



# **The influence of temperature on bacterial communities in arid soil microbiomes**

by

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submitted in partial fulfilment of the requirements for the degree of Magister Scientiae  
in the Department of Biochemistry, Genetics and Microbiology, Faculty of Natural and  
Agricultural Sciences, University of Pretoria

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February 2023

## **Declaration**

I, Mable Rosula Baluka, hereby declare that this dissertation submitted for the degree Magister Scientiae (M.Sc.) at the University of Pretoria, is my own work and has not previously been submitted for a degree at this, or other university.

MSc Candidate

A handwritten signature in blue ink, appearing to read 'Mable Rosula Baluka', enclosed within a blue oval.

Mable Baluka

February 2023

## **Acknowledgements**

I would like to express my appreciation to the following people and organizations for their continued support throughout this degree:

My gratitude extends to National Research Foundation (NRF) for the funding opportunity to undertake my studies at the Department of Biochemistry, Genetics, and Microbiology, University of Pretoria. Prof Thulani P. Makhalanyane, for his supervision, support, and suggestions to better this study. Dr Jessica E. Koopman, my co-supervisor, for her patience and guidance. Dr Surendra Vikram, Mrs Tidimalo Coetzee, and Mancha Mabaso for their assistance with data analysis. Mr Russel M. Mashile, for his assistance with the soil sample collection. Alex Mukiibi, for his assistance with the soil physicochemical analysis. My friends Brian, Eva, Gciniwe, Henry, Marvellous, Paul, Percy, and Oluwatayo, who provided stimulating discussions as well as happy distractions to rest my mind outside of my research. My family for their love, and encouragement. The Almighty God, for seeing this to completion.

## **Dedication**

I dedicate this work to my mother, Mrs Alaziya Kiirya, who taught me much about determination and perseverance.

“That dark laboratory we call the soil” – Aldo Leopold

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## List of Abbreviations

|                                 |                                             |
|---------------------------------|---------------------------------------------|
| ACC                             | 1-Aminocyclopropane-1-Carboxylate Deaminase |
| AI                              | Aridity Index                               |
| amoA                            | Ammonia Monooxygenase Subunit A             |
| amoB                            | Ammonia Monooxygenase Subunit B             |
| ANOVA                           | Analysis of variance                        |
| ASVs                            | Amplicon Sequence Variants                  |
| C                               | Carbon                                      |
| Ca                              | Calcium                                     |
| CCA                             | Canonical Correspondence Analysis           |
| CO <sub>2</sub>                 | Carbon Dioxide                              |
| Cu                              | Copper                                      |
| db-RDA                          | Distance-based Redundancy Analysis          |
| DCA                             | Detrend Correspondence Analysis             |
| DNA                             | Deoxyribonucleic Acid                       |
| Fe                              | Iron                                        |
| Fe <sub>2</sub> <sup>+</sup>    | Ferrous Iron                                |
| Fe <sub>3</sub> <sup>+</sup>    | Ferric Iron                                 |
| IPCC                            | Intergovernmental Panel on Climate Change   |
| K                               | Potassium                                   |
| KCl                             | Potassium Chloride                          |
| KCr <sub>2</sub> O <sub>7</sub> | Dichromate                                  |
| KSMs                            | Potassium Solubilizing Microorganisms       |
| Mg                              | Magnesium                                   |
| Mn                              | Manganese                                   |

|                              |                                                 |
|------------------------------|-------------------------------------------------|
| N                            | Nitrogen                                        |
| N <sub>2</sub>               | Dinitrogen                                      |
| N <sub>2</sub> O             | Nitrous Oxide                                   |
| Na                           | Sodium                                          |
| NH <sub>4</sub> <sup>+</sup> | Ammonium                                        |
| nirK                         | Copper-Containing Nitrite Reductase Gene        |
| nirS                         | Nitrite Reductase                               |
| NMDS                         | Non-metric Multidimensional Scaling             |
| NMDS                         | Non-metric Multidimensional Scaling             |
| NO                           | Nitric Oxide                                    |
| NO <sub>2</sub> <sup>-</sup> | Nitrite                                         |
| NO <sub>3</sub> <sup>-</sup> | Nitrate                                         |
| nosZ                         | Nitrous Oxide Reductase Gene                    |
| NWU                          | North-West University                           |
| OC                           | Organic Carbon                                  |
| P                            | Phosphorous                                     |
| P/PET                        | Precipitation to Potential Evapotranspiration   |
| PCR                          | Polymerase Chain Reaction                       |
| PERMANOVA                    | Permutational Multivariate Analyses of Variance |
| PGP                          | Plant Growth-Promoting                          |
| PGPB                         | Plant Growth-Promoting Bacteria                 |
| PGPR                         | Plant Growth Promoting Rhizobacteria            |
| PSMs                         | Phosphate Solubilizing Microorganisms           |
| RCPs                         | Representative Concentration Pathways           |
| RDA                          | Redundancy Analysis                             |
| rRNA                         | Ribosomal Ribonucleic Acid                      |

|       |                                                     |
|-------|-----------------------------------------------------|
| SA    | South Africa                                        |
| SAW   | South African Services                              |
| SD    | Standard Deviation                                  |
| SOC   | Soil Organic Carbon                                 |
| UNCCD | United Nations Convention to Combat Desertification |
| UNEP  | United Nations Environmental Programme              |
| UV    | Ultraviolet                                         |
| Zn    | Zinc                                                |

## **Abstract**

The predicted increases in the mean global temperatures will substantially impact the functioning of several ecosystems, including drylands. The effects of climatic changes on the biodiversity of macrofauna have been clearly demonstrated, but we lack mechanistic insights regarding the effects of these changes on the diversity and function of microbial communities. This knowledge deficit is in part due to the fact that microbial communities and their response to change may vary at global, regional, and local scales. Soil microorganisms are essential mediators of biogeochemical cycling and are important for plant growth. Although previous studies suggest that changes in the temperature regime may directly impact microbial ecosystem services, the precise effects are poorly characterized, especially in soils associated with invasive plants in arid zones. In this study, an experimental manipulation (microcosms) was used to investigate the effects of temperature increase on the structure, composition, and diversity of bacterial communities in arid soils. Fifteen soil physicochemical properties were used to assess the role played by nutrients in driving bacterial community structure based on 16S rRNA gene amplicon sequencing. The experimental manipulation used soils collected from below an *Acacia eriobola* tree canopy and those from adjacent soils. To assess the effect of temperature increases, soils were incubated at 30°C and 40°C, respectively, for forty days. Soils from three replicated microcosms were destructively sampled from the 30°C and 40°C incubators on days 0, 1, 2, 8, 16, 24, 32, and 40. In contrast to expectations that temperature increases would alter bacterial communities, we found

that soil physicochemical variables were more significant drivers of community structure. Our analysis revealed that temperature effects across microhabitats appeared to be unnoticeable, and not significant. Five bacterial phyla were dominant across all soils including Actinobacteria (39.1%), Firmicutes (36.1%), Proteobacteria (9.9%), Acidobacteria (4.6%), Chloroflexi (4.5%). The results suggest that microhabitat was linked to bacterial composition, with higher relative abundances of Firmicutes (in canopy soils) and Actinobacteria and Chloroflexi (in open soils), respectively. Distance-based redundancy analysis (db-RDA) showed that iron (Fe) was the major determinant of bacterial diversity in canopy soils, while Cu and pH were the major drivers of bacterial diversity in open soils. The results of this study suggest that physicochemical variables may buffer the effects of short-term temperature increases on soil bacterial abundance and diversity in arid soils.

**Keywords:** 16S rRNA gene, bacterial diversity, drylands, microcosms, microhabitat, soils, soil physicochemical variables, temperature

## Chapter 1 – Introduction

Anthropogenic emissions have led to unprecedented increases in global CO<sub>2</sub> levels (Cavicchioli *et al.*, 2019; Keenan and Williams, 2018; Sheikh *et al.*, 2014). Estimates from the Fifth Assessment Report of the IPCC suggest that global temperatures may increase by 1.5°C before the end of this century (IPCC, 2013a). This increase in average global temperatures may lead to the expansion of fragile and sensitive dryland ecosystems (Fu and Feng, 2014; Huang *et al.*, 2016; Koutroulis, 2019). The Paris Agreement of 2015 established measurable targets for reducing global emissions (UNFCCC, 2015). Yet, despite these targets, global temperatures continue to increase (Lawrence and Schäfer, 2019). The increased temperatures have been shown to result in ecological effects on biodiversity (García *et al.*, 2018). For instance, studies by Hyvönen *et al.* (2007); Ontl and Schulte (2012) have demonstrated clear effects on nutrient sequestration. While other studies suggest that microbial communities may represent potential negative or positive feedbacks for greenhouse gas emissions further aggravating the effects of climate change (Singh *et al.*, 2010). However, we lack a clear mechanistic understanding of potential microbial contributions, especially in dryland ecosystems.

There is an increased interest in soil microbial communities in dryland ecosystems (Delgado-Baquerizo *et al.*, 2017a; Fierer, 2017; Maestre *et al.*, 2015). Studies have focused on elucidating the phylogenetic and functional diversity of bacterial communities in drylands (Fierer *et al.*, 2012a; Le Bagousse-Pinguet *et al.*, 2019). Bacterial communities are essential components of maintaining soil quality and ecosystem services (Delgado-Baquerizo *et al.*, 2016; Liu *et al.*, 2017; Philippot *et al.*,

2013b; Wagg *et al.*, 2014). These ecosystem services include, for instance, the decomposition of litter and the maintenance of soil organic carbon (Bani *et al.*, 2018). In addition, soil microbes provide N to plants through nitrification by oxidizing ammonia to nitrate (Trivedi *et al.*, 2019), while bacterial community structure is correlated to N-mineralization and CO<sub>2</sub> sequestration (Balser and Firestone, 2005; Deng *et al.*, 2016; Li *et al.*, 2019).

The effects of global warming on dryland ecosystem services are becoming increasingly clear (Huang *et al.*, 2012; Huang *et al.*, 2017; Ji *et al.*, 2014). Several soil microcosm studies have investigated the effects of warming at temperatures above the average temperature (Bárcenas-Moreno *et al.*, 2009; Habtewold *et al.*, 2021; Müller *et al.*, 2022; Pietikäinen *et al.*, 2005). Researchers have reported reductions in soil microbial diversity and abundance with increasing temperature (Maestre *et al.*, 2015; Rodriguez-Caballero *et al.*, 2018). Moreover, initial high growth rates may be followed by substantial reductions due to substrate depletion and variations in temperatures (Pold *et al.*, 2017; Walker *et al.*, 2018). However, some studies have revealed contradictory results. For example, no shifts in soil bacterial community structure were observed in vineyard soils subjected to temperature variations over a one-year period microcosm experiment (Corneo *et al.*, 2014; Schultz, 2016). In contrast, short-term effects of temperature on soil bacterial communities reported strong community shifts (e.g., increase in Actinobacteria in warmed and inundated soils) over different time scales (Riah-Anglet *et al.*, 2015; Waring and Hawkes, 2015; Xiong *et al.*, 2014; Yuste *et al.*, 2014). These phylogenetic shifts may be due to a reduction in the available resources for decomposers as the sustained higher microbial activity in warmed soils may result in

labile substrate losses (Rousk *et al.*, 2012; Wang *et al.*, 2012). Moreover, the phylogenetic shifts also appear to affect soil function (Frey *et al.*, 2008; Hu *et al.*, 2017; Liu *et al.*, 2017). Hu *et al.* (2021) reported that a reduction in the dominant prokaryotic taxa resulted in a loss of special functions (e.g., nutrient cycling contributed by ammonia-oxidizing archaea of the phylum Thaumarchaeota) performed by these taxa.

*Acacia* trees were previously reported to alter bacterial community composition and function by changing soil chemical properties in fynbos soils (Keet *et al.*, 2021). The changes in soil function and bacterial community composition were correlated to shifts in the phosphatase enzymatic activity under *Acacia* invasion. The distribution of these trees is expected to increase in line with the anticipated transition to greater aridity. However, the effects of their increase on the regional ecology remain unclear. Because plants are known to substantially influence the diversity and function of microbiota, it is important to determine the effects of *Acacia* species on the diversity of soils below their canopy. Such predictions are crucial for modelling the effects of climate change on microbial communities in arid zones.

## Chapter 2 – Literature Review

### 2.1 The importance of drylands

Drylands constitute over 40% of the Earth's land surface area (Eldridge *et al.*, 2020; Maestre *et al.*, 2016b); Právělie (2016). Several studies have demonstrated the importance of drylands for lower- and middle-income countries in arid areas (Abduletif, 2019; Stavi *et al.*, 2021; Suich *et al.*, 2015). Current estimates suggest that just over two billion people live in drylands and depend directly on the ecosystem services they provide for sustenance through agriculture and pastoralism (Abdi, 2015; Cherlet *et al.*, 2018; Lee and Diop, 2009; Liu *et al.*, 2022). However, due to climate change and anthropogenic activities, these dryland ecosystem services are still under threat (Mooney *et al.*, 2009; Peng *et al.*, 2020; Zeng *et al.*, 2021). Several recent reports have documented the range of severe environmental conditions which characterize dryland ecosystems at a global scale (Fu *et al.*, 2021; Liu *et al.*, 2022; Moreno-Jiménez *et al.*, 2019; Reed *et al.*, 2016). These conditions include low and erratic precipitation, high ultraviolet (UV) A/B levels, and temperature variations (Daramola and Xu, 2022; Korell *et al.*, 2021; Liu *et al.*, 2022; Whitford and Duval, 2019). In addition, drylands are generally oligotrophic with sparse vegetation and generally low plant diversity (Choi *et al.*, 2022; Delgado-Baquerizo *et al.*, 2013; Jordaan *et al.*, 2022; Saco *et al.*, 2018). Despite appearing to be mostly heterogenous, drylands across the globe differ markedly in several traits which results in highly variable levels of aridity (Choi *et al.*, 2022; Greve *et al.*, 2019; Olsoy *et al.*, 2017).

A widely used measure of aridity used to define drylands is the aridity index (AI). This index was adopted by the United Nations Environmental Programme (UNEP) in their landmark world atlas on desertification (Middleton and Thomas, 1997). The aridity index expresses aridity as a ratio of precipitation to potential evapotranspiration (P/PET) and indicates aridity at a given location (Gao *et al.*, 2015). This index can be used to define four zones: arid (0.05-0.2), hyper-arid (0.05), semiarid (0.2-0.5), and dry-subhumid (0.5-0.65), (Figure 1.1) (Makhalanyane *et al.*, 2015a).

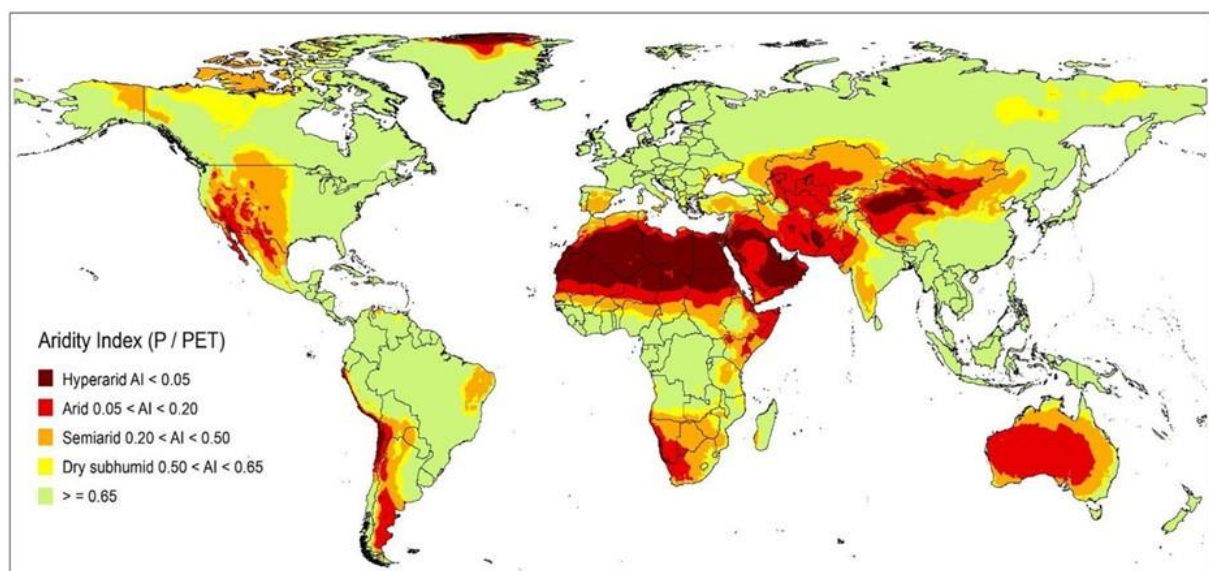


**Figure 2.1.** A global representation of Drylands and their aridity zones, (Hu *et al.*, 2017).

## **2.2 Increased carbon dioxide (CO<sub>2</sub>) emissions and temperature may exacerbate desertification in drylands**

Based on the aridity index, there is evidence that aridity may increase under higher CO<sub>2</sub> emission levels (Fu and Feng, 2014; Sherwood and Fu, 2014). Changes in CO<sub>2</sub> concentrations caused by anthropogenic warming (Figure 1.2), may result in

substantial changes in biodiversity and ecosystem function (Cramer *et al.*, 2001; Greve *et al.*, 2019). The projected increase in global temperature, whether by 1.5, 2, or 4°C above pre-industrial levels, has rightfully resulted in widespread concern (Arnell *et al.*, 2019; Gillett *et al.*, 2021; Liu *et al.*, 2019; Randalls, 2010; Warren *et al.*, 2021). The Paris Agreement predicts that CO<sub>2</sub> emissions, which are twice the pre-industrial levels, will result in temperatures 4.5°C above pre-industrial times (UNFCCC, 2015). The recent Intergovernmental Panel on Climate Change (IPCC) report has provided empirical evidence of the effects of these increases on several ecosystems, including drylands (IPCC, 2021).

