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Space, Time and Change: Investigations of Soil Bacterial Diversity and its Drivers in the Mongolian Steppe

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Abstract
Microorganisms are the most diverse life forms on Earth and are the foundation of any ecosystem. As estimates of microbial diversity rapidly increase with advances in sequencing technologies, so does the need to identify the drivers of such overwhelming diversity. This is particularly true in soil—the most biodiverse habitat on the planet and the key component of terrestrial ecosystems, which are being altered by changes in climate and land use. In order to understand the potential consequences of these changes, we conducted a multi-year experiment to test the effects of global change on soil bacterial communities in northern Mongolia, a region where air temperatures have increased by 1.7 °C since 1960, and traditional land-use patterns are shifting with socio-economic changes. Set in the semi-arid steppe, our global change experiment allowed as to evaluate responses to multiple stressors at once over a range of spatial and temporal scales. Over the course of three years, we investigated soil bacterial diversity at two positions (upper and lower) along a south-facing slope and documented the response of these communities to three experimental treatments: a Watering experiment (upper slope only), a Grazing experiment (lower slope only) and a Climate Manipulation experiment (both slopes). We measured diversity using both the number and abundance of distinct bacterial taxa in a soil sample and then correlated these findings with corresponding measurements of biotic and abiotic factors, which included plant richness and biomass, as well as plant available N, pH, soil moisture and soil temperature. We found that temporal and spatial factors explained much of the variation in the bacterial communities. After accounting for temporal and spatial variation, soil moisture content was the primary driver structuring bacterial diversity across the landscape and within experimental treatments. In particular, the effects of climate change on these semi-arid grasslands may act primarily through soil moisture content. Concomitant shifts in key members of the bacterial community may ultimately be bioindicators of a drier future for Mongolia.

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SPACE, TIME AND CHANGE: INVESTIGATIONS OF SOIL BACTERIAL DIVERSITY AND ITS DRIVERS IN THE MONGOLIAN STEPPE

For my family. Every word.

"We find that in science, it is always essential to keep a clear mental distinction between reality and the model that one develops to describe reality .... teaching and learning ... are made easier by making this distinction between the model, in which mathematical relationships are simple, in which tangents and points slide around with the greatest of ease; and reality, infinitely more complex ...."

Anderson and Crerar, *Thermodynamics of Geochemistry: The Equilibrium Model,*
Oxford University Press, 1993

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Finally, I thank Vinayak for being my other half.

ABSTRACT

SPACE, TIME AND CHANGE:

INVESTIGATIONS OF SOIL BACTERIAL DIVERSITY AND ITS DRIVERS IN THE MONGOLIAN STEPPE

Aurora A. MacRae-Crerar

Brenda B. Casper

Microorganisms are the most diverse life forms on Earth and are the foundation of any ecosystem. As estimates of microbial diversity rapidly increase with advances in sequencing technologies, so does the need to identify the drivers of such overwhelming diversity. This is particularly true in soil—the most biodiverse habitat on the planet and the key component of terrestrial ecosystems, which are being altered by changes in climate and land use. In order to understand the potential consequences of these changes, we conducted a multi-year experiment to test the effects of global change on soil bacterial communities in northern Mongolia, a region where air temperatures have increased by 1.7 °C since 1960, and traditional land-use patterns are shifting with socio-economic changes. Set in the semi-arid steppe, our global change experiment allowed as to evaluate responses to multiple stressors at once over a range of spatial and temporal scales. Over

the course of three years, we investigated soil bacterial diversity at two positions (upper and lower) along a south-facing slope and documented the response of these communities to three experimental treatments: a Watering experiment (upper slope only), a Grazing experiment (lower slope only) and a Climate Manipulation experiment (both slopes). We measured diversity using both the number and abundance of distinct bacterial taxa in a soil sample and then correlated these findings with corresponding measurements of biotic and abiotic factors, which included plant richness and biomass, as well as plant available N, pH, soil moisture and soil temperature. We found that temporal and spatial factors explained much of the variation in the bacterial communities. After accounting for temporal and spatial variation, soil moisture content was the primary driver structuring bacterial diversity across the landscape and within experimental treatments. In particular, the effects of climate change on these semi-arid grasslands may act primarily through soil moisture content. Concomitant shifts in key members of the bacterial community may ultimately be bioindicators of a drier future for Mongolia.

TABLE OF CONTENTS

ACKNOWLEDGMENTS ..... **IV**

ABSTRACT ..... **V**

LIST OF TABLES ..... **IX**

LIST OF ILLUSTRATIONS ..... **X**

INTRODUCTION **1**

Theoretical Background **4**

Global Change **6**

Mongolia **7**

Dissertation chapters **8**

CHAPTER ONE: ECOLOGICAL DETERMINANTS AND IMPLICATIONS OF SOIL BACTERIAL COMMUNITY STRUCTURE IN A MONGOLIAN CLIMATE AND LAND USE CHANGE EXPERIMENT **11**

1.1 ABSTRACT **11**

1.2 Introduction **13**

1.3 Methods **16**

1.4 Results **22**

1.5 Discussion **26**

CHAPTER TWO : HIGH AND DRY: SOIL BACTERIAL BIOINDICATORS OF CLIMATE CHANGE FORESHADOW A DRIER FUTURE FOR THE MONGOLIAN SEMI-ARID STEPPE **40**

2.1 ABSTRACT **40**

2.2 Introduction **41**

2.3 Methods **44**

2.4 Results **53**

2.5 Discussion **55**

CHAPTER THREE: ONE CORE OR MORE? INSIGHTS ON CHOOSING AN OPTIMAL SAMPLING STRATEGY FOR STUDIES IN SOIL MICROBIAL ECOLOGY **65**

3.1 ABSTRACT **65**

3.2 Introduction **66**

3.3 Methods **68**

3.4 Results **75**

3.5 Discussion **76**

DISCUSSION **85**

CONCLUSION **89**

APPENDIX **93**

BIBLIOGRAPHY **120**

LIST OF TABLES

Table 1.1: Summarized effects of climate manipulation, grazing, year and slope location on soil bacterial communities.....36

Table 3.1: Analysis of variance using Bray-Curtis dissimilarities to measure differences in bacterial community composition.....81

LIST OF ILLUSTRATIONS

Figure I.1: CAP Map of Mongolia.....10

Figure 1.1: CAP ordination of the soil bacterial communities.....37

Figure 1.2: The CAP ordination based on Bray-Curtis dissimilarities.....38

Figure 1.3: CAP ordination of soil bacterial communities based on phylogenetic (UniFrac) relatedness.....39

Figure 2.1: Structural equation models, testing direct and indirect effects of experimental treatments on environmental conditions and plant and bacterial communities over time.....62

Figure 2.2: Box-and-whisker plots of indicator taxa analysis.....64

Figure 3.1: Rarefaction curves examining cumulative number of observed OTUs.....82

Figure 3.2: Rarefaction and phyla accumulation curves for soil samples from control plots on the Upper and Lower Slopes.....83

Figure 3.3: Plots comparing the bacterial community composition between two soil Sample Types (three individual cores v. mixed core).....84

INTRODUCTION

The search for truth is in one way hard and in another way easy, for it is evident that no one can master it fully or miss it wholly. But each adds a little to our knowledge of nature, and from all the facts assembled there arises a certain grandeur.

Aristotle, whose name is carved in stone over the walkway when you enter Leidy Laboratory

***

[M]icrobial ecology… is the most necessary and fruitful direction to guide us in organizing our knowledge of that part of nature which deals with the lowest limits of the organic world, and which constantly keeps before our minds the profound problem of the origin of life itself.

Martinus Willem Beijerinck, 1905

(Translated by van Niel, 1949; republished by Woese, 2006).

*

There are many, arguably infinite, avenues to the exploration of diversity, the cornerstone of our ecosystems and economies. Each gives a different perspective and brings us closer to the truth. Scientific studies have explored diversity from many different perspectives (Magurran 1988). Our ecological understanding of diversity has evolved over time. Almost twenty years before Darwin published *On the Origin of the Species* (1859), he sketched the first conceptual tree of life. About 30 years later, Haekel introduced the first phylogenetic tree of life, which was based on shared morphologies and was rooted in a common origin (Dayrat 2003). More than a century later, Whittaker expanded upon this tree and classified life into five kingdoms and two domains—the prokaryotes and the eukaryotes (Hagen 2012). Soon after, Woese shattered the conventional wisdom of the bipartite divide and published the first "universal tree of life"

using molecular techniques (Woese et al. 1990). By investigating a region of the highly conserved ribosomal RNA (rRNA) sequence present in all living organisms, Woese constructed a molecular phylogeny that divided life on earth into three domains—*Bacteria, Archaea* and *Eucarya*. To this day, the universal phylogenetic tree of all known life is rooted in this work (Pace 1997; Madigan 2005) and is the primary reference for studies of biological diversity, or at least it was until very recently. In April of 2016, researchers published a dramatically expanded tree of life. Across the three domains, this tree revealed the profound dominance of bacterial diversification, even more than previously estimated, and the substantial portion of diversity lacking isolated representatives (Hug et al. 2016). This tree illustrates our ever-expanding understanding of diversity and its depths.

Though scientists have been studying microbes for more than a century, it is only recently that microbial ecologists have had the knowledge and tools necessary explore their tremendous diversity. This is because the majority of known microorganisms have eluded traditional culture methods on agar plates, a difficulty called the "Great Plate Count Anomaly" (Staley and Konopka 1985). Indeed, more than 70% of bacterial phyla characterized by molecular techniques have no cultured representatives (Achtman and Wagner 2008); this percentage has increased because culture techniques lag behind high-throughput sequencing innovations (Hug et al. 2016). Since the groundbreaking findings of Woese et al. (1990), on which the most recent and comprehensive tree of life is based (Hug et al. 2016)**,** the characterization of microbial communities by their rRNA fingerprints has become the most widely used method in studies of microbial diversity

(Olsen et al., 1986; Pace, 1997). Using this method, thousands of microorganisms have been catalogued, even if they have not been cultured, in a wide array of databases, e.g. The Ribosomal Database Project (Wang et al., 2007) and Greengenes (DeSantis et al. 2006).

In light of ever-increasing estimates, the study of microbial diversity has been called a Sisyphaean task (Fierer and Lennon 2011b). Do the ever-expanding catalogs of microbial sequences truly increase our understanding of microbial diversity? If we do not understand what the microbes associated with these sequences are doing, then are these sequence surveys advancing our understanding of microbial ecology? Or, perhaps more important, are these studies enhancing the state of science? Woese (2006) responds to these questions in the third edition of *The Prokaryotes*:

> Microbial ecology is no longer the *faux* ecology it had been—when defining a niche in organismal terms was not an option. Today the field rests on a par with plant and animal ecology and exceeds them in importance, for it is in the microbial realm that the base and fount of the global ecosystem lie. Studying microbial diversity used to be the equivalent of hunting through antique shops for curios—which resulted in a collection of species no more connected to one another than the items in a bower bird's nest. Now all organisms sit on the well-ordered tips of branches on the universal phylogenic tree (Woese 1987; Maidak et al. 1994), and the study of one, far from being an isolated adventure, can contribute to the study of all.

Though the metabolic potential and function of a microorganism cannot be fully understood until it is cultured, sequencing techniques have provided profound insights

into the breadth of the microbial world and the overall organization of life on Earth (Gilbert et al. 2011). Such insights are crucial for constructing a foundation upon which overarching hypotheses can be built. A solid knowledge of microbial diversity is essential for understanding the vital role these organisms, invisible to the naked eye, play in our ecosystems.

Theoretical background

Discerning temporal or spatial patterns in biodiversity is at the core of ecology (Legendre and Legendre 1998). Microbes make up most of the biodiversity on the planet (Rossello-Mora and Amann 2001) yet most of our practical and theoretical knowledge of biodiversity comes from studies of plants and animals (Martiny et al. 2006). As a result, ecological theory is underdeveloped in the field of microbial ecology (Prosser et al. 2007) as compared to "macro-bial" ecology**.** The ecological causes and consequences of macro-organismal biodiversity have been investigated for more than half a century (Hooper et al. 2005). How, and the extent to which, biodiversity affects ecosystem function remains a hotly contested question in the field (Loreau et al. 2001; Naeem 2002). For example, as the primary producers in most terrestrial ecosystems, plants have been the main subjects of field experiments investigating the importance of biodiversity (Loreau et al. 2001; Tilman et al. 2006). Overall, these studies conclude that increased diversity—number of different plant species—results in increased ecosystem stability—decreased variation in plant biomass (Zavaleta et al. 2010). Though these conclusions are still debated, they have advanced the state of ecological thought over the past century. In order for the field

of microbial ecology to provide similar theoretical insights, comprehensive data on how microbial diversity is correlated with environmental factors and ecosystem processes is needed.

With advances in technology, scientists are beginning to confront the theoretical impasse between microbial and macrobial ecology in a way that was not possible even a decade ago (Caporaso et al. 2012; Ram et al. 2011). Guided by the theories garnered from studies of macroorganisms, a framework for microbial biogeography is developing based on maps of microbial diversity at various temporal and spatial scales (Martiny et al. 2006). Such work is enabling microbial ecologists to explore fundamental hypotheses that were previously out of their reach, such as "the biogeography of microorganisms is similar to the biogeography of macroorganisms" (Martiny et al. 2006). It is investigations of hypotheses such as these that will lead to a more developed theoretical cannon for the ecology of microorganisms and ultimately a better understanding of ecosystem health. The biogeography of soil microorganisms is an especially complex and important area of research. The most biodiverse habitat on Earth is soil (Fierer and Lennon 2011a). Soil microbial diversity is vast—one gram of soil can contain over ten billion microbes (Rossello-Mora and Amann 2001; Torsvik and Øvreås 2002), while one ton of soil can contain 4×10^6 coexisting bacterial taxa (Curtis et al., 2002). The key to a healthy terrestrial ecosystem is healthy soil; in turn, the key to healthy soil lies in the diversity of organisms that live in, recycle and maintain that soil. The definition of *soil health* from the USDA (USDA 2010) is simply "the capacity of a soil to function." These functions

include supporting animal and plant life, maintaining or enhancing air and water quality and sustaining human health and habitation (USDA 2005).

These essential ecosystem services are dependent on microorganisms, which decompose organic matter and recycle nutrients needed for optimal plant and ecosystem productivity. Hence, microbes are vital for the maintenance of healthy soil and, in turn, form the foundation of the ecosystem services that all other life forms need to thrive. Because of their extraordinary diversity, very little is known about the structure (who's there) and function (what they're doing) of soil microbial communities—let alone how these communities will be affected by either climate or land-use change. This large gap in the scientific canon is both overwhelming and awe-inspiring. Though daunting, it is imperative for the health of our natural ecosystems that this knowledge gap be filled. Microorganisms are the ubiquitous engines of the planet's biogeochemical cycles (Falkowski et al. 2008). Understanding how bacterial diversity will respond to global change is key to gauging the future of our ecosystems.

Global change

Changing climate and altered land use are major components of global change (Vitousek 1994; Sala et al. 2000). Effects of climate change can be exacerbated by changes in land use (Meyer and Turner 1992; Vitousek 1994; Mantyka-Pringle et al. 2012; Oppenheimer et al. 2014), potentially increasing carbon emissions and global temperatures (Cubasch et al. 2013). Though we know that soil microbial diversity can be influenced by a wide range of abiotic and biotic factors, (Horner-Devine et al. 2003;

Schweitzer et al. 2008), there is still a large gap in our understanding of how microbes respond to environmental change (DeAngelis et al. 2015). In the face of climate change, average temperatures are projected to increase by 1.5 – 2.5ºC over this century and precipitation patterns are expected to be greatly altered (Easterling et al. 2000; IPCC 2014). As the climate changes, so does the relationship between abiotic and biotic factors, which can potentially result in drastic ecosystem consequences, such as high levels of extinction, rising sea levels and extreme weather patterns (IPCC. 2014).

Rising temperatures are projected to result in wide-spread decreases in biodiversity for a large range of organisms, potentially leading to declines in key ecosystem functions, e.g. carbon sequestration processes, nutrient cycling and disease regulation (Stearns 2009; Oppenheimer et al. 2014). Investigations of bacterial diversity and how it responds to climate change are vital to understanding our planet's future environmental trajectory (Reid 2011; Treseder et al. 2012; DeAngelis et al. 2015) and determining potential strategies to mitigate any of the harmful consequences climate change may have on Earth's ecosystems (Nie et al. 2013; Evans and Wallenstein 2014).

Mongolia

To investigate the potential ecosystem consequences of global change, I participated in an experiment on the effects of climate change and shifting land-use practices in the rangelands of northern Mongolia (Fig. I.1). Overall, rangelands constitute up to half of the world's terrestrial surface area (Lund 2007) and are of large societal and economic importance across the globe (Klein et al. 2007; Suttie 2005). Mongolian

nomadic herders are among the many global communities that depend on the steppe as rangelands for their livelihoods and may be adversely affected by climate change. Some of the greatest increases in temperatures associated with global warming are projected for northern Mongolia and the neighboring taiga forests of southern Siberia (Dagvadorj et al., 2011; Namkhaijantsan 2006). Already, the average annual temperature at this site has increased by 1.7ºC since 1963 (Namkhaijantsan 2006). Set in the semi-arid steppe, our global-change experiment included three experimental treatments: a Climate manipulation treatment, a Watering treatment and a Grazing treatment. These were monitored over multiple years and at two distinct positions across the landscape.

Dissertation Chapters

The central question of my dissertation is: What is the structure of microbial diversity and how is it influenced by climate change? Within the context of our global change experiment, I explore how soil bacterial diversity is affected by changes in abiotic and biotic factors over time and space. In Chapter 1, I characterize the temporal and spatial variability in the bacterial diversity of our experiment and investigate correlations between diversity and abiotic factors. I examine this diversity using measures of both taxa number and abundance over the course of two years and at two different locations on a south-facing slope. In Chapter 2, I investigate diversity on the same spatial scale, but a smaller temporal scale—between two months. Within this context, I examine putatively causal relationships between bacterial diversity, our experimental treatments and both abiotic and biotic environmental factors. I then identify specific bacterial taxa that may

act as bioindicators of climate change. In Chapter 3, I investigate spatial variability at several scales. I compare bacterial diversity between slope positions, between plots within an experimental treatment and between individual and mixed soil cores.

Figure I.1: Map of Mongolia. Our field-site, denoted by the yellow star, was located on the western side of Lake Hövsgöl (51°01.405'N, 100°45.600'E

CHAPTER ONE:

ECOLOGICAL DETERMINANTS AND IMPLICATIONS OF SOIL BACTERIAL COMMUNITY STRUCTURE IN A MONGOLIAN CLIMATE AND LAND-USE CHANGE EXPERIMENT

1.1 Abstract

Changing global climate and land-use practices are altering vital ecosystem functions. Despite the reliance of important ecosystem processes on soil microbes, much remains unknown about how microbial structure will be affected by changes in temperature and changes to livestock grazing. We undertook a multi-year experiment to test the effects of anthropogenic disturbances on soil bacterial communities in northern Mongolia, a region where air temperatures have increased by 1.7°C since 1960, and traditional land-use patterns are shifting with socio-economic changes. We examined how soil bacterial communities vary within the landscape between years and with climate and land-use change. Experimental treatments included manipulation of grazing and warming in plots that were deployed at two locations on a topographical gradient in 2010 and 2011. Plant-available nitrogen, soil temperature and moisture were measured for each plot. Bacterial community composition was determined by Illumina sequencing barcoded 16S rDNA amplicon reads. Year and moisture were robust factors structuring the bacterial community as revealed by both taxonomic (Bray-Curtis) and phylogenetic (UniFrac) analyses, while the importance of slope location, climate manipulation, temperature and nitrogen varied between analyses. In the taxonomic analysis,

Verrucomicrobia, Planctomycetes and Firmicutes correlated most closely with temperature, moisture and nitrogen, respectively, suggesting distinct ecological attributes of these broad phylogenetic groups. The phylogenetic analyses found some evidence of the importance of nitrate and moisture at the community level. Overall, this work provides insights into the temporal, spatial and environmental factors that influence soil bacterial diversity and community composition within the context of global change.

1.2 Introduction

Soil microbes are the engines of global biogeochemical cycles and are, therefore, vital for life on Earth (Falkowski et al. 2008). Soils are recognized as providing some of the most diverse habitats on the planet and supporting incredibly abundant and diverse microbial metabolisms (Roesch et al. 2007; Fierer and Lennon 2011). Yet, very little is known about the ecological factors that shape microbial diversity in soils and how this diversity responds to agents of global change. Exploring how soil microbial communities vary in composition is essential for further understanding the foundations of Earth's ecosystems (Xu 2006; Heimann and Reichstein 2008; McGuire and Treseder 2010). Such knowledge is imperative for mitigating consequences of global change, such as biodiversity loss, erosion of ecosystem services and degradation of soil organic matter, all of which can result in increased greenhouse gas emission to the atmosphere (Mooney et al. 2009; Midgley 2012; Oppenheimer et al. 2014).

Changing climate and altered land use are major components of global change (Vitousek 1994; Sala et al. 2000). Global average surface temperatures are predicted to increase between 1.8-4ºC over the next 100 years (IPCC 2007). Rising temperatures are projected to result in wide-spread decreases in biodiversity for a large range of organisms, potentially leading to declines in key ecosystem functions, e.g. carbon sequestration processes, wetland water purification, nutrient cycling and disease regulation (Chivian and Bernstein 2008; Oppenheimer et al. 2014). Effects of climate change can be exacerbated by changes in land use (Meyer and Turner 1992; Vitousek 1994; Mantyka-Pringle et al. 2012; Oppenheimer et al. 2014), potentially increasing carbon emissions

and global temperatures (Cubasch et al. 2013). Climate and land-use changes can also affect the structure and ultimately the function of the microbial community (Singh et al. 2010), though the link between structure and function is not well resolved (Nannipieri et al. 2003; Fuhrman 2009; Philippot et al. 2010).

When studying factors that structure soil microbial communities, it is important to consider potential responses to simultaneous stressors, while concurrently considering existing community variation over time scales, such as between years, and across the landscape (Martiny et al. 2006). Studies examining how the same system will respond to the simultaneous global change stressors of climate and land use (Klein et al. 2004, 2007; Luo et al. 2010; Spence et al. 2014) are limited, and especially so for belowground systems (Strebel et al. 2010).

There are experiments showing that warming (Zogg et al. 1997; Luo et al. 2014; Xiong et al. 2014) and grazing (Klumpp et al. 2009; Zhou et al. 2012) affect microbial community composition and diversity, but few incorporate variation over both temporal or spatial scales. Soil bacterial communities can exhibit extreme levels of variability across even small distances (Torsvik and Øvreås 2002), geochemical gradients (Nemergut et al. 2008; Philippot et al. 2010) and periods of time (Shade et al. 2013); hence, in order to have a better understanding of how global change may affect these communities, spatial and temporal variation must be taken into account.

With this in mind, we undertook an experiment in the montane steppe of Mongolia to understand the effects of global change on the composition of soil bacterial communities. Mongolia is experiencing acute alterations in both climate and land use.

Large increases in temperatures associated with global warming are projected to occur in northern Mongolia and the surrounding area of Siberia (Namkhaijantsan 2006; Dagvadorj et al. 2011). Already, the average annual temperature in this region has increased by 1.7ºC since 1963 (Namkhaijantsan 2006). In addition, land-use patterns are changing. Pastoralism has been widely practiced on the Mongolian steppe since the early 13th century (Fernandez-Gimenez 2006). Now urbanization is leading to a transition from traditional nomadic pastoralism toward more sedentary lifestyles, resulting in the cessation of expansive grazing practices that have characterized the Mongolian steppe for more than a millennium (Bradley 2012).

In this study, we focus on the structure of the soil bacterial communities with the intent to explore the mechanisms underlying their compositional make-up. Such investigations help unravel questions of taxon distribution and coexistence as well as provide a foundation to guide explicit hypotheses for more fruitful investigations of community function in the future. Specifically, we investigated the effects of topography, climate manipulation and grazing cessation on the soil bacterial community across two years and at two distinct topographical locations. Climate was manipulated using passive warming chambers, which caused both warming and drying of the soil. These were deployed at the top and bottom of a south-facing slope. At the bottom of the slope, we also examined microbial community responses to cessation of grazing. Correlated responses in community structure with soil temperature, moisture and plant- available nitrogen were also examined. This study aims to (1) shed light on the factors underlying the considerable variation seen among soil bacterial communities within the context of climate change and (2) unearth potential functional attributes of community members at

higher taxonomic levels. Since moisture content can be strongly influenced by temperature and in turn can have a strong influence on nutrient mineralization (Castro et al. 2010; Cregger et al. 2012; Manzoni and Schimel 2012), we hypothesized that bacterial diversity and composition would change the most with water stress, which would increase with rising temperatures. We predicted that bacterial richness would be lower on the warmer and drier upper slope in comparison to the wetter and cooler lower slope and that the community composition would differ between the two slope locations. Similarly, we expected there to be significant differences in the community between the warmer and drier climate manipulation treatment in comparison to the control. We predicted that grazing on the lower slope would accentuate the effect of climate manipulation, as grazed plots have less litter, higher temperatures and lower moisture content. Lastly, we hypothesized that any notable changes in phyla abundance would occur along these water stress gradients, which would suggest broad functional attributes for each phylum.

