SOIL CARBON SEQUESTRATION: FACTORS INFLUENCING MECHANISMS, ALLOCATION AND VULNERABILITY

by

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AN ABSTRACT OF A DISSERTATION

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Abstract

Increasing atmospheric CO_2 concentrations and other greenhouse gases have been linked to global climate change. Soil organic C (SOC) sequestration in both agricultural and native ecosystems is a plausible option to mitigate increasing atmospheric CO_2 in the short term. Laboratory and field studies were conducted to (1) understand the influence of soil water content on the temperature response of SOC mineralization (2) investigate burn and nutrient amendment effects on biogeochemical properties of tallgrass prairie and (3) assess perennial and annual plant management practices on biophysical controls on SOC dynamics. The laboratory study was conducted using soils collected from an agricultural field, currently planted to corn (C_4 crop), but previously planted to small grain (C_3) crops. The changes in cultivated crops resulted in a $\delta^{13}C$ isotopic signature that was useful in distinguishing older from younger soil derived CO_2-C during SOC mineralization. Soils were incubated at 15, 25 and 35 ^oC, under soil water potentials of -1, -0.03 and -0.01 MPa. Soil water content influenced the effect of temperature on SOC mineralization. The impact of soil water on temperature effect on SOC mineralization was greater under wetter soil conditions. Both young and older SOC were temperature sensitive, but SOC loss depended on the magnitude of temperature change, soil water content and experiment duration. The first field experiment investigated burn and nutrient amendment effects on SOC in a tallgrass prairie ecosystem. The main plots were burned (B) and unburned (UB) tallgrass prairie and split plots were nutrient amendments (N, P or

N+P including controls). Vegetation was significantly altered by burning and nutrient amendment. Treatment effects on either TN or SOC were depth-specific with no impact at the cumulative 0-30 cm depth. The P amendment increased microbial biomass at 0-5 cm which was higher in unburned than burned prairie. However, at 5-15 cm depth N amendment increased microbial biomass which was higher in burned than unburned prairie. In conclusion, SOC in both burned and unburned tallgrass prairie may have a similar trajectory however; the belowground dynamics of the burned and unburned tallgrass prairie are different. Another field experiment assessed SOC dynamics under perennial and annual plant management practices. The main plots were grain sorghum (*Sorghum bicolor*) planted in no-tillage (NT) or continuous tillage (CT), and replanted native prairie grass, (*Andropogon gerardii*) (RP). The spit plots were phosphorus (+P) and control without P (-P). The P amendment was used to repress arbuscular mycorrhizal fungi (AMF), known to influence soil aggregation. The macroaggregate >250 μm, SOC and TN were higher in RP and NT than CT. The relative abundances of AMF and saprophytic fungi were greater with less soil disturbance in RP and NT than in CT. Therefore, less soil disturbance in RP and NT increased AMF and fungal biomasses. The higher relative abundances of AMF and fungi with less soil disturbance increased macroaggregate formation in RP and NT, which resulted in higher SOC sequestration in RP and NT than CT.

SOIL CARBON DYNAMICS: FACTORS INFLUENCING MECHANISM, ALLOCATION AND VULNERABILITY

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To my dad, Mfombep Joseph Ekangwo for sowing the seeds of perseverance and quest for knowledge which have sustained me in times when he could not hear, see or understand what became of the seeds he sowed …

CHAPTER 1 - GENERAL INTRODUCTION

Over the past 30 years, mean global surface temperatures have increased by about 0.2°C per decade (Hansen et al., 2006). In addition, the mean global surface temperature is projected to increase 1.4 - 5.8°C between the years 1990 and 2100 (IPCC, 2001; Luo et al., 2001; IPCC 2007b). The global mean surface temperature is the simplest metric for global climate change. A comprehensive package of global climate change indicators include both increasing (air temperature near surface, specific humidity, ocean heat content, sea level, sea surface temperature, temperature over oceans and temperature over land) and decreasing (snow cover, glaciers and sea-ice) indicators (NOAA, 2010). The increasing mean global temperatures have been linked to greenhouse gases (GHG), which includes increases in atmospheric carbon dioxide (CO_2) (IPCC, 1995; IPCC, 2007b).The increase in atmospheric CO_2 has been mainly from fossil fuels and land-use change (IPCC, 2007b; Rice, 2006), such as changes in agricultural practices and extensive ecosystem disturbance (IPCC, 2001; Vitousek, 1994).

Several solutions have been proposed to mitigate global climate change which include CO_2 capture and storage, switching from fossil fuels to biofuels and alternative energies (coal technologies, hydrogen fuels) or removal of atmospheric CO_2 (Foley et al., 2005; IPCC, 2001; Rice, 2006). While storage in geological structures is riddled with uncertainties, technologies for alternative energies are still under development. Although biofuels crops on agricultural land has a mitigating potential (Cole et al., 1997), there are debates about the impact to soil (Fargione et al., 2008; Searchinger et al., 2008) and environmental impacts (Costello et al., 2009). Biochar as a mitigating option has also

been proposed (Woolf et al., 2010), but would be an effective strategy only if it is added and stored in soil.

While CO_2 removal strategies such as injection into geological structures may have uncertain consequences, C sequestration in soils is cheaper and a technology immediately deployed (Wander and Nissen, 2004) with ancillary benefits. Soil C sequestration increases soil organic C (SOC) with the added advantage of increasing soil fertility, as well as reducing soil erosion, improving water quality and general ecosystem function. Increasing SOC in degraded land will also enhance global food security and offset emission from fossil fuels (Lal, 2010). Therefore removal of atmospheric CO_2 and storage in soil is currently the most feasible of the proposed strategies.

Global carbon cycle and global climate change

The C cycle has always been important in the global biogeochemical cycles because, not only is C the building block of life on earth (Rice, 2002), but it is also the currency of energy transfer between and within ecosystems. Actively circulating C revolves within and between the atmosphere, biota, SOC and ocean (Janzen, 2004). About 80% of earth's C ($8.06 X10^7$ Pg C) is found as organic or inorganic compounds buried in sedimentary rocks of which 4000 Pg C constitutes fossil fuels (organic C that has been locked-up for thousands of years). The largest active pool occurs at the ocean surface containing $4 X 10^4$ Pg C (Janzen, 2004; McCarl et al., 2007), as dissolved inorganic C which acts to buffer atmospheric CO_2 and at equilibrium is 56 times the atmospheric pool (750 Pg C). Currently, the atmospheric pool contains more C than earth's vegetation (560 Pg C), while soil has the largest terrestrial pool with 1500 Pg C which is about three times C storage in vegetation and two times in the atmospheric pool

(Schlesinger, 1997). There is continuous exchange of C between the various C pools. The atmospheric pool is the most dynamic pool, exchanging about 20% CO_2 with the ocean and terrestrial vegetation per year. Without anthropogenic emissions, sources and sinks of active C are almost balanced (Friedlingstein and Prentice, 2010). The circulation of C within and between the pools had been stable for millennia (Janzen, 2004) until human activities during the recent century have distorted that established stability. Therefore, the amount of C at any given time in each pool is an indicator of earth's health, a proxy of which is earth's climate.

There have been changes in CO_2 flux resulting from changes in land use for agriculture with losses of 40 - 90 Pg (Houghton, 1999; Lal and Bruce, 1999) which represents a loss rate of about 1.6 Pg per year (Smith, 2008). Therefore, any small changes in global soil C will impact the atmospheric C concentration. Despite this agricultural release of CO_2 to the atmosphere, fossil fuel combustion currently supersedes agriculture by adding 6.5 Pg y^{-1} (Lal and Bruce, 1999). Fossil fuels represent C that had been out of active circulation for millions of years, but is being currently returned into active circulation (Janzen, 2004). In addition there is reducing sink strength to absorb the excess CO_2 from fossil fuels (Lal and Bruce, 1999), because the soil sink is not infinite.

The complex interaction of environment and climate system results in local and regional variations of global climate change effects (IPCC, 2007a). Surprisingly, there are still skeptics to the global climate change phenomenon, probably because of the regional variations in global climate change effects. The Intergovernmental Panel on Climate Change (IPCC, 2001) defines climate change as "a statistically significant variation in either the mean state of the climate or variability, persisting for an extended period

(typically decades or longer)". Atmospheric CO_2 concentration has increased exponentially from 280 ppm before 1750, to current levels at 394 as of October, 2013 (NOAA). The mean global temperature has increased 0.6 °C during the 20th century (IPCC, 2001); therefore by the IPCC definition, there is climate change because the globe is warming up and has been for decades.

Mitigating global climate change

The alarm on change in global climate was realized in 1988 resulting in the UN establishing the IPCC (Intergovernmental Panel on Climate Change) with a mission to assess global warming and suggest strategies for mitigation. This panel published its first assessment report in 1990 which was the basis for the United Nations Framework Convention on Climate Change (UNFCCC). The Kyoto protocol was the first international agreement to mitigate climate change (Dumanski, 2004).

Mitigating global climate change includes any measure to reduce GHG emissions or improve sinks for GHGs. Several mitigation options have been proposed to reduce GHGs in general and CO_2 in particular. Agriculture has been targeted because agricultural lands constitute 37% of earth's land surface. This land area produces 52 and 84% of global anthropogenic methane and nitrous oxide emissions respectively and about 5% of CO_2 emissions (Cole et al., 1997; Rice, 2006; Smith et al., 2007). Agricultural lands have lost 50 Pg C within the last half century which creates a potential to restore some C. Mitigating CO_2 emission is of particular interest because while CO_2 contributes 72% of global warming, current increases primarily due to fossil fuels cannot be readily absorbed due to reduced sink strength from land use change. Removal of atmospheric CO_2 is currently the most feasible of proposed strategies.

Removal of CO_2 is partly attainable by C sequestration in soils. Lal, (2004a) defines carbon sequestration as the transfer of atmospheric CO_2 into long-live soil pools for secure storage without immediate reemission. With 80% of total terrestrial C stored in soils (Amundson, 2001), and the loss of soil C resulting from agricultural soils of 50 Pg C within the last half century (Amundson, 2001; IPCC, 2000; Paustian et al., 1998), soil has a large potential to sequester as much as 0.4-1.2 Pg C y^{-1} (Lal, 2004b). Given that the stable (humic) soil C pool has a residence time of centuries to thousands of years, getting some CO_2 into this pool will sequester C from the atmosphere.

Increasing C into soil organic matter can be attained using three broad strategies; increase plant biomass entering the soil, reducing soil organic C decomposition through less soil disturbance (McCarl et al., 2007; Smith et al., 2008b) and improving soil organic C storage (Jastrow et al., 2007). These strategies are not mutually exclusive and will be different for various soils due to variation in soil properties, climate, and management practices.

Soil carbon sequestration

The basic process of C exchange between the atmosphere and terrestrial ecosystems is photosynthesis and respiration. Plants fix CO_2 amounting to 62 Pg C y^{-1} from the atmosphere during photosynthesis, which enters the soil as detritus and is stored in soil organic matter. Carbon dioxide is returned to the atmosphere through respiration (autotrophic and heterotrophic) and fire, which constitutes 62 Pg C y^{-1} (Falkowski et al., 2000). Therefore CO_2 fluxes due to photosynthesis and respiration are in equilibrium, at least for natural undisturbed ecosystems. Therefore SOC is a balance between belowground inputs and outputs (Davidson and Janssens, 2006). Soil organic C can be

grouped into three soil pools; the active (labile), slow (intermediate) and stable (passive or inert) (McGill, 1996; Parton et al., 1993; Smith et al., 1997). Carbon in these pools has varied mean residence times depending on its biochemical composition; ranging from less than one year for the labile fraction to more than 1000 years for the stable fraction. Soil management practices which improve gains in SOC will result in reduced atmospheric CO_2 concentration.

Organic matter enters soil through root exudates, root turnover and plant litter which are delivered to the upper layers of the soil. The organic matter is decomposed by the activities of soil microfauna, bacteria and fungi and redistributed between soil C pools. Therefore soil management options which increase SOC residence time will improve soil C sequestration and include biochemical alteration and physicochemical protection (Jastrow et al., 2007). While biochemical alteration includes humification, physicochemical protection constitutes stabilization mechanisms (sorption, organomineral complexation, and occlusion within aggregates). The effectiveness of each mechanism is attained by limiting soil decomposer activities on SOC mineralization. Therefore management practices which influence soil moisture, temperature, pH, nutrient availability will influence mineralization, residence time, hence soil C sequestration (Paustian et al., 2000).

Physical protection as a stabilization mechanism, which emphases SOC occlusion within aggregates is important in soil C sequestration. Several authors have noted that increased residence time is attained when biochemically altered C is physically separated from decomposer activity repressed through physical control of gaseous exchange and moisture limitations (Berg et al., 2010; Elliott and Coleman, 1988; Elliott et al., 1980;

Jastrow et al., 2007; Six et al., 2002; Sollins et al., 1996; Young et al., 1998). This implies that soil structure plays a pivotal role in soil C sequestration. Soil structure has been defined as the arrangement of particles and the associated pores (Oades, 1993). This definition however, does not emphasize the dynamic nature of soil structure. Hence soil structure can be re-defined as an arrangement of particles in soil as sand, silt and clay, bound into aggregates sizes by organic and inorganic means with varying life span influenced by environmental conditions. Soil aggregation and aggregate turnover influences the residence time of C in soil and is influenced by both environmental factors as well as soil management practices.

The hierarchical nature of soil is made possible by binding agents of either transient (readily decomposable organic materials of microbial and plant derive polysaccharides) or temporary binding agents of primarily fibrous roots and hyphae. These particles are bound into silt-sized microaggregate (2-20 μm diameter), which are bound by fungal and plant debris to larger microaggregates (20-250 μm). The primary microstructures are bound by persistent (humic or long-chained polysaccharides sorbed to clays) binding agents. Roots and hyphae are more important in forming macroaggregates than other organic residues. These hierarchical descriptions may suppose a sequential formation of aggregates, but decomposition has been identified as the cause of microaggregate formation. Therefore, aggregates may form simultaneously depending on vegetation type, management practices, and degree of protection from decomposition (Miller and Jastrow, 1990; Miller and Jastrow, 1992)

Soil microbiota in C sequestration

Soil microbes play a major role in influencing soil activities and function and will determine the desired outcome of sequestering C. Soil is a biogeophysicochemical system whose function depends on the interplay of all its components. Microbial community structure can influence changes in soil processes, including C sequestration, resulting from microbial community change even when microbial biomass is constant (McGuire and Treseder, 2010). Although models that described SOC mineralization bypass the role of microbial communities (Balser and Wixon, 2009), global changes result from cumulative effects of processes mediated by local microbial communities. The activities of soil microbial communities alter soil physical, chemical and biological function. Soil microbes mediate processes involved in CO_2 and other biogenic gas fluxes between soils and atmosphere (Schimel and Gulledge, 1998). Therefore current interest in managing soil to sequester C would not be complete without due consideration of the role of microbes in this process. Soils constitute a diverse microbial community of which 90 - 99% has not been characterized due to non-culturability (Hill et al., 2000). Thus soil microbes are usually discussed based on broad groupings such as fungi, bacteria and actinomycetes.

There are two main organic matter decomposition pathways; fungal-based and bacterial-based. These different pathways (bacterial and fungal), support different faunal communities (de Vries et al., 2006; Wardle and Lavelle, 1992). Fungi and bacteria are considered most important when discussing soil C stabilization because of their different C utilization efficiencies (CUE) also referred to as microbial growth efficiency (MGE) (Six et al., 2006). Carbon utilization efficiency refers to proportion of metabolized C released as CO_2. Therefore, a lower CUE implies greater proportion of metabolized C

lost as CO_2. In this regards, research has shown that bacteria have a lower CUE relative to fungi (Claus et al., 1999), with a magnitude as high as 26 times (Suberkropp and Weyers, 1996), although no difference has also been reported (Payne, 1970). In addition, while fungal cell walls consist of polymers of melanin and chitin, bacterial cells contain a constant proportion of their biomass as phospholipids (Balkwill et al., 1988). Chitin and melanin, considered more recalcitrant, will have a greater residence time in soil (Guggenberger et al., 1999); while phospholipids degrade more readily (Frey et al., 2001).

Further dominance of fungi relative to bacteria with regards to soil C stabilization is offered by the specific group of fungi known as arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal fungi are symbiotic associations between plant roots and fungi, involving over 80% of all land plants in all terrestrial ecosystems (Bago et al., 2002; Wilson et al., 2001). AMF can improve soil C sequestration through living, dead and residual hyphal biomass (Treseder and Allen, 2000). Arbuscular mycorrhizal fungi improve plant nutrient uptake and also enhances C assimilation (Eissenstat et al., 1993), which increases biomass production. The biomass is subsequently added to the soil which is an important step in soil C sequestration. While research have estimated that AM hyphae could accumulate up to 80% of plant's P, and 25% N (Wilson et al., 2001), these fungi may also enhance below ground C allocation by obtaining up to 20% of net C assimilated by the host plant (Jakobsen and Rosendahl, 1990). Plant sugars available to fungi, are converted and stored in intraradical fungal structures as lipids and translocated to extraradical mycelium (Pfeffer et al., 1999); this C source represents a large carbon sink within soil (Jakobsen, 1990). In addition hyphae function in translocating C away

from the rhizosphere to bulk soil where C mineralization is lower thus increasing the C residence time. The variation in response to AMF colonization by plant and nutrient availability has been exploited to assess the influence of AMF on soil structure and C sequestration potential (Wilson et al., 2009). Benefits to soil are mainly structure formation and stabilization i.e. they can be considered soil engineers. Arbuscular mycorrhizal fungi hyphal exudates a hydrophobic protein (glomalin related soil proteins), resistant to mineralization, also serve to glue soil particles, and increase soil aggregate stability, hence C sequestration.

Soil organic carbon mineralization and global temperature change

How SOC responds to global climate change will determine the effectiveness of mitigation strategies. With increasing temperatures SOC is expected to release CO_2 causing a positive feedback on climate change (Hansen et al., 2006; Kirschbaum, 1995). However there remain uncertainties to the extent of this feedback. These uncertainties are demonstrated in models that assess the temperature response of SOC decomposition, which varies in amount of additional CO_2 by 2100 ranging between 20 and 200 ppm (Friedlingstein et al., 2006). Despite these uncertainties, optimizing soil C sequestration requires a better understanding of the processes and mechanisms by which C is allocated to the various pools which are greatly influenced by soil physical, chemical and biological properties.

While there is consensus that global temperatures have been increasing (Balser and Wixon, 2009), there is lack of consensus on the vulnerability of SOC to this temperature change. Various means have been used to assess temperature sensitivity of

SOC. Temperature sensitivity of SOC referred to as Q_{10} represents the factor by which respiration rates differ with temperature intervals of $10^{o}C$ or the sensitivity to temperature variation of soil respiration (decomposition of SOC) (Fang and Moncrieff, 2001; Smith et al., 2008a). The Q_{10} may have significantly different values depending on the phenomenon measured even though the terms are used interchangeably. Some of the terms include actual temperature or short-term sensitivity (Kirschbaum, 2006), apparent temperature sensitivity (Davidson and Janssens, 2006), seasonal or mean annual temperature change (Reichstein et al., 2005), and SOC sensitivity to long-term climate change (Giardina and Ryan, 2000). The differences in terms arise from the theories on which different measurements are based. Though some studies suggest that recalcitrant C is not sensitive to temperature variation (Giardina and Ryan, 2000), others suggest that both recalcitrant and labile fractions are equally sensitive (Fang et al., 2005), yet others (Vanhala et al., 2007), found that old soil C is more temperature sensitive than young soil C (Knorr et al., 2005). Several factors may influence SOC sensitivity to temperature. The soil microbial community mediates the SOC response to temperature. At the same time, the soil microbial community is influenced by soil water content. Soil water not only plays a role in influencing soil microbial activity, but also influences the heat distribution in soil from temperature changes.

Various methods have been used to estimate temperature vulnerability of SOC which are either field or laboratory-based methods. There is controversy in linking short term and long term response of SOC to temperature. However, SOC response to temperature has been described using various models such as exponential or Arrhenius equations (Lloyd and Taylor, 1994), linear models (Rochette et al., 1991) and logistic

models (Jenkinson et al., 1991) and a summary of various kinetic models of biological relevance (single first-order, parallel first-order, parallel first- and zero-order, second-order and monod kinetic models) described by Sleutel et al. (2005). Various models have shown differences in Q_{10} of short term versus long term SOC response to temperature. Therefore, improved understanding of C dynamics in response to temperature changes is necessary.

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CHAPTER 2 - EFFECT OF TEMPERATURE ON SOC MINERALIZATION MODULATED BY SOIL WATER CONTENT

ABSTRACT

The stability of soil organic C (SOC) under changing climate is critical to the understanding of climate feedback effects on atmospheric CO_2. Most studies have focused on temperature impacts on SOC mineralization. However, changing climate will likely impact soil water content. Therefore coupled changes in soil water content and temperature will be important influences on SOC feedback on atmospheric CO_2. The objective of this study was to understand the influence of soil water content on the temperature response of microbial respiration, microbial community composition and SOC mineralization in an agricultural soil. Our microcosm study involved incubation of soil collected from the Kansas State University North Agronomy Farm, Manhattan, KS. The soil was a Fine-silty, mixed, superactive mesic Cumulic Hapludoll. The experimental plot was tilled and had previously been cropped to C_3 plants and is currently in continuous corn, a C_4 crop established in 1990. This crop change implies that the soil has a natural isotopic label, but not a very discrete label at this time. We used three incubation temperatures (15, 25 and 35°C), and for each temperature, soils were incubated at 3 soil water potentials (-1 MPa, -0.03 MPa, and -0.01 MPa). Incubation duration was 120 d. Head space gas was analyzed for CO_2 on days 3, 5, 7, 9, 14, 19, 24, 29, 36, 43, 50, 57, 85, 120 and for $\delta^{13}C$-CO_2 on days 7, 29, 57 and 120. Soils were destructively sampled and analyzed for phospholipid fatty acids (PLFA), SOC, and permanganate oxidizable C (POXC) on days 7, 29, 57 and 120. Potential mineralizable C (C_o) increased with

increasing temperature from 15 to 35^{o}C and also increased with increasing soil water content. The temperature sensitivity (Q_{10}) was highest for -0.01 MPa when temperature increased from 15 to 25 ^{o}C. Labile C estimated as POXC for 25 ^{o}C was significantly greater at -0.01 MPa than -1 and -0.03 MPa and for 35 ^{o}C was significantly greater at -1 MPa than -0.03 or -0.01 MPa. Progressively older SOC was mineralized at 15 ^{o}C under dry condition, but SOC was optimally mineralized at 25 ^{o}C under moderate soil water conditions. Soil microbial biomass (MB) was reduced with increasing soil water content; however % of potentially mineralizable C as microbial biomass reduced with increasing temperature. In conclusion, soil water content influenced temperature effects on SOC mineralization which was higher at higher than lower temperatures. Both young and older SOC were temperature sensitive, but SOC loss will depend on magnitude of temperature change, soil water content and duration of environmental condition. Soil MB was directly influenced by soil water content, while activity was indirectly influenced by temperature.

