The Effect of Leaf Litter Legacies and Nutrient Additions on Microbial Function

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Abstract

Microorganisms are integral to ecosystem carbon and nutrient cycling, yet we still lack a holistic understanding of the roles microorganisms play in these ecosystem processes. This thesis explores the role microbial communities play in leaf litter decomposition, as well as how micronutrient additions influence the function of microbial communities.

The way that microbes decompose litter can be assessed via the indexes of home-field advantage and functional breadth. Often, past studies have focused on home-field advantage and ignored functional breadth as it is believed that the two are interchangeable. We performed a full factorial leaf litter by soil mesocosm experiment to compare how different microbial communities decompose substrates. We found an unimodal relationship between home-field advantage and functional breadth, as well as several other correlated variables that may help explain the presence or absence of these indexes in other scenarios. This unimodal relationship suggests that these two indexes, while related to each other are not ultimately the same, and should each be considered carefully in future studies.

There is relatively little known about how micronutrients influence microbial communities, compared to the influence of macronutrients. In order to study the influence of micronutrients on microbial communities, we added micro- and macronutient fertilizer inputs to field soils and measured differences in microbial function (i.e., carbon mineralization, substrate-induced respiration, catabolic response profiles). We found that microbial communities responded to the addition of multiple types of fertilizer, rather than just one single type. This response to different nutrients suggests there is a possible colimitation of nutrients.

Understanding how microbial communities regulate decomposition and nutrient cycling is a fundamental question for soil scientists. This work on the relationship between home-field advantage and functional breadth provides insight into a key component of the decomposition process. Similarly, this research on how macronutrients and micronutrients influence microbes shines light on what factors regulate microbial community assembly and diversity. Combined this work provides important insight into the roles and importance of microbial communities in global carbon cycling and ecosystem function.

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Dedication

I would like to dedicate this thesis to my incredible and supportive family who stood by me and encouraged me through the worst of my struggles. My parents, David and Michelle Hoch were always only a phone call away anytime I needed them. My brothers, Scott and (David) DJ helped me to be less serious and the frequent chats with my dear Grandparents, John and Jeannette Barbuscak helped me focus on what is important. Finally, I would like to thank the wonderful people of St. Augustine's Catholic Center for being a family here in Idaho. Thank you all.

Table of Contents

Authorization to Submit ii
Abstract iii
Acknowledgments iv
Dedication v
Table of Contents vi
List of Tables vii
List of Figures viii

Chapter 1: Introduction 1
1.1 Soil Nutrient Cycling 1
1.2 Microbial Communities Role in Leaf Litter Decomposition 2
1.3 Abiotic Soil Matrix 5
Chapter 2: The Role of Functional Breadth and Home-Field Advantage in Leaf Litter Decomposition 8
2.1 introduction 8
2.2 Materials and Methods 11
2.3 Results 18
2.4 Discussion 29
Chapter 3: Effects of Macro- and Micronutrient Deposits on Soil Carbon, Microbial Community Biomass, and Function in a Coastal Grassland 35
3.1 Introduction 35
3.2 Materials and Methods 38
3.3 Results 41
3.4 Discussion 47
Literature Cited 49
Appendix A: Chapter 2 55
Appendix B: Chapter 3 56

List of Tables

Table 2.1: Sample Leaf Litter Chemistry....12
Table 2.2: Correlation Matrix of Test Variables....23
Table B.1: Linear mixed effect model results for soil pH....56
Table B.2: Linear mixed effect model results for above-ground plant biomass....57
Table B.3: Linear mixed effect model results for carbon mineralization....58
Table B.4: Linear mixed effect model results for substrate-induced respiration....59
Table B.5: Linear mixed effect model results for particulate organic matter carbon....60
Table B.6: Linear mixed effect model results for mineral associated organic matter carbon....60
Table B.7: Linear mixed effect model results for total soil organic matter carbon....61
Table B.8: Pairwise comparisons of catabolic response profile results with NP....61
Table B.9: Pairwise comparisons of catabolic response profile results without NP....62

List of Figures

Figure 1.1: Thesis Topics 1
Figure 2.1: Proposed Models of Home-field Advantage Relationship to Functional Breadth 11
Figure 2.2: Cumulative CO_2 Production from the Full Factorial Microcosm 18
Figure 2.3: Home-field Advantage, Functional Breadth, and Quality Index 19
Figure 2.4: Linear Models of Microbial Influence Index Relationships...................................22
Figure 2.5: Linear Models of Home-field Advantage to Other Test Variables 26
Figure 2.6: Linear Models of Functional Breadth to Other Test Variables 27
Figure 2.7: Linear Models of Quality Index to Other Test Variables 28
Figure 3.1: Soil pH Across Time Course 42
Figure 3.2: Carbon Mineralization Across Time Course 43
Figure 3.3: Substrate Induced Respiration Across Time Course 44
Figure 3.4: Carbon Content Across Different Soil Fractions 45
Figure 3.5: Non-metric Multidimensional Scaling Plots of Catabolic Response Profile 46
Figure A.1: Average inoculum cumulative CO2 production 55

Chapter 1: Introduction

1.1 Soil Nutrient Cycling

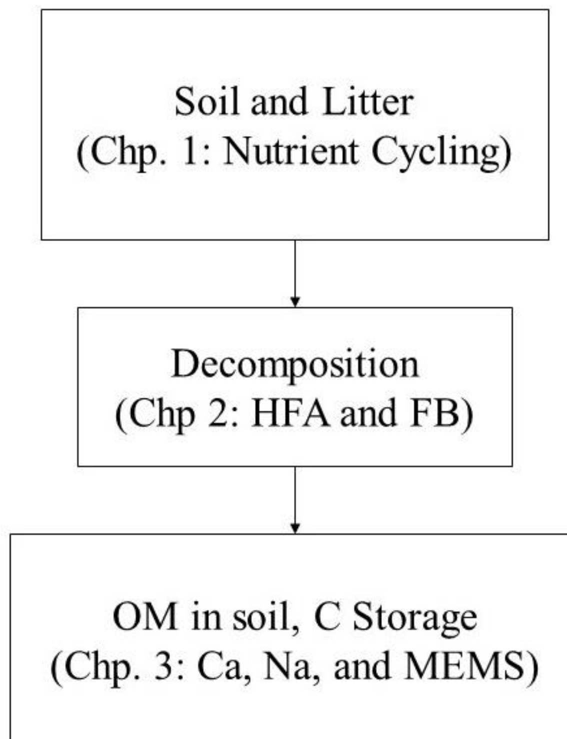

Figure 1.1: Thesis topics discussed in each chapter. The first chapter is an introduction to nutrient cycling in terrestrial systems, as well as the specific topics covered in greater detail in the following chapters. The second chapter is a study involving leaf litter decomposition and the mechanisms (home-field advantage (HFA) and functional breadth (FB)) related to the role of microbial communities. The third chapter is an investigation of organic matter (OM) in soil and what roles calcium and sodium play in the microbial efficiency-matrix stabilization (MEMS) framework.

Soil is critical to life on earth (Amundson et al. 2007). Soil provides many essential services such as the production of 99.7% of food calories worldwide (Pimentel and Burgess 2013). Other services include habitat provisioning (Weber 2007), water filtration (Wall et al. 2004), erosion control (Lavelle et al. 2006), and biological control of pests and diseases (Porter et al. 2009; Dominati et al. 2010). Soil is also a key component of carbon and nutrient cycling; it is estimated that on average 120 gigatons of carbon (C) is sequestered each year as organic matter inputs to soil (Kirchman 2018). However, much of this sequestered C is also returned to the atmosphere through organic matter degradation and one of the main processes of this degradation is leaf litter decomposition.

Decomposition is the breakdown of leaf litter by physical and chemical processes to its elemental chemical constituents (Aerts 1997). The rate of leaf litter decomposition is regulated by hierarchically organized, interacting factors (Lavelle et al. 1993; Adair et al. 2008; Wall et al. 2008). Of these factors, three of the most influential include climate, litter chemistry, and soil biota. Climate and litter chemistry are often considered to be more important in determining leaf litter decomposition despite the fact that microbial communities are the agents of decomposition (Lavelle et al. 1993; Cornwell et al. 2008; Currie et al. 2010; Carrillo et al. 2012). One aspect that could be leading to this belief is that microbial communities are often thought to be functionally homogenous, meaning that decomposition by microbial biota is determined solely by contemporary environmental conditions rather than the historical conditions of the site (Cardinale et al. 2007; Jiang 2007; Verity et al. 2007). However, there is growing evidence to suggest that microbial communities play a more dynamic and influential role in leaf litter decomposition than was previously considered (Hattenschwiler and Gasser 2005; Ayres et al. 2009; Schimel and Schaeffer 2012; Strickland et al. 2015).

1.2Microbial Communities' Role in Leaf Litter Decomposition

Microbes are everywhere doing nearly everything (Buol et al. 2011; Kirchman 2018). Microorganisms serve a regulatory role for the vital processes of non-symbiotic nitrogen fixation, nitrification, and denitrification in soil (Rosswall 1982; Hodge 2010). Plant growth is stimulated by the presence of microbes in the rhizosphere in the form of mycorrhizal fungi (Jeffries et al. 2002) and rhizobacteria are capable of promoting plant growth by colonizing the plant root (Hayat 2010). Fungi and other microorganisms can alter pore size distribution and increase the volume of mesopores (Strong et al. 1998; Chenchu and Cosentino 2011). Microorganisms also serve an important role as the foundation of food chains for other soil organisms (Mikola and Setala 1998). All of the above functions are important and have been the subject of much research, but of particular interest to this thesis is the role microbes play in the degradation of organic matter.

Two distinct and complementary mechanisms have been proposed for microbial regulation of decomposition rates. The first mechanism is home-field advantage (HFA). This

suggests that a microbial community displaying high HFA will have an advantage when decomposing litter that it shares a common history with, much in the same way that a sports team is hypothesized to have an advantage when playing in their home stadium (Gholz et al. 2000; Strickland et al. 2009b). The first usage of HFA in the context of microbial communities decomposing litter was by Gholz et al. (2000), where they found that litter from Central American broadleaf species decomposed much faster than pine litter from North America in the broadleaf habitat. This suggested a potential HFA effect in which the microbial community was historically adapted to better decompose its co-occurring litter as compared to a novel litter. This is in direct contrast to the general theory that contemporary climatic conditions are more important drivers of rates of decomposition. Similarly, Strickland et al. (2009b) performed a common garden experiment measuring decomposition of two different types of litter with different soil inocula. Microbial communities from different habitats decomposed litter at differing rates, suggesting that litter quality alone cannot predict rates of decomposition, but rather that microbial communities are important predictors as well. Veen et al. (2015) performed a meta-analysis of 35 studies that examined HFA effects based on climate and litter quality and found a general global trend of the HFA effect. However, there are also a number of studies which find no HFA effect or even a negative HFA effect (Gießelmann et al. 2011, John et al. 2011, Kagata & Ohgushi 2013, Veen et al. 2015). Though home-field advantage likely plays an important role in litter decomposition globally, we still lack an understanding of why HFA is sometimes present and sometimes not.

The second mechanism for how litter communities regulate decomposition is functional breadth (FB). Functional breadth suggests that a microbial community capable of decomposing recalcitrant litter is a “better decomposing community” because of a suite of characteristics and therefore will be able to decompose a wider array of litters (Van der Heijden et al. 2008; Keiser et al. 2011, 2014). Van der Heijden et al. (2008) was the first to hypothesize that microbial communities in nutrient-poor systems are functionally more diverse than communities in nutrient-rich environments. This is because microbes in nutrient-poor environments need specific adaptions to obtain resources. Keiser et al. (2013) found that across an elevational gradient, soil communities derived from the nutrient-poor higher

elevation sites were able to decompose different litters more evenly than the communities derived from the nutrient-rich lower elevation sites which had difficulty decomposing recalcitrant litter, providing support for the FB hypothesis. In a full factorial study of 3 inoculum communities sourced from different ecosystems of varying leaf litter input, it was found that communities which were gathered from areas with recalcitrant litter inputs (rhododendron and pine) were able to mineralize the other litters relatively well, while communities from labile litter inputs (grass) were poor at decomposing litter aside from grass, lending more weight to the FB hypothesis (Strickland et al. 2009a; Keiser et al. 2014).

Recently, there has been a tendency to consider HFA and FB to be interchangeable (Palozzi & Lindo 2018) as HFA effects have been observed to be most prominent when microbial communities are decomposing recalcitrant litter (Milcu & Manning 2011) such as with FB. However, there is evidence to suggest that HFA and FB, although potentially related, are separate mechanisms. In 2014, Keiser et al. found that by calculating HFA and FB using modified sporting statistics, the expressions of HFA and FB were not the same for each microbial community, lending evidence to the notion that these are unique mechanisms which describe different aspects of the microbial communities' role in decomposition. Understanding the relationship between these two mechanisms is an important next step in determining the role microbial communities play in leaf litter decomposition.

1.3Abiotic Soil Matrix

Soil microorganisms, while being the agents of leaf litter decomposition and critical to plant diversity and productivity, are influenced by the abiotic factors of the soil (Fierer and Jackson 2006; Andrew et al. 2017). Abiotic factors are the nonliving characteristics of soil (e.g., pH, temperature, soil moisture, nutrient content). Fierer and Jackson (2006) assert that edaphic characteristics are the primary control on microbial biogeography with pH, in particular, explaining a high level of differences in microbial community diversity and richness. Angel et al. (2010) examined microbial community diversity along a precipitation gradient in Israel and found that soil water content was correlated with the distribution of bacteria and archaea. A study of soil communities along an elevation gradient in a wetland on the Gulf of Mexico found that community diversity was influenced by salinity and soil water

content (Lee et al. 2019). While a broad range of abiotic factors can influence microbial communities, my work focuses on how soil communities and their function are shaped by the availability of macro- and micro-nutrients.

There have been numerous studies that highlight how microbial communities are influenced by macronutrients. Jonasson et al. (1995) found that when arctic soils were fertilized with nitrogen, phosphorus, and potassium (NPK), there was a 30% increase in soil respiration. A 20-year fertilization study on microbial communities in paddy fields found that the addition of NPK influenced the microbial communities' functional diversity (Chen et al. 2015). A study on fertilizer application over a 54-year long experiment found that different NPK volumes had little impact on the functionality of the microbial community, but there was an impact on bacterial community composition (Pan et al. 2014). While there has been a great deal of attention brought to the impacts of NPK, there have been relatively few studies on how micronutrient content influences soil communities and their functions.

Micronutrients are any nutrient that is important for life, however in trace amounts (Fageria et al. 2002). In grassland ecosystems, calcium (Ca) and sodium (Na) are considered micronutrients that can have complex ecosystem effects (Osimani et al. 2017). Sodium is necessary for plant life, but at high concentrations, it can become toxic (Jennings 1976; Kronzucker et al. 2013). Calcium is well known for its role in plant nutrition as it is involved in membrane stability and cell integrity maintenance (Bussler 1972). However, too much Ca reduces membrane permeability which restricts the flow of solutes in plants, leading to potential toxicity (Kirkby 1984; Daniel et al. 2007). These two elements also play important roles in soil systems. Excessive sodium ions at the root surface disrupt plant potassium nutrition which restricts plant growth (Zhu 2001). Increasing levels of Na beyond what is needed for growth can stress microbial communities and reduce both microbial biomass and function (Rietz and Haynes 2003). Calcium acts as a polyvalent cation which can form bridges between negatively charged soil organic matter (SOM) and phyllosilicates (Six et al. 2004; Cotrufo et al. 2013). These bridges result in a more stable soil matrix which may lead to greater microbial biomass. However, the benefits of Ca inputs on microbial growth may be diminished due to increased chemical stabilization of dissolved organic matter (DOM) (Cotrufo et al. 2013).

Understanding what regulates decomposition and nutrient cycling is fundamental to soil science. In this thesis, I will examine how microbial community composition can influence rates of decomposition, and how nutrient availability shapes soil communities and their function. Understanding the relationship between HFA and FB will provide insight into a key component of the decomposition process. Knowledge of how macronutrients and micronutrients influence microbes will shine light on what factors regulate microbial community assembly and diversity. This work can also be incorporated into climate models to increase our accuracy in predicting C cycling globally. As critical as microorganisms are to the function of ecosystems worldwide and the services they provide, there is relatively little known about such an important aspect of the planet. Combined this work will provide critical insight into the roles and importance of microbial communities in global C cycling and ecosystem function.