1.3 Materials and methods

Site description and experimental design

The study site is located in montane steppe on a south-facing slope in the Dalbay River Valley, on the eastern shore of Lake Hövsgöl (51° 01.405' N, 100° 45.600' E). Elevation ranges from 1660 to 1800 m, with a gentle to flat incline at the bottom of the slope and becoming much steeper (20^{o} incline) at the top. The average annual air temperature in this region is -4.5°C, with the coldest average monthly (January) temperature of -21°C and warmest (July) of 12°C (Nandintsetseg et al. 2007). Permafrost is not present on the south-facing slope, but is found in a nearby riparian zone and on

north-facing slopes under taiga forest (Sharkhuu et al. 2007). The soil is sandy loam texture of alluvial origin, classified as a non-carbonated Dark Kastanozem (Aridic Boroll or Typic Ustolls). Bedrock consists of Cenozoic volcanic deposit (Batkhishig 2006)**.** The steppe vegetation is a mixture of sedges (e.g., *Carex pediformis*, *Carex dichroa*), grasses (e.g., *Festuca lenensis*, *Koeleria macrantha*, *Agropyron cristatum*, *Helictotrichon schellianum*, *Stipa krylovii*) and short forbs (e.g., *Aster alpinus*, *Potentilla* spp., *Artemisia commutata*). Forbs and graminoids dominate the upper and lower slopes respectively. At present, the valley is grazed by yaks, horses, and a mixed herd of sheep and goats, mainly in late summer and autumn.

We established experimental plots in 9 × 9 m blocks at two elevations, roughly 1660 and 1750 m.a.s.l., with seven blocks on the upper slope and eight on the lower. Blocks on each slope were spaced 30 m apart and fenced throughout the year to prevent grazing. Each block contained a climate manipulation plot and a control plot. We manipulated climate using passive warming open top chambers (OTCs), installed from the first of June until mid-August each year (see Liancourt et al. 2012). Constructed of Sun-Lite® HP fiberglass, the hexagonal OTCs were 40 cm tall, 1 m wide at the top and 1.5 m wide at the bottom (Marion et al. 1997). Controls consisted of a hexagonal area of the same footprint. OTCs affected both air temperature and soil moisture; hence, it is not just a warming treatment, but instead a climate manipulation treatment. In comparison to controls, OTCs increased mean daytime temperatures by 1.5ºC and decreased volumetric soil moisture content by 1.6 to 4.1 percent (Liancourt et al. 2012a).

On the lower slope, blocks also included a grazing treatment crossed with the climate treatment. Grazing by yaks, horses, sheep and goats was allowed in a 5 × 9 m

area contiguous with the fenced 9 × 9 m block. Each 5 × 9 m area contained its own OTC and control plots. This area was fenced during the growing season (June – August) to protect experimental plots from stray yaks and horses; hence, grazing occurred after the removal of the fences and OTCs in mid-August and before OTCs were re-installed in June. See Spence et al. (2014**)** for more detailed information about grazing at this site.

Collection, extraction and sequencing of soil samples

We collected soil cores to analyze the bacterial community from seven replicate blocks (three upper, four lower) on July 9, 2010, and all 15 replicate blocks (seven upper, eight lower) on June 7 and July 29 – 30, 2011. Each soil sample consisted of three homogenized soil cores (2 cm diameter, 5 cm deep) taken along a one-meter transect within each OTC and control plot. There were 68 samples in total, but one anomalous sample, which had a relatively low number of 16S rDNA sequences, was dropped, resulting in 67 samples being used in our analyses (Table A1). We transported samples in a cooler at ~ 4ºC days during transit from Mongolia to the United States before being stored at -80ºC. The brief transit times (≤ seven days) should have prevented significant changes in the phylogenetic structure and diversity of the soil microbial community (Lauber et al. 2010). As a further precaution, we stored all samples in bacteriostatic LifeGuard Solution (MoBio, Carlsbad, CA, U.S.A.), which inhibits further microbial activity and prevents DNA or RNA degradation.

We measured total plant-available nitrogen and soil moisture and temperature in all of our sampled plots each year. We used plant-root simulator (PRS™) probes, consisting of ion-exchange membranes, to measure plant- available NO_3^- and NH_4^+

(Western Ag Innovations, Saskatoon, SK, Canada; http://www.westernag.ca/innov/prs-probes). We employed two cation probes and two anion probes in each experimental plot and left them in place for 21 days during the middle of the growing season, from July 1-July 22. We brushed soil from the probes in field and washed them with deionized water in the lab before analysis by Western Ag Innovations. We report nitrogen concentrations as μg per 10 cm^2 ion-exchange surface per day. We used a portable probe (WET-2 sensor, Delta-T Devices Ltd) to measure the volumetric moisture content (θ) and temperature (ºC) of the surface soil from each experimental plot over the course of the growing season, from June to August in 2010 and 2011. Measurements were taken on at least 15 days in each month. On each day and in each plot, temperature and moisture was measured three times and then averaged. These averages were used in subsequent analyses of the soil samples.

DNA extractions were done at Argonne National Laboratory in Lemont, IL, U.S.A. Soil samples were thawed soil to 4ºC and centrifuged to separate and then discard the LifeGuard preservation solution. DNA was extracted using standard EMP protocols (http://www.earthmicrobiome.org/emp-standard-protocols/). We isolated DNA from 0.20 g of soil per extraction using the manufacturer-suggested protocol for PowerSoil-htp 96 Well Soil DNA Isolation Kit (MoBio), with the modification that we heated the extraction at 65°C for ten minutes prior to the initial bead beating and cell lysis step.

We performed the 291 bp length V4 region amplification using the 515F primer and the 806R Golay-barcoded reverse primers (for a full list of these primers visit http://www.earthmicrobiome.org/emp-standard-protocols/). Each 25μl PCR reaction contained 12μl of MoBio PCR Water (Certified DNA-Free), 10μl of 5 Prime

HotMasterMix (1x), 1µl of Forward Primer (5uM concentration, 200pM final), 1µl Golay Barcode Tagged Reverse Primer (5µM concentration, 200pM final), and 1µl of template DNA. We performed PCR under the following conditions: 94°C for three minutes to denature the DNA, with 35 cycles at 94°C for 45sec, 50°C for 60sec, and 72°C for 90 sec, with a final extension of ten minutes at 72°C to ensure complete amplification. Each PCR reaction was performed in triplicate for each sample and then pooled together as one representative PCR product as a method to restrict PCR bias. The DNA concentration of each aggregate PCR product was then quantified using PicoGreen (Invitrogen) and a microplate reader. Once quantified, we performed a second pooling, where different volumes of the discrete sample pools with the same DNA concentrations were combined in a single tube. This allowed for an equal amount of 16S rDNA amplicon sequences from all samples in our study. We cleaned the final pool using the UltraClean® PCR Clean-Up Kit (MoBio). Sequencing was completed on the Illumina HiSeq2000 platform.

Bioinformatic processing of DNA sequences

We quality trimmed 16S rDNA amplicon sequences using SolexaQA, eliminating regions that fell below a Q-score of 18 (Cox et al. 2010), which corresponds roughly to 98.4% or higher accuracy for each base position. We only retained the longest continuous fragment for each sequence. We discarded trimmed reads that fell below 75% of the expected sequence length (< 112bp) from further analyses. We assigned sequences to samples by searching the corresponding 12bp barcode sequence against the PCR primer tags assigned to each sample with BLAST+ (blastn, word size=4) (Camacho et al. 2009). We allowed up to one mismatch of the 12bp barcode sequences when assigning sample

IDs. We used the open software package Quantitative Insights Into Microbial Ecology (QIIME) for further downstream analyses of this data (Caporaso et al. 2010). We clustered sequences, de-novo, into Operational Taxonomic Units (OTUs) at 97% identity using the Uclust algorithm (Edgar 2010). We discarded singletons (OTUs with only one representative sequence) from further analysis, as they commonly result from chimeric PCR products or sequencing error, and are usually non-informative in the overall analysis (Zhou et al. 2011).

Representative sequences were selected for each OTU by taking the longest available sequence and then assigned taxonomic identification using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007). Representative sequences were aligned using the Python Nearest Alignment Space Termination Tool (PyNAST) as implemented in QIIME. These aligned sequences, along with an OTU abundance matrix, were used to calculate beta diversity by means of weighted UniFrac distances with the QIIME script beta_diversity.py. By coupling the OTU abundance matrix with taxonomic classification of each OTU, a taxonomic summary was generated for each general taxonomic level (i.e. phylum, class, order, family, genus).

Statistical Analysis

We used constrained analysis of proximities (CAP) to determine if bacterial community composition varied with year, slope, or experimental treatment (Anderson and Willis 2003). We also examined how microbial communities, as visualized on the first two CAP axes, covaried with abundances of bacterial phyla, plant-available NO_3^- and NH_4^+, soil temperature and soil moisture.

We conducted two analyses, each using a different measure of proximities. The first analysis employed Bray-Curtis dissimilarity measures after data were square-root transformed and Wisconsin standardized (Jones 2013). The second set of analyses used weighted UniFrac distances. Unlike Bray-Curtis, UniFrac quantifies phylogenetic relatedness by weighting each taxon by its branch length on a phylogenetic tree (Lozupone et al. 2006). We performed all analyses using the vegan package in R (Oksanen et al. 2016). CAP was done using the capscale function and patterns of co-variation were examined using the envfit function. Significance tests were done using permutation tests. All figures were constructed using the R vegan package (Oksanen et al. 2016) and Microsoft® PowerPoint software.

We constructed rarefaction curves to estimate OTU richness and determine the cumulative number of genera detected as a function of sequencing effort in a given treatment combination (Fig. A1). First, we compared the number of genera on the lower and upper slopes within 2010 and 2011. Second, we compared the number of genera in OTC and control plots within 2010 and 2011. These two treatment combinations were chosen because the factors from each were significant in the taxonomic (Bray-Curtis) and phylogenetic (UniFrac) analyses of community composition, respectively.

1.4 Results

Rarefaction curves as a function of year and slope reveal that genera richness approaches saturation at the sequencing depth used in all cases except for the 2010 samples from the upper slope (Fig. A1a). The curves show that more genera were present in 2011 than in 2010. Within a year, more genera were observed on the lower slope than

the upper. Similarly, for rarefaction curves grouped by OTC treatment and year (Fig. A1b), more genera were observed in 2011 than in 2010, and control samples consistently show a larger number of genera than samples from OTC plots, though the separation between treatments is small (Fig. A1b).

As explained more fully below, both Bray-Curtis and UniFrac analyses indicated that year consistently influences the soil bacterial community structure, while grazing appears to have no significant influence (Table 1.1). The two analyses produced different results for effects of slope location and climate manipulation. There were no significant interactions in either analysis with the exception of an interaction between year and slope in the Bray-Curtis analysis (Table A2a).

Constrained analysis of proximities based on Bray-Curtis distances

While both slope and year significantly affect community composition ($P<0.01$ for both, Tables 1.1 and A2a), slope appears to have the greater influence of these two factors (Fig. 1.1). There were a total of 11 CAP axes. The first CAP axis, which is strongly associated with slope, explains 40.1% of the constrained variation (Fig. 1.1, Table A3). The second axis, which co-varies with year, explains an additional 23.4% of the variation (Table A3). The effect of slope also depends on the year, as there is a significant year × slope interaction ($P<0.03$, Table A2a); the difference in community structure between slopes is greater in 2010 than 2011 (Fig. 1.1). In contrast, neither the climate nor the grazing treatment, as either main effects or interactions, significantly affected the soil bacterial community (Table 1.1, Fig. A3).

All factors showed significant co-variation with community structure. Total plant-available nitrogen, which is a combination of available NH_4^+ and NO_3^-, co-varied with the first two axes of the CAP ordinations of bacterial community composition (Table A4a; Fig. A2). Significant gradients in total plant-available nitrogen ($p < 0.006$, Fig. A2a), NH_4^+ ($p < 0.001$, Fig. A2b), and NO_3^- ($p < 0.001$, Fig. A2c) were observed, as illustrated by contour lines and a dash-arrow vector representing the mean direction of those lines. Total plant-available nitrogen increased from 2010 to 2011 and from the lower to upper slope ($p<0.006$, Table A4a, Fig 1.2a). Soil temperature increased the most with changes in community structure from the lower to upper slope, but also slightly from 2010 to 2011 ($p<0.001$, Table A4a, Fig. 1.2b). The soil moisture also increased with changes in community structure from 2010 to 2011 and, in contrast to total plant-available nitrogen and temperature, from the upper to the lower slope ($p<0.001$, Table A4a, Fig. 1.2c).

Three phyla co-varied significantly with the CAP ordination (Table A5). In order of significance, these phyla are Verrucomicrobia ($p <0.001$), Planctomycetes ($p <0.003$), Firmicutes ($p <0.005$). Phyla abundance varied between year and slope location (Fig. 1.2). Verrucomicrobia, was more abundant on the upper slope than the lower slope. Planctomycetes was more abundant in 2011 than 2010. Firmicutes was more abundant in 2010 and on the lower slope.

The vectors for these phyla represent abundance gradients and co-vary with total plant-available nitrogen, soil temperature and soil moisture (Fig. 1.2)**,** indicating that these three environmental variables could potentially account for some of the temporal and spatial variation in bacterial community composition. Firmicutes abundance shows a strong negative correlation with increasing levels of plant-available nitrogen, a smaller

negative correlation with temperature and no correlation with moisture (Fig. 1.2a-c). Verrucomicrobia shows a strong positive correlation with temperature, and weaker yet notable correlations with both total plant-available nitrogen and moisture (Fig. 1.2a-c). Finally, Planctomycetes shows no correlation with temperature and slight correlations with moisture and total plant-available nitrogen (Fig. 1.2a-c).

Constrained analysis of proximities based on weighted UniFrac distances

Like the analysis based on Bray-Curtis distances, this analysis showed a significant effect of year ($p<0.01$), but no significant effect of grazing (Tables 1.1 and A2b). Out of a total of seven axes, the first two axes separate the two years and together account for 80.66% of the constrained variation (Table A3b). Unlike the Bray-Curtis analysis, however, UniFrac CAP analysis identified a significant difference between the OTC treatment and control plots ($p<0.01$**)** but not between the upper and lower slope locations (Tables 1.1 and A2b).

The co-variation of plant-available NO_3^- on the CAP ordination of bacterial community composition showed a significant gradient ($p<0.02$) and greater concentrations of NO_3^- in 2011 than in 2010 (Table A4b, Fig. 1.3a). The soil moisture gradient ($p<0.001$) but not soil temperature was also significant and like NO_3^-, moisture increased from 2010 to 2011 (Table A4b, Fig. 1.3b). Overall, the samples from the OTC plots were associated with a greater range of NO_3^- and soil moisture concentrations in comparison to the control plots (Fig. 1.3c, 3d). Unlike analysis based on Bray-Curtis distances, no significant gradients in phyla abundance were found.

1.5 Discussion

Our results clearly show that the composition of soil bacterial communities in the Mongolian steppe is highly dynamic over time. Overall, community composition varies more between years than with slope location or with the experimental manipulations of grazing or climate. The strong effect of year holds for both the taxonomic (Bray-Curtis, BC) and phylogenetic (UniFrac, UF) analyses. The former analysis also shows a large and significant effect of slope location while the latter shows a significant effect of the climate manipulation. These findings highlight the differences in the two analyses (Anderson et al. 2011; Lozupone et al. 2011; Parks and Beiko 2012). Taxonomic measures are strictly structural, i.e. OTU number and abundance. This measure (e.g., BC) is used to get a count of what OTUs have been replaced, irrespective of a difference in implied function. Phylogenetic measures can imply broad functions in that they take evolutionary relatedness into account (Philippot et al. 2010). Hence, the phylogenetic differences we observed between years and with the climate treatment are likely indicative of functional differences between the communities as well. Though by no means conclusive, phylogenetic analysis allows for fruitful speculation of what a bacterial community may be doing. Additionally, the taxonomic analysis identified bacterial taxa whose abundance varied with total plant-available nitrogen, temperature and moisture, while the phylogenetic analysis did not. Thus, our combining both taxonomic and phylogenetic approaches contribute to a more complete understanding of factors structuring these bacterial communities.

Our findings provide a strong case for the need to include temporal sampling in studies of bacterial diversity. For example, while we hypothesized that there would be

significant effects of our climate and grazing manipulations on the bacterial community composition, temporal variation strongly overshadowed these effects. Much of the temporal effects seem to be linked to changes in available N, soil temperature and moisture values, which were higher in 2011 than 2010. We speculate that these combined environmental trends may account for the higher levels of genera richness seen in 2011 (Fig. A1). In particular, moisture may be especially important in structuring the bacterial community in our system at different scale, as higher richness is associated with higher moisture concentrations. The positive association between taxa richness and moisture holds whether comparing between years, slope locations or the OTC treatment and its control (Fig. A1). In contrast to the analysis based on taxonomic differentiation, the analysis based on phylogenetic differences in the community composition suggests that functional differences between years are important. A possible explanation for this observation could be that the community in 2010 was composed of bacteria that were more resilient to drought, while a different community of bacteria were able to thrive in a wetter environment (Schimel et al. 2007). In line with our study, a meta-analysis spanning a larger range of habitats has also shown that variation of environmental conditions across time significantly affects both the taxonomic and phylogenetic composition of bacterial communities (Shade et al. 2013). This study, like ours, underscores the importance of considering temporal variation in any study of microbial ecology.

Our finding that phylogeny does not play a role in how small-scale spatial variation structures soil microbial communities (Table 1.1) suggests that function, not phylogeny, is important over small spatial scales. Our topography gradient (Casper et al.

2012) is characteristic of many others, in that the lower slope is higher in soil moisture, organic matter and primary plant productivity (Hook and Burke 2000; Xu and Wan 2008; Zhang et al. 2013). As predicted, we found that bacterial richness was higher on the lower slope than the upper slope. This could be because the water-stressed environment of the upper slope is not conducive to a large range of organisms, especially those with low stress tolerances. Other studies with similar topography gradients have shown differences in the bacterial community (Corre et al. 2002; Liu et al. 2007; Tsai et al. 2007; Swallow et al. 2009; Zhang et al. 2013), though none distinguish between taxonomic and phylogenetic composition and therefore miss out on a more refined understanding of diversity. For example, we did see the predicted difference in community composition between slope locations, but only in the taxonomic sense. This highlights the distinct facets of diversity, and suggests that there can be large OTU turnover along a spatial gradient without a significant change in the functions performed by the bacterial community.

The phylogenetic differences in community composition we found in the climate manipulation treatment suggest that the OTCs may affect the function of the bacterial community. As predicted, bacterial richness in the warmer, drier OTC plots was lower than in controls. This result is more subtle, yet similar to the trend seen between the upper and lower slope. Again, this suggests that less water-stressed environments, in this case the control, can support a greater diversity of bacteria than the drier environment inside the OTCs. Though there are similarities between the topographic and OTC treatments, there are differences in how either treatment affects-community composition. These could, in part, be based on spatial distance— the separation between the OTC treatment and

control is only a few meters, where the distance between slopes is approximately 300 m. In the smaller scale climate manipulation treatment there may be too much variation in OTUs to observe significant differences in taxonomy, while the more sensitive phylogenetic measure is able to detect a change in community function.

Experimental warming, both passive (Yergeau et al. 2012) and active (Luo et al. 2014; Xiong et al. 2014), commonly influences bacterial community composition, and in some studies, functional consequences of compositional changes have also been demonstrated. For example, after ten years of active warming in Oklahoma, Luo et al. (2013) found that the bacterial genes that were most abundant in the warmed plots were driven by abundance increases in the majority of the taxa in the community. They concluded that the differences seen between warming and control treatments were not so much about the differential presence of a few bacterial taxa, but instead due to community-wide adaptations induced by warming. A passive warming study in the Antarctic (Yergeau et al. 2012) suggests that warming resulted in a more functionally homogeneous community, possibly by promoting bacteria with generalist life-strategies harboring genes shared by many other taxa, relative to specialist taxa with less pervasive functional traits, and were capable of carrying out a wider range of metabolisms. These (Yergeau et al. 2012; Luo et al. 2013) and other studies (Rinnan et al. 2007, 2009; Xiong et al. 2014) do not emphasize that warming treatments can also dry the soil, as is the case for our climate-manipulation treatment using OTCs. Hence, the phylogenetic differences we observed with the climate manipulation treatment and between years may be indicative of community-wide adaptation, but not increased functional heterogeneity.

Instead, the implied differences in function we observe may be a consequence of not only warming and but also drying.

That we found no effect of grazing on bacterial community compositions, using either diversity measure, is informative, especially in comparison to the large differences we observed between years (Table 1.1). Though grazing did not accentuate the effects of climate manipulation belowground, interestingly it did aboveground. Grazing increased forb flowering richness at our site (Spence et al. 2014). Hence, the lack of a response in the soil bacterial communities to the same grazing treatment is particularly surprising since the flower richness response was highly correlated to changes in the environmental edaphic factors. Relative to ungrazed plots, the aboveground vegetation in the grazed plots experienced higher soil temperatures and lower soil moisture concentrations, likely due to the removal of litter (Spence et al. 2014), which allows for greater sunlight penetration and less moisture retention. We conclude that bacterial communities are less responsive to grazing than the plant communities that inhabit the top of the areas where the samples were collected. This could be due to a delay in bacterial responsiveness to above-ground plant community shifts, as compared with differences caused by other acute changes in this study. Other studies investigating the effects of grazing on belowground microbial communities have yielded inconsistent results, ranging from increases in bacterial diversity with grazing cessation (Zhou et al. 2012) to insignificant changes in the community depending on the site, grazing intensities and grazer identities (Hodel et al. 2014). It is likely that longer timescales are needed to assess whether these results of non-significance are the result of successional lag or uncoupled co-occupation of microbial and plant groups.

Moving from community-level to phylum-level responses can provide more specific insights into compositional changes and functional attributes of a bacterial community. While there is still some debate about whether and which taxonomic levels indicate functional phenotypes in bacteria (Philippot et al. 2010), evidence suggests that differences in life histories and certain other traits differentiate some higher level taxonomic groups from others (Janssen 2006; Fierer et al. 2007; Philippot et al. 2010). Lastly, we hypothesized that any notable changes in phyla abundance would occur along a water stress gradient, which would suggest broad functional attributes for each phylum. Though, as hypothesized, the abundance of notable phyla did not strongly correlate with a water stress gradient, there were correlations with total plant-available nitrogen and soil temperature. From these trends, we were able to infer broad functional attributes of these phyla using the copiotroph-oligotroph continuum theory (Winogradsky 1924; Fierer et al. 2007). Copiotrophs are known to thrive in high resource environments where they can exhibit high growth rates, while oligotrophs are known as slow growers able to thrive in low-resource environments where they can outcompete less stress-tolerant copiotrophs (Fierer et al. 2007). In comparison to these fundamental resource requirements, much less is known about how environmental variables, such as soil temperature and moisture may define a copiotrophic or oligotrophic life style.

Of the phyla identified in this study, Verrucomicrobia is common in grasslands, especially those that are unperturbed, (Bergmann et al. 2011; Fierer et al. 2013) and thought to be on the oligotrophic end of the spectrum. This is likely due to the relative resistance of oligotrophic organisms in adapting to abrupt anthropogenic changes, such as caused by agricultural practices. In our system, Verrucomicrobia abundance is positively

correlated with plant-available nitrogen (Fig. 1.2), which is greater on the upper slope, but negatively associated with both total soil N and C content, which are greater on the lower slope (Casper et al. 2012). These trends support the hypothesis that this phylum is oligotrophic and thrives in areas with lower overall nutrient and carbon content, even if it is relatively more abundant in areas with high concentrations of specific plant-available nitrogen compounds.

As found previously (Ramirez et al. 2012), Firmicutes increased in abundance as Verrucomicrobia decreased (Fig. 1.2), implicating general life strategy differences between these two broad taxonomic clades. In a reciprocal pattern to its oligotrophic counterpart, Firmicutes abundance is negatively correlated with plant-available nitrogen concentrations and positively correlated with total soil N and C, supporting its classification as a copiotroph (Ramirez et al. 2012). The negative correlation between Firmicutes and Verrucomicrobia abundances provides a clear illustration of the copiotrophic-oligotrophic continuum. In addition to different C and N needs, each phyla may also have different moisture requirements. Firmicutes are gram-positive bacteria that sporulate (Onyenwoke et al. 2004), likely imparting drought avoidance (sensu Harris, 1981; Schimel et al., 2007) and possibly explaining the lack of correlation between Firmicutes abundance and soil moisture concentrations (Fig 1.2c). On the other hand, Verrucomicrobia are gram-negative bacteria known for drought acclimation (sensu Harris, 1981; Schimel et al., 2007). These observations suggest that different water-use strategies could help distinguish copiotrops from oligotrophs.

Much less is known about the ecology of Planctomycetes. Unlike either of the other two phyla, Planctomycetes does not show a particularly strong correlation with any

of the measured environmental variables (Fig. 1.2), but it could possibly fall between Firmicutes and Verrucomicrobia on the copiotrophic-oligotrophic continuum. Planctomycetes is closely related to Verrucomicrobia and is part of what has been deemed the PVC superphylum, along with Clamydamonis (Fuerst and Sagulenko 2011), [insert comma] making investigations comparing the ecology of Planctomycetes and Verrucomicrobia of special interest. Similar to Verrucomicrobia, Planctomycetes abundance does increase with plant-available nitrogen and decrease with total N and C. Nitrogen is one of the few edaphic variables with which Planctomycetes has been correlated, with taxonomic richness of the group increasing with soil nitrate heterogeneity (Buckley et al. 2006). Thus, at least some members of this group may thrive in environments lacking the consistent nutrient input that copiotrophs favor, and in general, Planctomycetes may be closer to the oligotrophic side of the continuum. Of course, such categories are broad oversimplifications, but they provide a solid framework with which to create and explore hypotheses about the ecological attributes of microbes.