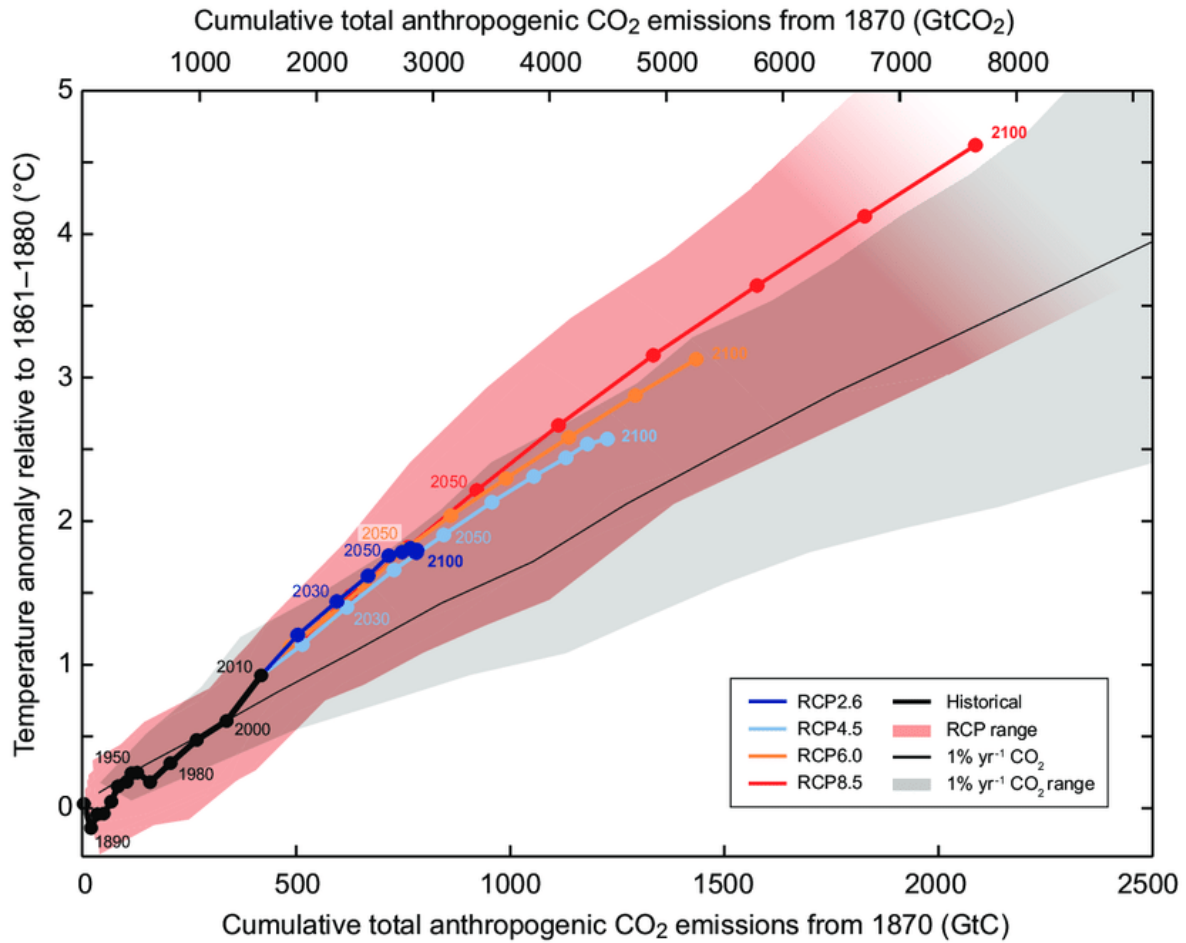


**Figure 2.2.** Global mean surface temperature increase as a function of the Representative Concentration Pathways (RCPs) emission scenarios (IPCC, 2013b).

In addition to rising CO<sub>2</sub> emissions, drylands are subject to further risks due to desertification (Al-Obaidi *et al.*, 2022; Yu *et al.*, 2022). Desertification is primarily due to decreases in rainfall, resulting in a drastic loss in vegetation cover (Costa and Soares, 2012; Li *et al.*, 2021a). This decrease in vegetation cover may lead to additional feedbacks including further temperature increases (Jeong *et al.*, 2011; Li *et al.*, 2021a). Desertification is defined by the United Nations Convention to Combat Desertification (UNCCD) as land degradation in arid, semi-arid, and dry sub-humid regions caused by several factors such as climatic variation and anthropogenic

activities (UNCCD, 2004). Together with expected global temperature projections by the year 2099, anthropogenic activities may further exacerbate desertification (Brevik, 2013; Huang *et al.*, 2016; Solomon *et al.*, 2007). Because dryland biomes have a reduced plant diversity and the proportion of plant cover is lower, compared to other terrestrial ecosystems, microbial communities are expected to be more important drivers of ecosystem services in drylands (Bardgett and Van Der Putten, 2014; Ferrenberg *et al.*, 2017; García-Vega and Newbold, 2020; Maestre *et al.*, 2021). However, there are comparatively few studies on global drylands compared to other terrestrial regions.

### **2.3 Advances in our understanding of arid soil microbiota**

Soils are complex and dynamic habitats which harbour high levels of phylogenetically diverse microorganisms (Aislabie *et al.*, 2013; Bardgett and Van Der Putten, 2014; Fierer *et al.*, 2012b; Jansson and Hofmockel, 2020; Thakur *et al.*, 2020). There is some evidence showing that microorganisms play important roles in ecosystem functioning (Saccá *et al.*, 2017). For instance, bacteria and fungi are known regulators of nutrient recycling and are important for organic matter decomposition, soil carbon (C) and nitrogen (N) sequestration (Condon *et al.*, 2010; Hewins *et al.*, 2017; Mueller *et al.*, 2015; Pagel *et al.*, 2020; Zheng *et al.*, 2019). However, until recently, there have been few mechanistic insights regarding the precise contributions of microbial communities (Cavicchioli *et al.*, 2019; Crowther *et al.*, 2019). The advent of modern molecular biology techniques, including the introduction of amplicon sequencing and shotgun metagenomics, has reduced these knowledge gaps (Callahan *et al.*, 2019; Jansson and Hofmockel, 2020; Zimmerman

*et al.*, 2014). There have been several recent initiatives such as the Earth Microbiome Project and BioDesert (Gilbert *et al.*, 2018; Maestre *et al.*, 2021), which provided new insights regarding microbiota composition and diversity in terrestrial ecosystems. These efforts have shown that microbial communities vary substantially across geographic scales and are driven by physicochemical variables, including pH (Feeser *et al.*, 2018; Guerra *et al.*, 2020; Maestre *et al.*, 2021; Van Horn *et al.*, 2013).

### **2.3.1 Culture independent techniques as a tools for investigating dryland microbial ecology**

The use of culture-independent techniques has greatly advanced our understanding of microbial ecology (Beng and Corlett, 2020; Seymour, 2019). Before the widespread application of culture-independent phylogenetics, most insights regarding microbial diversity and function were generated from laboratory isolates (Guerra *et al.*, 2021; Ranjard *et al.*, 2000). However, because traditional microbiological tools can only culture a small fraction of our planet's microbial diversity, we lack clear insights regarding the majority of bacteria and archaea (Molina-Menor *et al.*, 2021; Prosser, 2002; Ritz, 2007). Another challenge in cultivating environmental microorganisms is the difficulty in replicating the environmental conditions of the various microbes' natural habitats under standard laboratory conditions (Stewart, 2012). Furthermore, taking into account interspecies interactions and metabolic cooperation is key for microbes that would otherwise be reluctant to grow in standard laboratory conditions (Vartoukian *et al.*, 2010). In contrast to culture-based analysis, culture-independent techniques such as metagenomics and amplicon sequencing have expanded efforts to study a wider

array of habitats, as well as offered unique insights into relationships among microbial taxa (Burke *et al.*, 2011; Sabale *et al.*, 2019; Wilkins *et al.*, 2019). These studies have revealed the unprecedented extent of microbial diversity in soils and other ecosystems (Ranjard *et al.*, 2000).

Although culture-independent methods have facilitated an improved understanding of the depth and breadth of phylogenetic diversity, there are still gaps in our understanding. For example, microbial community distribution and diversity are known to vary remarkably based on specific abiotic variables (pH, temperature, nutrient availability). These variations are difficult to disentangle based purely on field-based sampling and require the application of laboratory-based simulations. There has been an expansion of laboratory-based experimental systems, such as soil transplants, microcosms, and mesocosms, which have enabled the simulation of various environmental conditions in order to study microbiomes under controlled settings (Barnard *et al.*, 2020; Ranjard *et al.*, 2000; Singer *et al.*, 2021; Stewart *et al.*, 2013). Such studies have been applied to both aquatic and terrestrial ecosystems (Senerpont Domis *et al.*, 2007; Wu *et al.*, 2012). In arid zones, an early study by Crits-Christoph *et al.* (2013) used an experimental system to monitor the mineralization of C-acetate by microbes from the Atacama Desert. The latter study found that although microorganisms in the driest soils of the Atacama Desert are mostly dormant if exposed to liquid water for an extended period of time, these microbiomes may metabolize C-acetate. In addition, several other studies also highlight the importance of microcosm analyses for understanding fundamental drivers of ecosystem change (Barnard *et al.*, 2020; Boyd *et al.*, 2018;

Delgado-Baquerizo *et al.*, 2017b; Langenheder and Prosser, 2008; Supramaniam *et al.*, 2016).

## **2.4 Drivers of ecosystem change in drylands**

Studies on arid soils have revealed that these ecosystems are dominated by a core group of phyla, including members of the Actinobacteria, Acidobacteria, and Proteobacteria (Fierer *et al.*, 2009; Maestre *et al.*, 2015; Makhalanyane *et al.*, 2015a; Makhalanyane *et al.*, 2015b; Vázquez-Dean *et al.*, 2020). There is also evidence from other studies showing that members belonging to the phyla Chloroflexi and Firmicutes may be present at relatively low abundances in arid soils (Makhalanyane *et al.*, 2013; Vikram *et al.*, 2016). According to a recent global scale study on arid environments, arid soils had a higher abundance of Actinobacteria and a lower abundance of Proteobacteria, Cyanobacteria, and Planctomycetes when compared to non-arid soils (Vázquez-Dean *et al.*, 2020). The adaptation to dryland conditions may explain differences in the relative abundances of microbial communities in arid soils (Fuentes *et al.*, 2020; Hwang *et al.*, 2021; Marasco *et al.*, 2022; Uritskiy, 2020). These adaptations include exposure to highly acidic or alkali pH, low and high temperatures, UV radiation, nutrient starvation, and oxidative stress (Eldridge *et al.*, 2018; Maestre *et al.*, 2015; Osborne *et al.*, 2020; Peng *et al.*, 2022).

## **2.5 Determinants of soil microbial diversity**

Studies suggest that bacterial diversity and composition may be influenced by a combination of abiotic and biotic factors (Andrew *et al.*, 2012; Larsen *et al.*, 2015). The effects of these factors vary considerably across different biomes and at

different spatial scales (Bahram *et al.*, 2018; Fierer *et al.*, 2012b; Lauber *et al.*, 2009). Recent studies show that the effects of these factors vary both globally and locally (Maestre *et al.*, 2015; Shi *et al.*, 2020). For instance, in mesic soils, pH is a major driver of phylogenetic diversity (Cho *et al.*, 2016; Fierer and Jackson, 2006). Some studies suggest that salinity may also be a substantial determinant of microbial diversity in arid regions (Van Horn *et al.*, 2013; Zhang *et al.*, 2019a). In their study, Zhang *et al.* (2019a) characterized soils along a natural salinity gradient in the Gurbatugut Desert (in China) and showed that salinity was a key determinant of soil microbial communities. Bacterial diversity is also influenced by other factors, including temperature, physicochemical properties, vegetation cover, and plant-microbe interactions (Ayangbenro and Babalola, 2021; Liu *et al.*, 2020; Mhete *et al.*, 2020; Zhou *et al.*, 2016). However, comparatively little is known regarding the influence of plant-microbe interactions in deserts (Daffonchio *et al.*, 2015; Heffernan and Fisher, 2012). In areas subject to increasing desertification, such as the arid regions of South Africa, it is important to understand the effects of plant-microbe interactions on the diversity and function of soils.

### **2.5.1 Vegetation (*Acacia*) effect on soil physicochemical properties**

The leguminous tree *Acacia eriobola* renamed *Vachellia eriobola*, is indigenous to Southern Africa and is widely distributed in Angola, Botswana, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe (Hyde *et al.*, 2018; Materechera and Materechera, 2001; Schachtschneider and February, 2013). This tree is a member of the pod-bearing subfamily Mimosaceae of the Fabaceae family, typically found in arid to semi-arid regions with an annual rainfall ranging between 40 to 900 mm. In

these regions, the trees are known to tolerate frosty winters and hot summers (Odirile *et al.*, 2019). As a so-called multipurpose tree, its pods, gum, leaves, and bark provide food for livestock and game and have been used to treat ear infections, headaches, and pneumonia (Butler *et al.*, 2010; Semanya and Maroyi, 2018). With a height of up to 26m and an umbrella-shaped canopy that spreads up to 18m, mature *Acacia* trees provide shade animals and people during hot dry seasons (Odirile *et al.*, 2019). In addition, the shade provided by the canopy may improve the microclimatic conditions in canopy soils. However, the extent to which this may alter soil microbes and their structure has not been investigated previously.

Canopy microclimate is characterized by lower irradiance and air temperature, and consequently, lower evapotranspiration demands compared with unvegetated open areas (Gómez-Aparicio *et al.*, 2005). Tree canopies may lower soil temperature by up to 7.1°C during the day under dry soil moisture conditions, in addition to intercepting water and windborne nutrients to soils below them (Lozano-Parra *et al.*, 2018; Puigdefábregas, 2005). *Acacia* trees, scattered in the sparse vegetation (Figure 1.4), were reported to diminish nutrient leaching and increase nutrient levels beneath their canopies in Mahikeng, in the North West province of South Africa (SA) (Materechera and Materechera, 2001). This increase in nutrient levels, reported for soils beneath tree canopies, is referred to as the 'islands of fertility' phenomenon (Van Miegroet *et al.*, 2000).



**Figure 2.3.** Picture showing *A. eriobola* trees on the North-West University (NWU) agricultural farm in Mahikeng, South Africa. Samples were collected from several trees in October 2018.

#### **2.5.1.1 Islands of fertility**

Previous studies suggest dryer climates lead to discontinuous vegetation cover dominated by soils (Escadafal, 2017). The discontinuity in vegetation cover is also reflected in the availability of nutrients in drylands through islands of fertility (Aguiar and Sala, 1999; Daffonchio *et al.*, 2015). Islands of fertility form when nutrients are concentrated around individual plants, via litterfall or when resources are transferred from unvegetated soils, which are also referred to as bare/open or interspace soils through fungal networks (Bachar *et al.*, 2012; Rudgers *et al.*, 2018). Litterfall is dead plant material that has fallen to the ground, and its inputs in drylands are relatively poor due to plant productivity constraints (Cortina and Maestre, 2005). However, litterfall has been shown to increase soil productivity and quality by connecting the tree canopy to the soils beneath it (Pitman, 2013).

## **2.5.2 The importance of litter production and decomposition processes in drylands**

Litter is an important component of the nutrient cycle, and its decomposition may act as nutrient sinks or sources (Dubeux Jr *et al.*, 2007; Lodge *et al.*, 1994). The effects of litter are often reported for traditional agricultural practices (Martínez-García *et al.*, 2021), including preventing soil freezing and erosion, preventing weed infestation, improving mine reclamation, conserving moisture, reducing evapotranspiration and improving the forest ecosystem function (Giweta, 2020). However, few studies have reported on the effects of litter on dryland ecosystem functions.

