ANALYSIS OF THE MICROBIAL DIVERSITY ASSOCIATED WITH THE LESOTHO HIGHLANDS THROUGH CULTURE-INDEPENDENT APPROACHES

by

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DECLARATION

I Jasmin Bharatkumar Patel (Student number: 1119140) am a student registered for Master in Science in the year 2020. I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
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PREFACE

Mountains are characterised by elevational gradients which exhibit dramatic changes in climate and biotic turnover across short geographical distances and thus represent suitable natural laboratories for the study of climate change. Studies relating to the impact of climatic factors in montane (i.e. mountain) environments has mainly been focused on the response of vegetation patterns in these environments, while little is known about the impact of these factors on soil microbial diversity and community structure. This study looked at microbial community structure on the warm north-facing slope, cold south-facing slope and summit plateau across a mountain peak near Kotisephola Pass, Drakensberg Mountains, Lesotho.

In chapter 1, relevant literature relating to the project covering the various aspects of the montane environment and its climate are reviewed. This includes the soil microbiota associated with mountains and their response to abiotic and biotic factors associated with these environments. Finally, the technological evolution of the study of microbial ecology and how it can be applied to studying montane ecosystems are discussed.

Chapter 2 engages with the soil samples collected from field sites, genomic DNA extraction, and amplicon sequencing that was performed of the bacterial (16S rRNA) and fungal (ITS) cohort across the mountain transect. The resultant data were analysed in terms of the soil microbiome structure, α- and β-diversity, and statistical analyses were undertaken to determine the relationship between microbial diversity and factors such as slope aspect and elevation across the mountain peak.

In chapter 3, an in-depth analysis of temperature data collected for the mountain peak was undertaken, prior to statistical evaluation of the effects that the distinct microclimates across the mountain summit have on bacterial and fungal diversity.

Finally, the implications of the study in terms of how it can contribute towards studying the effects of global climate change are discussed and future perspectives for further studies are provided.

DEDICATION

For my family

You are the reason behind what I am and what I have achieved today, your push to open doors I never thought could be opened has given me the opportunity to see the world as it is outside the comfort of my home.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE – Mountains and their soil microorganisms as models of climate change

1. Introduction

The rise in demand for fossil fuels and industrialisation has led to increased emission of greenhouse gases with the concomitant effect of global climate change (Anstey, 2013). These climatic effects include warming of the Earth's surface, leading to drastic changes in precipitation patterns, and the increased occurrence of extreme weather events (Barrett, *et al*, 2015). While these changes are experienced globally, the impact of climate change is predicted to have a far greater effect on higher elevation environments such as mountains (Lamprecht, *et al.*, 2018). Mountains themselves represent environmental niches showing large differences in climatic and biotic factors over a small geographical scale (Siles and Margesin, 2016; Yuan, *et al.*, 2014), with additional factors such as slope aspect and vegetation patterns further influencing these microclimatic gradients (Davies-Colley, *et al.*, 2000; Mayet, 2016). As such, these environments represent good natural laboratories for the study of climate change.

While several studies have examined the impact of climate change on vegetation patterns of mountains (Gatti, *et al.*, 2019; He, *et al.*, 2019; Hughes, *et al.*, 2000), soil microorganisms have also started gaining the limelight in studies aimed at gaining an understanding of the impact of climate change on a particular environment and its ecosystem (Dutta and Dutta, 2016). Furthermore, soil microorganisms serve as good indicators for the study of climate change and changing environments due to their response to changing climatic factors collectively (Castro, *et al.*, 2010).

While such studies have been conducted investigating the response of soil microorganisms to changes in abiotic and biotic factors due to climate change, data related to soil microorganisms in the Lesotho highlands and their response to these changes is sorely lacking. This study focuses on the fungal and bacterial components of the soil microbiome across a mountain summit in the Drakensberg, inland of the Sani Pass region near Kotisephola Pass. Despite the short geographic distance between the sampling points across the north- and south-facing slopes and the peak, they show remarkable variability in terms of their thermal and precipitation patterns and can thus serve as key indicators of the effects of changes in these abiotic factors on the key biotic component represented by the soil microbiota. Here literature pertinent to key aspects of the study will be discussed.

2. The mountain ecosystem

Mountains are generally defined as those surfaces of the Earth which are naturally elevated compared to the surrounding land, forming a peak (Gerrard, 1990). With over one million mountains around the world (PeakVisor, 2018), these landforms cover up to 27% of the total land surface (Blyth, 2002). Mountains are classified based on their elevation according to the United Nations Environmental Program with the lowest category containing mountains with elevations between 300 to 1,000 meters and the highest containing mountains with elevations above 4,500 meters (Blyth, 2002). Some of the world's highest mountains include Mount Everest in the Himalayan mountain range at 8,848 meters above sea level (m.a.s.l.) (Mathew and Sharma, 2020), K2 at 8,611 m.a.s.l. (Houston and Bates, 2008), and Kangchenjunga at 8,586 m.a.s.l. (Smythe, 2013), all of which are located in Asia.

Mountains represent some of the world's most unique environments, due to their upright dimensions which create climatic gradients across a slope, within which a range of the earth's climatic systems, such as alpine, tropical, and temperate can be found (Diaz, *et al.*, 2003; United Nations, 1992). These environments are an important source of water, energy, minerals, forest and agricultural resources, and natural regions of recreation (United Nations, 1992). Furthermore, mountains play a role in altering the local climate and controlling the flow of rivers within these regions (Zhang, *et al.*, 2009). Mountains are affected by various climatic factors such as precipitation, radiation, temperature (Yuan, *et al.*, 2014), and atmospheric pressure (Siles and Margesin, 2016). As a result of changing elevation on mountains, these climatic factors create low-temperature dependent environments due to temperature decreasing with increasing elevation and solar radiation exposure and precipitation increasing with increasing elevation (Donhauser and Frey, 2018). Furthermore, montane (i.e. mountain) regions have a variety of microclimates that occur over short distances (Davies-Colley, *et al.*, 2000), which are affected by factors such as shading, slope aspect, and types of vegetation, soil, and rock (Mayet, 2016). These microclimates thereby create a range of habitats over short distances and also influence ecosystem processes, function, and composition in mountain environments (Davies-Colley, *et al.*, 2000).

Montane environments are frequently characterised by harsh environmental conditions, particularly at higher elevations, where they experience cold temperatures and decreasing atmospheric pressure which results in decreased amounts of available oxygen as elevation increases (Peacock, 1998). Nevertheless, about 12% of the world's human population inhabit

mountain environments, with a further 10% and 40% of the global population, respectively depend directly or indirectly on resources provided by mountains (Schild, 2008). Mountains are also inhabited by a range of different flora and fauna species (Körner, *et al.*, 2017), endangered species (United Nations 1992), and above- and below-ground microorganisms (Adamczyk, *et al.*, 2019), all of which are encompassed within the various climatic systems found on mountains (United Nations 1992). While the plant and animal life of mountains has been the subject of substantive study (Lamprecht, *et al.*, 2018), this study will focus mainly on soil microorganisms and how the soil microbial community in mountains is shaped and changed by changing abiotic conditions.

3. Soil microorganisms at high elevations

Soil represents a rich habitat for microorganisms. The soil microbiome is crucial to terrestrial ecosystems due to the vital roles of soil-borne bacteria and fungi in nutrient cycling, decomposition of organic matter, and the formation of symbiotic relationships with higher organisms, such as plants and animals, inhabiting the same ecosystem (Ma, *et al.*, 2004). In any environment, soil microorganisms are affected by various abiotic and biotic factors experienced including temperature, moisture, vegetation type, pH, and soil type (Margesin, *et al.*, 2009; Zhang, *et al.*, 2013). These factors influence not only the community composition of the microorganisms, but their function and activity as well (Zhang, *et al.*, 2013). Mountain environments also harbour a range of soil microbial communities that are distinctly shaped by the environmental conditions present (Praeg, *et al.*, 2019). These environmental conditions are further influenced by geographical factors of mountains such as the orientation of the slope and elevation, which concomitantly affects soil microbial community composition and function (Figure 1) (Donhauser and Frey, 2018).

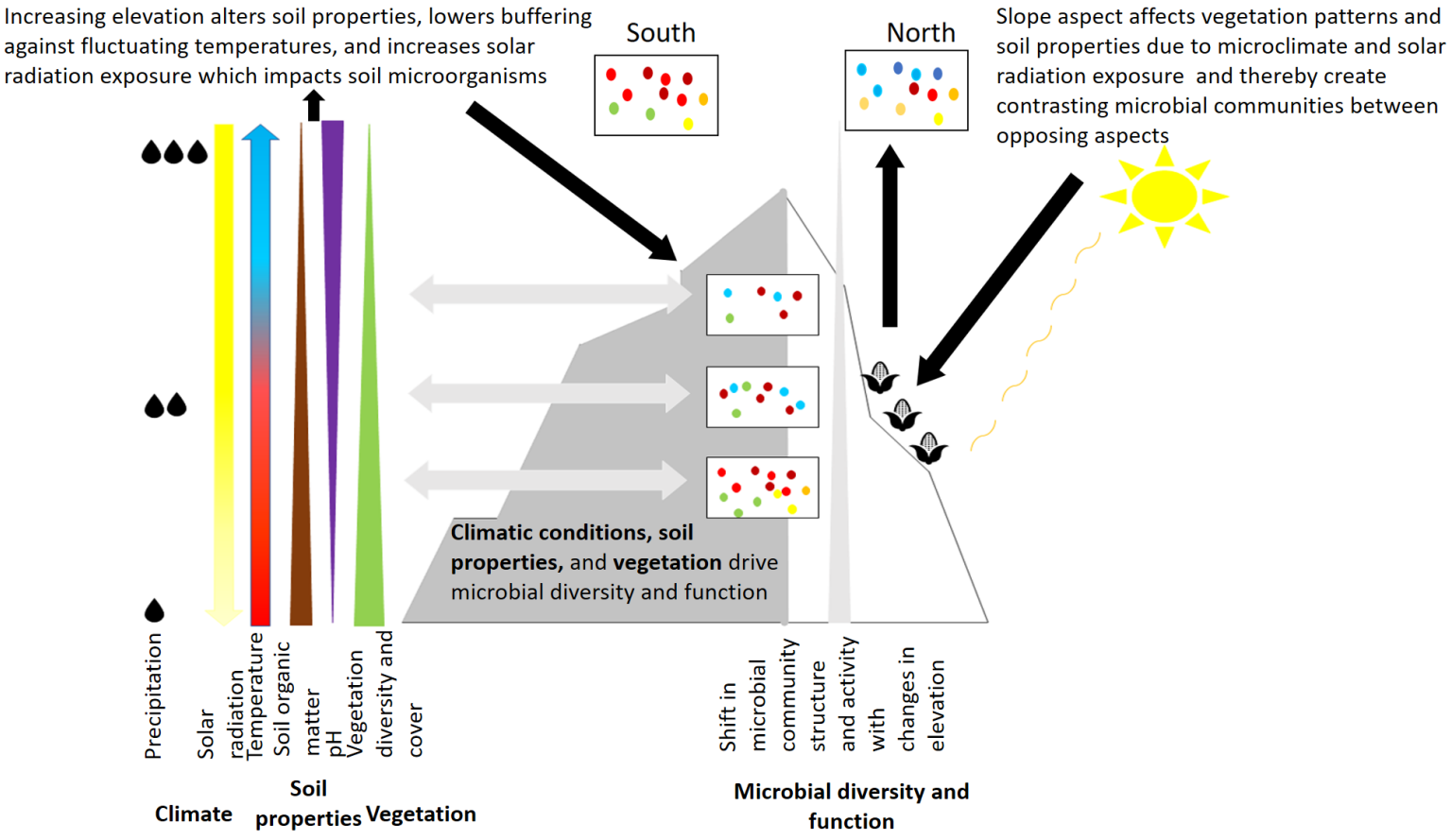

Figure 1: Abiotic and biotic factors affecting soil microorganisms community structure and function in mountain ecosystems. Geographical factors of mountains that affect soil microorganisms include elevation and aspect which determines the soil microclimate. Soil microclimates vary with temperature, solar radiation exposure, and precipitation levels experienced at various elevations of mountains across the various aspects. Changes in these abiotic properties influence biotic properties which further induce changes in soil microbial diversity and function. (Figure adapted from Donhauser and Frey, 2018)

In montane environments, the directional orientation of mountain slopes, known as aspect, plays a substantial role in the microclimate, vegetation distribution, and soil properties in these regions (Ghosh, *et al.*, 2014). Changes in these factors lead to differences in soil moisture, temperature, nutrient composition, quality, and, ultimately, the abundance in plant and microorganism species on the various aspects of mountains (Begum, *et al.*, 2013). For example, in the northern hemisphere, north-facing slopes of mountains tend to be more shaded and colder compared to the south-facing slopes which experience more sunlight and are warmer (Chu, *et al.*, 2016; Small and McCarthy, 2003). Vegetation cover in these areas also differs due to these conditions and this alters the microbial diversity within these regions (Small and McCarthy, 2003). With greater vegetation cover in warmer and favourable conditions causing an increase in nutrient availability through litter production in soil and thereby increasing microbial activity and community abundance (Wu, *et al.*, 2018).

Aspect furthermore determines the amount of solar radiation a mountain slope is exposed to which may, directly and indirectly, affect soil microorganisms on mountains (Kobler, *et al.*, 2019). Greater exposure to solar radiation may increase the photosynthetic capacity and carbon uptake by plants which, coupled with warming temperatures, can cause an increase in soil evaporation and transpiration rates (Kobler, *et al.*, 2019). This results in a decrease in water availability on slopes that are exposed to more radiation and indirectly affects the soil microorganisms present (Hernández, *et al.*, 2016; Kobler, *et al.*, 2019). High levels of solar radiation in montane environments furthermore directly affects soil microbial cell structure and functioning, resulting in the presence of more phototolerant microbial taxa in these regions compared to the other slope aspects of mountains (Hernández, *et al.*, 2016).

Apart from the slope aspect, changes in elevation on mountains severely affect abiotic factors such as temperature, soil properties, water availability, solar radiation, and precipitation (Anderson, *et al.*, 2018; Zhang, *et al.*, 2016). As elevation increases across a mountain slope, the temperature tends to decrease, often creating harsh environments that impact the survival of soil microbial communities, resulting in the dominance of psychrophilic microorganisms which can survive and function at lower temperatures (D'Amico, *et al.*, 2006; Kumar, *et al.*, 2019). For example, a study conducted in the Grossglockner Mountain area in the Austrian central Alps, incorporating two distinct climatic systems (alpine and subalpine, over an elevation gradient of 1,030 m.a.s.l.), observed an increase in the number of psychrophilic bacteria amongst culturable heterotrophic bacteria with an increase in elevation (Margesin, *et*

al., 2009). The increased presence of culturable psychrophilic bacteria together with fungal biomass was found to be particularly higher in the alpine soil which had significantly colder climatic conditions compared to subalpine soils (Margesin, *et al.*, 2009).

Lower temperatures on mountains further tend to impact the forms of precipitation experienced, which include snowfall or rainfall (Huss, *et al.*, 2017; Li, *et al.*, 2020). Lower temperatures influence the duration of snow cover at higher elevations and the snow thawing process in these regions (Armstrong, *et al.*, 2019). Snowfalls are the most important factor in the hydrological cycle on mountains during cold seasons (Li, *et al.*, 2020). Some mountains, such as on the Himalayas and the Andes mountains, tend to have permanent snow cover, in the form of glaciers, which are the gradual build-up of snow cover on mountains over decades and centuries (Armstrong, *et al.*, 2019; Huss, *et al.*, 2017). Other mountain regions tend to experience seasonal snowfall such as on the Drakensberg during cold seasons (Grab, *et al.*, 2017; Huss, *et al.*, 2017). Accumulated snow on mountains thus creates habitats for several microorganisms (Larose, *et al.*, 2013), whether it be for limited periods or permanently (Margesin and Collins, 2019).

While some microorganisms, such as psychrophiles, can thrive in these environments (Kumar, *et al.*, 2019), others adapt to these cold conditions (Schimel, *et al.*, 2007) and some rely on the protective snow cover against harsher conditions (Zhang, *et al.*, 2014). Depending on snow depth and density, warmer habitats can be created through the insulation of soil below the ground for soil microorganisms to function and survive, offering protection from much colder above-ground air temperatures (Zhang, *et al.*, 2014). Snow accumulation on mountains, however, leads to a decrease in water availability and temperatures below the ground surface, causing a reduction in microbial activity (Wipf, *et al.*, 2015). Furthermore, changes in snowfall and rainfall patterns can often cause either an increase or decrease in soil microorganism community diversity (Engelhardt, *et al.*, 2018; Wipf, *et al.*, 2015). At times this can lead to the dominance of microorganisms better suited for the changes in conditions (Engelhardt, *et al.*, 2018; Wipf, *et al.*, 2015). Winter snowfall at higher elevations with colder conditions assists psychrophiles to thrive, compared to lower elevations with warmer conditions that experience summer rainfall where their number would likely decrease (Kumar, *et al.*, 2019; Zhang, *et al.*, 2019).

Climatic variations, slope aspect, and elevation also affects the type of vegetation and where it grows on mountains (Egli, *et al.*, 2009). Typically, fewer plant taxa are able to grow at higher elevations, and the resultant low quantity and quality of plant litter negatively affects soil nutrient availability and concomitantly soil microbial composition and function at higher elevations (Donhauser and Frey, 2018; Egli, *et al.*, 2009). Microorganisms that do live at higher elevations have to survive in stringent soils with very limited moisture and nutrients; furthermore, poor plant cover exposes them to harsher environmental factors above the ground (Donhauser and Frey, 2018; Rime, *et al.*, 2015). Vegetation types also alter the availability of substrates, such as carbon and nitrogen, as well as the pH of the soil which affects the distribution of microorganisms and their community structure (Yuan, *et al.*, 2014).

In a study conducted on the south-facing slope of the Nyainqentanglha Mountains on the Tibetan Plateau (at elevations of 4,400 to 5,200 m.a.s.l.) which incorporated three different vegetation species in an alpine meadow, bacterial community compositions were found to be affected by changes in soil substrates (carbon and nitrogen), nitrogen compounds and pH in topsoil and subsurface soils (Yuan, *et al.*, 2014). Furthermore, soil bacterial communities were found to be influenced by the vegetation in the mountains (Yuan, *et al.*, 2014). In another study conducted on the highest mountain in north-eastern China (Changbai mountain; ranging from 530 to 2,200 m.a.s.l.), bacterial community structure was found to be influenced by vegetation types and soil substrates (carbon and nitrogen) (Shen, *et al.*, 2013). This region, however, incorporated vegetation types like those found in the temperate and frigid regions of the Eurasian continent and further inferred that the bacterial community structure may be affected indirectly by vegetation through changes in soil substrate levels (Shen, *et al.*, 2013).

In a study conducted in forests in the South Tyrol in the Italian Alps, across four vegetational zones (montane, sub-montane, alpine, and sub-alpine) with varying climatic conditions and elevational ranges, the impact of various abiotic factors on biotic factors and their overall impact on the soil microorganisms was determined (Siles, *et al.*, 2016). Abiotic factors studied included seasons, temperatures, and elevation and biotic factors included soil physiochemical properties and respiration (Siles, *et al.*, 2016). This study showed that soil nutrients and soil organic matter (SOM), comprised of dead plant and microbial matter (Neumann, *et al.*, 2014), increased with elevation and varied with seasons and vegetation zones. These changes thus, altered the abundance and community structures of soil microorganisms in the various regions (Siles *et al.* 2016).

The studies above highlight the broad range of biotic and abiotic factors that affect and shape the soil microbial composition associated with montane soils. Despite these changes, soil microorganisms have adapted to survive in these conditions. While some have adapted their cellular components to keep their membranes fluid and keep their enzymatic activity functional even in the much harsher cold conditions (Donhauser and Frey, 2018). Others have adapted to follow an oligotrophic lifestyle that allows them to survive under nutrient-limited conditions (Donhauser and Frey, 2018). Lastly, some soil microorganisms possess the ability to become dormant and decrease their metabolic activity to survive in such conditions (Sayed, *et al.*, 2020). However, changes in biotic and abiotic factors are further exacerbated by climate change which creates additional stress on soil microorganisms in mountain environments (Dutta and Dutta, 2016).

4. Mountains and climate change

Climate change is caused by increased emissions of greenhouse gases, such as nitrous oxide, methane, ozone, chlorofluorocarbons, and particularly carbon dioxide from increased industrial and anthropogenic activities (Anstey, 2013). This results in a range of effects which include warming of the planet, melting polar ice caps, the rise in ocean levels, and increased frequencies of droughts, floods, and other extreme weather events (Barrett, *et al.*, 2015). According to the latest Intergovernmental Panel on Climate Change report, climate change has already caused an increase in land surface air temperatures of about 1.53ºC from the period of 1850-1900 to 2006-2015 (IPCC, 2019; Jia, *et al.*, 2019). Projections of further changes of about 1.5ºC in surface temperatures between 2030 and 2052 are also expected if temperatures continue to increase at the current estimated rate of about 0.2 ºC per decade (IPCC, 2018).

While latitudinal gradients experience climatic zones over large distances, making it difficult to establish the true impact of climatic variables on biota (Graae, *et al.*, 2012), elevational gradients on mountains exhibit dramatic changes in climate (Siles and Margesin, 2016) and biotic turnover over short geographical distances (Yuan, *et al.*, 2014). As such, mountain ecosystems are very sensitive to changes in climate, making them suitable models for the study of how climate change could affect biota across a broader global context (Rogora, *et al.*, 2018). The impact of warming on montane ecosystems includes changes in precipitation patterns, snow cover, and water availability (Beniston and Stoffel, 2014), loss of biodiversity, degradation of existing habitats, and changes in vegetation patterns (Rogora, *et al.*, 2018).

While mountain plant species have been shown to provide important information as early indicators of climate change (Lamprecht, *et al.*, 2018), data relating to the changes in the soil microbiome related to climate change has also come to the fore (Dutta and Dutta, 2016). This is particularly apparent when considering their predominance in the soil environment and their pivotal biological role in this ecological niche (Dutta and Dutta, 2016). The following section will consider the montane soil microbiome and the effect of changing climatic conditions on this fundamental ecosystem.

4.1 Changing temperatures

Temperatures on mountains have been on the rise as a result of global climate change with higher elevation regions experiencing a far greater increase compared to lower lying areas (Lamprecht, *et al.*, 2018). Between 1961 and 2010, mean annual temperatures in higher elevation regions (> 500 m.a.s.l.) increased 1.24 times faster compared to lower elevation regions (< 500 m.a.s.l.) around the world (Wang, *et al.*, 2016). Since soil microorganisms have specific temperatures under which they grow and perform optimally (Fierer and Schimel, 2003), these increases in montane temperatures not only influence their growth and activity rates but also alter and shape their community composition (Pietikäinen, *et al.*, 2005). This includes an increase in those microbial taxa that are better adapted to warming conditions and the concomitant loss of those taxa that cannot adapt (Fierer and Schimel, 2003). Furthermore, with higher elevation regions experiencing greater levels of warming, species that dominate under warmer conditions at lower elevations in warmer conditions, outcompete those that initially inhabited the soils at higher elevations with previously lower temperatures (Castro, *et al.*, 2010). Reshaping of the soil microbiome further results in changes in biochemical cycles and crucial ecosystem processes such as photosynthesis and respiration (Donhauser and Frey, 2018). Higher temperatures lead to increases in processing and nutrient cycling rates as well as microbial activity, further affecting microbial community composition (Dutta and Dutta, 2016). Temperature changes on mountains are further coupled with changes in precipitation patterns and thereby soil moisture levels (Classen, *et al.*, 2015).

4.2 Changes in precipitation patterns and soil moisture

Rising winter temperatures have had a severe effect on precipitation patterns in montane environments. Several mountain regions, such as on the Tibetan Plateau and the Chinese Tienshan mountains (Li, *et al.*, 2020), are experiencing decreases in snowfall patterns, resulting in thinner layers or no snow cover at all (Wipf, *et al.*, 2015). Other mountain regions such as the European Alps and the Himalaya mountains (Rasul, *et al.*, 2020), which have more permanent snow cover have seen a decrease in areas with large volumes of snow, accelerated rates of snow melting, and complete loss of snow cover at lower elevations (Huss, *et al.*, 2017). Without the insulation of snow, soil temperatures on mountains fluctuate, resulting in the increased incidence of frequent freeze-thaw cycles in these regions (Liu, *et al.*, 2010).

Freeze-thaw cycles of snow can cause changes in the nutrient and water availability which further affect the microbial composition in montane soil (Xu, *et al.*, 2016). After the thawing of snow, an increase in nutrients is induced due to the death of microorganisms and the degradation of plant matter that occurs through the thawing process (Xu, *et al.*, 2016). This results in a decrease in microbial composition (Wipf, *et al.*, 2015). An increase in nutrients after the thawing of snow occurs, allowing surviving soil microorganisms to thrive. However, increases in freeze-thaw cycles of snow due to climate change lead to a substantial reduction in microorganisms that are better adapted to the much colder temperatures found at higher elevations on mountains such as psychrophiles (DeLuca, *et al.*, 1992; Skogland, *et al.*, 1988).