Though great progress is being made in understanding microbial diversity and its drivers, there is still much remaining to be explored, especially relative to ecological studies of plants and animals (Torsvik and Øvreås 2002; Martiny et al. 2006). To address this need, we examined how soil bacterial communities respond to treatments within a global change experiment in the Mongolian steppe. By simultaneously examining intrinsic (time and space), exogenous (i.e., climate manipulation and grazing cessation) and measured environmental factors, we are able to conclude that soil bacterial communities vary more over time than with slope location, climate manipulation or grazing and that plant-available nitrogen, soil temperature and soil moisture are

significantly involved in structuring these communities. In order to assess the pervasiveness of microbial response to experimental conditions, studies of soil microbial ecology should sample over multiple years and spatial gradients. The taxonomic approach identified significant abundance gradients of Verrucomicrobia, Planctomycetes and Firmicutes that suggest discrete ecological roles at the phylum level and provide fodder for the formation of overarching hypotheses that can guide future studies of incredibly complex soil systems. A more complete picture of bacterial diversity and structure requires knowledge of the taxonomic and phylogenetic relationships within the community. The divergent results from the phylogenetic analysis underscore the importance of both approaches, in that they give us an understanding of the degree to which intrinsic factors as well as exogenous manipulations may differentially influence the structure and implied function of soil bacterial communities. Future studies in microbial ecology that increase the number of measured environmental variables, while including both intrinsic and exogenous factors, will bring us closer to an understanding of how microbial communities are structured and how that structure may respond to global change.

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Table 1.1: Summarized effects of climate manipulation, grazing, year and slope location on soil bacterial communities based on permutation tests from constrained analysis of proximities (CAP) based on Bray-Curtis and UniFrac dissimilarities. Year indicates differences between 2010 and 2011; Slope, between upper and lower locations; Climate, between OTC versus control plots; Grazing between presence and absence of grazers. Supplementary Table A2 provides expanded results, which include the full model with all interactions. All analyses throughout the study were performed on the same set of 67 soil samples (Table A1).

	Bray-Curtis (Taxonomic)	UniFrac (Phylogentic)
Year	**0.01***	**0.01***
Slope	**0.01***	ns
Climate	ns	**0.01***
Grazing	ns	ns

*Significant p-values indicated in bold.

Figure 1.1: CAP ordination of the soil bacterial communities, based on taxonomic (Bray-Curtis) dissimilarities between years (2010, 2011) and topographical position (lower, upper slope). Each point represents the bacterial community composition of one soil sample. Separations of points are based on the statistical differences shown in Table 1.1.

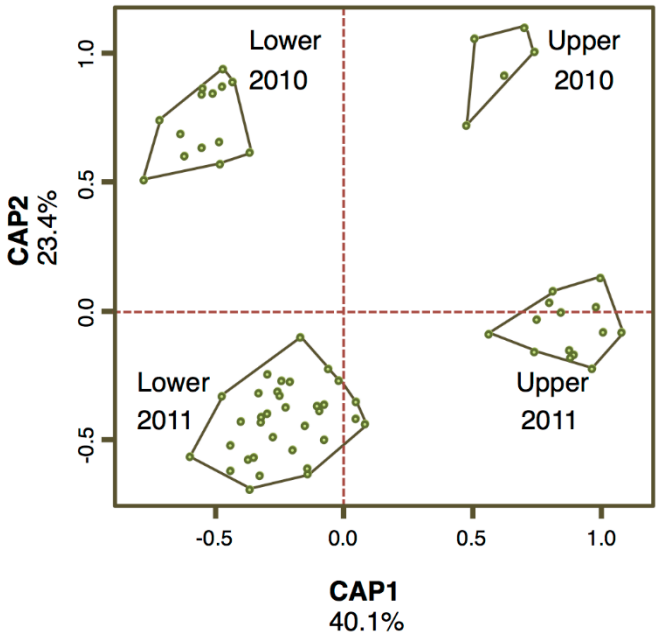


Figure 1.2: The CAP ordination based on Bray-Curtis dissimilarities (modified from Fig. 1.1) with the vectors for the three significant phyla and the environmental gradients that show significant co-variation with the ordination. Vectors for each phylum indicate the direction in which abundances increase (solid lines with full arrowheads). Contour lines represent the concentrations of each environmental variable across the gradient. The overall direction of the combined contour lines is represented by a vector (dashed line with thin arrowhead). The three significant environmental gradients are: (a) total plant-available N (a combination of plant-available NO_3^- and NH_4^+; µg per $10cm^2$ ion exchange surface per day), (b) soil temperature (ºC), and (c) soil moisture (volumetric moisture content, θ). The degree of correlation between two vectors is equivalent to the cosine of the angle between them, which is equivalent to r, the correlation coefficient. For example, there is strong negative correlation between Firmicutes abundance and total plant-available N (cosine ~ 180º; r ≅ -1).

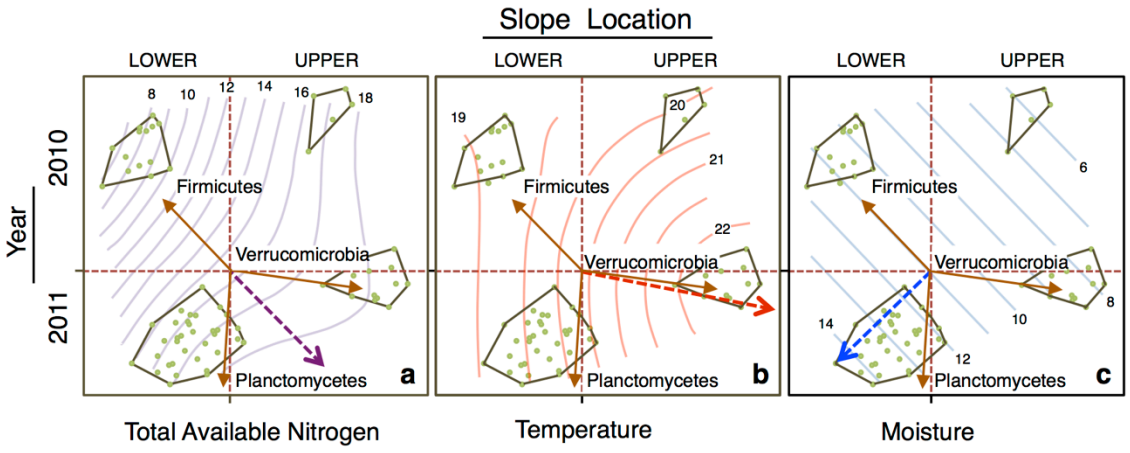
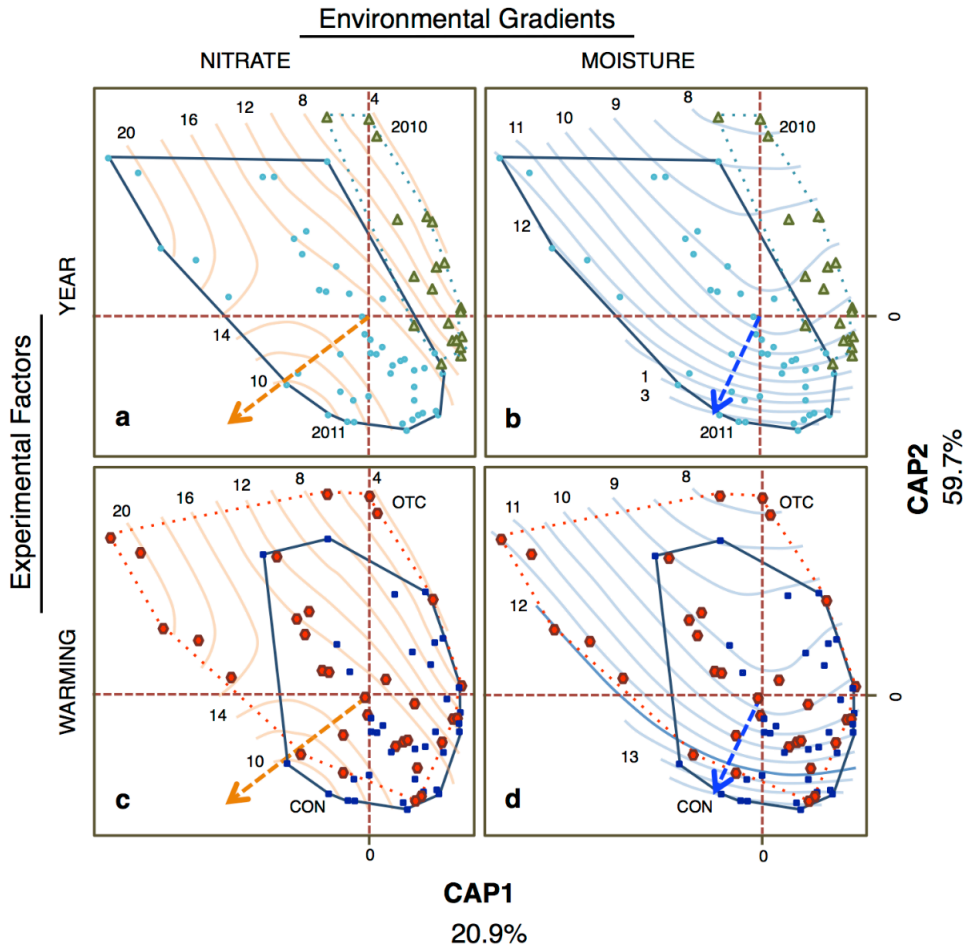


Figure 1.3: CAP ordination of soil bacterial communities based on phylogenetic (UniFrac) relatedness and separated by year (a, b) and climate manipulations (c, d). Contour lines indicate the concentrations of the only significant environmental variables: total plant-available NO_3^- (a, c) and soil moisture (b, d). For samples separated by year (a, b), triangles represent samples from 2010 and are surrounded by dashed lines, circles represent samples from 2011 and are surrounded by solid lines. For samples separated by the climate manipulation treatment (c,d), hexagons represent samples from OTC plots and are surrounded by dashed lines; squares represent samples from Control plots and are surrounded by solid lines. Each environmental gradient is represented by a vector (dashed line with thin arrowhead) that indicates the overall direction of the combined contour lines. Note that here were no significant differences in phyla abundance using UniFrac distances. Separations are based on the statistical differences shown in Tables 1.1 and A2b.



CHAPTER TWO:

HIGH AND DRY:

SOIL BACTERIAL BIOINDICATORS OF CLIMATECHANGE FORESHADOW A DRIER FUTURE FOR THE MONGOLIAN SEMI-ARID STEPPE

2.1 Abstract

As climate change intensifies with time, key organisms within a habitat—or bioindicators—can act as important sentinels of its effects, whether they be changes in aridity or other habitat-defining characteristics. To understand the effects of climate change on soil bacterial diversity, we conducted an experiment along two south-facing slope locations (lower versus upper) in the semi-arid steppe of northern Mongolia, a region experiencing significant shifts in temperature and moisture as a consequence of climate change. We used open-top chambers (OTCs) to create warmer and drier conditions, which were crossed with grazing and watering treatments on the lower and upper slope, respectively. Using structural equation modeling and indicator species analysis, we were able to characterize the connections between bacterial diversity and biotic and abiotic factors. Our results suggest that global change in the Mongolian steppe will act on bacterial communities by its effect on environmental variables and not through its effect on the surrounding plant community. In particular, the effects of climate change on these semi-arid grasslands may act primarily through soil moisture content. Concomitant shifts in key members of the bacterial community may ultimately be bioindicators of a drier future for Mongolia.

2.2 Introduction

Microbial communities are the foundation of Earth's ecosystems (Falkowski et al. 2008). Though we know that soil microbial diversity can be influenced by a wide range of abiotic and biotic factors, (Horner-Devine et al. 2003; Schweitzer et al. 2008), there is still a large gap in our understanding of how microbes respond to environmental change (DeAngelis et al. 2015). As climate changes, so does the relationship between abiotic and biotic factors, which can potentially result in drastic ecosystem consequences, such as high levels of extinction, rising sea levels and extreme weather patterns (IPCC. 2014). Investigations of bacterial diversity and how it responds to climate change are vital for understanding our planet's future environmental trajectory (Reid 2011; Treseder et al. 2012; DeAngelis et al. 2015) and determining potential strategies to mitigate any of the harmful consequences climate change may have on Earth's ecosystems (Nie et al. 2013; Evans and Wallenstein 2014).

Climate change is likely to act on soil bacterial communities through changes to key soil environmental factors, such as soil moisture and temperature, and plant diversity and biomass. In the face of climate change, average temperatures are projected to increase by 1.5 – 2.5ºC over this century and precipitations patterns are expected to be greatly altered (Easterling et al. 2000; IPCC. 2014). In addition to affecting each other, soil temperature and moisture can influence other abiotic factors (May et al. 2015), such as pH (Zárate-Valdez et al. 2006) and plant-available N (Sänger et al. 2011), which can play important roles in structuring microbial communities (Singh et al. 2009; Zeglin et al. 2009). Plant communities can have an impact on microbial diversity through both direct and indirect interactions and feedbacks (Schweitzer et al. 2008). Though studies of plant

and soil feedbacks have an important impact on soil microbial community composition, activity or ecosystem processes (Hobbie 1992; Bever et al. 1997; Hooper et al. 2000; Wardle et al. 2004; Wardle 2006) , much of this work focuses on the plants and not the microbes.

The bacterial community response to environmental change varies across ecosystems (Horner-Devine et al. 2004) and climate modification can occur through direct and indirect interactions between abiotic and biotic factors, which can vary across environmental gradients and over time (Harte and Shaw 1995; Klein et al. 2004; Elmendorf et al. 2012, Liancourt et al. 2012a,b; Spence et al. 2014). Hence, understanding the relative importance of the different soil environmental variables as factors altering the soil microbial community under climate change is challenging. Warming, for example, often reduces soil moisture through increasing evapotranspiration or through the inadvertent drying effects of experimental warming treatments themselves (Liancourt et al. 2012a), and both temperature and moisture can alter decomposition (Suseela et al. 2012; Craine et al. 2014) and nutrient availability (Leadley and Raynaud 2004; Long and Or 2009; Carson et al. 2010). Feedbacks are also expected between the plants and soil abiotic factors as plants use soil resources and alter microclimate. Altogether, such interactions make study of the consequences of climate change very complex.

In this work, set in the understudied Mongolian semi-arid steppe (Liancourt et al. 2012a) we examine how the experimental treatments of climate manipulation, grazing and location within the landscape affect soil bacterial diversity through altered soil

moisture, temperature, pH and available N. Here we define richness as the number of distinct taxa present and community composition as the taxa turnover between two communities as measured by Bray-Curtis dissimilarities. Climate was manipulated through open-top passive warming chambers, which also have a drying effect, and through supplemental watering. We employ structural equation modeling (SEM) to examine relationships among experimental treatments**,** environmental response variables (both biotic and abiotic) and bacterial richness. In comparison to analyses of other climate-change experiments (Arft et al. 1999), this method allowed us to move from a descriptive to a more causal understanding of the complex relationships between plant biomass and plant and bacterial richness, while accounting for variation in the abiotic environment. Using our SEM insights of the broader system, we were then able to employ indicator taxa analysis to identify specific bacterial phyla that may act as bioindicators of increased habitat aridity.

Bioindicators, also known as indicator species, can help the scientific community determine the best places to direct research efforts by representing the impact of environmental change on a habitat (McGeoch 1998; Hodkinson and Jackson 2005). Microbial bioindicators have been identified in agricultural studies of soil fertility (Visser and Parkinson 1992), but to our knowledge none have been identified in studies focused on climate change. As more researchers acknowledge the need to incorporate microbial responses in climate-change models (Allison et al. 2010; McGuire and Treseder 2010), the necessity of identifying specific bacterial taxa and their role as indicators of change becomes more apparent (Evans and Wallenstein 2014). As the first study to identify bacterial bioindicators within a well-characterized, multifactorial climate change

experiment, our study advances our understanding of how microbes both respond to and potentially forecast environmental change.

2.3 Materials and methods

Site description and experimental design

The study site was located in montane steppe on a south-facing slope in the Dalbay River Valley, on the eastern shore of Lake Hövsgöl (51° 01.405' N, 100° 45.600' E). Elevation ranges from 1660 to 1800 m, with a gentle-to-flat incline at the bottom of the slope and becoming much steeper (20^{o} incline) at the top. The average annual air temperature in this region is -4.5°C, with the coldest average monthly (January) temperature of -21°C and warmest (July) of 12°C (Nandintsetseg et al. 2007). Permafrost is not present on the south-facing slope, but is found in a nearby riparian zone and on north-facing slopes under taiga forest (Sharkhuu et al. 2007). The soil is sandy loam texture of alluvial origin, classified as a non-carbonated Dark Kastanozem (Aridic Boroll or Typic Ustolls). Bedrock consists of Cenozoic volcanic deposit (Batkhishig 2006). The steppe vegetation is a mixture of sedges (e.g., *Carex pediformis*, *Carex dichroa*), grasses (e.g., *Festuca lenensis*, *Koeleria macrantha*, *Agropyron cristatum*, *Helictotrichon schellianum*, *Stipa krylovii*) and short forbs (e.g., *Aster alpinus*, *Potentilla* spp., *Artemisia commutata*). Graminoids are far more abundant on the lower slope and, indeed, dominate. Yaks and horses are the main herbivores on the lower slope, and a mixed herd of sheep and goats forage preferentially on the upper slope.

We manipulated climate using passive warming open-top chambers (OTCs),

installed from the first of June until mid-August each year (see (Liancourt et al. 2012b). Constructed of Sun-Lite® HP fiberglass (Solar Components Corporation, Manchester, (NH), USA), the hexagonal OTCs were 40 cm tall, 1.0 m wide at the top and 1.5 m wide at the bottom (Marion et al. 1997). Controls consisted of a hexagonal area of the same footprint. OTCs decreased soil moisture as well and air and soil temperature; hence, it is not just a warming treatment, but instead a climate manipulation treatment. In comparison to controls, OTCs increased mean daytime air temperatures by 1.5ºC and decreased volumetric soil moisture content by 1.6 to 4.1 percent (Liancourt et al. 2012a).

We set up the experiment at two elevations, roughly 1660 and 1750 m.a.s.l. A watering treatment was applied with the climate manipulation treatment on the upper slope (higher elevation) and a grazing treatment on the lower slope (lower elevation). On the upper slope, treatment plots were organized in seven 9 · 9 m blocks, fenced year round, with one replicate of each of the four treatments per block (unwatered OTC, unwatered control, watered OTC, watered control). Water was collected from the river and applied once a week in the evening, using a watering can, to simulate a 4.5 mm rainfall event. On the lower slope, where climate manipulation was crossed with grazing, a block consisted of one OTC and control plot located inside a 9 · 9 m area, fenced year round, and a second OTC and control plot located inside an adjoining 3 · 9 m area that was fenced only in summer; there were eight blocks total. The fencing was removed from this smaller area when the OTCs were taken down in August, to allow grazing to take place, and reinstalled with the OTCs the following June. See Spence et al. (2014) for more detailed information about grazing at this site. Blocks at each elevation were spaced at least 30 m apart. These arrangements of treatment plots allowed three separate

experiments in our analyses. The first (Watering Experiment) examined all upper slope plots with climate manipulation and watering as experimental factors. The second (Slope Experiment) used unwatered and ungrazed OTCs and control plots on both slopes so that climate manipulation was crossed with slope location. The third (Grazing Experiment) examined all lower-slope plots so that climate manipulation was crossed with grazing.

Environmental variables and plant community composition

We wanted to understand how our experimental treatments affected environmental factors likely to influence plants and microbes. We measured total plant-available nitrogen and soil moisture, temperature and pH in all of our experimental plots. We looked for relationships between these environmental factors and the soil bacterial taxonomic richness and plant biomass and species richness. We used plant root simulator (PRS™) probes, consisting of ion exchange membranes, to measure plant available NO_3^- and NH_4^+ (Western Ag Innovations, Saskatoon, SK, Canada; http://www.westernag.ca/innov/prs-probes/). In late June, we deployed two cation probes and two anion probes in each experimental treatment plot and left them in place for 21 days before preparing them for processing. Probes were placed along the side of a plot but still within the 1.0 m wide opening of OTC. We brushed soil from the probes in field and washed them with deionized water in the lab before analysis by Western Ag Innovations. We report nitrogen concentrations as μg per 10 cm^2 ion exchange surface per day. We used a portable probe (WET-2 sensor, Delta-T Devices Ltd) to measure the volumetric moisture content (θ) and temperature (ºC) of the surface soil from each experimental plot during the time of sampling. Temperature and moisture were measured

three times per plot and then averaged. These averages were used in subsequent analyses of the soil bacterial communities. For determining pH, all samples were homogenized after collection, and 5 g of soil from each sample was dried and then aliquotted into 25 mL water. A slurry was made from which pH was measured using an OAKTON Waterproof pH Tester 30.

We harvested vegetation in all experimental treatments in the third week of July to determine above-ground plant biomass. For this harvest, vascular plants were clipped at the soil surface from within a 1.0 · 0.5 m area centered in each experimental plot. We used this biomass measure as a biotic factor in our models, even those constructed for bacterial communities contained in soil samples collected in June. Plant-species richness was determined by counting the number of species within 2 cm of each soil core removed for soil bacterial community profiling. Because we combined soils from three soil cores collected at a particular time (see below), we summed the number of plant species found within the 2 cm diameter of any of the three soil cores. We used these numbers as a measure of plant species richness in our models, with separate richness values collected at the time of soil sampling in early June and early July.

Collection, extraction and sequencing of soil samples

We collected soil cores to analyze the bacterial community from all experimental plots both in early June and early July. Blocks on the lower slope (nos. 1-8) and blocks on the upper slope (nos. 9-15) were sampled on June 6 and June 7, respectively and again on July 8 and 9. The order in which blocks were sampled within a slope location was randomized. Each soil sample consisted of three soil cores (2 cm diameter, 5 cm deep)

taken along a one-meter transect within each plot and homogenized within one sterile Whirl-Pak® bag. This resulted in 120 soil samples in total, but after sequencing, five of those samples were dropped because of low 16S rDNA sequences counts relative to the other samples. As a result, 115 samples are used in our analysis (Table B1). We transported samples in a cooler at ~ 4ºC during transit from Mongolia to the US, before storing them at -80ºC. The brief transit times (≤ 7 days) should have prevented significant changes in the phylogenetic structure and diversity of the soil microbial community (Lauber et al. 2010). As a further precaution for, we stored all samples in bacteriostatic LifeGuard Solution (MoBio, Carlsbad, CA, USA) directly after taking soil cores in the field, which inhibits microbial activity and prevents DNA or RNA degradation.

DNA extractions were performed at the University of Pennsylvania, PA, U.S.A. Soil samples were thawed to 4ºC and centrifuged to separate and then discard the LifeGuard preservation solution. DNA was extracted using standard EMP protocols (http://www.earthmicrobiome.org/emp-standard-protocols/). We isolated DNA from 0.20 g of soil per extraction using the manufacturer-suggested protocol for PowerSoil® DNA Isolation Kit (MoBio), with the modification that we heated the extraction at 65°C for 10 minutes prior to the initial bead beating and cell lysis step.

We preformed the 291 bp length V4 region amplification using the 515F primer and the 806R Golay-barcoded reverse primers (for a full list of these primers visit http://www.earthmicrobiome.org/emp-standard-protocols/). Each 25µl PCR reaction contained 12µl of MoBio PCR Water (Certified DNA-Free), 10µl of 5 Prime HotMasterMix (1x), 1µl of Forward Primer (5uM concentration, 200pM final), 1µl Golay Barcode Tagged Reverse Primer (5µM concentration, 200pM final), and 1µl of template

DNA. We performed PCR under the following conditions: 94°C for three minutes to denature the DNA, with 35 cycles at 94°C for 45 sec, 50°C for 60 sec, and 72°C for 90 sec, with a final extension of ten minutes at 72°C to ensure complete amplification. Each PCR reaction was performed in triplicate for each sample and then pooled together as one representative PCR product in order to restrict PCR bias. The DNA concentration of each aggregate PCR product was then quantified using PicoGreen (Invitrogen) and a microplate reader. Once these were quantified, we performed a second pooling, where different volumes of the discrete sample pools with the same DNA concentrations were combined in a single tube. This allowed for an equal amount of 16S rDNA amplicon sequences from all samples in our study. We cleaned the final pool using the UltraClean® PCR Clean-Up Kit (MoBio). Sequencing was completed on the Illumina MiSeq platform.

Bioinformatic processing of DNA sequences

We quality trimmed 16S rDNA amplicon sequences using SolexaQA, eliminating regions that fell below a Q-score of 18 (Cox et al. 2010), which corresponds roughly to 98.4% or higher accuracy for each base position. We then retained only the longest continuous fragment that remained after trimming. If this fragment fell below 75% of the expected sequence length (i.e., < 112bp), we discarded it completely from further analyses. The 12bp barcode sequence enabled us to assign sequences to samples by searching against the PCR primer tags assigned to samples using BLAST+ (blastn, word size=4) (Camacho et al. 2009). We allowed up to one mismatch of the 12bp barcode sequences when assigning sample IDs. We used the open software package Quantitative

Insights Into Microbial Ecology (QIIME) for downstream analyses of these data (Caporaso et al. 2010). We clustered sequences, de-novo, into Operational Taxonomic Units (OTUs) at 97% identity using the Uclust algorithm (Edgar 2010). We discarded singletons (OTUs with only one representative sequence) from further analysis, as they commonly result from chimeric PCR products or sequencing error, and are usually non-informative in the overall analysis of composition (Zhou et al. 2011).