Unlike forest ecosystems, comparatively little litter accumulates on the interspace soils among plants in dryland ecosystems (Ding and Eldridge, 2021; Ochoa-Hueso *et al.*, 2018). Instead, most of the litter accumulates under the plant (Chuckran *et al.*, 2020; Housman *et al.*, 2007; Throop and Archer, 2009; Throop and Belnap, 2019). While litter quality (the relative quantity of soluble C compounds, non-lignified cellulose/hemicellulose, and lignin) and precipitation may strongly influence decomposition rates in forests ecosystems; soil temperature and the activity of soil biota may have a stronger influence on decomposition rates in drylands (Aragão *et al.*, 2009; Tidiane *et al.*, 2016). In addition to the abovementioned factors, the ratio of carbon to nitrogen is also an indicator of N mineralization and has a strong influence on the rate of litter decomposition (Liu *et al.*, 2018; Yang *et al.*, 2017).

### **2.5.2.1 Soil C/N ratio as a predictor of decomposition**

Soil C/N ratio is defined as the ratio of organic carbon to total nitrogen in the soil, with an optimum ratio of 10 - 15:1 for dryland soils (Plaza *et al.*, 2018). High C/N ratios correlate with low organic matter decomposition and mineralization rates; while

low C/N ratios mean that organic matter may be decomposed and rapidly mineralized (Xu *et al.*, 2019). Materechera and Murovhi (2011) estimated a mature *A. erioloba* tree's annual litter biomass to be 39 kg, with a C/N ratio of 13:1. The high C/N ratio indicates that the biomass of *A. erioloba* trees may quickly decompose, releasing the nutrients contained within, and resulting in higher nutrient levels in canopy soils compared with those beyond the canopy. In addition to the abovementioned factors, litter decomposition is driven by photodegradation in drylands (Maestre *et al.*, 2016a). Findings from a meta-analysis suggest that UV exposure affects both biotic (microbial) and abiotic (photodegradation) litter decomposition processes, with the combined effects resulting in a higher litter decomposition rate (Almagro *et al.*, 2017).

#### **2.5.2.2 Grazing effects on decomposition rates**

Overgrazing is considered one of the main drivers of desertification in drylands (Abahussain *et al.*, 2002; Becerril-Piña and Mastachi-Loza, 2021; Mganga *et al.*, 2018; Middleton, 2018). Grazing may directly alter microclimatic conditions by reducing plant cover, increasing the exposure of litter to UV radiation, and hence, decomposition via photodegradation (Becerril-Piña and Mastachi-Loza, 2021; Chillo *et al.*, 2017; Hidalgo-Galvez *et al.*, 2022; Montenegro-Díaz *et al.*, 2022). By affecting plant species composition, which modifies litter quality and quantity, grazing may also indirectly affect decomposition rates (Li *et al.*, 2022; Roy and Bagchi, 2022; Semmartin *et al.*, 2010; Su *et al.*, 2022; Zhu *et al.*, 2021). In addition, soil analysis consistently revealed that, among the land-use practices, the concentrations of nutrients (including N, P, Ca, Mg, Zn, and Mn) and soil biological properties (organic carbon, particulate organic matter, microbial biomass nitrogen, and microbial

biomass carbon) were highest in fallow, followed by grazing, and bare land (Murovhi and Materechera, 2006). This finding corroborates the idea that grazing, through dung and urine inputs, increase nutrient availability and thus decomposition rates (Naeem *et al.*, 2022; Vaieretti *et al.*, 2013).

### **2.5.3 Plant-microbe interactions**

Plants provide microorganisms with a variety of habitats, including the rhizosphere (Berg *et al.*, 2005; Buee *et al.*, 2009; Pronk *et al.*, 2022; Saad *et al.*, 2020). The rhizosphere is the region of soil surrounding living roots that is under the influence of plant root exudates (De Zelicourt *et al.*, 2013; Philippot *et al.*, 2013a). Within the rhizosphere, mutualistic interactions exist between rhizobacteria and the roots of leguminous plants which improves soil fertility and promotes plant growth (Beukes *et al.*, 2019; Gupta *et al.*, 2015; Igiehon and Babalola, 2018; Zamioudis and Pieterse, 2012). Such bacteria are known as plant-growth promoting rhizobacteria (PGPR) (Jha and Saraf, 2015; Malik *et al.*, 1997; Santoyo *et al.*, 2021). These bacteria can do so by directly promoting the acquisition of nutrients, and by producing phytohormone (Martínez-Viveros *et al.*, 2010; Richardson *et al.*, 2009). Alternately, these bacteria may act indirectly through the induction of resistance in host plants against plant pathogens and abiotic stresses (Gopalakrishnan *et al.*, 2015).

Nutrient deficiency is a concern in drylands. Plant-microbe interactions increase the bioavailability of nutrients that are not readily available for plant use because they exist as either insoluble precipitates or bound to soil organic acids and inorganic components. Plant-growth promoting (PGP) bacteria use various traits for nutrient

mobilization which aid nutrient uptake by plants. The PGP traits exhibited by bacteria in arid and semi-arid regions are listed in Table 1.

**Table 2.1.** Plant growth-promoting traits of bacteria from arid and semi-arid regions (Ayangbenro and Babalola, 2021)

| Organism                                                                                                                                                                                                                                                                                                                           | Host                                                                                                                                                                                                                                                                                                               | PGP Traits                                                                                                                             | Location                                                                                                               |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|
| <i>Bradyrhizobium spp</i> and <i>Serratia marcescens</i>                                                                                                                                                                                                                                                                           | <i>Arachis hypogaea</i>                                                                                                                                                                                                                                                                                            | Nitrogen fixation; phosphorus solubilization                                                                                           | Northern part of Egypt                                                                                                 |
| <i>Bradyrhizobium</i> , <i>Burkholderia</i> , <i>Mesorhizobium</i> <i>Rhizobium</i> and <i>Paenibacillus</i>                                                                                                                                                                                                                       | <i>Matricaria chamomilla</i> , <i>Calendula officinalis</i> , and <i>Solanum distichum</i>                                                                                                                                                                                                                         | Nitrogen fixation                                                                                                                      | Desert region of Egypt                                                                                                 |
| <i>Pelomonas saccharophila</i> , <i>Methylosinus trichosporium</i> , <i>Sinorhizobium sp.</i> , <i>Methylococcus capsulatus</i> and <i>Azorhizobium caulinodans</i>                                                                                                                                                                | <i>Lasiurus indicus</i>                                                                                                                                                                                                                                                                                            | Nitrogen fixation                                                                                                                      | Thar Desert, India                                                                                                     |
| <i>Bacillus</i> , <i>Enterobacter</i> , and <i>Pseudomonas</i>                                                                                                                                                                                                                                                                     | <i>Zygophyllum simplex</i> , <i>Peganum harmala</i> , <i>Notoceras bicornis</i> , <i>Cassia italica</i> , <i>Glinus lotoides</i> , <i>Calotropis procera</i> , <i>Tamarix amplexicaulis</i> , <i>Capparis spinosa</i> , <i>Citrullus colocynthis</i> , <i>Tribulusterrestris</i> and <i>Haloxylon salicornicum</i> | Nitrogen fixation; phosphate and zinc solubilization; hydrogen cyanide, siderophore, indole acetic acid, and salicylic acid production | Almadinah Almunawarah, Kingdom of Saudi Arabia                                                                         |
| <i>Pseudomonas poae</i> , <i>Pseudomonas fluorescens</i> , <i>Pseudomonas trivialis</i> , and <i>Pseudomonas spp</i>                                                                                                                                                                                                               | <i>Hippophae rhamnoides</i>                                                                                                                                                                                                                                                                                        | Phosphate solubilization                                                                                                               | Lahaul and Spiti in the trans-Himalayas                                                                                |
| <i>Duganella violaceusniger</i> , <i>Bacillus megaterium</i> , <i>Paenibacillus dendritiformis</i> , <i>Paenibacillus amylolyticus</i> , <i>Pseudomonas lini</i> , <i>Pseudomonas monteilii</i> , <i>Pseudomonas thivervalensis</i> , <i>Psychrobacter fozii</i> , <i>Stenotrophomonas maltophilia</i> and <i>Stenotrophomonas</i> | <i>Triticum aestivum</i>                                                                                                                                                                                                                                                                                           | Potassium and zinc solubilization; nitrogen fixation; hydrogen cyanide, siderophore and indole acetic acid production                  | Gwalior, Sagar and Indore region of Madhya Pradesh, Jhansi region of Uttar Pradesh and Kota region of Rajasthan, India |

### **2.5.3.1 Nitrogen fixation**

Nitrogen is an essential element for life, particularly for plant growth (Leghari *et al.*, 2016; Richardson *et al.*, 2009; Vitousek *et al.*, 1997). Although recent evidence suggests that drylands store a great amount of N in large subterranean nitrate pools, N is still considered the second most important factor after water that limits biomass production and biological activity in drylands (Ramond *et al.*, 2022). This is because much of this N pool exists in unavailable forms for plant and microbial guilds. To circumvent this limitation, symbiotic relationships between bacteria and plants have evolved. For example, symbiotic relationships exist between leguminous plants and bacteria in the rhizosphere soils. In the rhizosphere, plant root exudates produce metabolites (sugars, amino acids, proteins), which free-living bacteria (*Rhizobium* and *Frankia*) use to convert atmospheric nitrogen (N<sub>2</sub>) to ammonia (NH<sub>3</sub>) through a complex enzyme system known as nitrogenase, thus fixing N for plant uptake (Parray *et al.*, 2016). Examples of rhizosphere diazotrophs detected in hot and cold deserts include bacterial phyla Cyanobacteria, Actinomycetota, Bacillota, and Pseudomonadota and the Euryarchaeota archaeal phylum (Ramond *et al.*, 2022).

### **2.5.3.2 Phosphate solubilization**

Phosphorous (P), after nitrogen (N), is the second limiting macronutrient needed for the development of plants. It is necessary for biological plant processes, including photosynthesis and N-fixation, and is contained in nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids (Kumar *et al.*, 2018; Sharma *et al.*, 2013). Phosphorous is present in soils in both insoluble organic and inorganic forms and only becomes soluble when it is bound to calcium, iron, and aluminium metal

cations ( $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ) (Sharma *et al.*, 2013). When  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Al}^{3+}$  cations bind to P, they form either calcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) in calcareous/alkaline soils or ferrous phosphate ( $\text{FePO}$ ) and aluminium phosphate ( $\text{AlPO}$ ) in acidic soils. The metal phosphate complexes render phosphate insoluble and unavailable for plant utilization. Phosphate solubilizing microorganisms (PSMs) improve P solubility in soils through the action of phosphatase enzymes and the production of organic acids by plant roots (Tian *et al.*, 2021). Therefore, as soil pH decreases, phosphate solubility increases. Phosphate solubilizing microorganisms decrease soil pH in a process known as acidolysis by producing organic acids, which are products of oxidative respiration or fermentation when glucose is used as a carbon source (Billah *et al.*, 2019; Satyaprakash *et al.*, 2017). The carboxylic or hydroxyl groups of the organic acids chelate with the metal cations bound to the phosphate compound and immobilize P. The organic acids chelate to the metal cation bound to the phosphate compound through their carboxylic or hydroxyl groups and immobilize P. The *Bacillus*, *Pseudomonas*, and *Rhizobium* genera of bacteria are known to be the most effective PSMs, which primarily produce gluconic acid and 2-ketogluconic acids for phosphate solubilization (Kalayu, 2019; Naseem *et al.*, 2018).

### **2.5.3.3 Potassium solubilization**

Along with nitrogen and phosphorous, potassium (K) is the third most essential plant nutrient, and its limitation in soils is considered a deficiency (Raghavendra *et al.*, 2016). Apart from being an essential plant nutrient, K also confers resistance to biotic (e.g., pathogens, insects, and weeds) and abiotic (e.g., drought, salinity, cold, frost, and waterlogging) factors that can hinder plant growth and development (Wang *et al.*, 2013). Potassium exists in three forms in soils, namely: unavailable K, slowly

available K, and readily available K. However, 90-98% of K in soils is from mineral sources (e.g., feldspars and micas) which are tightly bound and inaccessible to plants (Prajapati and Modi, 2012). Despite being a slow process that occurs over long periods, the weathering of these minerals releases some K into soils. However, the primary mechanism of potassium solubilization in soils is carried out by potassium solubilizing microorganisms (KSMs). Like with P, KSMs mobilize K by acidolysis, complexolysis, and chelation that release K from organic acids (Yadav and Sidhu, 2016). *Arthrobacter*, *Burkholderia*, *Bacillus*, *Pseudomonas*, and *Paenibacillus* are examples of PGP potassium-solubilizing rhizobacteria (Ayangbenro and Babalola, 2021).

#### **2.5.3.4 Trace elements solubilization**

In contrast to the aforementioned macronutrients (N, P, K), trace element pools in terrestrial ecosystems are low. The accumulation of trace elements in soils is mainly due to anthropogenic sources than natural deposition (Glina and Bogacz, 2013). Soil trace elements are critical for cell growth and act as cofactors in photosynthesis and other biochemical pathways (Moreno-Jiménez *et al.*, 2019). The solubilization of trace elements (Cu, Fe, Mn, and Zn) in the soil is facilitated by plant growth-promoting microorganisms through several mechanisms, including a decrease in soil pH and the use of chelating agents (Ayangbenro and Babalola, 2021). In its ferrous ( $\text{Fe}^{2+}$ ) form, iron is soluble at a soil pH of 7.35–7.40, yet insoluble in its ferric ( $\text{Fe}^{3+}$ ) form (Bou-Abdallah, 2010). In the rhizosphere soil, where microorganisms and plants compete for Fe, PGP rhizobacteria acquire Fe through siderophore production (Jian *et al.*, 2019). Genera belonging to the phyla Actinobacteria, Proteobacteria, and Firmicutes were shown to produce siderophores with a high affinity and specificity for

iron complexation, evidence of their PGP activities (Ferreira *et al.*, 2019; Wang *et al.*, 2014). Siderophores capture  $\text{Fe}^{3+}$  and reduce it to ferrous  $\text{Fe}^{2+}$ , increasing its availability (Colombo *et al.*, 2014). Apart from binding with ferric iron, siderophores are critical in the chelation of metals such as  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Eu}^{3+}$ , and  $\text{Pb}^{2+}$  (O'Brien *et al.*, 2014; Saha *et al.*, 2013; Schalk *et al.*, 2011).

#### **2.5.3.5 Plant-microbe interactions alleviate abiotic stress**

Plant-growth promoting rhizobia (PGPR) genera like *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Paenibacillus*, and *Bacillus*, were shown to increase plant stress resistance in drylands (Sandhya *et al.*, 2010; Saul-Tcherkas and Steinberger, 2009; Zhang *et al.*, 2010). Several mechanisms are used by PGPR to impart stress tolerance in bacteria, (Figure 1.5). For example, the production of exopolysaccharides (EPS) that are responsible for the structure and integrity of bacterial biofilms. They are made up of mostly water and impart protection against desiccation (Bhaskar and Bhosle 2005). Three *Bacillus* species strains identified by 16s rDNA sequence analysis as *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis* were isolated from the rhizosphere of plants grown for food in various semi-arid regions of India and subjected to water stress (Vardharajula, 2021). An increased EPS production in all three *Bacillus* species strains with increasing water stress indicated their tolerance to drought stress. Other mechanisms for alleviating abiotic stress in drylands include the production of phytohormones, the production of heat shock proteins, antioxidant defence, accumulation of osmolytes, and antioxidant defence (Kaushal and Wani, 2016).

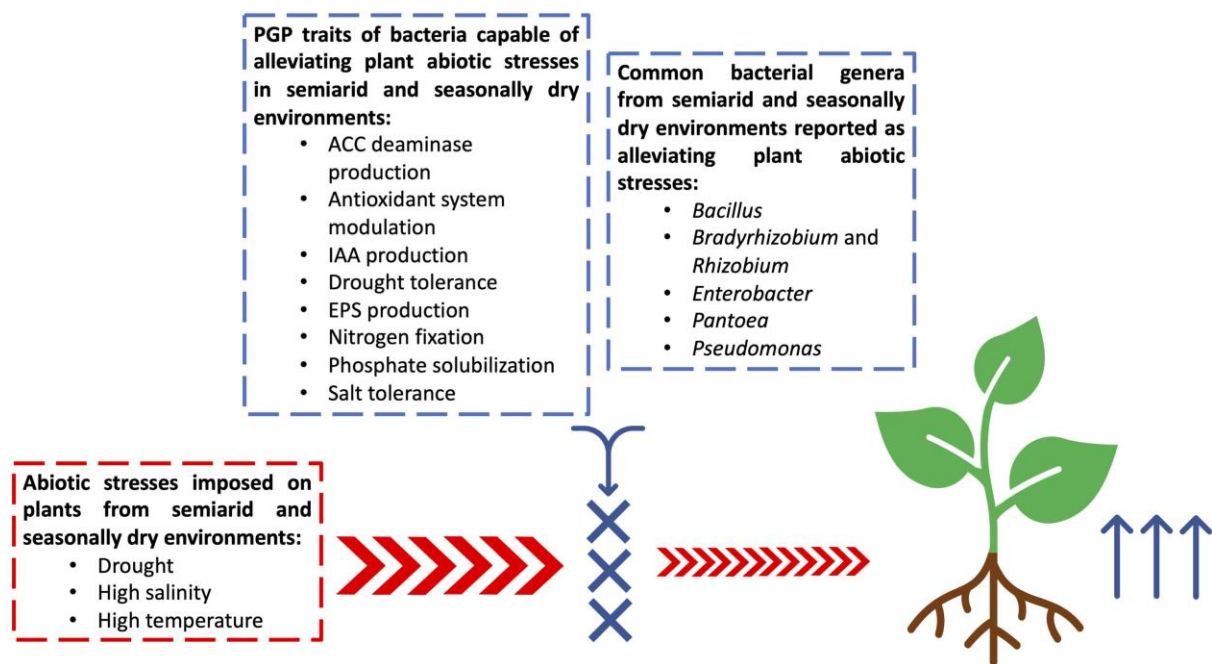


**Figure 2.4.** Plant growth-promoting bacteria from semi-arid, semiarid and seasonally dry conditions, as well as their plant growth-promoting traits, can alleviate drought and salinity stress (Bonatelli et al., 2020).

