2012

SOIL MICROBIAL COMMUNITY RESPONSE TO CLIMATE CHANGE: RESULTS FROM A TEMPERATE KENTUCKY PASTURE

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

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ABSTRACT OF THESIS

SOIL MICROBIAL COMMUNITY RESPONSE TO CLIMATE CHANGE: RESULTS FROM A TEMPERATE KENTUCKY PASTURE

Climate change is likely to alter plant species composition and interactions between plants and soil microbes that together dictate the quantity and quality of forage produced in pastures, the base of animal production in central Kentucky. This study assessed the seasonal dynamics of soil microbes and their response to increased temperature (+3^{o}C) and growing season precipitation (+30% of the mean annual). Total soil microbial biomass, community composition, enzyme activities, potential carbon mineralization, and catabolic responses to selected substrates were measured seasonally in the different climate treatments. In this system, seasonal variability was a dominant driving factor for all the soil microbial characteristics that I investigated. Summer maxima and winter minima were identified in the active microbial biomass, while soil microbial community structure differed between each season. Extracellular enzyme activities were generally highest in either the spring or summer, while seasonal patterns for each substrate were unique across catabolic response profiles. Climate treatments produced few significant main or interactive effects on the soil microbial biomass and function. This resiliency, coupled with evidence of functional redundancy, suggests that central Kentucky pasture ecosystems may be well-equipped to handle future environmental stress associated with climate change and to maintain critical ecosystem services.

KEYWORDS: Climate change, Pasture, Soil microbial communities, Phospholipid fatty acid analysis, Extracellular enzyme assays

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July 25, 2012

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To my sisters, Jennifer and Leeann.

ACKNOWLEDGEMENTS

The intellectual and personal development fostered during the production of this thesis and which made the following work possible resulted from the influence and contributions of many people. I would first like to thank my advisor, Dr. Rebecca McCulley, both for her ability to lead by example and inspire others to perform to their best advantage, and for her tireless encouragement, insight, and patience throughout this project. I am extremely grateful for the laboratory and field assistance provided by Jim Nelson and Elizabeth Carlisle, which was invaluable to the completion of this thesis, and also for the statistical support provided by Kristen McQuerry. This work was also made possible by the collaborative effort and warm support of Dr. Michael Weintraub, and the assistance of his laboratory staff at the University of Toledo, Ohio.

In addition, I would like to thank Dr. Paula Gale, who cultivated my passion for soil science and encouraged me to continue my pursuit of knowledge in graduate school.

Most importantly, I would like to thank my sisters, to whom I dedicate this thesis: My eldest sister Jennifer, who blazed the trail to higher education in our family and continues to embody the independent and intellectual spirit to which I aspire; and my twin sister Leeann, whose deep friendship and understanding have been a constant source of support throughout my lifetime.

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Chapter 1: Introduction

The soil microbial community is an important biological component of soil function, valued for its role in improving soil quality and regulating nutrient availability, and thereby influencing plant production for agriculture and other purposes (Kennedy and Smith, 1995; Papendick and Parr, 1992). For example, the soil microbial community composition affects the belowground dynamics and fate of photosynthetically-fixed carbon, which can influence fertility (Bradford et al., 2007). Soil microbial communities can also affect the interaction between plants and important aboveground macrofauna. For example, the presence of nematodes and microoganismal inoculants has been shown to reduce aphid populations, significant herbivores and vectors of disease, in a mixture of mid-succession grassland species (Bezemer et al., 2005). Singh et al. (2011) reviewed the role of soil microorganisms in the development of sustainable agriculture, and showed that plant growth promoting rhizobacteria and cyanobacteria often result in increased crop production and ecosystem health. Because of the importance of soil microbes for agricultural and plant production systems, understanding the effects of climate-related stressors, such as increased temperatures and altered precipitation, on the soil microbial community is necessary in order to better understand likely agricultural ecosystem responses to predicted climate change.

The soil microbial community is expected to be impacted by various facets of global climate change, such as increased atmospheric [CO_2], altered temperature and precipitation patterns, and increased frequency of extreme climate events (IPPC, 2007). Some notable examples of extreme, widespread climate events that negatively impacted agriculture in the U.S. southeast region, and presumably had some effect on the soil

microbial communities, included the 1986 summer heat wave and drought and the 1998 winter and spring flooding due to El Niño (Rosenzweig et al., 2000). According to a report by the U.S. Global Change Research Program, the average annual temperature in the southeast has risen by approximately 1.1^{o}C since 1970, with the greatest increase occurring in the winter months, while average annual rainfall has declined by 7.7%, despite a 3.3% increase in rainfall during the summer months (GCCI, 2009). The report predicts an increase of 2.5^{o}C in average annual temperatures in the southeast U.S. by the 2080s under a low CO_2 emissions scenario, and an increase of 5^{o}C under a high emissions scenario. These higher temperatures, combined with variable alterations in total average rainfall and difficult to predict changes in the seasonality and nature of precipitation events, may lead to increased frequency, intensity, and duration of droughts in the region (GCCI, 2009). Soil microbial community structure and function are known to be sensitive to changes in both temperature and water availability (Hartel, 2005), and are, therefore, predicted to be responsive to such alterations to the climate system.

As part of the microbiome, soil microorganisms could potentially play an important role in contributing to the development of ecosystem resistance to abiotic stresses, such as increased temperature and precipitation, and increasing resiliency in agricultural systems (Pankhurst et al., 1996). The soil microbial community also plays a significant role in the global exchange of C between the biosphere and atmosphere via organic matter decomposition and utilization (Schimel and Holland, 1995). Because the factors exerting the greatest control on global C fluxes (e.g., photosynthesis and respiration) are also those which strongly affect heterotrophic microbial activity (e.g., temperature, moisture, and nutrient availability), soil microorganisms and their responses

to changing biotic and abiotic factors will ultimately control whether ecosystems help mitigate or further exacerbate increases in atmospheric [CO_2], which can have direct feedbacks to future climate change (Schimel and Holland, 1995). Insights from research in microbial ecology should therefore be incorporated into ecosystem models to better predict how changes such as warming and altered precipitation regimes may affect global carbon cycling (Treseder et al., 2011). Multiple reviews and studies highlight the need for multifactor, long-term experimental approaches for assessing soil microbial response to climate change (Bardgett et al., 2008; Balser, 2010; Butenschoen et al., 2011; Docherty and Gutknecht, 2012).

1.1 Importance and Dynamics of the Soil Microbial Community

The soil ecosystem is a complex network composed of the interactions of thousands of organisms, of which the soil microbial community is a particularly important component (Brady and Weil, 2002). Soil microbial biomass and abundance is regulated by the quality and quantity of available substrate in an environment. Additional factors that can also affect soil microbial biomass include: physical factors, such as temperature and moisture; biotic factors, such as trophic interactions; and chemical factors, such as pH (Brady and Weil, 2002). It is important to understand how these factors affect soil microbes to manage soils in a manner that fosters an abundant, diverse community, which is essential to support plant growth, recycle nutrients, and other ecosystem services.

1.1.1 Regulation of Nutrient Use and Availability

Soil microorganisms are responsible for decomposing organic matter and regulating nutrient availability and turnover in the soil (Swift et al., 1979). As such, the

soil microbial community is widely recognized as an integral component of nutrient cycling through the atmosphere-plant-soil continuum, regulating how essential nutrients such as carbon, nitrogen, and phosphorus become available to plants and other soil biota. With regard to the cycling of carbon, microbes control the decomposition of soil organic matter and soil carbon pool dynamics, in part through their ability to produce and exude extracellular enzymes which are capable of breaking down complex compounds.

1.1.1.1 Soil Organic Matter & Decomposition In light of concerns about increasing atmospheric [CO_2], researchers are increasingly interested in the global carbon (C) cycle, in which soil plays a critical role (Wolf and Wagner, 2005). As seen in Figure 1.1, it is estimated that organic C stored in soil organic matter (SOM) accounts for 1550 Pg C in the total global C stock (Lal, 2008), which is over twice the amount of C in the atmosphere (Wolf and Wagner, 2005). SOM can be anything from decayed plant and faunal debris to microbial exudates, and consists of elements that are vital for life such as nitrogen (N), phosphorus (P) and sulfur (S), but the largest single component is organic C (Dungait et al., 2012). One of the most important functions of the soil microbial community is the breakdown and turnover of soil organic matter, converting the C contained in this material back to CO_2, which enters the atmospheric CO_2 pool. Carbon entering the atmosphere is cycled back into terrestrial ecosystems via photosynthesis by plants and other autotrophic organisms (Figure 1.1). Given the size of the terrestrial SOM-C pool, small increases or decreases in microbial conversion of SOM-C to CO_2 can have a dramatic impact on atmospheric [CO_2].

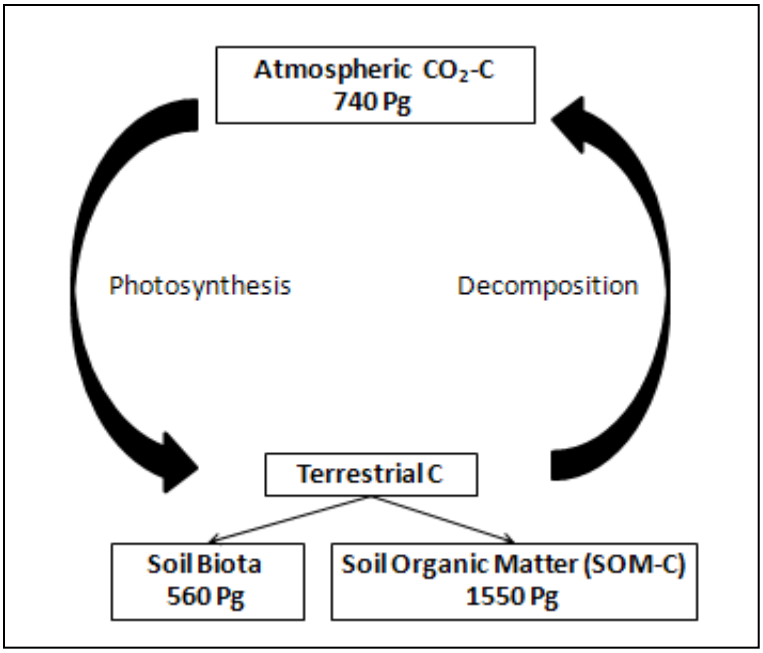


Figure 1.1. Conceptual diagram of global carbon (C) flux between the atmosphere and terrestrial ecosystems. Modified from Wolf and Wagner (2005; adapted from Post et al., 1990). Carbon flux from terrestrial to atmospheric C pools by decomposition includes the breakdown of plant residue, litter, and soil organic matter by soil organisms, which is balanced by the net assimilation of carbon via photosynthesis in ecosystems that are in steady state. Values for soil carbon pools are from Wolf and Wagner (2005) and Lal (2008). All units are in Pg C (10^{15} g).

Soil organic matter provides many beneficial biological, chemical and physical functions in the soil, including supplying slowly-available C-compounds and energy to microbes and other organisms, increasing cation exchange capacity (CEC) and pH buffering, decreasing bulk density, and increasing water-holding capacity (Wolf and Wagner, 2005). During SOM decomposition, some of the C is incorporated into and cycled through the soil microbial biomass (Rinnan and Baath, 2009). Organic matter is also related to stable soil aggregates in that the supply and decomposition of SOM by microorganisms contributes to the formation of soil aggregates, while aggregate stability also helps stabilize SOM (Watts et al., 2001; 2005).

There is a variety of C-substrates found in soil and utilized by the soil microbial community, primarily coming from plant litter degradation, SOM decomposition, microbial biomass turnover, and/or plant root exudation. Conceptually, these compounds are often divided into 'labile' and 'recalcitrant' substrate pools, depending on the ease with which they are broken down by soil microorganisms and exoenzymes. Simple sugars, fats, and proteins are easily decomposed by bacteria in soil, and are considered labile, while more complex carbohydrates, like cellulose, lignin, waxes, and oils, are degraded slowly and regarded as recalcitrant (Alexander, 2005). To put this into perspective, it has been found that in surface soil horizons the half life of simple sugars and amino acids may be less than one hour (Boddy et al., 2007; Hill et al., 2008). However, it takes one month to decompose 60% of cellulose added to soil, with another three months necessary to break down an additional 7% (Derrien et al., 2007), with the remaining 33% incorporated into long-term soil carbon pools (Gleixner et al., 2002;

Quenea et al., 2005). For a highly recalcitrant compound, such as lignin, it may take five years to decompose the majority (Thevenot et al., 2010).

It is often assumed in environmental microbiology that: (1) the soil microbial community can always be depended on to degrade a wide range of organic molecules; (2) nearly every soil has the capacity to decompose any substrate (Dungait et al., 2012). However, many studies have suggested that microorganisms may be preferentially adapted to break down specific C substrates found in their soil environment to maximize efficiency (Grayston et al., 2004; Hamer and Marschner, 2005; Orwin et al., 2006). If the diversity of the C substrates entering the soil is increased, possibly due to plant succession or disturbances, the microbial community could exhibit a similar increase in functional diversity by adapting to degrade those substrates, which could potentially affect the rate of C flux from the soil (Grayston et al., 1998). Similarly, if the microbial community does not adapt, then there will be different implications for the fate of this new C.

Because liberation of CO_2 from the soil is often the terminal product of microbial utilization of C, the quantitative rate at which CO_2—C is emitted from soil, or respiration, is commonly used as a proxy for microbial activity (Wolf and Wagner, 2005). While microbial activity via soil respiration is important as a feedback to the atmospheric [CO_2] pool and is thus commonly measured, one specific mechanism which greatly affects the efficiency by which microorganisms decompose SOM is extracellular enzyme production.

1.1.1.2 Extracellular Enzymes One of the ways that microbes control the rate at which they obtain energy from organic and inorganic compounds in the environment is through the production and use of enzymes to catalyze metabolic reactions (Fuhrmann, 2005). Enzymes are utilized throughout all spheres of soil biota (flora, fauna, micro- and macroorganisms) to degrade complex substances such as cellulose and chitin into simple nutrients that may be ingested directly by microorganisms via diffusion (Burns, 1982). One group of enzymes, called extracellular enzymes, are released outside the microbial cell and are generally used to catalyze the reactions that decompose polymeric compounds that are too large to pass through the cellular membrane. The term 'extracellular enzyme' is often used interchangeably with 'exoenzyme', though exoenzymes specifically catalyze reactions that remove terminal monomers from polymeric compounds and are usually released extracellularly (Fuhrman, 2005).

Specific extracellular enzymes are named for the reactions which they catalyze. Two major classes that are important for microbial metabolism include oxidoreductases, which aid respiration and fermentation pathways by catalyzing oxidation-reduction reactions, and hydrolases, which facilitate cleavage of chemical bonds via hydrolysis. Example subclasses of these enzymes include oxidases and peroxidases, which are oxidoreductases, and peptidases and phosphatases, which are hydrolases (Fuhrman, 2005).

Because of the complex chemical and biological strategies involved in microbial metabolism, soil enzymes are as diverse and prevalent as the soil microorganisms that employ them for nutrient-cycling processes. As the "proximate agents of organic matter decomposition," understanding the activity of certain enzymes provides valuable insight

into the metabolic function and decomposition of soil organic matter (Sinsabaugh et al., 2008). Assaying the activity of extracellular enzymes allows researchers to estimate microbial demand for the compounds they degrade, such as carbon, nitrogen, and phosphorus (Sinsabaugh and Moorhead, 1994). Enzyme activity and production may also be responsive to altering the concentrations of relevant compounds or substrates. Because extracellular enzyme activity is sensitive to changes in temperature and moisture, though the degree of sensitivity depends on individual enzymes and interactions with the substrates they degrade, their activity may be influenced by climate change (Conant et al., 2011; Henry 2012).

1.1.2 Soil Microbial Communities within Pasture Ecosystems

The diversity and composition of the aboveground plant community influences the soil microbial community primarily through the supply, timing, and composition of residues and exudates (Scherber et al., 2010). Thus, it is intuitive that grass-dominated systems, such as pastures, would support a unique soil microbial community compared to other biomes dominated by different plant species, such as forests or croplands, and microbial communities have indeed been shown to vary distinctly and predictably across biomes (Fierer et al., 2009). For example, grasslands are generally considered to contain a less diverse microbial community but higher biomass than is typically found in forests (Brady and Weil, 2002). Temperate grasslands can also exhibit higher soil C than forests, due to greater C inputs belowground derived from the high root:shoot ratios typical of grasses and potentially limited decomposition (De Deyn et al., 2008). Grassland ecosystems dominated by fungal-based soil food webs have shown elevated resilience and adaptability to drought in terms of microbial evenness and reduced C and

N loss, compared to bacterially-dominated wheat soils (De Vries et al., 2012). Much of the literature investigating soil microbial community dynamics has focused on cropped and forested ecosystems, despite the fact that pastures comprise a large area within the U.S., are often important providers of ecosystem services, and support animal production (Sanderson et al., 2009). This thesis will focus on literature concerning microbial dynamics in pasture and other grass-dominated ecosystems not utilized for agricultural row-cropping.

Many grass-dominated ecosystems experience dramatic inter- and intra-annual variability in precipitation and temperature (Craine et al., 2012). Such fluctuations in climate, from year-to-year or season-to-season, could have significant effects on soil microbial communities, directly via effects on soil moisture and temperature, or indirectly via effects on plant growth. For example, AMF (arbuscular mycorrhizal fungi) composition has been shown to vary seasonally, with distinct differences between winter and summer AMF communities in a temperate UK grassland (Dumbrell et al., 2011). Microbial biomass in temperate grasslands is known to vary significantly across seasons, with the greatest differences often exhibited between summer and fall (Bardgett et al., 1999b). Microbial fatty acid abundance has also been shown to be affected by both season and soil moisture (Bardgett et al., 1999b). Many grassland studies suggest that maximum microbial biomass and activity occurs in the spring, early in the growing season (Patra et al., 1990; Ross et al., 1995; Sarathchandra et al., 1988). Grassland systems also exhibit a high degree of year-to-year variation in plant production (Parton et al., 1995), often correlated to climate variability, and such variation may result in equally variable soil microbial communities.

Given that climate change will occur in conjunction with seasonal and inter-annual variability in temperature and precipitation, and all are likely to influence plant growth and soil microbial communities, evaluating the potential effects of climate change on grassland ecosystem response will be challenging. Year-to-year variation can alter the way manipulative climate treatments affect the soil microbial community (Saiya-Cork et al., 2002). In addition, numerous studies have demonstrated that seasonal variation (e.g., changes in weather) heavily influences soil microbial community structure and function (Ebersberger et al., 2003; Jin and Evans, 2007; Luo et al., 1996; Wolf et al., 2007). Seasonal variation in the soil microbial community may interact with climate change factors, such that warming effects on microbial activity in the spring differ from those observed in the fall. The use of long-term, multifactor studies can help determine whether the effects of warming and altered precipitation, predicted to occur as a result of climate change, on soil microbial community structure and function are seasonally dependent and/or vary across years.

1.2 Climate Change Effects on the Soil Microbial Community

Climate change factors such as increased atmospheric [CO_2] and altered temperature and precipitation regimes are expected to affect the soil microbial community in various ways. The alteration of surface soil temperature and moisture regimes is likely to have direct effects on soil microbes (Balser et al., 2010), as studies show community structure and function are responsive to changes in environmental extremes (Waldrop and Firestone, 2006). However, if plant activity, allocation, exudates, or community composition are altered by the climate change factors, then the microbes may be indirectly effected via changing substrate availability and potentially micro-

climatic effects (Figure 1.2; Singh et al., 2010). The current state of knowledge on the direct and indirect effects of three key climate change factors (atmospheric [CO_2], temperature, and precipitation) and their interactions with the plant community and each other will be explored.