From among the sequences assigned to an OTU, we chose a representative sequence to give the OTU a taxonomic identity. To do this, we chose the longest available sequence and then assigned taxonomic identification using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007). Representative sequences were aligned using the Python Nearest Alignment Space Termination Tool (PyNAST) as implemented in QIIME. These aligned sequences, along with an OTU abundance matrix, were used to calculate beta diversity by means of weighted UniFrac distances with the QIIME script beta_diversity.py. By coupling the OTU abundance matrix with taxonomic classification of each OTU, a taxonomic summary was generated for each general taxonomic level (i.e. phylum, class, order, family, genus).

Statistical analysis

We used structural equation modeling to discern the relationships between the experimental treatments, environmental factors and the biotic factors (bacterial and plant communities) in our system. We constructed a separate model for each of the three experiments (Water, Slope, and Grazing) and each collection date (June and July) for six models total. We first constructed hypothetical models for how the experimental

treatments, environmental factors and the biotic factors would be related, with the idea that our experimental treatments would act on biotic factors via changes to the measured environmental factors of available N and soil moisture, temperature and pH. This approach, employing hypothetical connections identified significant links between factors, enabled us to refine the models in subsequent iterations.

Our initial hypotheses about the relationships between environmental factors and biotic factors were the same for every model, regardless of the experiment and whether for June or July. We hypothesized that soil pH would only affect bacterial phyla richness, while available N, soil moisture and soil temperature could each affect plant species richness, plant biomass, and bacterial phyla richness. Because of shading effects from plant canopies, we hypothesized that plant biomass could, in turn, affect soil moisture and temperature. We hypothesized that environmental factors could be related to each other, with soil moisture affecting pH and available N, and soil moisture and soil temperature co-varying. We also realized that the biotic factors could influence each other and hypothesized co-varying connections between plant species richness and bacteria phyla richness, between plant species richness and plant biomass, and between plant biomass and bacteria phyla richness.

Based on measurements made in prior studies, we expected the OTC treatment to affect both soil moisture and soil temperature, and used those connections in all models. In the Watering experiment, we expected the watering treatment to affect soil moisture. In the Slope experiment, we expected slope location to impact soil moisture, temperature, and pH. Based on our own observations, we also hypothesized that slope location would

affect plant biomass directly. In the Grazing experiment, we expected grazing to impact available N, soil moisture, and soil temperature.

All analyses were performed in R using the vegan, lavaan and labdsv packages on OTU tables rarified to 31,708 sequences per sample. We used the *spec* function in vegan, which sums the number of taxa in a plot, to calculate plant and bacteria richness at the species and phyla level. We used lavaan's *sem* function to construct structural equation models (SEM) (Fig. 2.1) between experimental treatments and environmental and biotic factors. In order to identify the most robust relationships, or links, between factors, we ran three iterations of the models. The first iteration was based on our own hypotheses, and included all hypothesized links (outlined above) between each factor (Fig. 2.1a, b, c). A second iteration used the significant links, but not the insignificant links, from the initial model. In both the first and second iterations, all reciprocal arrows (both variables affecting each other) were included as one, double-ended link (recursive links measuring covariance) rather than two distinct arrows in order to allow an assessment of the strength of the individual, component links. In the final iterations of the models, we assessed the individual components of the recursive, double-sided relationships, by splitting any remaining recursive links into non-recursive links. This allowed us to determine the individual influence of each factor within the co-varying relationship. We present the non-recursive models (Fig. 2.1d, e, g, h), except where the non-recursive model did not run due to over-specification. In these cases, the recursive model is presented as the final model (Fig. 2.1f, i).

We were interested in particular bacterial phyla that responded most strongly to our experimental treatments. With the same datasets (Table B1), we used the *indval* function

in the labdsv package to identify "indicator taxa" (Dufrene and Legendre 1997) in the bacterial community, at the phylum level, that were associated with our treatments (Table B2).

2.4 Results

Relationships between treatments and abiotic and biotic variables

Overall patterns: SEM models show no significant links between plant richness (species level) or plant biomass and bacteria phyla richness in any of the three experiments for either June or July (Fig. 2.1). Bacteria phyla richness seldom responded to experimental treatment except in the Watering experiment conducted on the drier upper slope. Soil moisture often responded to experimental treatments (Fig. 2.1d-i), but soil temperature did not. In particular, the OTC often reduced soil moisture, especially later in the season.

Upper slope – Watering experiment: In June, soil moisture was increased by the watering treatment and decreased by plant biomass (Fig. 2.1d). Soil moisture proved to be a factor of major importance, acting on other environmental and biotic factors. Available N, soil temperature and bacteria phyla richness were all negatively related to soil moisture. Plant species richness was, in turn, negatively related to available N. Soil temperature showed no relationship with plant species richness, plant biomass or bacteria phyla richness.

In July, the OTC reduced soil moisture, but there was no detected effect of the watering treatment on soil moisture, and neither watering nor the OTC affected soil temperature (Fig. 2.1g). Just as in June, plant biomass reduced soil moisture, and soil

moisture reduced available N. Plant richness was positively related to soil moisture. In contrast to June observations, bacterial phyla richness was reduced by increasing soil temperature, but showed no relationship with soil moisture.

Both slopes – Slope experiment: In both months, the upper slope had significantly lower soil-moisture values in comparison to the lower slope (Fig. 2.1e, h). No environmental factor we measured influenced bacteria phyla richness. In June, soil moisture and available N were inversely related (Fig. 2.1e), just as they were for the upper slope experiment. The June model also shows that slope explained the variation in plant biomass, which was lower on the upper slope.

In July, the OTC reduced soil moisture (Fig. 2.1h), and soil moisture again reduced available N. Soil temperature was positively associated with soil moisture and higher temperatures reduced plant biomass. The upper slope had higher pH levels, but pH did not affect any other environmental or biotic factor.

Lower slope – Grazing experiment: The grazing experiment on the lower slope is the only one for which there is no direct or indirect connection, even if insignificant, between the experimental treatments and any of the biotic response factors (Fig. 2.1f, i). In June, the OTC reduced soil moisture, and soil moisture and soil temperature were inversely related (Fig. 2.1f). However, soil moisture did not link to available N as it did in other models. Available N reduced soil bacteria phyla richness, and no other environmental factor impacted bacteria phyla richness.

In July, bacteria phyla richness did not link to any other factor. Both grazing and the OTC reduced soil moisture, but soil moisture was not linked to any other abiotic or biotic factor (Fig. 2.1i). Plant biomass was inversely related to plant species richness and

soil temperature.

Indicator phyla

In examining the representation of bacteria phyla as a function of experimental treatments, the largest range of indicator phyla was found when comparing the upper and lower slopes in the Slope experiment. However, no particular phyla stood out as they did for the watering vs. control treatments for the Watering experiment and for the climate manipulation vs. control treatments for the Grazing experiment. In separate months, Elusimicrobia and Verrucomicrobia were both indicators of the controls in the Watering experiment ($p < 0.014$ in June and $p< 0.04$ in July, respectively, Table B2) and indicators of the OTC treatment in the Grazing experiment ($p<0.047$ in July and $p<0.021$ in June, respectively, Table B2). Compared to controls, watering depressed the relative abundance of Elusimicrobia in June and Verrucomicrobia in July (Fig. 2.2). Similarly, the OTC elevated the relative abundance Verrucomicrobia in June and slightly elevated the relative abundance of Elusimicrobia in July. No indicator species were identified for the climate manipulation treatment in either the Slope experiment or the Watering experiment. Within the Grazing experiment, the two indicator phyla identified were inconsistent between month and grazing vs. control treatments.

2.5 Discussion

Our experiments identify soil abiotic factors as primary in structuring soil bacterial composition in the Mongolian steppe. Soil bacteria phyla richness was influenced, at different locations within the landscape and at different collection dates, by soil moisture, temperature and available N, but we never observed a direct influence by

plant biomass or plant species richness. Only in June in the Watering experiment on the upper slope did plant biomass link indirectly to bacterial phyla richness through effects on soil moisture. Additionally, we found evidence that modulation of soil moisture may favor particular taxa in the bacterial community. The relative abundance of two indicator phyla, Verrucomicrobia and Elusimicrobia, decreased in wetter conditions and increased with soil drying.

Thus, we found no strong evidence that plant communities influence soil bacterial communities in our system. The effect of plant diversity on microbial diversity varies—while some studies report linkages (Zak et al. 2003; Lamb et al. 2011; Schlatter et al. 2015), others do not (Chabrerie et al. 2003; Nunan et al. 2005; Lamb et al. 2011). Compared to these studies, ours is unique in that it uses structural equation models (SEMs) to test the direct and indirect multivariate relationships between above- and below-ground communities through variation in abiotic factors. So-called "second generation" SEM analysis goes beyond the descriptive nature of "first-generation" multivariate methods, including multiple regressions and non-metric multidimensional scaling (Fornell 1982; Grace 2006), by allowing researchers to test causal relationships within a system (Eisenhauer et al. 2015; Shao et al. 2015). Hence, our viewing plant and bacterial communities after experimental manipulation of environmental factors adds a mechanistic understanding to other, more descriptive, studies investigating the relationships between plant and microbial diversity. In particular, our study may help illuminate the underlying mechanisms supporting a broad pattern, such as the one reported in a meta-analysis conducted across four continents by Prober et al. (2015), which found no relationship between the alpha diversity of grassland plant and microbial

communities. Such findings indicate that plant and microbial communities can exhibit distinctly different profiles under the same abiotic factors (Tedersoo et al. 2014; Prober et al. 2015).

In our experiment, soil moisture, soil temperature and available nitrogen affected both bacteria phyla and plant richness, but through different pathways at different times and in different places (Fig 2.1). Such findings underscore the importance of accounting for natural variation over time and across the landscape. By taking both temporal and spatial variation into account, our climate-change experiment documents a range of biotic and abiotic responses that would have otherwise been overlooked. Though other studies have taken existing sources of variation into account (Klein et al. 2004, 2007; Xiong et al. 2014), there are still many open questions about how such variation affects ecosystem-wide responses to climate change, especially in terms of microbial communities (Classen et al. 2015). Previous studies within our experiment have concluded that climate change will not produce consistent consequences throughout the growing season or across the landscape for either the environmental response variables or plant species (Liancourt et al. 2012a, 2013). As this study demonstrates, such conclusions can be applied to the microbial communities as well.

That the upper slope is drier and warmer is a potential explanation for why the model examining the OTC and watering treatment on the upper slope was the only one in which both plant and bacterial diversity were indirectly, yet consistently, affected by our treatments (Fig. 2.1). Hypothetically, this could mean that organisms on the upper slope are more responsive to environmental fluctuations compared to those on the cooler, wetter lower slope, which is a potentially more buffered environment. Consistent with

this interpretation, water is the only significant experimental treatment in the wetter and cooler month of June, as it could push conditions to the higher end of the soil moisture spectrum, resulting in decreased bacteria phyla richness. In contrast, the OTC, which both warms and decreases soil moisture content, is the only significant experimental treatment in the warmer, drier month of July, again causing decreased phyla richness.

Abiotic factors similarly explained variation in plant-species richness on the upper slope only, with different factors, such as available N and soil moisture, identified in June and July respectively. As the vegetation consists of mostly clonal, long-lived perennials, it is unlikely that the plant community changed in composition between the two sampling dates. More likely, greater richness occurs in locations characterized by less N early in the season and greater soil moisture later in the season. Interestingly, soil moisture decreased with increasing plant biomass both months, which suggests significant water loss through transpiration. The wetter, cooler environment on the lower slope apparently buffers biotic responses to experimental manipulation. While both the OTC and grazing reduced soil moisture on the lower slope for at least one sampling date, there was never a linkage between soil moisture and any of the biotic responses. Presumably grazing reduces soil moisture through the removal of plant litter (Spence et al. 2012), which otherwise provides shades and reduces evaporation.

Large differences exist in the ecology of the two slope locations. Differences in moisture between the two are evident in the Both Slopes model for June and July. Additionally, models show differences between the two locations in pH and plant biomass. Unexpectedly, pH showed no influence on any biotic response. This was surprising because pH has can have a large effect in structuring both bacterial (Lauber et

al. 2009) and plant communities (Schuster and Diekmann 2003). Perhaps the variation in pH was too low to find a response, or perhaps it is not important when accounting for other abiotic or experimental variables. Regardless, slope location directly affects both soil moisture and plant biomass and influences the impact of experimental treatments on biotic responses.

As soil moisture, and not temperature, was the only abiotic factor directly affected by our experimental treatments, future changes in rainfall may have an especially large impact on the semi-arid Mongolian steppe. Few studies have deployed OTCs in semi-arid systems (Liancourt et al. 2012a), and other studies in wetter systems have focused on the temperature effects of OTCs, reporting only minor moisture effects (Marion et al. 1997; Klein et al. 2004), with a few exceptions (Aerts 2006; Dabros et al. 2010, Liancourt et al. 2012a). By demonstrating that OTCs may act more through moisture than temperature in relatively arid regions of the globe, our experiment showcases the importance of performing passive warming studies across a range of habitats in order to get a more refined understanding of how systems will respond to changes in temperature and precipitation. Our results may be indicators of a positive feedback loop in which Mongolia becomes continually drier over time, consistent with climate models of the region (Dagvadorj et al. 2011).

Ecologists are generally interested in whether the current distribution of species over spatial gradients in moisture and temperature projects the species success with changes in climate over time (Blois et al. 2013). By conducting our climate manipulation experiment at two locations within the landscape, we found bacterial bioindicators which suggest that by drying the experimental plots, the OTC treatment pushes the lower slope

environment to become similar to the upper slope environment. In turn, the Watering treatment pushes the upper slope environment to be similar to that of the lower slope. These changes in phyla are consistent with a space-for-time substitution model (Blois et al. 2013). Indicator analysis revealed no corresponding indicator species from the plant community. Because of their rapid generation times, bacterial communities likely respond much faster than plant communities to changes in the environment. It follows that shifts in bacterial indicators may be the earliest sentinels of climate change.

Our findings that Verrucomicrobia and Elusimicrobia thrive in dry conditions provide insight on the elusive ecology of these phyla. Both have been documented in a range of terrestrial and aquatic environments, yet have very few cultured representatives (Geissinger et al, 2009; Bergmann et al, 2011, respectively). Those cultured are associated with oligotrophic characteristics, such as small genome size and a propensity for low-nutrient environments (Fierer et al. 2013). In contrast to their copiotrophic counterparts, oligotrophs are able to thrive under environmental stress (Fierer et al 2007). Thus our results suggest that these oligotrophic stress tolerators may become more prominent members of bacterial communities as habitats dry out due to climate change. Further investigations are required to understand the potential functional consequences of such shifts in the bacterial community on an ecosystem-level (Barnard et al. 2013; Evans and Wallenstein 2014) and whether shifting ecological strategies of bacteria, including oligotrophy, can provide important insights on how changes to these communities may alter surrounding ecosystem processes.

Our results suggest that climate change in the Mongolian steppe will act on bacterial communities by its effect on environmental variables and not through its effect

on the surrounding plant community. Our experiments altered water regimes more than temperature patterns, suggesting that the effects of climate change on these semi-arid grasslands may act primarily through soil moisture content. Concomitant shifts in key members of the bacterial community may ultimately be indicators of a drier future for Mongolia.

Figure 2.1: Structural equation models, testing direct and indirect effects of experimental treatments on environmental conditions and plant and bacterial communities over time. Hypothesized models for datasets A-C: a) upper slope samples including OTC and watering treatments, b) samples from both slopes including only OTC treatments, and c) lower slope samples including OTC and grazing treatments (see Table B1 for details). Arrows and their associated values indicate relationships, or links, between treatments, abiotic and biotic factors. The values above each arrow indicate the direction and magnitude of the relationship. Links that were not significant in the hypothesized models, do not appear in the corresponding final models, which are analyzed by collection month (d-i). Gray links were significant in the hypothesized model, but are not in the final model. Black links indicate a significant relationship. Stars specify p-values: * $p<0.05$, ** = $p<0.005$, *** $p<0.0005$. All final models broke down recursive links (double-headed arrow) into non-recursive links (two opposing single-headed arrows) in order to parse out which covariate had a bigger influence and in what direction, except Dataset C displays only recursive models, as the non-recursive model produced erroneous results. Dashed arrows represent links that were not significant in either the hypothesized or final model, but were automatically added back into the model by the labdsv package.

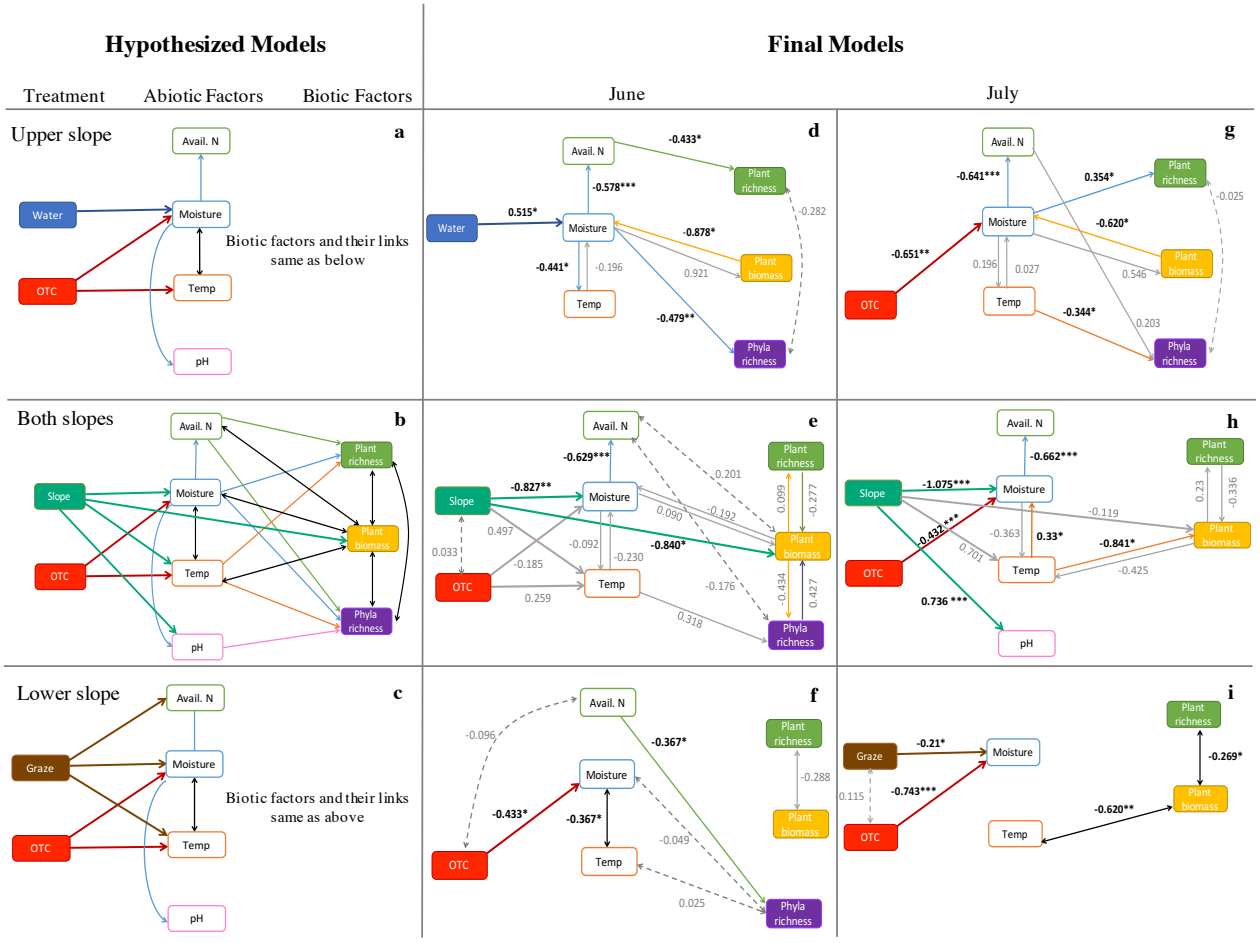
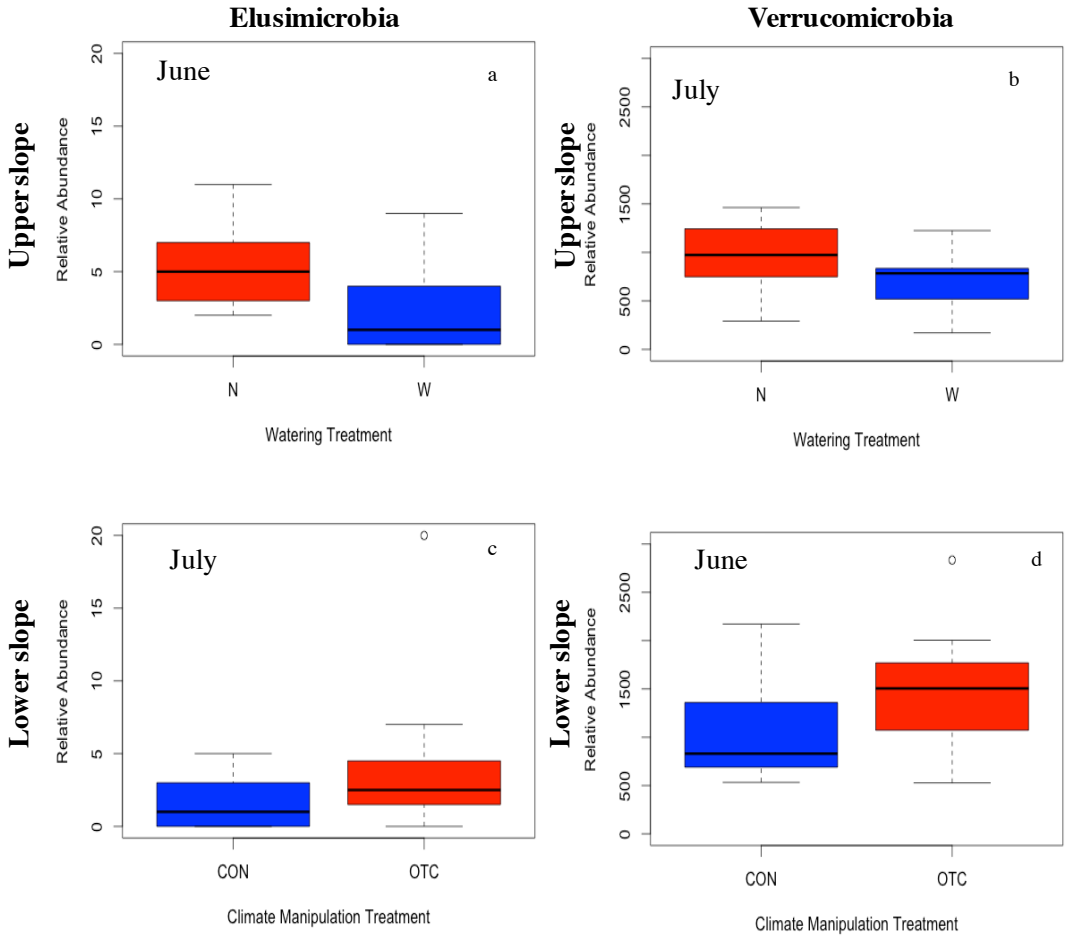

Hypothesized Models
Final Models
Treatment
Abiotic Factors
Biotic Factors
June
July
Upper slope
a
Avail. N
Water
Moisture
OTC
Temp
pH
Biotic factors and their links same as below
d
-0.433*
-0.578***
0.515*
-0.878*
-0.441*
-0.196
0.921
-0.479**
-0.282
Plant richness
Plant biomass
Phyla richness
g
-0.641***
0.354*
-0.620*
-0.651**
0.196
0.027
0.546
-0.344*
0.203
-0.025
Both slopes
b
Slope
e
-0.629***
-0.827**
0.201
0.099
-0.277
0.497
0.090
-0.192
-0.840*
0.033
-0.092
-0.230
-0.185
0.259
-0.176
-0.434
0.427
0.318
h
-0.662***
-1.075***
0.23
-0.336
-0.119
-0.363
0.33*
-0.841*
-0.432***
0.701
-0.425
0.736 ***
Lower slope
c
Graze
f
-0.096
-0.367*
-0.433*
-0.367*
-0.049
0.025
-0.288
i
-0.21*
0.115
-0.743***
-0.269*
-0.620**

Figure 2.2: Box-and-whisker plots of indicator taxa analysis, illustrating two significant bacterial indicator phyla in watering and climate manipulation treatments on the upper and lower slope in June and July. Box plots with higher mean relative abundance values specify the treatment or control for which either Elusimicrobia or Verrucomicrobia is considered an indicator phylum (see Table B2 for significance values). Upper slope (a,b): W, Water; N, No Water. Lower slope (c,d) : OTC, Open Topped Chamber; CON, Control.



CHAPTER THREE:

ONE CORE OR MORE? INSIGHTS ON CHOOSING AN OPTIMAL SAMPLING STRATEGY FOR STUDIES IN SOIL MICROBIAL ECOLOGY

3.1 Abstract

Microbial communities can vary widely across spatial scales, especially within soil, arguably the most heterogeneous and biodiverse habitat on Earth. Hence, any study of soil microbial diversity necessitates careful consideration of scale. There is an array of studies investigating the spatial breadth of microbial diversity, but much less attention has been given to methodological studies of soil sampling. By convention many studies of soil microbial diversity pool multiple soil cores into one sample in an effort to capture the highest levels of diversity. We investigated if and how different sampling schemes affected measurements of bacterial diversity within the context of a climate-change experiment set in the steppe of northern Mongolia. We made two main comparisons: In Dataset A, we compared three individual soil cores compared to one "mixed" sample composed of equal subsamples from each of the three individual cores. In Dataset B, we compared the same mixed sample to only one of the three individual cores from which it was composed. We found that within the context of our experiment, patterns of diversity were the same whether comparing the bacterial community in one soil core, or across a set of three individual cores, to a mixed core. Despite high levels of soil heterogeneity, our work provides evidence that pooling soil cores diversity may be an exercise in redundancy in certain studies of bacterial diversity.