## 2.5.4 Key edaphic factors that affect soil bacterial diversity

### 2.5.4.1 Soil pH

Soil pH is frequently reported as a driver of bacterial diversity across ecosystems. A plausible explanation is its effect on rhizosphere interactions by changing plant root exudation patterns. Although root exudation patterns are not fully understood, they are linked to soil nutrient mobilization. Soil pH affects the availability of macronutrients and micronutrients in soils. Acidolysis and complexolysis are two mechanisms that improve the solubilization of nutrients in soils upon the secretion of organic acids by plant roots. By decreasing the pH in rhizosphere soils, these mechanisms improve the chemical properties of soil, which control the composition

and diversity of soil bacterial communities in agricultural and dry ecosystems (Zhang *et al.*, 2019b).

#### **2.5.4.2 Soil texture**

After soil pH, soil texture is the second most important factor affecting the diversity of microbial communities in soils (Schulz *et al.*, 2012; Xia *et al.*, 2020; Xue *et al.*, 2018). Soil texture refers to the relative proportions of sand, silt, and clay particles in soils, known to control soil processes such as structure formation, carbon sequestration, nutrient retention, and water infiltration and storage (Twarakavi *et al.*, 2010; Xia *et al.*, 2020). Physicochemical properties (e.g., carbon, nutrients, aeration, proton concentration, and water availability) and soil microbes vary in the ecological niches formed between soil aggregates, which are groups of soil particles built from mineral and organic compounds with stronger bonds between them than adjacent particles (Mills, 2003; Yudina and Kuzyakov, 2019). Seaton *et al.* (2020) reported bacterial diversity in drier soils to be higher in clay and slit soils than in sandy soils. This is due to the small pores sizes of clay soils compared to sandy soils, which have a high water retention capacity necessary for resource diffusion and bacterial migration (Xia *et al.*, 2020). In a case study of the Laiwu experimental site in China, shallow soil bacteria were mainly affected by mineral components (Zhang *et al.*, 2020). The redundancy analysis (RDA) results of the study showed that amphibole and clay minerals were positively correlated with the dominant bacterial taxa Actinobacteria, Proteobacteria, and Acidobacteria.

#### 2.5.4.3 Soil temperature

The end of this century is expected to see a rise in global temperatures, with drylands experiencing higher temperature increases than humid areas (Huang *et al.*, 2017; Solomon, 2007). This will affect C and N biogeochemical cycling by affecting microbially mediated soil processes, thus altering microbial structure and diversity. Dai *et al.* (2020) demonstrated that irrespective of vegetative cover, increases in temperature enhances the microbial transformation of organic N to its mineralized forms. The temperature did not affect ammonia monooxygenase subunit A (*amoA*) or ammonia monooxygenase subunit B (*amoB*), while functional copper-containing nitrite reductase genes (*nirK*, *nirS*) and nitrous oxide reductase genes (*nosZ*) were upregulated. The elevated rates of nitrification and denitrification resulting in equivalent nitrous oxide emissions have positive climate warming feedbacks (Griffis *et al.*, 2017).

While N mineralization is dependent on the C/N ratio of the decomposable material, decomposition is independent of N mineralization (Dai *et al.*, 2020). In terrestrial ecosystems, plant litter is an important source of soil organic carbon (SOC) (Zeng *et al.*, 2017). Temperature increases accelerate litter decomposition resulting in C losses from soils as atmospheric CO<sub>2</sub>. According to Link *et al.* (2003), soil warming and drying resulted in a 32% reduction in soil carbon over a five-year period, which is much faster than the reductions in soil C that have been observed due to increased tillage. However, when microbes move from using labile C types to more recalcitrant C types, the decline in C content can be mitigated over time (Frey *et al.*, 2013). Additionally, the available C type may select for the succession of microbial communities capable of utilizing the specific C type (Waldrop and Firestone, 2004).

Analysis of 16S rRNA gene composition of nucleotide analog-labeled DNA by PhyloChip microarrays revealed that Proteobacteria and Actinobacteria were the predominant phyla that increased in relative abundance with labile organic substrates (i.e., glycine, sucrose) (Goldfarb *et al.*, 2011).

## **2.6 Microbial responses to dryland conditions**

Several theories have been used to explain the factors that drive microbial community composition following environmental disturbance (Coveley *et al.*, 2015; Liu *et al.*, 2021; Song *et al.*, 2017). For instance, there is some evidence showing that microbial communities may be resistant or resilient to ecosystem changes (Allison and Martiny, 2008; Bardgett and Caruso, 2020; Shade *et al.*, 2012). While resistance is defined as an ecosystem's ability to resist a disturbance; resilience is defined as an ecosystem's ability to recover following a disturbance, hence regaining its original structure and function (Chambers *et al.*, 2014; Connell and Ghedini, 2015; Griffiths and Philippot, 2013; Nimmo *et al.*, 2015). Alternatively, microbial communities may exhibit functional redundancy (Allison and Martiny, 2008; Huang *et al.*, 2020; Philippot *et al.*, 2021), which occurs when multiple distinct organisms perform similar metabolic functions (Louca *et al.*, 2018; Trivedi *et al.*, 2020). Ultimately, how microbial communities respond to environmental disturbances depends on the disturbance, community, and habitat properties that modulate the effects of community assembly processes (Figure 2.5) (Philippot *et al.*, 2021).

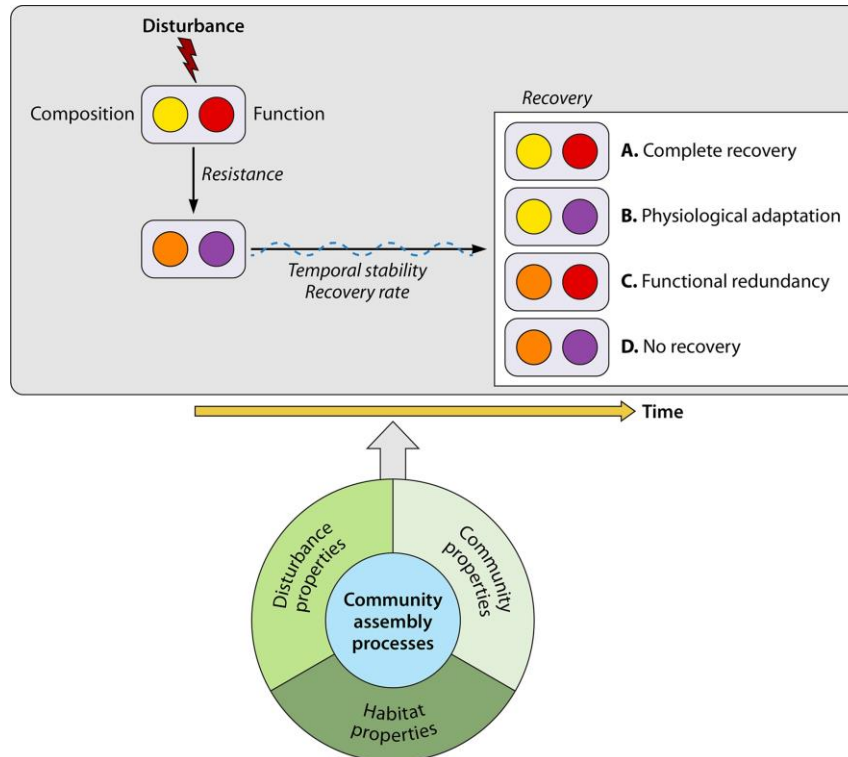


**Figure 2.5.** A schematic representation of how a disturbance can change microbial composition and function over time. Following an environmental disturbance, microbial communities may over time A. completely recover, B. physiologically adapt (only composition recovers but not function), C. become functionally redundant (only function recovers but not composition), and D. not recover (Philippot *et al.*, 2021).

### 2.6.1 Microbial capacities in determining resistance and resilience to dryland conditions

The extent to which dryland microbial communities may be able to circumvent the effects of climate change varies. This variation is determined by their capacity to undergo dormant states, inherent dispersal rates, metabolic diversity, and evolutionary life strategies (Philippot *et al.*, 2021; Shade *et al.*, 2012). Bacteria can display a wide spectrum of life strategies depending on resource availability. As a

result, bacteria can modify their life strategy to maximise their chances of survival under different biotic and abiotic conditions (Beattie and Lindow, 1999; Hatton *et al.*, 2021; Hibbing *et al.*, 2010; Noll *et al.*, 2005). These strategies have been referred to as *r*- and *k*-selection strategies, or copiotrophic and oligotrophic strategies (Fierer *et al.*, 2007; Guo *et al.*, 2022; Ho *et al.*, 2017; Malik *et al.*, 2020). While *r*- and *k*-selection explains microbial population growth rates, copiotrophic and oligotrophic strategies describe their affinity for substrate availability (Fierer *et al.*, 2007; Ho *et al.*, 2017; Li *et al.*, 2021b). Copiotrophs grow faster (*r*-strategists), rely on resource availability, and have an affinity for nutrient-rich soils, whereas oligotrophs (*k*-strategists) efficiently exploit resources in nutrient-poor soils at the expense of growth rate (Chen *et al.*, 2021; Koch, 2001; Song *et al.*, 2017).

The prevalence and prominence of members of the phyla Proteobacteria, Acidobacteria, and Actinobacteria, which constitute an average of 39%, 20%, and 13% of soil bacterial communities, respectively, may be linked to their life strategies (Ho *et al.*, 2017; Janssen, 2006). Previous studies suggest that Acidobacteria may be associated with oligotrophic environments (Fierer *et al.*, 2007; Kielak *et al.*, 2016; Yergeau *et al.*, 2012). Fierer *et al.* (2007) demonstrated that Acidobacteria predictably meets the presumption of an oligotroph by predominating in carbon-poor soils and in the bulk soil, where bioavailable resources are present at relatively lower concentrations than in the rhizosphere. This was confirmed based on empirical evidence from soils sampled at different locations ( $n = 71$ ), in addition to the results of a meta-analysis. The ability to use complex substrates, such as xylan, hemicelluloses, pectin, starch, and chitin, further corroborates the finding that Acidobacteria are oligotrophs (Belova *et al.*, 2018; Costa *et al.*, 2020).

In addition to carbon, nitrogen also influences the predominant life strategies of soil microbial communities (Fierer *et al.*, 2012a; Männistö *et al.*, 2016; Ramirez *et al.*, 2012). The phylum Proteobacteria is a metabolically diverse group capable of using both carbon and nitrogen. For instance,  $\gamma$ -Proteobacteria may metabolise a diverse array of C compounds, yet they have been found at low abundances in comparatively C-poor bulk soils (Fierer *et al.*, 2007; Goldfarb *et al.*, 2011). In contrast, the relative abundances of  $\gamma$ -Proteobacteria, Bacteroidetes, and Actinobacteria were associated with rapid growth in response to the addition of labile organic matter or nitrogen, indicative of a copiotrophic life strategy (Fierer *et al.*, 2007; Fierer *et al.*, 2012a; Goldfarb *et al.*, 2011; Leff *et al.*, 2015). Several  $\alpha$ - and  $\beta$ -Proteobacteria are known for their N-fixing capabilities (Burbano *et al.*, 2015; Velázquez *et al.*, 2017; Wolińska *et al.*, 2017) further supporting findings that members of this phylum are metabolically diverse.

Despite the finding that the application of nitrogen increases the abundance of members of the phylum Actinobacteria in agricultural soils, their prevalence in nutrient-poor soils suggests that some may be oligotrophic (Dai *et al.*, 2018; Song *et al.*, 2017; Yan *et al.*, 2021). This metabolic versatility often results in inconsistencies when reporting microbial life strategies but may also reflect the adaptative strategies of these phyla in coping with resource scarcity. In addition, several Actinobacteria have been recovered from extremely hot habitats such as hyper-arid and arid regions (Maestre *et al.*, 2021; Makhalanyane *et al.*, 2015a; Mohammadipanah and Wink, 2016). This is unsurprising given their capacity for sporulation, a specialized developmental program employed by a diverse set of bacteria, which culminates in

the formation of dormant cells displaying increased resilience to stressors (Beskrovnaya *et al.*, 2021; Makhalanyane *et al.*, 2015a; Rateb *et al.*, 2011).

Although well represented in arid and semi-arid soils, Chloroflexi and Firmicutes are found in relatively low abundances in these regions (Janssen, 2006; Mhete *et al.*, 2020). The extremely low abundance of these phyla may be due to their ability to adopt dormant states (Ortiz *et al.*, 2021). The ability to form heat-resistant endospores allows members of the phylum Firmicutes to survive environmental challenges, including osmotic pressure, heat, desiccation, and high UV radiation (Bukar *et al.*, 2019; Makhalanyane *et al.*, 2015a; Sharmin *et al.*, 2013). Members of the phylum Chloroflexi may also withstand desiccation due to their complex cell wall structure (Sutcliffe, 2011). In addition, Chloroflexi are best known as photoheterotrophs due to their capacity to preserve energy while dormant by using light as an alternative energy source (Ward *et al.*, 2018; Wu *et al.*, 2009).

## **2.7 Summary**

Drylands are characterized by water deficit, sparse vegetation cover, and extreme temperatures (Berg and McColl, 2021; Bouallala *et al.*, 2020; Middleton and Sternberg, 2013). Due to these environmental conditions, microbial communities are expected to play important functional roles in C and N biogeochemical cycling (Davidson and Janssens, 2006; Ochoa-Hueso *et al.*, 2016; Schimel and Schaeffer, 2012). There is evidence that microbial diversity in drylands is influenced by biotic and abiotic factors, which include soil physicochemical properties and temperature (Pajares *et al.*, 2016; Vargas-Gutierrez, 2015). As anthropogenic CO<sub>2</sub> levels continue to rise, global temperatures will continue to increase (Al-Ghussain, 2019;

McGowan *et al.*, 2020). This will significantly impact ecosystem resources and functions in drylands. Vegetation cover is suggested to mitigate the effects of rising temperatures through interactions with soil bacterial communities to influence the distribution of soil nutrients and impart resistance to abiotic and biotic stress. Furthermore, bacterial communities in dryland soils have developed adaptive mechanisms which allow them to persist under temperature perturbations (Ye *et al.*, 2020).

## **2.8 Problem statement**

Climate change and global warming have led to drastic effects on the biodiversity and function of ecosystems (Bouazid *et al.*, 2014; Burrell *et al.*, 2020; Loo *et al.*, 2015; Ochieng *et al.*, 2016; Tietjen *et al.*, 2017). According to the 6<sup>th</sup> assessment report of the IPCC, anthropogenic greenhouse gas emissions resulted in approximately 1.1°C of warming between 1850 and 1900. In addition, the report states that average global temperatures are predicted to increase on average above 1.5°C during the next 20 years (Arias *et al.*, 2021; IPCC, 2021). The effects of this increase may be increased incidents of extreme heatwaves, prolonged summers, and shorter winters. The latter is expected to have direct impacts on human health and the sustainability of agriculture (Mbokodo *et al.*, 2020; Wang *et al.*, 2021). The impacts of these changes have been the subject of extensive studies over the past decade (Bronstert, 2003; Dawson *et al.*, 2003; Piao *et al.*, 2010; Turner *et al.*, 2010). The insights from these studies are crucial for future planning (Araos *et al.*, 2016; Jones *et al.*, 2012; Watson *et al.*, 2012). Yet remarkably, we lack comparable insights regarding the effects of temperature increases on the function and diversity of soil microbiomes in

arid environments. This understanding is crucial for ensuring the sustainability of soil ecosystem services in arid environments.