1.2.1 Atmospheric [CO_2]

The direct effect of elevated [CO_2] in stimulating aboveground biomass production has been extensively studied (Pan et al., 1998). This increase in aboveground net primary production (ANPP) has been shown to increase C supply belowground and stimulate soil biological activity (Pendall et al., 2004). The effects of increased atmospheric [CO_2] on bacterial biomass, richness, and community composition have been shown to vary between ecosystems, resulting in no common trends, except a 3.5-fold decrease in the relative abundance of Acidobacteria Group 1 bacteria (Dunbar et al., 2012). Results from free-air [CO_2] enrichment (FACE) studies in pasture ecosystems in Europe found that elevated [CO_2] induced changes in soil microbial community activity, biomass and composition in both rhizosphere and bulk soil, but those changes were largely dependent on the plant species that was sampled (Marilley et al., 1999; Montealegre et al., 2002). A meta-analysis by van Groenigen et al. (2011) found that the most potent effects of elevated atmospheric [CO_2] on the soil system were increased soil gas emissions, such as N_2O, and that these effects became larger over time. However, other research has suggested that the effects of elevated atmospheric [CO_2] on the soil microbial population will diminish with time, as plant-soil feedbacks may have a balancing effect on carbon dynamics, though the effects of changing temperature and precipitation intensify with time, as permanent changes in temperature and water

availability may cause permanent changes in soil microbial population size and composition (Niklaus et al., 2003; Blankinship, 2011).

In recent years, research exploring how grassland ecosystems will respond to climate change has escalated in response to the potential for grassland C-sequestration and efflux dynamics to mitigate increasing atmospheric [CO_2] (Bahn et al., 2008; Gill et al., 2002). However, because the main driver of C sequestration in the soil is the balance between plant productivity (C-inputs) and microbial activity (C-outputs), it has been suggested that long-term soil C-input and sequestration can only be maintained with fertilization to sustain the plant community (De Graaff et al., 2006). Much uncertainty still surrounds the effect of elevated [CO_2] on long-term carbon dynamics, especially in conjunction with changes in temperature and precipitation.

1.2.2 Temperature

Because temperature is generally known to exhibit a strong influence on microbial activity, understanding the effects of temperature change, such as is likely to accompany climate change (i.e., warming), is important for assessing impacts on the soil microbial community and predicting its response. Increased temperature is generally known to increase decomposition of organic matter (Wallenstein et al., 2012). Increased temperature often affects 'recalcitrant' SOM more than 'labile' SOM (Bauer et al., 2008), because warming increases the likelihood of passing the critical activation energy (Ea) needed for decomposing resistant compounds (Dungait et al., 2012). This may allow soil microorganisms to access older soil C stocks under climate warming and release more C into the atmosphere. However, temperature sensitivity of soil respiration has also been shown to acclimate to warming conditions over time (Luo et al., 2001), which could

mitigate the initial release of C into the atmosphere that is typically observed with soil warming.

One possible mechanism by which microbes may acclimate to warming is by altering their physiology, specifically their carbon use efficiency (CUE) or the amount of carbon utilized by the soil microbial community that is allocated to growth (Allison et al., 2010). If microbial CUE is reduced with warming because microbial activity (respiration) is increased and prevents C-allocation to microbial growth (biomass), then respiration losses may initially be higher, but may decline over time as decomposer microorganismal biomass is reduced, which may reduce future C-losses from respiration over time (Allison et al., 2010). However, warming effects on CUE and the resulting amounts of CO_2 released from decomposition vary and may even differ based on quality and complexity of substrates, suggesting that research on temperature and CUE responses, as well as energy use for microbial maintenance and growth, is incomplete (Dijkstra et al., 2011; Steinweg et al., 2008). Research has shown that microbial biomass increases initially in direct response to heat, but may actually decrease over time as microbial growth efficiency is altered (Bardgett and Shine, 1999; Schimel et al., 2007).

While increasing temperature is generally known to increase decomposition of soil organic matter, biological responses to temperature, such as enzyme activities (Koch et al., 2007) and substrate utilization patterns (Dell et al., 2012), can also affect temperature sensitivity of decomposition (Wallenstein et al., 2012). Long-term warming could induce changes in plant species composition, which can significantly affect soil microbial production of extracellular enzyme activity (Henry, 2012). Changes in extracellular enzyme activities and production may influence which compounds are most

effectively utilized by soil microorganisms under warming conditions and potentially result in altered nutrient pools. Taken together, these studies suggest that the effects of elevated temperature on the soil microbial community activity, biomass, and function as related to decomposition, and the resulting influence on C stocks and soil nutrients are more complex than previously thought and may vary over time.

1.2.3 Precipitation

Unlike temperature, in which climate change predictions suggest increases (i.e., warming) will occur for most of the planet (IPCC, 2007), changes in precipitation are likely to be more regionally variable, with some areas experiencing increases in total quantity and others reductions, as well as the possibility of altered spatial or temporal patterns of distribution. More specific projections of precipitation regimes vary between climate models, especially by region, making it difficult to assess the potential impacts of precipitation on a biologically meaningful scale (Weltzin et al., 2003).

Less is known about the variable and complex soil microbial community response to alterations in precipitation or moisture than that of temperature or increased atmospheric [CO_2], although it is widely accepted that precipitation, in how it alters soil moisture regimes, regulates decomposition in periods of water stress (drought) or anoxia (wet) (Balser et al., 2010).

Microbial communities are able to adapt to local precipitation regimes and respond to moisture stressors such as drying/rewetting in different ways, in part depending on the historical variability of the system. This complexity of response inhibits generalizations about microbial response to soil moisture across biomes (Balser et al., 2010; Evans and Wallenstein, 2011; Fierer et al 2003). Despite this, soil moisture

has often been found to be the primary variable associated with microbial community structure and function and enzyme activities (Brockett et al., 2011; Guenet et al., 2012; Kardol et al., 2010), even dominating activity in other spheres of soil biota such as microarthropod populations (Kardol et al., 2011).

In mesic grassland systems, the pattern of precipitation (e.g., increased extremity and greater intervals between rainfall events) can be more important to aboveground plant production or soil microbial responses than the total amount of precipitation received (Knapp et al., 2008). Increasing the time between rainfall events has been shown to reduce soil microbial respiration (C-output) in a temperate tallgrass prairie system, but simultaneously, increased carbon inputs via leaf photosynthesis resulting in net C uptake and storage (Fay et al., 2008). Large precipitation events (>10 mm day^{-1}) have been shown to influence net ecosystem productivity (NEP) in a shortgrass steppe ecosystem by increasing soil water content, which increases plant photosynthesis and contributes to overall gains in daytime CO_2 uptake (Parton et al., 2012). Small precipitation events (<10 mm day^{-1}) caused overall losses in NEP by increasing nighttime, in part soil microbial, respiration losses over daytime CO_2 uptake, (Parton et al., 2012). Grassland ecosystem responses to altered precipitation regimes are likely to vary depending on the interactive effects of timing and duration of events, intensity, and total amount of rainfall delivered. All of which will influence plant-microbe ecosystem functions, such as plant productivity and soil microbial respiration, and determine ecosystem carbon dynamics (Fay et al., 2008). Predicting soil microbial responses to altered precipitation is difficult and will depend on a variety of factors such as regional and historical variation, and changes in the pattern, intensity and total amount of rainfall, and the response of the existing vegetation.

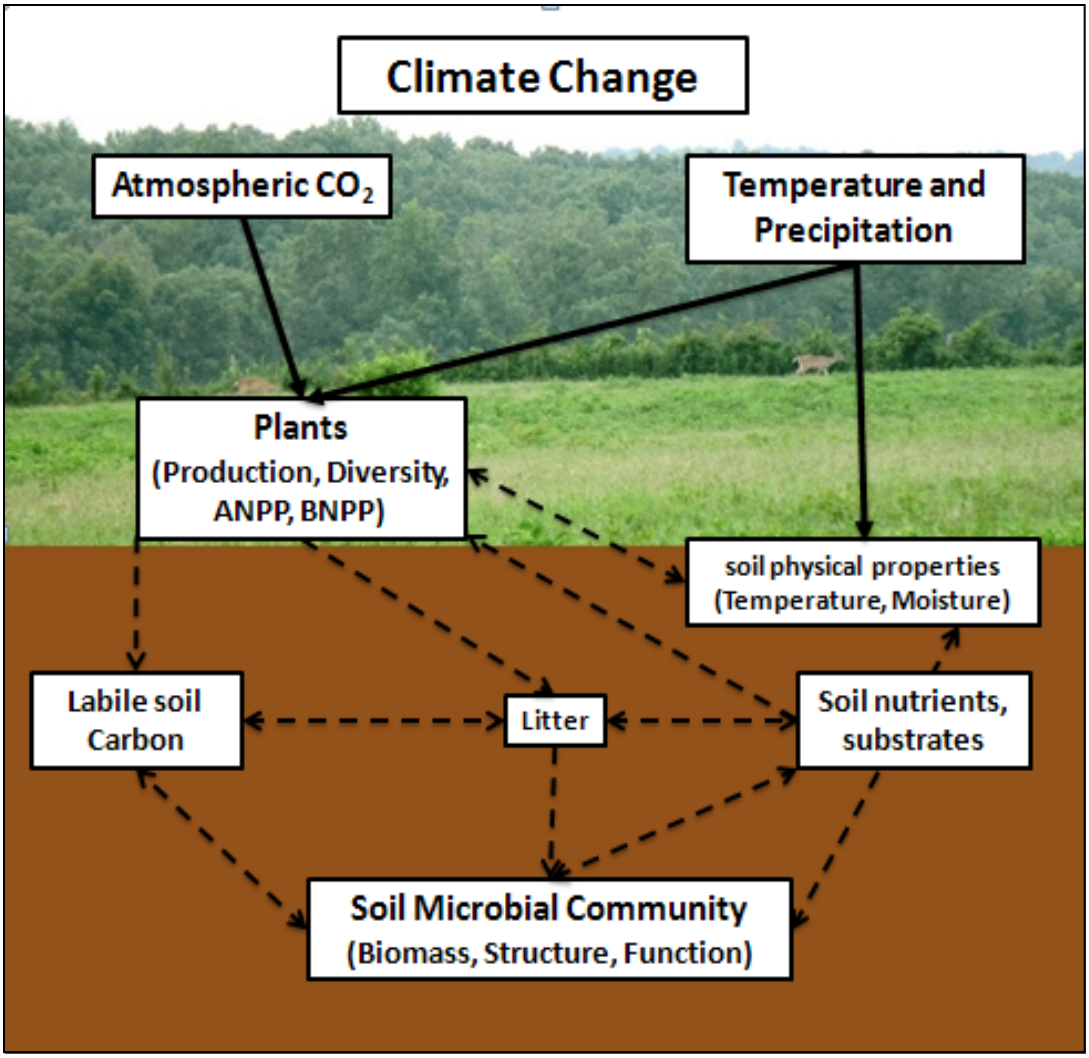


Figure 1.2: Illustration of direct (solid line) and indirect (dashed line) effects of climate change factors on a pasture ecosystem. Adapted from Balser et al. (2010). Most direct effects of these factors on soil microbes will occur either at the soil surface via drought stress and response to changes in surface temperature or aboveground by influencing plant productivity and species composition, crop selection and nutrient management dynamics (Dixon 2009; Tylianakis et al., 2008). The belowground effects of altered [CO_2], temperature and precipitation are largely indirect, and are related through resource availability and use between plants and the microbial community.

1.2.4 Indirect Effects Through Plant Communities

Consideration of the response of aboveground plant communities is very important for understanding belowground responses to climate change (Kardol et al., 2010). Often in multi-factor climate change studies, plant and soil communities change simultaneously, making it difficult to tease apart direct and indirect effects of the climate change factors or even obscuring the response (Dermody, 2006). Feedbacks moderating plant community responses in climate change studies are also difficult to assess if changes occur slowly over time (Wu et al., 2012). Climate change effects on soil ecosystem functioning such as enzyme activities and nematode abundance and community structure can be significantly affected by shifts in plant communities (Kardol et al., 2010). It is likely that changes in plant species composition, such as C_3—C_4 competition, will alter or mediate ecosystem responses to climate change, sometimes by altering water use efficiency and water availability (Morgan et al., 2004; Wan et al., 2005).

In addition to plant community composition and diversity, specific plant traits are important for assessing plant-soil feedback and ecosystem response to shifts in plant communities as a result of climate change. Traits such as relative growth rate influence leaf and litter quality, which thereby affect the quality of substrates entering the soil, and drive microbial biomass and C-cycling responses (Orwin et al., 2010). In temperate grasslands, plant species traits and productivity may exhibit a larger effect on soil biological properties (e.g., microbial biomass and activity) than direct addition of nitrogen fertilizer (Bardgett et al., 1999a). Plant communities can also influence the soil microbial community through changes in plant root exudates, which may favor certain

microbial groups (Bever et al., 2012; Kardol et al., 2007). One study showed that plant diversity affected microbial community composition and function, though C-mineralization was more affected by plant diversity-driven changes in microbial biomass rather than community composition (Carney and Matson 2005). In contrast to these studies, Marshall et al. (2011) found no effect of plant functional group on microbial community substrate utilization. However, studies have shown microbial community diversity and ecosystem functions can influence aboveground plant communities (Bonkowski and Roy, 2005). Competition between microbial communities for plant and soil resources can also affect plant-soil feedbacks in response to stress, generally through negative effects of plant pathogens (Bever et al., 2012).

1.2.5 Interactive Effects of Climate Change Factors

Climate change factors such as increased atmospheric CO_2, altered temperature or precipitation regimes, and shifts in plant species composition are unlikely to act individually, as often they will be varying simultaneously. The interactive (e.g., additive, subtractive, or multiplicative) effects of these conditions are powerful drivers of soil microbial responses (Paul and Clark, 1996a). The effects of soil moisture in particular may drive the effects of multiple climate change factors such as increased atmospheric [CO_2] or warming, as both atmospheric [CO_2] and warming have ramifications for soil moisture availability (Zavaleta et al., 2003).

Increased temperature and elevated [CO_2] have been shown to influence plant species composition. Pendall et al. (2011) found that [CO_2] enrichment and warming (+2^{o}C) favored C_4 species over C_3 species in Australian temperate grasslands, with increased soil carbon under C_4 species with only warming. They also found that warming

decreased decomposition, possibly due to limited soil moisture, but [CO_2] enrichment increased decomposition (Pendall et al., 2011).

Abundant soil moisture in conjunction with increased temperature has been shown to accelerate belowground decomposition, apparently due to increased microbial activity and efficiency (Bontti et al., 2009). Precipitation often regulates the effects of temperature—if precipitation is not limiting (i.e., abundant soil moisture) then increased temperature will improve microbial activity and efficiency, accelerating root or litter decomposition and carbon flux from grasslands; if precipitation is limiting (i.e., low soil moisture) then elevated temperature is likely to further inhibit microbial function, decelerating root or litter decomposition and potentially increasing carbon storage in grasslands (Bontti et al., 2009; Butenschoen et al., 2011). Similarly, belowground net primary productivity (BNPP), which is a primary substrate for microbes in these systems, has been shown to increase with warming and clipping in a tallgrass prairie when moisture is not limiting (Xu et al., 2011). In another study, warming and altered precipitation regimes affected plant functional composition (which, as previously discussed, can have effects on the soil microbial community) but did not significantly increase herbaceous biomass (Hoeppner and Dukes, 2012). Microbial metabolic efficiency of litter decomposition can also increase in concordance with plant diversity when soil moisture is abundant, but remains unchanged with limited soil moisture even with increased plant diversity (Butenschoen et al., 2011). This suggests that climate change effects on soil moisture may even overwhelm the benefits of plant diversity on microbial ecosystem functions (Butenschoen et al., 2011).

When studied with elevated [CO_2], soil moisture was the main factor explaining variations in microbial community structure and enzyme activities, though elevated [CO_2] increased acid phosphatase activity (Guenet et al., 2012). However, soil moisture levels induced no changes in soil carbon stocks (Guenet et al., 2012).

Relatively few studies have examined the effects of increased atmospheric [CO_2], warming, and altered precipitation together. Those that have often report that water was the primary driver for changes in ecosystem functions such as plant or microbial community composition or enzyme activities, rather than changes resulting directly from warming or [CO_2] enrichment (Castro et al., 2010; Henry, 2012; Kardol et al., 2010). Given the previously discussed complex interactions between temperature and moisture and their influence on the soil microbial community, it is likely that these factors will be the climate change factors of greatest importance in temperate grassland ecosystems. Elevated atmospheric [CO_2] by influencing plant water use and biomass production may have secondary, largely indirect effects. The specific effects of altered precipitation and temperature are likely to vary depending on local historical regimes and responses of vegetation and management. More long term studies are required which include manipulations of both temperature and moisture in various ecosystems (Balser et al., 2010; Bardgett et al., 2008; Butenschoen et al., 2011; Docherty and Gutknecht, 2012).

1.3 Objective and Hypotheses

The objective of this study was to quantify the seasonal responses of the soil microbial community to increased temperature and precipitation treatments, utilizing a manipulative field climate change study in a temperate pasture ecosystem. Soil microbial

response was measured in terms of total biomass, community composition, extracellular enzyme activities, soil microbial activity, and substrate utilization.

Hypothesis 1: There will be significant differences in total biomass, microbial composition and function, and extracellular enzyme activity across seasons. Specifically, soil microbial community structure and function will differ across seasons in ways that reflect the activity of the aboveground plant community. For my predominantly cool-season forage pasture, such changes may occur with new growth in spring vs. late growth in the fall vs. dormancy in the winter.

Hypothesis 2: While natural seasonal variation may significantly affect measured parameters such as microbial biomass or microbial fatty acid abundance, climate treatment effects would be significant enough to cause differences in microbial biomass and community structure. Given that soil moisture has been shown to regulate the effects of heat, I hypothesized there would be significant differences between treatments that receive only heat, and treatments which are a combination of warming and added precipitation. Warming treatments receiving added moisture would support enhanced microbial biomass and functions, such as enzyme activities and catabolic responses, whereas warming only treatments would have reduced responses due to water limitation that accompanies this treatment. Increased microbial catabolic response to recalcitrant substrate additions, such as lignin and cellulose, was expected in all warming treatments.

Hypothesis 3: Given the temperate location of my site and distinct seasonality of the climate, I hypothesized that season would modify the climate treatment soil microbe responses. For example, heated plots may stimulate microbial activity in months when temperature is generally considered limiting at the site and when precipitation is naturally

more abundant, such as during spring and winter. Precipitation treatments may boost activity in drier, warmer months when water may be the limiting factor, such as late summer.

Chapter 2: Materials and Methods

2.1. Site Description

The study area was located at the University of Kentucky Spindletop Research Farm in Lexington, Kentucky (38° 06'29.24"N; 84°29'29.72"W), at 281 m above sea level. The area was an upland pasture ecosystem and has a 30 year long-term annual precipitation of 1163 mm, with a 30 year mean annual summer temperature of 23.8^{o}C and a mean annual winter temperature of 1.6^{o}C (Ferreira et al., 2010). The underlying soil series was a Bluegrass-Maury silt loam complex with a 2 to 6 percent slope, which is a well drained, fine-silty, mixed, active, mesic Typic Paleudalf that formed from silty non-calcareous loess over clayey residuum derived from phosphatic limestone (USDA Soil Conservation Service, 1967).