3.2 Introduction

Spatial heterogeneity is crucial to any study of an ecological community (Legendre and Legendre 1998)**.** As an incredibly heterogeneous, diverse and complex medium, issues of scale are especially pertinent to studies of soil and the microbial communities it houses (Ettema and Wardle 2002; Mummey et al. 2006). Soil microbial diversity is vast—one gram of soil can contain more than ten billion microbes (Rossello-Mora and Amann 2001; Torsvik and Øvreås 2002), while one ton of soil can contain 4 x 10^6 coexisting bacterial taxa (Curtis et al. 2002)**.** Measuring such tremendous diversity is a formidable task that starts with the significant challenge of determining the relevant scale at which to take a sample (Mummey et al. 2006).

A sample scheme depends first and foremost on the scientific question at hand. We were interested in how soil bacterial diversity was affected by climate change. To explore this question, we conducted a climate-change experiment in the semi-arid steppe of northern Mongolia, a region that has seen a substantial rise in temperatures over the last 40 years (Namkhaijantsan 2006). Across the landscape, we manipulated climate using open-top chambers, which have both a warming and drying effect (Liancourt et al. 2012).

Reliable and representative results from an experimental field site depend on a sampling strategy that accounts for the spatial heterogeneity within the experimental design (Webster 1979; van Elsas and Smalla 1997). Unfortunately, as (Bending et al. 2006) and (Rodríguez-Cruz et al. 2006) point out, many soil studies do not take fine-scale variability into account (Gawlik et al. 2003). Specific research on the sampling

methodologies necessary to explore soil microbial diversity, especially using high-resolution molecular techniques, is still in its infancy (Baker et al. 2009; O'Brien et al. 2016).

The biogeography of microbes is a burgeoning field (Martiny et al. 2006), which extends across continents (Fierer et al. 2009, 2012, 2013; Bru et al. 2011; Barberan et al. 2012; Liu et al. 2014; Whitaker et al. 2014), within ecosystems (Klironomos et al. 1999; Noguez et al. 2005; Franklin and Mills 2009; Philippot et al. 2009; Ushio et al. 2010; Barberan et al. 2012; Correa-Galeote et al. 2013), and even, in a few limited cases, along centimeter scales (Morris 1999; Grundmann and Debouzie 2000; Franklin and Mills 2003; Oline et al. 2006; Keil et al. 2011). Though incredibly important for understanding the spatial breadth and general patterns of microbial diversity across scales, many of these studies do not address the sampling schemes with the same meticulous methodologies as either Baker et al. (2009) or O'Brien et al. (2016). While these studies recommend pooling samples taken randomly within an experimental plot, they caution that any strategy should be calibrated to the ecosystem, experiment and researcher's questions.

In order to investigate the best scale and sampling procedure in which to explore soil bacterial diversity, within the context of our climate change experiment, we asked the question: Bounded by our experimental treatments, is the diversity found in a set of individual soil cores the same as that found in a "mixed" soil core, composed of subsamples from the associated set of individual cores? In other words: Is taking a single sample, or a set of them, as good as taking a mixed sample?

We compared both alpha and beta diversity measurements between Sample Types, or individual and mixed soil cores. Here we classify alpha diversity as the number of bacterial taxa within a plot (Whittaker 1972), which we measured at both the smallest taxonomic scale of observed OTUs and the largest taxonomic scale of phyla. We characterize beta-diversity as community composition, or the pairwise comparison of bacterial phyla between plots (Whittaker 1972; Anderson et al. 2011), measured by Bray-Curtis dissimilarities. We were particularly interested in comparing the alpha diversity of individual and mixed soil samples from the upper and lower slopes and OTC and control plots. For beta-diversity we wanted to know if the patterns in community composition, relative to the slope and Climate Manipulation treatments, differed between individual and mixed soil samples. Finally, we investigated the contribution of specific phyla to the difference between communities in either Sample Type.

3.3 Materials and methods

Site description and experimental design

The study site was located in montane steppe on a south-facing slope in the Dalbay River Valley, on the eastern shore of Lake Hövsgöl (51° 01.405' N, 100° 45.600' E). Elevation ranges from 1660 to 1800 m, with a gentle-to-flat incline at the bottom of the slope and a much steeper (20^{o} incline) at the top. The average annual air temperature in this region is -4.5°C, with the coldest average monthly (January) temperature of -21°C and warmest (July) of 12°C (Nandintsetseg et al. 2007). Permafrost is not present on the south-facing slope, but is found in a nearby riparian zone and on north-facing slopes

under taiga forest (Sharkhuu et al. 2007). The soil is sandy loam texture of alluvial origin, classified as a non-carbonated Dark Kastanozem (Aridic Boroll or Typic Ustolls). Bedrock consists of Cenozoic volcanic deposit (Batkhishig 2006). The steppe vegetation is a mixture of sedges (e.g., *Carex pediformis*, *Carex dichroa*), grasses (e.g., *Festuca lenensis*, *Koeleria macrantha*, *Agropyron cristatum*, *Helictotrichon schellianum*, *Stipa krylovii*) and short forbs (e.g., *Aster alpinus*, *Potentilla* spp., *Artemisia commutata*). Graminoids are far more abundant on the lower slope and, indeed, dominate. Yaks and horses are the main herbivores on the lower slope, and a mixed herd of sheep and goats forage preferentially on the upper slope.

We manipulated climate using passive warming open-top chambers (OTCs), installed from the first of June until mid-August each year (see Liancourt et al. 2012). Constructed of Sun-Lite® HP fiberglass (Solar Components Corporation, Manchester, NH, U.S.A.), the hexagonal OTCs were 40 cm tall, 1.0 m wide at the top and 1.5 m wide at the bottom (Marion et al. 1997). Controls consisted of a hexagonal area of the same footprint. OTCs decreased soil moisture as well and air and soil temperature; hence, it is not just a warming treatment, it is a Climate Manipulation treatment. In comparison to controls, OTCs increased mean daytime air temperatures by 1.5ºC and decreased volumetric soil moisture content by 1.6 to 4.1 percent (Liancourt et al. 2012).

We set up the experiment at two elevations, roughly 1660 and 1750 m.a.s.l. On the upper slope, treatment plots were organized in seven 9 x 9 m blocks with one Climate Manipulation treatment replicate per block (OTC and control). Blocks at each elevation were spaced at least 30 m apart. Blocks were fenced year round to prevent access by grazers.

Collection, extraction and sequencing of soil samples

In 2012, we collected soil cores to analyze the bacterial community in each experimental plot. Blocks on the lower slope (nos. 1-8) and blocks on the upper slope (nos. 9-15) were sampled on June 6 and June 7, respectively. The order in which blocks were sampled at each slope location was randomized. A set of three soil cores was collected from each experimental plot for a total of six cores per Block. For analysis, a complete set of soil samples included the three individual cores taken from each plot plus one mixed sample, which was a composite of subsamples from each of the individual cores, for a total of four samples per plot. The cores were 2 cm diameter, 5 cm deep, and taken 30 cm apart along a 90 cm transect. Each soil core was placed in a sterile Whirl-Pak® bag and kneaded in order to homogenize the contents. Once out of the field, 4 g of soil was subsampled from each of the three individual cores in a set, placed in a single sterile Whirl-Pak® bag and homogenized in order to create a "mixed core." This resulted in 120 soil samples in total, but after sequencing, five sample sets were dropped because at least one of the cores had low 16S rDNA sequence counts relative to the other samples. Thus, 100 samples are used in our statistical analysis (Table C1).

We transported samples in a cooler at ~ 4ºC during transit from Mongolia to the United States, before storing them at -80ºC. The brief transit times (≤ 7 days) should have prevented significant changes in the phylogenetic structure and diversity of the soil microbial community (Lauber et al. 2010). As a further precaution, we stored both individual and mixed cores in bacteriostatic LifeGuard Solution (MoBio, Carlsbad, CA,

U.S.A.), which inhibits microbial activity and prevents DNA or RNA degradation.

DNA extractions were performed at the University of Pennsylvania, PA, U.S.A. Soil samples were thawed to 4ºC and centrifuged to separate and then discard the LifeGuard preservation solution. DNA was extracted using standard EMP protocols (http://www.earthmicrobiome.org/emp-standard-protocols/). We isolated DNA from 0.20 g of soil per extraction using the manufacturer-suggested protocol for PowerSoil® DNA Isolation Kit (MoBio), with the modification that we heated the extraction at 65°C for ten minutes prior to the initial bead beating and cell lysis step.

We performed the 291 bp length V4 region amplification using the 515F primer and the 806R Golay-barcoded reverse primers (for a full list of these primers visit http://www.earthmicrobiome.org/emp-standard-protocols/). Each 25µl PCR reaction contained 12µl of MoBio PCR Water (Certified DNA-Free), 10µl of 5 Prime HotMasterMix (1x), 1µl of Forward Primer (5uM concentration, 200pM final), 1µl Golay Barcode Tagged Reverse Primer (5µM concentration, 200pM final), and 1µl of template DNA. We performed PCR under the following conditions: 94°C for three minutes to denature the DNA, with 35 cycles at 94°C for 45 sec, 50°C for 60 sec, and 72°C for 90 sec, with a final extension of ten minutes at 72°C to ensure complete amplification. Each PCR reaction was performed in triplicate for each sample and then pooled together as one representative PCR product in order to restrict PCR bias. The DNA concentration of each aggregate PCR product was then quantified using PicoGreen (Invitrogen) and a microplate reader. Once quantified, we performed a second pooling, where different volumes of the discrete sample pools with the same DNA concentrations were combined in a single tube. This allowed for an equal amount of 16S rDNA amplicon sequences

from all samples in our study. We cleaned the final pool using the UltraClean® PCR Clean-Up Kit (MoBio). Sequencing was completed on the Illumina MiSeq platform.

Bioinformatic processing of DNA sequences

We quality trimmed 16S rDNA amplicon sequences using SolexaQA, eliminating regions that fell below a Q-score of 18 (Cox et al. 2010), which corresponds roughly to 98.4% or higher accuracy for each base position. We then retained only the longest continuous fragment that remained after trimming. If this fragment fell below 75% of the expected sequence length (i.e., < 112bp), we discarded it completely from further analyses. The 12bp barcode sequence enabled us to assign sequences to samples by searching against the PCR primer tags assigned to samples using BLAST+ (blastn, word size=4) (Camacho et al. 2009). We allowed up to one mismatch of the 12bp barcode sequences when assigning sample IDs. We used the open software package Quantitative Insights Into Microbial Ecology (QIIME) for downstream analyses of these data (Caporaso et al. 2010). We clustered sequences, de-novo, into Operational Taxonomic Units (OTUs) at 97% identity using the Uclust algorithm (Edgar 2010). We discarded singletons (OTUs with only one representative sequence) from further analysis, as they commonly result from chimeric PCR products or sequencing error, and are usually non-informative in the overall analysis of composition (Zhou et al. 2011).

From among the sequences assigned to an OTU, we chose a representative sequence to give the OTU a taxonomic identity. To do this, we chose the longest available sequence and then assigned taxonomic identification using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007). Representative sequences were

aligned using the Python Nearest Alignment Space Termination Tool (PyNAST) as implemented in QIIME. These aligned sequences, along with an OTU abundance matrix, were used to calculate beta diversity by means of weighted UniFrac distances with the QIIME script beta_diversity.py. By coupling the OTU abundance matrix with taxonomic classification of each OTU, a taxonomic summary was generated for each general taxonomic level (i.e. phylum, class, order, family, genus).

Statistical Analysis

We compared the soil bacterial diversity between Sample Types in two datasets: Dataset A compared replicates of the complete set of three individual cores to the associated mixed core. Dataset B compared replicates of one arbitrarily chosen individual soil core to the associated mixed soil sample. We used the same three overarching analyses for both datasets: 1) analysis of variance of distance matrices, 2) rarefaction and taxa accumulation curves and 3) taxonomic differences based on Bray-Curtis dissimilarities.

All analyses were performed in R using the vegan package (Oksanen et al. 2016). In order to investigate whether the patterns in community composition across our experiment differed for Dataset A (and again for Dataset B), we used the *adonis* function to perform an analysis of variance using Bray-Curtis distance matrices (Table 3.1). All comparisons were crossed with the Climate Manipulation treatment (OTC v. CON) and slope location.

In order to compare alpha diversity (defined above) between Sample Types, we constructed multiple rarefaction and taxa accumulation curves organized by different

taxonomic levels and experimental treatments (Fig. 3.1-3.2; Fig. C1-C4). We used QIIME (Caporaso et al. 2010) to produce collated files quantifying the observed number of OTUs (97% similarity cut-off) starting at ten and ending at 55,280 sequences per sample, with a step size of ~ 5,000 sequences. For each step there were ten iterations of random subsampling. The qiimer package in R (Bittinger 2015) was used to create the rarefaction curves. Alpha diversity was then compared between the average observed OTUs organized by slope location and Sample Type using rarefaction curves (Fig. 3.1; Fig. C3). Additionally, Sample Type comparisons were also organized by Climate Manipulation treatment and slope location (Fig. C1 and Fig. C2).

In addition to observed OTUs, the smallest and most specific taxonomic grouping, sequences were also analyzed at the phyla level, the broadest taxonomic grouping within the bacterial domain. At this broad level, we used the *rarecurve* and *specaccum* functions in vegan to create rarefaction and taxa accumulation curves (Fig. 3.2; Fig. C4). Analyses were done separately for the upper and lower slopes with only the control, and not the OTC, samples.

In order to investigate beta diversity (defined above), we used the *simper* function in vegan to compare the contribution of each bacterial phylum to the difference in community structure, measured with Bray-Curtis dissimilarities, between Sample Types (Table C.2 - C.3). We used the Kendall rank correlation coefficient to quantify the correlation of phyla contribution with a) phylum presence, which we defined as the percentage of soil samples in which each phylum was found, and b) phylum abundance or the average number of 16S DNA sequence reads/sample for each phylum, (Fig. 3.1; Fig. C5).

3.4 Results

There is no significant difference in the bacterial community composition, here defined as beta diversity, of individual versus mixed cores. The only significant difference in beta diversity in our soil samples can be attributed to slope, but not to Sample Type or Climate Manipulation treatment (Table 3.1). This holds whether comparing three individual cores to one mixed core (Dataset A) or one individual core to one mixed core (Dataset B).

There is a notable difference in OTU richness between slope locations, but not between individual and mixed core Sample Types (Fig. 3.1, Dataset A). Though the shape of the curves is slightly different, the same result holds when comparing one individual core to one mixed core (Fig. C3, Dataset B). The upper slope has higher OTU richness than the lower slope. The difference between Sample Types is also negligible when comparing the OTCs and controls on either slope and with either Dataset A or B (Fig. C1-2).

Overall phyla richness is greater on the upper slope compared to the lower slope when analyzing either Dataset A (Fig. 3.2) or B (Fig. C4). The average phyla richness across all mixed soil samples is similar to that across all individual cores on either slope, especially the lower (Fig. 3.2 b, d).

There is low Kendall rank correlation (τ) between the phylum contribution to the difference in Bray-Curtis distance between Sample Types and a) the percentage of soil samples in which a phylum is present (Fig. 3.3a, $\tau = 0.33$) or b) the relative abundance of each phylum, measured by the total number of sequence counts within a Sample Type

(Fig. 3.3b, $\tau = 0.30$). Though slight, there is a positive trend for both Kendall rank correlations. This suggests that phylum contribution—to the difference in community composition between Sample Types—may increase with relatively more common and abundant phyla. The most common phyla, Proteobacteria, drives this trend in that it shows the largest contribution between Sample Types for both Dataset A (Table C2) and B (Table C3; Fig C5).

3.5 Discussion

In the Mongolian steppe, we show that taking multiple soil samples and pooling them is unnecessary for analysis of bacterial richness and community composition. Within the context of our climate change experiment, a single core yielded the same experimental results as pooling three cores. Pooling samples is a standard practice in order to integrate spatial heterogeneity in the microbial community (Baker et al. 2009; O'Brien et al. 2016), but spatial heterogeneity at the decimeter scale at which we worked was inconsequential.

Heterogeneity in bacterial composition clearly exists at the landscape scale because slope location differed significantly in our analyses. The difference we uncovered at the scale of ~ 300 m could reflect any number of differences in abiotic properties, including soil carbon, N availability, and soil aridity, as well as differences in the plant community (Spence et al. 2014). Variation in the abiotic and biotic environment is not nearly so strong within our experimental plots.

Our work contributes to a small existing body of literature that examines spatial heterogeneity in soil bacterial communities. A variety of sampling techniques has been recommended for a range of soil studies (Petersen and Calvin 1965; Parkinson et al. 1971; Wollum 1994). While many studies have historically focused on the physiochemical aspects of the soil matrix (Bramley and White 1991; Klironomos et al. 1999), the advent of molecular microbial ecology has enabled more research on the biological component of soil ecology (Baker et al. 2009; O'Brien et al. 2016). The value of sample pooling is a fundamental question in both abiotic and biotic soil sampling studies (Bramley and White 1991). In our study, accumulation curves suggest that we captured similar amounts of phyla diversity whether we pooled the cores into a mixed sample or left them as individual soil cores (Fig. 3.2; Fig. C4), making the follow-up pooling step unnecessary. While this saves time and energy in terms of soil collection, there is a trade-off with subsequent molecular work and cost. Depending on the circumstances of a study, mixed soils samples may lessen the burden of DNA extraction and maximize the number of sequences per sample.

Our studies of alpha diversity show that the upper slope has higher phyla richness than the lower slope. This is true for both Datasets A and B at the smallest (Fig. 3.1-3.2; Fig. C1-C3) and largest (Fig. 3.2; Fig. C4) taxonomic levels of the bacterial domain. Such taxonomic consistency emphasizes the reproducibility of our results, which show that an individual soil core, or a set of them, has the almost same number of bacterial taxa as a mixed soil sample. The increased OTU richness on the relatively hotter and drier upper slope, as compared to the lower, is consistent with the theory of pore connectivity,

which links low soil pore connectivity, a result of low water content, with high bacterial diversity (Tiedje et al. 2001). Compared to wet conditions, bacterial motility and substrate diffusion are decreased in dry soil (Leadley and Raynaud 2004; Long and Or 2009). The theory posits that this decrease in fluidity may allow less competitive organisms to thrive in areas where they would otherwise be outcompeted for substrate by more mobile competitors. Hence, changes in moisture due to slope location may influence patterns of alpha diversity more than differences of Sample Type within the same slope.

Research questions are inextricably linked to the scale of the experimental design (Legendre and Legendre 1998). While the specific aim for this study was to explore diversity differences between Sample Types, the overarching question of our climate-change experiment was how the bacterial community responded to our experimental treatments. The data show that these response patterns did not change whether we were comparing a set of three individual cores (Dataset A) or only one individual core (Dataset B) to a mixed core (Table 3.1). The significant effect of slope, but not Sample Type or treatment, suggests that differences in community composition occur at relatively large (>100m) scales, between the upper and lower slope, but not at intermediate (<10m) scales, between the OTC and control plots, or small scales (<1m), between individual or mixed core. These findings are supported by (O'Brien et al. 2016), who found most distinct patterns in the composition of bacterial communities at the decimeter scale within an ecosystem (> 10m), relative to smaller (cm) or larger (km) scales. In contrast to that study, as well as others (Morris 1999; Baker et al. 2009; O'Brien et al. 2016), it appears

that compositing soil samples from the same plot is not necessary when investigating questions of community structure within our experimental design.

One caution of pooling soil cores into one sample is that information on an individual taxa's contribution to the community could be lost (Klironomos et al. 1999; Shade et al. 2013). In order to investigate this issue, we first asked if either rare or common taxa drove differences in community composition by quantifying the contribution of distinct phyla to the difference in community composition between Sample Types (individual vs. mixed cores). We then correlated these contributions with presence (Fig. 3.3a; Fig. C5a) and abundance (Fig. 3.3b; Fig. C5b) data, which are both measures of how common one phylum is relative to another. Using these results we could finally investigate if phyla specific information was lost upon pooling by comparing Dataset A (3 individual cores vs. 1 mixed core) to Dataset B (1 individual core vs. 1 mixed core).

Both datasets reached similar conclusions—there is a weak positive correlation between phylum contribution and how common that phyla are relative to others. For both datasets, this trend is driven by the most common phylum Proteobacteria, while the rarest phyla contribute the least to differences in community composition between Sample Type (Table S2-3). If the results were different for Datasets A and B, it would suggest that comparing one individual core to one mixed core was very different than comparing a set of three individual cores to one mixed core. Instead, our rigorous analysis methods suggest that no matter the sampling method, information on community structure will not be lost.

Motivated by the desire to produce reliable and representative results, all sound ecological studies should investigate the best scale at which to account for the spatial heterogeneity inherent to any ecosystem. Our results suggest that taking only one soil core is necessary to answer the questions we posed about bacterial diversity within the context of our climate-change experiment. Implementing these findings as practice can make for a much more efficient sampling strategy, not only in our study but possibly other similarly sized field experiments (within the bounds of 300 m^2). We hope that such studies may be able benefit from ours, by using it as a guide to determine the sampling scheme that is right for their experimental question and design.

Table 3.1: **Analysis of variance using Bray-Curtis dissimilarities to measure differences in bacterial community composition** between two Datasets: A) three individual cores compared to a mixed core and B) one individual core compared to a mixed core. All comparisons are crossed with the Climate Manipulation treatment (OTC v. CON) and analyzed by slope location. Community composition analyzed at the phylum level.

slope location	Dataset	Sample Type	Treatment	slope
Both slopes	A	0.709	0.095	**0.001*** **
	B	0.300	0.836	**0.001*** **
Upper slope	A	0.935	0.328	N/A
	B	0.721	0.739	N/A
Lower slope	A	0.779	0.303	N/A
	B	0.671	0.790	N/A

There were no significant interactions. Numerical values indicate Pr(>F). Significance codes: '***' 0.001; '**' 0.01; '*' 0.0.

Figure 3.1: Rarefaction curves examining cumulative number of observed OTUs, clustered at a 97% similarity threshold, as a function of the number of sequence reads in samples grouped by slope location (Lower v. Upper) and Sample Type (three individual cores v. one mixed core). These curves show OTU richness and how well sequence coverage characterized the community. Additional curves comparing the observed OTUs between only one individual soil core and one mixed core show similar results (**Figure C3**).

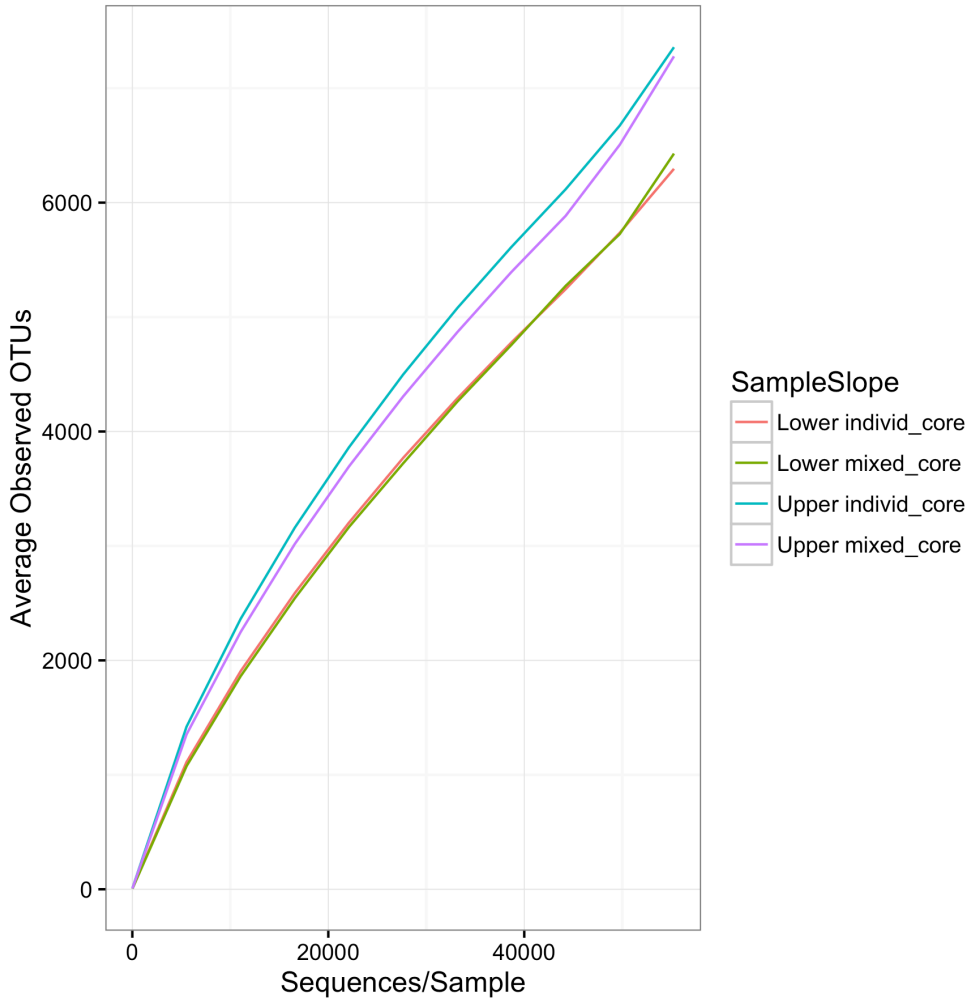


Figure 3.2: Rarefaction and phyla accumulation curves for soil samples from control plots on the Upper (a, b) and Lower (c, d) slopes. Rarefaction curves (a, c) examine phyla richness for each soil sample as a function of the number of sequences in individual cores soil cores (blue) and mixed soil cores (red). Phyla accumulation curves (b,d) quantify the average phyla richness of individual soil cores (blue) and mixed soil cores (red) as a function of the number of samples examined.

