UNIVERSITY OF CALIFORNIA,
IRVINE

Impacts of Urbanization and Drought on Soil Microbial Communities

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Andie Nugent

Dissertation Committee:
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2023

DEDICATION

To

My parents

and husband

in recognition of their endless support, love,

and encouragement throughout this journey

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To Steve – Thank you for many years of excellent mentorship, and for fostering such a fun and welcoming lab. Your advocacy for social change in academia and beyond has been inspiring, and you have taught me to be creative and ambitious about the impact my work can have. Thank you for the opportunity to join your group and for helping me grow so much as a scientist.

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ABSTRACT OF THE DISSERTATION

Impacts of Urbanization and Drought on Soil Microbial Communities

by

Andie Nugent

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2023

Professor Steven D. Allison, Chair

Soils support many vital ecosystem services including water filtration, pollution remediation, carbon sequestration, nutrient cycling, and increased biodiversity. Microbial communities are key regulators of these soil processes and are functionally responsive to shifts in environmental conditions. Global changes including climate change and urbanization are altering soil properties and soil microbial activity. The resulting feedbacks could increase GHG emissions and nitrogen leaching into water systems from both urbanized and natural soils. The aim of this dissertation is to investigate the impact of global changes on the soil microbiome. First, I addressed the impacts of urbanization on soil ecosystems by synthesizing prior literature and developed a framework to assist researchers in answering key questions about the urban soil microbiome. I argue that urban soils offer an excellent opportunity to study fundamental questions about microbial community structure and function under different environmental conditions, with the additional benefit finding methods to improve urban sustainability.

Next, I conducted a field experiment applying this framework to soils in a local neighborhood. I constructed a chrono-sequence of yards built across four decades, and characterized the soil and microbial community to provide insight into how urban soils recover from disturbance, and how irrigation, fertilization, and plant type may alter microbial processes compared to an adjacent undeveloped ecosystem. I found that these yard soils, particularly under turfgrass, are wetter and more nutrient-rich compared to undeveloped soils. The chrono-sequence also revealed that urban soils gain more abundant and active microbial communities over time which may result in accumulated soil carbon.

Finally, my last chapter explores the impact of drought and nitrogen addition, as may result from fossil fuel burning, on a natural grassland ecosystem. I characterized the microbial community of bulk soils across experimental treatments down to 30cm, and explored the effects of depth, drought, and fertilization on microbial community composition and potential function. I found that depth was the most consistent driver of microbial function, while microbial functions were more resilient to drought and fertilization. An interesting finding from this work was that community composition did not respond to treatments while potential function did. This suggests a need for a trait-based approach to describe microbial communities and predict function from their structure.

INTRODUCTION

Soil microbes (bacteria, fungi, archaea) lie at the heart of many ecosystem processes. Soil microbes cycle key nutrients, support plant growth and plant diversity, and regulate soil carbon storage (Aislabie & Deslippe, 2013). The activity and function of soil microbial communities can depend on community composition as well as environmental conditions (Martiny et al, 2015; Evans & Wallenstein, 2014, Glassman et al, 2018; Waldrop & Firestone, 2006). Global changes, including urbanization and climate change, are altering soil conditions and soil microbiomes (Jansson & Hofmockel, 2020; Wang et al, 2021; Pouyat et al, 2008). The goal of this dissertation is to determine the consequences of climate change and urbanization for soil microbial processes and implications for ecosystem health.

Urbanization involves extreme disturbance during development, and often repeated disturbances for land maintenance. Physical disturbances and alteration of soil nutrient and moisture levels can affect soil microbial processes. The initial development phase of urban soils tends to trigger significant carbon loss, but with careful management over years to decades, soils can become carbon sinks again (Golubiewski et al, 2006; Chen et al, 2013). To maintain lush greenery and a wealth of non-native plants, land managers often irrigate and fertilize the soil. Nitrogen amendments have been shown to alter microbial community composition and either increase or reduce microbial biomass and activity depending on the ecosystem (Treseder, 2008; Cusack et al, 2011; Zhang & Han, 2012). Increased precipitation tends to promote microbial decomposition which can accelerate soil carbon loss (Zhang et al, 2013; Ren et al, 2017). Additionally, urban landscapes often host a wide

variety of plant species, many of which are non-native. Plants are known to be strong drivers of microbial community assembly in soils, and novel plant communities may therefore foster different microbial communities than native plant communities (Hui et al, 2017; Reese et al, 2018; Kourtev et al, 2002).

It is unclear how all the factors described above are interacting in urban soils and how they influence the urban soil microbiome. If urban soil function has been significantly altered, careful management of the soil microbiome may be necessary to restore desired processes. Thus far, few studies have described the urban soil microbiome and its role in soil function. The studies which do exist employ different sampling and experimental approaches, which makes it difficult to compare results and draw overall conclusions. Therefore, in **Chapter 1**, I developed a conceptual framework to guide the study of microbial ecology in urban soils. I used this conceptual framework to synthesize the small amount of information we do know, and to propose important questions for future research. Finally, I proposed that urban ecosystems can be a convenient environment for studying sharp gradients and environmental extremes, and research on urban soils can improve our fundamental theoretical understanding of biogeochemical processes and the link between microbial community structure and function.

In my **second chapter**, I applied my framework to a local urban ecosystem. Most urban microbiome research in the United States has been conducted in arid, temperate, and subtropical climates (e.g. Delgado-Baquerizo et al, 2021; Li et al, 2018; Wafula et al, 2015; Joyner et al, 2019) and less is known about urban microbiomes in the semi-arid Mediterranean climate of the west coast of the United States. I compared a Southern California suburban neighborhood to a nearby ecological preserve to understand how soils

and microbial communities change in response to the major disturbance of development and an altered resource regime thereafter. I found that some soil and microbial characteristics correlate with soil age, indicating that urban ecosystems undergo succession after development. I also found that soil moisture and nutrient content were the primary variables differentiating urban from non-urban soils. Soil moisture in particular tends to be much higher in urban soils compared to natural soils in dry climates, which can have drastic effects on microbial function such as respiration (Koerner & Klopatek, 2002). In this study, we found that moisture and high nitrogen and carbon availability supported a diverse and highly active microbial community. Soil respiration rates were high, but urban soils still accumulated carbon over time.

One particular concern about the sustainability of urban ecosystems is the ubiquitous presence of turfgrass which is almost always managed through irrigation and fertilization (Groffman et al, 2016; Milesi et al, 2005). In Chapter 2, I also compared soil beneath turfgrass with soil beneath other vegetation types and found that turfgrass soil does have higher moisture and nitrogen levels but without additional benefit to microbial communities. Therefore, I recommend a reduction in turfgrass, or lower resource use for turfgrass management, to conserve water and minimize greenhouse gas (GHG) emissions while still receiving the benefits associated with diverse microbial communities.

The impacts of urbanization extend beyond city limits. On a regional scale, urbanization increases atmospheric nitrogen deposition which can impact undeveloped land adjacent to cities (Fenn et al, 2003; Ochoa-Hueso et al, 2011). Nitrogen deposition can alter soil microbial growth and activity, which can result in greater carbon sequestration or loss depending on ecosystem (Waldrop et al, 2004). In Southern California, nitrogen

deposition is increasingly likely to be coupled with drought as climate change progresses. Water availability in soils is important not only for direct use by plants and microorganisms, but also for dissolving and transporting crucial nutrients (Tecon & Or, 2017). When soil microbes have difficulty accessing water and nutrients, the microbes may need to shift strategies from growth to survival, leading to reduced activity and decomposition (Malik et al, 2020). Precipitation patterns can also affect microbial response to nitrogen deposition in semi-arid grasslands like those found in Southern California. (Nelson et al, 2015; Stursova et al, 2006).

In Chapter 3, I investigated how nitrogen deposition and drought independently and interactively impact the soil microbiome which will be important for anticipating soil-climate feedbacks and improving land management decisions. I sampled grassland soils from the Loma Ridge Global Change Experiment and quantified microbial functional genes related to carbon and nitrogen cycling. I also sampled at three different depths to determine whether global change impacts affect soil in a depth-dependent manner. I found that microbial community composition and potential function differed most across soil depths. Drought and fertilization also had some effects on potential function, but the magnitude and direction of these effects usually varied with soil depth. This chapter provides novel insight into the nutrient-cycling abilities of bulk soil microbiomes and suggests that depths greater than 10cm should be sampled to best capture the complex biogeochemical dynamics occurring under global change.

As cities expand and climate change progresses, understanding the effects of these global changes on soil processes will only become more crucial. This dissertation fills knowledge gaps of how climate change and urbanization are impacting the soil ecosystem

of Southern California and likely beyond. My findings can contribute to improved models of soil nutrient dynamics and predictions of whether semi-arid ecosystems will likely be carbon sources or sinks depending on the extent of human impact. Ultimately, it is my hope that this dissertation can contribute to greater sustainability of urban and adjacent natural ecosystems for the benefit of people and planet.

CHAPTER 1

A Framework for Soil Microbial Ecology in Urban Ecosystems

Introduction

Human impacts on the environment range in type, intensity, and scale. The effects of agriculture, mining practices, heavy metal pollution, and climate change on ecosystem structure and function have been thoroughly studied over the past several decades. Initially, plants and animals were the organisms of focus. Recently, microorganisms have gained more attention as key drivers of ecosystem processes. Yet even as microbiomes and their responses to human disturbances have come into greater focus, one major type of human impact has been largely overlooked: urbanization. Microbially driven processes such as carbon and nitrogen transformations have been studied in urban soils. However, we lack research linking these processes directly to microbial community membership and activity. Given that they lie along a steep human impact gradient, more focus on urban ecosystems would bolster fundamental understanding of microbial and ecosystem responses to disturbance (**Figure 1.1**).

Urbanization has drastic impacts on geochemistry, climate, and biota, including diverse microbiomes. Although urban areas currently occupy less than 0.5% of global land area (Schneider et al., 2009), urban land cover continues to expand, which could have substantial consequences for environmental health and sustainability (Seto et al., 2012). Urbanization causes landscape fragmentation, which can reduce plant and animal biodiversity (Delaney et al., 2010; Liang et al., 2008; Su et al., 2011). Urban light and sound pollution can alter animal behavior, disrupt species interactions, and cause shifts in species richness and composition (Ciach & Fröhlich, 2017; Firebaugh & Haynes, 2016; Francis et

al., 2009; Longcore & Rich, 2004). Soils in cities are often contaminated with organic pollutants and heavy metals. These contaminants can stress plants, contaminate plant tissues, impact soil and pollinator animal communities, and pose health risks for human residents (Hernández & Pastor, 2008; Pan et al., 2018; Pavao-Zuckerman & Coleman, 2007; Wang et al., 2013). The environmental impact of urban land use can reach far beyond city limits through greenhouse gas emissions (Pichler et al., 2017), atmospheric nitrogen deposition (Fenn et al., 2003), and water pollution (Overbo et al., 2021; Wright et al., 2011).

At the same time, urban environments sustain critical ecosystem processes. For example, sprawling urban areas continue to provide sufficient habitat, resources, and dispersal routes to support a high level of biodiversity (Angold et al., 2006; Wenzel et al., 2020). Insect pollinators can thrive in urban landscapes, which has made them a focus of urban conservation efforts (Baldock et al., 2019; Hall et al., 2017). Urban green spaces can help to offset impacts of urbanization by filtering air, regulating climate, and slowing runoff (Bolund & Hunhammar, 1999; McPhearson et al., 2015). Urban soils support nutrient cycling processes and, with proper management, may be effective at sequestering carbon (Brown et al., 2012; Pouyat et al., 2009). While urban landscapes appear quite different from their natural counterparts, cities continue to support diverse and functional ecosystems. Understanding these novel urban ecosystems can help inform management strategies and maintain vital ecosystem processes that make cities more sustainable.

In addition to flora and fauna, soil microorganisms are essential for ecosystem functioning and services. While urban microbial research has a long history (e.g., Blaschke-hellmessen, 1969; Passarelli et al., 1949), only in the last decade or two have funding opportunities, cross-disciplinary interest, and technological advances positioned the field

to grow rapidly. Within the recent wave of microbial ecology studies, the vast majority address human impacts such as climate change and pollution outside of urban systems. Although there has been extensive work on soil microbiomes in agricultural systems, if we want to understand how humans drive microbial community structure and function, we need to extend microbial ecology beyond "natural" and agricultural lands. Only recently has there been a push to understand the impact of urbanization on the soil microbiome (Antwis et al., 2017), and we do not yet know how insights from natural and agricultural systems apply to urban soils. A comparative approach is potentially useful (**Figure 1.1**); both urban and agricultural systems experience physical disturbance of surface soils, altered water regimes, high nutrient inputs, and introduction of novel plant communities. As with agricultural ecosystems, a deeper scientific understanding of urban ecosystems will become increasingly relevant as the human population expands.

Urban soils have been defined by the World Reference Base as Technosols, which are soils whose properties have been largely determined through human activity and often contain materials that would not be present without human intervention (Rossiter, 2007). Here, we define urban soils more broadly as any soil affected by or created through land development for human housing, commercial spaces, and workplaces. This definition encompasses rural towns and major cities. Urban soil may be new or old, and may be closely managed (e.g., park soil) or generally unmanaged (e.g., soil beneath parking lots). Urban soils may also be endemic or could be trucked in from other locations and may experience few or repeated disturbances. Thus, urban development creates a highly heterogenous soil matrix across both space and time. However, this heterogeneity and

extreme disturbance regime present excellent opportunities to understand microbial functioning under changing conditions.

There is a growing body of work investigating microbial communities and their functions in the built environment, such as within air conditioning systems and on hospital surfaces (e.g., Bonetta et al., 2010; Chaoui et al., 2019). Additionally, there is ongoing research addressing microbial function in urban aquatic systems (e.g., Calderón et al., 2017; Chaudhary et al., 2018). Still, urban waterways do not include all the heterogeneity driving microbial communities within urban ecosystems. Soils have only recently gained attention as crucial habitat for microorganisms in cities, despite the long-established importance of soil microbiomes in other ecosystems. Microorganisms can contribute to urban soil genesis and nutrient availability by breaking down minerals and organic matter and fixing nitrogen, shaping the soils upon which people live (Kaviya et al., 2019). These soil–microbe feedbacks are a rapidly emerging area of research, and to our knowledge, there is not yet an overarching conceptual framework for effectively developing and answering critical questions about urban soil microbial communities.

In this paper, we propose a new framework to advance research on urban soil microbial communities and their ecosystem functions. We apply our framework to synthesize previous findings and discuss the implications of urban soil microbes for ecosystem and human health. We find that, strikingly, there has been very little work done to link microbial taxa to functioning in urban soils—information that could guide urban sustainability efforts and improve our fundamental understanding of microbial structure–function relationships. Finally, we offer recommendations for research priorities and practices to guide the field of urban microbial ecology in answering these crucial questions.

We emphasize the need for collaboration between many experts and stakeholders, including ecologists, biogeochemists, urban planners, landowners, engineers, landscapers, and social scientists to gain a holistic understanding of microbes and their interactions with humans in the urban environment (Aronson et al., 2017; Shifflett et al., 2019).

Framework for Urban Soil Microbial Ecology

Many ecosystem processes depend on soil microbiomes that contain a diverse and abundant array of bacteria, fungi, and archaea (Reese et al., 2016; Wang et al., 2018). Soil microbial communities drive the cycling of key nutrients including carbon, nitrogen, and phosphorus within ecosystems (Aislabie et al., 2013), thereby supporting primary producer growth and diversity. The soil microbiome additionally affects soil health by immobilizing heavy metals, degrading organic pollutants, and altering physical soil structure (Kaviya et al., 2019). Microbiologists and microbial ecologists have therefore tried to understand how the environment drives microbial community activity to predict the direction and magnitude of microbial consequences for soil and ecosystem function.

Our proposed framework (**Figure 1.2**) draws on previously published ideas but fills a knowledge gap by emphasizing the intersection between humans and microbial function in urban ecosystems. Humans create and intensively manage urban environments and are thus a key component of our framework. Human society, including economies, cultures/values, policies, technologies, and resources, determines how the urban environment is structured and how it functions (Alberti, 1999; Byrne, 2009). However, these factors are difficult to capture quantitatively and are generally outside the wheelhouse of microbial ecologists. To address this challenge, we draw from Pickett and Cadenasso's (2009) analysis of altered resources, disturbance, and heterogeneity as the key

mechanisms through which humans shape urban soils (Arrow A). Ecologists are already well-equipped to study these mechanisms, which have consequences for microbial community composition and function (Arrow B) and in turn cause shifts in environmental resource pools and fluxes (Arrow C) (Hall et al., 2018). Finally, the environmental changes driven by microbial activity feedback to human society through the creation of environmental services or harms (Arrow D). Humans may adjust policy and behavior accordingly, which starts the cycle over again.

Our framework is useful because it synthesizes existing knowledge on disturbance ecology, urban ecology, and microbial ecology. We develop and discuss key questions to address knowledge gaps in our framework that limit fundamental understanding of urban microbial ecology and microbial ecology more broadly. We also emphasize the need for collaboration among ecologists, biogeochemists, and social scientists to understand how the human–environment–microbe feedback loop plays out in cities around the world. Such collaboration will improve our decision-making and management strategies in urban spaces with the ultimate goal of promoting sustainability and environmental justice.

Disturbance

Disturbance in the urban environment is practically unavoidable, especially during initial land conversion. As land is developed, soil layers are removed, mixed, and sometimes entirely replaced with soil from other locations (Craul, 1985). This disturbance can result in altered soil horizons, mineral composition, and chemistry (Huot et al., 2017). In other cases, soils may be sealed under concrete with little soil mixing resulting in not only less physical disturbance but also reduced interactions between soil and air. The nature, frequency, and scale of soil disturbances vary widely across urban soils, which

presents a challenge for retroactively defining baseline soil conditions and determining postdisturbance impacts. Urban soils may be more or less functional compared with their predisturbance state (Graham et al., 2021).

Using chronosequences of urban sites at different ages since land conversion, we can begin to assess how soil and soil microbial communities respond over time to disturbance. Microbial diversity may remain consistent across soil ages, indicating some resilience to disturbance (Yao et al., 2006). However, older soils have more abundant and active microbial communities and higher rates of carbon and nitrogen mineralization than newer urban soils (Scharenbroch et al., 2005). Golubiewski (2006) additionally found that it may take several decades for soil carbon and nitrogen storage to recover to predevelopment levels. Therefore, microbial function may be resilient on longer timescales than expected.

The above-mentioned studies focused on differences in microbial communities based on urban soil age. Crucially, because few studies have compared microbial communities pre- and postdevelopment in a single location, it is difficult to determine whether these communities have truly "recovered" or whether they might be novel in composition and functioning. Thus, it is unclear how quickly microbial communities recover after disturbance to urban soils. Even if microbial communities bounce back quickly, there may be a substantial lag in the recovery of soil geochemical properties, which may have implications for soil management. Rather than attempting to restore urban soils to an uncertain predevelopment state, it may be more practical to accept them as fundamentally altered and prescribe management techniques aimed at achieving realistic soil health benchmarks (Simenstad et al., 2006).

Soil bulk density may be one important factor driving response to disturbance. The bulk density of recently developed residential soils is significantly higher than old residential and park soils (Scharenbroch et al., 2005). Additionally, soils under turfgrass lawns are more compacted than soils under trees (Edmondson et al., 2011). Dense soils limit the flow of oxygen, water, and nutrients through the soil matrix, which in turn changes the resources to which microbes have access. Higher density soils may favor anaerobic bacteria, which correlate with higher denitrification potential (Chamindu Deepagoda et al., 2019; Hartmann et al., 2014; Longepierre et al., 2021). Heavily compacted soils have also been associated with increased CO_2 and methane emissions (Hartmann et al., 2014). On the contrary, compacted soils generally have lower microbial abundance, enzyme activity, organic carbon, and total nitrogen (Li et al., 2011; Pengthamkeerati et al., 2011; Torbert & Wood, 1992; Zhong et al., 2019). Therefore, high bulk density may help explain the reduced microbial abundance and activity observed in recently developed urban soils. In an agricultural system, a negative response to soil compaction was observed across bacterial phyla, rather than impacting only particular taxa (Longepierre et al., 2021). In urban soils, compaction may likewise have a widely distributed impact on the microbial community and consequently its function.

Compaction is a known problem for urban soils, and therefore, heavily trafficked urban green spaces such as athletic fields are frequently aerated and resurfaced to loosen soil and promote air and water flow. However, this frequent disturbance regime, much like agricultural tilling, can reduce soil carbon sequestration by disrupting soil structure and exposing soil organic matter to microbial decomposition (Balesdent et al., 2000; Townsend-Small & Czimczik, 2010). Therefore, it may be important to aerate soils enough

to combat severe compaction while allowing enough time between disturbances to re-sequester the carbon lost after each aeration event. Understanding how disturbance regimes impact soil health will better enable land developers and managers to prevent unnecessary soil damage and accelerate recovery.

Altered Resources and Soil Chemistry

Urbanization may alter the resources that microbial communities need to survive and grow. From nonurban systems, we know that a shift in resource availability, whether to the microbes' benefit or detriment, will often cause microbial communities to change in activity, and this change can have ecosystem consequences (Chung et al., 2007; Malik et al., 2020; Tiemann & Billings, 2011). Among the most important soil chemical characteristics and resources for microbial growth are pH, carbon, nitrogen, and water. In many urban soils, levels of these resources are considerably different from rural or unmanaged soils. Urban landscapes are also exposed to heavy metal deposition, organic pollutants, soil sealing, and novel plant communities. Here, we explore the impacts of these factors on the urban soil microbiome. Interactions between these variables make it challenging to predict their combined impact on microbial communities and activity. Teasing apart the individual and combined effects of these variables will be important to appropriately manage urban soils and promote healthy soil microbiomes.

pH

Due to the narrow optimal pH range for many taxa, soil pH is a strong driver of microbial community composition and function (Aciego Pietri & Brookes, 2008; Glassman et al., 2017; Rousk et al., 2010; Zhalnina et al., 2015). Generally, bacterial communities are more diverse and enzymatically active in neutral than in acidic soils (Acosta-Martínez &

Tabatabai, 2000; Fierer & Jackson, 2006; Liu et al., 2014). However, lower pH may promote some desirable microbial functions such as increased carbon storage (Malik et al., 2018). While natural soils range in pH from acidic to neutral, urban soils are often alkalized (Lorenz & Kandeler, 2006). Urban soil alkalinity is primarily attributed to the leaching of calcareous substances from construction materials such as concrete (Yang & Zhang, 2015). Increased pH in urban soil has been associated with decreased microbial function (Caravaca et al., 2017). However, the role of pH in driving microbial community structure and function in urban soils is largely unknown and requires further study.