2.2 Experimental Design

The UK Forage Climate Change Study was established in Spring 2008 as a hay-managed pasture uniformly seeded with Kentucky bluegrass (*Poa pratensis*), tall fescue (*Festuca arundinacea*), red clover (*Trifolium pretense*), and white clover (*Trifolium repens*). Bermuda grass (*Cynodon dactylon*) was plugged in August 2008 from established sod located in a nearby pasture. The site is mowed three times a year; mowing began May 2009. During a mowing event, all plant biomass above 7.6 cm from the soil surface was removed from the site. Harvest dates during the study year were May 23, 2011 (spring), July 25, 2011 (summer), and October 10, 2011 (fall).

Twenty, 5.8 m^2 hexagonal plots were identified within the pasture in Spring 2009 and were divided into five blocks with four treatments assigned at random: +Heat, +Precipitation, +Heat+Precipitation, and Control (Figure 2.1). Prior to treatments being

imposed, individual plots were similar with regard to plant community composition (unpublished data). Data collected from two permanent vegetation subplots (0.25 m^2) within each plot show that, at the time this study was conducted (2011), plant communities had diverged in response to the climate treatments. While varying seasonally, Control and +Precip plots tended to be dominated by C3 perennial grasses (tall fescue, bluegrass) while +Heat and +Heat+Precip plots were dominated by C4, annual (crabgrass [*Digitaria sanguinalis]*) and perennial (Bermuda) grasses and a mixture of forbs (Appendix 1, 2).

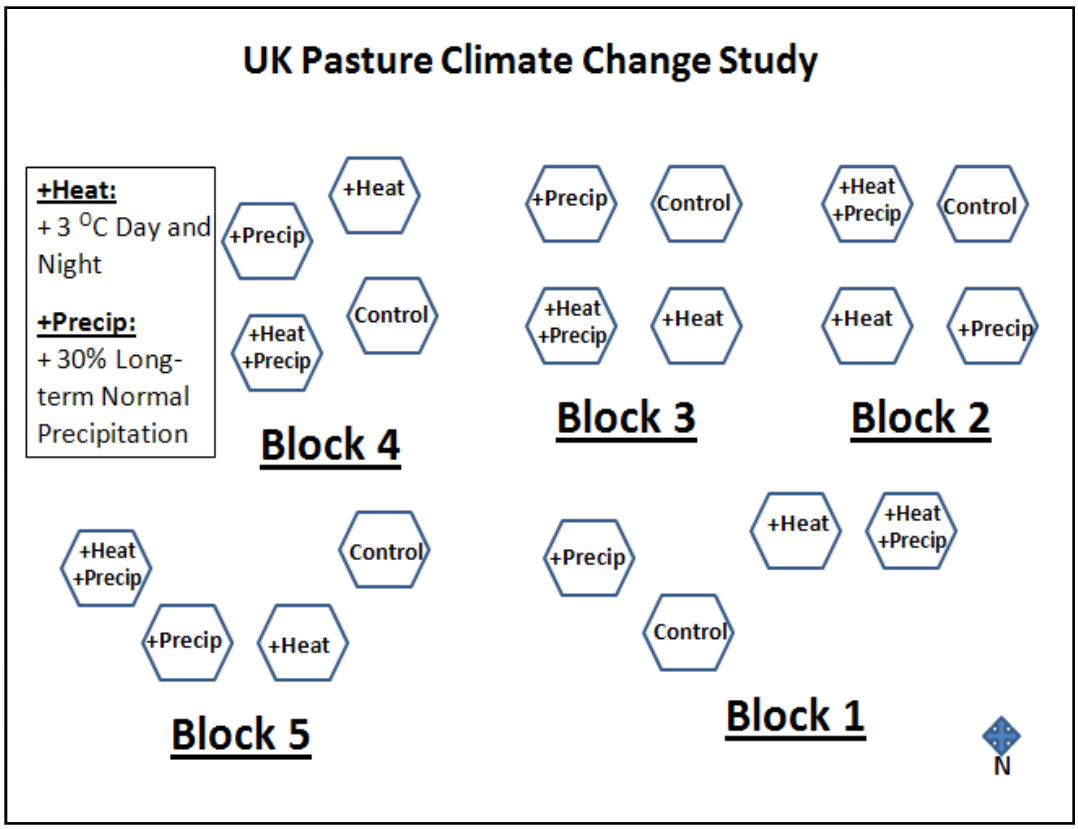


Figure 2.1. Randomized complete blocking design of the UK Forage Climate Change Study, located at the University of Kentucky Spindletop Research Farm in Lexington, Kentucky, USA. Four treatments (Control, +Heat, +Precip, +Heat+Precip) were assigned randomly to 5.8 m^2 plots within each of the 4 blocks. +Heat and +Heat+Precip treatments are maintained at 3°C above ambient air temperature day and night. +Precip and +Heat+Precip treatments receive an additional 30% of the long-term mean precipitation, added during the growing season. The control treatment experienced ambient conditions at the site.

Treatments for the project began on May 1, 2009, and have been applied continuously since. A temperature of +3^{o}C above ambient was maintained at plant canopy level day and night, year-round in the +Heat plots (Kimball et al., 2008). Constant heating in a uniform distribution throughout the plot areas was achieved using twelve, 1000W Salamander infrared heaters (Mor Electric Heating Assoc., Comstock Park, MI). The heaters were arranged around the edges of each plot, maintained at a 120 cm height above the plant canopy and angled at 45^{o} toward the ground at the center of each plot. Treatment plots that did not receive added heat (+Precip, Control) were surrounded by heater housing units that lacked the infrared heaters to account for any shading from the units. The +Precip plots received an additional 30% of the long-term mean precipitation applied primarily on rainy days (2 per month) throughout the growing season (April—September). The exact amount of added precipitation was determined by long-term monthly trends. Rain was collected from precipitation events on site, stored in a water tank, and applied using metered wands. To prevent lateral movement of water between plots, aluminum flashing was established around each plot at 0.5m depth. Control treatment plots remained at ambient conditions, with no added heat or precipitation, while the +Heat+Precip treatment plots received both the +Heat and +Precip treatments described above.

To ensure effectiveness and consistency of treatments, air temperature, soil temperature, and soil moisture have been continuously monitored since treatments began in 2009. To assess the treatment effects on the soil microenvironment, soil temperature measurements were recorded every 15 minutes in each plot using thermocouples at a depth of 5 cm (soil). Soil moisture between 0 and 15 cm in depth was measured as

volumetric water content using time domain reflectrometers (TDR) and was also recorded in each plot every 15 minutes.

2.3 Sample Collection and Storage

This study occurred in the third growing season of the project and lasted approximately one year. Soil samples were harvested once during all four seasons, and were collected approximately one week before each of the plant biomass harvest dates, except in winter, when there was no plant harvest. Soil harvest dates during the study period were May 18, 2011 (spring), July 19, 2011 (summer), October 3, 2011 (fall), and February 4, 2012 (winter). Three 1.5 cm diameter soil cores were taken to a depth of 15 cm in each plot, composited, placed in plastic bags, and put on ice in a cooler for immediate transport to the University of Toledo, Ohio, where extracellular enzyme assays and chloroform fumigation extractions were performed on hand-homogenized sub-samples. Once these analyses were completed, the remaining soil was transported back to the University of Kentucky, where the material was sieved to 2 mm and stored at -80^{o}C while awaiting further analyses.

2.4 Microbial Biomass

2.4.1 Chloroform Fumigation Extraction

Soil microbial biomass was measured using the chloroform fumigation extraction described in Rinkes *et al.* (2011), which was modified from Brookes *et al.* (1985) and Scott-Denton *et al.* (2006). For each sample plus three soil-free blanks, extractions were performed by adding 15 ml of 0.5 M potassium sulfate to 5 g of fresh soil (or a blank) and shaking on an orbital shaker for 1 hour, then vacuum filtering the extracts through Pall A/E glass fiber filters and freezing at -20^{o}C until total carbon could be measured,

usually within one week of extraction (Rinkes et al., 2011; Weintraub et al., 2007). For fumigated samples, 2 ml of ethanol-free chloroform was added to 5 g of fresh soil in a stoppered 250 ml Erlenmeyer flask, swirled gently to mix, and then incubated for 24 hours at room temperature in a fume hood. After the incubation period the flasks were allowed to vent for 30 minutes, and then extractions were performed as described above. Total dissolved organic carbon (DOC) was quantified for all extractions using a Shimadzu total organic carbon (TOC-VCPN) analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). The difference in DOC between the fumigated and non-fumigated samples represented extractable microbial biomass carbon (MB-C), expressed as µg-C g dry soil^{-1}.

2.4.2 Phospholipid Fatty Acid Analysis

Soil microbial biomass was also measured in terms of phospholipid fatty acid (PLFA) content following the procedure described by Findlay and Dobbs (1993) for assessing microbial communities via lipid analysis. For each sample, methylene chloride, phosphate buffer (50 mM), methanol and nanopure water were added to 5 g of thawed, sieved, field moist soil to extract and separate total lipids in a single phase. Soil dry weights were determined after lipid extraction by drying each sample at 105^{o}C for 2 days. Silicic acid chromatography (SiOH columns) was used to isolate the neutral lipids, glycolipids, and phospholipids with chloroform, acetone, and methanol eluents, respectively, keeping only the phospholipid fraction. Phospholipids were hydrolyzed and transmethylated to fatty acid methyl esters (FAMEs) using solutions of methanol: toluene (1:1) and 0.2 M potassium hydroxide (KOH) in methanol. FAMEs were purified using reverse-phase, solid phase extraction (SPE) column chromatography (C18 columns), then

dissolved in hexane and quantified using capillary gas chromatography (GC) with flame ionization detection (FID) (Shimadzu, Columbia, MD) and a RESTEK Rtx-1 column (30 m long, .25 µm thick, and .32 mm id, Bellefonte, PA). The GC measured a 1 µl injection of sample FAMEs at 250^{o}C, with an initial column temperature of 80^{o}C increased to the detector temperature of 260^{o}C at a rate of 3.0^{o}C/minute. A Supleco 37 component FAME mix (Sigma-Aldrich Co., St. Louis, MO) standard was run between every 4 samples to identify peaks and confirm column function. The total extractable PLFA quantified in each sample represented microbial biomass, expressed as nmol PLFA g soil^{-1}.

2.5 Microbial Community Structure

Phospholipid fatty acid analysis (PLFA), described above, was also used to assess microbial community structure by identifying individual FAMEs (Findlay and Dobbs, 1993). FAMEs were identified by comparing retention times for individual peaks to those in other published works and against the Supleco 37 standard (Zelles, 1999).

PLFA nomenclature is assigned to fatty acids based on the number of carbon atoms, number and location of double bonds, and other structural differences. An “n” followed by a number shows the location of the double bond, if present, from the aliphatic end of the C-chain. The prefix “cy” indicates that the FAME is a cyclopropane fatty acid. Cis or trans configurations are denoted by the suffixes “c” and “t”, while branching at the iso and anteiso positions are indicated by the prefixes “i” and “a”, respectively. A number followed by “Me” refers to a methyl group located that many C positions away from the carboxyl group (Sundh et al., 1997).

Certain FAMEs are used as biomarkers of broad taxonomic microbial groups, such as gram positive bacteria—a15, i15, i16 (Zelles, 1999); gram negative bacteria—

18:1n7c, cy19, 18:1n5c, 16:1n9c (Zelles, 1999); *Desulfovibrio*, a gram negative sulfate-reducing bacteria—i17:1n7c (D'Angelo et al., 2005); gram negative proteobacteria—11Me18:1 (Rowe et al., 2000); general bacteria—16:0, 18:0, 16:1n9c (Zelles, 1999); actinomycetes—10Me18 (Zelles, 1999); and general fungi—18:1n9c, 18:2n6c (Zelles, 1999).The relative abundance of all individual FAMEs was calculated for each sample. Seventy-eight FAMEs were identified from each sample. Of those, 20 FAMEs were present in >1% mole abundance in all samples.

2.6 Extracellular Enzyme Activity

Extracellular enzymes (a.k.a. exoenzymes), produced by soil microbes, are important in governing the rate of decomposition of soil organic matter and cycling of N and P. I assessed extracellular enzyme activities using assays, described in Saiya-Cork et al. (2002) and Weintraub et al. (2007). Table 2.1 describes the six exoenzymes that were assayed using 96-well microplates, and their substrates: 1.) β-1,4, Glucosidase (BG); 2.) β-1,4-N-Acetyl-glucosaminidase (NAG); 3.) Leucine amino peptidase (LAP); 4.) Acid phosphatase (PHOS); 5.) Phenol oxidase (Phenox) and 6.) Peroxidase (Perox). BG, NAG, LAP and PHOS were fluorimetrically assayed on black microplates. Phenox and Perox were assayed colorimetrically on clear microplates (Weintraub et al., 2007).

Table 2.1. Extracellular enzymes assayed in this study, their functions, and the substrates that were used to assess their activity. Adapted from Weintraub et al. (2007).

Enzyme	Function	Substrate
β-1,4, Glucosidase (BG),	Enables hydrolysis of 1,4-linked β-D-glucose residues from compounds such as cellobiose, a short-chain cellulose oligomer, to release β-D-glucose.	4-MUB-β-D-glucoside
β-1,4-N-Acetyl-glucosaminidase (NAG)	Facilitates hydrolysis of N-acetyl-β-D-glucosaminide residues with 1,4-β linkages in chitin and chitin-derived oligomers.	4-MUB-N-acetyl-β-D-glucosaminide
Leucine amino peptidase (LAP)	Enables hydrolysis of the peptide bonds adjacent to free amino groups, heavily targeting leucine while also breaking down other amino acid amines and methyl esters.	L-Leucine-7-amino-4-methylcoumarin
Acid phosphatase (PHOS)	Hydrolyzes phosphoric ester bonds to mineralize organic P into phosphate.	4-MUB-phosphate
Phenol oxidase (Phenox)	Uses oxygen to break down aromatic carbon compounds such as benzendiols into semiquinones (free radical).	L-3,4-dihydroxyphenylalanine (L-DOPA)
Peroxidase (Perox)	Reduces H_2O_2 to catalyze oxidation reactions, and is commonly considered a lignolytic enzyme due to its ability to break down erratically-structured molecules.	L-3,4-dihydroxyphenylalanine (L-DOPA)

For each sample, 1 g of fresh soil was homogenized with 125 mL of 50 mM sodium bicarbonate buffer (adjusted to match soil pH at harvest, 6-6.5) to make sample slurries using a Biospec Tissue Tearer for 1 min. From the continuously-stirred sample slurries, 200-µl aliquots were pipetted into 96-well microplates, with 16 replicate wells for each sample and enzyme (Weintraub et al., 2007). For the fluorimetric assays (BG, NAG, LAP, PHOS), 50 µl of 50 mM substrate solution was added to each sample well. Blank wells for each sample and enzyme used only 50 µl of the sodium bicarbonate buffer solution and 200 µl of soil slurry. Negative control wells for each sample and enzyme received 50 µl of the corresponding substrate and 200-µl of the buffer solution. Quench standard wells, to correct for the interference of slurry particulates with absorbance readings, received 50 µl of standard (7-amino-4-methylcoumarin for LAP, 10 mM4-methylumbelliferone for BG, NAG and PHOS) and 200 µl of soil slurry. Reference standard wells received 50 µl of standard and 200 µl of buffer solution. Blank, negative control and quench standard wells had 8 replicates each (Weintraub et al., 2007). Black microplates were incubated at 20^0C for up to 4 hours, after which fluorescence was measured on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) at 365 nm excitation and 460 nm emission filters. Quench and negative control readings were used to correct enzyme activity, which was calculated as nmol activity h^{-1} g $soil^{-1}$. For the colorimetric assays (PHENOX, PEROX), clear microplates received sample and substrate solutions in a similar manner, except that they both used 25 mM L-DOPA as the substrate solution, and PEROX received an added 10 µl of 0.3% H_2O_2 in the substrate, blank, and negative control wells. Clear microplates were incubated at 20^oC for up to 2 hours, after which absorbance was measured on the Bio-Tek Synergy HT

microplate reader (Bio-Tek Inc., Winooski, VT, USA) with 460 nm emission filters. PHENOX and PEROX activities are expressed as μmol activity h^{-1} g $soil^{-1}$, with net Peroxidase activity, the difference between calculated Phenol oxidase activity and Peroxidase activity, reported as PEROX (Weintraub et al., 2007; Burke et al., 2011).

2.7 Substrate Availability and Use

2.7.1 Carbon Mineralization Assay

To measure potential carbon mineralization for each sample, a static soil incubation assay was modified from Fierer et al. (2003) and Iqbal et al. (2012). Six grams dry weight equivalent of thawed, sieved, field moist soil was weighed into 50 ml plastic centrifuge tubes with a septum and O-ring installed in each cap. The samples were adjusted to and maintained at 65% water holding capacity, and incubated at 20^0C throughout the assay period of 70 days. CO_2 concentration in the headspace was measured in 24 hour "snap shot" incubations during the assay period. An initial (T_0) headspace CO_2 sample was taken from each capped tube by plunging three times with a syringe, then drawing 10 ml of headspace gas and measuring CO_2 concentration in parts per million (ppm) on a PP Systems EGM-4 soil respirometer (Amesbury, MA). After a 24 hour incubation, headspace CO_2 was measured with the same procedure, and the T_0 measurement was subtracted from this value. This procedure was repeated periodically until a steady rate of CO_2 emission was observed in each sample over time. Between CO_2 measurement incubations, samples were left uncapped in the incubator at 20^oC and 100% moisture atmosphere. Throughout the assay period, CO_2 emission was measured 1, 2, 3, 4, 5, 8, 15, 22, 29, 36, 43, 50, 57, 63 and 70 days from when the first T_0

measurement was taken. Cumulative mineralization of CO_2 from each sample was expressed as µg CO_2-C g $soil^{-1}$ (Iqbal et al., 2012).

2.7.2 Catabolic Response Profiles

To evaluate soil microbial function in terms of metabolic potential, catabolic response profiles were conducted for each sample based on the procedure developed by Degens and Harris (1997) and modified by Degens and Vojovodic (1999), in which multiple compounds are used to induce respiration responses from the soil microbial community. This study included nine substrates which represented a broad range of complexity and chemical structure: two simple sugars (75 mM glucose, 75 mM sucrose), two carboxylic acids (100 mM oxalic acid, 100 mM citric acid), one amino acid (15 mM L-glycine), one biological substrate (48 mg ml^{-1} autolysed yeast cells), one complex, somewhat recalcitrant polysaccharide (48 mg ml^{-1} cellulose), and two recalcitrant carbon compounds (42 mg ml^{-1} lignin, 48 mg ml^{-1} chitin). Deionized water was also included to adjust for basal respiration. Before use, all substrate solutions were adjusted to pH 6.0 by adding either HCl or NaOH. One gram equivalent dry weight of thawed, sieved, field-moist soil for each substrate and sample was weighed into 50 ml plastic centrifuge tubes with septum and O-rings installed in the caps. Next, 2 ml of substrate was added to the respective samples, and the tubes were allowed to incubate for 1 hour on a horizontal shaker at room temperature, uncapped. After shaking, the tubes were capped and headspace CO_2 measured using the same protocol as for the carbon mineralization assays. An initial (T_0) measurement was taken by plunging three times with a syringe, then extracting 10 ml of headspace gas to measure the CO_2 concentration on a PP Systems EGM-4 soil respirometer (Amesbury, MA). The tubes were incubated at 20^0C

for 4 hours (labile compounds: sugars, carboxylic acids, glycine and yeast) or 24 hours (recalcitrant compounds). Final headspace CO_2 concentrations were measured in the same manner after the incubation period. Total CO_2 produced from each substrate was calculated by subtracting the T_0 values from the final values, then subtracting the total CO_2 evolved from basal respiration (deionized water). Respiration induced by each substrate was expressed as μg CO_2-C g $soil^{-1}$ h^{-1}.

