Soil and microbial response to manipulated precipitation and land management

by

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B.S., Lincoln University (MO), 2012
M.S., Kansas State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

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Abstract

Microorganisms play a vital role in maintaining plant and soil health. Within soils, microorganisms are responsible for nutrient cycling and organic matter decomposition. Because soil microbes are sensitive to environmental conditions, they are early indicators of changes in soil health. Soil microbes depend heavily on moisture and substrate availability for growth and survival. Limitation of either factor can alter the survival rates and microbial community function. The objectives of this study were to understand better soil microbial responses to manipulated precipitation and land management by (i) assessing the microbial community composition and soil properties after over 25 years of irrigation in annually burned native prairie; (ii) assessing the effect of long-term irrigation on microbial community respiration response to moisture and substrate addition; and (iii) assessing the soil microbial community and soil structure of degraded agricultural soil under conventional tillage sorghum (CT), no-till sorghum (NT), and replanted big bluestem (RP). The first two objectives were based on a long-term study in a tallgrass prairie where ambient and irrigated transects have been maintained for 25 years. Soil samples were collected four times per year and assessed for soil chemical properties and microbial community structure. The third study used a long-term (17-y) study of ecosystems (replanted big bluestem, and no-till and tilled sorghum). Soil C and N, aggregate structure and microbial community structure were measured. Contrary to previous research, long-term irrigation did not significantly impact the overall microbial community; however, there was a seasonal effect on the microbial community. Fungal PLFA biomarkers increased at the end of each growing season. Because greater soil water content and carbon inputs are known to contribute to fungal dominance, this increase can generally be attributed to seasonal plant growth cycles. Bacterial PLFA biomarkers peaked during the middle of the plant growth cycle

indicating the influence of plant inputs on microbial growth. Microbial response to water and glucose addition in the laboratory was significant, indicating a historic effect of irrigation on community composition. For the third objective, soil macroaggregate formation was directly correlated to changes in land management. Macroaggregate fractions were greatest in replanted big bluestem soils, followed by no till and conventional till grain sorghum. No significant differences in soil organic carbon or total nitrogen were observed between ecosystems. Microbial biomass was greatest in RP soils. However, no significant differences were observed between NT and CT soils. This indicates that reduced tillage increased soil aggregation. Because microbial properties are controlled by vegetative growth, as well as soil properties and land management, incorporating known soil health improvement practices may allow nutrient resources and soil structure to improve toward near prairie soil health. Additional research is needed to define further linkages between microbial community composition, microbial function, and overall soil health.

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Approved by:

Major Professor
Dr. Charles Rice

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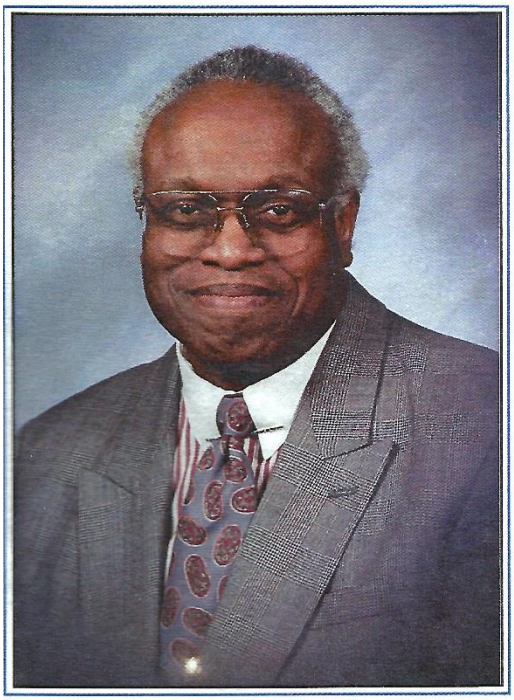
Finally, I would like to thank my husband, Kevin, and our daughters, Kaylee and Karlee, for their love, support, and sacrifice. You three mean the world to me. I love you more than words can say!

Dedication

This dissertation is dedicated to the memory of my grandfather

Donald E. Carter, Sr.

I hope you are still proud.



Chapter 1 - General Introduction

Tallgrass prairie once covered over 10% of the continental US (Samson and Knopf, 1994; Fierer et al., 2013). As agriculture expanded across the continent, fertile prairie soils were converted to cropping systems and livestock production (Stephens et al., 2008; Fargione et al., 2009; Wright and Wimberly, 2013). Today, uncultivated grassland soils occupy a small portion of US land mass, but contribute to over 30% of soil organic carbon (Sombroek et al., 1993; FAO and ITPS, 2015; Conant et al., 2017) and over 40% of soil inorganic carbon (Guo et al., 2006). Because prairie soils are often relatively high in organic matter, nutrient efficient, and water conservative, these soils are known to be highly fertile.

Grasslands serve a variety of functions including feed for livestock production and the storage of significant quantities of carbon within their soil. Perennial grasses are known to distribute a significant amount of carbon to their roots (CAST, 2004; Ma et al., 2014). This results in a high rate of C transfer from roots to the soil. The fine roots of perennial grasses contribute to soil aggregate formation via root exudates and physical binding. Grasslands are known to have intricate root systems that promote microbial activity, retain soil moisture, protect and retain nutrients via soil aggregates, and promote the formation of soil organic matter (Hinsinger et al., 2009; Kong and Six, 2010; McSherry and Ritchie, 2013). Due to their highly fertile and sustainable nature, grassland soils are often used as a benchmark for both soil health and agricultural land restoration.

Water Efficiency of the Prairie

Grasslands are known to be adaptable and are often resilient to water stress (Williams, 2007). Soil water is often a primary limiting factor in both agricultural and grassland ecosystems as available soil water is required for plant growth (Debaeke and Aboudrare, 2004; Ciais et al.,

2005; Mueller et al., 2010). In agricultural systems, available soil water is crucial for productivity, as crop yields are correlated with available water in soils (Jones et al., 2009; Shaxson, 2009; Mueller et al., 2010). The retention of plant residue can slow water run-off and assist the soil in retaining water, thereby contributing resilience in areas susceptible to drought conditions (De Vita et al., 2007; Turmel et al., 2015). De Vita et al. (2007) found that soil water content was significantly greater under no-tillage systems, indicating reduced water evaporation as compared to the conventional tillage system. Crop production and soil health are threatened by drought (Mishra and Singh, 2010). Nutrient cycling is ultimately impacted by desiccation as reduced available water for plants and microbial communities reduce productivity.

Drought impacts C cycling by reducing plant productivity, and subsequent transfer to the soil as biomass and root exudates. Drought conditions also impact soil nutrient availability by reducing the delivery of dissolved nutrients. This, in turn, reduces microbial activity. It is well documented that microbes mediate soil nutrient cycling (Van Der Heijden et al., 2008; Hobbie and Hobbie, 2013). Soil water plays a key role in the survival of microbes. Because of the broad functional capacities of diverse soil microbes, a soil microbial community can retain function during highly variable soil water conditions, though this may come at a physiological cost.

Prairie Plant Productivity

In addition to their sustainable and fertile nature, prairies are known to be diverse ecosystems. Studies have indicated that grassland species richness and productivity are positively correlated (Roscher et al., 2005). For example, Bullock et al. (2007) found that restored grasslands with greater species diversity had higher yields than grasslands with less species diversity. After eight years, the more diverse plots averaged 43% higher yield than the less diverse plots (Bullock et al., 2007). The amount of carbon stored within the soil is a function of

various factors including soil texture, soil mineralogy, soil disturbance, soil microbial activity, decomposition of root exudates, and plant biomass inputs (Torn et al., 2009; Lange et al., 2015). Thus, increases in carbon storage as a result of plant diversity represent increased productivity or slower microbial decomposition, or both (Jastrow et al., 2007; Torn et al., 2009).

Increased inputs of plant biomass provide substrate for soil microbes, enhancing community function and abundance (Lange et al., 2015). Though microbial communities are known to shift in response to nitrogen (N) and phosphorus (P) addition, the underlying mechanisms behind these shifts are not fully understood. However, several studies have suggested mechanistic explanations, such as increased plant inputs enhancing carbon storage and microbial activity via microbial necromass accumulation (Liang et al., 2011; Lange et al., 2015). Other studies have suggested that increased plant biomass inputs augment the decomposition of existing soil carbon pools via the positive priming effect (Guenet et al., 2010; Kuzyakov, 2010; Zimmerman et al., 2011). Though there is no single explanation for increased fertility and productivity in the prairie, the combination of plant species diversity and microbial community diversity are contributing factors.

Soil Microbial Community

Microorganisms play a vital role in maintaining plant and soil health. Within soils, microorganisms are responsible for the decomposition of organic materials and nutrient cycling (Hobbie and Hobbie, 2013). Soil microorganisms are some of the early indicators of changes in soil health, as microbes are sensitive to changes in soil condition (Murphy et al., 2016). The growth and survival of soil microbes depend upon a variety of factors including soil moisture and nutrient substrate availability. The limitation of any of these factors can alter the survival rate and mechanisms of the microbes affected. Like plant systems, most microbial communities

are limited by one or more essential growth factors. Soil microbes are often limited by either substrate availability, water availability, or both.

Microbial growth and activity depend heavily upon substrate availability. Microbial growth can only continue as long as adequate substrate is available as an energy source to fuel the community. In a system unlimited by substrate availability, microbes within the community breakdown complex organic compounds into smaller, usable compounds, transport them into the cell and use the nutrients to conduct a series of internal reactions resulting in the formation of new biomass (Lynch and Hobbie, 1988; Hobbie and Hobbie, 2013). It is likely that microbes within the soil are in a constant state of a "starving-survival lifestyle" (Hobbie and Hobbie, 2013). Several studies, across various ecosystems, have demonstrated that substrate limitation reduces microbial activity (Allen and Schlesinger, 2004; Demoling et al., 2007). Microbes have adapted various physiological techniques for survival under substrate limited conditions. Some bacterial species can tolerate these periods by entering a dormant or low activity state. When substrate is added, they return to their metabolically active state (Hobbie and Hobbie, 2013). Some bacterial species form endospores to protect DNA from destruction during unfavorable conditions (Nicholson et al., 2000). Some fungi form symbiotic relationships with plants to acquire a C source. These fungal species generally receive a photo-synthetically derived carbon source in exchange for extending fungal hyphae to seek out plant required nutrients.

Fluctuation in soil water content is a common occurrence in soils. The soil water potential is related tothe amount of energy a microbial cell must expend to obtain water from the soil (Voroney and Heck, 2015). Aerobic microbial activity is generally optimal when 30-50% of the soil pore space is filled with water and microbes must match the environmental water potential to survive (Harris, 1981; Voroney and Heck, 2015). Different microbial community

members may respond uniquely to environmental conditions. Some microbes respond to a reduction in water potential by accumulating compatible solutes and osmolytes within the cell, such as glycerol, amino acids, or mannitol (Estop-Aragonés and Blodau, 2012; Kakumanu and Williams, 2014; Voroney and Heck, 2015). Other microbial groups adjust the properties of their cell wall to prevent osmotic shock as a result of drying/rewetting cycles (Voroney and Heck, 2015). Under normal conditions, gram-negative bacterial cells, due to the thinner nature of their cell walls, are unable to withstand high pressure compared to the thicker-walled gram-positive bacteria. A sudden change in pressure, due to a wetting event, would cause gram-negative bacterial cells to burst. Adjustments to match the environmental water potential are made at a cost to the microbe. Rather than using energy for growth and reproduction, energy is expended making solutes or other physiological changes necessary for survival. As water availability is reduced by drought, substrate access is also reduced (Stark and Firestone, 1995).

In addition to water and substrate, soil microbial communities may also be impacted by nutrient limitation. More specifically, soil microbial activity is highly dependent upon N and P. Nitrogen is required for enzyme activity, p[rotein synthesis, and the synthesis of nucleic acids. Phosphorus is required for microbial cell growth and metabolism (Hartman and Richardson, 2013). Varying N and P levels in soils is a direct function of ecosystem N and P sources (Vitousek et al., 2010). Griffiths et al. (2012) reported that C:N:P ratios of microbial biomass were stable at optimum soil conditions, indicating that changes in substrate ratios or soil water may impact soil microbial activity.

Extreme Weather Events

Extreme weather events are a global environmental challenge. The International Panel on Climate Change (IPCC) has released reports that summarize evidence of scientific consensus that

weather patterns are changing as a result of human activity. More specifically, the changes are due to anthropogenic greenhouse gas emissions (Oreskes, 2005).In 2016, it was estimated that agriculture was responsible for 8.6% of total U.S. greenhouse gas emissions (US Environmental Protection Agency (EPA), 2018). More specifically, land management, enteric fermentation, crop cultivation, fertilization, liming, and manure management particularly impact greenhouse gas emissions (CAST, 2004; US Environmental Protection Agency (EPA), 2018). Outside of the agriculture sphere, other human activities such as transportation, industry, energy, and alteration of land use also contribute to greenhouse gas emissions. The gases of primary concern include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O).

Among other indications, changing weather patterns are expected to alter precipitation patterns. Geographic trends indicate that precipitation in the northern hemisphere will likely increase with greater amounts of precipitation falling within shorter time periods (IPCC, 2007; Hatfield et al., 2013). Intense thunderstorms and rapid runoff should be expected as a result of changing precipitation patterns (Easterling et al., 2000; Knapp et al., 2002; IPCC, 2007). Intense storms and rapid runoff may also be expected to offset precipitation projections, leading to less available soil water via flash flooding. Drought-like conditions can be expected due to long periods of time between precipitation events. Additionally, global temperature has consistently increased by 0.2°C (Hansen et al., 2006) and will continue to increase globally (IPCC, 2007). This is noteworthy because global temperature increases may alter plant growing seasons, alter microbial activity, and influence soil water retention thus influencing terrestrial ecosystem function.

There are a variety of factors that can impact terrestrial ecosystems and microbial activity, but water availability is a primary control of all biota. There is an intrinsic network

within terrestrial ecosystems composed of microorganisms, plants, and soil that is physically connected by soil pore water. An increase in precipitation variability will impact the overall function of microbial communities and ultimately impact terrestrial ecosystems (Chimner and Welker, 2005; Harris, 1981; Manzoni et al., 2012a). The effects of changing weather events are often linked to soil health (Doran, 2002; Lal, 2004; Gray and Bishop, 2016). Healthy soils are vital to our ability to combat global climate issues and sustain food production systems.

Soil Health

Soil health, often synonymous with soil quality, has been defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). The definition implies that the ability of a soil to perform depends upon the ecosystem boundaries or limitations placed upon the soil by the ecosystem. Those boundaries can be expounded by soil management and amendment. However, land management and soil amendments often have short-term effects on vital soil properties. Admittedly, it can be difficult to determine an exact assessment of soil quality due to the varied range of agricultural soils across the US (Stocking, 2003). It is, therefore, important that an assessment of soil health or quality encompasses the evaluation of soil characteristics reflective of the soil's intended function and planned management practices (Bünemann et al., 2018).

There is overwhelming evidence that soil health, soil physicochemical properties, and soil microbial community structure and function are related (Doran and Zeiss, 2000; Doran, 2002; Bastida et al., 2008). Several soil health indicators have been suggested to assess soil quality and infer a path forward in terms of expounding soil ecosystem boundaries (Stocking, 2003; Mukherjee and Lal, 2014). While several physical, chemical, and biological factors can be

considered indicators of soil health, carbon (C) and nitrogen (N) stocks, bulk density and compaction, soil aggregation, and the soil microbiome are known to be primary soil health indicators. It should also be acknowledged that these and other factors interact and are therefore interdependent.

Soil C

Carbon cycling within soils is the backbone of terrestrial ecosystems. Global soil carbon stocks have been estimated to contain over 2000 Pg of C, outnumbering combined biotic and atmospheric pools (Lal, 2004). Carbon is an essential element required for life and is a vital component of soil function, soil productivity, and a key contributor to soil health.

Carbon sequestration has been defined as the transfer of atmospheric CO_2 to long-lasting pools within the soil (Lal, 2004). Large quantities of C can be found sequestered in soil, primarily as organic matter. The amount of C stored in soil is the direct result of the balance between the rate of organic carbon inputs and the rate of carbon mineralization (Post and Kwon, 2000). Accumulation rates of soil organic carbon (SOC) can be correlated with various soil factors and vary amongst land usages and environmental conditions. For example, Post & Kwon (2000) found the rate of change in SOC pools varied as vegetation and management practices changed. They also determined that several factors and processes impact SOC increases including organic matter inputs, changing organic matter decomposability, depth of organic matter within the soil, and enhancement of physical protection of organic matter (Post and Kwon, 2000; Murphy et al., 2016). The physical protection of organic matter is particularly key to the long-term storage of SOC.

Soil physiochemical cycles regulate the balance between retained organic carbon compounds and compounds released to the atmosphere as CO_2 or CH_4 (Fig. 1.1). Soil

management practices play a critical role in determining whether carbon remains in the soil or is released into the atmosphere. In temperate regions, converting prairie to agricultural land can deplete as much as 60% of the SOC pool. The physical disturbance of the soil through cultivation reduces soil structure and exposes protected SOC in soil aggregates to microbial activity. The consequences of SOC depletion include reduced soil quality, reduced water retention, and overall loss of biomass productivity (Lal, 2004).

Agricultural land management practices also impact the quantity and composition of soil C pools (Rice, 2002). The established connection between land management practices and nutrient cycling indicate that changes in agricultural practices affect soil C, and thus impact agricultural productivity. Tillage practices influence the amount of C stored within soils. Murphy et al. (2016) observed an increase in C stored within macroaggregates of no-till systems as compared to conventionally tilled soils (Murphy et al., 2016). Several other studies have found that retained residue, as in no-tillage systems contribute to increased C inputs and retention (Ghimire et al., 2017; Smith et al., 2012). The continuous use of recommended land management practices, such as reduced or no-tillage, supports the increase of SOC pools. Carbon is sequestered when land management practices add large amounts of biomass, conserve soil and water, improve soil structure, reduce soil disturbance, and enhance microbial diversity and function (Post and Kwon, 2000; Lal, 2004; Derner and Schuman, 2007).

Soil N

Similar to C, nitrogen (N) is also a vital life element. Though N is a key component of soil function, due to its role as a necessary and often limiting nutrient for all biological activity and growth, the cycling process is more complex (Fig. 1.1). The N cycle is mediated by soil microbial activity that works to convert N inputs into the plant available N forms, ammonium

(NH_4^+) and nitrate (NO_3^-). However, these critical soil N forms, NH_4^+ and NO_3^-, may be subject to various fates within the soil (Weil and Brady, 2019).

Ammonium (NH_4^+) in soils is primarily subject to six soil processes: (1) fixation between layers of 2:1 clay minerals; (2) immobilization by soil microbes; (3) nitrification by microbes which oxidize ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-); (4) removal by plant root uptake; (5) anammox by the anaerobic oxidation of ammonium (NH_4^+) and nitrite (NO_2^-) to produce nitrous oxide (N_2O); and (6) volatilization of ammonium (NH_4^+) to ammonia (NH_3).

Nitrate (NO_3^-) in soils is also subject to six primary soil processes: (1) immobilization by soil microbes; (2) removal by plant root uptake; (3) denitrification by microbes which reduce nitrate (NO_3^-) to nitrite (NO_2^-) to nitric oxide (NO) to nitrous oxide (N_2O) and then to dinitrogen gas (N_2); (4) reduction by soil microbes to nitrite (NO_2^-) followed by conversion to nitrous oxide (N_2O) via anammox; (5) dissimilatory reduction by soil microbes to ammonium (NH_4^+) and (6) leaching out of the root zone by draining water sources.

In cropping systems, N lost via leaching is both a consequence of changing weather patterns and a major concern for crop production. Residue retention on the soil surface can reduce N losses. Murphy et al. (2016) found that surface residue contributed to an increase of approximately 40% of soil N, derived from fertilizer, retained within the crop and soil system. Nitrogen is key element in in various organic compounds including peptides, proteins, amino acids, nucleic acids, amino sugars, and phospholipids. It is therefore critical that soil conditions be monitored to ensure favorable cycling and nutrient availability for productive agricultural systems.

Bulk Density and Compaction

Soil bulk density is defined as the weight of dry soil within a unit of volume. More specifically, bulk density is used as metric to determine the level of compaction within a soil. Bulk density is highly variable within soils and is impacted by several factors including soil type, soil texture, and soil organic matter content. Healthy soils that are rich in organic matter generally have low bulk densities due to their loose and porous nature. An ideal agricultural soil should be strong enough to maintain its structure for crop cultivation and resisting compaction and erosion; but should be weak enough to allow easy root penetration and the propagation of soil organisms (Reynolds et al., 2002). In contrast, a higher bulk density generally indicates a compacted soil with little pore space, and thus reduced ability to sustain cropping systems.

Soil compaction has been defined as "the process by which the soil grains are rearranged to decrease void space and bring them into closer contact with one another, thereby increasing the bulk density" (SSSA, 2008). Recently, soil compaction has become of great concern to soil sustainability (Hamza and Anderson, 2005). Compaction has been termed one of the most serious environmental concerns caused by conventional agriculture (De Neve and Hofman, 2000; McGarry, 2001). Compacted soils are known to be highly restrictive of root growth and cannot ultimately sustain plant life (Hamza and Anderson, 2005).

Compacted soils have also been known to have a slight influence on microbial mediated processes and nutrient availability by reducing soil pore space, decreasing aeration, limiting water retention, and increasing bulk density. For example, De Neve and Hofman (2000) found when fresh leaf residue was added to soil, C mineralization rates in more compacted soils had less readily mineralizable C than soils with lower bulk densities. Changes in soil physical properties, such as bulk density, are often impacted by various environmental factors (Dam et al.,

2005; Martín et al., 2017; Sequeira et al., 2014). The magnitude of impact depends upon the preceding environmental conditions and the soil condition at the time of sampling.

Aggregate Stability

Soil aggregation has been defined as "the process by which aggregates of different sizes are joined and held together by different organic and inorganic materials" (Amézketa, 1999). The stability of soil aggregates can be defined as the ability of soil aggregates to withstand disintegration under disruptive forces or conditions. This definition implies that aggregates are only considered stable if they can withstand external forces, such as wind, water, or soil disturbance. The formation and stability of aggregates is highly dependent on a variety of soil characteristics, including SOC, soil biota, clay content, soil carbonates, land management, and ionic bridging (Bronick and Lal, 2005; Congreves et al., 2015; Eynard et al., 2005; Zhu et al., 2017).

The stability of soil aggregates has been correlated with the organic matter content of soil (Chenu et al., 2000). Planned land management practices, such as reduced or no-tillage, have been known to store large amounts of C by stabilizing soil aggregates and conserving organic matter within larger aggregate fractions (Ghimire et al., 2017). Aggregate stability and size are associated with pore size distribution (Nimmo, 2004). Varied pore size associated with soil aggregates, in addition to appropriate aeration for root growth, also allows for water infiltration to plant roots. Overall, changes in aggregate stability may be indicative of a change in soil health.

Soil Microbial Function

Changes in microbial activity or composition may have an impact on soil health. The current knowledge gap between predictability of microbial response and resulting ecosystem

conditions can be filled by gaining information about microbial growth controls under contrasting water content conditions (Manzoni et al., 2012b; Zeglin et al., 2013). Further understanding of soil microbial function can indicate the susceptibility of cropping system soils to loss of drought resilience, which may also be affected by land management practices and changing weather patterns. Understanding the microbial mechanisms related to soil vulnerability is necessary.

Nutrient cycling is mediated by enzyme activity and growth of soil microbes. In recent years, there has been increasing interest in linking changes in soil microbial community composition with microbial function. Bulk molecular extraction methods, such as bulk-extracted cell wall lipids (PLFAs), enable the general classification of microbial groups at a low resolution, such as the ratio between fungi and bacteria in the soil, which is positively associated with soil aggregation and fertility. With a higher taxonomic resolution, DNA sequencing methods reveal the connection between community composition and function, particularly in the case of carbon cycling (Hartman et al., 2017; Howe et al., 2016; Jansson and Hofmockel, 2018), and N transformation (Taylor et al., 2012).

Microbial function, as expressed via enzyme and metabolic activity, can be linked to the soil microbiome (Hirsch et al., 2010; Jansson & Hofmockel, 2018). β-glucosidases, proteases, and phosphatases are some of the most prominent soil enzymes involved with releasing nutrients from the soil organic matter into the plant-available pool. For example, β-glucosidases contribute to the decomposition of 1–4 glucosidic bonds in plant cellulose (Aragón et al., 2014). While β-N-acetylglucosaminidase controls amino sugar degradation in soils, leucine and other hydrophobic amino acids are hydrolyzed from polypeptides by leucine aminopeptidase (Hsiao et al., 2018; Sinsabaugh et al., 2008), both releasing available N. Alkaline and acid phosphatases

release inorganic P from soil organic matter producing biologically available forms. Oxidase enzymes, such as Phenol oxidases and Peroxidases facilitate lignin degradation and make N and P bound in complex organic matter more accessible (Aragón et al., 2014; Hsiao et al., 2018).

Summary

A broad understanding of microbial ecology within soils and the factors that play a role in its influence are the keys to understanding the potential effects of global climate change. This understanding can only be achieved through a multifaceted research approach. A thorough understanding of soil microbial ecology is vital to the ability to determine terrestrial carbon and climate feedbacks. It is difficult to determine the exact mechanisms due to the complex relationship between climate changes and the effects they have on the soil microbial community. The consideration of both the indirect and direct effects of climate change on the soil microbial community is necessary for a complete determination.

Soils likely contain microbes that are in a physiological stressed state at any point in time. Stable and optimal water content results in lower stress on soil microbial communities. Thus water availability has a significant influence on the physiological state of microbes within the soil community. Though soil water availability is a critical resource for soil microbes, less is known about the effect of wet-dry cycles on the composition of the soil microbial community. There is a need for multifactor experiments that provide insight into the soil microbial community composition and assess their responses to potential climate extremes.

Research Objectives

Chapter 2: Effects of Long-Term Rainfall Manipulation on the Soil Microbial Community of Native Tallgrass Prairie

The objective of this study was to understand the relationship between long-term changes in precipitation and the soil microbial community by comparing (i) microbial biomass; (ii) microbial community composition; (iii) differences in C and N pools; and (iv) other factors related to the understanding of long-term changes in native grasslands.

Chapter 3: Impact of wetting and drying cycles on microbial community respiration of a long-term rainfall manipulated grassland

The objectives of this study were to: i) better understand the impact of desiccation and rewetting on microbial respiration responses in time; ii) compare the response of different microbial populations to desiccation conditions and substrate addition.

Chapter 4: Long-Term Ecosystem Management Effects on the Soil Microbial Community and Soil Structure

The objectives of this study were to: (i) assess factors related to soil health after long-term perennial and annual plant management practices; (ii) characterize the soil microbial community in these systems; (iii) better understand the effects of land management on soil structure.

