SPACE, TIME AND CHANGE:

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

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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.

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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

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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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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"

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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

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(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

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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

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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

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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.

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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).

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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

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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.

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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,

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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.

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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

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