Chapter 2: The role of functional breadth and home-field advantage in leaf litter decomposition

2.1 Introduction

Leaf litter decomposition is central to global nutrient cycling and is a major contributor to ecosystem respiration (Raich & Schlesinger 1992). Rates of litter decomposition are controlled by three hierarchically-organized, interacting factors – climate, litter quality, and soil biota (Adair et al 2008). Soil biota, microbial communities, in particular, have been thought of as beholden to the effects of climate and substrate quality (Keiser et al. 2017) thus their contribution has been minimized. However, recent research suggests that soil biota may be of equal or greater importance to climate and litter quality in determining the rate of decomposition (Strickland et al. 2009b, & Ayres et al. 2009a, Glassman et al. 2018, & Bradford et al. 2017).

Due to their important role in the decomposition process, microbial communities are beginning to be incorporated into biogeochemical models (Bradford et al. 2017 & Strickland et al. 2009a, Weider et al. 2015). Yet, before this incorporation into models should be undertaken, we must first clearly identify the indexes which describe the microbial influence on decomposition dynamics. One such index is home-field advantage (HFA). This index posits that microbial communities may decompose litter faster than expected if they share a common history with the litter being decomposed (Gholz et al. 2000). For example, a microbial community sourced from a pine forest should decompose pine litter more rapidly than a community-sourced from a grassland or a rhododendron stand. While HFA has been used to describe decomposition rates in a variety of studies (Ayres et al. 2009b, Chomel et al. 2015, Strickland et al. 2009b, & Yu et al. 2015), there are some studies, such as Fanin et al. 2016, which dispute its importance, claiming that <5% of decomposition is explained by HFA. There are other studies where the presence of HFA or even an inverse HFA effect has been found (Veen et al. 2015). The absence of HFA has often been attributed to factors such as litter quality or plant successional stage (Palozzi & Lindo 2018).

Another index of microbial influence is functional breadth (FB). Functional breadth suggests that a microbial community that is capable of decomposing recalcitrant litter is more

functionally wide and therefore will be able to decompose a wider array of litters (Keiser et al. 2011, 2014). The presence of FB is associated with the recalcitrance of leaf litter input, the hypothesis being that the more complex a litter is the more functionally diverse a community will need to be in order to mineralize the various compounds in the litter (Van Der Heijden et al. 2008). Conversely, if a community is associated with litter considered to be higher quality and therefore less complex in composition, that community will not develop the same kind of wide functionality, but rather will favor a strategy where the community adapts to decomposing that particular type of litter quickly to compete with other microbes trying to decompose that litter.

As with FB, HFA has also been associated with complex and/or recalcitrant litter substrates (Milcu & Manning 2011, Veen et al. 2015 & Palozzi & Lindo 2018). Since an association of HFA to leaf litter chemistry complexity has been recorded, FB is often mistaken as a symptom of communities that exhibit HFA (Veen et al. 2015 & Palozzi & Lindo 2018). However, Keiser et al. (2014) found evidence to suggest that decomposer communities exhibiting HFA are not synonymous with communities exhibiting a high degree of FB. These results indicate that invoking FB as the same as HFA is incorrect and that, in fact, these are independent (or semi-independent) characteristics of microbial communities. A clear mechanism that accounts for the presence, absence, or inverse of HFA has yet to be proposed and experimentally verified. The key to the presence or absence of HFA may ultimately lie in the functionality of the microbial community.

Research Objectives

Here we explore the relationship between HFA and FB, demonstrating that it is more complex than a positive linear relationship. We predict a unimodal relationship between HFA and FB, with HFA being greatest at intermediate levels of FB (Fig. 2.1). This prediction is based on the assumption that HFA is indicative of the relative increase in decomposition that a microbial community exhibits on its ‘home’ litter versus ‘away’ litters, and as such if a microbial community has a high degree of FB, then the community will be so good at decomposing any kind of litter that the ‘home’ litter may elicit no benefit in terms of the rate of decomposition. Alternatively, a community that exhibits very low FB will only be capable of decomposing a small array of litter compounds and will exhibit no HFA because it is poor

at decomposing even a litter it has an association with. At intermediate levels of FB we expect to observe the greatest HFA because FB will not override the manifestation of HFA. We also investigated separate factors that could be used to indicate the presence or absence of HFA and FB, such as edaphic attributes, litter chemistry, and microbial community identity.

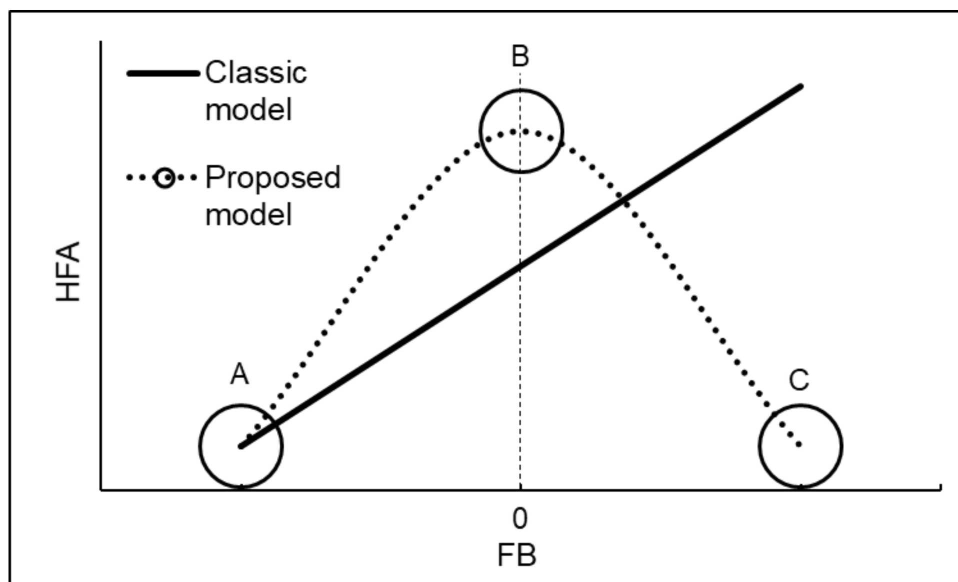

Figure 2.1: A comparison of the "classic model" and our new proposed conceptual model of how home-field advantage (HFA) and functional breadth (FB) are related to one another. We propose that HFA will be greatest at intermediate levels of FB and lowest at both high and low levels of FB. This is contrasted by the linear relationship of the "classic model" in which higher FB is will mean higher HFA. At low FB (A), the community performing decomposition will not be able to decompose a wide array of litter including the community's home litter and will therefore not exhibit HFA. At intermediate FB (B), HFA will be expected to be highest because FB will not be overriding. And at high FB (C), the microbial community will be able to decompose litter equally well without HFA.

2.2 Materials and Methods

Site and sample description

To determine the potential drivers of home-field advantage and functional breadth, we conducted a full factorial litter by soil inoculum mesocosm experiment. We collected soil and litter samples from 6 unique locations across the United States. The species include blue bunch wheatgrass (*Pseudoroegneria spicata*) collected from the Hudson Biological Reserve at Smoot Hill, WA (46°49' N, 117°14' W), trembling aspen (*Populus tremuloides*) from University of Idaho's Arboretum, ID (46°43' N, 117°1' W), and ponderosa pine (*Pinus

ponderosa) from University of Idaho's Agricultural Experiment Station, ID (46°55' N,116°49' W), rhododendron (*Rhododendron maximum*) collected from Pandapas farm, Montgomery County, VA (37°17' N, 80°28' W), tulip poplar (*Liriodendron tulipifera*) and white pine (*Pinus strobus*) both collected from Kentland farm, Montgomery County, VA (37°11' N, 80°34' W). All samples were collected from sites where the named plant species created the dominant leaf litter substrate, although the tulip poplar site was also a mixed forest stand. We selected litter to vary in chemical complexity from labile to recalcitrant (Table 2.1).

Table 2.1: Leaf litter chemistry of the species collected for the study. The range in quality is based on the lignin to nitrogen ratio.

Species	% Lignin	Lignin/Nitrogen	% Nitrogen	% Carbon
Bluebunch wheatgrass	2.8	8.6	0.3	40.2
Trembling aspen	11	12.4	0.9	46.2
Ponderosa pine	14.7	21.8	0.7	49.5
Rhododendron	11.1	33.2	0.3	48.6
Tulip poplar	13.2	11.8	1.1	45.8
White pine	16.7	33.8	0.5	50.3

Trembling aspen, ponderosa pine, rhododendron, tulip poplar, and white pine litters were collected as recent litterfall and blue bunch wheatgrass litter was collected as standing-dead material. In the laboratory, litter samples were sorted to remove unwanted additions (seed, fruits, etc.), air dried, and milled (4mm). Litter was sterilized in an autoclave (121°C, 30 min).

Microbial inocula sources were collected as 5-6 soil cores (5 cm depth) at each site with a standard soil auger 8 cm in diameter. The BBW site soil is a from the Tekoa series classified as an Argixeroll. The TA site soil is a Lathco-Thatuna complex classified as an Argixeroll. The PP site soil is from the Taney series classified as an Argixeroll. The RM site soil is from the Jefferson series classified as a Hapludult. The TP and WP site soils are a Wurno-Newbern-Faywood complex classified as a Eutrudept. Soil samples were passed through a no. 4 sieve, homogenized, and then stored at 5°C until analysis. Each microbial community derived from soils is named after the dominant plant species present at the site of collection, the same as the litter substrates.

Mesocosm Design

In order to calculate HFA and FB, we created a full factorial litter by microbial inocula incubation to measure each litter's decomposition in the presence of each microbial inocula. Six litter species were crossed with six microbial inocula with five replicates each (n=5) to create a total of 180 experimental units. We placed the experimental units in 50mL centrifuge tubes, where 1g of litter substrate was inoculated with 0.25g of dry mass equivalent soil for the inoculum source. We maintained the mixture at 65% water holding capacity (WHC) and 20ºC to facilitate microbial activity during the 150-d incubation. We determined litter decomposition by measuring CO_2 production over the course of 150 days. We took respiration measurements on days 2, 3, 7, 10, 14, 17, 22, 24, 28, 35, 46, 51, 57, 64, 72, 80, 86, 93, 101, 107, 122, 136, and 150, using a static incubation technique where all units were capped and then flushed with CO_2 free air and allowed 24 hrs to incubate, after which the headspace CO_2 was measured using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA). Total litter decomposition (cumulative CO_2-C (mg g dry wt litter^{-1})) was calculated by integrating CO_2 production values across time.

Determining initial leaf litter & edaphic characteristics

Prior to our mesocosm experiment, we determined litter quality and chemistry for all litter types in order to be investigated as potential indicators of HFA and FB. We analyzed total C and N of each litter using an ECS 4010 Nitrogen / Protein Analyzer (Costech, Valencia, CA, USA). Litter pH was determined in water (2:1 ratio of DI water: litter) using a benchtop pH meter (Mettler Toledo, Columbus, OH, USA). Lignin, calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, and molybdenum content were determined by DairyOne Laboratories through their forage lab (Ithaca, NY, USA) using traditional wet chemistry procedures (Ibanez and Bauer 2014).

We determined gravimetric soil moisture (GVM), 100% WHC, soil pH, and soil bulk density on all soil samples. Both GVM and WHC (after wetting to field capacity) were determined by drying soil at 105ºC for 24 h as per Strickland et al. 2009a. Soil pH was determined using the same methods as the leaf litter pH.

Determining initial microbial inocula characteristics

The microbial community composition of initial soil inocula was determined for bacterial and fungal communities using a 16S/ITS metabarcoding technique. For each microbial community, we extracted DNA using the MoBio© PowerSoil kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's protocols. We amplified ribosomal marker genes using 2 step PCR in accordance with the Earth Microbiome Project protocol for 16S and ITS sequencing (www.earthmicrobiome.org). We used the ITS1F/ITS2 and the 515f/806r primer pairs for fungi and bacteria, respectively. After the first round of PCR, sequences were cleaned using ExoSAP-ITTM PCR clean-up reagent (Affymetrix Inc., Santa Clara, CA, USA), according to the manufacturer's protocol. During the second round of PCR, unique barcoded primers were added to each sample. After the second round of PCR, we cleaned and normalized samples using SequelPrepTM 96-well plates (Invitrogen, Carlsbad, CA, USA). We pooled equimolar DNA, and these amplicon pools were sequenced on an Illumina MiSeq instrument using 2 × 300 bp sequencing kits at the IBEST sequencing facility at the University of Idaho. Controls were used throughout the laboratory process to ensure there were no contaminants

Raw sequences were first demultiplexed by the IBEST genomic resource core using the program dbcAmplicons (Uribe-Convers et al. 2016). This process also removed barcodes and primers from sequences. We processed paired sequences using the DADA2 pipeline (Callahan et al. 2016), which is designed to resolve exact biological sequences from Illumina sequence data and does not involve sequence clustering (Leff et al. 2018). Paired sequences were trimmed to uniform lengths, dereplicated, and the unique sequence pairs were denoised using the 'dada' function, accounting for errors through the model generated with the 'learnErrors' command. We then merged paired-end sequences and removed chimeras. We assigned taxonomy using the Silva (ver. 132, Quast et al. 2013) and the UNITE dynamic general release (ver 01.12.2017, Abarenkov et al. 2010) databases for bacteria and fungi, respectively. To account for differences in sequencing depths, we rarified samples to 3160 and 6400 sequences per sample for fungi and bacteria, respectively.

To determine total active microbial biomass, we measured substrate-induced respiration (SIR) as per Strickland et al. (2015). Briefly, SIR was determined using soil slurries (4 g dry mass equivalent soil) of microbial community source that were incubated for

5 h with autolyzed yeast as the substrate. After the 5 h incubation, respiration was determined using the static incubation technique used for the mesocosm described above. Then, in order to determine community function, including catabolic evenness, a catabolic response profile (CRP; Degens and Harris 1997) was performed using the same protocol for SIR except with six different substrates (DI water, glucose, oxalic acid, glycine, cellulose, and chitin; as per Strickland et al. (2017). We shook inoculated substrates for 1 hour then flushed with CO_2 free air. After flushing each substrate was incubated for a specific period of time. The DI water, glucose, oxalic acid, and glycine substrates were incubated for 5 h while the cellulose and chitin were incubated for 24 h. Respiration measurements were taken at the conclusion of the appropriate incubation period. The chosen substrates represent a variety of different classes of C compounds.

To determine bioavailable C of each initial microbial community, we determined C-mineralization through weekly measurements of respiration produced during a 30-day incubation using the same static incubation technique described above. Units for the incubation were 6 g of inoculum source soil with no substrate maintained at 20ºC and 65% WHC, which is considered favorable conditions for microbial growth. We calculated total mineralizable C (cumulative CO_2-C (mg g dry wt litter^{-1})) by integrating CO_2 production values across time.

Statistical Analysis

We analyzed cumulative litter mineralization as CO_2 production from the mesocosm using an ANOVA to determine if differences existed between litter types and inocula and if there was an interaction between litter and inocula. The cumulative CO_2 data was then used as the input for the model created by Keiser et al. (2014) to calculate HFA and FB. This model states that carbon mineralization (Yi) is equal to litter ability (βl) plus soil ability (γs) plus a home interaction term (ηh):

$$Y_i = \alpha + \sum_{l=1}^{N} \beta_l \text{ Litter}_{l_i} + \sum_{s=1}^{M} \gamma_s \text{ Soil}_{s_i} + \sum_{h=1}^{k} \eta_h \text{ Home}_{h_i} + \varepsilon_i$$

Where Y_i is the carbon mineralization for observation i, β_l is the ability of litter species l (from species 1 to N), γ_s is the ability of the soil community s (from community 1 to M), η_h is the HFA of h (from home combinations 1 to K), and $Home_h = Litter_l$ * Soils when l and s are home-field pairings. The parameters to be estimated are β_l, γ_s and η_h. The intercept term is defined by α and represents the average carbon mineralization rate for all observations in the dataset after controlling for litter, soil and home-field pairings. Negative parameter estimates indicate lower carbon mineralization than the average rate observed across all samples. The error term is defined by ε. Using this model, we calculated the ability of soil microbial communities (FB), and the interactions between litter and soil (HFA) on net cumulative carbon mineralization. This model also creates a quality index term (QI), which is a measure of the litter quality determined from the average decomposition from microbial communities, or rather it is the quality of litter from the perspective of the microbial communities.