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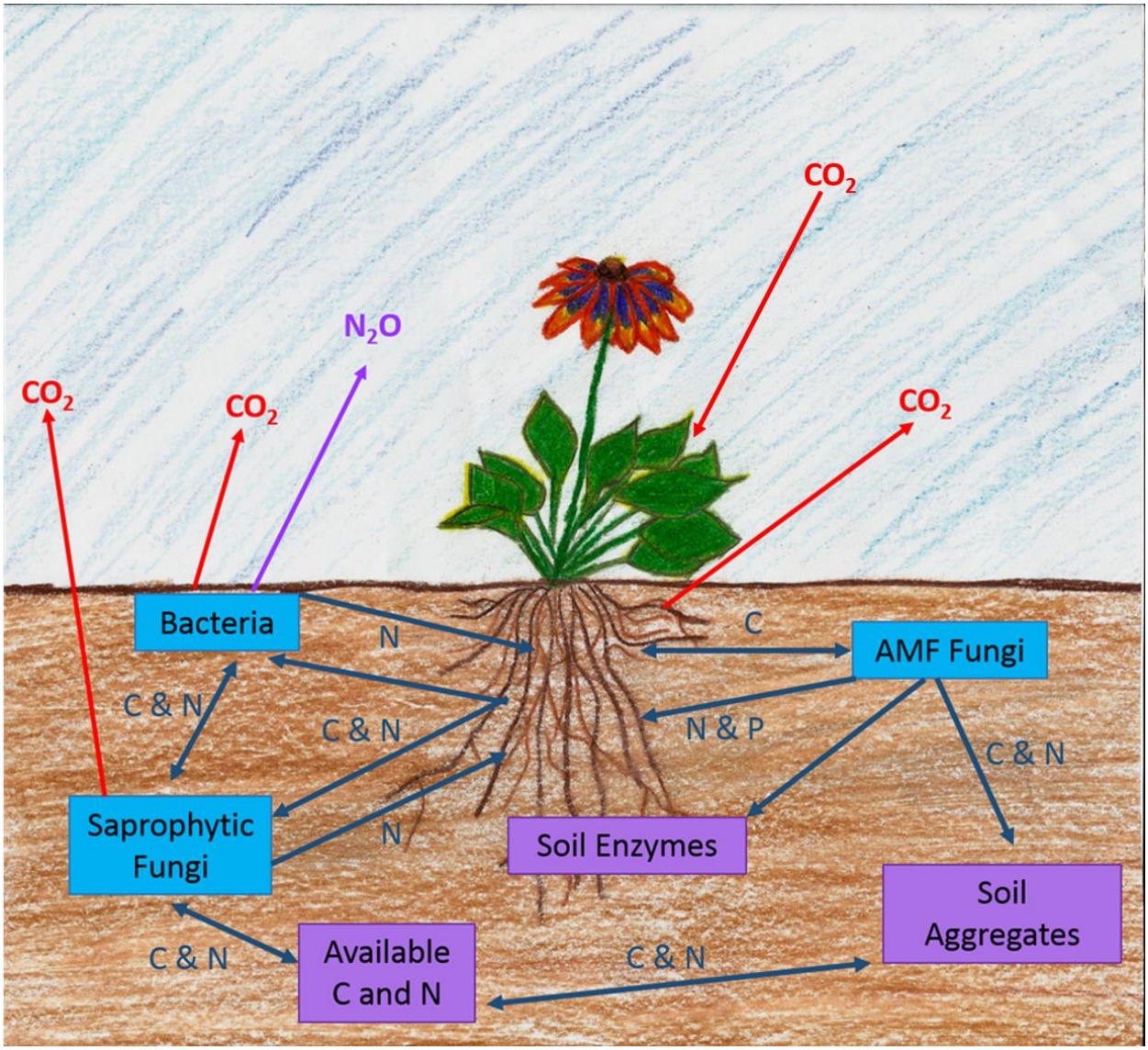

Figure 1.1. Simplified schematic representation of C and N flow through the plant-soil

Chapter 2 - Effects of Long-Term Rainfall Manipulation on the Soil Microbial Community of a Native Tallgrass Prairie

Abstract

Increasing variability in global environmental cycles is a growing concern. Changes in environmental patterns will change soil water and impact the overall function of microbial communities and ultimately terrestrial ecosystems. Variability in seasonal and annual precipitation is a natural component of tallgrass prairie. Tallgrass prairie is often viewed as a model for agricultural systems due to its soil health and resilience to change. Using tallgrass prairie as a model, soil management options need to be developed for agricultural systems. Microorganisms are a vital role in maintaining soil health. Though soil microbes have developed mechanisms to adapt to environmental changes, fluctuations in soil water potential can have a significant effect on the soil microbial community composition and function. Thus, understanding the impact of altered precipitation patterns and land management regimes is necessary to infer how ecosystems will respond to future environmental changes. The objectives of this study were to (1) better understand the relationship between long term precipitation changes and the soil microbial community and (2) assess biophysical properties of agricultural soil as influenced by long-term land management practices by assessing the microbial community response to over 25 years of enhanced soil moisture. Soil samples were collected from a long-term (25 yrs) irrigation transect located within Konza Prairie Biological Station in eastern Kansas. The experimental design consisted of two moisture regimes: irrigated (relatively consistent soil water) and control (non-irrigated, ambient precipitation). Two replicate plots were sampled in both an upland and lowland portions of the field. Soil samples were taken at four time points within the 2016 and 2017 growing seasons. Phospholipid fatty acid analysis was

conducted to compare differences within the microbial community composition and microbial biomass. We also measured inorganic N, total C, total N and soil cations to assess overall soil quality. In 2016, precipitation reduced the need for irrigation. We found no evidence of a legacy effect of water regime on the soil microbial community after 25 years of increased precipitation. However, we found differences in soil C between irrigated and control soils in the 0-10 cm depth of both upland and lowland soils, as well as significant differences in total N between irrigated and control soils at both topographical positions and at both depths. Based on our findings, we conclude that seasonality was the primary driver of differences in microbial community structure. An overall shift in plant community composition may have masked legacy effects of the soil moisture regime.

Introduction

Increasing variability in weather patterns is a growing global challenge. There is scientific consensus that weather patterns are changing as a result of human activity (IPCC, 2007). More specifically, agricultural practices, transportation, energy production, and land use alteration contribute to the increased emission of greenhouse gases. Among other predicted consequences of elevated greenhouse gases, changing weather patterns are expected to alter precipitation patterns. It has been projected that changing weather patterns will alter water availability and water demand globally (IPCC, 2007). Climate models predict that a majority of the United States will experience increased drought conditions due to intense thunderstorms, rapid runoff and long periods of time between precipitation events (IPCC, 2007; Hatfield et al., 2013; Cook et al., 2016). Additionally, global temperatures have consistently increased to unprecedented levels (Hansen et al., 2006) and will continue to increase globally (IPCC, 2014). The four warmest years on record have occurred since 2014 (Sanchez-Lugo et al., 2018). Though various factors influence terrestrial ecosystems, water availability is a primary control on all biota.

The ecological significance of water in temperate grasslands has been immensely documented (Knapp et al., 2001; Harpole et al., 2007; Niu et al., 2008). Variability in seasonal and annual precipitation is a natural component of tallgrass prairie. Tallgrass prairie is often viewed as a reference for agricultural systems due to its soil health and resilience to change (Lemaire et al., 2015). Using tallgrass prairie as a reference, soil management options can be developed to improve and sustain agricultural systems. Thus, understanding the impacts of contrasting precipitation patterns on the tallgrass prairie will assist scientists in inferring how grasslands, and perhaps agricultural ecosystems, will respond to future environmental changes.

More specifically, microbial life depends on adequate water supplies for both survival and function (Ruehr et al., 2009; Zeglin et al., 2013; Evans and Wallenstein, 2014; Barnard et al., 2015). An increase in precipitation variability will likely impact the overall function of microbial communities and ultimately impact terrestrial ecosystems (Harris, 1981; Chimner and Welker, 2005; Manzoni et al., 2012a).

The effects of changing weather events are often linked to soil health (Doran, 2002; Lal, 2004; Gray and Bishop, 2016). Healthy soils are vital to our ability to combat global climate issues and sustain food production systems. Microorganisms are key drivers of ecosystem function in soils. Consequently, microorganisms play a vital role in maintaining soil health and the microbial community composition is often linked to the environment and plant interactions.

Due to the role of soil water in nutrient transport, cellular metabolism, microbial cell motility, and plant growth, changes in water availability may impact the community structure and physiological processes of soil microbes. It is well documented that aerobic microbial activity is generally optimal when 30-50% of soil pore space is filled with water. Because microbes must respond to the environmental water potential to survive, microbial community members have developed unique methods to respond to changing environmental conditions (Harris, 1981; Voroney and Heck, 2015). For example, some microbes respond to a reduction in water potential by accumulating solutes and osmolytes within the cell, such as glycerol, amino acids, and mannitol (Estop-Aragonés and Blodau, 2012; Kakumanu and Williams, 2014; Voroney and Heck, 2015). Other microbial groups adjust their cell wall properties to prevent osmotic shock (Voroney and Heck, 2015). More specifically, gram-negative bacterial cells, due to the thinner nature of their cell walls, are known to be unable to withstand high turgor pressures, while gram-positive bacterial cells adjust their osmotic potential to survive

desiccation. Physiological adjustments in response to the environmental water potentials are made at a cost to the microbial cell. Rather than using energy for population growth, energy is expended making solutes for survival. As water availability is reduced by drought, nutrient access is also reduced (Stark and Firestone, 1995). Decreased plant productivity, both above and below-ground, in water-limited tallgrass prairies is an example of the ability of soil water to influence ecosystem properties that impact soil microbial community structure. The interdependence of soil microbes on terrestrial plants indicates that changes in root-derived exudates may impact soil microbial communities (Broeckling et al., 2008; Lange et al., 2015).

Because soil microorganisms are key drivers of ecosystem function, it is key that we understand how changing environmental factors will influence the soil microbial community. Currently, most studies of the effects of changing water availability are short-term manipulations. These often lead to incorrect conclusions that do not necessarily represent microbial community dynamics that may occur in the long-term (over a decade). Previous research has suggested that microbial community structure and function are sensitive to long- and short-term changes in water availability.

The long-term irrigation experiment used in this study has been the subject of various prior investigations. Williams and Rice (2007) evaluated the response of the soil microbial community through a growing season after 7 years of enhanced soil water availability. They observed a decrease in cyclopropyl to ω7-precursor PLFA biomarkers in irrigated soils indicating that microbial stress was reduced in those soils (Williams and Rice, 2007). They also observed greater fungal to bacterial biomass ratios in consistently moist soils. However, over time, plant composition has shifted (Wilcox et al., 2016a). A shift in plant community composition may influence C:N ratios, nutrient availability, and ultimately the soil microbial

community and function. Based on the previous research, the specific objective of this study was to assess the microbial community response over two growing seasons following over 25 years of enhanced soil moisture.

The objective of this study was to understand the relationship between long-term changes in precipitation and the soil microbial community by comparing (i) microbial biomass; (ii) microbial community composition; (iii) differences in C and N pools; and (iv) other factors related to understanding long-term responses to altered precipitation regimes in native tallgrass prairie. We predicted: i) fungal to bacterial ratios would be lower in the irrigated transects than in the control transects, ii) that soil microbial diversity would be greater in the surface soil than in the subsurface soil, and iii) Gram-positive bacteria, fungi, and actinomycetes will be more dominant in control soils than irrigated soils.

Materials and Methods

Research Site

The study was conducted in a native tall-grass prairie located at the Konza Prairie Biological Station (39°05'N, 96°35'W)in the Flint Hills of eastern Kansas, USA. The average monthly air temperature ranged from -2.7°C in January to 26.6°C in July. The mean annual precipitation was 835 mm. Soils at the site are silty clay loams. The upland site was the Clime (fine, mixed, active, mesic Udorthentic Haplustoll) -Sogn (loamy, mixed superactive, mesic Lithic Haplustoll) complex, while the lowland site of the transects was Irwin (fine, mixed, superactive, mesic Pachic Argiustol). The aboveground net primary productivity (ANPP) at Konza Prairie Biological Station was 536 g m^{-2} (Wilcox et al., 2016a) and the dominant vegetation includes native big bluestem (*Andropogon gerardii*),indiangrass (*Sorghastrum*

nutans), and switchgrass (*Panicum virgatum*). The site was burned annually in the spring and landscape was generally characterized by soils overlaying limestone and shale.

A precipitation manipulation experiment was set-up in the early 1990s to manipulate water availability to minimize water limitations. Two separate irrigation transects were established, one each in 1990 and 1993. The two replicate transects (140 meters long by 30 meters wide), were comprised of an upland and lowland field portion. This study included 2 irrigated and 2 ambient (control) transects. The irrigated transects were watered as needed to minimize water stress through the growing season via a series of 1-meter tall sprinkler heads arrayed along each transect. Irrigated treatments receive supplemental water when the measured volumetric water content (VMC) drops below 0.25 VMC to bring the water content to 0.30 VMC. Field moisture was maintained within the irrigation transects yearly from May to September. Ambient plots received no irrigation in addition to natural precipitation events. The site was burned annually and was protected from grazing for over 35 years. This experimental site has been previously referenced in the literature (Knapp et al., 1994b, 2001; Williams and Rice, 2007; Wilcox et al., 2016a).

Sample Collection

Soil samples were collected during the 2016 and 2017 growing seasons. Soil cores were randomly collected using a manual, 1.9 cm diameter, soil punch probe at depths of 0-10 and 10-20 cm from four sampling plots at each topographic position on each transect (n=8). Soil cores taken from within each sampling location were then pooled, mixed thoroughly, and passed through an 8-mm sieve to remove plant roots and rocks. The sieved soil samples were stored in zip lock bags at 4°C for further analysis. A subsample of each soil was freeze-dried for lipid analysis. Figure A2.1 contains a schematic image of the research site and sampling areas.

Ancillary Measurements

On each collection date, gravimetric soil water content was measured by weighing 10g of moist soil into a tin and oven drying at a temperature of 105°C for 48 hours. Samples were then re-weighed and the gravimetric soil water content was calculated as mass of water lost as a percentage of the oven dry soil mass using the equation below.

$$\%\ Soil\ Moisture = \frac{Moist\ Soil\ Sample\ (g) - Oven\ Dry\ Soil\ (g)}{Oven\ Dry\ Soil\ (g)}\ x\ 100$$

Soil pH was determined using a 1:10 soil-water suspension. All temperature and precipitation data were obtained from the US Climate Reference Network site located on Konza Prairie near the experimental site. The meteorological station was located within the general vicinity of the study site and was maintained by the National Oceanic and Atmospheric Administration's National Centers for Environmental Information. Figure A2.2 contains air temperature during the study years. Figure A2.2 contains average rainfall throughout the study.

Mehlich-3 Extractable Phosphorus

Air-dried soil subsamples were extracted with a solution of glacial acetic acid, ammonium nitrate, ammonium fluoride and nitric acid. A Lachat Quickchem 8000 was used to perform the colorimetric assay. The extraction and colorimetric assay were performed by the Kansas State University Soil Testing Lab as described by Frank et al. (1998).

Soil Extractable Cations

Air-dried soil subsamples were used to determine extractable cations (Ca, K, Mg, & Na). Cations were determined by the ammonium acetate (1M, pH 7.0) method with the use of low-sodium filter paper (Warncke and Brown, 1998). Analysis was done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, manufactured

by Varian Australia Pty Ltd, Mulgrave, Vic Australia and a Model AAnylyst 200 (AA) Spectrometer from Perkin Elmer Life and Analytical Sciences, Shelton, CT. The extraction and the analysis were performed by the Kansas State University Soil Testing Lab.

Soil Organic Carbon and Total Nitrogen

Soil subsamples were air-dried and all root biomass was removed via tweezers. Samples were then ground using a mortar and pestle and passed through a 250 µm sieve. Samples were analyzed for soil organic C (SOC) and total N (TN) by dry combustion using a C/N Elemental Analyzer gas chromatograph with a thermal conductivity detector (Thermo Finnegan Flash EA1112, Milan, Italy).

Soil Extractable Nitrogen

Inorganic soil nitrogen (NO_3-N and NH_4-N) was determined by KCl extraction. Briefly, within 24 hours of each collection, 100 mL of 1M KCl solution was added to 25g of field-moist soil to yield a ratio of 1:4 (Soil:1M KCl). Samples were shaken at 300 rpm on an orbital shaker for 60 minutes. Samples were then filtered through Whatman No. 42 filter paper and decanted into 20 mL scintillation vials. Vials were then submitted to the K-State Soil Testing Lab for colorimetric analysis (Keeney and Nelson, 1982; Gelderman and Beegle, 1998).

Microbial Phospholipid Analysis

The total lipids were extracted from the freeze-dried soil using a modification of the Bligh and Dyer lipid extraction method (Bligh and Dyer, 1959; White and Rice, 2009). Briefly, phospholipid fatty acids (PLFA) were separated from the total lipid extract using silicic acid chromatography. The fatty acids were cleaved from the glycerol backbone using KOH saponification, and the harvested fatty acids were methylated to form fatty acid methyl esters (FAME). The resulting FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ mass

spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30m x 250 µm i.d. x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA). The FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Tentative assignments of FAME peaks not present in the BAME mix were made by mass spectral interpretation. Peak concentration was quantified using the internal standard methyl nonadecanoate.

Fatty acids were grouped into gram-positive bacteria (i15:0, a15:0, i15:0, i17:0, and a17:0), Gram-negative bacteria (19:0:delta9,10, 17:0:delta9,10, C10:0:2-OH, C12:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH, C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10Me18:0), arbuscular mycorrhizal fungi (C16:1:11), and fungi (C18:2:9,12) (White and Rice, 2009).

Statistical Analysis

The data were analyzed as a split-plot design with precipitation manipulation (ambient or irrigated), depth or location, and sampling date as model class factors. The analysis used a split-plot model (Milliken and Johnson, 2009) and the Mixed covariate dependency procedures in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Unless otherwise stated, all results were considered significant at the $p < 0.10$ level.

Results

Total growing season precipitation (May–October) in 2016 and 2017 was 708 and 455 mm, respectively. Average air temperature during the growing season in 2016 and 2017 was 22.1 and 21.2, respectively (Fig. A2.2). In 2016, there were 2 irrigation events during which a total of 62.2 mm of irrigation was added to the upland position and 59.6 mm of irrigation was added to the

lowland position. In 2017, there were 5 irrigation events during which a total of 105.3 mm of irrigation was added to the upland position and 100.6 mm of irrigation was added to the lowland position. During the 2017 growing season, the irrigation system was set-up later in the season due to reconfiguration which caused the soil to be drier than normal..

Soil Moisture Content and pH

Soil pH was significantly higher in irrigated soils than in control soils (Table 2.1; Table 2.5). No significant differences were observed between topographic upland and lowland positions or between depths in the lowland soils. The source of the irrigation water was from local groundwater in limestone bedrock which likely caused the increase in soil pH.

In surface soils (0-10 cm), soil water content was not significantly different between irrigated and ambient plots of the upland and lowland topographic positions across all dates (Table 2.2). However, surface soil water content was significantly different between topographic positions across dates, as lowland soils retained more soil water than the upland soils ($p= 0.0014$; Table 2.2). A significant irrigation by time interaction ($p= 0.0676$) was observed in the surface soils, with more fluctuation in soil water content observed in irrigated soils than control soils, and irrigated soils being wetter than control soils on specific dates (Table 2.2). A significant location by time interaction ($p= 0.0323$) was also observed in the surface soils. This was likely due to the ability of deeper lowland soils to retain moisture despite seasonal soil water content fluctuations. Soil water content was significantly influenced by time as the soil water content decreased during the middle of the growing season ($p= 0.0481$). This was likely caused by increased vegetative demand during warmer summer months.

When comparing surface soils (0-10 cm) to subsurface soils (10-20 cm) in the lowland position, a significant 3-way interaction between irrigation, depth, and time ($p= 0.0330$) was observed. Surface soils maintained a higher soil water content than subsurface soils. This was

particularly true in irrigated soils, as they maintained higher soil water levels, while control soils experienced longer periods of desiccation. A significant depth*time interaction (p= 0.0123) was also observed between surface and subsurface soils (Table 2.6). Though vegetative growth reduced soil water content levels toward the middle of the growing season, surface soils were able to maintain more soil water than subsurface soils. There was a significant effect of irrigation treatment (p= 0.0816), with irrigated soils containing slightly more soil water than the control soils (Table 2.6). Soil water content between surface and subsurface soils was also significantly influenced by time (p= 0.0443).

Soil Organic C, Total N, and C/N Ratio

Both irrigation and topographic location had significant independent effects on surface (0-10 cm) SOC levels (p= 0.0367 and p= 0.0638). However, there was no significant interaction between topographic location and time. Irrigated treatments contained higher SOC levels than the control soils and upland soils contained more SOC than lowland soils (Table 2.1). Lowland surface soils (0-10 cm) contained more SOC than subsurface soils (10-20 cm), though there were no significant differences between irrigated and control treatments (Table 2.5).

Similar to SOC, both irrigation and topographic location had significant independent effects on surface (0-10 cm) TN (p= 0.0528 and p= 0.0396). There was no significant interaction of irrigation and topographic location. Surface soils in the lowland position had significantly higher levels of TN than subsurface soils (p= 0.0137). There was also a significant effect of irrigation treatment between lowland soil depths, with irrigated soils containing significantly more TN than control soils (p= 0.0723; Table 2.5). Thus, a significant irrigation by depth interaction was also observed (p= 0.0961).

When comparing upland and lowland surface soils (0-10 cm), the soil C/N ratio was significantly influenced by an interaction between irrigation treatment*location (p= 0.0937; Table

2.1). In lowland soils, the C/N ratio was significantly influenced by depth and was higher in the surface than subsurface soil ($p<.0001$; Table 2.5).

Mehlich-3 Extractable P

Available soil P was assessed in May 2016, at the start of the study. Available P was higher in the lowland soils than the upland soils; however, this difference was not significant (Table 2.1). At the lowland topographic position, available soil P was higher at the 0-10 cm soil depth than the 10-20 cm soil (Table 2.5). Available P did not differ significantly with landscape position, irrigation, or depth.

Soil Extractable Cations

When comparing upland and lowland surface soils, cations (Ca^{+}, Mg^{+2}, Na^{+}, and K^{+}) were not significantly affected by irrigation or topographic location (Table 2.1). When comparing across depths in the lowland position, both Ca^{+} and Na^{+} levels were significantly influenced by irrigation ($p= 0.0802$ and $p <.0001$) with irrigated soils containing higher levels of both Ca^{+} and Na^{+}. The higher levels of Ca^{+} and Na^{+} may be due to these cations in the irrigation water. There was also a significant effect of depth detected when comparing Na^{+} in the 0-10 cm and 10-20 cm depths ($p= 0.0020$; Table 2.5), as more Na^{+} was found in the lower depth.

Soil Inorganic N

There was no significant effect of irrigation treatment or topographic location on soil NH_4^{+} in surface soils. Only sampling time had a significant effect on soil NH_4^{+}, as soil NH_4^{+} levels generally decreased over time ($p <.0001$; Table 2.2). There were no significant effects of irrigation treatment, topographic location, or sampling time on soil NO_3^{-} on upland or lowland surface soils (Table 2.2).

When comparing the soil NH_4^{+} levels across lowland surface and subsurface soils, a significant irrigation by depth by time interaction ($p<.0001$) was observed. In 2016, a wet year, control soils had higher NH_4^{+} levels in the surface while irrigated soils had higher NH_4^{+} levels in the

subsurface. In 2017, a drier year, surface soils of both irrigated and control soils contained higher levels of NH_4^+ than subsurface soils. Soil NH_4^+ levels were also significantly influenced by an irrigation by depth interaction (p= 0.0588). Throughout the study, NH_4^+ levels were always lower at the end of the vegetative growing season, thus a significant effect of time was observed (p<.0001; Table 2.6).

Higher NO_3^- levels in surface soils, coupled with an overall decrease in soil NO_3^- levels throughout the growing season caused a significant depth by time interaction (p= 0.0270). Soil NO_3^- levels at 0-10 cm were higher in May but then decreased with time while at 10-20 cm, soil NO_3^- levels were consistently low. The consistent decrease in soil NO_3^- resulted in a significant effect of time (p= 0.0146; Table 2.6).

Microbial Lipids

Irrigation treatment, time, or topographic location were not independently significant, Total PLFA biomass in the surface 0-10 cm was affected by the interaction between irrigation and time (p= 0.0731; Fig. 2.1; Table 2.3) at both landscape positions. There was an overall increase in microbial biomass throughout the growing season but increased more in the irrigation treatment. The effect of irrigation was greater in 2017 which was a drier year.

Gram-positive bacterial PLFAs were significantly influenced by a 3-way interaction of irrigation, location, and time (Fig. 2.2; p= 0.0744). In 2016, a wet year, upland soils contained more gram-positive PLFA biomarkers than the lowland soils. However, in 2017, a dry year, the trend reversed with lowland soils containing more PLFA biomarkers. Additionally, the concentration of gram-positive bacteria was significantly influenced by time (p= 0.0600), with higher levels of gram-positive bacterial biomarkers found toward the end of the growing season.

Gram-negative bacteria (Fig. 2.3) and actinomycetes (Fig. 2.4) PLFAs were significantly influenced by time (p=0.0664 and p=0.0039 respectively). Gram-negative bacteria and

actinomycetes PLFAs increased with plant growth. Though 2017 was a drier year than 2016, the trend was similar.

For fungi, there were no significant effects of irrigation treatment, topographic location, or time detected for AMF when comparing the upland and lowland surface soils (Fig. 2.5; Table 2.3). Saprophytic fungi levels in both upland and lowland surface soils followed similar trends throughout growing seasons (Fig. 2.6). There was a significant 3-way interaction of irrigation, location, and time on saprophytic fungi levels ($p= 0.0977$; Table 2.3). In 2016, a wet year, upland soils contained more saprophytic fungal PLFA biomarkers than lowland soils. However, in 2017, a dry year, the trend reversed with lowland soils containing more fungal PLFA. There were also significant 2-way interactions of irrigation*location ($p= 0.0795$) and irrigation*time ($p=0.0063$). Topographic location and irrigation treatment were not singularly significant factors for saprophytic fungal PLFAs (Table 2.3). Sampling time was significant ($p= 0.0799$), as fungal biomarkers fluctuated with time, ultimately increasing from the beginning to the end of the growing season.

The fungal to bacterial ratio of upland and lowland surface soils was only significantly influenced by irrigation treatment with irrigated soils having a higher fungal to bacterial ratios than the control soils (Fig. 2.7; Table 2.4).

When comparing depths at the lowland position, there was a significant 3-way interaction of irrigation, depth, and time ($p= 0.0030$) and a significant 2-way interaction of depth*time ($p= 0.0014$) for total PLFAs (Table 2.7). Total PLFA of surface soils (0-10 cm) was higher than subsurface soils (10-20 cm). Though total PLFAs fluctuated slightly during the peak of vegetative growth, there was an overall increase observed from beginning to end (Fig. 2.8).

Additionally, total PLFA biomarkers decreased with increasing soil depth, leading to a significant effect of depth in lowland soils ($p=0.0135$; Table 2.7).

There was a significant 3-way interaction of irrigation, depth, and time ($p= 0.0114$) and a significant 2-way interaction of depth and time ($p= 0.0039$) observed for gram-positive PLFAs across lowland soil depths (Fig. 2.9; Table 2.7). Similar to the total PLFAs, gram-positive bacterial PLFAs were higher in surface soils than subsurface soils. Though gram-positive PLFAs fluctuated slightly during vegetative growth, biomarker levels were generally highest at the end of the growing season (Fig. 2.9). Additionally, both irrigation and depth were singularly significant factors ($p=0.0731$ and $p=0.0461$ respectively). At the 0-10 cm depth, control soils contained more gram-positive PLFA biomarkers. However, at the 10-20 cm depth, irrigated soils contained more gram-positive PLFA biomarkers.

Gram-negative bacteria levels were significantly influenced by a 3-way interaction between irrigation, depth, and time ($p=0.0723$). Though variation was large, gram-negative PLFAs generally increased from the beginning to the end of the growing season. Gram-negative bacterial PLFAs fluctuated more in the subsurface than in the surface depth and were thus significantly influenced by depth as a singular factor ($p=0.0132$; Fig. 2.10; Table 2.7). The concentration of actinomycetes was significantly influenced by the 2-way interaction of depth and time ($p= 0.0566$) and the single effect of time ($p= 0.0094$; Table 2.7). Actinomycetes levels generally increased over time regardless of sampling depth, though depth was not a singular significant effect (Fig. 2.11). Only a significant effect of sampling depth was detected for AMF, with surface soils containing higher levels than subsurface soils ($p= 0.0002$; Fig. 2.12; Table 2.7).

The 3-way interaction of irrigation, depth, and time was significant for saprophytic fungi ($p= 0.0082$). Surface soils generally contained higher levels of saprophytic fungal than subsurface soils, thus depth was a singularly significant factor ($p=0.0221$; Fig. 2.13; Table 2.7). In 2016, both surface and subsurface soils followed a similar pattern of general increase at the end of the season compared to the beginning. However, in 2017, a dry year, the trend differed in the surface soils, as the increase from the beginning of the season to the end was more apparent. There were also significant 2-way interactions of irrigation and time ($p= 0.0195$) and depth*time ($p=0.0448$). Irrigation treatment as a single factor was not significant (Table 2.7). However, time as a singular factor was significant, as fungal biomarkers ultimately increased from the beginning to the end of the growing season ($p= 0.0845$; Table 2.7).

The fungal to bacterial ratio of lowland soils was significantly influenced by sampling time ($p=0.0690$; Table 2.8) and a 2-way irrigation and time interaction ($p= 0.0849$; Table 2.8). Though the fungal to bacterial ratio was steady throughout the growing season, there was a dramatic increase in July 2017. This was likely due to the dual impact of a substantial increase in AMF and a slight decrease in gram-positive bacterial PLFAs that month (Fig. 2.14). Sampling depth was also a significant factor as the fungal to bacterial ratio generally decreased with depth ($p= 0.0112$; Fig. 2.14).

Discussion

Previous studies at this site have addressed questions concerning consistent soil water. After 7 years of the irrigation treatment, Williams and Rice (2007) found that soil microbial community structure and physiological characteristics were influenced by both water availability and sampling time. Reduced stress in irrigated soils was indicated by a decrease in known PLFA stress biomarkers. Further, increased water availability resulted in a 53% greater fungal to

biomass ratio and a 65% increase in fungal PLFA biomarkers. They attributed most structural and functional changes in the soil microbial community to cumulative effects of water regime on the ecosystem or legacy effects.

Contrary to the findings of Williams and Rice (2007), we found no cumulative legacy effect of water regime on the soil microbial community composition after over 25 years of increased precipitation. Most of the observed differences in microbial community structure were attributed to a combination of seasonality and other environmental factors such as landscape position or sampling depth. Wilcox et al. (2016b) suggest that both vegetation and biogeochemical properties in the irrigated plots were in a transitional state. The fungal to bacteria ratio did increased with irrigation. This increase in F:B ratio may be due to a change in plant community composition and C and N inputs. Changes in plant species within terrestrial ecosystems have been known to cause changes in soil microbial communities and in the rates of organic matter decomposition causing potential consequences across ecosystem processes (Spehn et al., 2000). Wilcox et al. (2016) documented a shift in plant species composition over time, from the inception of the field experiment to present, at this same research site. With irrigation, there was an increase in the abundance of *Solidago canadensis*, a common C3 forb, and *Amorpha canescens*, a C3 legume. They also documented a decrease in *Sorghastrum nutan*, a C4 grass. These shifts in plant community composition likely altered both quality and rates of belowground C and N inputs. Altered C inputs from increased root biomass in the irrigated soils may have favored fungi and actinomycetes over bacteria (Williams and Rice, 2007).