Apart from changes in snowfall patterns, climate change is also causing changes in rainfall patterns with several mountain regions experiencing an increase in extreme rainfall events (Lin, *et al.*, 2017), while others have experienced drought conditions (Bollig and Feller, 2014; Rosbakh, *et al.*, 2017). For example, in 2003 the European Alps experienced the highest temperatures recorded in over 500 years, which impacted the growing seasons of vegetation at elevations of 700 to 2,800 m.a.s.l. (Jolly *et al.* 2005). Drought on mountains results in decreased soil moisture, which negatively impacts nutrient availability as well as soil microbial activity and growth rates (Felsmann, *et al.*, 2015). Decreased soil moisture can cause changes in nutrient diffusion, soil pore size, and oxygen availability, which further impact the soil microbiome and its functioning on mountains (Stres, *et al.*, 2008). Rainfall in these mountains following droughts causes increased soil microbial activity due to greater levels of nutrients from the decomposition of SOM (Mikha, *et al.*, 2005). Increases in SOM in mountain soil result from the hydration of soil microbial cells that could not survive the drought and death of those

soil microorganisms that managed to survive the drought but could not survive the additional stress of rainfall (Borken and Matzner, 2009). Furthermore, microorganisms that had previously adapted to drought conditions need to readjust to soil with increased moisture and ultimately this affects microbial community composition and activity in montane soil (Mikha, *et al.*, 2005).

Although precipitation and soil moisture are prerequisites for microbial growth, extreme rainfall events (Lin, *et al.*, 2017) and soil moisture above optimal levels can lead to decreases in oxygen availability and subsequent reshaping of the microbial community inhabiting montane soil (Liu, *et al.*, 2009; Stres, *et al.*, 2008). Furthermore, the decomposition rate and activity of soil microorganisms are affected on mountains (Liu, *et al.*, 2009; Stres, *et al.*, 2008). This occurs as nutrient availability decreases as a result of increased water availability, subsequently leading to a decrease in microbial activity and decomposition rates (Ma, *et al.*, 2012).

4.3 Shifts in vegetation and nutrients on mountains

Mountain vegetation is thought to survive under a limited temperature range. Thus, changes in their distribution patterns may be directly correlated to changes in temperatures (Jurasinski and Kreyling, 2007). Plant response to temperature changes may either be an adaptation to the conditions or a shift in distribution to a habitat better suited for growth and functioning (Walther, *et al.*, 2005). The shift in plant taxa as a result of climate change has been observed on several mountains, globally (Beckage, *et al.*, 2008). For example, a study conducted in two alpine valleys in the Sikkim state, which is part of the eastern Himalaya, over the elevation zones between 4,000 and 5,500 m.a.s.l., showed an upwards shift in 87% of endemic plant species studied due to warming (Telwala, *et al.*, 2013). Similarly, in a study conducted in a valley on the Sikkilsdalen mountain summit in central Norway between 992 and 1,778 m.a.s.l., more vegetation species were found to shift upwards compared to downwards (Felde, *et al.*, 2012).

Changes in plant distribution have a profound effect on soil microorganisms and their function in mountain ecosystems (Eisenhauer, *et al.*, 2010). Vegetation type influences the composition and activity of soil microorganisms through several factors such as changes in the quality and quantity of litter produced (Bluhm, *et al.*, 2019), the microclimate, and through the symbiotic relationships between vegetation and soil microorganisms on mountains (Wu, *et al.*, 2018).

The biochemical composition of distinct plant species varies, and therefore changes in their distribution patterns and diversity affect the quality and quantity of the nutrients and litter produced, and ultimately soil microbial community composition and activity in mountain environments (Eisenhauer, *et al.*, 2010).

Changes in vegetation distribution on mountains due to climate change could either result in the dominance of slower-growing plants at higher elevations (Cornelissen, *et al.*, 2007) and thus, a reduction in soil microbiome complexity or it could result in the dominance of faster-growing plants that would promote soil microorganism composition and activity (Bardgett, *et al.*, 2008). Apart from providing nutrients to soil microorganisms, plant litter also plays a role in maintaining soil fertility, as greater SOM tends to improve soil properties such as soil structure and its ability to retain more moisture (Li, *et al.*, 2019). Thus, these shifts in vegetation distribution on mountains also disrupt the equilibrium that has been maintained between existing plant species and soil microorganisms (Jin, *et al.*, 2019), as well as soil fertility and soil properties in these regions which ultimately impact soil microorganisms (Li, *et al.*, 2019).

While various abiotic and biotic factors can be expected to have substantive effects on the montane soil microbiome, other non-climatic influences can also impact microbial community composition and activity and thus should also be considered. The key to this is the increased anthropogenic use of montane environments.

4.4 Other factors affecting the montane soil microbiome

The increased need for livestock products around the world has led to an increase in more land for grazing, such that several mountain regions such as the Tibetan Plateau have reported degradation in grasslands due to overgrazing (Lu, *et al.*, 2017). Grazing in mountain environments leads to a decrease in plant availability and diversity, resulting in reduced plant cover (Lu, *et al.*, 2017; Xun, *et al.*, 2018) and exposing soil microorganisms to environmental stressors (Rime, *et al.*, 2015). Furthermore, overgrazing of certain regions can lead to changes in vegetation types, with an increase in faster-growing plant and grass species which are tolerant to grazing (Liu, *et al.*, 2012; Yang, *et al.*, 2019). These changes in vegetation thereby have an impact on the composition and activity of soil microorganisms in these regions through the quality and quantity of plant litter produced (Yang, *et al.*, 2019).

Grazing of mountain environments also leads to changes in soil physical and chemical properties which affect soil microorganisms (Apollo, *et al.*, 2018). Through trampling by

livestock and wild herbivores (Mysterud, 2006), topsoil in mountain environments may be compacted, affecting properties such as moisture, water retention capacities, and aeration (Yang, *et al.*, 2019). Grazing can furthermore affect the nutrient composition of soil through the production of faecal matter and urination by animals (Liu, *et al.*, 2012; Mysterud, 2006). This affects the amount and composition of SOM which has an impact on the rate at which soil microorganisms can decompose organic matter and the availability of nutrients in the soil (Liu, *et al.*, 2012).

While studies pertaining to the impact of climate change in ecosystems and environments are mainly focused on changes in biotic and abiotic factors, studies determining the impact of climate change as well as changing abiotic and biotic factors on soil microorganisms have come into the limelight (Dutta and Dutta, 2016). While there are several techniques and technologies available to investigate the impact of these factors on soil microorganisms, these studies have mainly been made easier and more comprehensive through advancements in the metagenomic field (Wooley and Ye, 2009). The following section provides further consideration of available metagenomic techniques and their practical usage in studies pertaining to soil microorganisms on montane environments.

5. Studying the microbial ecology of montane environments

Since soil represents a rich environment for microorganisms, it has been the subject of extensive studies to elucidate the bacterial and fungal counterparts inhabiting this environment (Maron, *et al.*, 2018). Traditionally, soil microbial ecology studies relied on the isolation of microorganisms from the soil, culturing them on artificial growth medium prior to their identification and characterisation (Franco-Duarte, *et al.*, 2019; Houpikian and Raoult, 2002). Culture-dependent studies have been used to study montane environments and these have shown that soils in these regions harbour a range of culturable bacterial and fungal isolates. For example, in a study conducted in forest soil with hemlock vegetation on the Tatachia Mountain in central Taiwan, over an elevational range of 1,800 to 3952 m.a.s.l., 10^5 to 10^7 colony forming units (CFUs) per gram of culturable bacteria and 10^3 to 10^5 CFUs per gram of fungi were isolated from topsoil (Yang, *et al.*, 2006). In a study conducted on the Eastern Himalayan range of Northeast India, over an elevational range of 24 to 2,000 m.a.s.l., with various vegetational and climatic zones, 20.08 x 10^3 CFUs per gram of fungi was isolated below 500 m.a.s.l. and 5.48 x 10^3 CFUs per gram of fungi in soils at elevations of 1,500 to 2,000 m.a.s.l. (Devi, *et al.*, 2012).

Although these techniques can provide both quantitative and qualitative information about microorganisms, they are quite time-consuming and laborious (Franco-Duarte, *et al.*, 2019; Kirk, *et al.*, 2004). Furthermore, a key observation in microbial ecology studies over the years has been that > 99% of microorganisms present in the natural environment are not readily culturable under standard conditions (Amann, *et al.*, 1995). As such, the reliance on microbial cultivation from soil creates a bottleneck in understanding their diversity and function in an environment (Hugenholtz and Tyson, 2008). This has resulted in the development of a range of culture-independent techniques, where microbial communities can be identified *in situ* (Houpikian and Raoult, 2002).

Early culture-independent techniques included a range of molecular and biochemical approaches, each with their own benefits and drawbacks based on the type of study they are required in (Franco-Duarte, *et al.*, 2019). Biochemical approaches that can be used to identify microorganisms include Mass spectrometry, with techniques such as Gas chromatography (GC), Liquid chromatography (LC), and Nano-scale secondary ion mass spectrometry (NanoSIMS) (Franco-Duarte, *et al.*, 2019; Oehler, *et al.*, 2010) and Spectrometry with the Raman Spectroscopy technique (Franco-Duarte, *et al.*, 2019; Huang, *et al.*, 2009). Molecular techniques include both Polymerase Chain Reaction (PCR)-based methods such as terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), and quantitative PCR (qPCR) (Franco-Duarte, *et al.*, 2019; Su, *et al.*, 2012) and non-PCR-based methods such as fluorescence *in situ* hybridization (FISH), microarray (Su, *et al.*, 2012), fatty acid methyl ester (FAME), and phospholipids fatty acid analyses (PLFA) (Stefanis, *et al.*, 2013).

Many of these culture-independent techniques have been utilised in studies conducted in mountains. For example, a study conducted on the Changbai Mountain in north-eastern China, across the elevations 740 to 2,691 m.a.s.l. incorporating climatic and vegetational zonation of montane, subalpine, and alpine, PLFA showed that the total microbial biomass across various locations varied (Zhang, *et al.*, 2013). The total microbial biomass accounted for 125 to 129 nmol per gram of soil at elevations of 1,204, 1,974, and 2,295 m.a.s.l. and 302 nmol per gram of soil at a lower elevation of 807 m.a.s.l. (Zhang, *et al.*, 2013). In another study conducted on the North Tiashan Mountain in China, at elevations of 800 to 4,480 m.a.s.l., incorporating climatic and vegetational zonation of montane, subalpine, and alpine, T-RFLP analysis

determined the presence of 233 main Terminal Restriction Fragments (T-RF) representing the diversity of methanotrophs at various locations (Wu, *et al.*, 2013).

Many of these techniques, however, have more recently been superseded by the development of DNA sequencing technologies such as metagenomics which allows for the direct analysis of all the genomes present within an environmental sample, without the need for culturing (Thomas, *et al.*, 2012). In its early form, this technique involved the extraction of genomic DNA from an environmental sample, followed by cloning into a suitable vector and transformation into a heterologous host (Handelsman, 2004). This still forms the basis of functional metagenomic approaches, but with the development of next-generation sequencing (NGS) technologies, a novel avenue of research has evolved. This technological advance has made it possible to sequence the complete metagenome derived from an environmental sample (Thomas, *et al.*, 2012). Furthermore, apart from the study of genes through metagenomics, it has allowed for the introduction of several other meta-omics sequencing techniques such as metatranscriptomics, metaproteomics, and metametabolomics. These allow for the study of transcripts, proteins, and metabolites present within microbial communities (Segata, *et al.*, 2013).

While whole metagenome sequencing can provide excellent insights into the composition of a microbial community and its functional capacity within a given environment (Ghosh and Das, 2018), it is as yet, very costly, time-consuming, and requires extensive computing capacity to complete (Oulas, *et al.*, 2015). This has led towards the advancement of metagenomic techniques which enable random sequencing across the entire genome of microbial communities known as shotgun sequencing and targeted sequencing of gene markers (Suenaga, 2012). Targeted sequencing of gene markers can provide insight into microbial community composition and structure at a fraction of the price (Suenaga, 2015). This technique, amplicon-based metagenomics or metataxonomics has become a mainstay approach in microbial ecology studies over the years (Hilton, *et al.*, 2016).

5.1 Metataxonomics for the study of montane microbiomes

Metataxonomic functions on the basis of selective amplification and massive parallel sequencing of microbial marker genes directly from an environmental sample (Hilton, *et al.*, 2016). A typical metataxonomic study (Figure 2) involves the collection of an environmental sample from which genomic DNA is extracted, which should represent all the cells present in

the sample (Thomas, *et al.*, 2012). Subsequently, the conserved fragment of DNA from the collective genomes retained in the genomic DNA is selectively amplified using *en masse* PCR amplification (Hilton, *et al.*, 2016). Marker genes are preferred for phylogenetic studies as they are universally found in the population with hypervariable regions that allow for the differentiation of species and are flanked by conserved regions that can be targeted by universal primers (Breitwieser, *et al.*, 2017).

The ribosomal RNA (rRNA) gene is one of the most common marker sequences used as it is highly conserved across several taxa (Breitwieser, *et al.*, 2017) and these include the 16S rRNA gene of bacteria and archaea and the internal transcribed spacer (ITS) region of fungi (Gallon, *et al.*, 2019). PCR amplification of these conserved regions results in amplicon libraries that can be sequenced using available NGS platforms (De Maayer, *et al.*, 2014). Resultant sequences then need to be assessed for quality control to remove any sequencing errors (De Maayer, *et al.*, 2014) and then compared against databases containing reference sequences of known microorganisms (Thomas, *et al.*, 2012). These databases include SILVA (Glöckner, *et al.*, 2017), Ribosomal Database Project (RDP) (Cole, *et al.*, 2005), probeBase, and, GreenGenes (Sambo, *et al.*, 2018) which contains genes from several million species covering a greater range compared to genome databases (Breitwieser, *et al.*, 2017).

Through these means, microorganisms from an environmental sample can then be classified into groups known as operational taxonomic units (OTUs) by clustering sequences with a predetermined similarity level (Schloss and Handelsman, 2004; Thomas, *et al.*, 2012). These OTUs can then be used to determine abundance data representing the number of sequences observed in a sample for a particular taxon which can then be used for statistical analysis (Calle, 2019). A number of pipelines have been developed for the processing of metataxonomic data to assist in identifying and characterising microbial sequences isolated from samples (Dudhagara, *et al.*, 2015). These platforms include MG-RAST, MOTHUR, IMG/M, and QIIME that provide integrated platforms for the analysis, management, and storage of metataxonomic data (Oulas, *et al.*, 2015). Some of these platforms such as MOTHUR, QIIME, as well as R packages (Galloway-Peña and Hanson, 2020) further allow for statistical analysis of sequenced data.

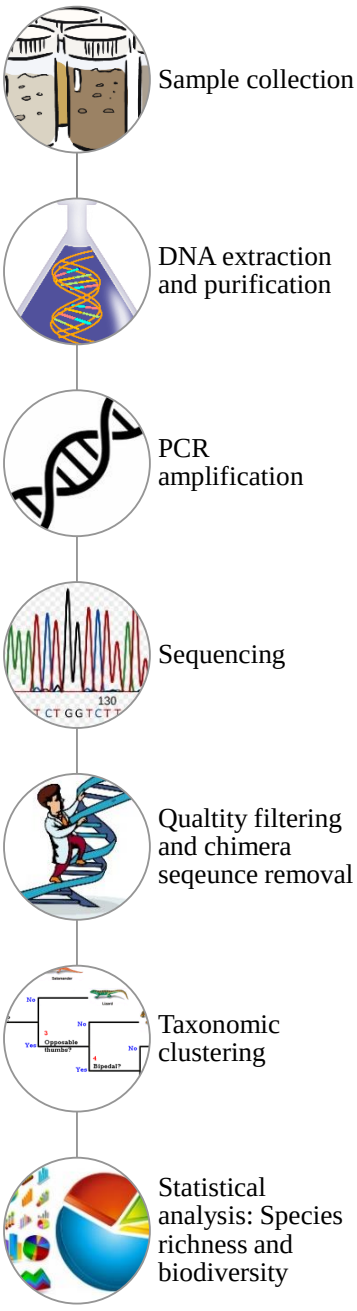

Figure 2: Representation of a typical metataxonomics workflow. (Diagram adapted from De Maayer, *et al*., 2014)

Metataxonomics has further become cheap and easy, such that metataxonomic studies have been conducted on a broad range of environments, including oceans (Hilton, *et al.*, 2016), deserts, temperate environments (Fierer, *et al.*, 2012), and within the human body (Hilton, *et al.*, 2016). Metataxonomics has also been applied to several fields such as agriculture, food science, forensics, and even pharmaceuticals (Hiraoka, *et al.*, 2016). Furthermore, comparative studies can be conducted using metataxonomics across a spatio-temporal scale or to test different abiotic and biotic conditions in various environments (Mascarenhas, *et al.*, 2020). These types of studies can be conducted using the measures of diversity within a community of microorganisms (α-diversity), compared to the diversity between communities of microorganisms (β-diversity) (Galloway-Peña and Hanson, 2020; Goodrich, *et al.*, 2014).

Likewise, metataxonomics has been employed to study several mountain ecosystems and the impact of factors such as elevational gradients, seasons, and mountain abiotic and biotic variables on the diversity and community structures of soil microorganisms. For example, a recent metataxonomic study by Adamczyk and associates conducted in the Swiss Alps, across ten different summits ranging in elevation from 2,360 to 3,212 m.a.s.l., across alpine grasslands and scarcely vegetated nival biomes identified a total of 10,406 bacterial and 6,291 fungal taxa present in these soils (Adamczyk, *et al.*, 2019). The study further found that the microbial community composition was mainly influenced by soil pH. Furthermore, bacterial communities were found to be strongly affected by changes in abiotic and biotic factors compared to fungal communities which responded weakly (Adamczyk, *et al.*, 2019).

This technique has also been employed to study the microbial composition in extreme mountain environments such as the West Kunlun Mountains on the edge of the Tibetan Plateau (Yang, *et al.*, 2016). Nine samples were collected from the Chongce Ice Cap from both glacial snow and glacial soil across an elevational range of 5,756 to 6,127 m.a.s.l., from which 96,012 bacterial taxa were identified (Yang, *et al.*, 2016). Bacterial diversity across glacial snow was also found to be lower than that in glacial soil, with bacterial communities also being different across the two habitats, thereby, providing insight on the response of bacteria to glacial conditions and habitats in these regions (Yang, *et al.*, 2016).

A metataxonomic analysis of the high Altiplano region spanning elevations between 3,870 and 4,480 m.a.s.l., located below the Lascar volcano which experiences frequent eruptions, creating extreme conditions, in the Atacama Desert identified 4,775 distinct bacterial taxa (Maza, *et al.*, 2019). A comparison of this data to that obtained from other environments showed the

taxonomic profile of the Altiplano region to resemble that of cold arid soil from the Arctic desert (Maza, *et al.*, 2019). Furthermore, Altiplano bacterial communities incorporated a range of taxa which are of potential biotechnological value which was determined through comparisons of bacterial isolates from this region to known species on the RDP database (Maza, *et al.*, 2019). Such studies highlight the versatility of metataxonomics in studying the impact of various environments and factors on soil microorganisms.

6. Rationale

Studies integrating metataxonomics in mountain environments are conducted across the world to study soil microorganisms present in these environments and their response to various abiotic and biotic factors. However, in the Lesotho highlands, located in southern Africa, such studies focussing on the soil microbiome are substantially lacking with no further knowledge available on their response to climate change in these regions. In this study, the main region of focus was particularly on a summit inland of the Sani Pass region of Drakensberg near Kotisephola Pass, also known as the Black Mountain Pass, in the Lesotho highlands. These regions have the presence of different vegetation patterns, aspects, and microclimates along an elevational gradient creating a unique environment to study the impact of various abiotic and biotic factors on soil microorganisms in the highlands.

As such the main aim of this study was to first characterise the total microbial diversity associated with a mountain peak and its flanking slopes in the Lesotho highlands using metataxonomics. Secondly, it was to determine the impact of factors such as slope aspect, elevation, and ground surface temperature changes on the diversity and community structures of these microorganisms.

This study would not only provide the first insight into the soil microbiome present on the selected summit in the Lesotho highlands but also their response to changing environmental and climatic conditions on the mountain due to variations in elevation and slope aspect. This kind of information could be used to compare regions with similar conditions in the Lesotho highlands and globally. Furthermore, this study could provide the opportunity to determine the ability of soil microorganisms to survive and respond in such extreme mountain environments that are currently changing due to climate change.

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CHAPTER TWO – Microbial community structure across a mountain summit in the Lesotho highlands

1. Introduction

Southern Africa comprises of approximately 10% mountain regions which either forms part of the Great Escarpment or are freestanding in various parts of the country, this is despite having lower numbers of mountains regions compared to the rest of Africa and the world (McDonald, *et al.*, 2019; Shroyer and Blignaut, 2003). One of the highest regions in southern Africa is the mountainous kingdom of Lesotho, located between the latitudes 28° 30′ to 30° 40′ south and longitudes 27° 00′ to 29° 30′ east (Grab, *et al.*, 2017). This region covers covering a total land area of 30,355 km^2 (Grab, *et al.*, 2017) and is completely bordered by the South African provinces Kwa-Zulu Natal, the Free State, and the Eastern Cape (Fitchett, *et al.*, 2016). With all land lying at elevations greater than 1,400 m.a.s.l. (Roberts, *et al.*, 2013), Lesotho is classified into four physiogeographical regions, namely the western lowlands, the foothills, the Senque River Valley, and the eastern highlands. The latter highlands account for a total of 80% of all land area and incorporates several summits that extend to elevations greater than 3,000 m.a.s.l. (Boelhouwers and Meiklejohn, 2002; Fitchett, *et al.*, 2016), which includes the highest point in southern Africa, Thabana Ntlenyana, which reaches a height of 3,482 m.a.s.l. (Grab, *et al.*, 2017). The climate in the Lesotho highlands ranges from semi-arid to sub-humid (Grab and Nash, 2010) and generally experiences cool and mild summers and cold winters (Mills, *et al.*, 2009a).

Precipitation in the highlands varies with regions experiencing frequent bouts of rain- and snowfall throughout the year (Mulder and Grab, 2009; Sene, *et al.*, 1998). Frontal perturbations from the southwestern part of the African continent further contribute to cold conditions in the highlands lasting for several days and at times leading to snowfall or severe frost at higher elevations between the months of May and September (Grab and Nash, 2010). Temperatures in the Lesotho highlands are further moderated by elevation and slope aspect, such that the higher elevation regions tend to be colder compared to the lower-lying areas across southern Africa, and the north-facing slopes of mountains being considerably warmer compared to the south-facing slopes (Roberts, *et al.*, 2013). These changes in the highlands bring about microclimatic variations which have a substantial impact on vegetation types that inhabit the slopes of mountains (Parker, *et al.*, 2011; Zhang, *et al.*, 2013).

Vegetation types on the summits in Lesotho vary across both elevation and slope aspect (Roberts, *et al.*, 2013). Plants utilising the C_4 photosynthetic pathway mainly grow on the north-facing slope at lower elevations with warmer temperatures, while C_3 vegetation predominates

at elevations higher than 2,745 m.a.s.l. due to colder temperatures (Parker, *et al.*, 2011; Roberts, *et al.*, 2013). On the south-facing slope, C_3 vegetation grows at elevations lower than 2,745 m.a.s.l. due to colder temperatures (Parker, *et al.*, 2011; Roberts, *et al.*, 2013). Some of the common vegetation occurring on the summit of Lesotho include tussock grasses and low shrubs generally from the genera *Merxmuellera* and *Helichrysum*, respectively (Knight and Grab, 2014; Morris, 2017). Both of these vegetation types follow the C_3 photosynthetic pathway and as a result, could indicate high levels of CO_2 in the highlands as these types of plants tend to be more sufficient in high CO_2 environments at colder temperatures (Brand, *et al.*, 2015; Morris, 2017). Thus, this C_3 vegetation could be used as a potential indicator of climate change in the Lesotho highlands (Morris, 2017). Variations in these vegetation types could have an impact on the community composition and activity of soil microorganisms through nutrient availability and soil properties in these regions (Ren, *et al.*, 2018).

While a number of studies have focused on the geomorphology, climate, and vegetation of the Lesotho highlands (Fitchett, *et al.*, 2016; Mills, *et al.*, 2009; Morris, 2017), there is a dearth of knowledge relating to the soil microbiome associated with this montane environment.

This chapter aims to characterise the total bacterial and fungal diversity across a mountain peak and its flanking slope in the Lesotho highlands and determine the impact of slope aspect and elevation on the diversity and community structure of these microorganisms. This was achieved through the following objectives:

1. Soil sample collection and DNA extraction
2. Amplicon sequencing of partial bacterial 16S rRNA and partial fungal ITS2 amplicon genes
3. Metataxonomic analysis to characterise the microbial diversity of the selected sites
4. Linkage between microbial diversity and community structure, slope aspect, and elevation using statistical tests

These objectives were used to test the hypothesis that microbial diversity varies with slope aspect as distinct environmental and microclimatic conditions are created on the various slopes (temperatures, soil properties, solar radiation exposure, and vegetation). Furthermore, the hypothesis of slope aspect and elevation influencing bacterial and fungal diversity and community structures on mountains was tested as increasing elevation creates harsher environmental conditions.

This study would provide first insight on the bacterial and fungal diversities present on the selected summit in the Lesotho highland and the impact of slope aspect creating various microclimatic conditions on diversity and community structure. Furthermore, it serves as an insight in understanding how these microorganisms survive in harsh environmental conditions present on mountains whether it be in soil under snow or soil exposed to extreme solar radiation.