Soil microbiomes may potentially mitigate the effects of climate change (Jansson and Hofmockel, 2020). The Earth Microbiome Project and recent advancements in DNA sequencing have improved our understanding of the biotic and abiotic factors that control microbial community structure across different geographies (Thompson *et al.*, 2017). However, despite these efforts, many African soil resources, in particular organic matter, and nutrient stocks remain largely unmapped (Guerra *et al.*, 2020; Hengl *et al.*, 2021; Jones *et al.*, 2013; Mutsaers *et al.*, 2017; Voortman, 2010). Soils vary markedly in their biotic and abiotic properties, making it difficult to generalize the impact of climate change on soil microbiomes across different spatial scales (Alzarhani *et al.*, 2019; Amelung *et al.*, 2020; Delgado-Baquerizo *et al.*, 2020; Islam *et al.*, 2020). For example, in comparison to other drylands, Australian dryland soils are more acidic and nutrient deficient with unique microbial communities (Eldridge *et al.*, 2018). In addition, soil pH was reported to vary across different soil layers in the hilly region of Chongqing, southwestern China (Zhang *et al.*, 2019b). It has been established that the topsoil, temperature, precipitation, and topography substantially influence soil pH variation. In the layers beneath the topsoil, parent material (generally bedrock) influenced soil pH. In other studies, vegetation and topography explained the distribution of SOC stocks at a local scale, while temperature and precipitation explained the distribution of SOC stocks at a regional scale (Oueslati *et al.*, 2013; Wiesmeier *et al.*, 2013). It is, therefore, crucial to study climate change effects at local scales, as the microbial composition may differ across various spatial scales subject to a range of biotic and abiotic factors. This study

hopes to elucidate the influence of temperature on the bacterial communities in arid soil microbiomes in Mahikeng.

### **2.8.1 Aim**

To determine the influence of temperature on the bacterial communities in arid soil microbiomes.

### **2.8.2 Hypothesis**

We hypothesize that temperature increases may induce shifts in the structure of arid soil bacterial communities.

### **2.8.3 Objectives**

1. To determine the influence of temperature increases on the arid soil bacterial community structure in samples retrieved below an *Acacia* canopy and those in adjacent soils, using 16S rRNA gene amplicons.
2. To assess the influence temperature increase has on soil physicochemical properties in samples retrieved below an *Acacia* canopy and those in adjacent soils.

## Chapter 3 – Materials and Methods

### 3.1 Study area, plot establishment and soil sampling

This study area was located on the North-West University agricultural farm (25°4 S and 25°36 E) (also known as Molelwane Farm) outside Mahikeng in the North West Province of South Africa, (Figure 2.1). The farm, located 260 km west of Johannesburg, shares an international border with Botswana. Molelwane Farm is comprised of open veld at an elevation of 1 500m, north of the Upper Molopo River (Ngoma *et al.*, 2013). Mahikeng is located in a semi-arid climate with mean annual precipitation ranging from 500 to 600mm. Most of this rain falls during summer between October and April (Munyati and Drummond, 2020; Thomas *et al.*, 2007). According to the South African Weather Services (SAWS) (SAWS, 2019), the average minimum and maximum temperatures in October for the years 2015, 2016 and 2017 were 17.3°C and 33.2°C, 15.8°C and 31.4°C, 2.7°C and 29.1°C, respectively.

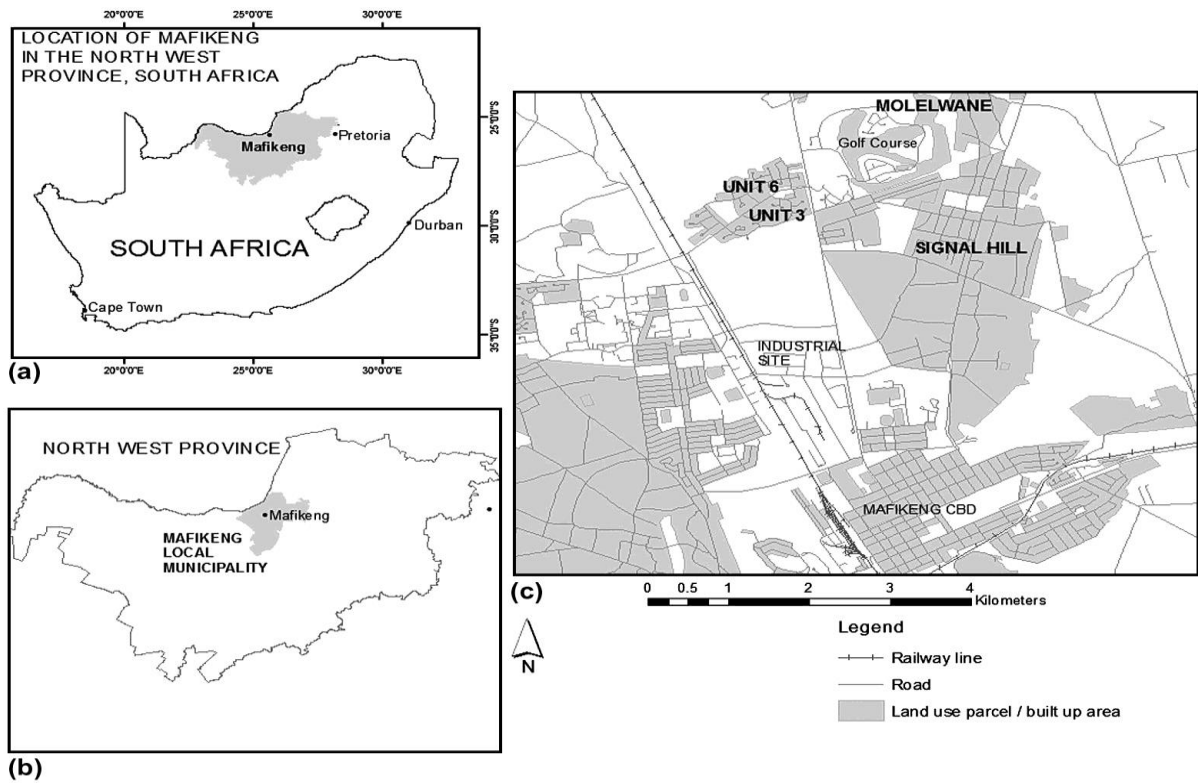


**Figure 3.1.** The location of Mafikeng renamed Mahikeng in (a) South Africa, (b) in the North West Province, and (c) the location setting of Molelwane suburb utilised in the study (Munyati and Motholo, 2014).

We established a 40 x 40m plot and recorded the three dominant plant species namely, *A. erioloba*, *A. tortilis*, and *A. herbeclada*. Soil samples were collected from an uncultivated area (Figure 2.2.a) on the farm on 19 October 2018. We collected surface soil samples below the canopy of an *A. erioloba* tree (Figure 2.2.b) and from unvegetated soils located 10m from the tree (Figure 2.2.c). For this study, these samples are referred to as the canopy and open soils, respectively. The collected soils were placed in sterile Whirl- Pak® bags (NASCO, U.S.A) and stored on ice before being transported to the laboratory. Ten sub-samples were collected at a sampling depth (0 – 15cm) from randomly selected spots at each location. The sub-samples, of each location, were bulked to obtain two composite soil samples of

roughly 4kg each. The bulk soils were air-dried, sieved through a 2mm sieve, and stored until further analysis. The soils were used to construct experimental systems to assess the effects of temperature.



**Figure 3.2.** (a) Sampling site on the NWU agricultural farm (b) Soil sampling of canopy soils from an *A.eriobola* tree (c) Soil sampling of open soils 10m away from the canopy of an *A.eriobola* tree.

### 3.2 Microcosm experiments

The microcosms consisted of sterile PVC piping (4 x 5.5 cm) with the base covering made of sterile aluminium foil secured with cable ties. A total of 102 soil microcosms, established in triplicates containing approximately 70g of soil each, were constructed to determine the effects of elevated temperatures on microbial communities. Of the 102 soil microcosms, 34 soil microcosms were set aside and maintained at 25°C to be used as a baseline for comparison between those incubated at control (30°C) and treatment (40°C) temperatures. Experimental manipulations were conducted over forty days. For this study, destructive sampling is defined as the removal of soil microcosms from incubators for DNA extraction and soil physicochemical analysis. Destructive sampling, at pre-determined times, was done to assess the effects of temperature changes on soil microbiomes. Samples were taken 5 days, (-5) prior to

the start of the experiment, and on days 0, 1, 2, 8, 16, 24, 32, and 40. Each microcosm was randomly assigned to the baseline (25°C) or control (30°C) or treatment (40°C) group to reduce the possibility that any difference found between the three groups may be due to the treatment or any other confounding variable. The control was chosen based on the average day-time temperature, during October and November from 2016 – 2017, of the sampling location (SAWS, 2019). The controls from both canopy and open soils were maintained at this average temperature throughout the experiment. The treatment microcosms were subjected to a constant temperature, which was approximately 10°C higher than their average temperature for the entire experiment. The treatment temperature was applied by a 1.8°C increment per day, for five days, before the beginning of the experiment. Triplicate microcosms from each temperature treatment (25°C, 30°C, 40°C) were removed from the incubators on experimental days and stored at -80°C for subsequent analysis.

### **3.3 Soil physicochemical analysis**

Soil samples were analysed for pH, extractable inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ), organic carbon, extractable elements (K, Ca, Mg, Na, Fe, Cu, Zn, Mn, P), and soil particle size as outlined by the Agri Laboratory Association of Southern Africa (AgriLASA) – Soil Handbook (AgriLASA, 2004).

#### **3.3.1 Soil pH**

Soil pH was measured by placing a 1:2.5 soil-to-water ratio suspension on a mass basis (Sparks *et al.*, 2020). 10g of dried soil was placed in a glass beaker and 25 cm

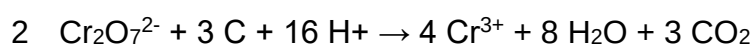
of deionized water was added. The solution was stirred rapidly for 5 seconds with a glass rod and allowed to stand for 50 minutes. The solution was then stirred and allowed to stand for a further 10 minutes. pH was measured by dipping electrodes in the supernatant.

### **3.3.2 Extractable nitrogen**

Inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ) was extracted by adding 4g of soil to 40 ml of 2M potassium chloride (KCl). The mixture was agitated for 1 hour on a shaker, filtered, and analysed using a spectrophotometer within 24 hours as detailed in the standard protocols (AgriLASA, 2004).

### **3.3.3 Soil organic carbon**

The amount of organic carbon present in the soil was quantified using the Walkley-Black chromic acid wet oxidation method (Walkley and Black, 1934). Dichromate ( $\text{KCr}_2\text{O}_7$ ) oxidized the organic matter. The heat produced when mixing two volumes of  $\text{H}_2\text{SO}_4$  with one volume of  $\text{KCr}_2\text{O}_7$  aided the reaction, according to the following reaction:



The remaining  $\text{KCr}_2\text{O}_7$  was titrated with iron (II) ammonium sulphate solution. The reduced dichromate was assumed to be equivalent to the organic C present in the sample.

### **3.3.4 Extractable elements**

The following elements K, Ca, Mg, Na, Fe, Cu, Zn, Mn, and P were analysed using Mehlich 3 (M3) extraction solution (AgriLASA, 2004). Mehlich 3 extract is composed of 0.2 M glacial acetic acid, 0.25 M ammonium nitrate, 0.015 M ammonium fluoride, 0.013 M nitric acid, and 0.001 M ethylene diamine tetraacetic acid (EDTA). Three grams of soil was added to 30ml of M3 extraction solution, agitated for 5 minutes, filtered, and analysed using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry, Spectro Genesis, SPECTRO Analytical Instruments GmbH & Co. KG, Germany).

### **3.3.5 Soil texture**

The distribution of particle sizes (% sand, clay, and silt) in soil was determined with a hydrometer in a non-destructive soil suspension undergoing settling (Bouyoucos, 1962). The hydrometer analysis uses a dispersing agent, Calgon (35.7g of sodium hexametaphosphate and 7.94g of sodium carbonate in 1L deionized water), that is considered the most effective dispersing agent (Kaur and Fanourakis, 2016). Soil texture was obtained from the particle size distribution by using a textural triangle. A soil texture triangle is used to classify the texture class of soil. The sides of the soil texture triangle are scaled for the percentages of sand, silt, and clay and are read from left to right.

### **3.4 Molecular ecology analysis**

#### **3.4.1 DNA extraction**

Approximately 0.25g per soil sample was used for DNA extraction using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications, with the following amendment: DNA was stored in 50uL of elution buffer to increase yields. DNA concentrations were measured using a NanoDrop Spectrophotometer 2000 (Thermo Scientific, USA). The concentrations of the DNA were determined by the optical density reading generated, and the quality was determined by 260/280 and 260/230 absorbance ratios. The DNA was also visualized on Agarose gels to assess quality as detailed in section 2.4.3.

#### **3.4.2 Polymerase Chain Reaction (PCR) to determine DNA quality**

All PCR reactions were performed at a final volume of 20 µl per reaction. Briefly, 3 µl of DNA, 1.0 µl of forward and reverse primers, as well as 6.5 µl of water were added to the Quick-LoadTaq2X Master Mix (New England Biolabs Inc, USA). The universal bacterial/archaeal 16S rRNA gene primers were used, 341F (5'CCTACGGGAGGCAGCAG-3') and 908R (5'-CGTCAATTCMTTGTGAGTT-3') to amplify fragments. The PCR reaction was performed on a BioRad T100TM Thermocycler (BioRad, Munich, Germany) as detailed previously (Lebre *et al.*, 2021). The amplification conditions consisted of 30 cycles of denaturation (95°C for 5 minutes 30 seconds), annealing (55°C for 30 seconds), and extension (72°C for 1 minute 30 seconds), followed by a final extension (10 minutes at 72°C) and an infinite hold of 4°C.

### **3.4.3 Agarose gel electrophoresis**

PCR products and DNA was assessed using a 1% (w/v) agarose gel stained with 6X loading dye (Thermo Scientific, USA), run at 90V for 45 minutes, and visualized on a Gel Doc XR system (BioRad, Germany).

### **3.4.4 16S rRNA gene sequencing**

All DNA samples were sent to Laragen, Inc for sequencing (Laragen Inc, USA). The V4 - V5 variable regions of the 16S rRNA gene were amplified using PCR primer pair 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada *et al.*, 2016; Quince *et al.*, 2011). PCR amplification was performed using Platinum Hot Start PCR Master Mix from ThermoFisher with cycle conditions: initial denaturation of 94°C for 3 minutes, followed by 35 cycles of 94°C for 45 seconds, 50°C for 60 seconds, 72°C for 90 seconds, followed by a final elongation step of 10 minutes was performed. Amplicons were confirmed on an agarose gel, and samples with an expected band size of 300 - 350bp were pooled and purified according to the manufacturer's instructions using the MoBio UltraClean PCR Clean-Up Kit. The 16S rRNA gene amplicons (2 x 300bp) were then sequenced on a Miseq platform (Illumina, San Diego, USA) following the manufacturer's guidelines.

### **3.4.5 Sequence analysis**

Sequence data were processed using the QIIME2 pipeline v 2017.6.0 (Bolyen *et al.*, 2019). Sequences were demultiplexed, quality filtered, denoised and processed to remove chimeric sequences using the DADA2 pipeline (Bolyen *et al.*, 2018). Low

quality reads were removed by truncating reads to 280bp in length at an average quality score of 30. Quality sequences were then clustered into amplicon sequence variants (ASVs). Representative sequences were aligned using a multiple sequence alignment program (MAFFT) (Katoh and Toh, 2008). A phylogenetic tree was computed using fasttree (Katoh and Toh, 2008). All bacterial taxonomies were assigned to an ASV using SILVA 138 database (Quast *et al.*, 2012).

### **3.4.6 Statistical analysis**

All statistical analyses were performed using the R package version 3.6.2 (R core Team 2019). Soil physicochemical variables were visually represented using ggplot2 (v3.3.3) in R (Wickham *et al.*, 2016). The `geom_signif` and the `stats_compare_means` function were used to determine significant differences between microhabitats and across the different incubation temperatures. Alpha diversity parameters (Observed, Shannon and InvSimpson) were calculated using the phyloseq (v1.30.0) R package (McMurdie and Holmes, 2013). Analysis of variance (ANOVA) was carried out to test for significant differences in diversity between each group using the vegan (v2.5-6) R package (Oksanen *et al.*, 2013). A Venn diagram, showing the number of shared and unique ASVs, was generated using the `comb` function in the vegan R package. The `prune_phyla` function of the phyloseq package in R was used to select the major phyla with a relative abundance of greater than 1%. The relative abundance of the major phylum across each microhabitat was visualized as stacked bar plots, to infer putative relevance.