2.8 Statistical Analysis

This experiment was designed to explore the effects of increased heat and precipitation on multiple soil microbial characteristics in a pasture ecosystem and the seasonality of these treatment effects. A mixed effects linear model procedure (proc mixed) (9.3 SAS Institute Inc., Cary, NC) was used to test for differences in data across all seasons and treatments for enzyme activities, total microbial biomass from both chloroform fumigation extraction and PLFA, cumulative carbon mineralization, and respiration responses from each substrate in the catabolic response profiles. Season (Spring, Summer, Fall and Winter) and treatment (Control, +Heat, +Precip, and +Heat+Precip) were designated as fixed effects, and a repeated effect of the treatments within blocks was specified. Type 3 Hotelling-Lawley-McKeon (HLM) statistics (McKeon, 1974) with an unstructured (un) covariance matrix were used to compare the means of each treatment and season across all levels of heat and precipitation, as the HLM output is more useful for small sample numbers than the default F-statistic from typical ANOVA tables (Wright, 1994). If the data was unable to converge, the covariance matrix was adjusted to a first-order autoregressive (ar(1)) type. In addition to the Least Squares Means (LSMeans) statement, the Estimate statement was used to test

the effects of individual heat and precipitation levels within each significant main effect or interaction. Hypotheses tested using the estimate statement produced the same significance values generated by LSMeans, but in a customized format (9.3 SAS Institute Inc., Cary, NC).

An additional statistical approach was used to further explore the PLFA data: the relative abundance of individual FAMEs with >1% average abundance (n=20) from PLFA extractions were also included in a non-metric multidimensional scaling ordination (NMS; PC-ORD version 4.41, MjM Software, Gleneden Beach, OR). The Multi-Response Permutation Procedure (MRPP) was used to statistically compare the differences between pairs of seasons (e.g., Spring vs. Summer; Control vs. +Heat) or pairs of treatments for each ordination, and a significant p-value was defined using the Bonferroni adjusted error rate (P = 0.05, divided the by the number of intended comparisons). Bonferroni adjustment is considered the default method of accounting for the family-wise error rate between multiple comparisons because it is the most conservative estimate. For MRPP comparisons, a Bonferroni adjusted error rate of P=0.008 (P = 0.05, divided by 6 comparisons between either 4 treatments or 4 seasons = 0.008) was used to determine significance. Ordinations were plotted by sample (as designated by sample and treatment) and by species (individual FAMEs). Enzyme activity and catabolic response profile data obtained from each sample were used as environmental variables, and overlaid on ordination plots to assess correlation with the ordination axis values.

Chapter 3: Results

3.1 Soil Temperature and Moisture

Overall, there were significant main effects of season and treatment on daily soil temperatures averaged across 30 days prior to each soil harvest, and a significant interaction of season and treatment (Table 3.1). Seasonal trends in soil temperature (Figure 3.1A) averaged across treatments for the year of study included a summer maxima of 25.2^{o}C and a winter minima of 7.3^{o}C. Reflecting the intended heat treatments, +Heat and +Heat+Precip plots were consistently 1—3^{o}C warmer than ambient controls for all seasons, with the greatest differences observed in winter (+3.4^{o}C for +Heat and +1.6^{o}C for +Heat+Precip over Controls; Figure 3.1A). In addition, soil temperatures in +Heat plots were 1.9—3.8^{o}C higher than in +Precip plots across seasons, with the greatest difference seen in the summer (+3.8^{o}C for +Heat plots over +Precip; Figure 3.1A). With the exception of spring, soil temperatures in +Heat plots were generally higher than in +Heat+Precip plots, with the greatest difference seen in summer (+1.8^{o}C for +Heat over +Heat+Precip; Figure 3.1A). As such, the added moisture applied to the +Heat+Precip treatment appeared to have slightly mitigated soil warming associated with elevated heat, although the effect was not significant in any season.

There were also significant main effects of season and treatment, and a significant interaction between season and treatment, on continuous surface soil moisture, i.e. volumetric water content (%), measured over the 30-days preceding soil harvests (Table 3.1). In general, winter months had the most abundant soil moisture (32.5%), followed by spring (31.5%), fall (30.0 %), and summer (25.5%) (Figure 3.1B). Reflecting the drying influence of the +Heat treatment, volumetric soil moisture tended to be lowest in the

+Heat plots, averaging 3.3% lower than Control and +Precip plots (Figure 3.1B). The additional precipitation given to the +Heat+Precip plots tended to ameliorate this heat-associated drying effect to some degree in the summer and fall seasons (+3.4% for +Heat+Precip over +Heat, averaged across summer and fall), although the effect was not significant in either season. +Precip plots had the highest soil moisture throughout the year, but never differed significantly from ambient controls. The greatest differences in soil moisture across the treatments occurred in the summer, primarily between +Precip and +Heat plots (+9.0% for +Precip over +Heat; Figure 3.1B). Subtle differences in statistical significance across treatments in spring, summer, and fall, coupled with the fact that there was no difference in soil moisture across treatments in the winter, contributed to the significant season × treatment interaction for this parameter.

Table 3.1. Significance tests for soil temperature (^{o}C) and soil moisture, as volumetric water content (%), over the sampled seasons and climate treatments. Bolding indicates statistical significance ($P < 0.05$).

Effect	DF n, d	**Soil Temperature**		**Soil Moisture**	
		F	P	F	P
Season	3,10	2243.22	**<0.0001**	6.82	**<0.0001**
Treatment	3,12	15.65	**0.0003**	0.10	**0.0477**
Treatment*Season	9,12.9	4.93	**0.0073**	2.97	**0.0056**

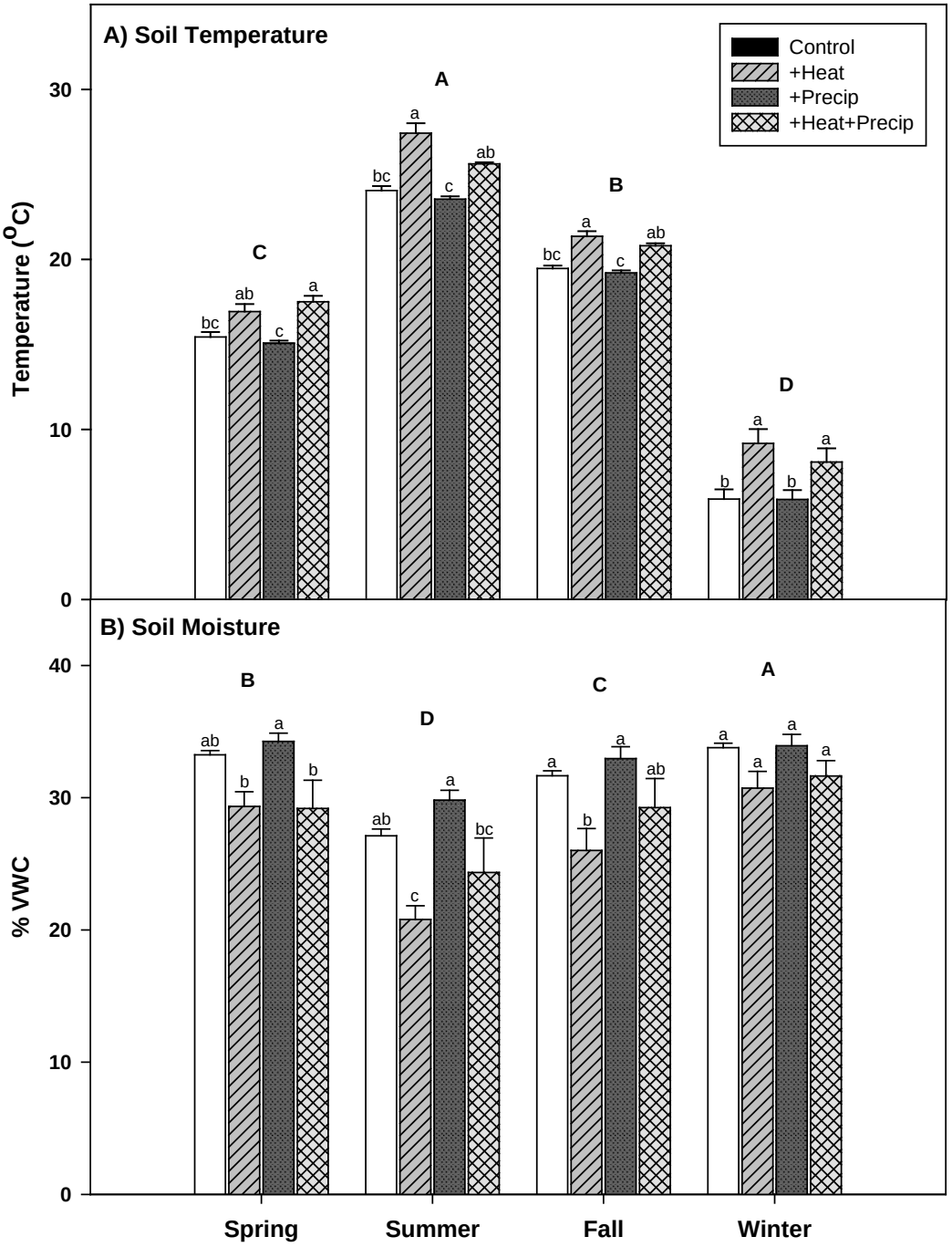


Figure 3.1. Daily soil temperature (^{o}C) (A) and soil volumetric water content (% VWC) (B) averaged across the month preceding seasonal soil sampling for each treatment. Although a significant treatment x season interaction was found for both parameters, for ease of interpretation, the main effect of season is represented by capital letters ($P < 0.05$), and within a season, the effects of treatment are indicated by small letters ($P < 0.05$). Bars represent average ± 1 S.E.

3.2 Soil Microbial Biomass

3.2.1 Chloroform Fumigation Extraction

There were significant main effects of season and treatment for microbial biomass as measured by chloroform fumigation extraction (CFE), but no significant interaction (Table 3.2). Microbial biomass C in spring and winter was, on average, 16 µg C g^{-1} dry soil higher than in summer and fall (Figure 3.2A). This seasonal effect was consistent across treatments. While +Heat tended to have the highest CFE biomass, +Heat and +Heat+Precip were not significantly different from each other, and both averaged +165 µg C g^{-1} dry soil more than the Control and +Precip treatments (Figure 3.2B).

3.2.2 Phospholipid Fatty Acid Analysis

Unlike microbial biomass measured by CFE, microbial biomass as quantified by phospholipid fatty acid analysis (PLFA) exhibited only significant seasonal effects, with no significant treatment main effect or interaction between season and treatment (Table 3.2). Also in contrast to the CFE data, microbial biomass by this estimate was highest in the summer (83 nmol PLFA g^{-1} dry soil) and lowest in the winter (46 nmol PLFA g^{-1} dry soil), with spring and fall being intermediate and not significantly different from each other (Figure 3.2C).

Table 3.2. Significance tests for soil microbial biomass estimates obtained from Chloroform Fumigation Extraction (CFE; μg C g^{-1} dry soil) and Phospholipid Fatty Acid Analysis (PLFA; nmol g^{-1} soil) over the sampled seasons and climate treatments. Bolding indicates statistical significance ($P < 0.05$).

Effect	DF n, d	**CFE**		**PLFA**	
		F	P	F	P
Season	3,10	4.00	**0.0414**	118.80	**<0.0001**
Treatment	3,12	4.00	**0.0345**	0.61	0.6215
Treatment*Season	9,12.9	1.60	0.2137	0.70	0.7033

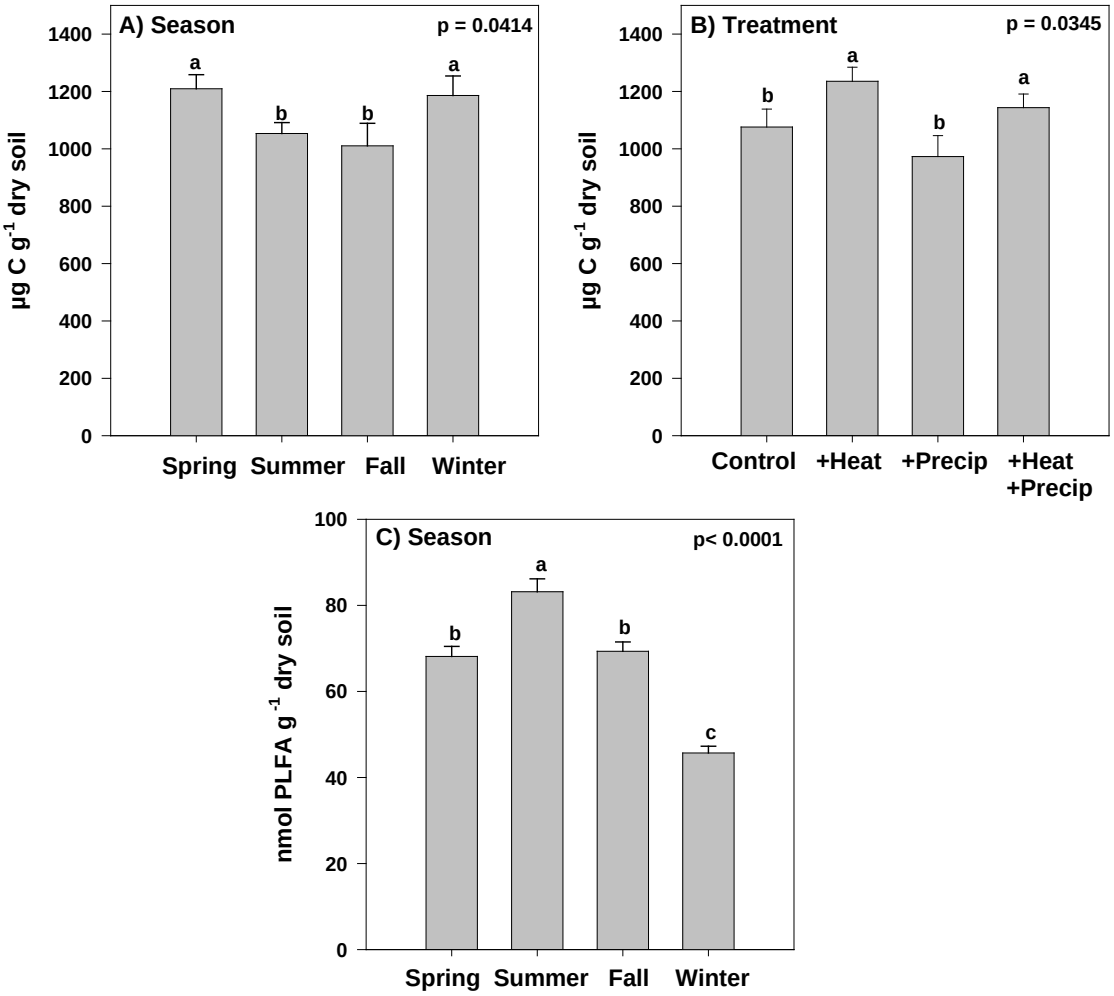


Figure 3.2. Microbial biomass results from Chloroform Fumigation Extraction (CFE) for significant main effects (A—Season; B—Treatment), and Phospholipid Fatty Acid Analysis (PLFA) results for the only significant main effect (C—Season). Bars represent averages ± 1 S.E., and significant differences within panels are denoted by different letters ($P < 0.05$).

3.3 Soil Microbial Community Structure

Non-metric multidimensional (NMS) scaling ordination analyses of the 20 most abundant PLFAs showed significant seasonal effects on soil microbial community composition overall and within each treatment. When all samples were included in the same ordination, strong seasonal separation was observed (Figure 3.3A). Multi-response permutation analysis of all the seasonal pairwise comparisons contained in this ordination indicated that lipid profiles for each season were distinct (Table 3.3). In the spring, abundant lipid biomarkers in the soil microbial community included a mix of mostly gram-negative and non-specific bacterial biomarkers, such as cy19, 18:0, 18:1n7c, 16:1n9c, 16:1n7c and 16:1n5c. In the summer, the population shifted more strongly toward general bacteria 18:0 and 16:0 biomarkers, and also toward gram-positive bacteria biomarkers such as i15 and i16. The relative abundance of lipid biomarkers in the fall, while significantly different from spring and summer, fell in between these two seasons in ordination space, indicating some overlap in lipid profiles. The winter population strongly favored gram-negative bacteria biomarkers such as 16:1n9c, 16:1n7c and 16:1n5c (Table 3.4).

NMS ordination (Figure 3.4A,C,E,G) and MRPP testing (Table 3.5) revealed significant seasonal separation of PLFA profiles in each climate treatment. For all treatments, the relative abundance of lipid biomarkers indicated that summer and winter microbial communities differed significantly from each other and from those observed in spring and fall, which where were statistically similar in all treatments. Specific FAMEs driving NMS ordinations of the seasonal effects for each treatment are outlined in Appendices 3—6. In general, the lipid biomarkers responsible for driving the overall

seasonal differences observed when all treatments were run in the same ordination (Figure 3.3A; Table 3.5) were also important for each treatment. However, multiple FAMEs that were not significant in the seasonal NMS ordination for all samples were found to be important in treatment-dependent ways (Appendix 3—6). Fungal biomarkers (18:1n9c and 18:2n6c) were present in each treatment, and appeared to drive seasonal separation of spring and fall samples from that of summer or winter in the Control and +Heat plots (Appendix 3,4), but appeared to drive separation of winter samples from the other seasons in the +Precip and +Heat+Precip plots (Appendix 5,6). The biomarker i17:1n7 for a sulfate-reducing bacteria, *Desulfovibrio* (D'Angelo et al., 2005), became significant in both the +Heat and +Precip plots and appeared to slightly drive spring samples (Appendix 4,5). The biomarker 11Me18:1, which has been described for lipids in various groups of gram negative proteobacteria (Rowe et al., 2000), was positively correlated with Axis 1 in the Control and +Heat+Precip ordinations, in areas associated with winter samples (Appendix 3,6).

NMS ordination analyses of the 20 most abundant PLFAs showed that there were no treatment effects on the soil microbial community composition (Figure 3.3B). Additional ordinations performed on each season separately and multi-response permutation analyses on these ordinations confirmed this result (Figure 3.5; Tables 3.3 & 3.6). Lipid profiles of the soils associated with the four climate treatments were similar to each other in all measured seasons.