2. Methods and materials

2.1 Field sites and soil collection

The Plateau (P), North-facing (N) and South (S)-facing slopes on a summit inland of the Sani Pass region in the Drakensberg, and located near Kotisephola Pass (also known as the Black Mountain Pass; (Figure 3), were selected for this study. This region falls between the longitudinal coordinates 29° 30' 46 to 29° 30' 52 south and latitude 29° 13' 09 east. The elevational sampling range over which this study was conducted was 3,330 to 3,350 m.a.s.l.. This is equivalent to about a 20-meter increase in elevation from the highest to the lowest point at which sampling was conducted. Despite the short elevational range, the distance across which sampling was conducted was ~ 27 m from the rock scarp on the south-facing slope to the bottom of the slope. This included the three distinct climatic and vegetational zonation visible across the south-facing slope and the steep gradient noted, as the slope seemed to level off after this distance. Similarly, the north-facing slope was sampled across ~ 27 m from the top to the bottom of the slope, to incorporate the zonation in vegetation observed across the slope. The plateau included zones close to the northern and southern ends of the summit as well as the middle and this also incorporated three distinctly different regions in terms of soil coverage and vegetation.

Three sampling sites (A, B, and C) were selected for soil sample collection along with the Plateau (P), North-facing slope (N), and South-facing slope (S) axis, respectively (Figure 4; Table 1), given a total of nine distinct sampling locations. At each of the nine locations, four soil samples were collected from each corner of a 4 m x 4 m plot. Plant material, debris, stones, and pebbles were scraped away from the sample sites and soil samples were collected at a depth of 5-10 cm below the surface. Soil samples were collected in sterile Greiner tubes which were sealed and transported back to the laboratory at room temperature prior to storage at 4ºC. All soil samples were processed within two weeks of collection.

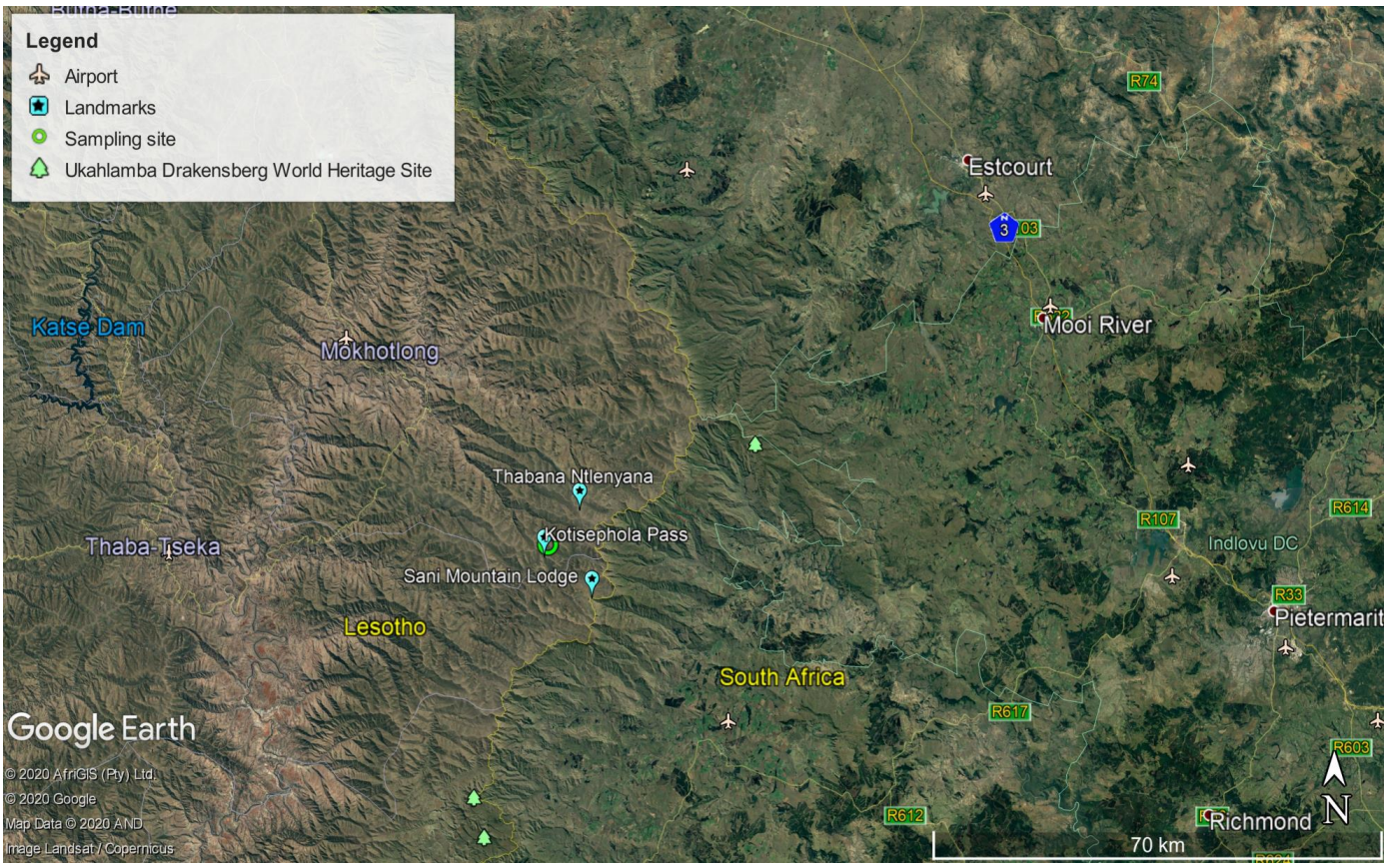


Figure 3: Geographical location of the study site in Lesotho. The sample site is located between the highest mountain in southern Africa, Thabana Ntlenyana, and the Kotisephola Pass (Map adapted from Google Earth, n.d.).

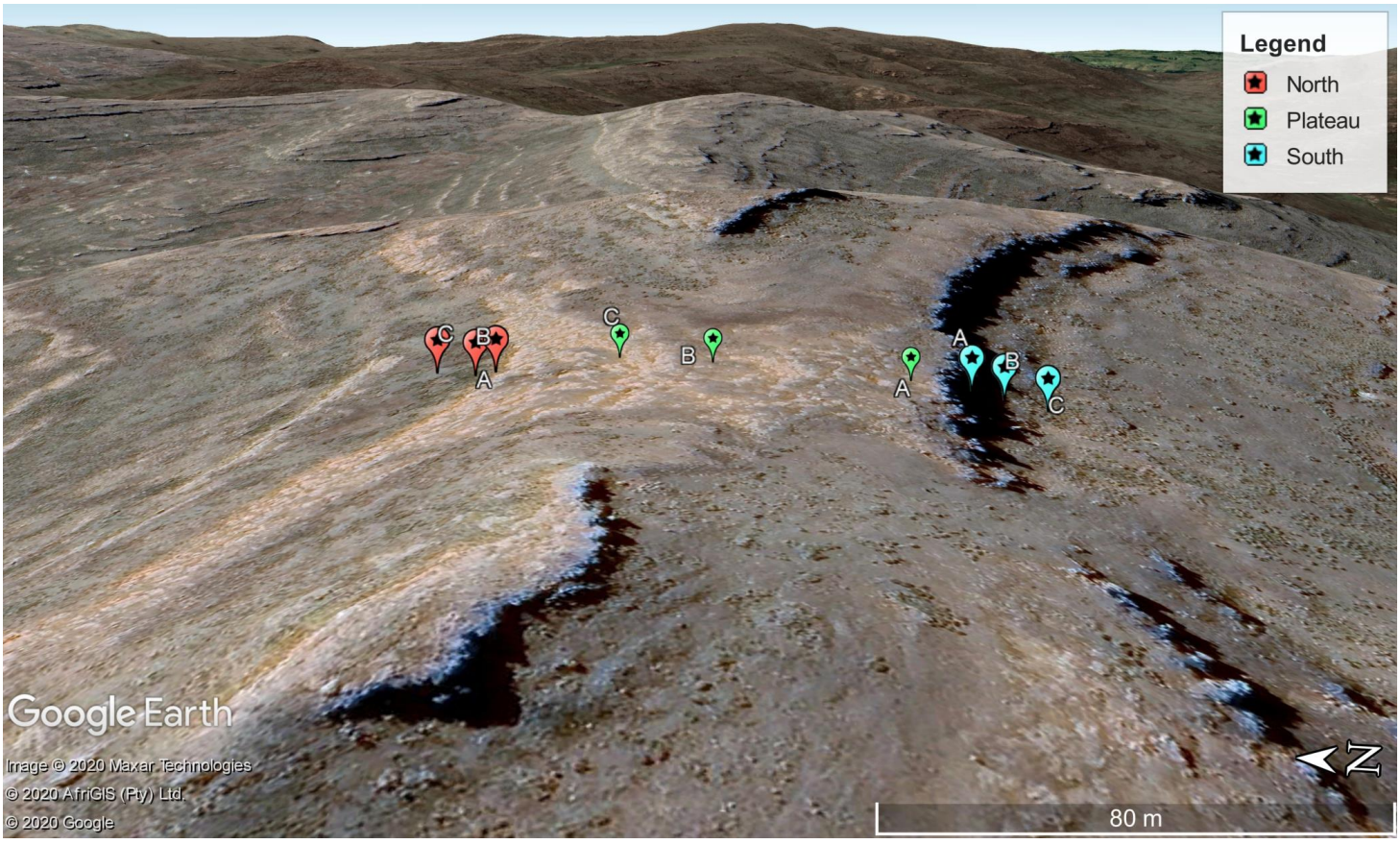


Figure 4: Sampling sites across the three montane aspects (plateau, north- and south-facing slope) on the selected summit

(Adapted from Google Earth, n.d.)

Table 1: Overview of the sampling sites and their location on the selected summit

Primary site	Secondary site	Site location	Replicates	Elevation (m.a.s.l.)	Latitude and longitude
South	South A	Top of the slope	SA1	3,338	29°30'51.9"S 29°13'09.3"E
			SA3	3,336	29°30'52.1"S 29°13'09.2"E
			SA4	3,336	29°30'52.0"S 29°13'09.4"E
	South B	Middle of the slope	SB1	3,332	29°30'52.2"S 29°13'09.3"E
			SB2	3,335	29°30'52.1"S 29°13'09.5"E
			SB4	3,334	29°30'52.3"S 29°13'09.3"E
	South C	Bottom of the slope	SC2	3,334	29°30'52.6"S 29°13'09.3"E
			SC3	3,330	29°30'52.7"S 29°13'09.0"E
			SC4	3,329	29°30'52.7"S 29°13'09.1"E
Plateau	Plateau A	Southern end of summit	PA1	3,348	29°30'51.4"S 29°13'09.1"E
			PA3	3,348	29°30'51.2"S 29°13'09.2"E
			PA4	3,347	29°30'51.3"S 29°13'09.4"E
	Plateau B	Midway of plateau	PB1	3,349	29°30'49.5"S 29°13'09.2"E
			PB2	3,349	29°30'49.4"S 29°13'09.3"E
			PB3	3,349	29°30'49.6"S 29°13'09.4"E
	Plateau C	Northern end of summit	PC1	3,349	29°30'48.6"S 29°13'09.4"E
			PC3	3,348	29°30'48.4"S 29°13'09.5"E
			PC4	3,348	29°30'48.4"S 29°13'09.4"E
North	North A	Top of the slope	NA1	3,335	29°30'47.5"S 29°13'09.2"E
			NA2	3,338	29°30'47.5"S 29°13'09.4"E
			NA4	3,335	29°30'47.3"S 29°13'09.2"E
	North B	Middle of the slope	NB1	3,335	29°30'47.1"S 29°13'09.3"E
			NB2	3,335	29°30'47.1"S 29°13'09.3"E
			NB3	3,335	29°30'47.2"S 29°13'09.3"E
	North C	Bottom of the slope	NC2	3,333	29°30'46.7"S 29°13'09.6"E
			NC3	3,333	29°30'46.6"S 29°13'09.6"E
			NC4	3,332	29°30'46.6"S 29°13'09.5"E

2.2 Genomic DNA extraction

Genomic DNA (gDNA) was extracted from ~0.4 g soil aliquots using the MoBio Powersoil DNA isolation kit (Qiagen, USA) as per manufactures instructions. DNA was quantified using the Nanodrop (Thermo Fisher Scientific, Massachusetts, USA). Extracted DNA was stored at -4ºC until further analysis was performed.

2.3 PCR amplification and sequencing

From the 36 samples of extracted DNA, a total of twenty-seven samples, three from each of the nine locations were selected at random (Table 1) and were sent to Molecular Research LP (www.mrdnalab.com, Shallowater, TX, USA) for amplicon sequencing. Both amplicon sequencing of the 16S rRNA and ITS2 gene regions were performed for the study of the bacterial and fungal counterparts, respectively. Partial bacterial 16S rRNA gene amplicons were generated using the 27F (5'-AGRGTTTGATCMTGGCTCAG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') primers, which target the hypervariable regions V1-V4 and the partial fungal ITS2 gene amplicons were generated using the ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers which target the ITS2 hypervariable region (Valverde, *et al.*, 2016). PCR amplification was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) using the conditions as per the manufacturers' instructions. Amplicon products were pooled based on their molecular weight and DNA concentration and thereafter, purified using Ampure XP beads. Following library preparation amplicon sequencing was performed using the Illumina MiSeq platform using the paired-end read approach (2 x 150 bp).

The raw sequence data were processed using the MR DNA analysis pipeline (www.mrdnalab.com, Shallowater, TX, USA). Initial processing of the reads included joining of paired-end reads, removal of barcodes, removal of reads less than 150 base pairs in length, quality filtering of trimmed sequences and, denoising of sequences in which sequence errors were rectified. Subsequently, chimeric sequences were removed using UCHIME (Edgar, *et al.*, 2011). The trimmed and filtered reads were clustered according to their Operational Taxonomic Units (OTUs) on the basis of 97% sequence identity using USEARCH (Edgar, 2010). The OTUs were taxonomically classified using BLASTn against a curated database which was derived from the Ribosomal Database Project (www.rdp.cme.msu.edu) as well as the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.gov).

The processed sequences were further analysed using R v 3.6.1 (R Foundation for Statistical Computing; http://www.R-project.org) and RStudio (RStudio Team 2014). First, singleton sequences were removed. Since certain diversity measures are dependent on sample size, standardisation of sample sizes should be undertaken using a rarefaction approach (Su, *et al.*, 2012).

Prior to rarefaction, two fungal sample replicates, SB2 (7,599 sequences) and NC3 (19,403 sequences) were excluded from the analysis due to low numbers of sequences before singleton removal. Low sequence counts could have resulted from either the lack of ITS2 amplicon genes in the sample or insufficient detection of these genes even though they were present during sequencing (Cameron, *et al.*, 2020). Furthermore, since very low sequence counts may decrease the overall data quality and precision of diversity measures during subsequent analysis (Cameron, *et al.*, 2020), fungal sample replicates SB2 and NC3 were removed. Each of the samples was then rarefied using the function *rarefy_obs* in metacoder (CRAN.R-project.org/package=metacoder). The bacterial 16S rRNA reads were rarefied to 22,818 reads whereas the fungal ITS sequences were rarefied to 45,378 reads. These represented the minimum number of sequences remaining in a single sample after the removal of low abundance counts. Rarefaction curves were created using the rare curve function in vegan (CRAN.R-project.org/package=vegan).

2.4 Data analysis

2.4.1 Bacterial and fungal α-diversity

The bacterial and fungal richness and diversity within the nine locations selected were observed by computing observed richness (number of different species observed in a particular area) and Shannon-Weaver index (relative abundance of species with a greater focus on species richness and evenness in a particular area) (Kim, *et al.*, 2017; Wagner, *et al.*, 2018). A phylogenetic sequence object, which contained OTU, taxonomic, and sample data, was created using the function *as_phyloseq* from phyloseq (https://bioconductor.org/packages/phyloseq/). This object was then used to compute the diversity and observed OTU richness measures using the function *estimate_richness*. Apart from richness, α-diversity indices provide information on the evenness of communities (a measure of the equability of various species in a community) (Wagner, *et al.*, 2018). As such to observe the evenness of the bacterial and fungal species within the nine locations selected together with the richness other α-diversity measures were computed. These included Simpson (the probability of selected individuals originating from

different species) (Wagner, *et al.*, 2018) and Inverse Simpson (inverse of the Simpson index as a measure of diversity) (Morris, *et al.*, 2014), Chao1 (the ratio of singletons and rare species present within sites, focussing on richness) (Prehn-Kristensen, *et al.*, 2018), and Pilou's evenness index (equality of the number of individual species in an area) (Thukral, 2017).

2.4.2 Linking α-diversity to slope aspect and elevation

In order to determine the impact of various factors on bacterial and fungal diversity, the distribution of the data first needed to be determined to evaluate which test to use for comparison. Thus, the Shapiro-Wilk test from STAT was used (CRAN.R-project.org/package=STAT). Based on the Shapiro-Wilk test results, bacterial data were found to be non-normally distributed, and therefore the Kruskal-Wallis test, the non-parametric equivalent test of Analysis of Variance (ANOVA), was used to determine the impact of slope aspect on bacterial observed OTU richness and diversity since it was a categorical variable. The slope aspect in this study was the direction of the mountain on which sampling was conducted, the north- and south-facing slope, as well as the plateau and the elevations used, were those recorded at each of the sampling points. Elevation data was used as opposed to the slope position, distance of sampling sites from the rock scarps, on the north- and south-facing slope to include the variations in bacterial and fungal diversities across both the north- and the south-facing slope as well as the plateau. The use of the slope position would require variations in bacterial and fungal diversity on the plateau from this factor to not be considered due to the lack of distance from a rock scarp in this region, as no rock scarp was present.

The general linear model following Quasipoisson distribution was used to determine the impact of elevation on bacterial diversity using the function *glm* from STAT since it is a continuous variable. For fungal diversity, a general linear model following the Gaussian distribution was used to determine the impact of elevation and ANOVA and Tukey's Honest Significant Difference test (Tukey's HSD) to determine the impact of slope aspect on diversity and observed OTU richness individually as it was found to follow a normal distribution. Tukey's HSD was obtained from agricolae (CRAN.R-project.org/package=agricolae).

2.4.3 Bacterial and fungal β-diversity

Bacterial and fungal β-diversity was analysed using Bray-Curtis dissimilarity based on the relative abundance of OTUs. The impact of slope aspect and elevation individually were determined using permutational ANOVA (PERMANOVA) with 1, 000 permutations. Pairwise β-diversity indices were calculated using the *vegdist* function and then the *adonis* function from vegan. Similarities between community structures were further visualised using non-metric multidimensional scaling (nMDS) with Bray-Curtis distance. The dispersion of the groups was further determined using the *betadisp* function.

3. Results

3.1 Characterisation of microhabitats across field sites

The three primary sampling areas: Plateau (P), South-facing slope (S), and North-facing slope (N) varied distinctly in terms of vegetation, elevation, and microclimatic conditions along with slope aspects. Elevational readings on the plateau remained above 3,340 m.a.s.l. with the highest elevation at 3,349 m.a.s.l., while elevations on the north- and south-facing slope remained below 3,340 m.a.s.l. with the lowest elevation recorded at 3,329 m.a.s.l. on the south-facing slope. The south-facing slope had a large rock scarp that ran along the summit, resulting in long-lasting shade across most of the slope and snow accumulation below the scarp (Figure 5a). Vegetation in this region started with low shrubs along the top (SA) and the middle of the slope (SB), and tall tussock grass along the bottom of the slope (SC) (Figure 5b). The plateau (PA-PC) was exposed to enhanced levels of solar radiation with no rock scarp to provide shade (Figure 5c). The soil was bare, with scant regions covered in low shrubs and very little tussock grass (Figure 5d).

The north-facing slope also had a rock scarp present, however, it is not as high or steep as the one present on the south-facing slope, leading to no shade and the slope being exposed to more solar radiation compared to the south-facing slope (Figure 5e). Vegetation along the northern slope comprises of tall tussock grass with small patches of low shrubs in between at the top of the slope (NA), after which low shrubs started dominating and occurring more towards the bottom of the slope, with little to no tall tussock grass in certain areas (Figure 5e and 5f). The low shrubs present in all three sites belonged in the main to the genus *Helichrysum* and the tall tussock grass was *Merxmuellera drakensbergensis*. Furthermore, the presence of feacal matter from livestock grazing was visible across the north- and south-facing slope and scattered along certain regions of the plateau.

Figure 5: Photographs of the sampling sites. The images represent the different microclimatic conditions and microhabitats occurring at various elevations on the plateau and two slope aspects. a. large rock scarp on the south-facing slope (SA), b. the vegetation across the south-facing slope (SC), c. bare soil and rocks on the plateau (PA), d. vegetation scattered on the plateau (PB), e. the north-facing slope with no rock scarp and tall tussock grass at the top of the slope (NA), f. low shrubs on the north-facing slope at lower elevations (NC).

3.2 Taxonomic composition of microorganisms across the sites

A total of 1,750,536 sequences were retained for further analysis after quality filtering, singleton removal, and sample rarefaction for the 27 samples (3 replicates for each of the nine sample sites). The number of sequences obtained for the bacterial 16S rRNA gene (616,086 sequences) was lower than that obtained for the fungal ITS assay (1,134,450 sequences). However, while fungi appear to be more abundant across the sampled mountain summit, there is greater diversity among the bacterial counterpart, with a total of 4,443 distinct bacterial OTUs and 1,961 fungal OTUs.

The bacterial communities across all sample sites were classified into a total of 22 phyla and were predominated by Proteobacteria (28%; mostly Alphaproteobacteria - 10%, Betaproteobacteria - 8%, and Deltaproteobacteria - 8%), Acidobacteria (21%, mainly from the class Acidobacteria - 19%), Verrucomicrobia (15%), Actinobacteria (11%) and Bacteroidetes (8%). These numbers represent the average percentages across all nine sites on the plateau, north-facing, and south-facing slopes. However, when comparing the different montane aspects, some differences in abundance is observed (Figure 6a). The microbial communities on the south-facing and north-facing slopes incorporated proportionately more Proteobacteria (11% and 10%) than the plateau (7%). By contrast, the plateau included a greater relative abundance of Acidobacteria (8%) and Verrucomicrobia (6%), compared to the north- (5% and 4%) and south-facing slopes (6% and 4%), respectively.

The fungal community on the sampled mountain belongs to ten distinct phyla, along with one labelled as "general classification of Fungi". The fungal soil microbiome across all three montane aspects is predominated by members of the phyla Ascomycota (49%, mainly from the classes Sordariomycetes - 12%, Leotiomycetes - 11%, Dothideomycetes - 7%, and Eurotiomycetes -7%), Chytridiomycota (21%, primarily the class Chytridiomycetes - 21%) and Basidiomycota (15%, mainly the class Agaricomycetes - 12%). As with the bacteria, differences could be noted between the sampled montane aspects (Figure 6b).

The plateau fungal microbiome incorporated a higher relative abundance of Ascomycota (19%) and Chytridiomycota (8%) compared to the south- (16% and 6%) and north-facing slopes (14% and 7%), respectively. The south-facing slope lacked representatives of the phylum Monoblepharidomycota and a very low abundance of Blastocladiomycota (0.01%) compared to the north-facing slope (0.01% and 0.21%) and the plateau (0.01% and 0.24%) where these

were only found in certain replicates. Mucoromycota and Neocallimastigomycota were higher on the north-facing slope (0.09% and 0.52%) compared to the south-facing slope (0.04% and 0.42%) and the plateau (0.03% and 0.26%), although all of these phyla were found at relatively low abundance on each aspect.