Microbial biomass tended to increase with the growing season. Differences in irrigation were greater during 2017, a drier year. Microbial communities are known to respond to external factors on a variety of time scales (Allison and Martiny, 2008; Zeglin et al., 2013; Carson and

Zeglin, 2018; Schmidt et al., 2018). We also observed a decline in soil water content and inorganic N (Tables 2.2; 2.6). This response was likely due to plant growth.

Tallgrass prairie is known for its distinct temporal changes (Knapp et al., 1994a) that are highly correlated with microbial community responses (Bell et al., 2008; Fox et al., 2017). Several studies have documented environmental factors as the primary drivers of soil microbial communities across grasslands (Ramette and Tiedje, 2007; Yao et al., 2018). Carson and Zeglin (2018) found changes in soil microbial community composition were more closely related to long-term management of burning. Several laboratory studies have indicated that drying and rewetting cycles in the soil stimulate microbial activity and may cause shifts in microbial community structure (Mikha et al., 2005; Manzoni et al., 2012). Because wetting and drying cycles are frequent in grassland soils, we infer that this may have been a primary driver in the lack of microbial community composition differences (Xiang et al., 2008; Borken and Matzner, 2009; Williams and Xia, 2009; Kaiser et al., 2015).

Wilcox et al. (2016) found no differences in soil C or total N between irrigated and ambient treatments. Unlike Wilcox et al. (2016), we found differences in soil C between irrigated and control soils in the 0-10 cm depth of both upland and lowland soils (Table 2.1). We also found significant differences in total N between irrigated and control soils in both topographical positions and in both depths. Further, we found no significant difference between irrigation treatments in the lowland subsurface (10-20 cm) soils (Table 2.5). Variability in precipitation will alter plant community production. Increased primary production with irrigation will increase C inputs thus causing the increase in soil C.

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Though significant shifts in plant community composition have occurred over time and across irrigation treatments, Wilcox et al. (2016b) found no change in functional composition. This indicates that changes in plant community may be stabilized by functional redundancy, ultimately leading to few observed differences in legacy irrigation effect as no functional changes have occurred (Collins et al., 2012). Further, soil nutrients may not change at the same scale as plant community composition. Additinoally, it is possible that the impacts of irrigation were small in comparison to other vegetation dynamics. The confounding effects of soil and plant interactions affect the microbial community structure (Reynolds et al., 2003; Herrera Paredes and Lebeis, 2016). Although we did not observe evidence of legacy irrigation treatment effects on the soil microbial community composition, it is possible then these effects are masked by the shift in plant community composition.

Conclusion

The simultaneous need for soil resources by both plants and microbes leads to competition in the rhizosphere. This is common in tallgrass prairie as feedback dynamics result in soil microbial community shifts. After over 25 years of increased precipitation, differences between the microbial community of irrigated and control plots could not be attributed to legacy effects of precipitation manipulation. Further, differences in C and N resources are likely due to a shift in plant production. The results of this study indicate that the confounding effects of plant community composition shift and temporal weather pattern govern microbial community composition and nutrient availability in this grassland. We suggest that additional research include longer-term studies to better predict future changes in microbial community structure and function as a result of changes in climate patterns.

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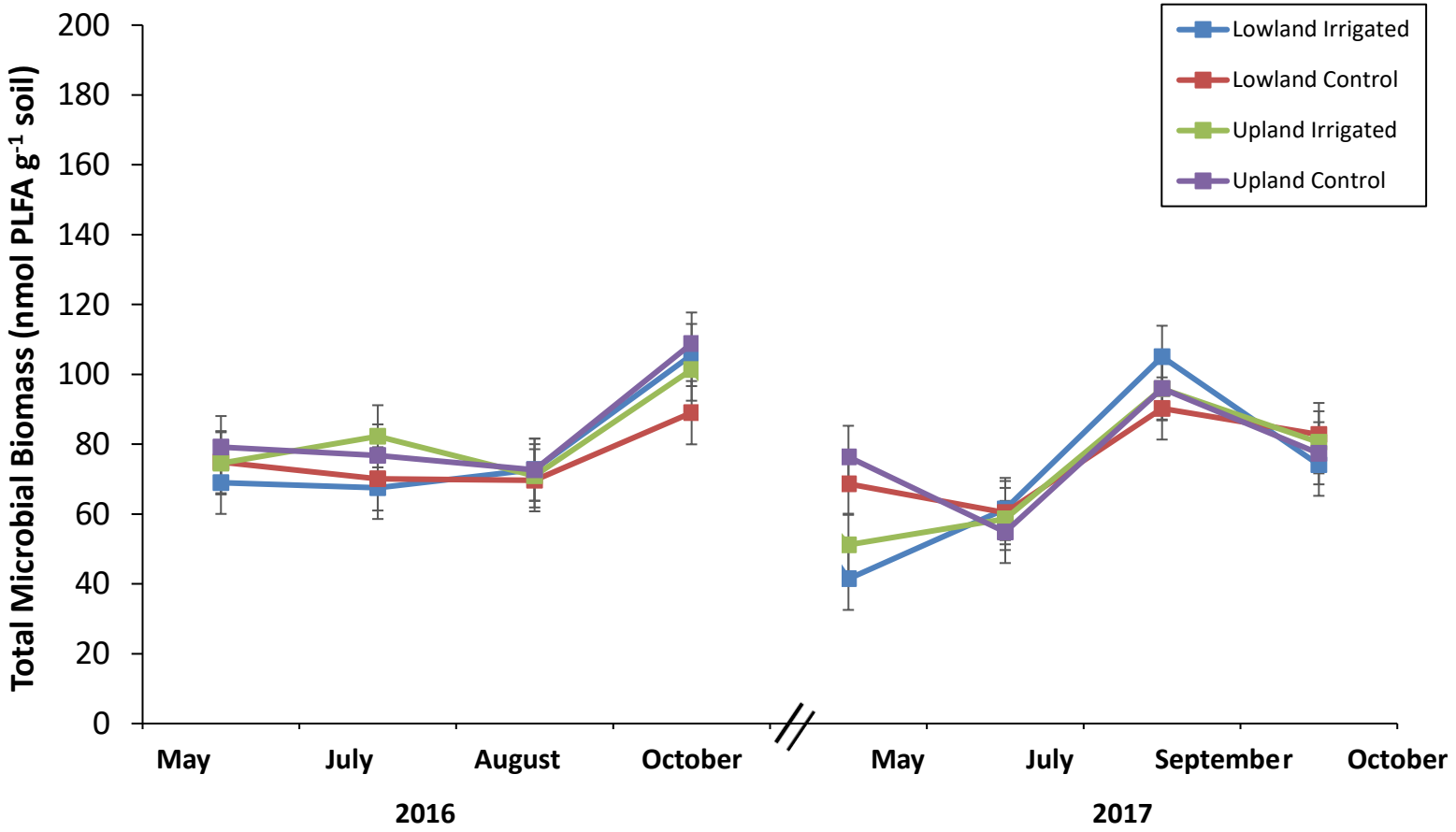

Figure 2.1. Total microbial biomass phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth. Error bars represent standard error of the mean (n=8).

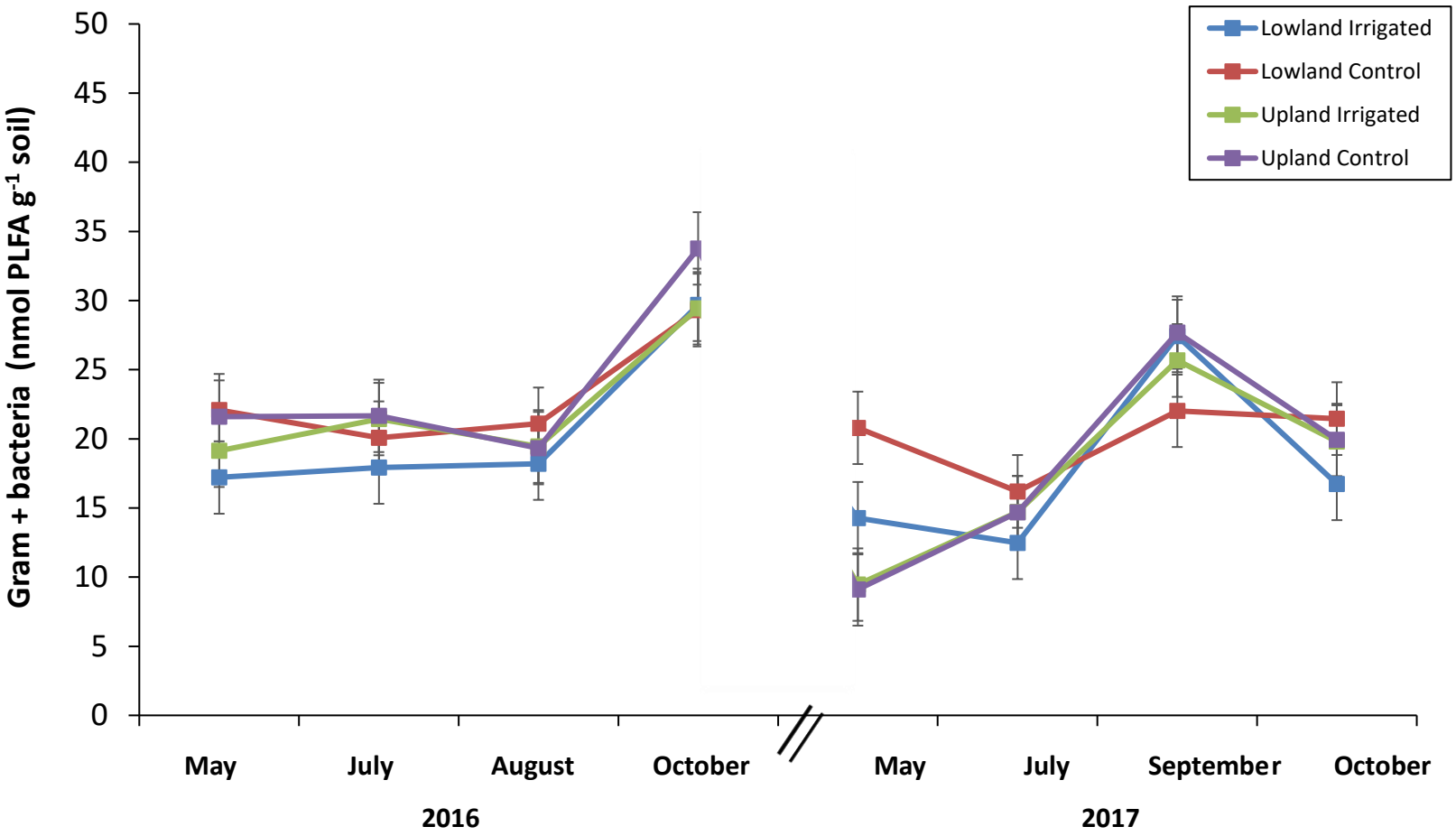

Figure 2.2. Gram-positive bacteria phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth. Error bars represent standard error of the mean (n=8).

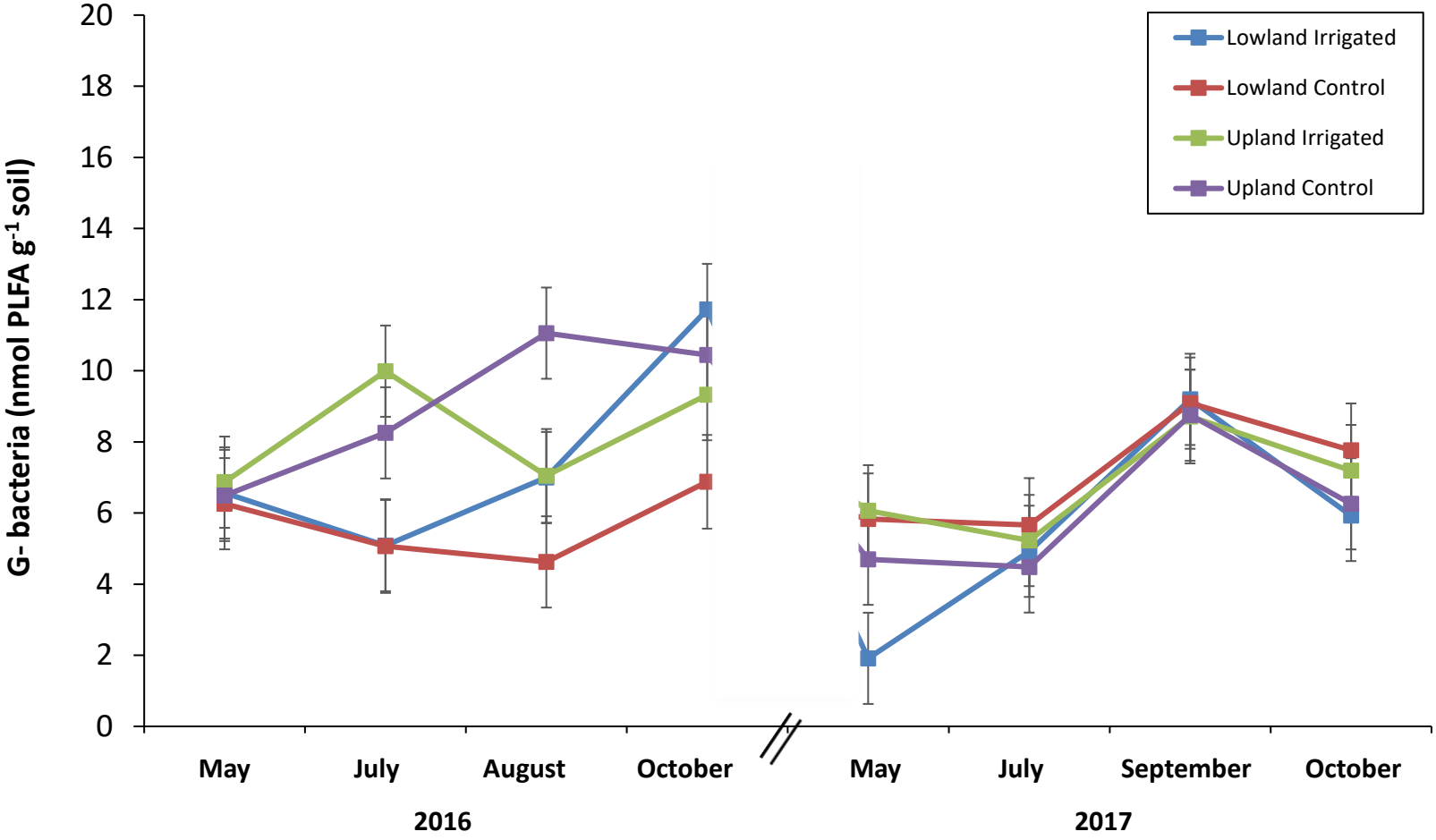

Figure 2.3. Gram-negative bacteria phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth. Error bars represent standard error of the mean (n=8).

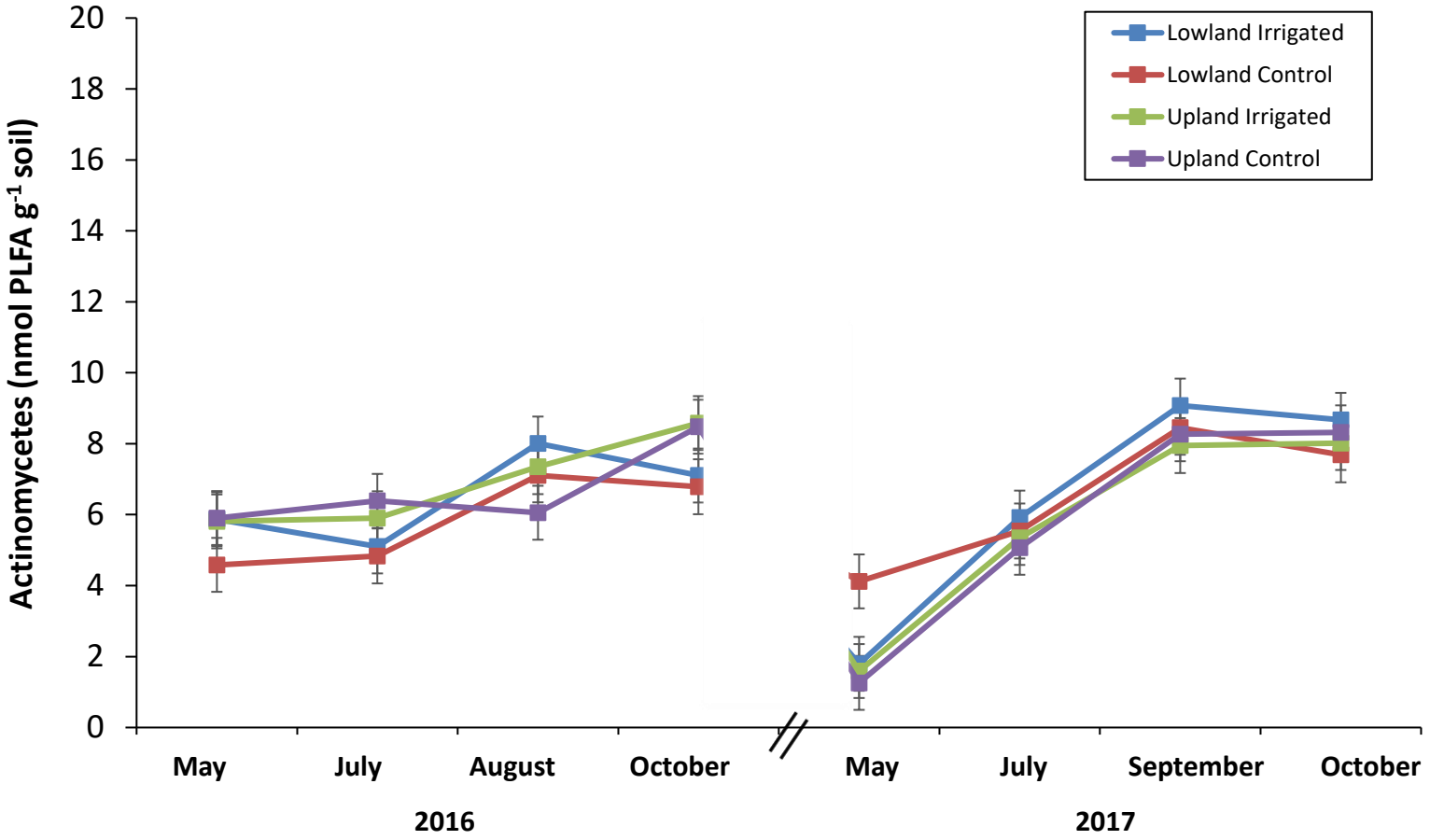

Figure 2.4. Actinomycetes phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth. Error bars represent standard error of the mean (n=8).

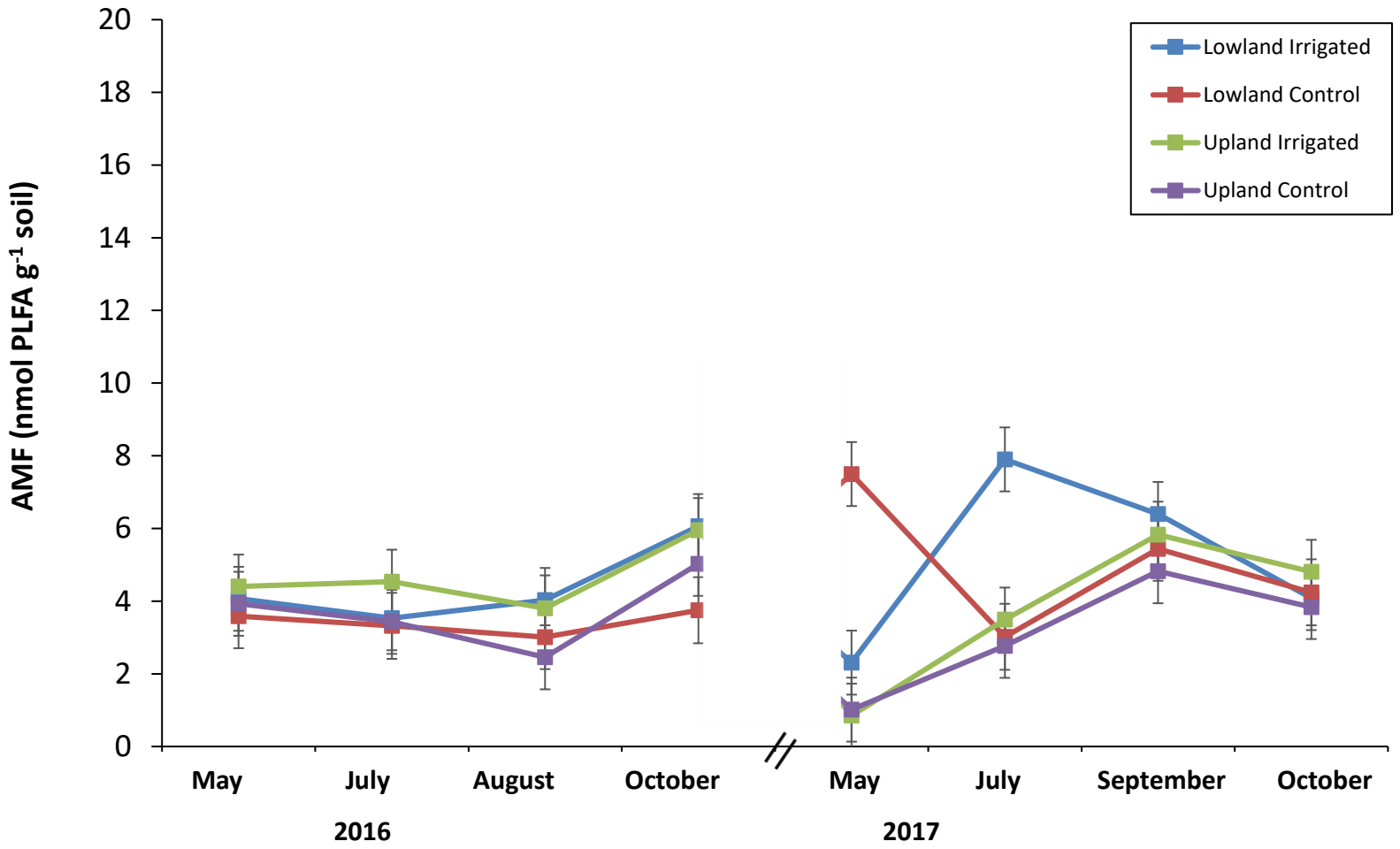

Figure 2.5. Arbuscular mycorrhizal fungi (AMF) phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth. Error bars represent standard error of the mean (n=8)

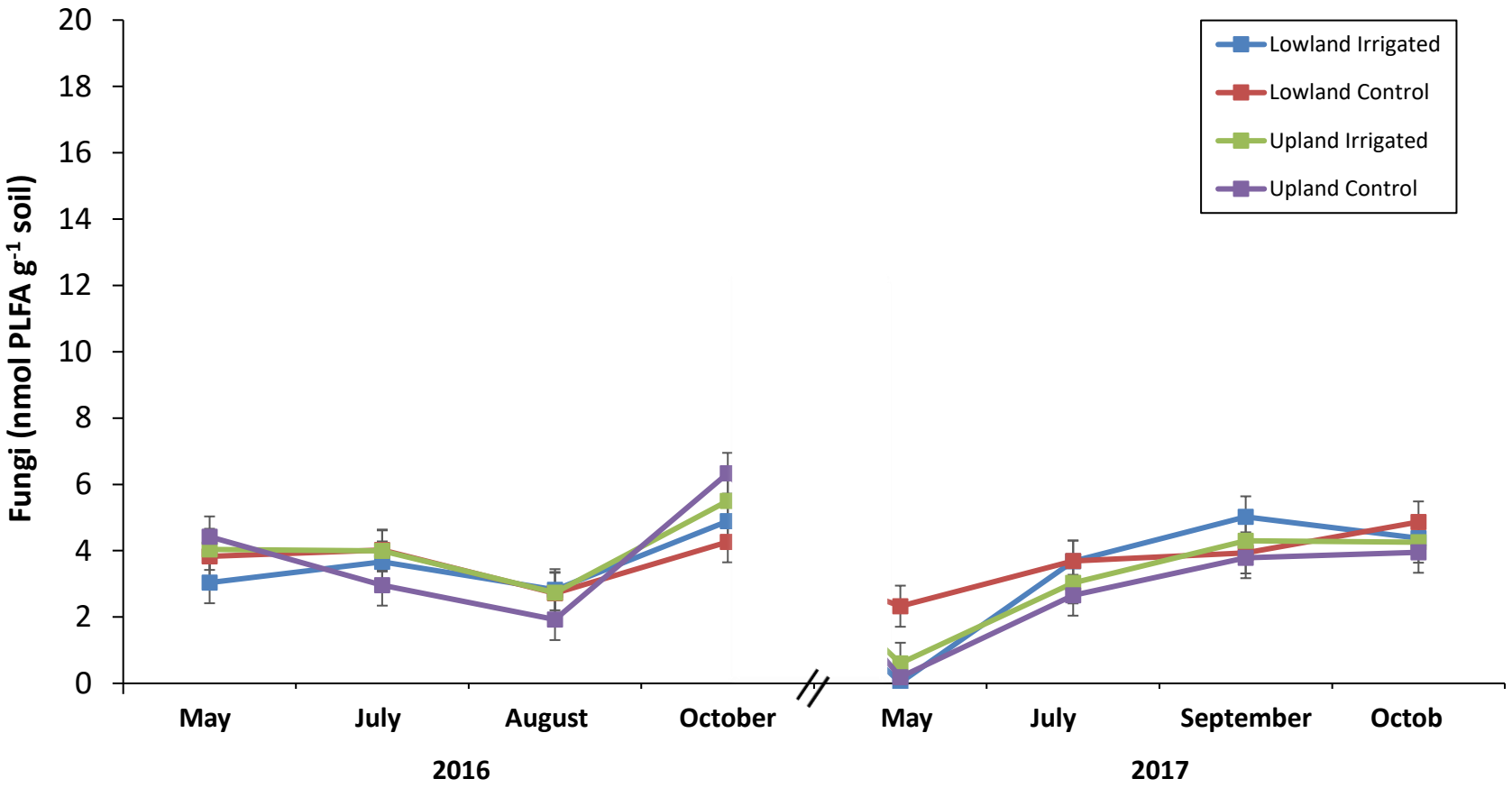

Figure 2.6. Fungal phospholipid fatty acid (PLFA) concentration across landscape positions and irrigation treatments at 0-10 cm depth.Error bars represent the standard error of the mean (n=8).

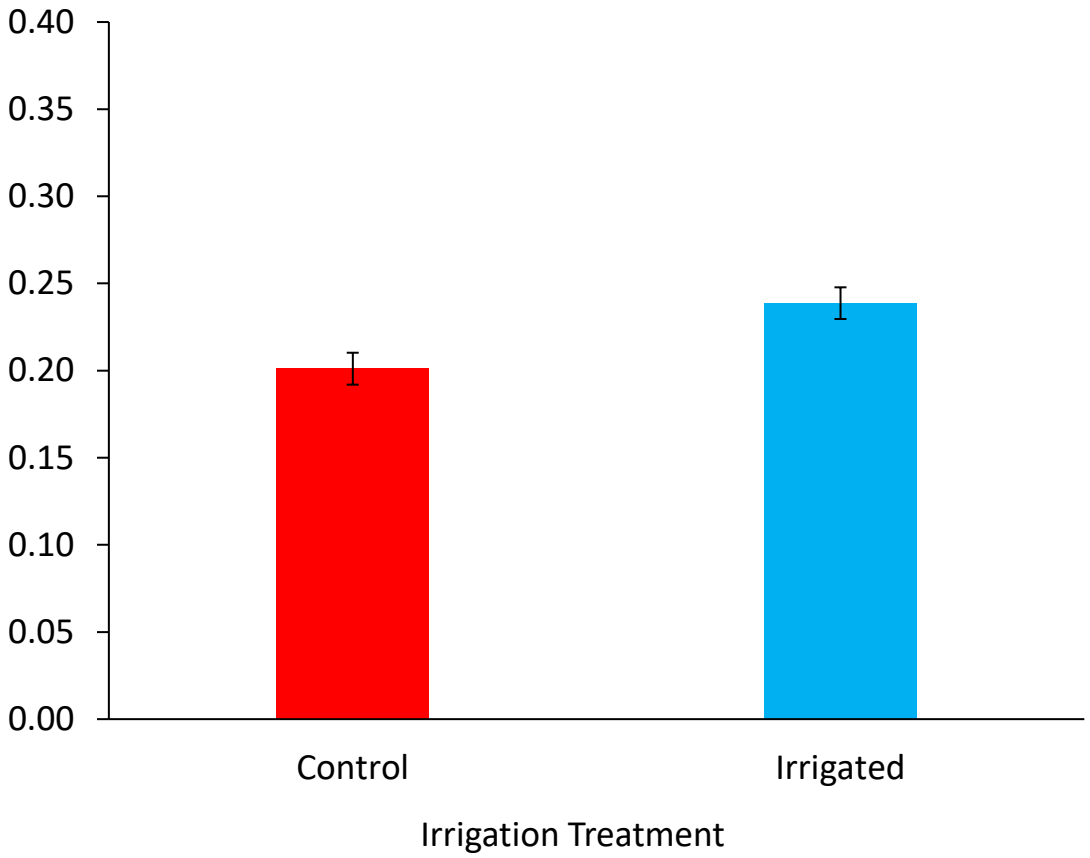

Figure 2.7. Mean fungal to bacterial ratio of phospholipid fatty acid (PLFA) concentration for by irrigation treatment at 0-10 cm depth. Error bars represent standard error of the mean (n=8).

