NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY

# PLANT INFLUENCES ON TAXONOMIC AND FUNCTIONAL DIVERSITY OF SOIL MICROBIAL COMMUNITIES AND SOIL BIOGEOCHEMISTRY IN A HYPER-ARID DESERT

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November 2024

**DECLARATION**

I, Elise Ndatulumukwa Ndahafa Nghalipo, hereby declare that the work contained in the thesis entitled: “Plant Influences on Taxonomic and Functional Diversity of soil microbial communities and Soil Biogeochemistry in a hyper-arid Desert” is my original work and that I have not previously in its entirety or part submitted to any university or higher education institution for the award of a qualification.

November 30, 2024

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**DEDICATION**

This dissertation is dedicated to my baby brother, George Nghilifa Nghalipo, affectionately known as Uncle G, G-man, G, Bro G. I wrote this thesis during such a challenging time in his life, a circumstance that deeply affected our entire family. There were moments when the difficulties seemed insurmountable, and I had no strength to continue; I even contemplated (on several occasions) putting this thesis on hold until things improved. But then I thought, what would Uncle G have me do regardless of his struggles? He would certainly want me to keep pushing and finish this degree because he was my biggest cheerleader and research assistant during this journey. Before he became unwell, he excitedly kept asking, when are you finishing, Dr? So, Uncle G, you were my motivation during my write-up, this is for you, Nghelo! I love you dearly and cannot wait for you to get well and read this thesis.

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**Philippians 1:6**

Being confident of this very thing, that he who began a good work in me will complete it until the day of Christ Jesus.

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Rolf has been such a strong support system at NUST, from defending my research against bureaucratic hurdles to persistently advocating for the necessary approvals for my laboratory experiment travels to South Africa. His dedication went beyond the science itself; he consistently checked on my well-being, ensuring that I received timely payments during my early days at NUST. His support often required knocking on the same doors and making phone calls multiple times; I know it was uncomfortable, but he continued to do so. Had it not been for his support, this project would not have taken off. Rolf, thank you for allowing me to work in your shadow while you were taking up all the heat. Baie dankie, Captain!

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The DNA extraction was done at the Centre for Microbial Ecology and Genomics (CMEG), University of Pretoria. I have made multiple trips to CMEG, and every time I mentioned the need to travel for DNA extraction, Don always welcomed me into CMEG with open arms and offered his postdocs to support me in every way possible. Don, thank you for being so welcoming and for allowing me to learn science in your space.

Maximiliano Ortiz taught me DNA extraction, and we have spent long hours and late nights in the lab trying to extract enough DNA for sequencing. My soil samples had a high salt concentration, which made DNA extraction difficult. Still, after many days of frustration, multiple attempts, and a lot of effort and guidance from Max, we eventually obtained enough DNA for sequencing, which was used in this thesis. Max sacrificed his nights while I was in Pretoria to spend time working with me in the laboratory. When we spent a full day extracting DNA and realized that the concentration was still low, we would sigh and laugh about it, and he would say, "It is okay; we will get the right concentration one day; today wasn't just that day." When we worked till late at night, he would escort me to my guesthouse to ensure I arrived safely, then return to the lab to continue the experiment. On several occasions, Max even slept in the lab to finish the experiment. He taught me the basics of bioinformatics, and we had Zoom meetings to get me started with data processing, which took a lot of time and effort. Max, thank you for your sacrifice, guidance, and assistance, which have shaped this project into what it is today, and for this, I am forever indebted to you.

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**LIST OF FIGURES**

**CHAPTER 1**



**CHAPTER 2**

**CHAPTER 3**



**CHAPTER 4**

**LIST OF TABLES**

**CHAPTER 3**



**CHAPTER 4**

**ACRONYM**

| | |
|---|---|
| AET | Actual Evapotranspiration |
| ANOVA | Analysis of Variance |
| AOA | Ammonia-oxidizing Archaea |
| ASVs | Amplicon Sequence Variants |
| C | Carbon |
| CCA | Canonical Correspondence Analysis |
| CCA | Constrained Correspondence Analysis |
| CEC | Cation exchange capacity |
| $CO_2$ | Carbon Dioxide |
| CT | Collection Time |
| DNA | Deoxyribonucleic Acid |
| DRAM | Distilled and Refined Annotation of Metabolism |
| EDTA | Ethylenediaminetetraacetic acid |
| GTDB-Tk | Genome Taxonomy Database Toolkit |
| ITS | Internal Transcribed Spacer |
| K | Potassium |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| KO | KEGG Orthology |
| LDA | Linear Discriminant Analysis |
| LEfSe | Linear discriminant analysis effect size |
| LP | Litter Placement |
| LT | Litter Type |
| MAG | Metagenome-Assembled Genomes |
| N | Nitrogen |
| NADPH | Nicotinamide Adenine Dinucleotide Phosphate |
| ORF | Open Reading Frames |
| OTU | Operational Taxonomic Unit |
| PCA | Principal Component Analysis |
| PCoA | Principal Coordinate Analysis |

| | |
|---|---|
| PCR | Polymerase Chain Reaction |
| PERMANOVA | Permutational Multivariate Analysis of Variance |
| PGPR | Plant Growth-Promoting Rhizobacteria |
| QIIME | Quantitative Insights into Microbial Ecology |
| RDA | Redundancy Analysis |
| RNA | Ribonucleic Acid |
| RPKM | Reads Per Kilobase Million |
| SLM | Soil-Litter Mixing |
| SOC | Soil Organic Carbon |
| TCA | 2 Tricarboxylic Acid Cycle |
| TN | Total Nitrogen |
| UV | Ultraviolet |
| WPS-2 | Wittenberg Polluted Soil-2 |

## OUTLINE OF CHAPTERS

This work is divided into 5 chapters. Each of the chapters is introduced separately, and a reference list is provided at the end of each chapter.

**Chapter 1: General Introduction**

This chapter provides a general introduction and motivation for the study. The objectives of the study are stated.

**Chapter 2: Plant influences on soil microbial composition and diversity in the Skeleton Coast National Park, Namib Desert**

This chapter presents the results of an investigation into the composition and diversity of the soil microbial communities (bacteria, Archaea & fungi) associated with plant hummocks. It determines how soil microbial communities compare between plant hummock soils and bare soils (unvegetated: windward slope and gravel plain).

**Chapter 3: Plant influences on microbial functional capacity in the Skeleton Coast National Park, Namib Desert**

This chapter presents the results of an investigation into the functional capacity of the soil microbial communities associated with plant hummocks and the metabolic strategies underlying the ability of soil microbes to thrive and perform ecosystem functions and services in hyper-arid ecosystems.

**Chapter 4: Influence of vegetation patches on litter decomposition in the Skeleton Coast National Park, Namib Desert**

This chapter presents the results of an investigation into the influence of vegetation patches on litter decomposition rates.

**Chapter 5: Conclusions and Future Work**

This chapter provides the final conclusion and further prospects of this study.

**TABLE OF CONTENTS**

**GENERAL ABSTRACT**

Dryland ecosystems are characterised by extreme environmental conditions, including large temperature fluxes, infrequent and highly variable rainfall patterns, and soil deficiency in organic matter. Consequently, vegetation in these regions is sparse and unevenly distributed, which creates unique microhabitats that harbor distinct microbial assemblages. In hyper-arid desert ecosystems, vegetation plays a pivotal role in shaping the taxonomic and functional diversity of soil microbial communities, thereby influencing soil biogeochemical processes. Generally, vegetation can play a significant role in regulating the temporal and spatial variation of soil microbial activities within the vegetation canopy by influencing various abiotic factors, such as soil moisture, solar radiation and temperature fluctuations, therefore creating specific niches that support diverse microbial communities. While previous studies have investigated soil microbiomes in the Namib Desert, these studies primarily focused on the central gravel plains, leaving the Skeleton Coast National Park, part of the northern Namib Desert, largely unexplored; thus, there is limited understanding of the soil microbiomes in this hyper-arid coastal region. This thesis aimed to provide insights into the influence of vegetation on the taxonomic and functional diversity of soil microbial communities and the associated biogeochemical processes by amplicon-based analyses and litterbag techniques, respectively, in this hyper-arid desert.

**Chapter 2:** Total environmental DNA was extracted to evaluate the microbial communities associated with plant hummocks in the Skeleton Coast National Park. The V3-V4 region of the bacterial (and archaeal) 16S rRNA gene and the ITS-1 and ITS-2 regions of the fungal internal transcribed spacer (ITS) rRNA regions were amplified and sequenced to assess bacterial and fungal communities associated with plant hummocks and determine how soil microbial communities compare between plant hummock soils and bare soils (unvegetated: windward slope and gravel plain). The findings revealed that vegetated hummocks and their surrounding soils of the Skeleton Coast National Park possess qualitatively distinct soil microbiomes. Notably, vegetated hummock soils harbored a significantly higher number of observed species relative to bare soils. This suggests that vegetation plays a crucial role in enhancing microbial diversity in this hyper-arid environment. Such diversity is vital, as soil

microbial communities are integral to ecosystem functions, including nutrient cycling and organic matter decomposition.

**Chapter 3:** The functional capacity of bacterial communities was predicted based on whole-shotgun metagenomic datasets to evaluate the microbial functional potential in three sampling locations: vegetated hummock, unvegetated windward slope, and gravel plains. Additionally, these datasets were used to investigate metabolic strategies that underlie the ability of these soil microbes to thrive and perform ecosystem functions in this hyper-arid ecosystem. The metagenomic analyses identified functions related to carbon fixation, carbon degradation, ammonium oxidation, methane metabolism, and sulfur assimilation. Vegetated hummock soils had higher enrichment of functional capacity relative to bare soils, suggesting that vegetation patches significantly influence microbial functional potential. Moreover, diverse taxa with the potential to utilise unique metabolic strategies were identified, enabling them to thrive and perform essential ecosystem functions in this hyper-arid ecosystem. For instance, the detection of marker genes such as NiFe hydrogenase Hyd-1 and *norBC* suggests metabolic pathways involved in atmospheric hydrogen oxidation to fix $CO_2$ and adaptations to environmental stress in hyper-arid environments.

**Chapter 4**: The study employed the litterbag technique to evaluate the influence of vegetation patches on litter decomposition rates. It compared the decomposition of two contrasting litter types (shrub and grass) under both vegetated and unvegetated patches. Additionally, the study examined how litter chemical composition, including nitrogen and carbon content, as well as the C: N ratio, affects decomposition rates. The results indicated that litter decayed more rapidly in unvegetated patches than in vegetated ones, with shrub litterbags retaining less mass than grass litterbags, regardless of the patch type. Moreover, shrub litter, which had higher nitrogen content, lower carbon and a lower C: N ratio than grass litter, decomposed at a faster rate. These findings provide insights into the mechanisms driving litter decay rates in drylands, which are crucial for predicting litter decay in hyper-arid ecosystems.

This work highlights that vegetation in hyper-arid deserts is a key determinant of soil microbial diversity and function. Through its influence on microbial communities, vegetation drives

essential biogeochemical processes that sustain ecosystem productivity. Understanding the interactions between vegetation and soil microbes in these ecosystems is essential for comprehending biogeochemistry and the functioning and dynamics of dryland ecosystems. Findings from this study contribute to the literature on how hyper-arid deserts such as the Namib Desert are microbially mediated and how the various edaphic communities adapt to extreme conditions in these regions. Furthermore, this study enhances our understanding of how different decomposition mechanisms influence litter decay rates and will ultimately aid in predicting litter decay rates in hyper-arid ecosystems.

## CHAPTER ONE: GENERAL INTRODUCTION

*"The desert tells a different story every time one ventures on it."*
*Robert Edison Fulton Jr.*

# CHAPTER ONE: GENERAL INTRODUCTION

## 1. BACKGROUND

### 1.1 General overview of drylands

Drylands are defined as ecosystems for which the aridity index, that is, the ratio between average annual precipitation and potential evapotranspiration, is less than 0.65 (Berdugo et al., 2020). Drylands include sub-humid, semi-arid, arid, and hyper-arid ecosystems (Berdugo et al., 2020). Such ecosystems have a climate characterised by large temperature fluxes, infrequent and highly variable rainfall patterns (Whitford, 2002), and soils with little organic matter (Whitford, 2002; Chanal et al., 2006; Collins et al., 2008). Drylands constitute the largest biome on Earth (Schimel, 2010), representing about 45% of the terrestrial land surface (Prăvălie, 2016). Despite the low soil organic carbon (SOC) content in dryland soils compared with more mesic environments, drylands account for approximately 32% of the global SOC pool (Plaza et al., 2018) and they play a significant role in global C cycles (Ahlström et al., 2015). Drylands are, therefore, of primary importance when projecting future climate change and its impact on terrestrial ecosystems (Safriel et al., 2005). Despite having little organic matter (Pointing & Belnap, 2012) and being considered relatively unproductive, drylands are socio-economically important, supporting more than 38% of the global human population (Reynolds et al., 2007; Dobie, 2001) through ecosystem services and agricultural practices, such as livestock and crop farming. As a result of these agricultural activities, drylands undergo frequent land disturbance (Global Land Project, 2005). The consequences of land disturbance in drylands are estimated to directly affect about 250 million people in developing countries (Reynolds et al., 2007). Additionally, disturbance significantly alters the structural and functional attributes of terrestrial ecosystems, such as plant productivity, nutrient cycling, and microbial communities.

Recent research showed that global drylands expanded by 4% between 1991 and 2005 alone, and this expansion is expected to continue in the face of projected climate change and human population growth (Reynolds et al., 2007; Dobie, 2001; Feng & Fu, 2013; IPCC, 2013; Spear et al., 2018). With the expected expansion, dryland systems are likely to cover ~60% of the land area of developing countries by the end of the twenty-first century (Huang et al., 2015). The

expansion of drylands is expected to involve the combined effects of changing rainfall patterns, longer periods of desiccation, and larger temperature fluctuations. These changes may drastically reduce ecosystem services and impact millions of human livelihoods, especially for populations inhabiting these dryland systems (Schlaepfer et al., 2017). This is especially true for developing countries where people are particularly dependent on land for livestock and crop farming (Global Land Project, 2005; Huang et al., 2015). Africa and Asia constitute the continents with the largest dryland areas, with these dryland portions occupying nearly 31% of the Earth's terrestrial land surface (Prăvălie, 2016). The countries with the most extensive dryland systems in Africa include Saharan countries (Western Sahara, Mauritania, Morocco, Algeria, Mali, Niger, Tunisia, Libya, Chad, Egypt, Sudan) and the southern African countries across the Kalahari, Namib, and Karoo Deserts (Botswana, Namibia, South Africa).

The Namib Desert, on the western coast of Namibia, is one of the oldest deserts in the world, with an estimated age of 80 million years (Prestel et al., 2008). Like any other desert ecosystem, the Namib Desert is characterized by long periods of desiccation, strong winds, low nutrient status, and large temperature fluxes (Whitford, 2002; Lester et al., 2007), leading to a limited diversity of higher plants and animals in this system (Makhalanyane et al., 2015). In drylands, vegetation cover is spatially discontinuous and patchy (Schlesinger et al., 1990) that is interspersed by areas of essentially bare ground (Thompson, 2010). In certain sandy, hyper-arid regions of the northwestern Namib Desert, spatially discontinuous patchy vegetation is present primarily in ‘hummocks’ where sand and litter accumulate around the base of vascular plants (Fig. 1.1). Plant hummocks create diverse microclimates and provide a microhabitat for many organisms (including soil microbes), thus promoting biological activities underneath vegetation canopy relative to bare ground (Ochoa-Hueso et al., 2018; Maestre et al., 2021). Plants influence soil microbial communities via litter and root exudate inputs and by regulating temporal and spatial patterns of microbial activity through control of moisture content, solar radiation, and temperature (Han et al., 2014; Wang et al., 2013). Thus, plant hummocks may be part of suitable niches for abundant microbial colonization in this hyper-arid where a wide variety of environmental stressors, including large temperature fluxes and long periods of desiccation, impose severe limitations on life.

Figure 1. 1: A hummock with *Arthraerua leubnitziae*. Photograph: A. R. Derr.

Microbial communities are composed of diverse microorganisms, ranging from viruses to microeukaryotes (Fierer & Jackson, 2006; Delgado-Baquerizo et al., 2017). Soil is regarded as the main reservoir of an extensive range of microbial communities representing the three domains of life (Archaea, Bacteria, and Eukarya) (Daniel, 2005; Berg & Smalla, 2009; Fierer et al., 2012; Fierer, 2017). Out of these three domains of life, the bacterial community has been described as the most dominant group, both in abundance and diversity (Delgado-Baquerizo et al., 2018; Li et al., 2022), the majority of which remain undescribed (Delgado-Baquerizo et al., 2018) because they cannot yet be cultured in laboratory settings (Wade, 2002; Konstantinidis et al., 2017).

Soil microbial communities are fundamental to life on earth, as they drive multiple ecological processes and the provision of essential ecosystem services (Pointing & Belnap, 2012; Makhalanyane et al., 2015; Ji et al., 2021). A key aspect of these communities is their relatively high level of functional redundancy, which is the presence of multiple microbial species capable of performing similar ecological roles (Chen et al., 2022). Functional redundancy ensures that critical soil functions remain stable even when specific microbial populations decline due to environmental stresses (Wei et al., 2016; Chen et al., 2022). Functional redundancy is particularly vital in drylands ecosystems, where studies have shown that

increasing environmental stresses lead to a reduction in soil microbial diversity, potentially compromising their adaptation ability and, in turn, affecting ecosystem functions and productivity (Wei et al., 2016; Kumaresan et al., 2017).

Soil microbial communities are instrumental in C and nutrient cycling processes and promote plant growth and productivity (Bardgett & Van Der Putten, 2014; Bahram et al., 2018). Approximately 20,000 plant species are thought to be entirely dependent on microbial symbionts for growth and survival; this is especially true in nutrient-poor ecosystems where plant symbionts facilitate the acquisition of limiting nutrients to plants (Van Der Heijden et al., 2008).

Previous studies have investigated the biotic and abiotic factors that shape soil microbial communities, allowing us to predict how microbes may respond to the changing environment (Singh et al., 2009; Andrew et al., 2012). Several studies have shown soil moisture availability to be a primary driver of microbial activity (Wu et al., 2011; Schnecker et al., 2014; Maestre et al., 2016), as soil moisture determines oxygen availability in the soil (Franzluebbers, 1999) and transport nutrients (Xue et al., 2017), which serve as energy sources for microbial communities. Soil pH has also been described as a factor strongly affecting soil bacterial communities (Fierer & Jackson, 2006; Zhao et al., 2018; Li et al., 2022). On the other hand, soil microbial communities are also shown to be shaped by vegetation through complex interactions (Habekost et al., 2008; Lange et al., 2014). Vegetation patches regulate the temporal and spatial patterns of soil microbial activities by controlling various abiotic factors, such as available soil moisture, solar radiation, and temperature (Han et al., 2014; Wang et al., 2013). Additionally, vegetation patches influence the taxonomy and functional diversity of soil microbial communities by modifying resource availability, particularly the quality and quantity of litter inputs into the soil within their vicinity (Han et al., 2007; Throop & Archer, 2007).

Compared with more mesic environments, dryland soils have been studied less intensively, possibly due to the relatively low rates of biological activity and sparse macrobiota in these systems (Schimel, 2010). However, the past two decades have seen a complete revolution in understanding microbial diversity in dryland soil habitats (Cowan et al., 2014), with the

increasing application of modern molecular phylogenetic and metagenomic methods to identify microbial communities. The bacterial (and Archaeal) 16S rRNA gene and the fungal internal transcribed spacer (ITS) rRNA regions have become the universal phylogenetic marker sequences for analysis of bacterial and fungal communities, respectively (Buchan et al., 2002; Martin & Rygiewicz, 2005; Janda & Abbott, 2007). However, deep metagenomic sequencing is increasingly used to examine complex microbial communities since it provides simultaneous access to phylogenetic data, whole genome assemblies, and the metabolic capabilities of the microbial community (Walker et al., 2014; Wang et al., 2013). Metagenomic approaches can also be used to predict how microbial diversity and the processes they mediate (such as litter decomposition and mineralisation of organic matter and primary production) may vary across terrestrial biomes in the face of projected climate change. This is crucial as soil microbes, mainly bacteria and fungi, are the principal decomposers of litter in drylands (McBride et al., 2023), a role that is amplified due to the relatively low plant litter inputs and small nutrient pools (Moorhead & Reynolds, 1991). Therefore, soil microbial composition affects the energy flow and material circulation in dryland ecosystems.

Plant litter is an important source of energy and nutrients in terrestrial systems. Litter decomposition is an essential component of biogeochemical cycles that strongly controls nutrient availability, primary production, and vegetation community composition (Throop & Archer, 2009; Prescott, 2010; Osanai et al., 2012; Giweta, 2020). In terrestrial systems, litter decomposition is a primary pathway for returning nutrients to the soil (Karberg et al., 2008) and, therefore, strongly controls ecosystem productivity (Throop & Archer, 2009). Additionally, litter decomposition determines C turnover and, therefore, controls the release of carbon dioxide ($CO_2$) into the atmosphere, which strongly influences biogeochemical cycling (Krishna & Mohan, 2017). Decomposition is crucial in dryland ecosystems where litter pools are heterogeneous across the landscape and soil nutrients are typically lower than those in mesic systems (Moorhead & Reynolds, 1991). Models predicting decomposition rates have been developed in mesic systems (Meentemeyer, 1978; Taylor et al., 1989; Parton et al., 2007); however, these models under-predict litter decomposition in dryland systems as observed rates of litter decomposition in drylands ecosystems are faster than what is estimated by the models (Mackay et al., 1994; Vanderbilt et al., 2008). This suggests that there may be unique interactions between abiotic drivers and biotic decomposition processes in

these systems (Throop & Archer, 2009; King et al., 2012). Considering that drylands account for approximately 45% of the terrestrial land surface (Prăvălie, 2016) and play a significant role in global C cycles (Ahlström et al., 2015), an improved understanding and accurate representation of litter decomposition in dryland systems are imperative.

Leaf litter decomposition has also been assumed to be driven by litter quality and the composition of the decomposer community (Hättenschwiler et al., 2005; Gessner et al., 2010; Lin et al., 2021). However, litter decomposition is also influenced indirectly by vegetation, such as plant structural influences on microclimate (Mack & D'Antonio, 2003). Plant canopy structure can potentially influence litter quality, physiochemical properties of the soil, microbial communities, soil moisture, and temperature regimes (Aanderud et al., 2008; Osanai et al., 2012; Predick et al., 2018). These variables can potentially interact directly with incoming solar radiation to mediate decomposition indirectly (Predick et al., 2018).

Research on litter decomposition has grown steadily since the invention of the *litterbag technique* in the 1960s, which enabled the measurement of mass loss and estimation of decomposition rates through the calculation of the decomposition constant or k value (Prescott, 2010). Studies on litter decomposition have used the litterbag technique to explore several key drivers of litter decomposition, such as microclimate, litter quality, litter–soil mixing, microbial activity, vegetation patches, and photodegradation (reviewed in Meentemeyer, 1978; Cepeda-Pizarro, 1993; Throop & Archer, 2009; Predick et al., 2018; Logan et al., 2022). The influence of vegetation patches on litter decomposition has mainly been studied in mesic ecosystems where plant litter cover is relatively spatially continuous (Vitousek & Sanford, 1986; Zhang et al., 2008; Mack & D'Antonio, 2003; Yang & Chen, 2009; Lu et al., 2017). The findings from mesic systems suggest that the continuous homogeneity of plant canopies and evenly distributed rainfall contribute to differences in litter decomposition rates compared to dryland systems (Zhang & Zak, 1995). A question arises in hyper-arid regions with discontinuous and patchy vegetation: How does this spatially discontinuous plant canopy affect litter decomposition rates? In general, litter decomposition in dryland ecosystems has been less studied than in mesic ecosystems (Throop & Archer, 2009; Carvalhais et al., 2014; Poulter et al., 2014). Most of the work on litter decomposition conducted in drylands is focused on semi-arid systems, with virtually no work on hyper-arid

systems. In hyper-arid systems, decomposition rates are likely to be lower and more variable compared to semi-arid systems due to infrequent and unevenly distributed precipitation. Rainfall events strongly regulate ecological processes in drylands (Schwinning et al., 2004). Additionally, the mechanisms (e.g., photodegradation) driving decomposition in hyper-arid systems may fundamentally differ from that of semi-arid systems (Throop & Archer, 2009). However, there is limited data available to fully understand decomposition in hyper-arid systems. Current data paucity on litter decomposition rates in dryland ecosystems inhibits our ability to accurately predict ecosystem C dynamics and response to environmental change at local-to-global scales, contributing to significant uncertainties in projecting global C cycles under future climate change. An improved understanding of litter decomposition in drylands is therefore critical for understanding how these systems may respond to changing climate and providing information for potential climate feedback that may advance our ability to refine climate models.

## 1.2. STUDY OVERVIEW

It is well-established that microbial life in dryland ecosystems is highly diverse, with strong links to ecological processes and the provisioning of essential ecosystem services (Fierer et al., 2012; Pointing & Belnap, 2012; Makhalanyane et al., 2015). However, studies linking microbial diversity and ecological functions in drylands are limited (Bhatnagar & Bhatnagar, 2005; Maestre et al., 2016), particularly those on how this relationship is influenced by biotic factors (i.e., vegetation patches).

In the coastal Namib Desert, previous studies have explored abiotic (such as available soil moisture) and biotic factors (i.e., vegetation) that shape soil microbial community structure (using metabarcoding techniques) (Valverde et al., 2016; Doniger et al., 2020;) and functions (using extracellular activities of five enzymes) (Scola et al., 2017), and the litter decomposition process (Logan et al., 2022). For instance, Valverde et al. (2016) examined the bacterial and fungal communities inhabiting the rhizosphere of *Welwitschia mirabilis*, enhancing our understanding of the root-associated microbes in the Namib Desert. Additionally, Treonis et al. (2024) found that soils associated with *Welwitschia* are dominated by bacterial-feeding nematode communities, highlighting the vital role plants play in shaping the microbial

community in extreme environments. While these studies have advanced our understanding of plant-microbe interactions, the methods used in these studies did not allow a deeper holistic investigation of the taxonomic diversity of soil microbial communities, the functional capacity of these communities, and the associated biogeochemical processes. Consequently, we need a more comprehensive understanding of how biotic factors such as vegetation patches influence the taxonomic and functional capacity of soil microbial communities, as vegetation patches have been shown to influence soil microbial communities through complex interactions (Habekost et al., 2008; Lange et al., 2014). Moreover, studies conducted in the Namib Desert have primarily focused on the central gravel plain regions. The Skeleton Coast National Park region, which forms part of the northern Namib Desert, is largely unexplored, and there is limited understanding of soil microbiome and their functional capacity, as well as litter decomposition rates in this hyper-arid coastal region of the Namib Desert.

This study aimed to evaluate the soil microbiomes in vegetated hummocks in the coastal region of the Skeleton Coast, northern Namib Desert, using amplicon sequencing and shot-gun metagenomics. The 16S rRNA gene and the ITS1 and ITS2 sequences were used to evaluate the taxonomic diversity of bacterial (and Archaeal) and fungal communities, respectively. Furthermore, shot-gun metagenomic data were used to explore the functional capacity of soil bacterial (and Archaeal) and fungal communities. Additionally, the study aimed to investigate how vegetation patches influenced litter decomposition rates using the litterbags technique over 14 months. The influence of vegetation patches on litter decomposition was investigated by comparing decomposition rates under plant hummocks in relation to bare (unvegetated) patches. Two contrasting litter types of plant species that occur in the Namib Desert, namely *Stipagrostis sabulicola* (hereafter, “grass”) and *Zygophyllum stapffii* (hereafter, “shrub”), were used. Understanding the litter decomposition process and how abiotic and biotic drivers may influence this process is essential to accurately describe C balance in hyper-arid systems and reduce knowledge gaps in predicting decomposition rates in dryland systems. Combining data from soil microbiomes and decomposition may provide the opportunity to understand ecosystem drivers and their interactions, thus improving our understanding of C loss and ecosystem functions in dryland systems.

### 1.2.1. Research objectives

- To investigate the composition and diversity of the soil microbial communities (bacteria, Archaea and fungi) associated with plant hummocks,
- To determine how soil microbial communities compare between plant hummock soils and bare soils,
- To evaluate the functional potential of soil microbial communities,
- To evaluate how soil microbes are genetically equipped to survive in hyper-arid systems,
- To investigate the influence of vegetation patches on litter decomposition rates in hyper-arid systems.

### 1.2.2. Research questions

- What is the composition and diversity of the microbial communities (bacteria, Archaea & fungi) associated with plant hummocks?
- How do microbial communities compare between plant hummock soils and bare soils?
- What is the functional potential of soil microbial communities?
- How are soil microbes genetically equipped to survive in hyper-arid systems?
- How do vegetation patches influence litter decomposition rates in hyper-arid systems?

## 1.3. JUSTIFICATION OF THE STUDY

The Skeleton Coast National Park is one of the most arid parts of Namibia and, indeed, one of the most arid places in Africa, south of the Sahara (Ministry of Environment and Tourism, 2013). Like any other hyper-arid ecosystem, the park is characterized by extreme environmental conditions, including long periods of desiccation, strong winds, and large temperature fluxes (Ministry of Environment and Tourism, 2013). The harsh conditions have created a challenging environment for terrestrial life, limiting macrofaunal and plant diversity (Makhalanyane et al., 2015). The park is a meeting point of the coastal and desert ecosystems, resulting in a distinct ecological mosaic with a unique blend of biodiversity (Ministry of Environment and Tourism, 2013). However, there is limited understanding of soil microbiomes that play a pivotal role in shaping the functionality of this unique coastal ecosystem. Specifically, there is limited data about the soil microbiomes and their functions,

as well as the biogeochemical processes, such as litter decomposition rates, and how these may shape this hyper-arid coastal region of the Namib Desert. This knowledge deficit limits our ability to describe soil microbiome and litter decomposition processes globally, especially as dryland climatic conditions change. Addressing the knowledge gap on soil microbiomes and biogeochemical processes is crucial not only for understanding hyper-arid systems but also for advancing our understanding of dryland ecosystems and their significance for global C cycling in the face of changing environmental factors.

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# CHAPTER TWO: PLANT INFLUENCES ON SOIL MICROBIAL COMPOSITION AND DIVERSITY IN THE SKELETON COAST NATIONAL PARK, NAMIB DESERT

*"How can I stand on the ground every day and not feel its power? How can I live my life stepping on this stuff and not wonder at it?"*

*William B. Logan*

# CHAPTER TWO: PLANT INFLUENCES ON SOIL MICROBIAL COMPOSITION AND DIVERSITY IN THE SKELETON COAST NATIONAL PARK

## 2.1. ABSTRACT

Top-soil microbiomes are major contributors to the critical ecological processes and ecosystem services in dryland ecosystems, particularly in hyper-arid regions of the globe, such as the Namib Desert. While several studies have explored soil microbiomes in the Namib Desert, these studies primarily focused on the central gravel plain regions. The Skeleton Coast National Park, which forms part of the northern Namib Desert, is largely unexplored, leading to a limited understanding of the soil microbiomes of this hyper-arid coastal region. Moreover, the Skeleton Coast National Park is a unique coastal ecosystem due to its extreme and variable environmental conditions, which create diverse microhabitats that shape soil microbial communities in distinctive ways. Using 16S rRNA genes and ITS sequences high-throughput sequencing, this study evaluates the soil microbiome in plant hummocks in the coastal Namib Desert by 1) investigating microbial communities associated with plant hummocks and 2) establishing how microbial communities compare between plant hummocks and bare soils. Soil surface (0-5 cm depth) zones from five *Arthraerua leubnitziae* vegetated hummocks were sampled in three sampling locations: hummocks, windward soils, and open gravel plains in the Skeleton Coast National Park, northern Namib Desert. Taxonomic barcoding was used to profile the microbial ecology of these samples. The results showed that the vegetated hummocks and surrounding soils of the Skeleton Coast National Park possess qualitatively different soil microbiomes. Additionally, the results show that plant hummock soils harbour a significantly higher number of observed species relative to bare soils. This study has allowed the identification of microbial communities associated with vegetation that might be particularly vulnerable to a decline in vegetation cover, as may be induced by climate change. Considering the vital role of prokaryotes in soil ecosystem services, this could lead to changes in microbial structure, potentially reducing microbial functional capacity, ecosystem functions, and resilience.