Carbon and nutrients

Carbon content in urban soils often changes following initial land conversion and may depend on ongoing land management methods. Particularly in urban turfgrass systems, frequent mowing and clipping may alter soil organic matter dynamics and microbial function (Thompson & Kao-Kniffin, 2019). Grass clipping can stimulate microbial activity by increasing root exudation. Returning the clippings to the soil can provide nutrients to soil microbes as the clippings decompose, reducing the need to fertilize with nitrogen. Removing the clippings, on the contrary, may cause microbes to rely more on existing soil organic matter and decrease the soil's ability to act as a nitrogen sink. Removal of plant biomass has also been shown to decrease microbial biomass and respiration and cause microbes to rely on more recalcitrant forms of carbon, indicated by an increase in recalcitrant carbon and nitrogen cycling genes in the community (Wang et al., 2011; Xue et al., 2016).

Nutrients such as nitrogen and phosphorus are often added directly to urban green spaces as fertilizer or are unintentionally added from runoff and atmospheric deposition.

These inputs may be high enough to trigger symptoms of nitrogen saturation in urban soils (Chen et al., 2010; Taylor et al., 2005; Yang & Toor, 2016). In studies of nonurban systems, nitrogen amendments generally reduce microbial respiration, biomass, and extracellular enzyme activity while altering community composition (Ramirez et al., 2012; Treseder, 2008). Consequently, nitrogen deposition may promote soil carbon storage, although the mechanisms for this observation are unclear (Zak et al., 2017). On the contrary, nitrogen deposition can also promote carbon loss from low-nutrient environments (Koceja et al., 2021). Therefore, soil type can have a strong impact on microbial response to nitrogen inputs.

At the watershed scale and within parks and lawns, we know that urban systems are capable of cycling nitrogen at rates comparable to or greater than nonurban systems (Enloe et al., 2015; Pouyat et al., 1997; Reisinger et al., 2016). The microbial contribution to urban nitrogen transformations has been less studied. Microbial genes related to nitrogen cycling are abundant in urban park soils (Wang et al., 2018), indicating that urban soil microbes are highly active in nitrogen cycling. Wang, Marshall, et al. (2017) presented one of the first studies to identify relative nitrogen cycling activity among microbial taxa in urban soils. They found that ammonia-oxidizing archaea may play a greater role in nitrification within urban soils than in rural soils. Additionally, they found a high abundance of microbes containing the nosZ clade II gene, which has been negatively correlated with soil N_2O emissions (Xu et al., 2020). This may present opportunities for managing urban microbial communities to reduce greenhouse gas emissions from soil.

In concert with the high nitrogen cycling activity of their microbial communities, urban soils remain significant sources of nitrogen runoff (Taylor et al., 2005; Yang &

Toor, 2016) and nitrous oxide (N_2O) (Kaye et al., 2004; Townsend-Small & Czimczik, 2010; van Delden et al., 2016). Microbes may reach a stoichiometric limit to the amount of nitrogen they can take up. Birt and Bonnett (2018) found that additional nitrogen stimulated microbial extracellular enzyme activity related to carbon acquisition, indicating that carbon may become a limiting resource if nitrogen is readily available. Therefore, if ecosystem management goals include increasing microbial denitrification rates and soil nitrogen uptake, it may be necessary to supplement fertilized soils with additional carbon sources.

Atmospheric CO_2

Carbon availability in urban areas is affected by the "CO_2 dome," an area of increased atmospheric CO_2 due to the local and concentrated burning of fossil fuels. With CO_2 levels rising globally, many researchers have investigated the impact of CO_2 enrichment on soil communities. For instance, He et al. (2014) and Yu et al. (2018) observed that CO_2 enrichment stimulated microbial functional genes involved in carbon and nitrogen cycling. Carney et al. (2007) found that doubling CO_2 levels resulted in higher activity of microbial carbon-degrading enzymes leading to an overall loss of soil carbon despite potential CO_2 benefits for plant growth. Increased CO_2 may also alter community composition and diversity (Jia et al., 2020; Jin et al., 2020; Wang, Marsh, et al., 2017). These impacts of CO_2 on microbial community structure and function are likely occurring indirectly through changes in plant inputs, nitrogen availability, soil pH, and moisture (Deltedesco et al., 2020; Gao et al., 2020; Wu et al., 2021).

Together, these studies have implications for microbial carbon cycling in cities, with the concern that carbon loss could be accelerated in urban soils due to increased microbial

enzyme activity. Carbon losses may be exacerbated in soils exposed to warming and irrigation (Carrillo et al., 2018; Thakur et al., 2019; Yu et al., 2021), potentially leading to interactions between CO_2 enrichment and urban management strategies such as irrigation and temperature regulation. To our knowledge, though, no studies have specifically investigated the impact of CO_2 domes on urban soil microbiome function. We recommend this topic as a priority for future studies.

Water

Variation in water availability may impact the activity and function of urban soil microbes. Many urban soils are irrigated, and some receive substantial irrigation to support lush greenery in arid regions. Meanwhile, urban soils in more mesic regions tend to be drier due to increased runoff from features such as impervious surfaces and drainage systems (Pickett & Cadenasso, 2009). Green and Oleksyszyn (2002) compared irrigated lawns, xeriscaped (reduced irrigation) lawns, and unmanaged desert patches and found that irrigated lawns showed the highest invertase and cellulase activities, indicating that irrigation promotes microbial breakdown of carbon sources in arid climates. This result is consistent with Orchard and Cook's (1983) findings that wetter soils contribute to higher microbial respiration and soil carbon loss, potentially offsetting the carbon sequestration benefits of increased plant biomass in irrigated urban spaces. Irrigation also makes nitrogen more accessible to microbes, while drier soils decrease diffusion of substrates through the soil, limiting microbial activity (Stark & Firestone, 1995). The combination of irrigation and fertilization results in greater N_2O and NO fluxes from urban soils (Hall et al., 2008; Kaye et al., 2004). Balancing the combined use of fertilizer and irrigation may

therefore be important for managing urban green spaces while minimizing greenhouse gas efflux (Bijoor et al., 2008).

Heavy metals

Heavy metal pollution is an unfortunate consequence of human activities such as smelting and fossil fuel combustion (Benin et al., 1999; Luo et al., 2015; Rodríguez Martín et al., 2015). Roadsides and industrial areas are hotspots for heavy metal pollution in soils. As soil toxicity from heavy metals increases, microbial biomass and activity generally decrease (Azarbad et al., 2013; Oliveira & Pampulha, 2006; Papa et al., 2010). Some microbial taxa are impacted more than others by heavy metals, with consequences for soil greenhouse gas emissions (Ma et al., 2021; Oliveira & Pampulha, 2006). It will be important to further study the impacts of heavy metal pollution on soil communities and consequently on ecosystem functions, allowing us to explore new ways to reduce soil pollutants and restore vital microbial processes.

Organic pollutants

To maintain idyllic urban green spaces and reduce damage from insects and weeds, pesticides are often applied to urban soils. There have been recent efforts to understand the impacts of these chemicals on soil health, including the functioning of soil microorganisms. Several reviews have found mixed effects of pesticides on microbial communities and their functions (Imfeld & Vuilleumier, 2012; Kalia & Gosal, 2011; Riah et al., 2014). Depending on the pesticide, impacts on microbial biomass and enzyme activity may be negative, neutral, or positive. Effects may be short-lived or more long-term, and microbial interactions with pesticides may depend on other factors such as temperature, soil fertilization, and soil carbon content (García-Delgado et al., 2018; Muñoz-Leoz et

al., 2012; Reedich et al., 2017). Additionally, because most pesticide studies focused on agricultural systems or laboratory microcosms, little is known about how in situ urban microbial communities respond to pesticide application and what the response means for soil health and function.

Persistent organic pollutants (POPs) are also present in many urban soils and can have profound effects on ecosystem health. Such pollutants include polycyclic aromatic hydrocarbons, polychlorinated biphenols, and polybrominated biphenyl ethers, which can originate from e-waste processing, vehicle emissions, electronic insulation, lubricants, and other industrial sources. Although the use of these hazardous compounds is regulated and has generally decreased over time, their persistence in the environment still poses a tremendous challenge. These pollutants alter soil microbiome structure and favor taxa, which can tolerate and break down POPs (Girardot et al., 2020; Wu et al., 2020; Zhang et al., 2010). Heavily polluted urban sites such as brownfields can continue to host diverse, active microbial communities that mitigate pollutants. However, it remains unclear whether there is a trade-off between POP tolerance and other ecosystem-relevant functions. The ability of microbes to break down POPs may also depend on temperature, salinity, and nutrient availability, providing an opportunity to optimize soil conditions to promote bioremediation (Varjani & Upasani, 2017).

Soil sealing

A considerable amount of urban soil is sealed under impervious surfaces such as buildings, roads, sidewalks, and pavement. As of 2011, around 4.4% of the land area of European Union nations was artificially covered, half of which was sealed beneath impervious surfaces (Prokop et al., 2011). Within the United States, impervious surfaces

cover 17.5% of urban land area, and this fraction can be much higher in particularly dense cities (Nowak & Greenfield, 2012). Soil sealing rates may be outpacing population growth in many regions (Munafo et al., 2010; Prokop et al., 2011). As urban soil sealing continues, studies limited to open urban soils may not be sufficient to gain a comprehensive understanding of urban ecosystem functioning.

Impervious surfaces create a barrier that inhibits the exchange of substances between the soil, surrounding environment, and atmosphere. The resulting sealed soils contain less carbon and nitrogen than open soils and have reduced microbial activity (Lu et al., 2020; Raciti et al., 2012; Wei et al., 2014). Sealed soils may also have decreased microbial diversity and altered community structure (Hu et al., 2018; Yu et al., 2019). The impact of soil sealing on ecosystem function had been largely ignored until recently, but now researchers are emphasizing the need to include sealed soils in overall urban carbon budgets and models of urban geochemical dynamics (e.g., Bae & Ryu, 2020; Hu et al., 2018; Wei et al., 2014).

Novel plant communities

As in nonurban systems, soil microbial communities in urban green spaces appear to be shaped, at least in part, by plant inputs and diversity (Hui et al., 2017). Urban ecosystems are often home to novel plant communities, including many non-native plant species (Kowarik, 2011). Since plants can be major drivers of microbial community assembly, novel plant communities may foster microbial communities different from those typical in soils with native vegetation. Urbanization also facilitates the spread of invasive plant species (Lechuga-Lago et al., 2017; Marques et al., 2020; Skultety & Matthews, 2017), and invasive plants have been shown to alter the soil microbiome, in turn impacting native

plant survival and causing shifts in ecosystem processes (e.g., Batten et al., 2006). Even noninvasive exotic plants can alter the soil microbiome, shifting microbial community structure and function (Kourtev et al., 2002). More research should be done on how soil microbial communities and functioning respond to common exotic or invasive plants versus native plants. The impact of overall plant diversity on microbial communities should also be studied within urban systems.

Heterogeneity

At first glance, cities may appear to be a homogenous sea of concrete. However, the urban environment is composed of a highly diverse array of land-use types, ranging from parks and lawns dominated by turfgrass, to busy commercial centers with a mix of concrete and greenery, to large industrial complexes mainly characterized by impervious surfaces and polluted soils. It may be important to distinguish between these land uses to more wholly understand microbial function in cities and devise appropriate soil management approaches.

These land-use patches tend not to exist along a clear gradient but are instead jumbled together to create a complex habitat mosaic, which may create a novel context for studies of microbial biogeography and dispersal (**Figure 1.3**; Zhou et al., 2018). Along with variation in land-use types, there is also heterogeneity of climate within urban spaces. Cities tend to be hotter than their surrounding environment, a phenomenon known as the urban heat island (e.g., Imhoff et al., 2010; Li et al., 2017; Oke, 1995). Within this heat island, a variety of microclimates exist due to the position and size of buildings, density of trees, and green infrastructure (Liao & Heo, 2018; Pincebourde et al., 2016). Soils within a city can be trucked in from multiple nonlocal sources, and can vary in nutrient load,

irrigation, heavy metal and pesticide pollution, and other characteristics depending on the management and development history of that land (De Kimpe & Morel, 2000; Karim et al., 2014; Zhiyanski et al., 2017; Ziter & Turner, 2018).

How does the heterogeneity of urban habitats impact soil microbial community assembly, dispersal, and function? Understanding the role of landscape heterogeneity for microbial communities has only recently become a priority in microbial ecology. There is evidence that microbial communities vary with habitat heterogeneity (Horner-Devine et al., 2004). However, due to microorganisms' small size, their dispersal and survival may be constrained by different factors from macroorganisms (Martiny et al., 2006), and therefore, microbial response to habitat heterogeneity and patchiness, and the distance between patches, may not be predictable using our current theoretical frameworks based on macroorganism studies (Mony et al., 2020). In urban ecosystems, altered hydrology and foot and vehicle traffic may facilitate microbial dispersal at a more rapid rate and over greater distances than is typical in natural environments. On the contrary, vast swathes of impervious surfaces between green spaces may create a barrier to dispersal. No studies to our knowledge have investigated mechanisms of dispersal between soil patches in cities; this should be a focus in future studies.

While dispersal of urban microbial communities is poorly understood at this time, research has characterized communities within urban habitats such as bioswales, parks, green roofs, and residential soils. In general, these studies found differences in microbial composition and diversity by habitat (Gill et al., 2020; Wang et al., 2018). Microbial litter decomposition also differs between urban soils, indicating that microbial function may be affected by habitat type (Vauramo & Setälä, 2011). Heterogeneity likely has an impact on

the assembly and function of urban microbial communities, and future studies should investigate how microbial communities respond to patch type, size, edginess, and distance between patches.

It will also be important to track the impacts of temporal variation in environmental variables. In nonurban systems, microbial activity often varies with seasonality, rain pulses, or ecological succession (e.g., Cong et al., 2015; Deng et al., 2017; Tomar & Baishya, 2020). Several studies have found that soil respiration in cities likewise follows seasonal trends, with higher respiration in warmer, wetter months (e.g., Decina et al., 2016; Goncharova et al., 2017; Tao et al., 2016). As summarized in "Disturbance" section, there is also evidence of some microbial functional succession after initial land conversion. Still, it is unclear how the altered resources and disturbance regimes in cities interact with seasonality, heat waves, or extremes in precipitation to drive microbial community structure and function. This interaction should be explored in future work.

While cities may be highly heterogenous at small to medium scales, it is possible that cities reduce environmental variation at regional and global scales. The "urban convergence" hypothesis states that urban areas are more similar to each other than to their surrounding rural environments. Some studies have found evidence for this trend with biological, geochemical, soil, and microclimate variables, as well as in urban streams and waterways (Booth et al., 2016; Groffman et al., 2017; Hall et al., 2016; Herrmann et al., 2020; Kaye et al., 2006; McKinney, 2006; Pearse et al., 2016; Polsky et al., 2014). Recently, Delgado-Baquerizo et al. (2021) found evidence for the homogenization of soil microbial taxa and functional genes in urban green spaces across the globe. Homogenization of soil communities was related to economic metrics, climate, and land

management practices. This work used metagenomic data to draw conclusions about community function. Moving forward, it will be important to validate these conclusions with complementary methods such as transcriptomics, proteomics, and extracellular enzyme assays that can reveal the in situ consequences of community homogenization for soil processes and ecosystem health. With a high degree of heterogeneity at neighborhood and city scales, and homogenization likely occurring at regional and global scales, urban soil microbial function should be analyzed at all these scales.

Priorities for Future Research and Recommended Approaches

There is a crucial need for sustainable and equitable design of urban spaces to benefit humans and the environment from local to global scales. To best harness the power of microbial communities to achieve this goal, we have identified the following essential questions in urban microbial ecology and biogeochemistry. Furthermore, addressing these questions will help advance these disciplines more broadly, including in nonurban ecosystems. We summarize the current research providing insight into these questions thus far and recommend approaches for future research.

1. *Are urban soil microbial communities taxonomically and/or functionally distinct from nonurban soil microbial communities, and how much variation exists within the urban environment?*

Microbial phyla most found in soils include the following: α-Proteobacteria, β-Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes, Planctomycetes, and Bacteroidetes (Fierer et al., 2007; Zhang & Xu, 2008). At the phylum level, taxa dominating soils from parks, schoolyards, gardens, road medians, and other urban green spaces are consistent with those observed in nonurban soils (Huot et al., 2017; Lysak &

Lapygina, 2018; Reese et al., 2016; Wang et al., 2018). However, relative abundances of these phyla differ within urban soils and along urban–rural gradients (Hui et al., 2017; Stephanou et al., 2021; Stoma et al., 2020; Tan et al., 2019). Overall, diversity sometimes increases with urbanization (Naylo et al., 2019; Tan et al., 2019), sometimes decreases (Rai et al., 2018), and often remains the same but with shifts in composition (Huot et al., 2017; Joyner et al., 2019; Reese et al., 2016; Yao et al., 2006). Additionally, there is tremendous diversity within these major phyla, which can influence community function. Understanding how microbial diversity and community composition change within urban soils is an important first step, but it is also important to understand what drives community assembly and the consequences of varying community composition for ecosystem function, hence the next two questions.

2. *If differences in microbial taxa and function exist, what are the associated drivers?* (**Figure 1.2**, Arrows B and C)

Although we are only just starting to determine which microbes reside in urban soils, it is becoming clear that there are differences between urban and rural communities, as well as among soil communities within the urban matrix. What environmental variables are driving these differences? How do different taxa respond to these drivers? Answers to these questions are essential to manage for healthy and beneficial microbial communities. Urban microbes may be affected by the same environmental variables as nonurban microbes, but there may be differences in the intensity of these factors and the magnitude of interactions between the drivers and the microbial taxa present.

Questions 1 and 2 can, and ideally should, be answered in conjunction. With careful sampling design, it is possible to characterize urban soil microbial communities while simultaneously identifying major drivers of community composition. One common approach has been to establish urban–rural gradients using factors such as human population density, neighborhood income, and pollution levels (e.g., Azarbad et al., 2013; Chen et al., 2010; Zhao & Guo, 2010). This method allows the identification of large-scale effects of urbanization on soil function. However, gradients may be less effective at fine-to-medium scales due to the high levels of heterogeneity and patchiness across the urban landscape. Temporal trends in temperature and precipitation should also be considered as microbial drivers both among and within cities.

A second major approach has been to focus on particular land-use types within the urban matrix, for example, soils along roads, under impervious surfaces, or beneath turfgrass lawns and parks (e.g., Hu et al., 2018; Law & Patton, 2017; Lorenz & Kandeler, 2006; Papa et al., 2010; Yao et al., 2006; Zhao et al., 2013). Since factors such as dominant plant cover, pH, moisture content, and nutrient content can be among the largest drivers of microbial community composition and may differ drastically across these sites, this approach may be helpful to link microbial taxa and functioning with multiple environmental factors. Focusing on particular land-use types may also enable researchers to generate more site-specific management recommendations to improve urban soil function.

3. *How much does taxonomic composition* versus *functional plasticity play a role in urban soil microbial community function?* (**Figure 1.2**, Arrow C)

A major topic of interest in microbial ecology is the link between taxonomic composition and function. If composition is sufficient to predict microbial community function, then sequencing communities and measuring microbial biomass would facilitate the prediction of microbial community impacts on ecosystem dynamics. To an extent, metagenomic analysis has been useful for understanding and predicting microbial community's functional roles (e.g., Fierer et al., 2012; Graham et al., 2016, Amend et al., 2016). While some functions are phylogenetically conserved, studies have also found that soil microbial communities exhibit functional plasticity and can shift ecological and resource acquisition strategies depending on pressures from the environment (Evans & Wallenstein, 2014; Martiny et al., 2015; Morrissey et al., 2017). Microbial taxa may also be redundant, where the loss of one taxon can be compensated by the function of another (Allison & Martiny, 2008). This research is still developing, and we do not yet understand the direct consequences of most microbial taxa in any ecosystem.

In urban soils, studies explicitly linking specific microbial taxa to function have only recently been conducted. Research on urban microbial communities has been limited primarily to describing composition and functional gene abundance, without directly linking community genetics to in situ ecosystem variables. Bledsoe et al. (2020) and Bonetti et al. (2021) recently used urban constructed wetlands to link microbial community structure to greenhouse gas emissions and quantify microbial contributions to ecosystem services. To manage urban soils and boost ecosystem services, it will be important to understand the functional roles and limitations of the microbial communities in a wider variety of urban soils. This knowledge will have implications for how soil communities can be manipulated by managing environmental factors, or whether inoculation of the soil with

novel microbes will be needed to achieve desirable results. Furthermore, urban soils can serve as model systems for studying fundamental questions about structure–function relationships in microbiomes.

Studies of urban microbiomes could enhance the understanding and societal relevance of ecological science (Forman, 2016). Urban areas experience many environmental extremes within a small geographic area. This variation provides an opportunity to study how variables such as pH, heavy metals, and precipitation impact organisms while controlling for other state factors such as geography, elevation, and seasonality (Jenny, 2012). With many major research laboratories located in urban areas, there is scientific expertise and infrastructure available to set up local observational networks and reveal long-term dynamics (Sparrow et al., 2020; Wang et al., 2021). Urban ecosystem health, including soil microbiome health, could also be monitored through partnerships with community organizations and volunteers (Bliss et al., 2001). As part of this urban ecosystem monitoring effort, it might be feasible to combine field, common garden, and laboratory studies to more explicitly link microbial taxa to function and better understand how microbial communities respond to changes over time.