We determined the relationship between HFA and FB using a linear model: HFA ~ FB + FB^2. We squared FB to create a polynomial fit. We performed an ANOVA and Tukey HSD on HFA and FB to test for significance between microbial communities. We created a correlation matrix using Pearson's correlation coefficient in order to identify if initial leaf litter and edaphic characteristics are associated with HFA and FB serving as indicators. Correlations that were found to be significant were further tested using linear models. We performed all statistical tests using R software (version 3.1.1, The R Foundation for Statistical Computing, Vienna, Austria) and SAS software (version 9.0, SAS Institute, NC, USA).

2.3 Results

Litter mineralization, Home Field Advantage, Functional Breadth, and Litter Quality

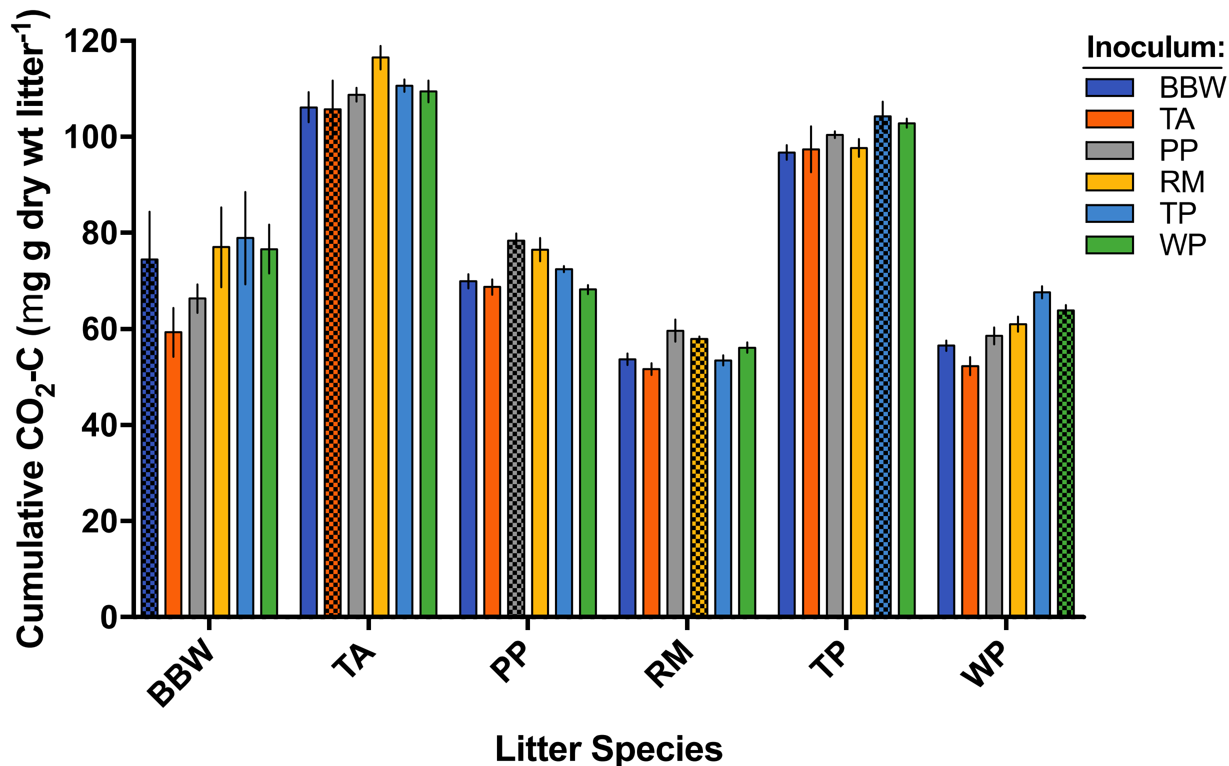

Fig 2.2: Cumulative CO_2 production (mg g dry wt litter^{-1}) from the full factorial microcosm. Each bar represents a different inoculum as it decomposes each type of litter as shown in groups along the x-axis (blue bunch wheatgrass (BBW), trembling aspen (TA), ponderosa pine (PP), rhododendron (RM), tulip poplar (TP), and white pine (WP)). Values are means ± SE. Main effects of litter type and inoculum were significant (Litter; $P<0.001$, Inoculum; $P<0.001$). No significant interaction between litter and inoculum was detected for cumulative CO_2 production. Bars displaying inoculum that share a historical association with the litter are patterned.

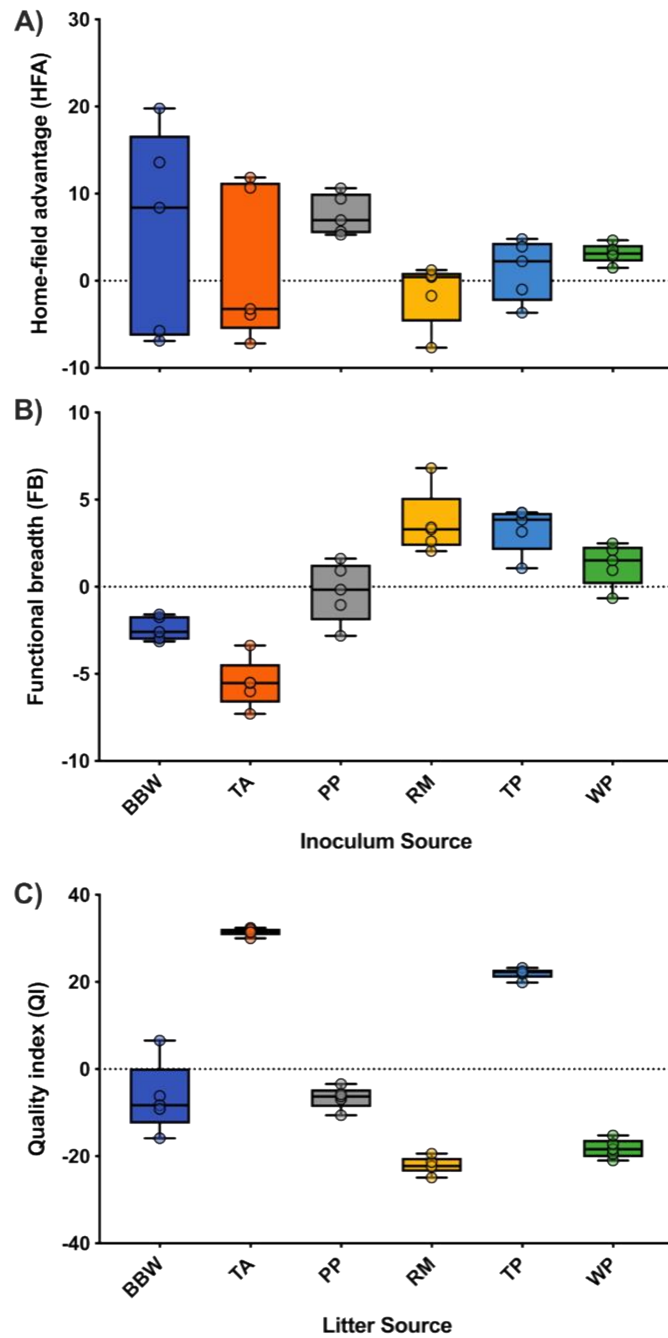

Fig 2.3: Boxplots describing home-field advantage (A) and functional breadth (B) of each inoculum source (blue bunch wheatgrass (BBW), trembling aspen (TA), ponderosa pine (PP), rhododendron (RM), tulip poplar (TP), and white pine (WP)) as parameter estimates calculated using the quantitative model approach from Keiser (2014). Also displayed are boxplots describing the QI (C) of each litter substrate which shares names with inoculum source. The line in the box represents the median. The circles represent data points.

In order to examine differences between inocula and litter, we monitored litter mineralization across 150 days. We observed both a significant inoculum effect ($P < 0.001$, Figure 2.2) and a litter effect for cumulative CO_2 produced during this 150-day experiment ($P < 0.001$, Figure 2.2). No significant inoculum by litter type interaction was observed (Figure 2.2). The litter effect was likely driven by greater cumulative mineralization associated with both TP and TA, low mineralization for RM and WP, and intermediate respiration for BBW and PP. The inoculum effect was primarily attributed to lower cumulative mineralization associated with TA compared to all the other inocula except for BBW (Fig A.1).

Using the model proposed by Keiser et al. (2014), we found no significant HFA term (Fig 2.3 A; $P = 0.3086$). While there may not have been a significant HFA term, inocula still exhibited a range in HFA. Additionally, both the PP and WP inocula were significantly greater than zero exhibiting a positive HFA, while the other inocula exhibited no HFA (Fig 2.3 A). We did observe a significant FB term (Fig 2.3 B; $P<0.01$), with both BBW and TA inocula exhibiting a negative FB, and RM and TP inocula values exhibiting a positive FB. The FB term corresponds with the differences observed in litter mineralization for the inocula (Fig A.1). We also observed a significant QI term as all litters were significantly different from zero except for BBW (Fig. 2.3 C; $P<0.01$). The TA and TP litters exhibited positive QI values, and the PP, RM, and WP exhibited negative QI values. The QI index corresponds with the differences observed in litter mineralization for the litter species (Fig 2.2).

Relationships between HFA, FB, and QI

We examined relationships between HFA, FB, and QI. We found that only the HFA by FB relationship was significant when including all of the data. Specifically, we observed a unimodal relationship between HFA and FB ($r^2 = 0.8256$, Figure 2.4 A). This unimodal relationship was due to the greatest HFA observed at intermediate FB (i.e. when FB is ~ 0), and the lowest HFA observed for both high and low values of FB. While the relationship between QI and FB was not significant ($r^2 = 0.19$; Fig 2.4 B), this appeared to be driven by a single data point (i.e. TP). After removing TP from the analysis, we observed a significant negative linear relationship between QI and FB ($r^2 = 0.85$; $P<0.05$; Fig 2.4 B) whereby lower QI values were associated with greater FB values. We observed no significant relationship between HFA and QI (Fig 2.4C).

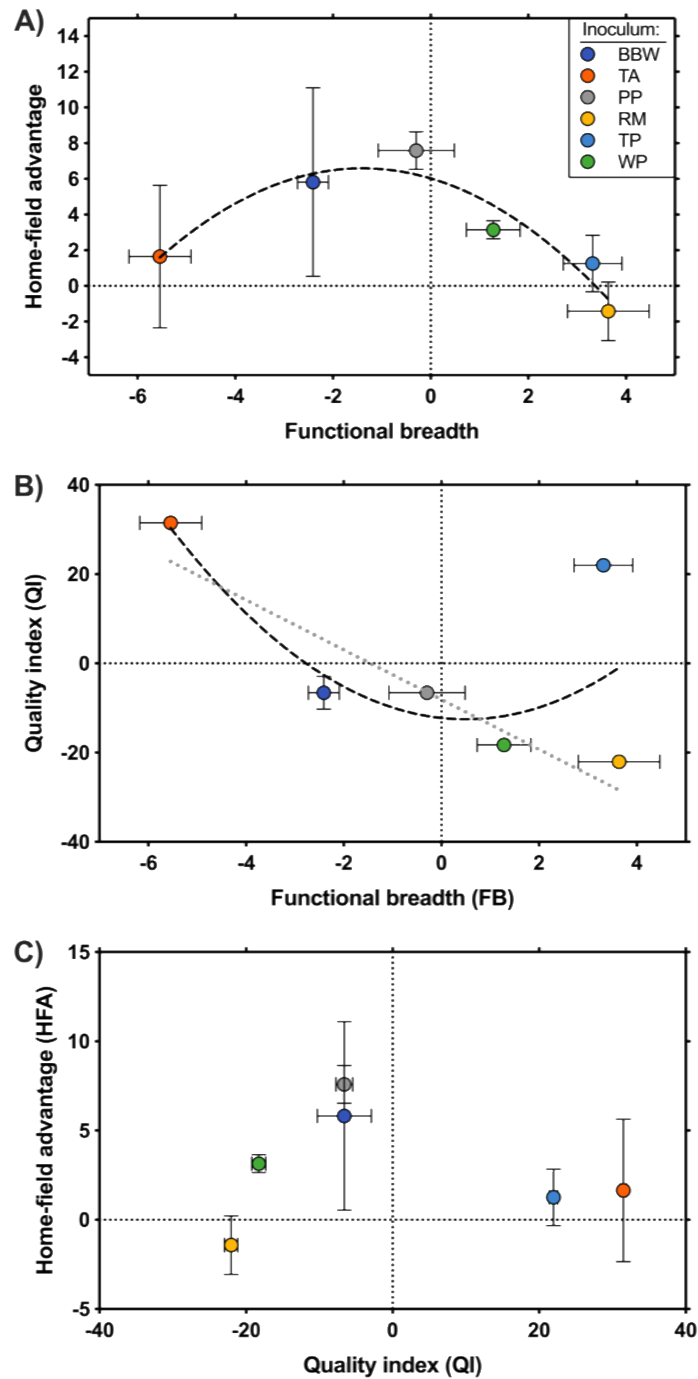

Figure 2.4: Relationship between the parameter estimates of HFA and FB (A; Adjusted r^2:0.82; $P < 0.05$), QI~FB (B), and HFA~QI (C). Each point represents the different inoculum sources or litters that were studied. BBW is blue bunch wheatgrass, TA is trembling aspen, PP is ponderosa pine, RM is rhododendron, TP is tulip poplar, and WP is white pine. Error bars are included for each axis. The grey line in B is the potential negative linear relationship observed if TP is removed. The black unimodal line in B is not significant. The methods used were non-linear models (A; $HFA \sim FB + FB^2$/ B; $QI \sim FB + FB^2$/ C; $HFA{\sim}QI{+}QI^2$).

Relationship of indexes to starting inoculum characteristics and litter chemistry

Table 2.2: Pearson's correlation coefficients of home-field advantage (HFA), functional breadth (FB), and quality index (QI) by catabolic response profile results, initial inoculum, and litter characteristics. Values are colored blue based on the degree of correlation, with values closer to correlation having stronger colorization. Values considered to be correlated (> 0.8 and < -0.8) are in bold. All correlations with microbial taxon use rarefied relative abundances of the taxon. The taxon included are the 95% most abundant taxon in the initial microbial communities. Bacteria are identified to phyla except for α-Proteobacteria, δ-Proteobacteria, and γ-Proteobacteria which are classes of the phyla proteobacteria. All fungi are identified to class.

	HFA	FB	QI
	correlation coefficient		
Bacteria			
Planctomycetes	0.24	-0.48	**0.84**
Actinobacteria	0.78	-0.35	-0.19
Gemmatimonadetes	**0.85**	-0.35	0.28
Verrucomicrobia	**0.86**	-0.20	-0.17
Acidobacteria	-0.60	**0.94**	-0.48
Bacteroidetes	-0.54	-0.51	0.27
Proteobacteria	0.50	-0.42	-0.24
α-Proteobacteria	0.31	0.41	-0.68
δ-Proteobacteria	0.07	**-0.88**	0.64
γ-proteobacteria	-0.05	-0.06	0.12
Fungi			
Dothideomycetes	0.07	-0.63	0.27
Pezizomycetes	0.00	0.09	-0.34
Sordariomycetes	0.06	-0.25	0.71
Leotiomycetes	-0.27	-0.53	**0.86**
Mortierellomycetes	-0.32	-0.50	0.75
Agaricomycetes	0.22	0.28	-0.66
Catabolic Response Profile			
Chitin	-0.49	-0.51	0.71
Cellulose	**-0.83**	0.30	0.54
Glucose	0.60	0.40	-0.45
Glycine	0.65	**-0.84**	0.21
Oxalic acid	-0.55	0.65	-0.67
Catabolic Evenness	-0.71	-0.19	0.68
Substrate Induced Respiration	**0.81**	-0.14	-0.31
Litter Characteristics			
% Nitrogen	-0.11	-0.06	**0.85**
Carbon/Nitrogen	-0.14	0.26	**-0.85**
% Phosphorus	0.07	-0.72	**0.89**
% Sodium	0.34	**-0.97**	0.46
PPM Iron	0.16	**-0.93**	0.75

To observe potential indicators of HFA, FB, and QI we examined correlations between these three indexes and microbial taxa, CRP, edaphic properties, and litter characteristics of the source location. Overall, we observed multiple significant correlations associated with both the initial inoculum and litter characteristics. We found fewer correlations with HFA than with FB and QI. For HFA we found correlations with the relative abundance of two bacterial taxa, the proportional respiration of one CRP substrate, and active microbial biomass (i.e. SIR). Specifically, for bacterial taxa, HFA was positively correlated with the relative abundance of Verrucomicrobia ($r^2 = 0.74$; $P<0.05$; Figure 2.4A), and the relative abundance of Gemmatimonadetes ($r^2 = 0.72$; $P<0.05$; Figure 2.4A). For CRP, HFA was negatively correlated with the respiration of cellulose ($r^2 = 0.69$; $P<0.05$; Figure 2.4B). That is the greater the percent contribution of cellulose to the overall CRP profile of the starting inoculum, the lower the HFA index. For SIR, an indicator of active microbial biomass, we observed a positive relationship with HFA ($r^2 = 0.66$; $P<0.05$; Figure 2.4C).