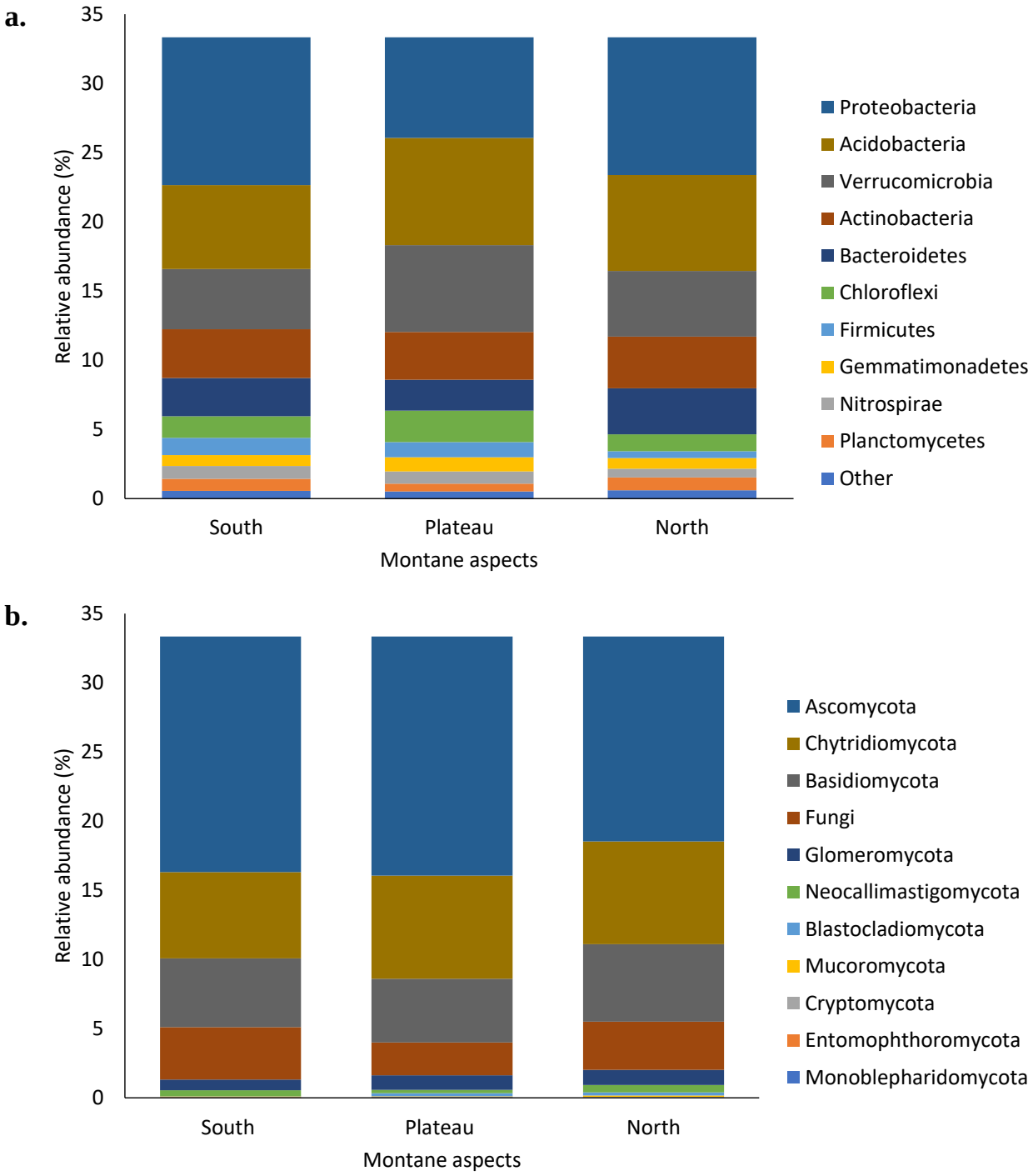
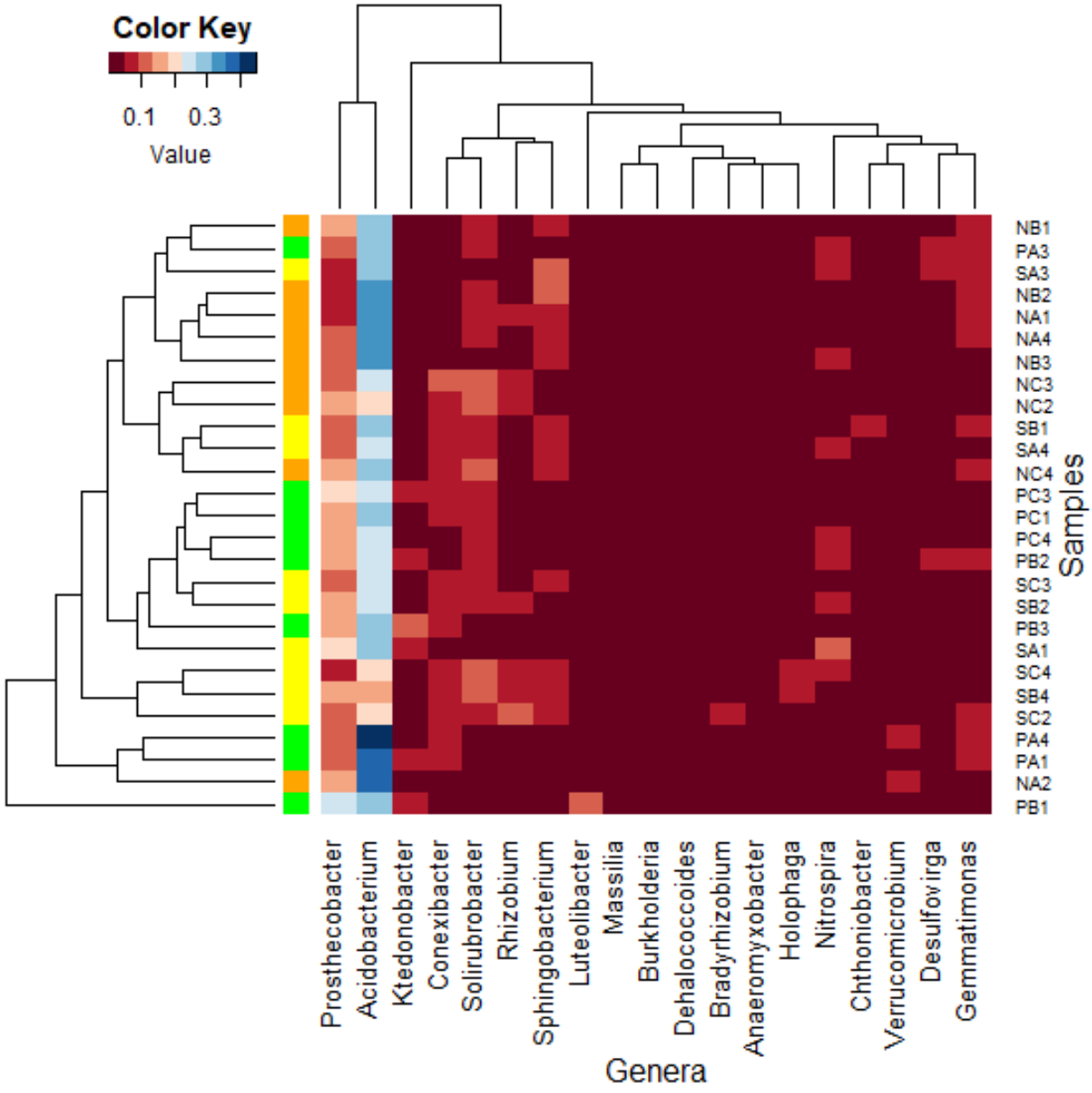


Figure 6: Relative abundance of bacterial and fungal phyla across the three montane aspects. The bar charts represent the most abundant a. bacterial and b. fungal sequences classified at the phylum level per montane aspect. The total relative abundance of the three montane aspects together sum up to a 100%, with each montane aspect having a total abundance of 33.33% bacterial and fungal phyla, each.

At the genus level, a total of 339 distinct bacterial genera were identified. However, 227 of these genera each contributed less than 0.1% of the total abundance. Across the three montane aspects, the most abundant genera included *Acidobacterium* (17%), *Prosthecobacter* (8%), *Solirubrobacter* (4%), *Sphingobacterium* (3%), and *Conexibacter* (3%). While all three aspects had similar relative abundances for most genera (Figure 7a), the plateau incorporated a greater proportion of taxa belonging to the genera *Acidobacterium* (7%) and *Prosthecobacter* (4%) compared to the south- (6% and 2%) and north-facing slopes (4% and 2%), respectively.

For the fungal counterpart, 475 distinct fungal genera were identified in the combined montane sample sites, of which 322 contributed < 0.1% of the total abundance. The most abundant genera included *Mortierella* (9%), *Phlyctochytrium* (6%), *Leptodontidium* (4%), *Coniochaeta* (3%), and *Fusarium* (3%). All three montane aspects had similar relative abundance for most fungal genera, however, variations in the various replicates were noted (Figure 7b), with the north-facing slope having a greater proportion of taxa belonging to the genus *Phlyctocytrium* (3%) compared to the south-facing slope (2%) and the plateau (1%). The plateau had a greater proportion of *Leptodontidium* (3%) and *Fusarium* (2%) compared to the south- (1% and less than 0.50%) and the north-facing slopes (0.50% and 1%). In general, relatively good congruence was observed in terms of taxon abundance of bacteria and fungi between the replicates for each of the nine montane aspect sites.

a.


Color Key
0.1
0.3
Value
NB1
PA3
SA3
NB2
NA1
NA4
NB3
NC3
NC2
SB1
SA4
NC4
PC3
PC1
PC4
PB2
SC3
SB2
PB3
SA1
SC4
SB4
SC2
PA4
PA1
NA2
PB1
Samples
Prosthecobacter
Acidobacterium
Ktedonobacter
Conexibacter
Solirubrobacter
Rhizobium
Sphingobacterium
Luteolibacter
Massilia
Burkholderia
Dehalococcoides
Bradyrhizobium
Anaeromyxobacter
Holophaga
Nitrospira
Chthoniobacter
Verrucomicrobium
Desulfovirga
Gemmatimonas
Genera

b.

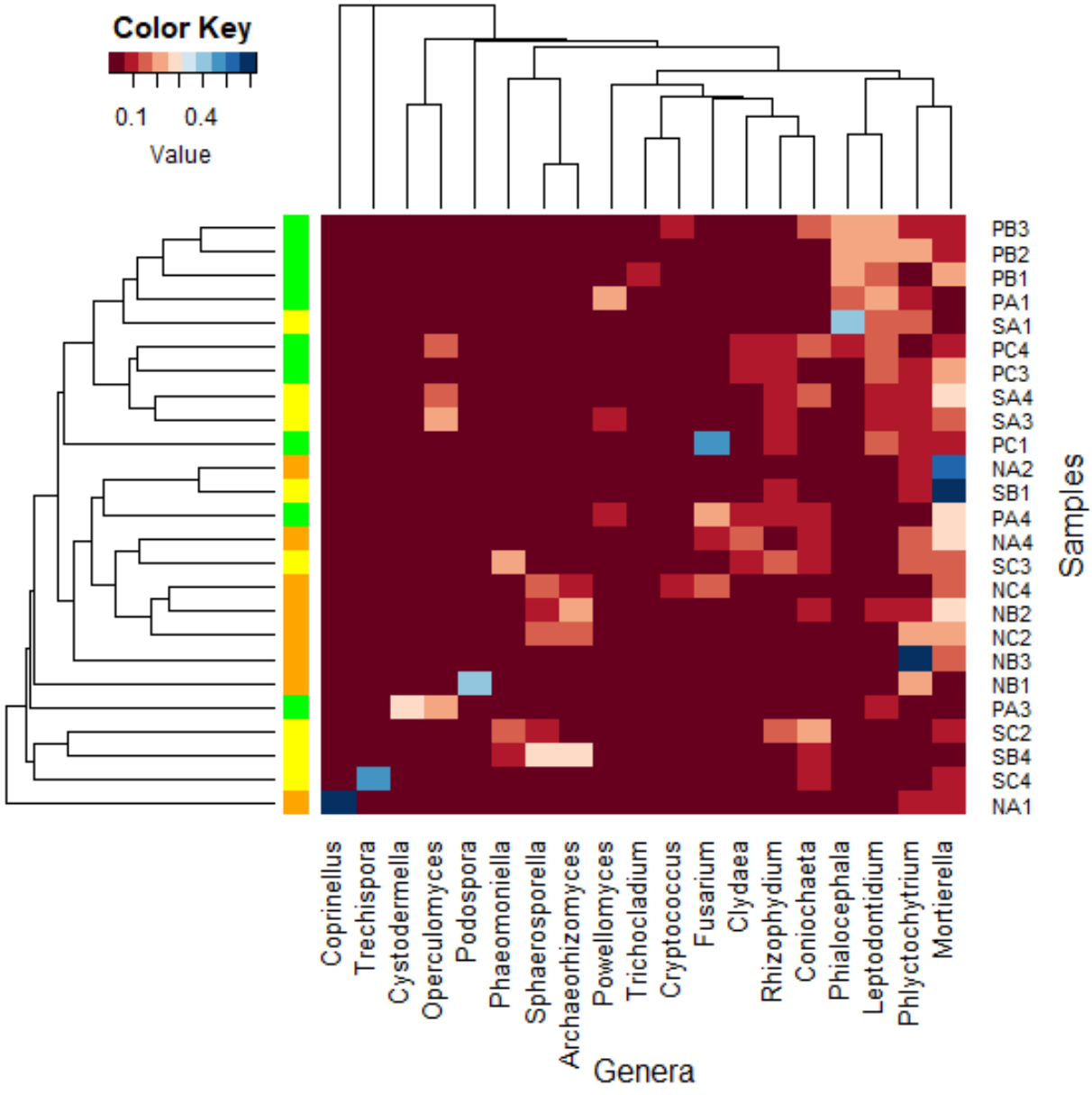


Figure 7: Abundance of bacterial and fungal genera across the montane aspects. The heatmaps display the most abundant a. bacterial and b. fungal genera present across the various replicates at the nine locations selected. Samples were clustered based on sample replicates as shown in the rows and the relative abundance of the genera as shown in the columns. The colour key indicates the percentage relative abundance with dark red shades indicating absence of a specific genus, with increasing percentage relative abundance represented by the various shades of blue. The first row colours represent the sample replicate clusters, with yellow indicating replicates on the south-facing slope, green for replicates on the plateau, and lastly, orange for replicates on the north-facing slope.

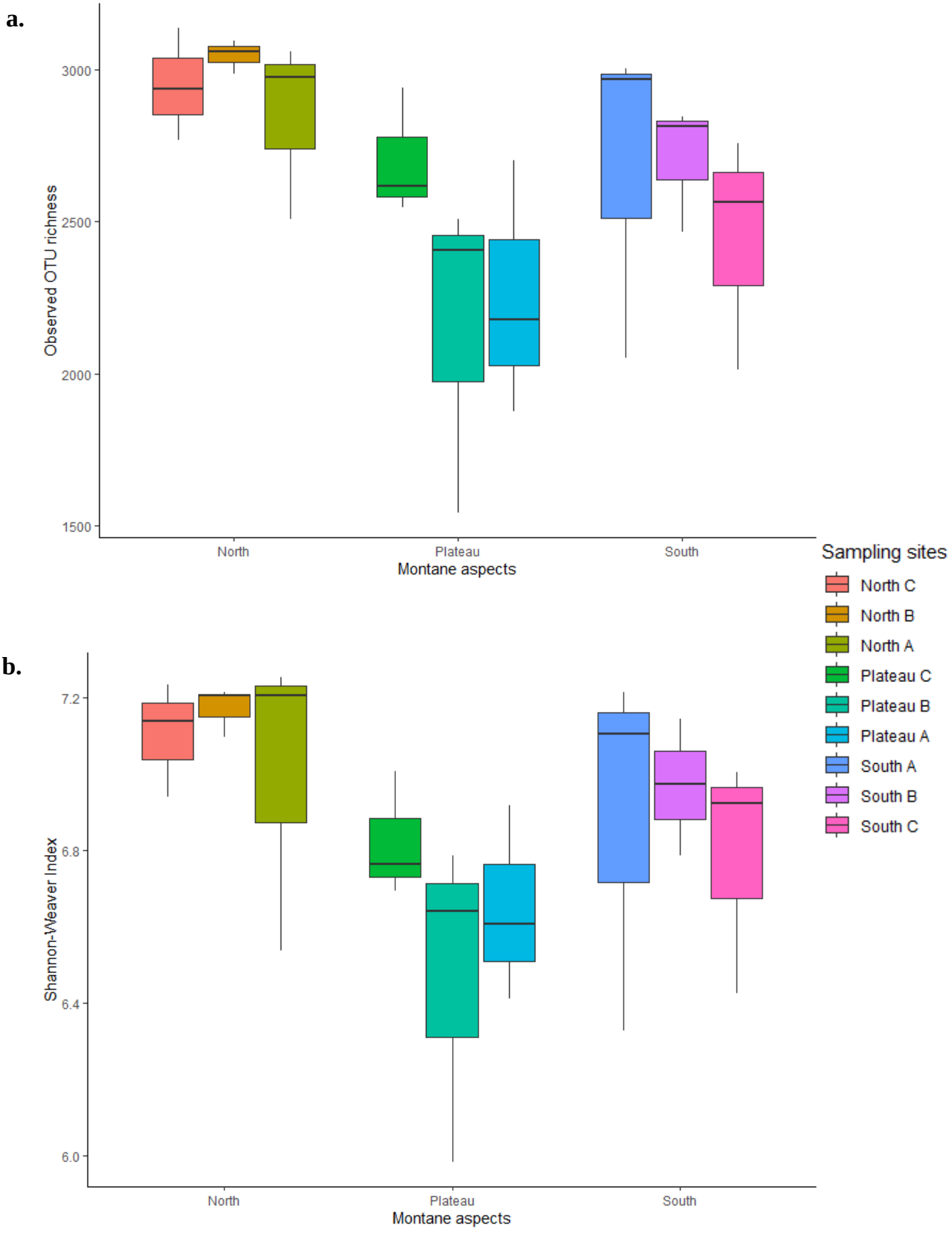
3.3 Bacterial and fungal α-diversity

Prior to any statistical analysis, singleton sequences were removed. Bacterial and fungal sequences were rarefied to 22,818 and 45,378, respectively, to reflect the lowest counts found in sample PB1 (for bacteria) and SB1 (for fungi), respectively. Rarefaction curves indicated that most members of the bacterial and fungal communities had been sampled in most of the replicates (Appendix Figure A1). There were sufficient counts to capture most of the diversities within the sites which could be seen by the plateau in the rarefaction curves of most of the replicates. Only two samples, namely SC2 (23, 199) for bacteria and NC2 (48, 053) for fungi, did not reach a plateau as they had lower counts compared to the rest of the replicates, although they had slightly higher counts than PB1 and SB1 of bacteria and fungi.

To determine the diversity of bacterial and fungal species within each of the montane sample sites, OTU richness and diversity indices were determined. Relatively greater bacterial diversity could be observed for the north-facing slope compared to the south-facing slope and the plateau, while compared to the south-facing slope, much lower bacterial diversity could be observed for the plateau (Figure 8b). For both the north-facing and south-facing slope, the highest diversity could be observed at the top (NA and SA), followed by the middle (NB and SB) and bottom (NC and SC) of the slopes. When looking at the sample sites along the plateau, the highest diversity was observed for the sample site nearest the northern slope (PC) and nearest the sample point (NA) with the highest observed diversity. Similarly, bacterial taxon richness was higher on the north-facing slope, compared to the south-facing slope, which decreased down the slope and the plateau (Figure 8a).

With the exception of the middle of the south slope (SB), the lowest fungal diversity was also observed across all three sampling points along the plateau (Figure 8d). Fungal diversity was also greater on the north-facing slope compared to the south-facing slope and the plateau. By contrast to the bacterial diversity of the north-facing slope, the lower portion (NC) showed the greatest fungal diversity, followed by the middle (NB) and upper slope (NA) sampling points. Similarly, fungal taxon richness decreases down the south-facing slope (from SA to SC) (Figure 8c). It must be noted, however, that two samples, SB2 (7,599 sequences) and NC3 (19,403 sequences) were excluded from the analysis due to low numbers of sequences before singleton removal. This may have resulted in the lower Shannon-Weaver diversity indices observed in particular for the middle of the south-facing slope (SB).

Additionally, the α-diversity indices Pilou's evenness, Simpson and Inverse Simpson as well as the Chao1 indices, were calculated for both the fungi and bacteria for all three sampling sites for each montane aspect. These indices follow the same trend for both bacteria (Appendix Table A1) and fungi (Appendix Table A2) as demonstrated by the observed richness and Shannon-Weaver plots.


a.
Observed OTU richness
3000
2500
2000
1500
North
Plateau
South
Montane aspects
Sampling sites
North C
North B
North A
Plateau C
Plateau B
Plateau A
South A
South B
South C
b.
Shannon-Weaver Index
7.2
6.8
6.4
6.0
North
Plateau
South
Montane aspects

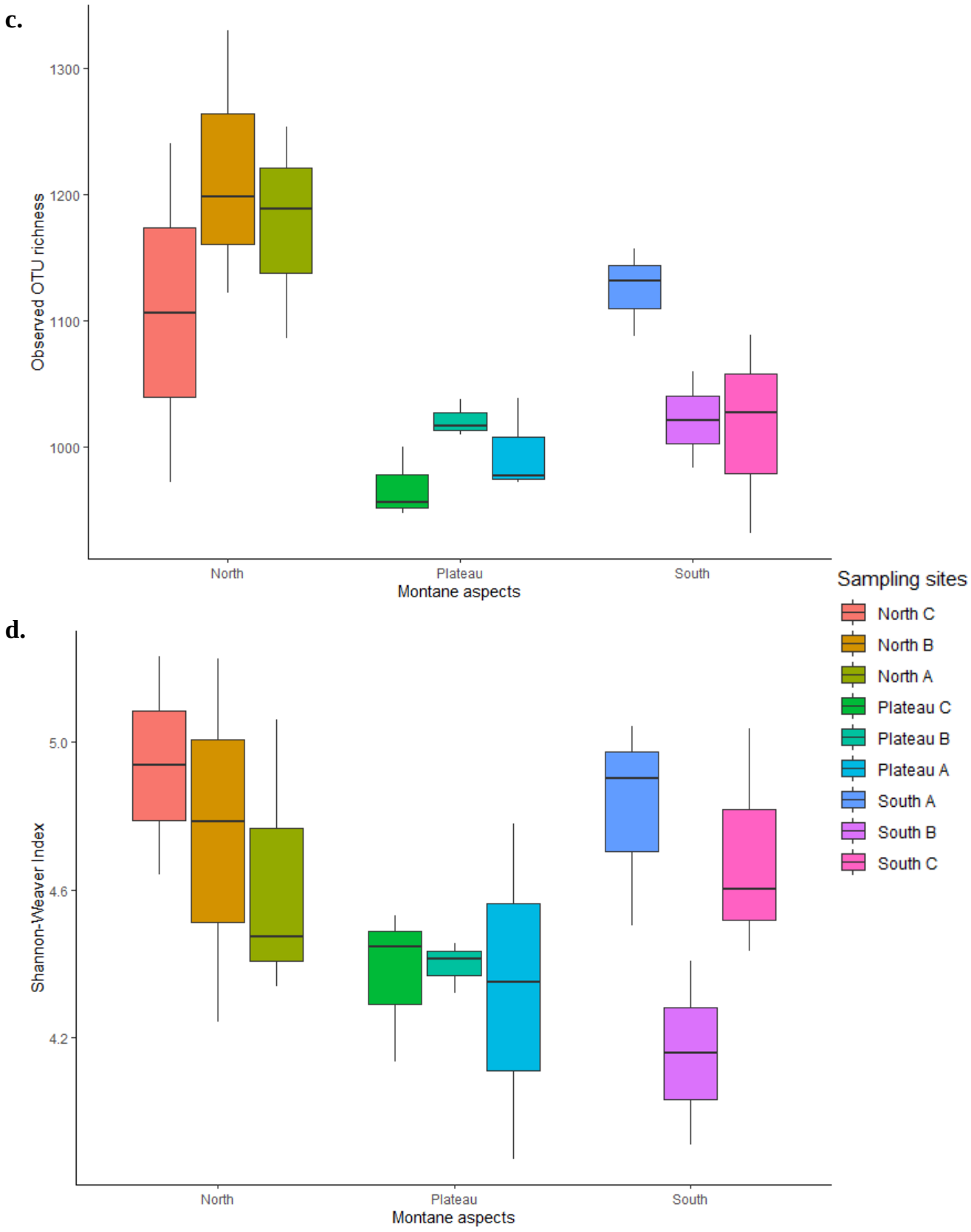


Figure 8: Variation in α-diversity indices for bacterial and fungal communities on the sampled mountain. The boxplots represent the observed OTU richness and Shannon-Weaver diversity indices for the different sample points for the bacterial (a and b) and fungal counterparts (c and d), respectively. North A and South A were located at the top of the mountain followed by North B and South B in the middle and North C and South C at the bottom of the mountain slope. Plateau A was the edge on the southern end and Plateau C was the edge on the northern end of the mountain with Plateau B in middle. Each sample point incorporates three replicates at each site, with the exception of NC and SB for fungi, where one replicate was eliminated due to low read counts.

To determine the impact of the slope aspect on the bacterial richness and Shannon diversity, the non-parametric equivalent of ANOVA, Kruskal-Wallis test was used as the Shapiro-Wilk test for normality indicated that bacterial data followed non-normal distribution (Appendix Table A3). Bacterial diversity and evenness indices were observed to be significantly different from a normal distribution, assuming non-normal distribution based on the Shapiro-Wilk test (p-value < 0.05), except for Inverse Simpson diversity. Shapiro-Wilk test used for fungal richness and Shannon diversity, however, indicated that this data was not significantly different from normality thereby following normal distribution (p-value > 0.05). Simpson diversity and Pilou's evenness for fungal data, however, followed non-normal distribution (p-value < 0.05) (Appendix Table A4). Unlike the bacterial counterpart, ANOVA and Tukey HSD was used to determine the impact of the slope aspect on the fungal richness and Shannon diversity.

A significant difference in bacterial OTU richness and Shannon diversity of the plateau, north- and south-facing slope was noted using the Kruskal-Wallis test (Observed OTU richness: chi-square = 11.58 and p-value = 0.003, Shannon diversity: chi-square = 9.91 and p-value = 0.007, degrees of freedom (df) = 2). This indicated that the slope aspect did have a significant impact on the diversity of bacteria across the sampled mountain. The differences between the various slope aspects and their impact on bacterial diversity were further determined using the Wilcoxon Rank Sum test, which indicated that bacterial OTU richness was significantly different between the north-facing slope and the plateau (p-value = 0.004) and between the north- and south-facing slope (p-value = 0.028). There was, however, no significant difference in bacterial OTU richness found between the plateau and the south-facing slope (p-value = 0.200). A significant difference in the Shannon diversity was also noted on the north-facing slope and the plateau (p-value = 0.008), however, no significant difference between the north- and south-facing slope (p-value = 0.077) and the plateau and the south-facing slope (p-value = 0.077) were noted.