INTRODUCTION

Increasing atmospheric concentrations of CO_2 with other greenhouse gases (N_2O and CH_4) have been implicated in global climate change (Townsend et al., 1997; IPCC, 2007). Climate models project 2.4 to 6.7^oC increase in global surface temperatures by end of the 21st century (IPCC, 2007). The rise in global temperatures may accelerate soil organic C (SOC) mineralization which would exacerbate atmospheric CO_2 concentrations because of the large size of the soil C pool (Lal, 2004; Hayes et al., 2011). Therefore mineralization of SOC may result in a positive feedback to climate change (Knorr et al., 2005; Kirschbaum, 2006; Agren and Wetterstedt, 2007). These concerns have generated interests in SOC mineralization with respect to the global C cycle (Kirschbaum, 1995; Trumbore et al., 1996; Giardina and Ryan, 2000; Schlesinger and Andrews, 2000; Thornley and Cannell, 2001; Fang et al., 2005; Knorr et al., 2005; Reichstein et al., 2005; Fierer et al., 2006; Vanhala et al., 2007; Conant et al., 2008b; Hartley and Ineson, 2008).

The response of SOC mineralization to global climate change is still not clearly understood despite extensive research (Davidson and Janssens, 2006). Temperature is an important determinant of SOC mineralization, but the nature of this relationship with regards to climate change is still unclear (Bardgett et al., 2008). The heterogeneous nature of SOC complicates its mineralization. Soil OC is often placed in three pools; fast, slow and passive in the widely used CENTURY model (Parton et al., 1987). In laboratory studies, SOC is conceptually described as either labile (younger) or stable (older) (Xu et al., 2010). The younger SOC is more easily mineralized (Shaver et al., 2006), while the older fraction is more resistant to mineralization (Hartley and Ineson, 2008). The different SOC pools exhibit different inherent temperature sensitivities (Davidson and Janssens, 2006). Studies have suggested that temperature sensitivity of younger SOC is

less than (Fierer et al., 2005; Knorr et al., 2005; Conant et al., 2008b) or similar to (Giardina and Ryan, 2000; Fang et al., 2005; Conen et al., 2006) older SOC.

Temperature is not the sole driver of SOC mineralization. Soil water content and temperature interact with microbial activity that drives SOC mineralization. Most studies on climate feedback have examined single factors (elevated CO_2, warming or water availability) as influencing SOC mineralization (Bardgett et al., 2008). However, the soil water-temperature interaction could have antagonistic effects on SOC mineralization. Therefore, understanding the sensitivity of SOC mineralization to climate change requires a multifactor approach which should include the role of factors that act concurrently to influence SOC mineralization.

Alongside temperature, global climate change has been predicted to alter precipitation (Weltzin et al., 2003; IPCC, 2007; Knapp et al., 2008; Koerner et al., 2013) which will lead to variations in soil water content. Even with more precipitation, higher temperatures will increase evapotranspiration thus lower soil water content. Soil water has been reported to limit the temperature sensitivity of SOC mineralization (Carlyle and Than, 1988). Soil OC mineralization has been found to be insensitive to soil drying (Reichstein et al., 2005), yet reported to be most sensitive to temperature at intermediate soil water content (Craine and Gelderman, 2011).

The physiological activity of soil microbes as influenced by soil water content could modify the SOC mineralization response to temperature change (Allison et al., 2010; Exbrayat, 2013). Soil water content can affect SOC through microbial dynamics either directly though correlation with microbial activity or indirectly through the shifts in microbial community structure resulting from differential functional response to soil

water (Zeglin et al., 2013). Soil water content and microbial activity may not be linearly related if physiological tradeoffs occur between C assimilation and respiration (Schimel et al., 2007) or differences in some physiological traits (Aanderud and Lennon, 2011).

Bacteria and fungi, largely responsible for SOC mineralization, have different C use efficiencies and respond differently to soil water content (Jastrow et al., 2007). Fungi have been reported to dominate early stages of plant residue and low quality material (Beare et al., 1990; Feng et al., 2007), Gram+ bacteria prefer low quality substrates (Griffiths et al., 1999; Fierer et al., 2003) and Gram- bacteria depend on younger OC (Kramer and Gleixner, 2006; Potthoff et al., 2006).

Therefore, the assessment of temperature as the driver of SOC mineralization (Fang et al., 2005; Knorr et al., 2005) and the assumption of a single rate for SOC mineralization are not sufficient to explain SOC sensitivity to the changing climate. Changes in soil water content needs to be included with temperature when evaluating climate change impacts on SOC.

For this reason, we questioned whether soil water content could influence the impact of temperature on SOC mineralization by assessing: (1) the response of SOC mineralization as influenced by temperature and soil water interaction; (2) the impact of temperature and soil water content on soil microbial communities; and (3) the sensitivity of younger and older C to temperature as influenced by soil water content.

MATERIALS AND METHODS

Site description and sample collection

Soil was collected in 2011 at Kansas State University North Agronomy Farm, Manhattan, KS. The soil was a moderately well drained Kennebec silt loam (Fine-silty,

mixed, superactive mesic Cumulic Hapludoll). The field had been previously cropped to wheat and other small grains (C_3 plants) for at least 60 y prior to cultivation of continuous corn (*Zea mays* L.) a C_4 plant established in 1990. Aboveground vegetation and surface litter were removed before sampling. Surface soil was sampled in six (10 cm x 10 cm) areas to 20 cm depth after corn harvest. Nitrogen was applied annually as urea at a rate of 168 kg N ha^{-1}. The ^{13}C isotopic signature of the soil was determined to be -16.75 ‰ by dry combustion on a Carlo Erba Flash EA 1110 CHN connected to Gas bench II GC Quest Delta Plus (Thermo Finnigan, Bremen, Germany). After field collection, the soil samples were cold-dried (4°C) and sieved using 2-mm mesh sieve, while removing visible plant material. Soil was stored in sterilized and sealed polypropylene bags in the dark at 4°C until use.

Soil incubation (C mineralization)

Initial soil water content was assessed gravimetrically. Soil water retention curves were estimated using the method described by (Cassel and Klute, 1986). Root-free soil (25 g) was placed in Erlenmeyer flasks (microcosms). The flasks were placed in three groups of 48 and each group brought to soil water potentials of -1, -0.03, and -0.01 MPa equivalent to 0.13, 0.22 and 0.30 g H_2O g^{-1} dry soil, respectively. Flasks were weighed and sealed using parafilm to enable gas exchange and allowed to equilibrate for 10 days in the dark at 10°C. After equilibration, flasks were re-weighed to ascertain soil water content and placed in 1 L Mason jars containing 30 mL water (to maintain humidity and prevent soil drying) and covered using lids fitted with rubber septa. Flasks were incubated at 15, 25 and 35°C for 120 days. Head space gas was collected from four replicates of each treatment on the following days; 3, 5, 7, 9, 14, 19, 24, 29, 36, 43, 50,

57, 85, 120 to assess C respired to determine SOC mineralization kinetics. Head space gas was also sampled on days 7, 29, 57 and 120 to estimate changes in $\delta^{13}C\text{-}CO_2$. Destructive soil sampling was also done on days 7, 29, 57 and 120 for soil phospholipid fatty acid (PLFA), SOC, and permanganate oxidizable C (POXC). Soils were frozen (-20^{o}C) immediately after sampling, freeze-dried (lyophilized) and ground prior to analysis.

Control samples containing 30 mL water and an empty Erlenmeyer flask were included in the experiment. Headspace CO_2 was sampled through the Mason jar lid septa using gas tight 30 mL and 1 mL plastic syringes. To measure CO_2 concentration, one sample (0.5 mL using the 1 mL syringe) was injected directly into a Schimdazu Gas Chromatograph-8A (Kyoto, Japan) equipped with a thermal conductivity detector (TCD) and a 2-m Porapak column. The column temperature was 65^{o}C and the carrier gas was He at a flow rate of 20 mL min^{-1}. A second sample (25 mL) using a 30 mL syringe was transferred into an evacuated and sealed evacutainer (Labco Limited, Ceredigion - UK) for $\delta^{13}C$ determination. Mason jars were ventilated for 20 min after each gas sampling to allow headspace equilibration with the atmosphere. Water was added as required to maintain samples at the initial water potential. This was done by weighing the experimental setup and adding distilled water by spraying the required volume on the soil surface to bring the soil to the original soil water content. Care was taken to minimize soil disturbance during sampling.

$\delta^{13}C\text{-}CO_2$ estimation

The headspace CO_2 (25 mL) sampled through the Mason jar lid septa using 30 mL syringe was transferred into an evacuated and sealed evacutainer for $\delta^{13}C\text{-}CO_2$

determination on days 7, 29, 57 and 120. The ^{13}C-CO_2 was analyzed using a Thermo Finnigan Gas Bench II with CombiPAL autosampler (Thermo Finnigan, Bremen, Germany.

Permanganate oxidizable C

Permanganate oxidizable C (POXC), which represents the active fraction of SOC, was assessed using the method of Weil et al. (2003). Briefly, 2.5 g soil samples were placed into 50 mL plastic conical test tubes with screw caps. To the soil was added 20 mL of 0.02 *M* $KMnO_4$ solution (prepared in 0.1*M* $CaCl_2$). The soil suspension was shaken on a horizontal shaker at 120 strokes/ minute for 2 min, and then allowed to settle for 10 min. A 1 mL aliquot of the supernatant was diluted to 50 mL and absorbance measured at 550 nm using a GENESYS 20 spectrophotometer (Thermo Fisher Scientific Inc., Walthan, MA, USA). Standard solutions containing 0.0*M* (blank), 0.005*M*, 0.01*M* and 0.02*M* $KMnO_4$ were prepared. The soil C oxidized after reaction with $KMnO_4$ solution was determined by the change in concentration of $KMnO_4$ solution. The assumption was that 1 mol MnO_4 was consumed (reduced from Mn^{+7} to Mn^{+4}) in the oxidation of 0.75 mol (9000 mg) of C. Permanganate oxidizable C was expressed as mg C oxidized kg^{-1}soil.

Microbial community structure: Phospholipid fatty acid analysis

Changes in soil microbial community were assessed by measuring phospholipid (PLFA) of soil samples. The PLFA are indicators of viable microbial biomass (Kennedy and Gewin, 1997). The method of (Bligh and Dyer, 1959) as modified by (White and Ringlberg, 1998) was used to extract soil total lipids. The lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution for 3h from 5g of ground

lyophilized soil. The total lipids extracts were then separated into neutral lipids (NLFA), polar lipids (PLFA) and glycolipids by silicic acid chromatography using pre-conditioned disposable silica gel columns (J.T. Baker, Phillipsburg, NJ, USA). The neutral and polar lipids were then cleaved by alkaline methanolyis (KOH saponification and methylation) which cleaves the fatly acids from the glycerol backbone replacing it with methyl groups to form fatty acid methyl esters (FAMES) (Allison, 2005). The resulting FAMES were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Scientific, Germany) with a DB5-MS column (30 m × 250 μm i.d. × 0.25 μm film thickness). Helium was the carrier gas (1.0 mL min^{-1} constant flow). The temperature program was: 50 to 170°C at 20°C per min^{-1}; from 170 to 270°C at 2°C min^{-1}. The injection temperature was 220°C. Analysis was conducted in the electron impact (70 eV) mode and mass spectrometer scanning m/z^{+} was from 200 to 400. Peaks were identified using retention times of commercially available bacterial acid methyl esters (BAME; Matreya 1114) standard mix. Tentative assignments of methyl ester peaks not present in the BAME mix were made through mass spectral interpretation by comparison with spectra from a library (Wiley 138K mass spectral database). Sample peaks were quantified based on comparison of the abundance with an internal standard - nonadecanoic acid methyl ester (19:0). The abundance was expressed as nmol g^{-1} dry soil but also converted to mole % before statistical analysis. Nomenclature of identified fatty acids (FA) was as described by (Bossio and Scow, 1998), whereby FA were designated ***a:b***, where ***a*** is the total number of carbons and ***b*** the number of double bonds. An ***ω*** indicates the position of a double bond from the aliphatic end of the FA. The prefixes ***a*** and ***i*** refer to ***anteiso*** and ***iso*** branching, while the suffixes ***c*** and ***t*** refers to ***cis*** and ***trans***

isomers (conformations). Presence of methyl groups are indicated by ***aMe***, where ***a*** indicates the position of the methyl group. Fatty acids were grouped based on criteria by (McKinley et al., 2005) whereby Gram positive bacteria were i15:0, a15:0, 10Me16:0, i17:0 and a17:0. Gram negative bacteria as 18:1ω7c and cy19:0, while actinomycetes is 10Me18:0 and 10Me17:0, and fungi 18:2 ω 6,9c and 18:1 ω 9c.

Experiment design and statistical analysis

The microcosm experiment was a 3 x 3 x 4 factorial design with three temperatures (15°C, 25°C, 35°C), three levels of soil water potentials (-1 MPa, -0.03 MPa and -0.1 MPa), four sampling times. Each treatment consisted of four replicates to assess ^{13}C-CO_2, PLFA, POXC and TOC. Carbon mineralization was fitted to both single first and parallel first order kinetic models. The models used the Marquardt option NLIN, a nonlinear curve fitting procedure model (SAS 9.1). The first procedure was used to fit a one-pool model (De Neve and Hofman, 2002; Stanford and Smith, 1972; Tian et al., 1992) to determine cumulative mineralizable C (C_m) and mineralization rate (*K*). The model was:

$$C_m = C_o [1 - \exp(-kt)]$$

Where C_m = mineralized C (μg CO_2-C g^{-1} soil) at time t

C_o = potentially mineralizable C (μg CO_2-C g^{-1} soil)

k = rate constant of mineralization (d^{-1})

t = time (d)

The single first order kinetic model estimated two parameters; potential mineralizable C (C_o) and C mineralization rate (k).The second procedure also used the Marquardt option in NLIN to fit the parallel (mixed) first order kinetic model (Brunner and Focht, 1984; Sleutel et al., 2005) which was:

$$C_m = C_o [1 - \exp(-k_s t) - -k_f t^2/2)]$$

Where C_m = mineralized C ($\mu g\ CO_2$-C g^{-1} soil)

C_o = potentially mineralizable C ($\mu g\ CO_2$-C g^{-1} soil)

K_f = first rate constant of mineralization (d^{-1})

k_s = second rate constant of mineralization (d^{-1})

t = time (d)

The first rate constant is the rate constant for the fast pool, while the second rate constant represents the rate constant of the slow pool. The parallel first order model, estimated three parameters; C_o and two mineralization rates (K_f and K_s). The K_f and K_s represent rate constants for younger and older pools respectively. The C_o is the amount of C that can be mineralized under specific soil conditions.

To estimate the respiratory quotient (Q_{10}), a proxy for sensitivity of SOC to temperature change, the equation used was:

$$Q_{10} = \frac{\text{Respiration rate at T2 (°C)}}{\text{Respiration rate at T1 (°C)}}$$

Where Q_{10} = respiratory quotient

T1 = lower temperature (^oC)

T2 = higher temperature (^{o}C)

Analysis of variance (ANOVA) was performed using PROC MIXED in SAS 9.1 (SAS Institute, Cary, NC) and means were considered as significantly different at $P < 0.05$ except indicated otherwise. PROC REG in SAS 9.2 was also used to establish the relationship between temperature sensitivity of SOC (Q_{10}) and % TC respired.

RESULTS

Carbon mineralization

Cumulative CO_2 respired was fitted to first and mixed order models. The best fit was the mixed order model as assessed by comparing root mean square errors (RMSE) (Table A2.1). The first order kinetic model over-estimated C_o relative to the mixed order model. Therefore all further mineralization kinetics will be reported and discussed based on the mixed order kinetic model.

The estimates of potentially mineralizable C (C_o) was significantly ($P<0.0001$) influenced by a temperature x water interaction (Table 2.1). Overall, mineralizable C was highest for 35^{o}C at -0.01MPa (1301 μg g^{-1}soil) and least for 15^{o}C at -1MPa (194 μg g^{-1}soil) (Table 2.1). Although C_o increased with increasing temperature, the difference in C_o was larger at lower (15-25 ^{o}C) than higher (25-35 ^{o}C) temperature ranges. The temperature response of C_o increased with increasing temperature but was moderated by soil water content. When soil water content increased from -1 to -0.03 MPa, the change in C_o was greater than 55 %. However, when soil water increased from -0.03 to -0.01 MPa, change in C_o was less than 20 % (Fig 2.1) for all temperatures. These results indicate

potentially greater mineralizable SOC in higher latitude (cooler) soils under wetter soil conditions.

The rate constant, K_f, for the labile pool was significantly ($P<0.02$) affected by temperature but not by soil water (Table 2.1). The K_f decreased with increasing temperature (Fig. 2.2). However, the rate of change was significantly higher when temperature increased from 15-25 ^oC, but not significantly different when temperature increased from 25-35 ^oC. This suggests rapid exhaustion of the labile pool at higher temperatures (Fig. A 2.1). There was a significant effect of temperature and soil water on K_s but no significant interaction (Table 2.1). The effect of temperature on K_s was such that mineralization rate increased with increasing temperature (Fig. 2.3). This indicates that the stable pool which constitutes the greater proportion of SOC may be vulnerable under warmer soil conditions. The effect of soil water on K_s increased with increasing soil water content (Fig. 2.4). Although K_s increased when water content increased from -1 to -0.03 MPa, the rate was not significantly different when water content increased from -0.03 to -0.01 MPa (Fig. 2.4). This may indicate that stable pool mineralization rates may not be responsive to wetter soil conditions.

Respiratory quotient (Q_{10})

Temperature and water interacted significantly ($P<0.03$) to influence the sensitivity of SOC mineralization to temperature change expressed as the respiratory quotient (Q_{10}) (Table 2.2). As temperature increased from 15 to 25 ^oC the Q_{10} was highest under wetter soil conditions (Fig. 2.5). However, the influence of water was not significant when temperature increased from 25 to 35 ^oC. Therefore, at lower temperatures soil water content may be more influential than at higher temperatures. The

lower Q_{10} values at higher temperatures may indicate rapid exhaustion of labile C at higher temperatures.

Permanganate oxidizable C (POXC)

The POXC was significantly affected by a 3-way (Day x Temperature x Water) interaction (Table 2.3) (Fig. A2.4). On Day 7 there was a significant ($P< 0.05$) temperature x water interaction (Table 2.4). On Day 7, POXC for 25 ^{o}C was significantly highest at -0.01 MPa. However, for 35 ^{o}C POXC was highest at -1 MPa, but there was no significant water content effect at 15 ^{o}C (Fig 2.6). Results for Day 120 indicated a significant ($P<0.02$) water effect on POCX (Table 2.5). The water effect on Day 120 was such that -0.01 MPa was higher than -1 and -0.03 MPa which were similar (Fig 2.7). These results suggest that soil water content may be more influential in rendering SOC labile than temperature.

The $\delta^{13}C$-CO_2 respired

The initial $\delta^{13}C$ of bulk soil was -16.75 ‰ PDB indicating dominant C_4 plant contribution to SOC. Therefore the C_4-C constitutes more of the labile (younger) SOC, being the current vegetation in this experimental plot. The $\delta^{13}C$ of respired CO_2 was significantly ($P<0.0001$) influenced by a 3-way temperature x water x day interaction (Table 2.6). When analyzed by day, a significant ($P<0.0001$) temperature x water interaction for the $\delta^{13}C$-CO_2 on incubation day 7 (Table 2.9). The $\delta^{13}C$-CO_2 on day 7 indicates that progressively older SOC was mineralized with increasing temperature at -1 MPa (Fig. 2.8). For 15 and 35 ^{o}C, the influence of water was not significantly different at higher soil water content, while for 25 ^{o}C the -0.03 MPa was more depleted in $\delta^{13}C$-CO_2 than -0.01 MPa (Fig. 2.8). While older SOC for 15 and 35 ^{o}C mineralized similarly at

higher soil water content, for 25 ^{o}C it was greatest under moderate soil water content. Although older SOC may be more vulnerable with increasing temperature under drier soil conditions, the dominant SOC pool mineralized at any temperature was influenced by the soil water content.

The $\delta^{13}C$-CO_2 on incubation day 29 also showed a significant ($P<0.001$) interaction of temperature and water (Table 2.9). On day 29, the oldest SOC mineralized at 25 for -1 MPa, while the youngest mineralized at 15 ^{o}C for -1 MPa. However there was no influence of soil water content on SOC pool mineralized at 35 ^{o}C (Fig. 2.9). These results suggest that optimal SOC mineralization could occur at 25 ^{o}C under dry soil conditions. The temperature effect on day 53 indicated that the oldest SOC was again mineralized at 25 ^{o}C and the youngest at 15 ^{o}C (Fig. 2.10a). The soil water content effect for incubation days 53 indicated that -1 MPa was highest while -0.01 and -0.03 MPa were similar (Fig. 2.10 b). The trend for temperature and soil water effect on day 120 was similar to day 53 (Fig. 2.11a and Fig. 2.11b). The rate of change for $\delta^{13}C$-CO_2 when temperature changed from 15 to 25 ^{o}C was more than twice the rate of change when temperature increased from 25 to 35 ^{o}C on both days 53 and 120. The $\delta^{13}C$-CO_2 dynamics over incubation time was bi-directional for all temperatures (Fig. 2.12). The trends show that $\delta^{13}C$-CO_2 values initially reduced during the first 29 days and stabilized towards the end of incubation period. The trends suggest that the younger C_4-C might have been quickly exhausted by the 29th day of incubation resulting in a slowed mineralization of older SOC (Fig. 2.12).

Microbial community structure

The microbial community was not responsive to temperature but was affected by soil water content (Table 2.4). The changes in soil microbial community were assessed at various intervals during the 120 d incubation period, but there were no significant effect of incubation time on microbial groups as assessed using PLFA. However, the proportion of total PLFA as mineralizable C, was significantly ($P<0.0001$) influence by temperature x day x time interaction (Table 2.9). The proportion of total PLFA as C_o generally reduced over incubation time. However on day 7 and 29 this proportion was highest for all temperatures under -1 MPa (Fig. 2.18). This could indicate greater microbial biomass turnover under drier soil conditions (Fig. 2.18). The total microbial biomass as estimated by PLFA was significantly ($P<0.0001$) influenced by soil water content but not temperature. Total PLFA decreased with increasing soil water content (Fig 2.13). While reduction in soil water potential from -1 to -0.03 MPa reduced total PLFA by 8%, reduction in soil water potential from -0.03 to -0.01 MPa resulted in a 19% reduction in total PLFA.