Bacterial community structure was visualized using non-metric multidimensional scaling (NMDS) ordination plots using unifracs distances in the R phyloseq package

(McMurdie and Holmes, 2013) and visualized using ggplot2 (Wickham, 2016). Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations was determined using the Adonis (Anderson, 2001) function in R to test whether temperature or microhabitat contributed to differences in bacterial community composition. The detrended correspondence analysis (DCA) function in phyloseq was used to determine which ordination model to use, canonical correspondence analysis (CCA) or redundancy analysis (RDA), based on whether the data was linear or unimodal in distribution.

Soil physicochemical data were standardized, and pair-wise distances were computed based on Euclidean distances. Microbial relative abundances were log10 transformed, and the Bray-Curtis distance measure was used to generate a dissimilarity matrix. A distance-based redundancy analysis (db-RDA) plot was generated to identify the significant environmental variables that contributed to the variation in bacterial community composition. ANOVA was used to determine which soil physicochemical properties contributed to significant differences in bacterial diversity.

## Chapter 4 – Results

### 4.1 Microcosm Experiment and Soil Physicochemistry

Microcosm experiments were established to determine the influence of temperature on the bacterial communities in arid soil microbiomes. Temperature and relative humidity were measured over the entire forty-day period of the experiment. The temperature for the control samples (30°C) and treatment (40°C) did not deviate substantially from the set temperatures, (Figure A1, **Appendix 1**). Fifteen soil physicochemical variables were measured throughout the microcosm experiment on days 0, 1, 2, 8, 16, 24, 32, and 40. We tested the influence of increasing temperatures and microhabitats on the soil physicochemical variables.

#### 4.1.1 The influence of temperature on soil physicochemical properties

Temperature effects on the soil physicochemical properties across both microhabitats (canopy and open soils) appeared to be unnoticeable, and not significant between soils subjected to control (30°C) and treatment (40°C) temperatures (Kruskal-Wallis,  $p > 0.05$ ). However, with reference to the baseline (25°C) temperature, we observed significantly higher variations in the C content, Cu, and  $\text{NH}_4^+$  concentrations in open soils subjected to control (30°C) and treatment (40°C) temperatures, (Figure 3.1.a, b & c). A comparison of open soils incubated at the control (30°C) and treatment (40°C) temperatures revealed that the C content and  $\text{NH}_4^+$  concentration decreased with increasing temperature, (Figure 3.1.a & c). However, the observed decrease was not significant. In contrast, although not

significant, we observed an increase in the Cu concentration with increasing control (30°C) and treatment (40°C) temperatures in open soils, (Figure 3.1.b).

In canopy soils, we observed significantly higher variations in the Fe concentration between the baseline (25°C) and treatment (40°C) temperature groups, (Figure 3.1.d). Zinc concentrations showed significantly higher variations with temperature increases between the baseline (25°C) and control (30°C) or treatment (40°C) temperature groups, (Figure 3.1.e).

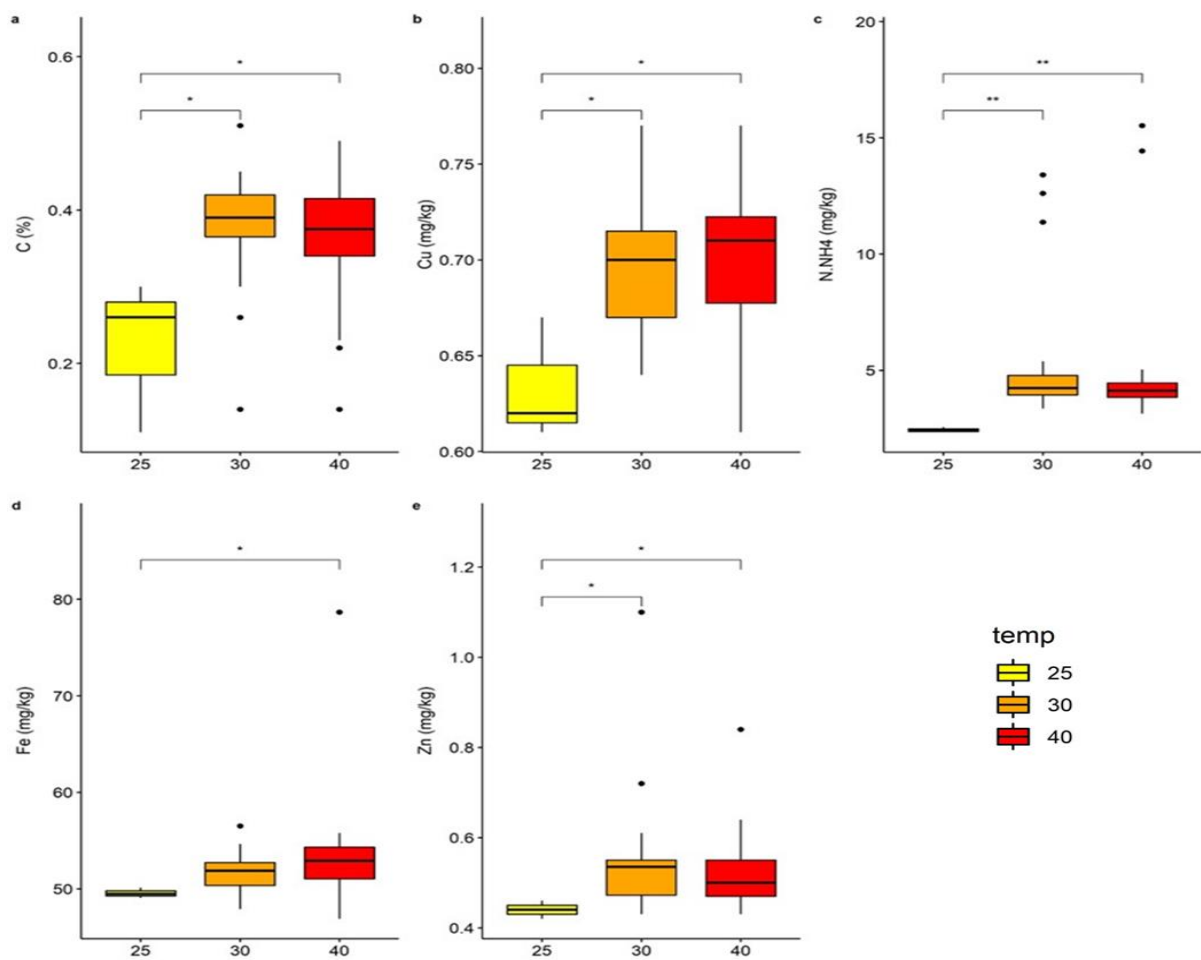


**Figure 4.1.** Box plots of differences in soil physicochemistry in open soil samples [(a) C - 25/30°C & 25/40°C: Kruskal-Wallis,  $p = 0.015$ ,  $p = 0.033$ , respectively), (b) Cu - 25/30°C & 25/40°C: Kruskal-Wallis,  $p = 0.019$ ,  $p = 0.027$ , respectively), (c)  $\text{NH}_4^+$  - 25/30°C & 25/40°C: Kruskal-Wallis,  $p = 0.0063$ ,  $p = 0.0061$ , respectively)] and in canopy soil samples [(d) Fe - 25/40°C: Kruskal-Wallis,  $p = 0.024$  (e)

25/30°C & 25/40°C: Kruskal-Wallis,  $p = 0.021$ ,  $p = 0.011$ , respectively] at control (30°C) and treatment (40°C) temperatures with reference to uninoculated/, baseline (25°C) soils. The interquartile range is represented by the outer bound of the boxes, the median is represented by the middle line and the outliers by the dots. The whiskers represent the minimum and maximum values. Significant differences between the temperature groups are indicated by asterisks at the top of the bars.

#### **4.1.2 The influence of microhabitat on soil physicochemical properties**

Soil physicochemical variables differed substantially between microhabitats (Figure 3.2). Generally, the results show that nearly all soil physicochemical variables in the canopy and open soils were significantly different, except for Na and  $\text{NO}_2^-$  (Wilcoxon,  $p > 0.05$ ).

The pH values for the soil were close to neutral and had average pH values of 7.3 and 7.9 for canopy and open soil, respectively. The pH was significantly higher in open soils compared to canopy soils (Wilcoxon,  $p < 0.0001$ , Figure 3.2.a).

The average  $\text{NH}_4^+$  (Canopy/Open: 9.38mg/kg / 4.96mg/kg, Figure 3.2.b),  $\text{NO}_3^-$  (Canopy/Open: 7.37 mg/kg / 4.34mg/kg, Figure 3.2.c), available P (Canopy/Open: 12.65mg/kg / 10.30mg/kg, Figure 3.2.d) concentrations and C (Canopy/Open: 0.67% / 0.37%, Figure 3.2.e) content was significantly higher under the canopy of *A. eriobola* compared to the adjacent open soils (Wilcoxon,  $p < 0.0001$ ).

Similarly, the average Ca (Canopy/Open: 437.64mg/kg / 350.81mg/kg, Figure 3.2.f), K (Canopy/Open: 235.15mg/kg / 122.74mg/kg, Figure 3.2.g), Mg (Canopy/Open: 102.29mg/kg / 106.80mg/kg, Figure 3.2.h) cation concentrations were significantly higher under the *A. eriobola* canopy compared with the adjacent open soils (Wilcoxon,  $p < 0.0001$ ).

The average Fe (Canopy/Open: 52.45mg/kg / 42.97mg/kg, Figure 3.2.i) and Zn (Canopy/Open: 0.53mg/kg / 0.05mg/kg, Figure 3.2.j) metal ion concentrations were significantly higher under the *A. eriobola* canopy compared with the adjacent open soils (Wilcoxon,  $p < 0.0001$ ). In contrast, the average concentrations of Al (Canopy/Open: 314.37mg/kg / 418.74mg/kg, Figure 3.2.k), Cu (Canopy/Open: 0.38mg/kg / 0.70mg/kg, Figure 3.2.l), and Mn (Canopy/Open: 59.87mg/kg / 116.60mg/kg, Figure 3.2.m) were significantly higher in the open soils in comparison to the *A. eriobola* canopy soils (Wilcoxon  $p < 0.0001$ ).

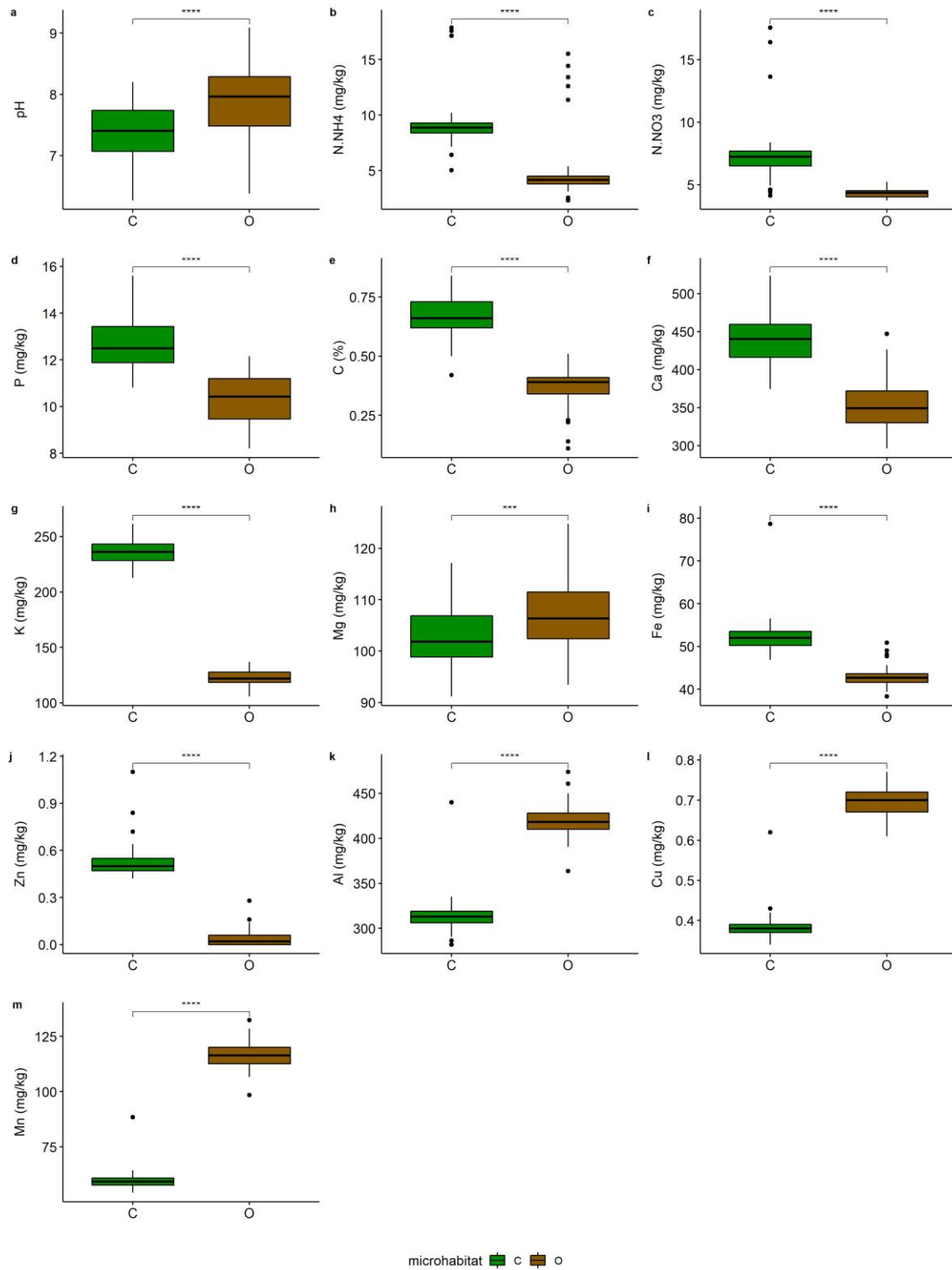


**Figure 4.2.** Box plots of differences in soil physicochemical variables in canopy and open soil samples. The interquartile range is represented by the outer bound of the boxes, the median is represented by the middle line and the outliers by the dots. The whiskers represent the minimum and

maximum values. Asterisks at the top of the bars indicates significant differences between microhabitats.

## **4.2 Sequence analysis**

Following the extraction and sequencing of metagenomic DNA, 6,403,639 reads were obtained from 100 samples. These ranged from 24,225 to 101,538 sequences per sample. After quality filtering and chimera removal, the number of sequences decreased from 5,161,091 to 4,541,096, respectively. The remaining number of sequences after denoising was 4,034,743, whereas the lowest number of sequences in a sample was 14,272. According to rarefaction analysis, which shows whether or not the sampling depth was adequate to estimate the diversity, the number of ASVs (Amplicon Sequence Variants) for 16S rRNA genes plateaued after 15000 sequences at 99% similarity. This suggests that the sequencing depth (18,141) was sufficient to capture the diversity and richness of the samples. Following rarefaction to a sampling depth of at least 18,141 sequences per sample, the total number of sequences decreased to 1,777,818. All samples were rarefied to the same number of sequences (22, 584) per sample. As a result, we lost all the samples with a sampling depth of less than 18, 141 sequences per sample and retained samples with a sampling depth of 22, 584 sequences per sample. After the clean-up of the sequences of the 100 samples, two samples were lost, and the remaining 98 samples were used for downstream analysis. The lost samples originated from the canopy and open soils with a sequence number of 15,116 and 14, 272 each, respectively. The number of ASVs detected after quality filtering, rarefaction, and singleton removal, against the SILVA138 database, was 12,066. The number of

sequences per sample is plotted as a function of the number of ASVs and is shown as a curve after rarefaction, (Figure A2, **Appendix 1**).

### **4.3 Compositional differences in bacterial diversity between canopy and open soils**

Significant (ANOVA,  $p < 0.001$ ) compositional differences in bacterial diversity were observed between canopy and open soils (Table 3.1). However, these differences were not observed between temperature treatments. The five most dominant bacterial phyla across all soil samples were Actinobacteria (39.1%), Firmicutes (36.1%), Proteobacteria (9.9%), Acidobacteria (4.6%), Chloroflexi (4.5%), (Figure 3.2.a). Members of the Gemmatimonadota and Myxococcota were detected in lower abundances (<1%). The relative abundance of Proteobacteria and Firmicutes was higher in canopy soils compared with those in open soils. In contrast, Acidobacteria, Actinobacteria, and Chloroflexi were more abundant in open soil than in canopy soil (Figure 3.2.a). Acidobacteria, Actinobacteria, Firmicutes, Proteobacteria, Chloroflexi, and WPS-2 were detected across all soil samples. Gemmatimonadota, WPS-2, and Myxococcota were detected in relatively low abundances in both canopy and open soils (<1%).

Thirty different bacteria, at the genus level (relative abundance >1%), were revealed across all samples. Apart from the unknown and uncultured genera, the most abundant bacteria at the genus level were *Bacillus* (27%), *Conexibacter* (3.1%), TK10 (2.7%), *Acidothrmus* (1.5%), and *Sporosarcina* (1.5%), (Figure A3, **Appendix 1**). A high abundance of *Bacillus* was detected in the canopy (35%) and open (19.3%) soils. *Conexibacter* (5.3%) and TK10 (5.1%) were only detected in the open

soil, whereas *Sporosarcina* (2.8%) and *Lysinibacillus* (2.2%) were exclusive to canopy soils (Figure A3, **Appendix 1**).