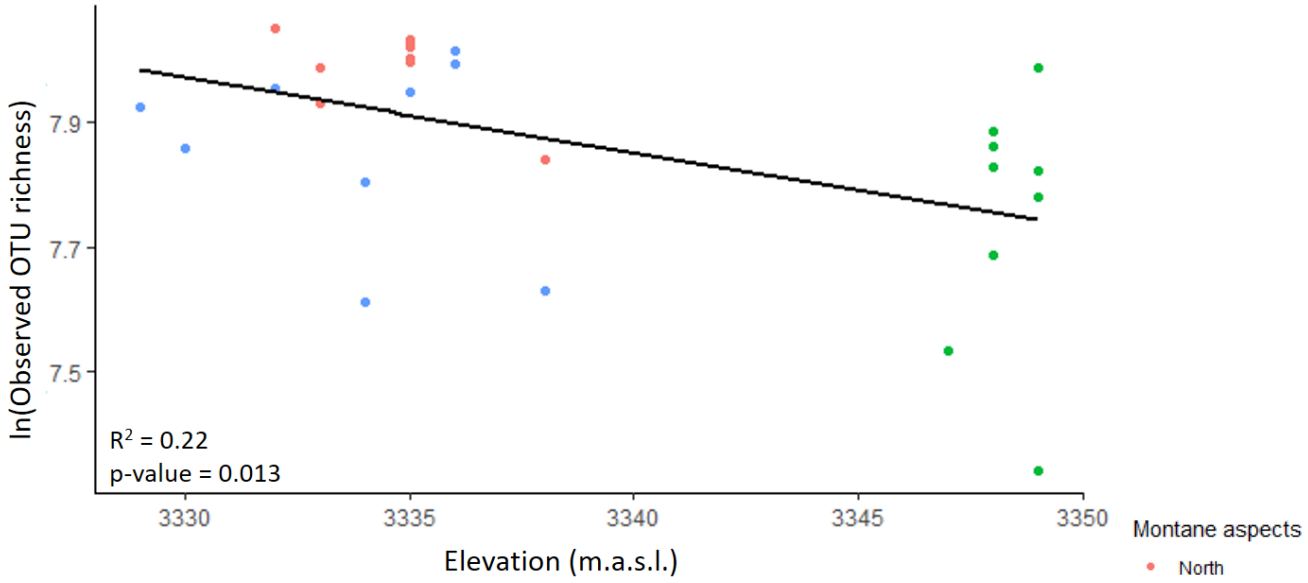
A significant difference in fungal OTU richness was also noted in the various slope aspects (F-value = 13.51, p-value = 0.0001) using ANOVA. However, Shannon diversity was not significantly affected by the plateau, north- and south-facing slope (F-value = 2.67, p-value = 0.092). The difference between the various aspects and their impact on OTU richness and diversity was further determined using Tukey HSD, which indicated that fungal OTU richness was significantly different between the north-facing slope and the plateau (p-adjusted = 0.0003), and between the north- and south-facing slope (p-adjusted = 0.029), as with bacterial

OTU richness. A significant difference between the plateau and the south-facing slope, however, was not noted (p-adjusted = 0.183). While a significant difference in Shannon diversity was also noted between the north-facing slope and the plateau (p-adjusted = 0.076), no significant difference was noted between the north- and the south-facing slope (p-adjusted = 0.665) and between the plateau and the south-facing slope (p-adjusted = 0.354).

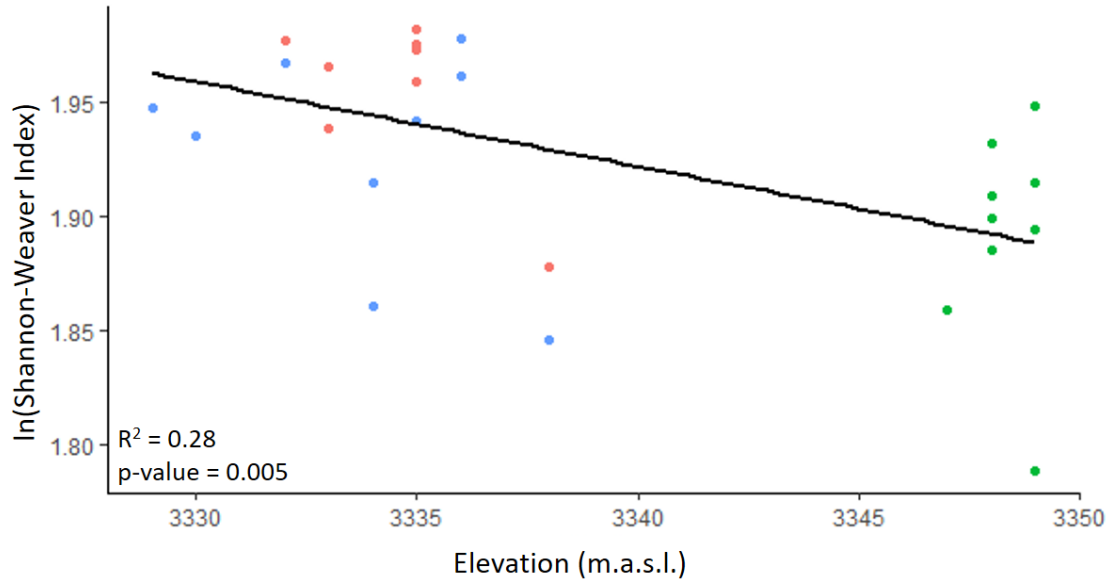
A general linear model with Quasipoisson (log) distribution was used to determine the impact of elevation, recorded at each sampling site, on bacterial OTU richness and diversity. The bacterial OTU richness (t-value = -2.74, p-value < 0.05) and Shannon diversity (t-value = -3.11, p-value < 0.05) were significantly affected by elevation and decreased with an increase in elevation (Figure 9a and b) as seen by the negative slope (Appendix Table A5). As independent variables, slope aspect and elevation both affected bacterial diversity and OTU richness.

The general linear model using Gaussian distribution was used to determine the impact of elevation on fungal OTU richness and diversity. While both fungal OTU richness and Shannon diversity decreased with an increase in elevation (Figure 9c and d), only OTU richness (t-value = -2.82, p-value < 0.05) was significantly affected by elevation (Appendix Table A5). As independent variables, only OTU richness appears to be affected by both slope aspect and elevation.

a.


ln(Observed OTU richness)
7.9
7.7
7.5
R² = 0.22
p-value = 0.013
3330
3335
3340
3345
3350
Elevation (m.a.s.l.)
Montane aspects
North
Plateau
South

b.


ln(Shannon-Weaver Index)
1.95
1.90
1.85
1.80
R² = 0.28
p-value = 0.005
3330
3335
3340
3345
3350
Elevation (m.a.s.l.)

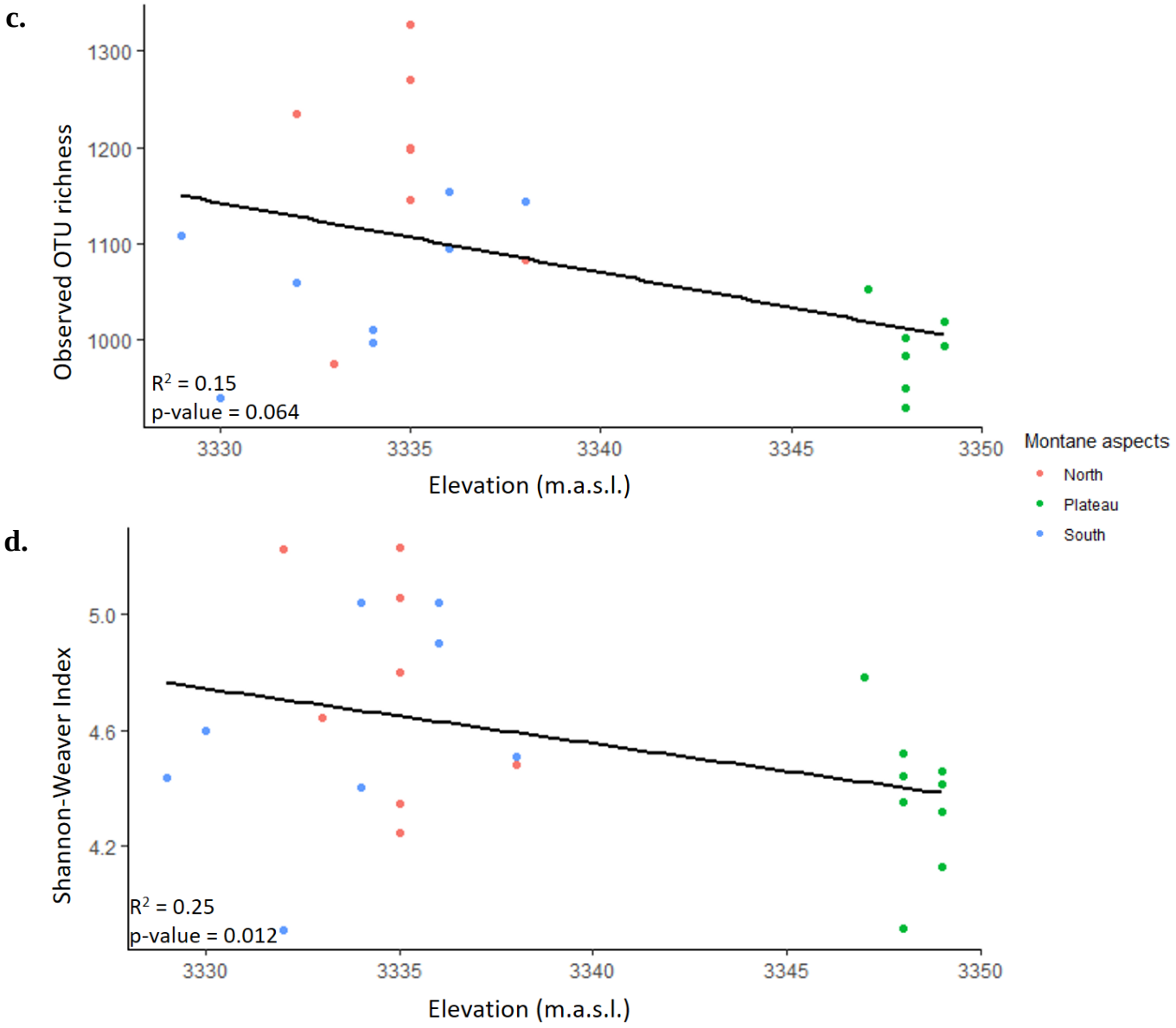


Figure 9: Relationship of operational taxonomic unit richness and diversity with elevation on the summit. The scatterplots represent the relationship of a. bacterial OTU richness, b. bacterial Shannon diversity, c. fungal OTU richness, and d. fungal Shannon diversity concerning changes in elevation on the selected summit. R^2 values on each represent that the degree to which the data is explained by this relation is low, which could be due to the variability in elevation values. p-values appeared to be < 0.05 for all graph except for fungal Shannon diversity.

3.4 Bacterial and fungal β-diversity

The variation between the bacterial and fungal soil microbiomes across the different montane aspects sampled were determined using PERMANOVA based on Bray-Curtis dissimilarity. The bacterial community was significantly influenced by both slope aspect (F-model = 3.80, R^2 = 24%, p-value < 0.001) and elevation (F-model = 5.28, R^2 = 17%, p-value < 0.001) acting individually, however, the slope aspect had a slightly greater influence as it accounted for higher variance (R^2) compared to elevation. nMDS plots were used to visualise community structures of both bacteria and fungi (Figures 10a and b). Bacterial communities across the different sample sites on the north-facing slope clustered more closely together compared to the south-facing slope and the plateau and fully and partially overlapped with the south-facing slope and the plateau, respectively (Figure 10a). Variation in the bacterial communities was greatest along the south-facing slope transect, with particular samples from the middle (SB) and bottom (SC) separated from the main cluster of the three montane aspects.

Fungal community structure, like its bacterial counterpart, was significantly affected by both slope aspect (F-model = 2.26, R^2 = 17%, p-value < 0.001) and elevation (F-model = 3.00, R^2 = 12%, p-value < 0.001) individually, with the slope aspect having a slightly greater influence with higher variance compared to elevation when determined using PERMANOVA. Similar to the bacterial community, the fungal communities of all sample sites across the north-facing slope clustered, but with the fungi, there was also an observable clustering of fungal communities across the plateau (Figure 10b). As observed with the bacteria, the fungal communities along the south-facing slope also differed the most, with the same sample points along the bottom (SC) and middle (SB).

Variations in bacterial and fungal community structure along the distinct montane aspects were further determined using beta dispersion. This showed that there were no differences in the variance of bacterial community structures between the plateau, north- and south-facing slopes (p-value > 0.05). Differences in the variance of fungal community structure were observed between the north- and south-facing slopes as well as the plateau and south-facing slope (p-value < 0.05). No differences were observed between the plateau and the north-facing slope. These would account for the overlaps and dispersion of replicates on the plateau, north- and south-facing slopes observed (Figure 10a and b).

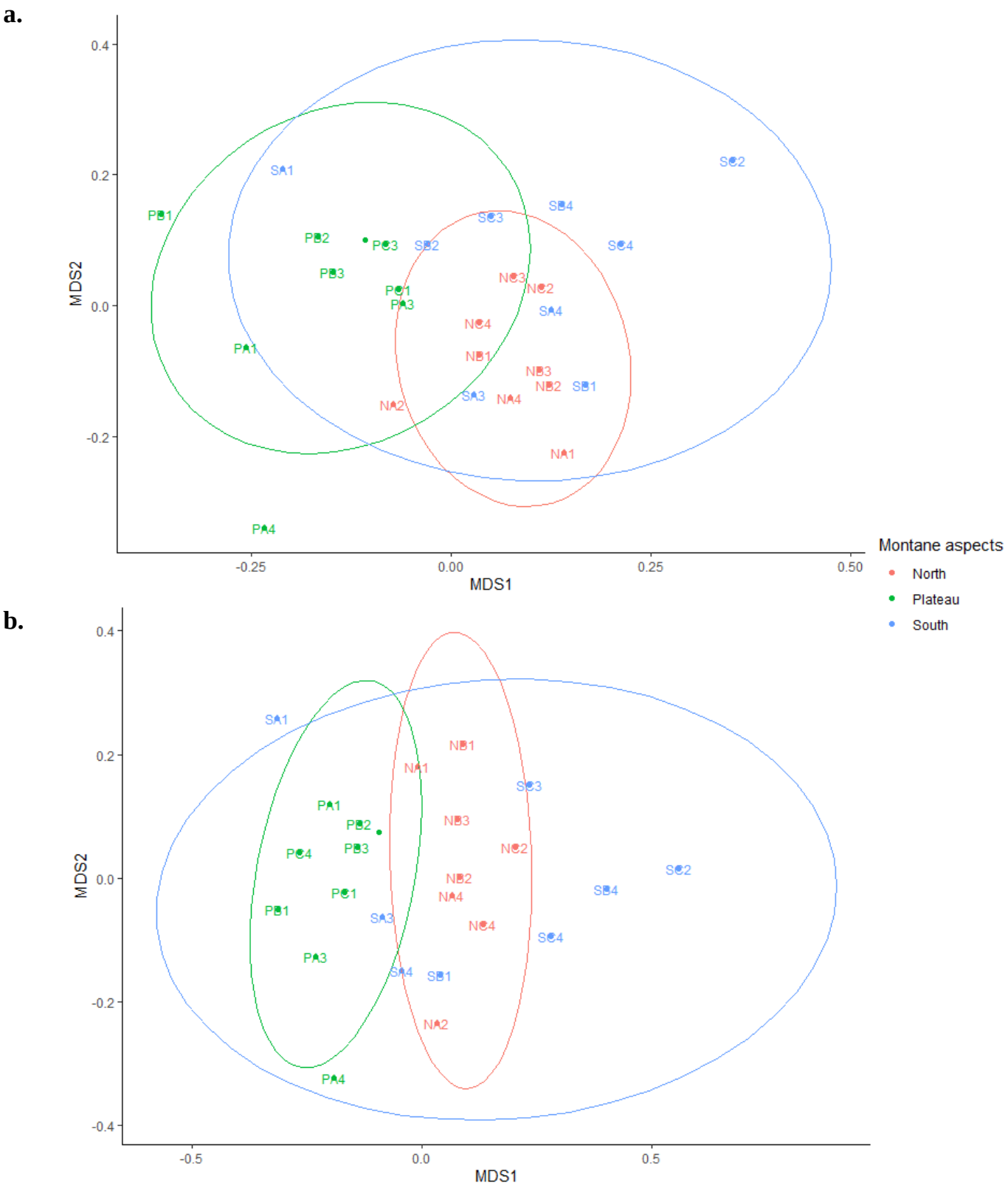


Figure 10: Bacterial and fungal community structures across all sample sites for the three montane aspects. The nMDS plots represent a. bacterial and b. fungal community structures across the montane aspects based on Bray-Curtis distance. The distance between points is indicative of the relative dissimilarities in community structures with the ellipses indicating dispersion regions of each site. Bacterial stress solution was reached at about 0.16 and fungal at 0.21, both of which were relatively low.

4. Discussion

The Lesotho highlands have been the focus of a broad range of studies relating to their geomorphological composition, unique and variable climate, and the diverse vegetation present in these environments (Grab and Nüsser, 2001). Research related to the soil microbial diversity in these highlands, however, is significantly lacking. Here a metataxonomic study of the bacterial (16S rRNA) and fungal (ITS) soil communities across a mountain summit has been undertaken. A total of 1,750,536 sequences were obtained from the summit, with fungal taxa accounting for 65% of these.

The most common bacterial phyla found on the plateau, north- and south-facing slopes included Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia, which accounted for >80% of the bacteria isolated from the summit. These bacterial phyla have been shown to be dominant in soil across a broad range of biomes including the Changbai and Shennongjia Mountains in China as well as the Italian Alps and the Tibetan Plateau (Shen, *et al.*, 2013; Siles and Margesin, 2016; Yuan, *et al.*, 2014; Zhang, *et al.*, 2015).

While these phyla predominated across the sampled mountain summit, some variance at the phylum level could be observed on the different mountain aspects. The soil microbiome on the plateau incorporated a greater number of Acidobacteria, Chloroflexi, Gemmatimonadetes, and Verrucomicrobia compared to the north- and south-facing slopes. The north- and south-facing slopes had a greater abundance of Proteobacteria, particularly Alphaproteobacteria and Betaproteobacteria, and Bacteroidetes. This variation could have resulted due to the life strategies followed by these bacterial phyla. Acidobacteria, Chloroflexi, Gemmatimonadetes, and Verrucomicrobia (Ho, *et al.*, 2017; Yao, *et al.*, 2017) are generally classified as oligotrophic microorganisms (able to survive in nutrient-limited environments) (Tada, *et al.*, 1995), whereas, Proteobacteria are copiotrophic microorganisms (survive in nutrient-rich environments) (Koch, 2001; Yao, *et al.*, 2017). This correlates with the observation that the plateau had very dry, bare soil with little or no plant cover, while the north- and south-facing slopes had a diverse range of vegetation and soil coverage.

The most common fungal phyla across the sampled summit were mostly comprised (98% of all phyla) of Ascomycota, Basidiomycota, Chytridiomycota, Fungi, and Glomeromycota. These results correspond with studies conducted on the Taibai Mountain in China, montane forests in South America, as well as retreating glaciers in the North American Arctic Transect

(Geml, *et al.*, 2014; Ren, *et al.*, 2018b; Timling, *et al.*, 2014). Variations in the numbers of these phyla were also noted across the montane aspects, such that the soil microbiome on the plateau and the north-facing slope incorporated a greater number of Chytridiomycota, Basidiomycota, and Glomeromycota compared to the south-facing slope. All the montane aspects sampled, further had a greater number of Ascomycota, with the plateau having the highest abundance.

While many of these phyla are generally known for the essential roles they play in the soil, such as nutrient cycling (Ascomycota, Basidiomycota, and Glomeromycota) (Krishnamoorthy, *et al.*, 2015; Schoch, *et al.*, 2009; Wang, *et al.*, 2020), decomposition of organic matter produced by plants and animals (Ascomycota and Basidiomycota) (Schoch, *et al.*, 2009; Wang, *et al.*, 2020), and forming symbiotic relationships with other plant species (Glomeromycota) (Krishnamoorthy, *et al.*, 2015), variations in their numbers could be related to the lifestyle strategies followed. Certain saprotrophic fungi such as Chytridiomycota may possess characteristics of copiotrophic microorganisms due to their need for nutrient-rich environments for decomposition (Ho, *et al.*, 2017; McConnaughey, 2014). Other phyla such as Glomeromycota and Basidiomycota may be classified as oligotrophic microorganisms (Ho, *et al.*, 2017). While the presence of these oligotrophic microorganisms corresponds with their ability to survive in the less vegetated and bare soil on the plateau and the more vegetated north- and south-facing slope, the presence of Chytridiomycota on the plateau at a higher number may be indicative of other potential drivers of this phylum in this region as they have copiotrophic characteristics. Members of the Ascomycota may be classified as either copiotrophic- or oligotrophic microorganisms, regardless of them being saprotrophic fungi (Yao, *et al.*, 2017), such that the isolated genera *Fusarium* and *Penicillium* are oligotrophic microorganisms, while *Alternaria* and *Cladosporium* are copiotrophic microorganisms (Ho, *et al.*, 2017). This correlates with the high numbers at which this phylum and their represented genera were found overall the montane aspects sampled, regardless of the conditions present.

The presence of the rare phylum Neocallimastigomycota was also observed at higher numbers in the middle of the north-facing slope (NB) and the bottom of the south-facing slope (SC). Members of this phylum are generally restricted to the digestive systems of herbivores animals; however, they may be found as resistant structures in the soil at times (Liggenstoffer, *et al.*, 2010). The presence of this phylum may correlate with livestock feacal matter, which was

scattered across the montane aspects, with larger quantities observed mainly at lower elevations on the north- and south-facing slopes.

Vegetation patterns and soil microbiome structure in the Lesotho highlands are influenced by livestock grazing apart from abiotic factors (Nüsser and Grab, 2002). A decrease in potential agricultural land and degradation of pastures for livestock at lower elevations in Lesotho has led to the use of the highlands for these purposes (Grab and Linde, 2014). Flocks of sheep, goats, cattle, and horses are generally moved to the highlands for grazing during the summer months at elevations between 2,750 and 3,100 m.a.s.l. and then further moved to selected pastures at higher elevations during winter (Nüsser, 2002). Furthermore, visible signs of livestock grazing were observed on the north-facing slope by the presence of more trampled lower-lying grass and shrub patches at lower elevations compared to the other sampling sites.

Overall, bacterial and fungal α-diversity (observed richness and Shannon-Weaver index) was greater on the north-facing slope and lower on the plateau. Bacterial diversity was particularly lower on the southern end (Plateau A) and the middle (Plateau B) of the plateau, whereas, fungal diversity was lower on the northern end (Plateau C). This could be a result of the harsh conditions present on the plateau, where reduced plant diversity and cover would cause these microorganisms to be exposed to significant solar radiation. Increased exposure to solar radiation for prolonged periods of the day has a profound effect on microbial growth and diversity (Alonso-Sáez, *et al.*, 2006). A study conducted in the Salar del Huasco wetland (>3,800 m.a.s.l.) in Chilean Altiplano has observed changes in bacterial community composition when exposed to variations in daily solar radiation (Molina, *et al.*, 2016).

Bacterial and fungal community structures (β- diversity) also differed across the different summit aspects as determined by PERMANOVA and beta dispersion. While no difference was noted between the variance of bacterial community structure on the plateau, north- and south-facing slopes, a significant difference was noted between the variance of fungal community structure on the north- and south-facing slope and the plateau and the south-facing slope. Bacterial α-diversity and community structure seemed to significantly be affected by the slope aspect (north, south, and plateau) of the summit. This corresponds with studies conducted on Mt. Cardrona, in New Zealand which showed that the slope aspect strongly impacts bacterial diversity over short distances of < 1,000 m on a montane summit rather than the elevation (Wu, *et al.*, 2017).

Bacterial diversity and community structure across the studied summit were significantly affected by changes in elevation, with a decrease in diversity observed with an increase in elevation. A decrease in bacterial diversity has been noted in several studies as increased elevation usually leads to an increase in environmental harshness with colder conditions and more stringent soil environments (Adamczyk, *et al.*, 2019; Margesin, *et al.*, 2009; Shen, *et al.*, 2015). However, studies have also highlighted that microbial diversity and structure at times do not follow elevational patterns as observed in plants and animals (Siles and Margesin, 2016; Zhang, *et al.*, 2013). This was observed in the case of fungal diversity in this study, where although a decrease in diversity was observed with increased elevation, diversity was not significantly affected by changes in elevation.

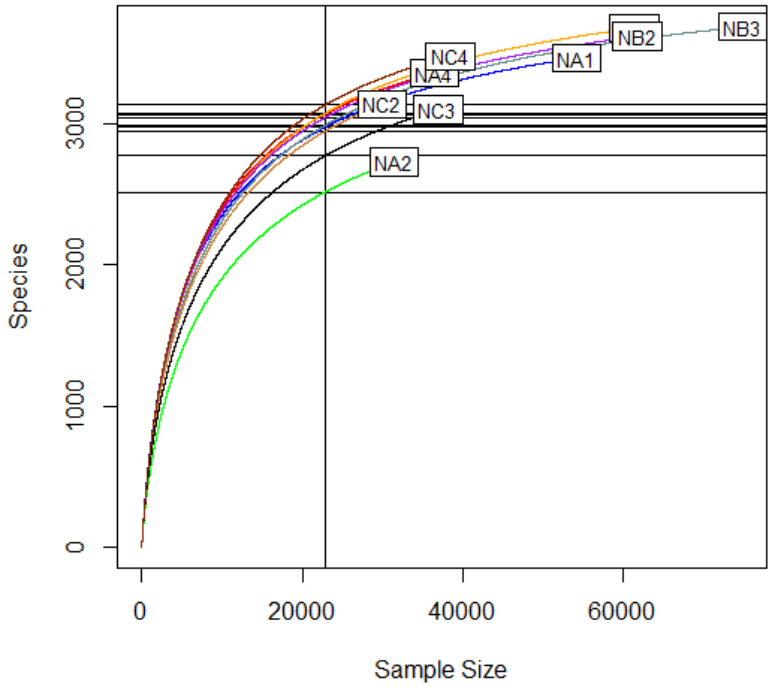
It should, however, be noted that across the sampled mountain, the elevation (3,330 to 3,350 m.a.s.l.) only varied across a relatively short range and as such could have had a limited impact on bacterial and fungal diversity and community structure. Due to the limited impact of elevation, the interactions between elevation and slope aspect on bacterial and fungal diversity and community structures to account for the nested impacts and the presence of non-independent variables within the data were not considered (Adamczyk, *et al.*, 2019). Together with elevation, the impact of slope position, the distance of sampling sites from the rock scarp, on the north- and south-facing slope could have also affected bacterial and fungal diversity and community structures noted. However, further research is required to determine the microclimatic variations experienced across the slopes due to the slope position with measures of solar radiation exposure and shading, soil physiochemical properties, vegetation types, and temperatures experienced taken into consideration to determine the overall impact of this factor on bacterial and fungal communities across these slopes.

