limitation and historical or evolutionary processes, are predicted to be dominant drivers of community

dynamics (Hubbel, 2001; Horner-Devine *et al*., 2007). Random patterns in species co-occurrence are expected to dominate communities under such stochastic conditions (Bell, 2005).

Conclusion

Taken together, we have demonstrated that the edaphic bacterial community in the Namib Desert gravel plains may reflect seasonal variations resulting from K^+ and Mg^{2+} (soil salinity), soil moisture, P and stochastic and/or unmeasured factors. A significant difference between communities in the same season was identified over the year (autumn), suggesting that patterns of community change observed at a single time point, may not hold across multiple time points. These differences highlight the need for long-term monitoring of the Namib Desert edaphic communities. In addition, we observed a significant increase in community turnover (β diversity) over time, however these differences were not related to temporal variation in soil variables. Thus, our study clearly shows the need to move away from snapshot investigations that have dominated so far, to achieve a more comprehensive understanding of edaphic bacterial communities in desert environments. Finally, our findings advance understanding of hot desert edaphic communities by showing that temporal patterns of change, which have largely been related to climate, may also be influenced by microenvironmental conditions.

Chapter 5: Conclusions and future prospects

The principal aim of this thesis was to investigate the temporality of the edaphic bacterial community in the Namib Desert gravel plain soils, notably in response to seasonal soil environmental conditions. T-RFLP fingerprinting and fine-scale microenvironmental characterization were employed to investigate the bacterial community dynamics over 1 year.

In chapter 3, we observed bacterial communities to be more similar within habitats than among habitats, where habitats represented environments with similar soil structure and microenvironmental parameters. These observations are consistent with the concept of habitat filtering (Van der Gught *et al*., 2007), which suggest that the composition of communities is driven by the local environmental conditions (Lagenheder & Székely, 2011). Habitat filtering includes selection of taxa by abiotic conditions (for example, higher temperatures selecting for specific communities; Garcia-Pichel *et al*., 2013) and interspecific competition (e.g., between fungal and bacterial communities). Indeed, we found communities to be shaped by variation in soil pH across relatively short spatial scales (< 200 m). While this outcome is in line with the prevailing view in soil microbial ecology (Fierer & Jackson, 2006; Lauber *et al*., 2009; Rousk *et al*., 2010; Chu *et al*., 2010; Osborne *et al*., 2011; Griffiths *et al*., 2011), this study presents the first evidence of this occurrence across spatial scales in the Namib Desert.

In chapter 4 we showed that the edaphic communities demonstrated seasonal variations in bacterial community composition over 1 year. We also found the microenvironment to be relatively stable, whereas the community dissimilarity (β diversity) increased over time. In addition, soil moisture, P, Mg^{2+} and K^{+} were shown to be important factors shaping the temporal variability of the edaphic bacterial community, potentially regulated by fog events in the gravel plains. Soil moisture has been shown to influence the physiology of edaphic microorganisms and to impact soil physiochemical properties (Castro *et al*., 2010). Furthermore, water availability has been demonstrated to be a dominant factor in shaping desert microbial communities (Pointing *et al*., 2007; Bell *et al*., 2009; Pasternak *et al*., 2012). Recent work suggests that the environmental history plays important roles in shaping the structures of indigenous microbial communities (Belnap *et al*., 2004; Martiny *et al*., 2006; Allison & Martiny, 2008). Variability in precipitation has been shown to be a dominant factor shaping bacterial and fungal community structure and function (Castro *et al*., 2010). Future research into microbial community structure and specific functional responses to water bioavailability could elucidate important mechanisms of ecosystem functioning in the Namib Desert. This may include RNA-based quantification of nutrient cycling genes, such as amoA, nifH, nirK and nifK, cbbL and mcrA (Yergeau *et al*., 2007) and enzyme activities such as aminopeptidases, phosphatases, phytases, β-glucosidases (Singsabaugh, 1994).