4. *What consequences do soil microbial communities have for urban ecosystem function and human well-being?* (**Figure 1.2**, Arrows C and D)

Urban microbial communities may have significant effects on urban ecosystem processes, including soil genesis, greenhouse gas fluxes, soil nutrient dynamics, and plant growth. However, it remains unclear to what extent microbial communities drive these

processes as opposed to plants and other organisms. Studies that parse out the functions of soil microbes will help clarify where to invest management efforts to improve soil services.

Soil microbial communities drive ecosystem processes that in turn affect human populations. On regional and global scales, soil microbes have the potential to help mitigate or exacerbate the climate crisis by regulating soil carbon uptake and release (Cavicchioli et al., 2019). On the scale of a city or a neighborhood, however, little is known about how soil microbes affect human communities. Some human health studies have recently found that exposure early in life to a diverse environmental microbiome can reduce asthma and allergy rates, and there has been a push to "rewild" cities with diverse plant- and soil-associated microbes (Mills et al., 2017, 2020; Rook, 2013; Sandifer et al., 2015; Selway et al., 2020). In cities, green spaces are generally the source of diverse environmental microbiomes. Green spaces are not evenly distributed throughout cities and tend to be more common in wealthier neighborhoods. On the contrary, urban soils can also house pathogenic microbes and may serve as reservoirs for antibiotic resistance (Li et al., 2018; Xiang et al., 2018). Therefore, urban soil microbiomes have the potential to help or harm humans, and these benefits and burdens may not be evenly distributed across cities.

Microbiome services raise a question of environmental justice: are wealthier, often white, communities benefitting more from access to green space microbiomes than low-income and minoritized communities? And are there other microbial community functions that benefit or harm some human communities over others? A recent analysis by Schell et al. (2020) found that a history of systemic racism in cities remains a strong determinant of how urban ecosystems are structured. The urban environment may have a patchy distribution of goods and harms that continue to correlate with race and income.

Understanding how microbial functioning is different across the urban landscape and how that affects human communities should be a priority in urban microbial ecology. This research would benefit from collaborations with human geographers, social and environmental justice experts, city officials, and community members to identify impacts of urban soil microbiomes on people and develop ways to improve the urban environment through better understanding and valuing of microbial services.

5. *How might urban areas be better designed/managed to boost ecosystem services by soil microbial communities while minimizing harms?* (**Figure 1.2**, Arrow A)

Efforts are being made to improve ecosystem benefits in cities. Much of this work focuses on conserving or restoring native habitat (e.g., De Sousa, 2003; Marzluff & Ewing, 2008). While restoring urban land to a predevelopment state may provide ecological benefits, there has been a recent push to investigate the ecological roles that novel urban ecosystems play and to consider whether they might also provide important ecosystem services, act as reservoirs for biodiversity, and convey other environmental benefits (Klaus & Kiehl, 2021; Kowarik, 2011; Planchuelo et al., 2019). Pavao-Zuckerman (2008) points out that urban soils can be deliberately manipulated as part of ecosystem management and restoration. While habitat restoration may be the preferred and conventional way to manage ecosystem processes in some locations, it may be unfeasible in urban ecosystems, and fostering a novel but more functionally beneficial ecosystem might be a better use of management effort and resources. To this end, it will be important to form multidisciplinary collaborations with conservationists, city planners, landscape architects, and engineers when managing urban soils.

Cities have already been taking advantage of novel ecosystems to improve sustainability and promote ecosystem services. For instance, green roofs have been designed to help cool buildings and reduce air conditioning needs (Takebayashi & Moriyama, 2007). Bioswales filter debris and pollution out of stormwater and recharge groundwater sources (Li & Davis, 2009). Phytoremediation takes advantage of plant uptake of heavy metals in order to clean up polluted soils (e.g., Ali et al., 2013; Cheng, 2003). Only recently has attention been paid to the role of microbes in these processes (e.g., Cui et al., 2017; Hrynkiewicz & Baum, 2014), and a better understanding of microbial function could allow us to improve on green infrastructure technologies. It is possible that urban green space cover is underestimated (Zhou et al., 2018), so there might be more opportunity than expected to boost ecosystem services in cities. A study of three Swedish cities found that 22.5% of urban area was covered in turfgrass lawns (Hedblom et al., 2017). In addition to that already substantial area of green space, Rupprecht and Byrne (2014) estimated that "informal" green spaces such as vacant lots, brownfields, and road verges made up between 4.8% and 6.3% of cities, presenting additional and undervalued land area that can be utilized to improve urban sustainability.

While most green infrastructure has focused heavily on plants, microbes themselves may have the potential to reduce the negative impacts of urbanization, either independently or in conjunction with plants. For example, microbial communities in green roof soils help plants tolerate and recover from environmental stress (Fulthorpe et al., 2018; Hoch et al., 2019). Additionally, permeable reactive barriers have been designed to intercept and remove nitrates from groundwater by promoting microbial denitrification within the barriers (Vallino & Foreman, 2008). Soil microbes also influence the breakdown

of pesticides, although the efficacy of this microbial degradation depends on community composition and environmental conditions (Reedich et al., 2017). Several studies have tracked and modeled microbial pesticide degradation to prevent pesticides and their harmful breakdown products from leaching into groundwater and aquatic systems (e.g., Soulas & Lagacherie, 2001; Verma et al., 2014; Yale et al., 2017). A more thorough understanding of microbial communities and their functions may allow us to "micromanage" microbial services (Peralta et al., 2014) and develop new technologies, infrastructure, and management practices to improve urban soil health and ecosystem processes.

Conclusion

We propose a new conceptual framework for urban microbial ecology that will help focus research questions and advance knowledge about microbial communities and ecosystem functioning. By identifying key drivers, we provide a path forward to link human actions with changes in the soil microbiome. Feedback loops connect microbes back to human society through the provisioning of environmental goods and harms, which brings attention to microbial consequences for human well-being. We argue that microbial ecologists and biogeochemists should take advantage of the heterogeneity and sharp environmental gradients in urban ecosystems for future study. Not only do microbial communities represent convenient systems for fundamental research on urban biogeochemistry, microbiomes could also play a role in creating healthy, equitable, and sustainable cities. Overall, urban ecosystems deserve more attention from microbial ecologists, and urban ecology would benefit from a greater focus on microbes.

Figure 1.1. Comparison of human impact through land development on urban and agricultural ecosystems. Soil microbiomes in rural and agricultural landscapes have been well studied, while those in more urban landscapes have been largely overlooked.

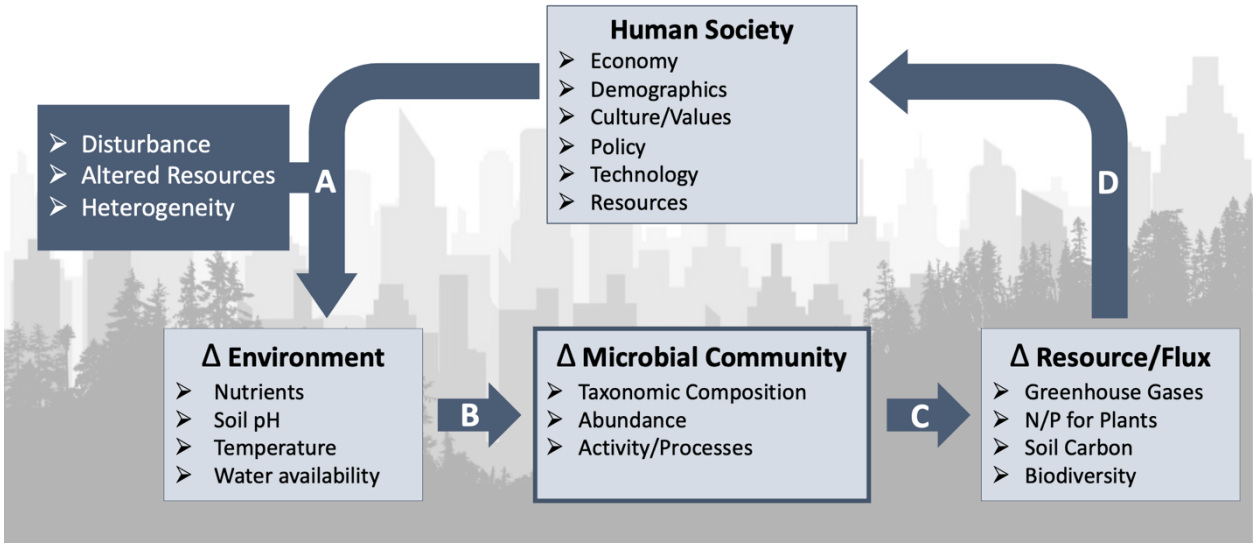

Figure 1.2. Authors' framework for studying soil microbial communities in human-impacted environments.

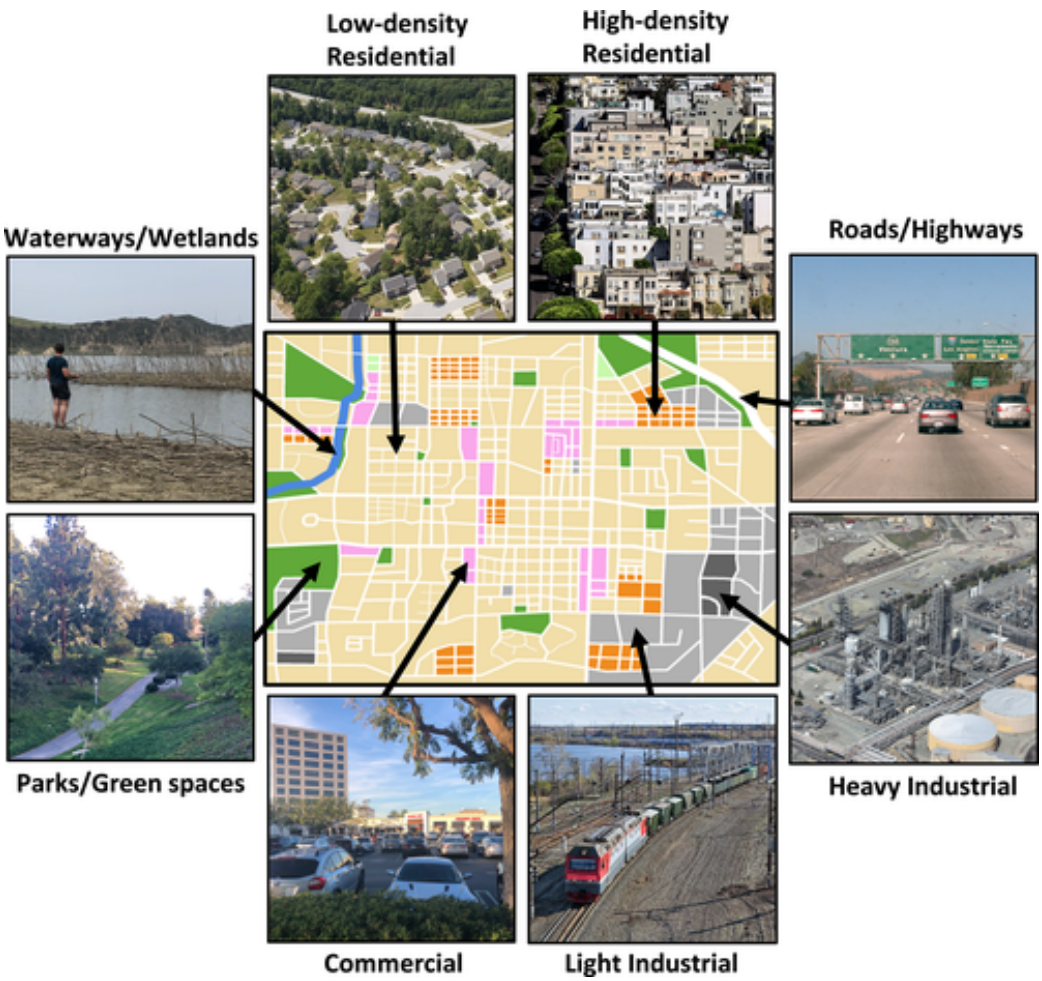

Figure 1.3. Conceptual diagram of an urban matrix, based on a zoning map of Santa Ana, CA. Colors indicate major land-use types.

CHAPTER 2

The Dirt on Your Backyard: Effect of Property Age and Management on Soil Function in a Southern California Neighborhood

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Abstract

Southern California is experiencing rapid urbanization which is drastically altering ecosystem processes. In particular, suburban residential neighborhoods comprise novel ecosystems with water and nutrient inputs that differ greatly from the surrounding natural landscape. This generates concern over the sustainability of these new urban ecosystems, especially whether they will contribute to an increase or reduction in regional greenhouse gas emissions in the coming decades. In this study, we characterized the soils of a Southern California residential neighborhood including the microbial community which mediates vital biogeochemical processes including nitrogen cycling and carbon sequestration. We established a chronosequence of yards to understand how urban soils change over time after development, and compared different vegetation types to understand the potential impacts of our landscaping choices. We found that yard soils were highly nutrient and moisture enriched compared to an adjacent undeveloped ecosystem, and turfgrass in particular was associated with high levels of water and nitrogen. Despite high respiration rates, yard soils did accumulate carbon and nitrogen over time. This was largely driven by non-turfgrass soils which suggests that transitioning away from turfgrass may benefit urban sustainability goals by reducing water and nitrogen needs and promoting carbon storage over time as soils recover from development.

INTRODUCTION

Urban soils are rich in organismal and functional diversity and provide a variety of ecosystem services including carbon sequestration, pollution remediation, and water filtration (Brown et al, 2012; O'Riordan et al, 2021; Elmqvist et al, 2015). Despite this importance, urban soils are not well studied which presents a problem for managing to optimize ecosystem services with minimal resource waste. One challenge for this research is the heterogeneity of urban soils. Although there is increasing evidence that urban ecosystems are converging functionally across the globe (Trammell et al, 2020; Groffman et al, 2014), there is also a high level of complexity across relatively small scales within urban landscapes including differences in land history, use, and management (Pickett & Cadenasso, 2009; Mao et al, 2014; Kendal et al, 2012). Therefore while urban ecosystems may contain similar motifs across regional and global scales, we continue to lack a strong understanding of soil function at management-relevant scales.

Urban soil function, mediated by organisms such as microbes, comprise a feedback cycle of which we require a better predictive understanding (**Figure 2.1A**). Urban soils are also unique in their disturbance history compared to most other soil ecosystems, which may affect ecosystem functional trajectories and appropriate management changes. Upon initial development, the original soil profile is heavily disrupted and often removed completely, and later backfilled with homogenized soil that may come from another location. After a massive but short-term disturbance, soils may undergo succession in response to state factors, and eventually return to a steady state functionally reminiscent of the pre-development soil (Huggett 1998; Horn, 1974). However, urban soils are rarely left alone after development. Humans usually introduce novel plant communities and, to

maintain these plant communities, amend the soil with high levels of nutrients and water (Pavao-Zuckerman, 2008; Pouyat et al, 2010). Particularly in urban green spaces, these new and sustained resource inputs to the ecosystem may result in very different soil communities and functions compared to the pre-developed soil (**Figure 2.1B**).

The outcome of this new resource regime on soil function may have repercussions for urban sustainability. Many research groups have focused on the carbon sequestration potential of urban soils to partially offset local greenhouse gas emissions. Depending on climate, land use, and management practices, urban soils can indeed store high quantities of carbon and may even be considered global hotspots of carbon sequestration (Pouyat et al, 2006; Brown et al, 2012; Vasenev and Kuzakov, 2018). Especially in semi-arid ecosystems, urban soils may be effective at accumulating carbon over time (Sapkota et al, 2020). To prescribe appropriate management strategies for improved ecosystem services such as carbon sequestration while conserving water and limiting inputs of synthetic fertilizer, we require a more thorough understanding of how urban soils function while also serving human needs and values (Nugent & Allison, 2022).

Among urban land use types, green spaces such as parks, golf courses, and residential yards receive the most attention for sustaining biological diversity and ecosystem services within cities while also promoting human cultural, physical, and mental well-being. Urban greenspaces tend to be exposed to similar management regimes, but recent findings suggest that urban soils also respond to a historical legacy of prior land use (Ziter & Turner, 2018; Raciti et al, 2011). Plant communities present in urban greenspaces can also influence soil properties both directly (e.g. reduction in bulk density, soil organic matter inputs) and through their management needs (irrigation and fertilization).

Therefore, green space soils may have differing soil ecology that result from dynamic interactions between land use history, soil age, management, and dominant vegetation.

While some characteristics of soil may be slow to change over time such as pH and bulk density, other components of the soil ecosystem are more dynamic within short time frames and are likely to respond to even small changes in soil management. In particular, soil microbial communities can rapidly change in abundance, structure, and function when exposed to new environmental conditions (Andrade-Linares et al, 2021; Chase et al, 2021; Lau and Lennon, 2012). Soil bacteria and fungi mediate many important soil processes including carbon and nitrogen cycling, and thus focusing more explicitly on microbes has become a high priority in urban ecology and biogeochemistry studies (Antwis et al, 2017; King, 2014; Yang & Zhang, 2015).

In this study, we analyzed a residential neighborhood and an adjacent ecological preserve to characterize the impact of urban development on soil and microorganisms. Within the residential yards, we also distinguished soils from two plant groups: Turfgrass, and "mixed-vegetation" (any non-turfgrass plants). This enabled us to investigate how traditional turfgrass lawns support ecosystem function compared to landscaping alternatives. We assessed these functions along a chronosequence of yards established between 1980-2020, allowing us to use a space-for-time approach to explore how soils and their microbiota may change as soils recover from development. While we did not replicate neighborhoods or chronosequences in this study, this work is beneficial for demonstrating helpful methodology for examining urban biogeochemistry, and provides actionable information for local land managers.

We measured a suite of soil variables known to be influenced by urbanization and, in turn, to influence microbial communities including bulk density, pH, moisture, and nutrient content. We sequenced bacterial 16s rRNA and fungal ITS2 to determine community composition. As metrics for microbial function, we also measured soil respiration and potential extracellular enzyme activity (EEA). Respiration provides a metric for microbial activity and decomposition, while potential EEA enables us to determine which substrates the communities are likely targeting to satisfy stoichiometric requirements for growth. As microbial communities respond to changing environmental variables, soil respiration and EEA can provide insight into the potential fate of carbon and nitrogen in soil ecosystems (Morrissey et al, 2014; Ullah et al, 2019; Ramin & Allison, 2019).

We developed three hypotheses around soil and microbial characteristics in urban soils. **First**, we predicted that residential soils will have distinct soil characteristics and harbor a different microbial community compared to adjacent undeveloped soils. **Second**, microbial activity and diversity will increase over time since development, correlating with an increase in C, N, and pH and a decrease in bulk density. **Third**, we predicted that turfgrass will affect soil and microorganisms differently than landscaping alternatives, resulting in diverging microbial communities and soil characteristics between these urban vegetation types over time. In particular, turfgrass soils will have higher moisture content, nitrogen, and respiration compared to soils under other vegetation. To test these hypotheses, we sampled soils in a residential neighborhood on the University of California, Irvine, campus called University Hills which contains homes reserved for faculty and staff. All residents are on one email list-serve, which made it convenient to recruit participants

for the study. We sampled soils from 45 yards of homes built across a 40-year timespan, as well as a neighboring ecological preserve which was historically used for cattle grazing but has been a conserved natural space since the 1980s.

METHODS

Study Site

We visited 45 yards at the homeowners' convenience between March 2022 and May 2022 (**Figure 2.1**). During this time, weather conditions and temperature were relatively consistent and sampling of the yards occurred at random so that geographic locations and time of property development were spread across the sampling timeframe. The pre-development soils at University Hills were predominantly silty clay, atop paralithic bedrock (Soil Survey Staff - Web Soil Survey, 2023). During development, backfill may have resulted in the introduction of new soil types. We did not characterize soil type for each property in this study, accepting that novel soil types may be a source of background variation.

Field Collection and Sample Preparation

At each property, we collected soil from two vegetation types if both were present: "turfgrass" and "mixed vegetation" which included any non-turfgrass plants. This allowed us to differentiate between the effect of traditional turf lawns on soil compared to common alternative landscaping options. We determined the post-development soil age for each property, using a combination of satellite imagery and construction records from the University Hills management offices. We used this information to establish a chronosequence which allows us to understand how soils and microbial communities may change over time after the major disturbance of urban development (Walker et al, 2010). In

the ecological preserve, we deliberately sampled from under both grasses and shrubs to make those samples more comparable to yards with regard to plant functional groups that may influence soil properties.

At each sampling location, the litter layer was carefully removed to expose the bulk soil. Only bulk soil was sampled, with the O-horizon excluded. Three 10cm cores within the same vegetation type from each site were collected and homogenized together. Distance between cores varied based on the size of the yard and location of vegetation types, but were always at least 3 meters apart. One third of the homogenized soil, by mass (the equivalent of 1 core), was removed and used to quantify bulk density. The remainder was sieved to remove particles such as rocks and roots larger than 2mm. Subsamples were weighed out for each downstream analysis. Fresh soil was used for pH measurement, flow cytometry quantification of bacterial cells, inorganic nitrogen extraction, and measurement of gravimetric water content. Another portion of soil was frozen at -20C for extracellular enzyme assays and DNA sequencing, and the remainder was oven dried at 60C for elemental analysis of total carbon and nitrogen.

Soil pH

To measure soil pH, we added 30 mL of deionized water to 15g field-moist soil in a 50ml Falcon tube. Tubes were shaken to form a slurry, and then caps were removed and tubes were allowed to stand at room temperature for 30 minutes while soil settled to bottom and the solution equilibrated with atmospheric CO_2. Supernatant pH was measured with a Mettler-Toledo SevenEasy pH probe, calibrated using standards of pH 4, 7, and 10.

Bulk Density

Soil was allowed to air dry to improve the ease of sieving. Soil was then sieved to separate particles larger than 2mm. The fine soil (<2mm) was oven dried at 60ºC and dry mass was recorded. The volume of the large particles (>2mm) was determined by displacement of water in a graduated cylinder. That volume was subtracted from the core volume in calculations. Bulk density was calculated as the mass of dry fine soil divided by the adjusted core volume, in grams per cubic centimeter.