5. Conclusion

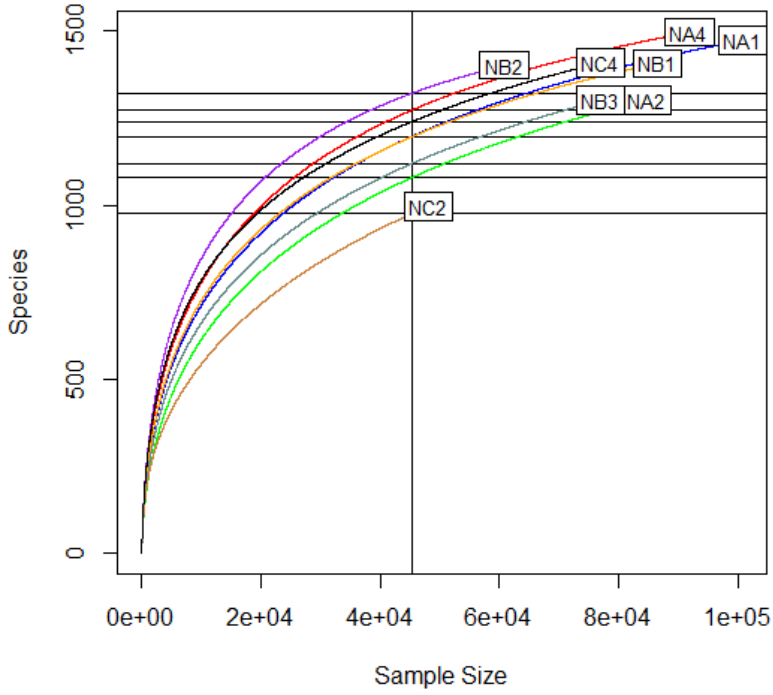
This study has provided the first insight into the soil fungi and bacteria found on a summit in the Lesotho highlands. Several of the bacterial and fungal phyla found in this region are related to essential ecosystem processes and function and their abundance can be used as indicators of changes in certain environmental conditions. This study further looked at the influence of the slope aspect and elevation on the fungal and bacterial diversity of the summit. Elevation appeared to have a very limited effect on microbial diversity. However, only a very limited elevational gradient was considered here, and as such this variable was not considered in further analysis in this study. By contrast, the slope aspect was found to support the hypothesis of having a substantial impact on diversity as it had a significant effect on both bacterial and fungal diversity. This is likely due to the substantively different climatic conditions, such as distinct precipitation patterns and temperatures, experienced by the north- and south-facing slopes as well as the plateau. Further research is required to test the role of these abiotic drivers on the fungal and bacterial communities inhabiting this summit.

6. Appendix

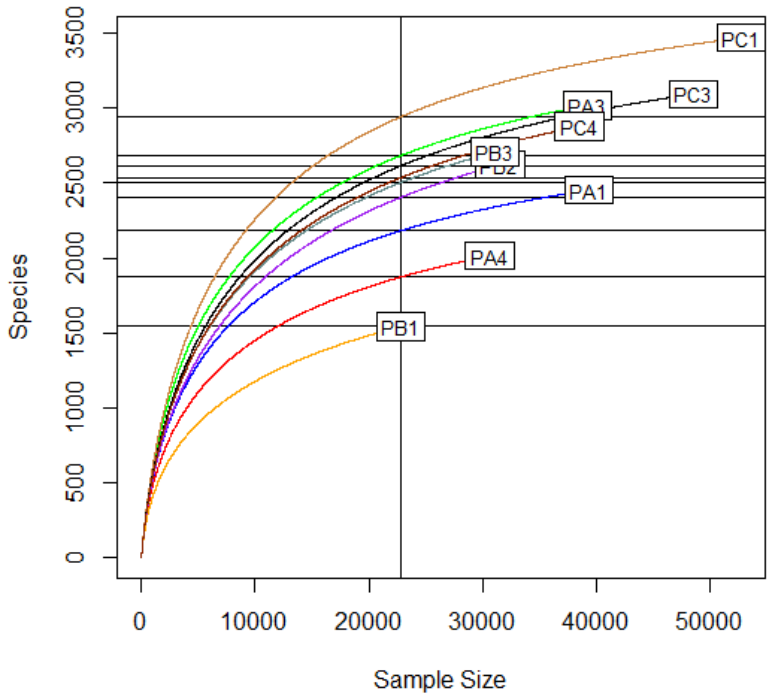
a.



b.



c.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1500
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2500
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PC1
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PA3
PC4
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PA1
PA4
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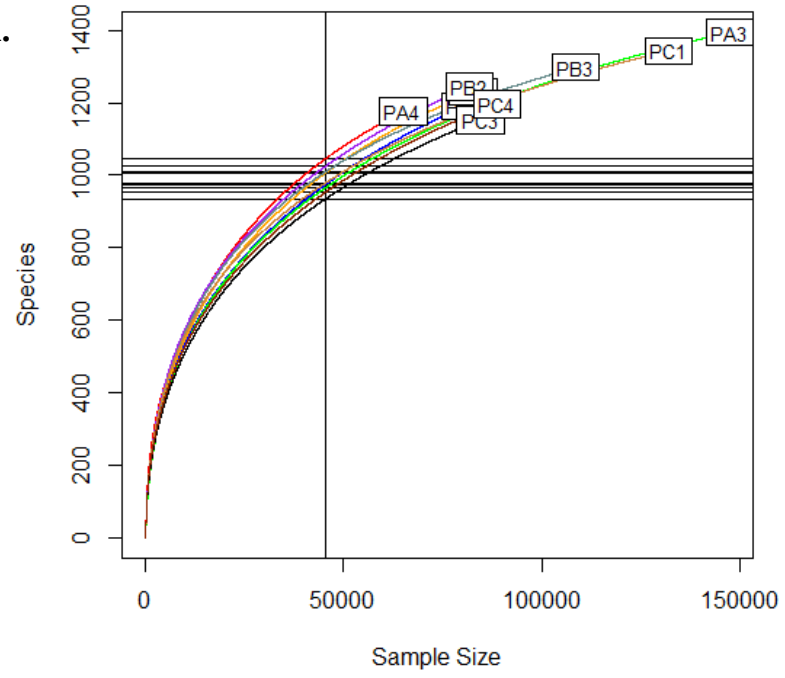
d.


Species
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50000
100000
150000
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PC1
PB3
PA4
PC4

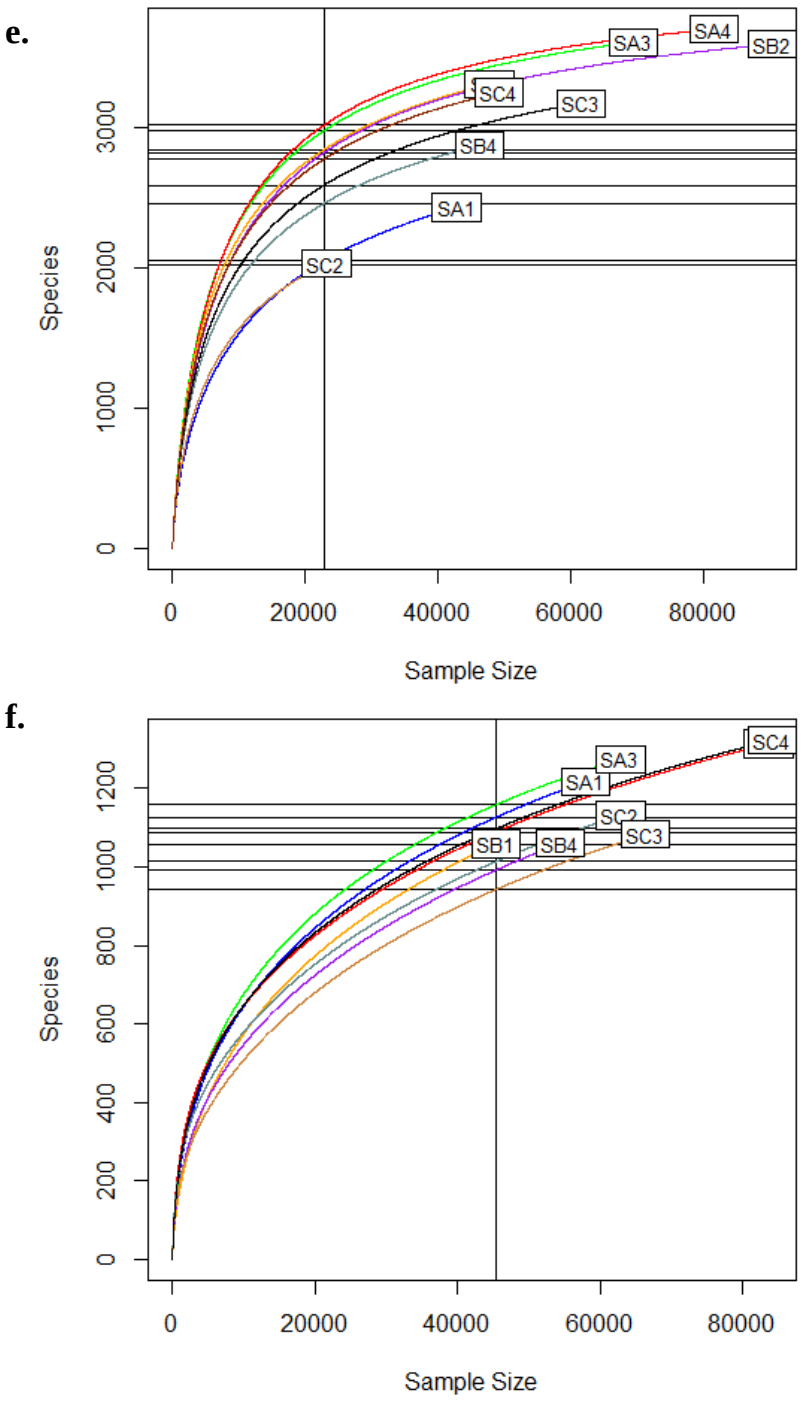

Figure A1: Bacterial and fungal rarefaction curves. Rarefaction curves represent the rarefied samples of bacterial OTUs to 22818 and fungal OTUs to 45378 on the north-facing slope (a. to b.), on the plateau (c. to d.), and on the south-facing slope (e. to f.), respectively.

Table A1: Bacterial diversity indices computed for the sample replicates

Replicates	Observed	Chao1	Standard error Chao1	Shannon	Simpson	Inverse Simpson	Pilou's Evenness
SA1	2,061	2,641.76	61.03	6.31	1.00	213.91	0.83
SA3	2,981	3,510.03	51.47	7.22	1.00	674.55	0.90
SA4	3,024	3,626.61	56.60	7.10	1.00	445.23	0.89
SB1	2,852	3,332.72	48.48	7.15	1.00	584.67	0.90
SB2	2,827	3,388.01	54.35	6.98	1.00	435.15	0.88
SB4	2,471	2,934.89	48.92	6.80	1.00	330.52	0.87
SC2	2,019	2,437.09	47.96	6.42	1.00	217.62	0.84
SC3	2,571	3,124.19	56.73	6.92	1.00	459.26	0.88
SC4	2,772	3,297.76	52.00	7.00	1.00	374.68	0.88
PA1	2,167	2,578.04	47.42	6.60	1.00	283.00	0.86
PA3	2,693	3,203.08	51.46	6.92	1.00	374.87	0.88
PA4	1,874	2,358.41	56.51	6.41	1.00	277.28	0.85
PB1	1,542	2,015.54	58.71	5.98	0.99	146.34	0.81
PB2	2,406	2,987.19	58.02	6.64	1.00	309.87	0.85
PB3	2,511	3,087.39	57.39	6.79	1.00	364.97	0.87
PC1	2,974	3,553.82	54.82	7.01	1.00	350.12	0.88
PC3	2,591	3,175.56	57.40	6.74	1.00	271.75	0.86
PC4	2,495	3,037.34	54.92	6.69	1.00	267.39	0.85
NA1	3,014	3,539.10	52.24	7.26	1.00	694.17	0.91
NA2	2,516	3,129.89	60.47	6.54	0.99	192.23	0.83
NA4	3,089	3,610.60	51.03	7.21	1.00	560.14	0.90
NB1	3,108	3,761.98	60.17	7.22	1.00	624.07	0.90
NB2	3,077	3,597.31	50.37	7.20	1.00	557.83	0.90
NB3	3,002	3,544.23	51.76	7.09	1.00	463.98	0.89
NC2	2,938	3,480.43	52.24	7.14	1.00	589.93	0.89
NC3	2,782	3,363.17	55.56	6.95	1.00	402.19	0.88
NC4	3,134	3,635.66	48.15	7.22	1.00	573.29	0.90

Table A2: Fungal diversity indices computed for the sample replicates

Replicates	Observed	Chao1	Standard error Chao1	Shannon	Simpson	Inverse Simpson	Pilou's evenness
SA1	1,129	1,628.24	69.50	4.50	0.96	23.95	0.64
SA3	1,160	1,499.25	47.97	4.90	0.98	47.73	0.70
SA4	1,092	1,508.40	60.02	5.04	0.98	60.23	0.72
SB1	1,059	1,515.25	63.06	3.91	0.92	11.96	0.56
SB4	995	1,548.58	79.07	4.40	0.96	28.33	0.64
SC2	1,031	1,452.17	59.25	5.04	0.99	67.88	0.73
SC3	944	1,384.01	63.15	4.60	0.97	37.84	0.67
SC4	1,079	1,502.14	61.86	4.45	0.92	13.22	0.64
PA1	959	1,389.63	62.74	4.34	0.97	30.81	0.63
PA3	965	1,331.31	53.63	3.90	0.92	12.65	0.57
PA4	1,038	1,418.24	53.35	4.78	0.98	49.85	0.69
PB1	1,015	1,424.52	57.09	4.46	0.97	31.28	0.64
PB2	1,021	1,481.05	66.96	4.42	0.96	27.29	0.64
PB3	995	1,360.63	54.20	4.31	0.97	28.60	0.62
PC1	973	1,315.88	51.19	4.12	0.92	12.70	0.60
PC3	927	1,365.10	64.66	4.45	0.97	36.39	0.65
PC4	963	1,474.65	73.93	4.53	0.98	40.19	0.66
NA1	1,201	1,532.93	46.80	4.33	0.91	11.46	0.61
NA2	1,083	1,484.47	57.36	4.47	0.97	28.66	0.64
NA4	1,268	1,540.53	39.71	5.05	0.98	52.15	0.71
NB1	1,194	1,477.88	42.22	4.80	0.97	36.51	0.68
NB2	1,318	1,553.73	36.21	5.23	0.98	62.32	0.73
NB3	1,135	1,456.45	46.44	4.24	0.92	12.94	0.60
NC2	976	1,451.15	68.11	4.64	0.98	40.01	0.67
NC4	1,235	1,530.49	44.45	5.23	0.99	70.63	0.74

Table A3: Bacterial data normality and impact of slope aspect on diversity indices

	Shapiro-Wilk test[1]		Kruskal-Wallis test[2]			Differences in groups[3]		
Diversity indices	**W**	**p-value**	**Chi-squared**	**df**	**p-value**	**North X Plateau**	**North X South**	**Plateau X South**
Observed	0.90	0.016	11.58	2	**0.003**	**0.004**	**0.028**	0.200
Chao1	0.90	0.016	10.67	2	**0.005**	**0.004**	**0.028**	0.222
Shannon	0.91	0.030	9.91	2	**0.007**	0.008	0.077	0.077
Simpson	0.88	0.005	9.22	2	**0.010**	0.008	0.162	0.094
Inverse Simpson	0.96	**0.376**	9.22	2	**0.010**	0.008	0.162	0.094
Pilou's evenness	0.85	0.001	11.05	2	**0.004**	0.006	**0.021**	0.222

[1] Normal data distribution determined using Shapiro-Wilk test with p-value > 0.05, indicated in bold

[2] Significant impact of slope aspects tested using Kruskal-Wallis test with significant p-value < 0.05, indicated in bold

[3] Significant differences between slope aspects tested using Wilcoxon Rank Sum test with significant p-value < 0.05, indicated in bold

Table A4: Fungal data normality and impact of slope aspect on diversity indices

	Shapiro-Wilk test[1]		ANOVA[2]			Differences in groups[3]		
Diversity indices	**W**	**p-value**	**F-value**	**df**	**p-value**	**North X Plateau**	**North X South**	**Plateau X South**
Observed	0.93	**0.080**	13.51	2	**0.000**	**0.000**	**0.029**	0.183
Chaos1	0.97	**0.751**	10.11	2	**0.001**	**0.001**	0.957	**0.002**
Shannon	0.96	**0.325**	2.67	2	0.092	0.076	0.665	0.354
Simpson	0.80	0.000	0.03	2	0.971	0.961	0.983	0.996
Inverse Simpson	0.93	**0.095**	0.58	2	0.569	0.545	0.937	0.759
Pilou's evenness	0.91	0.025	1.31	2	0.290	0.314	0.688	0.801

[1] Normal data distribution determined using Shapiro-Wilk test with p-value > 0.05, indicated in bold

[2] Significant impact of slope aspects tested using ANOVA with significant p-value < 0.05, indicated in bold

[3] Significant differences between slope aspects tested using Tukey HSD test with significant p-value < 0.05, indicated in bold

Table A5: Impact of elevation on bacterial and fungal diversity indices

	Bacteria[1]		Fungi[2]	
Diversity indices	**t-value**	**p-value**	**t-value**	**p-value**
Observed	-2.74	**0.011**	-2.82	**0.010**
Chao1	-2.69	**0.013**	-3.56	**0.002**
Shannon	-3.11	**0.005**	-1.94	0.064
Simpson	-2.79	**0.010**	-0.01	0.994
Inverse Simpson	-3.29	**0.003**	-0.96	0.346
Pilou's evenness	2.67	**0.013**	1.28	0.214

[1] Significant impact determined using general linear model with Quasipoisson (log) distribution with significant p-value < 0.05, indicated in bold

[2] Significant impact determined using general linear model with Gaussian distribution with significant p-value < 0.05, indicated in bold

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CHAPTER THREE - Impact of environmental conditions on bacterial and fungal communities in the Lesotho highlands

1. Introduction

Climate change over the last century has led to a rise in global air and surface temperatures and changes in precipitation patterns (Walker, *et al.*, 2019). Southern Africa has already seen increases in surface temperatures (van Wilgen, *et al.*, 2016) and further increases in temperatures of ~4ºC across the interior regions of southern Africa and > 6ºC across the central, northern, western regions of southern Africa have been projected by 2100 (Chersich and Wright, 2019). Concomitant with this increase in temperature is the occurrence of extreme weather events with drought, flood, and heatwave events becoming more prominent across several regions in the country (Chersich and Wright, 2019).

Increases in daily minimum and maximum temperatures together with changes in precipitation patterns are expected to occur at a far greater rate in montane (i.e. mountain) environments compared to regions at lower elevations (Rangwala and Miller, 2012). These changes are predicted to change the landscape as well as hydrological systems on mountains and result in habitat loss and a decrease in the diversity of the many life forms that inhabit mountain regions (Price, 1995; Rogora, *et al.*, 2018). At present, little is known about the effect of climate change on mountain ranges in southern Africa, due to the inaccessibility of many of the mountain ranges in the country and the lack of available climate data (Mukwada and Manatsa, 2018). For the montane environment in the current study in the Lesotho highlands, mean annual temperatures were observed to increase by approximately 0.76ºC between the years 1970 and 2000, with further increases of about 1ºC to 2ºC expected by 2050 and 2.5ºC to 3.5ºC by 2080 (Morris, 2017). Apart from rising temperatures, the number of days experiencing frost activity and the frequency of severe snowfall events are expected to decrease in the future (Morris, 2017).

Frost activity is quite a common occurrence throughout the year at higher elevations in the Lesotho highlands (Sumner 2004), together with needle-ice formation (van Zinderen Bakker and Werger, 1974), small needle-like crystal accumulation under the surface of the ground, tend to disrupt soil in the highlands (Boelhouwers and Meiklejohn, 2002; Grab, 2001). Due to the frost activity experienced coupled with the freeze-thawing activity that takes place in the highlands, this region has been classified as marginal- to sub-periglacial (Mills, *et al.*, 2009a). Apart from this, precipitation in the highlands occur in the form of summer rainfall between the months of October and March which accounts for 80% of the total precipitation in the region (Grab and Nash 2010), and snowfalls between the months of April and October (Mills,

et al., 2009b). The Lesotho highlands experience approximately eight snowfall events per year, resulting in approximately 183 frost days per annum, which tend to last for several weeks on the ground (Mills, *et al.*, 2009b; Nel and Sumner, 2008).

Changes in climatic conditions could have a substantial impact on the community diversity and structure of soil microorganisms inhabiting the mountain slopes in the Lesotho highlands. In the previous chapter (Chapter 2), the fungal and bacterial diversity across the aspects (north-facing and south-facing slope and summit) of a mountain peak in the Lesotho highlands was studied and correlated distinct diversity indices to differences in slope aspect. In this chapter, the microbial diversity data have been combined with available ground surface temperature data. Temperature and humidity data-loggers were placed at the nine sample points highlighted in Chapter 2, during the sampling excursion in 2019. However, due to the Covid-19 pandemic and border closures between South Africa and Lesotho, the collection of these temperature and humidity data-loggers has not been possible. Instead, the ground surface temperature data collected in 2018 from the north-facing and south-facing slopes of the same peak have been used.

The aim of this chapter was thus, to link the microbial diversity characterised across the north- and south-facing slope of the selected mountain in the Lesotho highlands with available ground surface temperatures to determine the impact of temperature variations on microbial diversity and community structures. This was achieved through the following objectives:

1. Statistical analysis of ground surface temperatures from 2018
2. Linkage between microbial diversity and community structure, and ground surface temperatures from 2018 using statistical tests

These objectives were used to test the hypothesis of the microbial diversity varying between the north- and south-facing slope. This occurs due to the presence of snow cover leading to constant temperatures on the south-facing slope as opposed to reduced snow cover leading to fluctuating temperatures on the north-facing slope. Furthermore, the hypothesis of these temperature fluctuations influencing bacterial and fungal diversity and community structures on mountains was tested as temperature fluctuations influence bacterial and fungal activity rate and as a result diversity.

This study highlights the profound impact that large temperature variations can have on microbial community structure and diversity across a short geographic distance and can serve as a primer for understanding the effects of climate change on all forms of life.

2. Methods and materials

2.1 Meteorological data collection and analysis

Ground surface temperature data were collected by Professor Stefan Grab from the School of Geography, Archaeology, and Environmental Studies, the University of the Witwatersrand during a field trip in May 2018. Tinytag Plus 2 TGP-4020 data loggers (Gemini, Chichester, UK) were installed with a thermistor probe at ground level. The Tinytag data loggers utilised could measure temperatures from -40ºC to +125ºC with a resolution of ±0.02ºC. They were installed at two locations, between the longitudes 29˚30′47 S and latitudes 29˚13′09 E on the north-facing slope and between longitudes 29˚30′52 S and latitudes 29˚13′09 E on the south-facing slope (Figure 11) and were located close to North A, North B, South A, and South B from which microbial diversity samples were obtained during the field trip in May 2019.

The Tinytag data loggers measured the actual, minimum, and maximum temperatures hourly from 18 May to 24 September 2018 on the north-facing slope and from 18 May to 03 November 2018 on the south-facing slope. The variations in the duration of the recordings between the north- and south-facing slope was due to the presence of snow accumulation on the south-facing slope preventing the data loggers from being accessed for removal. Thus, the data loggers were removed over two field trips, in September for the north-facing slope and November for the south-facing slope. After removal, recorded data from the data loggers were extracted and manipulated for further studies in RStudio (RStudio Team 2014).