## 2.2. INTRODUCTION

Numerous and diverse microorganisms are associated with humans, fauna, flora, and soils around the globe (The Human Microbiome Consortium, 2012; Delgado-Baquerizo et al., 2018). Soils are considered to be the main reservoir of the most diverse and complex microbiome on earth, with often more than 0.5 mg of microbial biomass C and >50,000 species per gram of soil (Daniel, 2005; Berg & Smalla, 2009; Fierer et al., 2012; Bardgett & Van Der Putten, 2014; Fierer, 2017). Earlier studies focused on describing microbial communities; however, the focus has shifted from just describing these communities to interest in linking microbiome composition and diversity to functions (Graham et al., 2016; Antwis et al., 2017; Fierer, 2017). This is not surprising, considering that microbes impact all living organisms and play a pivotal role in many biogeochemical cycles on earth, driving global C and nutrient cycling with direct feedback effects on ecosystem functioning and productivity (Maestre et al., 2016; Delgado-Baquerizo et al., 2017; Wagg et al., 2019).

The role of microbial communities on ecosystem functioning and productivity is amplified in drylands, particularly in hyper-arid systems. This is especially true as microbial communities are considered to be the predominant drivers of ecological processes in hyper-arid systems due to the limited macrofaunal and plant biodiversity (Pointing & Belnap, 2012; Makhalanyane et al., 2015; Vikram et al., 2016; Neilson et al., 2017). Previous experiments and observational studies have shown that soil microbial communities play an important role in ecological processes and functions in hyper-arid systems (Vikram et al., 2016; Delgado-Baquerizo et al., 2017; Leung et al., 2020; Ortiz et al., 2021). For instance, in a study done in the hyper-arid Namib Desert, Vikram et al. (2016) reported the presence of numerous key photosynthetic genes. In another study done in the hyper-arid desert of Antarctica, Ortiz et al. (2021) reported diverse members of bacteria and archaea with specialist energy and C acquisition strategies and ammonium and nitrite oxidizers. Underlying their ecosystem roles, soil microbes have developed unique metabolic strategies to tolerate and thrive in extreme conditions in hyper-arid environments (Soussi et al., 2016; Ortiz et al., 2021; Vikram et al., 2016).

The soil microbial diversity and community composition in dryland ecosystems are influenced by a range of environmental variables, e.g., moisture availability, edaphic variables, extreme dryness, and vegetation cover (Ronca et al., 2015; Montiel-González et al., 2017). Vegetation cover is closely linked to soil microbial community diversity and structures, with vegetation providing shelter (e.g., decreased radiation and temperature) and energy (in the form of organic matter) and with the microbial community providing a wide range of critical services (including plant litter degradation, nitrogen fixation, nitrification, and denitrification; biocontrol of pathogens) to the plants through soil biogeochemical processes (Buyer et al., 2016; Singh et al., 2016; Sherman et al., 2019). Increasing evidence suggests that the microbial community may promote plant growth, improve drought tolerance, facilitate pathogen defence, and even assist in environmental remediation (reviewed in Jones et al., 2019). In hyper-arid ecosystems where there is limited resource availability, the vegetation actively influences the composition of the overall microbiome within its vicinity by recruiting specific taxa that are enriched in specific functions. For instance, in the hyper-arid desert of the Namib Desert, soils under vegetation patches are enriched with a microbiome responsible for ammonia-oxidizing (*Nitrososphaera*), anoxygenic photosynthesis, and the ability to use energy derived from atmospheric $H_2$-oxidation to fix $CO_2$ (WPS-2 (Wittenberg Polluted Soil-2) or Eremiobacterota) and plant productivity (Glomeromycota) (Chapter 2).

Prior studies have examined the interaction between vegetation and soil microbes (Van Der Heijden et al., 2008; Saul-Tcherkas et al., 2013; Bruto et al., 2014; Soussi et al., 2016); however, these studies have been conducted with respect to individual (or small collections of) microbial species (Jones et al., 2019). Additionally, the focus has often been on how microbes negatively or positively impact the plant and not how a plant may influence the selection of the microbiome under biotic or abiotic stress (reviewed in Jones et al., 2019). The selection of the microbiome beneath the plants is not only fundamental in driving vegetation patterns but can also shape biogeographical patterns (reviewed in Maestre et al., 2021), particularly in hyper-arid systems where vegetation is discontinuous and tends to form islands, or 'patches,' surrounded by bare soil (Schlesinger et al., 1990; Thompson, 2010). It is, therefore, crucial to understand how plants influence the soil microbiome in order to predict how ecological functions and biogeographical patterns may be altered under future climate change. Addressing how plants may influence the selection of the microbiome beneath their

canopy can help us to better link biotic interactions with ecosystem structure and functioning in hyper-arid environments and determine spatial and biodiversity patterns in these environments (Maestre et al., 2021).

Here, high-throughput amplicon sequencing was used, specifically targeting the 16S rRNA genes and ITS region, to examine the bacterial, archaeal, and fungal community diversity and composition in three sampling locations (vegetated hummock, unvegetated windward slope, and unvegetated gravel plains) in the Skeleton Coast National Park, Namib Desert. Two questions were addressed: 1) What is the composition and diversity of the microbial communities (bacteria, archaea and fungi) associated with vegetated hummocks? 2) How do microbial communities compare between vegetated hummock soils and bare (unvegetated: windward slope and gravel plains) ground? The working hypotheses were: 1) that vegetated hummocks would harbour a higher composition and diversity of soil microbial taxa than unvegetated patches (windward slope and gravel plain) due to the modified microclimate around plants, which also can influence resource availability and 2) the taxonomic composition of microbial communities would be heterogeneous (or distinct) across the three sampling locations.

## 2.3. MATERIALS AND METHODS

### 2.3.1 Site description

The study was conducted in the Skeleton Coast National Park, in the northern part of the Namib Desert in Namibia (Fig. 1.1). The Park covers over 1.6 million hectares between latitudes 17$^{o}$10'S and 21$^{o}$10'S and longitudes 11$^{o}$45'E & 14$^{o}$00'E (Braine, 1993). The soils are strongly alkaline, about 98% sand, and low in organic C and total nitrogen. The area is classified as the northern Namib Desert vegetation biome, characterised by extreme aridity, with an average of less than 50 mm of rain per year (Mendelsohn et al., 2002). The most common plant species associated with hummocks are *Acanthosicyos horridus, Arthraerua leubnitziae*, *Barleria solitaria*, *Ectadium virgatum var. rotundifolium*, *Salsola nollothensis*, and *Zygophyllum stapffii*. The grass species are mostly *Stipagrostis ramulosa* and *Stipagrostis uniplumus*. The temperature is highly variable; the daily minimum temperature ranges

between 9°C and 13°C in winter and 12°C and 15°C in summer. The daily maximum temperature ranges between 24°C to 29°C (Meteoblue, 2018).

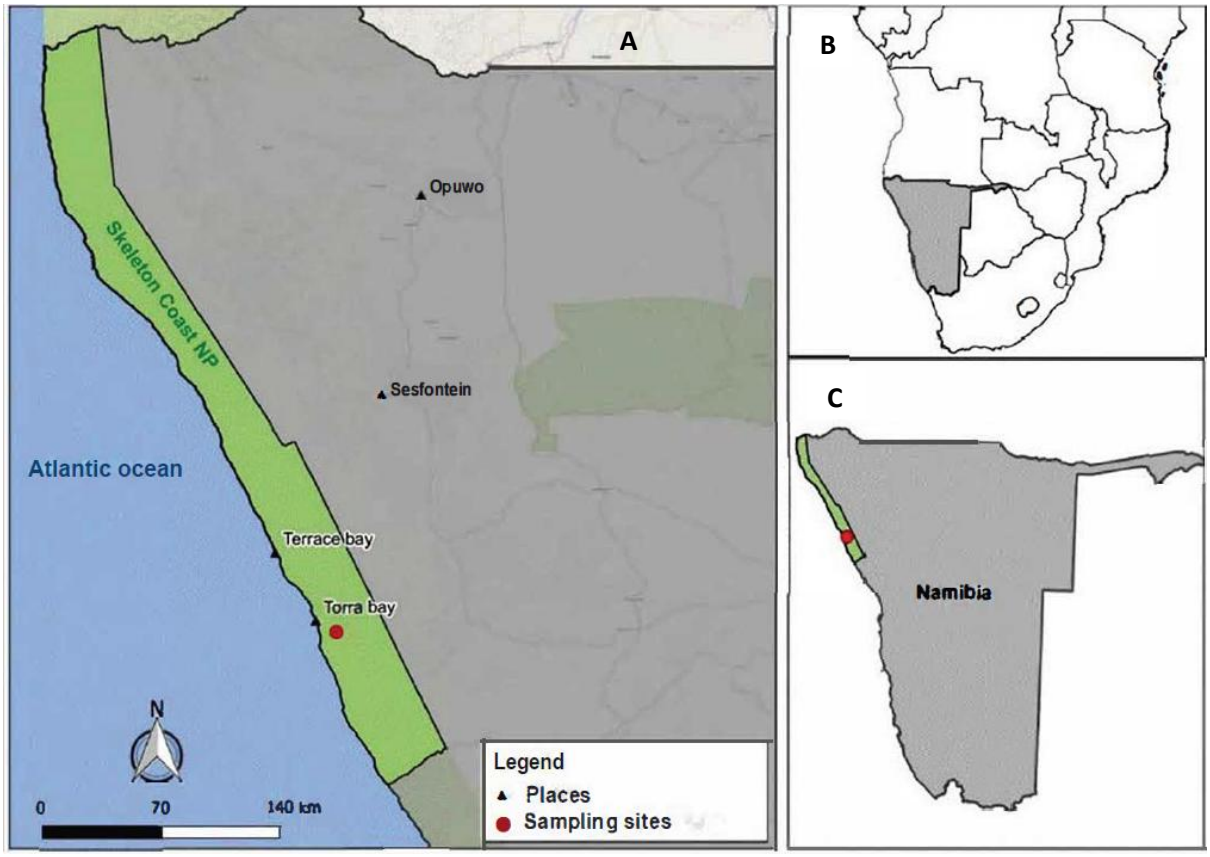


Figure 2. 1: Map showing the **A)** sampling sites in the Skeleton Coast National Park relative to **B)** southern Africa and **C)** Namibia.

### 2.3.2 Soil Sampling Strategy

Five *Arthraerua leubnitziae-vegetated* hummocks were sampled in the Skeleton Coast National Park in September 2018. A stratified sampling design was used to select plant hummocks within a study area (20.3711°S, 13.1818°E). The population of *Arthraerua leubnitziae-vegetated* plant hummocks in the study area was divided into distinct strata based on the size (width and length); five vegetated hummocks with a length >4 m and width > 4 m were selected. About 5 m away from the sampled plant hummock, a respective vegetation-free patch was located for sampling. The reason for sampling a vegetation-free patch was to reduce the effect of vegetation impact on soil microbial composition and abundance and allow comparison among sites. Surface soil samples (0-5 cm depth) were collected from three

sampling groups: *vegetated hummock, unvegetated windward slope, and unvegetated gravel plains* (Fig. 2.1). The hummock sample was obtained from the vegetated area of the hummock, the windward sample was obtained from the unvegetated windward slope of the hummock, and the gravel plain sample was obtained from the open, vegetation-free patch area (Fig. 2.1). Each sample was a composite of four soil scoops (0-5 cm deep) that were obtained with a trowel, recovered from within a 1 $m^2$ virtual quadrant. Branches and rocks larger than 1 cm in diameter and disturbed areas (e.g., with footprints, animal burrows, etc.) were avoided during sampling. To avoid contamination, the hand trowel was sterilised with 70 % ethanol after the recovery of each sample. Soil samples were aseptically collected into separate sterile Whirl-Pak® plastic bags (Nasco, Fort Atkinson, USA) and stored at 4 °C for subsequent soil DNA extraction.


Figure 2. 2: The hummock with *Arthraerua leubnitziae*. Filled circles represent the sampling points from the three groups (vegetated hummock, windward slope, and gravel plains).

## 2.4. SOIL DNA EXTRACTION, FRAGMENT AMPLIFICATION, AND HIGH-THROUGHPUT SEQUENCING

### 2.4.1. Bacterial/Archaeal and fungal sequencing

Deoxyribonucleic Acid (DNA) was extracted from 5 g of soil using the DNeasy PowerSoil Kit (QIAGEN, USA) following the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a Nanodrop spectrophotometer ND 2000 (ThermoFisher, USA) and 1 % agarose gel electrophoresis. DNA samples were send to a commercial supplier for sequencing (Omega Bioservices Laboratories, Norcross, GA, USA) for sequencing of the V3-V4 region of the 16S rRNA gene and the ITS-1 and ITS-2 regions of the internal transcribed spacer (ITS) sequences. Bacterial/Archaeal 16S rRNA gene fragments were amplified using forward-primer 341F (5’ -CCTACGGGAGGCAGCAG-3’) and reverse primer 785R (5’- GACTACHVGGGTATCTAATCC-3’). Fungal ITS regions were amplified using forward-primer ITS1F (5’-TCCGTAGGTGAACCTGCGG-3’) and reverse primer ITS4 (5’-TCCTCCGCTTATTGATATGC-3’). The regions of interest were amplified using the KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA) and subsequently processed with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA). Sequencing (2 x 300 bp paired-end reads) was performed using an Illumina MiSeq platform (Ravi et al., 2018).

### 2.4.2. Amplicon Sequencing Processing

The raw sequence reads were filtered, trimmed, and clustered into amplicon sequence variants (ASVs) using the QIIME2 pipeline (v.2020.2), as described by Bolyen et al. (2019). Briefly, raw paired-end reads were trimmed, denoised, merged, and filtered (to remove chimeric sequences) using DADA2, as described by Callahan et al. (2016). Reads were truncated to remove sequences below a quality score of 20. For 16S rRNA gene amplicons, the reads were truncated 15 bps at the 5’-end, with forward and reverse reads truncated after 290 and 200 bp at the 3’-end, respectively. For the ITS reads, the truncation lengths were 15 bps at the 5'-end and 181 and 185 bp at the 3’-end of the forward and reverse reads, respectively. Processed reads were clustered into ASVs and taxonomically classified using a trained classifier of the SILVA 138 (release 12-2019)(Quast et al., 2013) database for

prokaryotic species and the UNITE fungal database (release 11-2018) (https://unite.ut.ee/) (Kõljalg et al., 2005).

### 2.4.3. Soil Physicochemical Analyses

The soil samples were air-dried and passed through a 2 mm sieve prior to chemical analyses. Soil pH was determined in a 10 g soil slurry (1:2.5 soil/deionised water ratio) using a pH meter (pH 510, Eutech Instruments, Singapore). The colourimetric Walkley–Black method was used to measure soil organic C content (Walkley & Black, 1934). Total nitrogen (TN) was determined by combustion analysis using an elemental analyser (LECO Corporation, St Joseph, MI, USA). The Olsen method (Olsen et al., 1954) was used to quantify extractable phosphorous (P). Soil texture (sand/silt/clay proportions) was determined using the pipette method (Gee & Bauder, 1986), using sodium hexametaphosphate, water, and sodium carbonate as dispersing agents. Soil exchangeable cations (K, Ca, Mg, and Na) were extracted using ammonium acetate, and the concentration was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Fisher Scientific, Cambridge, UK). Metals (Fe and Mg) were extracted from 10 g of soil using EDTA buffer and quantified by ICP-OES after filtration through a 0.45 µm Millipore filter (EMD Millipore Corporation, Billerica, MA, USA). Cation exchange capacity (CEC) was determined using ammonium acetate as the extractant for the exchangeable cations. All analyses were conducted at the Soil Laboratory of the Ministry of Agriculture, Water and Land Reform, Namibia.

## 2.5. STATISTICAL ANALYSES

### 2.5.1 Soil microbial taxonomic distribution, diversity, and community structure

Microbial diversity metrics (alpha-diversity) and community structures (beta-diversity) were calculated using the estimated richness and distance function in R, using the phyloseq (version 1.16.2) (McMurdie & Holmes, 2013), microbiome (Callahan, Sankaran, et al., 2016), tidyverse (Wickham et al., 2019) and vegan (version 2.6.2) (Oksanen et al., 2020) packages (R Core Team, 2022). Observed richness was used as a metric to assess differences in alpha-diversity among the sampling locations. Before calculating alpha-diversity values, the ASV tables were rarefied to a read depth of 20000 and 10000 reads for prokaryotic and fungal ASVs,

respectively. The normality of the data was tested with the Shapiro-Wilk test (Royston, 1982). Kruskal-Wallis rank sum test (Ostertagová et al., 2014) was used to compare mean differences in diversity among sampling locations (adj. *p*-value < 0.05).

Microbial community dissimilarity was calculated using the Bray-Cutis beta-diversity index from the transformed rarefied ASV tables using the "vegdist" function of the vegan package. The distribution of samples was visualized on a principal coordinate analysis plot (PCoA) (Jolliffe, 1986). Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2017) was used to test for statistically significant variance among sampling locations; this was performed with 999 permutations using the "adonis" function of the vegan package.

Linear discriminant analysis effect size (LEfSe) was performed to determine biomarkers differentially represented among the sampling locations (Segata et al., 2011). Specifically, LEfSe combined the standard tests for statistical significance (Kruskal-Wallis test and pairwise Wilcoxon test) with linear discriminate analysis to compare the significant abundant differences among taxa and estimate the effect size of the abundant difference of these taxa in different sampling locations. The threshold on the logarithmic linear discriminant analysis (LDA) score for discriminative features was set to 2.0, and log10 normalized the LDA scores.

### 2.5.2. Relation of microbial community and soil physiochemical properties

The explanatory importance of soil physiochemical parameters on the microbial community composition was estimated using constrained correspondence analysis (CCA) (McArdle & Anderson, 2001). The soil physiochemistry data was first *z*-score standardised and tested for multicollinearity using the "vif" function. The best models for explanatory parameters that presented significant impacts (*p*-value < 0.01) on the microbial community composition were calculated using the forward stepwise regression model selection with the ordistep function in the vegan package. Then, the significance of the best-fitted models was calculated using the Analysis of Variance (ANOVA) permutation test for CCA (Legendre et al., 2011) with 1000 permutations.

## 2.6. RESULTS

### 2.6.1. Soil taxonomic distribution in and outside the vegetated hummocks

For 16S sequencing, the hummock samples produced a total number of 667,763 raw reads, averaging 133,553 reads per sample. The windward slope samples generated 301,608 raw reads, with an average of 60,322 reads per sample, while the gravel plains samples yielded 578,731 raw reads, averaging 115,746 reads per sample. After quality filtering, the hummock samples retained 343,652 ASVs, the windward slope samples retained 158,742 ASVs, and the gravel plains samples retained 275,471 ASVs. In the case of ITS sequencing for the fungal community, the hummock samples produced a total of 427,660 raw reads, averaging 85,532 reads per sample. The windward samples generated 15,973 raw reads, with an average of 3,195 reads per sample, while the gravel plains samples yielded 39,528 raw reads, averaging 7,906 reads per sample. After quality filtering, the hummock samples retained 263,814 ASVs, the windward samples retained 8,132 ASVs, and the gravel plains samples retained 17,301 ASVs.

The overall taxonomic analyses showed a total of 41 prokaryotic (Fig. 2.3) and 5 fungi phyla (Fig. 2.4) present across the three sampling locations, with nine and two phyla showing relative abundances greater than 1 %, respectively. For prokaryotic phyla, Actinomycetota, with a mean relative abundance of 24 %, dominated the soils in all sampling locations, followed by Pseudomonadota (22 %), Bacillota (20 %), and Bacteroidota (8 %). Relatively low abundances of Chloroflexota (4 %), Gemmatimonadota (4 %), Planctomycetota (3 %), Myxococcota (2 %) and Verrucomicrobiota (1.9 %) were found (Fig. 2.5A).

For fungi, unknown or unclassified phyla dominated the soils with a mean relative abundance of 54 %, followed by Ascomycota (37 %) and Basidiomycota (8 %) (Fig. 2.5B). Rozellomycota and the plant-symbiont mycorrhizal phylum Glomeromycota were detected at much lower relative abundances, cumulatively less than 1 % of fungal ASVs, and occurred exclusively in hummock soils (Fig. 2.4).

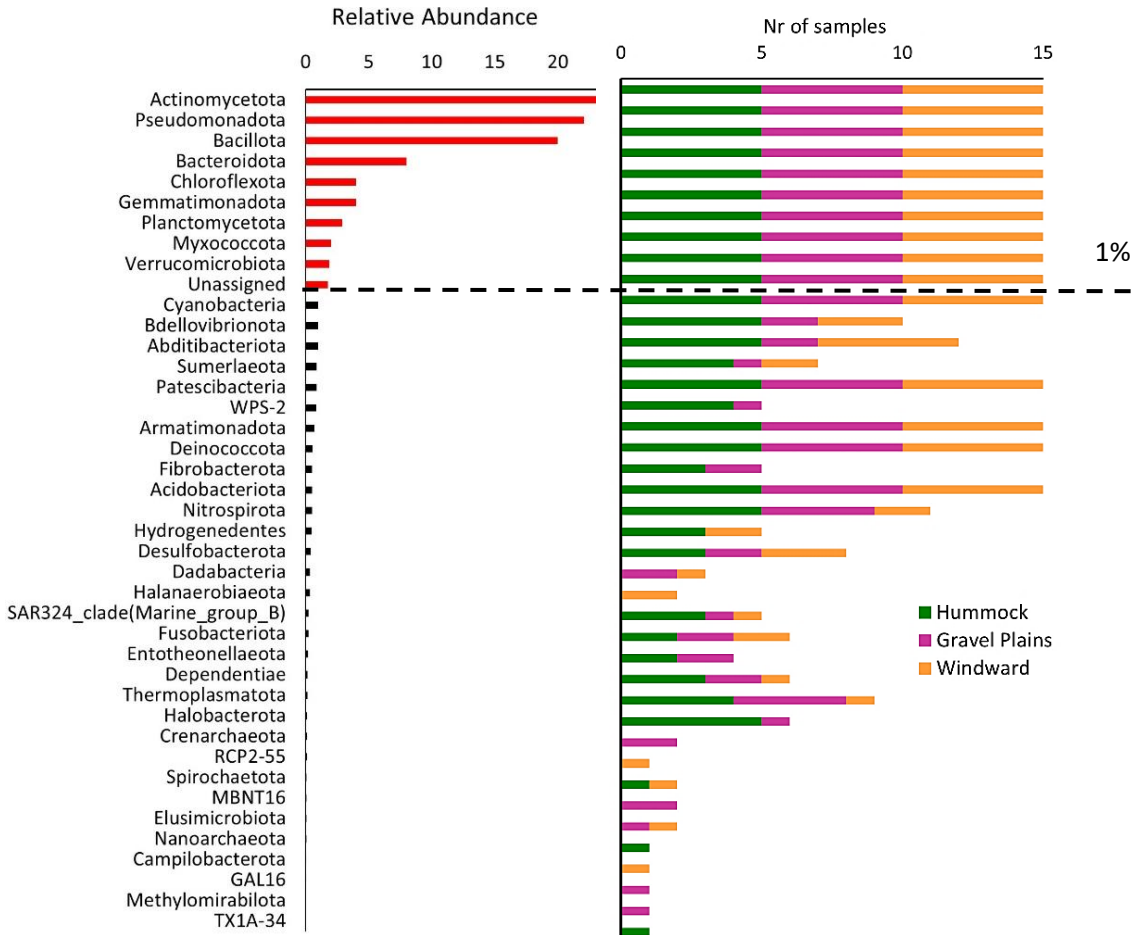


Figure 2. 3: Mean relative abundances (expressed as a fraction of total abundance) of prokaryotic phyla in the different soil samples and the number of samples in which they were identified. Dominant phyla, defined as phyla with more than 1 % mean relative abundance, are highlighted in red above dashed black lines, representing the threshold between dominant and rare taxa.

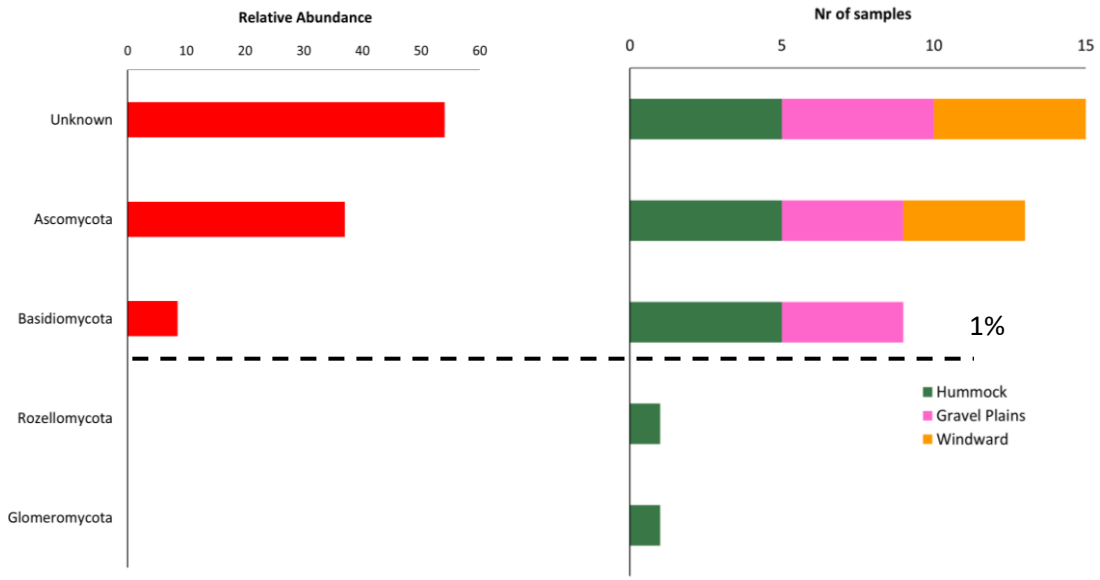


Figure 2. 4**:** Mean relative abundances (expressed as a fraction of total abundance) of fungal phyla in the different soil samples and the number of samples in which they were identified. Dominant phyla, defined as phyla with more than 1% mean relative abundance, are highlighted in red above dashed black lines, representing the threshold between dominant and rare taxa.

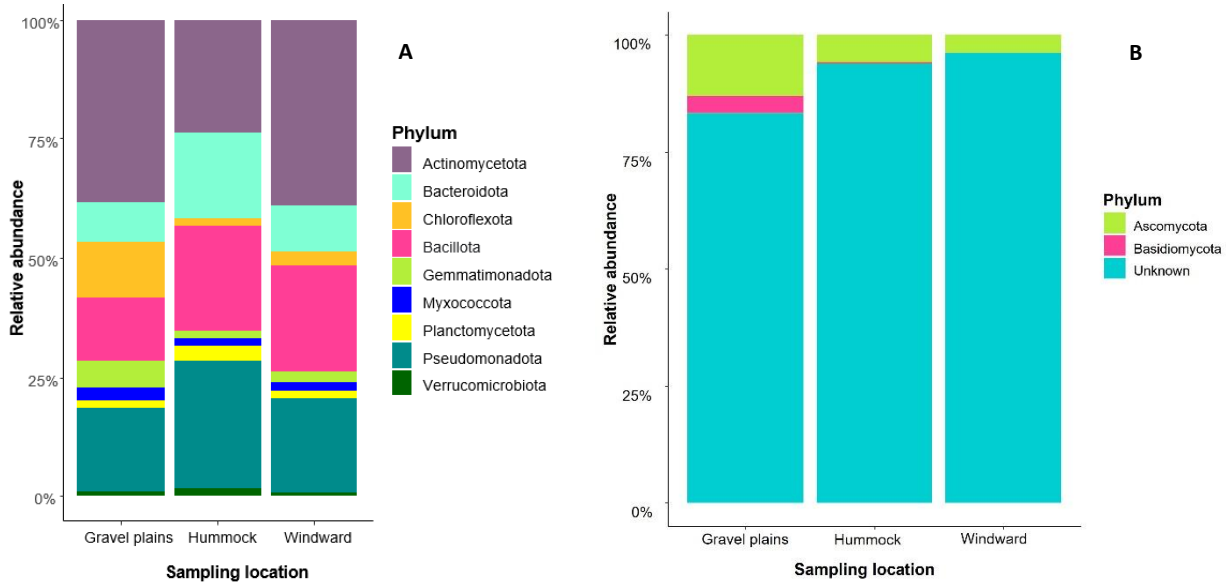


Figure 2. 5: Relative abundance of the major phyla based on **A)** 16S rRNA gene and **B)** ITS sequences across sampling locations.

The LEfSe analysis of taxa among sampling locations yielded significant results, showing 9 and 1 differentially abundant taxa at the genus level (LDA score ≥ 2) for the bacterial/archaeal and fungal communities, respectively (Fig. 2.6). This analysis revealed overrepresented bacterial

genera, including Pontibacter, Kocuria (phylum Actinomycetota) and Planococcus (phylum Bacillota) in vegetated hummock soils, compared to the windward slope and gravel plains samples. Pseudomonas (phylum Pseudomonadota, previously known as Proteobacteria) and Cutibacterium (phylum Actinomycetota) were overrepresented in windward slope soils relative to vegetated hummock and gravel plains samples. The genus Longimicrobiacea (phylum Gemmatimonadota), known for thriving in oligotrophic environments, was overrepresented in gravel plains soils. The gravel plains soils were enriched with members from the Agaricomycetes class in the fungal community.

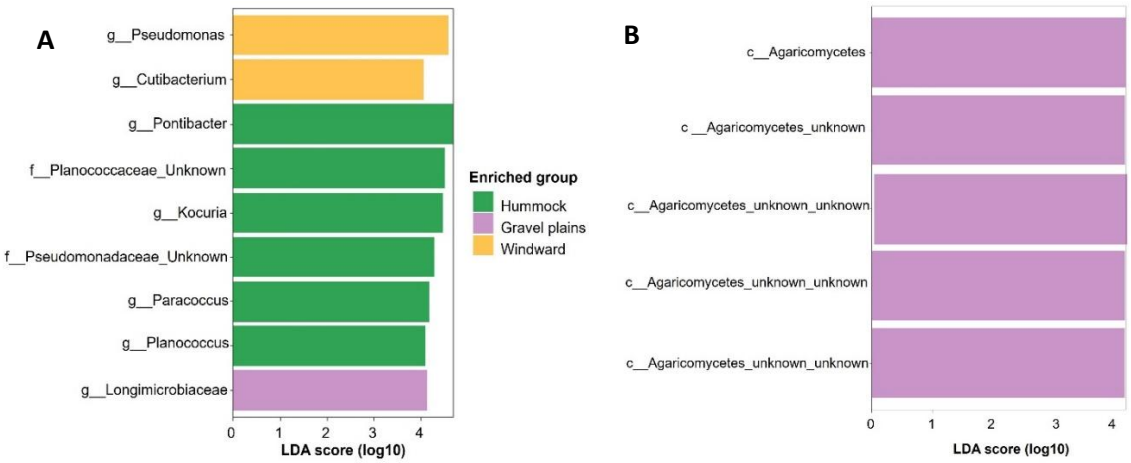


Figure 2. 6: Linear discriminant analysis (LDA) effect size (LEfSe) for taxa in different sampling locations, **A)** Bacteria and **B)** Fungi). Horizontal bars represent the effect size for each taxon. The length of the bar represents the log10 transformed LDA score. The name of the taxon level is abbreviated as k-kingdom, p-phylum, c-class, o-order, f-family, and g-genus.

### 2.4.2 Soil microbial diversity and community structures in and outside the vegetated hummocks

Based on the 16S rRNA gene and ITS sequence data, the alpha-diversity of soil samples was measured and compared among sampling locations. Alpha-diversity results revealed that sampling locations exhibited significantly (*p*-value < 0.01) different levels of microbial community richness as indicated by the number of observed species (Fig. 2.7A & C). To understand community structure (beta-diversity), samples were clustered using the Bray-Curtis index to explore further the differences in microbial community structures among sampling locations. The resulting scores were presented on a principal coordinate analysis

(PCoA) plot and compared using the PERMANOVA test of significance (Fig. 2.7B & D). Samples were separated along the principal component analysis (PCA) axes, which significantly explained (adjusted *p*-value < 0.001) the percentage of variation in community structure for the bacterial/archaeal ($R^2$ = 0.269) and fungal ($R^2$ = 0.14) fractions.