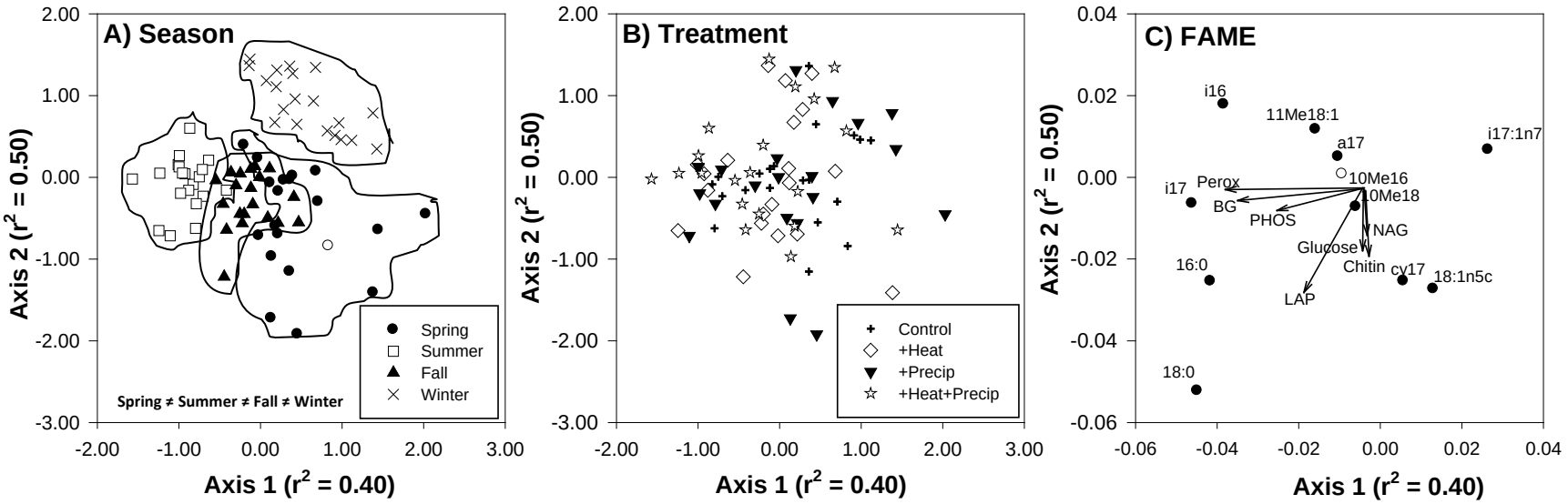

A) Season
Axis 2 (r^2 = 0.50)
Axis 1 (r^2 = 0.40)
Spring
Summer
Fall
Winter
Spring ≠ Summer ≠ Fall ≠ Winter
B) Treatment
Control
+Heat
+Precip
+Heat+Precip
C) FAME
i16
11Me18:1
a17
i17:1n7
i17
Perox
BG
PHOS
10Me16
10Me18
NAG
Glucose
Chitin
cy17
18:1n5c
16:0
LAP
18:0

Figure 3.3. Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for all samples collected during this study. The samples are grouped based on Season (A) and Treatment (B). The stress value for the ordination was 14.537, which indicates the ordination is an accurate two-dimensional representation of the data, and the amount of variation explained by each axis (r^2) is included in each axis title. (A) Text and circles indicate significant separations ($P < 0.008$) between seasons (Table 3.3) in the panel. There were no significant separations across treatments (B). (C) Displays weighted cumulative average of individual FAME scores from the NMS ordination, and includes correlated vectors of two environmental variables, activity of selected enzymes (where BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; and Perox = Peroxidase) and catabolic response to substrates (glucose and chitin). Axes in panel (C) are zoomed in from those of (A) and (B) for clarity. Not all 20 FAMEs included in the ordination are shown in (C) because they were located outside of the zoomed in axes; however, if these nine missing FAMEs were important in explaining the variation in this ordination, they are shown in Table 3.4.

Table 3.3. Significance tests for the Multi-Response Permutation Procedures (MRPP) used to perform pairwise comparisons between seasons and treatments for the Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for all samples. A-values represent the within-group agreement statistic corrected for chance, where higher values mean differences are less likely due to chance. Bolded P-values indicate statistical significance, based on a Bonferroni-adjustment (P < 0.05 / 6 comparisons < 0.008).

Paired	**Season**		**Paired**	**Treatment**	
Comparison	**A**	**P**	**Comparison**	**A**	**P**
Spring *vs.* Summer	0.216	**<0.0001**	Control *vs.* +Heat	0.015	0.0778
Spring *vs.* Fall	0.067	**<0.0001**	Control *vs.* +Precip	-0.007	0.7298
Spring *vs.* Winter	0.207	**<0.0001**	Control *vs.* +Heat+Precip	0.020	0.0449
Summer *vs.* Fall	0.149	**<0.0001**	+Heat *vs.* +Precip	0.015	0.0748
Summer *vs.* Winter	0.343	**<0.0001**	+Heat *vs.* +Heat+Precip	-0.004	0.5518
Fall *vs.* Winter	0.260	**<0.0001**	+Precip *vs.* +Heat+Precip	0.012	0.1198

Table 3.4. FAMEs driving the NMS ordination of all samples combined (Figure 3.3). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, All Samples

Axis 1 (r^2=0.40)			**Axis 2 (r^2=0.50)**		
FAME	r-value	Classification	FAME	r-value	Classification
18:1n7c	0.74	Gram-negative bacteria	16:1n7c	0.697	Gram-negative bacteria
16:1n7c	0.691	Gram-negative bacteria	16:1n5c[1]	0.658	Gram-negative bacteria
16:1n9c	0.663	Non-specific bacteria	16:1n9c	0.647	Gram-negative bacteria
16:1n5c1	0.556	Gram-negative bacteria	a15	0.588	Gram-positive bacteria
16:0	-0.74	Non-specific bacteria	cy19	-0.85	Gram-negative bacteria
i16	-0.674	Gram-positive bacteria	18:0	-0.681	Non-specific bacteria
i15	-0.637	Gram-positive bacteria			
18:0	-0.572	Non-specific bacteria			

Table 3.4 (cont'd). FAMEs driving the NMS ordination of all samples combined (Figure 3.3). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, All Samples					
Axis 1 (r^2=0.40)			**Axis 2 (r^2=0.50)**		
Overlay	r-value	Classification	Overlay	r-value	Classification
BG[2]	-0.606	Exoenzyme	Chitin[3]	-0.479	Recalcitrant carbon
PHOS[2]	-0.505	Exoenzyme	Glucose[3]	-0.461	Simple sugar
Perox[2]	-0.619	Exoenzyme	LAP[2]	-0.553	Exoenzyme
LAP[2]	-0.443	Exoenzyme			

[1]Has also been used as a biomarker for arbuscular mycorrhizal fungi.
[2] Assayed extracellular enzymes, (BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase).
[3]Substrate used in Catabolic Response Profiling.

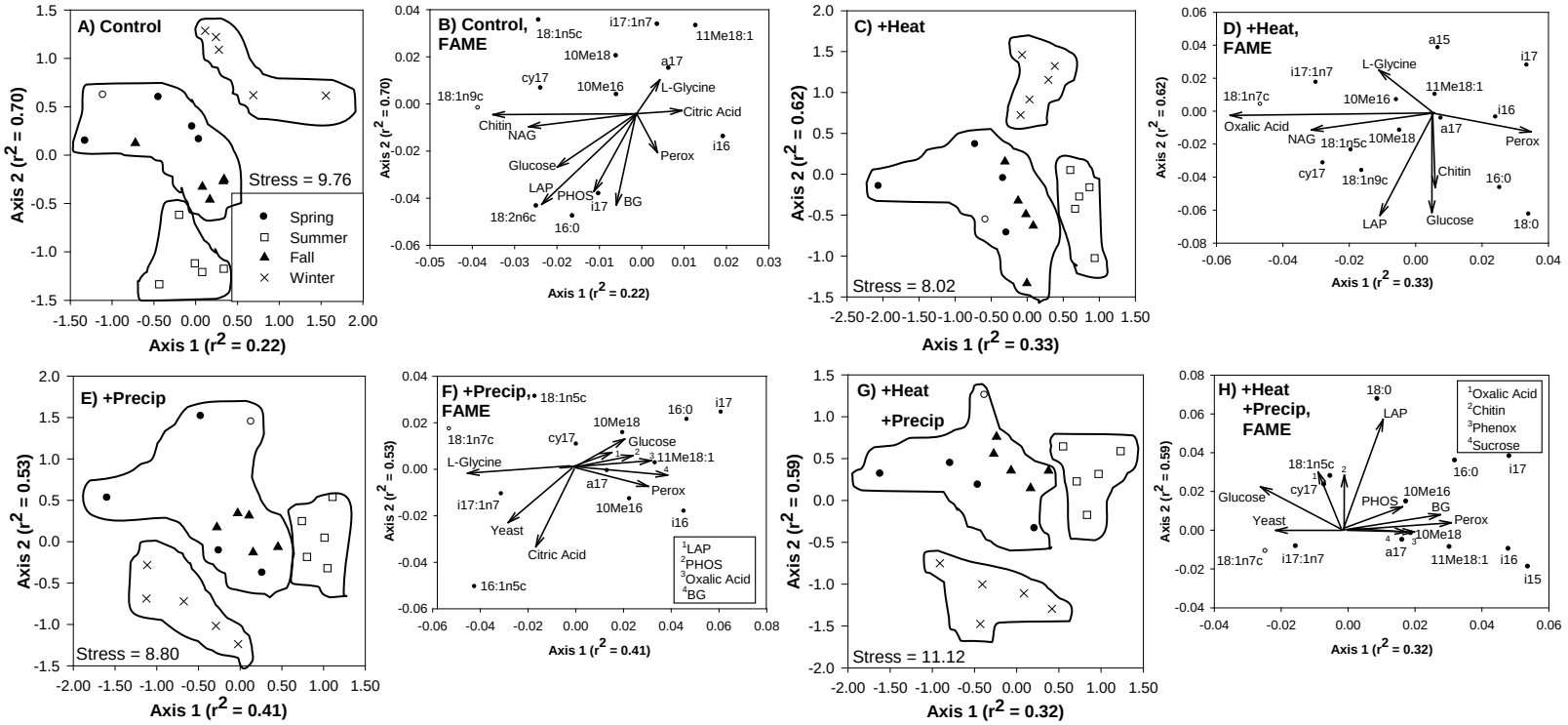

A) Control
Stress = 9.76
Spring
Summer
Fall
Winter
Axis 2 (r^2 = 0.70)
Axis 1 (r^2 = 0.22)
B) Control, FAME
18:1n5c
i17:1n7
11Me18:1
10Me18
a17
cy17
10Me16
L-Glycine
18:1n9c
Citric Acid
Chitin
NAG
i16
Perox
Glucose
LAP
PHOS
i17
BG
18:2n6c
16:0
Axis 2 (r^2 = 0.70)
Axis 1 (r^2 = 0.22)
C) +Heat
Stress = 8.02
Axis 2 (r^2 = 0.62)
Axis 1 (r^2 = 0.33)
D) +Heat, FAME
a15
i17
L-Glycine
i17:1n7
11Me18:1
18:1n7c
10Me16
i16
Oxalic Acid
NAG
18:1n5c
10Me18
a17
Perox
cy17
18:1n9c
Chitin
16:0
LAP
Glucose
18:0
Axis 2 (r^2 = 0.62)
Axis 1 (r^2 = 0.33)
E) +Precip
Stress = 8.80
Axis 2 (r^2 = 0.53)
Axis 1 (r^2 = 0.41)
F) +Precip, FAME
18:1n5c
16:0
i17
10Me18
18:1n7c
cy17
Glucose
L-Glycine
11Me18:1
a17
Perox
i17:1n7
10Me16
i16
Yeast
Citric Acid
16:1n5c
1LAP
2PHOS
3Oxalic Acid
4BG
Axis 2 (r^2 = 0.53)
Axis 1 (r^2 = 0.41)
G) +Heat +Precip
Stress = 11.12
Axis 2 (r^2 = 0.59)
Axis 1 (r^2 = 0.32)
H) +Heat +Precip, FAME
18:0
LAP
1Oxalic Acid
2Chitin
3Phenox
4Sucrose
18:1n5c
16:0
i17
cy17
Glucose
PHOS
10Me16
BG
Yeast
Perox
10Me18
18:1n7c
i17:1n7
a17
11Me18:1
i16
i15
Axis 2 (r^2 = 0.59)
Axis 1 (r^2 = 0.32)

Figure 3.4. Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for all seasons within each Treatment (Control—A, +Heat—C, +Precip—E, +Heat+Precip—G). Stress values are included in each panel indicating the accuracy of the two-dimensional representation (lower is better), and the amount of variation explained by each axis (r^2) is included in axis titles. (A, C, E, G) Circled groups indicate significant separations (Table 3.5), where Summer and Winter samples differ from each other and from Spring and Fall samples combined. (B, D, F, H) Displays weighted cumulative average of PLFA scores from the NMS ordinations, and includes correlated vectors ($r > 0.40$) of two environmental variables, the activity of selected enzymes (where BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase.) and catabolic response to substrates (Glucose, Sucrose, Yeast, L-Glycine, Citric and Oxalic Acids, and Chitin). Axes in panels (B, D, F, H) are scaled down from those in (A, C, E, G) so that individual PLFA scores and environmental variables are easier to read. This scaling down resulted in some of the 20 PLFAs included in the ordinations not being shown because they were located outside the range of the zoomed in axes; however, if these missing FAMEs were important in explaining the variation in the ordinations, they are shown in Tables A1.2 – 1.5.

Table 3.5. Significance tests for the Multi-Response Permutation Procedures (MRPP) used to perform pairwise comparisons between seasons for Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for each treatment (Figure 3.4). A-values (A) represent the within-group agreement statistic corrected for chance. Bolded P-values indicate statistical significance, with a Bonferroni-adjusted acceptable P-value of <0.008.

Compared	**Treatment**							
	Control		**+Heat**		**+Precip**		**+Heat+Precip**	
	A	**P**	**A**	**P**	**A**	**P**	**A**	**P**
Spring *vs.* Summer	0.262	**0.0016**	0.246	**0.0017**	0.197	**0.0034**	0.209	**0.0023**
Spring *vs.* Fall	0.127	0.0149	0.050	0.0818	0.041	0.1668	0.065	0.0412
Spring *vs.* Winter	0.203	**0.0014**	0.293	**0.0020**	0.176	**0.0065**	0.217	**0.0033**
Summer *vs.* Fall	0.176	**0.0023**	0.158	**0.0020**	0.187	**0.0017**	0.184	**0.0034**
Summer *vs.* Winter	0.389	**0.0018**	0.386	**0.0017**	0.407	**0.0017**	0.359	**0.0020**
Fall *vs.* Winter	0.282	**0.0022**	0.336	**0.0020**	0.274	**0.0015**	0.338	**0.0016**

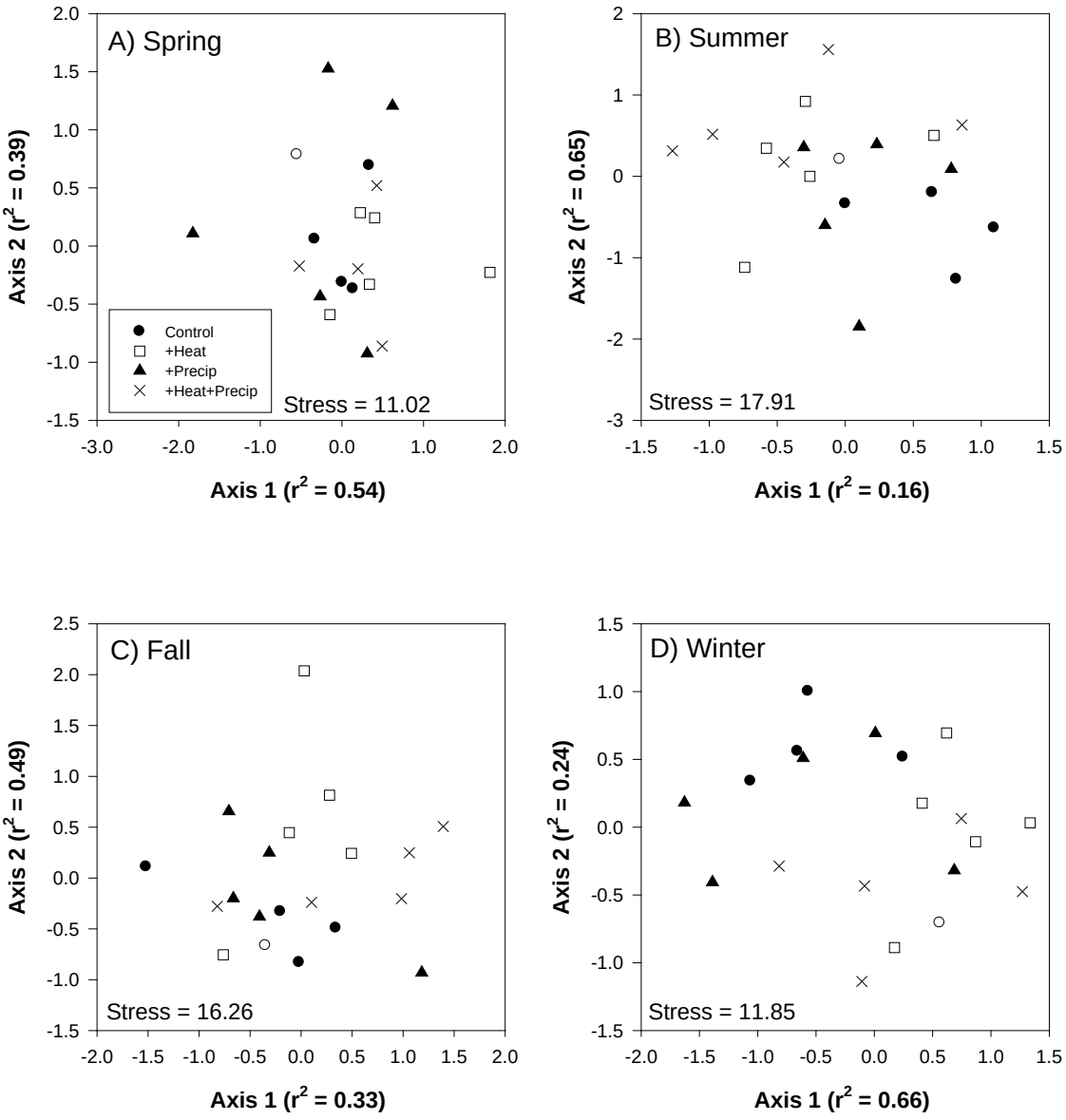


Figure 3.5. Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for all samples by treatment during each season (Spring - A, Summer - B, Fall - C, Winter - D). Stress values are included in each panel indicating the accuracy of the two-dimensional representation, and the amount of variation explained by each axis (r^2) is included in each axis title.

Table 3.6. Significance tests for the Multi-Response Permutation Procedures (MRPP) used to perform pairwise comparisons between treatments for Non-metric multidimensional scaling ordination of the 20 most abundant lipid biomarkers for each season (Figure 3.5). A-values (A) represent the within-group agreement statistic corrected for chance. No P-values were statistically significant, with a Bonferroni-adjusted acceptable P-value of <0.008.