We found correlations between the FB index and the relative abundance of two bacterial taxa, the proportional respiration of another CRP substrate, and two components of litter chemistry. Specifically, for the bacteria taxa, FB was positively correlated with the relative abundance of Acidobacteria ($r^2 = 0.88$; $P < 0.01$; Figure 2.5A) and negatively correlated to the relative abundance of δ-Proteobacteria ($r^2 = 0.77$; $P < 0.05$; Figure 2.5A). For CRP, FB was negatively correlated with the respiration of glycine ($r^2 = 0.71$; $P<0.05$; Figure 2.5B). For litter chemistry components, FB was negatively correlated with the percent of Na in the litter ($r^2 = 0.95$; $P<0.01$; Figure 2.5C) and the PPM of Fe in the litter ($r^2 = 0.86$; $P<0.01$; Figure 2.5D).

Finally, we found correlations between the QI with two microbial taxa (one bacteria and one fungi) and with three litter chemistry components. The QI was shown to be positively correlated to the relative abundance of Planctomycetes ($r^2 = 0.71$; $P<0.05$; Figure 2.6A) and the relative abundance of Leotiomycetes ($r^2 = 0.75$; $P<0.05$; Figure 2.6B). For litter chemistry, QI was positively correlated with the percent of N in litter ($r^2 = 0.72$; $P<0.05$; Figure 2.6C). There was a negative correlation between QI and the ratio of C to N in litter ($r^2 = 0.73$; $P<0.05$; Figure 2.6D). The last litter chemistry component was the percent of P in litter which was positively correlated with QI ($r^2 = 0.79$; $P<0.05$; Figure 2.6F).

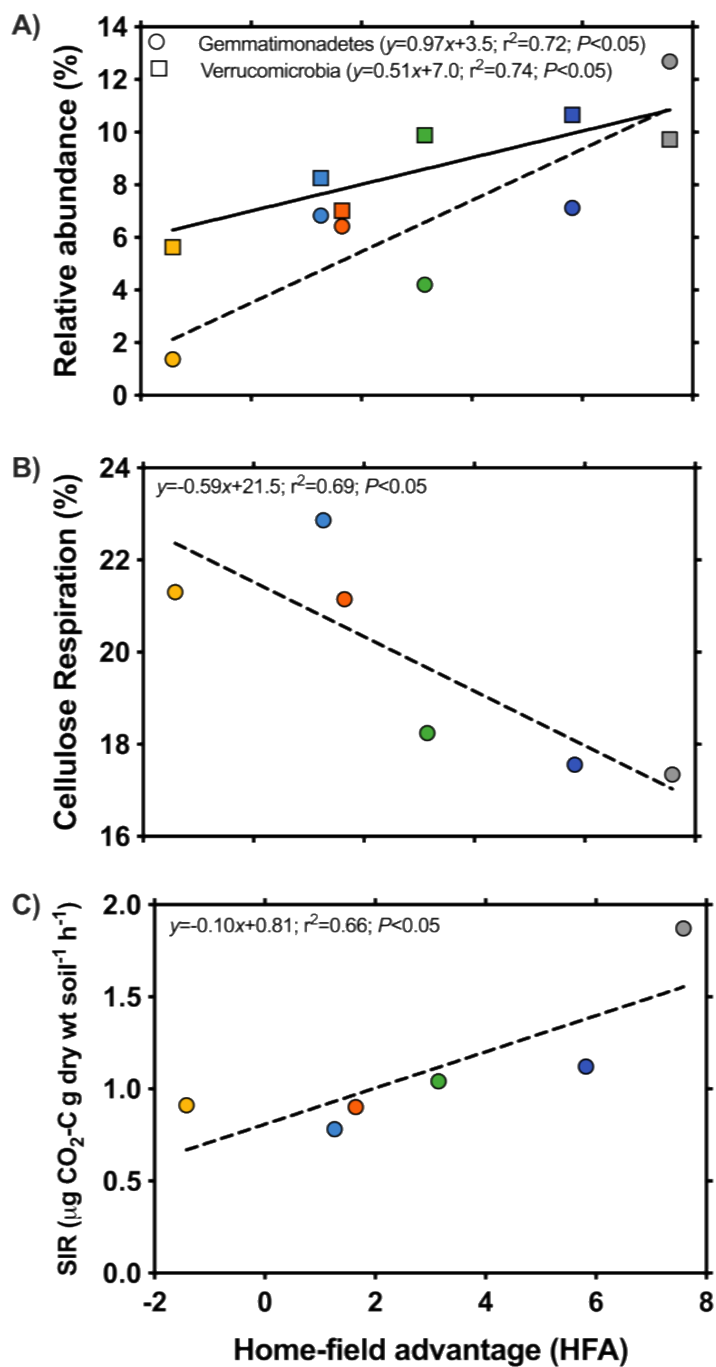

Figure 2.5: Linear models of the relationships of home field advantage (HFA) and the relative abundances of Verrucomicrobia and Gemmatimonadetes (A), the respiration of cellulose (B), and the substrate-induced respiration (SIR; C). Each point represents the different inoculum sources that were observed. blue is blue bunch wheatgrass, orange is trembling aspen, grey is ponderosa pine, gold is rhododendron, light blue is tulip poplar, and green is white pine.

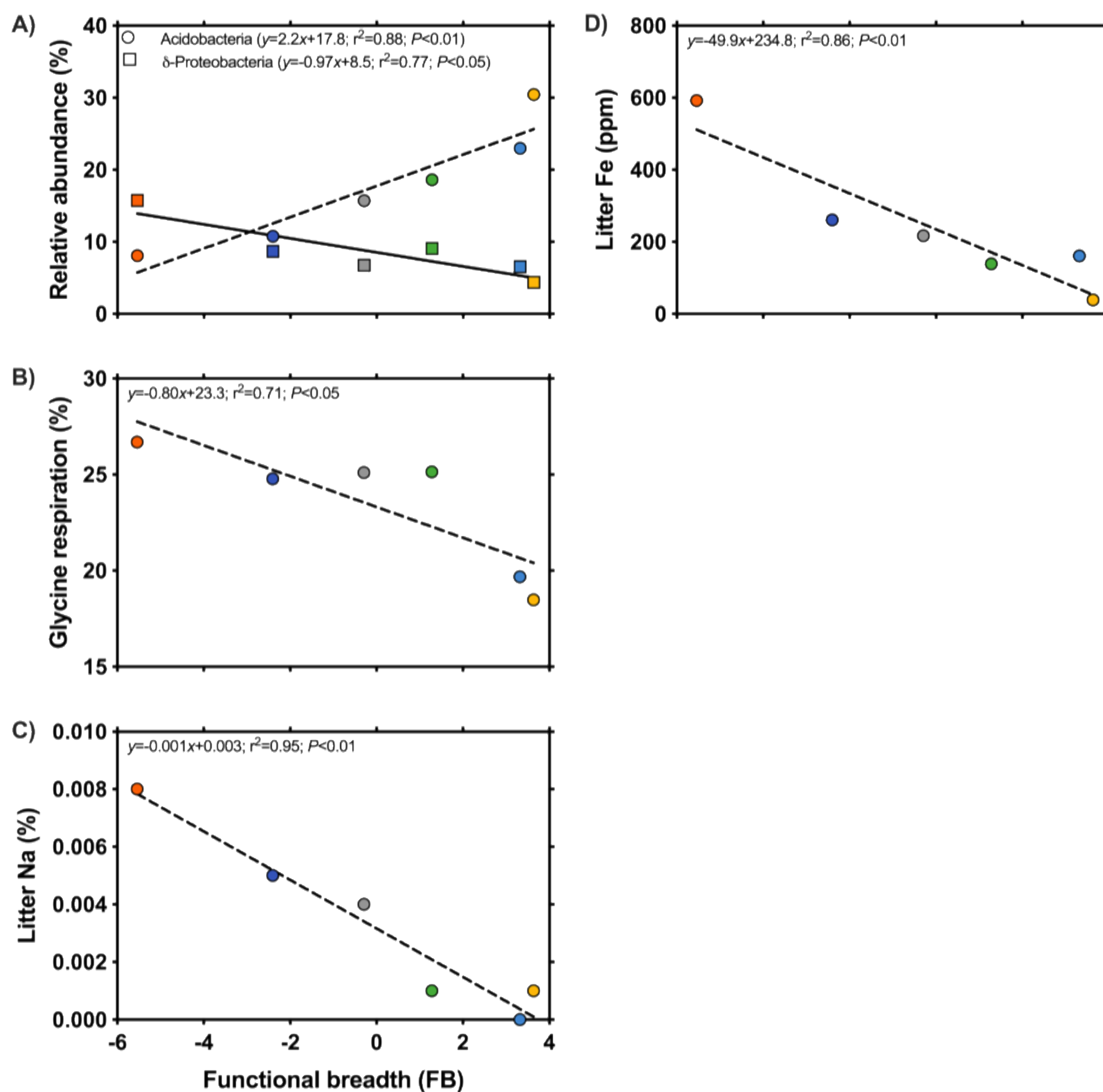

Figure 2.6: Linear models of the relationships of functional breadth (FB) to the relative abundances of Acidobacteria and δ-Proteobacteria (A), the respiration of glycine (B), the percent of sodium in litter (C), the ppm of iron in litter (D). Each point represents the different inoculum sources that were observed. blue is blue bunch wheatgrass, orange is trembling aspen, grey is ponderosa pine, gold is rhododendron, light blue is tulip poplar, and green is white pine.

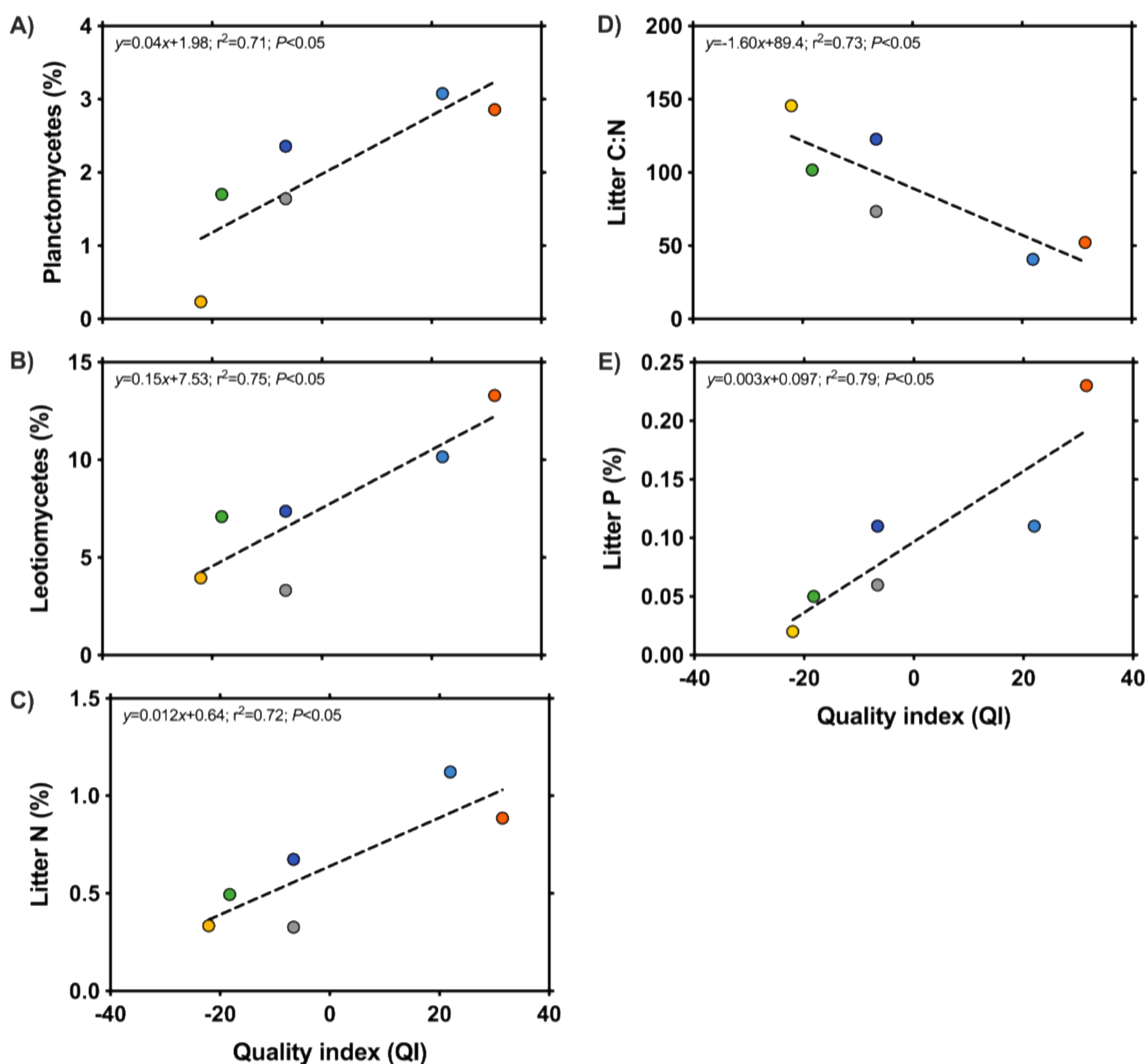

Fig 2.7: Linear models of the relationships of quality index (QI) to the relative abundances of Planctomycetes (A) and Leotiomycetes (B), the percent nitrogen in the litter (C), the ratio of carbon to nitrogen in the litter (D), and the percent of phosphorus in the litter (E). Each point represents the different inoculum sources that were observed. blue is blue bunch wheatgrass, orange is trembling aspen, grey is ponderosa pine, gold is rhododendron, light blue is tulip poplar, and green is white pine.

2.4 Discussion

We examined relationships between HFA, FB, and, QI as well as the relationships of these indices to initial inoculum and litter characteristics. Comprehension of these relationships is critical to understanding the role microbial communities play in litter decomposition and how these communities are likely to respond to future perturbations (e.g. overstory tree species shifting north, forest harvest, land-use change, etc.). We argued that

HFA and FB are not interchangeable. That is a high level of FB is not necessarily indicative of a community that also exhibits strong HFA, in fact, communities that exhibit strong or weak FB may actually exhibit no HFA.

Relationships between HFA, FB, and QI

Of the relationships between HFA, FB, and QI examined, the relationship between HFA and FB was significant (Fig 2.4A). The HFA index was least at the highest and lowest expressions of FB because FB was masking the HFA effect. For example, communities with high FB such as RM and TP have such high functionality that they did not show a HFA effect as these communities decomposed all leaf litter species equally including the litter these communities are historically associated with. Conversely, communities with extremely low FB such as TA and BBW did not have high HFA because the communities are so poor at decomposition that they have no advantage even when decomposing litter that they are associated with. Communities that had high HFA in response to intermediate FB such as PP and WP were caused by FB not being too high or low and therefore not masking HFA. Although there are a few studies that present HFA and FB as the same (Milcu & Manning 2011 and Palozzi & Lindo 2018) it is evident from our findings that high FB does not necessarily equate to high HFA (Keiser et al. 2014).