Gravimetric Water Content

10-15g of field-moist sieved soil was weighed then oven-dried for several days at 60ºC, then weighed again. The loss in mass was determined to be the water content of the soil. Gravimetric water content was calculated as the mass of evaporated water divided by the remaining dry mass of soil.

Bacterial Cell Count via Nycodenz Extraction and Flow Cytometry

Field-fresh soil was stabilized in 1% glutaraldehyde (GTA) buffer for up to 30 days before analysis. Bacterial cells were then extracted from the soil following the methods in Khalili et al (2018). Extracted cells were stained with 1% Sybr Green in tetrasodium pyrophosphate (TSP) buffer and quantified via a Novocyte Flow Cytometer. Unstained and stained Pi-buffered GTA were also run on the Flow Cytometer to account for environmental autofluorescence and to check for contamination. Absolute counts of samples minus background counts were then converted from counts/ml to counts/g dry soil.

Extracellular Enzyme Activity (EEA)

Extracellular enzyme activity was assayed following German et al (2012). We homogenized 0.30 grams of soil in 100ml of 25mM maleate buffer using a Tissue Tearor 985370-07 Homogenizer. In a 96-well black plate, we created a serial dilution of 7

synthetic substrates that fluoresce when cleaved in a hydrolytic reaction by enzymes of interest (**Table 1**). Two plate columns contained standards (7-Amino-4-methylcoumarin, 4-Methylumbelliferone) and a final column contained ultrapure water. 125ul of sample homogenate was added to each well. A buffer plate was also set up, adding a 125ul of 25mM maleate buffer in place of the sample homogenate. After 4 hours of incubation at room temperature, plates were read using a Bio Tek Synergy H1 Microplate Reader at 360 nm/460 nm (excitation/emission).

We removed outliers from each curve, and activities were fit to the Michaelis Menten equation (below) using the nonlinear least squares (nls) function in R following German et al (2012).). V is the reaction velocity, K_m is the substrate concentration at half V_{max}, and V_{max} is the maximum velocity. We utilized the V_{max} to determine maximum potential activity in umol/min/mg for each enzyme. Data were log transformed (natural log (x+1)) to improve data normality .

Michaelis-Menten Equation for enzyme activity:

$$\mathbf{V = V_{max}[substrate\ concentration] / K_m + [substrate\ concentration]}$$

Units: V = μmol / min/ mg

Ratios of enzymes targeting different nutrients can provide insight into which elements are potentially limiting for microbial growth (Zhao et al, 2018; Sinsabaugh et al, 2009). The ratio of BG/NAG+LAP represents carbon-to-nitrogen acquisition. BG/AP represents carbon-to-phosphorus. NAG+LAP/AP represents nitrogen-to-phosphorus acquisition.

DNA Extraction and Sequencing

DNA was extracted from the soil using the Qiagen DNeasy PowerSoil Pro Kit (Cat No. 47014) following manufacturer instructions. Successful extraction of DNA was confirmed using a Nanodrop Spectrophotometer (ND-1000, Thermo Scientific). DNA was then diluted 1:10 in RNase/DNase-free water. For bacteria, we amplified the V4–V5 region of the 16S rRNA, following the same procedure and using the same primers as Barbour et al (2023). We confirmed successful amplification via gel electrophoresis and pooled our PCR products based on gel image band strength: 2 µL for high concentration, 4 µL for medium concentration, and 6 µL for low concentration. For fungi we used custom 5.8S-Fun and ITS4-Fun primers optimized for Illumina Sequencing that target the ITS2 rRNA region (length of 267 and 511 base pairs)(Taylor et al, 2016; Willing et al, 2021). Successful amplification was confirmed with gel electrophoresis, and libraries were pooled in the same manner as the bacterial 16S libraries.

Our pooled bacterial and fungal amplicon libraries underwent a Speed Bead Magnetic Carboxylate cleanup (GE Healthcare UK Limited, Buckinghamshire, UK) to remove primers. After checking the quality of each pool with qubit and Bioanalyzer each library was sequenced in separate Illumina MiSeq v.3 (2 x 300bp) runs at the UC Irvine Genomics Research and Technology Hub (Irvine, CA, USA).

Bioinformatics and Taxonomic Assignment

For bacteria, forward reads were demultiplexed in Qiime2. Primers were removed using Trimmomatic v0.39-1, and sequences were quality filtered and dereplicated in the Qiime2 pipeline using DADA2 (reverse reads were excluded due to low sequencing quality). 81% of sequences passed quality control (filtered, denoised, and non-chimeric) in Qiime2. Amplicon Sequence Variants (ASVs) were picked at 100% identity level, and

taxonomy was assigned using the SILVA 138 SSU Ref NR99 database. Non-bacterial sequences were then removed, along with sequences that could not be assigned at the phylum level, resulting in 21,036 final bacterial ASVs. The bacterial taxonomic table was then rarefied to a sequencing depth of 3211 prior to downstream analysis, using the EcolUtilis package in R studio. For fungi, sequencing data was processed following the methods of Willing et al (2021). Briefly, we used AMPtk (Jusino et al, 2019) to merge and trim reads, remove chimeras and singletons, and assign taxonomy. A total of 1,268,379 reads were mapped onto 4,359 ASVs. ASVs that were non-fungal were removed, and we rarefied data to 4800 reads per sample.

Soil Nutrient Concentrations

Inorganic nitrogen was extracted from soil in 2M KCl and frozen at -20C until analysis. We followed Allison et al (2008) for ammonium and nitrate assays in 96-well plates modified from Weatherburn (1967) and Doane and Horwath (2003). KCl blanks were included in the assays to account for and subtract out background nitrogen in the reagents.

To quantify percent carbon and nitrogen, soil samples were dried and finely ground in preparation for elemental analysis. 10-15mg of dry soil were packed in 8x5mm tin capsules, then combusted in a Flash 2000 Organic Elemental Analyzer by CE Elantech, using aspartic acid for the standard.

Statistical Analyses

All data analysis was performed in R version 4.3.1 (R Core Team, 2023). Spearman correlations between variables were determined in R using the *cor.test* function. Environmental variables and microbial activities were compared with *t*-tests between

ecosystems and between vegetation types. To determine whether ecosystem or vegetation type had a significant effect on microbial community composition, we conducted PERMANOVAs, using an adonis2 model from the *vegan* package, on Bray-Curtis distance matrices with 999 permutations. When the PERMANOVA indicated a significant effect, we used a SIMPER test to determine which taxa contributed most to differences between communities. Microbial Shannon diversity index was calculated using the "diversity" function from the R package *vegan* (Oksanen et al, 2020).

Enzyme activity was not normally distributed even after performing normalization transformations (multiplied by percent carbon, and natural log transformed), and therefore we used Wilcoxon Signed Rank tests to compare means between groups (Ecosystem or Vegetation).

Figures were made using ggplot2 in R studio (Wickham, 2016). In all statistical analyses within this study, a p-value of <0.05 was considered significant.

RESULTS

Soil Properties - Residential vs. Undeveloped Soil

We found statistically significant differences between residential yards and the ecological preserve for most variables measured (**Figure 2.3**). Soil percent C, percent N, and C:N ratio were higher in residential yards. Despite higher overall nitrogen in yards, soil nitrate concentration was significantly higher in the preserve. We did not find statistically significant differences in bulk density, pH, or ammonium concentration between ecosystems.

Soil Properties Along the Chronosequence

Time was significantly correlated with many factors across residential yards in University Hills, indicating that urban soils do undergo succession (**Figure 2.3**). Immediately after development, soil pH was alkalized but gradually decreased to neutral and was comparable to the ecological preserve toward the end of the chronosequence. Percent carbon and nitrogen both increased significantly over time after development, beginning at or below the levels of the ecological preserve immediately after development, and surpassing the ecological preserve within 10-15 years. Soil moisture was higher in the residential soil compared to the preserve soil immediately after development, and continued to increase over time. Bulk density trended down with borderline significance ($p=0.052$) as developed soil aged but did not diverge considerably from the ecological preserve over the 40 years captured in our study.

Microbial Function - Residential vs. Undeveloped Soil

Extracellular enzyme activity (uMol/mg/min) may provide insight into microbial strategies for nutrient acquisition. Total EEA is reduced in University Hills compared to the Ecological Preserve, suggesting a lower investment in overall resource acquisition. Ratios of carbon-to-phosphorus, nitrogen-to-phosphorus, and carbon-to-nitrogen enzyme activity are all close to 1:1 in the Ecological Preserve, indicating that microbes are investing in these three nutrients to a similar extent. University Hills potential EEA has a higher ratio of BG/AP and NAG+LAP/AP compared to the Ecological Preserve with the ratios trending above 1:1. This suggests that University Hills may be more carbon or nitrogen limited compared to the ecological preserve.

Respiration was also measured as an indicator of potential microbial activity and decomposition rates. Soil respiration was very low in the ecological preserve and had low

variation across samples. Respiration was higher in yards compared to the ecological preserve.

Microbial Function Along the Chronosequence

Soil respiration in yards trended up over time with borderline significance ($p=0.0503$), and was consistently higher than the ecological preserve throughout the chronosequence. Ratios of extracellular enzyme activity did not change over the chronosequence to indicate shifts in resource limitation for microbes. The total enzyme activity gradually increased with soil age, likely in response to increased nutrient and water availability which also responded positively to soil age.

Microbial Community Diversity and Composition

The two contrasting ecosystems harbored distinct microbial communities. We observed separation of both bacterial and fungal communities between the ecological preserve and residential yards (**Figure 2.4**), and differences in major phyla can be seen in **Figure 2.5.** Ecosystem type significantly contributed to community differences for both bacteria and fungi, explaining 16.6% of differences in bacterial composition and 15% for fungi (PERMANOVA; $p<0.05$). The top three bacteria phyla that contributed to community differences between ecosystems were *Actinobacteria* (3.8% of difference, higher abundance in ecological preserve), *Proteobacteria* (2.2% of difference, higher abundance in yards), and *Acidobacteria* (1.6% of difference, higher abundance in ecological preserve). For fungi, *Ascomycota* (higher in ecological preserve), *Basidiomycota* (higher abundance in yards), and *Mortierellomycota* (higher abundance in yards) were the top three phyla contributing to community differences (6.4%, 4.7%, and 1.1% respectively).

We tested for a correlation between microbial Shannon diversity and soil age to determine whether diversity changed over time after development in yard soils. Fungal diversity increased significantly with soil age, but bacterial diversity did not. Fungal alpha diversity was already similar to the Ecological Preserve soon after development, and continued to increase over time. Bacterial diversity remained comparable to the ecological preserve over the span of the chronosequence. We also tested correlations between the relative abundance of major taxa with soil age to determine whether the taxa contributing most to community differences also respond to the chronosequence. For both bacteria and fungi, we observed shifts in the abundance of major taxa across soil age: Among common bacteria, *Acidobacteria* increased with soil age, while *Proteobacteria* decreased. Among common fungi, *Ascomycota* decreased, and *Mortierellomycota* increased.

Influence of Yard Vegetation on Soil Properties and Microbial Communities

Turfgrass soils were significantly more moist and nitrogen-rich compared to the soils with other plant types. We did not observe differences in microbial function (total EEA or respiration) or Shannon diversity when comparing soils from turfgrass and non-turfgrass vegetation. Vegetation type did have a significant effect on bacterial community composition, explaining 8% of variation among University Hills bacterial communities. Bacterial phyla that particularly responded to vegetation type included *Actinobacteria*, *Proteobacteria*, and *Acidobacteria* (3.8%, 2.2%, and 1.6% contribution to community differences, respectively). Fungal beta-diversity did not respond to vegetation type.

We also tested for differentiated response of vegetation type to time along the chronosequence. We found that changes in several variables over time along the chronosequence were driven by the mixed vegetation samples. Significant correlations

between variables and time often disappeared when examining turfgrass samples alone. These variables included percent C, percent N, pH, nitrate, and bacterial alpha diversity (**Figure 2.6**).

DISCUSSION

Urban Soils as Novel Ecosystems

Urban soils often have very different characteristics compared to the undeveloped soils they replaced, creating a novel ecosystem which we are only just beginning to study and understand. As we manage these ecosystems, we create a feedback loop where anthropogenic inputs alter soil processes leading to environmental benefits or harms which we then respond to with evolving management goals. The aim of this study was to determine how management of a Southern California neighborhood is impacting soil metrics and microbial function, which will allow local land managers to make informed changes for improved sustainability of urban land. To do this, we established a chronosequence within the neighborhood to examine how urban soils respond to and recover from development. We found that suburban soils in Southern California undergo succession after development but become more moist and nutrient rich compared to analogous undeveloped soils. This results in a novel ecosystem with different biota and ecological functions than what the Southern California climate and geology would naturally support (**Figure 2.1B**).

Urban Soil Characteristics and Recovery from Disturbance

Soils are often compacted and alkalized after recent development (De Kimpke & Morel, 2000; Yang & Zhang, 2015; Scharenbroch et al, 2005). Here, we found that bulk density and pH did not differ overall between the residential yards and ecological preserve contrary to our hypothesis. The chronosequence revealed that pH started off more alkalized immediately after development, and became more neutral-to-acidic over time. Bulk density decreased with borderline significance over time, likely as a result of soil modification by plants and microbes. While bulk density did not differ between ecosystems within the timespan captured in this study, it's possible that bulk density may continue to decrease as properties age on a longer time scale, leading to lower bulk density in urban soils compared to unmanaged soils in future decades (Sharenbroch et al, 2005).

In line with our predictions, the soil characteristics that differed most notably between the suburban and undeveloped ecosystems in our study were soil moisture and nutrient content. Differences in soil moisture between urban and rural soils can vary heavily by land use and region, but in general, urban greenspace soils in semi-arid climates such as Southern California tend to have higher water content due to frequent irrigation. Our results support this trend, and we found that soil moisture immediately started higher in residential yards compared to the ecological preserve and continued to increase with soil age. This may be a result of tree and shrub canopies expanding to provide more shade and cooling, reducing evaporation from soil (Edmondson et al, 2016). Declining bulk density and increased OM content as plants establish may also increase the water-holding capacity of the soil (Cogger, 2005; Cannavo et al, 2014).

Soil carbon and nitrogen in residential yards were comparable to the ecological preserve immediately after development, but quickly increased above undeveloped soil

levels. We did not observe a shift in C:N ratio over time, indicating that these nutrients increased in urban soils at a similar rate. C:N ratio was higher overall in residential compared to ecological preserve soils but still within typical range for vegetated soils. Urban ecosystems in drier climates have become N-enriched, resulting in concerns over GHG emissions and nitrate leaching (Zhu et al, 2006; Bytnerowicz & Fenn, 1996). However, carbon inputs to the soil in this study appear to be keeping pace with nitrogen inputs, and the nutrients maintain a similar balance over time. Nitrate concentrations were also higher in the ecological preserve compared to the residential soils, suggesting that urban greenspaces in semi-arid climates may be better capable of cycling excess nitrogen compared to natural soil. We did not measure nitrogen fluxes from soil to determine whether nitrate in the urban soils is being converted to biomass or is predominantly lost back to the atmosphere or leached at a higher rate than in the ecological preserve. Overall, we find that residential soils are nutrient rich and moist compared to adjacent natural soils, and are likely to become even more so over time.

Bacterial and Fungal Communities in Residential Soils

Changes in the abundance of two major phyla contributed most to differences between residential soil communities and undeveloped soil communities. Relative abundance of *Actinobacteria* decreased substantially in yard soils, and were replaced primarily by *Proteobacteria*. Long-term nitrogen fertilization has been found to promote growth of *Proteobacteria*, and therefore the higher availability of nitrogen in urban soils including residential soils in this study may contribute to their increased relative abundance (Dai et al, 2018). *Actinobacteria* were found by Eisenlord and Zak (2010) to decrease in abundance under nitrogen fertilization and increased soil organic matter.

However other studies have found a positive response of *Actinobacteria* abundance to nitrogen addition, particularly when there is sufficient soil organic carbon (Liu et al, 2017; Ramirez & Fierer, 2012). These inconsistencies across studies indicate that focusing at the phylum level may be too broad to draw meaningful functional conclusions about these taxa and their response to environmental change.

Among fungal phyla, both ecosystems were heavily dominated by *Ascomycota* followed by *Basidiomycota*. *Ascomycota* had reduced abundance in urban soils, while *Basidiomycota* increased. This shift in relative abundance between these taxa may be due to higher moisture levels in residential soils. Zhang et al (2014) found that along a gradient of ecosystems with different moisture regimes, *Ascomycota* had reduced abundance in wetter ecosystems, while *Basidiomycota* increased in abundance in wetter ecosystems. However in response to nitrogen fertilization, *Ascomycota* increases while *Basidiomycota* decreases (Nie et al, 2018; Ye et al, 2020). The shift in relative abundance between these taxa in our study may indicate that the increased moisture availability outweighs the effects of increased nitrogen on the fungal community. *Ascomycota* also significantly decreased in abundance over the chronosequence as soils aged, coinciding with an increase in both soil moisture and nitrogen, lending further evidence to the likelihood that moisture is driving *Ascomycota* relative abundance rather than nitrogen availability in these ecosystems.

In addition to characterizing the microbial community composition, we also measured soil respiration and EEA to determine whether there may be microbial functional consequences as a result of urbanization. Soil respiration is frequently found to be higher in urban landscapes compared to the natural environment and degraded greenspaces can

further add to this CO2 flux (Decina et al, 2016; Li & Wang, 2021; Ng et al, 2015). Li and Wang (2021) suggest that a higher degree of urban greening can reduce soil temperature and improve carbon sequestration in cities. In the residential yards in our study, soil respiration was still highly elevated compared to the nearby undeveloped soils, and total microbial enzyme activity also increased, indicating that microbial communities may have increased decomposition activity in these soils resulting in high carbon losses despite high inputs from vegetation. Respiration and EEA also increased with soil age. Sapkota et al (2020) found that older urban soils in an arid climate did continue to store more soil organic matter until reaching a threshold at 50 years, and our study also suggests that soil carbon continues to increase for at least 40 years. Therefore, long-term soil carbon storage may outweigh the higher respiration and decomposition rates present in urban soils in arid and semi-arid climates. A more thorough analysis of carbon and nitrogen stocks and their turnover within these ecosystems over time will be important to determine whether these soils are ultimately a carbon source or sink. It is also crucial that we conduct more extensive life-cycle analyses on the GHG consequences of water and synthetic fertilizer inputs, as a large proportion of GHG emissions from these resources may occur before they are even applied to soil.

Vegetation Type - Turfgrass or Alternative Landscaping for Improved Ecosystem Management?

When it comes to urban green spaces and concerns over carbon sequestration or losses from these soils, turfgrass is often a focus of the discussion. In addition to cultural and aesthetic value, turfgrass can provide ecosystem services including water filtration, erosion control, and fire protection (Monteiro 2017; Thompson and Kao-Kniffin, 2019). A

drawback is that turfgrass requires significant water input, particularly in semi-arid and arid climates. Some find that turfgrass has high carbon storage potential (Bandaranayake et al, 2003; Pouyat et al, 2009). Others point out that after accounting emissions associated with management and nitrous oxide emissions in addition to carbon dioxide, turfgrass is likely to be a source of GHGs rather than a sink particularly when water and nitrogen are applied excessively (Townsend-Small & Czimczik, 2010; Kong et al, 2014). Our research finds that turfgrass does have higher water and nitrogen content compared to other vegetation found in residential yards. Respiration was similar between the vegetation types, indicating that microbial activity in turfgrass soils is not significantly elevated compared to other landscaping options. Measuring GHG fluxes will be crucial in future work to understand whether this turfgrass is able to act as a carbon sink over time, or if it ultimately leads to greater emissions.

We also found that soil characteristics under turfgrass do not respond to time in the chronosequence and are likely much more affected by turfgrass management. This may be due to rapid establishment of turfgrass, while other vegetation types such as trees and shrubs take years to decades until they reach maximum size and fully establish their root networks. This lack of response to time highlights the importance of careful turfgrass management, as management and land use change are the main drivers to which turfgrass soils can respond (Yao et al, 2006).

One concern about turfgrass is a reduction in biodiversity. Baldi et al (2023) found that turfgrass has reduced bacterial biodiversity compared to native gardens, and Mills et al (2020) found that lawns harbored lower fungal diversity compared to park, revegetated, and remnant forest soils. We found that bacterial composition responded slightly to

vegetation type, but there was no difference in fungal composition. Alpha diversity was similar between the vegetation types as well as metrics of microbial function including respiration and EEA. It's possible that microbial communities and their functions are not negatively impacted by turfgrass in this ecosystem, or that yards are small enough that microbial dispersal from other vegetation types may promote higher biodiversity in lawn soils. Regardless, conserving scarce resources such as water and promoting other ecosystem benefits including pollinator and bird biodiversity may support the need to convert some turfgrass to other vegetation (Billeisen et al, 2022; Larson et al, 2017; Elderbrock et al, 2020).

CONCLUSION

Urban soils are sensitive to management, and non-turf soils follow a successional timeline where moisture, nutrients, and bacterial diversity increase for at least 40 years. We found that microbial communities in these suburban soils are more functionally active compared to the natural soils. There is growing concern over urban greenspace management and its ability to support ecosystem services and biodiversity. Our results suggest that managed residential soils in southern California are capable of hosting diverse soil microbiota regardless of vegetation type, but that further work is needed to understand the role of these soils in regional greenhouse gas budgets, water use, and nitrogen pollution. We recommend that future research into urban ecosystems includes multiple time points to capture finer-scale temporal trends, and detailed surveys of irrigation and fertilization schedules to better correlate human land management with soil function. Many people are interested in making their homes more sustainable, and this

research can enable homeowners and land managers to take an active role in promoting environmental health in their communities.