Compared	**Season**							
	Spring		**Summer**		**Fall**		**Winter**	
	A	**P**	**A**	**P**	**A**	**P**	**A**	**P**
Control *vs.* +Heat	-0.016	0.6718	0.049	0.0757	0.083	0.0392	0.135	0.0156
Control *vs.* +Precip	-0.015	0.5484	-0.023	0.7572	-0.015	0.6418	-0.026	0.6798
Control *vs.* +Heat +Precip	0.012	0.3300	0.097	0.0178	0.085	0.0424	0.060	0.1045
+Heat *vs.* +Precip	0.004	0.3968	0.004	0.4069	0.033	0.1529	0.164	0.0120
+Heat *vs.* +Heat +Precip	-0.013	0.6308	-0.005	0.5327	0.049	0.1030	0.007	0.3527
+Precip *vs.* +Heat +Precip	-0.026	0.6707	0.029	0.1335	0.060	0.0872	0.057	0.1128

3.4 Extracellular Enzyme Activity

All of the assayed extracellular enzymes were significantly affected by season (Table 3.7). No significant main effect of treatment or interaction between treatment and season were observed for any of the measured enzymes (Table 3.7). Similar seasonal dynamics were recorded for β-1,4-glucosidase (BG) and acid phosphatase (PHOS), with both having highest activity in the summer (161 and 275 nmol activity h^{-1} g^{-1} soil, respectively) followed by dramatic declines in activity in fall and winter (Figure 3.6 A, C). β -1, 4-N-acetyl glucosaminidase (NAG) differed from BG and PHOS in that the highest activity was measured in the spring instead of the summer (Figure 3.6 D). For leucine amino peptidase (LAP), differences in activity between spring, summer, and fall were less dramatic than that observed for the other exoenzymes; however, similar to the rest of the hydrolases (BG, PHOS, NAG), winter activity was much reduced, dropping to 19 nmol activity h^{-1} g^{-1} soil (Figure 3.6 B). In contrast, the oxidoreductases, Peroxidase (Perox) and Phenol oxidase (Phenox), had lowest activities in the spring (12 µmol activity h^{-1} g^{-1} soil) and fall (14 µmol activity h^{-1} g^{-1} soil), respectively (Figure 3.6 E, F). Similar to the hydrolases, both enzymes had the highest activity in the summer.

Table 3.7. Significance tests for extracellular enzyme activities assayed over the sampled seasons and climate treatments (BG = β-1, 4, Glucosidase; LAP = Leucine amino peptidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase). BG, LAP, NAG and PHOS values were measured as nmol activity h^{-1} g^{-1} soil; Perox and Phenox values were measured in µmol activity h^{-1} g^{-1} soil. Bolding indicates statistical significance ($P < 0.05$).

Effect	DF	**BG**		**LAP**		**NAG**	
	n, d	F	P	F	P	F	P
Season	3,10	140.74	**<0.0001**	216.8	**<0.0001**	330.44	**<0.0001**
Treatment	3,12	0.14	0.9371	0.82	0.5079	0.71	0.5661
Treatment * Season	9,12.9	1.25	0.3457	0.68	0.7134	0.97	0.5046

Effect	DF	**PHOS**		**Perox**		**Phenox**	
	n, d	F	P	F	P	F	P
Season	3,10	314.63	**<0.0001**	9.19	**0.0032**	23.45	**<0.0001**
Treatment	3,12	0.23	0.876	0.56	0.6489	0.51	0.6805
Treatment * Season	9,12.9	0.86	0.5796	0.68	0.7176	0.89	0.5606

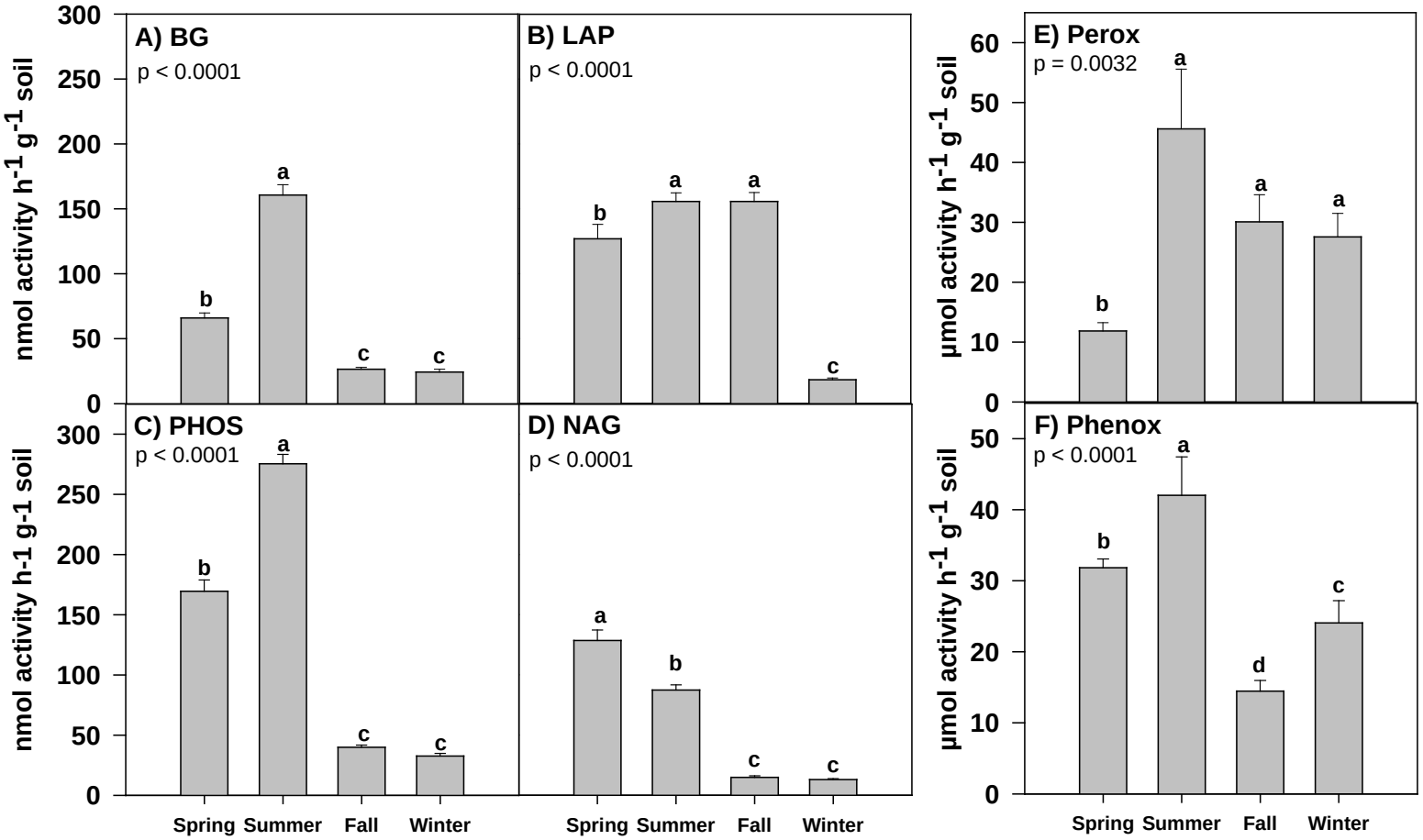

A) BG
p < 0.0001
B) LAP
p < 0.0001
E) Perox
p = 0.0032
C) PHOS
p < 0.0001
D) NAG
p < 0.0001
F) Phenox
p < 0.0001
nmol activity h-1 g-1 soil
µmol activity h-1 g-1 soil
Spring
Summer
Fall
Winter

Figure 3.6. Extracellular enzyme activities with significant seasonal main effects (P-values shown) (A) BG = β-1, 4, Glucosidase; (B) LAP = Leucine amino peptidase; (C) PHOS = Acid phosphatase; (D) NAG = β-1, 4-N-Acetyl-glucosaminidase; (E) Perox = Peroxidase; and (F) Phenox = Phenol oxidase. Bars represent average ± 1 S.E., and within each panel, columns with differing letters are significantly different ($P < 0.05$).

3.5 Substrate Availability and Use

3.5.1 Carbon Mineralization Assay

A significant main effect of season and treatment x season interaction was found for the amount of respired carbon measured via the carbon mineralization assay (CMA); however, no significant treatment effect was identified (Table 3.8). Averaged across treatments, soils from each season respired significantly different amounts of carbon (Figure 3.7), with the greatest amount measured in the spring (185 μg CO_2-C g $soil^{-1}$) and decreasing throughout the growing season to 120 μg CO_2-C g $soil^{-1}$ in the fall. However, labile soil carbon pools appeared to increase from fall to winter, as respired carbon measured via CMA increased to 134 μg CO_2-C g $soil^{-1}$ in the winter soils.

There was only one season where significant treatment differences were identified. In winter, the amount of carbon respired in +Heat plots was 40 μg CO_2-C g $soil^{-1}$ less than that measured in Control or +Heat+Precip plots (Figure 3.7). This general trend (+Heat < Control, +Heat+Precip) was also apparent in the summer, although it was only marginally significant ($P \leq 0.075$). However, the +Heat plots had greater respired carbon in the spring than the other treatments, although again this comparison was not statistically significant. These trends explain the significant treatment x season interaction for this parameter.

Table 3.8. Significance tests for carbon mineralization assays (CMA) over the sampled seasons and climate treatments, measured as cumulative µg CO_2-C g^{-1} soil produced over a 70 day incubation period. Bolding indicates statistical significance ($P < 0.05$).

Effect		**CMA**	
	DF n, d	F	P
Season	3,10	6.82	**0.0088**
Treatment	3,12	0.10	0.9585
Treatment*Season	9,12.9	2.97	**0.0375**

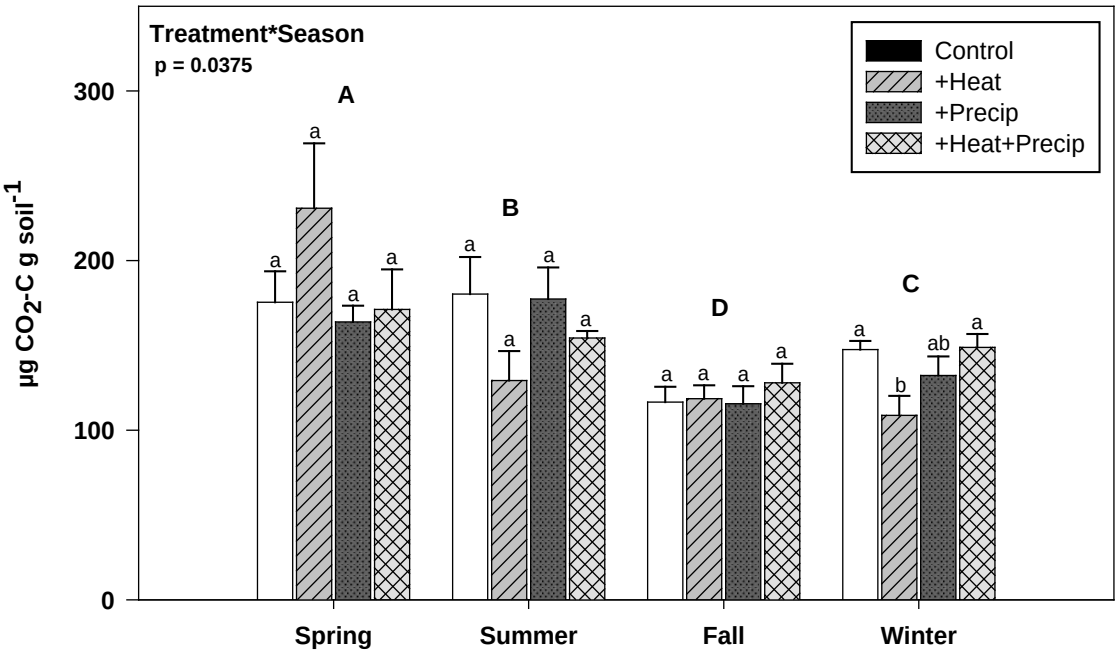


Figure 3.7. Carbon Mineralization Assay (CMA) results showing the seasonal effects and treatment effects within season. Values are cumulative soil respiration (µg CO_2-C g^{-1} soil) produced over a 70 day incubation period for each sample. Although a significant treatment x season interaction was found, for ease of interpretation, the main effect of season is represented by capital letters, and within a season, the effect of treatment is indicated by small letters ($P < 0.05$). Bars represent average ± 1 S.E

3.5.2 Catabolic Response Profiles

In assessing the microbial communities' ability to utilize nine substrates with a wide range of digestibility, a significant main effect of either season or treatment was observed for all substrates except oxalic acid (a carboxylic acid) and yeast (a biological substrate) (Table 3.9). There were no significant interactions between treatment and season for any of the substrates tested.

Three labile substrates (glucose, citric acid, and L-glycine) and three recalcitrant carbon substrates (cellulose, chitin and lignin) exhibited significant seasonal patterns (Table 3.9). Glucose, cellulose, and chitin had similar seasonal patterns in utilization, with spring being highest, winter lowest, and summer and fall in between (Figure 3.8A, D, E). Citric acid induced the largest microbial utilization response of the labile substrates, but unlike glucose, another labile compound, utilization of this substrate increased throughout the growing season (Figure 3.8B). The response to L-glycine additions was relatively low compared to the other labile substrates and seasonal patterns were subtle (Figure 3.8C). Of the recalcitrant compounds, lignin additions elicited the strongest microbial utilization response (averaged across seasons, 2.02 μg CO_2-C g $soil^{-1}$ h^{-1} for lignin vs. 0.69 μg CO_2-C g $soil^{-1}$ h^{-1} and 0.27 μg CO_2-C g $soil^{-1}$ h^{-1} for cellulose and chitin, respectively), and this response was most pronounced in the fall, which had significantly greater utilization than spring, summer and winter for this substrate (Figure 3.8F).

Only two substrates exhibited significant treatment effects: sucrose, a labile simple sugar, and cellulose, a more recalcitrant compound (Table 3.9). Treatment effects for sucrose additions consisted of increased microbial utilization in +Heat plots, inducing

an average of +0.34 µg CO_2-C g $soil^{-1}$ h^{-1} more respiration in +Heat than from soils of Control, +Precip, or +Heat+Precip plots (Figure 3.9A). Cellulose was similarly affected by treatments, except in this case, the stimulation of the microbial response was observed from both +Heat and +Heat+Precip plots, producing an average of +0.14 µg CO_2-C g $soil^{-1}$ h^{-1} more respiration than the Control or +Precip plots (Figure 3.9B).

Table 3.9. Significance tests for catabolic response profiles over the sampled seasons and climate treatments, where soil microbial response is measured as µg CO_2-C g $soil^{-1}$ h^{-1}. Substrates are grouped based on whether they are considered to be labile or recalcitrant compounds. Bolding indicates statistical significance ($P < 0.05$).

Effect	DF	Labile Substrates					
		Glucose		Sucrose		Citric Acid	
	n, d	F	P	F	P	F	P
Season	3,10	30.15	**<0.0001**	0.18	0.9068	33.06	**<0.0001**
Treatment	3,12	1.18	0.3585	7.13	**0.0052**	0.84	0.4957
Treatment* Season	9,12.9	1.29	0.3289	0.87	0.5717	1.15	0.3964

Effect	DF	Labile Substrates					
		Oxalic Acid		L-Glycine		Yeast	
	n, d	F	P	F	P	F	P
Season	3,10	1.76	0.2420	4.17	**0.0131**	2.52	0.1174
Treatment	3,12	0.25	0.8626	0.23	0.8713	1.09	0.3925
Treatment* Season	9,12.9	0.55	0.7988	1.08	0.4038	1.5	0.2453

Effect	DF	Recalcitrant Substrates					
		Cellulose		Chitin		Lignin	
	n, d	F	P	F	P	F	P
Season	3,10	4.22	**0.0359**	36.84	**<0.0001**	35.11	**<0.0001**
Treatment	3,12	4.56	**0.0237**	1.52	0.2596	2.57	0.1030
Treatment* Season	9,12.9	0.71	0.6944	0.89	0.5578	1.28	0.3318

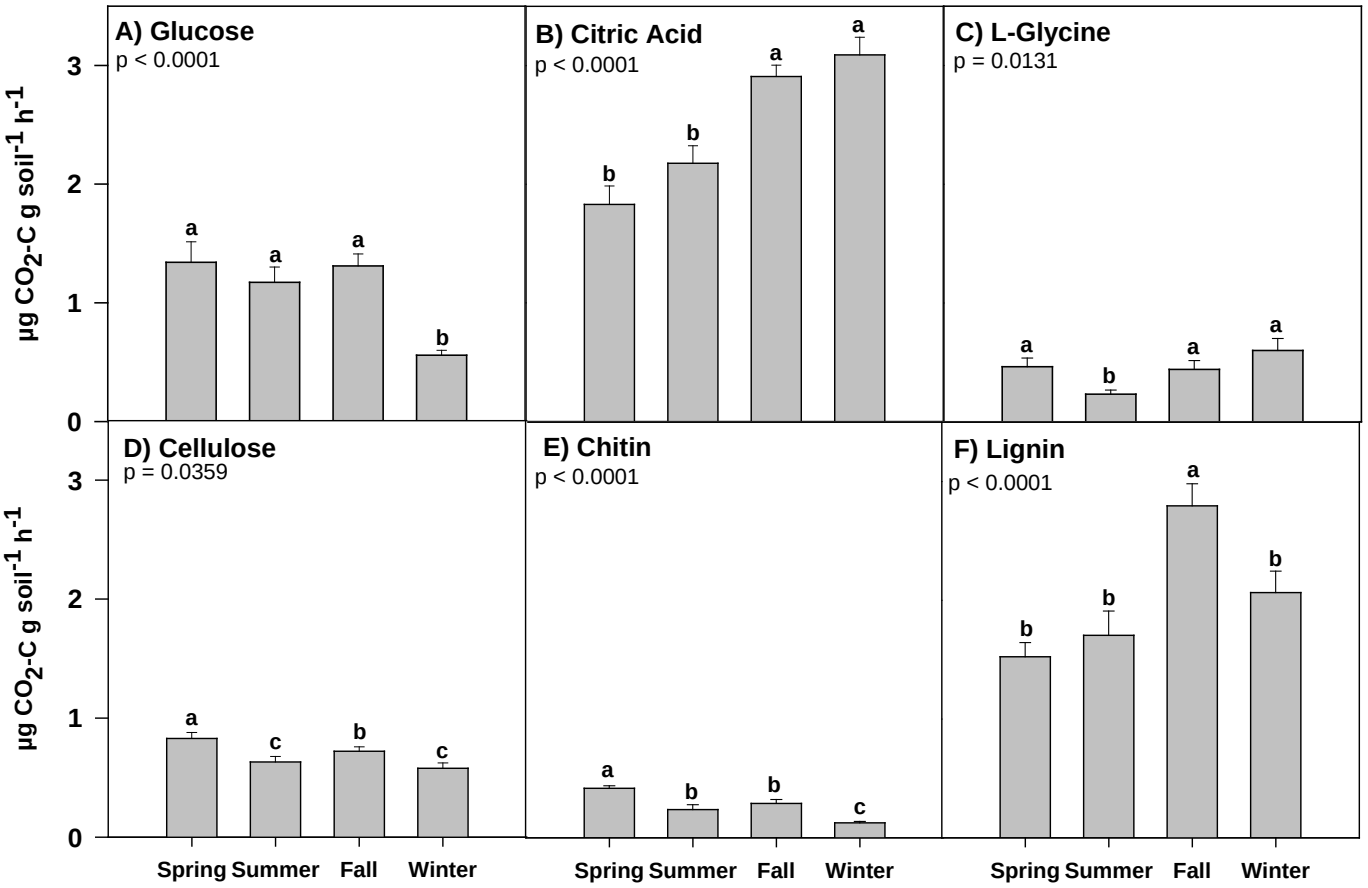

A) Glucose
p < 0.0001
B) Citric Acid
p < 0.0001
C) L-Glycine
p = 0.0131
D) Cellulose
p = 0.0359
E) Chitin
p < 0.0001
F) Lignin
p < 0.0001
µg CO2-C g soil-1 h-1
0
1
2
3
a
b
c
Spring
Summer
Fall
Winter

Figure 3.8. Catabolic response profile results showing the main effect of season for each substrate. Labile substrates are: A) Glucose; B) Citric Acid; and C) L-Glycine. Recalcitrant substrates are: D) Cellulose; E) Chitin; and F) Lignin. Values are microbial respiration induced by additions of each substrate on a per hour basis (µg CO_2-C g $soil^{-1}$ h^{-1}). Bars represent average ± 1 S.E., and within each panel, columns having no common letter are significantly different from each other.