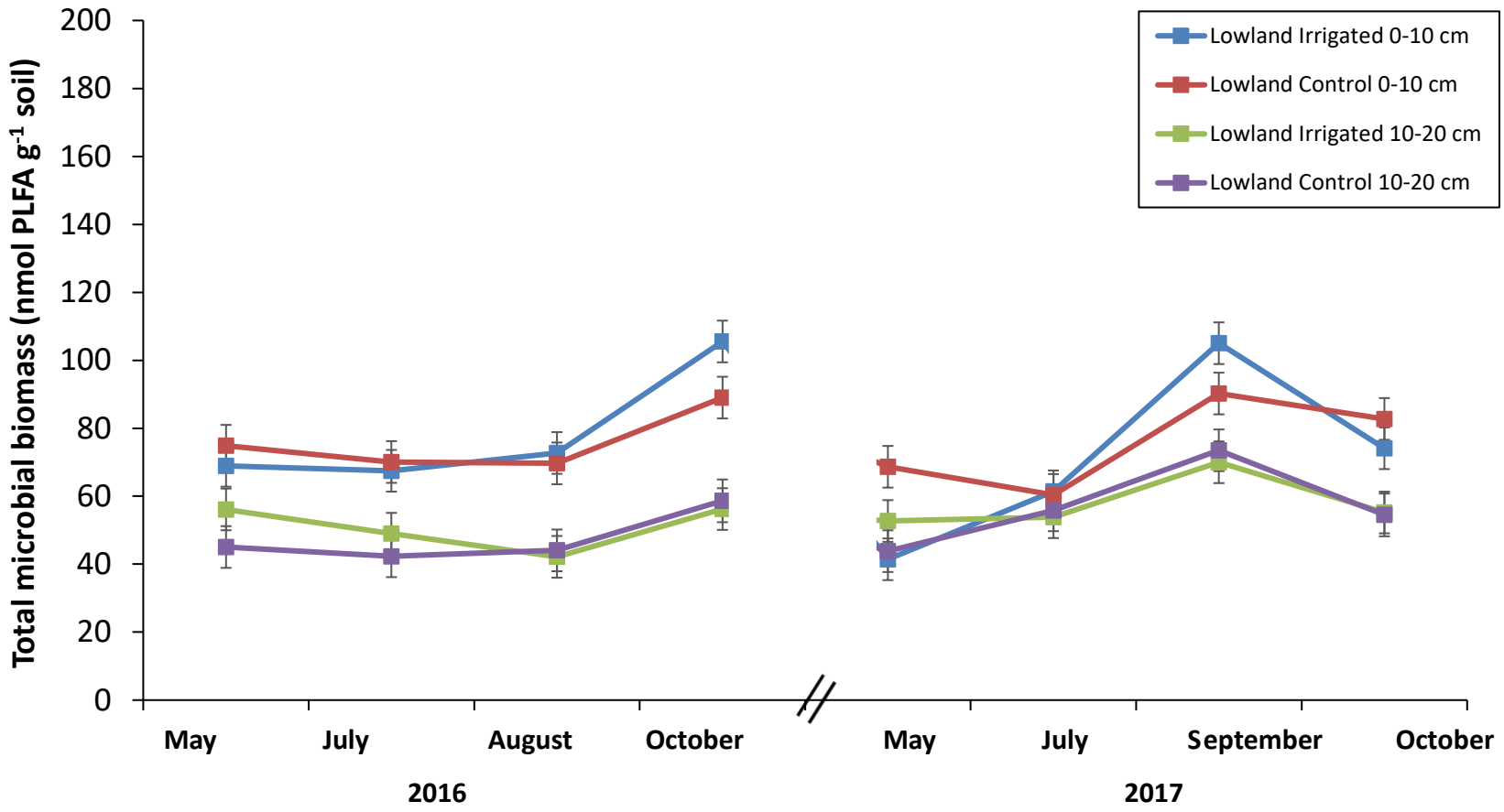

Figure 2.8. Total microbial biomass phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

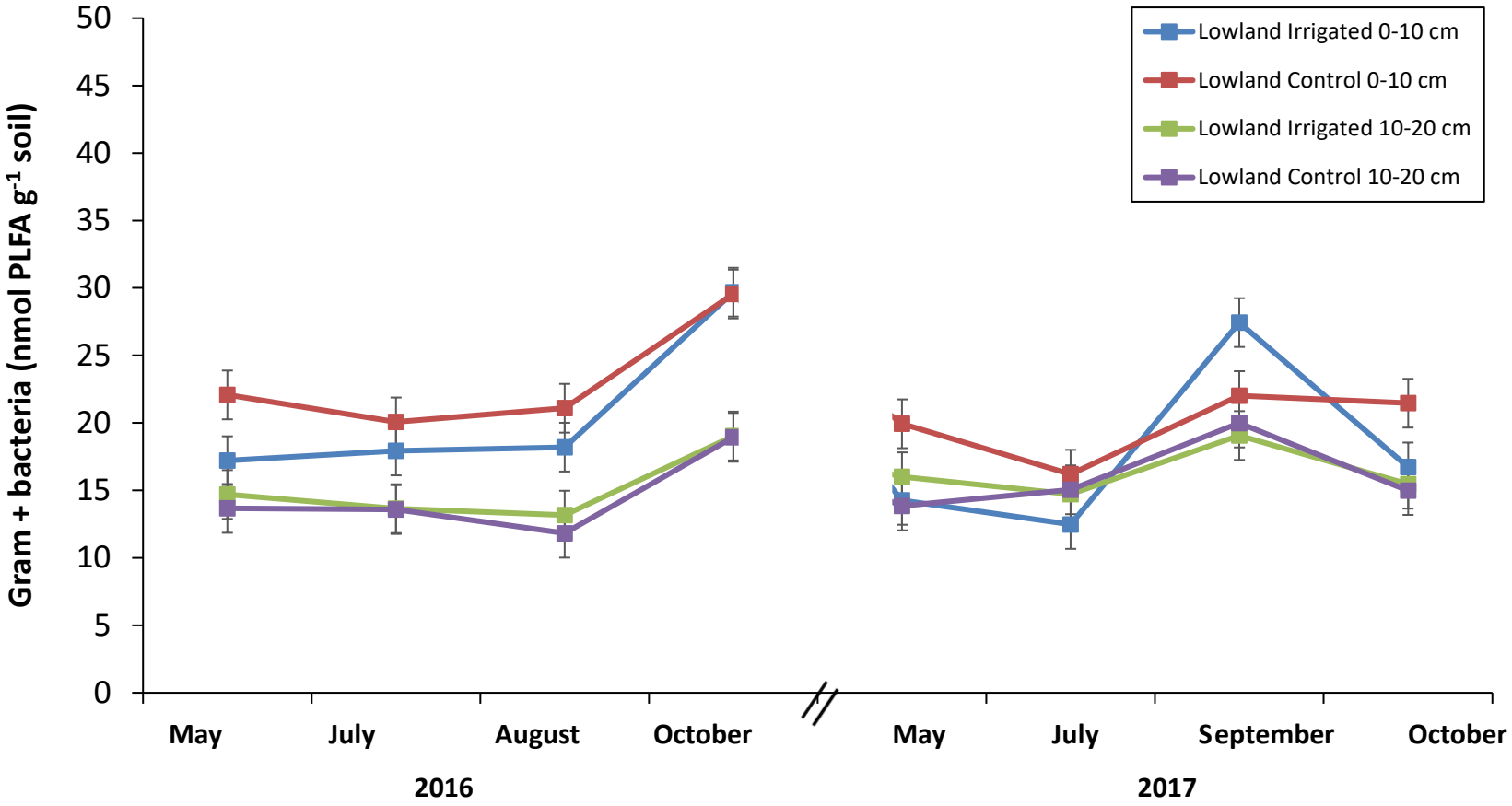

Figure 2.9. Gram-positive bacteria phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

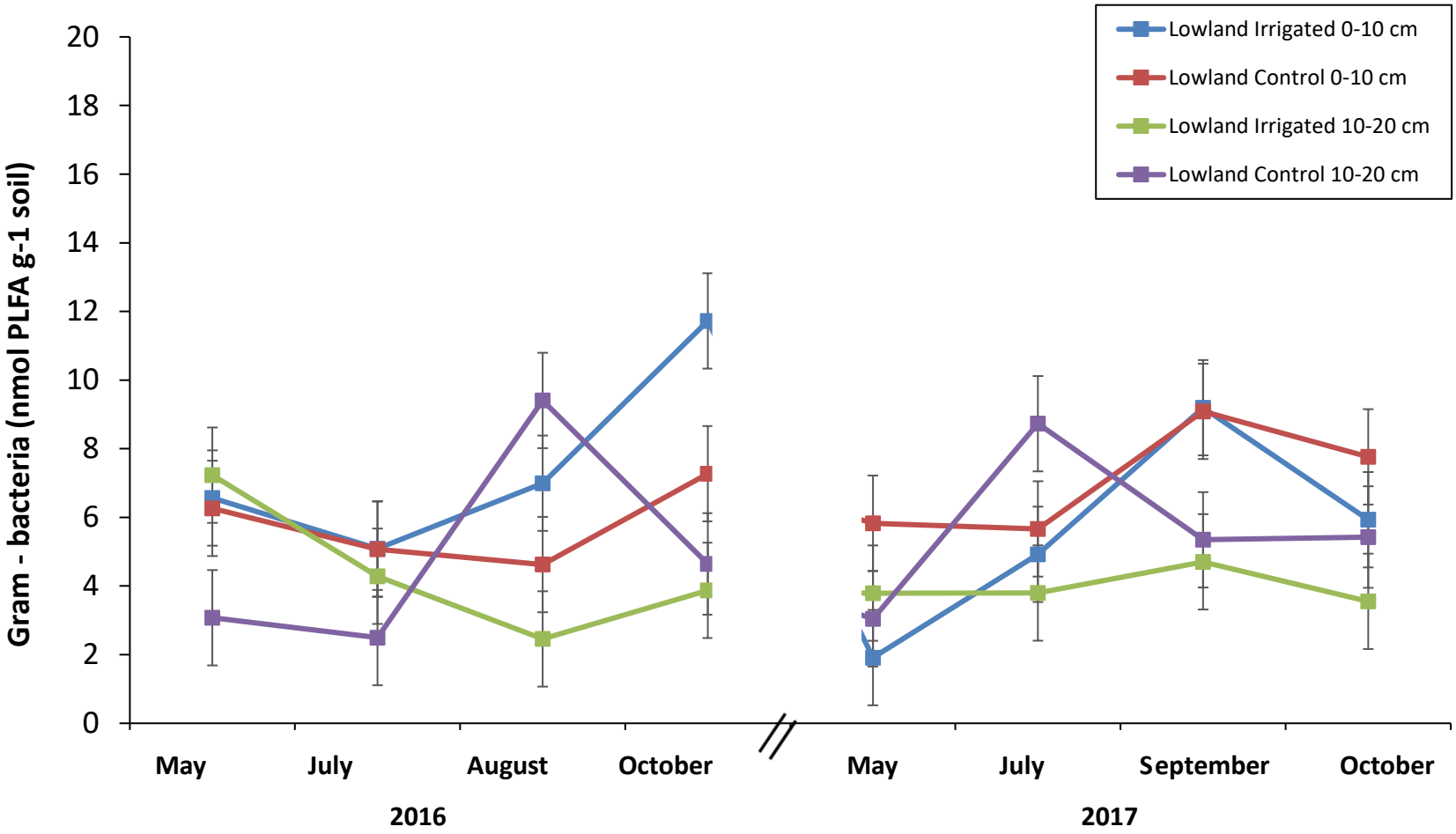

Figure 2.10. Gram-negative bacteria phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

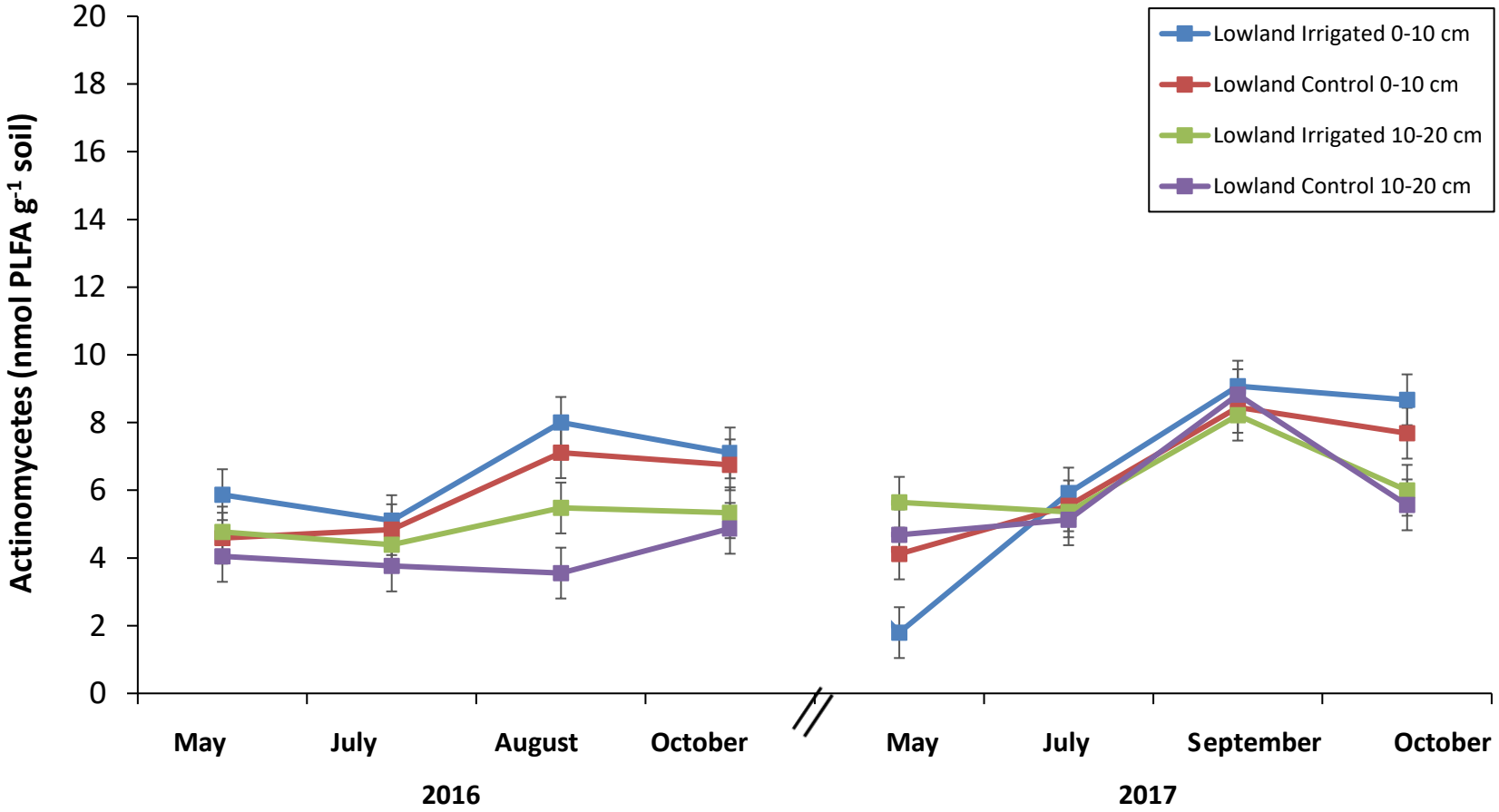

Figure 2.11. Actinomycetes phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

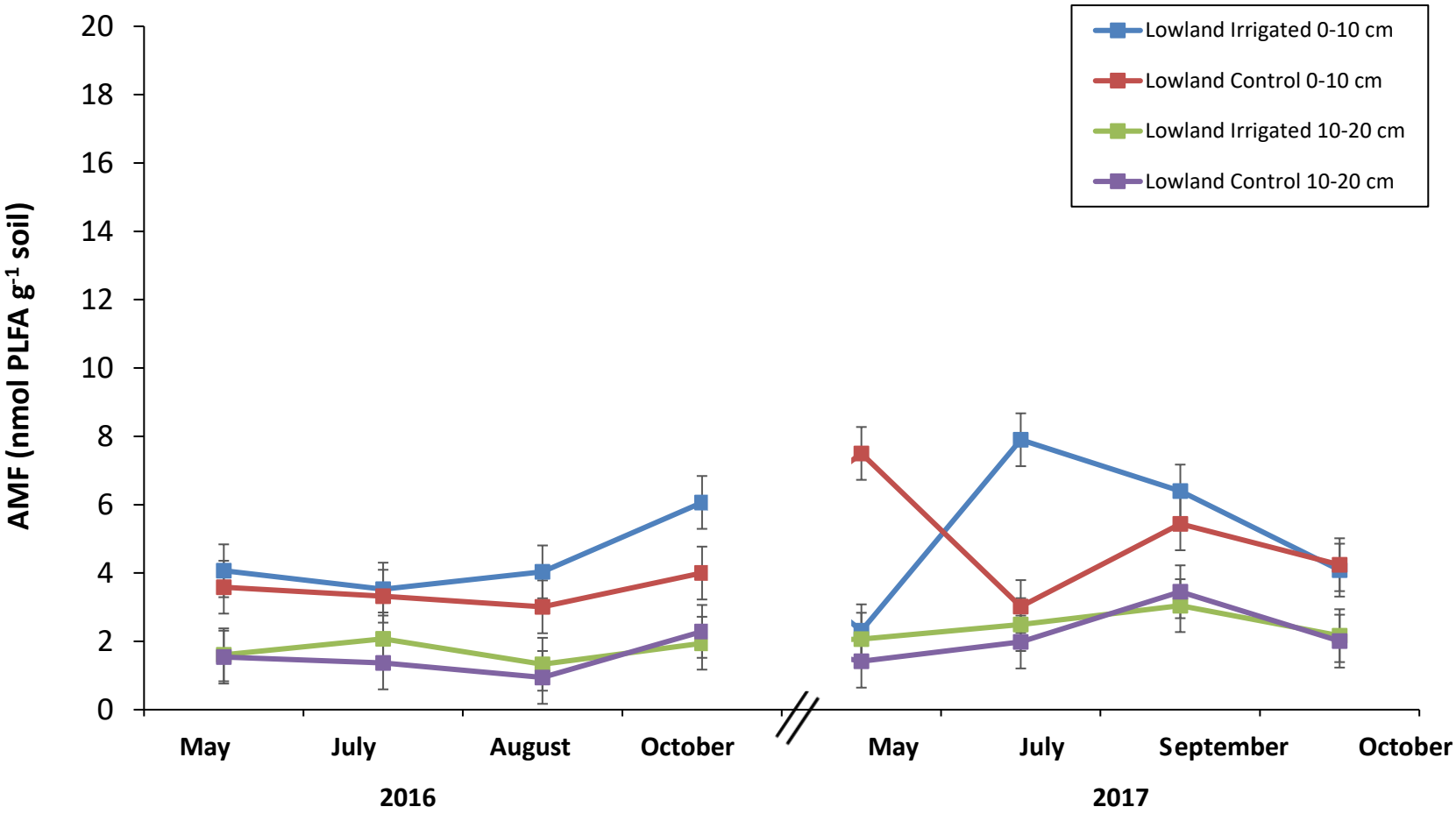

Figure 2.12. Arbuscular mycorrhizal fungi (AMF) phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

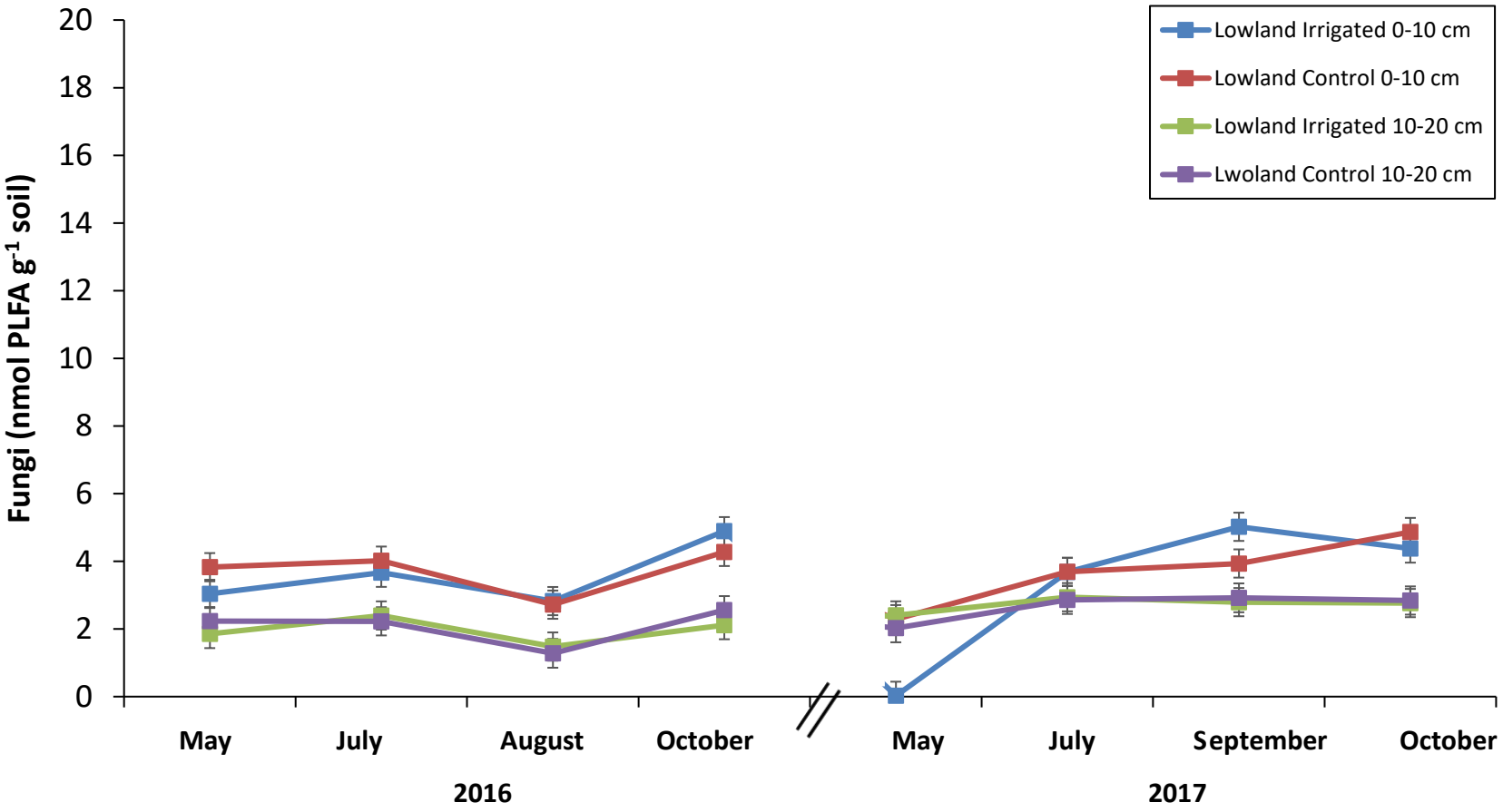

Figure 2.13. Fungi phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

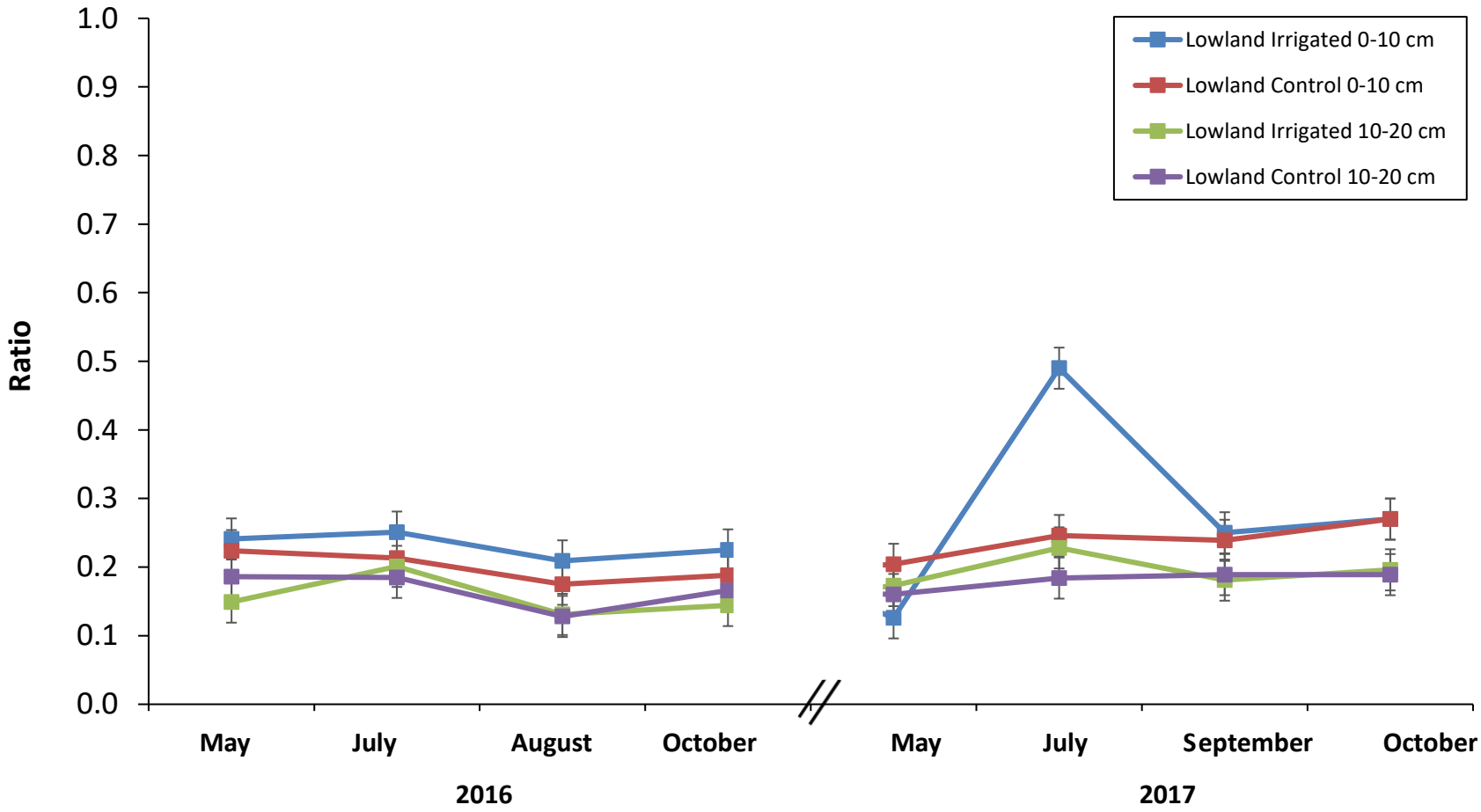

Figure 2.14. Fungal to bacterial ratio of phospholipid fatty acid (PLFA) concentration by depth and treatment in lowland soil. Error bars represent standard error of the mean (n=8).

Table 2.1. Ancillary soil measurement means (n=8) and p-values across landscape positions and irrigation treatments at 0-10 cm depth. All measurements were taken at the beginning of the study. Significant p-values are highlighted in bold type.

	Total N	Soil Organic C	C:N	pH	Ca	Mg	Na	P	K
	---------g kg^{-1}---------				----------µg g^{-1} soil----------				
Upland Control	2.58	33.6	13.1	6.51	3492	597	44.2	2.99	344
Upland Irrigated	2.83	36.5	12.9	7.32	4225	682	41.0	3.01	378
Lowland Control	2.05	27.3	13.3	6.82	3840	670	40.2	3.29	382
Lowland Irrigated	2.53	34.6	13.7	7.44	4332	673	56.5	3.66	384
	----------p-values----------								
Irrigation	**0.0528**	**0.0367**	0.5573	**<.0001**	0.1092	0.1852	0.5207	0.7161	0.8143
Location	**0.0396**	**0.0638**	0.1776	0.2256	0.4885	0.7026	0.5712	0.6248	0.5563
Irrigation*Location	0.3978	0.2170	**0.0937**	0.1274	0.7055	0.2210	0.3546	0.7492	0.6700

Table 2.2. Soil water content and soil nitrogen means (n=8) and p-values across landscape position and irrigation treatments at 0-10 cm depth. Significant p-values are highlighted in bold type.