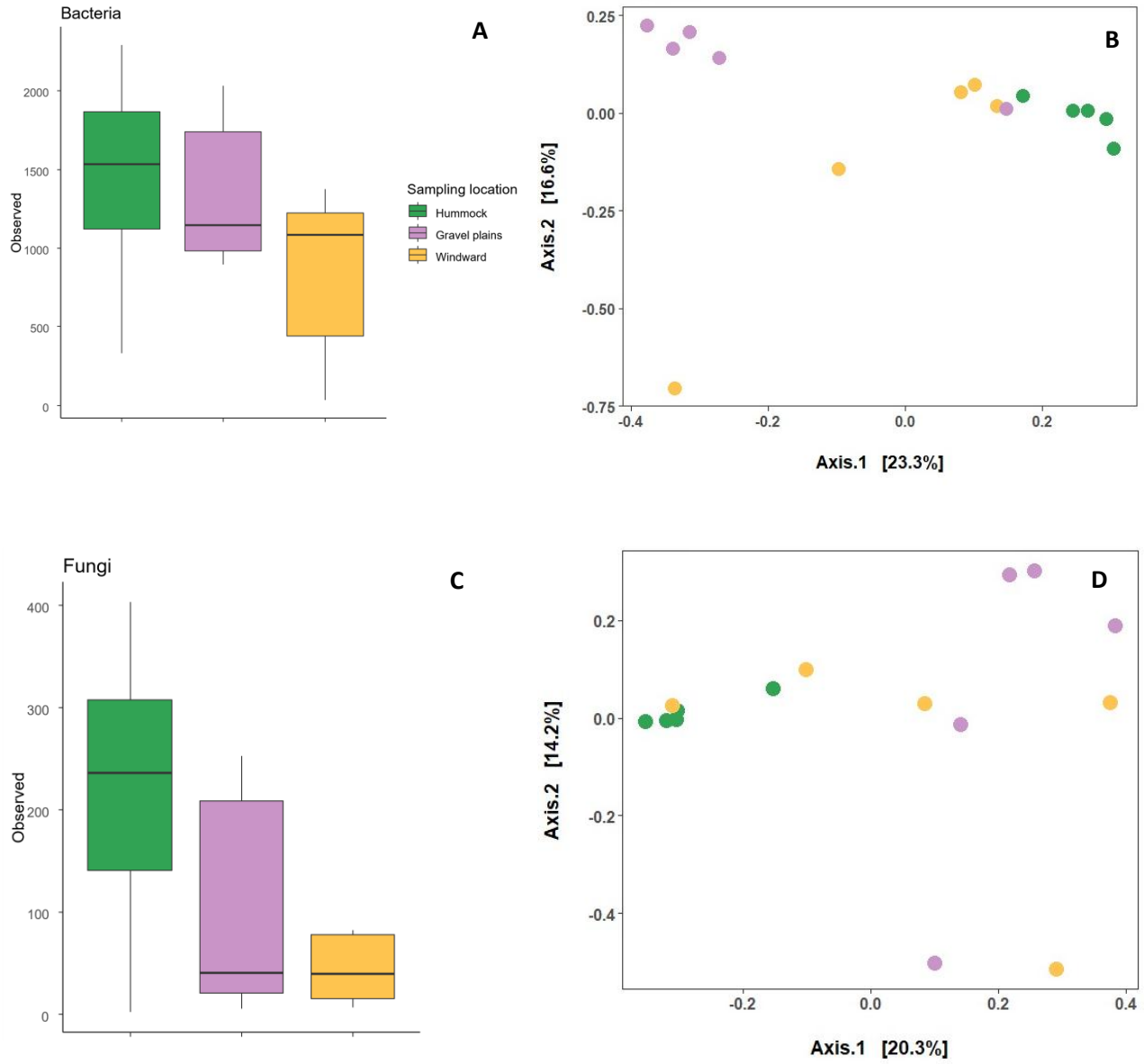


Figure 2. 7: Alpha-diversity and beta-diversity of microbial communities according to sampling location. Alpha-diversity was calculated as the observed number of species per sample and visualised using box-plots for the different fractions of the community (Bacteria (and Archaea) /Fungi). Beta-diversity was calculated using the Bray-Curtis index and visualized as PCA ordination plots.

### 2.4.3 Soil physicochemical drivers of soil microbial communities in hummocks

A stepwise model-building approach for constrained ordination models was used to assess the relative importance of physiochemical soil parameters on the soil microbial community composition and structure. The canonical correspondence analysis (CCA) results showed that bacterial/archaeal community composition and structure were significantly affected by only two parameters (adj. p-value < 0.01), namely potassium (K) and cation exchange capacity (CEC) (Fig. 2.8). In contrast, physiochemical properties did not significantly affect the fungal community.

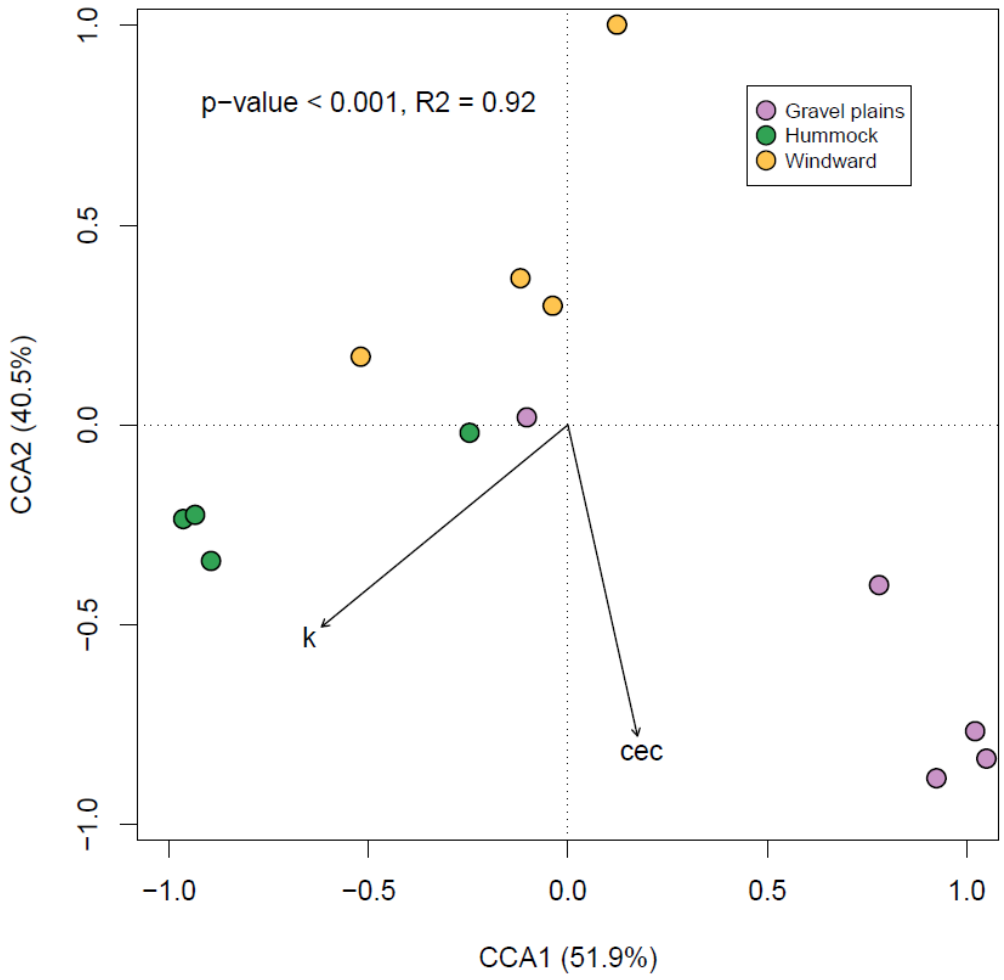


Figure 2. 8: Redundancy analysis (RDA) showing the correlation between responsive variables (environmental factors) and microbial taxonomic composition (explanatory variables). The arrows represent the two significant environmental variables explaining the variability in microbial community structure.

## 2.7. DISCUSSION

Soil microbiomes across most terrestrial environments are believed to be dominated by a relatively small number of prokaryotic phyla (Connon et al., 2007; Lester et al., 2007; Fierer et al., 2009; Delgado-Baquerizo et al., 2018b; Fierer, 2017) whose members are involved in mediating key ecosystem processes. This study revealed a similar pattern for the Namib Desert soils, with only 9 of 41 prokaryotic phyla and 3 of 5 fungal phyla representing the dominant fraction. In this study, the bead-beating and silica-based purification method was used for DNA extraction. This method was used due to the complexity of desert soils, which contain low microbial biomass, tough microbial cell walls, and humic substances that inhibit the activity of PCR and restrict enzymes. The bead-beating method is efficient at lysing microorganism cells and removing inhibitors (Schrader et al., 2012; Dineen et al., 2010). While different DNA extraction methods have been shown to influence microbial diversity signatures in the samples (Wüst et al., 2016), the bead-beating method is one of the effective and recommended methods for desert soils, as it is efficient at cell lysing and removing PCR inhibitors (Niu et al., 2022).

### 2.7.1 Soil taxonomic distribution in and outside the vegetated hummocks

The ubiquitous presence of phyla such as Actinomycetota, Pseudomonadota, Bacillota, Bacteroidota, Chloroflexota, Gemmatimonadota, Planctomycetota, Myxococcota and Verrucomicrobiota across the three sampling locations was expected, as these phyla have been consistently reported to be the most dominant phyla detected in desert soils worldwide (Fierer et al., 2012; Valverde et al., 2016; Armstrong et al., 2016; Soussi et al., 2016; Wei et al., 2016), indicating that common members of these phyla are probably well adapted to survive in desert soils. Not surprisingly, the Actinomycetota phylum dominated the gravel plains and windward slope soils relative to the vegetated hummock soils (Fig. 2.5A). The overall dominance is most probably linked to their adaptations to oligotrophic environments and numerous Ultraviolet (UV) repair mechanisms (Belov et al., 2018). In desert soils where nutrients are often limited, certain species from the *Rubrobacter* lineages have been found to have evolved metabolic strategies such as trace gas oxidation (Jordaan et al., 2020) as critical energy sources supporting the productivity and resilience of desert ecosystems (Ortiz

et al., 2021). Additionally, some members of the Actinomycetota have the capacity for metabolic plasticity and the ability to produce a wide range of secondary metabolites (Makhalanyane et al., 2015; Zoccarato & Sher, 2022), allowing them to thrive in challenging desert conditions.

Pseudomonadota was the most dominant phylum in the hummock soils relative to the gravel plains and windward slope soils (Fig. 2.5A). Plants frequently serve as hosts for many members of Pseudomonadota, including *Bacillus, Rhodobacteraceae*, *Pseudomonas, Enterobacteriaceae, Rhizobium*, and *Azospirillum* (Yousaf et al., 2011; Bruto et al., 2014; Wang et al., 2016; Neilson et al., 2017; Zoccarato & Sher, 2022), which may explain the abundance of this phylum in the hummock soils. Several studies have reported that these members may enhance plant growth and development. For instance, some members of the *Rhizobium* genus can form nitrogen-fixing symbiotic associations with plants (Turner et al., 2013; Aislabie & Deslippe, 2018). Additionally, members of the genus *Pseudomonas* play a role in enabling the degradation of organic compounds such as 1-aminocyclopropane-1-carboxylic acid (an immediate precursor to ethylene, which is a plant hormone involved in various stress responses, growth, and developmental processes) (Blaha et al., 2006; Lugtenberg & Kamilova, 2009; Bruto et al., 2014), potentially enhancing plant resistance to environmental stresses (Lugtenberg & Kamilova, 2009; Soumare & Diédhiou, 2021).

Although representing less than 1% relative abundance, some rare phyla that were identified in the samples may play important roles in community structure and function. These included Fibrobacteres, a phylum with several genera, such as *Fibrobacter*, whose members are considered major degraders of cellulosic plant biomass (Ransom-Jones et al., 2012). The predatory bacterial phylum Bdellovibrionota was also observed across 10 of 15 samples. Bdellovibrionota consists of several classes, including Oligoflexia, whose members have been proposed to prey on a broader spectrum of microbes in marine environments, thus acting as biological control agents (Li et al., 2020). Another rare phylum identified across the hummock (4 samples) and open gravel plains (1 sample) samples was WPS-2 (or Candidate Phylum Eremiobacterota); this phylum includes several members capable of anoxygenic photosynthesis (Holland-Moritz et al., 2018; Ward et al., 2019) and others that are associated with the ability to use energy derived from atmospheric $H_2$-oxidation to fix $CO_2$ (Ji et al., 2016;

Sheremet et al., 2020; Ji et al., 2021). Ecologically important archaeal taxa were also identified in five samples, including the ammonia-oxidizing archaeon *Nitrososphaera* (Tourna et al., 2011; Lu et al., 2020), which occurred exclusively in hummock soils.

Within the fungal community, Rozellomycota and the plant-symbiont mycorrhizal phylum Glomeromycota were detected at much lower relative abundances, cumulatively less than 1% of fungal ASVs, and occurred exclusively in hummock soils (Fig. 2.4). These results are consistent with previous studies showing that some members within the Glomeromycota phylum form a symbiotic association with most terrestrial plants (van de Voorde et al., 2010; Behie et al., 2013), therefore, are crucial to plant nutrition and productivity (Van der Heijden et al., 1998a; Klironomos et al., 2000; Klironomos & Hart, 2002; Smith & Read, 2008).

LEfSe analysis showed that the genus *Pontibacter* from phylum Bacteroidota was overrepresented in vegetated hummock soils (Fig. 2.6A). This genera includes N-fixing members that have been shown to have a positive association with plants (Zhang et al., 2008; Xu et al., 2014) and promote plant growth (Dastager et al., 2011; Liu et al., 2022). Additionally, the overrepresentation of genus *Kocuria* in vegetated hummock soils may be attributed to their ability to inhabit the plant rhizosphere and root endosphere, therefore promoting plant development and enhancing their resilience against extreme environmental conditions in dryland ecosystems (Mukhtar et al., 2021). The overrepresentation of the genus *Longimicrobiacea* (phylum Gemmatimonadota) in the gravel plains samples is an interesting observation, as the Gemmatimonadota taxon has also been added to the list of bacterial phyla containing anoxygenic phototrophic species (Koblížek et al., 2020; Zeng et al., 2021; Mujakić et al., 2021; Demergasso et al., 2023). This can have important ecological implications in desert environments as anoxygenic phototrophic bacteria can support their heterotrophic metabolism with energy from light, enhancing their growth efficiency, thereby meeting their energy demands (Ortiz et al., 2021; Demergasso et al., 2023). For the fungal community, the gravel plains soils were enriched with *Agaricomycetes*; members of this class are known to have the ability to thrive in low N habitats, with relative abundances inversely related to nitrification rates (Lilleskov et al., 2002) (Fig. 2.6B).

### 2.7.2 Soil microbial diversity and community structures in and outside the vegetated hummocks

Samples showed preferential clustering based on the sampling location (vegetated hummock/gravel plains/windward slope), suggesting that different sampling locations harbour distinct soil microbial communities (Fig. 2.7B & D). This supports the hypothesis that the taxonomic composition of microbial communities would be heterogeneous (or distinct) across sampling locations. The findings support the concept of niche partitioning, which is the natural selection that drives competing microbial taxa into colonising different patterns of an environment based on the resource availability (Johnson et al., 2017). Niche partitioning has been reported in previous studies as a critical process in the assembly of microbial communities in the Namib Desert (Gombeer et al., 2015; Ronca et al., 2015; Ramond et al., 2014; Johnson et al., 2017). For instance, Ronca et al. (2015) found that different habitats within the Namib Desert exhibited habitat-specific microbial communities based on their abiotic characteristics, highlighting the role of niche partitioning in shaping the distribution and composition of microbial taxa.

The distinct soil microbial communities observed across sampling locations in my study suggest that distinct environmental conditions and resource availability at each sampling location create specific niches promoting distinct microbial taxa in each location. The results suggest that niche partitioning plays a significant role in the assembly of microbial communities in the soils of the Skeleton Coast National Park. However, it is also important to highlight that while sampling locations accounted for some of the observed variations in the microbial community, a large percentage of the variation could not be explained by sampling location. Specifically, more than 70 % of the variance in bacterial and archaeal, and in the case of fungal communities, over 80 % cannot be attributed to sampling location. This suggests that other factors such as micro-scale environmental variations, temporal dynamics, or stochastic processes, may also play significant roles in shaping these communities (Mitchell et al., 2012).

Vegetated hummocks exhibited the highest bacterial/archaeal and fungal biodiversity (Fig. 2.7A &C) of the three sampling locations. This confirms our initial hypothesis that hummock

soils would harbor a significantly higher diversity of soil microbial taxa relative to the bare (unvegetated: windward slope and gravel plain) ground. This higher observed diversity within the vegetated hummock soils may be attributed to the modified microclimate around plants, which can affect resource availability. However, this observation should be interpreted with caution as the number of soil samples on which this finding was based was limited. It's therefore important that future research aims to include larger sample sizes to enhance the statistical power of the results.

Vegetated hummocks appear to be selective environments due to the interactions between the plant and microbes within the hummock, therefore harbouring a specialised microbial community. This was highlighted by the fact that the soils under vegetation patches are enriched with specific microbiomes responsible for specific functions such as ammonia-oxidizing (*Nitrosococcus* and *Nitrosospira*), anoxygenic photosynthesis, and the ability to use energy derived from atmospheric $H_2$-oxidation to fix $CO_2$ (WPS-2) and plant productivity (Glomeromycota). This finding is supported by previous studies showing that plants exert positive impacts on soil microbial communities by harboring specific bacterial and fungal taxa(Andrew et al., 2012; Acosta-Martınez et al., 2008; Marschner et al., 2001). Additionally, plants are known to produce secondary metabolites that may hinder the success of certain bacteria, thus harbouring a subset of a specialised microbial community beneficial for their development and growth in hyper-arid ecosystems (Hartmann & Schmid, 2009).

### 2.7.3 The effect of the environment on the diversity of the soil microbiome

The physicochemical soil analysis revealed low nutrient levels, with values similar to other studies conducted in the Namib Desert (Frossard et al., 2015; Armstrong et al., 2016; Naidoo, 2020). The results showed that the variation in the composition and structure of microbial communities among the sampling locations is explained by two factors, potassium and CEC (Fig. 2.8). This is unexpected, as it is well known that C and nitrogen content and pH are key factors that commonly shape the composition and structure of bacterial/archaeal and fungal communities in soils (Schnecker et al., 2014; Zeng et al., 2017; Ren et al., 2018). A plausible explanation for this observation may be that among the sampling locations, microbial diversity in these soils may be driven more by temporal dynamics and micro-scale

environmental variables than chemical variables, highlighing the importance of spatial variability in desert soil microbial assembly. These findings are similar to previous studies, where environmental variables were found to have a greater influence on microbial community than physiochemical properties in China (Zhang et al., 2022; Zhang et al., 2018).

## 2.8. CONCLUSION

The objectives of this part of the research project were to understand 1) the diversity of the microbial communities (bacteria, Archaea & fungi) associated with vegetated hummocks and 2) how microbial communities' composition compares between vegetated hummock soils and bare (unvegetated: windward slope and gravel plains) ground. Microbial taxonomic alpha-diversity (observed diversity) was significantly different among the sampling locations, which supports the initial hypothesis that vegetated hummock soils would harbor a higher diversity of soil microbial taxa relative to the bare (unvegetated: windward slope and gravel plain) ground due to the modified microclimate around plants, which also can influence resource availability. The findings show that the vegetated hummock exhibited the highest number of observed bacterial/archaeal and fungal species relative to bare soils. Soil microbial community composition (beta-diversity) differed among the sampling locations, confirming the second hypothesis. Samples showed preferential clustering based on the sampling location (vegetated hummock/gravel plains/windward slope), suggesting that different sampling locations harbor distinct soil microbial communities.

Furthermore, the vegetated hummock appears to be a selective environment that hosts specific taxa that are enriched in specific functions such as ammonia-oxidizing (*Nitrososphaera*), anoxygenic photosynthesis, and the ability to use energy derived from atmospheric $H_2$-oxidation to fix $CO_2$ (WPS-2) and plant productivity (Glomeromycota). This is particularly important considering climate change projections, where climate change impacts are projected to reduce vegetation cover in drylands. Reduced vegetation cover may, in turn, lead to reduced soil microbial diversity and functions and subsequently affect the biogeochemical processes they mediated. Overall, this study provides insights into microbial ecology associated with vegetated sand hummocks in this oligotrophic ecosystem.

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# CHAPTER THREE: PLANT INFLUENCES ON SOIL MICROBIAL FUNCTIONAL POTENTIAL IN THE SKELETON COAST NATIONAL PARK, NAMIB DESERT

*Essentially, all life depends upon the soil. There can be no life without soils and no soil without life; they have evolved together.*

*Charles E. Kellogg*

# CHAPTER THREE: PLANT INFLUENCES ON MICROBIAL FUNCTIONAL CAPACITY IN THE SKELETON COAST NATIONAL PARK

## 3.1. ABSTRACT

Soil microorganisms serve as an essential foundation of dryland environments, particularly hyper-arid ecosystems, where they play a key role in driving crucial ecosystem functions and services. Given the important roles of soil microbes in hyper-arid systems, it is critical to understand how vegetation influences the soil microbiome beneath their canopy to better link biotic interactions with ecosystem structure and functioning in hyper-arid environments. This will improve our ability to determine spatial and biodiversity patterns in these environments. This study evaluates the soil microbiome in vegetated hummocks in the coastal Namib Desert by 1) establishing the functional potential for microbial communities and how they compare between vegetated hummock and bare soils (unvegetated: windward slope and gravel plains) and 2) investigating metabolic strategies that underlie the ability of these soil microbes to thrive and perform ecosystem functions in this hyper-arid ecosystem. Here, shotgun metagenomics sequencing technology were used to evaluate the microbial functional potential in three soil samples collected from three sampling locations: vegetated hummock, unvegetated windward slope, and gravel plains. The metagenomic analyses revealed functions related to carbon (C) fixation, C degradation, ammonium oxidation, methane metabolism, and sulfur assimilation. The vegetated hummock soils had more enrichment of microbial functions relative to bare soils, suggesting that vegetation influences the microbial functional potential. Moreover, our findings revealed diverse taxa with unique metabolic strategies to tolerate and thrive in hyper-arid environments. For instance, the detection of marker genes such as NiFe hydrogenase Hyd-1 and *norBC* suggests metabolic pathways involved in atmospheric $H_2$ oxidation to fix $CO_2$ and the adaptation to environmental stress in hyper-arid environments. Overall, this study highlights the influence of vegetation in shaping the microbial communities and their functional dynamics, which ultimately contribute to the overall ecosystem's functioning. Furthermore, the study provides insights into metabolic strategies that enable soil microbial communities to thrive in these extreme desert environments.

## 3.2. INTRODUCTION

Soil microorganisms are the functional backbones of dryland environments (Li et al., 2023), particularly hyper-arid ecosystems as they are predominant ecosystem drivers underpinning crucial ecosystem functions and services (Pointing et al., 2010; Neilson et al., 2012; Vikram et al., 2016; Leung et al., 2020). Contrary to historical belief, drylands harbour a wide range of active microbial taxa that have adapted and successfully colonized the various habitats, from UV-exposed desert pavements to cryptic refuge niches (Cowan et al., 2020; Ray et al., 2022). Even the most extreme dryland ecosystems such as those in the hyper-arid regions of the Atacama and Namib Deserts harbour diverse and active microbial taxa (Bull & Asenjo, 2013; Schulze-Makuch et al., 2018). The adaptation of microbial communities to a range of abiotic stresses in desert environments may include survival of water deficiency, high daily and seasonal temperature fluctuations, high soil salinity and high UV-radiation, which would require specific and/or unusual adaptive mechanisms (Heulin et al., 2017). The central question remains: What metabolic strategies underlie the ability of soil microorganisms to perform crucial ecosystem functions and services in these depauperate ecosystems?

Generally, desert microbiomes are considered to be functionally distinct compared with those found in other biomes (Fierer et al., 2012; Noronha et al., 2017), in part due to unique abiotic deterministic factors (such as soil chemistry, solar radiation, precipitation) that shape desert soil microbial assemblages (Johnson et al., 2017; Neilson et al., 2017). In hyper-arid systems, the role of microbial communities on ecosystem functioning is more amplified relative to semi-arid systems due to limited macrofaunal and plant biodiversity (Pointing & Belnap, 2012; Makhalanyane et al., 2015; Vikram et al., 2016; Neilson et al., 2017). Microbial communities drive ecosystem functions including the cycling of key biogeochemical elements such as C, nitrogen (N) and sulfur (S). The cycling of essential elements has important implications for the ecosystem productivity and sustainability (Cowan et al., 2014; Zheng et al., 2019), as this influences nutrient availability and contributes to the overall health and resilience of desert ecosystems. For instance, some microbes (such as members of genus *Arthrobacter*) are known for their ability to degrade organic compounds therefore contributing to nutrient cycling and overall ecosystem functioning in arid environments (Lüneberg et al., 2018; Ayangbenro & Babalola, 2021; Mukhia et al., 2021).

There is considerable evidence in the literature that the dryland soil microbiome encompasses a diverse functional gene pool (Lakshmanan et al., 2014; Makhalanyane et al., 2016; Vikram et al., 2016; Noronha et al., 2017; Chen et al., 2021; Naidoo et al., 2021). However, further research is needed to understand the full extent of the functional potential of microbial communities in hot deserts and its implications for key biogeochemical cycling processes (Makhalanyane et al., 2015). Therefore, a global effort is crucial to evaluate microbial communities of the Earth's hot deserts, by cataloguing and understanding their functional capacity, and the way in which microbial functional capacity may affect ecosystem services under future projected climate change. This is particularly relevant, given that climate models often lack microbially-mediated data (Treseder et al., 2012; Jansson & Hofmockel, 2020).

Microbial functional capacity can be described as the potential of microbial communities to carry out various functions based on their genetic and enzymatic makeup (Kumaresan et al., 2017). Zak et al. (1994) suggested that the high environmental variability and resource heterogeneity associated with desert ecosystems may contribute to increased functional capacity of microbial communities. However, the functional capacity may differ across different desert ecosystems due to variability in abiotic and biotic factors such as moisture availability, edaphic variables, extreme dryness and vegetation cover (Ronca et al., 2015; Montiel-González et al., 2017). For instance, in poor-nutrient ecosystems where there is limited resource availability, vegetation patches may actively shape the overall microbiome composition within its vicinity (Andrew et al., 2012). In the semi-arid Kalahari, Lan et al. (2021) found that bacterial species with a specialized niche, rather than a generalized lifestyle, were mainly restricted to soil surfaces beneath shrubs. While there have been many studies conducted to examine vegetation-soil microbe interactions (Van Der Heijden et al., 2008; Saul-Tcherkas et al., 2013; Bruto et al., 2014; Soussi et al., 2016), these studies have been conducted with respect to individual (or small collections of) microbes (Jones et al., 2019). Moreover, the focus has often been on how microbes negatively or positively impact the plant and not how a plant may influence the functional potential of the microbiome under biotic or abiotic stress (reviewed in Chu et al., 2011; Jones et al., 2019). Consequently, our understanding of how vegetation influences the soil microbes and their functional capacity in

desert ecosystems, especially in hyper-arid environments, remains unclear despite their fundamental importance in biogeochemistry and functioning of these environments.

Given the critical roles of soil microbiomes in hyper-arid systems in providing a wide range of ecosystem services, it is essential to understand how vegetation influences the soil microbiome in order to predict how ecological functions may be altered under future climate-change projections. Since drylands represent the most dominant biome on Earth, covering approximately 45 % of the planet's terrestrial surface (Prăvălie, 2016), a comprehensive understanding of the functional capacity of the different microbial communities in this biome is highly relevant at the planetary scale (Ramond & Cowan, 2022). Additionally, addressing how vegetation may influence the selection of the microbiome beneath their canopy can help us to better link biotic interactions with ecosystem structure and functioning in hyper-arid environments and determine spatial and biodiversity patterns in these environments (Maestre et al., 2021).

In this study, shotgun metagenomics sequencing was used to investigate the functional potential of soil microbial communities in three sampling locations (vegetated hummock, unvegetated windward slope, and unvegetated gravel plains) in the Skeleton Coast National Park, Namib Desert. Shotgun metagenomics allows the deep exploration of microbial functional potential, which can be very important in understanding ecosystem productivity and resilience (Tappu, 2016; Castañeda & Barbosa, 2017; Newell, 2022). To investigate the functional potential of soil microbial communities, two basic questions were addressed: 1) How does the functional potential for microbial communities compare between vegetated hummock soils and bare (unvegetated: windward slope and gravel plains) ground? 2) What metabolic strategies underlie the ability of soil microbes to thrive and perform ecosystem functions and services in hyper-arid ecosystems? The working hypotheses were: 1) that the vegetated hummock soils would have more functional capacity relative to the bare (unvegetated: windward slope and gravel plain) ground due to plant-microbe interactions and the modified microclimate around plants, and 2) microbial communities in the Skeleton Coast possess genes encoding enzymes vital for thriving and performing ecosystem functions and services in hyper-arid ecosystems. To investigate these hypotheses, two basic questions were addressed: 1) How does the functional potential for microbial communities compare between

vegetated hummock soils and bare (unvegetated: windward slope and gravel plains) ground? 2) What metabolic strategies underlie the ability of soil microbes to thrive and perform ecosystem functions and services in hyper-arid ecosystems?

## 3.3. MATERIALS AND METHODS

### 3.3.1 Site description

Refer to chapter 2 (page 27-28)

### 3.3.2 Soil Sampling Strategy

Five *Arthraerua leubnitziae*-vegetated hummocks were sampled in the Skeleton Coast National Park, northern Namib Desert, in September 2018. A stratified sampling design was used to select the *Arthraerua leubnitziae*-vegetated hummocks (20.3711$^{o}$S, 13.1818$^{o}$E). Fifteen surface soil samples (0-5 cm depth) were collected from three sampling locations (one sample per sampling location): the vegetated hummock, the unvegetated windward hummock slope, and unvegetated open gravel plains (Fig. 3.1). The vegetated hummock sample was obtained from the vegetated area of the hummock, the unvegetated windward sample was obtained from the windward slope of the hummock and the unvegetated gravel plain sample was obtained from the open, vegetation-free patch area (Fig. 3.1). Each sample was a composite of four soil scoops (0-5 cm deep) that were obtained with a trowel, recovered from within a 1 $m^2$ virtual quadrat. Branches and rocks larger than 1 cm and disturbed areas (e.g., with footprints, animal burrows, etc.) were avoided during sampling. To avoid contamination, the hand trowel was sterilized with 70 % ethanol after the recovery of each sample. Soil samples were aseptically collected into separate sterile Whirl-Pak® plastic bags (Nasco, Fort Atkinson, USA) and stored at 4 °C for subsequent soil DNA extraction.


Figure 3. 1: A hummock with *Arthraerua leubnitziae*. Filled circles represent the sampling points from the three groups (vegetated hummock, unvegetated windward slope, and unvegetated open gravel plains). Photograph: A. R. Derr

## 3.4. DNA EXTRACTION AND SEQUENCING

### 3.4.1 Metagenomics DNA extraction

The DNA extractions from each sampling group (three groups; *vegetated hummock,* unvegetated *windward slope,* and unvegetated *open gravel plains*) were performed using the DNeasy PowerSoil Kit (QIAGEN, USA) following the manufacturer's instructions with necessary modifications. The soils were pretreated due to their high salt concentrations (the detailed protocol is provided in the appendices section). The samples were pooled into one sample prior to whole-shotgun sequencing, resulting in one metagenome per group. DNA samples were sent to Omega Bioservices for library preparation and sequencing (Norcross, GA, USA). DNA concentration was measured using the QuantiFluor dsDNA System on a Quantus Fluorometer (Promega, Madison, WI, USA). Fifty (50) ng of genomic DNA was enzymatically sheared using the Biosystems HyperPlus kit (Kapa Biosystems, Wilmington, MA, USA) according to the manufacturer's instructions. DNA fragment ends were repaired, 3' adenylated, and ligated to adapters. The resulting adapter-ligated libraries were PCR-

amplified. The DNA denaturation was at 98°C for 45 sec, followed by 5 cycles of denaturation (98 °C, 15 sec), annealing (60°C, 30 sec), and extension (72 °C, 30 sec). The final elongation occurred at 72°C for 1 min. PCR product was cleaned up from the reaction mix with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA). The libraries were quantified and qualified using the D1000 ScreenTape on an Agilent 2200 TapeStation instrument. The libraries were normalized and pooled for multiplexed sequencing on an Illumina HiseqX10 sequencer (Illumina, San Diego, CA, USA) using the pair-end 150bp run format.