A large amount of variation remained unexplained by the environmental variables that were measured. This is not uncommon for ecological studies, due to the relatively large amount of variation present in species abundance data (Dumbrell *et al*., 2001) and the fact that specific

microbial communities exhibit contrasting responses to environmental factors (Drakare & Leiss, 2010). For example, contrasting diversity patterns have been shown for edaphic bacterial and fungal groups, which were driven by differences in soil pH and organic matter, respectively (Zinger *et al*., 2011). Plant-associated fungal communities (not targeted in this study) have further been shown to respond differently to environmental stress (e.g., limited bioavailable water) as compared to bacterial communities (de Vries *et al*., 2012). Moreover, fungal rather than bacterial species have been suggested to be the dominant degraders of recalcitrant (lignocellulosic) organic C in arid environments (Cregger *et al*., 2012). Decomposition of detritus could favour fungal dominance over bacterial due to the presence of recalcitrant compounds (such as lignin), as fungi have the necessary degrading enzymes (Baldrian *et al*., 2011). Fungal dominance could demonstrate higher resource competition in this oligotrophic region, potentially playing a role in shaping the bacterial community structure (Hanson *et al*., 2008). Targeting fungal communities in the Namib Desert would prove a fruitful area of future work.

While T-RFLP has been shown to detect only the most abundant organisms, simplifying the community profile (Bent & Forney, 2008; Verbruggen *et al*., 2012), it remains a useful tool for investigating microbial structure in natural environments (e.g., Fierer & Jackson, 2006; Besemer *et al*., 2012; Knight *et al*., 2012). While a link between community composition and function is often implied, DNA-based methods do not enable the measure of community function. The use of next-generation ultradeep sequencing, functional microarrays and/or soil enzyme activities measurements among others, could significantly improve our

understanding of the desert edaphic microbial communities (Makhalanyane *et al*., 2013). Functional gene markers, such as *nif*H for bacterial nitrogen fixation have successfully been employed in diverse habitats from marine to terrestrial environments (Caporaso *et al*., 2011; Dias *et al*., 2012). Rare species have been shown to have key functional roles in nutrient cycling, (e.g., methonogenesis; Thauer *et al*., 2008) and nitrogen fixation (Farnelid *et al*., 2011). Deep sequencing enables the observation of rare species, often undetected by molecular fingerprinting techniques such as T-RFLP. Functional microarrays such as GeoChips are considered to be powerful tools to characterize microbial communities (composition, function and diversity; He *et al*., 2007). Key genes relating to essential ecosystem processes such as biogeochemical cycling of C, N, P and S and stress responses can be targeted by GeoChips (He *et al*., 2007; McGrath *et al*., 2010). Furthermore, the use of enzyme activity potential has been shown to be a useful tool in desert terrestrial environments (Bell *et al*., 2009). Substrate utilization assays (Biolog and Fungilog) have enabled the study of the relationship between microbial functional diversity and ecosystem functioning (Butcher & Lanyon, 2005) and have furthermore been used to investigate desert microbial communities' response to environmental stress (Bell *et al*., 2009).

Finally, deserts are inherently susceptible to climate change (Seager *et al*., 2007), as a result of increasing atmospheric CO_2 levels, elevated temperatures and increased variation in pulse-precipitation events (Adeel *et al*., 2005). Changes in water availability have been shown to impact the composition fungal and bacterial communities, resulting in different functional responses of these communities (Barnard *et al*., 2013). Furthermore, increasing

temperatures have been shown to cause increased heterotrophic microbial activity, processing and turnover of essential nutrients (Bardgett *et al*., 2008). This process has been suggested to select for species adapted to higher temperatures, leading to the extinction of other essential community members (Garcia-Pichel *et al*., 2013). The stability of edaphic microbial communities in extreme environments such as the Namib Desert could have important effects on global ecosystem functioning, as these environments constitute approximately one-third of the earth's surface (Collins *et al*., 2008).

Taken together, the findings presented here support the use of a temporal framework when studying the variation of edaphic microbial communities in natural environments (Knight *et al*., 2012; Jansson & Prosser, 2013). Overall, this study has contributed to a better understanding of how the structure of edaphic bacterial communities responds to seasonal changes in environmental conditions.

APPENDICES

Appendix A: Microenvironmental variables measured in soil samples over 57 days in Sites A, B and C (vegetation-covered and vegetation-free study sites) in the Namib Desert gravel plains.