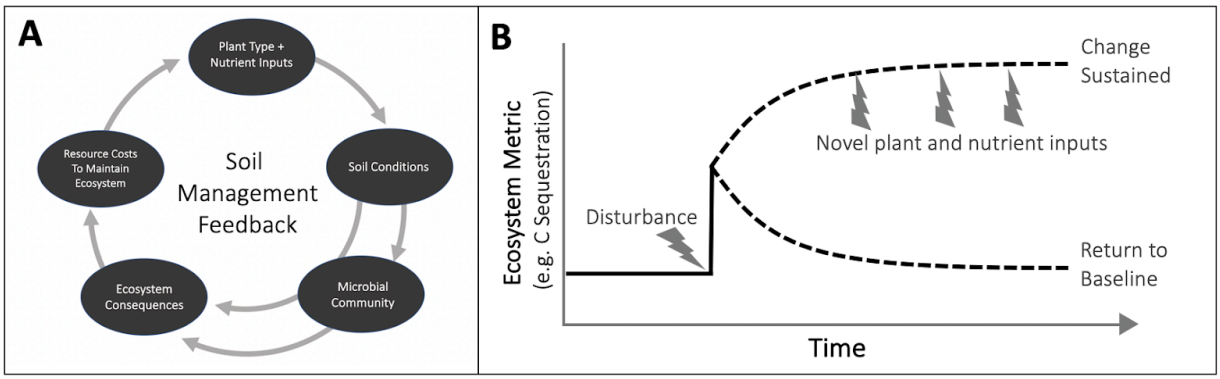

Figure 2.1. A) Conceptual framework of soil management feedback cycle. B) After a major disturbance, ecosystem metrics (e.g. carbon storage, microbial diversity) may return to baseline values or may have sustained new values. We propose that in urban ecosystems, repeated resource inputs will sustain a novel ecosystem after development. Framework adapted from Shade et al (2012).

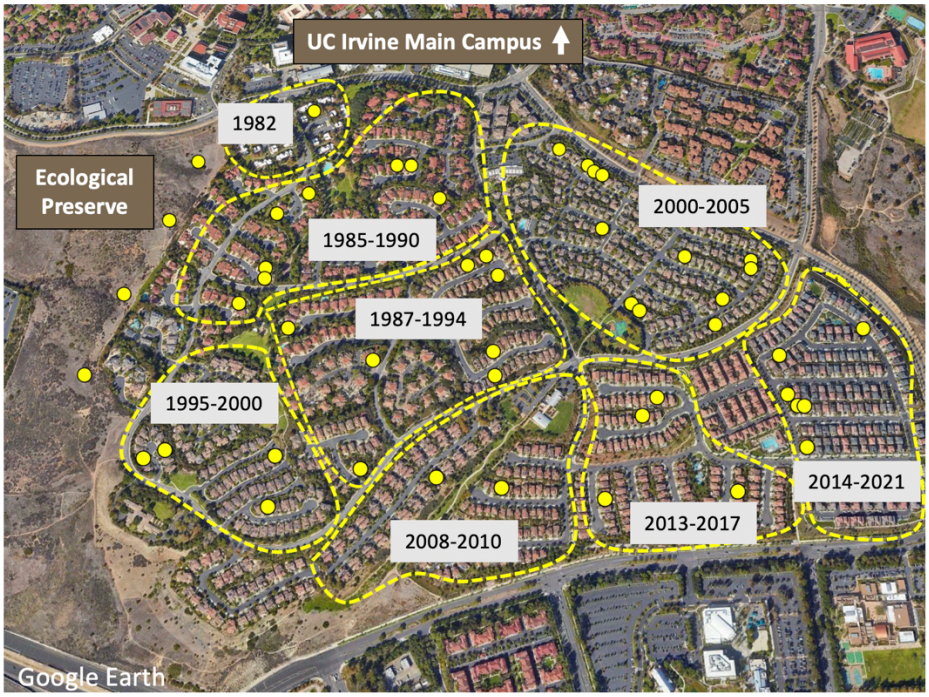

Figure 2.2. Satellite image of the study area from Google Earth. Yellow dots represent sampling locations. Yellow dashes outline the developmental phases completed within the years indicated on the image. The ecological preserve is located immediately to the west of University Hills.

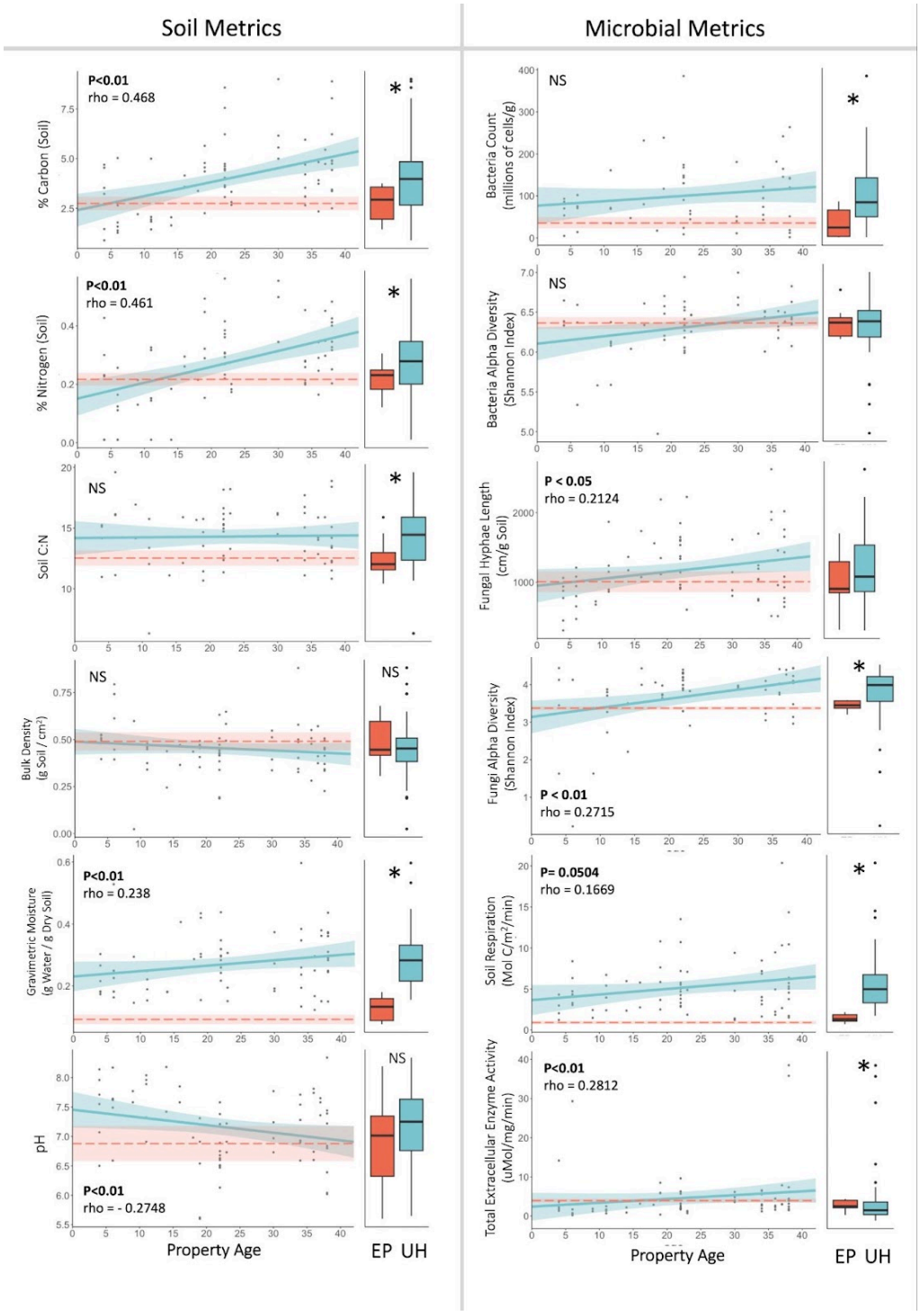

FIGURE 2.3. Scatter plots indicate response of soil and microbial metrics in University Hills to soil age (years), with a fitted regression line (blue) and correlation values. The red dotted line represents the average value for the ecological preserve. Shaded areas around both lines represent standard error. Boxplots to the right of the scatterplots are comparisons of the soil or microbial metric between the Preserve and University Hills ecosystems. Asterisks indicate significant differences between ecosystems using a T-test. NS = not significant.

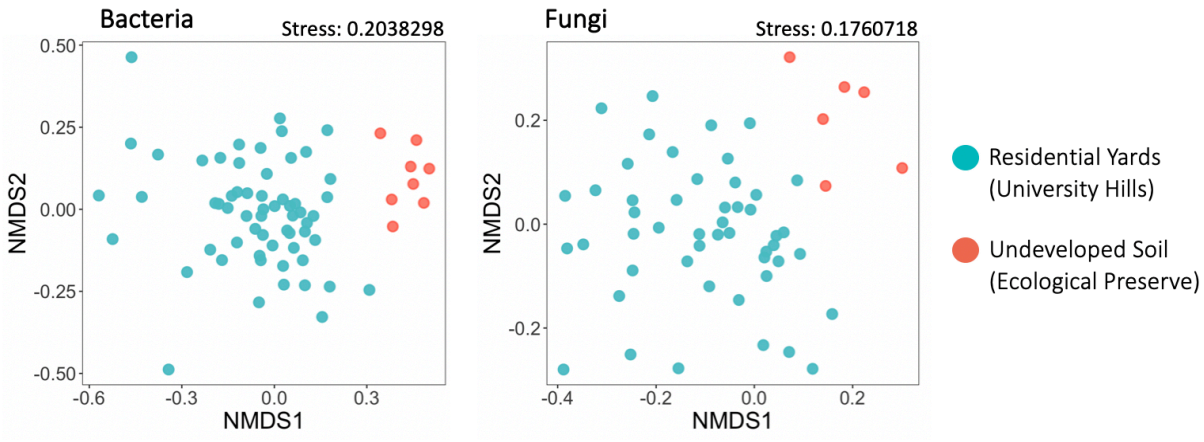

Figure 2.4. NMDS plots of bacterial bray curtis distances (left) and fungal Bray Curtis distances (right) color coded by ecosystem.

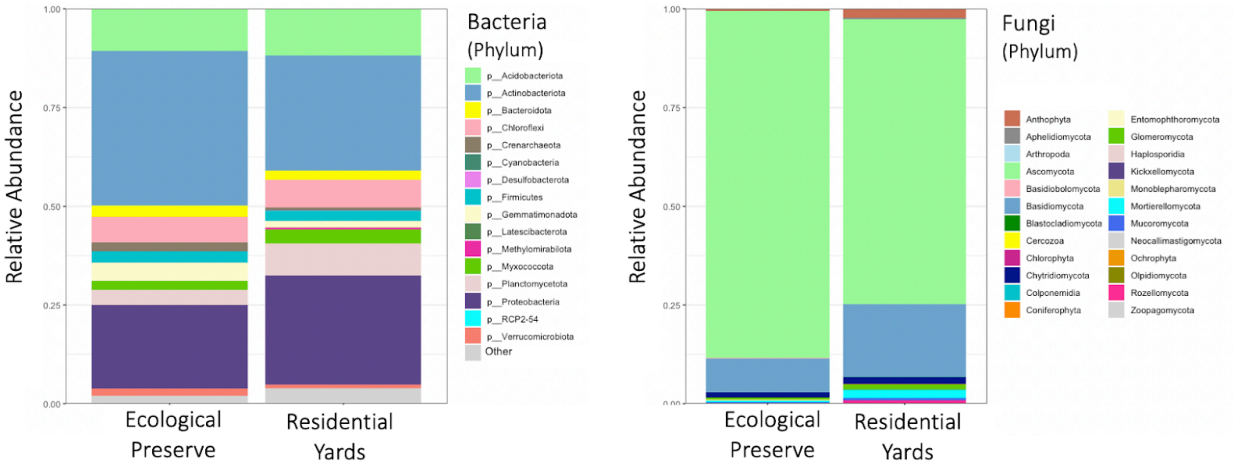

Figure 2.5. Taxa bar plots of relative abundance of bacterial phyla (left) and fungal phyla (right) in the ecological preserve and residential yards (University Hills)

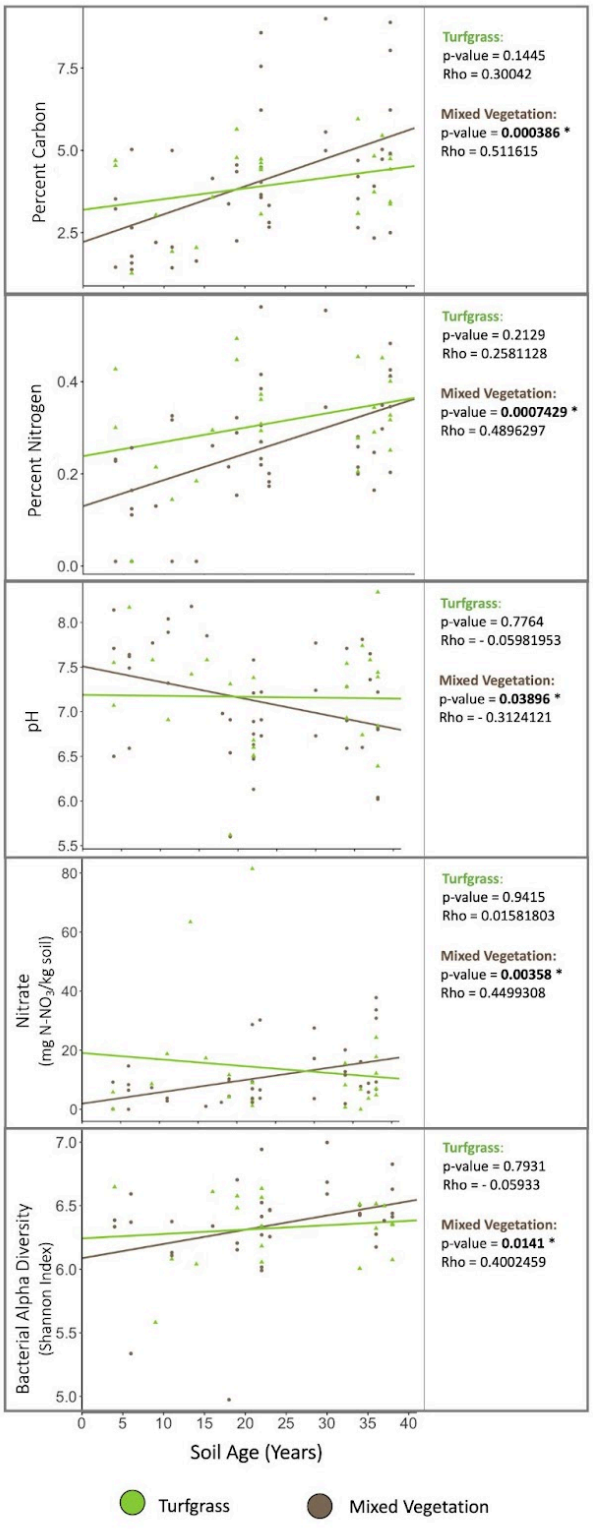

Figure 2.6. Spearman Correlation results of soil and microbial variables over time separated by vegetation type, with linear regressions of Variable vs. Soil Age.

Table 2.1. Enzymes assayed for this study, and their functions and substrates.

Enzyme	Abbreviation	Function	Substrate
α-glucosidase	AG	Starch degradation	4-MUB-α-D-glucopyranoside
acid phosphatase	AP	Mineralizes organic P into phosphate	4-MUB Phosphate
β-glucosidase	BG	Cellulose degradation	4-MUB-β-D-glucopyranoside
β-xylosidase	BX	Hemicellulose degradation	4-MUB-β-D-xylopyranoside
cellobiohydrolase	CBH	Cellulose degradation	4-MUB-β-D-cellobioside
leucine aminopeptidase	LAP	Peptide Breakdown	L-leucine-7-amido-4-methlcourmarin hydrochloride
N-acetyl-β-D-glucosaminidase	NAG	Chitin degradation	4-MUB-N-acetyl-β-D-glucosaminide

Adapted from German *et al.*, 2007

CHAPTER 3

Effects of Drought and Nitrogen Pollution on Microbial Nutrient Cycling in the Bulk Soil of a Semi-Arid Grassland

Authors: Andie Nugent, Alberto Barron Sandoval, Nikki Fiore, Michael Goulden, Steven D Allison

ABSTRACT

Climate change and urbanization are resulting in increased droughts and nitrogen pollution across Southern California, and conserved natural ecosystems may be impacted. In particular, drier and more nitrogen-rich soils may alter microbial communities and their function in biogeochemical cycles. The Loma Ridge Global Change Experiment was established in 2007 to simulate future drought and nitrogen pollution scenarios. Using this ecosystem-scale experiment, many prior studies have characterized litter microbial communities, their traits, and responses to global change. However few studies have examined the microbiome of bulk soil which may still contribute considerably to ecosystem nutrient cycling. We sampled bulk soil at three depths (0-10 cm, 10-20 cm, 20-30 cm), and sequenced the metagenome to determine the consequences drought and nitrogen pollution may have for microbial functional potential. We found that depth had the strongest influence on gene abundance related to carbon and nitrogen cycling. Drought sometimes affected nutrient cycling, often in a depth-dependent manner. Fertilization never had a direct effect on potential microbial function and direction of influence depended on interactions with depth and drought. Despite these changes to functional potential under global change, microbial community composition only responded to depth which suggests

that traditional taxonomic characterization of communities by phylum or genus may not be sufficient to capture functionally relevant groups with shared traits.

INTRODUCTION

Nitrogen and carbon cycling are vital ecosystem processes that may be affected by climate change, particularly the increased frequency and severity of droughts. Southern California has a Mediterranean climate which experiences a winter wet season and a hot, dry summer. Organisms here are adapted to some degree of short-term drought stress, but droughts are predicted to increase in frequency and length in this region which may test organisms' physiological limits (IPCC, 2022; Allison, 2023; Hueso et al, 2012). Additionally, Southern California natural ecosystems receive high rates of nitrogen deposition from fossil fuel burning in nearby urbanized landscapes. This excess nitrogen can impact biodiversity, facilitate invasive plant invasion, and endanger water systems through nitrate leaching (Fenn et al, 2003; Perry et al, 2010, He et al, 2011). Understanding the individual and combined effects of drought and nitrogen pollution on soil nutrient dynamics is therefore crucial in order to predict and mitigate future ecosystem consequences.

Major transformations in the nitrogen cycle are largely mediated by microorganisms. The rate at which various steps of the nitrogen cycle occur may depend on the composition of the microbial community. For instance, only a handful of taxa are known to perform nitrification and therefore the presence or absence of these taxa in the community can have a strong impact on the fate of nitrogen. Alternatively, the relative strength of each pathway may depend on environmental conditions conducive to the chemical reactions involved. Heavy rain which saturates the soil may create anoxic

conditions which enable higher activity from denitrifiers and thus a large pulse of N_2/N_2O from soil to atmosphere (Leitner et al, 2017; Hu et al, 2017). Under drought, there is instead reduction in nitrous oxide emissions and a buildup of ammonium pools (Homyak et al, 2017). Nitrogen deposition alone may increase nitrous oxide emissions from soil due to a surplus of ammonium which microbes then dissimilate, but the addition of drought can result in net nitrous oxide emission reductions (Aronson et al, 2019).

There is emerging evidence that microbial communities respond to global change, and that these responses may be phylogenetically conserved with consequences for potential nutrient cycling function (Canarini et al, 2021; Amend et al, 2015). However, communities can also be resistant to environmental change, and observed changes in ecosystem fluxes may not be linked to microbiome functional potential. For instance, Hartmann et al (2012) found that while nitrous oxide emissions were heavily reduced under drought, drought only led to minor changes in microbial functional potential related to denitrification. Nelson et al (2015) likewise observed that while global change may result in compositional shifts, microbial gene abundance linked to particular nitrogen cycle pathways did not respond to drought or fertilization. Nguyen et al (2018) did observe an effect of drought on community composition with some predictable functional consequences, but also found that concurrent nitrogen addition may reduce observable correlations between community structure and function. It remains difficult to predict how soil processes will respond to global change, and to what extent ecosystem nutrient dynamics can be linked to microbial community composition and functional potential.

Understanding the interactions between drought and nitrogen pollution on soil biogeochemical processes will become increasingly important in Southern California as

climate change progresses and urban areas continue to expand. The Loma Ridge Global Change Experiment (LRGCE) was constructed in 2007 to investigate the impacts of altered precipitation regimes and nitrogen pollution on Southern California grassland and shrubland ecosystems. Long term ecological field experiments such as the LRGCE allow researchers to disentangle the effects of global change factors on biotic and abiotic ecosystem processes while accounting for realistic variation in the field. Prior research at Loma Ridge has extensively characterized the leaf litter microbiome and the impact of simulated drought and nitrogen pollution on microbial physiology and decomposition rates (e.g. Finks et al, 2021; Matulich & Martiny, 2015; Malik et al, 2020). However little work has been conducted on bulk soil communities in this ecosystem, which likely have different functional responses to global change than litter communities (Manzoni et al, 2012). Barbour et al (2022) found that the impact of drought and fertilization had a reduced effect on microbial composition with depth, but the consequence for function in bulk soil remains unknown. Bulk soil below the O-horizon generally has reduced nitrogen compared to surface soil, but there is still a substantial nitrogen pool available for microbes to exploit (Whitney & Zabowski, 2004; Rovira & Vallejo, 2002).

We explored the effect of global change factors (drought and fertilization) on microbial communities and their nutrient cycling functional potential at the LRGCE. Specifically, we quantified functional genes involved in 8 pathways of the nitrogen cycle (Nelson et al, 2015), and genes encoding carbon-cycling enzymes important in decomposition (CAZymes)(Cantarel et al, 2015). We asked: **How do microbial communities and their potential function respond to global change factors in bulk soil, and does this response change with soil depth?** Based on prior research which

found a diverse microbial community in bulk soil, we expect that microbial communities in bulk soil will be functionally active in nutrient cycling, but this activity may be affected by drought and fertilization.

We characterized the microbial community of grassland soils down to a depth of 30 cm and quantified the effect of drought and nitrogen fertilization on potential function using extracellular enzyme activity (EEA) and nitrogen and CAZyme gene abundances from metagenomic data. **We hypothesized that (1)** Depth will have the strongest effect on microbial composition and function, but **(2)** drought will reduce overall potential nutrient-cycling function and **(3)** nitrogen fertilization will increase potential function for carbon acquisition. **(4)** Nitrogen fertilization will reduce gene abundance for assimilatory nitrogen-cycling pathways and promote dissimilatory function.