The biomarkers for various microbial groups (actinomycetes, Gram-, Gram+, AMF, fungi) responded significantly to soil water content but not temperature (Table 2.4). Overall, the abundances of the microbial groups decreased with increasing soil water content (Fig. 2.14), but varied in the percent change. Gram+ bacteria were higher than either Gram- or fungi throughout the incubation. Nutritional status of microbes can be assessed using either the total saturated to total monosaturated (14;0, 15:0, 16:0, 17:0, 18:0,19:0, 20:0)/ (16:1ω5, 16:1ω7, 16:1ω9;, 18:1ω7, 18:1ω9) fatty acid ratio or the cyclopropyl to their monoenoic precursor ratio (cy17:0/16:1ω7c and cy19:0/18:1ω7c). The former is expected to be higher in environments with limited C and nutrients, while

the later has a higher ratio when bacterial growth rates reduce due to increase limitation in C. The total saturated to monostaturated fatty acid ratio was significantly ($P<0.01$) influenced by temperature and highest at 25 ^{o}C indicating limited available C at higher temperatures. Meanwhile the cyclopropyl:monoenoic precursor ratio (cy19:0/18:1ω7) was significantly ($p<0.0003$) influenced by soil water content. The ratio was highest at -0.03 MPa. On the contrary, the cyclopropyl:monoenoic precursor (17:0/16:1ω7) was not affected by either temperature or soil water content.

Relationship among SOC, Q_{10}, Co, PLFA, POXC

There was a positive relationship between Q_{10} and % change in C_o (Fig. 2.15). However, Q_{10} explained 64 and 94% of total variation in the % change in C_o when temperature changed from 15 - 25 ^{o}C (Fig. 2.15a) and 25-35 ^{o}C (Fig. 2.15b) respectively. Also the % change in C_o or every 0.1 change in Q_{10} was about 5x greater when temperature changed from 25-35 ^{o}C (Fig 2.15b) than change from 15 to 25 ^{o}C (2.15a). This could indicate that increases in temperature of cooler soils will result in faster mineralization rate but higher potential loss of C for warmer soils. The C_o:SOC ratio was also influenced by both temperature and soil water content. Potentially mineralizable C relative to TOC increased with increasing temperature. The ratio ranged from 0.72 to 5.25 and was lowest at 15 ^{o}C (-1 MPa) and highest for 35^{o}C (0.01 MPa) but not significantly different between 25 and 35 ^{o}C. The C_o:TOC ratio increased with increasing soil water content. The relationships between PLFA and SOC was significantly ($P<0.03$) influenced by a day x temperature interaction and a significant ($P<0.056$) temperature x water interaction (Table 2.5). On day 29, the percent PLFA of SOC was highest at 25 and 35 ^{o}C while on day 120 it was higher at 15 ^{o}C (Fig. 2.16). The percent PLFA of SOC at

25 ^{o}C was highest at -1 MPa but highest at 35 ^{o}C for -0.01 MPa (Fig. 2.17). The PLFA/ C_o and POXC /C_o relationships were both significantly influenced by 3-way interactions (Day x Temperature x Water) (Table 2.5). The PLFA:C_o ratio decreased with increasing temperature (Fig. 2.18). The ratio for incubation days 7 was higher at -1 MPa for all temperatures, while for day 29 it was higher at -1 MPa for 15 and 25 ^{o}C (Fig. 2. 18). There was no significant difference of PLFA:C_o ratio on day 57, however, the 15 ^{o}C (-1 MPa) treatment had highest value on day 120 (Fig. 2.18). The POXC proportions of C_o result indicated that on day 7 the -1 MPa soil water was highest for all temperatures (Fig. 2.19) while on the other days it was highest for 15 ^{o}C (-1 MPa) (Fig. 2.19). Therefore, wetter soils will produce higher potentially mineralizable SOC, but SOC turnover is faster under drier soil conditions.

DISCUSSION

Potentially mineralizable C (C_o) increased with increasing temperature, but the difference was larger at lower (15-25 ^{o}C) than higher (25-35 ^{o}C) temperature ranges and higher under drier than wetter soil conditions (Fig. 2.1). Similar results have been reported (Conant et al., 2008a; Curtin et al., 2012). It had been speculated that C_o is fixed for each soil independent of incubation temperature and soil water (Stanford and Smith, 1972; Campbell et al., 1984). This school of thought could have driven proponents of no climate change-related consequences of SOC mineralization. However, Zak et al. (1999) hypothesized that temperature and soil water could affect C_o resulting from variation in OC fluxes to MB through diffusion. In our study, the % C_o of SOC ranged from 0.7% for 15 ^{o}C (- 1 MPa) to 5% for 35 ^{o}C (- 0.01 MPa) (Table 2.9). Although these values were

lower than 11 to 17% reported by Fabrizzi (2006), they were higher than 0.3 to 5.2 % reported by Bouckaert et al. (2013) and 2% reported by Riffaldi et al. (1996) for a range of agricultural soils. This indicates that the mineralizable C is different for each soil and could change due to changes in environmental conditions.

The higher C_o under wetter soil could be due to several reasons. The C-acquiring enzyme activities have been reported as higher in moist than drier soil (Zeglin et al., 2013). The differences in C_o for different soils have also been related to soil texture (Motavalli et al., 1994; Curtin et al., 2012). Differences in soil texture can influence soil water holding capacity and flux of OC to microbial biomass (Zak et al., 1999), implying that although the C_o could be higher under wet soil conditions, mineralization of SOC would depend on the microbial SOC utilization. Therefore increasing temperature in cold regions under drier soil conditions has the potential to increase soil respiration and reduce soil C stocks.

The response of ^{13}C-CO_2 to interaction of temperature and soil water content indicated that temperature either caused changes in SOC mineralization kinetics or shifts in the C pool being mineralized. Different results have been reported for sensitivity of different SOC pools to temperature; older SOC more sensitive (Fierer et al., 2005; Knorr et al., 2005; Conant et al., 2008a) and no difference between older and younger OC (Giardina and Ryan, 2000; Fang et al., 2005; Conen et al., 2006). However, younger and older SOC could be utilized either concurrently or sequentially (Andren and Paustian, 1987; Dalias et al., 2001) and influenced by soil water content as evident from our $\delta^{13}C$-CO_2 results. The $\delta^{13}C$-CO_2 indicated progressively older SOC mineralized with increasing temperature at -1 MPa (Fig. 2.8). However, oldest SOC was mineralized at 25

^{o}C at -0.03 MPa (Figs. 2.10 and 2.11). Overall, the δ^{13}C-CO_2 results indicated that there seem to be a threshold between 25 and 35^{o}C at which SOC can be optimally mineralized under moderate soil water conditions. Using C isotopic ratios (Bol et al., 2003) reported that old SOC mineralized more efficiently at higher temperatures. In addition, preferential utilization of older SOC at higher temperatures by arctic soil microbes has been reported (Biasi et al., 2005). However, utilization of ^{13}C by soil microbes has also been reportedly influenced by the soil water status (Yao et al., 2012). Our results therefore suggest that although older SOC may be more vulnerable with increasing temperature under drier soil conditions, the dominant SOC pool mineralized at any temperature is influenced by the soil water content. Hence, vulnerability of younger or older SOC will depend on the magnitude of temperature change and soil water content.

The soil water content, but not temperature significantly influenced MB which decreased with increasing soil water content (Figs. 2.13 and 2.14). However, our results indicated a temperature influence on microbial physiological status noted as changes in PLFA stress ratios, which was highest at 25 ^{o}C. The higher stress ratio at 25 ^{o}C could imply exhaustion of younger SOC, similar to the δ^{13}C-CO_2 results which indicated limited available C for microbial utilization from predominantly older SOC. Such reduced C availability could result from exhaustion of younger more available C. Our results indicated that the % PLFA of C_o (Fig. 2.18) and % POXC of C_o (Fig. 2.19) concurrently reduced and predominantly older SOC utilized after day 29. Therefore, the temporal decrease in MB as % PLFA of C_o could have resulted because MB is often energy-limited over incubation time. The reduction in MB with respect to changing substrate quality with increasing incubation duration is because SOC becomes chemically

recalcitrant (predominantly older SOC) and minimally accessible to microbes (Wagai and Sollins, 2002; Bettina et al., 2003;). There was also higher fungal:bacteria ratio at lower soil water content, which may indicate shifts in microbial community due to water stress, similar to reports by Zeglin et al. (2013). At lower soil water content substrate may be less available, which favors fungi which can access resources not available to bacteria through their hyphae resulting in higher fungal biomass relative to bacteria at lower soil water content.

Low MB with increasing soil water has been reported (Linn and Doran 1984, Freeman et al., 2001). Increasing soil water content could reduce O_2 diffusion as soil which may reduce available substrate, hence MB. The lack of field soil warming effect on microbial biomass assessed as PLFA has been reported (Zhang et al., 2005). However, C use by microbes due to temperature change has also been reported (Lopez-Urrutia and Moran, 2007). Microbes may respond to temperature changes by producing new enzymes and changing membrane fatty acids expressed as change in C use efficiency (CUE) (Steinweg et al., 2008). Therefore, although temperature changes, the decomposer community may remain the same, but could be in a transient state, which could explain the lack of direct temperature effect on MB in our study. Osmoregulation strategies by microbes may result in the high MB under drier soil while the higher fungal:bacteria ratio could result because certain fungi accumulate more compatible solutes than bacteria under water potential stress conditions (Schimel et al., 2007). These results indicate that MB could be lower under higher soil water conditions but have higher fungal biomass under drier soil which could potentially mineralize SOC in spite of temperature stress.

This may also explain the optimal SOC mineralization at 25 ^{o}C under moderate soil water conditions.

Although temperature may not have explicitly influenced MB following exhaustion of the younger SOC, heat energy could become important in enhancing activation of exo-cellular enzyme hydrolysis of the more abundant but older SOC, but at a slower rate (Godley, 2004). Older SOC has been shown to require higher activation energy, hence assumed to be more temperature sensitive than high quality younger SOC (Bosatta and Agren, 1999). The substrate quality of older or younger SOC could have also influenced mineralization rates. The mineralization rates of the younger SOC (K_f) and the older SOC (K_s) responded variedly to temperature and soil water. Therefore, the dominant SOC pool mineralized under different soil conditions could influence the mineralization rates of the labile (K_f) and stable (K_s) pools. Generally, SOC mineralization rate is predicted to increase with increasing temperatures (Schlesinger and Andrews, 2000). The K_f was responsive only to temperature and increased with increasing temperature, similar to the $\delta^{13}C$-CO_2 on day 7 under drier conditions when the younger SOC was the dominant source at lower temperature. However, the K_s was influenced by both temperature and soil water content similar to the $\delta^{13}C$-CO_2 results on days 53 and 120 when older SOC was the dominant SOC source. However, we suggest that the K_f may not depict the complete scenario. Rapid exhaustion of labile SOC at higher temperatures earlier in the incubation when integrated over incubation time could translate into lower K_f with increasing temperatures. Several confounding process rates influence mineralization rates; the rates at which decomposers take up substrate at their surface, rate at which substrate diffuses up to the surface of the decomposer, and rate at

which substrate is made available in the soil (Agren and Wetterstedt, 2007). All these processes require water for solubility or as transport medium, hence water should be potentially influential even as energy from temperature is also important for SOC mineralization.

The temperature sensitivity Q_{10}, assessed as the change in SOC mineralization rate with change in temperature (Fang and Moncrieff, 2001; Fang et al., 2005), should theoretically be higher for older than younger SOC due to increase in activation energy (Bosatta and Agren, 1999). However, Q_{10} has also been reported to decrease as soil water reduces ((Reichstein et al., 2002). Our reported Q_{10} ranged between 1.19 ± 0.06 and 2.54 ± 0.06. Although the temperature effect was larger than the water effect, the Q_{10} was higher at lower soil temperature, where it was highest at -0.01 MPa (Fig 2.5). Supposing that younger C is exhausted faster at higher temperatures, we could assume that the Q_{10} at lower temperature range 15-25 ^{o}C reflects the Q_{10} of younger SOC while at 25-35 ^{o}C represent Q_{10} of older SOC. However our data analysis of the relations between Q_{10} and % change in C_o indicated potentially greater SOC mineralized at higher than lower temperature ranges (Figs. 2.15a and b).The Q_{10} dynamics was similar to changes observed for $\delta^{13}C\text{-}CO_2$ results on day 29. The $\delta^{13}C\text{-}CO_2$ data indicate that older and younger SOC mineralization at different temperatures showed opposing trends with changing soil water content (Fig. 2.9).

Our Q_{10} results are similar to those reported by (Liu et al., 2006). Also, our Q_{10} range falls within the range of other studies (Kirschbaum, 1995); even at the ecosystem level (Janssens and Pilegaard, 2003; Fang et al., 2005). Similar to our results, the rate at which the rate constant increased with temperature has also been reported as greatest at

lower than higher temperatures (Kirschbaum, 1995; Conant et al., 2008b). However, interpreting Q_{10} results with regards to SOC mineralized is complicated because SOC is predicted to be more temperature-sensitive at higher temperature which is contrary to our findings. The Q_{10} estimates performed in various ways have produced varying results, which may also be biased because it may confound SOC mineralization due to temperature changes and effects of decomposer community in a transient state from temperature change (Wetterstedt et al., 2010).

A plausible explanation for the higher Q_{10} at lower than higher temperature ranges can be deduced from field experiments, where higher winter time than summer time Q_{10} of SOC mineralization have been reported (Janssens and Pilegaard, 2003), even though higher SOC mineralization occurs in summer than winter. This implies that temperature response of SOC mineralization should not be based only on relative changes in rates, but also on the absolute rate of SOC mineralization which is significantly lower at low than higher temperature (Fig. A-2.1). At low temperatures, the SOC mineralization rate is low, such that as temperatures increase, the difference in absolute mineralization rates is larger at lower temperature ranges. Therefore, the Q_{10} could also depend on the dominant SOC pool mineralized. If this be the case, younger SOC could persisted at lower temperatures, but rapidly exhausted at higher temperatures such that when integrated over the incubation period, could result in higher rates at lower than higher temperature ranges. Therefore, it may be difficult to tease out the various rates which could also be responsible for our rate hysteresis for Q_{10}, K_f and K_s.

The different parameter responses of SOC mineralization to temperature and soil water content may indicate differences in mechanisms responsible for SOC

mineralization or shifts in dominant pool mineralized under different environmental conditions. The results reflect the complexities involved in SOC mineralization.

CONCLUSION

The relationship between SOC mineralization, temperature and soil water content is complex, such that processes involved in SOC mineralization do not respond similarly to soil temperature and soil water content. Soil OC mineralization may involve a continuum of substrates with varying susceptibility to changes in temperature and influenced by soil water content. Our study results suggest a strong influence of soil water content on temperature response of SOC mineralization and therefore should be considered in models to appropriately depict climate change feedbacks on soil C dynamics. Although Q_{10} of the smaller labile SOC pool may be higher at lower temperature ranges, mineralization of both younger and older SOC may be occurring concurrently, sequentially or both. Therefore, global climate change could result in an increase of C released to the atmosphere from this agricultural soil. However, the contribution of either younger or older SOC will dependent on the magnitude of temperature shift, soil water content and duration of environmental conditions. We believe that mineralization of labile pool would not contribute much towards a positive feedback on climate change because it acts more like a revolving door. On the other hand, older C mineralization could be important in accelerating climate change because of the larger pool size, whereby even small changes over long time span may contribute significantly to climate feedback. It would be interesting to include more soil water and temperature intervals to assess the asymptote of temperature and soil water interaction

during SOC mineralization. It would also be informative to concurrently use different soil types in the same experiment to assess the effect of different soil properties on SOC mineralization.

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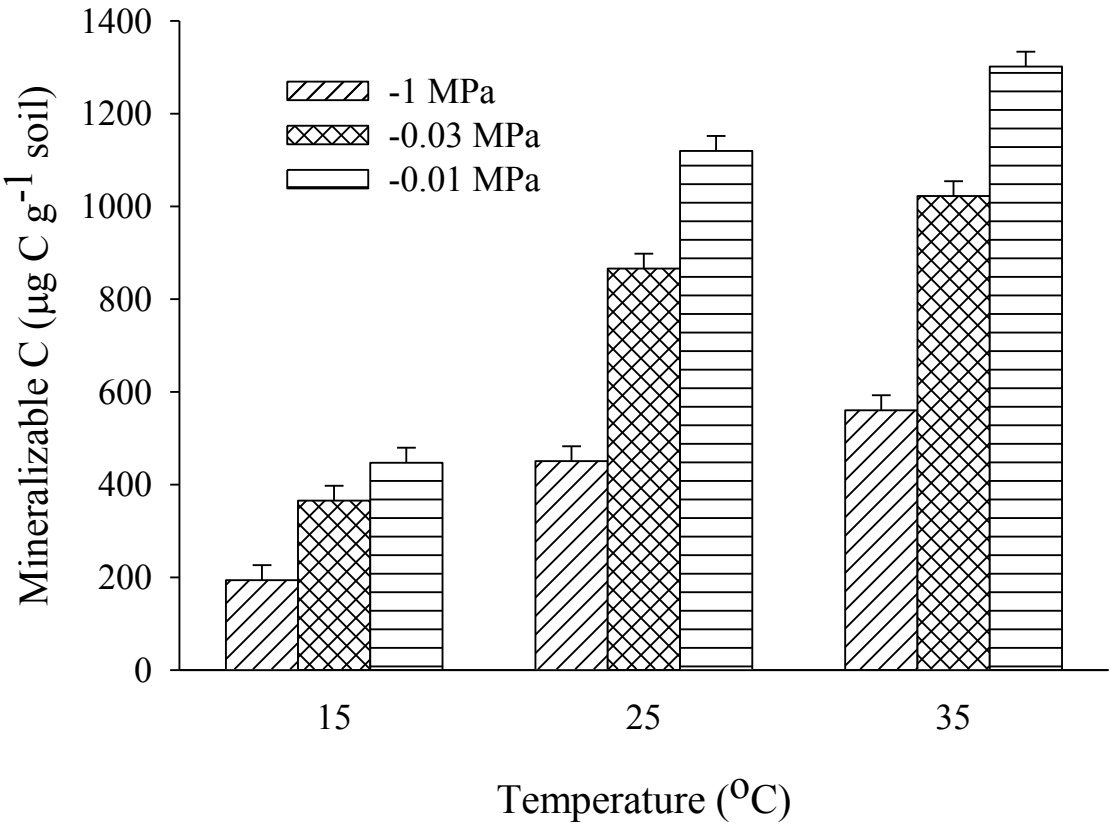

Figure 2.1. Temperature x soil water potential interaction means (P<0.0001) of potentially mineralizable C of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa). Error bars represent standard error of the mean (n = 4).

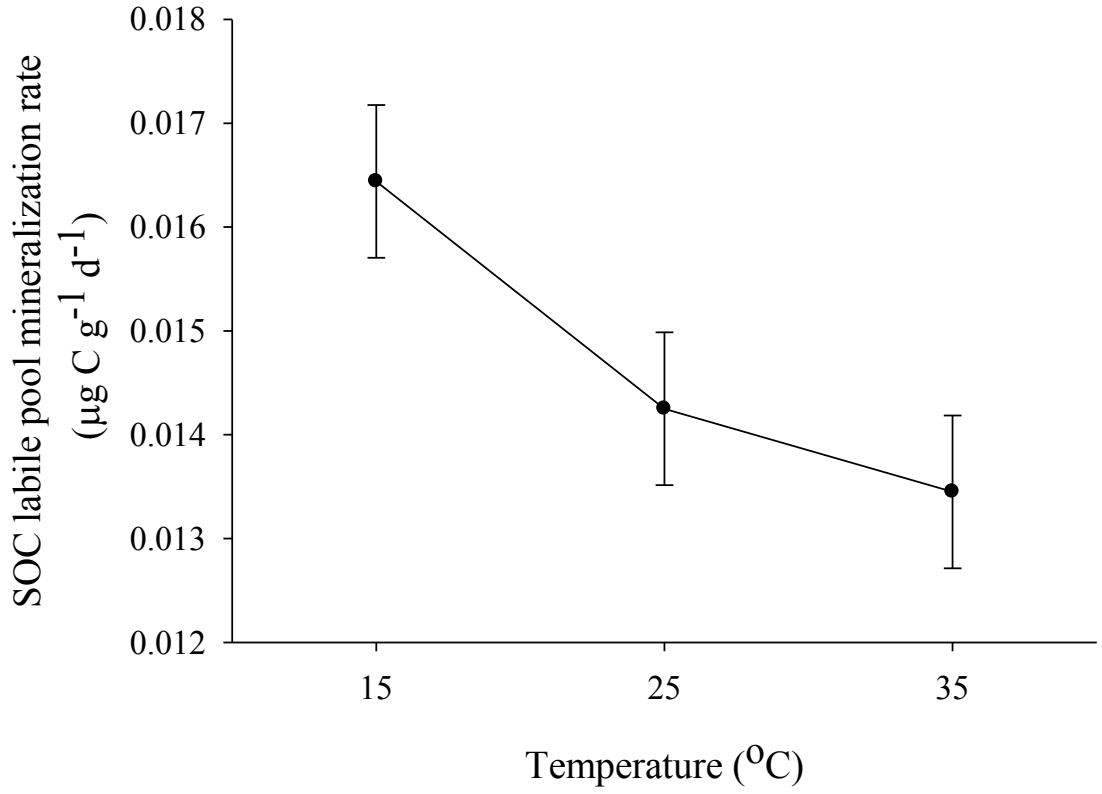

Figure 2.2. Temperature effect (P<0.02) on SOC younger pool mineralization rate (k_f) estimated from a mixed parallel first order kinetic model of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