Site		Sample	pH	C (%)	P (mg/kg)	NH_4^+ (mg N/g)	NO_3^- (mg N/g)	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)
A		sample1	9.46	0.11	1.25	1.82	1.27	1533.66	214.94	62.14	19.54
A		sample2	8.76	0.16	1.51	2.97	0.75	1248.66	298.04	60.56	113.73
A		sample3	8.95	0.06	1.07	2.55	1.31	1108.66	191.54	59.3	18.79
A	Day 0	sample4	8.7	0.1	0.97	2.38	0.99	1299.66	251.14	65.69	49.87
A		sample5	8.87	0.08	1.04	2.56	0.82	1160.66	191.74	65.73	16.63
A		sample6	7.44	0.16	1.43	2.69	1.48	1484.66	356.94	113	1364.63
A		sample7	8.7	0.1	1.23	3.3	0.78	1401.66	203.74	55.29	17.96
A		sample8	8.25	0.22	1.1	2.7	0.74	2399.66	249.24	72.32	39.37
A		sample9	8.98	0.04	1.45	1.8	1.19	1889.66	211.84	62.05	20.02
A		sample10	9.24	0.12	1.35	1.82	0.77	1108.66	194.14	61.41	20.78
A	Day 45	sample11	9.13	0.1	1.11	1.87	0.74	1745.66	185.34	56.93	17.9
A		sample12	8.77	0.09	1.24	2.95	0.82	958.76	201.24	59	19.62
A		sample13	8.59	0.07	1.2	1.88	0.96	1214.66	186.04	57.12	16.3
A		sample14	8.43	0.09	1.16	2.44	0.91	1170.66	187.74	58.93	21.87
A		sample15	8.42	0.09	1.15	2.42	0.58	1101.66	248.34	58.8	233.23
A		sample16	8.06	0.11	1.23	2.81	3.27	1421.66	211.34	57.39	20.22
A		sample17	8.72	0.1	1.19	2.73	0.55	2900.66	344.14	89.45	248.83
A		sample18	8.12	0.06	0.39	2.6	2.39	1190.66	180.24	54.33	20.21
A	Day 12	sample19	9.08	0.12	0.17	2.46	1.29	999.66	213.24	64.28	20.46
A		sample20	8.75	0.11	1.14	2.56	2.39	1108.66	194.94	57.78	20.16
A		sample21	8.53	0.11	1.33	2.21	0.77	1157.66	210.14	54.43	16.51
A		sample22	8.5	0.16	1.3	2.39	0.59	1368.66	166.44	52.56	14.24
A		sample23	8.5	0.07	1.15	3.13	2.61	1069.66	199.84	67.91	18.63
A		sample24	8.81	0.1	1.39	1.97	2.24	1335.66	190.54	61.89	18.56
A		sample25	9.4	0.11	1.25	2.37	0.52	1041.66	172.34	57.01	14.91
A		sample26	9.33	0.12	1.15	2.38	0.52	1116.66	209.94	62.83	23.19
A	Day 28	sample27	7.74	0.04	1.28	2.36	6.53	3087.66	482.04	116.3	0
A		sample28	9.01	0.06	0.46	1.58	0.38	1646.66	264.04	61.11	82.07
A		sample29	8.7	0.05	1.14	2.52	0.56	1136.66	197.94	61.15	24.62
A		sample30	9.1	0.05	1.31	2.15	0.59	1155.66	214.24	68.79	18.01
A		sample31	8.6	0.1	1.21	2.51	0.78	1229.66	190.94	62.36	18.94