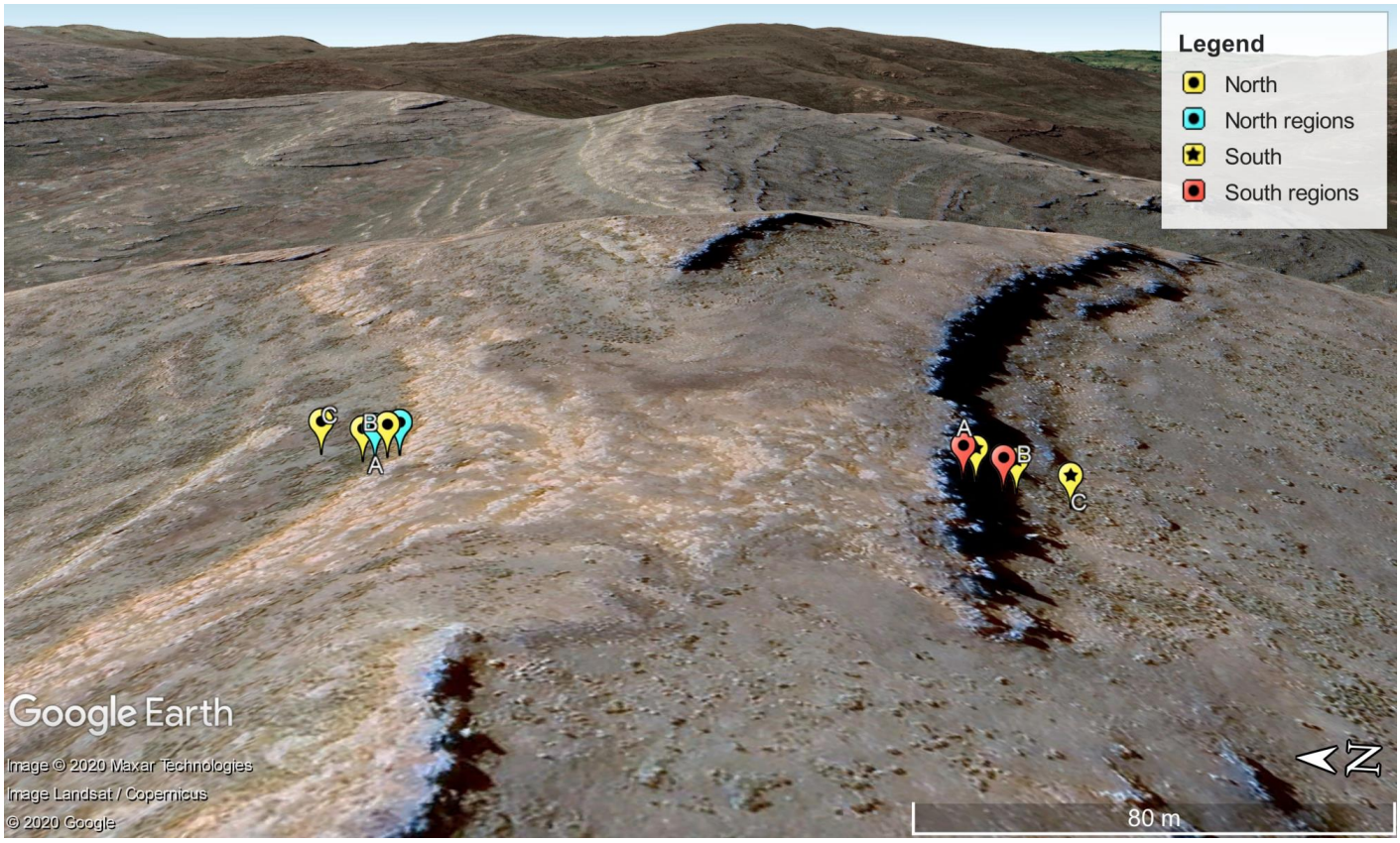

Figure 11: Locations of the Tinytag data loggers across the sampled summit. Tinytag loggers were installed in May 2018. Region A is located at the top, followed by region B in the middle of the north- and south-facing slopes, indicated by the blue and red landmarks, respectively. The location of these Tinytag loggers were close to the sites where sampling was conducted in May 2019, indicated by the yellow landmarks (Adapted from Google Earth, n.d.).

Actual minimum and maximum temperature data were manipulated to obtain the minimum and maximum recorded temperatures per slope between 18 May and 24 September 2018. Fluctuations in daily temperatures between 18 May and 24 September 2018 and the daily temperature fluctuation for the hottest days and the coldest days on both the north- and south-facing slopes were also determined. Since Lesotho experiences, an austral summer, autumn, winter, and spring (Grab and Nash, 2010), the fluctuations between late Autumn (18 May to 30 May 2018), winter (01 June to 31 August 2018), and early spring (01 September to 24 September 2018) were also determined.

Mean daily temperatures between 18 May and 24 September 2018 were calculated on the north- and south-facing slope. Mean daily temperature fluctuations between the period of 18 May to 24 September 2018 and the seasons on the north- and south-facing slope was also calculated using the mean of the daily range between minimum and maximum temperatures recorded.

2.2 Statistical analysis of the relationship between slope aspect, temperature, and season

ANOVA and Tukey HSD tests were used to determine whether slope aspect (north and south) impacts mean daily temperature and mean daily temperature fluctuations recorded between 18 May and 24 September 2018. These tests were conducted in R using the functions *aov* and *TukeyHSD* in the STAT package (CRAN.R-project.org/package=STAT). Similarly, the impact of the months' May to September and the season on mean daily temperatures and mean daily temperature fluctuations were determined for both the north- and south-facing slopes. The period of early spring was considered from 01 September to 24 September 2018 on both the north- and south-facing slope for comparisons of the impact of this season over an equal period. The impact of the variable slope aspect with months and seasons, interactively on mean daily temperatures and mean daily temperature fluctuations were further determined using general linear mixed models incorporating ANOVA and Tukey HSD.

2.3 Linking bacterial and fungal α- and β-diversity to meteorological data

The impact of daily temperature fluctuations on bacterial and fungal diversity (Shannon diversity and observed OTU richness) and on the measures Inverse Simpson, Simpson, Pilou's evenness, and Chao1, identified on the north- and south-facing slope was determined using general linear models following various distributions. As determined by the Shapiro-Wilk test ($p < 0.05$) in Chapter 2, the bacterial data followed a non-normal distribution. Therefore, the

general linear model following the Quasipoisson distribution was used to determine the impact of the daily temperature fluctuations and the mean daily temperature fluctuations calculated on bacterial diversity. This was done using the *glm* function from the STAT package in R, as they were all continuous variables. General linear mixed models following Quasipoisson distribution were then used to determine the impact of the interactions between daily temperature fluctuations and mean daily temperature fluctuations with the slope aspect on bacterial diversity.

Fungal data followed normal distribution according to the Shapiro-Wilk test ($p > 0.05$), thus, the general linear model following the Gaussian distribution was used to determine the impact of daily temperature fluctuations and daily mean temperature fluctuations on fungal diversity. The general linear mixed model using the default *glm* function was used to determine the impact of the interactions between daily temperature fluctuations and mean daily temperature fluctuations with the slope aspect on fungal diversity as they incorporated both categorical and continuous variables. These mixed models were used to control for both nested impacts and the presence of non-independent variables in the data (Adamczyk, *et al.,* 2019).

The impacts of daily temperature fluctuation and mean daily temperature fluctuations individually and as interaction with slope aspect on bacterial and fungal community structure, were determined using PERMANOVA, with 1,000 permutations. Furthermore, pairwise β-diversity indices were calculated to determine the variance between each variable using the *vegdist* function and then the *adonis* function from vegan (CRAN.R-project.org/package=vegan).

3. Results

3.1 Meteorological data analysis

Ground temperature data was recorded between 18 May to 24 September 2018, and between 18 May to 3 November 2018 on the north- and south-facing slope, respectively. Comparison of the temperature data for the shared period (18 May to 24 September 2018) demonstrated differences in both temperature range and fluctuations between the north- and south-facing slopes. The south-facing slope experiences substantially cooler daily mean temperatures than the north-facing slope (Figure 12). As such, the average daily mean temperature for the north-facing slope from May to September was 5.4ºC (Range: -0.46), while for the south-facing slope it was -1.39ºC (Range: -0.54) (Table 2). The average daily mean temperatures for the south-facing slope were below 0ºC for most of the months when the dataloggers were covered by snow as seen by the near-constant low temperatures and only rose above 0ºC in the extended recording period (October to November 2018) when the snow is expected to have melted.

While conditions are on average warmer on the north-facing slope, it does experience far more extensive temperature fluctuations compared to the south-facing slope (Figure 13). The lowest and highest temperatures, -9.86ºC and 44.83ºC were recorded on the north-facing slope on 02 July and 30 August 2018, respectively. By contrast, the lowest and highest temperatures recorded for the south-facing slope were -7.93ºC and 0.02ºC on 05 May and 08 August 2018, respectively. Furthermore, the highest daily fluctuation in temperature was also recorded on the north-facing slope (48.37ºC) on 05 July 2018 compared to the south-facing slope (4.83 ºC) on 08 August. By contrast, the lowest daily fluctuations in temperature were recorded on the south-facing slope (0.002ºC) on 10 September and 11 September 2018 compared to the north-facing slope (0.19 ºC) on 12 August.

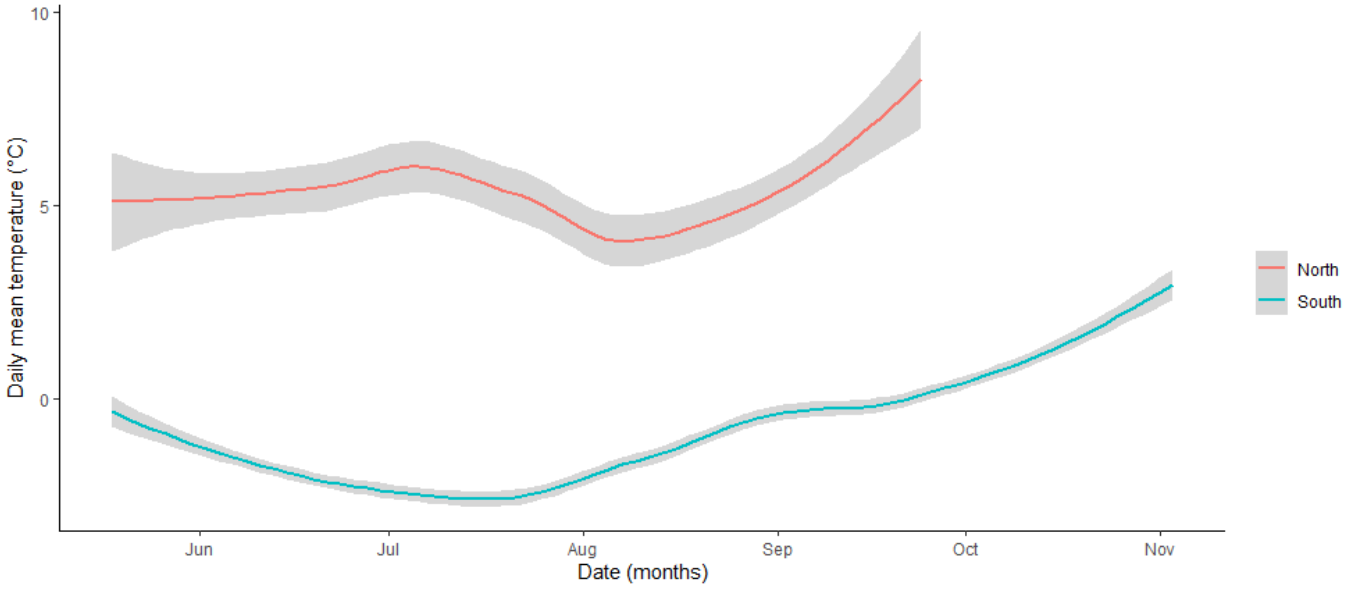

Figure 12: Mean daily temperatures recorded on the south- and north-facing slopes in 2018. Daily mean temperatures represent the ground surface temperatures recorded on the selected summit in the Lesotho highlands. The grey regions on each line represent the 95% confidence interval at which these graphs were obtained.

Table 2: Average daily temperature fluctuations and average mean daily temperature fluctuations over various periods and seasons

	North	South
Mean daily temperature per site (ºC)	5.40	-1.39
Daily temperature fluctuations (ºC)		
18 May to 24 September 2018	53.82	6.12
Late autumn (May)	35.56	2.08
Winter (June to August)	53.82	6.12
June	42.19	1.68
July	48.16	2.59
August	49.05	6.12
Early Spring (September)	41.84	0.37
Minimum temperature range	0.22	0.00
Maximum temperature range	45.88	3.62
Mean daily temperature fluctuations (ºC)		
18 May to 24 September 2018	25.02	0.38
Late autumn (May)	21.60	0.61
Winter (June to August)	26.03	0.43
June	27.82	0.22
July	34.00	0.48
August	16.33	0.59
Early Spring (September)	23.12	0.04

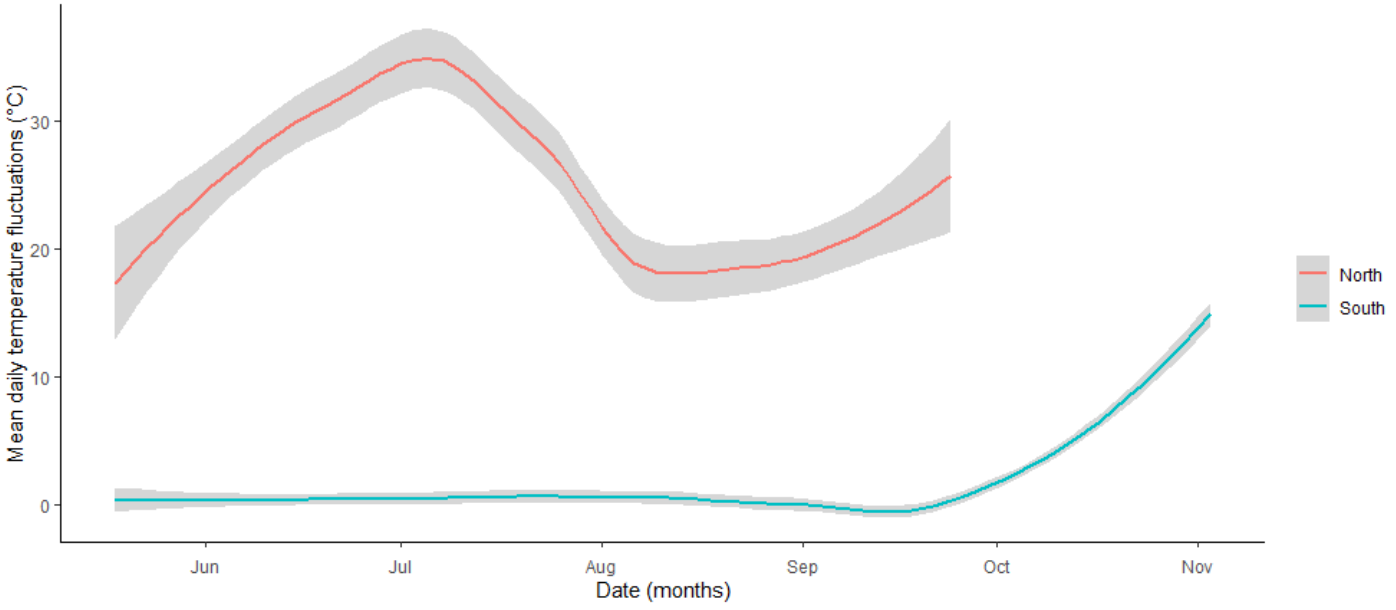

Figure 13: Mean daily temperature fluctuations obtained on the south- and north-facing slopes in 2018. Mean daily temperature fluctuations represent the range between the maximum and minimum ground surface temperatures recorded per day on the selected summit in the Lesotho highlands. The grey regions on each line represent the 95% confidence interval at which these graphs were obtained.

The largest average fluctuation in daily temperatures was recorded between 02 July and 30 August on the north-facing slope (53.82ºC) and between 08 May and 08 August 2018 on the south-facing slope (6.12ºC). While the fluctuation in daily temperatures on the north-facing occurred over two months, fluctuations on the south-facing slope occurred over one month. When considering the extended recording period for the south-facing slope, the largest average fluctuation in daily temperature (27.45ºC) occurred over three months, between 18 May to 03 November 2018. However, this is still substantially below the temperature fluctuation faced by the north-facing slope.

The period of 18 May to 24 September 2018 on the north- and south-facing slopes also incorporated the colder months of the year including the end of autumn, winter, and the beginning of spring. The highest temperature fluctuation recorded over the three seasons occurred in winter on both the north- and south-facing slope, with the north-facing slope having the highest fluctuation (53.82ºC) compared to the south-facing slope (6.12ºC). The lowest fluctuations in temperature were observed in early spring on the south-facing slope with fluctuations of < 1ºC. By contrast, the lowest temperature was observed in late autumn on the north-facing slope with fluctuations remaining comparatively high (> 35ºC).

3.2 Statistical analysis of the relationship between slope aspect, temperature, and season

The impact of slope aspect, month, and season independently and as interaction on slope aspect with season and month on mean daily temperatures and mean daily temperature fluctuations were determined using ANOVA. Differences between the individual months and seasons were further analysed using the Tukey HSD test. Independently, the slope aspect (north- and south-facing slope), season, and the months during which temperatures were recorded had a significant impact on the mean daily temperatures and fluctuations (Table 3). Significant differences in mean daily temperature during the three seasons were, however, only noted during winter and early spring (p-adjusted = 0.004), and none were noted in mean daily temperature fluctuation during the three seasons (p-adjusted > 0.05). Significant differences in mean daily temperatures were also noted between the various months, however, no significant difference was noted in mean daily temperatures, particularly between the month of August compared with the months of May (p-adjusted = 0.094), June (p-adjusted = 0.108) and July (p-adjusted = 0.179). Concerning mean daily temperature fluctuation, a significant difference was only found between the months of August and June (p-adjusted = 0.002).

In terms of the impact of the interaction of season and months with the slope aspect, only the interaction of slope aspect with months significantly affected mean daily temperatures and fluctuations. Significant differences in mean daily temperatures and fluctuations during the seasons and months across the north- and south-facing were observed (p-adjusted < 0.05) using Tukey HSD, indicating differences in mean daily temperatures and fluctuations between the two slopes. However, several comparisons of mean daily temperature and fluctuations over the seasons and months between the two data collection points on each slope were not significantly different from each other (p-adjusted > 0.05), highlighting the similarities in mean daily temperatures and fluctuations faced across each slope.

Table 3: Impact of slope aspect, seasons, and months on mean daily temperatures and mean daily temperature fluctuations[1]

	Mean daily temperature			**Mean daily temperature fluctuation**		
	df	**F value**	**p-value**	**df**	**F value**	**p-value**
Slope aspect	1	285.00	**<0.005**	1	169.83	**<0.005**
Season	2	8.43	**0.005**	2	0.02	0.981
Month	4	29.01	**0.000**	4	7.95	**0.005**
Slope aspect X Season	2	0.56	0.584	2	0.10	0.910
Slope aspect X Month	3	10.75	**0.002**	3	12.34	**0.002**

[1] Significant impact of slope aspects, month, and season independently and as interactions tested using ANOVA with significant p-value < 0.05, indicated in bold

3.3 Linking bacterial and fungal α- and β-diversity with meteorological data

A total of 772,974 sequences were retained for the north- and south-facing slope, with each slope having 136,908 bacterial and 249,579 fungal sequences after sampling rarefaction. Bacterial samples were rarefied to 22,818 and fungal samples to 45,378, which were the lowest number of sequences found in a sample. The bacterial and fungal communities on the north- and south-facing slopes both incorporated twenty-two phyla and eleven phyla (including a "general classification of Fungi"), respectively. However, the number of genera represented on each slope varied. A greater number of both bacterial (335) and fungal (453) genera were observed on the north-facing slope compared to the south-facing slope (331 and 441, respectively). Several bacterial genera such as *Rodopila* and *Rhodovibrio* unique to the north-facing slope and *Azospira* and *Rhodocyclus* unique to the south-facing slope were mainly from the phylum Proteobacteria (Appendix Table A6). Whereas, fungal genera such as *Lasiodiplodia* and *Tubaria* unique to the north-facing slope, and *Bacidia* and *Thelephora* unique to the south-facing slope were mainly from the phyla Ascomycota and Basidiomycota, respectively (Appendix Table A7). Several other fungal genera also from the phyla Ascomycota and Basidiomycota were further found to be unique to either the north-facing slope (twelve and six) or south-facing slope (ten and five), respectively. The abundance of these bacterial and fungal genera were however < 0.01%.

Bacterial and fungal diversity indices also varied between the north- and south-facing slope, such that the average bacterial diversity on the north-facing slope (Shannon index = 7.09 and OTU richness = 2,967.67) was higher than the south-facing slope (Shannon = 6.93 and OTU richness = 2,702.67). Similarly, average fungal diversity on the north-facing slope (Shannon = 4.69 and OTU richness = 1199.83) was higher than the south-facing slope (Shannon = 4.55 and OTU richness = 1087).

The impact of minimum and maximum recorded temperatures, as well as daily temperature fluctuations and mean daily temperatures and fluctuations on the α-diversity indices of both bacteria and fungi were determined using the general linear model following the Quasipoisson (bacterial) and the Gaussian distributions (fungal). These analyses indicated that minimum and maximum recorded temperatures on the north- and south-facing slope, had no significant impact on both bacterial and fungal diversity (p-value > 0.05) (Appendix Table A8 and A9). No significant impact on the other bacterial and fungal diversity and evenness measures were noted (p-value > 0.05). Furthermore, daily temperature fluctuations and mean daily

temperatures and fluctuations had no significant impact on bacterial and fungal diversity (p-value > 0.05). Only minimum recorded fluctuation in temperature had a significant impact on fungal observed OTU richness (t-test = 2.27, p-value = 0.05), while only fungal observed OTU richness was significantly affected by mean daily temperatures (t-test = 2.29, p-value = 0.048).

The impact of the interaction of minimum and maximum recorded temperatures, as well as daily temperature fluctuations and mean daily temperatures and fluctuations with slope aspect (north- and south-facing slopes) on bacterial and fungal diversity were also determined using general linear mixed models. The interaction of both slope aspect with minimum and maximum recorded temperatures and daily temperature fluctuations had no significant impact on bacterial and fungal diversity (p-value > 0.05) (Appendix Table A10 and A11). Similarly, the impact of both slope aspect with mean daily temperatures and fluctuations had no significant impact on bacterial and fungal diversity (p-value > 0.05).

Variations in bacterial and fungal community structures (β-diversity) due to minimum and maximum temperature, daily temperature fluctuations and mean daily temperatures and fluctuations, independently and interactively with the slope aspect were determined using PERMANOVA on the Bray-Curtis dissimilarity measure. The analyses indicated that bacterial and fungal community structures were significantly affected by maximum recorded temperature and daily temperature fluctuations independently (p-value < 0.05) (Table 4). The minimum recorded temperature, however, only significantly influenced fungal community structure and not bacterial community structure. Similarly, mean daily temperatures and fluctuations significantly affected bacterial and fungal community structure (p-value < 0.05) (Table 5). The interaction of slope aspect with minimum and maximum recorded temperatures and daily temperature fluctuations had no significant impact on bacterial and fungal community structures (p < 0.05). Similarly, the interaction of slope aspect with mean daily temperatures and fluctuations also had no significant impact on bacterial and fungal community structures.

Table 4: Impact of recorded temperatures and daily temperature fluctuations independently and interactively on bacterial and fungal community structure[1]

	Bacteria				Fungi			
	df	**F-model**	**R^2 (%)**	**p-value**	**df**	**F-model**	**R^2 (%)**	**p-value**
Minimum and maximum recorded temperature (ºC)								
Minimum temperature	1	1.65	14.17	0.073	1	1.64	15.39	**0.001**
Minimum temperature X Slope aspect	1	0.73	6.28	0.776	1	0.96	8.86	0.585
Maximum temperature	1	2.16	17.78	**0.012**	1	1.51	14.39	**0.003**
Maximum temperature X Slope aspect	1	0.89	7.58	0.568	1	1.30	12.05	0.062
Temperature fluctuations (ºC)								
Between 18 May to 24 September 2018	1	2.15	17.73	**0.008**	1	1.54	14.58	**0.001**
Between 18 May to 24 September 2018 X Slope aspect	1	0.84	7.18	0.635	1	1.09	10.10	0.254
Minimum	1	2.03	16.86	**0.014**	1	1.52	14.43	**0.001**
Minimum X Slope aspect	1	0.88	7.52	0.573	1	1.30	12.01	0.051
Maximum	1	2.12	17.48	**0.016**	1	1.53	14.57	**0.002**
Maximum X Slope aspect	1	0.94	8.02	0.517	1	1.30	12.05	**0.041**
Seasonal temperature fluctuations (ºC)								
Late autumn	1	2.10	17.36	**0.016**	1	1.53	14.50	**0.003**
Late autumn X Slope aspect	1	0.91	7.75	0.554	1	1.31	12.13	**0.044**
Early spring	1	2.13	17.56	**0.015**	1	1.52	14.45	**0.002**
Early spring X Slope aspect	1	0.89	7.64	0.555	1	1.30	12.08	0.055
Winter	1	2.15	17.73	**0.012**	1	1.54	14.58	**0.002**
Winter X Slope aspect	1	0.84	7.18	0.637	1	1.09	10.10	0.269

[1] Significant impact determined using PERMANOVA on the Bray-Curtis dissimilarity measure with significant p-value < 0.05, indicated in bold

Table 5: Impact of mean daily temperature and mean daily temperature fluctuations independently and as interactions on bacterial and fungal community structure[1]

	Bacteria				Fungi			
	df	**F-model**	**R^2 (%)**	**p-value**	**df**	**F-model**	**R^2 (%)**	**p-value**
Mean daily temperatures between 18 May and 24 September (ºC)								
Daily temperatures	1	2.15	17.67	**0.019**	1	1.49	14.21	**0.014**
Daily temperatures X Slope aspect	1	0.67	5.73	0.873	1	1.03	9.51	0.418
Mean temperature fluctuations (ºC)								
Between 18 May to 24 September 2018	1	2.11	17.41	**0.026**	1	1.52	14.47	**0.002**
Between 18 May to 24 September 2018 X Slope aspect	1	0.89	7.61	0.555	1	1.30	12.07	0.051
Mean seasonal temperature fluctuations (ºC)								
Late autumn	1	2.07	17.12	**0.018**	1	1.52	14.46	**0.004**
Late autumn X Slope aspect	1	0.88	7.56	0.574	1	1.30	12.04	0.054
Early spring	1	2.17	17.85	**0.011**	1	1.51	14.37	0.004
Early spring X Slope aspect	1	0.91	7.79	0.538	1	1.31	12.15	**0.033**
Winter	1	2.09	17.32	**0.016**	1	1.52	14.47	**0.003**
Winter X Slope aspect	1	0.89	7.62	0.558	1	1.30	12.07	0.054

[1] Significant impact determined using PERMANOVA on the Bray-Curtis dissimilarity measure with significant p-value < 0.05, indicated in bold

4. Discussion

Climate records for the Lesotho highland are quite scarce and usually limited to certain parts of the highlands, such as at Sani Pass (Fitchett, *et al.*, 2016). Here ground surface temperature data has been recorded and collected on the north- and south-facing slope of a single summit in the Lesotho highlands over a period of five and six months, respectively. Mean daily temperatures across the north- and south-facing slopes were significantly different from each other, with the north-facing slope experiencing higher mean daily temperatures above 4ºC compared to the south-facing slope which experienced mean daily temperatures below 0ºC. Significant differences were also noted in mean daily temperature over the months recorded across the two slopes. These differences in mean daily temperatures could be attributed to the levels of solar radiation and shading experienced by the north- and south-facing slopes (Grab, 1999; Mills, *et al.*, 2009b).