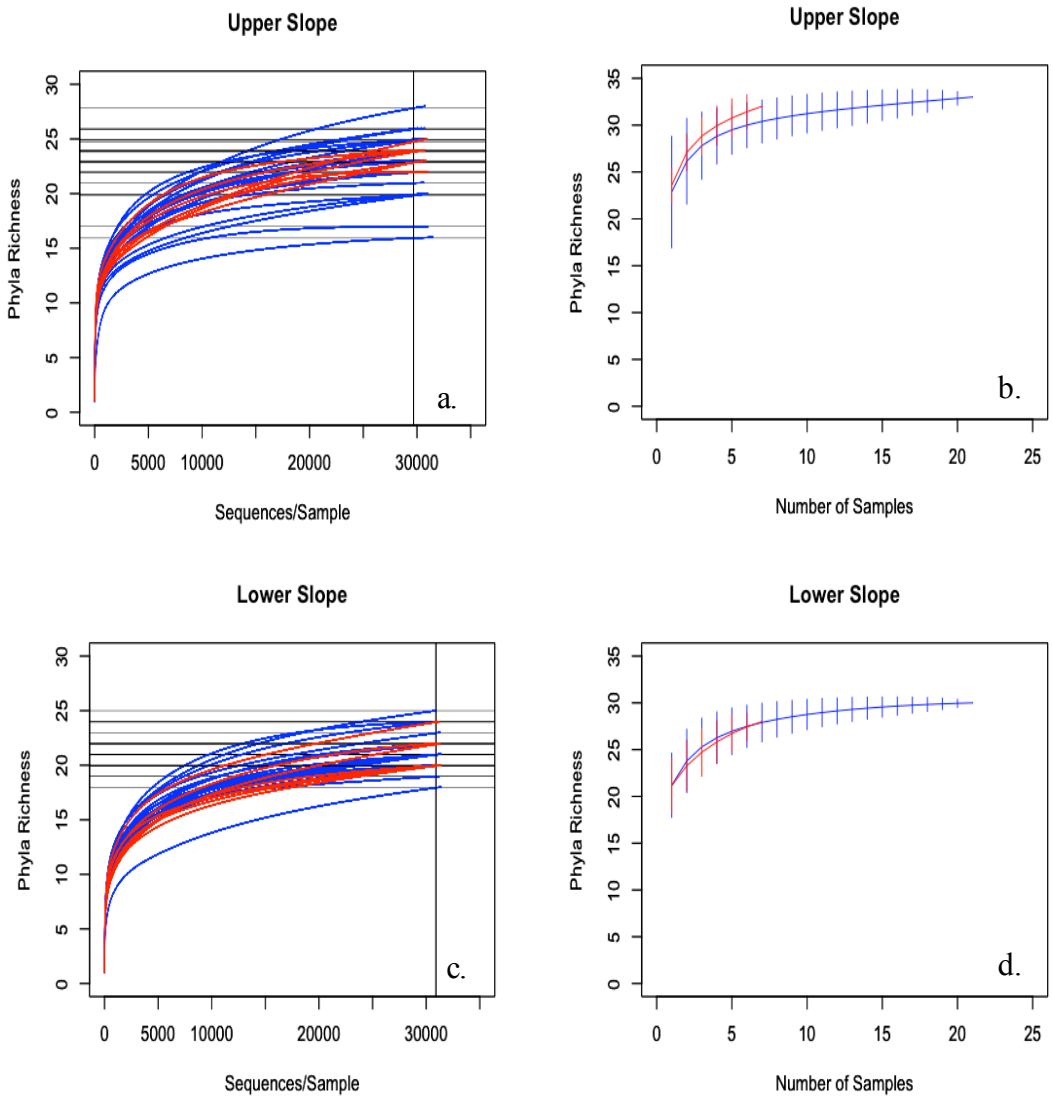
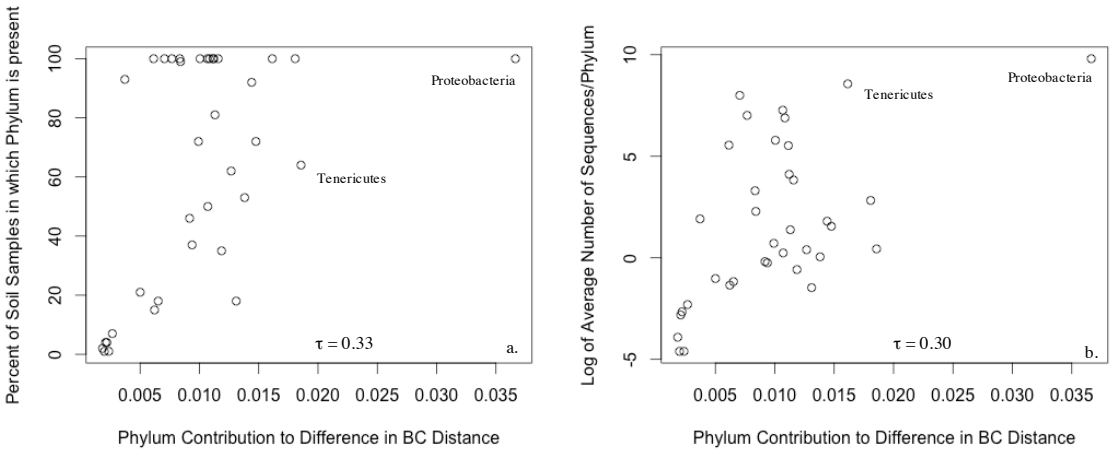


Figure 3.3: Plots comparing the bacterial community composition between two soil Sample Types (three individual cores v. mixed core). Community composition was measured using Bray-Curtis dissimilarities at the phylum level. The contribution values indicate the importance of each phylum to the difference in community composition between Sample Types. Each point represents a phylum detailed in Table C2**.** The two phyla with the highest contribution value are labeled. Plot illustrate the correlation between contribution values and phylum presence (a) and abundance (b). The Kendall correlation value is reported as τ. Phylum presence is measured by the percentage of the total number of soil samples in which each phylum is present. Abundance is represented by the average number of 16S DNA sequences per soil sample for each phylum.



DISCUSSION

There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.

(Darwin 1859)

Microorganisms are the most astounding example of life's immense diversity. Though we now know that they are found in all three domains of life (Woese et al. 1990), two of which are exclusively microbial, "men groped and fumbled for thousands of years without seeing things that lay right under their noses" (de Kruif 1940).

> Antonie van Leeuwenhoek (1632 – 1723), was the first person to ever observe microorganisms through a magnifying lens. He referred to them as "cavorting, wee beasties" and "animalcules."
>
> But Carl Linnaeus (1707 – 1778), the king of cataloguing the diversity of organisms, "threw up his hands at the very idea of studying the wee beasts. … 'They are too small, too confused, no one will ever know anything about them, we will simply put them in the class of Chaos,' " he said (de Kruif 1940).

Now almost three hundred years later, thousands of microbial taxa have been catalogued and the pace of discovery only increases with time (Hug et al. 2016).

The importance of microbes to the functioning of life on Earth is indisputable (Falkowski et al. 2008). Yet, for a more complete understanding of them, we must understand fundamental aspects of microbial diversity (Prosser et al. 2007). We need to know not only "Who is there?" but also, "Where and how abundant are they in time and

space?" As the field of microbial biogeography catches up to that of macrobial ecology (Martiny et al. 2006), the ecological theory surrounding these invisible communities develops basic, yet vital, hypotheses that can be tested (Prosser et al. 2007). With Next-Generation Sequencing techniques, we can explore similar hypotheses about where the highest diversity of microbes can be found in a particular habitat or how microbial communities will change with time. Such hypotheses help us navigate the sea of sequencing data produced by these techniques and provide the guidance necessary to enable future studies of intricately complex microbial systems.

My dissertation investigates the unparalleled bacterial diversity of soil (Fierer and Lennon 2011). This work is unique in that it spans multiple temporal and spatial scales within the context of a fully factorial global-change experiment. Set in northern Mongolia, our results document patterns of soil bacterial diversity in an understudied region of the Eurasian steppe (Liancourt et al. 2012), which is experiencing drastic climate and land-use changes (Namkhaijantsan 2006; Dagvadorj et al. 2011).

We explored the drivers of soil bacterial diversity from many angles. From broad to fine scales, we used a slew of metrics to investigate responses to multiple experimental treatments and correlations with an array of environmental factors and found that, above all else, changes in diversity are driven by time and space. Across all chapters, differences in bacterial diversity are seen consistently seen between slope positions, but not at the plot or soil core level. This reinforces the conclusion that spatial variation drives the structure of bacterial communities at relatively large (~300m), as compared to small (>10m), spatial scales. Chapters 1 and 2 suggest that the influence of spatial scale

on diversity is modulated by time. Over the course of two months, changes in bacterial diversity are primarily explained by differences in slope (Chapter 2), but this trend shifts at larger temporal scales. Over the course of two years, bacterial communities vary more with time than with slope location (Chapter 1). These findings underscore the necessity of accounting for spatial and temporal variability at multiple scales in any study of microbial ecology, whether it is rooted in experimental manipulations or not.

Out of the three experimental treatments, Climate Manipulation showed the largest effect on soil bacterial diversity. The differences in alpha diversity between OTCs and controls were negligible at both large and small scales (Chapters 1 and 3, respectively) and there was only difference in community composition when phylogeny was taken into account (Chapter 1). Structural equation modeling (Chapter 2) suggests that this response can be attributed to the OTCs decreasing soil moisture content. Though watering was investigated only in one chapter, it too affected bacterial diversity through its influence on soil moisture content. Hence, decreases or increases in soil moisture may be primary drivers of soil bacterial diversity. Grazing also reduces soil moisture, but this correlation appears to be much weaker, as this treatment it did not have an effect on bacterial diversity at any scale or using any metric across two studies (Chapters 1 and 2).

Soil bacterial diversity may be driven not only by soil moisture at the community level but also by soil moisture at the individual phylum level. Across all three years of data, our findings suggest that Verrucomicrobia thrives in dry habitats. While Chapter 1 reveals that Verrucomicrobia is significantly more abundant on the drier and hotter Upper Slope, Chapter 2 goes further and identifies Verrucomicrobia as an indicator taxon,

present in the relatively drier plots within the Watering and Climate Manipulation treatments. This phylum's consistent abundance patterns across an aridity gradient illustrate that there is ecological coherency, over a wide-range of temporal and spatial scales, at the broadest level of bacterial classification.

This work provides a guide to the search for the ecological significance of the most enigmatic and diverse forms of life on the planet. In our efforts to illuminate the depths of this invisible diversity, we were able to uncover consistent patterns, despite the extreme complexity of soil and the bacterial communities that live in it. Overall, we found that these patterns were dictated by temporal and spatial variation, followed by differences in moisture.

Our results show that, to put it simply, change is the interaction of time and space.

CONCLUSION

My dissertation research explores soil bacterial diversity and the environmental factors that drive the structure of various bacterial communities through time and across the landscape. With the guidance of my mentors, I examined how soil bacterial communities respond to treatments within a global change experiment in the Mongolian steppe. At different temporal and spatial scales, I investigated shifts in bacterial diversity across three experimental treatments: a Watering treatment (Upper Slope only), a Grazing treatment (Lower Slope only) and a Climate Manipulation treatment (both slopes). I measured diversity using both the number and abundance of distinct bacterial taxa in a soil sample and then correlated these findings with corresponding measurements of biotic and abiotic factors. At the community level, I documented patterns of alpha and beta-diversity. For the purposes of this work, I define alpha diversity as taxa richness within an experimental plot (e.g., a watered plot or its unwatered control). I define beta-diversity as the variation in community composition between plots, measured by pairwise dissimilarities using both taxa richness and abundance counts. These pairwise dissimilarities were quantified with taxonomic (Bray-Curtis) and phylogenetic (UniFrac) distances. Finally, I identified shifts in diversity at the individual phylum level. Below I summarize the overarching findings from each chapter of my dissertation.

Chapter 1: Comparing soil bacterial diversity between two slope locations (Upper and Lower) and two years (2010 and 2011)

I. The variation in soil bacterial communities is explained more by temporal and spatial factors than by our experimental manipulations. At the individual taxon level, significant abundance gradients of notable phyla were correlated with abiotic gradients between years and across the landscape.

   a. The greatest separation in alpha diversity was between the two years and the two slope locations.

   b. Year was the only factor in which differences in beta-diversity were significant for both taxonomic and phylogenetic measures. Community composition significantly differed by slope when using taxonomic dissimilarities, while community composition significantly differed with Climate Manipulation when using phylogenetic dissimilarities. Beta-diversity was not affected by grazing using either taxonomic or phylogenetic dissimilarities.

   c. The phylum Firmicutes was more abundant in 2010 on the Lower Slope and showed strong negative and positive correlations with total available nitrogen and soil temperature, respectively. Verrucomicrobia patterns were the opposite—this phylum was more abundant in 2011 on the Upper Slope and showed weak positive and negative correlations with total available nitrogen and soil temperature, respectively. Firmicutes

abundance showed absolutely no correlation with soil moisture gradients, while Verrucomicrobia showed a slight negative correlation.

Chapter 2: Comparing soil bacterial diversity between two slope locations (Upper and Lower) and between two months (June and July) in one year (2012)

II. Structural equation modeling suggests that slope location and moisture regime are the primary drivers drivers of bacterial diversity in our system. Indicator taxa analysis identified two phyla that may act as sentinels of progressive aridity stress.

   a. Soil bacteria phyla richness was influenced by abiotic factors but not by plant biomass or plant species richness. Though independent, there was a consistent response of bacterial and plant richness to the experimental treatments and abiotic factors on the hotter, drier Upper Slope, which was not seen on the cooler, wetter Lower Slope. There were differences in the responses to experimental treatments between months, though these were not as consistent as those between slopes.

   b. Across the landscape and through time, all experimental treatments consistently altered soil moisture and inconsistently altered temperature, total available N and pH.

   c. Relative abundance of two indicator phyla, Verrucomicrobia and Elusimicrobia, decreased in wetter conditions and increased with soil drying. Specifically, the abundance of both phyla was relatively lower in

d. Watered plots compared to the associated controls on the Upper Slope and was relatively higher in the Climate Manipulation plots compared to the associated controls on the Lower Slope.

Chapter 3: Comparing soil bacterial diversity between Sample Types (individual and mixed soil cores) across slope locations (Upper and Lower) within one month (June, 2012)

I. We found that there was a negligible difference in soil bacterial diversity between Sample Types–whether we compared a set of three individual soil cores or one individual soil core to a mixed soil sample (composed of equal subsamples from the associated set of three individual soil cores).

   a. There are relatively large differences in alpha diversity between the Upper and Lower Slope as compared to the negligible differences seen between Sample Types or within the Climate Manipulation treatment. This is true at the finest taxonomic level of observed OTUs and the broadest taxonomic level of phyla.

   b. Patterns of beta-diversity remain the same whether comparing three individual soil cores or one individual soil core to one mixed core. There is a significant difference in community composition between slopes, but not between Climate Manipulation treatments or Sample Types.

   c. The degree to which phyla are rare or common across soil samples has a weak positive correlation to the contribution each phylum plays in the differences in community composition between Sample Types.

APPENDIX

APPENDIX A: Chapter One

Table A1: List of the 68 samples collected for this study. Sample number 21* is an outlier with anomalously low number of sequences and OTUs; therefore, it was not included in the analyses. Climate manipulation (by Open Top Chamber, O; Control, C) was crossed with grazing (grazing, G; no grazing, N) on the lower slope in a fully factorial design. The grazing was not applied on the upper slope.

No.	Sample	Block	OTC	Grazing	Year	Slope
1	B01.CG.Y11	01	C	G	2011	Lower
2	B01.CN.Y11	01	C	N	2011	Lower
3	B01.OG.Y11	01	O	G	2011	Lower
4	B01.ON.Y11	01	O	N	2011	Lower
5	B02.CG.Y10	02	C	G	2010	Lower
6	B02.CN.Y10	02	C	N	2010	Lower
7	B02.OG.Y10	02	O	G	2010	Lower
8	B02.ON.Y10	02	O	N	2010	Lower
9	B02.CG.Y11	02	C	G	2011	Lower
10	B02.CG.Y11.1	02	C	G	2011	Lower
11	B02.CN.Y11	02	C	N	2011	Lower
12	B02.CN.Y11.1	02	C	N	2011	Lower
13	B02.OG.Y11	02	O	G	2011	Lower
14	B02.ON.Y11	02	O	N	2011	Lower
15	B03.CG.Y10	03	C	G	2010	Lower
16	B03.CN.Y10	03	C	N	2010	Lower
17	B03.OG.Y10	03	O	G	2010	Lower
18	B03.CG.Y11	03	C	G	2011	Lower
19	B03.CN.Y11	03	C	N	2011	Lower
20	B03.OG.Y11	03	O	G	2011	Lower
21*	**B03.ON.Y10**	**03**	**O**	**N**	**2011**	**Lower**
22	B04.CN.Y10	04	C	N	2010	Lower
23	B04.OG.Y10	04	O	G	2010	Lower
24	B04.ON.Y10	04	O	N	2010	Lower
25	B04.CG.Y11	04	C	G	2011	Lower
26	B04.CN.Y11	04	C	N	2011	Lower
27	B04.OG.Y11.1	04	O	G	2011	Lower
28	B04.ON.Y11.1	04	O	N	2011	Lower
29	B04.ON.Y11.2	04	O	N	2011	Lower
30	B05.CG.Y11	05	C	G	2011	Lower
31	B05.CN.Y11	05	C	N	2011	Lower
32	B05.OG.Y11	05	O	G	2011	Lower
33	B05.ON.Y11	05	O	N	2011	Lower

Table A1, continued.

No.	Sample	Block	OTC	Grazing	Year	Slope
34	B06.CG.Y10	06	C	G	2010	Lower
35	B06.CN.Y10	06	C	N	2010	Lower
36	B06.OG.Y10	06	O	G	2010	Lower
38	B06.CG.Y11	06	C	G	2011	Lower
39	B06.CN.Y11	06	C	N	2011	Lower
40	B06.OG.Y11	06	O	G	2011	Lower
41	B06.ON.Y11	06	O	N	2011	Lower
42	B07.CG.Y11	07	C	G	2011	Lower
43	B07.CN.Y11	07	C	N	2011	Lower
44	B07.OG.Y11	07	O	G	2011	Lower
45	B07.ON.Y11	07	O	N	2011	Lower
46	B08.CG.Y11	08	C	G	2011	Lower
47	B08.CN.Y11	08	C	N	2011	Lower
48	B08.OG.Y11	08	O	G	2011	Lower
49	B08.ON.Y11	08	O	N	2011	Lower
50	B09.CN.Y11	09	C	N	2011	Upper
51	B09.ON.Y11	09	O	N	2011	Upper
52	B10.CN.Y11	10	C	N	2011	Upper
53	B10.ON.Y11	10	O	N	2011	Upper
54	B11.CN.Y11	11	C	N	2011	Upper
55	B11.ON.Y11	11	O	N	2011	Upper
56	B12.CN.Y10	12	C	N	2010	Upper
57	B12.CN.Y11	12	C	N	2011	Upper
58	B12.ON.Y11	12	O	N	2011	Upper
59	B13.CN.Y10	13	C	N	2010	Upper
60	B13.ON.Y10	13	O	N	2010	Upper
61	B13.CN.Y11	13	C	N	2011	Upper
62	B13.ON.Y11	13	O	N	2011	Upper
63	B14.CN.Y11	14	C	N	2011	Upper
64	B14.ON.Y11	14	O	N	2011	Upper
65	B15.CN.Y10	15	C	N	2010	Upper
66	B15.ON.Y10	15	O	N	2010	Upper
67	B15.CN.Y11	15	C	N	2011	Upper
68	B15.ON.Y11	15	O	N	2011	Upper

Table A2: Permutation tests of the effects of Climate manipulation (OTC, control), Grazing (grazing, no grazing), Year (2010, 2011) and location on the south-facing Slope (upper, lower slope) on soil bacterial communities based on (a) Bray-Curtis and (b) UniFrac distances. The two analyses labeled Lower and Upper Slope were performed on the entire set of 67 soil samples (Table A1). Because the grazing manipulation, which was applied only on the lower slope, was a significant factor in the full model using Bray-Curtis distances, a second model, labeled Lower Slope Only and excluding the upper slope plots, was run. Grazing was not significant in this second model. In parallel with the Bray-Curtis analysis, a second model was performed using UniFrac dissimilarities though grazing was not significant in the first model.

a. Bray-Curtis: Taxonomic Analysis

	Lower and Upper Slope			Lower Slope Only		
	Df	Variance	Pr (>F)	Df	Variance	Pr (>F)
Climate (C)	1	0.0739	0.21	1	0.07710	0.28
Grazing (G)	1	0.1921	**0.01***	1	0.08368	0.15
Year (Y)	1	0.4121	**0.01***	1	0.35387	**0.01***
Slope (S)	1	0.5469	**0.01***	n/a	n/a	n/a
C:G	1	0.0639	0.48	1	0.06354	0.64
C:Y	1	0.0725	0.35	1	0.05822	0.78
G:Y	1	0.0665	0.40	1	0.05030	0.95
C:S	1	0.0554	0.75	n/a	n/a	n/a
Y:S	1	0.1074	**0.03***	n/a	n/a	n/a
C:G:Y	1	0.0481	0.91	1	0.05266	0.94
C:Y: S	1	0.0441	0.95	n/a	n/a	n/a
Residual	55	3.4483		40	2.74299	
Total	66	5.3212		47	3.48236	

Table A2, continued.

b. UniFrac: Phylogenetic Analysis

	Lower and Upper Slope			Lower Slope Only		
	Df	Variance	Pr (>F)	Df	Variance	Pr (>F)
Climate (C)	1	1.1679	**0.01***	1	0.07710	**0.01***
Grazing (G)	1	0.3250	0.12	1	0.08368	0.57
Year (Y)	1	1.9106	**0.01***	1	0.35387	**0.01***
Slope (S)	1	0.3789	0.10	n/a	n/a	n/a
C:G	1	0.1774	0.29	1	0.06354	0.89
C:Y	1	0.1478	0.35	1	0.05822	0.14
G:Y	1	0.1844	0.26	1	0.05030	0.80
C:S	1	0.2166	0.25	n/a	n/a	n/a
Y:S	1	0.1607	0.33	n/a	n/a	n/a
C:G:Y	1	0.0753	0.71	1	0.05266	0.98
C:Y: S	1	0.2130	0.25	n/a	n/a	n/a
Residual	55	8.9525		40	2.74299	
Total	66	13.9101		47	3.48236	

.*Significant p-values indicated in bold.

Table A3: Sources of variation due to of climate manipulation, grazing, year, and slope location on soil bacterial communities based on the CAP analyses using (a) Bray-Curtis and (b) UniFrac distances. The second model focused only on plots on the lower slope, where the grazing treatment was applied. Companion table for Table A2 and (a) Fig. 1.1 and (b) Fig. 1.3.

a. Bray-Curtis: Taxonomic Analysis

	Lower and Upper Slope		**Lower Slope Only**	
	Inertia	Proportion	Inertia	Proportion
Constrained	1.6830[1]	0.322	0.7390	0.212
CAP Axis 1	0.6749[1]	0.401[1]	0.3559	0.482
CAP Axis 2	0.3934	0.234	0.1105	0.149
Remaining Axes	0.6405	0.381	0.2725	0.369
Unconstrained	3.5480	0.678	2.7430	0.788
Total	5.2310	1.000	3.4820	1.0000

b. UniFrac: Phylogenetic Analysis

	Lower and Upper Slope		**Lower Slope Only**	
	Inertia	Proportion	Inertia	Proportion
Constrained	4.9580	0.3564	3.0710	0.3679
CAP Axis 1	2.9599	0.5970	2.2323	0.7269
CAP Axis 2	1.0393	0.2096	0.7025	0.2288
Remaining Axes	0.9588	0.1934	0.1362	0.0443
Unconstrained	8.9530	0.6436	5.2770	0.6321
Total	13.9100	1.0000	8.3480	1.0000

[1]The proportion of constrained variation of an axis equals the proportion of the total constrained variation (e.g., for CAP axis 1 in (a) Bray-Curtis, 0.6749/1.6830 = 0.401).

Table A4: Significance of environmental gradients in the CAP analyses based on (a) Bray-Curtis and (b) UniFrac distances. Each gradient is represented by contour lines and dashed-arrow vectors that indicate the overall direction of the combined contour lines in (a) Fig. 1.2 and (b) Fig. 1.3.

a. Bray-Curtis: Taxonomic Analysis

Environmental Factor	r^2	**Pr (>r)**
Total Nitrogen (NO_3^- + NH_4^+)	0.1482	**0.006***
Nitrate (NO_3^-)	0.2097	**0.001 ***
Ammonium (NH_4^+)	0.1745	**0.001 ***
Temperature	0.5669	**0.001 ***
Moisture	0.5921	**0.001 ***

b. UniFrac: Phylogenetic Analysis

Environmental Factor	r^2	**Pr (>r)**
Total Nitrogen (NO_3^- + NH_4^+)	0.0582	0.154
Nitrate (NO_3^-)	0.1177	**0.020***
Ammonium (NH_4^+)	0.0426	0.228
Moisture	0.2333	**0.001***
Temperature	0.0613	0.122

*Significant p-values indicated in bold.

Table A5: Significance of phylum abundance gradients in the CAP analysis based on Bray-Curtis distances. Each abundance gradient is represented by solid-arrow vectors in Fig. 1.2. Note that there were no significant phylum abundance gradients identified by the UniFrac analysis.