A		sample32	8.14	0.04	1.17	2.64	1.38	1640.66	303.64	70.05	268.13
A		sample33	9.39	0	1.18	2.59	0.88	1294.66	156.24	55.67	15.02
A		sample34	9.12	0.14	1.01	1.96	0.47	1136.66	166.44	57.6	14.53
A	Day 42	sample35	8.79	0.04	1.15	1.98	0.54	1229.66	177.94	55.39	17.15
A		sample36	8.38	0.05	1.08	3.33	0.82	1026.66	201.74	63.89	19.75
A		sample37	8.42	0.05	0.91	2.49	0.96	985.06	207.74	67.08	18.85
A		sample38	8.73	0.07	1.01	2.38	0.88	1228.66	195.34	56.6	19.16
A		sample39	9.05	0.02	0.95	1.78	0.75	1414.66	174.34	57.29	16.76
A		sample40	8.32	0.02	0.81	2.95	1.16	1157.66	185.64	57.62	20.6
A		sample41	9.14	0	0.84	0.98	0.34	1103.66	172.94	54.55	18.7
A		sample42	8.84	0.07	1.11	2.96	0.94	1178.66	212.94	72.14	21.01
A	Day 57	sample43	9.16	0.02	1.09	2.59	0.46	1404.66	196.74	58.88	20.56
A		sample44	8.17	0.02	0.5	1.04	13.42	3462.66	670.14	177.6	51.07
A		sample45	9.17	0.03	1.21	2.7	1.15	1303.66	195.04	67.43	18.62
A		sample46	8.94	0.03	1.14	2.29	0.72	1178.66	197.24	60.18	19.69
A		sample47	8.94	0.01	1.25	2.56	0.44	1366.66	189.94	65.4	18.77
B		sample48	7.06	0	0.4838	2.25	2.32	381.2	62.81	5.814	63.51
B	Day 0	sample49	6.94	0	0.7373	1.65	2.345	340.1	56.22	5.447	40.06
B		sample50	7.02	0	1.074	1.23	10.325	511	64.02	11.46	386.2
B		sample51	7.34	0.057	0.7434	1.78	3.73	523.6	64.31	8.019	144.3
B	Day 4	sample52	7.46	0	0.9293	1.88	2.21	366.4	62.98	6.825	68.54
B		sample53	7.42	0	0.8876	1.865	2.315	373.7	49.54	5.197	55.83
B		sample54	7.65	0.032	0.8043	1.355	1.32	367.5	40.49	5.271	24.2
B	Day 12	sample55	7.95	0	0.4443	1.265	2.56	431.9	58.43	6.29	61.18
B		sample56	7.83	0.082	0.9923	2.275	2.05	374.1	49.35	4.857	26.93
B		sample57	7.89	0	0.0557	1.395	1.835	404.3	64.98	8.094	51.67
B	Day 28	sample58	7.68	0.007	0.0509	1.64	4.3	542.8	75.51	9.901	155.48
B		sample59	8.02	0.997	0.0265	1.5	6.88	547.4	89.65	8.576	121
B		sample60	7.92	0	0.4985	2.34	3.19	381.2	66.65	6.451	48.19
B	Day 42	sample61	7.95	0.862	0.8346	1.495	3.09	562.6	87.03	9.937	425.6
B		sample62	7.9	0	1.027	1.565	2.83	402.3	60.51	5.99	44.19
B		sample63	8.2	0	0.9508	1.755	1.48	186.1	34.74	4.579	19.65
B	Day 57	sample64	8.19	0.137	0.8549	1.625	3.915	417.4	56.56	7.613	96.1
B		sample65	8.06	0.192	0.8499	1.86	2.02	354.5	44.39	5.282	29.24
C		sample66	7.18	0	0.8083	2.73	3.065	382.1	55.2	6.242	72.36
C	Day 0	sample67	7.21	1.177	1.019	1.505	1.38	270.2	38.27	5.019	27.52
C		sample68	7.19	0.192	0.9075	1.24	3.75	443.2	50.9	6.571	170.84
C		sample69	7.48	0	1.005	1.995	2.425	403.4	45.86	6.288	43.28
C	Day 4	sample70	7.82	0	0.9707	1.685	3.2	380.5	59.36	6.911	91.84
C		sample71	7.56	0	0.9912	1.685	1.59	364.6	39.46	5.103	30.9

C	Day 12	sample72	7.52	0	1.19	1.94	3.33	396.6	44.75	6.619	46.21
C		sample73	7.61	0	0.876	1.975	2.865	396.7	56.56	5.762	42.62
C		sample74	7.71	0	1.211	2.095	3.255	334.1	41.27	6.345	50.79
C	Day 28	sample75	8	0	0.0146	2.56	2.865	537	67.85	7.266	107.8
C		sample76	8.11	0	0.4548	1.285	2.786	402.4	69.99	8.14	87.84
C		sample77	8.2	0.617	0.3074	1.535	2.985	458.4	71.38	7.596	114.7
C	Day 42	sample78	8.01	0.447	0.0827	1.425	2.72	437.1	68.42	8.426	84.52
C		sample79	8.04	0	0.467	1.815	2.42	546.4	80.57	7.316	142.8
C		sample80	7.93	0	0.9636	1.845	2.05	404.8	45.58	5.537	36.79
C	Day 57	sample81	8.09	0.212	0.8558	1.72	4.35	399.1	55.02	7.842	53.68
C		sample82	7.85	0	1.145	1.565	3.98	464.4	58.95	8.886	253.6
C		sample83	7.92	0.347	1.155	1.765	2.43	273	35.52	5.233	52.93

Appendix B: Microenvironmental variables measured in soil samples over 1 year (in Site A) in the Namib Desert gravel plains.