There was a potential negative linear relationship between FB and QI, but it was only present if the TP data are ignored (Fig 2.4B). This potential relationship is not surprising because more complex litter will require a community to be more functionally diverse in order to mineralize the various compounds in the litter (Van Der Heijden et al. 2008) and QI is a measure of leaf litter quality based on average decomposition by microbial communities. The TP data point is unusual because it is both the second-highest quality litter and the second most functionally wide inocula. Of all the litters collected, TP was the only litter to be gathered from a mixed forest stand, which could mean that the FB index is not shaped solely by the most dominant type of litter, but rather FB is shaped by the aggregate of all the various litter components in the system. While only six inocula by litter combinations were examined in this study, our results indicate that the primary driver of the microbial community's influence on litter decomposition is its FB and not HFA. This is not to say that historical legacies do not shape the function of decomposer communities. On the contrary, FB is likely a

product of litter quality but this exposure to litters of high or low quality may lead to communities with high or low FB, respectively.

Relationship of indexes to starting inoculum characteristics and litter chemistry

The previously explored indexes of HFA, FB, and QI are useful in understanding how soil communities are influenced by changes in leaf litter substrate, but calculating these indices could be prohibitively time consuming an undertaking for every study and other more readily available information about a site could be useful as proxies or indicators to the presence and magnitude of HFA, FB, and QI. Thus, potential indicators were investigated by finding the relationships of other site variables with HFA, FB, and QI. These site variables included biological characteristics for each initial community, litter characteristics of each litter source, and edaphic characteristics. We found that HFA, FB, and QI were correlated with particular microbial taxa, the respiration of certain substrates from the CRP, and a number of litter characteristics. However, some of the variables tested were correlated with each other making it difficult to disentangle what is truly driving the relationship. There was minimal overlap between the variables correlated to HFA, FB, and QI suggesting further these are separate indexes (Table 2.2).

The HFA index was correlated with the relative abundance of Verrucomicrobia and Gemmatimonadetes, the respiration of cellulose, and the SIR (Fig 2.5). The positive correlation between HFA and Verrucomicrobia abundance could be present because high Verrucomicrobia abundance has been associated with soils that have experienced less disturbance (Strickland et al. 2017 & Fierer et al. 2013) and a site with less disturbance will lead to longer adaption time and in turn higher HFA. The reason we did not see this same correlation with FB is possibly because the specificity to a certain litter involved with HFA requires a different time scale for adaption to legacy effects. The positive relationship between HFA and Gemmatimonadetes is potentially due to Gemmatimonadetes being correlated with site moisture (DeBruyn et al. 2011) and the sites with higher quality leaf litter tended to be the sites with higher moisture, suggesting that there is some potential for a connection between HFA and litter quality. There were a few correlations which are more difficult to explain. The negative relationship between HFA and cellulose respiration could be a link to litter quality as cellulose content is often indicative of lower quality litter (Talbot and

Treseder 2012) and the increased respiration of cellulose might be indicative of a community with higher FB, which would mask the HFA index. The positive correlation between HFA and SIR, or community activity (Anderson and Domsch 1978; West and Sparling 1986), is possibly explained by microbial activity being negatively associated with site disturbance (Mummey, Stahl, & Buyer 2002; Banning & Murphy 2008), which could be another indication that a site needs time in order to develop HFA.

The FB index was found to be correlated with two bacteria taxa, Fe, Na, and glycine respiration from the catabolic response profile. These variables include the relative abundances of Acidobacteria, and δ-Proteobacteria, the decomposition of glycine, and the litter Fe and Na content (Fig 2.6). There is evidence to suggest that the phyla of Acidobacteria and Proteobacteria are oligotrophic and copiotrophic, respectively (Fierer, Bradford, and Jackson 2007). Oligotrophs are associated with poorer litter quality (Semenov 1991) and copiotrophs are associated with higher litter quality environments (Fierer, Bradford, and Jackson 2007; Singh et al. 2010). This shift in the relative abundance of these taxa could indicate the microbial community's direct response to litter quality and the subsequent FB. The negative correlation with the presence of Na and Fe in litter components is another example of FB's relationship to litter quality as high Fe and Na content are considered indicative of higher quality litter (Lousier and Parkinson 1978). A difficult relationship to disentangle was the negative relationship between FB and glycine respiration. This relationship could potentially be explained by the fact that glycine turnover is associated with high C-mineralization (McFarland et al. 2010) and therefore litter quality which would mean that communities able to respire glycine are from communities that share a historical legacy with high-quality litter, or in other words, communities with low FB.

The QI index was found to correlate with the relative abundances of Planctomycetes and Leotiomycetes and elemental litter characteristics (%N, C/N ratio, and %P (Fig 2.7)). The abundance of Planctomycetes is often related to the N cycling in systems, which is potentially an indicator of higher quality litter (Schlesner and Stackebrandt 1986; Strous et al. 1999; Isobe and Ohte 2014) and the abundance of Leotiomycetes has been correlated with higher quality litter substrates (Strickland et al. 2009b). A litter with a higher content of N, and therefore a smaller C/N ratio, and a higher content of P is also considered to be higher quality

(Lousier and Parkinson 1978; Melillo, Aber, and Muratore 1982; McClaugherty et al.1986). Given that the QI index is a measure of how microbial communities perceive litter quality, it is not surprising that litter chemistry could serve as an indicator of QI.

Our results reveal a distinction between HFA and FB as separate indices of a microbial community's influence on leaf litter decomposition. In previous studies, there has been a strong emphasis on HFA (Veen et al. 2015) and leaf litter quality (Meentemeyer 1978; Aerts 1997), with relatively few articles mentioning, let alone studying FB. Ignoring FB could lead to confounding HFA and FB with one another instead of considering them as separate indexes (Milcu & Manning 2011 and Palozzi & Lindo 2018). This confounding may also explain why in some studies HFA is observed and in some studies, it is not (John, Orwin, & Dickie 2011; Gießelmann 2011). It is therefore imperative that FB is considered as equally as HFA and litter quality when the role of microbial communities in the C cycle is considered.

It is through the understanding of HFA, FB, and QI collectively that we can learn more about the influence of historical legacy effects (Crowther et al. 2019) on microbial community composition and functioning in response to changes in litter input. The historical conditions of an ecosystem may influence microbial community composition and functioning for years or even decades after litter input has changed (Bond-Lamberty et al. 2016; Hawkes et al. 2017) by events such as deforestation, land-use change, and vegetation species shifting north (Schwartz, Iverson, & Prasad 2001; Iverson & Prasad 2002). These changes may influence communities differently based on their overall level of HFA or FB. For instance, to a community with high (or low) FB, it will matter relatively little if there is a change in leaf litter since the community is so well equipped (or poorly equipped) to decompose anything. However, if a community with high HFA is exposed to a dramatic transition in leaf litter, there could be a serious impact on overall C cycling because that community is specifically adapted to decomposing a particular kind of litter. Over time, microbial community composition and functioning will adjust to novel conditions, but not without a period of time where communities will be placed in unfamiliar and potentially unideal situations, affecting the entire process of carbon cycling in their own ecosystem and potentially worldwide if many ecosystems experience disruption in tandem.

Chapter 3: Effects of macro- and micronutrient availability on soil carbon, and microbial community biomass and function in a coastal grassland

3.1 Introduction

Soil microbial communities perform many functions as part of the belowground ecosystem such as mediating carbon (C) cycling (Kandeler, Stemmer, & Gerzabek 2005), regulating nitrogen (N) fixation (Rosswall 1982; Hodge 2010), and stimulating plant growth (Jeffries et al. 2002; Hayat 2010). Although microbial communities influence the environment, the environment also influences microbial community biomass and overall functioning. For example, sites with increased soil moisture have been found to exhibit greater microbial growth rates (Barros et al. 1995) and soil pH has long been considered a major determinant for microbial community composition and abundance, particularly for bacteria (Rousk et al. 2010). Of particular interest is the influence of nutrient abundance on microbial communities.

Numerous studies have highlighted how macronutrients shape microbial communities. Jonasson et al. (1995) found that when arctic soils were fertilized with plant macronutrients (i.e. N, P, and K), there was an increase in the inorganic microbial N and P concentrations. A 20-year fertilization study on microbial communities in paddy fields found that the addition of macronutrients significantly increased microbial biomass and functional diversity (Chen et al. 2015). A 54-year long experiment on fertilizer application found that different macronutrient volumes had little effect on the functionality of the microbial community, but there was an effect on bacterial community composition (Pan et al. 2014). While there has been a great deal of attention brought to the effects of macronutrients, there have been relatively few studies concerned with how micronutrients – elements required in small amounts to sustain an organism (Gernand et al. 2016) – influence soil communities and their functions.

Micronutrient additions can influence a range of ecosystem properties and processes, including plant biomass (Hepler 2005), decomposition rates (Kaspari et al. 2009, Powers and Salute 2011), soil aggregation (Six et al. 2004), and invertebrate herbivore abundance (Kaspari et al. 2009, Kaspari et al. 2017). In our study we consider Ca, Na, and K to be microbial micronutrients as they are not considered macronutrients (Kirchman 2012), but are

still important to microbial physiology (Wackett et al. 2004) and improving microbial functioning (Kaspari and Powers 2016). Excess soil Na can lead to stressed communities leading to biomass and function reduction (Rietz and Haynes 2003). The addition of Ca, however, forms cation bridges between negatively charged soil organic matter and negatively charged phyllosilicates which results in greater microbial biomass (Cortufo et al. 2013). Also, Ca in the form of $CaCO_3$ is well known to raise pH in a process known as liming (Derome et al., 1986; Lehto 1994). The role of K in microbial biomass could be relatively small (Turner & Wright 2014), but evidence suggests that greater K levels are linked with more stable soil environments (Belay, Claassens, & Wehner, 2002; van Groenigen, et al. 2006). However, there is still much unknown about the influence on micronutrients, in particular how they potentially limit microbial biomass and function.

Belowground community growth and function are limited by nutrient availability (Sinsabaugh, Hill, & Shah 2009; Hartman & Richardson 2013). Although, it is unknown if microbial communities are limited by a single nutrient or by a combination of nutrients. In ecology, the concept known as Liebig's law (Van der Ploeg & Kirkham 1999) maintains that a population of organisms is limited by one resource which has the highest demand to supply ratio. With regards to plant populations, however, Liebig's law has been contested recently in favor of co-limitation which is the concept that more than one resource is responsible for limiting populations simultaneously (Harpole et al. 2011, 2017; Fay et al. 2015). There is growing evidence to suggest the concept of co-limitation is not limited to plants alone (Raubenheimer & Simpson 2004; Sperfeld et al. 2012; Simpson et al. 2015; Kaspari & Powers 2016) and could also apply to terrestrial microbial communities (Mills et al. 2008; Zhang et al. 2015).

Here we test how macronutrients (N and P), micronutrients (Ca, Na, and K) and their interaction shape belowground community biomass and function, as well as the influence of these nutrients on soil C stores. We predict that the addition of Na will decrease microbial biomass and C mineralization in the belowground communities due to increased osmotic stress and dehydration (Galinski 1995; Oren 1999). In contrast, we predict that Ca additions will increase microbial biomass and decrease C mineralization ability because of the long-term soil organic matter stabilization provided by cationic bridges that are formed between

negatively charged soil organic matter and phyllosilicates (Cortufo et al. 2013; Rowley et al. 2018) which act to sequester C in the soil via entrapment. Furthermore, the addition of K will create a more stable soil environment because of increased plant root growth leading to an increase in microbial biomass and bioavailable C (Belay, Claassens, & Wehner, 2002; van Groenigen, et al. 2006). Finally, we predict that similar to herbivore and plant communities (Prather et al. 2018, Harpole et al. 2011, 2017; Fay et al. 2015), microbial communities will be co-limited by macro- and micronutrient concentrations (Sperfeld et al. 2016). Therefore, we predict enhanced functionality and substrate utilization in plots with all nutrients added, as compared to plots with single nutrient additions. Combined, this study provides an important first look at how varying levels of nutrient availability regulates grassland ecosystems belowground.

3.2 Materials and Methods

Site and sample collection

The sampling site location was a coastal tallgrass prairie in Texas at the University of Houston’s Coastal Center (UHCC; 29°23’26.96″ N; 95°1’51.95″ W; Prather et al. 2018). The site is a part of the Lake Charles soil series, classified as a Hapludert with a clay texture. Treatments for the study consisted of fertilizing with macronutrients (N and P combined) and three micronutrients (Ca, K and Na, each manipulated individually). Fertilization began in 2016. Hurricane Harvey occurred on-site in late summer of 2017 creating heavy rainfall which led to flooding of the study site. Separate N and P treatments were not feasible or needed for our study as the main focus was the influence of individual micronutrients rather than macronutrients. As per Prather et al. (2018), the experiment consisted of large fertilized (30 x 30 m^2) plots using a fully-crossed, factorial design: 2 macronutrient levels (ambient vs. fertilized) × Ca levels (ambient vs. fertilized) × K levels (ambient vs. fertilized) × Na levels (ambient vs. fertilized) × 8 replicates of each treatment combination for a total of 16 treatments and 128 plots across eight blocks. Each block contains a single replicate of each treatment. We collected three soil cores at 0-10 cm at each plot with a standard steel soil auger 8 cm in diameter.

Macronutrient fertilizer was in the form of a combination of granular monoammonium phosphate and urea. The micronutrients were added as granular calcium carbonate for Ca, granular potassium chloride for K, and granular soda ash for Na. The fertilizer was applied in late winter in 2016 and 2017 before the growing season. The macronutrients of N and P were added at a concentration of 10 g m^{-2}. The macronutrient concentration was chosen because it is common in fertilization experiments (e.g. Nutrient Network: Borer et al. 2014). Treatments with micronutrient additions were targeted to have concentrations ~ 30% higher than average ambient levels (≈ 1 standard deviation above the average) found in the top 10 cm of the soil. Accordingly, fertilizer was added to create micronutrient concentrations of 46.5 g m^{-2} for Ca, 3.1 g m^{-2} for K, and 6.2 g m^{-2} for Na.

Water holding capacity, pH, carbon mineralization, and plant biomass

We performed water holding capacity (WHC), gravimetric water content (GVM), soil pH, C-mineralization, and substrate-induced respiration (SIR) analyses each year. Soil pH was determined in water (2:1 ratio of DI water: litter) using a benchtop pH meter (Mettler Toledo, Columbus, OH, USA). In order to determine the correct volume of soil needed for the other analyses performed, we first calculated WHC and GVM of our soils. We determined both GVM and WHC (after wetting to field capacity) by drying soil at 105ºC for 24 h following the protocol outlined in Strickland et al. (2009a). Plant biomass was determined by taking five 0.25m^2 quadrats per plot. Plant biomass samples were then dried and weighed.

After we determined WHC and GVM, we performed soil respiration analyses. The first analysis we performed was C-mineralization, a measure of the bio-available C in each soil sample. This analysis was performed via 30 day incubations of each soil sample in 50 mL test tube where units were capped, flushed with CO_2 free air, allowed to incubate for 24 hours, and then the headspace gas was sampled for CO_2 content using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA) on a weekly basis. We weighed 6 g of inoculum source soil for each unit maintained at 20ºC and 65% WHC, which is considered favorable conditions for microbial growth (Strickland 2009a). Total mineralizable C was calculated by integrating CO_2 production values across time.

To test for total active microbial biomass, we measured SIR. This analysis involves a similar process to the one described for C-mineralization except that the incubation is one time only rather than over a 30-day period and an autolyzed yeast substrate is added. Four grams of dry weight equivalent soil was mixed with autolyzed yeast, which is considered to maximize the growth of microbial organisms, and then left for 5 hours to respire. After the 5 hours, CO_2 measurements were taken using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA).