### 3.4.2 Metagenomic sequence data processing

The raw Illumina reads in FASTQ format were quality-filtered, and the adaptors were removed using Trimmomatic's ILLUMINACLIP command (v0.36; SLIDINGINDOW: 4:15 MINLEN: 36) as described in Yang et al. (2016). Next, the trimmed Illumina paired-end reads were assembled using the NGS de novo *assembler,* MegaHit, using kmers 21 (minimum) and 91(maximum) with step of 20. The quality of each assembled metagenome (*n* = 3) was assessed using QUAST v5.2.0 (Mikheenko et al., 2018). All contigs shorter than 500 bp were removed, leaving an N50 value contigs of 981 for vegetated hummock samples, 891 for unvegetated windward slope samples and 890 bp for unvegetated open gravel plains soil samples. Subsequently, taxonomy classification of short reads was performed based on their annotations at the lowest taxonomic level using the Kraken2 taxonomic sequence classifier (Siegwald et al., 2017). Gene prediction was performed using Prodigal v2.6.3 (Hyatt et al., 2010), and protein files generated from the gene prediction were then used for functional annotation. GhostKAOLA was used to assign functions to the predicted Open Reading Frames (ORFs) (Takami et al., 2012). The predicted genes were functionally annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) bioinformatics database (Kanehisa & Sato, 2020).

### 3.4.2 Recovery of bacterial genomes

Genome binning was performed using MetaBAT2 (Kang et al., 2019). Binning yielded 117 MAGs (all from bacterial taxa), 9 of which were ≥75 % complete with ≤5 % contamination. These MAGs were considered to be of good enough quality for subsequent analysis. Resulting

MAGs were dereplicated using the Duplication, Aggregation and Scoring Tool (DAS_Tool) (Sieber et al., 2018) and consolidated into high-quality metagenome-assembled genomes (MAGs) using dRep (Olm et al., 2017). MAGs were purified using the MAGpurify tool to remove contamination and maintain completeness, as described in Nayfach et al. (2019). For all MAGs, completeness and contamination estimates were calculated using CheckM as described in Parks et al. (2015).

### 3.4.3 Taxonomic and functional profiling of MAGs

MAGs taxonomy was assigned with the Genome Taxonomy Database Toolkit (GTDB-Tk) (Chaumeil et al., 2020) by placing the MAGs into a domain-specific reference tree (Vasudeva et al., 2022). Prodigal was used to identify open reading frames (ORFs) and subsequently predict their amino acid sequences (Hyatt et al., 2010). Potential metabolic functions of the ORFs in each MAGs dataset were annotated by searching against Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KOs) using Distilled and Refined Annotation of Metabolism (DRAM) (Shaffer et al., 2020) to infer functional categories and pathways for MAGs (Kanehisa & Sato, 2020).

### 3.4.4 MAGs coverage and relative abundance

MAGs coverage was calculated by mapping the quality-filtered sequencing reads back to the dereplicated MAGs using CoverM v0.7.0 (https://github.com/wwood/CoverM). Coverage for each MAG was calculated as the average of all contig coverages within the MAG, weighted by their length (https://github.com/wwood/CoverM). Relative abundance was calculated as the proportion of a given MAG's coverage out of the sum of all present MAGs' coverage, per sample in the dereplicated MAG dataset. Abundance values were multiplied by the fraction of reads mapped to the MAGs to produce the relative abundance of each MAG within the entire dataset as described in Ray et al. (2022).

## 3.5. RESULTS

### 3.5.1 Metagenome assembly

Three metagenomes (60 million paired-end 150 bp read) were sequenced from three soil samples collected from three sampling locations: vegetated hummock, unvegetated open gravel plains, and windward slope. The primary assembly resulted in 1 056 032 contigs for the vegetated hummock sample, 1 121 092 for the open gravel plains sample and 1 035 498 for the unvegetated windward slope soil sample. The resulting assemblies had an average N50 of 981 bases for the vegetated hummock samples, 890 bases for the open gravel plains sample, and 891 bases for the windward slope sample. The read coverage of the metagenomes ranged from 87.1-95.4 %, highlighting that the majority of the raw read data was used in the assemblies (Table 3.1).

Table 3. 1: Metagenome assembly statistics for the three sampling locations (vegetated hummock, open gravel plains, and windward slope soils), highlighting assembly size and contig length, produced in Quast (Gurevich et al., 2013).

| Statistics without referer | Hummock | Gravel plains | Windward |
|---|---|---|---|
| # contigs | 1056032 | 1121092 | 1035498 |
| # contigs (>= 0 bp) | 2549722 | 3014487 | 2889266 |
| # contigs (>= 1000 bp) | 263538 | 246666 | 232468 |
| # contigs (>= 5000 bp) | 10188 | 6516 | 5467 |
| # contigs (>= 10000 bp) | 2537 | 1330 | 1025 |
| # contigs (>= 25000 bp) | 321 | 170 | 96 |
| # contigs (>= 50000 bp) | 47 | 41 | 15 |
| Largest contig | 132099 | 1230994 | 131842 |
| Total length | 1045130202 | 1029999567 | 943342965 |
| Total length (>= 0 bp) | 1592020030 | 1703960587 | 1601825337 |
| Total length (>= 1000 bp) | 511976678 | 443162656 | 404588440 |
| Total length (>= 5000 bp) | 94740359 | 62257918 | 45699211 |
| Total length (>= 10000 bp) | 43633059 | 27755649 | 16570518 |
| Total length (>= 25000 bp) | 12044010 | 11504451 | 3694928 |
| Total length (>= 50000 bp) | 3089738 | 7295673 | 967288 |
| N50 | 981 | 890 | 891 |
| N75 | 671 | 649 | 648 |
| L50 | 274234 | 322968 | 303708 |
| L75 | 602358 | 666270 | 618156 |
| GC (%) | 65.26 | 69.6 | 71.05 |
| Mismatches | | | |
| # N's | 0 | 0 | 0 |
| # N's per 100 kbp | 0 | 0 | 0 |

### 3.5.2 Metagenomic Community Profiling

Shotgun metagenomic reads were retrieved and classified to determine the community composition of the samples. The most abundant community members were from the organo-heterotrophic lineages within the bacterial phyla known to predominate in desert soils (Fierer et al., 2012; Delgado-Baquerizo et al., 2018) showing relative abundance greater than 1 % (Fig. 3.2). Actinomycetota, with a mean relative abundance of 58 %, dominated the soils in all sampling locations, followed by Pseudomonadota (35 %), Bacteroidota (2 %), and Bacillota (1.2 %). All four phyla were observed in both vegetated hummock soils and unvegetated gravel plains. However, in unvegetated windward soils, three phyla were observed, with Bacillota notably missing. The metagenome results show similar community profiles to those that were observed through 16S rRNA gene amplicon sequencing (Chapter 2, Fig. 2.5A). While the taxonomy results from the metagenome show a similar community profile to the 16S rRNA data regarding the dominant phyla, the latter data showed a higher diversity of dominant phyla consisting of nine phyla Actinomycetota (24 %), Pseudomonadota (22 %), Bacillota (20 %), Bacteroidota (8 %), Chloroflexi (4 %), Gemmatimonadota (4 %), Planctomycetota (3 %), Myxococcota (2 %) and Verrucomicrobiota (1.9 %); Chapter 2, Fig. 2.5A) as compared to the four phyla (Actinomycetota (58 %), Pseudomonadota (35 %), Bacteroidota (2 %), and Bacillota (1.2 %) observed in the metagenome data (Fig. 3.2).

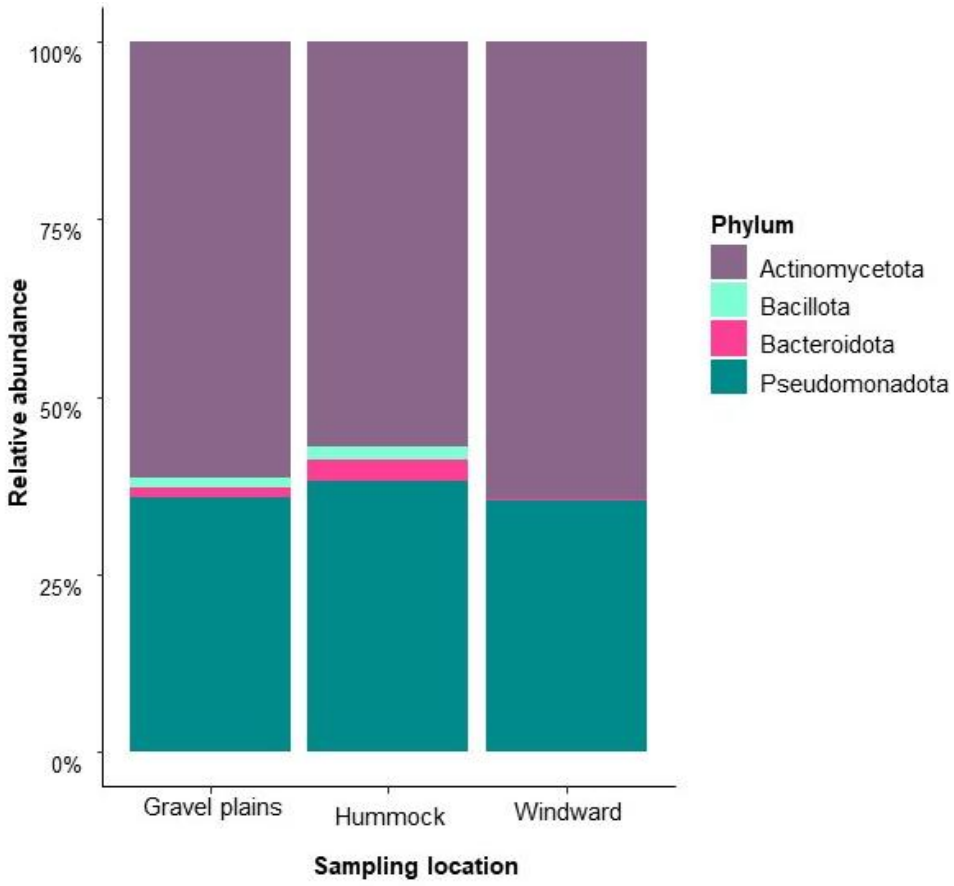



Figure 3. 2: Relative abundance of the major phyla based on based on shotgun metagenomic reads across sampling locations.

### 3.5.2 Metagenome functional annotation

#### 3.5.2.1 Metabolic pathways

KEGG functional prediction database was used to annotate ORFs from the metagenomes and investigate the functional potential of soil microbial communities in the vegetated hummock and surrounding soils. This study focused on energy-generating pathways (such as carbohydrate metabolism, C degradation, chemotaxis, glycoside metabolism and methane metabolism) and biogeochemical pathways (C fixation, nitrogen & sulfur cycle). All three sampling locations showed complete energy metabolism for glycolysis, TCA cycle, F-type ATPase, and gluconeogenesis pathways. For C fixation, RuBisCo and beta-N-acetylhexosaminidase pathways were complete in all the sampling locations, whereas 3-hydroxypropionate Bi-cycle and 4-hydroxybutyrate/3-hydroxypropionate pathways were also observed but incomplete (Fig. 3.3).

The complete nitrogen metabolism processes, i.e., nitrate reduction and denitrification, were observed in all metagenomes (vegetated hummock, unvegetated gravel plains, and unvegetated windward slope soils) with the presence of key enzymes, i.e., nitric oxide reductase, nitrous oxide reductase, nitrate reductase, and nitrite reductase. However, it is important to note that other key enzymes for nitrogen processing, i.e., ammonia oxidation (*amo/pmmo*), hydroxylamine oxidation, and nitrite oxidation, were exclusively observed in vegetated hummock soils (Fig. 3.3). Similarly, complete sulfur metabolism was observed in all three sampling locations, including both assimilatory and dissimilatory sulfate reduction and sulfate oxidation, except for sulfite hydrogenase, which was detected in unvegetated windward slope and open gravel plains soils but absent in vegetated hummock soils (Fig. 3.3). For methane metabolism, methanogenesis via the use of trimethylamine as a substrate was observed across all sampling locations. Metagenomes were also analyzed for the presence of proteins involved in secretion systems, revealing a number of complete pathways for secretion systems (Type IV, Type VI, and Type III) in hummock soils compared to unvegetated open gravel plains and windward slope soils.

In order to further explore the abundance of key pathways, the abundance of marker genes for the pathways discussed above was counted (Appendix 2; Table 1). Nitrate reduction genes such as *narG, narH, narZ, napA, napB,* and *nxrB* were detected in vegetated hummock and open gravel plains soils (Fig. 3.4). It is worth noting that a deficiency of nitrogen cycle-related genes (except for *nirD* and *nirB* genes) was observed in the windward soils. Furthermore, a higher number of genes related to sulfite reductase was observed in unvegetated soils (windward slope (7 genes) and gravel plain (9 genes)) relative to the vegetated hummock soils (Fig. 3.4), indicating a higher sulfate reduction potential in the in unvegetated soils. Ribulose-bisphosphate carboxylase (*rbcS*) and phosphoribulokinase (*prkB*) enzymes from C fixation pathways were also observed in all the sampling locations. However, it is worth noting that the C fixation and metabolism enzymes showed high abundance in unvegetated soils (windward slope and gravel plain) relative to the vegetated hummock soils (Fig. 3.4).

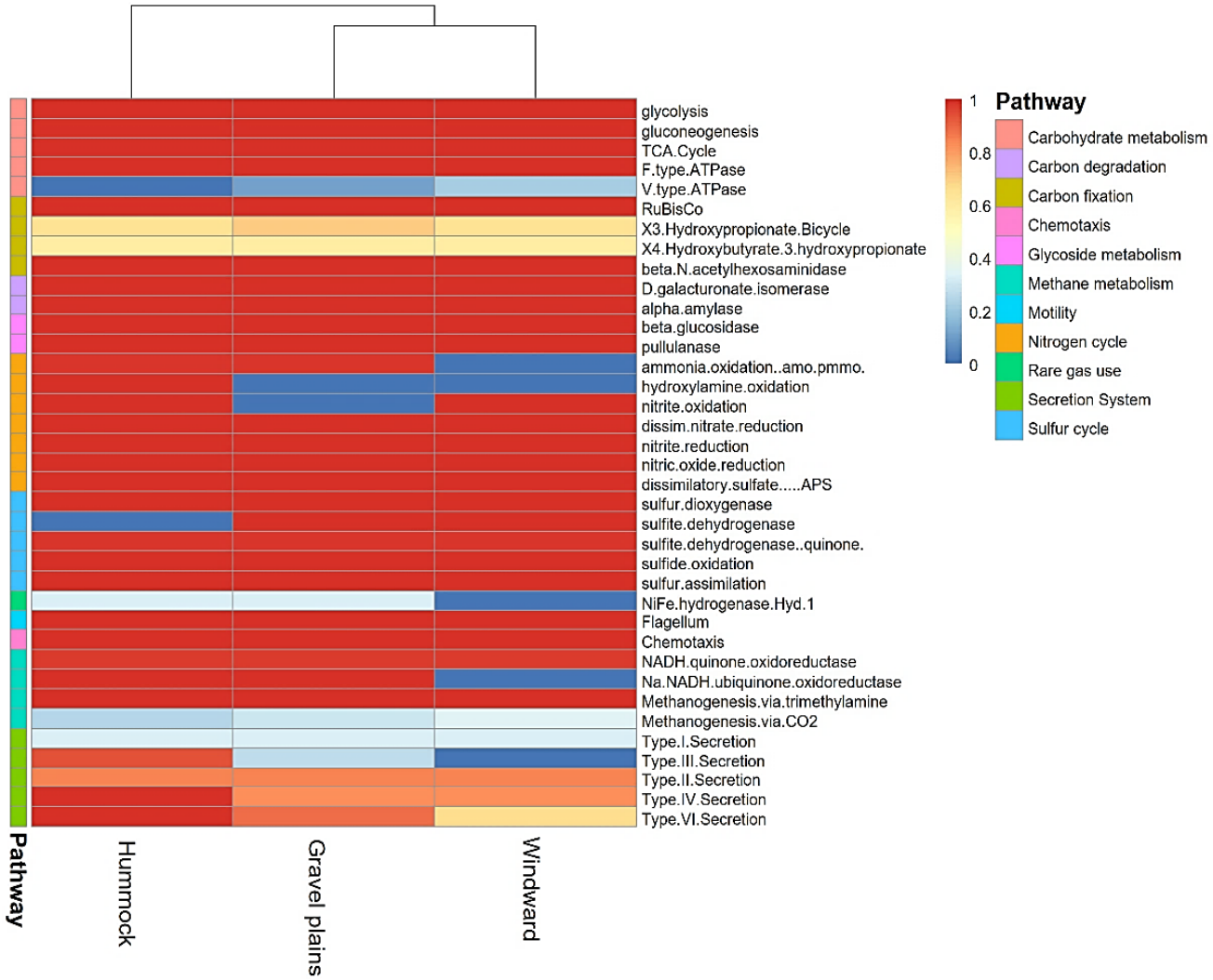



Figure 3. 3: Heatmap plot and functional clustering of the selected KEGG pathways for the predicted ORFs from the metagenomic reads for the vegetated hummock, unvegetated open gravel plains, and unvegetated windward slope. The color scaling represents the completeness of different KEGG pathways, with darker red indicating completeness level and darker blue indicating absent or incomplete pathways.

### 3.6.2.2 Phylogenetic analysis of marker genes for key pathways within the metagenome

Marker genes observed in vegetated hummock soils related to C, N, and S cycling were affiliated with bacterial classes within the Pseudomonadota (58 genes), Actinomycetota (15 genes), Bacillota (4 genes), Cyanobacteria (2 genes) and Bacteroidota (2 genes) phyla (Fig. 3.5A). In unvegetated gravel plains, most of the observed genes were affiliated with bacterial classes within the Pseudomonadota (34 genes), Actinomycetota (23 genes), Cyanobacteria (7 genes), Chloroflexota (4 genes) and Bacteroidota (1 gene) phyla (Fig. 3.5B). Furthermore, genes observed in unvegetated windward soils related to C, N, and S cycling were affiliated with bacterial classes within the Pseudomonadota (29 genes), Actinomycetota (26 genes),

Cyanobacteria (8 genes) and Chloroflexota (8 genes) and Bacillota (1 gene) phyla (Fig. 3.5C). In all the sampling locations, bacterial classes within the Pseudomonadota phylum contained the highest number of genes relative to all the observed phyla (Fig. 3.5A, B & C). It is worth noting that the unvegetated soils (both gravel plains and windward soils; Fig. 3.5B & C) had a relatively high number of genes affiliated with Actinomycetota phylum as compared to the vegetated hummock soils (Fig. 3.5A).

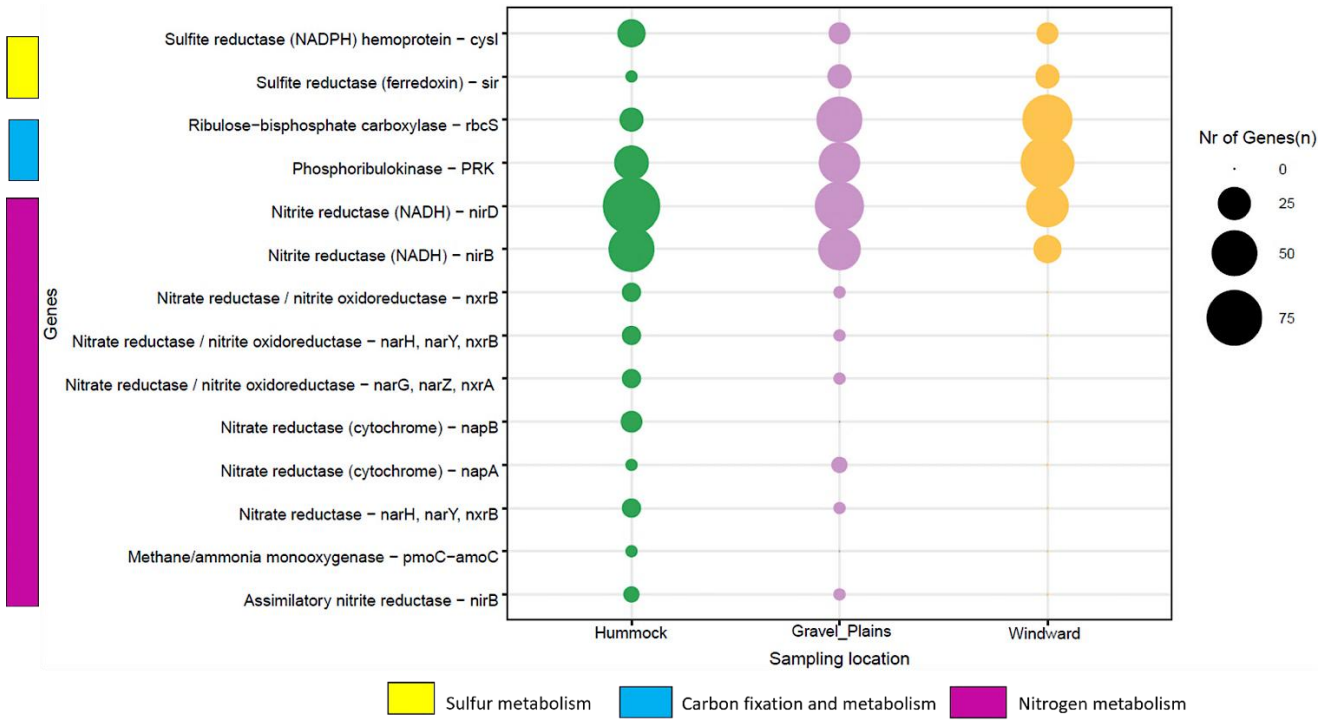
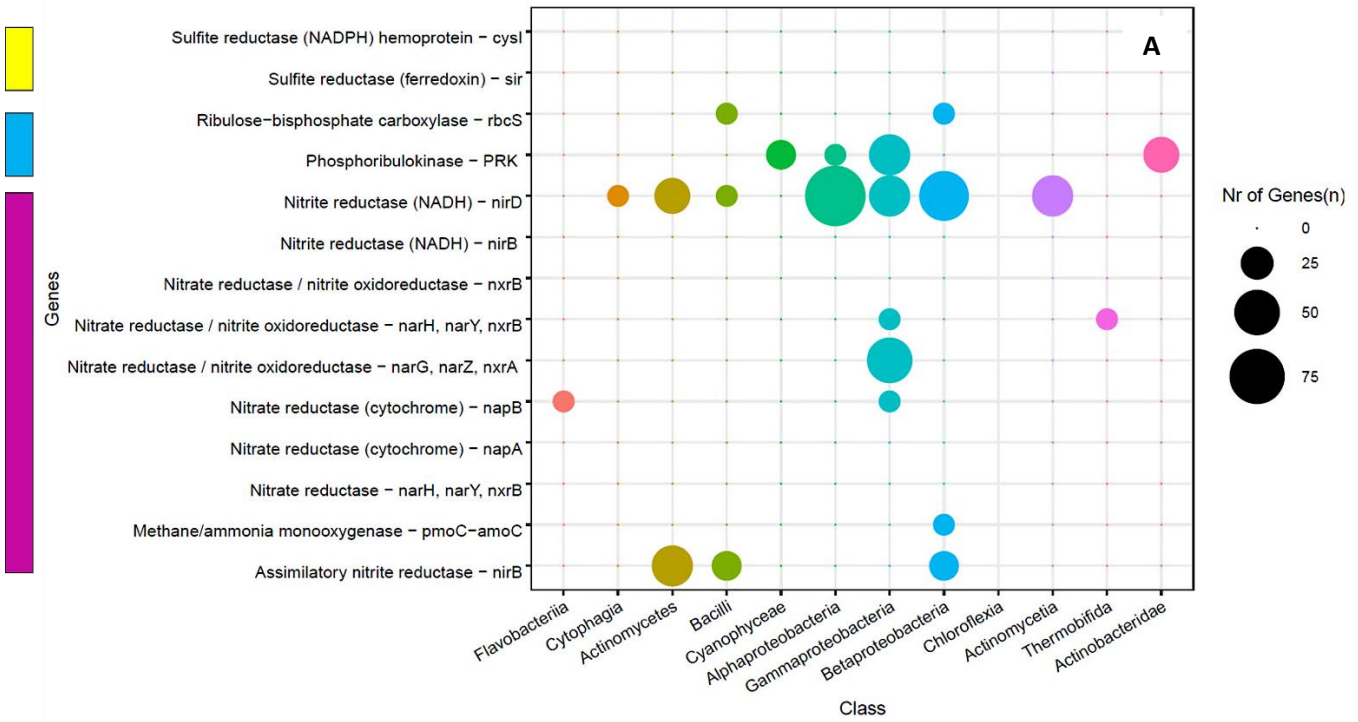
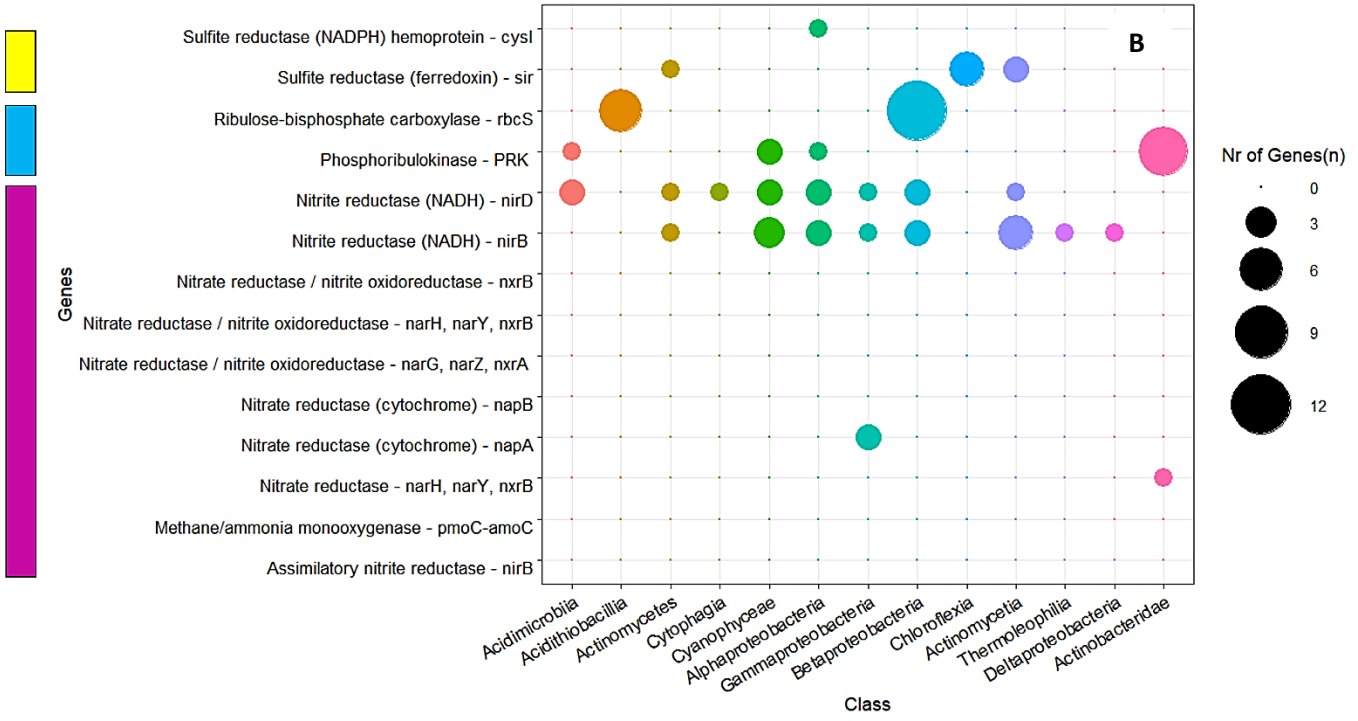



Figure 3. 4: Dot plot showing the presence and abundance of genes in the three (vegetated hummock, unvegetated open gravel plains and unvegetated windward slope soils) metagenomic samples.


A
Sulfite reductase (NADPH) hemoprotein − cysI
Sulfite reductase (ferredoxin) − sir
Ribulose−bisphosphate carboxylase − rbcS
Phosphoribulokinase − PRK
Nitrite reductase (NADH) − nirD
Nitrite reductase (NADH) − nirB
Nitrate reductase / nitrite oxidoreductase − nxrB
Nitrate reductase / nitrite oxidoreductase − narH, narY, nxrB
Nitrate reductase / nitrite oxidoreductase − narG, narZ, nxrA
Nitrate reductase (cytochrome) − napB
Nitrate reductase (cytochrome) − napA
Nitrate reductase − narH, narY, nxrB
Methane/ammonia monooxygenase − pmoC−amoC
Assimilatory nitrite reductase − nirB
Genes
Flavobacteriia
Cytophagia
Actinomycetes
Bacilli
Cyanophyceae
Alphaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Chloroflexia
Actinomycetia
Thermobifida
Actinobacteridae
Class
Nr of Genes(n)
0
25
50
75


B
Sulfite reductase (NADPH) hemoprotein - cysI
Sulfite reductase (ferredoxin) - sir
Ribulose-bisphosphate carboxylase - rbcS
Phosphoribulokinase - PRK
Nitrite reductase (NADH) - nirD
Nitrite reductase (NADH) - nirB
Nitrate reductase / nitrite oxidoreductase - nxrB
Nitrate reductase / nitrite oxidoreductase - narH, narY, nxrB
Nitrate reductase / nitrite oxidoreductase - narG, narZ, nxrA
Nitrate reductase (cytochrome) - napB
Nitrate reductase (cytochrome) - napA
Nitrate reductase - narH, narY, nxrB
Methane/ammonia monooxygenase - pmoC-amoC
Assimilatory nitrite reductase - nirB
Genes
Acidimicrobiia
Acidithiobacillia
Actinomycetes
Cytophagia
Cyanophyceae
Alphaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Chloroflexia
Actinomycetia
Thermoleophilia
Deltaproteobacteria
Actinobacteridae
Class
Nr of Genes(n)
0
3
6
9
12

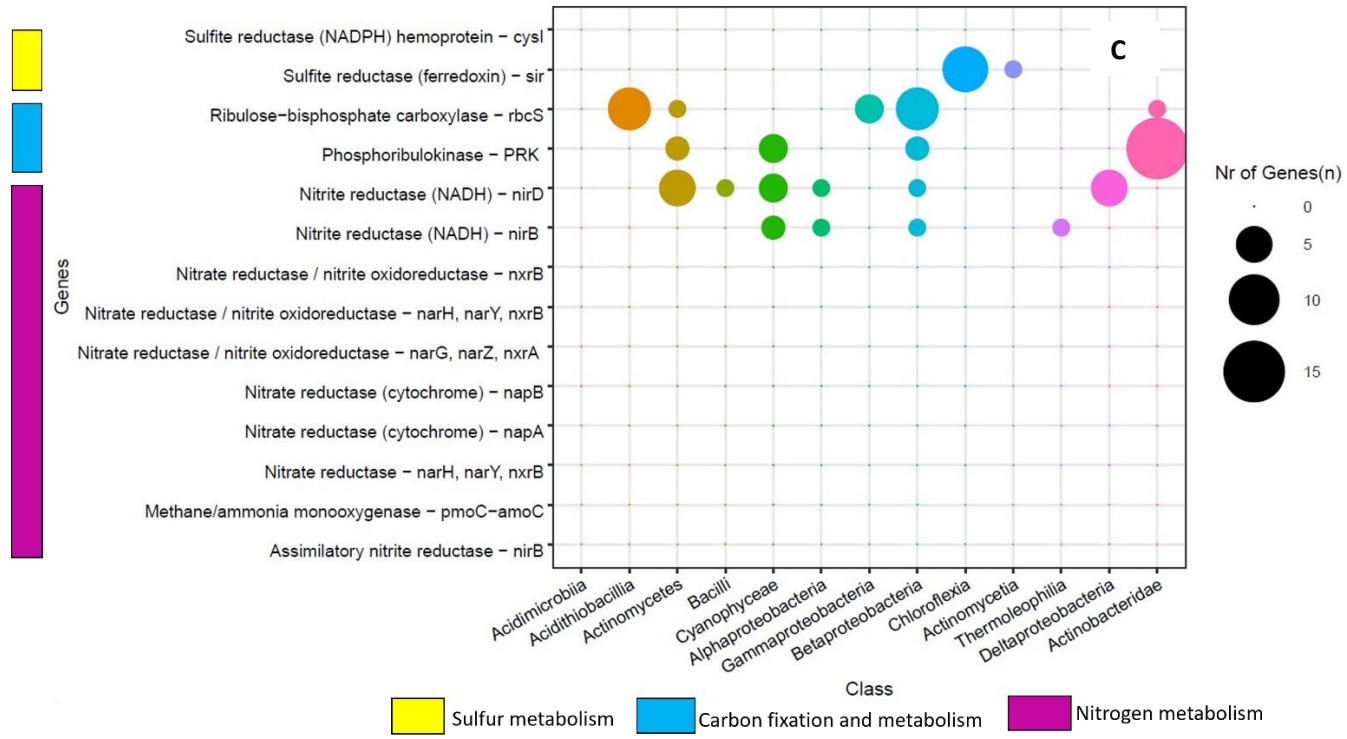



Figure 3. 5: Dot plot showing the metabolic potential of the **A**) vegetated hummock, **B**) unvegetated gravel plains and **C**) unvegetated windward metagenome. The size class of each point represents the number of genes in each class that encode the gene of interest, and the shading represents the average genome completeness.