		Gravimetric Soil Water Content		Ammonium (NH_4^+-N)		Nitrate (NO_3^--N)	
	Month	2016	2017	2016	2017	2016	2017
		g g^{-1} soil		µg NH_4^+-N g^{-1} soil		µg NO_3^--N g^{-1} soil	
Upland Control	May	34.6	28.7	1.46	0.74	0.27	0.07
	July	36.4	16.0	0.26	0.22	0.02	0.29
	Aug/Sept	24.9	14.1	0.42	0.35	0.09	0.06
	Oct	34.8	17.1	0.26	0.29	0.09	0.03
Upland Irrigated	May	40.0	30.7	1.14	0.67	0.18	0.07
	July	37.0	14.6	0.28	0.36	0.02	0.23
	Aug/Sept	30.8	21.3	0.43	0.38	0.07	0.07
	Oct	42.2	19.8	0.34	0.28	0.12	0.04
Lowland Control	May	34.7	30.9	1.44	0.71	0.19	0.12
	July	34.9	15.2	0.34	0.26	0.01	0.10
	Aug/Sept	28.9	21.1	0.53	0.54	0.04	0.08
	Oct	44.1	20.3	0.23	0.27	0.02	0.03
Lowland Irrigated	May	43.2	34.6	0.87	1.08	0.20	0.13
	July	34.6	15.2	0.29	0.26	0.01	0.10
	Aug/Sept	34.8	23.9	0.49	0.41	0.03	0.02
	Oct	45.8	20.6	0.29	0.32	0.01	0.02
		p-values					
Irrigation		0.1489		0.6395		0.5915	
Location		**0.0014**		0.3397		0.1407	
Irrigation*Location		0.7717		0.6832		0.7172	
Time		**0.0481**		**<.0001**		0.1729	
Irrigation*Time		**0.0676**		0.5715		0.8488	
Location*Time		**0.0323**		0.7276		0.3641	
Irrigation*Location*Time		0.2512		0.3759		0.4408	

Table 2.3. Phospholipid fatty acid (PLFA) means (n=8) and p-values across landscape position and irrigation treatments at 0-10 cm depth. Significant p-values are highlighted in bold type.

		Total biomass		Gram +		Gram -		Actinomycetes		AMF		Fungi	
	Month	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
		PLFA (nmol g^{-1} soil)											
Upland Control	May	79.2	76.4	21.6	9.11	6.49	4.70	5.91	1.26	3.93	1.02	4.42	0.19
	July	76.8	54.9	21.7	14.7	8.25	4.48	6.39	5.07	3.43	2.77	2.96	2.66
	Aug/Sept	72.7	96.0	19.3	27.7	11.1	8.75	6.05	8.27	2.45	4.82	1.92	3.79
	Oct	109	77.4	33.8	19.9	10.4	6.26	8.48	8.32	5.03	3.84	6.34	3.95
Upland Irrigated	May	74.5	46.6	19.1	9.52	6.87	5.04	5.81	1.78	4.40	0.64	4.04	0.46
	July	82.3	58.6	21.4	14.7	9.99	5.23	5.90	5.34	4.54	3.49	3.99	3.03
	Aug/Sept	70.9	95.9	19.4	25.7	7.04	8.71	7.35	7.95	3.80	5.83	2.74	4.30
	Oct	101	80.5	29.4	19.8	9.33	7.19	8.58	8.02	5.96	4.81	5.50	4.26
Lowland Control	May	74.9	63.9	22.1	19.7	6.26	5.40	4.59	3.87	3.59	5.92	3.83	2.01
	July	61.3	60.4	17.6	16.2	4.44	5.66	4.23	5.54	2.91	3.02	3.52	3.69
	Aug/Sept	69.7	90.2	21.1	22.0	4.62	9.09	7.11	8.45	3.01	5.44	2.72	3.94
	Oct	90.8	82.8	29.6	21.5	7.27	7.76	6.75	7.69	4.00	4.24	4.28	4.87
Lowland Irrigated	May	68.9	41.4	17.2	14.3	6.56	1.91	5.87	1.80	4.07	2.31	3.04	0.02
	July	67.5	61.4	17.9	12.5	5.08	4.92	5.10	5.92	3.53	7.90	3.66	3.68
	Aug/Sept	72.7	105	18.2	27.4	7.00	9.20	8.01	9.08	4.03	6.40	2.82	5.02
	Oct	106	74.1	29.7	16.7	11.7	5.93	7.10	8.67	6.07	4.08	4.89	4.38

	p-values					
Irrigation	0.5623	0.3149	0.9876	0.3764	0.1257	0.8587
Location	0.3398	0.8317	0.2455	0.9596	0.3322	0.7219
Irrigation*Location	0.4006	0.2586	0.8463	0.5425	0.9852	**0.0795**
Time	0.1099	**0.0600**	**0.0664**	**0.0039**	0.3242	**0.0799**
Irrigation*Time	**0.0731**	0.6844	0.7124	0.8477	0.2211	**0.0063**
Location*Time	0.8926	0.1348	0.8186	0.3179	0.5147	0.1411
Irrigation*Location*Time	0.2440	**0.0744**	0.2744	0.8786	0.5923	**0.0977**

Table 2.4. Fungal to Bacterial ratio means (n=8) and p-values across landscape positions and irrigation treatments at 0-10 cm depth. Significant p-values are highlighted in bold type.

		F:B	
	Month	2016	2017
-------Ratio-------			
Upland Control	May	0.25	0.07
	July	0.18	0.22
	Aug/Sept	0.13	0.19
	Oct	0.22	0.23
Upland Irrigated	May	0.27	0.04
	July	0.24	0.26
	Aug/Sept	0.19	0.24
	Oct	0.24	0.26
Lowland Control	May	0.22	0.20
	July	0.21	0.25
	Aug/Sept	0.18	0.24
	Oct	0.19	0.25
Lowland Irrigated	May	0.24	0.13
	July	0.25	0.49
	Aug/Sept	0.20	0.25
	Oct	0.23	0.27
------p-values------			
Irrigation		**0.0472**	
Location		0.1495	
Irrigation*Location		0.8033	
Time		0.2538	
Irrigation*Time		0.1295	
Location*Time		0.1930	
Irrigation*Location*Time		0.5421	

Table 2.5. Ancillary soil measurement means (n=8) and p-values by depth and irrigation treatment in lowland soils. All measurements were taken at the beginning of the study. Significant p-values are highlighted in bold type.

	Total N	Soil Organic C	C:N	pH	Ca	Mg	Na	P	K
	---------g kg^{-1}---------				----------µg g^{-1} soil----------				
Lowland 0-10 cm Control	2.05	27.3	13.3	6.82	3840	670	40.2	3.29	382
Lowland 0-10 cm Irrigated	2.53	34.6	13.7	7.44	4332	673	56.5	3.66	384
Lowland 10-20 cm Control	1.84	22.0	12.0	6.91	3909	751	50.2	1.64	318
Lowland 10-20 cm Irrigated	1.86	22.9	12.3	7.31	4340	670	83.4	2.33	346
	----------p-values----------								
Irrigation	**0.0723**	0.4046	0.1450	**<.0001**	**0.0802**	0.3669	**<.0001**	0.2926	0.7022
Depth	**0.0137**	0.4083	**<.0001**	0.7576	0.6443	0.3709	**0.0020**	0.2269	0.1615
Irrigation*Depth	**0.0961**	0.4067	0.8987	0.1181	0.7201	0.3291	0.1328	0.7546	0.4884

Table 2.6. Soil water content and soil nitrogen means (n=8) and p-values by depth and irrigation treatment in lowland soils. Significant p-values are highlighted in bold type.

		Gravimetric Soil Water Content		Ammonium (NH_4^+-N)		Nitrate (NO_3^--N)	
	Month	2016	2017	2016	2017	2016	2017
				µg NH_4^+-N g^{-1} soil		µg NO_3^--N g^{-1} soil	
Lowland 0-10 cm Control	May	34.7	30.9	1.44	0.71	0.19	0.12
	July	34.9	15.2	0.34	0.26	0.01	0.10
	Aug/Sept	28.9	21.1	0.53	0.54	0.04	0.08
	Oct	44.1	20.3	0.23	0.27	0.02	0.03
Lowland 0-10 cm Irrigated	May	43.2	34.6	0.87	1.08	0.20	0.13
	July	34.6	15.2	0.29	0.26	0.01	0.10
	Aug/Sept	34.8	23.9	0.49	0.41	0.03	0.02
	Oct	45.8	20.6	0.29	0.32	0.01	0.02
Lowland 10-20 cm Control	May	35.0	31.8	1.05	0.47	0.04	0.02
	July	30.6	14.7	0.29	0.26	0.01	0.02
	Aug/Sept	27.7	21.4	0.45	0.42	0.07	0.02
	Oct	30.8	17.9	0.26	0.34	0.01	0.02
Lowland 10-20 cm Irrigated	May	36.9	30.1	1.74	1.25	0.06	0.03
	July	32.6	13.8	0.39	0.31	0.12	0.03
	Aug/Sept	29.4	26.0	0.45	0.40	0.04	0.02
	Oct	37.1	17.6	0.25	0.35	0.02	0.02
		---------- p-values ----------					
Irrigation		**0.0816**		0.1840		0.4572	
Depth		0.1055		0.3820		0.1243	
Irrigation*Depth		0.2118		**0.0588**		0.4202	
Time		**0.0443**		**<.0001**		**0.0146**	
Irrigation*Time		0.1014		0.1698		0.4926	
Depth*Time		**0.0123**		0.3481		**0.0270**	
Irrigation*Depth*Time		**0.0330**		**<.0001**		0.7259	

Table 2.7. Phospholipid fatty acid (PLFA) means (n=8) and p -values by depth and irrigation treatment in lowland soils. Significant p-values are highlighted in bold type.

		Total biomass		Gram +		Gram -		Actinomycetes		AMF		Fungi	
	Month	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
		PLFA (nmol g^{-1} soil)											
Lowland 0-10 cm Control	May	74.9	63.9	22.1	19.7	6.26	5.40	4.59	3.87	3.59	5.92	3.83	2.01
	July	61.3	60.4	17.6	16.2	4.44	5.66	4.23	5.54	2.91	3.02	3.52	3.69
	Aug/Sept	69.7	90.2	21.1	22.0	4.62	9.09	7.11	8.45	3.01	5.44	2.72	3.94
	Oct	90.8	82.8	29.6	21.5	7.27	7.76	6.75	7.69	4.00	4.24	4.28	4.87
Lowland 0-10 cm Irrigated	May	68.9	41.4	17.2	14.3	6.56	1.91	5.87	1.80	4.07	2.31	3.04	0.02
	July	67.5	61.4	17.9	12.5	5.08	4.92	5.10	5.92	3.53	7.90	3.66	3.68
	Aug/Sept	72.7	105	18.2	27.4	7.00	9.20	8.01	9.08	4.03	6.40	2.82	5.02
	Oct	106	74.1	29.7	16.7	11.7	5.93	7.10	8.67	6.07	4.08	4.89	4.38
Lowland 10-20 cm Control	May	45.0	43.8	13.7	13.8	3.08	3.04	4.05	4.68	1.54	1.42	2.23	2.02
	July	42.3	55.8	13.6	15.0	2.50	8.73	3.76	5.13	1.37	1.98	2.23	2.86
	Aug/Sept	44.1	73.5	11.8	20.0	9.41	5.35	3.55	8.82	0.94	3.45	1.28	2.92
	Oct	58.6	54.5	18.9	15.0	4.64	5.43	4.88	5.57	2.29	2.01	2.55	2.84
Lowland 10-20 cm Irrigated	May	56.1	52.7	14.7	16.0	7.23	3.79	4.77	5.65	1.61	2.06	1.85	2.40
	July	49.0	53.8	13.6	14.7	4.28	3.80	4.39	5.36	2.07	2.49	2.40	2.94
	Aug/Sept	42.2	70.0	13.2	19.1	2.46	4.70	5.48	8.22	1.33	3.05	1.48	2.79
	Oct	56.2	55.2	19.0	15.5	3.87	3.55	5.34	6.00	1.95	2.17	2.11	2.77

	p-values					
Irrigation	0.6211	**0.0731**	0.6561	0.1196	0.1944	0.5481
Depth	**0.0135**	**0.0461**	**0.0132**	0.1484	**0.0002**	**0.0221**
Irrigation*Depth	0.8579	0.4357	0.5329	0.7334	0.5473	0.6552
Time	0.1056	0.1088	0.3482	**0.0094**	0.7603	**0.0845**
Irrigation*Time	0.5108	0.2773	0.7704	0.8908	0.2079	**0.0195**
Depth*Time	**0.0014**	**0.0039**	0.2930	**0.0566**	0.9957	**0.0448**
Irrigation*Depth*Time	**0.0030**	**0.0114**	**0.0723**	0.6171	0.3197	**0.0082**

Table 2.8. Fungal to Bacterial ratio means (n=8) and p-values by depth and irrigation treatment in lowland soils. Significant p-values are highlighted in bold type.

		F:B	
	Month	2016	2017
		----------Ratio--------	
Lowland 0-10 cm Control	May	0.22	0.20
	July	0.21	0.25
	Aug/Sept	0.18	0.24
	Oct	0.19	0.25
Lowland 0-10 cm Irrigated	May	0.24	0.13
	July	0.25	0.49
	Aug/Sept	0.20	0.25
	Oct	0.23	0.27
Lowland 10-20 cm Control	May	0.19	0.16
	July	0.19	0.18
	Aug/Sept	0.13	0.19
	Oct	0.17	0.19
Lowland 10-20 cm Irrigated	May	0.15	0.17
	July	0.20	0.23
	Aug/Sept	0.13	0.18
	Oct	0.14	0.20
		----------p-values--------	
Irrigation		0.2231	
Depth		**0.0112**	
Irrigation*Depth		0.1278	
Time		**0.0690**	
Irrigation*Time		**0.0849**	
Depth*Time		0.5229	
Irrigation*Depth*Time		0.4731	

Chapter 3 - Short-term impact of moisture and substrate addition on microbial respiration of prairie soil

Abstract

Global climate models predict environmental changes including changing precipitation patterns in the coming years. Changing precipitation patterns will alter soil water content, making it more variable. Precipitation events are expected to be interspaced with long dry periods, causing increased wetting and drying cycles in terrestrial soils. When dry soil is rewet, microbial respiration increases rapidly producing a phenomenon widely known as the "Birch effect". The community composition may alter the respiration response to rewetting events. The objective of this study was to assess the microbial community response to moisture and substrate addition in soils from a long-term precipitation manipulation in a grassland. Soil samples (0-10 cm) were collected from a long-term (25 yrs) irrigation transect located within Konza Prairie Biological Station in eastern Kansas. The experimental design consisted of two moisture regimes: irrigated (relatively consistent soil water) and control (non-irrigated, ambient precipitation). Substrate-induced respiration (SIR) was used to estimate the active microbial community and the contributions of fungi and bacteria to respiration. An extended version of the SIR measured respiration for 21 days. Extended substrate-induced respiration (SIR) results indicated that both moisture and substrate addition stimulated microbial community response, with little impact of historic irrigation treatment. However, short-term SIR indicated that historic irrigation influences may be expressed within the first few hours of incubation. These results suggest that historic irrigation treatment may impact soil microbial respiration but long-term effects can be masked by other environmental factors.

Introduction

Global climate models predict environmental changes within the coming years. More specifically, increased temperature, extreme weather events and changing precipitation patterns are expected to have profound impacts on terrestrial ecosystems (CAST, 2004; Oreskes, 2005; IPCC, 2014; Hatfield et al., 2013). Increased temperatures and altered precipitation patterns will impact carbon (C) cycle feedbacks potentially accelerating climate change (IPCC, 2013). Global soil respiration is a significant contributor to atmospheric CO_2 and has increased concurrently with global temperatures (Bond-Lamberty and Thomson, 2010; Hashimoto et al., 2015; Salazar et al., 2018). Though higher temperature generally increases respiration, soil water is another key driver of respiration. As global precipitation events become more intense and less frequent, we may also expect to see changes in respiration response (IPCC, 2013; Salazar et al., 2018). To better understand the impacts of changing environmental patterns on microbial communities and soil properties, we must better understand the mechanisms that control these responses, specifically wetting and drying cycles in soils.

Soil water availability is highly variable, ultimately depending on infrequent precipitation events interspaced with dry periods. The soil water potential fluctuates presenting various physiological challenges to soil microbial communities (Kakumanu and Williams, 2014). Microbial communities are known to respond to desiccation and low water potential by accumulating compatible solutes and osmolytes within the cell, such as glycerol, amino acids, and mannitol (Fierer and Schimel, 2003; Estop-Aragonés and Blodau, 2012). Some microbial groups adjust their cell wall properties to prevent osmotic shock during drying/rewetting cycles (Voroney and Heck, 2015). Generally, gram-negative bacterial cells are unable to withstand high turgor pressure compared to their thick-walled, gram-positive counterparts.

When dry soil is rewet, microbial CO_2 production increases rapidly (Birch, 1958). This phenomenon, widely known as the "Birch effect," has been documented in both field and laboratory studies (Fierer and Schimel, 2002; Mikha et al., 2005; Sawada et al., 2016). The overall duration and degree to which the Birch effect is observed depend on several factors including soil type, amount of water added, temperature, and microbial community composition (Huxman et al., 2004; Xiang et al., 2008; Borken and Matzner, 2009; Lado-Monserrat et al., 2014; Rossabi et al., 2018). This microbial CO_2 pulse has been attributed to a rewetting induced release of labile organic carbon, the release of microbial osmolytes from cells lysed by osmotic shock, rapid metabolism of microbial osmolytes by surviving cells, or a combination of these (Bottner, 1985; Lundquist et al., 1999; Williams and Xia, 2009; Unger et al., 2010; Jenerette and Chatterjee, 2012; Kakumanu and Williams, 2014). The impact of rewetting-associated CO_2 pulses are a key consideration in better understanding ecosystems subject to frequent drying and rewetting cycles, such as grasslands.

Soil water availability in grasslands is highly variable (Harper et al., 2005). This variability impacts grassland microbial communities by creating pulses of activity when water is available and periods of limitation when water is unavailable (Borken and Matzner, 2009; Manzoni et al., 2014).. In addition to their sensitivity to environmental changes, microbes are taxonomically and functionally diverse (Carson and Zeglin, 2018). The short generation time of microbes coupled with their diversity and changing environmental drivers have the potential to influence microbial responses on multiple time scales with differing levels of magnitude (Jones and Murphy, 2007; Cregger et al., 2012; Carson and Zeglin, 2018). Differing interactions between substrate availability, soil water, and microbial groups further confound the overall response to intermittent precipitation. Disentangling the microbial responses and biological

drivers of respiration pulses is complex because they are interrelated (Mikha et al., 2005; Schimel et al., 2007; Manzoni et al., 2014). Little is known concerning the effect of long-term changes in soil water availability on short-term responses to wetting-drying effects. It is therefore important to determine how matric potential differences of individual events may elucidate microbial responses to water stress conditions. Further, a better understanding of microbial community responses to short term changes will aid in the further understanding of long-term environmental changes and how they may impact the soil microbial community.

The objectives of this study were to: i)assess microbial respiration response to an individual drying and rewetting event after long-term precipitation manipulation; and ii) compare the response of different microbial populations to desiccation conditions and substrate addition. We hypothesized that irrigation history would affect soil CO_2 efflux following a dry-down and re-wet event, and specifically predicted that: (i) both water and carbon addition would induce respiration with the combination of the two inducing the most respiration, (ii) soil microbes from the historically irrigated plots would have a greater CO_2 response than control due to (iii) a greater abundance of bacterial populations in irrigated soils.

Materials and Methods

Research Site

The study was conducted form soil of a native tallgrass prairie located at the Konza Prairie Biological Station (39°05'N, 96°35'W)in the Flint Hills of eastern Kansas, USA. The average monthly air temperature ranged from -2.7°C in January to 26.6°C in July. The mean annual precipitation was 835 mm. Soils at the site were silty clay loams. The upland site was the Clime (fine, mixed, active, mesic Udorthentic Haplustoll) -Sogn (loamy, mixed superactive, mesic Lithic Haplustoll) complex, while the lowland site of the transects was Irwin (fine, mixed,

superactive, mesic Pachic Argiustol). The aboveground net primary productivity (ANPP) at Konza Prairie Biological Station is 536 g m^{-2} (Wilcox et al., 2016a) and the dominant vegetation includes native big bluestem (*Andropogon gerardii*), indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgat*um). The site was burned annually in the spring and landscape was generally characterized by soils overlaying limestone and shale.

A precipitation manipulation experiment was established in the early 1990s to manipulate water availability to minimize water limitations. Two separate irrigation transects were established, one each in 1990 and 1993. The two replicate transects (140 m long by 30 m wide), were comprised of an upland and lowland landscape positions. This study included 2 irrigated and 2 ambient (control) transects. The irrigated transects were watered as needed to minimize water stress through the growing season via a series of 1 m tall sprinkler heads arrayed along each transect. Irrigated treatments receive supplemental water when the measured volumetric water content (VMC) drops below 0.25 VMC to bring the water content to 0.30 VMC. Field moisture was maintained within the irrigation transects yearly from May to September. Ambient plots received no irrigation in addition to natural precipitation events. The site was burned annually and was protected from grazing for over 35 years. This experimental site has been previously referenced in the literature (Knapp et al., 1994b, 2001; Williams and Rice, 2007; Wilcox et al., 2016a).

Sample Collection

Soil samples were collected 4 June 2018 from the lowland position only. Soil cores were randomly collected using a manual, 1.9 cm diameter, soil punch probe to a depth of 0-5 cm from three sampling sites within each transect. Soil cores taken from within each site were mixed thoroughly and passed through an 8-mm sieve to remove plant biomass. The sieved soil samples

were air-dried in open ziplock bags at room temperature (24°C) for 72 hours. At the conclusion of the drying period, soils were stored at 4°C until further analysis (within 24 hours).

Gravimetric Soil Water Content

Gravimetric soil water content was measured at the start of the study by weighing 10g of field moist soil into a tin and oven drying at a temperature of 105°C for 48 hours. Samples were then re-weighed and the gravimetric soil water content was calculated as mass of water lost as a percentage of the oven dry soil mass using the equation below.

$$\%\ Soil\ Moisture = \frac{Moist\ Soil\ Sample\ (g) - Oven\ Dry\ Soil\ (g)}{Oven\ Dry\ Soil\ (g)}\ x\ 100$$

Soil Extractable Nitrogen

Inorganic soil nitrogen (NO_3-N and NH_4-N) was determined at the beginning of the study by KCl extraction. Briefly, within 24 hours of the sample date, 100 mL of 1M KCl solution was added to 25g of field moist soil to yield a ratio of 1:4 (Soil:1M KCl). Samples were shaken at 300 rpm on an orbital shaker for 60 minutes. Samples were then filtered through Whatman No. 42 filter paper and decanted into 20 mL scintillation vials. Vials were then submitted to the K-State Soil Testing Lab for colorimetric analysis (Keeney and Nelson, 1982; Gelderman and Beegle, 1998).

Soil Cations

Air-dried soil subsamples were used to determine extractable cations (Ca, K, Mg, & Na) at the beginning of the study. Cations were determined by the ammonium acetate (1M, pH 7.0) method with the use of low-sodium filter paper (Warncke and Brown, 1998). Analysis was done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, manufactured by Varian Australia Pty Ltd, Mulgrave, Vic Australia and a Model

AAnylyst 200 (AA) Spectrometer from Perkin Elmer Life and Analytical Sciences, Shelton, CT. The extraction and the analysis was performed by the Kansas State University soil testing lab.

Substrate Induced Respiration

To estimate the contributions of fungi and bacteria to respiration, selective inhibition of microbial respiration was conducted using a modified version of the classic protocol by Anderson and Domsch (1973). Briefly, 15 g of soil was placed into four sets of 160 mL serum bottles. One set of bottles received 15 mL of water and was capped. The other three sets of vials received a 15 mL solution containing 5 g L^{-1} D-glucose. One set of D-glucose bottles was capped and the other two sets of bottles received either 200 mg cycloheximide (a fungal inhibitor) or 100 mg chlortetracycline (a bacterial inhibitor). Earlier experiments conducted by Garcia (1992), Smith (1998), and Williams (2001) determined optimum rates of addition of glucose and selective inhibitors. Samples were shaken on an orbital shaker at 200 RPM and CO_2-C production was measured four times during a 5-hr incubation. The CO_2-C efflux from soil samples was assessed by taking a 0.5 ml sample of headspace gas from each vial and analyzing it using a Shimadzu gas chromatograph-8A (Shimadzu Inc., Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector and a 2-m Porapak column. The column temperature was 75°C and the injection temperature was 160°C. The carrier gas was He.

Extended Substrate Induced Respiration

The carbon use pattern of the total soil microbial community, in soils taken from both long-term irrigation and ambient conditions, was assessed by substrate-induced respiration. Responses to D-glucose substrate addition, water addition, or both were measured. Briefly, 30 g of air-dried soil, from both irrigated and ambient transects, was placed into four sets of 125 mL

Erlenmeyer flasks, for a total of eight flask sets. The first set of flasks received no amendments. The second set received 10 mL of deionized water. The third set received 0.5 g of dry D-glucose and the final set received 0.5 g of dry D-glucose and 10 mL of deionized water. After the addition of the amendments, flasks were sealed in 940 mL mason jars. Mason jar tops were modified with a septum to retrieve gas samples. Jars were incubated at 25°C for 21 days. Six replicate samples were used to assess the effects of each amendment. The CO_2-C efflux from soil samples was assessed by taking a 0.5 ml sample of headspace gas from each jar and analyzing it using a Shimadzu gas chromatograph-8A (Shimadzu Inc., Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector and a 2-m Porapak column. The column temperature was 75°C and the injection temperature was 160°C. The carrier gas was He.

Statistical Analysis

The data were analyzed as a split-plot design with precipitation manipulation (ambient or irrigated), and substrate treatment as main factors. The analysis used a split-plot model (Milliken and Johnson, 2009) and the Mixed covariate dependency procedures in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Unless otherwise stated, all results were considered significant at the $p< 0.05$ level.

Results

Extractable N and Soil Cations

Ancillary soil measurements were taken at the beginning of the study. Irrigated soils contained more soil water, extractable $NH_4^{+,}$ and soil cations than control soils (Table 3.1).

Substrate-Induced Respiration

In all treatments, CO_2 production gradually increased from the beginning to the end of the incubation ($p <0.0001$, Fig. 3.1). Historical irrigation treatment also affected SIR ($p= 0.0045$), as

irrigated soils respired more CO_2 than control soils in both the water only and glucose only incubations. Historical irrigation also caused differential contribution of fungi and bacteria to glucose-induced respiration. ($p<0.0001$), in that the response of respiration to the addition of glucose and the antibacterial compound chlorotetracycline was higher in control soils than irrigated soils (Fig 3.2 d). The response of respiration to the addition of glucose and the antifungal compound, cycloheximide, was significantly affected by the interaction of irrigation and treatment ($p <.0001$) where the irrigated soil had higher respiration than the control soil (Fig 3.2 c).

Extended Substrate Induced Respiration

The laboratory water and glucose amendments significantly increased respiration ($p= 0.0067$, <0.0001 respectively; Table 3.3). There was a positive effect of glucose (Glucose byTime, $p<0.0001$; Fig. 3.2 c and d) and of glucose coupled with water amendment (Water Amendment by Glucose; $p=0.0003$; Fig 3.2 d) on CO_2 production in both historically irrigated and control soils.

A significant 3-way interaction between irrigation, water, and glucose ($p=0.0002$; Table 3.3) was the result of historic irrigation increased CO_2 accumulation over time, but only in the dry, glucose-amended treatment. The 3-way interaction of Water Amendment, Glucose, and Time ($p <0.0001$), was due to an increased respiration above dry treatment level with the water amendment, more with glucose amendment (Fig. 3.2 c).

Discussion

We predicted that irrigation history would affect soil CO_2 efflux following a dry-down and re-wet event. More specifically, we predicted that both water and carbon addition would induce respiration with the combination of the two inducing the greatest response from the historically irrigated plots than the control due to a greater abundance of bacterial populations in irrigated soils.

Substrate-induced respiration (SIR) measures the response of the soil microbial community to substrate addition, representing the subset of microbial cells within the community that are in a metabolically active state at the time of assay (Bailey et al., 2002). The SIR procedure has been used to estimate active microbial biomass across a range of organic matter decomposition stages (Aira and Domínguez, 2010). The influence of water availability alone on soil microbial activity has also been documented: CO_2 production often peaks 48 hours after a rewetting event, then declines even if soil moisture is maintained (Schimel et al., 2007; Wang et al., 2015; Rossabi, 2018). In a laboratory incubation, 24 hours after rewetting, soil CO_2 production was 3 times higher than 72 hours after the rewetting event, though moisture levels remained consistent (Fierer and Schimel, 2003). This suggests that the instantaneous CO_2 pulse may be derived from lysed bacterial cells upon re-wetting. As the microbial substrate was depleted, respiration rates decline through the soil remains moist.

Reduced soil water content restricts the molecular diffusion of microbial substrates through soil pore space and limits microbial mobility, thus reducing substrate availability to microbial cells (Kakumanu and Williams, 2014). The restricted soil solute flow and may prevent both plants and microbes from assimilating necessary nutrients during soil drying, but can also cause an accumulation of unused C and nutrients in the dry soil. Fungi are generally more drought tolerant than bacteria and require less physiological adjustment to survive desiccation conditions. Therefore, microbial cells that are unable to adjust via osmoregulation may be subject to cell lysis.