The distribution of observed ASVs from both canopy and open soils is depicted in a Venn diagram (Figure 3.2.b). The results showed that the total richness of all sites was 11234, of which 884 (7.87%) of the ASV were shared by all sites. The number of unique ASVs for each site was: 5372 ASVs for canopy soils (47.8%) and 4978 ASVs for open soils (44.3%).

**Table 4.1.** Total relative abundance of bacterial phyla detected in canopy and open soil samples from Mahikeng. P-values marked with asterisk indicate significant differences (ANOVA,  $p < 0.001$ ).

| Phylum                | Canopy | Open  | p-value           |
|-----------------------|--------|-------|-------------------|
| <b>Actinobacteria</b> | 34.1%  | 43.9% | $<2e - 16$ * **   |
| <b>Firmicutes</b>     | 47.4%  | 25.2% | $<2e - 16$ * **   |
| <b>Proteobacteria</b> | 11.6%  | 8.3%  | $<3.4e - 16$ * ** |
| <b>Acidobacteria</b>  | 2.7%   | 6.5%  | $<2e - 16$ * **   |
| <b>Chloroflexi</b>    | 0.5%   | 8.3%  | $<2e - 16$ * **   |

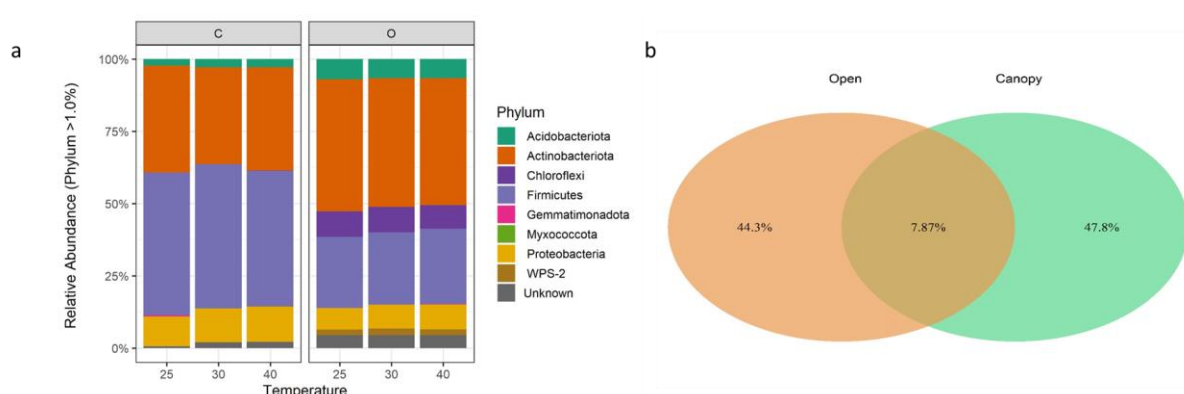


**Figure 4.3.** (a) The relative abundance bar plot showing the distribution of bacteria at phylum level in canopy and open soils across the three temperature treatments. (b) A proportional Venn diagram

showing the number of ASVs found only in the open soils (4978), only in the canopy soils (5372), or in both locations (884).

#### 4.4 Bacterial diversity and community structure in canopy and open soils

Alpha diversity refers to the mean species diversity within a particular ecosystem and is measured with respect to its richness, evenness, or both characteristics (Thukral, 2017). In contrast, beta diversity reflects the variation in species diversity between different ecosystems. Environmental filtering and dispersal limitation processes are two factors that influence beta diversity at local scales (Tan *et al.*, 2017).

##### 4.4.1 Alpha diversity

Microhabitat, and not temperature, significantly influenced alpha diversity (ANOVA,  $p < 0.05$ ). Diversity measures (Shannon, and InvSimpson index) were significantly higher in open soil than in canopy soil samples (Table 3.2 and Figure 3.3). The Observed index showed no significant differences between the canopy and open soils.

**Table 4.2.** Differences in bacterial diversity measures in canopy and open soil samples. Standard deviations (SD) from the means are indicated in parenthesis. P-values marked with asterisk indicate significant differences (ANOVA,  $p < 0.05$ ).

| Alpha diversity across microhabitats |                |               |                  |
|--------------------------------------|----------------|---------------|------------------|
| Diversity Index                      | Canopy         | Open          | p-value          |
| Observed                             | 406.9 (111.31) | 442.4 (95.22) | 0.124            |
| Shannon                              | 4.9 (0.30)     | 5.4 (0.27)    | $< 2e - 16$ * ** |

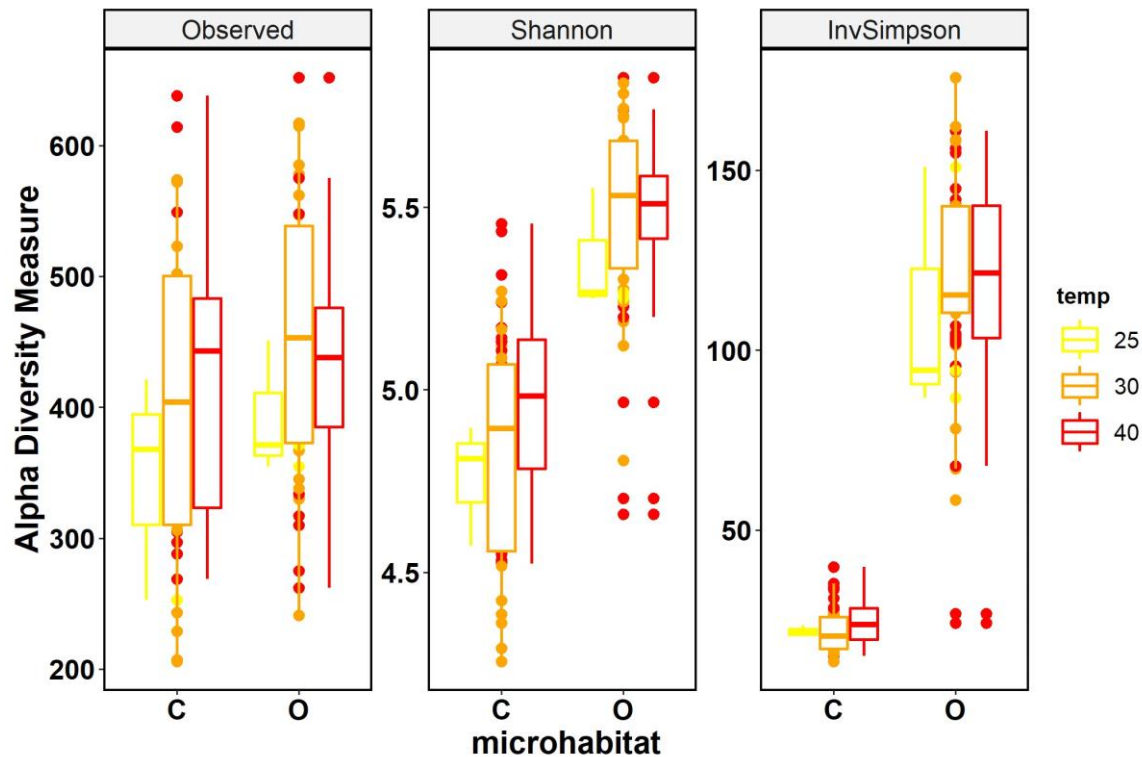


**Figure 4.4.** The alpha diversity indices Observed, Shannon, and InvSimpson are shown as box plots. Samples are grouped according to microhabitat. The interquartile range is represented by the outer bound of the boxes, the median is represented by the middle line and the outliers by the dots. The whiskers represent the minimum and maximum values.

#### 4.4.2 Beta diversity

The difference in bacterial community structure was visualized using a non-metric multidimensional scale (NMDS) plot based on the Bray-Curtis distance. We observed significant clustering according to microhabitat. Bacteria in canopy soils were differentiated from those in open soils (Figure 3.5). The significant differences between the canopy and open soils were confirmed by Adonis (permutations = 999,

$R^2 = 0.50$ ,  $p = 0.001$ ). However, no clustering according to temperature was observed.

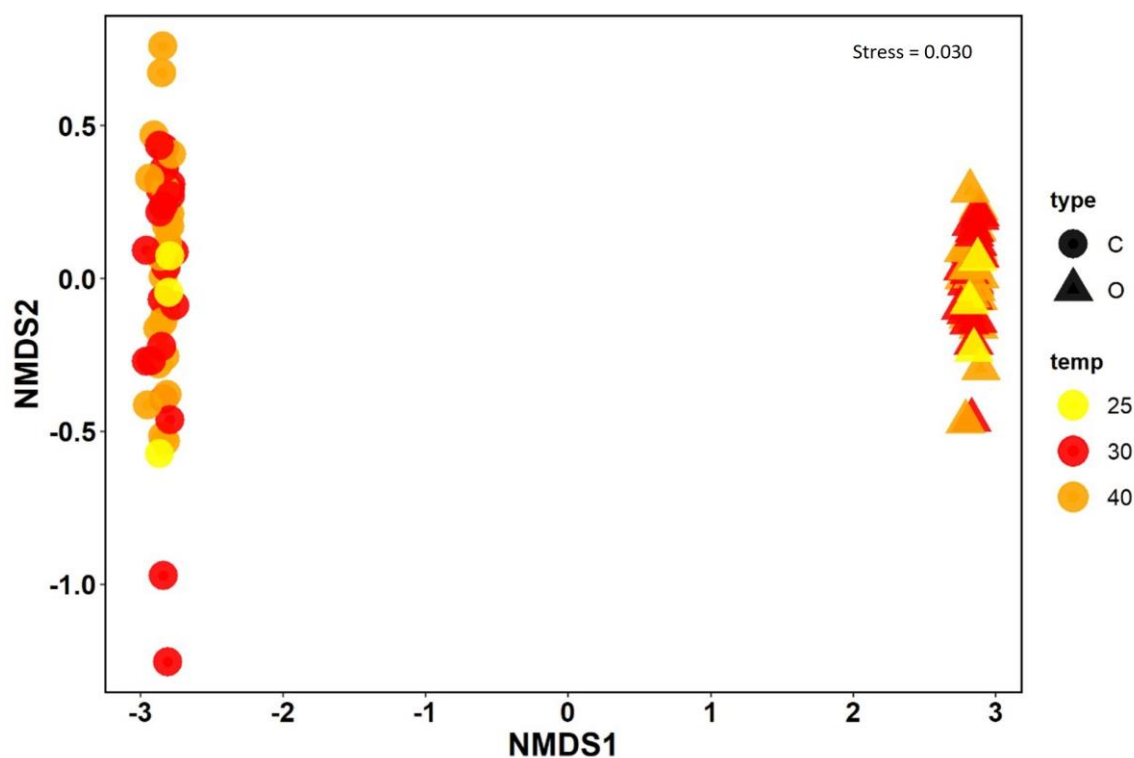


**Figure 4.5.** Non-metric multidimensional scaling (NMDS) ordination of soil bacterial communities.

The effect of soil chemistry on bacterial diversity was visualized using redundancy analysis (RDA) using the Bray-Curtis distance to understand the potential effects of soil physiochemistry on bacterial composition, (Figure 3.6). Individual selection of variables revealed that soil physicochemical variables: Al, C, Ca, Mg, Na, Fe, Cu, Zn, Mn, P,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  had no significant effect on the bacterial community composition (ANOVA,  $p$ -value = 0.05). In canopy soils, Fe was found to significantly drive bacterial community composition (ANOVA,  $p$ -value 0.001), while Cu and pH were found to significantly drive bacterial community composition in open soils (ANOVA,  $p$ -value = 0.029).

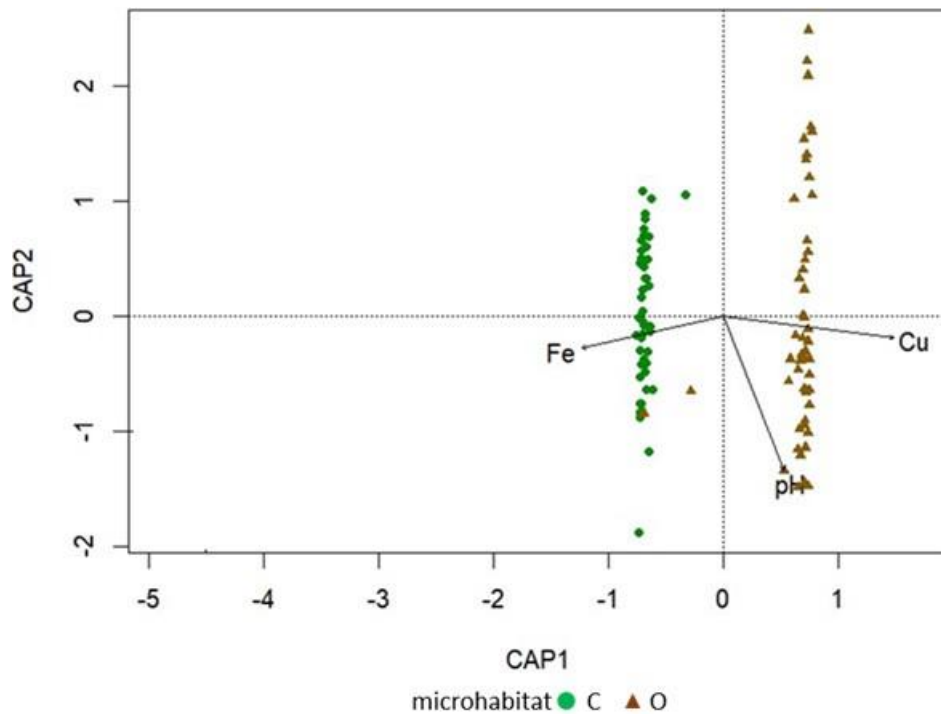


**Figure 4.6.** Redundancy analysis plot based on Bray-Curtis distance depicting the relationship between bacterial diversity and the soil physicochemical variables.

#### 4.5 Summary

We analysed the effect of temperature and microhabitat (canopy and open soils) on the bacterial microbiome and soil physicochemical variables in arid soils. The concentration of physicochemical properties varied significantly between microhabitats, except for Na and  $\text{NO}_2^-$ . We observed significantly higher variations in the Fe and Zn concentrations in canopy soils and the C, Cu, and  $\text{NH}_4^+$  concentrations in open soils in response to temperature increases between the baseline (25°C) and control (30°C) or treatment (40°C) groups. The dominant phyla across all soil samples were Actinobacteria, Firmicutes, Proteobacteria, Acidobacteria, and Chloroflexi. Microhabitat, and not temperature, significantly influenced alpha diversity. Diversity measures (Shannon and InvSimpson) were

significantly higher in open soils than in canopy soils. Iron was reported as the major driver of bacterial diversity in canopy soils, while Cu and pH were the major drivers of bacterial diversity in open soils.

## Chapter 5 – Discussion

The predicted increases in global temperature regimes are expected to disproportionately affect arid lands (Bathiany *et al.*, 2018; Spinoni *et al.*, 2021). The decrease in the diversity of macrofauna is expected to alter ecosystem dynamics in drylands. However, we lack clear mechanistic insights regarding the effects of temperature increases on microbiomes in arid lands. This knowledge deficit is especially true for plant-associated microbiomes in drylands. To gain predictive insights regarding the influence of temperature increase on arid soils we used soil microcosms, which offer tractable systems for testing the effects of environmental perturbations on microbial communities. Here, we provide insights regarding the influence of temperature as well as physicochemical variables on bacterial communities from open and canopy soil. Samples collected within the vicinity and underneath an *A. erioloba* tree canopy were subjected to incubations at 30°C and 40°C, for forty days.

### 5.1 Temperature does not appear to drive bacterial community structure

Our analysis revealed that temperature had no noticeable effect on bacterial community structure. Liu *et al.* (2017), studied the effect of experimental warming on microbial functionality in forest soils through a metaproteomics survey. It was reported that community functioning was more strongly influenced by temperature than taxonomic variation. There are several possible reasons explaining the lack of bacterial response to temperature. Firstly, bacterial communities have been shown to demonstrate some resilience to short-term temperature changes (Allison and

Martiny, 2008; Pérez-Guzmán *et al.*, 2020). The reasons for this resilience are varied and may include, for instance, the relatively high clay contents associated with semi-arid soils (Pérez-Guzmán *et al.*, 2020).