### 3.5.3 Metagenome-assembled genomes (MAGs)

#### 3.5.3.1. MAGs assembly and taxonomy

A total of nine medium to high quality genomes were assembled from the metagenomes, with a completeness percentage ranging from 78 to 99 % (Table 3.2). The assembled genomes included *Ralstonia insidiosa* (99 %) genome consisting of 18 contigs, *Pseudorhizobium* (96 %) with 161 contigs, *Paenibacillus* (92 %) with 440 contigs, *Rhodobacteraceae* (90 %) with 346 contigs, *Pseudomonas luteola* (89 %) with 426 contigs, *Nitriliruptoraceae* (88%) with 243 contigs, *Flavobacterium* (83 %) with 178 contigs, *Pseudarthrobacter* (82 %) with 353 contigs and *Mycobacteriales* (78 %) with 362 contigs. The results obtained by GTDBTk classification demonstrated that the MAGs were distributed in four different phyla. Three MAGs belonged to phylum Pseudomonadota, two MAGs were assigned to phylum Actinomycetota, and one MAG was assigned to phylum Bacteroidota and Bacillota. Similarly, in unvegetated open

gravel plains, one MAG was assigned to phylum Pseudomonadota whereas in unvegetated windward slope one MAG was assigned to phylum Actinomycetota (Table 3.2).

Table 3. 2: Summary of MAGs obtained from the vegetated hummock, unvegetated open gravel plains and unvegetated windward slope sampling locations. Genome completeness and contamination were estimated with CheckM. The name of the taxon level in marker lineage is abbreviated as k-kingdom, p-phylum, c-class, o-order, f-family, and g-genus.

| Bin ID | Marker lineage | Phylum | Completeness (%) | Contamination (%) | Number of Contigs | Longest contig |
|---|---|---|---|---|---|---|
| Open gravel plains | *s__Ralstonia insidiosa* | Pseudomonadota | 99 | 0 | 18 | 1230994 |
| Hummock | *g__Pseudorhizobium* | Pseudomonadota | 96 | 2.1 | 161 | 132099 |
| Hummock | *g__Paenibacillus* | Bacillota | 92 | 0.44 | 440 | 79667 |
| Hummock | *f__Rhodobacteraceae* | Pseudomonadota | 90 | 4.0 | 346 | 52234 |
| Hummock | *s__Pseudomonas luteola* | Pseudomonadota | 89 | 3.7 | 426 | 60289 |
| Hummock | *f__Nitriliruptoraceae* | Actinomycetota | 88 | 1.7 | 243 | 73780 |
| Hummock | *g__Flavobacterium* | Bacteroidota | 83 | 0.6 | 178 | 69907 |
| Hummock | *g__Pseudarthrobacter* | Actinomycetota | 82 | 0.9 | 353 | 24269 |
| Windward | *o__ Mycobacteriales* | Actinomycetota | 78 | 3.9 | 362 | 38229 |

### 3.6.3.2 MAGs coverage

All nine MAGs were represented in the three metagenomes. The relative abundance results show that unvegetated open gravel plains and windward slope soils are dominated by one MAG each: one belonging to the species *Ralstonia insidiosa* (24 %) and one MAG from the order *Mycobacteriales* (47 %), respectively (Fig. 3.6A). By contrast, vegetated hummock soils exhibited a more even distribution of MAGs (Fig. 3.6A). Looking at RPKM (reads per kilobase million) abundance values, the unvegetated windward slope exhibited the highest abundance of MAGs, while the vegetated hummock soils contained the lowest abundance of MAGs (Fig. 3.6B).

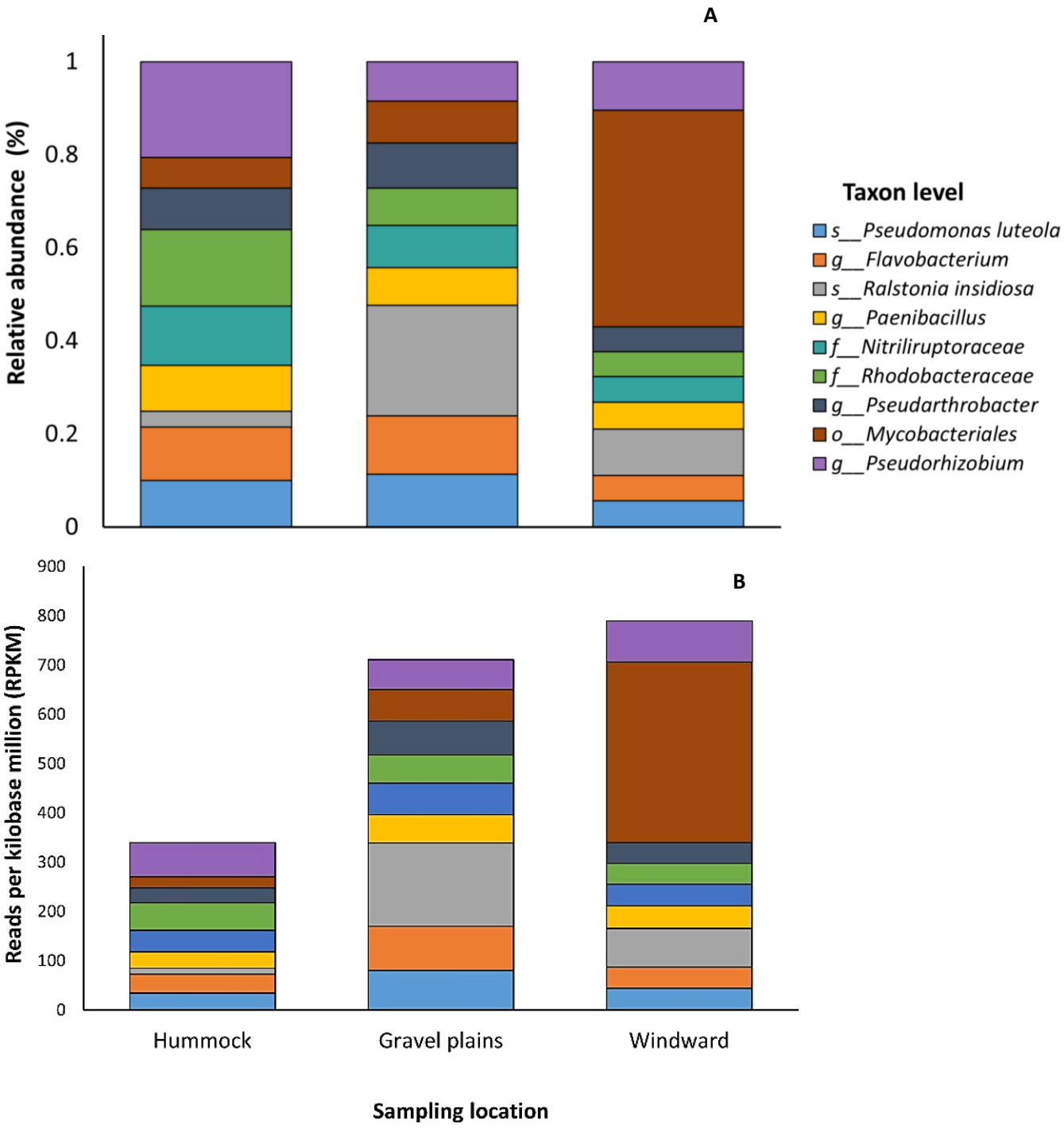



Figure 3. 6: Relative abundance **(A)** and reads per kilobase million (RPKM) **(B)** recovered in MAGs across the vegetated hummock, open gravel plains and windward slope samples. The name of the taxon level is abbreviated as k-kingdom, p-phylum, c-class, o-order, f-family, and g-genus.

### 3.6.3.3 Functional annotation of metagenome-assembled genomes

The MAGs obtained from vegetated hummock, unvegetated open gravel plains and unvegetated windward slope soils were compared in terms of key metabolic pathways to

assess their potential metabolic role in the soil microbiome. The functional annotation using the DRAM pipeline indicated the presence of various complete energy and biogeochemical metabolism pathways in the three sampling locations. Most of the MAGs showed complete energy metabolism for glycolysis, the Entner-Doudoroff pathway (except *Nitriliruptoraceae*), cytochrome c oxidase and F-type ATPase pathways. Gluconeogenesis pathway was present in four MAGs, namely *Flavobacterium, Rhodobacter, Pseudomonas luteola* and *Ralstonia insidiosa* MAGs. Additionally, the beta-N-acetylhexosaminidase pathway was complete in most of the MAGs, whereas the glucoamylase, alpha-amylase and beta-glucosidase enzyme groups were unique to specific groups of MAGs obtained from vegetated hummock soils. Specifically, glucoamylase pathway was complete in *Pseudomonas luteola* and *paenibacilus* MAGs; alpha-amylase pathway was complete in the *paenibacilus* MAG while beta-glucosidase showed complete enzymes in *Pseudomonas luteola, Paenibacilus, Pseudorhizobium, Rhodobacteraceae* and *Flavobacterium* MAGs.

The presence of C fixation, nitrogen, methane, and sulfur metabolism was also observed in the MAGs, indicating the diverse biogeochemical cycles prevailing in the Skeleton Coast National Park. For C fixation, MAGs from all three sampling locations contained genes for the TCA cycle pathway. Similarly, MAGs from all three sampling locations contained genes for 3-Hydroxypropionate Bicycle (except *Nitriliruptoraceae*) and 4-Hydroxybutyrate/3-hydroxypropionate (except *Pseudarthrobacter*) pathways, whereas *Ralstonia insidiosa, Pseudorhizobium*, *Rhodobacteraceae* and *Nitriliruptoraceae* MAGs contained genes for the Wood-Ljungdahl pathway. The enzymes responsible for nitrogen metabolism were exclusively observed in the *Pseudomonas luteola* and *Paenibacilus* MAGs belonging to phyla Pseudomonadota and Bacillota, respectively. The enzymes responsible for sulfur metabolism were observed in *Pseudomonas luteola, Nitriliruptoraceae, Ralstonia insidiosa*, *paenibacilus* and *Mycobacteriales* MAGs of phyla Pseudomonadota and Bacillota. Additionally, *Pseudarthrobacter, Pseudomonas luteola, and Ralstonia insidiosa* MAGs had a high abundance of sulfur-oxidizing enzymes (Fig. 3.7). The MAGs were also compared for the presence of the enzymes involved in secretion systems. Among all the recovered MAGs, *Pseudomonas luteola and Ralstonia insidiosa* belonging to Pseudomonadota suggesting the presence of genes associated with secretion system enzymes relative to other MAGs.

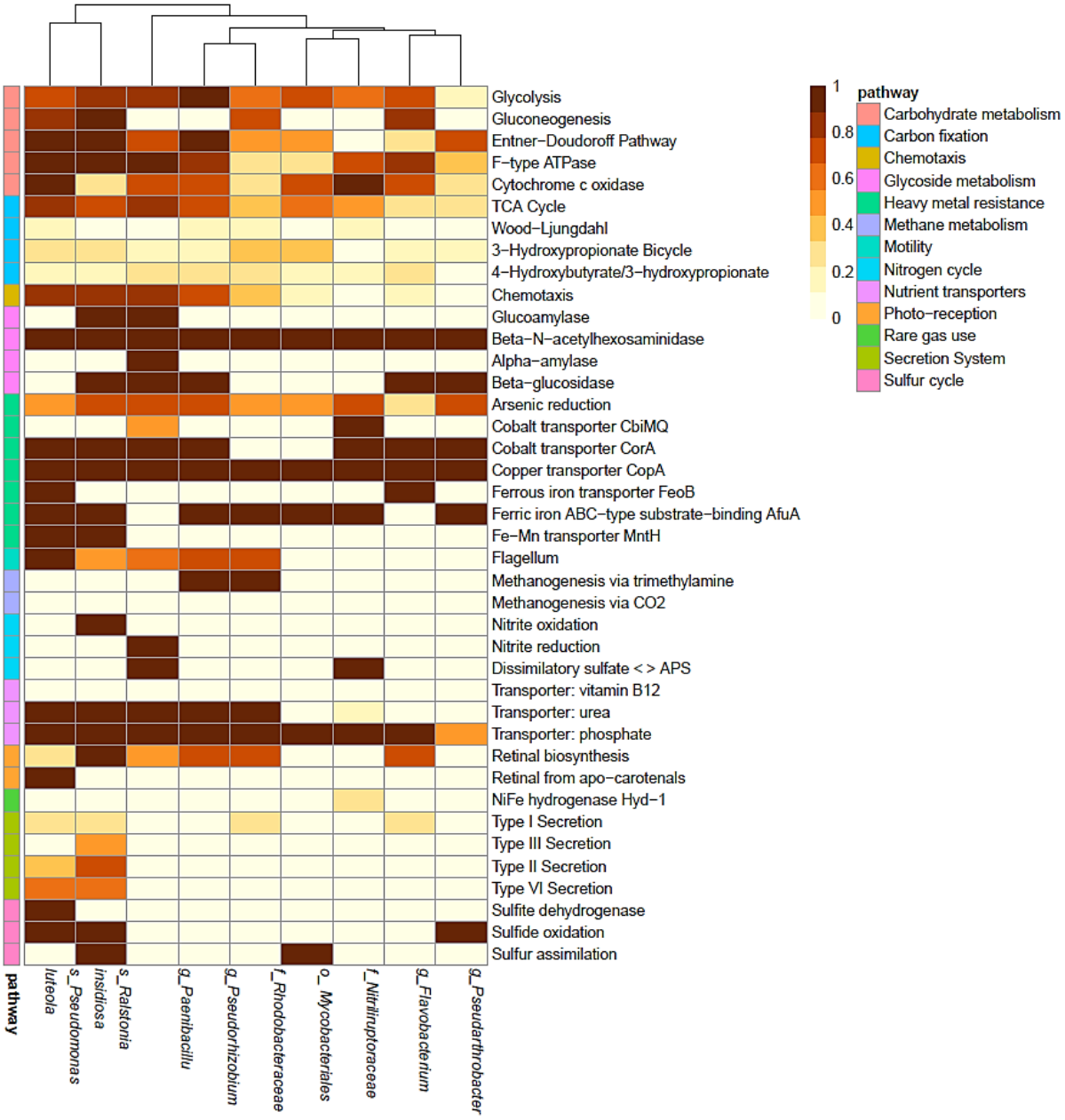



Figure 3.7: Heatmap displaying key functional pathways involved in biogeochemical cycles, autotrophy, and energy conservation across the 9 high-quality MAGs constructed from the metagenomic reads for the vegetated hummock, open gravel plains, and windward slope. The color scaling represents the completeness of different KEGG pathways in the MAGs, with darker brown indicating complete and cream indicating incomplete pathways.

## 3.6. DISCUSSION

Shotgun metagenome sequencing was used to evaluate the soil microbial functional potential in the Skeleton Coast National Park, Namib Desert. The results show that vegetated hummock soils appear to have more functional potential compared to the open gravel plains and windward slope locations (Fig. 3.1). The presence of vegetation has been observed to create

a more favourable environment for microbial activity, nutrient cycling, and soil stability (Han et al., 2007; Lan et al., 2021; Wang et al., 2022), therefore resulting in higher functional potential compared to unvegetated areas. This agrees with the trend observed in chapter 2 (Fig. 2.7A & C) where vegetated hummock soils are found to be associated with a higher number of observed microbial diversity relative to unvegetated open gravel plains and windward slope. Similar to Chapter 2, this observation should be interpreted with caution as the number of soil samples on which this finding is based was limited. This may suggest that there is a positive correlation between microbial diversity and the functional potential of these communities. This correlation was also corroborated, for example, by another study carried out in the Namib Desert (Naidoo, 2020), which showed a correlation between microbial diversity and functional potential. In another study, Chen et al. (2021) reviewed metagenomic data from 56 peer-reviewed publications from around the world and found a correlation between microbial diversity and microbial functions. As a whole, these results indicate that the overall functional profiles of these microbial communities seem to be predictable, at least to a certain extent, from the taxonomic community profiles.

### 3.6.1 Metagenome assembly

The metagenomic assembly showed comparable numbers of contigs, and the N50 values were similar across all three metagenome samples (Table 3.1). The contigs number exceeded one million for all the samples, suggesting a substantial degree of fragmentation in the assembly. This fragmentation may be attributed to the extreme conditions in the hyper-arid Skeleton Coast region from which the environmental samples were recovered, as well as the extraction method used for DNA extraction (Alawi et al., 2014; Hinlo et al., 2017). In this study, a standardised metagenomics DNA extraction protocol (the detailed protocol is provided in the appendices section) was used, which combines the enzymatic (lysozyme and proteinase K) and chemical (CTAB and $CaCl_2$) strategies to ensure efficient cell lysis and the use of PEG and isopropanol for precipitation of humic impurities-free DNA (Kumar et al., 2017). While this protocol has produced a higher yield of good quality metagenomic DNA from different types of soils collected from sites such as a garden, domestic waste dumps, cellulose waste dumps, sewage, and tannery waste sites (Kumar et al., 2017), it is important to note that soils used in this study are highly oligotrophic with high salt concentration (Appendix 1; Table 1).

Therefore, the protocol may be improved by evaluating different concentrations of lysozyme and proteinase K to determine the optimal combination for complete cell lysis to suit oligotrophic soils. Despite the fragmentation in the data, this study provided insight into the functional diversity of soil microbial communities in the soils of the Skeleton Coast.

### 3.6.2 Metagenomic Community Profiling

The metagenome samples were dominated by Actinomycetota, Pseudomonadota, Bacteroidota and Bacillota (relative abundance >1 %) (Fig. 3.2). This was not unexpected, as similar results have been found in other desert studies (Fierer et al., 2012; Valverde et al., 2016; Armstrong et al., 2016; Soussi et al., 2016; Wei et al., 2016). Among these bacterial phyla, Actinomycetota dominated the open gravel plains and windward slope soils, while the vegetated hummock soils were dominated by Pseudomonadota (Fig. 3.2). This could be due to Pseudomonadota and Actinomycetota belonging to the different ecological categories (Ru & Feng, 2020). Pseudomonadota (in particular Alpha- and Beta-Proteobacteria) encompass copiotrophic taxa that preferentially consume labile soil organic C pools (Fierer et al., 2007; Fierer et al., 2012), and soil samples from vegetated hummock areas contained higher soil organic C relative to open gravel plains and windward slope soils (Appendix 1; Table 1). Also, desert vegetation provides an essential energy source for soil microbial communities through the production of litter and secretion of root exudates (Andrew et al., 2012; Zhang et al., 2013), which may constitute another reason for the higher abundance of Pseudomonadota in vegetated hummock areas compared to Actinomycetota. In contrast, the phylum Actinomycetota includes several oligotrophic taxa (e.g., members of the *Marmoricola* and *Marmoricola* family) (Demergasso et al., 2023) with a higher adaptive capacity under poor-nutrients conditions (Fierer et al., 2007; Davis et al., 2011) which may explain the reason why this phylum dominated the open gravel plains and windward slope soils but not the vegetated hummock soils. Additionally, phyla including Fribrobacteres, Chlamydia, Gemmatimonadetes, Nitrospirae, Planctomycetes, Verrucomicrobia, and Candidatus Saccharibacteria (relative abundance <1 %) were also found in the metagenomes of all soil samples. Although reported in low abundance, some members of these phyla, such as *Fibrobacter* from Fribrobacteres phylum, are considered to be major degraders of cellulosic plant biomass (Ransom-Jones et

al., 2012). Moreover, the archaeon *Nitrososphaera* from *Nitrospirae* plays an ecological role as an ammonia-oxidizing Archaea (AOA) (Tourna et al., 2011; Lu et al., 2020).

### 3.6.3 Metagenome functional annotation in and outside the vegetated hummocks soils

Soil microbes are essential in driving ecological functions and biogeochemical processes such as litter decomposition and C, nitrogen (N) and sulfur (S) cycling (Madsen, 2011; Schimel & Schaeffer, 2012; Nelson et al., 2016). This study focused on energy-generating and biogeochemical pathways beneficial for ecosystem services. These included pathways related to carbohydrate metabolism, C degradation, methane metabolism, and C, N, and S cycling. The results showed enrichment of functional capacity in vegetated hummock soils (Fig. 3.3), suggesting that vegetation influences the microbial functional potential. The presence of nitrogen pathways in vegetated hummock soils, particularly nitrification, highlights the pivotal role of nitrification in providing nitrate for plant utilisation, thus influencing nutrient uptake, root system and abiotic stress tolerance in plants (Ayiti & Babalola, 2022). Genes such as NiFe hydrogenase Hyd-1, Na-NADH-ubiquinone oxidoreductase, and genes for Type III Secretion, Type IV Secretion, and Type VI Secretion were also detected, mainly in the vegetated hummock and open gravel plains soils; however, these pathways were not complete (Fig. 3.3). Although not complete, secretion system pathways are ecologically relevant as they play significant roles in microbial interactions, competition and survival (Green & Mecsas, 2016). Secretion system pathways allow bacteria to secrete proteins, including toxins and enzymes that promote bacterial virulence and contribute to cellular success in inter-taxon competition. (Green & Mecsas, 2016). Furthermore, NiFe hydrogenase Hyd-1 genes are involved in the chemotrophic scavenging of atmospheric trace gases as alternative energy sources (Leung et al., 2020; Ortiz et al., 2021). The presence of the NiFe hydrogenase Hyd-1 gene provides insights into how bacterial communities persist in extreme desert environments, where phototrophic primary producers are scarce, by utilising alternative energy sources.

The presence of genes related to C, N, and S cycling was used as a proxy to understand the potential relevance of each biogeochemical cycle at each sampling location. The ammonia-oxidizing marker gene methane/ammonia monooxygenase - pmoC-amoC was exclusively

detected in vegetated hummock soils (Fig. 3.4), suggesting the increased potential for oxidation of ammonia to nitrite and nitrate in vegetated hummock soils compared to unvegetated windward and open gravel plains soils. Ammonia-oxidizing processes require high energy input. Thus root exudates and the availability of mineral nutrients in vegetated hummock soils can serve as a source of energy to support the enzymatic reactions (Amoo & Babalola, 2017; Nakamura et al., 2020). This might offer a plausible explanation for why methane/ammonia monooxygenase - pmoC-amoC was exclusively detected in vegetated hummock soils. The detection of ammonia-oxidizing gene in vegetated hummock soils is supported by the presence of genera *Nitrosococcus* and *Nitrosospira* ammonia-oxidizers (Neilson et al., 2017), which were detected in metagenome samples recovered from vegetated hummock soils. This was also observed in amplicon sequencing data (chapter 2), which showed enrichment of these genera (*Nitrosococcus* and *Nitrosospira*) in vegetation hummock soils relative to the unvegetated open gravel plains and windward slope soils. These findings support the idea that plants play an essential role in driving the distribution of ammonia oxidisers in soils across the landscape at local and regional scales (Moreau et al., 2015; Thion et al., 2016).

Genes involved in denitrification (*narG, narH, narZ, napA, napB, nxrB, nirD* and *nirB*) were observed across all the sampling locations (Fig. 3.4), indicating the presence of microbial taxa capable of reducing various forms of nitrogen. Bacterial taxa known to be involved in the denitrification process were observed in the metagenome, and these belonged to genera such as *Bacillus, Flavobacterium, Neisseria, Pseudomonas, and Rothia* (Rosier et al., 2020). The presence of genes involved in the denitrification process suggests the significance of desert soils in global nitrogen regulation by transforming nitrates into nitric oxide and nitrogen gases, which are subsequently released into the atmosphere (Jin et al., 2015). Nitric oxide reductase plays a crucial role in the detoxification process, and its presence in the metagenomes suggests a beneficial impact of denitrifies in this hyper-arid system. The denitrification process has mostly been assumed to be important in wet and nutrient-rich environments (Peterjohn, 1991; Wu et al., 2021). However, recent studies have challenged this assumption by demonstrating that desert subsoil represents a large reservoir of bioavailable N in the form of nitrate (Walvoord et al., 2003; Al-Taani & Al-Qudah, 2013; Wu et al., 2021). Given the climate change projections in dryland systems, these systems have the

potential to significantly influence microbially mediated N cycling processes, including N mineralisation, nitrification, and denitrification. Several studies have found elevated $CO_2$ to increase the abundances of genes like *nirK* and *nosZ*, with *nirS*-containing denitrifiers being more sensitive to temperature increases than those containing *nirK* and *nosZ* genes (Lee et al., 2017; Xing et al., 2021; Wu et al., 2021; Porto et al., 2023; Hui et al., 2024). This highlights the ability of deserts to regulate nitrogen levels and thus affect global nitrogen cycling.

For C metabolism, *rbcS* and *PRK* genes were detected across the sampling locations (Fig. 3.4), indicating the presence of enzymes associated with the reductive pentose phosphate cycle (Calvin-Benson cycle), which is a crucial part of C metabolism in plants (Yamaoka et al., 2016). These genes encode two essential proteins: *rbcS* encodes the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme responsible for C fixation, while *PRK* genes encode phosphoribulokinase, which catalyses a key step in the regeneration of ribulose-1,5-bisphosphate (RuBP) within the cycle (Yamaoka et al., 2016; Meloni et al., 2023). Both *rbcS* and *PRK* genes were present across all three sampled locations (vegetated hummock, open gravel plains, and windward slope soils); however, both *PRK* and *rbcS* genes were less abundant in vegetated hummock soils (Fig. 3.5A) as compared to open gravel plains, and windward slope soils (Fig. 3.5B & C). More likely, the low abundance of *PRK* and *rbcS* genes observed in vegetated hummock soils may be attributed to the limited presence of chemotrophs capable of possessing these genes. For instance, bacterial classes such as *Thermoleophilia* and *Acidimicrobiia* are common chemotrophs in desert soils known to possess *PRK* and *rbcS* genes, and these were not observed in vegetated hummock soils (Fig. 3.5A). Notably, these classes were exclusively observed in open gravel plains and windward slope soils (Fig. 3.5B & C). The *rbcS* and *PRK* genes enable members of *Thermoleophilia* and *Acidimicrobiia* classes to contribute to chemotrophic activity, such as C fixation, in oligotrophic environments (Bay et al., 2021).

In the context of sulfur cycling, two genes involved in the enzymatic reactions associated with sulfite reduction were identified. These genes (*cysI* and *sir*) encode two key proteins: the assimilatory sulfite reductase [NADPH] hemoprotein and sulfite reductase (Askenasy et al., 2015; Naumann et al., 2018). These genes were present in low abundance across all three sampled locations when compared to the genes related to C and nitrogen metabolism (Fig.

5). The results are consistent with previous studies that found the abundance of genes implicated in sulfur metabolism lower in desert soils than in mesic systems due to moisture constraints (Fierer et al., 2012). Members of phylum Chloroflexota belonging to the class *Chloroflexia* showed the highest frequency of *sir* genes (Fig. 3.4). In contrast, *cysI* gene was exclusively associated with members of the Pseudomonadota phylum, specifically within the *Alphaproteobacteria* classes, suggesting a central role of this group in sulfur assimilation and cycling. Sulfur has significant implications for biogeochemical processes at the ecosystem level (Wasmund et al., 2017), as the sulfur cycle is tightly interconnected with other essential element cycles, such as C and nitrogen cycles (Wasmund et al., 2017).

### 3.6.4 MAG coverage in and outside the vegetated hummock soils

The relative abundance analysis based on RPKM results indicates that vegetated hummock soils have lower RPKM abundance than in open gravel plains and windward slope soils (Fig. 3.6B). However, vegetated hummock soils are characterised by a more uniform distribution of MAGs, indicating higher microbial evenness of species compared to open gravel plains and windward slope soils. The high evenness of MAGs in the vegetated hummock soils and the functional diversity of these MAGs suggest a higher metabolic complexity within the hummock system.

The Rhodobacteraceae family consists of members that have been reported to exhibit diverse symbiotic interactions with plants that are beneficial for the growth and survival of both the bacteria and the associated plants (Simon et al., 2017). The *Rhodobacteraceae* sp. MAG in this study's dataset possessed genes for nitrite reduction (*nirK* or *nirS*), nitric oxide reduction (*norBC*) and the cbb3 type cytochrome *c* oxidases. The presence of these genes indicates the potential for utilizing nitrite in nitrogen metabolism (*nirK* or *nirS*) and the ability to detoxify nitric oxide (*norBC*), thus impacting nutrient cycling and adaptation to environmental stress (Trutschel et al., 2022). Although *Rhodobacteraceae* is primarily known for its aquatic bacterial members, its broad ecological range suggests its adaptability to various environments, including hot deserts (Simon et al., 2017; Ma et al., 2022). *Pseudorhizobium* is a genus from *Rhizobiaceae* usually living in the soils associated with plant roots (Shrivastava et al., 2021), suggesting their potential role in plant-microbe interactions, thus potentially contributing to plant growth and nutrient acquisition (Lassalle et al., 2021). The prevalence of

the species *Ralstonia insidiosa* (24 %) in open gravel plains soils (Fig. 3.6A) suggests an inherent adaptability and versatility of this bacteria to thrive under harsh environmental conditions. Previous studies proposed that *Ralstonia insidiosa* species possess enzymes associated with trehalose synthesis, contributing to their tolerance of heat, oxidative stress, and osmotic stresses (Streeter & Gomez, 2006; Iturriaga et al., 2009; Macintyre et al., 2020). This adaptation mechanism promotes resilience to water stress, offering a plausible explanation for the observed overrepresentation of *Ralstonia insidiosa* MAG in open gravel plains soils in comparison to vegetated hummock and windward slope soils. A MAG from the *Mycobacteriales* order predominated in windward slope soils, which is supported by the observed prevalence of members of this order in water-stressed and low nutrients and organic matter (Bei et al., 2023), which suggests their ability to contribute to the resilience and functioning of ecosystems under extreme environmental conditions.

#### 3.7.4.1 MAG functional annotation in and outside the vegetated hummock soils

The reconstructed MAGs were analysed with the aim of gaining a deeper understanding of the potential role of taxa in vegetated hummocks and unvegetated open soils on the Skeleton Coast. This analysis showed that the recovered MAGs contained complete pathways related to carbohydrate metabolism, C degradation, methane metabolism, motility, and C, N, and S cycling (Fig. 3.7). Nitrogen and sulfur metabolism play a crucial role in chemolithotrophic by acting as electron donors and mostly prevailing in harsh conditions (Osburn et al., 2014). The Beta-N-acetylhexosaminidase pathway was complete across all nine MAGs (Fig. 3.7). This pathway has been shown to be universally distributed among most types of living organisms, including prokaryotic and eukaryotic (Slámová et al., 2010). Beta-N-acetylhexosaminidases have been intensively studied due to their important physiological role in cell wall recycling and participation in chitin degradation; thus, its presence indicates the potential for microbial communities to degrade complex organic compounds, particularly chitin in these soils (Slámová et al., 2010).

The presence of the Entner-Doudoroff pathway in eight of the MAGs (except in the *Nitriliruptoraceae* MAG) (Fig. 3.7) indicates its crucial role in nutrient cycling and organic matter turnover in soil ecosystems (Chen et al., 2016). Different reasons may be contributing

to the presence of the Entner-Doudoroff pathway in these MAGs. First, it has been hypothesised that the Entner-Doudoroff pathway involves lower protein cost and more favourable thermodynamic characteristics under extreme conditions (Flamholz et al., 2013; Klingner et al., 2015), which may be beneficial for soil microbes in an environment with limited resources such as the hyper-arid Skeleton Coast National Park. Secondly, the Entner-Doudoroff pathway has been reported to produce large quantities of NADPH, thus offering protection against oxidative stress (Klingner et al., 2015; Dijkstra et al., 2022). This may especially be beneficial to microbial communities in hyper-arid soils where there is high UV radiation exposure (Lester et al., 2007; Stomeo et al., 2013; Alsharif et al., 2020).