Carbon substrate inputs are key regulators of microbial activity (Wang et al., 2003; Jenerette and Chatterjee, 2012; Allison et al., 2014). Under favorable moisture and temperature conditions, substrate availability is the principal determinant of soil respiration (Wang et al., 2003). Plant net primary production has increased in response to irrigation, more so after a shift in dominant grass

composition (Wilcox et al., 2016). This likely lead to an increase in substrate availability to soil microbes, which may also change the microbial community as a result of legacy irrigation.

Long-term incubation soils with no glucose amendment displayed little difference in respiration pattern over time (Fig 3.2 a and b). Soils treated with both glucose and moisture addition appear to have reached a plateau in respiration potential after about 10 days regardless of historic irrigation treatment (Fig 3.2 d). Substrate-induced respiration indicated that microbial respiration was enhanced by long-term irrigation. As expected, alleviating substrate limitation through glucose addition was correlated with increased respiration.

The documented differences in SIR between the irrigated and control sites coincide with the findings of Williams and Rice (2007) that long periods of increased water additions via irrigation reduces microbial stress and advances microbial activity and function. However, the results of the extended SIR were strikingly different. It is possible that the extended SIR followed a similar trend in the early stages of respiration; however, our study did not record those early responses.

Sakamoto and Oba (1994) found that fungal/bacterial ratio significantly influenced respiration. Overall, respiration decreased as the fungal to bacterial ratio increased likely due to a higher substrate use efficiency of fungi compared to bacteria. The re-wetting of dry soil released C from the mineralization of soil organic matter (SOM) protected by soil aggregates. In our study, water addition, glucose, and the combination of both had a significant effect on historically irrigated soils. The response was likely mediated by a subset of microbes that maintained their metabolically active state.

The response of soils with glucose + chlorotetracycline addition was surprising. The lack of microbial respiration inhibition by chlorotetracycline amendment may suggest that the fungal/bacterial ratio was not affected. Though our study did not directly measure microbial biomass, it is possible that the microbial community in our soils was less susceptible to the anti-microbial

agents (Fry et al., 2018). It is also plausible that the proportion of metabolically active community members was large and impacts were minimal.

Conclusion

In summary, our results indicate that increased respiration was stimulated by both moisture and substrate addition with stronger responses in the active microbial community of historically irrigated soils. The study summarizes the evidence supporting a greater contribution of metabolically active bacteria in irrigated soils. (chapter 2).

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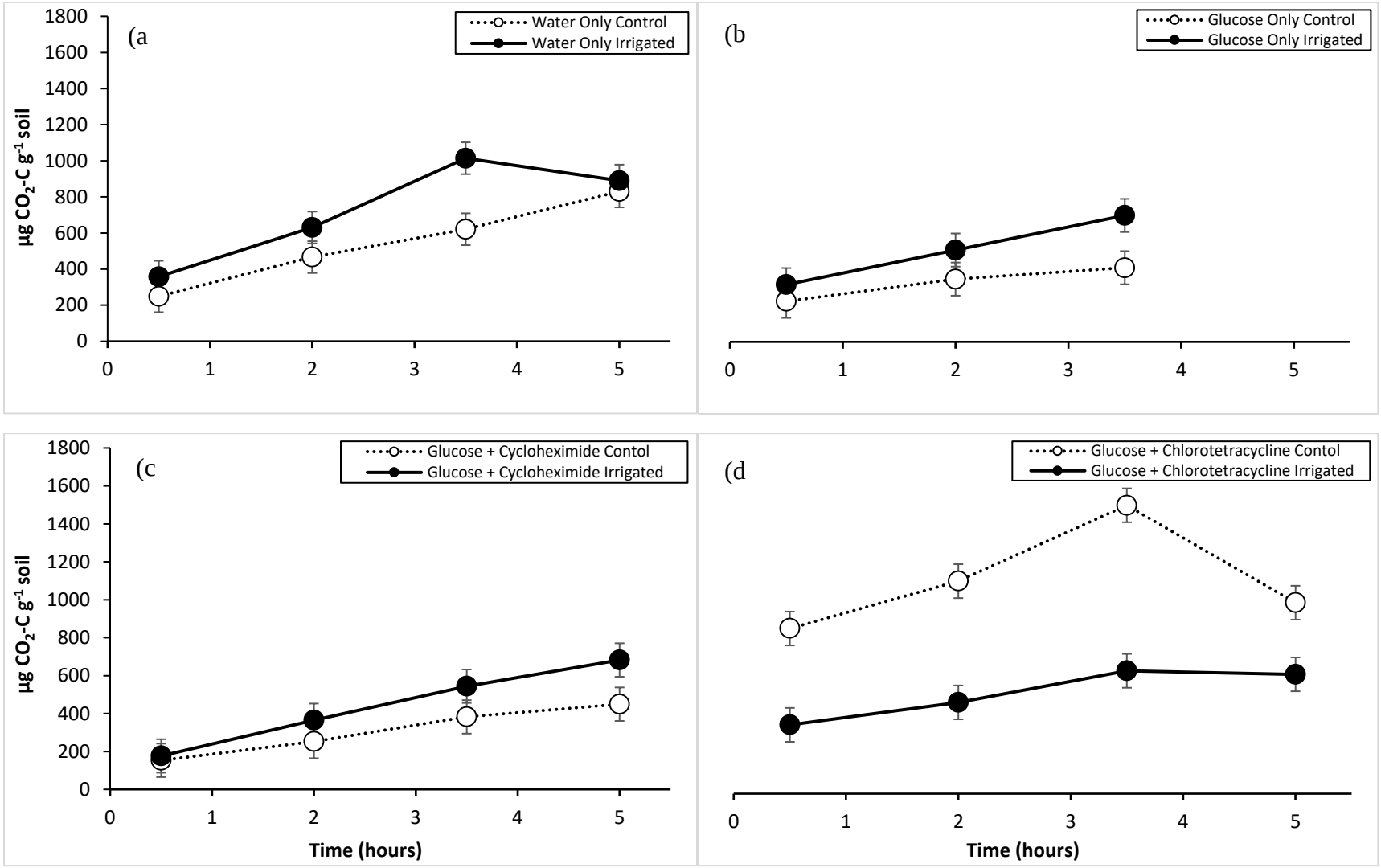

Figure 3.1. Mean substrate respiration induced by 4 soil additions in irrigated (——) and control (·····) soils over time. Error bars represent ± SE (n=4). Panel (a) shows the respiration of irrigated and control soils with water addition only. Panel (b) shows the respiration of irrigated and control soils with addition of 5 g L^{-1} D-glucose solution. Panel (c) shows the respiration of irrigated and control soils with addition of 5 g L^{-1} D-glucose solution and 200 mg cycloheximide. Panel (d) shows the respiration of irrigated and control soils with addition of 5 g L^{-1} D-glucose solution and 100 mg chlorotetracycline.

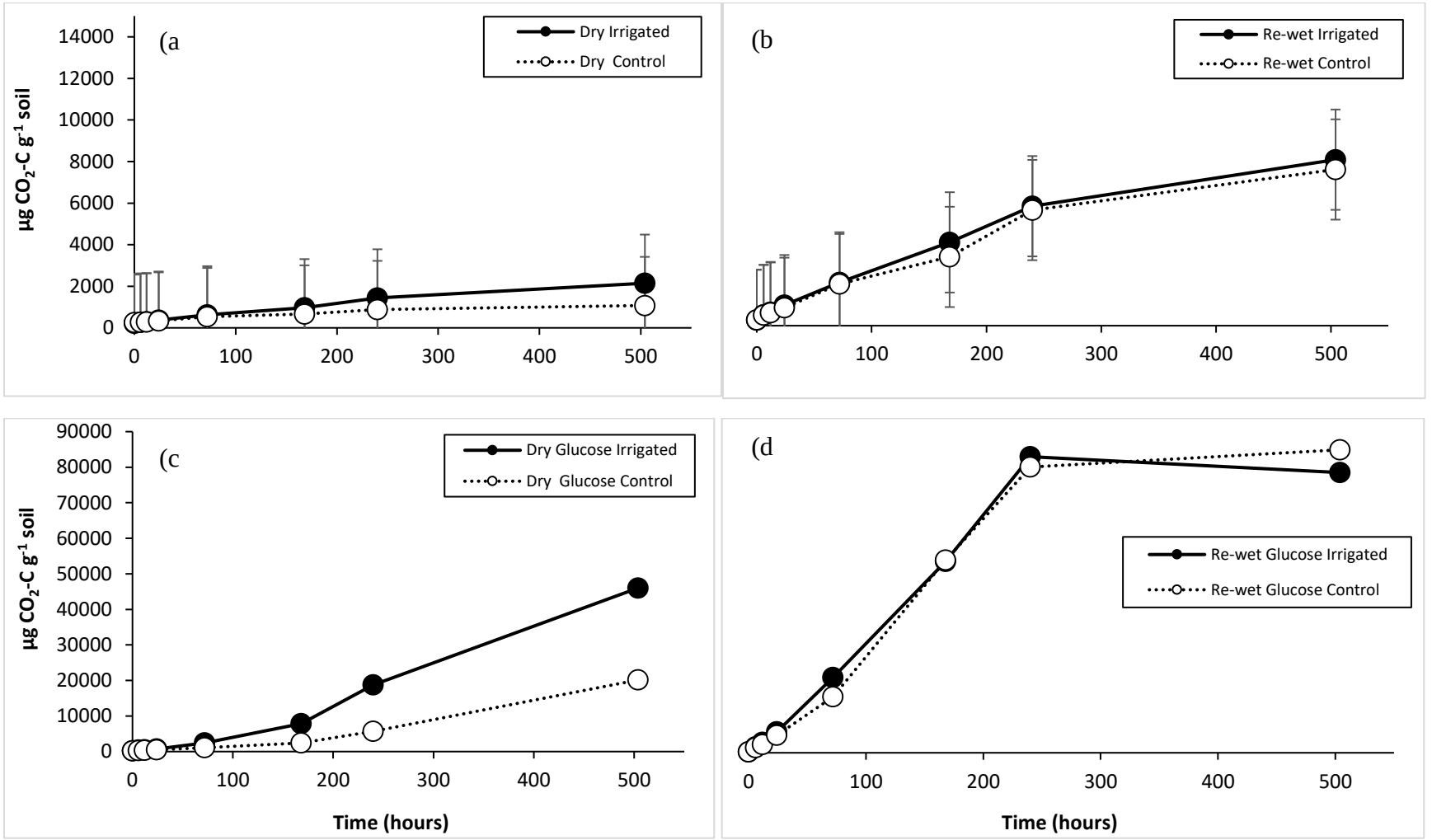

Figure 3.2. Mean substrate respiration induced by glucose and water addition in irrigated (——) and control (·····) soils over time. Error bars represent ± SE (n=6). Panel (a) shows the respiration of irrigated and control soils with no amendments. Panel (b) shows the respiration of irrigated and control soils with water addition only. Panel (c) shows the respiration of irrigated and control soils with addition of powdered D-glucose only. Panel (d) shows the respiration of irrigated and control soils with both water and D-glucose addition. Panels a and b are depicted on a smaller scale than panels c and d.

Table 3.1. Ancillary soil measurement means across irrigation treatments at 0-5 cm depth. All measurements were taken at the beginning of the study.

	Air Dry Soil Water Content	Ammonium	Nitrate	Ca	Mg	Na	K
	$g\ kg^{-1}$	$\mu g\ NH_4^+\text{-}N\ g^{-1}$ soil	$\mu g\ NO_3^-\text{-}N\ g^{-1}$ soil	----------$\mu g\ g^{-1}$ soil----------			
Lowland Control	16.4	0.62	0.04	4568	555	26.9	563
Lowland Irrigated	17.6	0.66	0.03	5241	684	45.2	570

Table 3.2. ANOVA results of mean substrate respiration induced by 4 soil additions in irrigated and contro soils over time.

Effect	p-value
Irrigation	**0.0045**
Lab Treatment	**<.0001**
Irrigation*Lab Treatment	**<.0001**
Time	**<.0001**
Irrigation*Time	0.7059
Lab Treatment *time	**0.0883**
Irrigation*Lab Treatment*time	0.1964

Table 3.3. ANOVA results of mean substrate respiration induced by glucose and water addition in irrigated and control soils over time.

Effect	p-value
Irrigation	0.3616
Water Amendment	**0.0067**
Irrigation*Water Amendment	0.3270
Glucose	**<.0001**
Irrigation*Glucose	0.2078
Water Amendment*Glucose	**0.0003**
Irrigation* Water Amendment*Glucose	0.2383
Time	**<.0001**
Irrigation*Time	**0.0433**
Water Amendment *Time	**<.0001**
Irrigation*Water Amendment*Time	**<.0001**
Glucose*Time	**<.0001**
Irrigation*Glucose*Time	0.1277
Water Amendment*Glucose*Time	**<.0001**
Irrigation*Water Amendment*Glucose*Time	**0.0002**

Chapter 4 - Long-term management on soil structure and microbial community of a degraded agricultural soil

Abstract

Microorganisms are critical to maintaining soil health and cultivating grain crops. Land management practices significantly affect the soil microbial community composition. The soil microbial community regulates nutrient cycles and soil structure. The objective of this study was to assess the relationship between long-term land management practices, soil microbial community composition, and soil structure. The ecosystems used in this study were grain sorghum (*Sorghum bicolor*) planted with either no-tillage (NT) or tilled (CT), and replanted (RP) big bluestem, (*Andropogon gerardii*). The experiment was located at the Konza Prairie Biological Station near Manhattan, Kansas. The experimental design was a split-plot design with cropping systems as the main effect and phosphorus amendment (+P) and no P (-P) as the subplot effect. Soil sampling was done at incremental soil depths (0-5, 5-10, 10-15, 15-30, 30-45, 45-60, and 60-90 cm) in the fall of 2017. We assessed water-stable aggregates (WSA), soil organic C, total N, phospholipid fatty acid analysis (PLFA), Mehlich-3 P, and soil cations. Macroaggregates (>250 µm) were significantly influenced by cropping system and depth. Macroaggregates in the RP was significantly greater than in NT and CT. Tillage did not significantly affect macroaggregates. There was a significant effect of depth on soil cations. No significant differences were observed between systems for the soil profile (0-90 cm) SOC or TN. This study found evidence that several mechanisms contribute to increased aggregation and nutrient levels in restored cropland. Our results also suggest that reduced tillage practices may increase soil aggregation faster than microbial community structure and soil C and nutrient storage.

Introduction

Soil health, often synonymous with soil quality, has been defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). Soils are multifunctional and the factors influencing soil quality are interdependent. In addition to supporting agricultural production, soils store nutrients, filter water, sustain biodiversity, and support various forms of vegetative life. As the global population increases, the agricultural sector faces the challenge of increasing global food sources while developing strategies to sustain agriculture through changing environmental conditions.

Besides climatic factors, land management can impact soil function. Due to the range of potential soil uses, an assessment of quality should evaluate the characteristics pertinent to the intended function and management practices of the soil (Stocking, 2003; Bünemann et al., 2018). While the indicators of soil health are numerous, soil microbial community structure, soil aggregation, and soil carbon (C) and nitrogen (N) are known to be primary soil health indicators (Bending et al., 2004).

Soils support a remarkable abundance of diverse microorganisms that benefit soil health through the activities of organic matter decomposition and nutrient cycling (Hobbie and Hobbie, 2013; Wall et al., 2015). Soil microbes are early indicators of soil health as they are sensitive to soil environmental changes (Murphy et al., 2016). Microbes have adapted various physiological survival mechanism to respond to environmental changes. For example, when nutrients are limited, some microbes enter a dormant state and return to an active state after substrate is added (Hobbie and Hobbie, 2013). Under water limited conditions, microbes accumulate compatible

osmolytes in the cell to avoid desiccation (Estop-Aragonés and Blodau, 2012; Kakumanu and Williams, 2014). When the soil is rewetted, microbes quickly dispose of excess osmolytes via CO2 respiration or exudates (Fierer et al., 2003). Thus changes in soil condition can impact the activity of soil microorganisms (i.e., global nutrient cycling).

Carbon and nutrient cycling are the backbone of terrestrial ecosystems. Estimated at over 2000 Pg of C, soil organic matter contains more C than the combined pools of global vegetation and the atmosphere (Lal, 2004). The total amount of C within the soil is a balance of organic inputs, C mineralization, and respiration (Post and Kwon, 2000). Soil organic carbon (SOC) is impacted by soil management practices and vegetation (Post and Kwon, 2000; Rice, 2002; Adkins et al., 2016; Nicoloso et al., 2018; McGowan et al., 2018). Soil N cycling is also a key soil function, as N is often the limiting nutrient for biological activity and growth. The N cycle is mediated by microbial activity converting organic N into plant available N forms. The soil C and N pools can be sustained or increased under ecosystems with adequate residue retention and minimal soil disturbance (Smith et al., 2012; Murphy et al., 2016; Ghimire et al., 2017b; Nicoloso et al., 2018).

Soil aggregation is "the process by which aggregates of different sizes are joined and held together by organic and inorganic materials" (Amézketa, 1999). The formation and stability of aggregates is a function of soil characteristics, including SOC, clay content, soil microbial community composition, soil disturbance and soil polysaccharide content (Chenu et al., 2000; Bronick and Lal, 2005; Eynard et al., 2005; Congreves et al., 2015; Zhu et al., 2017). Soil aggregation is the result of various chemical, physical, and biological soil interactions. More specifically, soil organisms, facilitate aggregation of mineral particles by chemical and physical mechanisms to form larger aggregates (Banwart, 2011). Pores between soil aggregates retain

moisture and oxygen, facilitating further microbial and vegetative growth. However, intensive agricultural cultivation has led to a decline in soil structure, agricultural productivity, and soil nutrient stability (Banwart, 2011; Regelink et al., 2015).

Improved land management practices have been promoted to reduce atmospheric CO_2, to enhance soil nutrient composition, improve soil structure and ultimately enhance soil health (Ontl and Schulte, 2012; Lal, 2013; Dignac et al., 2017). Reduced and no-tillage practices store large amounts of C by stabilizing soil aggregates and conserving organic matter within larger aggregate fractions (Ghimire et al., 2017a; Nicoloso et al., 2018). Aggregate stability and size are associated with pore size distribution (Nimmo, 2004). Varied pore size associated with soil aggregates, in addition to appropriate aeration for root growth, also allows for water infiltration to plant roots and soil microbes.

Understanding the elusive relationship between the impacts of conservative tillage practices on the microbial community composition in conjunction with soil structure is vital to sustaining the future of agriculture. This is particularly important as members of the soil microbial community, particularly arbuscular mycorrhizal fungi, are known to influence physical aggregation as well as chemical binding of soil aggregates via exudates (Rillig and Mummey, 2006; Hallett et al., 2009; Wilson et al., 2009). Determining the amount of C and N physically protected within soil aggregates is a key component of determining the effects of management practices on soil C dynamics thus aiding in the selection of best practices in land management and soil health restoration via the enhancement of SOC pools (Beare et al., 1994; Mikha and Rice, 2004; Zheng et al., 2018).

The objective of this study was to assess the impact of tillage and replanted big bluestem (Andropogon gerardii) on the biophysical properties of a C-depleted and disaggregated soil 13

years post intensive cultivation. More specifically, we aimed to: (1) to assess the effects of conservative continuous tillage and no-tillage, within grain sorghum (Sorghum bicolor), on soil aggregation and C pools associated with aggregate size and (2) to better understand the process of soil restoration via partial ecosystem restoration after the termination of intensive crop cultivation. We predicted that: (i) replanted big bluestem would increase rates of C inputs and increase macroaggregates compared to grain sorghum, (ii) increased macroaggregates would result in increased C associated with those macroaggregates, (iii) the termination of soil tillage would increase macroaggregates and soil C accumulation in NT as compared to CT plots, and (iv) increased macroaggregation would correspond to an increase in soil fungal communities, specifically arbuscular mycorrhizal fungi.

Materials and Methods

Research Site

The study was conducted in an agricultural field native tallgrass prairie located at the Konza Prairie Biological Station (39°05'N, 96°35'W) in the Flint Hills of eastern Kansas, USA. The soil at the study site was a fine, smectitic, mesic aquertic Argiudolls (Chase silty clay loam; Description C4.1).

Prior to the establishment of the experiment in 2004, the field was had been under intensive agricultural production of winter wheat (*Triticum aestivum*), a C3 crop, for over 20 years. The field was tilled and fertilized annually as part of the land management regime. In 2004, grain sorghum (*Sorghum bicolor*) was planted in either conventional tillage (CT) or no-tillage (NT) plots. The tillage systems were compared to replanted big bluestem (*Andropogon gerardii*) (RP). Tillage consisted of fall chisel plow and spring disked prior to planting in late May. The RP was burned annually in the spring. The prairie grass was planted in 2004 after

tillage of the entire experimental field. Grain sorghum (*Sorghum bicolor*) was planted at a density of approximately 75,000 seeds ha^{-1}. Urea Ammonium Nitrate (UAN) solution fertilizer was applied to grain sorghum at a rate of 134 kg N ha^{-1}. Nitrogen fertilization was discontinued for the replanted big bluestem in 2010 to mirror the normal management of prairie grasses. At initiation, half of each plot was amended with 90 kg P ha^{-1}, added in the spring. The P fertilizer was only applied to maintain higher levels of soil P to suppress mycorrhizal fungi. The other half of each plot was not amended with P to serve as a control. Herbicide application to the sorghum plots prior to planting included glyphosate, plus 2,4-D, plus Dicamba, and plus atrazine was applied to the sorghum plots prior to planting. Herbicide was not applied to the re-planted big bluestem. The big bluestem strips were burned annually, a common land management practice in the region.

Experimental Design

The field experiment was a split-plot design within a completely randomized block design. Ecosystem (CT, NT, RP) was used as the main plot factor. The P amendment with P (+P) or without P (–P) was the subplot factor. Each plot was 32 m2 (6m x 6m). All combinations of the main plot and subplot factors were represented within the replicated design.

Sample Collection

Soil samples were taken on 20 November 2017. With the exception of soil for water-stable aggregates, samples were acquired using a hydraulic, 5 cm diameter soil probe (Giddings Machine Company, Windsor, Colorado, USA). Samples were separated at incremental depths for analysis (0-5, 5-10, 10-15, 15-30, 30-45, 45-60, and 60-90 cm). Soils were stored in Ziplock bags

at 4°C for further processing and analysis. A subsample of each soil was freeze-dried for lipid analysis. A separate subsample of each soil was air dried for chemical analysis.

Samples for water-stable aggregate analysis were taken using a spade at incremental depths (0-5, 5-10, and 10-15 cm) to keep the extracted bulk soil intact. Samples were segregated at incremental depths (0-5, 5-10, and 10-15 cm). Soils were then stored in Ziploc bags at 4°C prior to air drying for soil aggregate analysis.

Mehlich-3 Extractable Phosphorus

Air-dried soil subsamples were extracted with a solution of glacial acetic acid, ammonium nitrate, ammonium fluoride, and nitric acid. A Lachat Quickchem 8000 was used to perform the colorimetric assay. The extraction and colorimetric assay were performed by the Kansas State University Soil Testing Laboratory as described by Frank et al. (1998).

Soil Cations

Air-dried soil subsamples were used to determine extractable cations (Ca, K, Mg, & Na). Cations were determined by the ammonium acetate (1M, pH 7.0) method with the use of low-sodium filter paper (Warncke and Brown, 1998). Analysis was be done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, (Varian Australia Pty Ltd, Mulgrave, Vic Australia) and a Model AAnylyst 200 (AA) Spectrometer (Perkin Elmer Life and Analytical Sciences, Shelton, CT). The extraction and the analysis was performed by the Kansas State University Soil Testing Lab.

Water Stable Aggregate Distribution

Soil samples were carefully separated along natural breaks into large aggregates. Soil was sieved through an 8mm sieve and air-dried prior to analysis. Water-stable aggregate size distribution was determined by wet sieving 50 g of air-dried soil through 20, 53, 250 and 2000 μm sieves with a

Yoder-type machine as described by Mikha and Rice (2004). Briefly, 50 g air-dried soil was placed on top of stacked 250 and 2000 μm sieves. Sieves were then submersed in water for 10 min (slaking phase) and subjected to 10 min of 4 cm length oscillations at a frequency of 0.5 Hz. The soil remaining on the sieves at the conclusion of the oscillation cycle was collected, allowed to settle, and dried at 60°C for 72 h. The soil that passed through both sieves was filtered through the 53 and 20 μm sieves. The soil remaining on these sieves was then collected, allowed to settle, and dried at 60°C for 72 h. The dried soil was weighed and used to estimate % aggregate size fraction within the soil. The larger aggregate fractions did not yield enough soil to correct for sand content. The average sand content for this soil type was 1-4% (Description C4.1).

Microbial Phospholipid Analysis

Samples from the top three depths were used: 0-5, 5-10, and 10-15 cm. Samples were frozen, lyophilized and ground with a mortar and pestle. Residual plant material was removed manually with tweezers. Total lipids were extracted from the freeze-dried soil using a modification of the method described by Bligh and Dyer (Bligh and Dyer, 1959; White and Rice, 2009). Briefly, phospholipid fatty acids (PLFA) were separated from the total lipid extract using silicic acid chromatography. The fatty acids were cleaved from the glycerol backbone using potassium hydroxide (KOH) saponification, and the harvested fatty acids were methylated to form fatty acid methyl esters (FAME). The resulting FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30m x 250 μm i.d. x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Tentative assignments of FAME peaks not present in the BAME mix were

made by mass spectral interpretation. Peak concentration was quantified using the internal standard methyl nonadecanoate.

Fatty acids were grouped into gram-positive bacteria (i15:0, a15:0, i15:0, i17:0, and a17:0), Gram-negative bacteria (19:0:delta9,10, 17:0:delta9,10, C10:0:2-OH, C12:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH, C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10Me18:0), Arbuscular mycorrhizal fungi (C16:1:11), and fungi (C18:2:9,12) (White and Rice, 2009).

Soil Organic Carbon and Total Nitrogen

Soil subsamples from each depth were air-dried and all plant material was removed via tweezers. Samples were then ground using a mortar and pestle and passed through a 250 µm sieve. Samples were analyzed for soil organic C (SOC) and total N (TN) by dry combustion using a C/N Elemental Analyzer gas chromatograph with a thermal conductivity detector (Thermo Finnegan Flash EA1112, Milan, Italy).

Statistical Analysis

The data were analyzed as a split-plot design within a completely randomized block design. Cropping system (CT, NT, RP) was used as the main plot factor, and P amendment (+P or –P) was used as the subplot factor. An analysis of variance was conducted using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis was performed by soil depth. The four blocks were considered as random factors. Post-hoc comparisons were made using Tukey adjustment. The degrees of freedom method used was Satterthwaite. Unless otherwise stated, means were considered significant at the $p = <0.05$ level.

Results

Mehlich-3 P and Soil Cations

There was a significant effect of depth across chemical properties (Table 4.1). With the exception of K, no effect of ecosystem was observed. The P treatment factor was not significant. However, surface P in the 0-5 cm depth was significantly higher than P at other depths (85.1 mg P kg^{-1}). Potassium (K) followed a similar trend, as the 0-5 cm depth had a significantly higher value (451 mg kg^{-1} soil) than all other depths. Magnesium and sodium increased significantly with depth ($p<0.0001$ and $p<0.0001$). Calcium was variable, ranging from 3045-3440, with no observed pattern.

Water Stable Aggregate Distribution

Macroaggregates (>250 µm) were significantly affected by the interaction between ecosystem x depth ($p = 0.0212$; Fig. 4.1). The macroaggregate fraction in RP was significantly higher than NT and CT. Differences between NT and CT were not significant at 0-5 cm or 5-10 cm. The interaction of P and depth was significant, particularly at 5-10 cm ($p = 0.0113$; Table 4.2; Fig 4.2) where P decreased aggregation at the 5-10 cm depth. Across ecosystems, macroaggregates (>250 µm) increased significantly with depth ($p <0.0001$).

Macroaggregates increased over time (Fig. 4.3). The mass of macroaggregates from RP approached that of the native prairie from the same soil (Mfombep, 2014). The observed trend and similarity to the native prairie suggest that the RP ecosystem was approaching equilibrium. A similar trend in NT suggests that this ecosystem may be nearing an equilibrium. An increase in macroaggregates with soil depth was observed for all ecosystems. Macroaggregates increased with decreasing soil disturbance (Table 4.2).

Further separation of macroaggregate fractions (> 2000 µm and 250-2000 µm) indicated that depth and ecosystem were individually significant. Both macroaggregate fractions were greater in RP than CT and NT. Further, these size fractions decreased with increasing soil disturbance (Table A4.1). There was no effect of P addition across ecosystems or depth.

Microbial Lipids

There was no significant 3-way interaction between ecosystem, depth, and P addition for any of the PLFA biomarkers. A significant 2-way interaction of ecosystem by depth was observed for AMF, fungi, and gram-positive bacterial biomarkers, as these biomarkers declined with increasing depth (Table 4.3). Actinomycetes and gram-negative bacteria were not significantly influenced by depth. Except for actinomycetes, ecosystem had a significant effect on all biomarkers (Table 4.3).

Total microbial biomass differed significantly among ecosystems ($p = 0.0008$) and across depths ($p < 0.0001$), though there was no significant interaction between ecosystem and depth. Microbial biomass was greater in RP than CT and NT (Fig. 4.4). No significant differences were found between the CT and NT sorghum. Microbial biomass in the surface soil (0-5 cm) was significantly higher than subsoil depths which were not significantly different from each other (Fig 4.5). The relative abundance of PLFA biomarkers followed a similar trend as the PLFA concentration (Table 4.4; Fig 4.6).