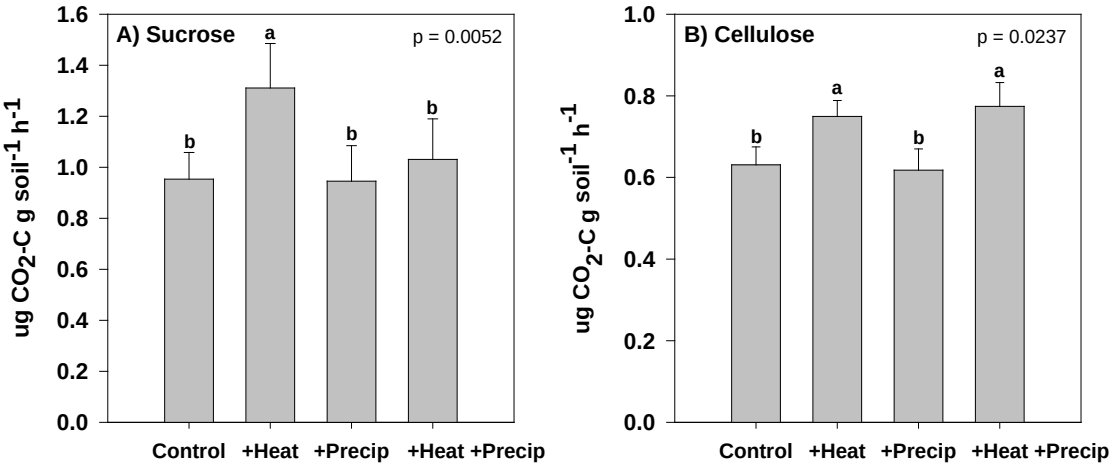


Figure 3.9. Catabolic response profile results showing the main effect of treatment for a labile substrate, (A) Sucrose, and a recalcitrant substrate, (B) Cellulose. Values for microbial response are presented as respiration induced by each substrate per hour (µg CO_2-C g^{-1} soil h^{-1}). Bars represent ± 1 S.E., and within each panel, columns having no common letter are significantly different.

3.6 Relationships Between Soil Microbial Community Structure and Function

To examine the relationships between structure and function of the soil microbial community, the PLFA results (community structure) and the results from exoenzyme activity assays and microbial responses to substrate additions (community function) were compared across seasons and treatments. For each PLFA NMS ordination, the corresponding extracellular enzyme activities and the catabolic responses to the various added substrates were added as potential environmental overlays that may correlate with the axes the ordination identified as explaining variability in the PLFA data (Figure 3.3C; Figure 3.4B,D,F,H). Significant correlations between individual FAMEs and these parameters may illustrate relationships between taxonomic groups of microbes and some aspect of function.

As described in Section 3.3, the NMS ordination and MRPP comparisons of the 20 most abundant lipid biomarkers for all samples collected during the study revealed significant separation of microbial community structure between seasons (Figure 3.3A; Table 3.3). The most highly correlated overlay variables for this ordination included exoenzymes BG, PHOS, Perox, LAP, and the CRP substrates, chitin and glucose (Table 3.5). Exoenzymes BG, PHOS, and Perox were correlated with the summer PLFA samples in the ordination (Figure 3.3A), when the activity of all three of these enzymes was highest. The overlay indicates that their activity was positively correlated to the abundance of FAME i17, a lipid biomarker for gram-positive bacteria (Figure 3.3C). Exoenzyme NAG and CRP substrates, glucose and chitin, were associated with spring PLFA profiles (Figures 3.3A,C), also when their activities/responses were highest, and were correlated with lipid biomarkers for gram-negative bacteria, cy17 and 18:1n5c

(Figure 3.3C). LAP was correlated with fall and spring PLFA samples and general biomarkers (16:0, 18:0). Interestingly, no exoenzymes or CRP substrates were correlated with the winter ordination space.

When examining the soil microbial community for each climatic treatment across seasons (Figure 3.4), MRPP revealed significant shifts in community structure between summer and winter, but similar communities in the spring and fall for each treatment (Table 3.5). In general, the FAMEs driving these lipid profile trends were similar across treatments (Appendix 3—6), and the correlated exoenzymes and CRP substrate responses also tended to be similar across treatments (Figures 3.4B,D,F,H), consistent with the fact that these parameters exhibited no significant season × treatment interaction in the ANOVA analyses (Tables 3.7, 3.9).

Functional parameters that were significantly correlated with ordination axes in every treatment included exoenzymes Perox and LAP, and the CRP substrate glucose (Figure 3.4B,D,F,H). In each treatment, the environmental vector for Perox was oriented towards the lipid biomarker for gram-positive bacteria, i16, and correlated with summer PLFA measurements. The environmental vectors for LAP and glucose correlated with summer PLFA measurements in the Control and +Precip plots, but for fall and spring, LAP and glucose correlated with PLFA measurements in the +Heat and +Heat+Precip plots. LAP activity was also associated with different individual FAMEs in the different treatments. For example, in the Control and +Heat plots, the environmental vector for LAP tended to point towards fungal biomarkers such as 18:2n6c and 18:1n9c, but in +Precip and +Heat+Precip plots, it tended to point towards non-specific bacterial biomarkers such as 18:0 and 16:0. The vector for glucose tended to be directed towards

the bacterial biomarker 16:0 (for all treatments except +Heat+Precip) and the fungal biomarker 18:2n6c (for +Heat and Control treatments) (Figure 3.4B,D,F,H).

A variety of exoenzymes and CRP substrates were not strongly correlated with the seasonal PLFA ordinations of specific treatments (Appendix 3—6). For example, the CRP substrate oxalic acid was not correlated with lipid profiles in the Control plots, and yeast was not correlated with either Control or +Heat ordinations. Chitin was absent from the +Precip ordination, and L-glycine was not correlated with the +Heat+Precip ordination. Citric acid utilization was correlated with Control and +Precip ordinations, but not +Heat and +Heat+Precip. The exoenzymes PHOS and BG were not correlated with +Heat plots, and NAG activity was not related to the lipid profiles of the +Precip or +Heat+Precip plots. However, correlated environmental vectors for the exoenzyme Phenox and the CRP substrate sucrose only appeared in +Heat+Precip plots. None of the seasonal ordinations for each treatment included correlated environmental vectors for CRP substrates cellulose and lignin, despite significant seasonal variation for these substrates (Table 3.9).

Citric acid and NAG were unique in that the environmental vectors correlated with different FAMEs between treatments when significant correlations occurred. In the Control plots, citric acid correlated with fall PLFA measurements but no specific FAMEs. In the +Precip plots, citric acid turned towards the winter PLFA measurements and gram-negative bacteria biomarkers such as 16:1n5c (Figure 3.4B,F). The vectors for NAG, while correlating with the spring and fall PLFA ordinations in both control and +Precip treatments, tended to point towards fungal biomarker 18:1n9c in Control plots but towards gram-negative bacteria biomarkers (18:1n7c, 18:1n5c) in +Heat plots.

Environmental vectors that showed consistent correlation with certain FAMEs or microbial taxonomic groups between treatments, included PHOS, BG, chitin, L-glycine, yeast, oxalic acid, sucrose, and Phenox. The vectors for PHOS and BG, where present, tended to point towards gram-positive and non-specific bacterial biomarkers i17 and 16:0 (Figure 3.4B,D,F,H). In both the Control and +Heat plots, the environmental vector for chitin was oriented towards fungal biomarkers such as 18:1n9c and correlated with the spring and fall PLFA measurements. The environmental vector for L-glycine, present in each treatment except +Heat+Precip, appeared to orient towards the lipid biomarker for gram-negative sulfate-reducing bacteria i17:1n7. Yeast also tended to correlate with i17:1n7. The vector for oxalic acid tended to point towards various gram-negative biomarkers (18:1n7c, 18:1n5c, cy 17, 11Me18:1) and to correlate with spring and fall PLFA measurements in +Heat and +Heat+Precip plots, but shifted towards summer PLFA measurements in the +Precip plots (Figure 3.4D,F,H). Unique to the +Heat+Precip plots, sucrose and Phenox correlated with the actinomycetes biomarker, 10Me18, and the gram-positive bacteria biomarker, a17 (Figure 3.4B,D,F,H).

Chapter 4: Discussion

In this study, seasonal patterns in the soil microbial community structure and function were more dramatic than effects produced by the climate change treatments. While every aspect of the soil microbial community assessed in this study exhibited significant seasonal variation, treatment effects were either relatively subtle or interacted with seasonal variation. Taken together, these results suggest the soil microbial community of this temperate Kentucky pasture may be quite resilient to potential future increases in heat and precipitation.

4.1 Seasonal Drivers of Soil Microbial Community Structure and Function

My first hypothesis, in which I expected to see significant differences across seasons in each measured microbial parameter, was verified by my data, but not always in the manner I had predicted. Prior work has shown that plant communities influence the soil microbial community through timing and composition of plant root exudates (Bever et al., 2012; Kardol et al., 2007; Scherber et al., 2010). My site, being temperate in climate, experiences distinct seasons of varying plant activity and community composition. Therefore, I expected to see significant seasonal variation in soil microbial community structure across all samples. This was observed, although for individual climate treatments, spring and fall communities were comparable, which is unusual given that there were distinct contrasts in plant community composition across treatments between spring (dominated by C3 grasses) and fall (dominated by C4 grasses) (Appendix 2). In spring the soil microbial community was composed of a mix of gram-negative and general bacteria. In summer, when the plant community was a mix of late growth C3 grasses, early growth of C4 grasses, and forbs, the soil microbial community was

dominated by gram-positive and general bacteria. While the fall plant community was composed largely of C4 grasses (as opposed to mainly C3 grasses in the spring), the soil microbial community composition was similar to that of spring, containing a mix of the previously mentioned bacterial groups. The soil microbial community was dominated by gram-negative bacteria in the winter, when plant activity was presumably lowest and no plant species harvest took place.

Although many studies consider C3 and C4 grasses as distinct plant functional groups (e.g., Burke et al., 1998; Morgan et al., 2011; Zak et al., 2003), in this system, C3 and C4 grasses appeared to provide similar feedbacks to the soil microbial community structure. This result contrasts with multiple studies that have observed differences in soil microbial communities associated with C3 and C4 grass-dominated plant communities, but most of these studies were conducted across multiple years and included plant community dynamics in response to altered CO_2 years in arid environments, where the impacts of CO_2 on water use and availability over time were potentially more influential than in a temperate environment with relatively abundant rainfall (Morgan et al., 2004; Morgan et al., 2011).

Other studies in long-term experiments have shown that plant diversity has significant effects on soil microbes, increasing microbial biomass and fungal abundance (Zak et al., 2003). However, Kowalchuk et al. (2002) demonstrated that while many studies assume that aboveground plant diversity drives belowground microbial diversity, this influence might be restricted to rhizosphere soil rather than the microbial community in bulk soil. Similar to my results, Marshall et al. (2011) found no effect of plant functional group on soil microbial community structure or substrate utilization, also using

bulk soil samples for analysis. The lack of distinction between the spring and fall soil microbial communities, despite large differences between plant functional composition in the spring and fall, suggests aboveground plant communities were not driving seasonal variation of soil microbial community structure at this site.

As hypothesized, microbial biomass expressed significant seasonal effects. However, the two measurements of this parameter (CFE and PLFA) provided different estimates of seasonal maxima and minima, presumably because the methods are measuring two different microbial components: carbon and lipids. PLFA measurements suggested summer maxima and winter minima, while CFE yielded spring/winter maxima and summer/fall minima. These results are in direct contrast to Bardgett et al. (1999b), who found spring maxima and fall minima with PLFA measurements, and summer maxima and winter minima with CFE at temperate grassland sites in the United Kingdom. Some studies have shown significant correlation between these two measurements of microbial biomass (Zelles et al., 1992; Zogg et al., 1997), while others have shown significant differences (Zak et al., 1996). Differences between the two methods might stem from the fact that the total amount of PLFA generally has a fast turnover rate and therefore, primarily reflects the amount of active microbial biomass (Tunlid et al., 1985; Tunlid and White, 1992; Zak et al., 1996), whereas CFE simply measures cytoplasmic microbial carbon in soil with no differentiation between pools of potentially active or dormant biomass (Paul and Clark, 1996b). My results suggest that, while microbial biomass as measured by CFE was highest in the spring and winter, the greatest active microbial community was present in the summer. Bardgett et al. (1999b) attributed spring maxima of microbial biomass to probable increases in soil temperatures,

root growth, and utilization of organic material accumulated over the winter, and such seasonal trends are supported by similar reports from other temperate grassland sites (Lovell et al., 1995; Sarathchandra et al., 1998; Ross et al., 1995). The summer maxima of microbial biomass measured at this site may also reflect increased soil temperatures (Figure 3.1A), although it is noteworthy that in general elevated temperature plots did not have enhanced microbial biomass over that measured in Control and +Precip plots.

4.2 Treatment Effects on the Soil Microbial Community

My second hypothesis, that I would find significant differences between climate treatments for each parameter, was largely unsupported by my data. I hypothesized that either heat or moisture stress would have some consistent effect on the soil microbial community despite seasonal variation. In this study, the only significant effects of climate treatments included increased microbial biomass C in warming treatments (+Heat and +Heat+Precip), and increased microbial response to CRP substrates sucrose and cellulose. In agreement with my second hypothesis, warming treatments with added moisture enhanced microbial biomass. Although this effect was only as measured by CFE, and warming only treatments did not exhibit reduced response due to water limitation. Also, I had predicted increased microbial response to recalcitrant substrates as a result of warming. I instead found increased response to one labile substrate (sucrose) and one recalcitrant substrate (cellulose) from warming treatments. Because I assayed microbial response to six labile substrates and three recalcitrant substrates, these results do not overwhelmingly suggest that warming treatments favored recalcitrant compounds over labile.

However, surprisingly, microbial community composition, soil extracellular enzyme activities, labile soil carbon, and seven out of nine catabolic substrates did not express significant treatment effects. Climate change effects on parameters such as enzyme activities and microbial lipid abundance have been overcome by year-to-year variation in other studies (Gutknecht et al., 2010; Gutknecht et al., 2012). As this study did not encompass multiple years, the effects of inter-annual variability remain unknown. It is possible seasonal variation at this temperate site would remain consistent across time or it may vary depending on yearly weather events.

4.3 Interactive Effects of Season and Treatment

My third hypothesis, that season would modify microbial response to climate treatments, was also largely unsupported by my data. The only parameter in this study that exhibited significant interactions between treatment and season was labile soil carbon, as measured by carbon mineralization assays. However, the only notable interactive effect was decreased labile soil carbon due to +Heat treatments but only in the winter. This interaction appeared relatively subtle, and could have resulted from variation within samples or precision of measurement associated with the technique.

While not explicitly analyzed for treatment x season interactions, results from the seasonal ordinations of PLFA with environmental overlay variables suggested that treatments did have some interactive effect on microbial community structure and function. When the seasonal effect of PLFA abundance and environmental overlay variables were examined within climate change treatments, some of the links observed between microbial community structure and function were altered by absence or presence

of certain correlated environmental variables or lipid biomarkers which were not observed in the seasonal ordination of all samples.

These results suggest that while no significant interaction between season and climate treatment was independently found in PLFA analysis, exoenzyme activities, or CRP, climate treatments may act subtly on each of these parameters to collectively influence the relationship between microbial community structure and function. This relationship merits more thorough exploration in future studies.

4.4 Functional Redundancy and Resilience of the Soil Microbial Community

Studies often utilize either extracellular enzyme activity or catabolic response profiles to assess changes in functional diversity or the capacity of the soil microbial community to degrade organic substrates for both microbial and plant utilization (Marx et al., 2001; Torsvik and Ovreas, 2002). It was surprising to find in this study no similarity in seasonal patterns of extracellular enzyme activity and catabolic response profiles, especially for enzymes and CRP substrates that should, intuitively, be related. For example, β-1,4, Glucosidase (BG) is known to release glucose residues from cellulose; β-1,4-N-Acetyl-glucosaminidase (NAG) is a chitinase, and Peroxidase (Perox) is widely recognized as a lignolytic enzyme (Table 2.1). Therefore, one might expect that the ability of the microbial community to utilize the substrates cellulose, chitin, and lignin over the year would parallel the measured activity of the enzymes BG, NAG and Perox, respectively, presuming said enzymes were responsible in part for digestion of these substrates. However, similar seasonal patterns in utilization and enzyme activity were not observed. Waldrop et al. (2000) also found extracellular enzyme activities did not correlate well with results from substrate utilization profiles. However, Waldrop et al.

(2000) assayed heterotrophic substrate utilization colorimetrically using BIOLOG microplates (Garland and Mills, 1991), rather than measuring CO_2 respiration response from incubation vessels (Degens and Harris, 1997) as I did in my study.

Because extracellular enzymes are often assayed using microplates, it is more convenient in some cases to also utilize a microplate method to assess substrate utilization profiles, such as BIOLOG plates (Garland and Mills, 1991). BIOLOG plates have been shown to account for only a small portion of the soil microbial community responsible for degradation of organic material (Smalla et al., 1998; Torsvik and Ovreas, 2002), while microplate methods for extracellular enzyme activities are thought to be a more useful indicator of functional changes in microbial communities, especially in relation to community composition (Waldrop et al., 2000; Torsvik and Ovreas, 2002). In addition, multiple studies have shown that unlike extracellular enzymes, substrate utilization results from BIOLOG plates often do not correlate well with PLFA results (Buyer and Drinkwater, 1997; Waldrop et al., 2000; Torsvik and Ovreas, 2002), but in this study, various extracellular enzymes and CRP substrates showed multiple correlations with seasonal PLFA ordination data across treatments. Because there are no known studies assessing both extracellular enzyme activity and substrate utilization using the same combination of microplate assays for enzyme activities and CO_2 efflux for catabolic response techniques used in this study, I can only speculate as to why seasonal variation of extracellular enzyme activities and catabolic response profiles did not show parallels.

My results suggest extracellular enzymes and catabolic response profiles accounted for separate fractions of the microbial communities' ability to breakdown and

utilize related substrates, which varied independently of each other on a seasonal basis. This indicates to me that this site exhibits a high degree of functional redundancy, i.e., multiple organisms are able to perform the same tasks in an ecosystem, contributing to the stability and resilience of the soil microbial community to perturbations, such as climate change may present (Brady and Weil, 2002). Soil microbial communities use functional redundancy to buffer biotic and abiotic stress and maintain ecosystem services, a concept becoming increasingly important in research dedicated to improving soil stability and ecosystem response to change (Griffiths and Philippot, 2012).

Functional redundancy in the microbial community at this site may also be observed in the measured patterns of microbial utilization of cellulose and lignin. Despite significant seasonal effects on microbial utilization of cellulose and lignin, neither parameter was well correlated with any of the seasonal PLFA overlays; even though lignin exhibited relatively high catabolic responses for each season. This suggests soil microbes at this site possessed the capacity to utilize lignin and cellulose, and this capacity varied seasonally but was not strongly linked to any specific taxonomic group of microorganisms, as determined by PLFA. Multiple microorganisms may contribute to these functions at this site at different times in the year.