Another reason for resilience is that soil bacterial communities have evolved mechanisms to withstand temperature stress (Berard *et al.*, 2015; Griffiths and Philippot, 2013). This was supported by the presence of Actinobacteria, Firmicutes, and Chloroflexi in our soils. These phyla are well known to possess adaptations to resist/tolerate environmental stressors such as desiccation, high UV radiation, and high salinity. We observed a high abundance of Actinobacteria and Chloroflexi in open soils. This is unsurprising given Actinobacteria's capacity for sporulation, wide metabolic capacity, competitive advantages via secondary metabolite synthesis, and multiple UV repair mechanisms (Makhalanyane *et al.*, 2015a; Mohammadipanah and Wink, 2016; Rateb *et al.*, 2011). Chloroflexi have also been found in high abundance in the Atacama Desert (Neilson *et al.*, 2012). A notable reason for this is their complex layered envelope structure. This structure demonstrates their resistance to desiccation in hyperarid deserts and consists of a distinct membrane construction with long-chain 1,2-diols and a cell wall with variable and atypical peptidoglycan contents (Neilson *et al.*, 2012; Wu *et al.*, 2009).

Secondly, in addition to climatic stress, the soils in drylands are known to have a very low nutrient status (Eldridge *et al.*, 2018; Moreno-Jiménez *et al.*, 2019; Trivedi *et al.*, 2019). This may result in an inability to support bacterial communities, as the accumulation of nutrients at specific soil depths has been shown to select microbial communities with associated cycling pathways (Jiao *et al.*, 2018). Although we found no direct effects on bacterial community structure, the temperature had a small effect

on the distribution of certain soil physicochemical properties in both canopy and open soils. Processes such as soil respiration and litter decomposition that regulate nutrient and carbon cycling in arid regions are influenced by litter quality, soil temperature, and moisture (Ranucci *et al.*, 2022; Souza and Billings, 2022). The distribution of soil nutrients can be attributed to the increased metabolic activities of microorganisms at higher temperatures, which are involved in processes that cycle nutrients and make them available for plant uptake (Jacoby *et al.*, 2017; Onwuka and Mang, 2018).

Our results show that the concentration of carbon increased with higher temperatures in open soils. Increasing temperature accelerates the decomposition of soil organic matter (Stuble *et al.*, 2019), which leads to a higher soil organic carbon output resulting in positive feedbacks on climate change (Ontl and Schulte, 2012). Although some of this carbon may be lost to the atmosphere, an alternative explanation is that warming elicits increases in the rate of physicochemical processes, which transfer organic carbon to recalcitrant soil carbon pools (Thornley and Cannell, 2001). The latter corroborates our findings where soil carbon percentage increased with increasing temperatures.

In addition, soil Cu concentration was found to increase with increasing temperature. Copper plays a key function in CO<sub>2</sub> assimilation and ATP production (Kumar *et al.*, 2021). It is an essential micronutrient for all living organisms and an important constituent of enzymes involved in photosynthesis and respiration (Moreno-Jiménez *et al.*, 2020; Ridge *et al.*, 2008; Zhang *et al.*, 2009). However, in its free form, Cu is insoluble (Argüello *et al.*, 2013). Cu ions dissolve as divalent ions (Cu<sup>2+</sup>) in soil solutions, where they form organic Cu complexes that absorb soil organic matter,

carbonates, phyllosilicates, and Al, Fe, and Mn hydrous oxides due to their high reactivity (Mengel *et al.*, 2001).

Two factors are known to affect Cu availability in soils, namely a high SOM content and temperature. Copper levels have been shown to increase in soils with a low SOM content. Therefore, Cu levels are expected to be higher in alkaline soils than in acidic soils. This explains our observation of high Cu concentrations in open soils, which had slightly higher/alkaline soil pH values than canopy soils. Due to the lack of a canopy, open soils are more exposed to high temperatures than canopy soils. High temperatures are known to enhance Cu sorption processes (Elkhatib *et al.*, 2007). Adsorption is a primary process in which heavy metals accumulate in the soil, while desorption is the process of its removal. Our results are consistent with the reports that temperature increases enhance cation adsorption and reduce anion adsorption (Barrow, 1992). A study on the effect of temperature on the absorption-desorption of  $\text{Cu}^{2+}$  in a Cu tailings yard reported that Cu adsorption increased with the elevation of temperature, while Cu desorption decreased (Wang and Zhang, 2010). Another study on the uptake of Cu by wheat plants at different soil temperatures reported that less Cu was taken up by plants at high incubation temperatures (Brennan *et al.*, 1980). As a result, Cu accumulated in higher amounts in the soil.

Our results are similar to the findings of Xue *et al.* (2018) and Schulz *et al.* (2012), who reported that temperature compared to soil properties or plant litter had a lesser impact on microbial distribution. This indicates that at local scales temperature effects might be masked by more significant variables, including vegetation cover and soil properties.

## 5.2 Microhabitat effects on bacterial communities

A typical feature of drylands is the discontinuous vegetation cover that is intermittently surrounded by open soils. The heterogenic landscape creates distinct microhabitats that support different microbial communities and control the accumulation of soil nutrients (Kushwaha *et al.*, 2021). In this study, microhabitat (canopy vs open soils) had a larger effect on bacterial community structure and soil physicochemical properties than temperature. This is mostly because a tree canopy in a dryland area affects the soil underneath by reducing irradiance and temperature, consequently lowering evapotranspiration demands compared to open soils with no canopies (Gómez-Aparicio *et al.*, 2005). Another possible reason is the islands of fertility phenomenon, previously described for several arid and semi-arid ecosystems (Novoa *et al.*, 2021; Saaed *et al.*, 2022). This theory states that soil nutrients due to litterfall incorporation may become concentrated below plants (Pitman, 2013). Accordingly, we found that most soil physicochemical properties were in higher concentrations in the canopy soils of the *A. eriobola* tree.

We found that pH was a driver of bacterial diversity in open soils, which agrees with previous studies (Delgado-Baquerizo *et al.*, 2018; Fierer and Jackson, 2006). Our soils had pH values just above neutral, with the mean pH for canopy soils being slightly lower than that of open soils. This could be attributed to root exudation action in which the secreted organic acids cause acidification of the soil, hence lowering soil pH (Msimbira and Smith, 2020). A mechanism by which soil pH affects bacterial diversity is through the regulation of soil nutrients and processes, including the mobilization of essential macro-and micronutrients (Neina, 2019). The effect of soil pH on sorption and complexation processes has been shown to control the

bioavailability of metal nutrients essential for plant growth. In particular, Cu bioavailability and toxicity in soils (Oorts, 2013). The resistance to Cu is regulated by a *copA* gene encoding a multi-copper oxidase that oxidizes Cu(I) to the less toxic chemical form of Cu(II) (Altimira *et al.*, 2012). Our RDA analysis revealed that Cu was a major driver of bacterial diversity in open soils. A higher relative abundance of Acidobacteria and Actinobacteria was observed in open soils compared to canopy soils. The phyla Acidobacteria and Actinobacteria are known to be Cu-tolerant, suggesting that open soils may have been selected for Cu-tolerant taxa (Berg *et al.*, 2012; Lejon *et al.*, 2008).

Iron (Fe) is the fourth most abundant element in soils, and like Cu, it is present in unavailable forms such as its ferrous form  $\text{Fe}^{3+}$ . The solubility of Fe depends on soil pH, having a higher solubility in acidic soils (Colombo *et al.*, 2014). In this study, we reported high Fe concentrations in canopy soils which had slightly lower pH values compared to open soils. We can deduce that the higher concentration of organic matter in canopy soils resulted in the lower pH values for these soils, thus improving Fe availability in canopy soils compared to open soils with slightly higher pH values. This highlights how plant activity, through root exudation of organic compounds used as a food source by bacteria, sustains interactions between bacteria and Fe minerals, which ultimately lowers the pH of the soil (Dhaliwal *et al.*, 2019). Furthermore, this points to existing evidence for Fe-stimulated decomposition during which the Fe-organic matter associations, formed during Fe oxidation and reduction, promote C release and mineralization (Chen *et al.*, 2020). In this sense, a coupled network exists between the Fe and C biogeochemical cycle (Duan *et al.*, 2020).

Our RDA analysis further revealed that Fe was the major driver of bacterial diversity in canopy soils. Bacteria synthesize low-weight molecules, called siderophores, that have a high affinity for ferric iron  $\text{Fe}^{3+}$  that is subsequently transformed at the membrane level to ferrous iron  $\text{Fe}^{2+}$  and released inside the cell (Gamalero *et al.*, 2020). Microorganisms able to synthesize siderophores are present in large amounts in the rhizosphere, including the phyla Proteobacteria and Firmicutes which we found in higher abundances in canopy soils than in open soils (Ali and Vidhale, 2013). This shows that arid environments select diverse bacterial communities that may be shaped through the presence of plants, and by resources provided by plant root exudates.

## Chapter 6 – Conclusion

This study sought to elucidate how climate change affects soil microbiota in arid lands. We observed that the interactive effects of climate and local factors in shaping microbial communities tend to yield contradictory results at the global and local scales. In contrast to our initial hypothesis, bacterial community structure was not significantly affected by temperature increases in the arid soils. Microhabitat, namely open / canopy, was identified as a significant factor in determining bacterial community structure. Furthermore, we observed differences in the soil physicochemical properties between the canopy and open soils because of plant cover influence. We showed that the relative abundances of major phyla differed between canopy and open soils, with a higher relative abundance of Firmicutes in canopy soils compared with Actinobacteria and Chloroflexi in open soils. Our data suggest that the effects of temperature increase at the local scale may be masked by more significant factors such as the presence of an *Acacia* tree and soil physicochemical properties. This study corroborates the idea that arid environments harbour diverse bacterial communities that are shaped by the microhabitat resources and are resistant to temperature stress perturbations. Therefore, plants may mitigate potential positive climate feedbacks through the selection and interaction of arid soil bacterial communities.

## **6.1 Recommendations**

- Incorporating diurnal temperature and light variations in microcosm experiments may provide additional insights regarding adaptations in bacterial communities from arid soils.
- Information on the functional attributes of these microbiomes is required to support predictions regarding ecosystem functioning in drylands.

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# Appendix 1

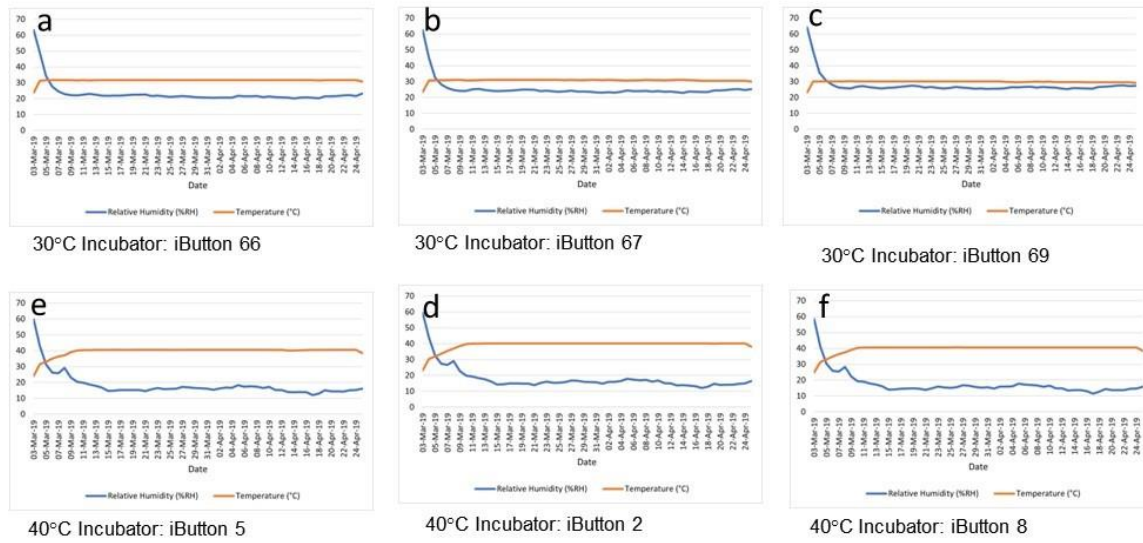


**Figure A.1.** Mean relative humidity and temperature in the control (30°C) ((a), (b), (c)) and treatment (40°C) ((d), (e), (f)) incubator for the duration of the microcosm experiment.

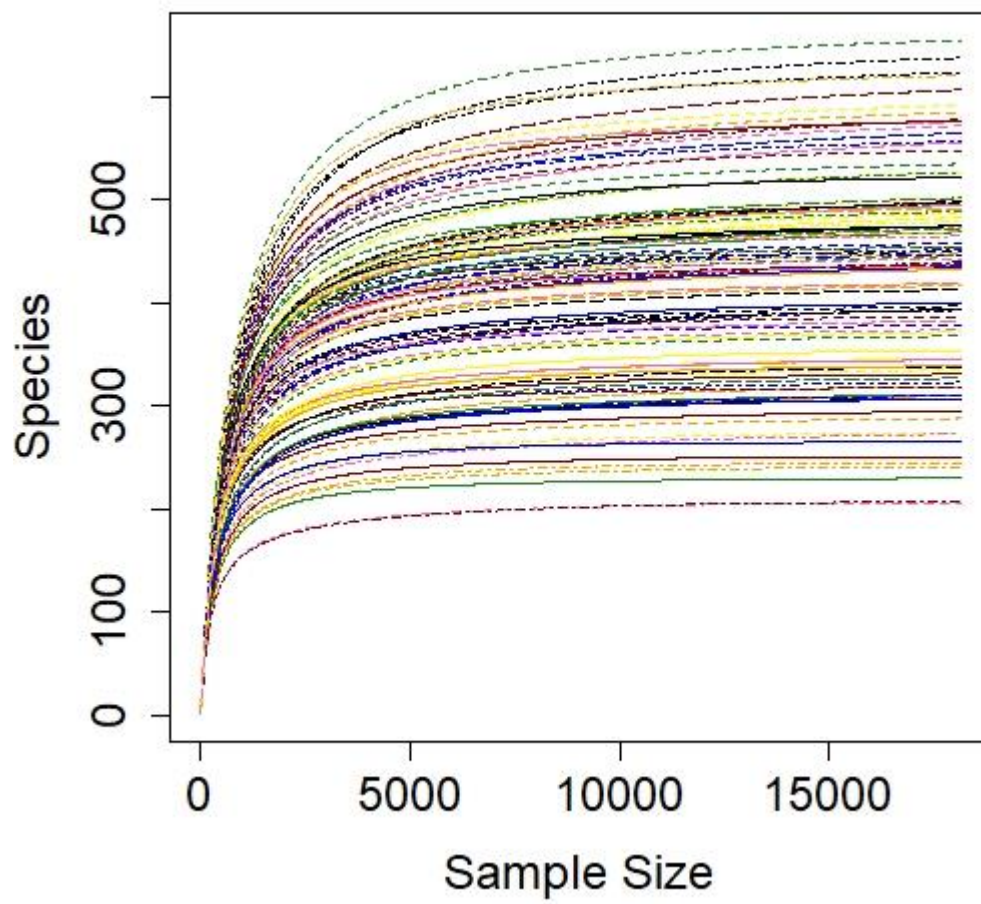


**Figure A.2.** Rarefaction curves of ASVs clustered at 99% similarity across different samples

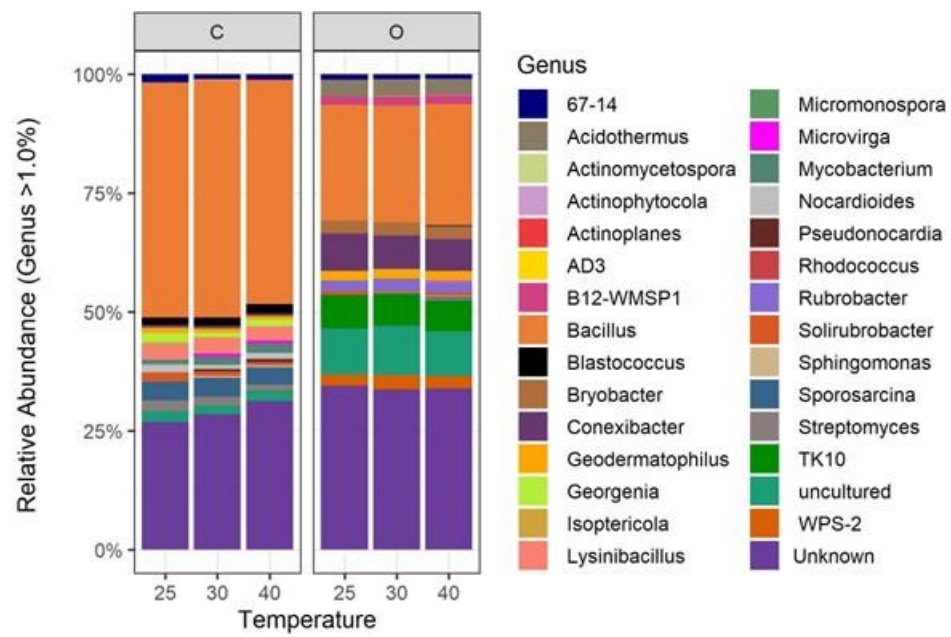


**Figure A.3.** The relative abundance bar plot showing the distribution of bacteria at genus level in canopy and open soils across three temperature treatments.