	Sample	pH	C (%)	P (mg/kg)	NH_4^+ (mg N/g)	NO_3^- (mg N/g)	CEC (cmol/kg)	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	MC (%)
Day 0	sample1	9.46	0.11	1.25	1.82	1.27	39.07	1533.66	214.94	62.14	19.54	7.5
	sample2	8.76	0.16	1.51	2.97	0.75	28.8	1248.66	298.04	60.56	113.73	0.9
	sample3	8.95	0.06	1.07	2.55	1.31	25	1108.66	191.54	59.3	18.79	8.6
	sample4	8.7	0.1	0.97	2.38	0.99	6.7	1299.66	251.14	65.69	49.87	7.1
	sample5	8.87	0.08	1.04	2.56	0.82	5.82	1160.66	191.74	65.73	16.63	7.5
	sample6	7.44	0.16	1.43	2.69	1.48	9.87	1484.66	356.94	113	1364.63	4.5
	sample7	8.7	0.1	1.23	3.3	0.78	8.16	1401.66	203.74	55.29	17.96	5.9
	sample8	8.25	0.22	1.1	2.7	0.74	6.29	2399.66	249.24	72.32	39.37	7.9
Day 4	sample9	8.98	0.04	1.45	1.8	1.19	6.1	1889.66	211.84	62.05	20.02	7.7
	sample10	9.24	0.12	1.35	1.82	0.77	8.07	1108.66	194.14	61.41	20.78	5.2
	sample11	9.13	0.1	1.11	1.87	0.74	13.02	1745.66	185.34	56.93	17.9	9.9
	sample12	8.77	0.09	1.24	2.95	0.82	6.84	958.76	201.24	59	19.62	7.7
	sample13	8.59	0.07	1.2	1.88	0.96	9.13	1214.66	186.04	57.12	16.3	1.4
	sample14	8.43	0.09	1.16	2.44	0.91	3.68	1170.66	187.74	58.93	21.87	6.6
	sample15	8.42	0.09	1.15	2.42	0.58	5.8	1101.66	248.34	58.8	233.23	7.72
Day 12	sample16	8.06	0.11	1.23	2.81	3.27	9.29	1421.66	211.34	57.39	20.22	5.3
	sample17	8.72	0.1	1.19	2.73	0.55	3.12	2900.66	344.14	89.45	248.83	7.1
	sample18	8.12	0.06	0.39	2.6	2.39	8.8	1190.66	180.24	54.33	20.21	5.7
	sample19	9.08	0.12	0.17	2.46	1.29	7.78	999.66	213.24	64.28	20.46	6.3
	sample20	8.75	0.11	1.14	2.56	2.39	4.62	1108.66	194.94	57.78	20.16	4.2
	sample21	8.53	0.11	1.33	2.21	0.77	3.91	1157.66	210.14	54.43	16.51	4.4
	sample22	8.5	0.16	1.3	2.39	0.59	2.53	1368.66	166.44	52.56	14.24	3.3
	sample23	8.5	0.07	1.15	3.13	2.61	2.21	1069.66	199.84	67.91	18.63	6.6
Day 27	sample24	8.81	0.1	1.39	1.97	2.24	2.51	1335.66	190.54	61.89	18.56	5.6
	sample25	9.4	0.11	1.25	2.37	0.52	6.48	1041.66	172.34	57.01	14.91	0.4
	sample26	9.33	0.12	1.15	2.38	0.52	3.03	1116.66	209.94	62.83	23.19	0.3