Catabolic response profiles and soil organic matter fractions

For samples collected in 2018, we measured the catabolic response profile (CRP), particulate organic matter (POM) C and mineral-associated organic matter (MAOM) C. The CRP analysis was performed to measure community function, including catabolic evenness (Degens and Harris 1997). The analysis was performed using the same protocol as SIR except that this analysis was performed four times, once for each of the different substrates (DI water, glucose, oxalic acid, and glycine; as per Strickland et al. (2017)). To determine mineral-associated and POM C and N pools, the fractionation method described in Bradford et al. (2008) was used. Briefly, duplicate soil samples (10 g of air-dry soil) from each plot were dispersed with NaHMP (30 mL sample^{-1}) via shaking (18 h) and then passed through a 53 μ sieve. Material <53 μ is considered mineral-associated and material >53 μ is considered POM. Both mineral and POM material were dried (105 °C), ball-milled to a fine powder, and percentage C determined using an ECS 4010 CHNS-O analyzer (Costech Analytical Technologies, Valencia, CA, USA). Of these two fractions, mineral-associated C pools are expected to turn over more slowly than POM C pools (Schlesinger & Lichter, 2001). Mineral-associated C pools are presumed to be primarily microbial-derived C whereas POM pools are primarily plant-derived (Grandy & Robertson, 2007).

Statistical Analysis

We performed linear mixed effect models for the time course data (pH, C-mineralization, and SIR). We then performed an ANOVA for the POM and MAOM data to test for differences across treatments from 2018 samples. Lastly, we performed a permutational MANOVA on the CRP data to test for significant differences across treatments

of 2018 samples divided by whether or not the treatments had NP additions. We performed all linear mixed effect models and ANOVA tests using R software (version 3.1.1, The R Foundation for Statistical Computing, Vienna, Austria) and the permutational MANOVA was performed using Primer 6 (version 6.0, PRIMER-e, Albany, New Zealand).

3.3 Results

pH, aboveground plant biomass, carbon mineralization, and substrate-induced respiration

For soil pH (Figure 3.1; Table B.1), we observed significant main effects of sample year ($P<0.001$) and Ca ($P<0.001$). We also observed significant interactions between Ca and sample year ($P<0.001$), and Ca, NP, and sample year ($P<0.01$). In general, we observed that treatments with added Ca exhibited an increase in soil pH. Additionally, it appears that the addition of NP tended to amplify the differences between treatments with Ca added and those that did not (Figure 3.1A). It also appears that when NP was added but Ca was absent, soil pH tended to decline through time (Figure 3.1A), but in the absence of NP and Ca soil pH tended to remain the same (Figure 3.1B).

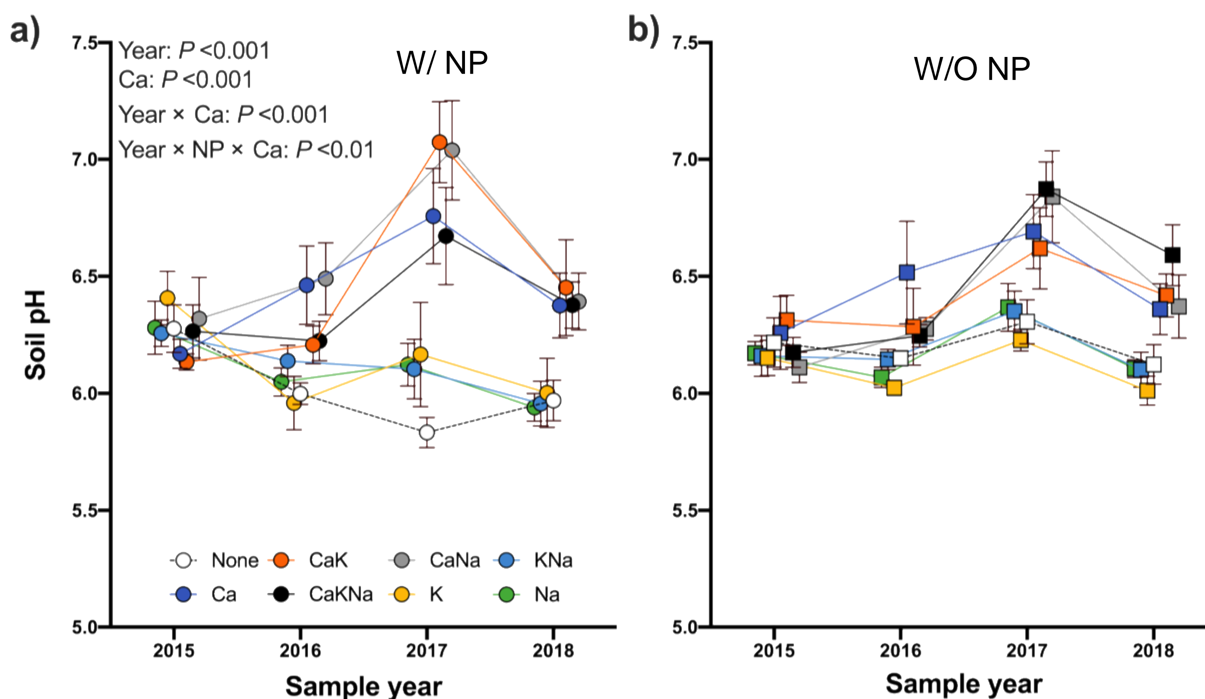

Figure 3.1: Soil pH of samples collected from 2015 to 2018. Each point represents the average pH of each treatment either with NP additions (A) or without NP additions (B). There is a noted difference in pH across sample years: P <0.001. Samples with Ca additions treatments tended to have higher pH: P <0.001. There are also two interactions noted. There is a two-way interaction between year and Ca: P <0.001, as well as a three-way interaction between year, NP, and Ca: P <0.01. Each treatment is labeled by what fertilizer additions it included, either being nothing (None), Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

For the above-ground plant biomass (Figure 3.2; Table B.2), we observed significant main effects of sample year (*P*<0.001) and NP (*P*<0.001). We also observed significant interactions between sample year and NP (*P*<0.001), and sample year, NP, K, and Na (*P*<0.05). Plant biomass was lowest in 2017. Additionally, we observed that treatments containing NP exhibited greater plant biomass compared to treatments that did not receive NP (Figure 3.2A and B). The interaction between sample year, NP, K, and Na appears to be because of an interaction between K and Na in 2017 for the plots receiving NP. Specifically, for the NP treatments in 2017, plant biomass was lowest for those treatments that received either K or Na, but greater for treatments receiving combinations of both K and Na or neither K or Na.

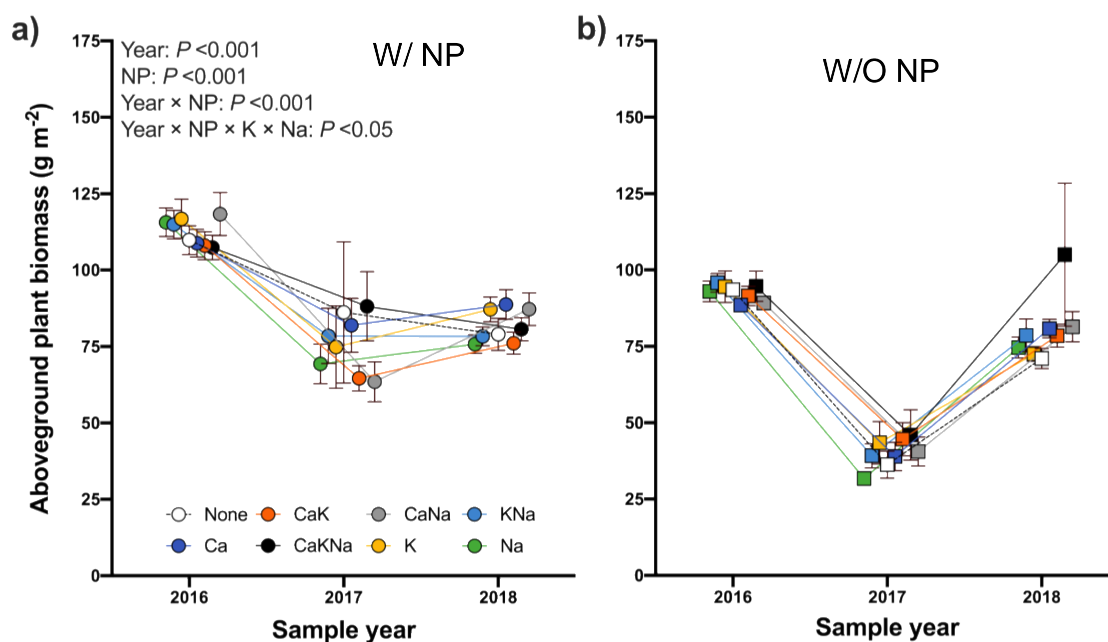

Figure 3.2: Aboveground plant biomass collected from 2016 to 2018. Each point represents the average plant biomass collected from each treatment either with NP additions (A) or without NP additions (B). There is a noted difference in plant biomass across sample years: P <0.001. Samples with NP additions tended to have higher plant biomass: P <0.001. There are also two interactions noted. There is a two-way interaction between year and NP: P <0.001, as well as a four-way interaction between year, NP, K, and Na: P <0.05. Each treatment is labeled by what fertilizer additions it included, either being nothing (None), Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

For mineralizable-C (Figure 3.3; Table B.3), an indicator of bioavailable C, we observed main effects of sample year ($P < 0.001$), NP ($P < 0.001$), and Ca ($P < 0.05$). For the sample year, we observed a general increase in mineralizable-C (Figure 3.3A and B). We also observed that the addition of NP (Figure 3.3A) tended to increase mineralizable C compared to treatments that did not receive NP (Figure 3.3B). The addition of Ca tended to decrease mineralizable C, regardless of the sample year or NP addition (Figure 3.3C).

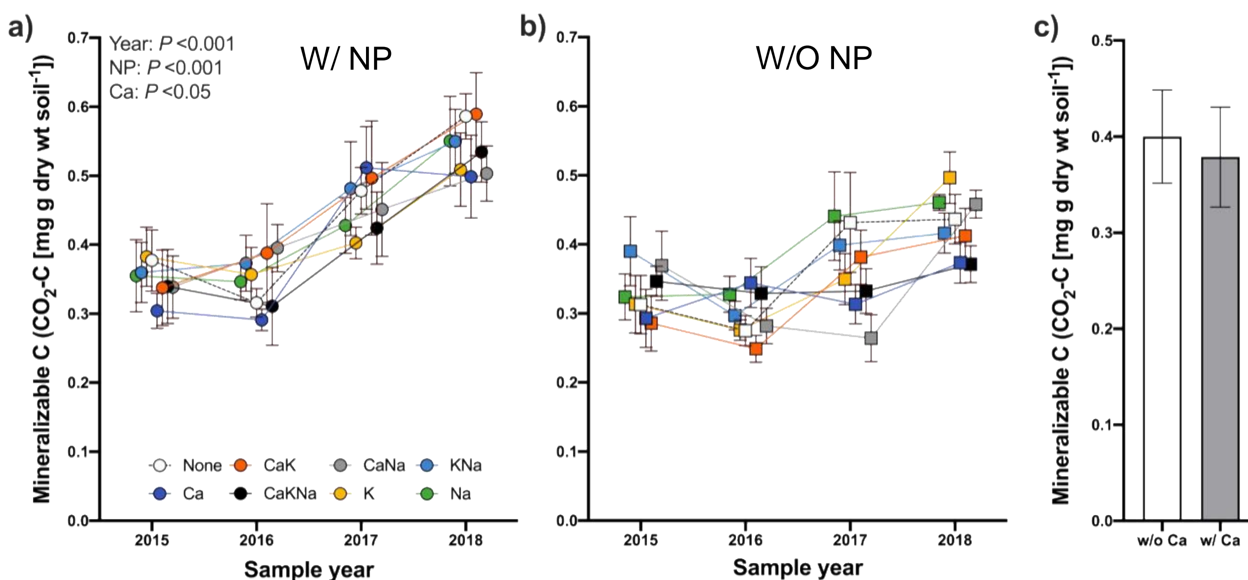

Figure 3.3: Carbon mineralization (mg g dry wt soil^{-1}) of samples from 2015 to 2018. Each point represents the average mineralization of each fertilizer treatment either with NP additions (A) or without NP additions (B). Panel C displays the total average carbon mineralization across the sample years divided by samples with and without Ca additions. Samples collected from later years had higher rates of mineralization: P <0.001. In addition, samples with NP additions had higher mineralization rates: P <0.001. Finally, it was found that samples with Ca additions displayed lower rates of mineralization: P <0.05. Each treatment is labeled by what fertilizer additions it included, either being nothing (None), Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

For SIR (Figure 3.4; Table B.4), an indicator of total active microbial biomass, we observed main effects of sample year ($P < 0.001$) and NP ($P < 0.01$). We also observed a significant interaction between sample year and NP ($P<0.01$). For the sample year, we observed that SIR was lower in the years 2015 and 2018, and generally greater in 2016 and 2017 (Figure 3.4A and B). The interaction between sample year and NP is caused by a less dramatic change in SIR across sample years for treatments that did not receive NP versus those that did receive NP (Figure 3.4B). For treatments receiving NP, we observed a more dramatic increase in SIR for 2016 and 2017 (Figure 3.4A).

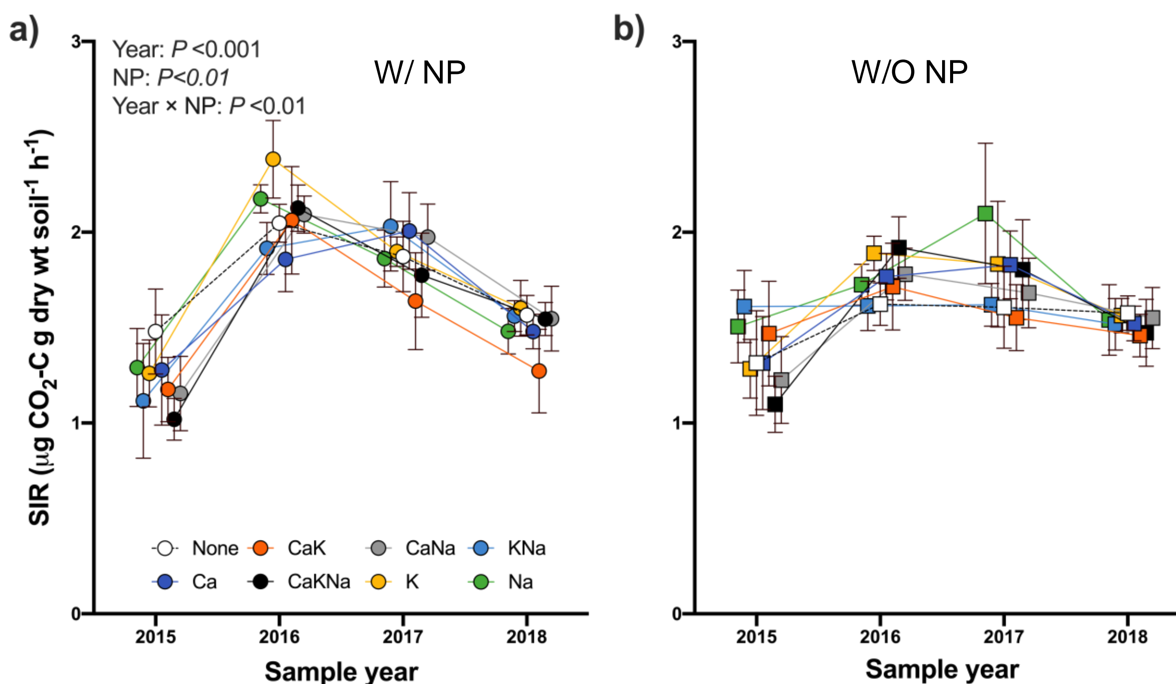

Figure 3.4: Substrate induced respiration (μg CO_2-C g dry wt soil^{-1} h^{-1}) of samples collected from 2015 to 2018. Each point represents the average substrate-induced respiration of each fertilizer treatment either with NP additions (A) or without NP additions (B). There is a significant difference in samples collected from different years: P <0.001 as well as samples collected from plots with or without NP additions: P <0.01. There is also an interaction between year and NP additions: P <0.01. Each treatment is labeled by what fertilizer additions it included, either being nothing (None), Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

Soil organic matter C and catabolic response profiles

For soil organic C fractions (Figure 3.5; Table B.5, B.6, and B.7) we observed main effects of NP ($P < 0.05$) and K ($P < 0.05$) for POM C. These main effects can be attributed to greater POM C with all treatments containing NP and all treatments containing K (Figure 3.5A). We observed no treatment effects for the mineral-associated organic matter (MAOM) C fraction (Figure 3.5B). However, for total soil organic C (Figure 3.5C; likely due to change in POM C) we observed a significant main effect of NP ($P < 0.05$), and a marginally significant effect of K ($P = 0.08$). These effects mirrored those observed for POM C.