Bray-Curtis: Taxonomic Analysis

Taxa	Pr(>r)*	MEDIAN*	MIN*	MAX*
Verrucomicrobia	**0.001**	947	129	18837
Planctomycetes	**0.003**	119	22	805
Firmicutes	**0.005**	22152	803	54232

Significant p-values indicated in bold are followed by the median, minimum and maximum number of 16S sequences representing each phylum's abundance across the study's 67 samples.

Figure A1: Rarefaction curves examining cumulative number of genera as a function of the number of sequence reads in samples grouped by (a) year and slope location or (b) year and climate manipulation treatment (OTC and CON). These curves show genera richness and how well sequence coverage characterized the community. OTC, Open Top Chamber; CON, Control.

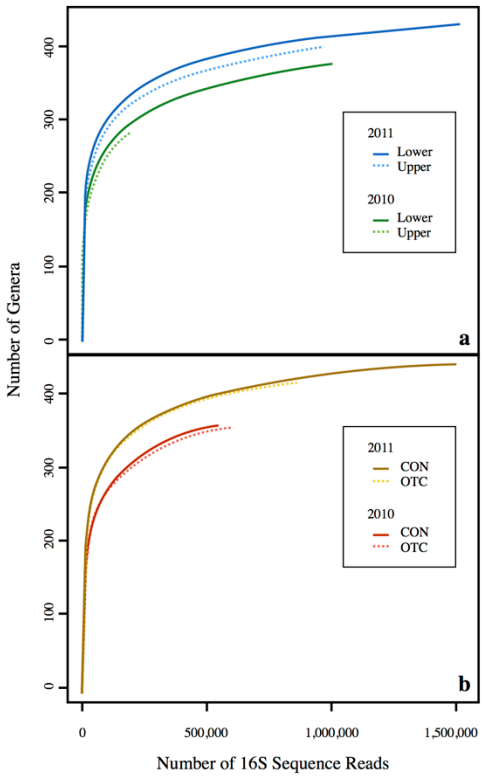


Figure A2: Three types of significant nitrogen gradients: (a) total plant available N, (b) NH_4^+ and (c) NO_3^-, represented by contour lines imposed upon CAP plots using Bray-Curtis (BC) distances. The overall direction of the combined contour lines is indicated by dashed-arrow vectors. Note: (a) is a combination of (b) and (c). Companion figure for Table A4a.

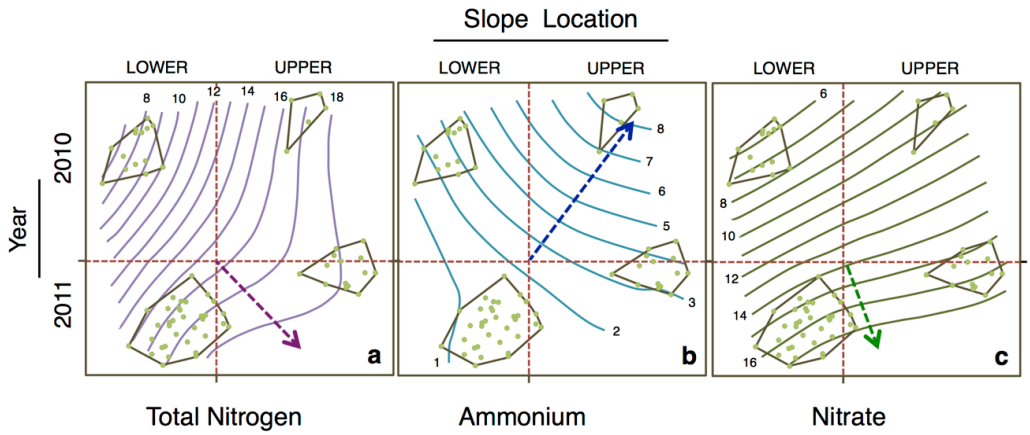
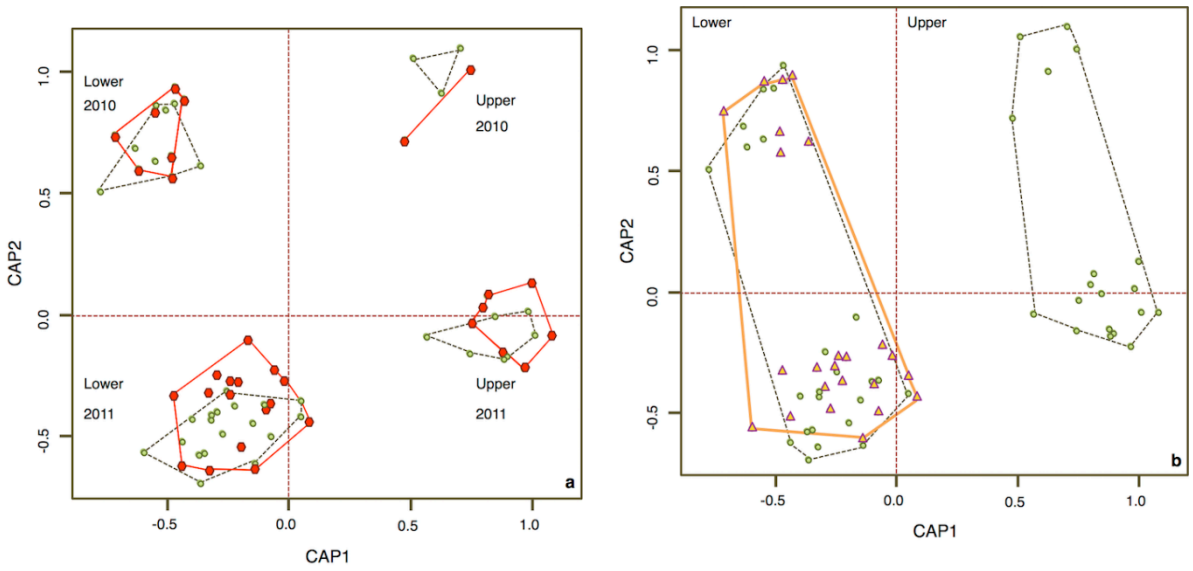


Figure A3: (a) Climate manipulation and (b) grazing treatments are shown here as insignificant factors in CAP plots of taxonomic community relatedness, based on Bray-Curtis distances: (a) hexagons encircled by solid lines represent samples from OTC plots, circles encircled by dashed lines represent samples taken from control plots, (b) triangles encircled by solid lines represent samples from grazed plots, circles encircled by dashed lines represent samples taken from control plots. Companion figure for Tables 1 and S2a.



APPENDIX B: CHAPTER TWO

Table B.1: List of the 120 samples collected for this study. Starred (*) samples had anomalously low number of sequences and OTUs; therefore, they were not included in the analyses. Climate manipulation (by Open Top Chamber, OTC; Control, CON) was crossed with watering on the upper slope (watering, W; no watering N) and grazing (grazing, G; no grazing, N) on the lower slope in a fully factorial design. Dataset: A) all samples from the upper slope, B) only climate manipulation samples from both slopes C) all samples from the lower slope, respectively.

Table B1 A – Upper Slope Samples including Watering Treatment

No.	PlotID	Month	Block	Slope	OTC	Water	Graze	Dataset
1	B09.CON.N.W	June	B09	Upper	CON	W	N	A
2	B09.OTC.N.W	June	B09	Upper	OTC	W	N	A
3	B10.CON.N.W	June	B10	Upper	CON	W	N	A
4	B10.OTC.N.W	June	B10	Upper	OTC	W	N	A
5	B11.CON.N.W	June	B11	Upper	CON	W	N	A
6	B11.OTC.N.W	June	B11	Upper	OTC	W	N	A
7	B12.CON.N.W	June	B12	Upper	CON	W	N	A
8	B12.OTC.N.W	June	B12	Upper	OTC	W	N	A
9	B13.CON.N.W	June	B13	Upper	CON	W	N	A
10	B13.OTC.N.W	June	B13	Upper	OTC	W	N	A
11	B14.CON.N.W	June	B14	Upper	CON	W	N	A
12	B14.OTC.N.W	June	B14	Upper	OTC	W	N	A
13	B15.CON.N.W	June	B15	Upper	CON	W	N	A
14	B15.OTC.N.W	June	B15	Upper	OTC	W	N	A
15	B09.CON.N.W	July	B09	Upper	CON	W	N	A
16	B09.OTC.N.W	July	B09	Upper	OTC	W	N	A
17	B10.CON.N.W	July	B10	Upper	CON	W	N	A
18	B10.OTC.N.W	July	B10	Upper	OTC	W	N	A
19	B11.CON.N.W	July	B11	Upper	CON	W	N	A
20	B11.OTC.N.W	July	B11	Upper	OTC	W	N	A
21	B12.CON.N.W	July	B12	Upper	CON	W	N	A
22	B12.OTC.N.W	July	B12	Upper	OTC	W	N	A
23	B13.CON.N.W	July	B13	Upper	CON	W	N	A
24	B13.OTC.N.W	July	B13	Upper	OTC	W	N	A
25	B14.CON.N.W	July	B14	Upper	CON	W	N	A
26	B14.OTC.N.W	July	B14	Upper	OTC	W	N	A
27	B15.CON.N.W	July	B15	Upper	CON	W	N	A
28	B15.OTC.N.W	July	B15	Upper	OTC	W	N	A

Table B1, continued

Table B1 AB – Upper Slope Samples also found in Both Slope Dataset

No.	PlotID	Month	Block	Slope	OTC	Water	Graze	Dataset
29	B09.CON.N.N	June	B09	Upper	CON	N	N	AB
30	B09.OTC.N.N	June	B09	Upper	OTC	N	N	AB
31	B10.CON.N.N	June	B10	Upper	CON	N	N	AB
32	B10.OTC.N.N	June	B10	Upper	OTC	N	N	AB
33	B11.CON.N.N	June	B11	Upper	CON	N	N	AB
34	B11.OTC.N.N	June	B11	Upper	OTC	N	N	AB
35	B12.CON.N.N	June	B12	Upper	CON	N	N	AB
36	B12.OTC.N.N	June	B12	Upper	OTC	N	N	AB
37	B13.CON.N.N	June	B13	Upper	CON	N	N	AB
38	B13.OTC.N.N	June	B13	Upper	OTC	N	N	AB
39	B14.CON.N.N	June	B14	Upper	CON	N	N	AB
40	B14.OTC.N.N	June	B14	Upper	OTC	N	N	AB
41	B15.CON.N.N	June	B15	Upper	CON	N	N	AB
42	B15.OTC.N.N	June	B15	Upper	OTC	N	N	AB
43	B09.CON.N.N	July	B09	Upper	CON	N	N	AB
44	B09.OTC.N.N	July	B09	Upper	OTC	N	N	AB
45	B10.CON.N.N	July	B10	Upper	CON	N	N	AB
46	B10.OTC.N.N	July	B10	Upper	OTC	N	N	AB
47	B11.CON.N.N	July	B11	Upper	CON	N	N	AB
48	B11.OTC.N.N	July	B11	Upper	OTC	N	N	AB
49	B12.CON.N.N	July	B12	Upper	CON	N	N	AB
50	B12.OTC.N.N	July	B12	Upper	OTC	N	N	AB
51	B13.CON.N.N	July	B13	Upper	CON	N	N	AB
52	B13.OTC.N.N	July	B13	Upper	OTC	N	N	AB
53	B14.CON.N.N	July	B14	Upper	CON	N	N	AB
54	B14.OTC.N.N	July	B14	Upper	OTC	N	N	AB
55	B15.CON.N.N	July	B15	Upper	CON	N	N	AB
56	B15.OTC.N.N	July	B15	Upper	OTC	N	N	AB

Table B1, continued

Table B1 BC – Lower Slope Samples also found in Both Slope Dataset

No.	PlotID	Month	Block	Slope	OTC	Water	Graze	Dataset
57	B01.CON.N.N	June	B01	Lower	CON	N	N	BC
58	B01.OTC.N.N	June	B01	Lower	OTC	N	N	BC
59	*B02.OTC.N.N	July	B03	Lower	OTC	N	N	BC
60	B02.CON.N.N	June	B02	Lower	CON	N	N	BC
61	B03.CON.N.N	June	B03	Lower	CON	N	N	BC
62	B03.OTC.N.N	June	B03	Lower	OTC	N	N	BC
63	B04.CON.N.N	June	B04	Lower	CON	N	N	BC
64	B04.OTC.N.N	June	B04	Lower	OTC	N	N	BC
65	B05.CON.N.N	June	B05	Lower	CON	N	N	BC
66	B05.OTC.N.N	June	B05	Lower	OTC	N	N	BC
67	B06.CON.N.N	June	B06	Lower	CON	N	N	BC
68	B06.OTC.N.N	June	B06	Lower	OTC	N	N	BC
69	B07.CON.N.N	June	B07	Lower	CON	N	N	BC
70	B07.OTC.N.N	June	B07	Lower	OTC	N	N	BC
71	B08.CON.N.N	June	B08	Lower	CON	N	N	BC
72	B08.OTC.N.N	June	B08	Lower	OTC	N	N	BC
73	B01.CON.N.N	July	B01	Lower	CON	N	N	BC
74	B01.OTC.N.N	July	B01	Lower	OTC	N	N	BC
75	*B02.CON.N	July	B02	Lower	CON	N	N	BC
76	B02.OTC.N.N	July	B02	Lower	OTC	N	N	BC
77	B03.CON.N.N	July	B03	Lower	CON	N	N	BC
78	B03.OTC.N.N	July	B03	Lower	OTC	N	N	BC
79	B04.CON.N.N	July	B04	Lower	CON	N	N	BC
80	B04.OTC.N.N	July	B04	Lower	OTC	N	N	BC
81	B05.CON.N.N	July	B05	Lower	CON	N	N	BC
82	B05.OTC.N.N	July	B05	Lower	OTC	N	N	BC
83	*B06.CON.N	July	B06	Lower	CON	N	N	BC
84	B06.OTC.N.N	July	B06	Lower	OTC	N	N	BC

Table B1, continued

Table B1 BC – Lower Slope Samples with grazing treatment

No.	PlotID	Month	Block	Slope	OTC	Water	Graze	Dataset
89	B01.CON.G.N	June	B01	Lower	CON	N	G	C
90	B01.OTC.G.N	June	B01	Lower	OTC	N	G	C
91	*B02.OTC.G.N	June	B02	Lower	OTC	N	G	C
92	B02.CON.G.N	June	B02	Lower	CON	N	G	C
93	B03.CON.G.N	June	B03	Lower	CON	N	G	C
94	B03.OTC.G.N	June	B03	Lower	OTC	N	G	C
95	B04.CON.G.N	June	B04	Lower	CON	N	G	C
96	B04.OTC.G.N	June	B04	Lower	OTC	N	G	C
97	B05.CON.G.N	June	B05	Lower	CON	N	G	C
98	B05.OTC.G.N	June	B05	Lower	OTC	N	G	C
99	B06.CON.G.N	June	B06	Lower	CON	N	G	C
100	B06.OTC.G.N	June	B06	Lower	OTC	N	G	C
101	B07.CON.G.N	June	B07	Lower	CON	N	G	C
102	B07.OTC.G.N	June	B07	Lower	OTC	N	G	C
103	B08.CON.G.N	June	B08	Lower	CON	N	G	C
104	B08.OTC.G.N	June	B08	Lower	OTC	N	G	C
105	B01.CON.G.N	July	B01	Lower	CON	N	G	C
106	B01.OTC.G.N	July	B01	Lower	OTC	N	G	C
107	B02.CON.G.N	July	B02	Lower	CON	N	G	C
108	B02.OTC.G.N	July	B02	Lower	OTC	N	G	C
109	B03.CON.G.N	July	B03	Lower	CON	N	G	C
110	B03.OTC.G.N	July	B03	Lower	OTC	N	G	C
111	B04.CON.G.N	July	B04	Lower	CON	N	G	C
112	B04.OTC.G.N	July	B04	Lower	OTC	N	G	C
113	B05.CON.G.N	July	B05	Lower	CON	N	G	C
114	B05.OTC.G.N	July	B05	Lower	OTC	N	G	C
115	B06.CON.G.N	July	B06	Lower	CON	N	G	C
116	B06.OTC.G.N	July	B06	Lower	OTC	N	G	C
117	B07.CON.G.N	July	B07	Lower	CON	N	G	C
118	B07.OTC.G.N	July	B07	Lower	OTC	N	G	C
119	B08.CON.G.N	July	B08	Lower	CON	N	G	C
120	B08.OTC.G.N	July	B08	Lower	OTC	N	G	C

Table B.2a: List of the bacterial phyla that were identified as indicators of the experimental treatments on the upper and lower slope in June and July (Datasets A and C respectively, see Table B1). Within each treatment, each indicator phyla are accompanied by its indicator value (IndVal) and probability score (*p*). Only significant phyla ($p < 0.05$) are reported. The letter in parenthesis directly after the name of each phyla specifies whether it is an indicator taxon for the treatment or its control (watering, W/no watering, N; OTC, O/ control, C; grazing, G/no grazing, N).

		JUNE			JULY		
Dataset	Treatment	Indicator Phylum	IndVal	*p*	Indicator Phylum	IndVal	*p*
Upper Slope: Watering Experiment (A)	Watering	Elusimicrobia (N)	0.6562	0.014	Nitrospirae (N)	0.6172	0.024
		WS3 (N)	0.5159	0.012	Acidobacteria (N)	0.5943	0.007
					Gemmatimonadetes (N)	0.5902	0.042
					Firmicutes (N)	0.5851	0.021
					Verrucomicrobia (N)	0.5817	0.04
					Actinobacteria (N)	0.5656	0.017
					Chloroflexi (N)	0.5649	0.044
					Planctomycetes (N)	0.5634	0.033
					Proteobacteria (W)	0.5402	0.001
	OTC	None			None		
Lower Slope: Grazing Experiment (C)	Grazing	Bacteroidetes (G)	0.57	0.049	Fibrobacteres (N)	0.3492	0.04
	OTC	Verrucomicrobia (O)	0.5948	0.021	WYO (O)	0.6375	0.019
					Elusimicrobia (O)	0.6263	0.047

Table B.2b: List of the bacterial phyla that were identified as indicators of the climate manipulation (OTC) or slope location treatments in June and July (Dataset B, see Table B1). Reported values are equivalent to those in Table B2a. The letter in parenthesis directly after the name of each phyla specifies whether it is an indicator taxa for the upper slope (U) in comparison to the lower slope (L) or for the OTC (O) compared to its control (C).

		JUNE			JULY		
Dataset	Treatment	Indicator Phylum	IndVal	*p*	Indicator Phylum	IndVal	*p*
Both Slopes: Slope Experiment (B)	Slope	TM6 (L)	0.8921	0.001	TM6 (L)	0.9333	0.001
		WS3 (L)	0.6877	0.005	Chlorobi (L)	0.7500	0.001
		Chlamydiae (L)	0.6587	0.022	Chlamydiae (L)	0.6829	0.008
		Proteobacteria (L)	0.5568	0.001	WS3 (L)	0.6759	0.010
		WYO (U)	0.846	0.001	Verrucomicrobia (L)	0.6506	0.001
		Gemmatimonadetes (U)	0.7818	0.001	Fibrobacteres (L)	0.3446	0.041
		Elusimicrobia (U)	0.7390	0.001	OP11 (L)	0.3077	0.033
		Actinobacteria (U)	0.7167	0.001	WYO (U)	0.7056	0.001
		Planctomycetes (U)	0.6983	0.001	Gemmatimonadetes (U)	0.6282	0.001
		Armatimonadetes (U)	0.6861	0.001	Actinobacteria (U)	0.6161	0.001
		Chloroflexi (U)	0.6813	0.001	Thermi (U)	0.3571	0.042
		Nitrospirae (U)	0.6798	0.002			
		TM7 (U)	0.6499	0.030			
		Acidobacteria (U)	0.6496	0.001			
		Bacteroidetes (U)	0.6218	0.001			
		Tenericutes (U)	0.5971	0.046			
		BRC1 (U)	0.5895	0.048			
		WS2 (U)	0.5075	0.048			
		OP3(U)	0.4298	0.048			
	OTC	None			None		

APPENDIX C: Chapter Three

Table C.1: List of the 120 samples collected for this study. Bolded samples had anomalously low number of sequences and OTUs; therefore, they were not included in the analyses. Climate Manipulation Treatment: Open Top Chamber, OTC; Control, CON. Soil sampling scheme: within an experimental plot, one "Mixed core" (MX) was composed of equal aliquots from the three "Individual cores" (1C, 2C, 3C).

No	Sample Name	Block	Slope	Treatment	Sample Type	Core
1	**B01.CON.1C**	**B01**	**Lower**	**CON**	**Individual**	**1C**
2	**B01.CON.2C**	**B01**	**Lower**	**CON**	**Individual**	**2C**
3	**B01.CON.3C**	**B01**	**Lower**	**CON**	**Individual**	**3C**
4	**B01.CON.MX**	**B01**	**Lower**	**CON**	**Mixed Core**	**MX**
5	**B01.OTC.1C**	**B01**	**Lower**	**OTC**	**Individual**	**1C**
6	**B01.OTC.2C**	**B01**	**Lower**	**OTC**	**Individual**	**2C**
7	**B01.OTC.3C**	**B01**	**Lower**	**OTC**	**Individual**	**3C**
8	**B01.OTC.MX**	**B01**	**Lower**	**OTC**	**Mixed Core**	**MX**
9	B02.CON.1C	B02	Lower	CON	Individual	1C
10	B02.CON.2C	B02	Lower	CON	Individual	2C
11	B02.CON.3C	B02	Lower	CON	Individual	3C
12	B02.CON.MX	B02	Lower	CON	Mixed Core	MX
13	**B02.OTC.1C**	**B02**	**Lower**	**OTC**	**Individual**	**1C**
14	**B02.OTC.2C**	**B02**	**Lower**	**OTC**	**Individual**	**2C**
15	**B02.OTC.3C**	**B02**	**Lower**	**OTC**	**Individual**	**3C**
16	**B02.OTC.MX**	**B02**	**Lower**	**OTC**	**Mixed Core**	**MX**
17	B03.CON.1C	B03	Lower	CON	Individual	1C
18	B03.CON.2C	B03	Lower	CON	Individual	2C
19	B03.CON.3C	B03	Lower	CON	Individual	3C
20	B03.CON.MX	B03	Lower	CON	Mixed Core	MX
21	B03.OTC.1C	B03	Lower	OTC	Individual	1C
22	B03.OTC.2C	B03	Lower	OTC	Individual	2C
23	B03.OTC.3C	B03	Lower	OTC	Individual	3C
24	B03.OTC.MX	B03	Lower	OTC	Mixed Core	MX
25	B04.CON.1C	B04	Lower	CON	Individual	1C
26	B04.CON.2C	B04	Lower	CON	Individual	2C

Table C1. (continued)

No	Sample Name	Block	Slope	Treatment	Sample Type	Core
27	B04.CON.3C	B04	Lower	CON	Individual core	3C
28	B04.CON.MX	B04	Lower	CON	Mixed Core	MX
29	**B04.OTC.1C**	**B04**	**Lower**	**OTC**	**Individual core**	**1C**
30	**B04.OTC.2C**	**B04**	**Lower**	**OTC**	**Individual core**	**2C**
31	**B04.OTC.3C**	**B04**	**Lower**	**OTC**	**Individual core**	**3C**
32	**B04.OTC.MX**	**B04**	**Lower**	**OTC**	**Mixed Core**	**MX**
33	B05.CON.1C	B05	Lower	CON	Individual core	1C
34	B05.CON.2C	B05	Lower	CON	Individual core	2C
35	B05.CON.3C	B05	Lower	CON	Individual core	3C
36	B05.CON.MX	B05	Lower	CON	Mixed Core	MX
37	B05.OTC.1C	B05	Lower	OTC	Individual core	1C
38	B05.OTC.2C	B05	Lower	OTC	Individual core	2C
39	B05.OTC.3C	B05	Lower	OTC	Individual core	3C
40	B05.OTC.MX	B05	Lower	OTC	Mixed Core	MX
41	B06.CON.1C	B06	Lower	CON	Individual core	1C
42	B06.CON.2C	B06	Lower	CON	Individual core	2C
43	B06.CON.3C	B06	Lower	CON	Individual core	3C
44	B06.CON.MX	B06	Lower	CON	Mixed Core	MX
45	B06.OTC.1C	B06	Lower	OTC	Individual core	1C
46	B06.OTC.2C	B06	Lower	OTC	Individual core	2C
47	B06.OTC.3C	B06	Lower	OTC	Individual core	3C
48	B06.OTC.MX	B06	Lower	OTC	Mixed Core	MX
49	B07.CON.1C	B07	Lower	CON	Individual core	1C
50	B07.CON.2C	B07	Lower	CON	Individual core	2C
51	B07.CON.3C	B07	Lower	CON	Individual core	3C
52	B07.CON.MX	B07	Lower	CON	Mixed Core	MX
53	B07.OTC.1C	B07	Lower	OTC	Individual core	1C
54	B07.OTC.2C	B07	Lower	OTC	Individual core	2C
55	B07.OTC.3C	B07	Lower	OTC	Individual core	3C
56	B07.OTC.MX	B07	Lower	OTC	Mixed Core	MX
57	B08.CON.1C	B08	Lower	CON	Individual core	1C
58	B08.CON.2C	B08	Lower	CON	Individual core	2C
59	B08.CON.3C	B08	Lower	CON	Individual core	3C
60	B08.CON.MX	B08	Lower	CON	Mixed Core	MX
61	B08.OTC.1C	B08	Lower	OTC	Individual core	1C
62	B08.OTC.2C	B08	Lower	OTC	Individual core	2C
63	B08.OTC.3C	B08	Lower	OTC	Individual core	3C