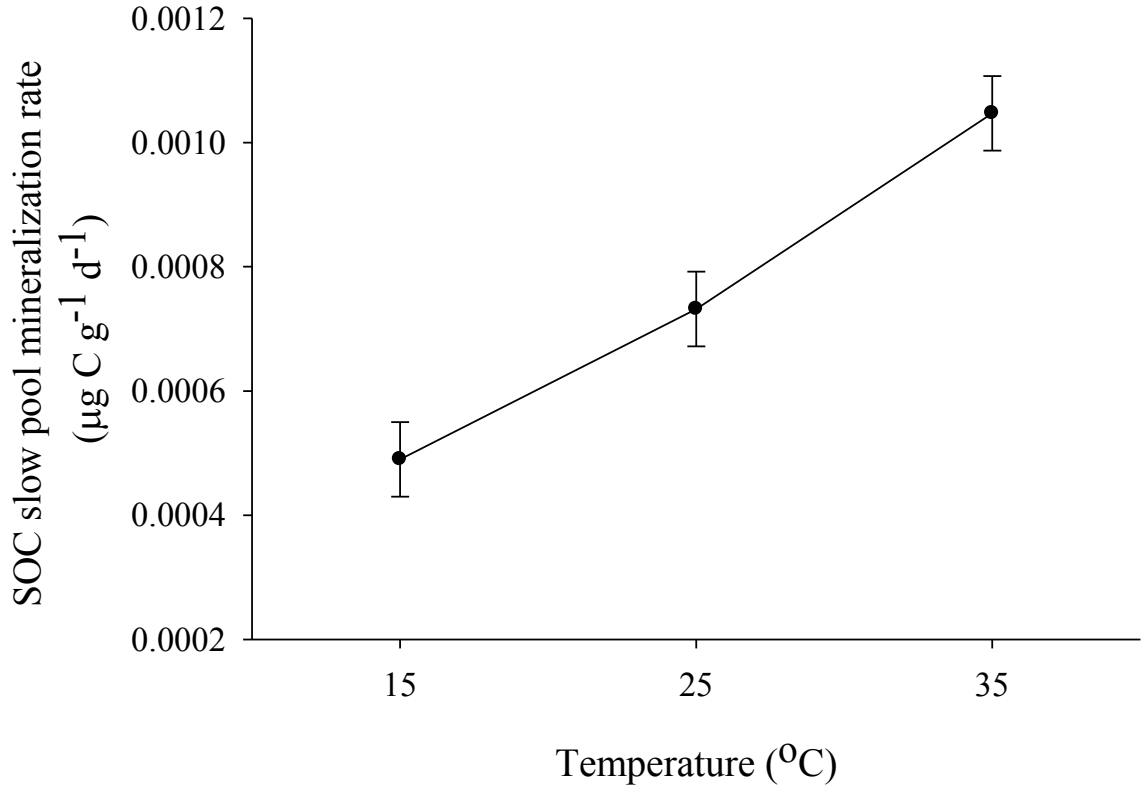

Figure 2.3.Temperature effect (P<0.0001) on SOC older pool mineralization rate (k_s) estimated from a mixed parallel first order kinetic model of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

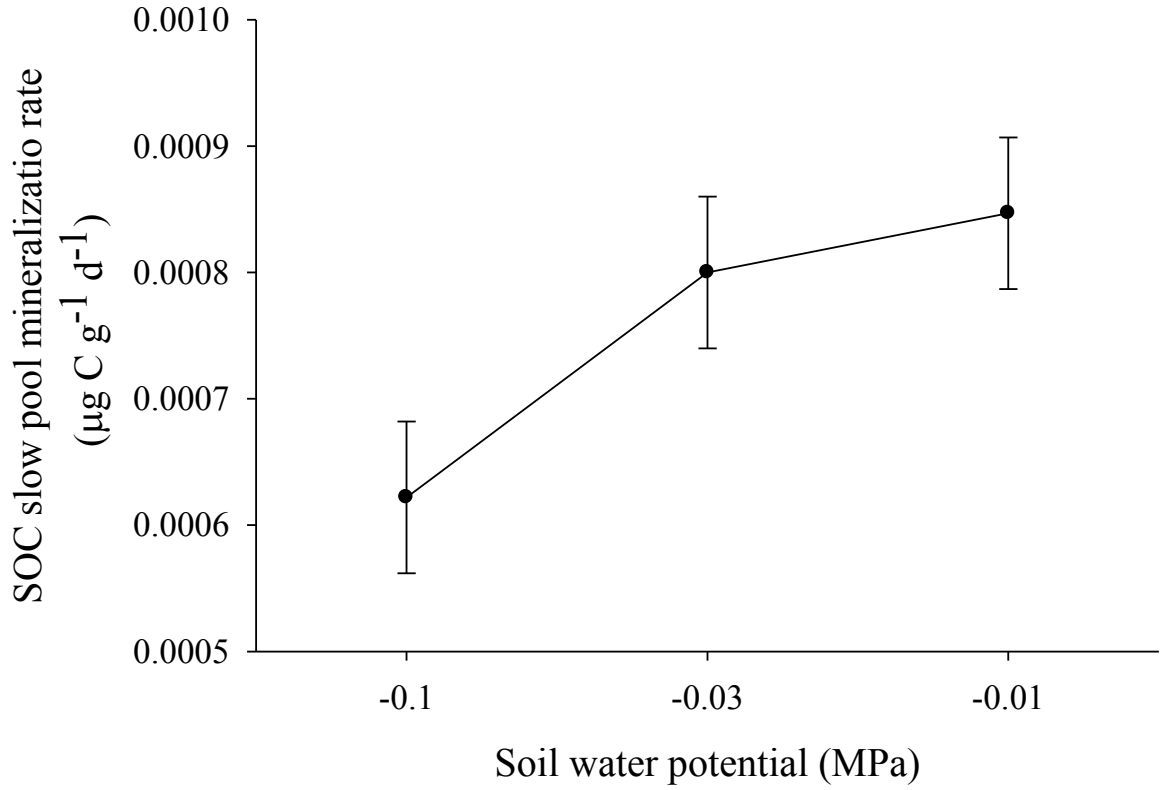

Figure 2.4. Soil water potential effect (P<0.03) on SOC older pool mineralization rate (k_s) estimated from a mixed parallel first order kinetic model of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

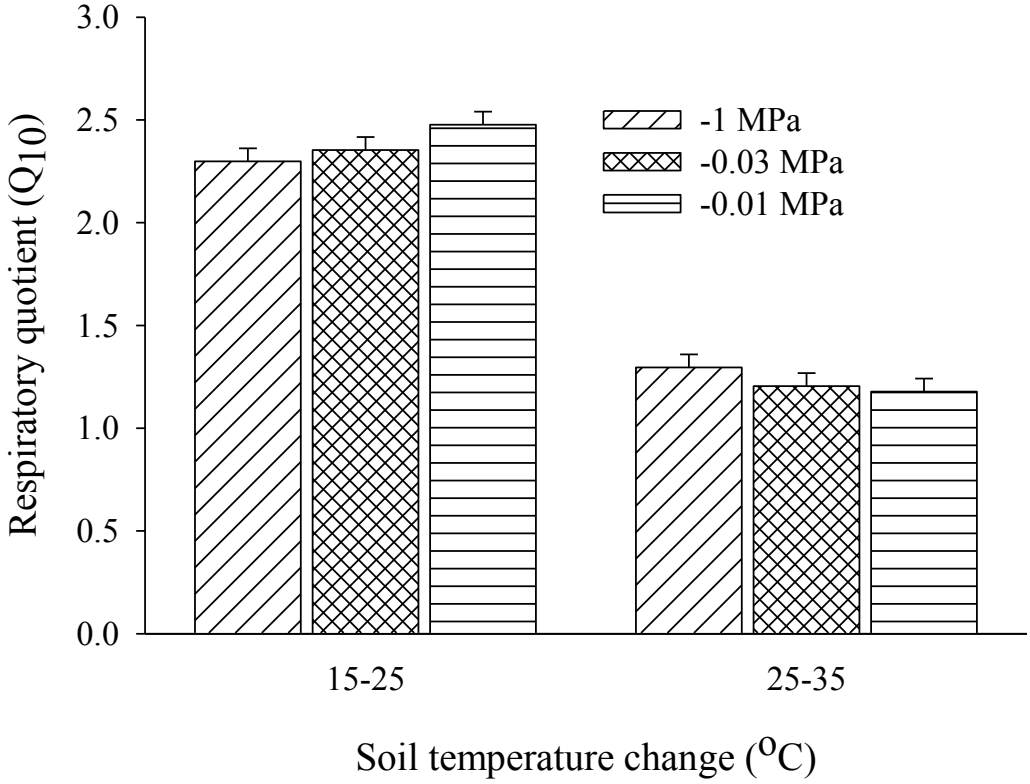

Figure 2.5. Temperature x soil water potential interaction means (P<0.04) of soil respiratory quotient (Q_{10}) estimated by incubating soil at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

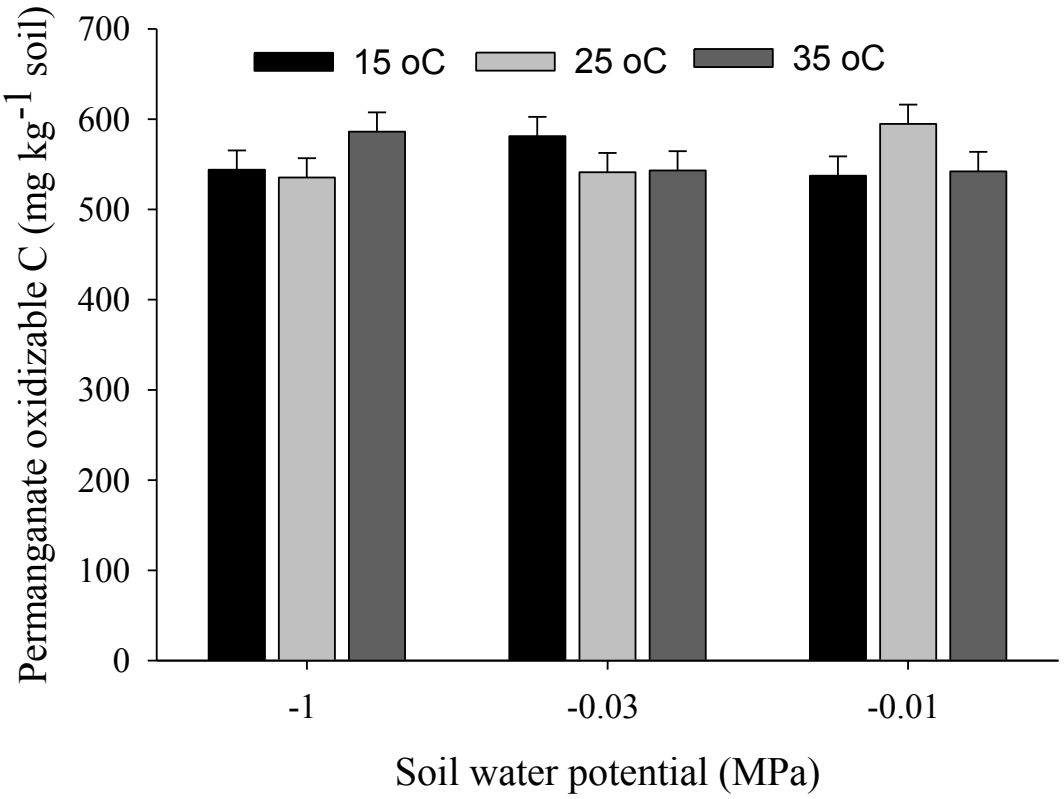

Figure 2.6.Temperature x water potential interaction means (P<0.05) of permanganate oxidizable C (POXC) for incubation day 7 of soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

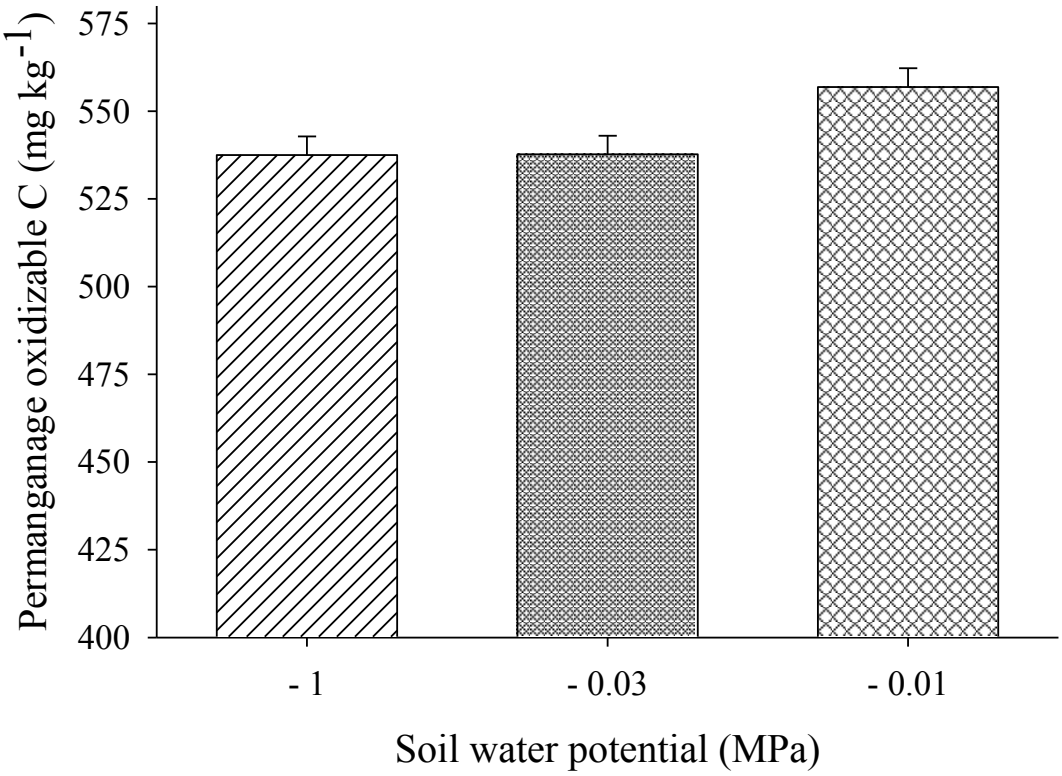

Figure 2.7. Soil water content effect means (P<0.02) of permanganate oxidizable C (POXC) for incubation day 120 of soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

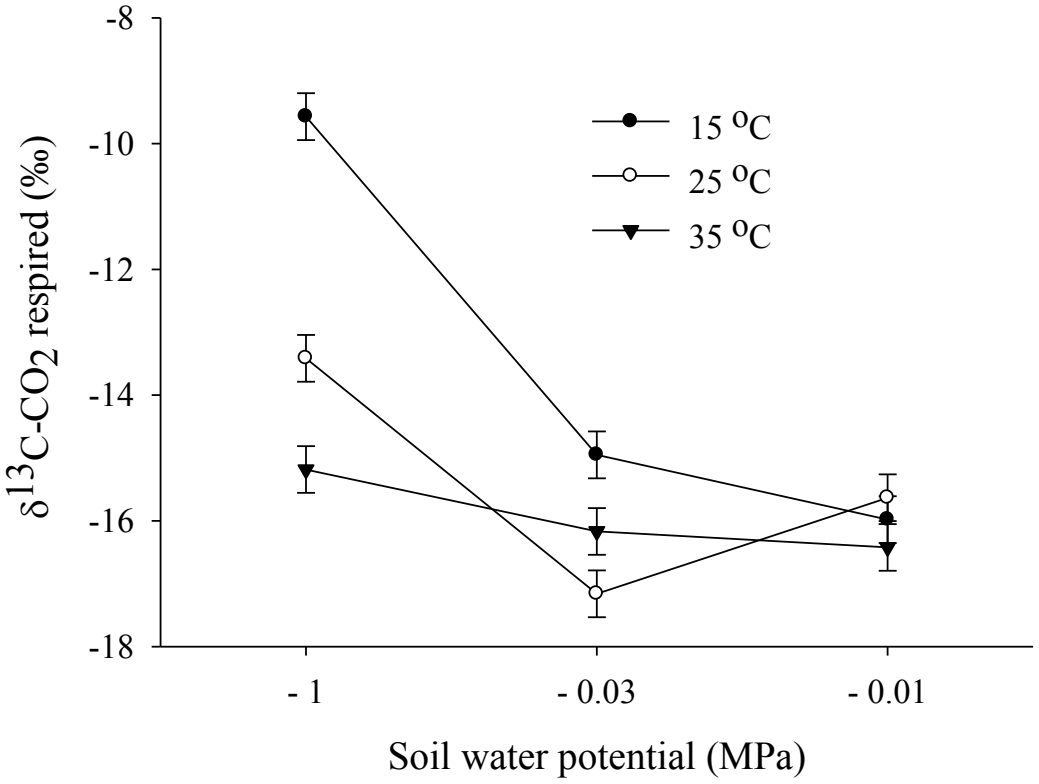

Figure 2.8. Temperature x soil water potential interaction means (P<0.0001) of $\delta^{13}C$-CO_2 for incubation day 7 of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

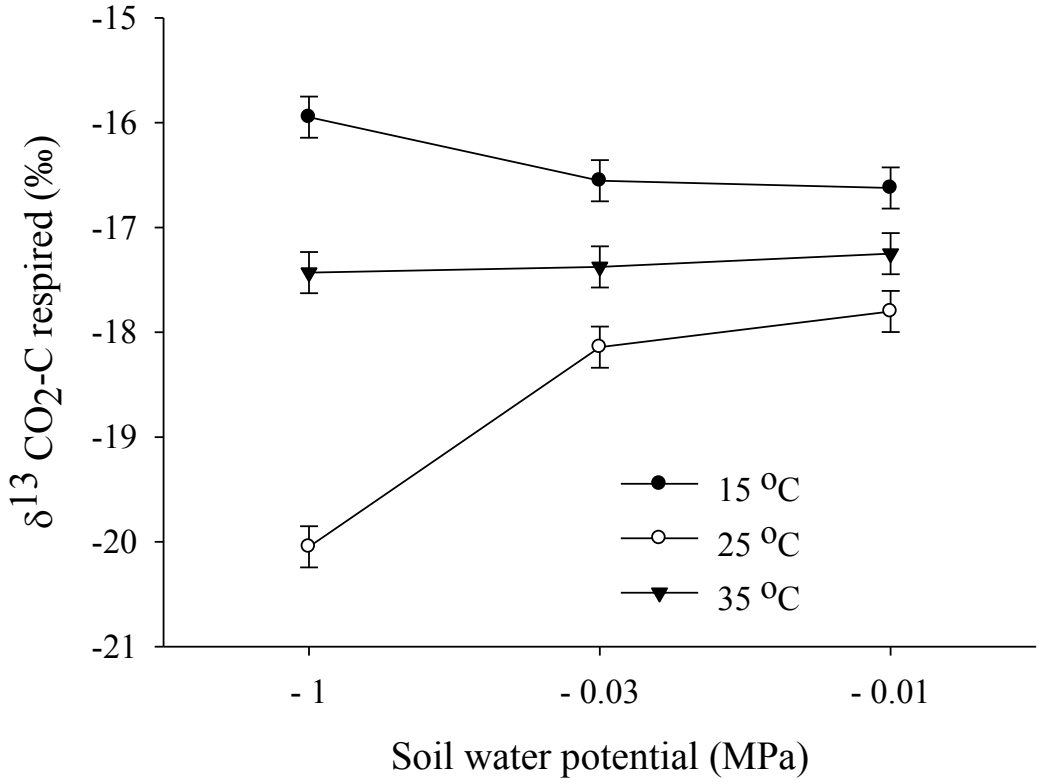

Figure 2.9. Temperature x soil water potential interaction means (P<0.0001) of $\delta^{13}C$-CO_2 for incubation day 29 of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

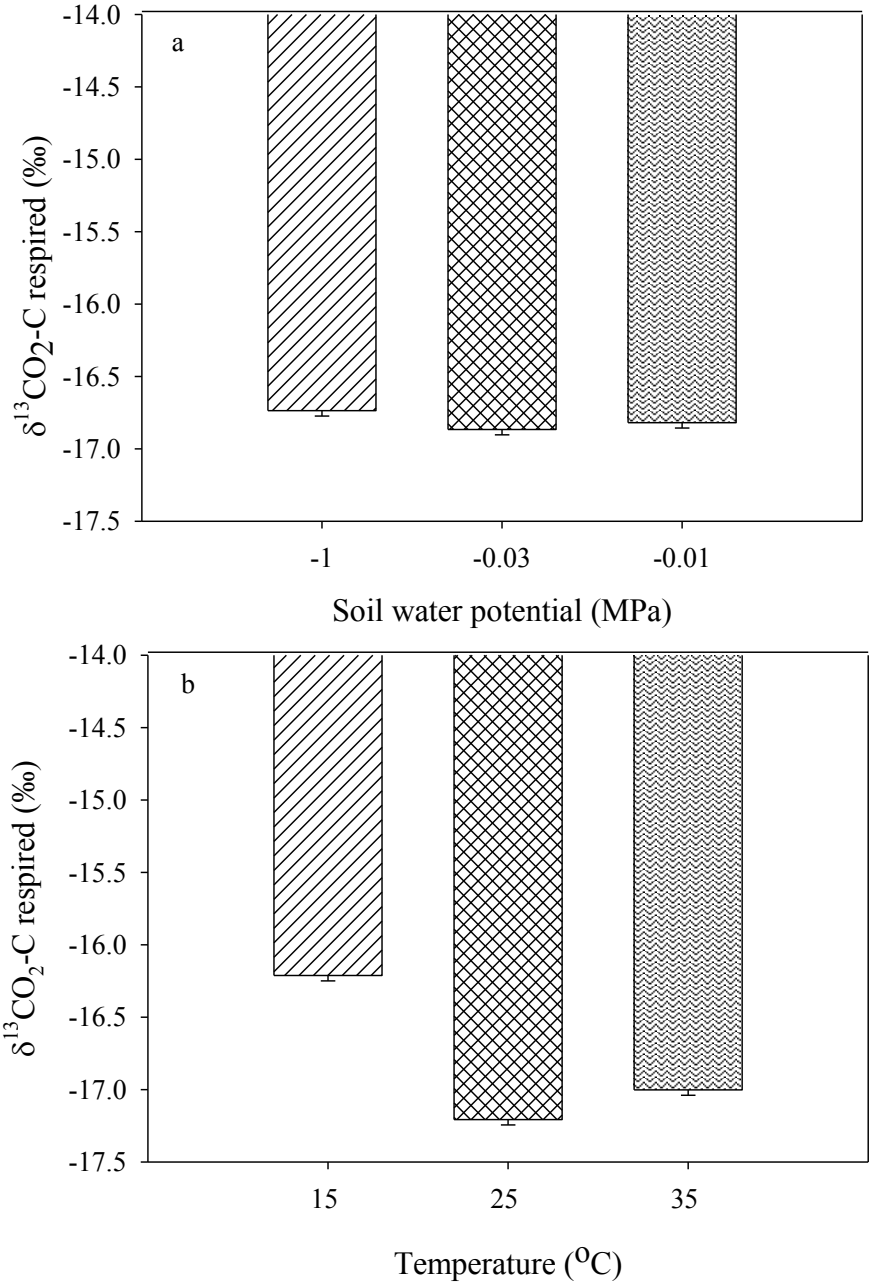

Figure 2.10. The means of (a) soil water potential(P<0.057) and (b) temperature (P<0.0001) of $\delta^{13}C$-CO_2 for incubation days 57 of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa). Error bars represent standard error of the mean (n = 4).