One of the ecologically important pathways detected was the nitrite oxidation pathway. This pathway was complete in the *Pseudomonas luteola* MAG recovered from vegetated hummock soils. *Pseudomonas luteola* has been identified as a significant contributor to nitrite oxidation in soils (Trivedi et al., 2019; Fudjoe et al., 2021). This species plays an important role by converting ammonia into forms that are readily available for plant uptake, therefore facilitating nutrient cycling in ecosystems (Amoo & Babalola, 2017). Thus, the recovery of *Pseudomonas luteola* MAG from the vegetated hummock soils suggests its active participation in nutrient cycling processes within the vegetated hummock system, ultimately supporting plant growth and overall ecosystem functioning in hot desert soils.

Methanogenesis via the use of trimethylamine was exclusively complete in *Pseudorhizobium* and *Rhodobacteraceae* MAGs, which were observed in high abundance in the vegetated hummock soils relative to the unvegetated open gravel plains and windward slope soils (Fig. 3.6A). This may be attributed to the presence of organic matter, particularly plant debris, which provides the necessary substrate for methanogenic microorganisms to thrive (Conrad, 2020). Additionally, methanogenesis has been reported to be significant for C cycling, acting as the final stage in the degradation of organic material (Maisie, 2019; Conrad, 2020; Amin et al., 2021), which is an important process in the degradation of organic matter, potentially explaining the occurrence of this pathway within the vegetated hummock soils. The presence of methanogenesis may, therefore, have implications for both the local and global C cycle over long timescales for both the degradation and recycling of organic matter (Maisie, 2019).

This highlights the role of methanogenesis pathways in completing the C cycle with the vegetated hummock system.

#### 3.7.4.2 MAGs enriched in the vegetated hummock soils

##### *a) Nitriliruptoracea*

The *Nitriliruptoraceae* MAG was recovered from all sampling locations but found to be in higher relative abundance in vegetated hummock soils as compared to the unvegetated open gravel plains and windward slope soils, suggesting an important interaction between plants and this MAG. This higher representation of *Nitriliruptoraceae* MAG in vegetated hummocks soils was also reflected in the amplicon sequencing and the metagenomic annotations (data not presented), where *Nitriliruptoraceae* was observed in higher abundance in vegetated hummocks soils than unvegetated open gravel plains and windward slope soils. The *Nitriliruptoraceae* family has been reported from hyper-arid ecosystems with alkaline saline soils (Sorokin et al., 2009; Neilson et al., 2012), including the Namib Desert (Walt et al., 2016), suggesting a unique adaptation of this family to arid ecosystems. Interestingly, Walt et al. (2016) found *Nitriliruptoraceae* members exclusively within the soils of dune and gravel plain fairy circles in the Namib Desert, which are circular patches of soil completely devoid of vegetation within a grass matrix. This contradicts my findings, where *the Nitriliruptoraceae* MAG was detected in vegetated hummock soils at a higher RPKM value relative to the unvegetated open gravel plains and windward slope soils. The association of *Nitriliruptoraceae* with the vegetated hummock soils in the hummock hyper-arid system suggests a broader habitat range for this family beyond open patches soils previously reported.

In this study, the *Nitriliruptoraceae* MAG contained marker genes involved in sulfide detoxification such as *psr*/*psh (*polysulfide/thiosulfate reductase), *sqr* (sulfide: quinone oxidoreductase) and ubiquinol-cytochrome c reductase iron-sulfur (Vavourakis et al., 2018; Jayasinghe et al., 2022). This suggests the crucial contribution of this group to sulfur assimilation and cycling in the vegetated hummock soils. The *Nitriliruptoraceae* family include a novel bacterial strain *Nitriliruptor alkaliphilus*, a notable obligate alkaliphilic and aerobic

heterotroph that specialises in nitrile degradation (Sorokin et al., 2009). Although the *Nitriliruptoraceae* MAG does not appear to contain the nitrile hydratase enzyme necessary for this process, collectively, these results support the potential for either aerobic or anaerobic oxidation of diverse reduced sulfur species in the vegetated hummock soils. This may support plant growth and overall ecosystem processes as sulfur cycling plays a crucial role in nutrient availability (Narayan et al., 2023), and its diverse oxidation contributes to sulfate production, a form of sulfur that plants can uptake for essential processes (Zhao & Kok, 2008; Chaudhary et al., 2023).

***b) Rhodobacteraceae***

A MAG belonging to the *Rhodobacteraceae* family was enriched in vegetated hummock soils (Fig. 3.6A). Beta-N-acetylhexosaminidases enzyme was one of the complete enzymes observed in this MAG. This pathway plays a crucial role in the survival and adaptation of *Rhodobacteraceae* in desert soils by enabling members of this family to degrade complex organic molecules into simpler forms, thus facilitating nutrient acquisition in nutrient-poor desert soils (Pham et al., 2017; Simon et al., 2017). The presence of more organic matter content in the vegetated hummock soils relative to the unvegetated (both open gravel plains and windward slope) soils could explain the enrichment of the *Rhodobacteraceae* family in this sampling location.

**c) *Paenibacillus***

A MAG from the genus *Paenibacillus* was also enriched in vegetated hummock soils (Fig. 3.6A). The enzyme Alpha-amylase was observed in the *Paenibacillus* MAG, which is commonly associated with starch degradation, thus allowing microorganisms to utilise starch as a C source in these arid soils (Hu et al., 2023). Glutathione reductase, an enzyme involved in the alpha-amylase pathway, was also detected in *Paenibacillus,* highlighting the central importance of NADPH as a driver in supplying the necessary reducing power in starch degradation enzymatic reactions (Klingner et al., 2015). This may suggest that life within the vegetated hummock system at least partially benefits from the enhanced NADPH supplied via the alpha-amylase pathway.

Another pathway that was present in the *Paenibacillus* MAG is the dissimilatory sulfate pathway, which plays a crucial role in sulfur cycling (Jørgensen et al., 2019; Shanquan et al., 2023) (Fig. 7). This pathway enables members of *Paenibacillus* to generate energy through the reduction of sulfate, contributing to their metabolic activities and survival in poor nutrient-hyper-arid environments (Kumar et al., 2018). Additionally, the dissimilatory sulfate pathway promotes the role of *Paenibacillus* members in metabolising sulfate, therefore contributing to soil health by participating in sulfur cycling, which is essential for nutrient availability and ecosystem functioning (Zhang et al., 2023). Moreover, this genus has some species capable of fixing atmospheric nitrogen, phosphorus solubilisation, and phytohormone production (Weselowski et al., 2016). In turn, plants secrete organic compounds into the soil, such as root exudates, which serve as a nutrient source for *Paenibacillus* species (Grady et al., 2016). This mutualist relationship could explain the enrichment of *Paenibacillus* genus in the vegetated hummock soils.

Moreover, *Paenibacillus* have been identified as plant growth-promoting rhizobacteria (Grady et al., 2016; Liu et al., 2020), as they can, directly and indirectly, improve plant growth and performance under stress via promoting nitrogen fixation, increasing nutrient uptake, improving soil properties, inhibiting plant pathogens and enhancing plant tolerance to drought (Marasco et al., 2012; Astorga-Eló et al., 2021). For example, an early study found that *Paenibacillus polymyxa* increased drought tolerance in *Arabidopsis thaliana* by regulating the expression of gene *ERD15* involved in the drought-stress response (Timmusk & Wagner, 1999). The ability of *Paenibacillus* members to promote plant growth under harsh conditions, modulate stress-related gene expression, and increase drought tolerance is particularly beneficial to plants in hyper-arid environments where there is water limitation, high levels of solar radiation and temperature fluctuations, along with soil salinity and nutrient deficiency (Alsharif et al., 2020). Therefore, the enrichment of the *Paenibacillus* genus in vegetated hummock soils suggests a beneficial microbial community associated with vegetation roots, which can promote plant growth and facilitate nutrient uptake, stress tolerance, and overall ecosystem resilience in this oligotrophic environment.

## 3.7. CONCLUSION

The objectives of this part of the research project were to investigate 1) the functional potential of soil microbial communities in three sampling locations (vegetated hummock, windward slope, and gravel plains) in the Skeleton Coast National Park, Namib Desert and 2) the metabolic strategies underlying the ability of soil microbes to thrive and perform ecosystem functions and services in hyper-arid ecosystems. The analysis of shotgun metagenome sequence data has provided an overview of the functional and metabolic potential of microbial communities in this system. The results showed more enrichment of microbial functions in vegetation hummock soils relative to the bare (unvegetated: windward slope and gravel plain) ground. The observed functions included those related to C fixation, C degradation, ammonium oxidation, sulfur assimilation, etc. This confirms the initial hypothesis that vegetated hummock soils would have more enrichment of microbial functions due to plant-microbe interactions, suggesting that vegetation influences the microbial functional potential.

Moreover, the findings reveal diverse taxa that have developed unique metabolic strategies to tolerate and thrive in extreme conditions in hyper-arid environments. For instance, some observed *Pseudomonadota* members employ anoxygenic photosynthesis, a vital strategy in water-limited environments like the Skeleton Coast region. Additionally, some phyla, such as WPS-2, have members that utilise atmospheric H2 oxidation to fix CO2, thereby meeting their energy needs and promoting the carbon cycle. Specific groups, like *Rhodobacteraceae*, possess genes for nitric oxide detoxification (norBC), which is crucial for adapting to environmental stress in harsh environmental conditions of the hyper-arid Skeleton Coast. The higher abundance of genes encoding ammonia oxidisation enzymes in the vegetated hummock soils suggests that ammonium likely serves as a primary energy source, sustaining primary production in the vegetated hummock system and supporting heterotrophic demand.

Most of the taxa recovered from vegetated hummock soils are known to be associated with plants. This suggests that the vegetated hummock may be a selective system, favouring microbial communities beneficial for their growth and survival in this system. Notably, the

presence of plant growth-promoting groups such *as Rhodobacteracea, Paenibacillus* and *Pseudorhizobium* is a clear indication of active plant-microbe interactions within the vegetated hummock system. In turn, desert vegetation provides an essential energy source for soil microbial communities through the production of litter and secretion of root exudates, which helps in sustaining mutualist relationships.

Overall, this study provides insights into the functional potential of microbial communities associated with vegetated sand hummocks in this oligotrophic ecosystem. Furthermore, the study highlights the influence of vegetation in shaping the microbial communities and their functional dynamics, which ultimately contribute to the overall ecosystem's functioning. The enrichment of functions assisted with plant microbial functioning in vegetated hummock soils emphasises the significance of understanding the roles of plant-microbe interactions in ecosystem processes and contributes valuable insights to the broader understanding of ecological interactions in hyper-arid environments. Further studies investigating soil microbiomes in this ecosystem are necessary to reveal the soil microbes across vegetated sand hummocks of different plant species that have adapted to this ecosystem, thus gaining a more complete understanding of the relationships among vegetation, soils, and microbial communities in hyper-arid ecosystems. The annotation of MAGs of both vegetated (hummock) and unvegetated (windward slope and gravel plain) soils indicated the microbiome’s diverse functional metabolic potential that balances the community’s geochemical and energy flux.

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## CHAPTER FOUR: INFLUENCE OF VEGETATION PATCHES ON LITTER DECOMPOSITION IN THE SKELETON COAST NATIONAL PARK

*The soil is not a mass of dead debris, merely resulting from the physical and chemical weathering of rocks; it is a more or less homogeneous system which has resulted from the decomposition of plant and animal remains. It is teeming with life.*

*Selman Waksman*

# CHAPTER 4: INFLUENCE OF VEGETATION PATCHES ON LITTER DECOMPOSITION IN THE SKELETON COAST NATIONAL PARK

## 4.1. ABSTRACT

Litter decomposition is an essential component of biogeochemical cycles that strongly controls nutrient availability and primary production. Decomposition is particularly important in dryland ecosystems where plant litter input and nutrient pools are relatively smaller than in mesic systems. Vegetation patches are known to influence litter decomposition, either directly or indirectly, via biological and physical mechanisms; however, how they affect litter decomposition is not well understood, particularly in hyper-arid ecosystems. Using the litterbag technique, this study investigated the influence of vegetation patches on litter decomposition rates in the hyper-arid Skeleton Coast National Park, Namib Desert, by 1) measuring decomposition differences of two contrasting litter types (shrub and grass) under vegetated and unvegetated patches, and 2) establishing how litter structure and chemical composition (N & C/N ratios) influence decomposition rates. The results showed that vegetated patch placements did not impact litter decay. Decomposition was positively correlated with soil infiltration into litterbags, which varied with litter placement and was lowest under vegetated patches. Shrub litter, which had lower N & C content and C/N ratio than grass litter, showed a higher decomposition rate than grass litter. This study provides insights into mechanisms that influence litter decay rates. Given that decomposition in drylands is driven by unique interactions between abiotic and biotic drivers, understanding how different decomposition mechanisms influence litter decay rates will ultimately aid in predicting litter decay rates in hyper-arid ecosystems.

## 4.2. INTRODUCTION

Plant litter decomposition is a key component of global biogeochemical cycles (Aerts, 1997; Almagro et al., 2017; Delgado-Baquerizo et al., 2013). Litter decomposition can influence ecosystem productivity by altering soil organic matter content (Predick et al., 2018). Decomposition is particularly important in dryland ecosystems where plant litter input and nutrient pools are relatively smaller than in mesic systems (Moorhead & Reynolds, 1991). However, our ability to accurately model decomposition dynamics in drylands lags behind that of mesic systems, suggesting a need to better account for unique interactions between abiotic drivers and biotic decomposition processes that may be important in these systems (Austin, 2011; King et al., 2012; Throop & Belnap, 2019).

Decomposition is driven by abiotic factors such as climate (that is, temperature and moisture) and biotic factors (Austin & Vitousek, 2000). For instance, in mesic systems, climate metrics such as actual evapotranspiration (AET) (Aerts, 1997) and litter chemistry (Hobbie, 1992) strongly control litter decomposition. In a review of litter decomposition data from the temperate regions (including both cool and warm temperate sites), the Mediterranean sites region, and the humid lowland tropics (excluding montane rain forests), Aerts (1997) found mean AET to have exerted a stronger influence on litter decomposition at a global scale than litter chemistry.

In drylands, decomposition is driven by unique mechanisms such as photodegradation, thermal degradation and soil-litter mixing (Austin & Vivanco, 2006; Brandt et al., 2007; Logan et al., 2022; Hewins et al., 2013; McBride et al., 2023). Additionally, resource availability can vary significantly across the landscape due to the discontinuous distribution of vegetation cover in drylands (Kéfi et al., 2007; Gonzalez-Polo & Austin, 2009). Vegetation patches in dryland systems reduce solar radiation and modify the microclimate around plants (Mack & D'Antonio, 2003), thus creating localised hotspots of moisture and nutrients. These hotspots often enhance the growth of specialised microbial communities that can influence decomposition rates by driving nutrient cycling (Jacobson et al., 2015). The interactions of these unique mechanisms could potentially explain, at least in part, why traditional models

typically underpredict decomposition rates in drylands and why dryland litter decomposition may fundamentally differ from those of mesic systems (Throop & Archer, 2009; Austin, 2011).

### 4.2.1. Photodegradation

Photodegradation has been suggested to be one of the important mechanisms of litter decay in dryland ecosystems (Austin & Vivanco, 2006; Almagro et al., 2016; Logan et al., 2022). Due to limited cloud cover and minimal shading from sparsely distributed vegetation, solar radiation exposure is high throughout the year (King et al., 2012). Exposure to UV radiation is much greater in unvegetated patches than in vegetated patches (Austin, 2011), where the vegetation canopy and accumulated litter and other organic matter beneath it protect against sunlight (King et al., 2012). Manipulative experiments have been conducted to quantify the role of photodegradation in dryland decomposition. For instance, in a semi-arid Mediterranean perennial grassland, Almagro et al. (2016) found that mass loss of S*tipa tenacissima* and *Retama sphaerocarpa litter* were higher in litterbags exposed to UV radiation compared to bags where most UV was blocked (10% of UV was blocked out). Similarly, in the hyper-arid Namib Desert, Logan et al. (2022) found greater rates of *Stipagrostis sabulicola* litter cuticle damage under near-ambient than reduced UV radiation or visible light.

### 4.2.2. Thermal degradation

Temperatures over 30°C have been reported to increase litter decay in drylands through a process known as thermal degradation (Lee et al., 2012; Almagro et al., 2016; Fishburn, 2019). For instance, in a semi-arid Mediterranean litterbags experiment, Fishburn (2019) assessed the impact of warming on the decay rate of mixed plant material and found that thermal degradation contributed ca. 56% to litter decomposition. Similarly, Gliksman et al. (2017) assessed the decay rate of grass species and found that thermal degradation contributed 12% to litter decomposition in a semi-arid Mediterranean system. Lee et al. (2012) suggest that the thermal degradation of plant litter happens through the physical and chemical breakdown of chemical bonds in the litter, and thermal degradation could also speed up photodegradation. Thus, thermal degradation is important in drylands, particularly in hyper-arid systems where it is hot and yet dry, leading to limited microbially mediated decomposition potential; plant litter can degrade via a thermal degradation mechanism.

### 4.2.3. Soil-litter mixing

While the climate and sparse vegetation patches of drylands create conditions of high solar radiation exposure near ground level, these environments also favour considerable surface soil movement via wind and water transport (Breshears et al., 2003; Okin et al., 2009; Throop & Belnap, 2019). Soil movement can partially cover and eventually bury plant litter on the soil surface, creating what is called “soil-litter mixing” (Throop & Archer, 2007). Soil-litter mixing can be more pronounced in unvegetated patches than in vegetated patches, particularly in desert ecosystems where the vegetation cover provides protection from wind and water (Hewins et al., 2013; Throop & Archer, 2007). For instance, in the northern Chihuahuan Desert, Smith & Throop (2018) found that litter decomposition was faster in unvegetated than vegetated microsites, where it was exposed to greater soil-litter mixing, ultimately increasing the rate of decomposition. Soil-litter mixing enhances litter decomposition via several mechanisms, such as acting as a vector for microbial colonisation of litter surfaces (McBride et al., 2023). Additionally, soil-litter mixing has been suggested to enhance litter decomposition by causing physical abrasion and thus increasing the litter surface area available for microbial colonisation (Throop & Archer, 2007; Throop & Archer, 2009) or buffering litter from extreme heat and aridity, thereby reducing photodegradation and temporarily extending the window of possible microbial activity (Lee et al. 2014; Joly et al., 2017). Microclimate buffering is particularly important in dryland decomposition processes due to the infrequency of suitable conditions for microbial activity and the pulsed nature of precipitation events (Throop & Archer, 2009).

### 4.2.4. Vegetation patches in dryland systems

Drylands experience sporadic precipitation events and are characterised by discontinuous distribution of vegetation cover, subsequently leading to patches of vegetation across the landscape (Kéfi et al., 2007; Gonzalez-Polo & Austin, 2009). Vegetation patches modulate abiotic controls and biotically driven processes such as litter decomposition directly and indirectly via biological and physical mechanisms (Ward et al., 2015; Liu et al., 2021). Vegetation patches in drylands increase the importance of wind and water as horizontal transport vectors of litter and surface soil due to large gaps that occur between plants in these

systems (Throop & Belnap, 2019), therefore suggesting a fundamental role for the spatial heterogeneity of vegetation patches in affecting ecosystem processes in drylands (Austin, 2011).

Vegetation exerts strong control over local decomposition through direct effects associated with changes in litter quality or quantity (Hobbie, 2000), while indirect effects are associated with differences in vegetation structure, such as canopy influences on microclimate (Mack & D'Antonio, 2003). Plant litter quality is often considered to be the key factor controlling the efficiency of the decomposition cycle and nutrient release at local scales (Hobbie 1992; Wickings et al., 2012; Gao et al., 2016; Zhao et al., 2022). Generally, the litter decomposition rate is positively correlated with the initial litter nitrogen (N) content. In contrast, it negatively correlates with C/N and lignin/N ratios of initial litter over various ecosystems (Taylor et al., 1989; Gao et al., 2016). Additionally, the physical structure of litter, such as size, thickness, and texture, can impact its ability to retain moisture and affect temperature. Different types of litter, such as grass, shrub, or tree litter, have varying chemical compositions (C & N, C/N ratio, and lignin/N ratio), which may influence the decomposition rate.

Vegetation canopy exerts indirect effects on litter decomposition by providing unique microenvironments for decomposing organisms (Jones et al., 1997; Hector et al., 2000), which may increase microbial pools and cycling of nutrients under vegetation patches. For instance, Sherman et al. (2019) reported high soil organic matter content in the vicinity of vegetation in the Namib Desert compared to unvegetated patches. High microbial pools were also concentrated in vegetated areas relative to unvegetated patches in the hyper-arid Namib Desert (Chapter 2; Figure 2.9). As a result of high microbial pools and the interplay of various factors, such as thermal degradation, photodegradation, and moisture dynamics under vegetation patches, litter decomposition rates may be enhanced under vegetation patches relative to unvegetated patches (Bachar et al., 2010). Moreover, vegetation patches in dryland systems form part of litter retention elements where the transported litter accumulates and can affect litter decay rates (Throop & Belnap, 2019). Litter accumulation around vegetation patches can enhance the capture of additional litter, leading to the aggregation of soil particles with partially decayed litter (Throop & Belnap, 2019),

subsequently creating stable litter crusts. This enhances moisture and reduces surface temperature, which, in turn, may affect litter decomposition (Jia et al., 2018).

Although the indirect effects of vegetation patches on decomposition have been identified in arid and semi-arid systems (Throop & Archer, 2007; Hewins et al., 2013; Molaeinasab et al., 2021) hyper-arid systems have received relatively less attention. Most existing research on litter decomposition in hyper-arid systems emphasizes the more widely recognised direct effects of moisture, temperature, and litter quality and quantity on decomposition. Thus, it is important to determine the influence of vegetation patches on litter decomposition, especially given the fact that these systems are characterized by spatial heterogeneity of vegetation, which has the potential to exert strong controls on litter decomposition and subsequently contribute to the spatial patterns of soil nutrients in the ecosystem and influence global C cycling. Understanding their influence on litter decomposition rates in hyper-arid systems is critical in developing models incorporating mechanisms that control litter decomposition in hyper-arid systems. Incorporating vegetation patches into existing litter decay models is important in improving our understanding of biogeochemical dynamics in drylands now and under future climate change scenarios.

### 4.3. Study Overview

Here, the litterbag technique was used to determine litter decomposition rates under vegetated hummock and unvegetated patches in the hyper-arid area of the Skeleton Coast National Park, Namib Desert. Two questions were addressed to determine litter decomposition rates: 1) How do vegetation patches influence litter decomposition rates in this hyper-arid system? 2) Does the chemical composition and physical structure of litter influence its decay rate in this hyper-arid system? The hypotheses were: 1) that the vegetated patches would have higher decomposition relative to the unvegetated patches due to the modified microclimate around plants, which also can influence resource availability and microbial community, 2) litter decomposition rate would differ among hummock species, and 3) litter with a low C/N ratio would decompose faster than litter with a high C/N ratio.

## 4.4. MATERIALS AND METHODS

### 4.4.1. Site description

Refer to Chapter 2 (pages 27-28)

### 4.4.2. Leaf litter collection

Senescent leaves were collected from two contrasting plant species: *Stipagrostis sabulicola* (Poaceae; hereafter "grass") and *Tetraena stapffii* (previously known as *Zygophyllum stapffii),* (Zygophyllaceae; hereafter "shrub"). *Stipagrostis sabulicola* was used as grass litter because it is the dominant grass biomass input in the Namib Desert, and it could provide enough grass litter for the experiment. Standing senescent litter material from *S. sabulicola* was collected in the central Namib Desert in January 2019 (Fig. 4.1A). For shrub litter, naturally senesced leaves were gently removed from shrubs in the Skeleton Coast National Park (Northern Namib Desert) in January 2019 (Fig. 4.1B). The leaves were air-dried in paper bags for 60 days before deployment.

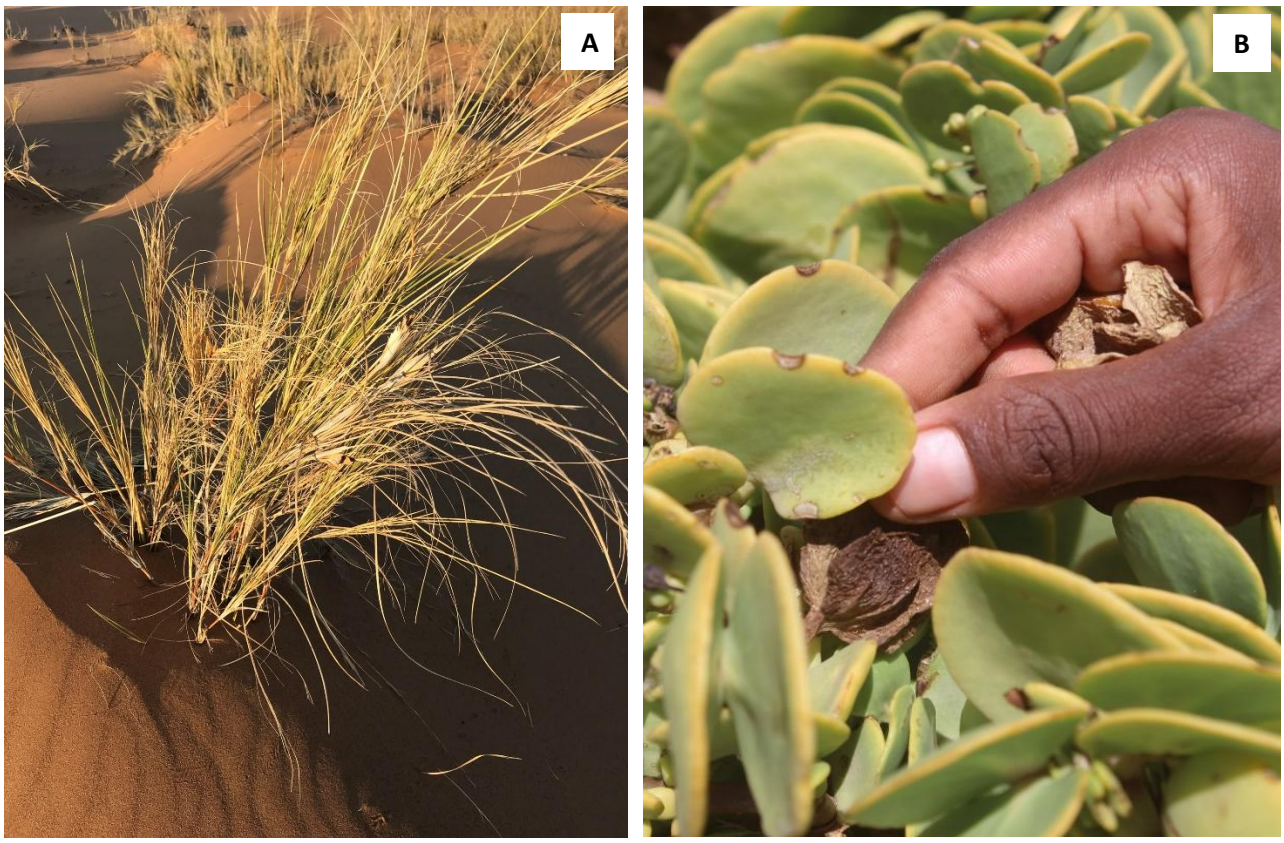


Figure 4. 1: (A) *Stipagrostis sabulicola* and (**B**) *Zygophyllum stapffii***,** where the senescent leaves were collected for the experiment. Photographs: (**A**) H.L. Throop, (**B**) E.N. Nghalipo.

### 4.4.3. Litterbag construction and deployment

Litterbags (10 cm x 15 cm) were constructed using fibreglass screens (1.4 mm mesh size). Approximately 4 g of air-dried leaf litter was put into each litterbag. The litterbags were deployed in mid-March 2019 on the soil surface in two vegetation placements representative of differing levels of radiant energy exposure: unvegetated and vegetated patches (Fig. 4.2). Unvegetated patches were in bare areas that received full sun and were at least 5 m away from the nearest shrubs (Fig. 4.3 A). Vegetated patch locations were in shaded areas beneath one of three possible plant canopy species (*Arthraerua leubnitziae, Ectadium rotundifolium,* and *Salsola nollothensis*; Fig. 4.3 B), all of which form hummocks. Vegetated patches of the three species were divided into distinct strata based on size (width and length), and selected vegetated patches had hummocks >3 m in length and >3 m in width. Patches (both vegetated and unvegetated) were excluded from consideration if they had signs of disturbance (e.g., rodent burrows or digging). There were two separate sampling locations based on species distributions, with *Salsola* closer to the coast and *Arthraerua* and *Ectadium* farther inland. Vegetation patch replicates were at least 200 m apart within the same sampling location and about 4.5 km between the sampling locations. Litterbags were secured to the soil surface with a metal peg. One litterbag of each litter type from each replicate placement × plant canopy species was retrieved at random for collection immediately after deployment. One additional litterbag from each litter type from placement replicate × plant canopy species was collected at 4, 8, and 14 months post-deployment. A total of 480 litterbags were deployed (2 litter types x 3 plant canopy species x 2 patch types x 4 collection times x 10 replicate locations). Of the 480 litterbags, 130 (72 grass and 58 shrub litterbags) were lost, leaving 350 litterbags for analysis.

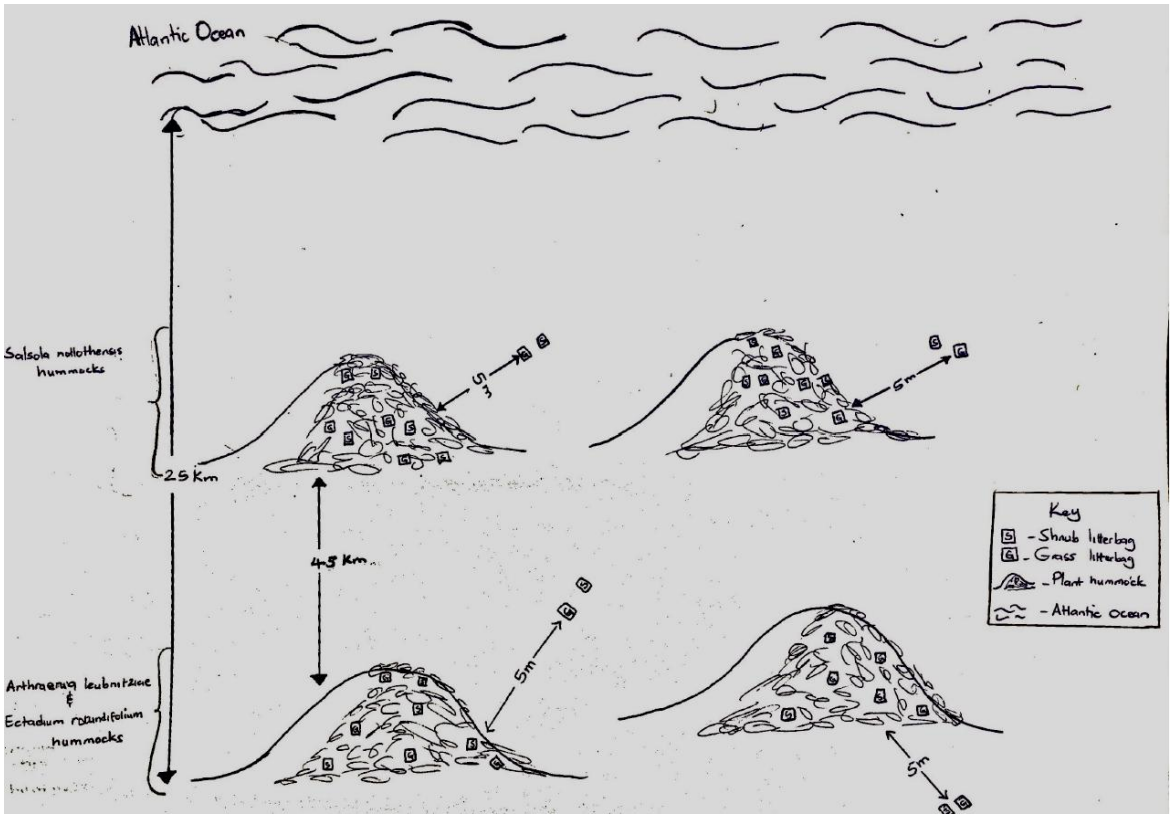


Figure 4. 2: Experimental layout of the litterbag deployment. The diagram illustrates where litterbags were deployed on different hummock species: *Arthraerua leubnitziae*, *Ectadium rotundifolium*, and *Salsola nollothensis*. *Arthraerua leubnitziae* and *Ectadium rotundifolium* were found in the same area, while the population of *Salsola nollothensis* was located approximately 4.5 km away. The separation was due to the absence of *Salsola* species in the area where *Arthraerua leubnitziae* and *Ectadium rotundifolium* were present.