	sample27	7.74	0.04	1.28	2.36	6.53	0.93	3087.66	482.04	116.3	0	18.8
	sample28	9.01	0.06	0.46	1.58	0.38	1.55	1646.66	264.04	61.11	82.07	0
	sample29	8.7	0.05	1.14	2.52	0.56	2.73	1136.66	197.94	61.15	24.62	0
	sample30	9.1	0.05	1.31	2.15	0.59	3.18	1155.66	214.24	68.79	18.01	0.71
	sample31	8.6	0.1	1.21	2.51	0.78	1.66	1229.66	190.94	62.36	18.94	1
Day 42	sample32	8.14	0.04	1.17	2.64	1.38	4.06	1640.66	303.64	70.05	268.13	7.1
	sample33	9.39	0	1.18	2.59	0.88	1.32	1294.66	156.24	55.67	15.02	87.1
	sample34	9.12	0.14	1.01	1.96	0.47	3.68	1136.66	166.44	57.6	14.53	95.1
	sample35	8.79	0.04	1.15	1.98	0.54	1.34	1229.66	177.94	55.39	17.15	53.2
	sample36	8.38	0.05	1.08	3.33	0.82	1.82	1026.66	201.74	63.89	19.75	48.7
	sample37	8.42	0.05	0.91	2.49	0.96	3.88	985.06	207.74	67.08	18.85	0
	sample38	8.73	0.07	1.01	2.38	0.88	4.1	1228.66	195.34	56.6	19.16	29.9
	sample39	9.05	0.02	0.95	1.78	0.75	3.99	1414.66	174.34	57.29	16.76	0
Day 57	sample40	8.32	0.02	0.81	2.95	1.16	3.5	1157.66	185.64	57.62	20.6	0
	sample41	9.14	0	0.84	0.98	0.34	4.79	1103.66	172.94	54.55	18.7	0
	sample42	8.84	0.07	1.11	2.96	0.94	3.04	1178.66	212.94	72.14	21.01	0
	sample43	9.16	0.02	1.09	2.59	0.46	4.72	1404.66	196.74	58.88	20.56	0
	sample44	8.17	0.02	0.5	1.04	13.42	4.2	3462.66	670.14	177.6	51.07	0
	sample45	9.17	0.03	1.21	2.7	1.15	5.31	1303.66	195.04	67.43	18.62	8.4
	sample46	8.94	0.03	1.14	2.29	0.72	4.06	1178.66	197.24	60.18	19.69	0
	sample47	8.94	0.01	1.25	2.56	0.44	6.65	1366.66	189.94	65.4	18.77	31.8
Day 88	sample48	8.72	0.07	1.33	1.8	2.19	10.98	1892.66	270.34	61.51	218.53	0.7
	sample49	8.62	0.09	1.39	0.76	0.49	4.05	1526.66	349.04	66.94	123.53	2.1
	sample50	8.84	0.07	1.03	1.96	0.47	4.63	1089.66	145.04	53.69	13.43	0
	sample51	8.68	0.08	1.1	2.13	0.73	7.58	1205.66	199.14	61.51	19.54	1.3
	sample52	8.5	0.12	0.95	1.46	1.03	4.97	2384.66	225.64	67.54	21.75	0.1
	sample53	8.68	0.06	1.2	2.32	1.22	3.85	1106.66	191.14	58.78	20.35	0.6
	sample54	8.75	0.09	1.04	1.93	0.35	4.18	1505.66	244.84	54.02	346.53	0.7
	sample55	8.86	0.05	1.22	1.71	1.33	3.91	1152.66	205.74	64.32	19.77	1
Day 118	sample56	8.72	0.09	1.24	1.79	0.4	3.82	999.66	182.14	61.99	20.22	0.7
	sample57	8.62	0.13	1.22	2.07	0.21	4.56	1528.66	203.84	64.91	19.37	0
	sample58	8.84	0.15	1.24	1.79	0.75	2.13	1147.66	212.34	68.31	19.55	0.6
	sample59	8.68	0.18	1.2	2.09	0.79	6.46	1178.66	156.14	56.89	15.71	1.1
	sample60	8.5	0.08	1.19	1.85	0.67	4.88	1108.66	141.34	53.08	14.17	0.8
	sample61	8.68	0.36	1.28	6.97	0.61	8.79	1885.66	212.94	65.34	37.97	1.3
	sample62	8.75	0.06	1.25	2.77	0.63	5.02	1168.66	190.64	62.28	18.25	0.7
	sample63	8.86	0.01	1.07	2.6	1.14	3.14	1721.66	216.54	58.9	24.09	1.2
Day 148	sample64	9.01	0.06	1.43	2.88	2.94	3.78	1205.66	215.84	71.18	22.1	1
	sample65	9.2	0.08	1.3	1.89	1.67	4.36	1351.66	192.34	66.86	21.22	0.5
	sample66	9.24	0.03	0.65	2.24	5.16	1.99	3240.66	556.94	151.4	0	0.6
	sample67	8.99	0.04	1.41	2.54	5.59	1.43	1354.66	211.74	65	25.08	0.3
	sample68	9.01	0.07	1.48	1.63	2.78	3.45	1250.66	219.14	73.42	25.04	0.6
	sample69	9.43	0.01	1.29	2.7	1.52	43.17	1206.66	205.44	69.61	24.12	0.3
	sample70	8.53	0.07	1.27	1.97	1.16	3.06	2467.66	238.14	69.72	39.55	1.1
	sample71	8.53	0.06	1.33	1.96	0.88	112.95	2487.66	237.94	70.96	42.69	1.2
Day 178	sample72	7.84	0	1.44	2.23	1.76	6.57	1285.66	197.64	67.46	18.99	1.1
	sample73	8.32	0.04	1.27	1.96	1.1	4.12	1309.66	207.34	67.09	24.62	0.7
	sample74	7.83	0.05	1.51	2.8	1.65	3.98	1163.66	248.14	68.63	58.96	0.7
	sample75	8.8	0.03	1.42	2.5	1.59	5.39	1136.66	199.94	69.48	26.8	0.9
	sample76	9.47	0.02	1.23	1.93	1.26	3.91	1384.66	190.24	66.61	18.97	1
	sample77	9.19	0	1.64	2.89	2.48	3.79	2408.66	259.64	75.32	67.17	1
	sample78	9.02	0.04	1.25	1.29	2.46	5.73	1282.66	178.64	59.12	19	2
	sample79	8.7	0.05	1.38	2.33	1.54	7.08	1392.66	215.54	59.48	45.32	0
Day 198	sample80	8.53	0.06	1.27	2.31	1.71	6.4	1164.66	211.54	64.02	21.32	0.3
	sample81	8.3	0.02	1.19	2.51	1.72	7.49	1766.66	303.24	71.63	357.23	0.6
	sample82	8.11	0.07	1.33	2.51	2.17	7.9	2163.66	229.84	73.38	26.75	0.6
	sample83	8.17	0.05	1.38	3.47	2.46	4.99	1355.66	231.14	67.06	37.44	0.9
	sample84	7.97	0.05	1.46	2.72	3.29	5.43	1242.66	209.64	68.54	19.69	3.3
	sample85	7.26	0.04	1.45	2.3	2.51	3.9	1267.66	222.14	69.74	22.16	0.4
	sample86	7.64	0.04	1.44	3.16	3.08	5.71	1240.66	222.04	67.8	21.92	0.7
	sample87	7.73	0.04	1.66	2.28	1.75	6.81	1228.66	201.84	63.6	19.89	0.4