Table C1. (continued)

No	Sample Name	Block	Slope	Treatment	Sample Type	Core
64	B08.OTC.MX	B08	Lower	OTC	Mixed Core	MX
65	B09.CON.1C	B09	Upper	CON	Individual	1C
66	B09.CON.2C	B09	Upper	CON	Individual	2C
67	B09.CON.3C	B09	Upper	CON	Individual	3C
68	B09.CON.MX	B09	Upper	CON	Mixed Core	MX
69	**B09.OTC.1C**	**B09**	**Upper**	**OTC**	**Individual**	**1C**
70	**B09.OTC.2C**	**B09**	**Upper**	**OTC**	**Individual**	**2C**
71	**B09.OTC.3C**	**B09**	**Upper**	**OTC**	**Individual**	**3C**
72	**B09.OTC.MX**	**B09**	**Upper**	**OTC**	**Mixed Core**	**MX**
73	B10.CON.1C	B10	Upper	CON	Individual	1C
74	B10.CON.2C	B10	Upper	CON	Individual	2C
75	B10.CON.3C	B10	Upper	CON	Individual	3C
76	B10.CON.MX	B10	Upper	CON	Mixed Core	MX
77	B10.OTC.1C	B10	Upper	OTC	Individual	1C
78	B10.OTC.2C	B10	Upper	OTC	Individual	2C
79	B10.OTC.3C	B10	Upper	OTC	Individual	3C
80	B10.OTC.MX	B10	Upper	OTC	Mixed Core	MX
81	B11.CON.1C	B11	Upper	CON	Individual	1C
82	B11.CON.2C	B11	Upper	CON	Individual	2C
83	B11.CON.3C	B11	Upper	CON	Individual	3C
84	B11.CON.MX	B11	Upper	CON	Mixed Core	MX
85	B11.OTC.1C	B11	Upper	OTC	Individual	1C
86	B11.OTC.2C	B11	Upper	OTC	Individual	2C
87	B11.OTC.3C	B11	Upper	OTC	Individual	3C
88	B11.OTC.MX	B11	Upper	OTC	Mixed Core	MX
89	B12.CON.1C	B12	Upper	CON	Individual	1C
90	B12.CON.2C	B12	Upper	CON	Individual	2C
91	B12.CON.3C	B12	Upper	CON	Individual	3C
92	B12.CON.MX	B12	Upper	CON	Mixed Core	MX
93	B12.OTC.1C	B12	Upper	OTC	Individual	1C
94	B12.OTC.2C	B12	Upper	OTC	Individual	2C
95	B12.OTC.3C	B12	Upper	OTC	Individual	3C
96	B12.OTC.MX	B12	Upper	OTC	Mixed Core	MX
97	B13.CON.1C	B13	Upper	CON	Individual	1C
98	B13.CON.2C	B13	Upper	CON	Individual	2C
99	B13.CON.3C	B13	Upper	CON	Individual	3C
100	B13.CON.MX	B13	Upper	CON	Mixed Core	MX

Table C1. (continued

No	Sample Name	Block	Slope	Treatmen	Sample	Core
101	B13.OTC.1C	B13	Upper	OTC	Individual	1C
102	B13.OTC.2C	B13	Upper	OTC	Individual	2C
103	B13.OTC.3C	B13	Upper	OTC	Individual	3C
104	B13.OTC.MX	B13	Upper	OTC	Mixed Core	MX
105	B14.CON.1C	B14	Upper	CON	Individual	1C
106	B14.CON.2C	B14	Upper	CON	Individual	2C
107	B14.CON.3C	B14	Upper	CON	Individual	3C
108	B14.CON.MX	B14	Upper	CON	Mixed Core	MX
109	B14.OTC.1C	B14	Upper	OTC	Individual	1C
110	B14.OTC.2C	B14	Upper	OTC	Individual	2C
111	B14.OTC.3C	B14	Upper	OTC	Individual	3C
112	B14.OTC.MX	B14	Upper	OTC	Mixed Core	MX
113	B15.CON.1C	B15	Upper	CON	Individual	1C
114	B15.CON.2C	B15	Upper	CON	Individual	2C
115	B15.CON.3C	B15	Upper	CON	Individual	3C
116	B15.CON.MX	B15	Upper	CON	Mixed Core	MX
117	B15.OTC.1C	B15	Upper	OTC	Individual	1C
118	B15.OTC.2C	B15	Upper	OTC	Individual	2C
119	B15.OTC.3C	B15	Upper	OTC	Individual	3C
120	B15.OTC.MX	B15	Upper	OTC	Mixed Core	MX

Table C.2: Comparison of phyla from three individual cores to one mixed core (Dataset A). Contribution denotes the average contribution of each phylum to the overall dissimilarity between the bacterial communities in the two Sample Types (individual v. mixed cores). Average sequence denotes the average number of 16S DNA sequence reads/sample for each phylum. Percent present denotes the percentage of soil samples in which each phylum was found.

Phyla	Contribution	Average Sequences/Sample	Percent
Proteobacteria	0.03664167	18114.02	100
Tenericutes	0.018566324	1.55	64
Chlorobi	0.018073229	16.86	100
Firmicutes	0.016141857	5252.23	100
TM6	0.014763859	4.72	72
Chlamydiae	0.014408731	6.06	92
OD1	0.013815273	1.05	53
OP11	0.013103082	0.23	18
WS3	0.012678155	1.49	62
WPS	0.011869009	0.56	35
WYO	0.011583278	46.09	100
Elusimicrobia	0.011310846	3.98	81
Nitrospirae	0.011216232	61	100
Gemmatimonadetes	0.01114608	251.22	100
Bacteroidetes	0.010854289	978.92	100
OP3	0.010708957	1.27	50
Verrucomicrobia	0.010672359	1434.63	100
Planctomycetes	0.010053828	326.1	100
BRC1	0.009920504	2.04	72
AD3	0.009381112	0.78	37
WS2	0.009175016	0.83	46
Cyanobacteria	0.008408246	9.83	99
Armatimonadetes	0.008343655	27.2	100
Acidobacteria	0.007673515	1110.33	100
Actinobacteria	0.007054664	2982.07	100
MVP	0.00652464	0.31	18
Thermi	0.006217112	0.26	15
Chloroflexi	0.006138805	256.56	100
Fibrobacteres	0.005007828	0.36	21
TM7	0.003713333	6.79	93
Fusobacteria	0.002661508	0.1	7
GOUTA4	0.002356062	0.01	1
SR1	0.002197666	0.07	4
NKB19	0.002086572	0.06	4
GAL15	0.0019836	0.01	1
GN02	0.001832853	0.02	2

Table C.3: Comparison of phyla from three individual cores to one mixed core (Dataset B). Same as Table C2, but with Dataset B: Comparison one individual core to one mixed core.

Phyla	Contribution	Average Sequences/Sample	% Presence
Proteobacteria	0.028053244	18114.02	100
Firmicutes	0.019824191	5252.23	100
Chlorobi	0.016213919	16.86	100
Chlamydiae	0.014808182	6.06	92
Tenericutes	0.014534922	1.55	64
TM6	0.012946336	4.72	72
WYO	0.01192203	46.09	100
OP11	0.011454933	0.23	18
Armatimonadetes	0.011442531	27.2	100
OD1	0.011412224	1.05	53
Elusimicrobia	0.011254515	3.98	81
MVP	0.011027448	0.31	18
OP3	0.010966521	1.27	50
TM7	0.010919574	6.79	93
Nitrospirae	0.010888601	61	100
WS3	0.010875701	1.49	62
Bacteroidetes	0.010712733	978.92	100
WPS	0.010457755	0.56	35
Verrucomicrobia	0.010452015	1434.63	100
Gemmatimonadetes	0.009777506	251.22	100
BRC1	0.009239986	2.04	72
Planctomycetes	0.008829813	326.1	100
AD3	0.008230999	0.78	37
WS2	0.007955744	0.83	46
Cyanobacteria	0.007448687	9.83	99
Thermi	0.007211977	0.26	15
Acidobacteria	0.006417631	1110.33	100
Chloroflexi	0.006363988	256.56	100
Fibrobacteres	0.005927632	0.36	21
Actinobacteria	0.005798623	2982.07	100
GN02	0.004979696	0.02	2
Fusobacteria	0.004363576	0.1	7
NKB19	0.002627154	0.06	4
GOUTA4	0.002125865	0.01	1
SR1	0.001938022	0.07	4
GAL15	0.001723427	0.01	1

Figure C.1: OTU richness comparison between three individual soil cores to one mixed core. Rarefaction curves examining cumulative number of observed OTUs, clustered at a 97% similarity threshold, as a function of the number of sequence reads in samples grouped by slope location (Lower v. Upper), Climate Manipulation treatment (OTC v. CON), Sample Type (three individual cores v. one mixed core). These curves show OTU richness and how well sequence coverage characterized the community.

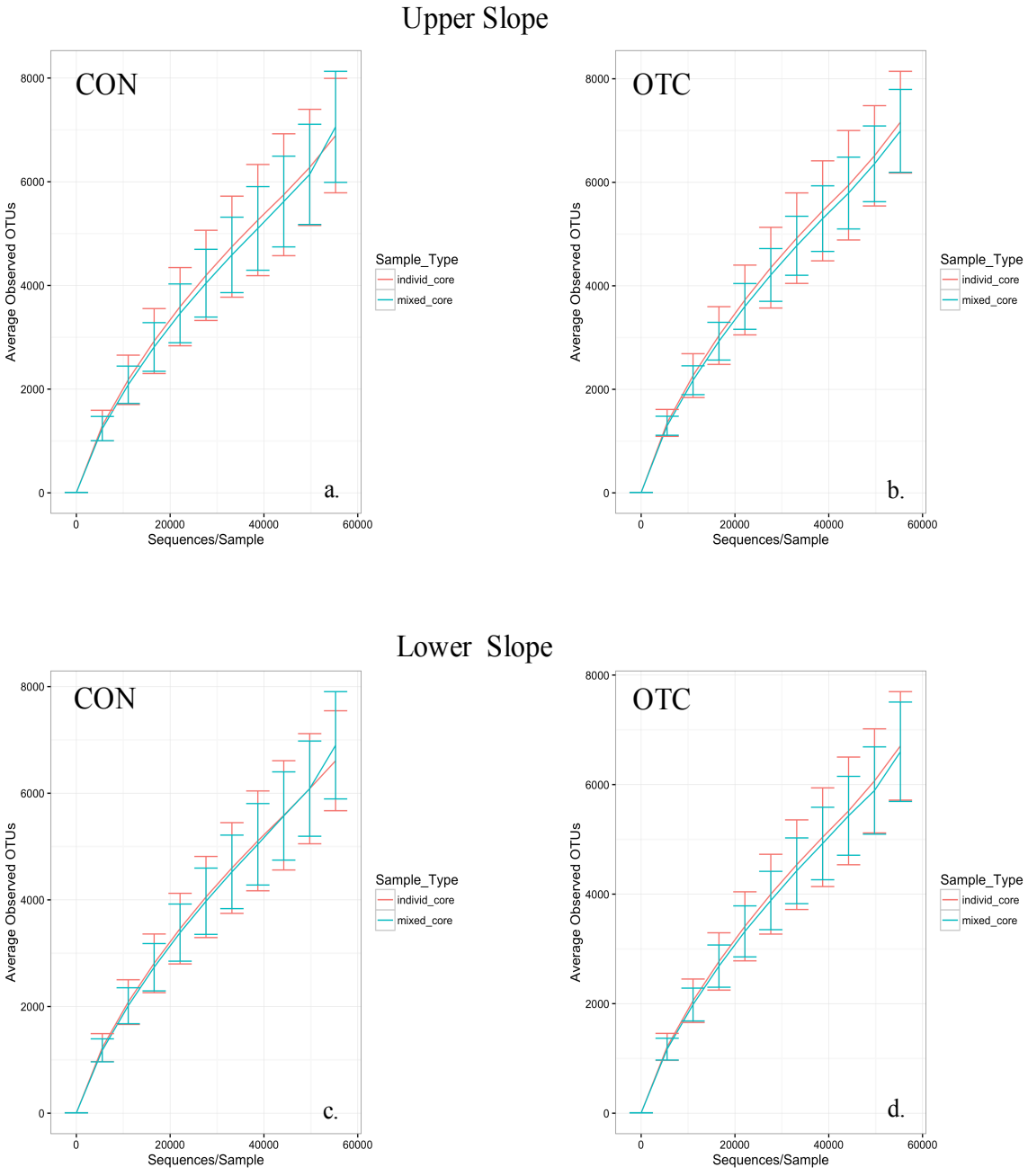


Figure C.2: OTU richness comparison between one individual soil core to one mixed core. Format same as Figure C1.

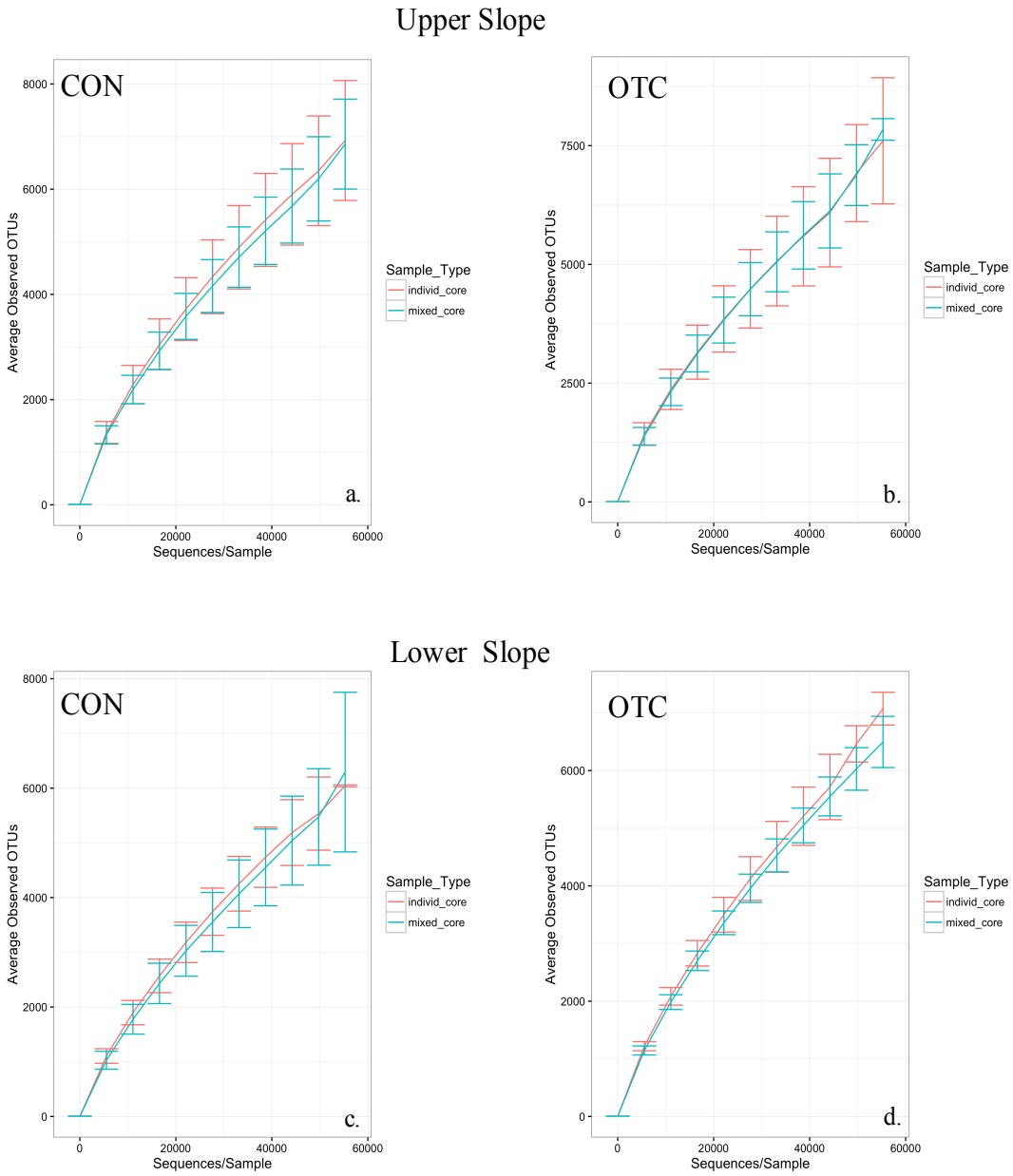


Figure C.3: Rarefaction curves grouped by slope and soil sample type comparing the observed OTUs between three individual soil cores and one mixed core (a) and only one individual soil core and one mixed core (b).

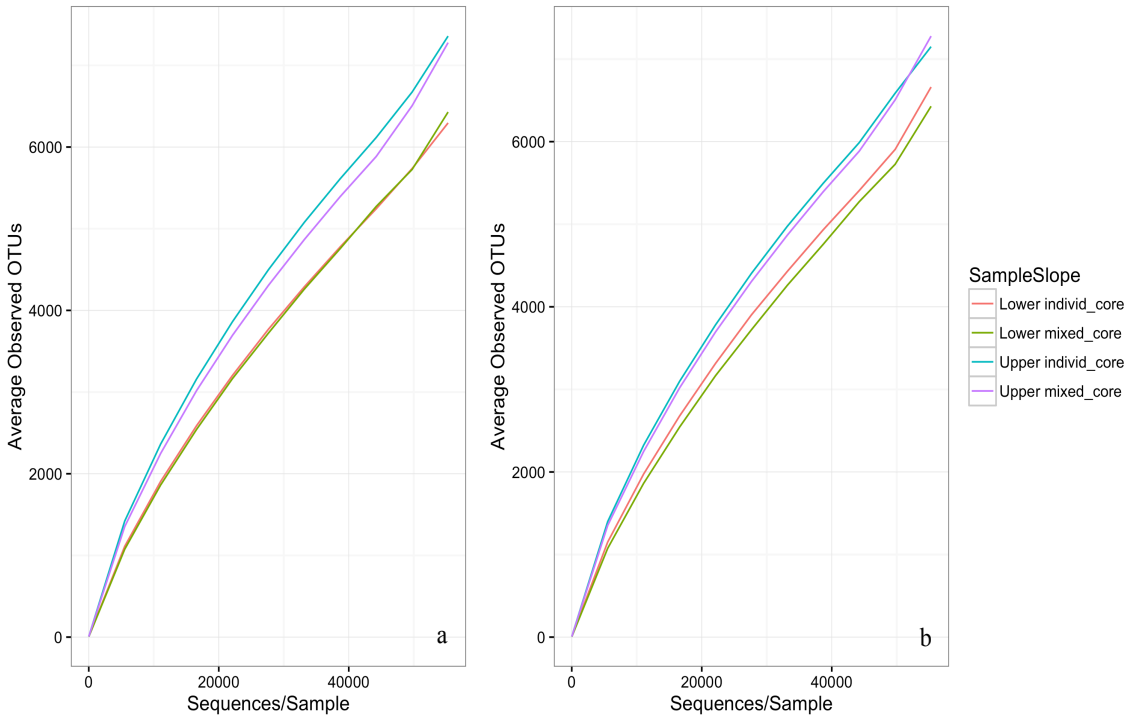


Figure C.4: Rarefaction and phyla accumulation curves for soil samples from control plots on the upper (a,b) and lower (c,d) slope. Complimentary to Figure 3.2, but for the comparison between one individual core and the mixed soil core. Rarefaction curves (a,c) examine phyla richness for each soil sample as a function of the number of sequences in individual cores soil cores (blue) and mixed soil cores (red). Phyla accumulation curves (b,d) quantify the average phyla richness of individual soil cores (blue) and mixed soil cores (red) as a function of the number of samples examined.

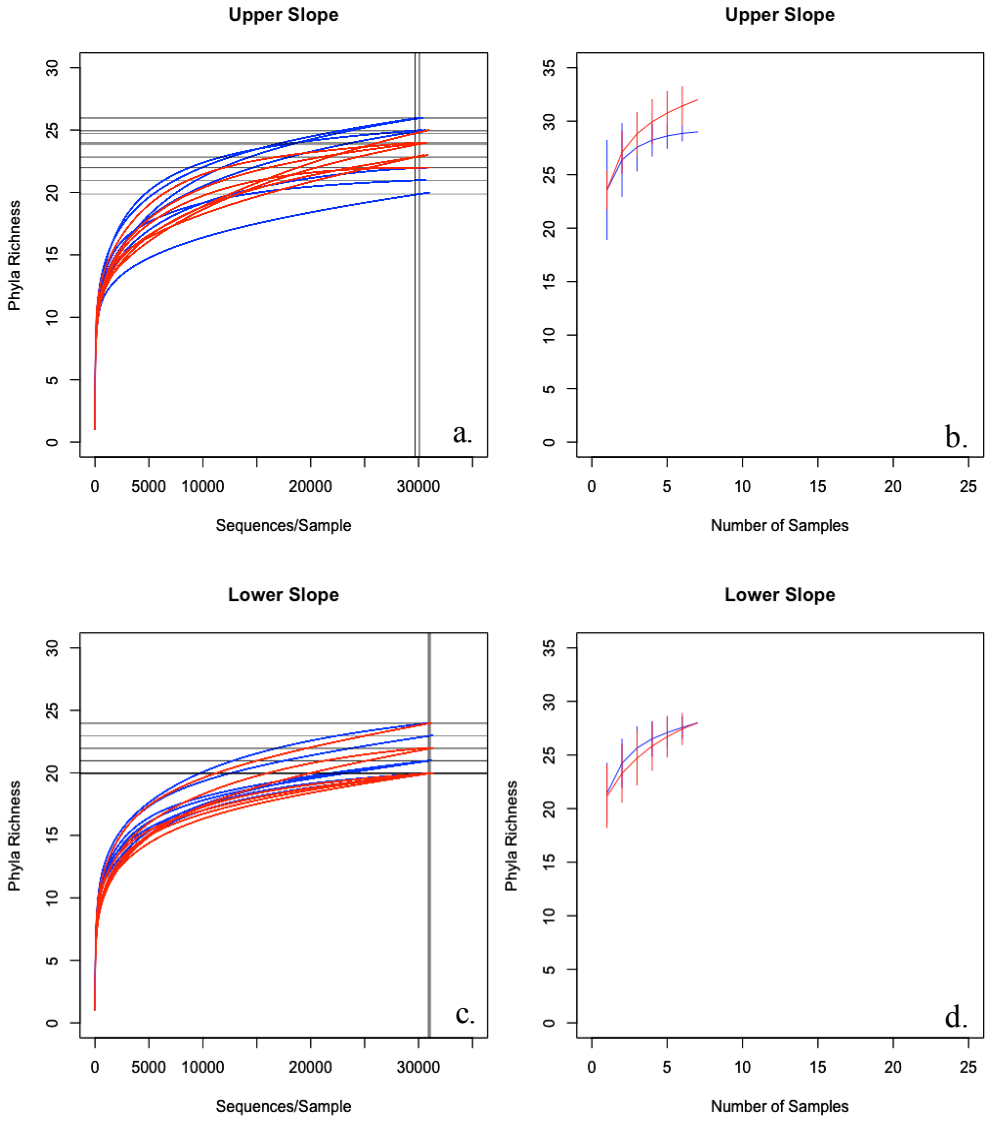
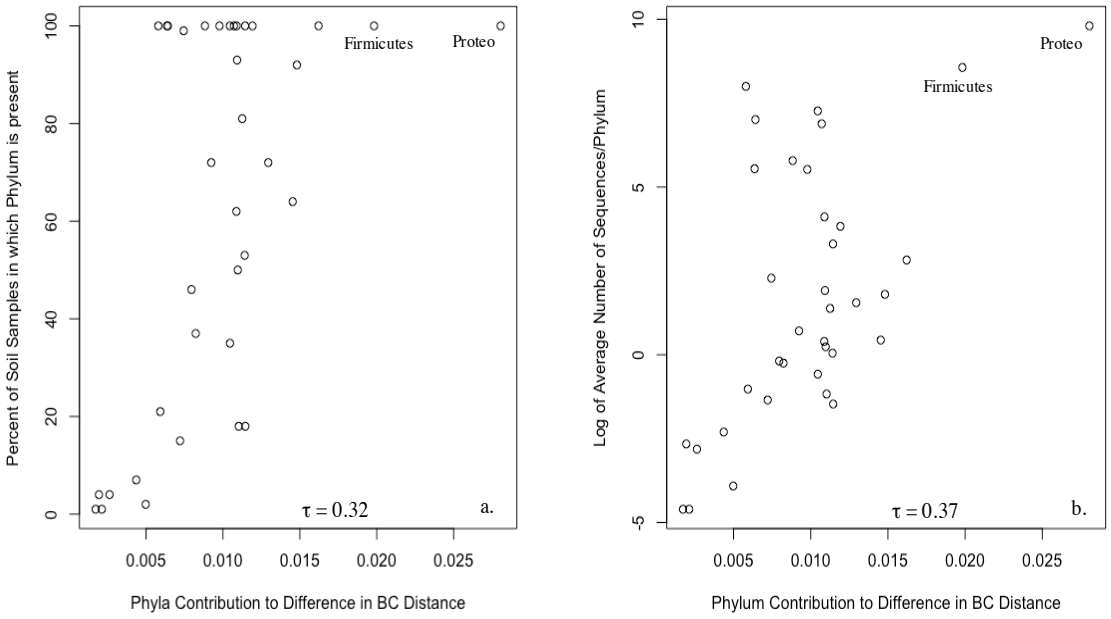


Figure C.5: Plots comparing bacterial community composition between one individual core to one mixed core. Same as those in Figure 3.3 except that they are based on the comparison between one individual core to one mixed core. Each point represents a phylum detailed in Table C3.



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