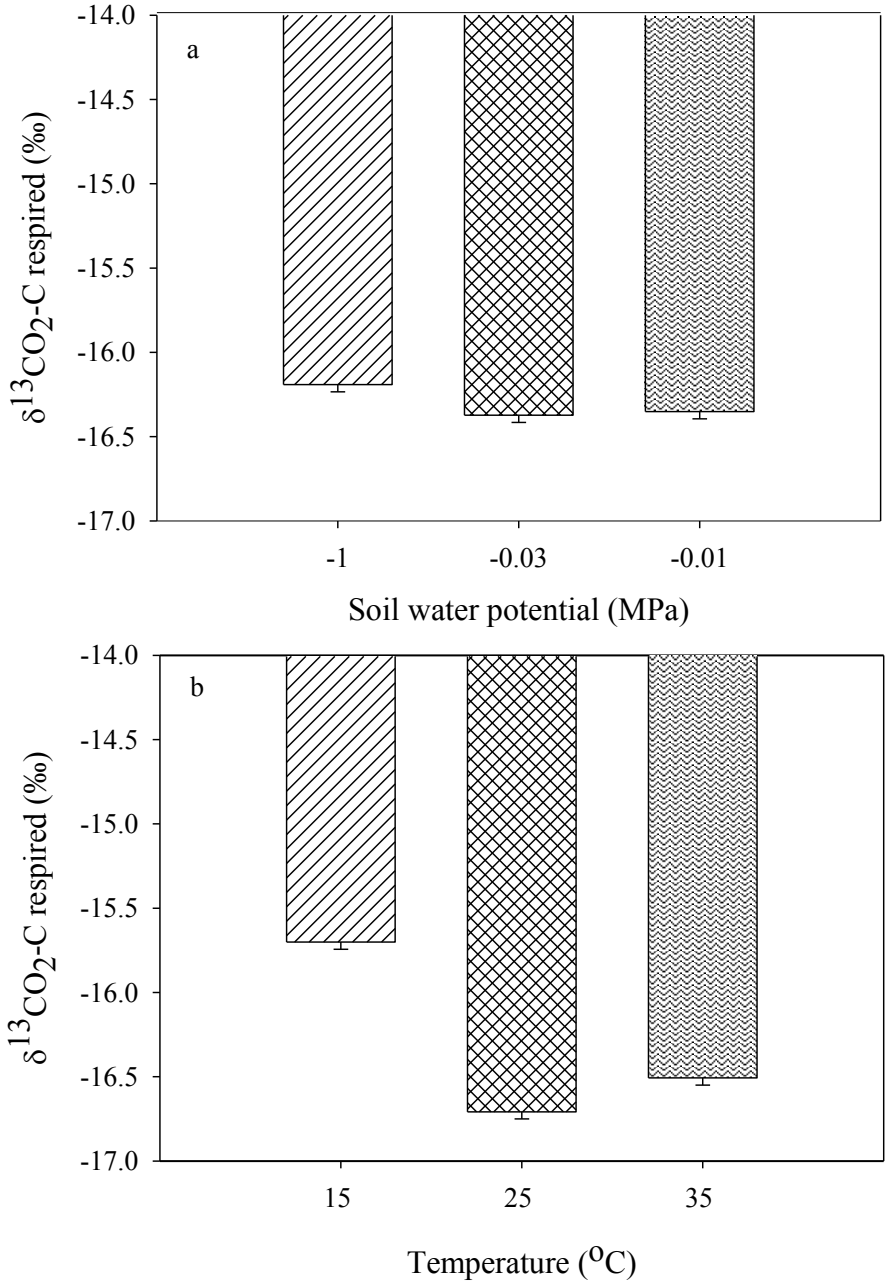

Figure 2.11. The means of (a) soil water potential (P<0.01) and (b) temperature (P<0.0001) of $\delta^{13}C$-CO_2 for incubation days 120 of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa). Error bars represent standard error of the mean (n = 4).

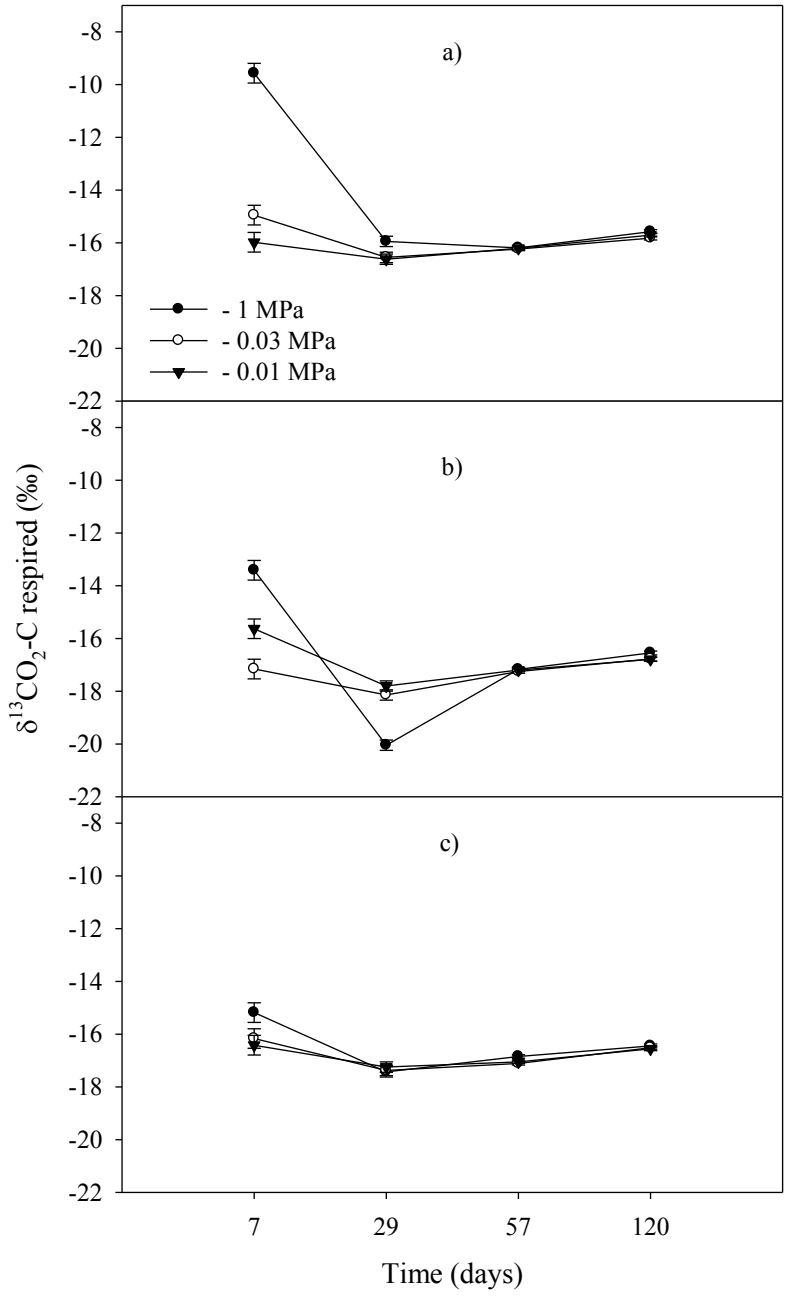

Figure 2.12. Dynamics of $\delta^{13}C$-CO_2 sampled on different incubation days of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at a) 15 ^{o}C b) 25 ^{o}C and c) 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

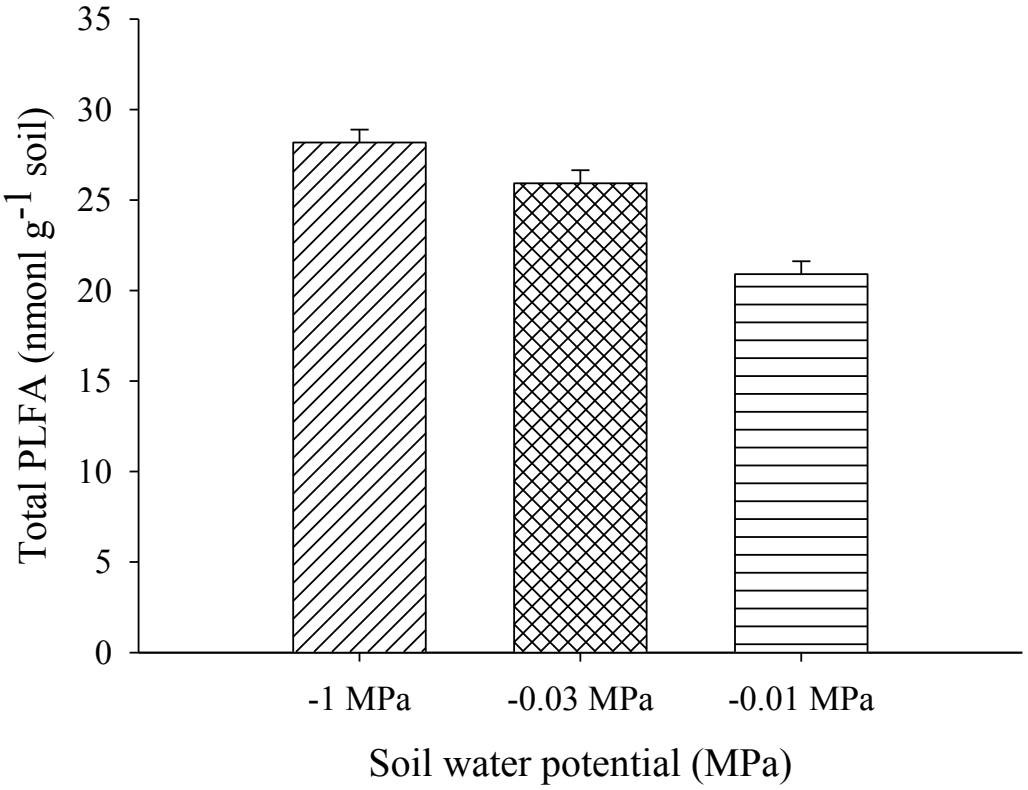

Figure 2.13. Soil water potential effect means (P<0.0001) on microbial biomass assessed as PLFA of soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

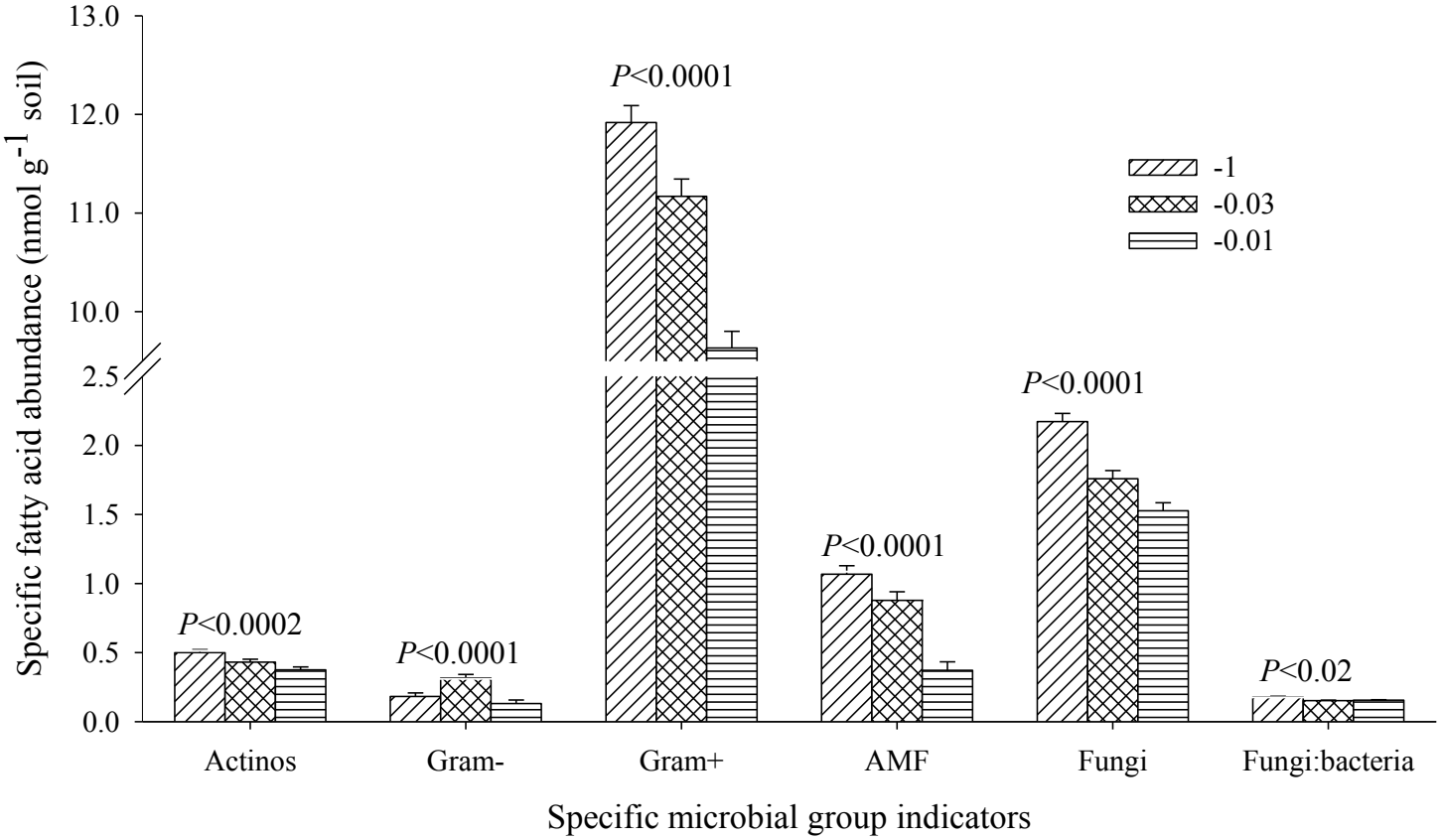

Figure 2.14. Soil water potential effect on relative abundance of microbial groups assessed as PLFA of a soil incubated at 15, 25 and 35 ^{o}C under various soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

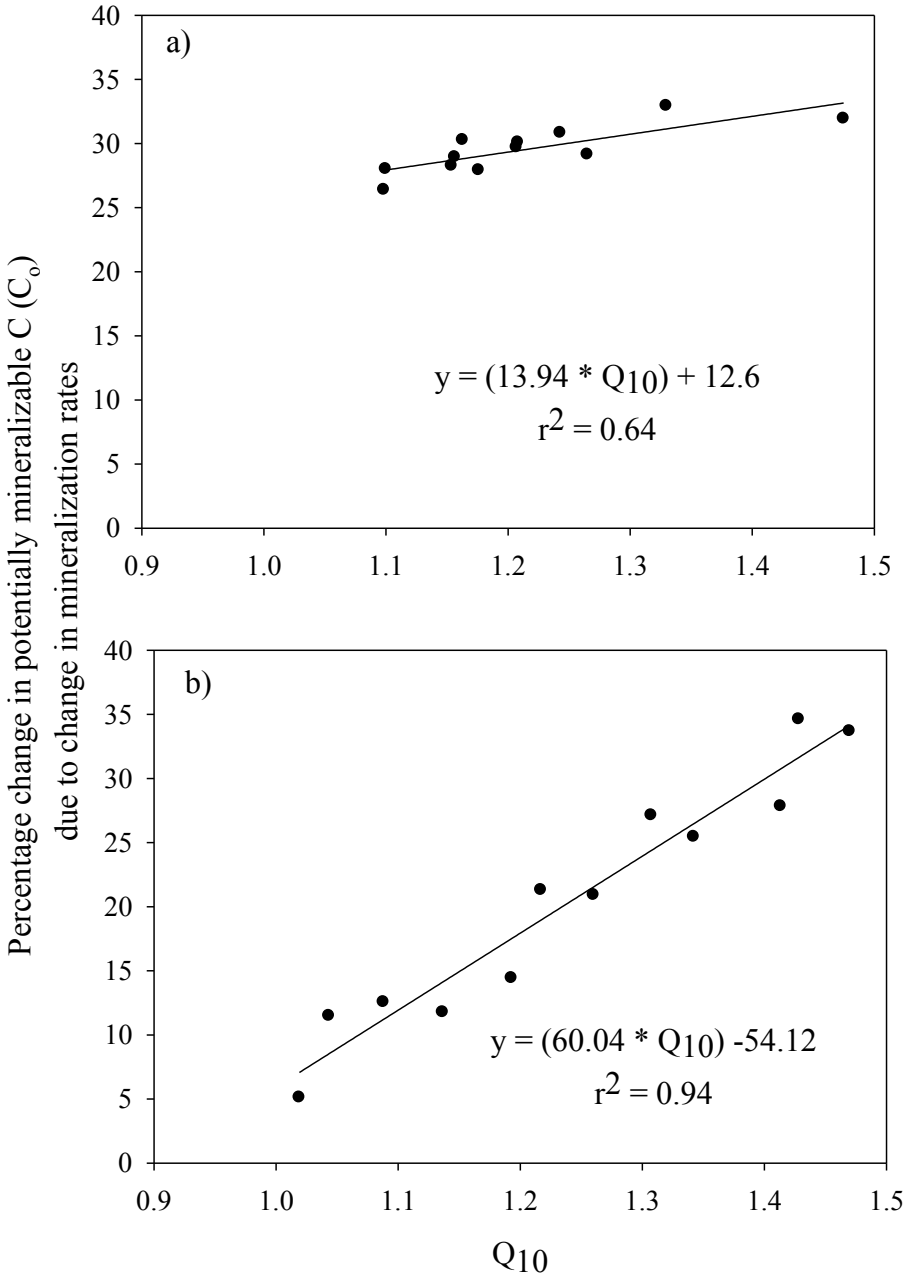

Figure 2.15. Temperature sensitivity (Q_{10}) against % C_o change with change in temperature of a soil incubated at 15, 25 and 35 ^oC under varying soil water potentials (-1, -0.03, -0.01 MPa). (a) Q_{10} when temperature changed from 15 to 25^oC (b) Q_{10} when temperature changed from 25 to 35 ^oC.

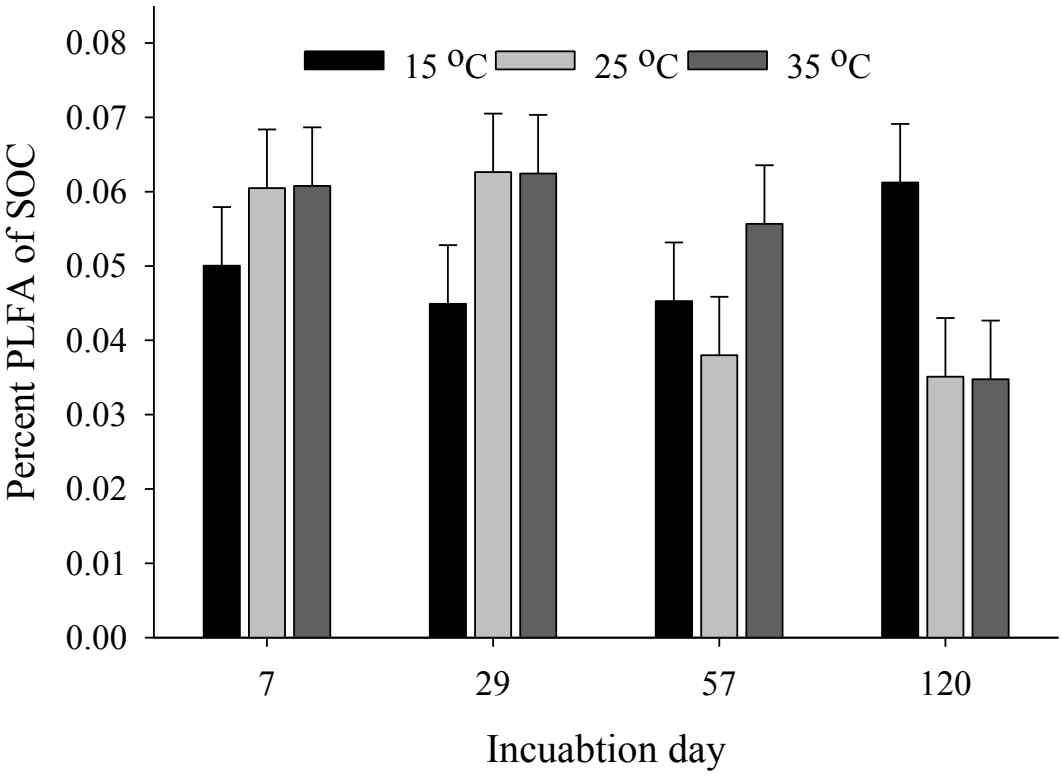

Figure 2.16. Incubation day x temperature effect means (P<0.03) of percent total microbial biomass of SOC assessed as phospholipid fatty acids (PLFA) of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