The contribution of functional redundancy to the resilience of the soil microbial community at this site potentially explains the lack of response to climate treatments in this study. Resiliency is the ability of an ecosystem to resume relatively normal functions after a perturbation (Scheffer and Carpenter, 2003). In this study, I considered increases in heat and precipitation to be possible perturbations to the soil microbial community that may result from future climate change. With the exception of a slight increase in

microbial biomass carbon and increased microbial utilization of sucrose and cellulose additions, there were no effects of climate treatments on microbial community composition or function. The resistance, resiliency and functional redundancy of the soil microbial community was recently reviewed by Allison and Martiny (2008), who found broad microbial taxa are generally not immediately resilient to disturbance. Allison and Martiny (2008) also showed the average length of studies finding soil microbes to be resistant to change in temperature lasted up to 2 years, while studies finding soil microbes to be sensitive to changes in temperature lasted up to 8 years. As such, the timeframe of microbial response can vary greatly. Because climate treatments have been in place for almost three years at this site, it is possible that either a) the soil microbial community is very resilient and has already resumed normal composition and function; or b) the soil microbial community has resisted climate treatments thus far, and more dramatic changes to increased heat or precipitation may be seen if the study is continued. Additional years of data would help answer these questions.

4.5 Altered Relationships Between Microbial Community Structure and Function

Despite the overwhelming influence of seasonal variation on the soil microbial community in this study, the effect of climate change treatments raised some interesting questions about microbial community structure and function. The fact that the soil microbial community exhibited little response to increased heat and precipitation with respect to active microbial biomass, community structure, extracellular enzyme activities, or catabolic response profiles suggests the interactive effects of biotic and abiotic feedbacks at this site may enable soil microbes to withstand stresses induced by climate change. Apparent lack of correlation or similar seasonal patterns between extracellular

enzyme activity and catabolic response profiles, both of which influence the capacity of the soil microbial community to utilize nutrients and organic material, implies functional redundancy may be one of the ways soil microbes mitigate stress induced by climate treatments. In addition, changes in the relationship between certain exoenzymes, CRP substrates, and seasonal ordination of microbial lipids either examined as a whole or when divided by climate treatment, promotes the need for further research evaluating microbial community structure and function and the nature of their relationship in response to environmental stress.

Chapter 5: Conclusions

This thesis assessed the seasonal soil microbial community structural and functional responses to increased temperature and precipitation in a Kentucky pasture ecosystem. In this study, seasonal variation was the primary driver of changes in the soil microbial community. Active microbial biomass was highest in the summer and lowest in the winter. Relative abundance of microbial lipid biomarkers across all samples indicated that the soil microbial community was dominated by a mixture of gram-negative and non-specific bacteria in the spring, gram-positive and non-specific bacteria in the summer, an overlapping mix of the previously mentioned bacterial groups in the fall, and a primarily gram-negative bacteria community in the winter. Activity of extracellular enzymes (β-1,4, Glucosidase, BG; β-1,4-N-Acetyl-glucosaminidase, NAG; Leucine amino peptidase, LAP; Acid phosphatase, PHOS; Phenol oxidase, Phenox; and Peroxidase, Perox) were generally highest in the spring or summer. Seasonal dynamics of catabolic response to substrates (glucose, sucrose, oxalic acid, citric acid, L-glycine, autolysed yeast, cellulose, lignin, and chitin) were highly variable and exhibited few generalities.

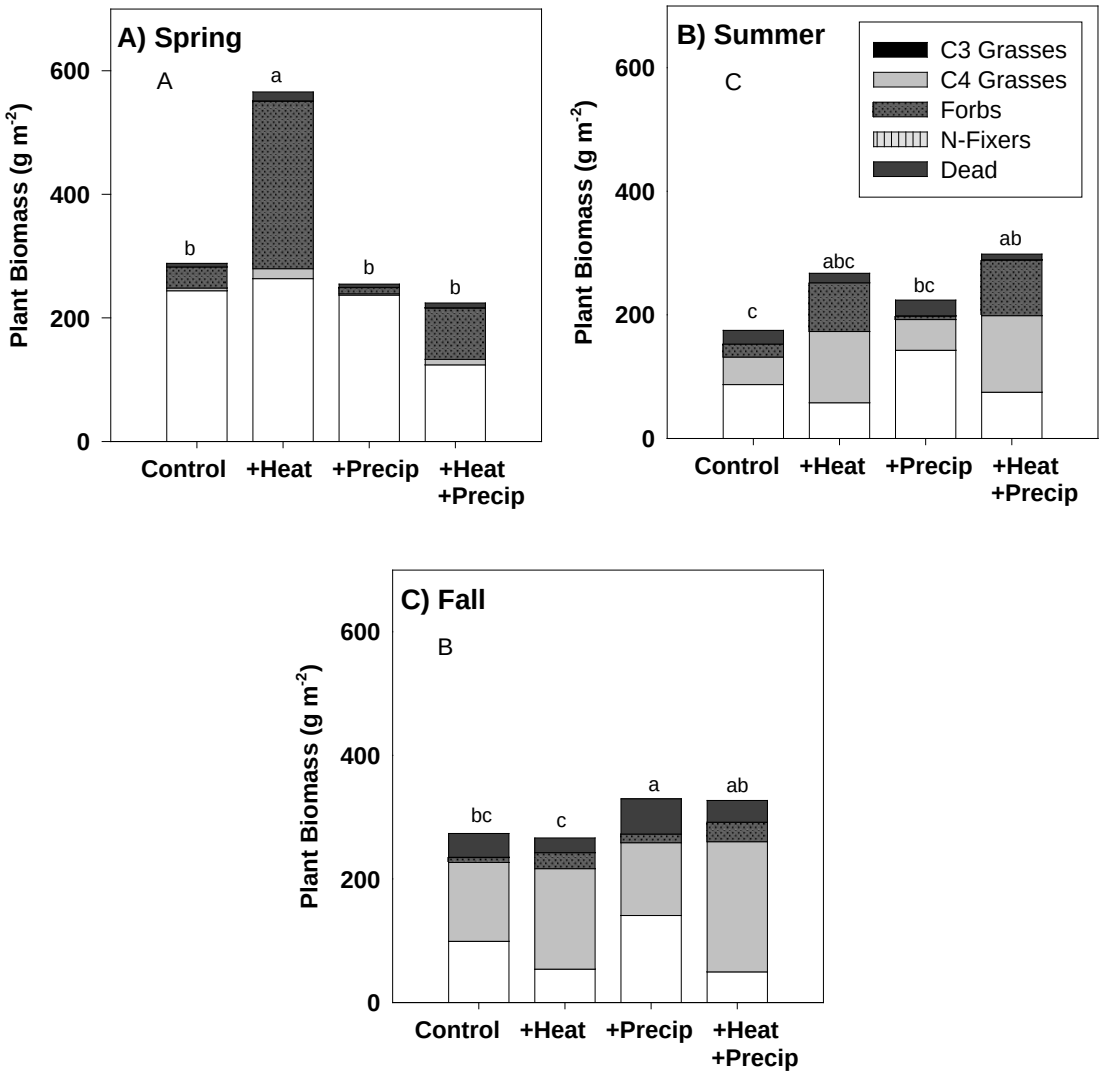
Because seasonal dynamics of enzyme activities and catabolic response profiles were dissimilar, my data suggest that the soil microbial community of this site contains a high degree of functional redundancy, which allows soil microbes to utilize various nutrients and organic materials during times of stress. Because microbial response to seasonal variation largely overwhelmed treatment effects in this study, I conclude that the soil microbial community is quite resilient to climate change factors,

However, some changes in the soil microbial community due to climate treatments were observed, such as increased microbial biomass carbon and increased catabolic response to sucrose and cellulose due to warming treatments. In addition, examination of microbial lipid abundance across seasons for individual climate treatments yielded different functional relationships with community structure than when lipid abundance was examined by season alone. These results lead me to conclude that, given the varying timeframe of microbial responses in other studies, more pronounced effects could arise over time due to potential changes in carbon dynamics and substrate utilization. These changes could further affect the aboveground plant community through alterations in available soil carbon and nutrients. Also, future changes in climate could alter specific relationships between soil microbial community structure and function, such as between microbial taxonomic groups and the functional processes they regulate.

More long-term, multifactor research is required to further investigate the relationship between microbial community structure and function and how the response to climate change may transform over time. This type of research will lead to more thorough understanding of ecological feedbacks in the soil microbial community and how they potentially affect both above and belowground ecosystem responses to change.

Appendix 1. Significance tests for total aboveground plant biomass (g m^{-2}) produced and collected over the sampled seasons of this project and climate treatments. Bolding indicates statistical significance ($P < 0.05$).

Effect		**Aboveground plant biomass**	
	DF n, d	F	P
Season	2,11	13.2	**0.0012**
Treatment	3,12	6.16	**0.0089**
Treatment*Season	6,13	24.21	**<0.0001**



Appendix 2. Aboveground plant biomass for the various functional groups (g m^{-2}; C3 Grasses, C4 Grasses, Forbs, N-Fixers, and Dead) produced in 2011, collected during each seasonal harvest (A - Spring, May 2011; B - Summer, July 2011; C - Fall, October 2011). Column height within each panel represents total biomass for each treatment, and the shaded areas within each column represents the amount of each plant functional group comprising the total (g m^{-2}). Although a significant treatment*season interaction was found, for ease of interpretation, the main effect of season for total biomass is represented by capital letters between panels ($P < 0.05$). Within panels, the effect of treatment for total biomass is indicated by small letters ($P < 0.05$).

Appendix 3. FAMEs driving NMS ordination of seasonal effects for the Control plots (Figure 3.5 A, B). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, Control

Axis 1 (r^2=0.22)			**Axis 2 (r^2=0.70)**		
FAME	r-value	Classification	FAME	r-value	Classification
a15	0.672	Gram-positive bacteria	16:1n7c	0.846	Gram-negative bacteria
i15	0.607	Gram-positive bacteria	16:1n9c	0.813	Gram-negative bacteria
i16	0.559	Gram-positive bacteria	16:1n5c[1]	0.803	Gram-negative bacteria
18:1n9c	-0.575	Fungi	18:1n7c	0.787	Gram-negative bacteria
cy19	-0.676	Gram-negative bacteria	a17	0.578	Gram-positive bacteria
			11Me18:1	0.572	Gram-negative bacteria
			18:1n5c	0.517	Gram-negative bacteria
			cy19	-0.579	Gram-negative bacteria
			a15	-0.599	Gram-positive bacteria
			i15	-0.707	Gram-positive bacteria
			i17	-0.765	Gram-positive bacteria
			18:0	-0.814	Non-specific bacteria
			16:0	-0.842	Non-specific bacteria

Appendix 3 (cont'd). FAMEs driving NMS ordination of seasonal effects for the Control plots (Figure 3.5 A, B). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, Control					
Axis 1 (r^2=0.22)			**Axis 2 (r^2=0.70)**		
Overlay	r-value	Classification	Overlay	r-value	Classification
Citric Acid[3]	0.407	Carboxylic acid	L-Glycine[3]	0.417	Amino acid
LAP[2]	-0.476	Exoenzyme	Glucose[3]	-0.536	Simple sugar
NAG[2]	-0.588	Exoenzyme	Perox[2]	-0.658	Exoenzyme
Chitin[3]	-0.674	Recalcitrant Carbon	PHOS[2]	-0.694	Exoenzyme
			LAP[2]	-0.755	Exoenzyme
			BG[2]	-0.757	Exoenzyme

[1]Has also been used as a biomarker for arbuscular mycorrhizal fungi.

[2] Assayed extracellular enzymes, (BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase).

[3]Substrate used in Catabolic Response Profiling.

Appendix 4. FAMEs driving NMS ordination of seasonal effects for +Heat plots (Figure 3.5 C, D). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Heat

Axis 1 (r^2=0.33)			**Axis 2 (r^2=0.62)**		
FAME	r-value	Classification	FAME	r-value	Classification
i15	0.874	Gram-positive bacteria	16:1n5c[1]	0.916	Gram-negative bacteria
i16	0.613	Gram-positive bacteria	16:1n7c	0.900	Gram-negative bacteria
cy17	-0.535	Gram-negative bacteria	16:1n9c	0.854	Gram-negative bacteria
i17:1n7	-0.806	*Desulfovibrio* and anaerobes	a15	0.573	Gram-positive bacteria
18:1n7c	-0.855	Gram-negative bacteria	cy17	-0.528	Gram-negative bacteria
			18:2n6c	-0.572	Fungi
			16:0	-0.714	Non-specific bacteria
			18:0	-0.761	Non-specific bacteria
			cy19	-0.946	Gram-negative bacteria

Appendix 4 (cont'd). FAMEs driving NMS ordination of seasonal effects for +Heat plots (Figure 3.5 C, D). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Heat					
Axis 1 (r^2=0.33)			Axis 2 (r^2=0.62)		
Overlay	r-value	Classification	Overlay	r-value	Classification
Perox[2]	0.622	Exoenzyme	L-Glycine[3]	0.435	Amino acid
NAG[2]	-0.463	Exoenzyme	Chitin[3]	-0.589	Recalcitrant Carbon
Oxalic Acid[3]	-0.716	Carboxylic acid	Glucose[3]	-0.669	Simple sugar
			LAP[2]	-0.680	Exoenzyme

[1]Has also been used as a biomarker for arbuscular mycorrhizal fungi.
[2] Assayed extracellular enzymes, (BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase).
[3]Substrate used in Catabolic Response Profiling.

Appendix 5. FAMEs driving NMS ordination of seasonal effects for +Precip plots (Figure 3.5 E, F). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Precip

Axis 1 (r^2=0.41)			**Axis 2 (r^2=0.53)**		
FAME	r-value	Classification	FAME	r-value	Classification
i17	0.849	Gram-positive bacteria	18:0	0.796	Non-specific bacteria
i16	0.793	Gram-positive bacteria	cy19	0.790	Gram-negative bacteria
16:0	0.788	Non-specific bacteria	18:1n5c	0.616	Gram-negative bacteria
i15	0.601	Gram-positive bacteria	18:2n6c	0.537	Fungi
cy19	0.546	Gram-negative bacteria	i15	-0.588	Gram-positive bacteria
18:0	0.509	Non-specific bacteria	16:1n7c	-0.669	Gram-negative bacteria
16:1n5c[1]	-0.562	Gram-negative bacteria	a15	-0.682	Gram-positive bacteria
i17:1n7	-0.579	*Desulfovibrio* and anaerobes	16:1n5c[1]	-0.733	Gram-negative bacteria
16:1n9c	-0.587	Gram-negative bacteria	16:1n9c	-0.794	Gram-negative bacteria
18:1n9c	-0.660	Fungi			
16:1n7c	-0.715	Gram-negative bacteria			
18:1n7c	-0.769	Gram-negative bacteria			

Appendix 5 (cont'd). FAMEs driving NMS ordination of seasonal effects for +Precip plots (Figure 3.5 E, F). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Precip

Axis 1 (r^2=0.41)			**Axis 2 (r^2=0.53)**		
Overlay	r-value	Classification	Overlay	r-value	Classification
BG[2]	0.670	Exoenzyme	Yeast[3]	-0.501	Biological substrate
Oxalic Acid[3]	0.641	Carboxylic acid	Citric Acid[3]	-0.633	Carboxylic acid
PHOS[2]	0.604	Exoenzyme			
Glucose[3]	0.569	Simple sugar			
Perox[2]	0.556	Exoenzyme			
LAP[2]	0.536	Exoenzyme			
Yeast[3]	-0.420	Biological substrate			
L-Glycine[3]	-0.580	Amino acid			

[1]Has also been used as a biomarker for arbuscular mycorrhizal fungi.
[2] Assayed extracellular enzymes, (BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase).
[3]Substrate used in Catabolic Response Profiling.

Appendix 6. FAMEs driving NMS ordination of seasonal effects for +Heat+Precip plots (Figure 3.5 G, H). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is indicated. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Heat+Precip (Figure 3.5 G, H)					
Axis 1 (r^2=0.32)			Axis 2 (r^2=0.58)		
FAME	r-value	Classification	FAME	r-value	Classification
i16	0.898	Gram-positive bacteria	18:0	0.874	Non-specific bacteria
i15	0.808	Gram-positive bacteria	cy19	0.874	Gram-negative bacteria
i17	0.789	Gram-positive bacteria	16:0	0.658	Non-specific bacteria
16:0	0.641	Non-specific bacteria	i17	0.572	Gram-positive bacteria
a15	0.606	Gram-positive bacteria	a15	-0.560	Gram-positive bacteria
11Me18:1	0.532	Gram-negative bacteria	16:1n5c[1]	-0.856	Gram-negative bacteria
a17	0.521	Gram-positive bacteria	16:1n9c	-0.876	Gram-negative bacteria
18:2n6c	-0.631	Fungi	16:1n7c	-0.898	Gram-negative bacteria
18:1n9c	-0.855	Fungi			

Appendix 6 (cont'd). FAMEs driving NMS ordination of seasonal effects for +Heat+Precip plots (Figure 3.5 G, H). The most correlated FAMEs (r > 0.500) with each ordination axis are shown, as well as the most correlated environmental overlay variables (r > 0.400). The amount of variation explained by each axis (r^2) is in**dicated**. Positive r-values for each FAME and Overlay correlate with the right-most area of Axis 1 or the upper-most area of Axis 2. Negative r-values for each FAME and Overlay correlate with the left-most area of Axis 1 or the bottom-most area of Axis 2. For FAMEs, Classification indicates the microbial taxonomic group for which each FAME is used as a lipid biomarker, whether the environmental overlay variable used was an extracellular enzyme (exoenzyme), or the type of compound used as a substrate.

NMS, +Heat+Precip (Figure 3.5 G, H)

Axis 1 (r^2=0.32)			**Axis 2 (r^2=0.58)**		
Overlay	r-value	Classification	Overlay	r-value	Classification
Perox[2]	0.603	Exoenzyme	LAP[2]	0.685	Exoenzyme
BG[2]	0.574	Exoenzyme	Oxalic Acid[3]	0.538	Carboxylic acid
Phenox[2]	0.527	Exoenzyme	Chitin[3]	0.485	Recalcitrant carbon
Sucrose[3]	0.441	Simple sugar			
PHOS[2]	0.432	Exoenzyme			
Yeast[3]	-0.472	Biological substrate			
Glucose[3]	-0.521	Simple sugar			

[1]Has also been used as a biomarker for arbuscular mycorrhizal fungi.
[2] Assayed extracellular enzymes, (BG = β-1, 4, Glucosidase; NAG = β-1, 4-N-Acetyl-glucosaminidase; LAP = Leucine amino peptidase; PHOS = Acid phosphatase; Phenox = Phenol oxidase; and Perox = Peroxidase).
[3]Substrate used in Catabolic Response Profiling.

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Lindsey Slaughter and Paula Gale. Practicality and Procedures for Composting Institutional Waste Streams. Poster presentation at the 2009ASA-CSSA-SSSA International Annual meetings. November 2, 2009 in Pittsburgh, PA. Available online http://a-c-s.confex.com/crops/2009am/webprogram/Paper53599.html.

Honors and Awards
- First place speaker, National Student Research Symposium Oral Contest, Session 1, American Society of Agronomy-Crop Science Society of America-Soil Science Society of America international annual meetings in Long Beach, California (2010).
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