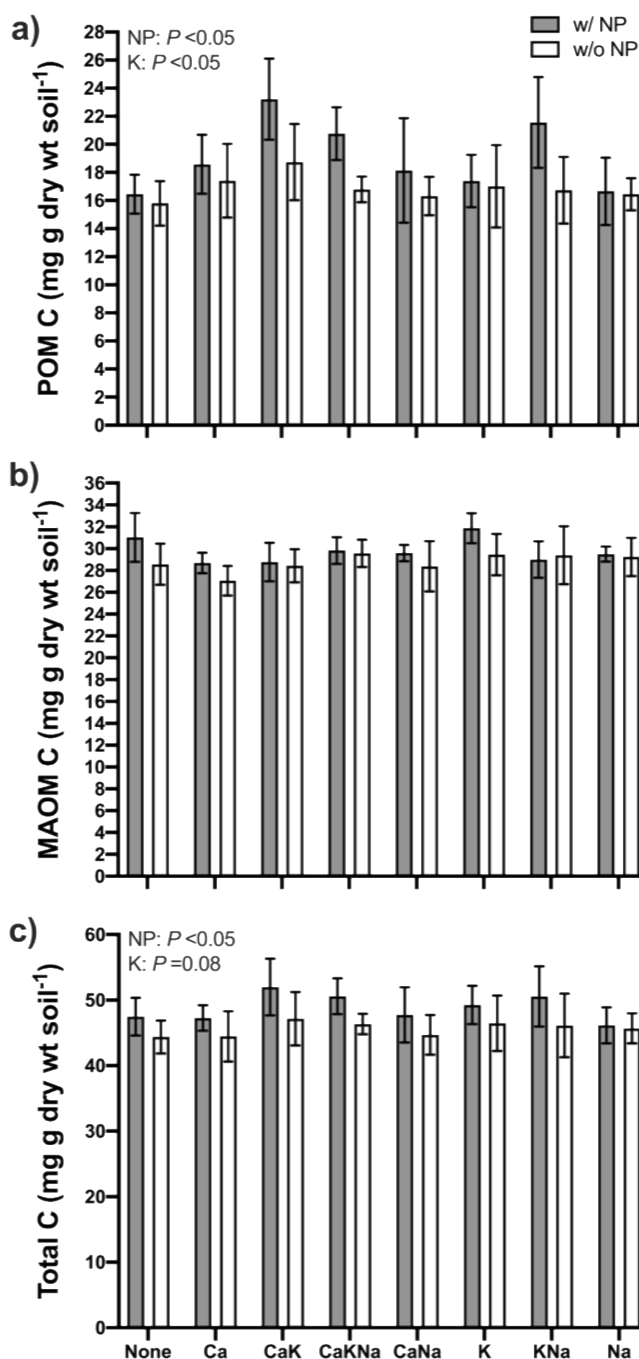

Figure 3.5: Different C fractions of particulate organic matter (POM) of 2018 samples from each treatment divided by whether samples included NP additions or did not. The C fractions include the particulate organic matter fraction (> 53µ A), the mineral-associated organic matter (MAOM) fraction (< 53 µ B), and the total C of those two fractions (C). In the particulate organic matter fraction, the samples with NP treatments showed higher C ($P < 0.05$) and the samples with K treatments showed higher C ($P < 0.05$). There was no difference among treatments in the mineral-associated organic matter fraction. The total C showed that samples with NP additions were still higher ($P < 0.05$), while samples with K treatments were marginally significantly higher still ($P = 0.08$).

For catabolic response profiles (Figure 3.6 and 3.7; Tables B.8 and B.9), an indicator of community function, we observed a main effect of NP additions ($P<0.001$), and a significant NP × Ca × K interaction ($P<0.01$). Examining this interaction further, we observed a significant main effect of Ca ($P<0.05$) and a Ca × K interaction ($P<0.05$) with NP. This interaction appears to be driven by similar profiles when Ca, K, or both (i.e. CaK) are present but distinct profiles when absent (Figure 3.6). Additionally, when Ca and/or K is absent, microbial communities tend to elicit greater mineralization rates of the three CRP substrates (glucose, glycine, and oxalic acid; Figure 3.6). When NP was absent, we again observed a significant Ca × K interaction ($P<0.05$) but this interaction appears largely due to distinct profiles associated with treatments containing only K versus treatments that received Ca or the combination of Ca and K (i.e. None), the Ca and CaK treatments were intermediate between K and None (Figure 3.7). The observed difference between K and None treatments appears to be driven by greater mineralization of all three CRP substrates for soils that received only K (Figure 3.7).

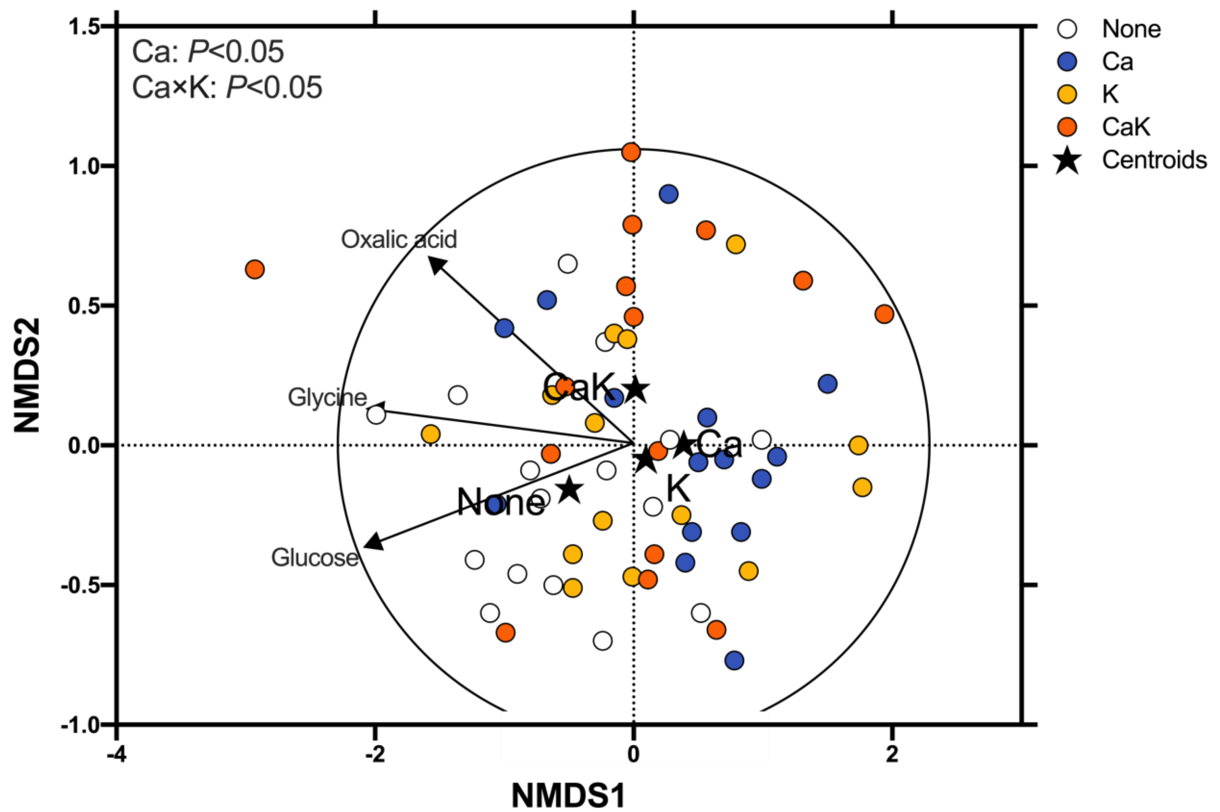

Figure 3.6: Non-metric multidimensional scaling plots of data derived from a permutational MANOVA of the catabolic response profile assay performed on 2018 samples with NP additions. There is a noted Ca effect on samples (P <0.05) as well as an interaction between Ca and K (P <0.05).

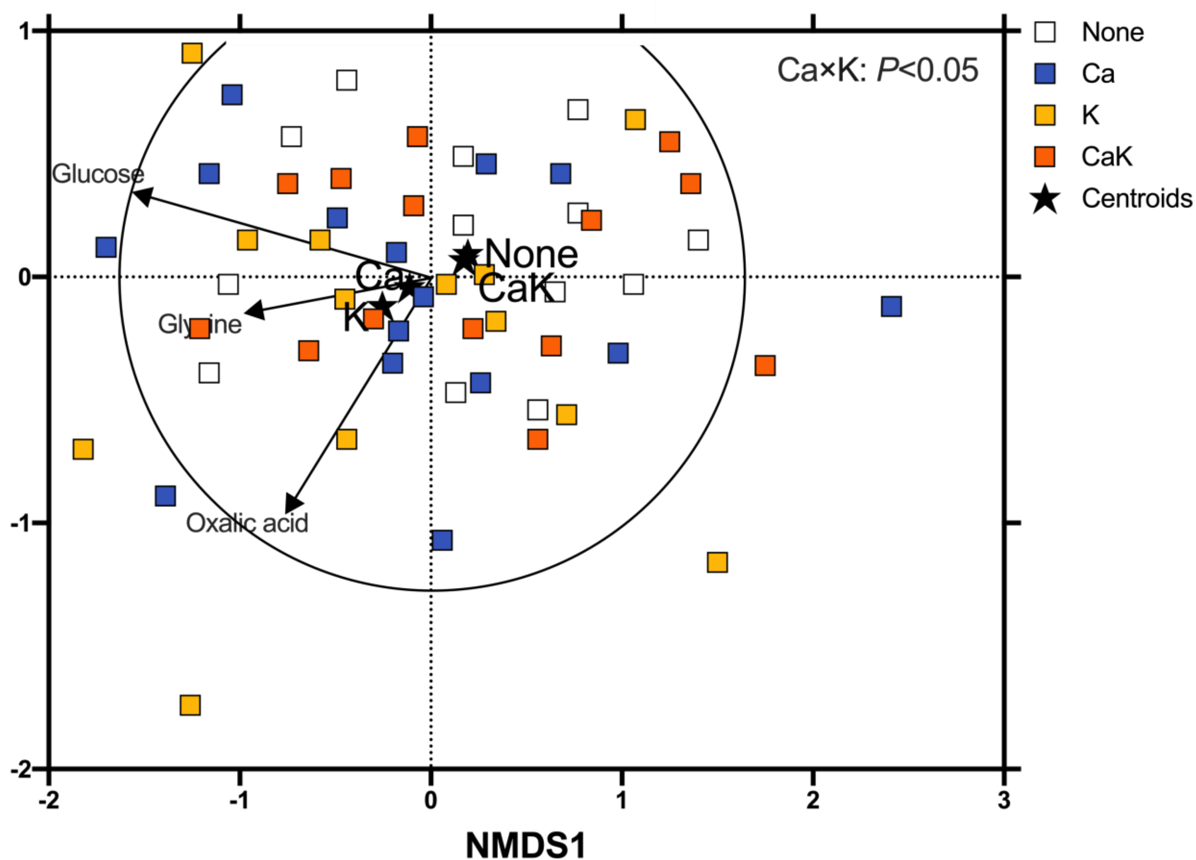

Figure 3.7: Non-metric multidimensional scaling plots of data derived from a permutational MANOVA of the catabolic response profile assay performed on 2018 samples with NP additions. There is a Ca by K interaction (P <0.05).

3.4 Discussion

In order to study the influence of micronutrients on microbial communities, we added micro- and macronutrient fertilizer inputs to field soils and measured differences in microbial function (i.e., carbon mineralization, substrate-induced respiration, catabolic response profiles), edaphic properties (soil pH and total soil C), and plant biomass. We found that microbial communities responded to the addition of multiple types of nutrient additions, rather than just one single type. This response to different nutrients suggests there is a possible co-limitation (the concept that more than one resource is responsible for limiting a characteristic of a population simultaneously (Harpole et al. 2011, 2017; Fay et al. 2015)) on belowground community function.

pH, aboveground plant biomass, carbon mineralization, and substrate-induced respiration

The significantly higher pH in plots with Ca additions was a consequence of the Ca fertilizer being Ca carbonate (i.e. lime). The application of Ca carbonate to raise pH is a well-known technique (Tisdale et al. 1993). The increased pH in plots with both Ca and NP additions is likely due to a chemical reaction between the basic Ca carbonate fertilizer and the acidic phosphate fertilizer (Bull et al. 1964). Inorganic phosphate fertilizers are known to be sorbed by Ca carbonate thereby depressing the solubility of the phosphate and preventing the acidic nature of the phosphate fertilizer to influence the soil. Since the phosphate fertilizer solubility is reduced there is a greater influence on soil pH of the basic urea and Ca carbonate fertilizers (Cole, Olsen, and Scott 1954). This also explains why there was a reduction in pH in plots with NP additions but no Ca additions, since the phosphate fertilizer is no longer being sorbed and will freely be able to acidify the soil. Soil pH is a major determining factor in microbial diversity, with diversity and richness of soil bacteria being highest in neutral pH soils and lowest in acidic soils (Fierer and Jackson 2005). The application of these fertilizers may alter the microbial diversity of these soils, but additional research aimed at assessing bacterial diversity is needed.

Unsurprisingly, additions of N and P increased aboveground plant mass. However, with NP additions there is a more distinct effect of the addition of other nutrients (i.e. Na and K) which is potentially difficult to disentangle. It seems that plant growth limitations are removed by adding NP fertilizer and therefor other nutrient additions had a more marked effect on plant biomass. For instance, we observed in 2017 that the addition of NP led to an interaction between K and Na, whereby individual additions of either micronutrient led to a decrease in plant biomass. When both K and Na were added together, or neither was added an increase in plant biomass was observed. The reduction in plant biomass with the addition of both Na and NP may be accounted for by increased herbivory. In fact, Prather et al. (2018) observed an increased abundance of grasshoppers, and likely increased herbivory, associated with these same treatments in 2017 which could account for the decrease in plant biomass associated with treatments receiving both NP and Na. However, Prather et al. (2018) did not observe a similar increase in herbivory with K but did note a shift in grasshopper community composition. While individually, additions of Na and K may decrease plant biomass via an

herbivory response, combined additions may increase plant biomass via increased photosynthesis (Krishnasamy et al. 2014). While it typically expected that additions of plant limiting nutrients will increase plant biomass, this expectation often fails to consider what additional factors, such as herbivory, might also increase with nutrient additions.

The enhanced bioavailable C created by NP additions was potentially caused by an increase in above and belowground plant biomass leading to more plant detritus and or root exudates entering the soil. Further, there was a reduction in bioavailable C in plots with Ca additions, likely a result of C being entrapped through organic-mineral interaction via cation bridging (Muneer and Oades 1989; Clough and Skjemstad 2000). This reduction in bioavailable C possibly means that more C is being successfully sequestered and there could be an opportunity to enhance C sequestration efforts with Ca additions, although more C might be freely available if there are NP additions present as well as the Ca additions.

Unsurprisingly, the consumption of increased inorganic nutrients led to higher total active microbial biomass in plots with NP additions (Roberge 1976). We expected micronutrient additions to also influence the active microbial biomass rather than there simply being a difference between the presence or absence of macronutrients. These results would indicate that the only limiting nutrient in terms of active microbial biomass is NP, although additional research should be conducted that determines the effect of micronutrient additions on microbial growth efficiency (Geyer et al. 2016). This may be especially important considering that multiple nutrients likely control microbial function (Wackett et al. 2004; Manzoni et al. 2012; see catabolic response profiles below).

Soil organic matter C and catabolic response profiles

Higher levels of POM fraction C in soils with NP additions have been linked to an increase in organic material inputs from enhanced plant biomass (Yan et al. 2007), which was reflected in our own study (Figure 3.2). The addition of K increased in POM C as well, possibly the result of stimulated root growth leading to greater belowground inputs of C (Belay, Claassens, & Wehner, 2002; van Groenigen, et al. 2006). There was no influence of fertilizer on the MAOM fraction because the mean residence time of the MAOM fraction is decades to centuries (Lavallee, Soong, & Cotrufo 2019). Such as with the POM C fraction,

the NP additions enhanced total soil organic matter C, and the K additions had a marginally significant influence. Since there was an increase in total soil C in soils with various nutrient additions, it might be possible to sequester more C through specific fertilization treatments (Yan and Gong 2010).