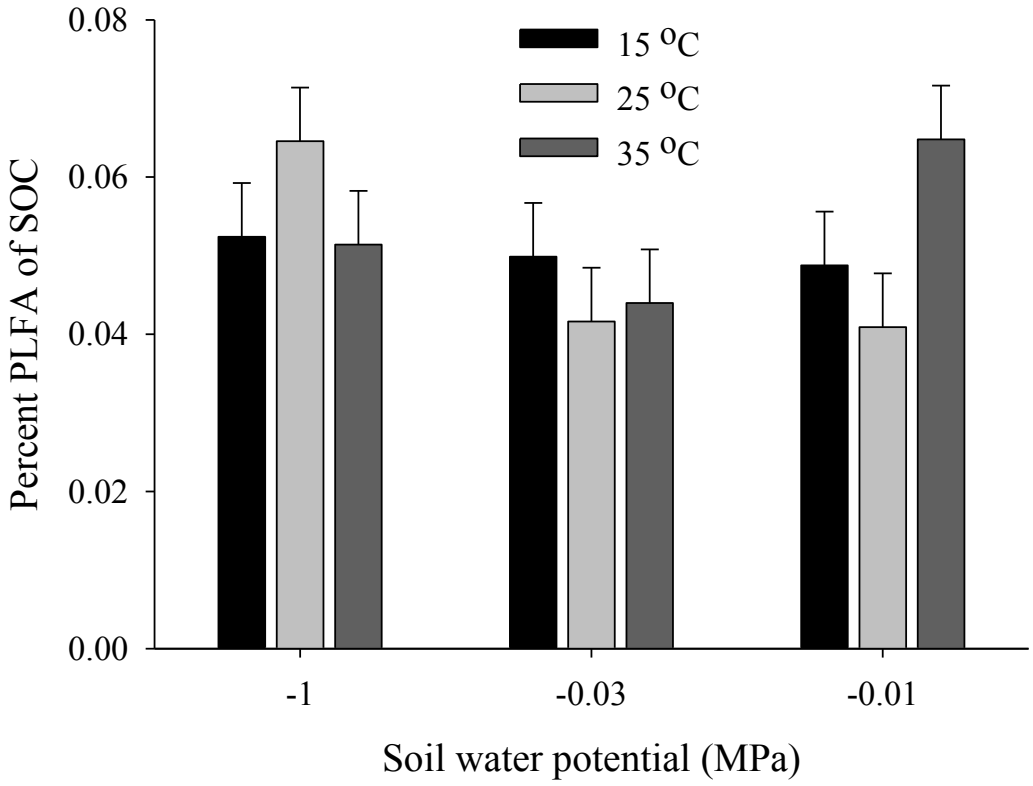

Figure 2.17. Temperature x soil water potential interaction means (P<0.056) of percent total microbial biomass of SOC assessed as phospholipid fatty acids (PLFA) of a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

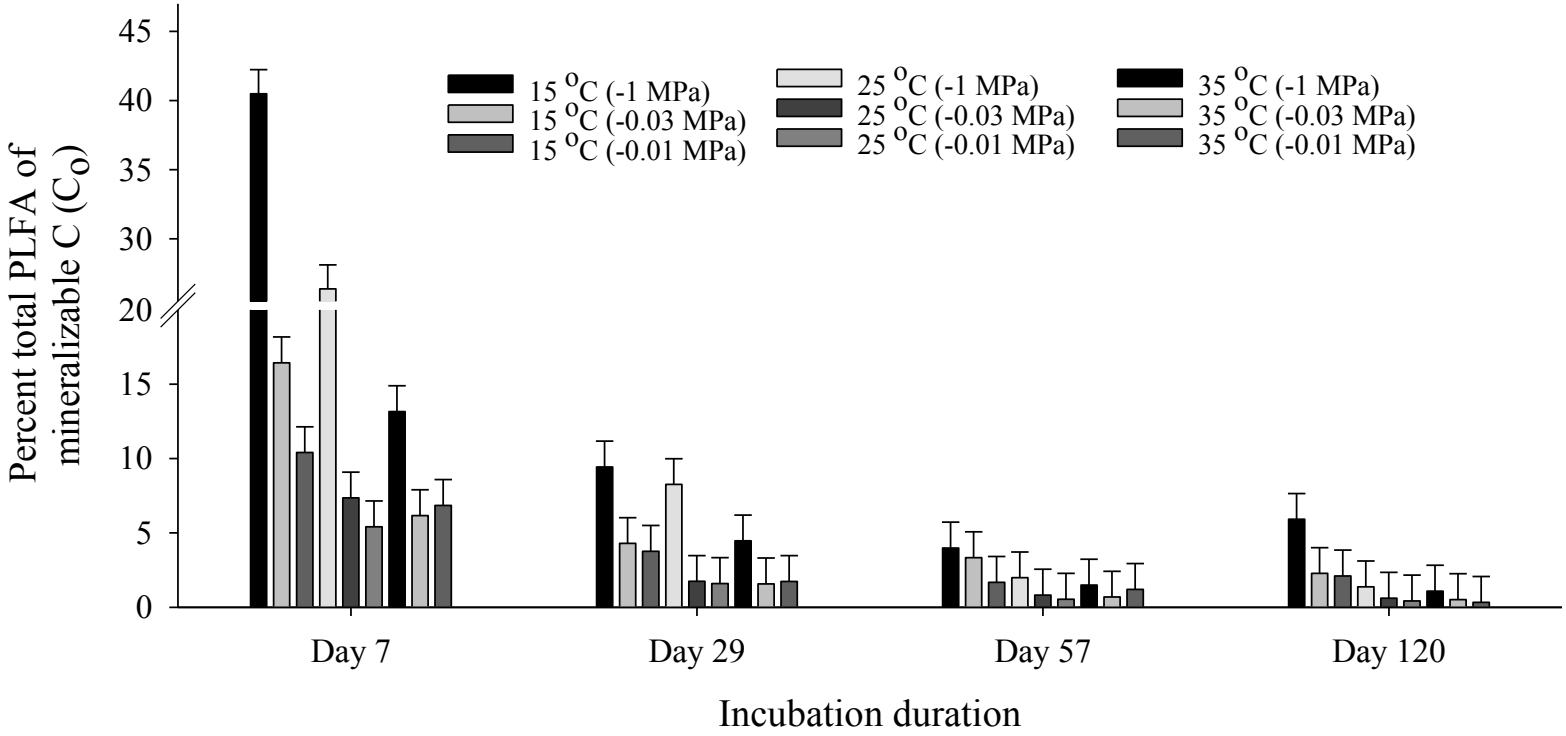

Figure 2.18. Incubation day x temperature x soil water interaction means (P<0.003) of percent total microbial biomass (assessed as phospholipid fatty acids (PLFA)) of mineralizable C (C_o)) for a soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

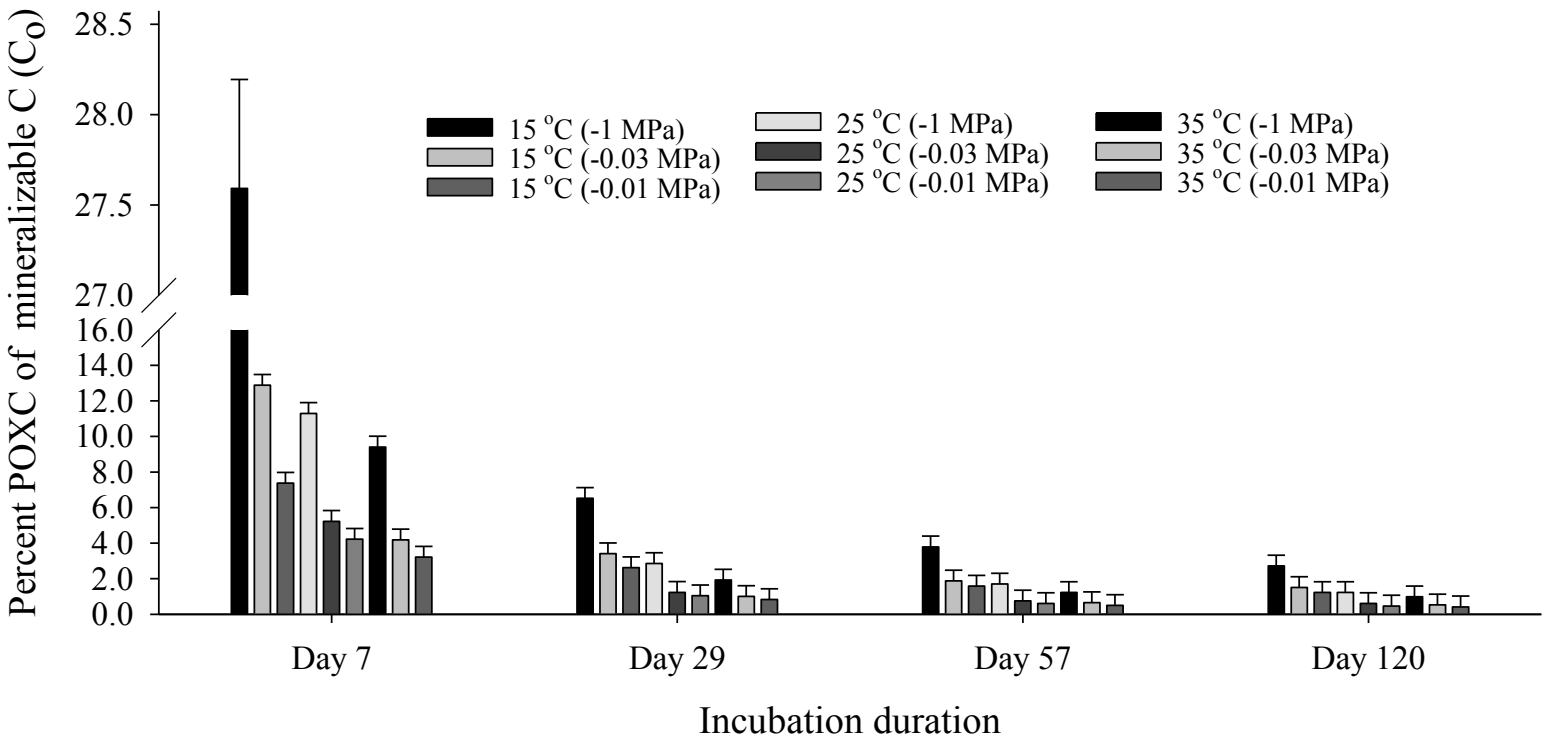

Figure 2.19. Incubation day x temperature x soil water potential interaction means (P<0.0001) of permanganate oxidizable C (POCX) of mineralizable C (C_o)) for a soil incubated at 15, 25 and 35 °C under varying soil water potentials (-1, -0.03, -0.01 MPa). Error bars represent standard error of the mean (n = 4).

Table 2.1. Parameters of mixed order model for C mineralization of a soil incubated at different temperatures (15 ^{o}C, 25 ^{o}C, 35 ^{o}C) with varying soil water potentials (-1, -0.03, -0.01 MPa).

	C_o	K_f	K_s
	μg C g^{-1}	---------- d^{-1} ----------	
15 ^{o}C (-1 MPa)	194 g§	0.0145	0.00054
15 ^{o}C (-0.03 MPa)	365 f	0.0158	0.00047
15 ^{o}C (-0.01 MPa)	447 f	0.0191	0.00046
25 ^{o}C (-1 MPa)	451 f	0.0149	0.00045
25 ^{o}C (-0.03 MPa)	866 d	0.0137	0.00090
25 ^{o}C (-0.01 MPa)	1120 b	0.0142	0.00084
35 ^{o}C (-1 MPa)	560 e	0.0134	0.00088
35 ^{o}C (-0.03 MPa)	1022 c	0.0132	0.00116
35 ^{o}C (-0.01 MPa)	1301 a	0.0138	0.00110
	---------- *P*-values ----------		
Temperature (T)	<.0001	0.0219	<.0001
15 ^{o}C		0.0164 a	0.00049 a
25 ^{o}C		0.0143 b	0.00073 b
35 ^{o}C		0.0135 b	0.00105 c
Water (W)	<.0001	0.2964	0.0335
-1 MPa			0.00062 b
-0.03 MPa			0.00085 a
-0.01 MPa			0.00080 a
T*W	<.0001	0.3357	0.1316

§ Different letters for same column means differences by temperature and soil water content (n=4).

Table 2.2. The respiratory quotient of SOC mineralization (Q_{10}) of soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

Respiratory quotient (Q10)		
	15-25	25-35
	--------Temperature change ^{o}C------	
-1 MPa	2.3 b§	1.3 c
-0.01 MPa	2.35 ab	1.2 c
-0.01 MPa	2.48 a	1.18 c
15-25 ^{o}C	2.43 a	
25-35 ^{o}C	1.24 b	
	----------*P*-values ----------	
Temperature (T)	<.0001	
Water (W)	0.6821	
T x W	0.0383	

§Different letters means significant differences within columns.

Table 2.3. Permanganate oxidizable C of soil incubated at different temperatures (15 ^{o}C, 25 ^{o}C, 35 ^{o}C) with varying soil water potentials (-1, -0.03, -0.01 MPa) as sampled on different days (7, 29, 57, 120).

	Permanganate oxidizable C (POXC)			
	Day 7	Day 29	Day 57	Day 120
	---------- mg POCX kg^{-1} ----------			
15 ^{o}C (-1 MPa)	544 bcde§	506 ghi	571 abcd	536 cdefg
15 ^{o}C (-0.03 MPa)	581 ab	500 efghi	535 cdefg	536 cdefg
15 ^{o}C (-0.01 MPa)	537 cdefg	509 fghi	564 abcd	544 bcde
25 ^{o}C (-1 MPa)	535 cdefg	504 i	570 abcd	544 bcde
25 ^{o}C (-0.03 MPa)	541 cdef	489 hi	541 bcde	536 cdefg
25 ^{o}C (-0.01 MPa)	595 a	517 efghi	569 abcd	564 abcd
35 ^{o}C (-1 MPa)	586 a	480 i	570 abcd	533 defgh
35 ^{o}C (-0.03 MPa)	543 cdef	495 hi	572 abc	541 cdef
35 ^{o}C (-0.01 MPa)	542 efghi	512 efghi	559 abcd	562 abcd
	----------*P* - values ----------			
Day (D)	<.0001			
Temperature (T)	0.7917			
D*T	0.8974			
Water (W)	0.0460			
D*W	0.5481			
T*W	0.1369			
D*T*W	0.0378			

§ Different letters represent significant differences among soil water content, temperature and day.

Table 2.4. Permanganate oxidizable C of soil incubated at different temperatures (15 ^{o}C, 25 ^{o}C, 35 ^{o}C) with varying soil water potentials (-1, -0.03, -0.01 MPa) as sampled on day 7.

	Permanganate oxidizable C (POXC) on day 7		
	-1	-0.03	-0.01
	----------MPa----------		
15 ^{o}C	544 ab§	535 b	586 ab
25 ^{o}C	581 ab	541 ab	543 ab
35 ^{o}C	537 ab	595 a	542 ab
	----------*P*-values----------		
Temperature (T)	0.9809		
Water (W)	0.9818		
T x W	0.0504		

§ Different letters represent significant differences among soil water content and temperature.

Table 2.5. Permanganate oxidizable C of soil incubated at different temperatures (15 ^{o}C, 25 ^{o}C, 35 ^{o}C) with varying soil water potentials (-1, -0.03, -0.01 MPa) as sampled on day 120.

	Permanganate oxidizable C (POXC) on day 120
	----------*P*-values----------
Temperature (T)	0.454
Water (W)	0.0242
T x W	0.7038
	----------mg kg^{-1}soil----------
-1 MPa	537 b
-0.03 MPa	538 b
-0.01 MPa	557 a

§ Different letters represent significant differences among soil water content and temperature.

Table 2.6. Respired $\delta^{13}CO_2$-C of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d as sampled on different days (7, 29, 57, 120).

	Soil respired $\delta^{13}CO_2$-C			
	Day 7	Day 29	Day 57	Day 120
	---------- $\delta^{13}CO_2$-C (‰) ----------			
15 ^{o}C (-1 MPa)	-9.57 a §	-15.95 def	-16.19 fj	-15.58 ce
15 ^{o}C (-0.03 MPa)	-14.95 c	-16.55 hij	-16.24 fjk	-15.82 dg
15 ^{o}C (-0.01 MPa)	-15.98 cdfh	-16.62 hik	-16.21 fj	-15.71 cdg
25 ^{o}C (-1 MPa)	-13.41 b	-20.05 s	-17.17 lop	-16.55 hm
25 ^{o}C (-0.03 MPa)	-17.16 ilmnopq	-18.14 r	-17.25 p	-16.78 i
25 ^{o}C (-0.01 MPa)	-15.63 cdf	-17.80 qr	-17.20 op	-16.79 i
35 ^{o}C (-1 MPa)	-15.18 cd	-17.43 ioq	-16.85 in	-16.45 hm
35 ^{o}C (-0.03 MPa)	-16.17 defhi	-17.38 opq	-17.11 lop	-16.52 hm
35 ^{o}C (-0.01 MPa)	-16.42 fghil	-17.25 nopq	-17.05 lo	-16.56 hm
	---------- *P*- values ----------			
Day (D)	<.0001			
Temperature (T)	<.0001			
D*T	<.0001			
Water (W)	<.0001			
D*W	<.0001			
T*W	<.0001			
D*T*W	<.0001			

§Different letters represent significant differences among soil water content, temperature and day.

Table 2.7. Respired $\delta^{13}CO_2$-C of a soil previously planted to C_3 crop and later to C_4 crop (corn) incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d as sampled on different days (7, 29, 57, 120).

	Soil respired $\delta^{13}CO_2$-C			
	Day 7	Day 29	Day 57	Day 120
	---------- $\delta^{13}CO_2$-C (‰) ----------			
15 ^{o}C	-13.5 b§	-16.37 b	-16.21 c	-15.70 a
25 ^{o}C	-15.4 a	-18.66 c	-17.21 b	-16.71 c
35 ^{o}C	-15.92 a	-17.35 a	-17 a	-16.51 b
-1 MPa	-12.72 b	-17.81 b	-16.74 a	-16.19 b
-0.03 MPa	-16.09 a	-17.36 a	-16.87 b	-16.37 a
-0.01 MPa	-16.01 a	-17.22 a	-16.82 ab	-16.35 a
	----------*P*-values ----------			
Temperature (T)	<.0001	<.0001	<.0001	<.0001
Water (W)	<.0001	0.003	0.0579	0.0105
T x W	<.0001	<.0001	0.4112	0.635

§Different letters represent significant differences among temperature and soil water for each day.

Table 2.8. Specific phospholipid fatty acids (PLFA) of a soil incubated for 120 d at different temperatures (15, 25, 35 ^{o}C) with varying soil water soil water potential (-1, - 0.03, -0.01 MPa) and sampled on several incubation days (4, 29, 53 and 120).

	Actino	Gram -	Gram +	AMF	Bacteria	Fungi	Fungi:baceria
	---------- PLFA (nmol g^{-1} soil) ----------						
-1 MPa	0.5 a§	1.16 a	6.66 a	1.07 a	7.97 a	2.17 a	0.28 a
-0.03 MPa	0.43 b	1.01 b	6.22 b	0.88 b	7.80 a	1.76 b	0.25 b
-0.01 MPa	0.38 b	0.80 c	5.27 c	0.37 c	6.09 b	1.53 c	0.25 b
	---------- *P* - values ----------						
Day (D)	0.4763	0.1719	0.8479	0.8722	0.5141	0.4115	0.7262
Temperature (T)	0.8462	0.5698	0.6362	0.5638	0.7047	0.8811	0.7168
D*T	0.1999	0.8778	0.6126	0.8652	0.3613	0.4496	0.7835
Water (W)	0.0002	<.0001	<.0001	<.0001	0.0193	<.0001	0.0232
D*W	0.9362	0.3879	0.3915	0.5209	0.0776	0.4083	0.1267
T*W	0.9849	0.247	0.5037	0.968	0.5577	0.9648	0.806
D*T*W	0.5895	0.1815	0.6658	0.9887	0.7938	0.4569	0.6401

§ Different letters in same column means differences by soil water content.

Table 2.9. Relationships between SOC, C_o, PLFA and POXC of a soil incubated for 120 d at different temperatures (15 ^oC, 25 ^oC, 35 ^oC) under varying soil moisture potentials (-1 MPa, - 0.03 MPa, -0.01 MPa) and sampled on several incubation days (4, 29, 53 and 120).

	$PLFA_{Total}$ /SOC	$PLFA_{Total}$ /C_o	POXC/C_o	C_o/SOC
	---------- % ----------			
15 ^oC (-1 MPa)	0.05 ab	14.96 a	10.16 a	0.72 h
15 ^oC (-0.03 MPa)	0.05 ab	6.58 c	4.92 b	1.37 g
15 ^oC (-0.01 MPa)	0.05 ab	4.49 cde	3.20 c	1.72 f
25 ^oC (-1 MPa)	0.07 a	9.5 b	4.27 b	1.63 f
25 ^oC (-0.03 MPa)	0.04 b	2.63 def	1.95 d	3.41 d
25 ^oC (-0.01 MPa)	0.04 b	1.99 f	1.58 d	4.38 b
35 ^oC (-1 MPa)	0.05 ab	5.05 cd	3.38 c	2.19 e
35 ^oC (-0.03 MPa)	0.04 b	2.24 ef	1.59 d	4.14 c
35 ^oC (-0.01 MPa)	0.07 a	2.53 ef	1.24 d	5.25 a
	----------P-values ----------			
Day (D)	0.0755	<.0001	<.0001	<.0001
Temperature (T)	0.7147	<.0001	<.0001	<.0001
D*T	0.0298	<.0001	<.0001	<.0001
Water (W)	0.1277	<.0001	<.0001	<.0001
D*W	0.6242	<.0001	<.0001	<.0001
T*W	0.0565	0.0003	<.0001	<.0001
D*T*W	0.7482	0.0036	<.0001	0.7510

§ Different letters within the same column represent significant differences by soil temperature and water for the relationship.

CHAPTER 3 - BURN MANAGEMENT AND NUTRIENT AMENDMENT EFFECTS ON SOIL C AND BIOGEOCHEMICAL PROCESSES OF TALLGRASS PRAIRIE.

ABSTRACT

Grasslands, one of the world's most productive ecosystems are being transformed by changes in historic burn regimes and by nutrient management practices. Such practices are changing aboveground vegetation and belowground processes which could affect ecosystem functions such as soil C storage with unpredictable consequences on climate change. The objective of our study was to investigate the effect of management (burning and nutrient amendment) on soil biogeochemical characteristics of a tallgrass prairie and effect on soil C. A long term experiment located at the Konza Prairie Biological Station was sampled. The main plots were burned (B) and unburned (UB) tallgrass prairie and sub plots were N, P or N+P including controls (no nutrient amendment). Aboveground vegetation and biomass were assessed. Soil samples were collected three times; post growing season (October 2009), pre-burned or pre-growing season (March 2010), and post-burned or mid-growing season (July 2010). Soil samples were collected at incremental 0-5, 5-15 and 15-30 cm soil depths and analyzed for total N (TN), soil organic C (SOC), potential enzyme activities, phospholipid fatty acids (PLFA), permanganate oxidizable C (POXC) and Mehlich available P. Burning and nutrient amendments affected vegetation distribution and aboveground biomass. The total aboveground biomass was similar between burned and unburned controls; however, grass biomass was higher in burned than unburned. There was a conspicuous absence of woody

plant in burned. Nutrient N and N+P amendment increased biomass in burned with no effect in unburned. The SOC in 2010 was similar to amounts in 1989, for both burned and unburned. Treatment effects on both TN and SOC were depth-specific with no impact at the cumulative 0-30 cm depth. There was no burn management effect on either SOC or TN at the 0-5 cm soil depth. However, nutrient N and N+P increased TN while P reduced TN at 0-5 cm. At 5-15 cm, SOC and TN were higher in unburned than burned. At the 15-30cm depth, P reduced SOC in unburned. The enzyme activities responded to seasonal changes. The C-acquiring β-glu was highest at the end of the growing season when substrate was most available, while the nutrient acquiring β-glsm and acidP and alkalineP phosphatases were highest in July when competition for nutrients between plants and microbes was highest. There was no seasonal effect for POXC at 0-5 cm depth; but at 5-15 cm, the POCX was significantly higher in July than in March and October. Available P was increased by P and N+P amendments at 0-5 cm depth. The available P was higher in burned than unburned at 0-5 cm depth but there was no significant nutrient amendment effect on available P at 5-15 cm depth. Microbial biomass was increased by P addition at 0-5 cm depth and was higher in unburned than burned, but at 5-15 cm depth, it was increased by N and higher in burned than unburned. Both burned and unburned managements were dominated by Gram+ bacteria; however, AMF and fungi were more abundant in burned than unburned. Therefore, C stocks in both burned and unburned may have similar trajectories in this mesic tallgrass prairie however; the biogeochemical processes that drive belowground C and N dynamics of the burned and unburned prairie are apparently different.