METHODS

Field Site and Sample Collection:

The LRGCE was established in 2007 and is located in the Santa Ana foothills of California (33.742 N, 117.704 W, elev. 365 m) (Potts et al, 2012). The experiment includes adjacent grassland and coastal sage scrub ecosystems, however only the grassland ecosystem was sampled for this study. Drought is simulated with shelters that reduce annual rainfall by 40-50%. The ambient precipitation and drought treatment plots are split into ambient and added nitrogen treatments. Therefore, the experiment allows the investigation of individual and interactive effects of drought and fertilization on ecosystem processes. Nitrogen is applied as $CaNO_3$ fertilizer annually in the wet season, and the most recent fertilization treatment prior to sampling in this study occurred in December

2018. Soil samples were collected in May 2019. After carefully removing the litter layer, we collected bulk soil cores (5.7cm diameter) at three depths (0-10 cm, 10-20 cm, 20-30 cm) from the grassland plots undergoing the following four treatments (**Figure 3.1**): Ambient water + ambient nitrogen; Ambient water + nitrogen fertilizer; Drought + ambient nitrogen; Drought + nitrogen fertilizer. We sampled four replicate plots of each treatment (n=4) in May 2019, after a rainy winter which broke a 7-year drought in Southern California. Cores were sieved immediately after collection to remove rocks and roots larger than 2mm. Soils were then kept frozen at -20°C until analysis.

Soil Characteristics

A subsample of soil was oven dried, and gravimetric moisture was calculated as grams of evaporated water divided by grams of remaining dry soil. An aliquot of soil was pulverized and combusted for elemental carbon and nitrogen content in a NC Soil Analyzer 2400 (Thermofisher). Bulk density was calculated as dry mass (g) of soil per core volume for each depth range. Soil carbon, nitrogen, and bulk density were originally reported by Fiore (2022) in a separate analysis of the soil samples used for this study.

Extracellular Enzyme Assays

We homogenized 0.3g of each soil sample in 100ml 25mM maleate buffer adjusted to pH 6.0 using NaOH. Using this homogenate, we followed the methods outlined by German et al (2011) to assay the soil samples for activity of 7 extracellular enzymes (**Table 2.1**).

Extracellular Enzyme activity may be determined by fitting data to a Michaelis-Menten curve. Outlier points were removed, and activities were fit to the equation below using the nonlinear least squares (nls) function in R following German et al (2012). V is the

reaction velocity, K_m is the substrate concentration at half V_{max}, and V_{max} is the maximum velocity. We utilized the V_{max} to determine maximum potential activity in umol/min/mg for each enzyme. Data were log transformed (natural log (x+1)) to improve data normality . Michaelis-Menten Equation for enzyme activity:

$$\mathbf{V = V_m[substrate\ concentration] / K_m + [substrate\ concentration]}$$

Units: V = μmol / min/ mg

Using ratios of enzymes targeting different elements, we can gain insight into what nutrients are potentially limiting for microbial growth in these soils (Zhao et al, 2018; Sinsabaugh et al, 2009). The ratio of BG/NAG+LAP represents carbon-to-nitrogen acquisition. BG/AP represents carbon-to-phosphorus. NAG+LAP/AP represents nitrogen-to-phosphorus acquisition.

Metagenomic Sequencing and Bioinformatics

DNA was extracted from soil using the Zymo Quick-DNA Miniprep Kit and following manufacturer's protocol. Samples were sent to the UCI Genomics High Throughput Facility for paired-end sequencing on a HiSeq 4000. The data underwent quality control using *FastQC*, and adapter sequences were removed using *Trimmomatic*. 75% of reads passed quality control and were retained for further analysis. Sequences were then processed following the bioinformatics pipeline described in Barrón-Sandoval et al, 2023. Briefly, sequences were pooled and assembled using MEGAHIT v1.2.9 with default parameters (Li et al. 2016). We then used Prodigal v2.6.3 to predict open reading frames (ORFs) which we annotated using the KEGG database and GhostKoala server. Abundance of each protein was calculated using Bowtie2 v2.5.1. We then mapped these results back onto the individual metagenomic libraries and filtered results for gene sequences involved in eight nitrogen

cycling pathways following Nelson et al (2015) (**Figure 3.2**). For a complete list of nitrogen-cycling functional genes used in this study, please see the supplemental material. We identified 15,225 ORFs that were predicted to encode nitrogen-cycling functional genes.

We also quantified CAZyme gene abundance. We ran the predicted ORFs from Prodigal through dbcan4 (run_dbcan 4.0.0) (Zheng et al, 2023), which used three annotation tools (HMMER, eCAMI, DIAMOND) to predict CAZymes from ORFs. We retained only the ORFs which were predicted to encode CAZymes by all three tools, resulting in 12,162 contigs for analysis.

Finally, all functional gene counts were normalized to Reads per Kilobase per Genome Equivalent (RPKG) calculated as (reads mapped to gene)/(gene length in kb)/(genome equivalents). Genome equivalents were calculated using MicrobeCensus v 1.1.1 (Nayfach and Pollard, 2015). For all results and discussion in this paper, "gene count" or "gene abundance" will refer to normalized RPKG**. These normalized gene count values indicate relative abundance, not absolute abundance.**

Taxonomic Identification

We used the default MG-RAST pipeline to join paired ends and assign taxonomic identity to sequences using the RefSeq database (Keegan et al, 2016). 70% of quality-filtered reads were assigned to OTUs in MG-RAST. Of those, 98% of sequences were identified as prokaryotic, and <1% were eukaryotic. Due to the low rate of fungal reads, all taxa were analyzed together as one community, with stronger emphasis on prokaryote results. Singletons and OTUs that could not be identified at the phylum level were removed,

and sequences were rarefied to a depth of 366,755 using the 'EcolUtils package' (Salazar, 2020). Finally, OTU counts were transformed to relative abundance for analysis.

Statistical Analysis

Extracellular Enzyme data were analyzed in R with a linear mixed effect (lme) model from the *nlme* package (v. 3.1-162), using Block as the random effect. The model was set up in R as follows:

lme(response_variable ~ Water*Nitrogen*Depth, random=~1|Block)

We then conducted an ANOVA on the linear mixed effects model to determine whether EEA differed by each treatment and if treatments had an interactive effect. To examine pairwise differences, we then conducted a least square means analysis on the lme model. We repeated this statistical approach for functional gene abundances binned into nitrogen-cycling pathways to determine how each pathway may respond to depth and treatment.

For the OTU counts retrieved from metagenomes, we translated relative abundance into a Bray-Curtis distance matrix and used PRIMER-e statistical software v. 7 (Clark et al, 2014) to conduct Type III PERMANOVAs on the distance values to test treatment and depth effects on microbial community composition. We then conducted a SIMPER test in PRIMER-e to identify which taxa contributed most to differences between communities sampled from each depth and treatment.

Figures were made using ggplot2 in R studio (Wickham, 2016). A p-value of < 0.05 was used as the threshold for significance in all statistical analyses.

RESULTS

Soil Characteristics

Percent carbon and nitrogen decreased significantly with depth and drought, but were unaffected by fertilization (**Figure 3.3**). Soil C:N ratio decreased with depth, and increased with fertilization. C:N ratio also responded to an interaction between fertilization and drought, where drought may decrease C:N ratio under ambient nitrogen conditions, but fertilization led to a recovery of C:N ratio in drought plots. Soil moisture did not change significantly with depth, but did decrease with drought and fertilization. Bulk density increased significantly with both depth and drought but was unaffected by fertilization.

Nitrogen Cycling Pathways in Loma Ridge Grassland Soils

Across all depths and treatments, nitrogen cycling pathway genes were dominated by ammonia assimilation, which were an order of magnitude more abundant than the other pathways. This is expected, as all microorganisms have the ability to incorporate nitrogen from ammonium into their biomass. **Figure 3.4** shows the relative gene abundance associated with each pathway by depth and treatment, excluding ammonia assimilation to enable better visualization of the remaining pathways. Among those pathways, the soil metagenome was dominated by assimilatory nitrite reduction. Denitrification was the next most abundant pathway by gene count, followed by nitrification. Assimilatory and dissimilatory nitrate reduction, dissimilatory nitrite reduction, and nitrogen fixation had very small representation in gene counts.

Extracellular Enzyme Activity - Effects of Depth and Treatment

Enzyme activity at Loma Ridge was highly variable. The ratio of BG/NAG+LAP estimates potential C:N acquisition. At Loma Ridge, this ratio was consistently higher than one across treatments and depths, indicating that carbon is more limiting than nitrogen (**Figure 3.5**). The ratio of NAG+LAP/AP can be used to compare nitrogen vs. phosphorus

acquisition. This ratio was consistently below 1, indicating that Loma Ridge soils are more phosphorus limited compared to nitrogen. The ratio of BG/AP indicates potential carbon vs. phosphorus limitation. This ratio increased with depth, driven by a reduction in AP activity, and responded to an interaction between fertilization and drought where soils experiencing both the drought and N-addition treatments had increased BG/AP activity. Responses of individual enzymes to depth and treatment can be found in the Supplementary Materials. Briefly, potential activity for phosphorus acquisition (AP) and nitrogen acquisition (LAP, NAG) decreased with depth but there were no other significant responses by individual enzymes to depth or treatment. Extracellular enzyme activity can fluctuate rapidly in response to current environmental conditions.

Response of Microbial Functional Gene Abundance to Depth

Depth affected gene count for all pathways of the nitrogen cycle, and CAZyme gene abundance ($P<0.05$, complete statistical results may be found in Appendix). Pathways which increased with depth, particularly between 0-10cm and 10-20cm, include: Ammonia Assimilation, Nitrogen Fixation, Dissimilatory Nitrite Reduction, Denitrification, and Nitrification (**Figure 3.6**). In all cases of a direct depth effect, gene abundance for nitrogen pathways increased with depth. Depth also interacted with treatments for several pathways, leading to increases with depth under some treatments, and decreases under other treatments.

Drought and Fertilization - Effects on Microbial Functional Genes

Drought treatment increased the relative abundance of nitrification genes, particularly as depth increased. Dissimilatory Nitrite Reduction genes decreased under drought. The effect of drought on other pathways involved interactions with depth and/or

fertilization. For Dissimilatory Nitrate to Nitrite genes, drought increased relative gene abundance in the surface layer, but then resulted in reduced gene abundance at 20-30cm. Drought and fertilization interacted, where fertilizer increased gene abundance under ambient water treatment, but reduced gene abundance under drought. Assimilatory Nitrate Reduction pathways similarly responded to complex interactions between drought, fertilization, and depth, where fertilizer had opposite impacts on gene count depending on water treatment and these effects also shifted throughout the soil profile (**Figure 3.6**).

We also compared the overall ratio of assimilatory processes (ammonia assimilation excluded) and dissimilatory processes. The ratio of Assimilatory:Dissimilatory pathways decreased from 0-10cm to 10-20cm, and in deeper soils, drought led to a further reduction in this ratio (**Figure 3.6**). Assimilatory:Dissimilatory pathways changed least with depth under the fertilized ambient-water plots, representing the lowest ratio in the surface layer and the highest ratio in deeper soils. These shifts in ratio were driven primarily by changes in assimilatory pathways; drought + fertilized plots had a rapid reduction in assimilatory pathways with depth, while ambient water + fertilized plots had increased assimilatory pathways with depth.

Finally, we also compared CAZyme gene abundance across depths and treatments to understand how microbial acquisition of carbon might be impacted by global change. There was an effect of depth, and an interaction between depth and drought. CAZyme gene count increased under drought at 0-10cm, but this difference was reduced in deeper soil. Overall, nitrogen cycling pathways responded least to treatment with regard to significance and variation at the surface, and most to treatment deeper in the soil, while CAZyme gene abundance was most affected by treatment toward the surface.

Taxonomic Composition

Next we explored whether the effects of depth and treatment on potential function were correlated with shifts in overall taxonomic composition. Starting with microbial alpha diversity, Shannon Index at the phylum level was reduced in fertilized plots, and increased with soil depth. Loma Ridge grassland soils were dominated by *Actinobacteria* (>50% relative abundance across treatments) and *Proteobacteria* (>30% relative abundance across treatments). We conducted PERMANOVAs at the phylum and genus levels to determine whether treatments and depth had an effect on community composition.

At the phylum level, only depth had a significant effect (**Figure 3.7**) and explained 38.8% of differences between communities, driven primarily by *Actinobacteria* which decreased slightly with depth and contributed 24.8% toward community differences observed. We did not find an effect of the individual treatments on composition at the phylum level through a PERMANOVA, but the increase in Shannon diversity with depth ($F=16.83$, $p<0.01$) may indicate that as a major group of bacteria (*Actinobacteria*) decreases in relative abundance, many less abundant taxa became more abundant.

At the genus level, depth also had the strongest effect on composition (**Figure 3.7**) and explained 35% of differences between communities. Nitrogen and water treatments did not have a significant effect at the genus level (water was borderline, $P = 0.061$). Nitrogen and water both interacted significantly with block, indicating that treatment effects on community composition may depend on other soil or plant properties which are more heterogeneous across Loma Ridge. *Geodermatophilus*, *Conexibacter*, *Streptomyces*, and *Mycobacterium* were the top genera that contributed to community shifts across depths. *Geodermatophilus* and *Mycobacterium* decreased with depth, while *Conexibacter*

and *Streptomyces* increased (**Table 3.1**). Due to the focus of this study on nitrogen cycling, we also pulled out key nitrifying taxa (*Nitrospira*, *Nitrobacter*, *Nitrococcus*, and others) to explore how their relative abundance responded to treatment using a Linear Mixed Effects Model and ANOVA ($p<0.05$). We found that nitrifiers significantly increased in relative abundance with depth consistent with nitrification gene counts, and decreased in relative abundance in fertilized soils which was not reflected in gene count data. Pairwise contrasts may be found in the supplemental materials.

DISCUSSION

Response of Microbial Communities to Depth

We hypothesized that depth would influence microbial composition and function, and this hypothesis was supported by our results. Gene abundance differed with depth for all eight nitrogen-cycling pathways and CAZymes examined in this study. Nitrogen-cycling functional potential generally increased with depth, particularly for dissimilatory pathways leading to a decrease in assimilatory:dissimilatory ratio. Within dissimilatory pathways, potential denitrification and nitrification increased with depth, but may decrease again in even deeper (below 30cm) soils (Vilain et al, 2014). The observed depth gradient in denitrification potential may result from rainfall bringing surface ammonium down into a denser and less oxygenated layer of soil where denitrification can more optimally occur (Venterink et al, 2003). Extracellular enzyme activity for nitrogen acquisition also responded to depth. The activity of LAP and NAG, which target peptides and chitin respectively, decreased with depth, which aligns with our findings of decreased nitrogen assimilation with depth.

Depth was also the only factor to which community composition responded. Barbour et al (2022) found that microbial community diversity (using Amplicon Sequence Variants) at Loma Ridge increased from the litter layer to the first 10 cm of bulk soil. They hypothesized that this difference in diversity is due to harsh environmental conditions and lower nutrient availability in litter compared to surface soil. We likewise found that diversity increased with depth ($F=16.83$, $P<0.01$), resulting from a slight decrease in *Actinobacteria* relative abundance, and an increase in more rare taxa. *Actinobacteria* abundance has been found to correlate with higher soil temperatures and reduced moisture, and these bacteria are capable of decomposing lignin, cellulose, and other tough substrates which may explain their higher abundance in more exposed topsoil (Siles & Margesin, 2016; Chaturvedi et al, 2019; Buresova et al, 2019; Yao et al, 2017). Surface soils had higher total carbon and nitrogen, likely due to inputs from the nearby litter layer, which may promote taxa specialized for decomposition of litter inputs. At the genus level, the top four taxa which changed most with depth were all members of the *Actinobacteria* phylum: *Geodermatophilus*, *Conexibacter*, *Streptomyces*, and *Mycobacterium*.

One low-abundance but functionally significant group of microbes which increased with depth are the nitrifiers, most of which are in the bacterial phylum *Proteobacteria* (e.g. *Nitrospira*, *Nitrobacter*) or archaea phylum *Thaumarchaeota* (e.g. *Nitrosopumilus*, *Nitrososphaera*). This increased representation of nitrifiers likely explains the increase in potential nitrification with depth, as nitrification function is highly conserved within that small group of taxa (Freitag et al, 2005; Siripong & Rittmann, 2007). Despite this level of functional conservation, nitrifier relative abundance and nitrification potential did not

respond similarly to global change factors, indicating a more complex set of interactions between drought, fertilization, composition, and function is at play.

Effects of Drought on Microbial Nutrient Cycling Potential

We predicted that drought would generally reduce potential nutrient-cycling functions, particularly more specialized pathways in the nitrogen cycle and for carbon acquisition. Nitrogen gene relative abundance did respond to drought, but in a depth-dependent manner. Drought boosted assimilatory potential in the first soil layer, but reduced assimilatory potential in deeper soils. Homyak et al (2017) found that ammonium accumulated in surface soils under drought conditions, which is consistent with the increased abundance of assimilatory genes in our study. Aronson et al (2019) observed a decrease in N_2O emissions under drought, suggesting a reduction in denitrification. However they also found that rain pulses after drought may result in high short-term N_2O emissions, and therefore drought may be altering the timing and strength of N_2O pulses. We also found that denitrification potential was not affected by drought indicating that environmental conditions, rather than microbial functional potential, may be most predictive of N_2O fluxes (Hartmann et al, 2013). Our findings of increased nitrification potential, and no change to denitrification potential, supports the notion that microbial communities under drought may efficiently transform ammonium to nitrate or N_2O when the first rains arrive after a prolonged dry period, resulting in a rapid loss of nitrogen from the ecosystem.

We also explored potential effects of drought on carbon cycling function. Drought increased CAZyme gene abundance in surface soil, which seems to contrast many observations that decomposition rates slow under drought (Deng et al, 2021; Walter et al,

2013). This increase in microbial investment for carbon may be due to a reduction in labile carbon and nitrogen pools, and an increase in low-nutrient litter requiring more enzymes to decompose (Sanaullah et al, 2012; Khalili et al, 2016). Enzyme efficiencies also decrease under drought, and thus microbes may need to invest in higher enzyme production in order to maintain nutrient acquisition rates (Alster et al, 2013). Thus increased CAZyme activity is unlikely to correspond to increased decomposition under drought, but rather higher CAZyme gene abundance supports greater microbial investment in enzymes for carbon acquisition to compensate for reduced efficiency. Extracellular enzyme activity, however, did not indicate that microbial investment in carbon was altered by drought regardless of depth. Our study was conducted after a particularly wet winter, and it is possible that enzyme production responded to a short-term increase in litter quality and moisture while community genetics maintained a legacy of long-term drought (Gao et al, 2021; Tiemann & Billings, 2011; Sinsabaugh et al, 2008).

Effects of Nitrogen Fertilization on Microbial Nutrient Cycling Potential

We predicted that $CaNO_3$ fertilization would promote potential carbon cycling function and dissimilatory nitrogen-cycling function, and would decrease the abundance of genes involved in nitrogen assimilation. Higher demand for carbon under fertilization may lead to a reduction in recalcitrant carbon sources, which has concerning implications for ecosystem carbon losses (Li et al, 2019). We found that fertilizer alone did not have a strong consistent effect on any measurements of potential microbial function, and we did not find evidence of significant carbon loss in fertilized soils. It is possible that this ecosystem is already nitrogen-enriched from urban nitrogen deposition, and therefore additional fertilizer does not significantly alter carbon storage or loss. Instead, nitrogen

amendments only affected microbial function through interactions with drought and depth. It may also be the case that plants are capable of utilizing this excess nitrogen and are buffering the soil against nitrogen pollution. Further research into the plant-soil-microbe feedbacks at Loma Ridge will be vital to understand the fate of nitrogen in this ecosystem.

Drought in combination with fertilization led to a greater reduction in nitrogen assimilation pathways at 20-30 cm compared to drought alone. Other studies have found that fertilization increases assimilatory nitrate reduction (Li et al, 2020; Enebe & Babalola, 2021) but fertilization appears to have the opposite effect when soils are also experiencing drought. This reduction in microbial nitrogen cycling potential may contribute to an accumulation of nitrates in the soil which, after a large rain event, could be rapidly lost from the ecosystem through leaching or denitrification (Austin et al, 2004).

Limitations and Future Directions

In this semi-arid grassland ecosystem, depth is a consistent driver of microbial community composition and some potential functions respond to drought and nitrogen pollution. Drought also had an impact on microbial functional potential, and drought effects often depended on soil depth. One limitation of our study was the manipulation of drought without a corresponding temperature increase, as the two are likely to coincide with climate change. We recommend that further work on microbial nutrient cycling include a warming treatment in concert with drought, as taxa may have different responses to combined stressors compared to one alone (Brzostek et al, 2012; Manzoni et al, 2012; Berard et al, 2015).

A notable result from our study is the lack of correlation between community composition and functional potential in response to drought and fertilization. The abundance of genes encoding CAZymes and assimilatory enzymes in the nitrogen cycle were responsive to drought particularly in deeper soil. No microbial taxa at the phylum or genus level responded significantly to drought or fertilization. This disconnect between taxonomic composition and potential function has been highlighted in other recent studies. Chen et al (2020) found that functional potential is more responsive to fertilization treatments than community composition and Krause et al (2014) argue that a deeper understanding of microbial traits is required to effectively link composition to observed functions. Measuring microbial diversity at the genus level, as we did here, may still be too broad for effectively linking traits to function. In the past two decades, "ecotype" has become an increasingly popular way to describe taxonomic groups occupying functionally relevant niches (Cohan, 2002; Rocap et al, 2003; Chase et al, 2018). This version of a species concept in the microbial world may better enable us to predict function from community genetics. Our findings suggest that nitrogen and carbon cycling function is likely conserved at a level which is difficult to detect through traditional taxonomic groupings (e.g. genus), and future studies on microbial function under global change may explore possible ecotype groupings which are more predictive of functional gene responses to environmental change.