Day 238	sample88	8.15	0.04	0.91	1.99	1.92	2.66	3254.66	378.34	81.56	174.03	0.3
	sample89	9.1	0.03	1.33	2.26	1.3	4.54	1163.66	195.74	67.97	34.95	0.5
	sample90	8.62	0.04	1.4	2.76	1.68	12.21	1838.66	202.14	67.49	27.42	0.6
	sample91	8.64	0.02	1.39	4.05	2.2	6.25	1201.66	187.34	64.56	24.91	0.7
	sample92	8.75	0.05	1.31	1.85	2.89	10.61	1716.66	197.14	64.36	19.93	1.2
	sample93	9.6	0.04	1.4	2.28	2.28	5.09	1129.66	208.94	70.26	26.12	0.5
	sample94	9.03	0.04	1.16	3.18	2.81	4.36	1206.66	193.84	70.79	22.02	0.4
	sample95	8.61	0	1.25	3.63	2	3.87	1265.66	198.04	63.8	18.43	0
Day 268	sample96	7.58	0.03	1.38	3.03	2.06	5.46	1228.66	203.14	71.15	23.57	0.5
	sample97	7.38	0.02	1.55	4.18	3.8	3.9	1355.66	203.44	70.5	20.86	0.7
	sample98	7.28	0.04	0.93	2.83	3.53	5.53	2193.66	270.84	73.07	1267.63	0.7
	sample99	9.54	0.02	1.41	3.01	3.21	2.64	1119.66	208.44	66.92	24.4	0.4
	sample100	9.3	0.04	1.39	2.21	3.78	4.28	1614.66	228.14	62.56	53.8	0.7
	sample101	9.23	0.03	1.28	4.36	2.21	3.31	1910.66	238.54	64.55	30.77	0.3
	sample102	8.29	0.41	1.5	3.29	3.64	0	1740.66	271.74	68.95	118.93	1.7
	sample103	8.45	0.03	1.42	3.58	1.61	3.59	1510.66	216.34	62.33	22.02	0.7
Day 298	sample104	9.38	0.01	1.32	1.49	1.68	4.69	1175.66	193.54	63.92	23.06	1
	sample105	9.12	0.05	1.19	2.01	2.49	7.63	1304.66	191.64	66.72	21.68	0.5
	sample106	9	0.06	1.41	3.08	1.84	5.1	1182.66	207.74	68.88	21.29	0.6
	sample107	8.77	0.06	1.46	3.22	1.81	6.46	1376.66	203.64	70.76	23.72	0.5
	sample108	8.6	0.04	1.59	1.11	1.63	5.99	1172.66	225.44	73.64	22.85	0.6
	sample109	8.35	0.01	0.77	1.67	2.98	5.34	2018.66	335.34	95.77	1383.63	0.5
	sample110	8.13	0	1.4	3.52	3.83	4.8	2448.66	209.64	73.66	74.48	0.6
	sample111	7.77	0.02	1.4	3.43	2.96	4	1212.66	212.84	69.49	23.07	0.4
Day 328	sample112	9.25	0.18	0	2.24	2.74	5.51	1183.66	208.14	71.24	22.46	0
	sample113	9.02	0.1	1.19	2.89	2.16	5.92	1172.66	206.14	69.82	25.35	0
	sample114	9.15	0.11	1.41	3.27	3.85	4.53	1145.66	225.64	71.6	21.66	0
	sample115	9	0.08	1.46	3.01	3.13	4.89	1590.66	208.24	68.24	20.84	0
	sample116	8.85	0.1	1.59	3.31	1.6	4.49	1283.66	204.34	66.2	21.99	0
	sample117	8.66	0.1	0.77	2.88	2.32	4.78	1449.66	201.04	67.38	21.01	0
	sample118	8.57	0.09	1.4	3.4	2.83	4.42	1389.66	214.74	72.75	20.04	0
	sample119	8.15	0.07	1.4	2.42	1.18	4.2	1304.66	212.54	72.25	23.06	0
Day 355	sample120	8.8	0.05	0.84	3.41	2.18	3.21	1605.66	373.14	120	767.53	1.3
	sample121	9.22	0.1	1.47	4.76	4.29	5.53	1259.66	235.14	77.36	156.13	0.8
	sample122	8.94	0.11	1.35	3.52	2.69	3.98	1360.66	192.84	67.82	19.14	0.4
	sample123	8.2	0.07	1.14	4.83	2.8	5.85	1854.66	192.04	55.85	17.1	1.3
	sample124	8.58	0.15	1.45	2.77	1.44	6.09	1172.66	211.64	69.73	18.9	1.3
	sample125	8.61	0.1	1.28	1.67	1.36	10.74	1407.66	217.84	68.91	21.83	0
	sample126	8.51	0.08	1.44	3.27	1.53	3.82	1468.66	205.04	67.78	20.59	14.3
	sample127	8.52	0.1	1.42	2.41	1.53	4.18	1699.66	250.44	73.25	27.98	1