North-facing slopes in the Lesotho highlands tend to experience warmer and drier climatic conditions due to the higher levels of solar radiation exposure they receive throughout the year (Boelhouwers 2003; Mills, *et al.*, 2009b). Furthermore, the south-facing slopes in the highlands experience excessive shading due to reduced solar radiation exposure in the southern hemisphere leading to much cooler conditions across these slopes (Grab, 1999). The south-facing slope also experienced lower mean daily fluctuations between various periods compared to the north-facing slope. This could further be attributed to the varying amounts of snow cover across the north- and south-facing slopes in the Lesotho highlands (Grab, *et al.*, 2017). Snow tends to accumulate for longer periods at higher elevations on the south-facing slope (Grab, *et al.*, 2017), insulating the soil below from freezing temperatures (Contosta, *et al.*, 2019) and thereby, protecting above- and below-ground biota from extensive freezing (Freppaz, *et al.*, 2007). Snow cover also reduces the occurrence of freeze-thaw cycles which could have substantial impacts on biota in these regions (Freppaz, *et al.*, 2007).

The presence of snow cover, especially below rocks scarps where snow tends to last longer, could have contributed to the near-constant mean daily temperature fluctuations recorded across the south-facing slopes over several months (~ 0ºC) compared to the north-facing slope. The absence of shading, a high and steep rocks scarp for the snow to accumulate under, and the increased levels of solar radiation exposure on the north-facing slope may contribute to the rapid melting of snow across the slope rather than providing the cover for insulation and maintenance of constant temperatures (Edwards, *et al.*, 2007; Grab, *et al.*, 2017). The south-

facing slope experienced an increase in mean daily temperature fluctuations from early October to November 2018 which could be indicative of snow-melting across the slope. This is commonly noted during spring in October and November resulting in the rapid melting of snow cover in the Lesotho highlands (Grab, *et al.,* 2017). However, due to climate change, the frequencies of snowfalls and periods of snow cover in the Lesotho highlands have varied from the previous century, with less snowfall being recorded and the duration of snow cover decreasing to several weeks rather than months in the last century (Grab, *et al.,* 2017).

It should however be noted that soil below the ground tends to be more buffered against changes in dramatic climatic conditions experienced above the ground, which as a result causes below ground microorganisms to experience different environmental conditions to those experienced by biota above the ground (Classen, *et al.,* 2015). Temperatures in this study were recorded above the ground which could influence the response bacterial and fungal diversity and community structure may have in this study. Furthermore, since snow cover provides insulation for soil, from freezing temperatures experienced above the ground, temperatures below the ground could have varied from those recorded in this study (Contosta, *et al.,* 2019; Freppaz, *et al.,* 2007).

Temperature fluctuation may cause changes in the community structure, diversity, and function of soil microorganisms. It can do so directly, through imposing stress on the bacterial or fungal cell, or indirectly through changes in the availability of nutrients required for growth and activity, altering soil physiochemical properties, and through selective pressure of microorganisms which can survive such large fluctuations in temperatures (Akbari and Ghoshal, 2015; Gray, *et al.,* 2011; Zhang, *et al.,* 2013). Higher temperature fluctuations lead to increased soil respiration rates, faster consumption, and depletion of available soil nutrients and subsequently lead to decreased microbial diversity and activity (Supramaniam, *et al.*, 2016). Furthermore, higher temperature fluctuations decrease soil moisture, thereby increasing osmotic stress and decreasing nutrient and substate availability for microorganisms (Supramaniam, *et al.*, 2016).

Several studies have indicated changes in soil microbial communities associated with rising temperatures over extended periods. This includes studies in a sub-arctic heath in Sweden (15 years) (Rinnan, *et al.,* 2007), on an elevational gradient in an alpine meadow on the Tibetan Plateau (7 years) (Yu, *et al.,* 2019) and in forest soil at the Harvard Forest Long-Term Ecological Research site in Massachusetts (20 years) (DeAngelis, *et al.,* 2015). However,

temperature changes considered in these studies occurred over years with increases of a few degrees Celsius a year, rather than large daily temperature fluctuations over short periods as seen in this study.

In the Lesotho highlands, soil microorganisms experience daily temperature fluctuations which may be as low as 0ºC on the coldest day on the south-facing slope and as high as 48.37ºC across the hottest day (24 hour period) on the north-facing slope. These rapid temperature fluctuations may have a substantial effect on bacterial and fungal communities inhabiting the soil in this montane ecosystem. These microbial communities experience similar changes in environmental conditions as those experiencing temperature fluctuation over longer periods, however at a much quicker rate (Edwards, *et al.*, 2007). The rapidity at which soil microorganisms adapt and respond to these changes is, however, dependent on their genetic capabilities and regulations which may influence their diversity and cause changes in their community structures (Jansson and Hofmockel, 2020). These changes may further influence the stability and resilience of microbial communities to future temperature fluctuations over the short-term (Jansson and Hofmockel, 2020).

Colder conditions on the south-facing slope coupled with the presence of snow for much of the recorded period may have influenced the lower bacterial and fungal diversity observed on the south-facing slope compared to the north-facing slope which had warmer climatic conditions. Increased microbial activity rates, growth, and soil respiration rates have all been observed to result from higher temperatures (Classen, *et al.,* 2015; Martins, *et al.,* 2017). In terms of bacteria, members of the class Alphaproteobacteria were found at greater abundance on the north-facing slope (2.34%) compared to the south-facing slope (2.28%). Furthermore, Acidobacteria and Actinobacteria were also found at higher abundance on the north-facing slope compared to the south-facing slope. Alphaproteobacteria, Acidobacteria, and Actinobacteria are commonly used as indicator taxa of warming and the increase in abundance of these taxa at higher temperatures has been observed in both long-term soil warming experiments in forest soil in the Harvard Forest Long-Term Ecological Research site in Massachusetts (DeAngelis, *et al.*, 2015) and short-term soil warming experiments in an alpine meadow on the Tibetan plateau at an elevation of 4,635 m.a.s.l. (Xiong, *et al.,* 2014).

These differences in bacterial taxa may be due to increased availability of nutrients and substrates in soil following high-temperature fluctuations which alter soil physiochemical composition, allowing copiotrophic microorganisms such as Proteobacteria with the ability to

grow faster to outcompete oligotrophic microorganisms such as Acidobacteria and Verrucomicrobia (DeAngelis, *et al.,* 2015; Ho, *et al.,* 2017). This corresponds to the lower abundance of these oligotrophic microorganisms on the south-facing slope compared to the north-facing slope. Similarly, among the fungi, the copiotrophic phylum Chytridiomycota (5.37%) and the oligotrophic phyla Basidiomycota (4.35%), and Glomeromycota (0.76%) were also found in greater abundance on the north-facing slopes compared to the south-facing slope (3.59%, 2.62%, and 0.62%), respectively. Furthermore, fungal taxa such as Basidiomycota can also be used as indicators of climate change as they are very sensitive to changes in environmental conditions (Xun, *et al.,* 2018). The occurrence of these bacterial and fungal taxa across both the north- and south-facing slope at varying abundances, despite experiencing various temperature fluctuations, are indicative of them being more physiologically flexible to these changes (Chan, *et al.,* 2016).

Bacterial and fungal community structures (β-diversity) were found to be significantly affected by minimum and maximum recorded temperatures, daily temperature fluctuations, mean daily temperatures, and fluctuations, independently. These results correspond with several studies conducted to determine the impact of both long-term temperature variations and short-term temperature variations in various environments on soil microbial communities which indicated that these changes in temperatures have a significant impact on both bacterial and fungal communities (DeAngelis, *et al.,* 2015; Deslippe, *et al.,* 2012; Wu, *et al.,* 2016; Xiong, *et al.,* 2014).

Bacterial and fungal α-diversity (observed OTU richness and Shannon-Weaver index), however, was not significantly affected by minimum and maximum temperatures recorded, daily temperature fluctuations, mean daily temperatures and fluctuations independently, and neither as interactions with slope aspect. These results correlate with those from studies conducted on an alpine meadow in the Yangtze River source and the Haibei Alpine Grassland Ecosystem Research Station, both on the Tibetan Plateau, where bacterial and fungal diversity was not affected by short-term changes in temperatures (Xiong, *et al.,* 2014; Zhang, *et al.,* 2016b).

5. Conclusion

Temperature analysis conducted in this study has provided the first insights into the response of the soil microbiome associated with a summit in the Lesotho highlands. The differences in daily temperature fluctuations over various periods of times on the north- and south-facing slope provided a platform to highlight the response of soil bacterial and fungal α- and β-diversity to temperature fluctuations independently and as interactions with other variables such as the slope aspect, which has never been studied before in this region. As such, daily temperature fluctuations and mean daily temperatures and fluctuations were found to have no impact independently nor as interactions with the slope aspect on bacterial and fungal diversity. However, it had a stronger significant impact on bacterial and fungal community structure independently. Variations noted in the diversity of bacteria and fungi indicate that it is plausible that other factors, such as soil physical and chemical properties, as well as vegetation, may have a more profound impact on the different community structures observed along the north-facing, south-facing slopes and the plateau. Further research is required to investigate other potential drivers for the presence of the different bacterial and fungal taxa across the Lesotho highlands summit.

6. Appendix

Table A6: Bacterial taxa unique to either the north- or south-facing slope

Phylum	Class	Order	Family	Genus	Abundance on South (%)	Abundance on North (%)
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	0	<0.01
Chloroflexi	Ktedonobacteria	Ktedonobacterales	Thermosporotrichaceae	*Thermosporothrix*	0	<0.01
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Rhodovibrio*	0	<0.01
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Rhodopila*	0	<0.01
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	0	<0.01
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Microcoleaceae	*Microcoleus*	0	<0.01
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Klebsiella*	0	<0.01
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Azospira*	<0.01	0
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Rhodocyclus*	<0.01	0
Actinobacteria	Actinobacteria	Actinomycetales	Actinospicaceae	*Actinospica*	<0.01	0

Table A7: Fungal taxa unique to either the north- or south-facing slope

Kingdom	Phylum	Class	Order	Family	Genus	Abundance on South (%)	Abundance on North (%)
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Deniquelata*	0	<0.01
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Hebeloma*	0	<0.01
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellales	*Derxomyces*	0	<0.01
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Sordaria*	0	<0.01
Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	*Eutypella*	0	<0.01
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Bolbitiaceae	*Conocybe*	0	<0.01
Fungi	Ascomycota	Pezizomycetes	Pezizales	Helvellaceae	*Helvella*	0	<0.01
Fungi	Basidiomycota	Agaricomycetes	Russulales	Lachnocladiaceae	*Asterostroma*	0	<0.01
Fungi	Ascomycota	Pezizomycetes	Pezizales	Chorioactidaceae	*Chorioactis*	0	<0.01
Fungi	Ascomycota	Ascomycota	Ascomycota	Ascomycota	*Tumularia*	0	<0.01
Fungi	Glomeromycota	Glomeromycetes	Diversisporales	Diversisporaceae	*Diversispora*	0	<0.01
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Dipodascaceae	*Sporopachydermia*	0	<0.01
Fungi	Ascomycota	Pezizomycotina	Pezizomycotina	Pezizomycotina	*Leptodiscella*	0	<0.01
Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Hyaloriaceae	*Tremiscus*	0	<0.01
Fungi	Ascomycota	Pezizomycetes	Pezizales	Ascodesmidaceae	*Cephaliophora*	0	<0.01
Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	*Phaeoisaria*	0	<0.01
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Corynesporascaceae	*Corynespora*	0	<0.01
Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Trichophaea*	0	<0.01
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporellus*	0	<0.01
Fungi	Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	*Tubeufia*	0	0.01
Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	*Exidia*	0	0.01
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Tubaria*	0	0.01
Fungi	Ascomycota	Leotiomycetes	Leotiomycetes	Pseudeurotiaceae	*Gymnostellatospora*	0	0.01
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Lasiodiplodia*	0	0.01
Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Coniosporium*	<0.01	0
Fungi	Basidiomycota	Agaricomycetes	Boletales	Pisolithaceae	*Pisolithus*	<0.01	0
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	*Postia*	<0.01	0
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Bipolaris*	<0.01	0
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Fibroporia*	<0.01	0
Fungi	Ascomycota	Lecanoromycetes	Teloschistales	Teloschistaceae	*Huea*	<0.01	0
Fungi	Ascomycota	Eurotiomycetes	Mycocaliciales	Mycocaliciaceae	*Chaenothecopsis*	<0.01	0
Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Phellodon*	<0.01	0
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe*	<0.01	0
Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Parmeliaceae	*Usnea*	<0.01	0
Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*	0.01	0
Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Ramalinaceae	*Bacidia*	0.01	0

Table A8: Impact of recorded temperatures and daily temperature fluctuations independently and interactively on bacterial diversity indices[1]

	Observed		Chao1		Shannon		Simpson		Inverse Simpson		Pilou's evenness	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Minimum and maximum recorded temperature (ºC)												
Minimum temperature	-1.17	0.271	-1.08	0.306	-0.96	0.358	-0.57	0.582	-0.65	0.534	1.30	0.223
Minimum temperature X Slope aspect	0.70	0.505	0.91	0.389	0.62	0.555	0.84	0.428	0.39	0.704	-0.63	0.549
Maximum temperature	1.41	0.190	1.37	0.200	0.92	0.382	0.40	0.699	0.76	0.463	-1.46	0.175
Maximum temperature X Slope aspect	0.03	0.974	-0.08	0.940	0.30	0.773	0.33	0.750	0.02	0.984	-0.16	0.876
Temperature fluctuations (ºC)												
Between 18 May to 24 September 2018	1.41	0.189	1.37	0.201	0.94	0.372	0.42	0.683	0.77	0.462	-1.47	0.172
Between 18 May to 24 September 2018 X Slope aspect	-0.60	0.564	-0.83	0.429	-0.43	0.677	-0.62	0.555	-0.34	0.746	0.50	0.633
Minimum	1.53	0.157	1.54	0.155	1.02	0.331	0.54	0.599	0.83	0.429	-1.57	0.148
Minimum X Slope aspect	0.06	0.957	-0.05	0.962	0.32	0.758	0.36	0.730	0.03	0.975	-0.18	0.860
Maximum	1.46	0.176	1.43	0.182	0.97	0.355	0.47	0.651	0.79	0.448	-1.51	0.162
Maximum X Slope aspect	-0.24	0.820	-0.42	0.688	0.02	0.983	-0.04	0.971	-0.13	0.902	0.11	0.918
Seasonal temperature fluctuations (ºC)												
Late autumn	1.48	0.170	1.46	0.174	0.98	0.351	0.48	0.641	0.80	0.443	-1.52	0.158
Late autumn X Slope aspect	-0.04	0.973	-0.17	0.873	0.23	0.822	0.24	0.816	-0.02	0.987	-0.10	0.926
Early spring	1.45	0.178	1.43	0.184	0.95	0.363	0.45	0.666	0.79	0.451	-1.50	0.165
Early spring X Slope aspect	0.01	0.992	-0.11	0.918	0.28	0.789	0.30	0.771	0.01	0.994	-0.14	0.892
Winter	1.41	0.189	1.37	0.201	0.94	0.372	0.42	0.683	0.77	0.462	-1.47	0.172
Winter X Slope aspect	-0.60	0.564	-0.83	0.429	-0.43	0.677	-0.62	0.555	-0.34	0.746	0.50	0.633

[1] Significant impact of independent variables determined using general linear model and interactions of variables determined using general linear mixed models both with Quasipoisson (log) distribution with significant p-value < 0.05, indicated in bold

Table A9: Impact of recorded temperatures and daily temperature fluctuations independently and interactively on fungal diversity indices[1]

	Observed		Chao1		Shannon		Simpson		Inverse Simpson		Pilou's evenness	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Minimum and maximum recorded temperature (ºC)												
Minimum temperature	-1.03	0.328	2.20	0.055	0.40	0.695	0.86	0.413	0.71	0.494	1.41	0.192
Minimum temperature X Slope aspect	0.71	0.504	0.78	0.459	0.51	0.623	0.32	0.757	0.47	0.650	0.09	0.928
Maximum temperature	2.17	0.058	-0.71	0.497	0.54	0.606	-0.22	0.831	-0.04	0.967	-1.05	0.320
Maximum temperature X Slope aspect	-1.35	0.220	-3.51	**0.010**	-1.86	0.105	-1.22	0.261	-1.29	0.239	-0.81	0.446
Temperature fluctuations (ºC)												
Between May to September	2.05	0.070	-0.83	0.427	0.45	0.665	-0.28	0.784	-0.11	0.916	-1.10	0.299
Between May to September X Slope aspect	-1.14	0.291	-2.14	0.069	-1.20	0.268	-0.79	0.458	-0.94	0.379	-0.42	0.689
Minimum	2.27	**0.050**	-0.63	0.544	0.59	0.573	-0.18	0.865	0.02	0.984	-1.02	0.332
Minimum X Slope aspect	-1.33	0.225	-3.50	**0.010**	-1.85	0.106	-1.22	0.263	-1.28	0.242	-0.81	0.447
Maximum	2.14	0.061	-0.77	0.460	0.49	0.634	-0.25	0.809	-0.07	0.948	-1.08	0.308
Maximum X Slope aspect	-1.46	0.188	-3.43	**0.011**	-1.86	0.106	-1.21	0.265	-1.33	0.227	-0.76	0.474
Seasonal temperature fluctuations (ºC)												
Late autumn	2.19	0.057	-0.70	0.501	0.54	0.602	-0.21	0.835	-0.03	0.979	-1.06	0.319
Late autumn X Slope aspect	-1.34	0.222	-3.57	**0.009**	-1.87	0.104	-1.24	0.255	-1.33	0.224	-0.81	0.445
Early spring	2.18	0.057	-0.69	0.505	0.55	0.599	-0.21	0.837	-0.03	0.978	-1.05	0.320
Early spring X Slope aspect	-1.31	0.232	-3.55	**0.009**	-1.85	0.106	-1.23	0.258	-1.32	0.228	-0.81	0.443
Winter	2.05	0.070	-0.83	0.427	0.45	0.665	-0.28	0.784	-0.11	0.916	-1.10	0.299
Winter X Slope aspect	-1.14	0.291	-2.14	0.069	-1.20	0.268	-0.79	0.458	-0.94	0.379	-0.42	0.689

[1] Significant impact of independent variables determined using general linear model and interactions of variables determined using general linear mixed models both with Gaussian distributions with significant p-value < 0.05, indicated in bold

Table A10: Impact of mean daily temperatures and mean daily temperature fluctuations independently and interactively on bacterial diversity indices[1]

	Observed		Chao1		Shannon		Simpson		Inverse Simpson		Pilou's evenness	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Mean daily temperatures between 18 May and 24 September (ºC)												
Daily temperatures	1.42	0.186	1.40	0.192	0.91	0.385	0.40	0.701	0.77	0.459	-1.46	0.175
Daily temperatures X Slope aspect	-0.57	0.584	-0.66	0.528	-0.70	0.505	-0.90	0.397	0.32	0.759	0.61	0.558
Mean temperature fluctuations (ºC)												
Between 18 May to 24 September 2018	1.47	0.172	1.46	0.176	0.97	0.355	0.47	0.648	0.80	0.445	-1.52	0.160
Between 18 May to 24 September 2018 X Slope aspect	0.02	0.984	-0.09	0.928	0.29	0.782	0.31	0.762	0.01	0.990	-0.15	0.885
Mean seasonal temperature fluctuations (ºC)												
Late autumn	1.51	0.163	1.50	0.164	1.00	0.341	0.51	0.620	0.81	0.435	-1.55	0.153
Late autumn X Slope aspect	0.04	0.969	-0.07	0.947	0.30	0.769	0.34	0.744	0.02	0.982	-0.17	0.871
Early spring	1.39	0.194	1.35	0.206	0.90	0.388	0.38	0.711	0.76	0.468	-1.44	0.179
Early spring X Slope aspect	-0.05	0.960	-0.19	0.856	0.22	0.835	0.22	0.833	-0.03	0.980	-0.08	0.939
Winter	1.48	0.169	1.47	0.172	0.98	0.349	0.49	0.638	0.80	0.441	-1.53	0.157
Winter X Slope aspect	0.02	0.985	-0.10	0.926	0.28	0.783	0.31	0.763	0.01	0.991	-0.15	0.886

[1] Significant impact of independent variables determined using general linear model and interactions of variables determined using general linear mixed models both with Quasipoisson (log) distribution with significant p-value < 0.05, indicated in bold

Table A11: Impact of mean daily temperatures and mean daily temperature fluctuations independently and interactively on fungal diversity indices[1]

	Observed		Chao1		Shannon		Simpson		Inverse Simpson		Pilou's evenness	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Mean daily temperatures between 18 May and 24 September (ºC)												
Daily temperatures	2.29	**0.048**	-0.56	0.588	0.64	0.539	-0.15	0.888	0.04	0.971	-1.00	0.345
Daily temperatures X Slope aspect	0.50	0.631	2.00	0.086	1.01	0.345	0.66	0.531	0.68	0.520	0.52	0.618
Mean temperature fluctuations (ºC)												
Between 18 May to 24 September 2018	2.20	0.055	-0.68	0.512	0.55	0.593	-0.21	0.842	-0.02	0.986	-1.05	0.322
Between 18 May to 24 September 2018 X Slope aspect	-1.30	0.234	-3.55	**0.009**	-1.85	0.107	-1.23	0.258	-1.32	0.230	-0.81	0.443
Mean seasonal temperature fluctuations (ºC)												
Late autumn	2.23	0.053	-0.66	0.529	0.57	0.582	-0.19	0.854	0.00	0.999	-1.04	0.327
Late autumn X Slope aspect	-1.29	0.238	-3.54	**0.009**	-1.84	0.108	-1.23	0.260	-1.31	0.232	-0.81	0.443
Early spring	2.13	0.062	-0.71	0.494	0.53	0.610	-0.23	0.827	-0.05	0.961	-1.06	0.319
Early spring X Slope aspect	-1.35	0.219	-3.57	**0.009**	-1.87	0.104	-1.24	0.255	-1.34	0.223	-0.81	0.446
Winter	2.21	0.055	-0.68	0.515	0.56	0.592	-0.20	0.845	-0.01	0.989	-1.05	0.323
Winter X Slope aspect	-1.30	0.234	-3.55	**0.009**	-1.85	0.107	-1.23	0.258	-1.32	0.229	-0.81	0.443

[1] Significant impact of independent variables determined using general linear model and interactions of variables determined using general linear mixed models both with Gaussian distributions with significant p-value < 0.05, indicated in bold

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SUMMARY

The Lesotho highlands provide a unique environment for the exploration of relationships between environmental factors and soil microorganisms due to the exceptional diverse mountain topography creating a range of microclimates and vegetation types over short spatial scales. This study has thus provided the first insights into the soil microbiome on a summit in the Lesotho highlands. Microbial diversity was studied at three distinct localities across the north- and south-facing slope and plateau top of a mountain summit. Meteorological data collected from both the north- and south-facing slopes highlighted the distinct climatic conditions across a short geographic distance. Hence, this study serves as a good model for studying the effect of climate change on microbial diversity.

Despite the harsh environmental conditions observed across the plateau, north- and south-facing slopes of the summit, the soil was found to harbour highly diverse bacterial and fungal communities. Most of the bacterial and fungal taxa found on the Lesotho highlands summit are essential for ecosystem processes. Bacterial diversity (p-value = 0.007) and community structures (p-value < 0.001 and R^2 = 24%) were found to be strongly influenced by the slope aspect, together with fungal community structure (p-value < 0.001 and R^2 = 17%). Analysis of temperature data further showed that the distinct temperature fluctuations, which included the maximum and minimum daily temperatures observed between 18 May and 24 September 2020 on the north-facing slope (48.37ºC and 0.22ºC) and the south-facing slope (3.62ºC and 0.002ºC), also affected bacterial (p-value = 0.016 and 0.014) and fungal (p-value = 0.002 and 0.001) community structures significantly. These results thus highlight the response of soil microorganisms on the summit to changes in environmental and climatic conditions. Furthermore, they could assist in evaluating possible changes in climatic conditions as a result of climate change and the response of soil microorganisms to such variations. Lastly, such responses could then be used to predict the overall response of soil microorganisms to climate change in extreme environments such as the Lesotho highlands, given changes in their environmental conditions.

Further research is, however, required to determine whether there may be other potential drivers on the summit, such as soil pH, nutrient and substrate availability, moisture content, vegetation type, and solar radiation exposure across the slopes that could have influenced the response of bacterial and fungal communities in this region. These data could then be linked with the microclimatic variations across the north- and south-facing slope due to the rock scarps

present on these slopes. As such, the slope position (distance from the rock scarp) and a larger elevational gradient could also potentially have a substantial impact on bacterial and fungal diversities and community structures across these slopes.