For microbial community function, as assessed via catabolic response profiles, we observed complex interactions between the additions of NP, Ca, and K. For instance, with NP additions, the further additions of K and/or Ca tended to lead to lower overall mineralization of the CRP substrates. This could potentially indicate that microbes are limited simultaneously by multiple nutrients (i.e. co-limitation) and that once these limitations are alleviated then mining of organic substrates decreases (Fontaine et al. 2004). While additions of Ca and/or K in combination with NP tended to suppress the mineralization of CRP substrates, K tended to stimulate mineralization in the absence of NP. This may suggest increased microbial mining of organic substrates with K additions, especially since K limits microbial cellulase activity (Kaspari et al. 2007). However, future research should further investigate the potential influence of multiple limiting nutrients on soil microbial community function, particularly with an eye towards whether such additions increase or decrease soil C stores.

We found that microbial communities and edaphic characteristics responded to the addition of multiple types of macro- and micronutrient additions, rather than just one single type. We found that the additions of NP combined with Ca and/or K led to a reduction in overall CRP substrate mineralization, suggesting that the need for mining organic substrates had been reduced. This response to different nutrients suggests there is a possible co-limitation of nutrients in microbial biomass and function. Future studies could improve this work by studying N and P separately as well as investigating differences in community composition in the presence of multiple micronutrient additions. The results of this study suggest that combinations of micro- and macronutrients impact microbial community function and biomass differently. As microbial communities are integral parts of global carbon cycling and ecosystem-wide nutrient cycling it is important to understand the magnitude nutrient availability will have on soil microbial communities and the ecosystem processes that these communities regulate.

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Appendix A: Chapter 2

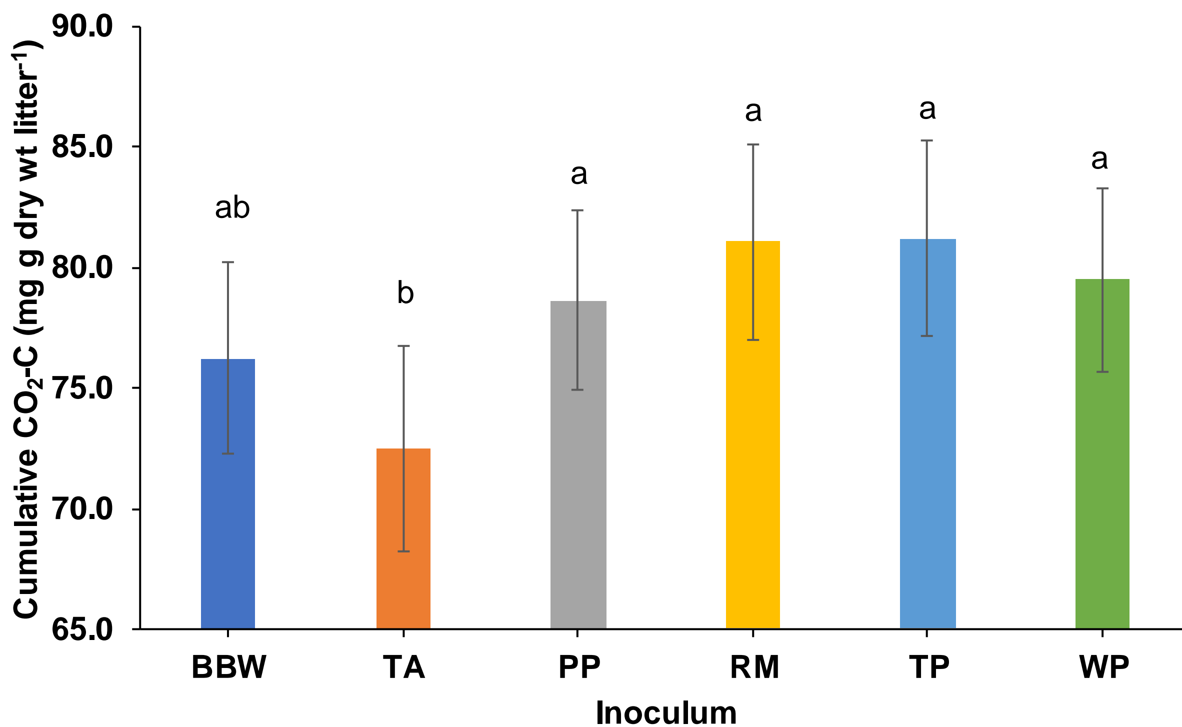

Figure A.1: Average inoculum cumulative CO_2 production (mg g dry wt litter^{-1}) from the full factorial microcosm of chapter 2. Each bar represents the average decomposition total of each inoculum which are named after the litters they are associated with (blue bunch wheatgrass (BBW), trembling aspen (TA), ponderosa pine (PP), rhododendron (RM), tulip poplar (TP), and white pine (WP)). Values are means ± SE.

Appendix B: Chapter 3

Table B.1: Linear mixed effect model results for soil pH in chapter 3. There were significant effects of Year and Ca. There was also a significant two-way interaction of Year: Ca and a significant three-way interaction of Year: NP: Ca. Each term in the left-most column is either a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients, or the influence of sample year.

	numDF	denDF	F-value	p-value
(Intercept)	1	224	142575.5	<.0001
Year	2	224	41.2	<.0001
NP	1	105	2.2	0.15
Ca	1	105	100.7	<.0001
K	1	105	0.1	0.71
Na	1	105	0.8	0.36
Year:NP	2	224	0.4	0.66
Year:Ca	2	224	12.1	<.0001
NP:Ca	1	105	4.8	0.03
Year:K	2	224	1.9	0.15
NP:K	1	105	0.0	0.87
Ca:K	1	105	0.4	0.54
Year:Na	2	224	0.7	0.48
NP:Na	1	105	0.1	0.78
Ca:Na	1	105	0.3	0.60
K:Na	1	105	0.0	0.91
Year:NP:Ca	2	224	3.0	0.05
Year:NP:K	2	224	0.5	0.60
Year:Ca:K	2	224	2.1	0.12
NP:Ca:K	1	105	1.3	0.26
Year:NP:Na	2	224	1.6	0.20
Year:Ca:Na	2	224	0.6	0.56
NP:Ca:Na	1	105	0.3	0.59
Year:K:Na	2	224	3.0	0.05
NP:K:Na	1	105	3.1	0.08
Ca:K:Na	1	105	0.2	0.68
Year:NP:Ca:K	2	224	0.0	0.99
Year:NP:Ca:Na	2	224	0.8	0.48
Year:NP:K:Na	2	224	1.5	0.22
Year:Ca:K:Na	2	224	0.1	0.92
NP:Ca:K:Na	1	105	0.3	0.57
Year:NP:Ca:K:Na	2	224	0.1	0.91

Table B.2: Linear mixed effect model results for above-ground plant biomass in chapter 3. There were significant effects of Year and NP. There was a significant two-way interaction of Year: NP and a significant four-way interaction of Year: NP: K: Na. Each term in the left-most column is either a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients, or the influence of sample year.

	numDF	denDF	F-value	p-value
(Intercept)	1	224	2297.6	<.0001
Year	2	224	184.5	<.0001
NP	1	105	71.0	<.0001
Ca	1	105	0.6	0.43
K	1	105	1.1	0.30
Na	1	105	0.3	0.57
Year:NP	2	224	27.7	<.0001
Year:Ca	2	224	2.9	0.06
NP:Ca	1	105	1.6	0.21
Year:K	2	224	0.2	0.81
NP:K	1	105	1.8	0.18
Ca:K	1	105	0.2	0.69
Year:Na	2	224	0.7	0.51
NP:Na	1	105	0.5	0.47
Ca:Na	1	105	1.7	0.19
K:Na	1	105	2.1	0.15
Year:NP:Ca	2	224	0.5	0.61
Year:NP:K	2	224	0.1	0.88
Year:Ca:K	2	224	0.2	0.83
NP:Ca:K	1	105	1.0	0.31
Year:NP:Na	2	224	1.2	0.30
Year:Ca:Na	2	224	0.3	0.77
NP:Ca:Na	1	105	0.0	0.91
Year:K:Na	2	224	2.1	0.12
NP:K:Na	1	105	0.1	0.82
Ca:K:Na	1	105	1.0	0.32
Year:NP:Ca:K	2	224	1.3	0.28
Year:NP:Ca:Na	2	224	0.0	0.97
Year:NP:K:Na	2	224	3.7	0.03
Year:Ca:K:Na	2	224	0.5	0.61
NP:Ca:K:Na	1	105	0.0	0.89
Year:NP:Ca:K:Na	2	224	0.4	0.64

Table B.3: Linear mixed effect model results for carbon mineralization in chapter 3. There were significant effects of Year, NP, and Ca. Each term in the left-most column is either a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients, or the influence of sample year.

	numDF	denDF	F-value	p-value
(Intercept)	1	224	1640.9	<.0001
Year	2	224	69.3	<.0001
NP	1	105	41.3	<.0001
Ca	1	105	4.8	0.03
K	1	105	0.1	0.78
Na	1	105	0.0	0.88
Year:NP	2	224	1.1	0.32
Year:Ca	2	224	0.7	0.51
NP:Ca	1	105	2.0	0.16
Year:K	2	224	0.1	0.87
NP:K	1	105	0.0	0.93
Ca:K	1	105	0.5	0.50
Year:Na	2	224	1.4	0.24
NP:Na	1	105	0.2	0.68
Ca:Na	1	105	1.2	0.27
K:Na	1	105	0.1	0.78
Year:NP:Ca	2	224	1.6	0.20
Year:NP:K	2	224	0.9	0.41
Year:Ca:K	2	224	1.7	0.19
NP:Ca:K	1	105	0.1	0.72
Year:NP:Na	2	224	0.0	0.98
Year:Ca:Na	2	224	1.7	0.18
NP:Ca:Na	1	105	0.0	0.90
Year:K:Na	2	224	1.4	0.24
NP:K:Na	1	105	0.0	0.99
Ca:K:Na	1	105	0.3	0.57
Year:NP:Ca:K	2	224	2.3	0.10
Year:NP:Ca:Na	2	224	0.4	0.64
Year:NP:K:Na	2	224	3.6	0.03
Year:Ca:K:Na	2	224	0.3	0.71
NP:Ca:K:Na	1	105	3.4	0.07
Year:NP:Ca:K:Na	2	224	1.4	0.26

Table B.4: Linear mixed effect model results for substrate-induced respiration in chapter 3. There were significant effects of Year and NP. There was a significant two-way interaction of Year: NP. Each term in the left-most column is either a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients, or the influence of sample year.

	numDF	denDF	F-value	p-value
(Intercept)	1	224	2342.9	<.0001
Year	2	224	26.3	<.0001
NP	1	105	7.6	0.01
Ca	1	105	0.8	0.36
K	1	105	0.2	0.70
Na	1	105	0.4	0.53
Year:NP	2	224	4.6	0.01
Year:Ca	2	224	0.2	0.82
NP:Ca	1	105	0.5	0.50
Year:K	2	224	1.1	0.35
NP:K	1	105	0.0	0.87
Ca:K	1	105	0.6	0.44
Year:Na	2	224	0.2	0.79
NP:Na	1	105	0.0	1.00
Ca:Na	1	105	1.3	0.27
K:Na	1	105	0.3	0.57
Year:NP:Ca	2	224	0.3	0.71
Year:NP:K	2	224	0.0	1.00
Year:Ca:K	2	224	0.4	0.70
NP:Ca:K	1	105	0.5	0.50
Year:NP:Na	2	224	0.1	0.89
Year:Ca:Na	2	224	0.9	0.40
NP:Ca:Na	1	105	0.3	0.56
Year:K:Na	2	224	0.9	0.40
NP:K:Na	1	105	0.1	0.81
Ca:K:Na	1	105	3.2	0.08
Year:NP:Ca:K	2	224	0.7	0.50
Year:NP:Ca:Na	2	224	0.0	0.98
Year:NP:K:Na	2	224	0.9	0.41
Year:Ca:K:Na	2	224	0.6	0.53
NP:Ca:K:Na	1	105	0.7	0.41
Year:NP:Ca:K:Na	2	224	1.0	0.38

Table B.5: Linear mixed effect model results for particulate organic matter carbon in chapter 3. There were significant effects of NP and K. Each term in the left-most column is a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

	numDF	denDF	F-value	p-value
(Interecept)	1	105	198.1	<.0001
NP	1	105	4.7	0.03
Ca	1	105	2.2	0.14
K	1	105	4.1	0.04
Na	1	105	0.0	0.88
NP:Ca	1	105	0.5	0.50
NP:K	1	105	1.5	0.23
CA:k	1	105	0.0	0.83
NP:Na	1	105	0.3	0.61
CA:Na	1	105	1.8	0.19
K:Na	1	105	0.0	0.98
NP:Ca:K	1	105	0.0	0.89
NP:Ca:N	1	105	0.2	0.63
NP:K:Na	1	105	0.2	0.64
Ca:K:Na	1	105	0.5	0.46
NP:Ca:K:Na	1	105	0.6	0.45

Table B.6: Linear mixed effect model results for mineral associated organic matter carbon in chapter 3. There were no significant effects of treatments. Each term in the left-most column is a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

	numDF	denDF	F-value	p-value
(Interecept)	1	105	1513.2	<.0001
NP	1	105	1.7	0.19
Ca	1	105	1.6	0.21
K	1	105	0.5	0.49
Na	1	105	0.0	0.93
NP:Ca	1	105	0.0	0.83
NP:K	1	105	0.2	0.64
CA:k	1	105	0.1	0.81
NP:Na	1	105	0.8	0.37
CA:Na	1	105	1.8	0.19
K:Na	1	105	0.1	0.73
NP:Ca:K	1	105	0.1	0.81
NP:Ca:N	1	105	0.5	0.46
NP:K:Na	1	105	0.0	0.96
Ca:K:Na	1	105	0.1	0.74
NP:Ca:K:Na	1	105	0.0	0.87

Table B.7: Linear mixed effect model results for total soil organic matter carbon in chapter 3. There were significant effects of NP and almost K. Each term in the left-most column is a treatment, either being Ca (calcium), K (potassium), Na (sodium), or any combination of those three nutrients.

	numDF	denDF	F-value	p-value
(Interecept)	1	105	619.3	<.0001
NP	1	105	4.7	0.03
Ca	1	105	0.1	0.73
K	1	105	3.0	0.08
Na	1	105	0.0	0.96
NP:Ca	1	105	0.1	0.73
NP:K	1	105	0.3	0.56
CA:k	1	105	0.1	0.79
NP:Na	1	105	0.0	0.91
CA:Na	1	105	0.0	0.84
K:Na	1	105	0.0	0.87
NP:Ca:K	1	105	0.0	0.97
NP:Ca:N	1	105	0.0	0.96
NP:K:Na	1	105	0.1	0.77
Ca:K:Na	1	105	0.1	0.74
NP:Ca:K:Na	1	105	0.2	0.66

Table B.8: Pairwise comparisons of catabolic response profile data of plots with NP additions as evaluated in primer for a nonmetric multidimensional scaling plot considering the groups as plots that contained Ca, K, both, or neither.

Groups	t	P(perm)	Unique perms
None, CaK	1.66	0.07	693
None, Ca	2.84	0.00	629
None, K	1.77	0.06	612
CaK, Ca	1.08	0.30	658
CaK, K	0.63	0.70	691
Ca, K	0.95	0.38	589

Table B.9: Pairwise comparisons of catabolic response profile data of plots without NP additions as evaluated in primer for a nonmetric multidimensional scaling plot considering the groups as plots that contained Ca, K, both, or neither.

Groups	t	P(perm)	Unique perms
None, CaK	0.58	0.79	9906
None, Ca	1.45	0.12	9923
None, K	1.66	0.06	9912
CaK, Ca	1.04	0.34	9920
CaK, K	1.33	0.17	9925
Ca, K	0.55	0.79	9928