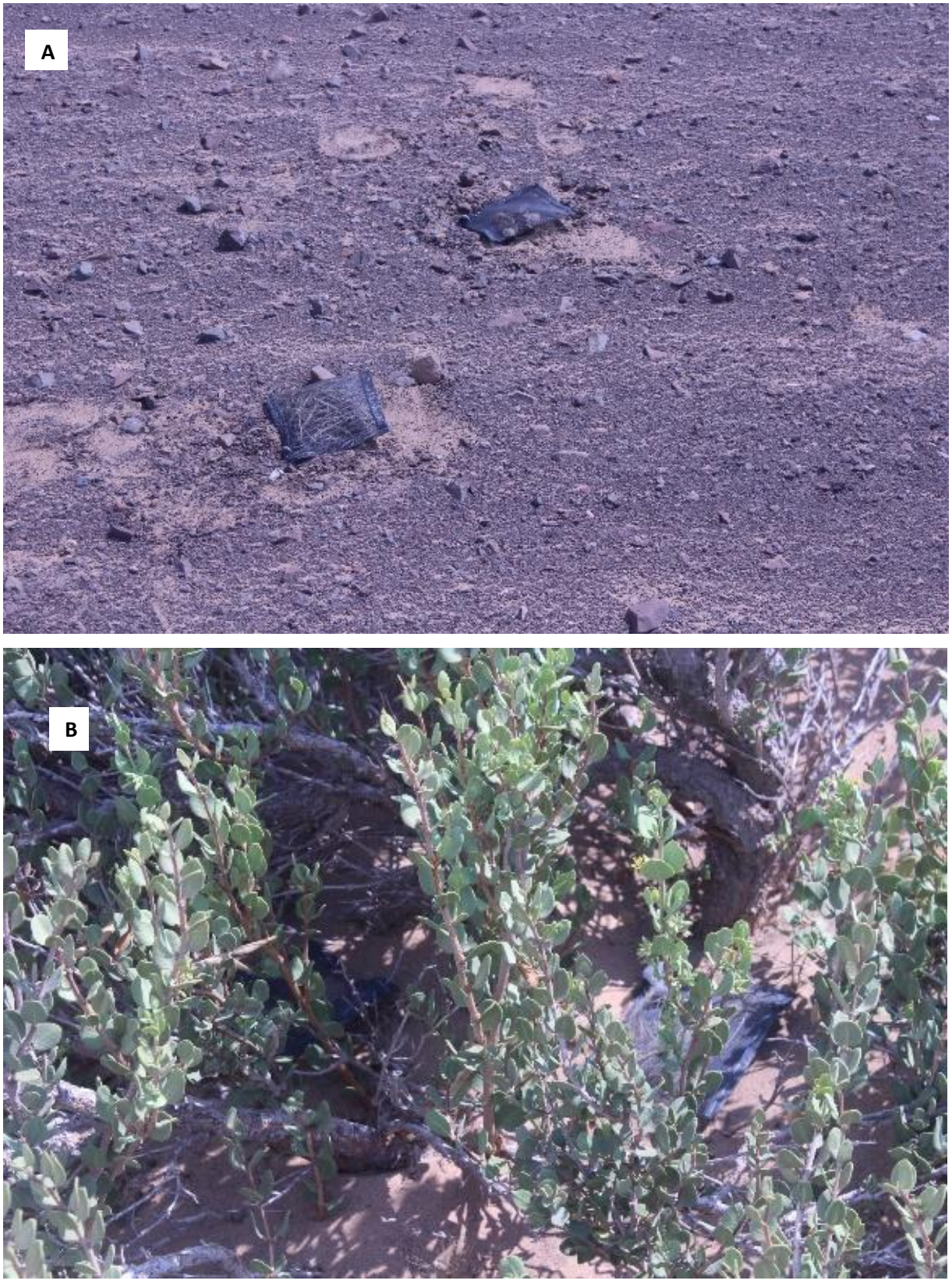


Figure 4. 3: Deployed litterbags in the **A**) unvegetated and **B**) vegetated patch placements of *Ectadium rotundifolium*. Photographs: (**A-B**) E.N. Nghalipo

Figure 4. 4: Vegetated patch placement *of Salsola nollothensis*. Photographs: E.N. Nghalipo

Figure 4. 5: Vegetated patch placements *of Arthraerua leubnitziae.* Photographs: E.N. Nghalipo

### 4.4.4. Sample Analyses

Litterbags were transported to the lab after field collection and litter was dried for 48 h at 60 °C. Following drying, the litter was carefully cleaned with brushes to remove soil particles attached to litter surfaces. Additionally, tweezers were used to remove materials that seeped through the bags that were not part of the initially deployed litter (Fig. 4.4 A & B). After cleaning, the litter samples were weighed and ground with a mortar and pestle (Fig. 4.4 C & D), followed by a ball mill (8000D Mixer/Mill, Spex Certiprep, Metuchen, NJ, USA). A 0.25 g subsample of ground litter from each litterbag was combusted in a muffle furnace at 550 °C for 6 h and 45 minutes to correct for mass gain from mineral soil that entered the bags. Other subsamples from each litterbag were analysed for C and nitrogen (N) content on an elemental analyser (ECS 4010; Costech Analytical Technologies, Valencia, California, USA). The percentage of ash remaining following combustion was used to express the percent litter mass remaining on an ash-free basis. Litter percent ash was also used as an index of soil-litter mixing, in which more soil being adhered to the litter was indicated by a greater percentage of ash (Throop & Archer, 2007).

Litter decomposition was assumed to be the proportional difference in ash-free dry mass between the initial (0 months) and subsequent litterbag collections. The decay constant, *k*, was determined for each litter type by placement using a single exponential decay model ($M_t = M_0 e^{-kt}$), where $M_t$ is the litter mass at time *t,* $M_0$ is the initial litter mass described in Olson (1963).

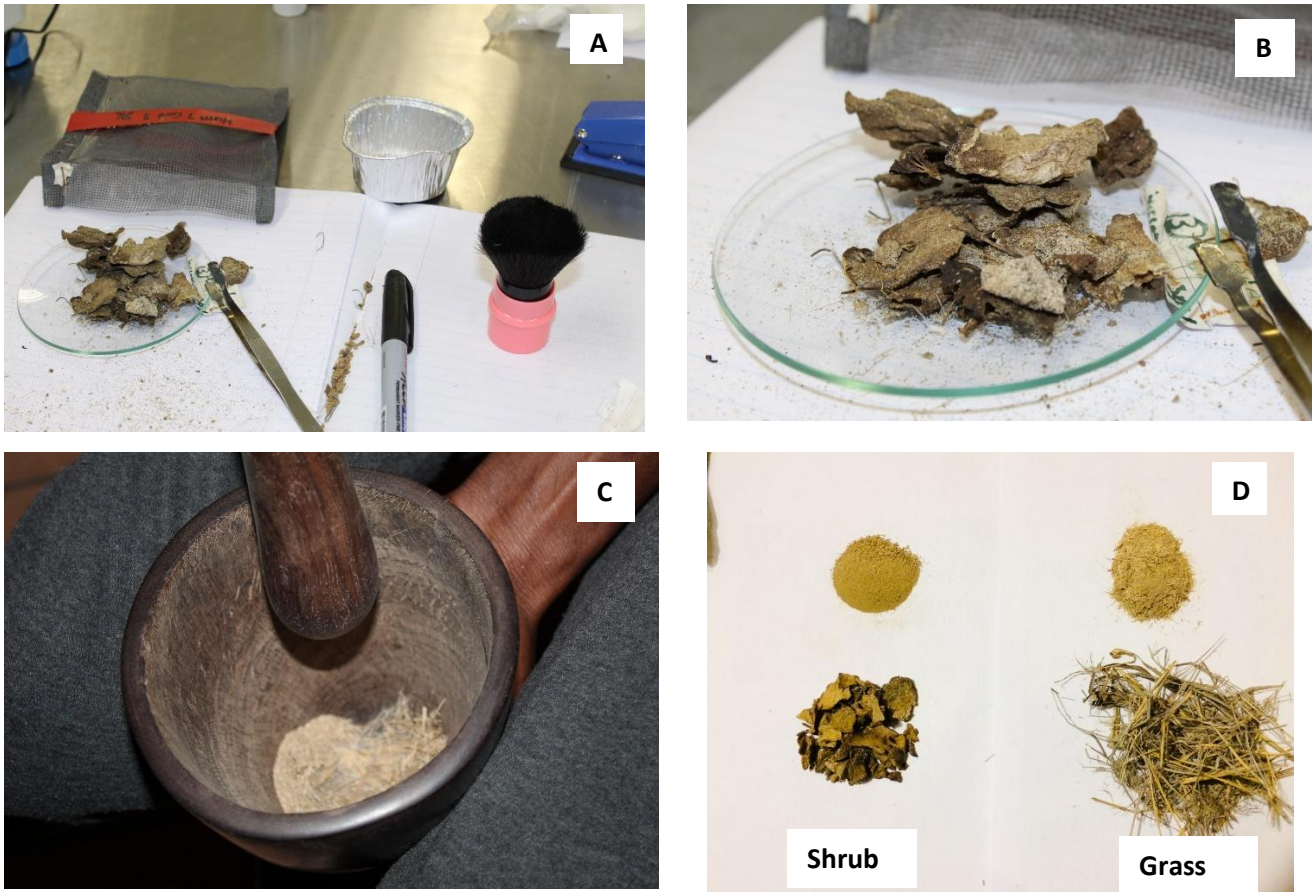


Figure 4. 6: (**A-B**) Litter weighing and cleaning process, (**C**) manual litter grinding, and (**D**) the ground litter types (shrub and grass litter). Photographs: (**A-D**) E.N. Nghalipo

### 4.4.5. Statistical analysis

Changes in litter mass remaining and ash-free percent N, C, and C/N ratio were analysed using a three-way, split-plot model ANOVA. Litter type (LT), hummock species and litter placement (LP) were considered the main effects, in addition to all possible interaction terms. Data were naturally log-transformed prior to analysis to improve normality. Decay constants (*K*) for percent mass remaining were analysed as a two-factorial, completely randomised design. Multiple decay constants (*K*) were obtained for each hummock species by averaging individual *K* values from litter type replicates to calculate a mean *K* value for each litter type and litter placement within the same hummock species. Each litter type replicate had its percent mass remaining over time, allowing for the calculation of separate *K* values. The exponential decay model included the main effects of litter type, hummock species, litter placement, and the interactions among them. The relationship between decay constants (*K*) and the litter type

was assessed with a linear model. Moreover, the relationship between litter percent mass remaining and litter percent ash was assessed with linear regression. All analyses were performed using R (R version 4.2.0) (R Core Team, 2022).

## 4.5. RESULTS

### 4.5.1. Mass Loss

After 14 months of exposure in the hyperarid Namib Desert, mean litter mass declined over time, with greatest mass loss rates occurring in the first four months of the experiment (Fig. 4.7 A&B). Hummock species was the only variable with a significant effect on mass loss at 14 months (Table 4.1), with mass loss differing significantly between *Ectadium rotundifolium* and *Salsola nollothensis* species. Litter decomposed faster under the *Ectadium rotundifolium* than *Salsola nollothensis* species on both vegetated and unvegetated patch placements (Fig. 4.7 A&B). Decomposition of both shrub and grass litter followed similar patterns. Litter mass loss did not consistently fit an exponential decay model well, with $R^2$ values ranging from 0.24 to 0.77 (Table 4.2). Analyses of the decay constants (*K*) for each litter type indicated there were significant effects on the decay rate of both litter types within *Ectadium rotundifolium* ($F_{1, 90}$ = 4.796, $P < 0.05$) and *Salsola nollothensis* species ($F_{1, 90}$ = 10.729, $P < 0.01$) with each hummock species contributing significantly to the variation in decay rates (Table 4.3).

Table 4. 1: Summary of results from ANOVAs of litter ash-free mass remaining in 14-month litterbags (shrub and grass) with main effects of litter type, litter placement, hummock species, and all possible interactions between them. Asterisks indicate statistical significance: *P<0.05, **P<0.01, ***P<0.001.

| Effect | F ratio |
|---|---|
| Litter type | $F_{1, 47}$ = 1.718 |
| Litter placement | $F_{1, 47}$ = 0.514 |
| Hummock species | $F_{3,47}$ = 6.730** |
| Litter type x Litter placement | $F_{1, 47}$ = 1.856 |
| Litter type x Hummock species | $F_{3, 47}$ = 0.068 |
| Litter placement x Hummock species | $F_{3, 47}$ = 0.341 |
| Litter type x Litter placement x Hummock species | $F_{3, 47}$ = 2.335 |

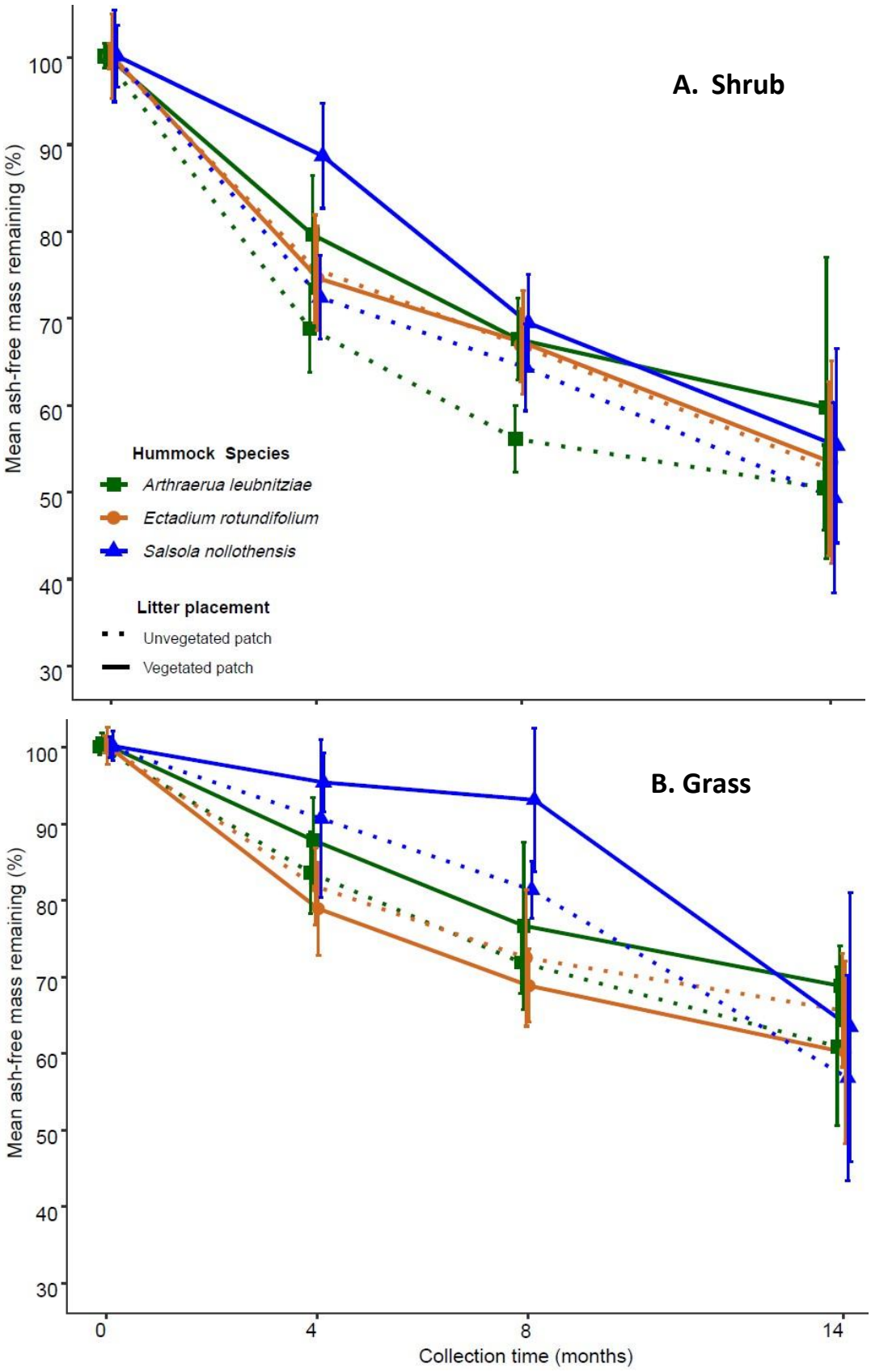


Figure 4. 7: Mean ash-free mass remaining from 0 to 14 months for (A) shrub and (B) grass litter in three hummock species (*Arthraerua leubnitziae, Ectadium rotundifolium* and *Salsola nollothensis*) under vegetated and unvegetated patch placements. Error bars represent standard errors.

Table 4. 2: Exponential decay constant *(K)* means ± SE and $R^2$ values by litter type (shrub and grass) under each placement within different hummock species. The $R^2$ values reflect the goodness of fit for individual regression models and are not averaged across groups.

| | | Shrub | | | Grass | | |
|---|---|---|---|---|---|---|---|
| | | *K* ($y^{-1}$) | | | *K* ($y^{-1}$) | | |
| **Species** | **Litter placement** | **Mean** | **SE** | **$R^2$** | **Mean** | **SE** | **$R^2$** |
| *Arthraerua leubnitziae* | Unvegetated patch | 0.364 | 0.149 | 0.770 | 0.543 | 0.106 | 0.582 |
| | Vegetated patch | 0.835 | 0.196 | 0.418 | 1.160 | 0.348 | 0.450 |
| *Ectadium rotundifolium* | Unvegetated patch | 0.765 | 0.116 | 0.734 | 0.455 | 0.196 | 0.243 |
| | Vegetated patch | 0.783 | 0.188 | 0.231 | 0.427 | 0.127 | 0.24 |
| *Salsola nollothensis* | Unvegetated patch | 0.717 | 0.206 | 0.503 | 0.566 | 0.160 | 0.374 |
| | Vegetated patch | 0.884 | 0.139 | 0.483 | 0.320 | 0.203 | 0.291 |

Table 4. 3: Results of ANOVAs of exponential decay constants *(K)* of litter type x litter placement and their interactions within each hummock species. A separate ANOVA was performed for each hummock species. Asterisks indicate statistical significance: *P<0.05, **P<0.01, ***P<0.001

| **Species** | **Effect** | **F ratio** |
|---|---|---|
| *Arthraerua leubnitziae* | Litter type | $F_{1,90}$ = 2.080 |
| | Litter placement | $F_{1,90}$ = 0.001 |
| | Litter type x Litter placement | $F_{1, 90}$ = 0.229 |
| *Ectadium rotundifolium* | Litter type | $F_{1, 90}$ = 4.796* |
| | Litter placement | $F_{1, 90}$ = 0.407 |
| | Litter type x Litter placement | $F_{1, 90}$ = 1.368 |
| *Salsola nollothensis* | Litter type | $F_{1, 90}$ =10.729** |
| | Litter placement | $F_{1, 90}$ = 2.631 |
| | Litter type x Litter placement | $F_{1, 90}$ = 0.065 |

### 4.5.2. Litter mass remaining vs. litter % ash

Litter ash content, a conservative index of soil accumulation on leaf surfaces and soil litter mixing (SLM) as used in this study, differed significantly with litter type ($F_{1,349}$ = 37.56, P< 0.001) and collection time ($F_{3, 349}$ = 93.04, P < 0.001) (Table 4. 4). Ash content was higher in the shrub

litterbags than in the grass litterbags (Table 4. 5). There was also a significant interaction between litter type and collection time ($F_{3, 349} = 5.38$, $P < 0.01$) (Table 4. 4), with shrub litter accumulating more soil as time progresses as compared to grass litter. Ash content did not differ significantly between litter placements ($F_{1, 349} = 0.094$, $P > 0.05$) (Table 4.4). Across the 14-month experiment, there was a relatively strong negative linear relationship between litter percent mass remaining and percent ash, with percent mass remaining decreasing as percent ash increases, for both shrub ($R^2 = 0.91$) and grass litter ($R^2 = 0.84$) (Fig. 4.6 A&B).

Table 4. 4: Summary of results from ANOVAs of percent ash content with main effects of litter type, collection month (time), litter placement, and all possible interactions between them. Asterisks indicate statistical significance: *P<0.05, **P<0.01, ***P<0.001

| **Effect** | **F ratio** |
|---|---|
| Litter type | $F_{1, 349} = 37.56$ *** |
| Litter placement | $F_{1, 349} = 0.094$ |
| Time | $F_{3,349} = 93.04$** |
| Litter type x Litter placement | $F_{1, 349} = 1.630$ |
| Litter type x Time | $F_{3, 349} = 5.381$** |
| Litter placement x Time | $F_{3, 349} = 0.177$ |
| Litter type x Litter placement x Time | $F_{3, 349} = 0.807$ |

Table 4. 5: Percentage of ash means and ± SE values in 14-month collection time litterbags (shrub and grass) under each placement within different hummock species.

| | | **Ash Content (% by mass)** | | | |
|---|---|---|---|---|---|
| | | Shrub | | Grass | |
| **Species** | **Litter placement** | **Mean** | **SE** | **Mean** | **SE** |
| *Arthraerua leubnitziae* | Unvegetated patch | 23.3 | 3.31 | 20.7 | 4.95 |
| | Vegetated patch | 32.8 | 4.22 | 16.9 | 3.97 |
| *Ectadium rotundifolium* | Unvegetated patch | 31.9 | 4.58 | 18.8 | 3.74 |
| | Vegetated patch | 35.4 | 4.40 | 18.9 | 3.79 |
| *Salsola nollothensis* | Unvegetated patch | 32.5 | 4.22 | 22.3 | 4.43 |
| | Vegetated patch | 29.7 | 4.22 | 17.8 | 4.05 |

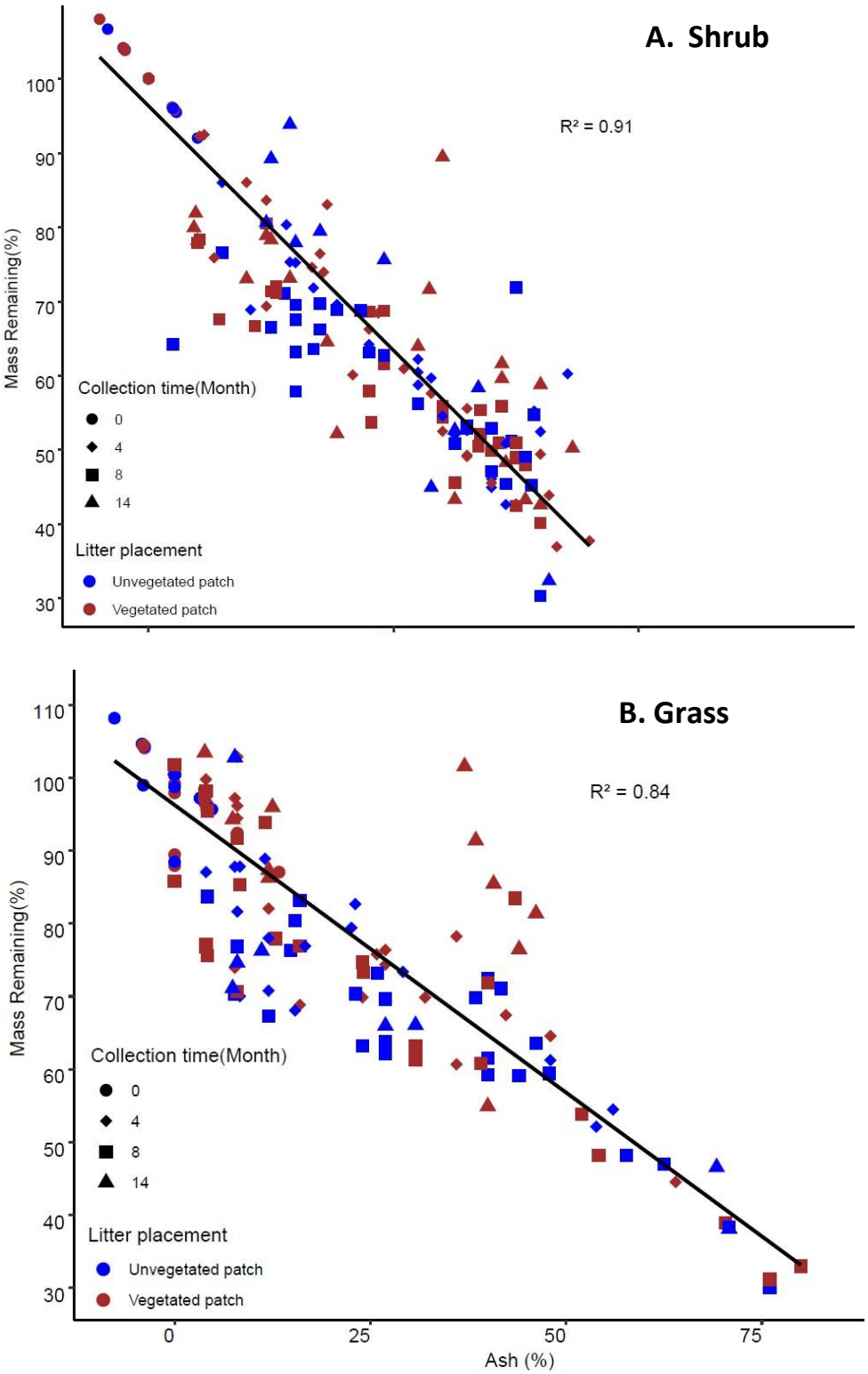


Figure 4. 8: Litter % mass remaining ash-free vs. litter % ash partitioned by collection time(month) for (**A**) shrub and (**B**) grass.

### 4.5.3. Litter N, C and C/N dynamics

To determine the influence of N & C content and C/N ratios on litter decomposition rates in hyper-arid ecosystems, I measured N and C content and C/N ratios of two contrasting litter types (shrub and grass) under vegetated and unvegetated patches. Mean ash-free percent N, ash-free percent C and the C/N remaining for the litter types was compared among hummock species and litter placements (Fig. 4.7, 4.8 & 4.9) for collection times 4, 8 and 14. Ash-free percent N decreased through time in all treatment combinations; however, it was not different between litter placements ($F_{1,199}$ = 0.008; P > 0.05) nor among hummock species ($F_{1,199}$ = 2.683; P > 0.05) (Fig. 4.7A & B). Additionally, ash free percent N was consistent between placements, with no significant interactions between placement and litter type for any hummock species. On the contrary, ash-free percent C remaining differed significantly between litter types, with shrub litter having significantly low ash-free percent C as compared to grass litter ($F_{1,199}$ = 146.752; P < 0.001; Fig. 4.8A & B). Additionally, ash-free percent C/N differed significantly between litter types (F1,199 = 146.752; P < 0.001), with shrub litter having significantly low mean ash-free percent C/N as compared to grass litter (Fig. 4.9A & B).

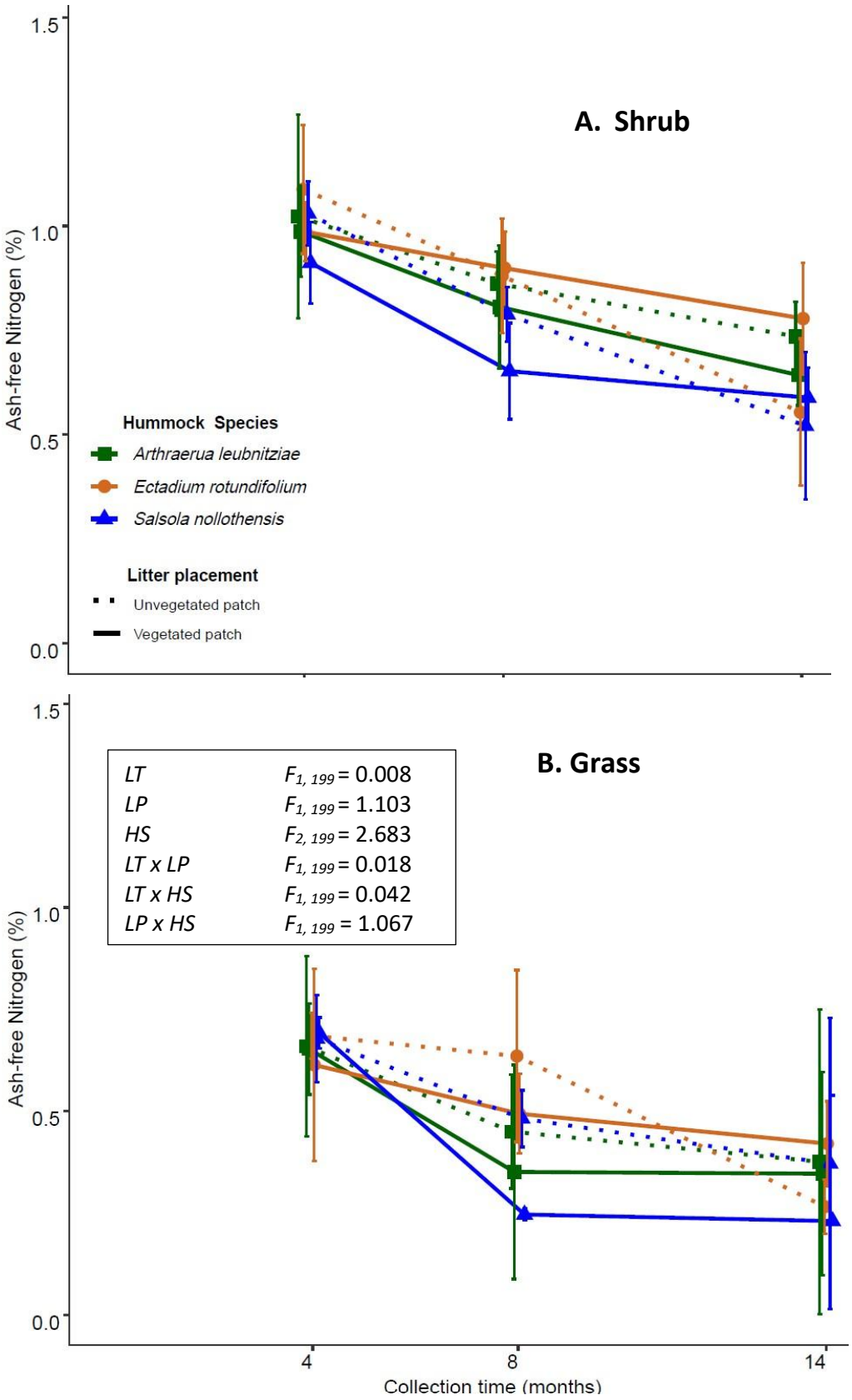


Figure 4. 9: Mean ash free percent N through time for litter type (shrub and grass) in vegetated and unvegetated patch placements within different hummock species. Error bars represent standard errors. The ANOVA table shows F and df values from significant terms in a three-way ANOVA where LT is litter type, LP is placement, and HS is hummock species.