INTRODUCTION

Worldwide, grasslands and savannas account for 30-35 % of terrestrial net primary production (Knapp et al., 2008). Additionally, grassland ecosystems cover 6.1-7.4% of global land area which stores 7.3-11.4% of soil OC (Zeglin et al., 2007). In the U.S., the tallgrass prairie grassland is a declining but major ecosystem which, like other grasslands, has been managed using fire. The use of fire maintains grasslands and prevents encroachment of woody species (Axelrod, 1985; Hibbard et al., 2001). Fire in grassland ecosystems remove surface litter (Knapp and Seastedt, 1986; Briggs et al., 2002), which provides light and energy required for early season growth of C_4 grasses. In the mesic tallgrass prairie, adequate soil water and fire has been shown to increase productivity of the perennial C_4 grasses (Knapp and Seastedt, 1986), while fire suppression has increased the proliferation of C_3 grasses, forbs and woody plants.

Additionally, experimental N amendment alongside burning increases productivity of tallgrass prairie which is greater than the increase due to N addition alone (Seastedt et al., 1991; Wilson et al., 2009). Annual burning increases root productivity and biomass (Johnson and Matchett, 2001) and has the potential to increase root and SOC C:N (Ojima et al., 1994; Fynn et al., 2003). More than two thirds of burned tallgrass prairie plant biomass exists belowground as roots and rhizome (Risser and Parton, 1982; Kitchen et al., 2009). Therefore, long term burning of this ecosystem could currently be important in light of climate change mitigation because of its capacity to absorb C from the atmosphere and channel it into the soil through the deep rooting systems of the dominant grass *Andropogon gerardii*. Root C has been recognized as a better means of stabilizing SOC than shoot C (Rasse et al., 2005). At the 0-5 cm depth however, higher %

C in unburned than burned tallgrass prairie has also been noted (Kitchen et al.; 2009). This may also suppose that long term burning of tallgrass prairie may reduce SOC as predicted by (Ojima et al., 1994) If this were the case, then burning will not be a recommended practice in terms of global climate change mitigation.

The tallgrass prairie plant productivity and vegetation composition are being altered by fire management and N amendment (Collins et al., 1998; Gibson et al., 1993; Hartnett et al., 1996; Towne and Kemp 2003; Kitchen et al., 2009), without any effect of P amendment (S.L. Collins and M. D. Smith, unpublished data) in (Zeglin et al., 2007). Literature on the effect of combined N and P amendment on tallgrass prairie productivity is sparse, although (Craine et al., 2008), made allusion to the possibility of nutrient co-limitation of productivity of grasslands, which can be difficult to detect. In addition, the degree of N-limitation on tallgrass prairie productivity has been reported to depend on the fire frequency (Blair, 1997).

Vegetation shifts related to changing climate, atmospheric CO_2 concentration, N enrichment, and alteration in management practices is altering grasslands (Briggs et al., 2005; Montane et al., 2010). Changes in plant communities can influence associated soil microbial communities through the types and amounts of C and nutrient inputs (Waldrop and Firestone, 2004), hence control SOC. The effect of aboveground changes on belowground processes that influence SOC dynamics is not well known. Several studies have addressed fire effects on soil N pools of tallgrass prairie (Rice et al.,1994; Seastedt et al., 1991; Blair, 1997; Turner et al., 1997). Other studies have reported higher plant available N in unburned relative to burned tallgrass prairie, attributed to decrease in net N mineralization rates in the absence of fire (Blair, 1997; Ojima et al., 1994; Johnson and

Matchett, 2001). Less productivity of unburned tallgrass prairie has also been attributed to relatively low N of senescent and dead grasses (Seastedt, 1985). Nitrogen is the most limiting nutrient of the prairie; therefore, low N in senescent unburned grasses may further exacerbate low productivity of unburned prairie. In addition, higher contributions of root biomass to soil C (Van Groenigen et al., 2006) in burned than unburned tallgrass prairie (Kitchen et al., 2009; Wilson et al., 2009), implies that SOC could be greater in burned than unburned tallgrass prairie. The differences in N dynamics in the burned and unburned could also be attributed to the shift in vegetation resulting from burning and N amendment.

The last few decades have heralded concerns about consequences of vegetation changes and the effects on global ecosystems (Vitousek, 1994; McCarron and Knapp, 2003). Any changes in plant community composition will likely alter the biological and chemical properties of soils which can significantly affect SOC and nutrient dynamics. Alteration of microbial mineralization and nutrient cycling controls long term ecosystems responses to environmental changes. Annual burning of tallgrass prairie has been reported to cause low SOC in spite of higher plant productivity relative to unburned (Parton et al., 1987).

Many studies have mostly addressed the potential effects of burning and nutrient enrichment of tallgrass prairie in terms of aboveground productivity (Lett and Knapp, 2003) and change in plant diversity (Collins et al., 1998; Knapp et al., 2002; Heisler et al., 2003). Few studies have assessed belowground microbial community processes, and the effect on SOC. The microbial community and its biochemical activities are less well understood, especially in the long term. Less is known about the below-ground impacts

of burning and nutrient addition on biogeochemical processes that drive the aboveground productivity and influence ecosystem function. The N amendment has been reported to reduce arbuscular mycorrhizal fungi (AMF) and the C:N of microbial communities (Garcia and Rice, 1994; Ajwa et al., 1999; Johnson et al., 2003; Egerton-Warburton et al., 2007). Burning has been reported to increase acid phosphatase but decreased β-glucosidase and alkaline phosphatase activities (Ajwa et al., 1999). Ecosystem function can be significantly altered from changes in plants with differing growth forms and their associated microbial communities.

Soil OC dynamics are controlled by soil microbes through extracellular enzyme activities (EEA) and dependent on substrate availability and nutrient requirements (Allison, 2005a; Fennessy et al., 2008; Manning et al., 2008) Enzyme production is N and energy intensive; hence microbial investment in enzyme production is a reflection of nutrient scarcity (Koch, 1997) . Available substrate concentrations have been negatively correlated with the corresponding enzyme activity (Pelletier and Sygusch, 1990; Sinsabaugh and Moorhead, 1994). On the other hand, nutrient scarcity stimulates microbial enzyme activities to release required nutrient from substrate (Harder and Dijkhuizen, 1983). Therefore, potential activity of extracellular enzymes is projected to reflect the microbial investment in nutrient acquisition (Sinsabaugh et al., 2002). As such, N availability should increase the enzymes involved in P and C acquisition relative to enzymes involved in N acquisition (Allison et al., 2007b). This model can be used to assess the effect of long term burn and nutrient addition on ecosystem changes that influence nutrient cycling.

The objectives of this study were:

(1) Investigate the effect of long term annual burn management and nutrient addition on SOC.

(2) Investigate the effect of long term annual burn management and nutrient addition effect on microbial community structure.

(3) Assess changes in enzyme activities involved in soil C, N and P cycling due to long term burn management and nutrient N and P addition of tallgrass prairie.

Materials and Methods

Site description and experimental treatments

The experiment was conducted at the Konza Prairie Biological Station, south of Manhattan (39.10 N, 96.61 W), Kansas, USA. The Konza Prairie Biological Station is a remnant of unplowed tallgrass prairie with a mixed vegetation dominated by C_4 grasses predominantly big bluestem (*Andropogon gerardii* Vitman), little bluestem (*A. scoparius* Michx.), and indiangrass (*Sorgastrum nutans* (L.) Nash). The field experiment was initiated in 1986 on an area whose soil is mapped as Irwin sitly clay loam (Fine, mixed, mesic, Pachic Argiustolls). In this area, rainfall is generally greatest in the spring, declining through the summer months. Temperature is generally lowest in January and peaks in July.

The experimental design is a split-plot organized as a randomized complete block design, where main plots are the burn treatment (annually burned and unburned), subplots are nutrient addition (N, P, N+P and control). This gave eight treatments: burned-N (B-N), burned-P (B-P), burned-N+P (B-N+P), burned-control (B-C), unburned-N (UB-N), unburned-P (UB-P), unburned-N+P (UB-N+P), unburned-control (UB-C). Each treatment had four field replications. Burning takes place in the late spring - last week of

April. Although maximum fire temperature during burning has been estimated at 240^{o}C (Hobbs et al., 1991), the temperature at the soil surface (< 5 cm) rarely exceeds 50 ^{o}C because the burning occurs fast (Ajwa et al. 1999). After burning, nutrients are usually applied first week of June. Nitrogen was added at a rate of 10 g N m^{-2} as $NH_4NO_3^-$ and P was added at a rate of 1 g P m^{-2} as phosphate. It should be noted that the unburned plots in this experiment have experienced invasion by shrubby plant species.

Soil sampling

Soil samples were taken from each plot at 0-5, 5-15, and 15-30 cm depths. Ten soil cores were randomly collected using a 2-cm diameter soil auger to a depth of 30 cm. Each core was cut into incremental segments from the surface of 0-5, 5-15 and 15-30 cm. Each depth was composited in separate sterile polypropylene bags. Three other soil cores were similarly collected for bulk density analysis. Samples were collected three times to represent different seasons; October 2009 - post growing season, March 2010 – pre-growing (pre-burned) season and July 2010 – mid growing season. Upon arrival in the lab, after the July sampling, composited soils were homogenized, a sub sample (10 g) collected, then frozen (-20^{o}C), later freeze-dried (lyophilized) and ground prior to microbial community assessments using phospholipid fatty acid analysis (PLFA).

Soil organic C and total N

Soil subsamples (10 g) were air-dried, ground to a fine powder using a mortar and pestle. The samples were analyzed for soil organic C (SOC) and total N (TN) by dry combustion using a CN Elemental Analyzer, gas chromatograph with thermal conductivity detector (GC-TCD) (Thermo Finnegan Flash EA1112, Milan, Italy.

Soil microbial community structure (phospholipid fatty acid analysis)

Changes in soil microbial community were assessed by measuring phospholipid (PLFA) of soil samples. The method of (Bligh and Dyer, 1959) as modified by (White and Ringlberg, 1998) was used to extract soil total lipids. The lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution for 3 h from 5 g of ground lyophilized soil. The total lipids extracts were then separated into neutral lipids (NLFA), polar lipids (PLFA) and glycolipids by silicic acid chromatography using pre-conditioned disposable silica gel columns (J.T. Baker, Phillipsburg, NJ, USA). The neutral and polar lipids were then cleaved by alkaline methanolyis (KOH saponification and methylation) which cleaves the fatly acids from the glycerol back bone replacing it with methyl groups to form fatty acid methyl esters (FAMES) (Allison, 2005b). The resulting FAMES were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer with a DB5-MS column (30 m × 250 μm i.d. × 0.25 μm film thickness). Helium was the carrier gas (1.0 mL min^{-1} constant flow). The temperature program was: 50 to 170°C at 20°C per min^{-1}; from 170 to 270°C at 2°C min^{-1}. The injector temperature was 220°C. Analysis was conducted in the electron impact (70 eV) mode and mass spectrometer scanning m/z^{+} was from 200 to 400. Bacterial acid methyl esters mix (BAME; Matreya 1114) was used to identify peaks. Tentative assignments of methyl ester peaks not present in the BAME mix were made by mass spectral interpretation. The internal standard methyl nonadecanoate was used to quantify the data. Sample peaks were quantified based on comparison of the abundance with an internal standard - nonadecanoic acid methyl ester (19:0). The abundance was expressed as nmol g^{-1} dry soil but converted to mole % before statistical analysis.Nomenclature of identified fatty acids (FA) was as described by Bossio and

Scow (1998), whereby FA were designated ***a:b***, where ***a*** is the total number of carbons and ***b*** the number of double bonds. An ***ω*** indicates the position of a double bond from the aliphatic end of the FA. The prefixes ***a*** and ***i*** refer to ***anteiso*** and ***iso*** branching, while the suffixes ***c*** and ***t*** refers to ***cis*** and ***trans*** isomers (conformations). Presence of methyl groups are indicated by ***aMe***, where ***a*** indicates the position of the methyl group. Fatty acidPs were grouped based on criteria by McKinley et al. (2005) whereby Gram positive bacteria were i15:0, a15:0, 10Me16:0, i17:0 and a17:0. Gram negative bacteria as 18:1ω7c and cy19:0, while actinomycetes is 10Me18:0 and 10Me17:0, and fungi 18:2 ω 6,9c and 18:1 ω 9c.

Soil enzyme activities

β-Glucosaminidase activity

The activity of N-acetyl-β-glucosaminidase (NAGase, EC 3.2.1.30) also known as β-glsm was assayed using the method described by Parham (Parham and Deng, 2000). In summary, 1 g ground soil was place in a 50-mL test tube to which was added 4 mL of 0.1 *M* sodium acetate buffer (pH 5.5). A 1 mL (10 mM) *p*-nitrophenol-*N*-acetyl-β-D-glucosaminide solution was added to the soil solution and incubated at 37^{o}C for 1 h. After incubation, 1 mL (0.5 *M* $CaCl_2$) and 4 mL (0.5 *M* NaOH) were added to the test tube. Samples were swirled to mix and filtered using Whatman no 2v filter paper. Intensity of the yellow colored filtrate was determined by measuring the absorbance using a spectrophotometer (GENESYS 10 spectrophotometer, Model 335902P-000, Thermo Electron Corp. Madison WI) against modified universal buffer blank at 405 nm. Each experiment had a control consisting of substrate without enzyme, which was subjected to

the same experimental conditions. A p-nitrophenol (PNP) standard was prepared containing 0, 10, 20, 30, 40 and 50 µg PNP.

β-Glucosidase activity (EC 3.2.1.21)

The activity of β-glu was assayed using the method described by (Eivazi and Tabatabai, 1988) except without the use of toluene. In summary, 1 g ground soil was place in a 50-mL test tube to which was added 4 mL of modified universal buffer (pH 6.0). A 1 mL (0.05 *M*) *p*-nitrophenyl-β-D-glucoside solution was added to the soil solution and incubated at 37^{o}C for 1 h. After incubation, 1 mL (0.5 *M* $CaCl_2$) and 4 mL (0.1*M*) THAM buffer (pH 12) were added to the test tube. Samples were swirled to mix and filtered using Watman no 2v filter paper. Intensity of the yellow colored filtrate was determined by measuring the absorbance using a spectrophotometer (GENESYS 10 spectrophotometer, Model 335902P-000, Thermo Electron Corp. Madison WI) against modified universal buffer blank at 400 nm after 30 min. Each experiment had a control consisting of substrate without enzyme, which; was subjected to the same experimental conditions. A PNP standard was prepared containing 0, 10, 20, 30, 40 and 50 µg PNP.

Acid and alkaline phosphatase activities

Phosphatase activity of soils was assayed using the method described by (Tabatabai, 1994). In summary, 0.05 *M* solution of *p*-nitrophenyl phosphate tetrahydrate was prepared in modified universal buffer at pH of either 6.5 for acid phosphatase (acidP) or 11 for alkaline phosphatase (alkalineP) activities. A 1 g ground soil was place in a 50-mL test tube to which was added MUB of appropriate pH. A 1 mL substrate solution was added to the flask and swirl to mix. Test tubes containing the mixtures were capped and incubated in a water bath at 37^{o}C for 1 h. After incubation, 1 mL of 0.5 *M* $CaCl_2$ and

4mL of 0.5 *M* NaOH were added to the test tube, filtered using Whatman #2 filter paper. Intensity of the yellow colored filtrate was determined by measuring the absorbance using a spectrophotometer (GENESYS 10 spectrophotometer, Model 335902P-000, Thermo Electron Corp. Madison WI) against modified universal buffer blank at 400 nm after 30 min. Each experiment had a control consisting of substrate without enzyme, which; was subjected to the same experimental conditions. A PNP standard was prepared containing 0, 10, 20, 30, 40 and 50 μg PNP.

Mehlich extractable phosphorus

The Mehlich extractable P was analyzed by the Soil Testing Lab in the Department of Agronomy at Kansas State University. Briefly, in the Mehlich III Phosphorus method, soil is extracted with a solution containing glacial acetic acid, ammonium nitrate, ammonium fluoride and nitric acid and colorimetric assay is performed using a Lachat Quickchem 8000. The extraction and colorimetric procedures are described in the handbook "Recommended Chemical Soil Test Procedures for the North Central Region".

Permanganate oxidizable C

Permanganate oxidizable C was assessed using the method of (Weil et al., 2003). In summary, 2.5 g soil samples were placed into 50 mL plastic conical test tubes with screw caps. To the soil was added 20 mL of 0.02*M* $KMnO_4$ solution (prepared in 0.1*M* $CaCl_2$). Soil suspension was shaken on a horizontally shaker at 120 strokes/ minute for exactly 2 min, and then allowed to settle in an upright position for 10 min. A 1 mL aliquot of the supernatant was diluted to 50 mL and absorbance measured at 550 nm using a GENESYS 20 spectrophotometer (Thermo Fisher Scientific Inc. Walthan, MA,

USA). Standard solutions containing 0.0 (blank), 0.005 *M*, 0.01*M* and 0.02*M* $KMnO_4$ were prepared. The soil C oxidized after reaction with $KMnO_4$ solution was determined by the change in concentration of $KMnO_4$ solution. The assumption is that 1 mol MnO_4 is consumed (reduced from Mn^{+7} to Mn^{+4}) in the oxidation of 0.75 mol (9000 mg) of C. Permangate oxidizable C (POXC) was expressed as mg C oxidized g^{-1}soil.

Statistical analysis

Analysis of variance (ANOVA) was performed with the Proc Mixed Procedure using SAS software (SAS 9.1, Cary, NC, USA). Differences were considered significant at $P< 0.05$ except indicated otherwise. When depth was a significant effect, data were analyzed by sampling depth. Principal component analysis (PCA) was conducted using SAS Proc PCA. During the PCA, values for TN and TOC were standardized prior to analysis due to differences in TN and TOC related to differences in both depth and bulk density.

RESULTS

Aboveground annual primary productivity (ANPP)

The ANPP data for 2009 and 2010 were extracted from the Konza LTER data base, established since 1986. Vegetation was separated into grasses, forbs and woody. A significant ($P<0.0001$) burn effect on grasses indicated greater amounts of grass biomass in burned than in unburned and a conspicuous absence of woody biomass in burned (Fig. 3.1). However, the average total biomass was similar in both burned and unburned controls (Fig. 3.2). Results also indicated a significant nutrient effect on grass ($P<0.0001$) and forbs ($P<0.0001$) but not for woody ($P>0.27$). The N+P increased forbs in both

burned and unburned. However, a significant ($P<0.0001$) burn x nutrient interaction effect indicated that N and N+P increased grass biomass in burned but not in unburned prairie (Fig. 3.3). Therefore, both burning and nutrient amendment caused shifts in vegetation type, without differences in total biomass between burned and unburned controls.

Soil organic C, total N and permanganate oxidizable C

The effects of burn management and nutrient amendment on assessed variables were depth- specific. Predictions that long term burn management and nutrient N amendment would reduce soil organic C (SOC) in burned tallgrass prairie were not evident from comparisons of SOC for 1989 and 2010 (Fig. B2-1). Soil OC was not significantly ($P>0.05$) influenced by any factor at the 0-5 cm depth (Table 3.1).

At 5-15 cm depth SOC was significantly ($P<0.02$) higher in unburned (27.2 ± 0.57 Mg C ha^{-1}) than burned (25.6 ± 0.57 Mg C ha^{-1}). A significant ($P<0.03$) burned x nutrient interaction effect for SOC at 15-30 cm revealed that, P reduced SOC by about 12% in unburned relative to control, but had no significant effect in burned (Fig. 3.4).

At 5-15 cm (Table 3.1) TN in unburned (3.36 ± 0.04 Mg N ha^{-1}) was significantly ($P<0.0003$) higher than in burned (3.14 ± 0.04 Mg N ha^{-1}).

At 15-30 cm depth, a significant ($P<0.03$) P addition reduced TN by 11 % relative to control (Table 3.2).

The soil C:N ratio was influenced by a significant interactions of burned x nutrient addition at the 0-5($P<0.0001$) and 5-15 ($P<0.014$) cm depths (Table 3.1). At both depths, the C:N ratio was higher in burned than unburned (Fig. 3.5). A ($P<0.04$) seasonal effect on the C:N ratio at the 5-15 cm depth was least in July (Table 3.3).

Permanganate oxidizable C indicated significant (P<0.0001) seasonal effect at 5-15 but no effect at 0-5 cm depth (Table 3.1). At the 5-15 cm, POXC was highest in July and least in March (Table 3.3). Therefore, labile substrate was similar for burn and unburned.

Enzyme activities

The extracellular enzymes (EEs) assayed were β-glucosaminidase (β-glsm), β-glucosidase (β-glu), acid phosphatase (acidP) and alkaline phosphatase (alkalineP), which are related to soil C, N and P cycling. Our data indicated significant (P<0.0003) seasonal and depth responses for all EEs assayed (Table A 3-2).

At 0-5 cm depth, β -glsm activity was highest in July (Fig 3.7). At 5-15 cm depth, β -glsm activity was similar for July and October, and least in March (Fig. 3.7). Nitrogen amendment significantly (P<0.03) reduced β-glsm activity at 5-15 cm (Fig. 3.8).

The β-glu activity at 0-5cm depth was highest in October which was twice the activity in both March and July (Fig. 3.9). A significant (*P*<0.05) burned x nutrient x season interaction for β-glu activity at 5-15 cm depth (Table 3.4), reveal that β-glu activity was highest in March for both burned and unburned, increased in burned by N+P but reduced by N+P in unburned relative to their controls. In July β-glu was reduced by N in burned, but increased by N in unburned, with N+P reducing activity in burned but similar to control in October (Fig. 3.10).