Finally, seasonal variation may affect microbial response to global change or may even overpower perceptible responses to global change, which we could not capture in this study with only one sampled time point (Matulich et al, 2015; Li et al, 2020). Many studies observe a rapid increase in microbial activity when soils are rewetted after a prolonged

drought, and therefore sampling across multiple seasons will improve our understanding and modeling of long-term nutrient cycling (Asensio et al, 2021; Hannula et al, 2019; Langenheder et al, 2012). While temporal variation may be high, we still observed effects of drought and fertilization on potential nutrient-cycling function at the end of the wet season, demonstrating lasting effects of global change on soil biogeochemistry beyond initial rewetting after summer.

CONCLUSION

Ours is among the first studies to investigate changes in microbial functional potential in bulk soil communities under drought and fertilization. Many microbial studies of bulk soil are limited to the first 10cm of the soil profile, and we found that deeper soil (down to 30cm) still has a high potential for nutrient cycling and may respond to global change differently than surface soils. The role of microbial communities in this deeper bulk soil should not be forgotten when building our conceptual understanding of soil ecosystems and impact of anthropogenic activity. We also found it challenging to link microbial community composition to potential function under global change, suggesting that we may need to pursue a trait-based approach that incorporates fine-scale taxonomic variation to relate community structure and function.

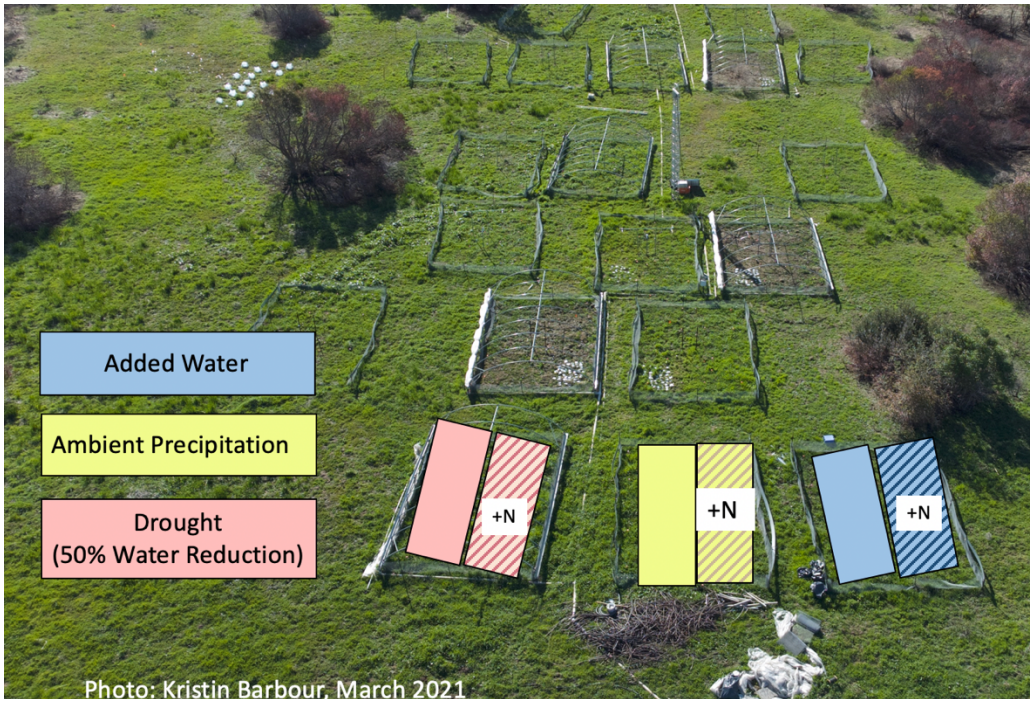

Figure 3.1. Experimental design at the LRGCE. Plots receive ambient, reduced, or added water. Each plot is split and half receives $CaNO_3$ fertilizer once annually.

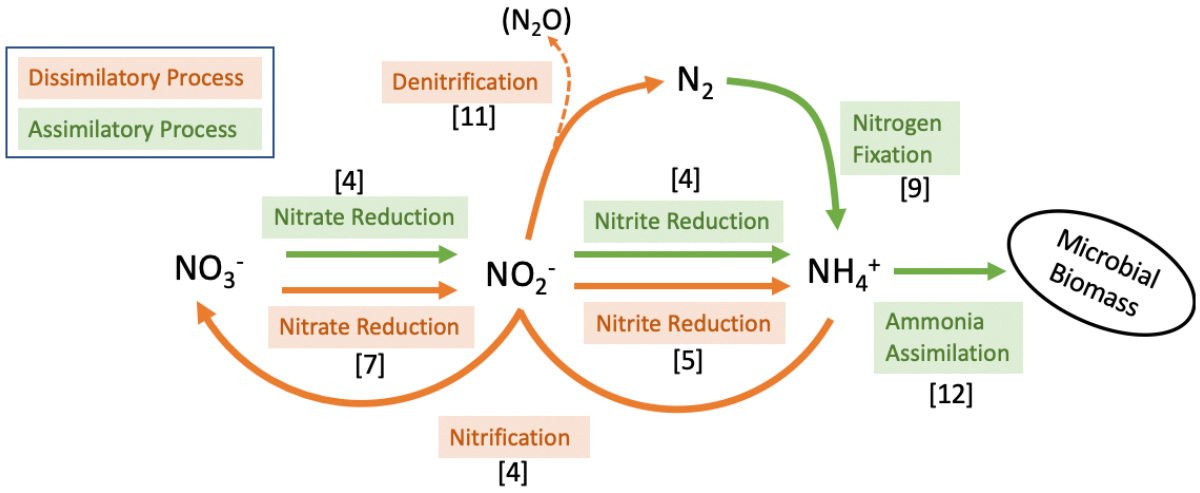

Figure 3.2. Nitrogen cycle pathways examined in this study. Values in brackets indicate number of genes associated with each pathway that could be matched to contigs through KEGG. Note that each KEGG ID may encompass multiple genes. See supplement for more information.

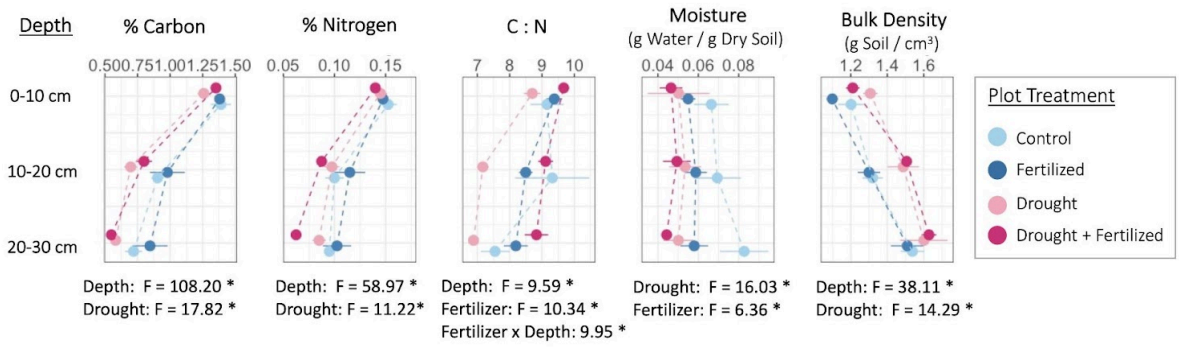

Figure 3.3. Soil characteristics of Loma Ridge grassland ecosystem (x-axes), at three depths (y-axis), with color representing experimental treatments. F-values are reported for linear mixed effects model results, with asterisks indicating a significant effect.

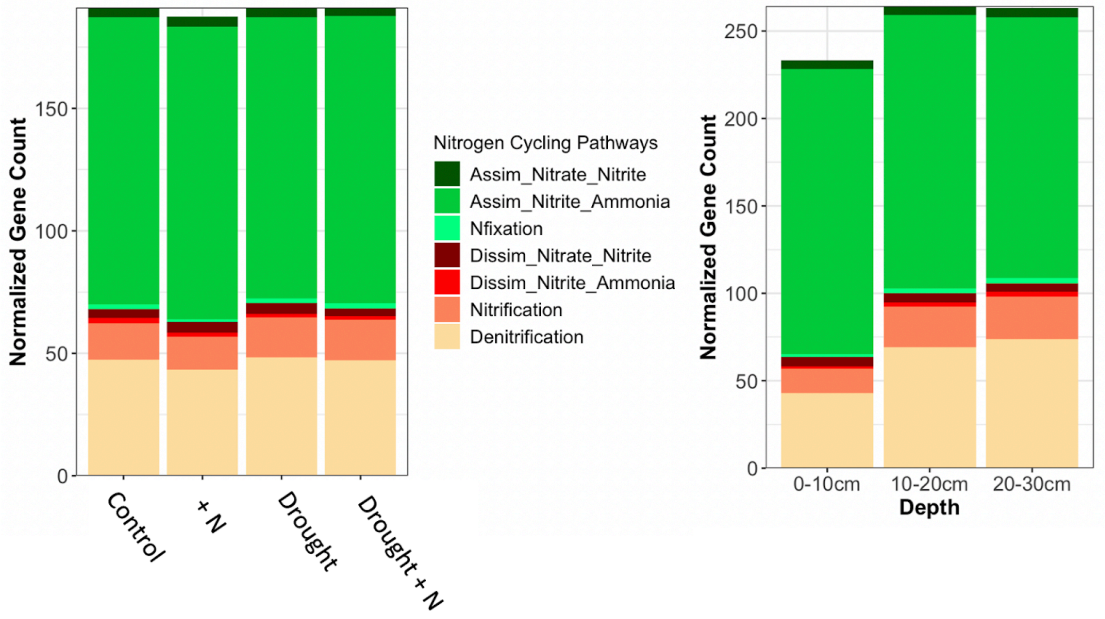

Figure 3.4. Stacked barplot of normalized gene counts for each nitrogen-cycling pathway by depth and treatment (Ammonia Assimilation was excluded due to its high abundance and ubiquitous presence across all microbial taxa)

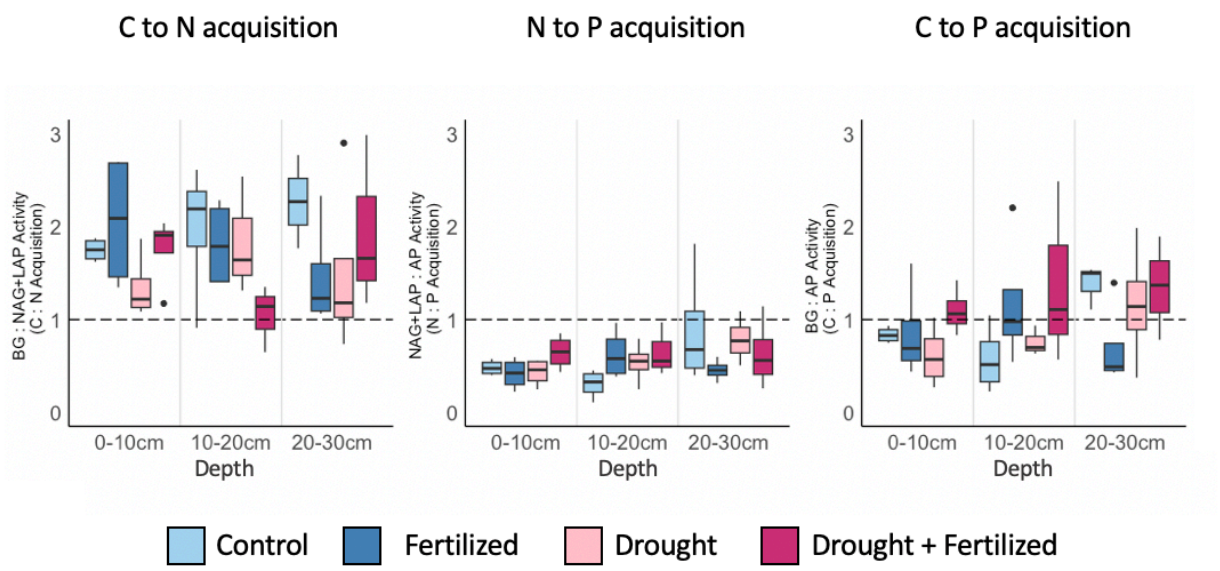

Figure 3.5. Ratios of extracellular enzymes for carbon, nitrogen, and phosphorus acquisition. Dotted line at y=1 indicates expected ratio if microbial investment in both elements are equal. BG = carbon acquisition, NAG+LAP = nitrogen acquisition, and AP = phosphorus acquisition.

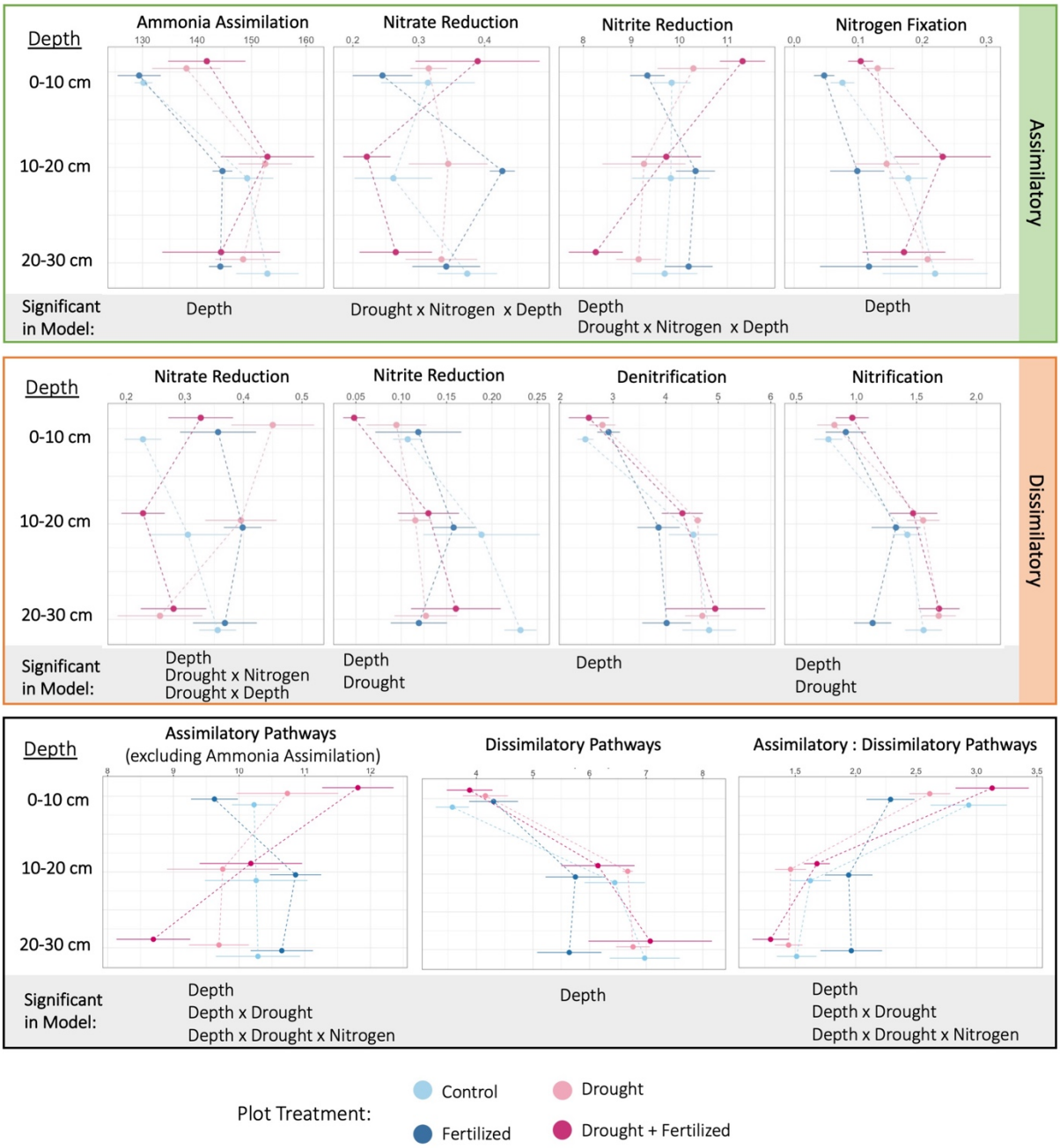

Figure 3.6. *Relative* gene abundances for nitrogen cycling pathways by depth and treatment. Solid horizontal lines are standard error, and dotted lines connect points between depths for easier visualization of change with depth. Factors with significant effects ($p<0.05$) on gene abundance using a linear-mixed-effects and ANOVA model are noted under each panel.

Table 3.1. Relative abundance of top 4 individual genera and grouped nitrifying genera in Loma Ridge soils, at 0-10cm vs. 20-30cm depths. Bolded values indicate higher relative abundance in the corresponding soil layer. SIMPER results indicate % contribution to differences in community composition depths. Differences in Nitrifiers between depths was confirmed ($p<0.05$) using a Linear Mixed Effects model and ANOVA rather than a SIMPER analysis.

Genus	**Relative Abundance (0-10 cm)** ± SE	**Relative Abundance (20-30 cm)** ± SE	**Contribution to Community Differences** (SIMPER results)
Conexibacter	0.1132 ± 0.0183	**0.127** ± 0.0236	1.4%
Streptomyces	0.0713 ± 0.0034	**0.0791** ± 0.0074	1.1%
Mycobacterium	**0.0614** ± 0.0031	0.0533 ± 0.0056	0.76%
Geodermatophilus	**0.0688** ± 0.0059	0.0349 ± 0.0069	1.2%
Nitrifiers	0.0755 ± 0.003	**0.1015** ± 0.0068	—

CONCLUSION TO THE DISSERTATION

In this dissertation, I demonstrate that soil responses to environmental stressors and resources can vary across degrees of urbanization. Rather than using a static definition of "urban" in an ecological context, I argue it is helpful to understand urbanization as a disturbance gradient, the particular characteristics of which may be context-dependent. While urban ecosystems tend to have similar features across the U.S. and even the globe (Groffman et al, 2014), the climate in which urban development takes place may determine ecosystem response to the novel resources associated with yards and other urban greenspaces. For instance, I found that the addition of water in a suburban yard had a greater effect on microbial community composition and function compared to the drought treatment at Loma Ridge. This indicates that in Southern California, soils are already adapted to arid conditions and are resilient to increased drought. The large influx of water and nutrients associated with urbanization, however, significantly shifted microbial composition and function and may generate a novel nutrient cycling regime compared to undeveloped soils. Therefore, while prior research in Southern California has been concerned with drought and warming effects on soils, it is equally vital that we understand the effects of urbanization as an ecological driver.

Urbanization can broadly be viewed through a disturbance lens, but large urban-to-rural gradients can fail to capture the impressive degree of variation that can exist within just one urban land-use type. In the suburban yards of University Hills, an array of plant types, irrigation, and fertilizer regimes resulted in heterogenous soils with a range of nutrient content and microbial activity. If more sustainable land management is a goal of urban ecological research, it is crucial that we understand urban soils at more

management-relevant scales. Thus, while a gradient approach may be helpful to generate hypotheses and uncover major environmental patterns, this work should be coupled with studies targeting particular land-use types at scales small enough to understand what human actions are driving observed ecological trends.

Finally, linking microbial taxa and function remains a challenge, particularly when predicting community response to environmental change. I found that a more comprehensive analysis of plant-soil-microbe feedbacks will be necessary to understand the role of microbial taxa in nitrogen cycling. While microbes may have some direct response to soil moisture, it is likely that microbial nutrient cycling is mediated by plant interactions. For natural systems, understanding these dynamics will allow us to more fundamentally understand ecosystem resilience to disturbance and prioritize areas of conservation. In urban systems, we may be able to take advantage of soil organism interactions to boost ecosystem services and promote urban sustainability through soil health.

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APPENDIX A – SUPPLEMENTAL MATERIAL FOR CHAPTER 3

Nitrogen-Cycling Genes Associated with 8 Pathways, from Nelson et al, 2015.