The RP ecosystem had significantly higher levels of fungi than the CT and NT sorghum indicating an increase in saprophytic fungi associated with big bluestem (Table 4.3; Fig 4.4). There was a significant ecosystem by depth interaction for both AMF and fungi, as biomass decreased significantly with depth (Table 4.3). There were no significant differences in PLFA concentration found for actinomycetes at any depth or across ecosystems.

Soil Organic Carbon and Total Nitrogen

Soil organic C was significantly affected by depth ($p<.0001$; Table 4.5). Except for the surface (0-5 cm) soil layer, SOC decreased with depth (Table 4.5). There was no significant effect of ecosystem, though RP had greater amounts of SOC in the surface layer and deeper in the soil profile (Fig 4.7). Soil TN followed the same pattern observed for SOC, with a significant effect of depth ($p<.0001$; Table 4.5). There was no significant effect of ecosystem observed for soil TN. However, RP had greater amounts of TN in the surface layer and deeper in the soil profile (Fig 4.8).

Discussion

After 14 years, replanted prairie had improved several indicators of soil health including aggregation, microbial biomass, and organic C and N. Further, components of the microbial community was elevated with the replanted prairie including Gram-negative bacteria and saprophytic and AM fungi. In a previous study at this site, Mfombep (2014) found that 7 years after project initiation macroaggregates had increased from less than 13% to 40% in CT, 50% in NT, and 59% in RP. Aggregation had not further improved after 14 years. It appeared that the RP and NT had reached equilibrium. Aggregation has been shown to recover rather quickly in restored grassland within 10 years in some cases (Bach et al., 2010; Scott et al., 2017). While no-till increases aggregation relative to CT (Mikha and Rice, 2004), macroaggregates in the NT system in this study had reached an equilibrium below the RP and native prairie. Nicoloso et al. (2018) found that C associated macroaggregates were at the levels of native prairie when NT and compost were combined. NT alone may improve aggregation but enhanced inputs may be

required to make further improvements in annual cropping systems. This could be achieved through intensification of cropping systems.

Greater microbial biomass and elevated levels of fungi and AMF were observed in RP in comparison to CT and NT. Differences in fungal biomass and AMF biomass between NT and CT were not significant. McGowan et al. (2019) observed an increase in microbial biomass, saprophytic fungi and AMF associated with miscanthus and switchgrass in comparison to annual crops. Several studies have demonstrated the negative effects of tillage and improvement of microbial biomass with reduced tillage (Feng et al., 2003; Allison et al., 2005; Mbuthia et al., 2015). For example, Feng et al. (2003) found that NT soils, of a long-term cotton tillage and rotation experiment, contained significantly greater levels of total PLFAs than CT soils. Contrary to our hypothesis, the difference in tillage did not significantly impact saprophytic fungi or AMF concentrations. The NT was expected to have an increased fungi concentration because tillage physically destroys both aggregates and fungal networks (Frey et al., 1999). The increase in microbial biomass in RP was expected and likely resulted in increased aggregate stabilization that was not fully provided to cultivated systems (Allison et al., 2005).

As for macronutrients, K and P are often required in large amounts by cropping systems. Because soil P and K are less mobile in soils, they often remain on the soil surface unless physically incorporated by tillage. In our study, surface levels of P and K were significantly higher than sub-surface levels. The lack of ecosystem response to P addition on microbial biomass was surprising. Previous studies at this site have shown that P addition reduced AMF biomass and root length colonization in both RP and NT (Mfombep, 2014). We found a significant interaction of P and depth at 5-10 cm indicating that soil P decreased aggregation in that depth. The increased concentration of extractable P in the surface layers was due to previous

P addition. As a soil nutrient, P is relatively immobile in soils. Studies have shown that both aggregate stability and macroaggregate proportions are highly dependent on fungal hyphae (Jastrow et al., 1998; Wilson et al., 2009; Zheng et al., 2011). The addition of P has been shown to reduce fungus hyphae density thus impacting soil aggregation (Mfombep, 2014).

Soil C is a crucial factor in soil aggregate formation and stabilization. It is well documented that land management practices, particularly soil tillage, degrade soil structure and reduce soil carbon. Plant inputs influence the rate of soil carbon change and thus influence both aggregate formation and C storage (Beare et al., 1994). Chemical properties within the topsoil affect C accumulation rates throughout the soil profile (de Oliveira Ferreira et al., 2018). It is therefore important to understand the driving factors that influence the transfer of C from the surface to subsoil horizons (Baer et al., 2010). McGowan et al. (2019) found evidence that increased fungal biomass and water stable aggregate percentage, as well as the positive correlation between fungal biomass and aggregate size, in perennial grasses may have increased protection of SOC within soil aggregates. An abundance of AMF has been known to increase macroaggregates in soil, which can contribute to an increase SOC pool (Jastrow et al., 2007; Wilson et al., 2009; Bach et al., 2010; McGowan et al., 2019).

Several studies have reported higher SOC and TN in prairie grass than in cultivated annual systems (Acosta-Martínez et al., 2007; Culman et al., 2010; Liang et al., 2012). Our results coincide with these earlier studies, as RP had higher SOC and TN than both the CT and NT. Though the NT ecosystem had higher SOC and TN than CT, they were not significantly different. In cultivated systems, differences in the microbial biomass and communities are often correlated with plant growth and land management (Frey et al., 1999; Liang et al., 2012). Perennial grassland plants allocate more resources belowground, compared to most annual

cropping systems, and fuel belowground soil food webs including those that fuel microbes (Liang et al., 2012). Root biomass inputs are considered one of the most important factors for predicting potential C storage in both natural and cultivated systems.

Reducing tillage intensity or eliminating tillage practices has been shown to improve soil health across various ecosystems (Doran, 1987; Mahboubi et al., 1993; Busari et al., 2015; Blanco-Canqui and Ruis, 2018; Nunes et al., 2018). There is evidence that as microbial diversity increases, so does ecosystem function, vegetative production, and carbon storage (Baer et al., 2015). Moreover, ecosystem temporal stability increases with improved diversity. Rosenweig et al. (2016) found that nutrient regulation and soil structure can be re-established within a few decades of soil restoration. However, C sequestration will recover over longer time scales (Scott et al., 2017).

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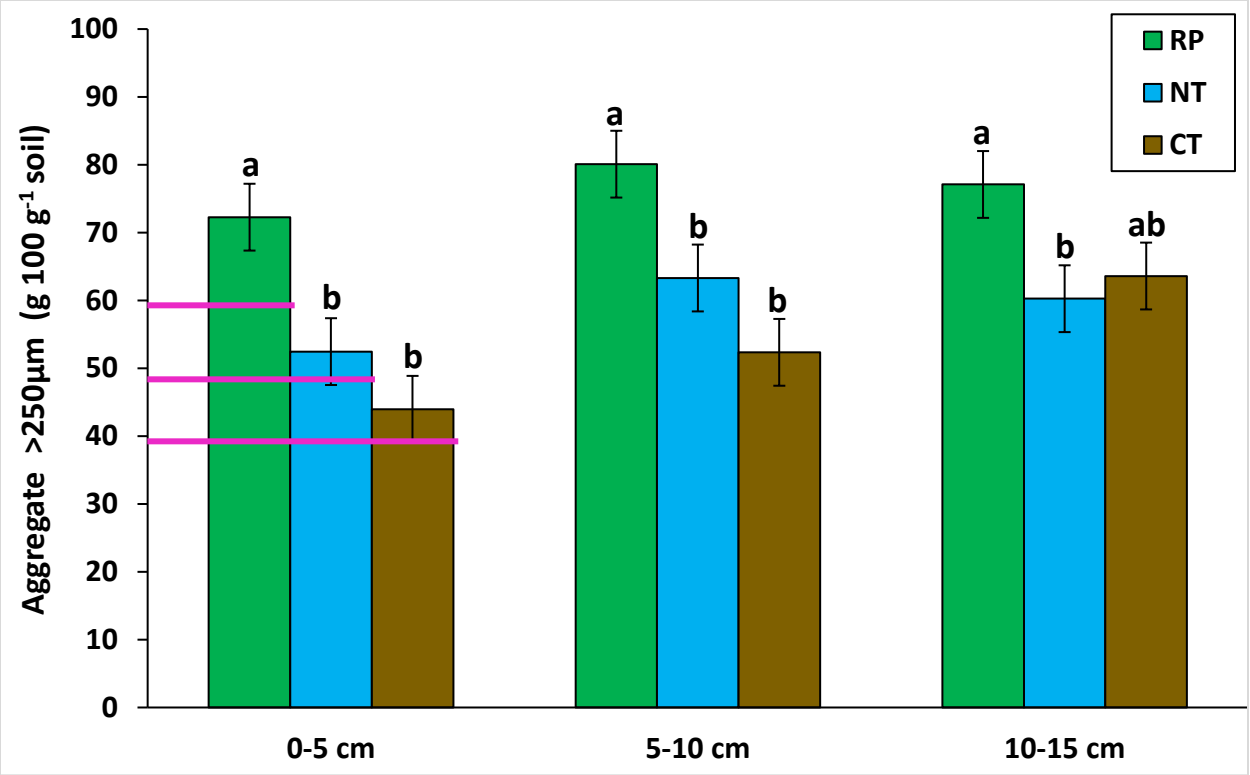


Figure 4.1. Distribution (%) of water-stable macroaggregates as influenced by ecosystem treatment (RP, CT, or NT). Error bars represent the standard error of the mean (n = 8). Different lower-case letters within an aggregate size fraction indicate significant differences between treatments. Pink lines indicate 2010 means.

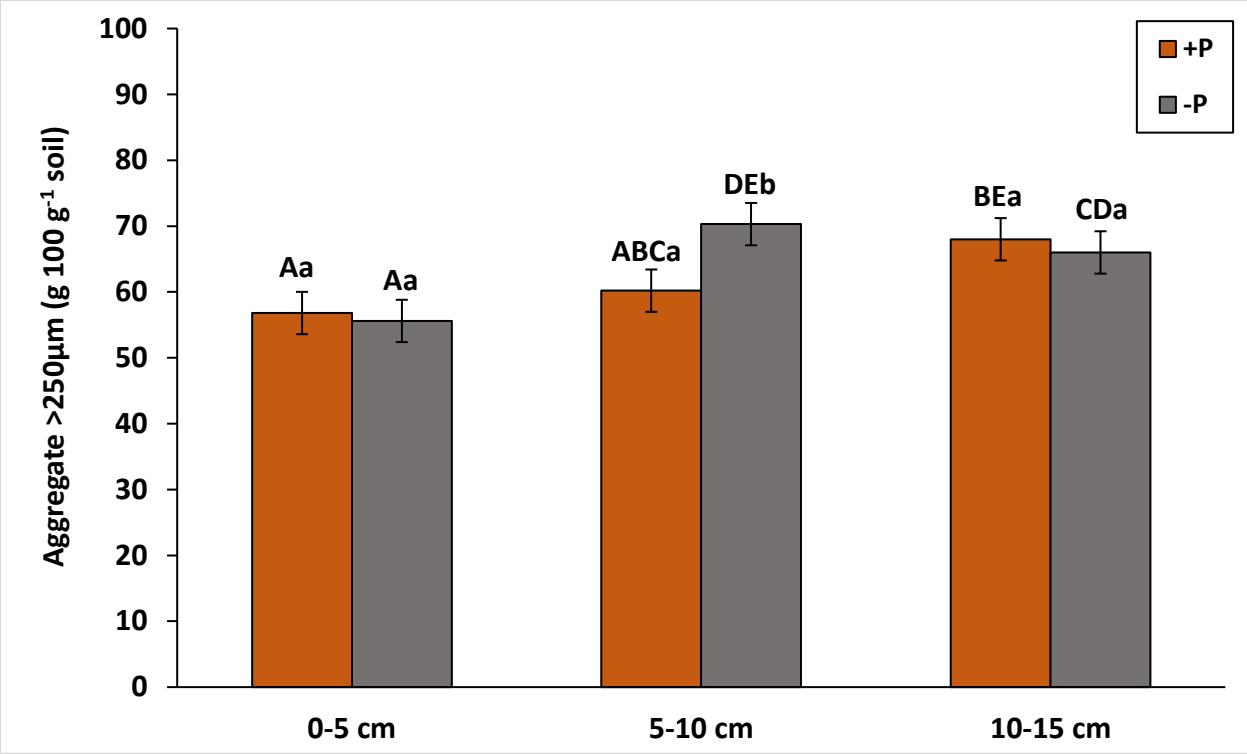


Figure 4.2. Effect of depth x P interaction (p=0.0113) means for water-stable macroaggregates (>250 µm) planted across ecosystem treatments (RP, CT, or NT). Different lowercase letters indicate significant difference within a depth. Different capital letters indicate significant differences between depths.

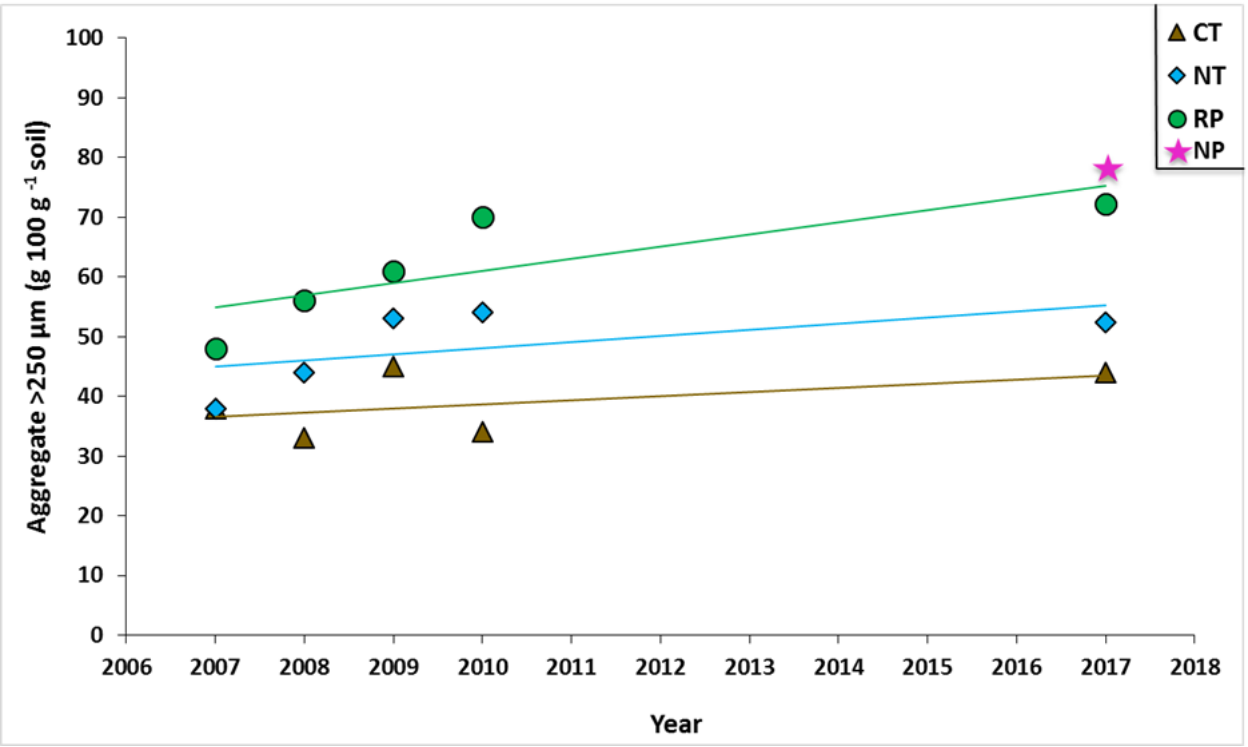


Figure 4.3. Regression of surface (0-5 cm) macroaggregates (>250µm) as influenced by ecosystem treatment (RP, CT, or NT). The local native prairie level is indicated by a pink star denoted NP.

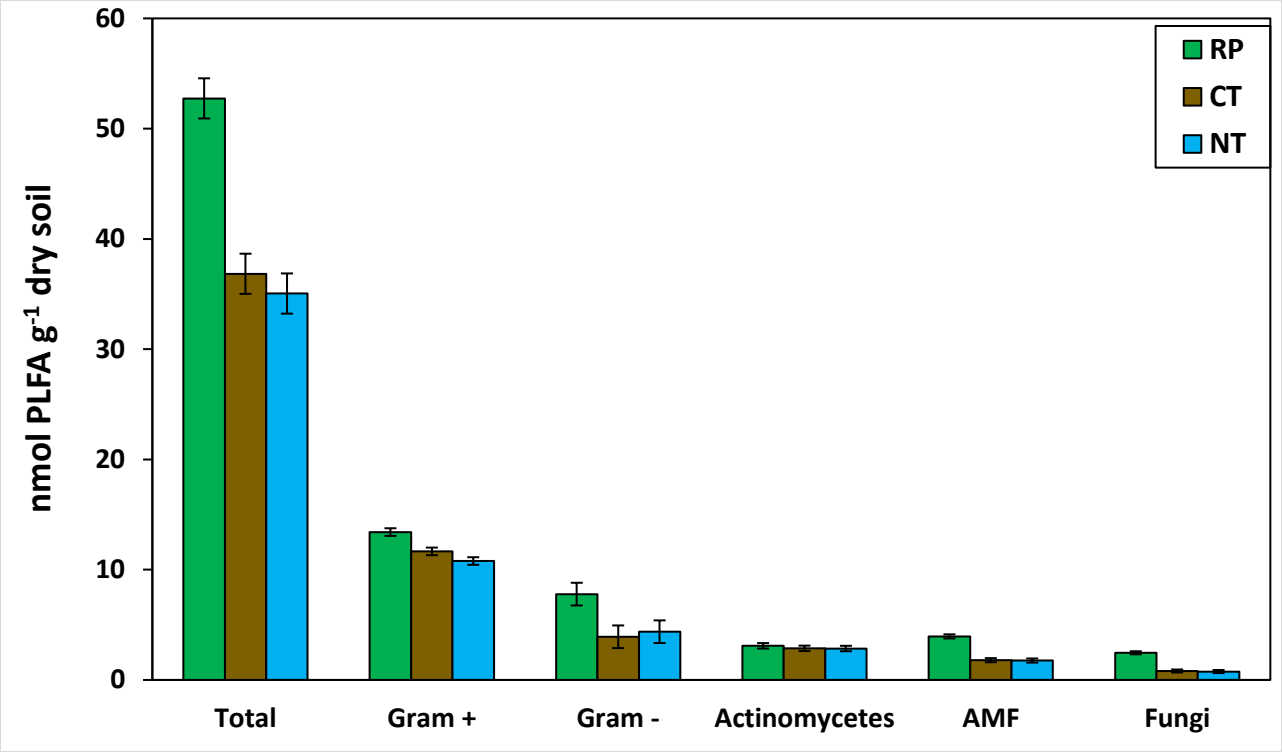


Figure 4.4. Effect of ecosystem, averaged over depth and phosphorus treatment, on total PLFA, gram-positive bacteria, gram-negative bacteria, actinomycetes, AMF, and fungi. Error bars represent standard error of the mean (n=24). Different lower-case letters within a microbial group indicate significant differences between means (p= <0.05).

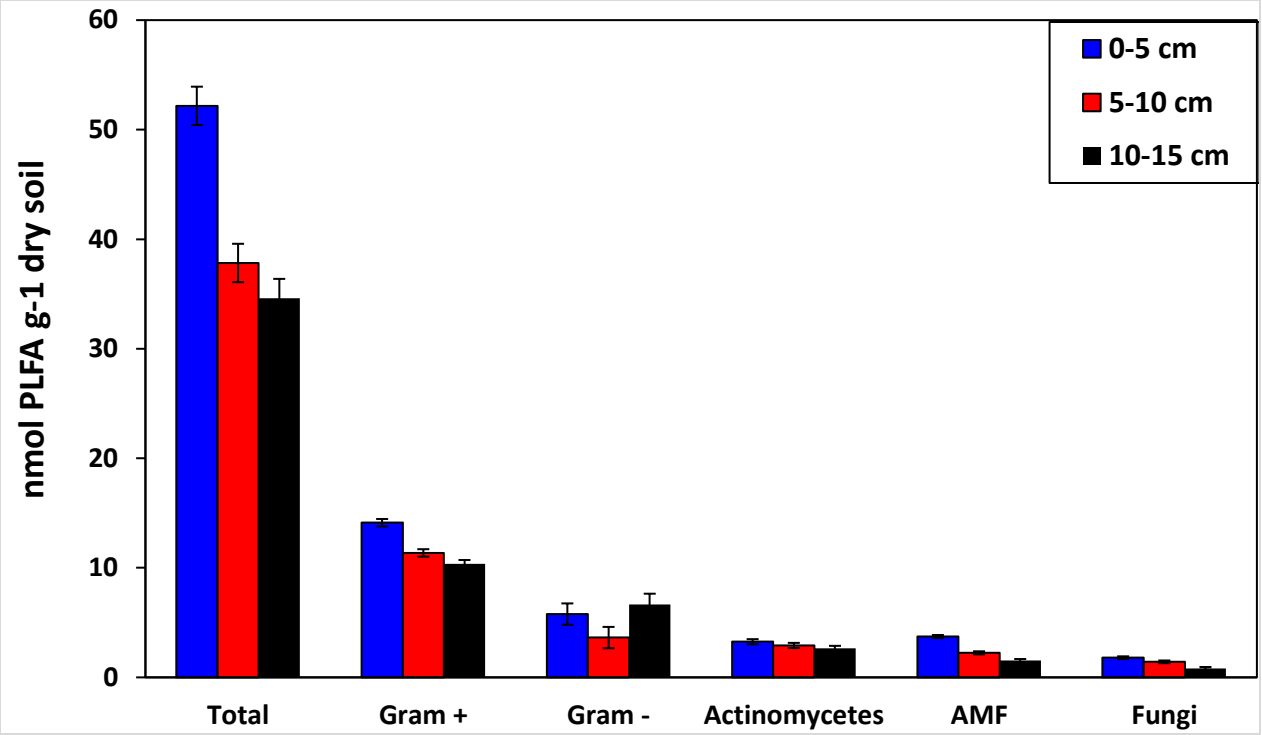


Figure 4.5. Effect of sampling depth, averaged over ecosystem and phosphorus treatment, on total PLFA, gram-positive bacteria, gram-negative bacteria, actinomycetes, AMF, and fungi. Error bars represent standard error of the mean (n=24). Different lower-case letters within a microbial group indicate significant differences between means (p= <0.05).

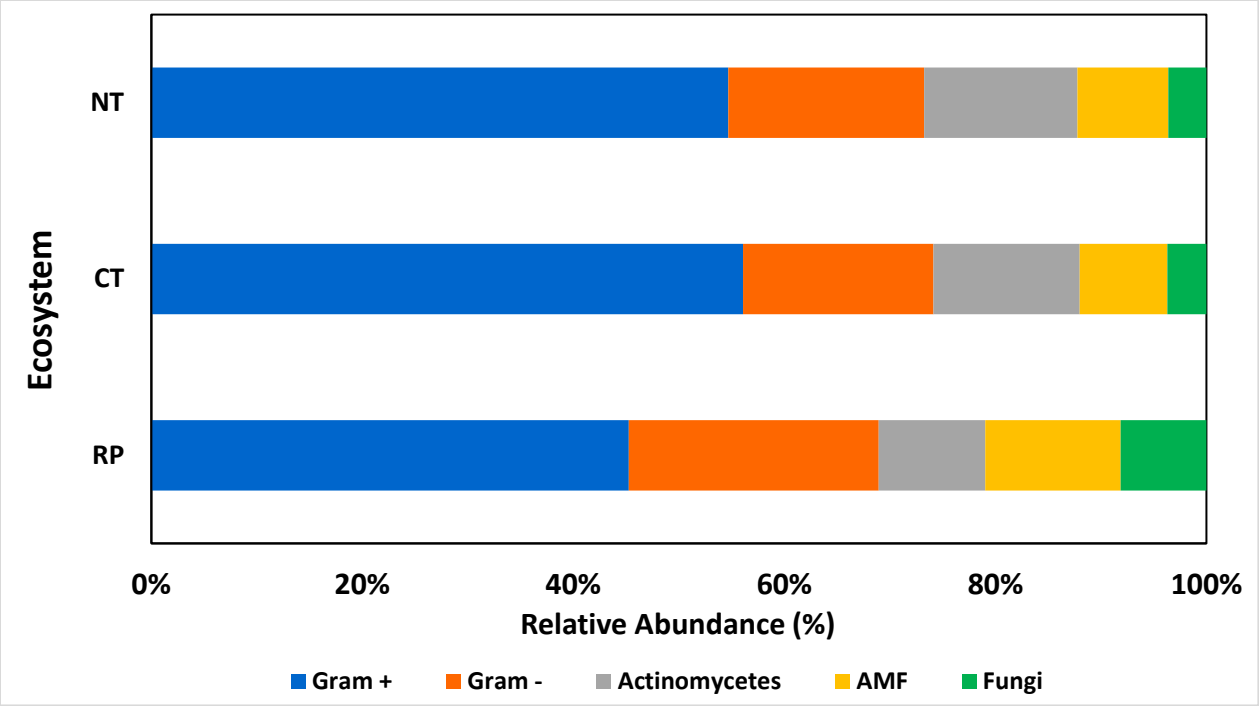


Figure 4.6. Relative abundance of microbial groups across ecosystems. With the exception of gram negative bacteria, ecosystem had a significant influence on the relative abundance of all microbial groups.

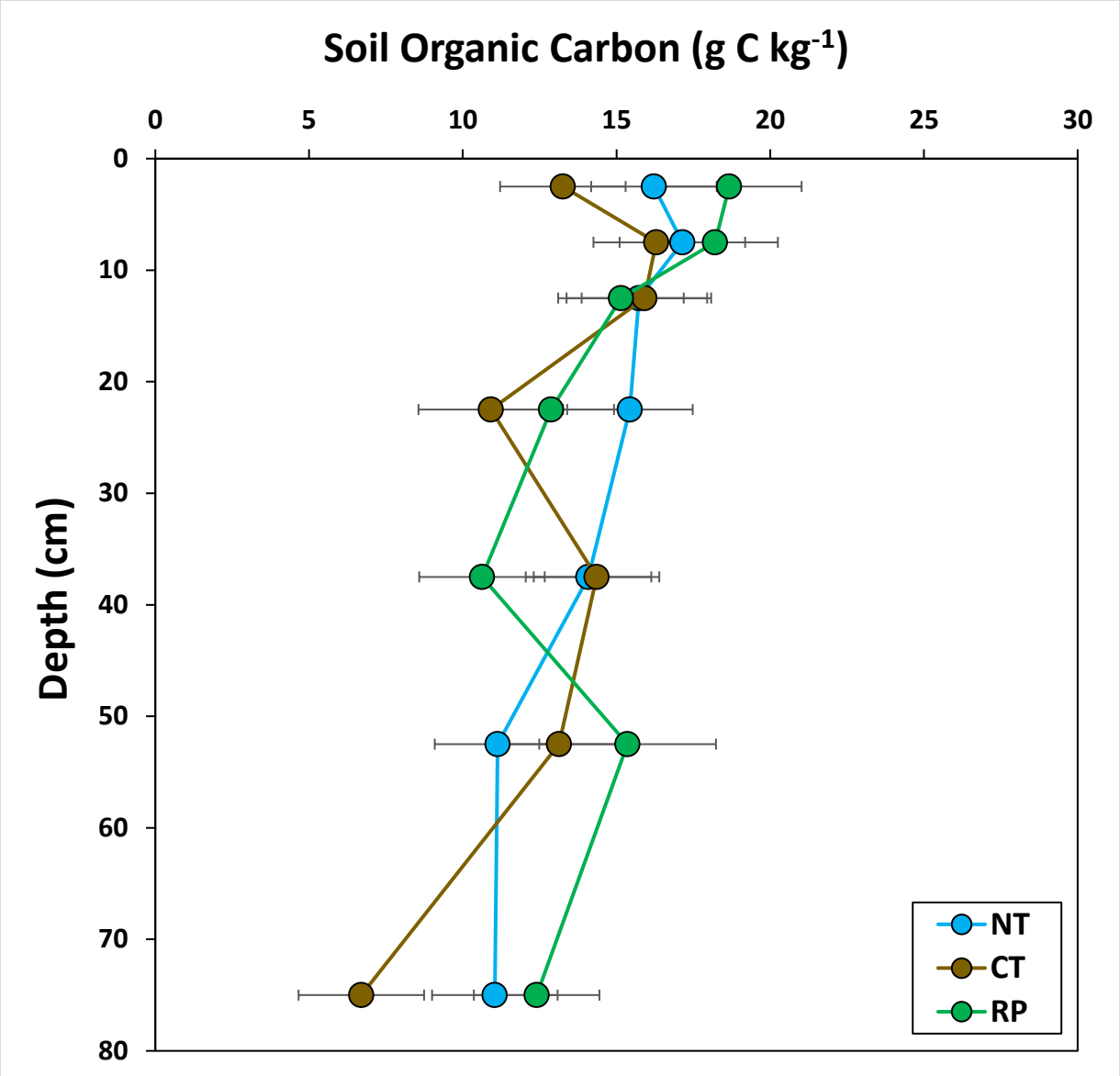


Figure 4.7. Soil organic carbon (SOC) by depth as influenced by ecosystem treatment (RP, CT, or NT). Error bars represent the standard error of the mean (n = 4). Depth was significant at p = <.0001.