Research outputs

International Travel

1. NASA-AMES-IMBM-GRTC. April 2011 and May 2012. Spaceward bound Research Expedition. Gobabeb Research and Training Centre, Namibia.

Conference Outputs

2. **Alacia Armstrong***, Jean-Baptiste Ramond, Angel Valverde, Marla I. Tuffin, Don A. Cowan (2013). *Seasonal dynamics of edaphic bacterial communities in the hyper-arid Namib Desert.* South African Society for Microbiology Conference. **Oral** *Presenting author

3. **Alacia Armstrong**, Jean-Baptiste Ramond, Angel Valverde, Marla I. Tuffin*, Don A. Cowan *(2013). Seasonal dynamics of edaphic bacterial communities in the hyper-arid Namib Desert.* University of the Western Cape Research Open Day. **Poster presentation**

4. **Alacia Armstrong**, Jean-Baptiste Ramond*, Marla I. Tuffin, Don A. Cowan (2013). *Seasonal dynamics of edaphic bacterial communities in the hyper-arid Namib Desert.* University of Pretoria (UP) Genomics Research Institute Colloquium Day. **Oral**

5. **Alacia Armstrong,** Jean-Baptiste Ramond*, Marla I. Tuffin, Don A. Cowan (2012). The effect of carbon input on the evolution of the soil microbial community in the central hyper-arid Namib Desert. International Society for Microbial Ecology, Symposium. **Poster presentation.**

6. **Alacia Armstrong**, Jean-Baptiste Ramond*, Marla I. Tuffin, Don A. Cowan (2012). *The effect of carbon input on the evolution of the soil microbial community in the central hyper-arid Namib Desert.* Extremophiles Meeting. **Poster presentation.**

Publications

7. **Alacia Armstrong**, Jean-Baptiste Ramond, Angel Valverde, Mary Seely, Marla I. Tuffin, Don A. Cowan. Temporal variability of edaphic bacterial communities in the hyper-arid Namib Desert. *International Society for Microbial Ecology*. ***In preparation***.

8. **Alacia Armstrong**, Jean-Baptiste Ramond, Angel Valverde, Mary Seely, Marla I. Tuffin, Don A. Cowan. temporal variability of hot desert edaphic bacterial communities and microenvironmental parameters. *Applied and Environmental Microbiology*. ***In preparation.***

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