KEGGid	Pathway	Symbol	Function
K00265	Ammonia assimilation	gltB	glutamate synthase
K01425	Ammonia assimilation	glsA, GLS	glutaminase
K00284	Ammonia assimilation	GLU, gltS	glutamate synthase
K01953	Ammonia assimilation	ansB, ASNS	asparagine synthase
K00261	Ammonia assimilation	GLUD1_2, gdhA	glutamate dehydrogenase
K15371	Ammonia assimilation	GDH2	glutamate dehydrogenase
K00266	Ammonia assimilation	gltD	glutamate synthesis
K00260	Ammonia assimilation	gudB, rocG	glutamate dehydrogenase
K00264	Ammonia assimilation	GLT1	glutamate synthesis
K05597	Ammonia assimilation	aspQ, ansB, ansA	glutamin-(asparagin-)ase
K00262	Ammonia assimilation	E1.4.1.4, gdhA	glutamate dehydrogenase
K01915	Ammonia assimilation	glnA, GLUL	glutamine synthesis
K00360	Assimilatory Nitrate to Nitrite	nasB	assim nitrate reductase
K10534	Assimilatory Nitrate to Nitrite	NR	nitrate reductase
K00372	Assimilatory Nitrate to Nitrite	nasC, nasA	Assim nitrate reductase
K15878	Assimilatory Nitrate to Nitrite	narB	nitrate reduction
K17877	Assimilatory Nitrite to Ammonia	NIT-6	
K00366	Assimilatory Nitrite to Ammonia	nirA	Nitrite reductase
K00362	Assimilatory Nitrite to Ammonia	nirB	Nitrite reductase
K00363	Assimilatory Nitrite to Ammonia	nirD	Nitrite reductase

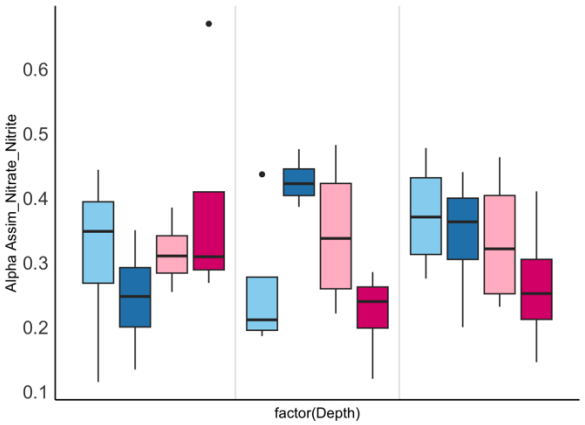
K00376	Denitrification	nosZ	N2O reductase
K04561	Denitrification	norB	NO reductase
K02305	Denitrification	norC	NO reductase
K15864	Denitrification	nirS	Nitrite reductase
K00368	Denitrification	nirK	Nitrite reductase
K00370	Denitrification	narG, narZ, nxrA	Nitrate reductase
K00371	Denitrification	narH, narY, nxrB	Nitrate reductase
K00374	Denitrification	narI, narV	Nitrate reductase
K02567	Denitrification	napA	nitrate reductase
K11264	Denitrification	norVW	nitrous oxide reductase
K02568	Denitrification	napB	nitrate reductase
K00373	Dissimilatory Nitrate to Nitrite	narJ, narW	nitrate reductase
K00374	Dissimilatory Nitrate to Nitrite	narI, narV	nitrate reductase
K00370	Dissimilatory Nitrate to Nitrite	narG, narZ, nxrA	nitrate reductase
K00371	Dissimilatory Nitrate to Nitrite	narH, narY, nxrB	nitrate reductase
K02567	Dissimilatory Nitrate to Nitrite	napA	nitrate reduction
K15879	Dissimilatory Nitrate to Nitrite	narC	nitrate reduction
K02568	Dissimilatory Nitrate to Nitrite	napB	nitrate reduction
K08361	Dissimilatory Nitrite to Ammonia	nasB	nitrite reductase
K04014	Dissimilatory Nitrite to Ammonia	nrfC	nitrite reduction
K03385	Dissimilatory Nitrite to Ammonia	nrfA	nitrite reduction
K04013	Dissimilatory Nitrite to Ammonia	nrfB	nitrite reduction
K04015	Dissimilatory Nitrite to Ammonia	nrfD	nitrite reduction
K10945	Nitrification	pmoB-amoB	ammonia to nitrite

K10944	Nitrification	pmoA-amoA	ammonia to nitrite
K10535	Nitrification	hao	ammonia to nitrite
K10946	Nitrification	pmoC-amoC	ammonia to nitrite
K02591	Nitrogen Fixation	nifK	nitrogen fixation
K02595	Nitrogen Fixation	nifW	nitrogen fixation
K00531	Nitrogen Fixation	anfG	nitrogenase, nitrogen to ammonia
K02588	Nitrogen Fixation	nifH	nitrogenase, nitrogen to ammonia
K02586	Nitrogen Fixation	nifD	nitrogenase
K22896	Nitrogen Fixation	vnfD	nitrogenase
K22897	Nitrogen Fixation	vnfK	nitrogenase
K22898	Nitrogen Fixation	vnfG	nitrogenase
K22899	Nitrogen Fixation	vnfH	nitrogenase

ANOVA results for Microbial Functional Potential

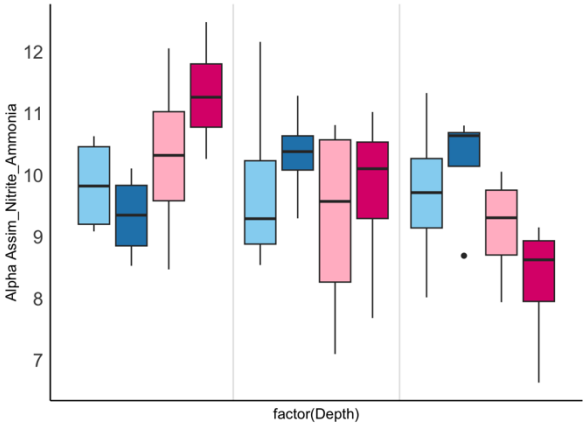
Assim_Nitrate_Nitrite

	numDF	denDF	F-value	p-value
(Intercept)	1	33	117.68500	<.0001
Depth	2	33	0.11448	0.8922
Water	1	33	0.30619	0.5838
Nitrogen	1	33	0.10439	0.7487
Depth:Water	2	33	2.49415	0.0980
Depth:Nitrogen	2	33	0.58994	0.5601
Water:Nitrogen	1	33	1.19404	0.2824
Depth:Water:Nitrogen	**2**	**33**	**5.03050**	**0.0124**



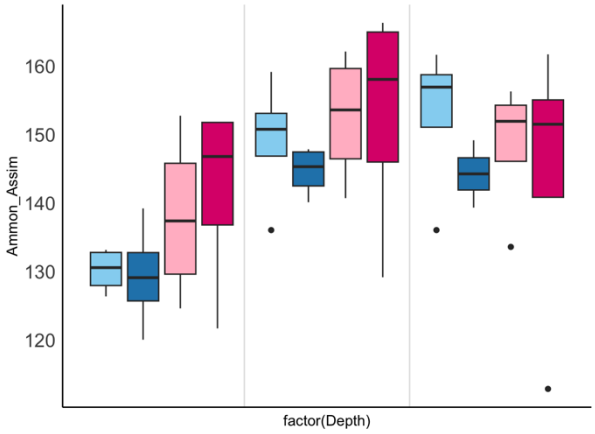
Assim_Nitrite_Ammonia

	numDF	denDF	F-value	p-value
(Intercept)	1	33	428.8559	<.0001
Depth	**2**	**33**	**4.8218**	**0.0146**
Water	1	33	0.7742	0.3853
Nitrogen	1	33	0.6456	0.4274
Depth:Water	**2**	**33**	**10.2620**	**0.0003**
Depth:Nitrogen	2	33	0.7756	0.4686
Water:Nitrogen	1	33	0.0043	0.9482
Depth:Water:Nitrogen	**2**	**33**	**3.3978**	**0.0455**



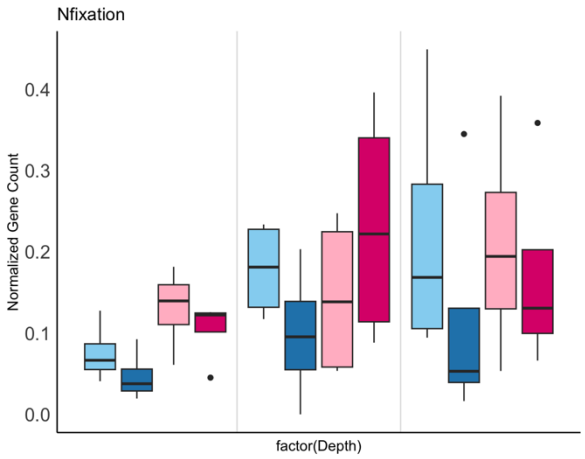
Ammonia Assimilation

	numDF	denDF	F-value	p-value
(Intercept)	1	33	2423.0238	<.0001
Depth	**2**	**33**	**9.2551**	**0.0006**
Water	1	33	2.2529	0.1429
Nitrogen	1	33	0.5718	0.4549
Depth:Water	2	33	1.3838	0.2648
Depth:Nitrogen	2	33	0.5448	0.5851
Water:Nitrogen	1	33	0.5864	0.4493
Depth:Water:Nitrogen	2	33	0.0004	0.9996



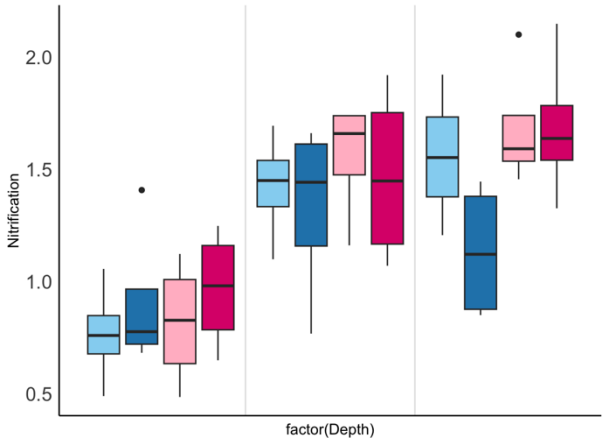
N Fixation

	numDF	denDF	F-value	p-value
(Intercept)	1	33	28.977614	<.0001
Depth	2	33	3.934383	0.0293
Water	1	33	2.299978	0.1389
Nitrogen	1	33	1.245713	0.2724
Depth:Water	2	33	0.143798	0.8666
Depth:Nitrogen	2	33	0.587174	0.5616
Water:Nitrogen	1	33	1.965962	0.1702
Depth:Water:Nitrogen	2	33	0.738475	0.4856



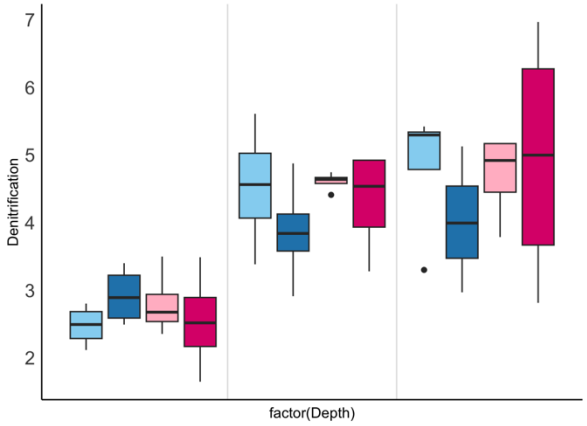
Nitrification

	numDF	denDF	F-value	p-value
(Intercept)	1	33	156.12860	<.0001
Depth	**2**	**33**	**33.29729**	**<.0001**
Water	**1**	**33**	**6.06605**	**0.0192**
Nitrogen	1	33	0.50811	0.4810
Depth:Water	2	33	1.42437	0.2551
Depth:Nitrogen	2	33	2.14941	0.1326
Water:Nitrogen	1	33	1.06114	0.3104
Depth:Water:Nitrogen	2	33	0.95053	0.3969



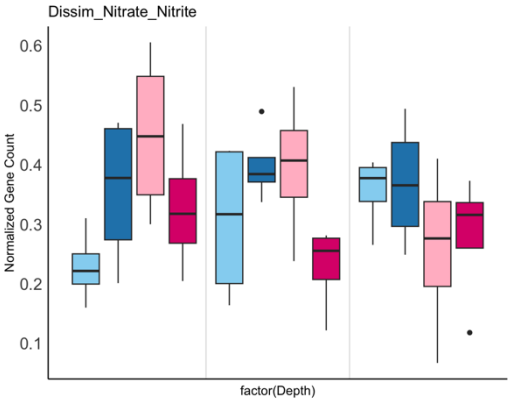
Denitrification

	numDF	denDF	F-value	p-value
(Intercept)	1	33	380.0934	<.0001
Depth	**2**	**33**	**26.3474**	**<.0001**
Water	1	33	0.8152	0.3731
Nitrogen	1	33	0.8888	0.3527
Depth:Water	2	33	0.2827	0.7555
Depth:Nitrogen	2	33	0.5028	0.6094
Water:Nitrogen	1	33	0.2655	0.6098
Depth:Water:Nitrogen	2	33	1.1779	0.3205



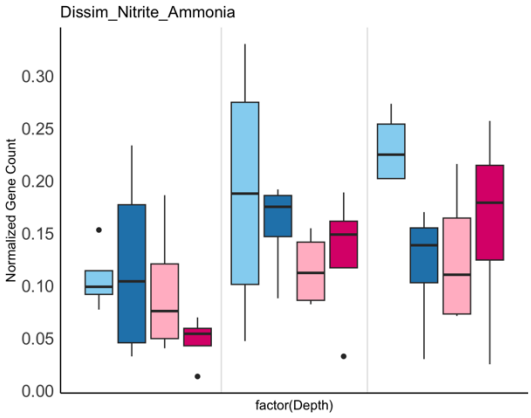
Dissimilatory Nitrate to Nitrite

	numDF	denDF	F-value	p-value
(Intercept)	1	33	429.6893	<.0001
Depth	2	33	0.2124	0.8098
Water	1	33	0.1506	0.7004
Nitrogen	1	33	0.0296	0.8645
Depth:Water	2	33	3.1423	0.0563
Depth:Nitrogen	2	33	0.2623	0.7709
Water:Nitrogen	**1**	**33**	**6.9141**	**0.0129**
Depth:Water:Nitrogen	2	33	1.9620	0.1566



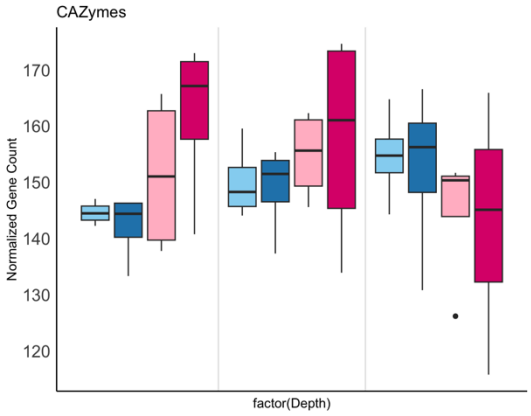
Dissimilatory Nitrite to Ammonia

	numDF	denDF	F-value	p-value
(Intercept)	1	33	86.87122	<.0001
Depth	2	33	4.61828	0.0170
Water	1	33	4.54194	0.0406
Nitrogen	1	33	1.25848	0.2700
Depth:Water	2	33	0.07717	0.9259
Depth:Nitrogen	2	33	0.22898	0.7966
Water:Nitrogen	1	33	1.29030	0.2642
Depth:Water:Nitrogen	2	33	2.29863	0.1163



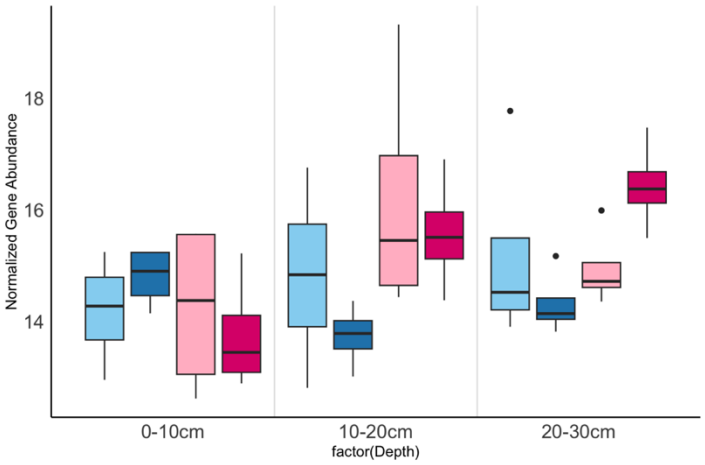
CAZymes

	numDF	denDF	F-value	p-value
(Intercept)	1	33	2007.0179	<.0001
Depth	2	33	0.5690	0.5715
Water	1	33	1.1203	0.2975
Nitrogen	1	33	0.0957	0.7590
Depth:Water	**2**	**33**	**4.4343**	**0.0197**
Depth:Nitrogen	2	33	0.2818	0.7562
Water:Nitrogen	1	33	0.8057	0.3759
Depth:Water:Nitrogen	2	33	0.3286	0.7223



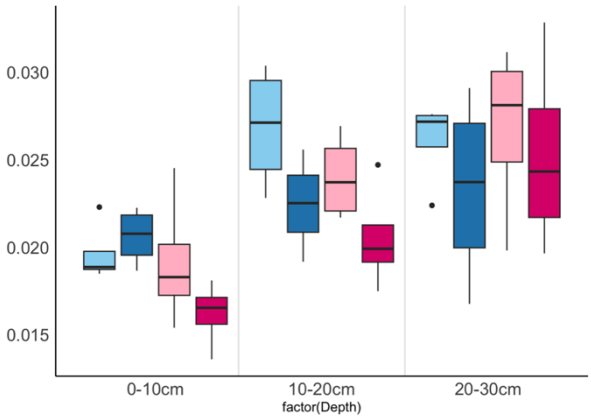
CAZymes : Assimilatory Genes

	numDF	denDF	F-value	p-value
(Intercept)	1	33	1651.1521	<.0001
Water	**1**	**33**	**4.9733**	**0.0327**
Nitrogen	1	33	0.2521	0.6190
Depth	**2**	**33**	**3.9920**	**0.0280**
Water:Nitrogen	1	33	0.9019	0.3492
Water:Depth	**2**	**33**	**4.1378**	**0.0249**
Nitrogen:Depth	2	33	1.3017	0.2857
Water:Nitrogen:Depth	2	33	2.6742	0.0839



Nitrifying Taxa - Relative Abundance

		numDF	denDF	F-value	p-value
(Intercept)		1	33	736.5193	<.0001
Water	1	33	1.4954	0.2300	
Nitrogen		**1**	**33**	**6.0240**	**0.0196**
Depth		**2**	**33**	**16.0563**	**<.0001**
Water:Nitrogen		1	33	0.0944	0.7606
Water:Depth		2	33	1.7126	0.1960
Nitrogen:Depth		2	33	0.8181	0.4500
Water:Nitrogen:Depth		2	33	0.7547	0.4781



PERMANOVA results for Community Composition

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	perms	Source	Estimate	Sq.root
De	2	124.15	62.073	11.457	0.003	998	S(De)	3.5409	1.8817
Ni	1	56.994	56.994	2.6615	0.184	363	S(Ni)	1.4825	1.2176
Wa	1	157.52	157.52	5.7177	0.102	367	S(Wa)	5.4153	2.3271
Bl	3	135.44	45.146	22.725	0.001	998	V(Bl)	3.5966	1.8965
DexNi	2	9.0184	4.5092	1.683	0.24	998	S(DexNi)	0.22874	0.47827
DexWa	2	24.09	12.045	2.0611	0.19	998	S(DexWa)	0.77512	0.88041
DexBl	6	32.508	5.418	2.7272	0.062	999	V(DexBl)	0.85784	0.9262
NixWa	1	9.614	9.614	0.39098	0.67	961	S(NixWa)	-1.248	-1.1171
NixBl	3	64.242	21.414	10.779	0.001	998	V(NixBl)	3.2379	1.7994
WaxBl	3	82.647	27.549	13.867	0.001	998	V(WaxBl)	4.2604	2.0641
DexNixWa	2	16.648	8.3238	4.1899	0.053	999	S(DexNixWa)	1.5843	1.2587
DexNixBl	6	16.075	2.6792	1.3486	0.281	999	V(DexNixBl)	0.3463	0.58847
DexWaxBl	6	35.064	5.844	2.9416	0.078	999	V(DexWaxBl)	1.9287	1.3888
NixWaxBl	3	73.768	24.589	12.377	0.003	999	V(NixWaxBl)	7.5343	2.7449
Res	6	11.92	1.9866				V(Res)	1.9866	1.4095
Total	47	849.69							

ANOVA and pairwise contrasts results for Nitrifier abundance

	numDF	denDF	F-value	p-value
(Intercept)	1	33	736.5193	<.0001
Water	1	33	1.4954	0.2300
Nitrogen	**1**	**33**	**6.0240**	**0.0196**
Depth	**2**	**33**	**16.0563**	**<.0001**
Water:Nitrogen	1	33	0.0944	0.7606
Water:Depth	2	33	1.7126	0.1960
Nitrogen:Depth	2	33	0.8181	0.4500
Water:Nitrogen:Depth	2	33	0.7547	0.4781

lsmeans(anovamodel, pairwise~Depth*Nitrogen, adjust="tukey")

contrast	estimate	SE	df	t.ratio	p.value
(0-10cm Added) - (10-20cm Added)	-0.003075	0.00166	33	-1.848	0.4504
(0-10cm Added) - (20-30cm Added)	**-0.005907**	**0.00166**	**33**	**-3.550**	**0.0138**
(0-10cm Added) - (0-10cm AmbN)	-0.000974	0.00166	33	-0.586	0.9913
(0-10cm Added) - (10-20cm AmbN)	-0.007035	0.00166	33	-4.229	0.0022
(0-10cm Added) - (20-30cm AmbN)	-0.008046	0.00166	33	-4.836	0.0004
(10-20cm Added) - (20-30cm Added)	-0.002832	0.00166	33	-1.702	0.5402

(10-20cm Added) - (0-10cm AmbN)	0.002101	0.00166	33	1.263	0.8025
(10-20cm Added) - (10-20cm AmbN)	-0.003960	0.00166	33	-2.380	0.1926
(10-20cm Added) - (20-30cm AmbN)	-0.004970	0.00166	33	-2.987	0.0543
(20-30cm Added) - (0-10cm AmbN)	0.004933	0.00166	33	2.965	0.0572
(20-30cm Added) - (10-20cm AmbN)	-0.001128	0.00166	33	-0.678	0.9832
(20-30cm Added) - (20-30cm AmbN)	-0.002139	0.00166	33	-1.285	0.7906
(0-10cm AmbN) - (10-20cm AmbN)	**-0.006061**	**0.00166**	**33**	**-3.643**	**0.0108**
(0-10cm AmbN) - (20-30cm AmbN)	**-0.007071**	**0.00166**	**33**	**-4.250**	**0.0021**
(10-20cm AmbN) - (20-30cm AmbN)	-0.001010	0.00166	33	-0.607	0.9898

Results of SIMPER analysis of microbial genera - top contributors to community differences between top and bottom soil depths (in descending order)

Genus	**Contribution to Differences**
Geodermatophilus	0.019654932
Conexibacter	0.014256305
Streptomyces	0.010734801
Mycobacterium	0.007643278
Nocardioides	0.005664782
Rubrobacter	0.004175773
Gemmatimonas	0.003405257
Bradyrhizobium	0.003315325
Candidatus.Solibacter	0.00284772
Micromonospora	0.002405605
Chthoniobacter	0.002362411
Anaeromyxobacter	0.002336823
Kribbella	0.002037952
Ktedonobacter	0.001909921
Methylobacterium	0.001836943
Candidatus.Koribacter	0.00181126

Sorangium	0.001681426
Amycolatopsis	0.001659727
Gemmata	0.00162652
Frankia	0.001508535
Arthrobacter	0.001388201
unclassified..derived.from.Verrucomicrobia.subdivision.3.	0.001368805
Thermomonospora	0.001157453
Mesorhizobium	0.001151854