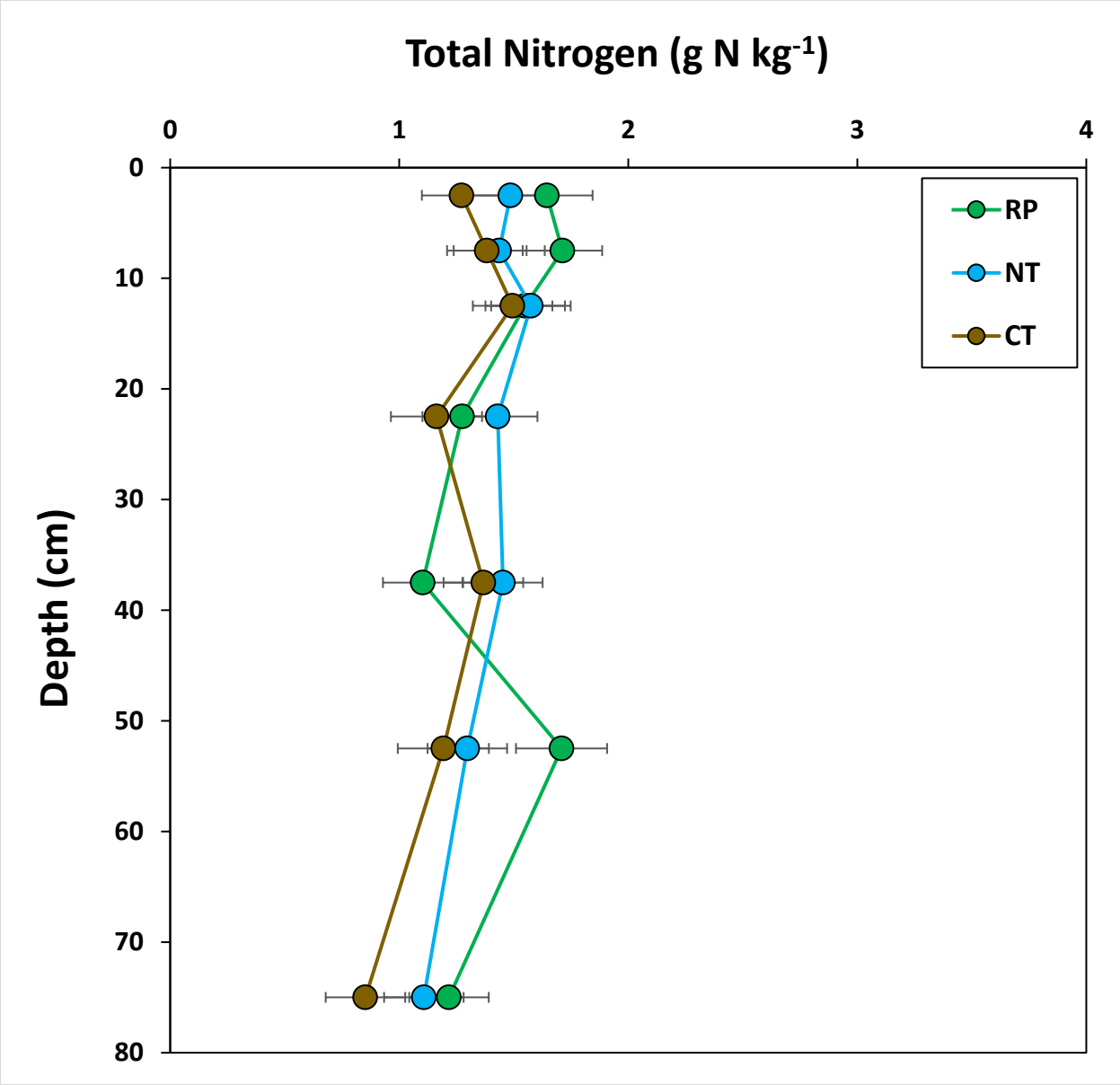


Figure 4.8. Total nitrogen (TN) by depth as influenced by ecosystem treatment (RP, CT, or NT). Error bars represent the standard error of the mean (n = 4). Depth was significant at p = <.0001.

Table 4.1. Average soil chemical values across ecosystems (RP, CT, and NT) and soil depths (0-5 cm, 5-10 cm, and 10-15 cm) in field since experiment initiation. Ecosystem or depth means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type. Values from 2004 and 2010 reported in previous research (Mfombep, 2014).

		Mehlich P	Ca	K	Mg	Na
		---------- mg kg^{-1} soil ----------				
2004		-	3127	392	269	-
2010		24	2748	382	270	-
2017						
	Big Bluestem (RP)	22.4 a	3456 a	362 a	330 a	29 a
	Conservation Till Sorghum (CT)	23.3 a	3309 a	316 ab	315 a	24 a
	No-till Sorghum (NT)	22.4 a	3189 a	295 b	311 a	26 a
	0-5 cm	85.1 a	3440 a	451 a	246 a	14.7 a
	5-10 cm	34.8 b	3223 ab	313 b	271 a	20.0 ab
	10-15 cm	14.4 b	3045 b	290 b	288 a	23.2 b
	15-30 cm	5.02 b	3377 ab	292 b	346 b	29.3 bc
	30-45 cm	3.59 b	3376 ab	310 b	363 b	29.4 bc
	45-60 cm	6.26 b	3350 ab	304 b	352 b	34.3 c
	60-90 cm	9.53 b	3414 a	310 b	364 b	33.3 c
2017		----------p-values----------				
	Ecosystem	0.9948	0.2207	**0.0350**	0.3643	0.1817
	P	0.5129	0.6699	0.7825	0.0962	0.3281
	Ecosystem*P	0.8918	0.4374	0.3212	0.5250	0.7607
	Depth	**<0.0001**	**0.0231**	**<0.0001**	**<0.0001**	**<0.0001**
	Ecosystem*Depth	0.9953	0.2469	0.0935	0.7436	0.5374
	P*Depth	0.7917	0.8530	0.6331	0.9840	0.1253
	Ecosystem*P*Depth	0.6227	0.9962	0.0952	0.7308	0.3506

Table 4.2. Aggregate size distribution (%) for pooled macroaggregates (>250 µm) and microaggregates (20-250 µm across ecosystems (RP, CT, and NT), soil depths (0-5 cm, 5-10 cm, and 10-15 cm), and in response to phosphorus addition. Means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type.

	>250 µm	20-250 µm
	Aggregate size distribution (%)	
Big Bluestem (RP)	76.5 a	17.5 a
Conservation Till Sorghum (CT)	53.3 b	38.1 b
No-till Sorghum (NT)	58.7 b	32.5 b
0-5 cm	56.2 a	34.4 a
5-10 cm	65.2 b	27.5 b
10-15 cm	67.0 b	26.2 b
Phosphorus		
0-5 cm	56.8	33.5
5-10 cm	60.2	32.0
10-15 cm	68.0	25.1
No Phosphorus		
0-5 cm	55.6	35.2
5-10 cm	70.3	23.0
10-15 cm	66.0	27.3
	----------p-values----------	
Ecosystem	**0.0124**	**0.0117**
P	0.2234	0.2970
Ecosystem*P	0.7069	0.3827
Depth	**<0.0001**	**0.0002**
Ecosystem*Depth	**0.0212**	**0.0160**
P*Depth	**0.0113**	**0.0070**
Ecosystem*P*Depth	0.2125	0.2179

Table 4.3. Specific phospholipid fatty acid (PLFA) values across ecosystems (RP, CT, and NT) and soil depth (0-5 cm, 5-10 cm, and 10-15 cm). Ecosystem or depth means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type.

	Total biomass	Gram +	Gram -	Actinomycetes	AMF	Fungi
	---------- PLFA (nmol g^{-1} soil) ----------					
Big Bluestem (RP)	52.7 a	13.4 a	7.78 a	3.10 a	3.95 a	2.46 a
Conservation Till Sorghum (CT)	36.8 b	11.7 b	3.91 b	2.87 a	1.79 b	0.81 b
No-till Sorghum (NT)	35.0 b	10.8 b	4.37 ab	2.85 a	1.76 b	0.76 b
0-5 cm	52.2 a	14.1 a	5.78 a	3.25 a	3.73 a	1.80 a
5-10 cm	37.8 a	11.4 b	3.63 b	2.91 a	2.24 b	1.42 b
10-15 cm	34.6 a	10.4 b	6.67 c	2.64 a	1.54 c	0.83 c
	----------p-values----------					
Ecosystem	**0.0008**	**0.0001**	**0.0313**	0.7395	**<0.0001**	**0.0001**
P	0.6297	**0.0412**	0.6140	0.8066	0.3806	0.5216
Ecosystem*P	0.7158	0.2208	0.3215	0.4816	0.1822	0.1237
Depth	**<0.0001**	**<0.0001**	0.0799	0.1795	**<0.0001**	**<0.0001**
Ecosystem*Depth	0.4813	**0.0442**	0.1512	0.2580	**<0.0001**	**0.0004**
P*Depth	0.9259	0.9392	0.9541	0.4984	0.9016	0.7256
Ecosystem*P*Depth	0.2440	0.4495	0.1627	0.1866	0.2958	0.9597

Table 4.4. Relative abundance of phospholipid fatty acid (PLFA) values across ecosystems (RP, CT, and NT) and soil depth (0-5 cm, 5-10 cm, and 10-15 cm). Ecosystem or depth means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type.

	Gram +	Gram -	Actinomycetes	AMF	Fungi
	----------Relative Abundance (%) ----------				
Big Bluestem (RP)	45.3 a	23.7 a	10.1 a	12.8 a	8.13 a
Conservation Till Sorghum (CT)	56.1 b	18.0 a	13.9 a	8.29 b	3.71 b
No-till Sorghum (NT)	54.5 b	18.6 a	14.5 a	8.61 b	3.62 b
0-5 cm	50.2 a	20.2 ab	11.4 a	12.3 a	5.85 a
5-10 cm	53.8 a	16.7 a	13.8 a	9.94 b	5.80 a
10-15 cm	52.1 a	23.3 b	13.3 a	7.47 c	3.80 b
	----------p-values----------				
Ecosystem	**<0.0001**	0.1872	**0.0492**	**<0.0001**	**0.0002**
P	0.1375	0.2790	0.8308	0.4387	0.7665
Ecosystem*P	0.4262	0.2045	0.3844	0.2416	0.1896
Depth	0.2261	**0.0468**	0.0614	**<0.0001**	**<0.0001**
Ecosystem*Depth	0.4408	**0.0185**	0.2218	**<0.0001**	**<0.0001**
P*Depth	0.9250	0.7287	0.5478	0.7697	0.6013
Ecosystem*P*Depth	0.2126	0.1144	0.2101	0.2178	0.7690

Table 4.5. Average soil organic carbon and total nitrogen values across ecosystems (RP, CT, and NT) and soil depths (0-5 cm, 5-10 cm, and 10-15 cm). Ecosystem or depth means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type.

	Total N gN kg^{-1} soil	Soil Organic C gC kg^{-1} soil
Big Bluestem (RP)	1.46 a	14.6 a
Conservation Till Sorghum (CT)	1.29 a	13.1 a
No-till Sorghum (NT)	1.35 a	13.8 a
0-5 cm	1.42 ab	14.9 ab
5-10 cm	1.60 a	16.7 a
10-15 cm	1.56 a	15.8 a
15-30 cm	1.40 ab	14.2 ab
30-45 cm	1.34 ab	13.5 abc
45-60 cm	1.22 bc	11.9 bc
60-90 cm	1.03 c	10.0 c
	----------p-values----------	
Ecosystem	0.1568	0.3666
P	0.9987	0.3523
Ecosystem*P	0.5728	0.5299
Depth	**<.0001**	**<.0001**
Ecosystem*Depth	0.4102	0.2950
P*Depth	0.0753	0.1449
Ecosystem*P*Depth	0.7192	0.5441

Chapter 5 - Summary

Increasing variability in weather patterns is a global concern. The effects of changing weather events are often linked to soil health. Healthy soils are vital to our ability to combat global climate issues and sustain food production systems. Changes in environmental weather patterns are expected to alter soil microbial community composition and function as studies have indicated that soil history dictates microbial community structure. It is well documented that microorganisms play a vital role in maintaining soil health. Though soil microbes have developed mechanisms to adapt to environmental changes, variation in soil water may have a significant impact on the soil microbial community composition and its overall function. Therefore, a better understanding of how the soil microbial community will respond to altered climatic conditions is needed.

Variability in seasonal and annual precipitation is a natural component of tallgrass prairie. Further, drying and re-wetting cycles in the soil have the potential to influence microbial community structure. Tallgrass prairie is often viewed as a model for agricultural systems due to its soil health and resilience to change. Using tallgrass prairie as a reference, soil management options can be developed to improve and sustain agricultural systems. Thus, understanding the interactive impact of contrasting precipitation patterns and different cropping systems on the soil microbial community are necessary to infer how ecosystems will respond to future environmental changes.

In chapter 2, our results show that after over 25 years of moisture addition, no legacy effect of water regime on the soil microbial community was detected. However, differences in soil C between irrigated and control soils in the 0-10 cm depth at both landscape positions were

observed in this study. Further, differences in total N between irrigated and control soils in both landscape positions and at both depths were also observed in this study.

The lack of irrigation legacy effect indicates that observed differences are driven by factors other than long-term moisture addition. This study found evidence for several contributing factors to microbial community composition and differences in C and N. Differences in microbial community composition were attributed to seasonal plant growth, landscape position or sampling depth. The differences observed in soil C and total N are also likely due to seasonal plant growth as plant communities are known to drive C and N inputs. Additionally, a documented shift in plant community composition since project inception may have masked any evidence of irrigation history.

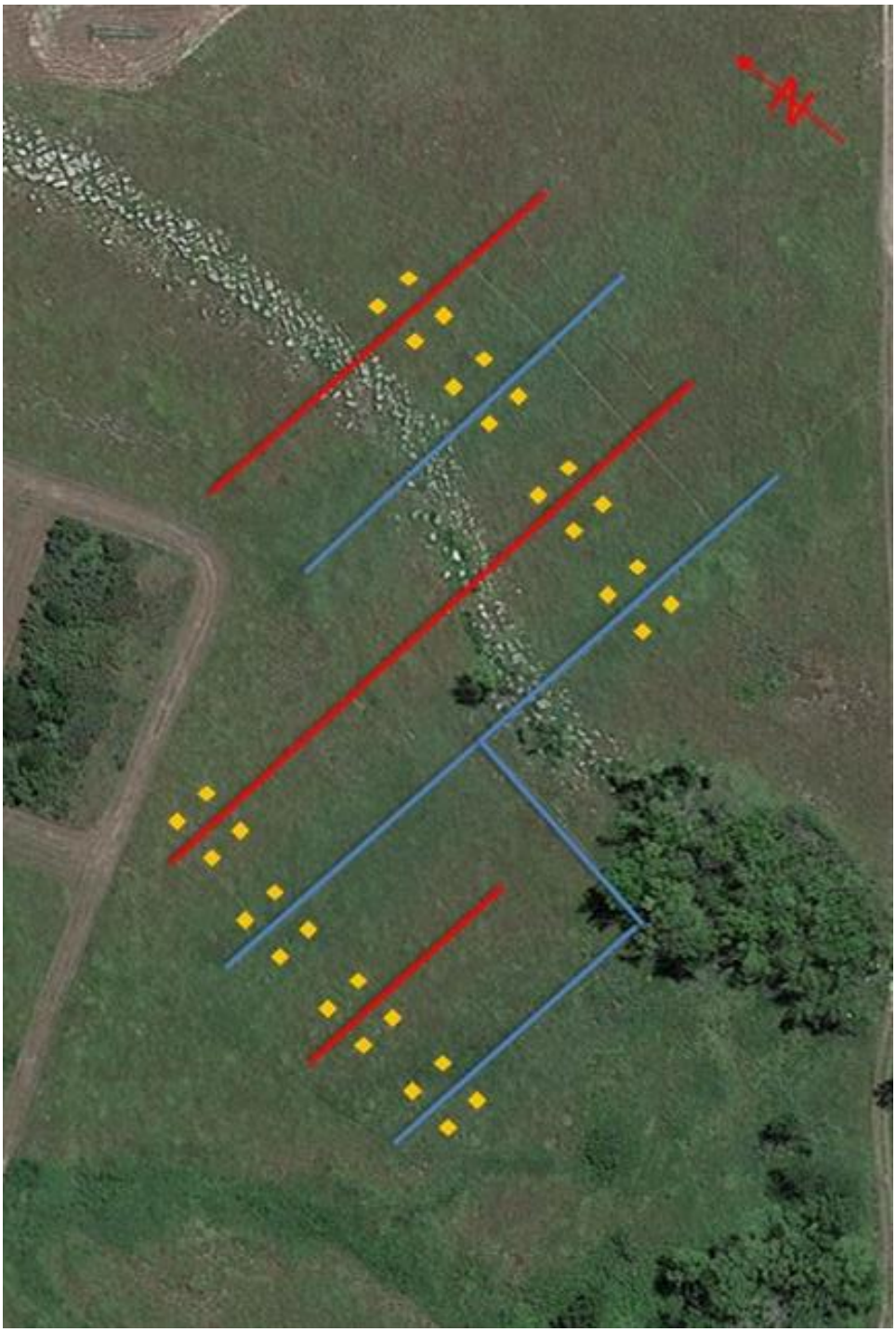
In chapter 3, we sought to better understand legacy moisture effects by inducing respiration in the short term. In the short-term, substrate induced respiration indicated that microbial respiration was enhanced under irrigated soil conditions. However, extended substrate induced respiration indicated little difference in respiration pattern between historic irrigation treatments. This finding coincides with the results of chapter 2 and is noteworthy because it supports the idea that historic irrigation treatment may impact soil microbial communities but the long-term effects can be masked by soil environmental factors.

The objective of chapter 4 was to assess the impact of tillage on grain sorghum (*Sorghum bicolor)* and partial ecosystem restoration via replanted big bluestem (*Andropogon gerardii*) on the biophysical properties of a degraded soil 13 years post-intensive cultivation. We found that after 13 years of altered land management, there was an increase in aggregation under NT relative to CT. Further, the RP had the greatest increase in aggregation. After 13 years, RP macroaggregates are near the levels of native prairie. We did not observe differences between

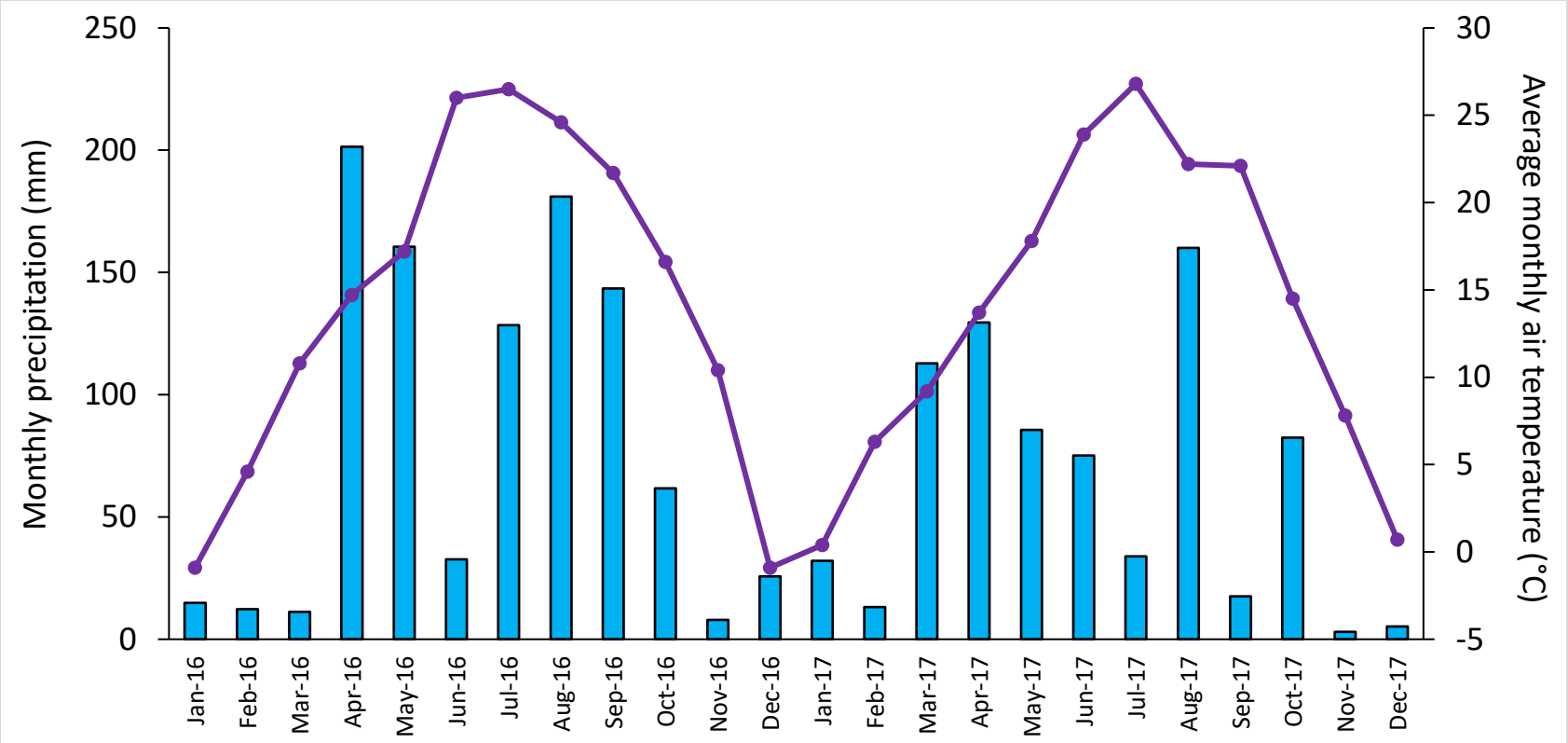
ecosystems for soil C or total N within the total soil profile (0-90 cm). Differences observed by depth may be due to inherent soil properties and not current vegetative inputs. Improved land management practices, such as NT, have been known to improve microbial diversity. Though microbial biomass was significantly greater in the RP, we did not find any significant difference between microbial groups in CT and NT grain sorghum. This indicates that microbial diversity may require more time to recover than soil structure.

Together, these results provide a glimpse into the effect of moisture addition on native prairie. Based on the findings of this study, microbial communities appear to be resilient to long-term changes in moisture in native prairie. However, careful management of cropping systems will be needed to mimic the resilience of the prairie in agricultural systems. More specifically, conservation agricultural practices are essential to prevent the loss of soil structure, microbial diversity, and microbial function in cropping systems. Additional research is needed to understand better how soil microbial communities will respond to predicted environmental changes. Future research should focus on further defining the linkage between microbial community composition, microbial function, and overall soil health. This linkage will be key to building resilient and sustainable agricultural systems.

Appendix A – Chapter 2



Appendix 2.1. Schematic map of the irrigation transects located on Konza tallgrass prairie. The blue parallel lines represent both the center of the irrigation transects and the irrigation pipeline used to water the transects. The red parallel lines represent the center of the ambient (control) transects. Approximate sampling locations are indicated by yellow diamonds.



Appendix 2.2. Monthly precipitation and average monthly air temperature at Konza tallgrass prairie for 2016 and 2017.

Appendix C – Chapter 4

Appendix 4.1. SAS code of data analysis in chapter 4

Block: replication 1-4
Ecosystem: CT, NT, BBS
Phosphorus: +P or –P
Depth: 5, 10, 15, 30, 45, 60, 90

```
proc mixed;
class Block Ecosystem Phosphorus Depth;
model "Variable" = Ecosystem|Phosphorus|Depth/ddfm = satterth;
random Block Block*Ecosystem Block*Ecosystem*Phosphorus;
lsmeans Ecosystem|Phosphorus|Depth/slice = Ecosystem
adjust=tukey;
lsmeans Ecosystem/diff adjust=tukey;
lsmeans Ecosystem*Depth/slice = Depth adjust=tukey;
run;
```

Appendix 4.2. Aggregate size distribution (%) for different aggregate size fractions (>2000 µm, 250-2000 µm, 53-250 µm, and 53-20 µm) across ecosystems (RP, CT, and NT) and soil depth (0-5 cm, 5-10 cm, and 10-15 cm). Ecosystem or depth means followed by different letters are significantly different at ($p < 0.05$). Significant p-values are highlighted in bold type.

	>2000 µm	250-2000 µm	53-250 µm	20-53 µm
	----------Aggregate size distribution (%) ----------			
Big Bluestem (RP)	32.4 a	44.1 a	12.9 a	4.54 a
Conservation Till Sorghum (CT)	5.58 b	47.7 a	31.8 b	6.24 a
No-till Sorghum (NT)	7.40 b	51.3 a	25.4 b	7.15 a
0-5 cm	16.0 a	40.3 a	26.3 a	8.06 a
5-10 cm	18.2 a	47.0 b	22.4 a	5.03 b
10-15 cm	11.2 a	55.8 c	21.4 a	4.85 b
	----------p-values----------			
Ecosystem	**0.0017**	0.2842	**0.0080**	0.1029
P	0.2239	0.7658	0.3235	0.9994
Ecosystem*P	0.5469	0.5941	0.2380	0.6696
Depth	0.0571	**<0.0001**	0.0572	**0.0023**
Ecosystem*Depth	0.4732	0.6851	**0.0102**	0.2105
P*Depth	0.0862	0.9540	**0.0461**	0.3209
Ecosystem*P*Depth	0.8614	0.1020	0.3351	0.9494

Appendix 4.3

***Adapted from Official Soil series Description**
<https://soilseries.sc.egov.usda.gov/OSD_Docs/C/CHASE.html>

Soil Series: Chase
Series Established: Riley County, Kansas, 1970
Classification: Fine, smectitic, mesic aquertic Argiudoll
Parent Material: Alluvium that has low sand content
Vegetation: Grain sorghum; Native vegetation is tall prairie grass
Drainage Class: Somewhat poorly or moderately well
Sand content: 1 to 4 percent
Clay content: 35 to 55 percent

CHASE SERIES

The Chase series consists of very deep, somewhat poorly drained and moderately well drained, soils that formed in alluvium on floodplains. Slopes range from 0 to 2 percent. Mean annual precipitation is about 86 centimeters and the mean annual temperature is about 13 degrees C (56 degrees F).

TAXONOMIC CLASS: Fine, smectitic, mesic Aquertic Argiudolls

TYPICAL PEDON: Chase silty clay loam - in a cultivated field. (Colors are for moist soils unless otherwise stated.)

Ap--0 to 15 centimeters; black (10YR 2/1) silty clay loam, dark gray (10YR 4/1) dry; moderate fine and medium granular structure; slightly hard, friable, slightly plastic, slightly sticky; few worm casts; moderately acid; clear smooth boundary.

A--15 to 36 centimeters; black (10YR 2/1) silty clay loam, dark gray (10YR 4/1) dry; moderate medium granular structure; slightly hard, friable; slightly plastic, slightly sticky; few fine irregular shaped iron-manganese concretions; moderately acid; gradual smooth boundary. (Combined thickness of A horizon is 20 to 51 centimeters)

BA--36 to 51 centimeters; black (10YR 2/1) silty clay loam, dark gray (10YR 4/1) dry; moderate fine and medium subangular blocky structure; hard, firm, plastic and sticky; few fine distinct dark brown (10YR 3/3) masses of iron accumulation; few fine rounded iron-manganese concretions; few fine worm holes; few worm casts; slightly acid; gradual smooth boundary. (10 to 25 centimeters thick)

Bt1--51 to 86 centimeters; very dark gray (10YR 3/1) silty clay, gray (10YR 5/1) dry; moderate medium and fine blocky structure; very hard, very firm, very plastic, very sticky; common medium distinct dark yellowish brown (10YR 4/4) irregular shaped masses of iron accumulations; few fine rounded iron-manganese concretions; few fine clay films on ped faces; few fine worm holes; few worm casts; slightly acid; gradual smooth boundary.

Bt2--86 to 107 centimeters; very dark brown (10YR 2/2) silty clay, dark gray (10YR 4/1) dry;

moderate medium blocky structure; very hard, very firm, very plastic, very sticky; common fine distinct yellowish brown (10YR 5/4) irregular shaped masses of iron accumulation; few fine rounded iron-manganese concretions; common fine clay films on ped faces; neutral; diffuse smooth boundary. (Combined thickness of the Bt horizons is 41 to 81 centimeters)

BC--107 to 137 centimeters; very dark brown (10YR 2/2) silty clay loam, dark gray (10YR 4/1) dry; very weak blocky structure; hard, firm, plastic and sticky; few fine distinct yellowish brown (10YR 5/4) irregular shaped masses of iron accumulations; few fine rounded iron-manganese concretions; neutral; diffuse smooth boundary. (0 to 41 centimeters thick)

C--137 to 203 centimeters; very dark grayish brown (10YR 3/2) silty clay loam, dark gray (10YR 4/1) dry; massive; hard, firm, plastic and sticky; few fine distinct yellowish brown (10YR 5/4) irregular shaped iron accumulations; few fine rounded black iron-manganese concretions; slightly alkaline.