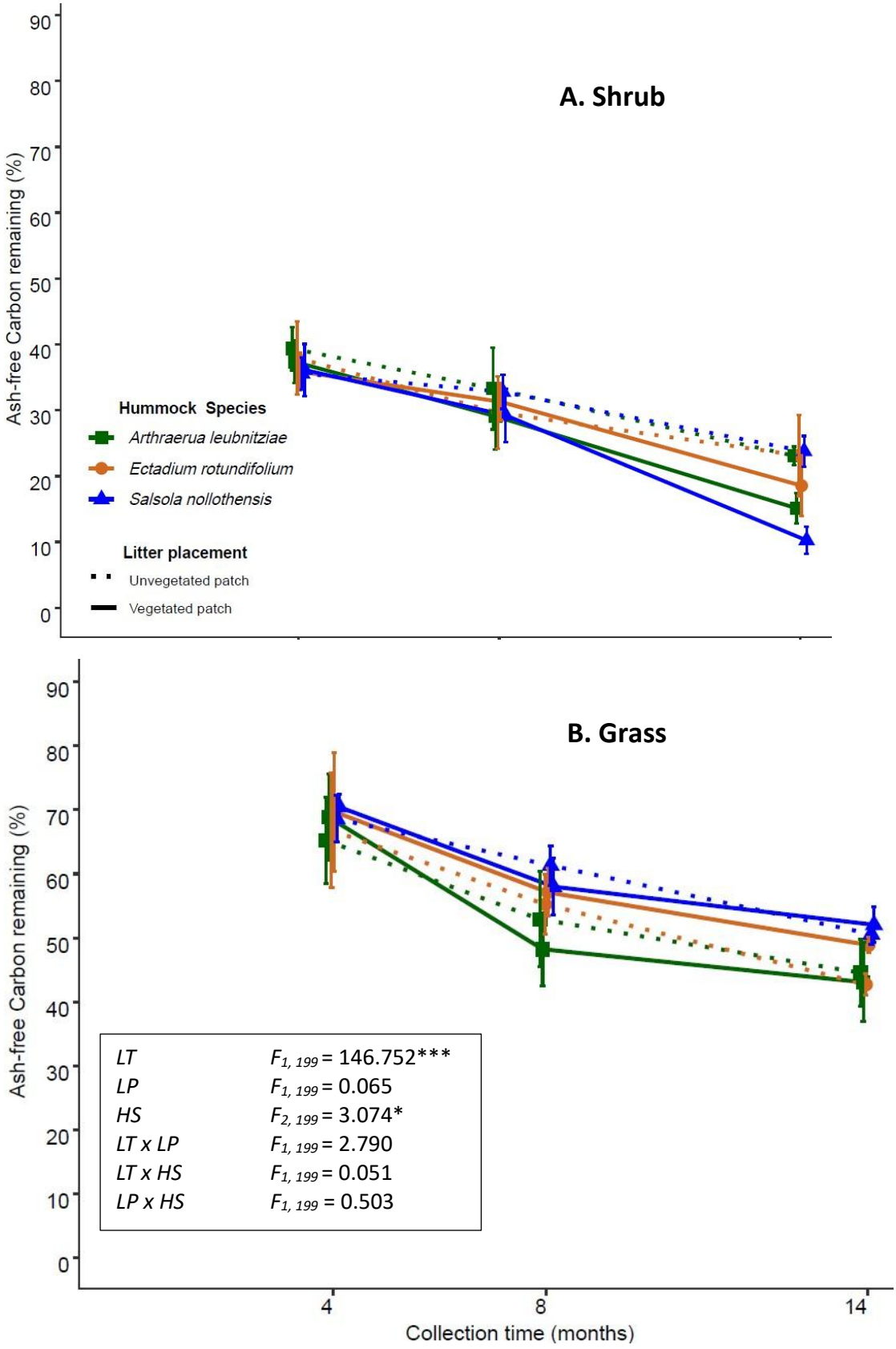


Figure 4. 10: Percent ash-free C remaining through time for litter type (shrub and grass) in vegetated and unvegetated patch placements within different hummock species. Error bars represent standard errors. The ANOVA table shows F and df values from significant terms in a three-way ANOVA where LT is litter type, LP is placement, and HS is hummock species. * P < 0.05; ** P < 0.01; *** P < 0.001.

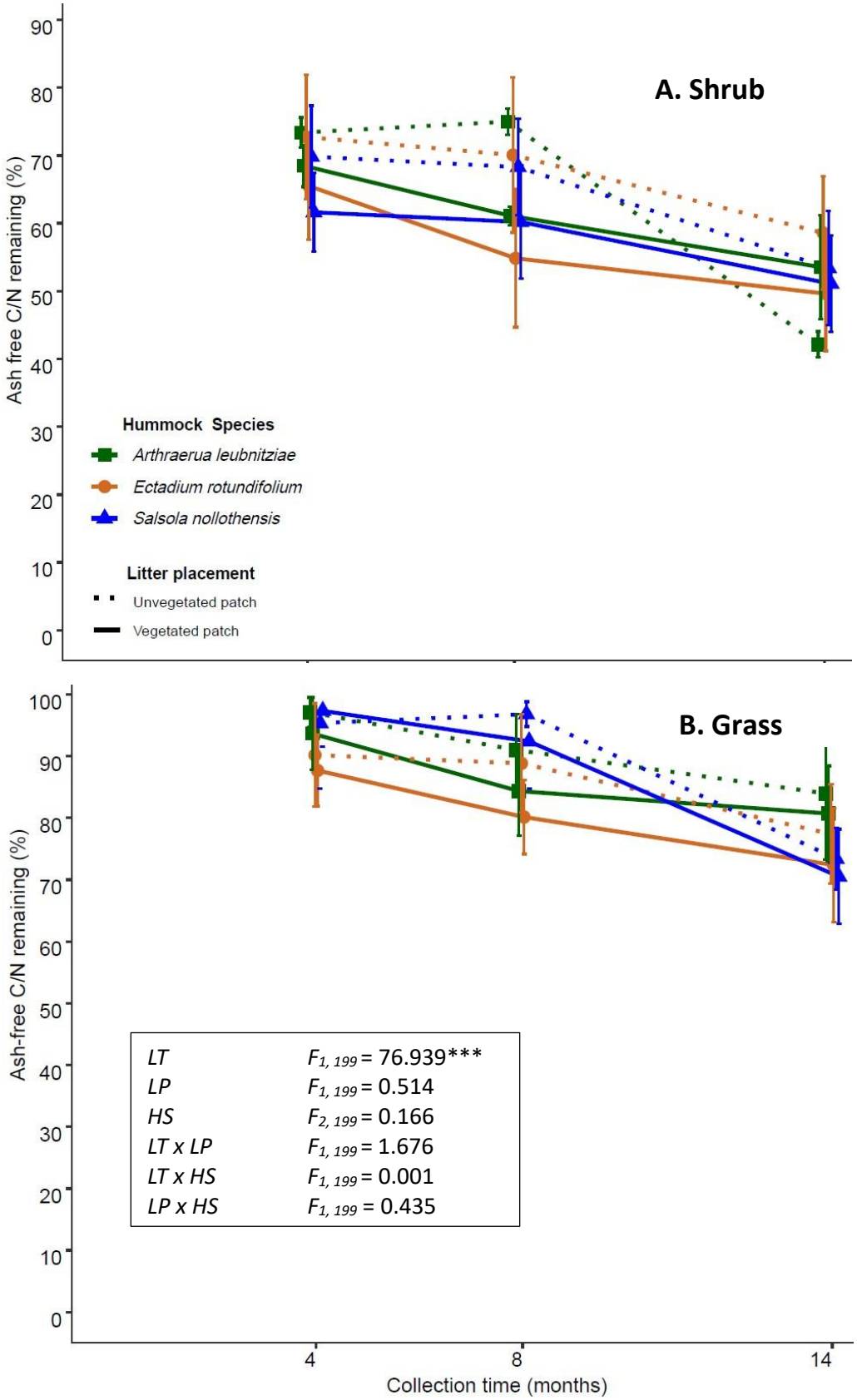


Figure 4. 11: Percent ash-free C/N remaining through time for litter type (shrub and grass) in vegetated and unvegetated patch placements. Error bars represent standard errors. The ANOVA table shows F and df values from significant terms in a three-way ANOVA where LT is litter type, LP is placement, and HS is hummock species. * P < 0.05; ** P < 0.01; *** P < 0.001.

## 4.6. DISCUSSION

The Namib Desert is characterised by spatial heterogeneity of vegetation, which has the potential to exert strong controls on litter decomposition and subsequently contribute to the spatial patterns of soil nutrients in the ecosystem and influence global C cycling. The litter decomposition technique provided insights into the influence of vegetated and unvegetated patches on decomposition rates in the hyper-arid area of the Skeleton Coast National Park, Namib Desert. To determine the influence of vegetation on litter decomposition rates in the hyper-arid ecosystems, the study measured decomposition differences of two contrasting litter types (shrub and grass) under vegetated and unvegetated patches in the Skeleton Coast National Park, Namib Desert. It was hypothesised that decomposition would be more rapid under vegetated patches than unvegetated patches. The results show that litter decay after 14 months did not differ between unvegetated patch placements and vegetated patch placements, which did not support my initial hypothesis. It was expected that the modified microclimate around plants, which influences resource availability and microbial communities, would increase decomposition rates under vegetated patches compared to unvegetated patch placements. However, factors such as non-rainfall-moisture, temperature fluctuations, and soil-litter mixing, which can also occur in unvegetated patches, may have enhanced decomposition similarly across the litter placements patches (Logan et al., 2022). Vegetated patches are known to be hotspots for microbial communities and potentially increase their activities such as decomposition rates (Gonzalez-Polo & Austin, 2009; Lu et al., 2017). This trend was observed in Chapter 2 (Figure 2.7), where high microbial pools were concentrated in vegetated areas relative to unvegetated patches. However, in this study, it appears that the influence of microbial decomposition associated with vegetation may not have been large enough to cause significant differences in the decay rates between the vegetated and unvegetated patch placements (Gliksman et al., 2017). Moreover, about 80% of the litterbags on the unvegetated patches were covered with sand by month 14 of collection (which was not the case for vegetated patch placements) and soil-microbial films were apparent on leaf litter at the month 4 sampling. This led to soil-litter mixing, which may have promoted litter decomposition through biological processes (Levi et al., 2020) and potentially equalizing the decomposition rates of unvegetated patches to vegetated patches.

### 4.6.1. Litter mass remaining vs. litter % ash

The strong relationship between decay rates and the percentage ash of retrieved litter suggests that soil infiltration into litterbags was a major driver of decomposition (Fig. 4.6A & B). A negative linear relationship between litter mass remaining and percent ash (a measure of inorganic material, i.e., soil) of shrub and grass litter suggests that the amount of soil adhered to the litter surface, or litter soil film development, increased in conjunction with decomposition-induced mass loss, supporting the importance of soil-litter mixing as a decomposition driver in this ecosystem (Throop & Archer, 2007; Barnes et al., 2012; Hewins et al., 2013; Liu et al., 2018; Levi et al., 2020). Soil particles adhering to litter not only provide a substrate for microbial colonisation but also enhance the physical interaction between soil and litter, therefore increasing litter decomposition (Jacobs et al., 2018). This may suggest the role of microbial activity, especially by the fungal community, in litter turnover in desert ecosystems (Jacobson et al., 2015).

### 4.6.2. Carbon, nitrogen and C/N ratio

Different litter types, such as grass, shrub, or tree litter, have varying chemical compositions (C, N, C/N ratio), which generally influence its decay rate. A low C/N ratio in shrub litterbags correlates with less mass remaining observed in these same litterbags. This supports my initial hypothesis that litter with a low C/N ratio would decompose faster than litter with a high C/N ratio. A low C/N ratio in shrub litter indicates that nitrogen is relatively abundant compared to carbon, a pattern also reflected in the mean ash-free percent N and mean ash-free percent C values shown in Figures 4.7 and 4.8. Shrub litter may have less complex structures, such as lignin and cellulose, needed to endure adverse environments. These structural compounds confer toughness on leaf litter, protect the litter from microbial degradation and constitute waterproofing properties of plant cell walls, slowing down physical abrasion (Su et al., 2022). Although C and N content and C/N ratios were the only litter-quality metrics I measured, lower decomposition rates of grass litter may suggest higher concentrations of cellulose (Piotr et al., 2017; Zhu et al., 2021; Zhao et al., 2022), which slows down the microbial activity and delaying the decomposition rate (Prescott et al., 2004). The rapid decomposition of low C/N shrub litter

as compared to high C/N grass litter suggests that litter quality is an important driver of decomposition in this system.

## 4.7. CONCLUSION

This part of the research project aimed to investigate the influence of vegetation patches on litter decomposition rates in hyper-arid systems. Although vegetated patches were expected to enhance decomposition compared to unvegetated patches due to their impact on microclimates and microbial communities, net litter decomposition was not impacted by vegetated patches. Other factors, such as non-rainfall moisture, temperature fluctuations, and soil-litter mixing, may have contributed to similar decomposition rates across both litter placement patches. The litter burial by sand and soil-microbial films on litter may have further equalised the decomposition processes between the patches. This highlights the complexity of decomposition dynamics in dryland ecosystems, where multiple factors interact to drive litter decomposition.

The study highlights the importance of chemical compositions (C & N, C/N ratios), which generally influence the decay rate. Given that decomposition in drylands is driven by unique interactions between abiotic and biotic drivers, understanding how different decomposition mechanisms influence litter decay rates will ultimately aid in predicting litter decay rates in hyper-arid ecosystems.

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# CHAPTER FIVE: CONCLUSIONS AND FUTURE WORK

## CHAPTER FIVE: CONCLUSIONS AND FUTURE WORK

Drylands constitute the largest biome on Earth, representing about 45% of the terrestrial land surface and one of the harshest environments. They are characterised by long periods of desiccation, strong winds, low nutrient status, and large temperature fluxes (Whitford, 2002; Lester et al., 2007), leading to a limited diversity of higher plants and animals in this system (Makhalanyane et al., 2015). As a result of limited macrofaunal plant biodiversity, soil microbial communities are considered to be predominant ecosystem drivers as they are major contributors to key processes vital for C and nutrient cycling (Pointing & Belnap, 2012; Makhalanyane et al., 2015; Vikram et al., 2016; Neilson et al., 2017). The spatially discontinuous and patchy vegetation cover in drylands has been shown to influence soil microbial communities via litter and root exudate inputs and regulate temporal and spatial patterns of microbial activity by controlling moisture content, solar radiation, and temperature (Schlesinger et al., 1990; Thompson, 2010). Therefore, vegetation patches in these environments have been suggested as good systems to evaluate the influence of vegetation on soil microbial communities and their functional capacity, which is largely unexplored in desert ecosystems, particularly in hyper-arid systems. Using 16S rRNA gene and ITS sequence high-throughput sequencing and shotgun metagenome sequencing, the taxonomic composition and functional capacity of soil prokaryotic and eukaryotic communities in vegetation hummocks in the coastal Namib Desert were investigated. The study aimed to test several hypotheses: 1) vegetated hummock soils would harbor a higher composition and diversity of microbial taxa than unvegetated patches (windward slope and gravel plain) due to the modified microclimate around plants, which also can influence resource availability, 2) the taxonomic composition of microbial communities would be heterogeneous (or distinct) across the three sampling locations, 3) vegetated hummock soils would be more enriched with functional capacity relative to the bare (unvegetated: windward slope and gravel plain) ground due to plant-microbe interactions, resource availability and the modified microclimate around plants, and 4) microbial communities in the Skeleton Coast possess genes encoding enzymes vital for thriving and performing ecosystem functions and services in hyper-arid ecosystems.

Alpha diversity of both bacterial and fungal communities was impacted by vegetated hummocks, with vegetated hummock soils harboring a high number of observed taxa relative to unvegetated bare ground soils, in agreement with the first hypothesis. Most of the taxa recovered from vegetated hummock soils are plant-growth-promoting taxa, suggesting that the vegetated hummock may be a selective system, favouring microbial communities beneficial for their growth and survival in this hyper-arid environment. Similarly, beta-diversity patterns differed significantly between the sampling locations, in agreement with the hypothesis that microbial communities would be heterogeneous (or distinct) across the three sampling locations. This supports the concept of niche partitioning, which involves the selection of microbial taxa capable of colonising an environment based on its abiotic characteristics (Johnson et al., 2017), suggesting that niche partitioning is a critical process in the assembly of microbial communities within the soils of the Skeleton Coast National Park. Altogether, these results indicate that vegetation patches serve as an important factor in shaping taxonomic attributes of the arid soil microbiome.

The use of shotgun metagenome sequencing in this study enabled an investigation into the nitrogen, C, sulfur cycling, and methane metabolism pathways through read annotation, genome binning, and functional pathway annotation. Functional marker genes provided evidence for microbial energy-generating and biogeochemical cycling potential, which cannot be investigated with taxonomic markers such as the16S rRNA gene. This study demonstrated that Skeleton Coast Park soils have the potential for microbial C fixation, heterotrophic C cycling, nitrogen fixation, nitrification, denitrification, methanogenesis, and sulfur oxidation and reduction. The study shows that microbes associated with heterotrophic nitrogen cycling were recovered from vegetated hummock soils, as this pathway requires labile nutrient stocks, which may be limited in the unvegetated windward slope and gravel plain. Additionally, the use of metagenomics in this study allowed the recovery of discrete MAGs from the sampling locations. In particular, several genomes related to alpha-amylase, TCA cycle, TCA cycle, entner-doudoroff, methanogenesis via trimethylamine, denitrifying, sulfur and sulfur-oxidizing microbes were recovered, highlighting these pathways and enzymes as potentially prevalent in the Skeleton Coast Park soils.

The presence of the NiFe hydrogenase Hyd-1 gene indicated the potential for chemotrophic microbes to scavenge atmospheric trace gases as alternative energy sources. This strategy may help microbes to rapidly replenish cell energy and C amid intense competition following hydration events, providing insights into how bacterial communities persist in this extreme hyper-arid system (Leung et al., 2020; Ortiz et al., 2021). Moreover, the presence of the ammonia-oxidizing marker gene methane/ammonia monooxygenase pmoC-amoC in vegetated hummock soils suggests the presence of microbes capable of oxidising ammonia to nitrite and nitrate in vegetated hummock soils compared to unvegetated windward and open gravel plains soils. Consequently, vegetated hummock soils may exhibit more efficient nitrogen cycling dynamics, thus contributing to ecological functionality and potential nutrient availability in this oligotrophic environment.

Litter decomposition is an essential source of energy and nutrients that strongly controls nutrient availability and primary production. This process is known to be influenced by vegetation patches; however, how they affect litter decomposition is not well understood, particularly in hyper-arid ecosystems. Given the fact that hyper-arid systems are characterised by spatial heterogeneity of vegetation patches, the Skeleton Coast National Park, Namib Desert, is a good system to investigate the influence of vegetation patches on litter decomposition rates. In this study, local litter from two dominant species was used to understand decomposition processes, which provided insights into unique ecological processes controlling nutrient cycling and energy flows within the hyper-arid Namib Desert ecosystem. Because both study species (shrub and grass) have occupied the Namib Desert for over 100 years, local litter inputs may likely have fostered a home-field advantage (Osburn et al., 2022) relative to global litter.

Using the litterbag technique, litter decomposition rates of two contrasting local litter types (shrub and grass) under vegetated and unvegetated patches were measured. This study aimed to test several hypotheses: 1) that the vegetated patches would have higher decomposition relative to the unvegetated patches due to the modified microclimate around plants, which also can influence resource availability and microbial community, 2) shrub leaf litter would decompose faster than grass leaf litter due to its larger surface areas, and 3) litter with low C/N ratios would decompose faster than litter with high C/N ratios. Litter

decomposition was not impacted by vegetated patches, contradicting the first hypothesis that vegetated patches would have higher decomposition relative to the unvegetated patches. The litter burial by sand and soil-microbial films on litter in unvegetated patches may have further equalised the decomposition processes between the patches, highlighting the complexity of decomposition dynamics in dryland ecosystems.

The study shows that decomposition was positively correlated with soil infiltration into litterbags, suggesting that the soil-litter mixing mechanism may be an important decomposition driver in this study. This interaction promotes microbial activity and other biological processes essential for litter decay (Levi et al., 2020). Additionally, the study found that high N, and low C content and C/N ratio in shrub litter somewhat increased decomposition rates, highlighting the role of chemical composition in litter decay rates. Using local litter materials to understand decomposition provides a more accurate representation of the complex interactions between biotic and abiotic factors in hyper-arid ecosystems. Altogether, these results provided insights into various mechanisms influencing litter decay rates, which will ultimately aid in predicting rates of litter decay in hyper-arid ecosystems.

Although this research has provided valuable insights into the taxonomic composition and function capacity of microbial communities as well as litter decomposition rates in the hyper-arid Skeleton Coast Park soils, it is hoped that this work will help to stimulate further research and probe several questions that require further investigation for future work, for example:

1. While amplicon and shotgun metagenomic sequencing provide insights into the microbial taxonomic composition (i.e., which microorganisms are present?) and what their potential functions are (i.e., what they might be doing?), an important question remains: how do the activities of microorganisms influence ecosystem functions in this hyper-arid system? To determine whether these communities are active, future research should employ metatranscriptomics to highlight which microbial communities are active and which are dormant. Transcriptomics involve sequencing the transcribed mRNA and goes one step further from metagenomics to identify which processes are active at a snapshot in time. This would be beneficial to understand which aspects of biogeochemical cycles are functioning and observe any changes in the ecosystem.

2. Do soil microbes express variable structural patterns of taxonomic composition and functional diversity at different soil depths? Given that soil microbial community is structured by soil depth due to variations in physicochemical properties, it would be interesting to compare soils from different depths to assess the influence of soil depths on the taxonomic and functional diversity of microbial communities and how depth shapes their abilities to contribute to the ecological functions in drylands. Understanding the microbial patterns specific to different soil depths and the factors shaping them is essential to better exploit the potential of soil functions and propose new strategies to enhance soil sustainability.

3. How do microbial functions respond to climatic shifts? Studies have demonstrated that aridity significantly alters the taxonomic composition of soil microbial communities, and since this study was only done in one area, it would be interesting to assess how microbial functional capacity responds to aridity gradient in this hyper-arid system. Investigating the response of microbial functional capacity to aridity is essential for gaining insights into the potential impact of climate change on desert ecosystem functions and processes.

4. Are there drought-resistant gene hotspot areas in the Skeleton Coast National Park? Most taxa observed from vegetated hummock soils are growth-promoting rhizobacteria; therefore, future research can investigate whether there are hotspot areas with drought-resistant genes. Given the projected increase in dryland aridity, identifying drought-resistant genes is crucial for crop improvement and sustainable agriculture in these regions.

5. What structural differences discriminate between low-affinity and high-affinity gas-oxidizing enzymes in desert systems? This will help to better resolve how electrons liberated by these enzymes are used for aerobic respiration and C fixation. Furthermore, this will provide insights into how trace gas oxidation is regulated at the cellular and ecosystem levels and how environmental factors affect these processes in oligotrophic environments.

5. What factors (abiotic and biotic) control the soil accumulation on litter at the microsite level? Exploring the factors that control soil accumulation on litter at the microsite level is crucial for understanding litter decomposition dynamics in hyper-arid systems, where environmental extremes can significantly influence ecological processes. By identifying

and analysing the interplay of these factors, we can better predict nutrient availability and ecosystem responses to climatic changes in hyper-arid regions, ultimately aiding in managing and conserving these fragile ecosystems.

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# APPENDIX

**Table 1:** Physicochemical analyses for sampling sites and sampling locations measurements.

| Sampling sites | Sampling location | pH | ecw | Om | P | K | Ca | Mg | Na | Fe | CeC | N | C | Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | uS/cm | % | ppm | ppm | ppm | ppm | ppm | ppm | meq/100g | % | % | | % | % |
| Windwardsample1 | Windward | 9.43 | 173 | 0.04 | 1.7 | 121 | 1691 | 109 | 211 | 52.8 | 4.11 | 0.004 | 0.023 | 99.2 | 0.4 | 0.4 |
| Windwardsample2 | Windward | 8.44 | 212 | 0.14 | 1.7 | 186 | 1805 | 124 | 378 | 60.9 | 4.9 | 0.007 | 0.081 | 99 | 0.5 | 0.5 |
| Windwardsample3 | Windward | 8.76 | 170 | 0.13 | 7.1 | 145 | 1342 | 107 | 246 | 52.5 | 4.74 | 0.002 | 0.076 | 98.8 | 0 | 1.2 |
| Windwardsample4 | Windward | 9.67 | 130 | 0 | 0.9 | 131 | 1431 | 98 | 231 | 55.2 | 4.06 | 0 | 0 | 99.2 | 0.4 | 0.4 |
| Windwardsample5 | Windward | 9.63 | 113 | 0.01 | 2.2 | 128 | 1535 | 101 | 241 | 57.5 | 3.45 | 0.03 | 0.006 | 99 | 0.4 | 0.6 |
| GravelPlainssample1 | Gravel Plains | 9.3 | 173 | 0.19 | 0.1 | 153 | 2701 | 178 | 183 | 49.3 | 6.17 | 0.002 | 0.112 | 96.4 | 1.5 | 2.1 |
| GravelPlainssample2 | Gravel Plains | 8.46 | 355 | 0.01 | 0.9 | 170 | 4065 | 170 | 597 | 48.2 | 8.42 | 0.007 | 0.006 | 94.3 | 3.9 | 1.8 |
| GravelPlainssample3 | Gravel Plains | 9.26 | 214 | 0.06 | 2 | 156 | 2688 | 171 | 224 | 50.2 | 7.08 | 0.01 | 0.032 | 97.2 | 2.6 | 0.1 |
| GravelPlainssample4 | Gravel Plains | 8.69 | 542 | 0.03 | 1.5 | 153 | 3112 | 139 | 215 | 40.9 | 8.08 | 0.002 | 0.02 | 97.2 | 0.5 | 2.3 |
| GravelPlainssample5 | Gravel Plains | 9.06 | 178 | 0.08 | 1.9 | 163 | 2648 | 194 | 164 | 42.6 | 6.62 | 0.007 | 0.048 | 96.2 | 2 | 1.8 |
| Vegetatedhummocksample1 | Hummock | 8.66 | 604 | 0.06 | 4.1 | 217 | 2081 | 164 | 340 | 58.9 | 9.69 | 0.005 | 0.04 | 96.7 | 2.1 | 1.2 |
| Vegetatedhummocksample2 | Hummock | 8.91 | 363 | 0.06 | 1.5 | 164 | 2254 | 155 | 310 | 61.2 | 5.74 | 0.004 | 0.034 | 98.3 | 0.2 | 1.5 |
| Vegetatedhummocksample3 | Hummock | 9.31 | 297 | 0.06 | 2.5 | 203 | 1864 | 151 | 304 | 101 | 6.28 | 0.001 | 0.036 | 97.6 | 2.13 | 0.27 |
| Vegetatedhummocksample4 | Hummock | 9 | 333 | 0.03 | 1.7 | 200 | 1913 | 189 | 403 | 67.2 | 5.82 | 0.001 | 0.02 | 97.2 | 1.12 | 1.7 |
| Vegetatedhummocksample5 | Hummock | 9.48 | 356 | 0.08 | 2.1 | 203 | 1997 | 204 | 418 | 66.7 | 5.9 | 0.003 | 0.049 | 98.8 | 0.6 | 0.6 |

**Appendix S1: Standardization of metagenomics DNA extraction protocol**

5 g soil samples (triplicate) from each soil sample were mixed with 10 ml of extraction buffer [100 mM Tris/HCl (pH 8.0), 100 mM EDTA (pH 8.0), 100 mM sodium phosphate buffer (pH 8.0), 1.5 M NaCl, 1% (w/v) CTAB, 100 mM CaCl2, 10 mg proteinase K/ml and 10 mg lysozyme/ml] in oakridge tubes and incubated at 37°C for 1h30 minutes in the incubator shaker at 200 rpm. After adding 4 ml of 10% (w/v) SDS, the mixture was incubated in water bath at 65°C for 2 h with invert mixing after every 10–15 min. The tubes were centrifuged at 7000g for 20 min at 4°C to collect the supernatant. The soil pellets were further extracted twice by adding 4.5 ml of extraction buffer and 0.5 ml of 20% (w/v) SDS, followed by incubation at 65°C for 15 min. The supernatants of three extractions were pooled and mixed with equal volume of chloroform/isoamylalcohol (24:1, v/v) and shaken gently. The tubes were centrifuged again at 14,000g for 20 min at 4°C to collect the upper aqueous phase. The crude DNA was precipitated by adding 0.1 volume 3 M sodium acetate along with 2 volumes of 30% (w/v) PEG-6000 (polyethylene glycol) + ethanol + NaCl (4 PEG 6000 + 20ml NaCl 5M + 75 ml 99% EtOH) solution to the aqueous phase and incubated overnight at -20°C. After the overnight incubation, crude DNA was pelleted by centrifugation at 14,000g for 30 min at 4°C, washed once with 70% (v/v) ethanol (room temperature) and air-dried. The dried pellets were dissolved in 1ml of 1X TE buffer and mixed with equal volume chloroform/isoamylalcohol (24:1, v/v). The aqueous phase was collected by centrifugation at 14,000g for 15 min at 4°C and DNA was precipitated using 1 volume iso-propanol followed by 2hrs incubation at -20°C. The DNA was pelleted again by centrifugation at 14,000g for 30 min at 4°C. The pellets were washed with 1 ml of 5 M NaCl, followed by 1 ml of 70% (v/v) ethanol and centrifuged at 14,000 g for 15 min at 4°C, and air-dried. The air-dried pellet was further re-precipitated. To re-precipitate, 0.1 volume of 3 M Sodium Acetate (pH 5.2) was added to each sample, followed by 2.5–3 volumes of 99% ethanol. The mixture was allowed to precipitate for one hour. The sample was then centrifuged at 14,000 g for 30 minutes to form a pellet. The pellet was washed with 70% ethanol and centrifuged again at 14,000 g for 15 minutes. After centrifugation, the pellet was air-dried before resuspended in 40 µl of TE buffer (EB).

**Table 2:** Summary of raw and trimmed reads (16S rRNA gene and ITS sequence) generated per sample.

| | 16S rRNA | | ITS sequence | |
|---|---|---|---|---|
| **Sampling group** | **Raw reads** | **Quality filtered reads** (ASVs) | **Raw reads** | **Quality filtered reads** (ASVs) |
| **Hummock** | 667,763 | 343,652 | 427,660 | 263,814 |
| **Windward slope** | 301,608 | 158,742 | 15,973 | 8,132 |
| **Gravel Plains** | 578,731 | 275,471 | 39,528 | 17,301 |

**APPENDIX 2**

**Table 1:** Abundance of marker genes for the observed pathways obtained from the vegetated hummock, open gravel plains and windward slope sampling locations.

| Marker Genes | Vegetated Hummock | Gravel plains | Windward slope |
|---|---|---|---|
| Assimilatory nitrite reductase - nirB | 8 | 0 | 0 |
| Methane/ammonia monooxygenase - pmoC-amoC | 1 | 0 | 0 |
| Nitrate reductase - narH, narY, nxrB | 0 | 1 | 0 |
| Nitrate reductase (cytochrome) - napA | 0 | 2 | 0 |
| Nitrate reductase (cytochrome) - napB | 2 | 0 | 0 |
| Nitrate reductase / nitrite oxidoreductase - narG, narZ, nxrA | 5 | 0 | 0 |
| Nitrate reductase / nitrite oxidoreductase - narH, narY, nxrB | 2 | 0 | 0 |
| Nitrate reductase / nitrite oxidoreductase - nxrB | 0 | 0 | 0 |
| Nitrite reductase (NADH) - nirB | 0 | 15 | 5 |
| Nitrite reductase (NADH) - nirD | 28 | 12 | 16 |
| Phosphoribulokinase - PRK | 10 | 12 | 22 |
| Ribulose-bisphosphate carboxylase - rbcS | 2 | 12 | 15 |
| Sulfite reductase (ferredoxin) - sir | 0 | 7 | 9 |
| Sulfite reductase (NADPH) hemoprotein - cysI | 0 | 1 | 0 |

**Table 2**: Summary of MAGs obtained from the vegetated hummock, open gravel plains and windward slope sampling locations. Genome completeness and contamination were estimated with CheckM.

| user_genome | fastani_reference | fastani_reference_radius | fastani_taxonomy | fastani_ani | fastani_af | closest_placement_reference |
|---|---|---|---|---|---|---|
| metabat_Windward slope_bins.25 | p__Actinobacteriota | c__Actinomycetia | *o__Mycobacteriales* | *f__* | *g__* | *s__* |
| metabat_Gravel Plains_bins.5 | p__Pseudomonadota | c__Gammaproteobacteria | *o__Burkholderiales* | *f__Burkholderiaceae* | *g__Ralstonia* | *s__Ralstonia insidiosa* |
| metabat_Hummock_bins.10 | p_ | c__Bacilli | *o__Paenibacillales* | *f__YIM-B00363* | *g__Paenibacillus_AE* | *s__* |
| metabat_Hummock_bins.13 | p__Pseudomonadota | c__Alphaproteobacteria | *o__Rhizobiales* | *f__Rhizobiaceae* | *g__Pseudorhizobium* | *s__* |
| metabat_Hummock_bins.14 | p__Pseudomonadota | c__Gammaproteobacteria | *o__Pseudomonadales* | *f__Pseudomonadaceae* | *g__Pseudomonas_B* | *s__Pseudomonas_luteola* |
| metabat_Hummock_bins.41 | p__Pseudomonadota | c__Alphaproteobacteria | *o__Rhodobacterales* | *f__Rhodobacteraceae* | *g__* | *s__* |
| metabat_Hummock_bins.44 | p__Bacteroidota | c__Bacteroidia | *o__Flavobacteriales* | *f__Flavobacteriaceae* | *g__Flavobacterium* | *s__* |
| metabat_Hummock_bins.48 | p__Actinobacteriota | c__Actinomycetia | *o__Nitriliruptorales* | *f__Nitriliruptoraceae* | *g__* | *s__* |
| metabat_Hummock_bins.50 | p__Actinobacteriota | c__Actinomycetia | *o__Actinomycetales* | *f__Micrococcaceae* | *g__Pseudarthrobacter* | *s__* |