At 5-15 cm a seasonal effect for acidP (Table 3.4) indicated highest activity in July, showing a 30 % reduction by October, which was 50 % higher than March (Fig. 3.11.).

At 0-5 cm, alkalineP activity was similar in July and October which were both higher than March. At the 5-15 cm depth, alkaline P activity was highest for July, but similar for both March and October (Fig. 3.12). A significant (P<0.001) nutrient amendment for alkalineP activity at 0-5 cm depth indicated that N reduced enzyme while P increased enzyme activity relative to the control (Fig. 3.13).

Enzyme activity ratios can also be used to assess potential microbial investments in C, N and P acquisition from soil organic matter (SOM) during nutrient or substrate limitation. The potential β-glsm activity ratio indicated that N acquisition was limiting in October at 0-5cm (Table A3-4.), while P amendment limited N acquisition at 5-15cm depth (Table. A3-5.).

At the 0-5 cm depth, β-glu activity ratios indicated that N limited C in burned while unburned had sufficient C substrate (Table A3-4). At 0-5 cm, burned was C-limited in October, but unburned was C-limited in March (Table A3-4). A burned x nutrient x season interaction for β-glu activity ratios indicated sufficient C except with P amendment in October. In unburned prairie, there was a nutrient effect on C sufficiency or limitation depending on the season (Table A3-5). Potential acidP phosphatases activity ratio at 5-15cm depth indicated that P acquisition was limiting in unburned and also showed limitation in July (Table. A3-5). At 5-15 cm depth, alkalineP activity ratio indicated that P was limiting for unburned in July (Table A3-5.). At 5-15 cm P amendment limited P acquisition in both July and October (Table A3-5). The enzyme activity results indicated that substrate availability and energy acquisition influenced enzyme activities depending on the season and depth.

Mehlich (available soil P)

Available P was assessed in March. The available P was significantly (P<0.0001) higher at 0-5 than 5-15 cm depth. At the 0-5 cm, P amendment increased available P by about 250% in burned and 100% in unburned (Fig. 3.15). Long-term annual burning of tallgrass prairie had about 33% more available P than unburned. Therefore, nutrient addition had a variable effect on P availability depending on depth, burned management and the type of nutrient added. Results indicated that P is immobile and P availability was reduced with N application which was greater when applied alone than in combination with N and significantly greater in burn than unburn.

Soil microbial community

Soils collected during the growing season in July were assessed for microbial community structure by measuring the phospholipid fatty acids (PLFA). The data was analyzed by depths (0-5 and 5-15 cm) because initial statistical analysis confirmed the expectation of significant depth effect on both PLFA (Table A-3.3) for most of the microbial groups.

At the 0-5 cm depth, nutrient amendment significantly influenced AMF (P<0.0001), Gram- bacteria (P<0.006) and fungi (P<0.0001) (Table 3.5). The results indicated that N and N+P reduced AMF, Gram- bacterial and fungal biomasses (Fig. 3.16).

At the 5-15 cm, fungal biomass was significantly (P<0.015) influenced by nutrient amendment (Table 3.5). At the 5-15 cm, P increased fungal biomass (Fig. 3.17). The total bacterial biomass was significantly (P<0.01) affected by a burned x nutrient interaction effect (Table 3.5). The result indicated that while N significantly increased

bacterial biomass in the burned, there was no influence of nutrient amendment in the unburned (Fig. 3.18). The fungal:bacterial (F:B) ratio was influenced by nutrient addition at 5-15 cm depth (Table 3.5). The trend showed P (0.34) > Control (0.3) > N+P (0.23) > N (0.16). The trend in F:B indicated that, P either increased fungal biomass and reduced bacterial biomass; while Neither reduced fungal biomass or increased bacterial biomass.

Total microbial biomass (MB) was higher in unburned than burned at 0-5 cm, but higher in burned than unburned at 5-15 cm depth (Table.3.7). When the effect of burning on individual PLFA biomarkers at 0-5 and 5-15 cm were compared, PLFA biomarkers that showed significant differences were generally greater at 0-5 cm depth for unburned than burned, and greater for burned than unburned at 5-15 cm depth (Table 3.6). When effect of nutrient amendment on individual PLFAs was assessed at 0-5 cm, when nutrient amendment was significantly different, P generally increased PLPAs relative to control (Table 3.7). However, the combined application of N+P reduced PLFAs when there were significant differences at 0-5 cm. At the 5-15 cm, only the fungal biomarker 18:1ω9c was influenced by nutrient addition (Table 3.8).

DISCUSSION

A major finding in our study was that SOC was not influenced by burning at the cumulative 0-30 cm depth. However, depth-specific differences were noted for SOC and TN between burn and unburned prairie. Long term burn management, contrary to model predictions (Ojima et al., 1994) did not reduce SOC stocks after 20yr. This result was surprising because vegetation had shifted due to burn management with possibly varying contributions to SOC. The vegetation shift due to burning and varying plant responses to nutrient addition may reveal differences in nutrient requirements and acquisition between

these ecosystems. However, we also reported similar total biomass for the burned and unburned controls. This result was similar to other reports from this mesic tallgrass prairie (Wilson et al., 2009).

The vegetation shift may explain the depth-related responses of SOC, TN and biochemical process in these ecosystems. Nitrogen and N+P amendment increased grass biomass but P had no effect in the burned treatment. Similar to our results, ANPP has been reported to increase with N but not P amendment in burned tallgrass prairie (S.L. Collins and M. D. Smith, unpublished data) reported by Zeglin et al. (2007). There was no effect of nutrient amendment on the grasses in unburned plots, similar to reports by Wilson et al. (2009). The reduction in aboveground biomass due to P amendment in unburned management could indicate that P may be a limiting nutrient for the woody species or that P may be exacerbating the N deficiency which could have resulted in the lower SOC at the 15-30 cm with P amendment.

The aboveground biomass in the annually burned is removed by fire; hence root biomass is the major residue source in burned while aboveground biomass is important in unburned. The active C pool assessed as permanganate oxidizable C (POXC) is contributed from aboveground litter and through-fall (Schwendenmann and Veldkamp, 2005), but another important source is belowground root and mycorrhizal turnover including rhizodepositon (Jones et al., 2009; Nguyen, 2003). However, POXC was similar between burned and unburned, indicating similar amounts of labile C, but from different sources between burned and unburned prairie.

The SOC and TN results did not meet our expectation of higher SOC with long term burning. The results at 0-5 cm are consistent with reports by Ajwa et al. (1999). Our

results for the 5-15 cm were similar to reports by Kitchen et al. (2009) who indicated significantly higher SOC in unburned than burned at 0-10 cm depth, and Reed et al., (2005) who reported no significant effect on TOC of burn regimes at 0-15 cm, but a species-specific effect of burn management on TOC. We did not observe nutrient effects on TOC at 0-5 cm consistent with reports by Wilson et al. (2009). The TN responded to nutrient addition, consistent with reports by Carson (2013). However our results were at variance with Wilson et al. (2009) and Zeglin et al. (2007) who reported no significant differences for TN at 0-5 and 0-20 cm depth respectively for burned and N amended tallgrass prairie soil.

The variation in depth distribution of SOC could be related to variation in means by which C enters and cycled in the burned and unburned prairie. In the burned prairie, residue is contributed principally by root biomass reportedly higher in burned than unburned prairie (Kitchen et al., 2009). The above ground litter in unburned also constitutes an important residue source whose accumulation on the soil surface influences the microclimatic conditions that regulates microbial activities and SOC mineralization. Our reported C:N ratio was always higher in burned than unburned, confirming the importance of root biomass in burned, which has been reported to have a higher C:N ratio (Kitchen et al., 2009).

Our observed values for C:N ratios at 0-5 cm were between 11.9 and 12.7 which were higher than 11.3 in 1994 (Ajwa et al., 1999).These differences indicated that treatments had either caused lower N or higher soil C content over time without causing changes in TOC. These differences in C:N ratios could also signify changes in litter quality entering mineral soil.

Total microbial biomass (MB) tended to be higher in unburned than burned at 0-5 cm depth, consistent with previous studies (Ajwa et al., 1999; Garcia and Rice, 1994). The MB was higher in burned than unburned at 5-15 cm depth which was also consistent with reports by Dell et al. (2005) for 0-15 cm depth. Specific biomarkers were significantly higher in unburned than burned at 0-5 cm, but higher in burned than unburned at 5-15 cm, indicating depth-related differences in microbial communities between burned and unburned prairie. Increase in MB at 5-15 cm due to burned treatment may result from increased soil C inputs of root biomass. Williams et al. (2000) reported increases in C inputs to soil from enhanced root growth and exudation. Also, increased immobilization of N in soils with burning could probably increase MB at 5-15 due to increased microbial N demand resulting from greater litter inputs from root biomass with wide C:N ratios (Dell et al., 2005). Root C:N is higher in burned than unburned (Kitchen et al., 2009). At 0-5 cm depth, P increased total microbial PLFA while at 5-15, it tended to be highest with N amendment.

In addition, the differences in residue quality and possibly quantity may accommodate different microbial communities, with varying mineralization capacities. The microbial groups responded variedly to nutrient amendment at both 0-5 and 5-15 cm depth, The AMF and fungi and Gram- bacteria were reduced by N and N+P amendment at 0-5 cm. The bacterial biomass indicated that N increased total bacterial biomass in burned at 5-15 cm depth with no effect in unburned. Nitrogen dynamics is reportedly regulated in burned tallgrass prairie through N conservation in microbial biomass (Garcia and Rice, 1994), while P may be conserved in microbial biomass in unburned creating a P limitations at lower depths and increased SOC mineralization which may reduce SOC.

Our results vary with the results of (Zeglin et al., 2007) who reported no difference in microbial biomass with N addition for annually burned prairie at 0-20 cm depth. The bacterial biomass contribution to MB could have been higher at lower soil depths due to preference of Gram+ bacteria for more recalcitrant C sources (Kramer and Gleixner, 2006), which would be higher in burned than unburned due to increased root biomass with N amendment. This difference in depth distribution of microbial biomass could indicate the differences in energy sources for microbes in burned and unburned tallgrass prairie. Total fungi and AMF were higher in burned than unburned. Research at the Konza prairie has reported fungal-dominated microbial activities in annually burned prairie (Rice et al., 1998). We also report that fungi were reduced at both depths with N+P amendment. Fungi and Gram+ bacteria seem to dominate at both depths. Shifts in microbial community composition with N amendment have been reported (Allison et al., 2007a; de Vries et al., 2007; Hogberg et al., 2003) towards bacterial dominance. In this study shifts in microbial composition was toward Gram+ dominance. This may indicate that the microbial communities of both the long term burned and unburned treatments might have resulted in shifts from dominance of r-strategists (Gram- bacteria) to k-strategists (fungi and Gram+). These changes in microbial community due to burning may have also been moderated by changes in soil resources in addition to changes in microclimate (altered temperature, soil water potential) and changes in host plants (Rice et al., 1998).

Literature on soil P availability of tallgrass prairie due to N, P and combination of N+P amendment is sparse. Available P was influenced by nutrient amendment indicating more available P in burned than unburned. This difference could be due to N being the

most limiting nutrient to primary productivity in tallgrass prairie (Ojima et al., 1994; Risser and Parton, 1982; Seastedt et al., 1991; Turner et al., 1997). Nitrogen has been reported to increase P-limitations in grasslands (Menge and Field, 2007). Our results indicate immobilization of P in soil and the higher coupling of N and P in burned tallgrass prairie than unburned. At 5-15 cm depth, available P was not responsive to nutrient amendment which could be due to low mobility of inorganic P in soils. Also P is second only to N in limiting productivity (Cross and Schlesinger, 2001; Ippolito et al., 2010; Lajtha and Schlesinger, 1988; McCulley et al., 2004); hence adding P together with N also reduces P limitation. Although inorganic P is the dominant form of soil P, biological P cycling also contributes significantly to available P (Turner et al., 2007; Vincent et al., 2010). Phosphorus is not very mobile in soil; therefore surface application does not guarantee non-limitation at higher soil depths. Biomining of P in soils have been reported (Jobbagy and Jackson, 2001). Therefore, biomining of P could also explain soil surface layer accumulation of P. The grasses, which are more abundant in burned, are highly mycotropic with extensive root systems, such that associated AMF hyphae could possibly mine P from lower depths to the soil surface, further increasing available P in burned than unburned.

Various studies have shown importance of microbial enzyme-mediated ecosystem nutrient acquisition (Sinsabaugh et al., 2002; van der Heijen, 2008), N-cycling (Bruns et al., 1998; Kowalchuk and Stephen, 2001), P-cycling (Cole et al., 1997; Singh et al., 1991) and C-cycling (Burns, 1982; Schimel and Weintraub, 2003). All enzymes assayed in this study were influenced by season at both depths. In line with our expectations, at 0-5 cm, β-glu activity was highest in October when substrates in the form of surface residue are

abundant. Our results are contrary to reports by Ajwa et al. (1999) from these plots indicating highest activity in August at 0-5 cm. The enzyme activity ratio for β-glu indicated C sufficiency at 0-5 cm for all treatments; however, at the 5-15 cm, β-glu activity results indicated C sufficiency for burned but deficiency for unburned. In October, N amendment created C deficiency for burned but sufficiency for unburned. Our result for β-glu in N-amended burned treatment is similar with the result of (Zeglin et al., 2007) who reported no difference in β-glu activity with N amendment of a burned tallgrass prairie at 0-20 cm depth. Similar to our results, Ajwa et al. (1999) reported higher β-glu activity with N amendment in unburned than burned.

The lack of burn effects on the activities of nutrient acquiring enzymes (β-glsm, acidP and alkalineP) at 0-5 cm depth was unexpected. Our results were at variance with (Ajwa et al., 1999) who reported that burning increased acidP and alkalineP activities at 0-5 cm depth. Our result is similar to Zeglin et al. (2007), who reported no effect of N-amendment on β-glsm activity due to burning at 0-20 cm. The potential β-glsm and alkalineP activities were highest in July, probably due to higher competition for N between microbes and plants. Dell and Rice (2005) reported a tight cycling of N within the root zone in tallgrass prairie. A similar competition has been reported for P, which is reported taken up more by microbes than plants in grasslands (Cole et al., 1997; Singh et al., 1991).Therefore the relationship between enzymes involved in N and P acquisition in burn and unburned emphasizes the complex mechanism that regulate nutrient supply and substrate mineralization, which has been reported to be asymmetrical for soil N and P (Olander and Vitousek, 2000).

CONCLUSIONS

Although the various treatments may not have indicated differences for TN or SOC at cumulative 0-30 cm soil depth, the mechanisms of TN and SOC dynamics seem to differ within different soil layers. The depth-specific differences may indicate different processes and mechanisms influencing TN and SOC, hence importance of sampling various soil layers when evaluating TN and SOC dynamics in this tallgrass prairie ecosystem. While N seemed to limit SOC in burned, it was P that limited SOC in unburned. Also, the mechanisms of C stabilization may differ at different soil depths due to differences in enzyme response to organic matter fractions. Therefore, it will be interesting to evaluate C in different soil fractions of the treatments in this experimental plots and their mineralization kinetics. Enzyme activity ratio could be a better measure of nutrient limitation than potential enzyme activity. Microbial biomass is an important nutrient store indicating more P being stored in microbial biomass in unburned and more N stored in microbial biomass in burned. The inherent differences in the C dynamics and nutrient cycling in this experiment require further investigation. The inherent complexity and diversity of microbial communities and the many ways by which they are affected by environmental factors hamper our ability to conclusively link microbial biomass to potential enzyme activities and soil C dynamics at the various soil depths Difficulties to explain patterns and controls of N and P on soil C dynamics leave many fundamental questions. One such question is whether N and P are synergistic or antagonistic with regards to soil C accumulation, and if both, then what could be the breakpoint? Further studies are required to examine depth-specific mechanisms that influence soil C and N in burned and unburned tallgrass prairie. Our data suggest that more research is required to

elucidate the range of processes and mechanisms that drive carbon dynamics in grasslands.

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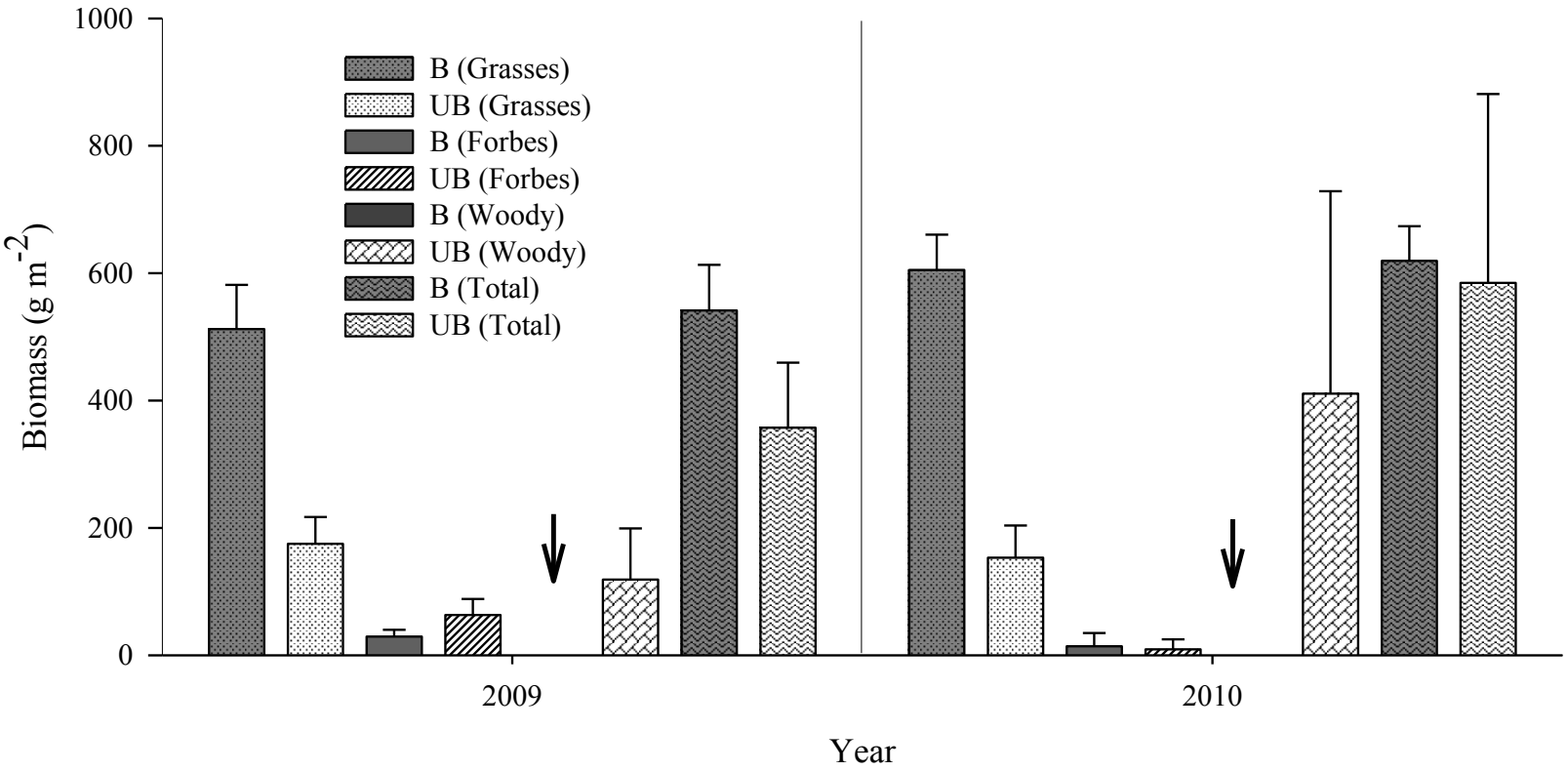

Figure 3.1. Burned effect on vegetation composition of a mesic grassland in 2009 and 2010. Arrows indicates absence of woody in burned prairie. Error bars represent standard error of means (n = 4).

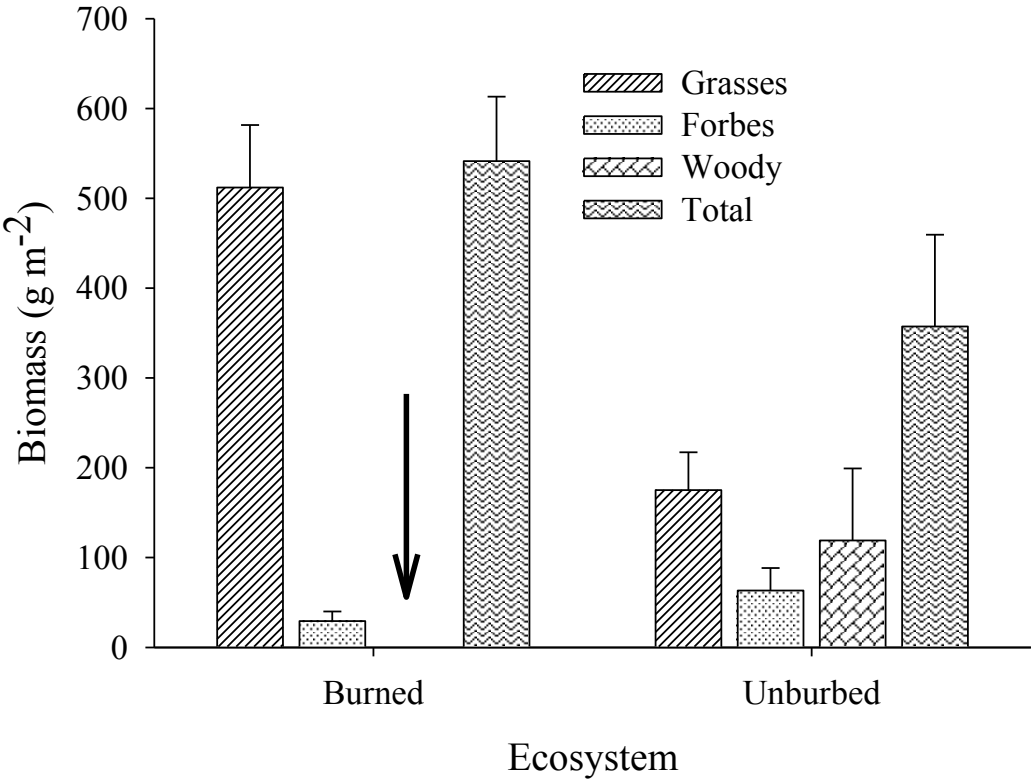

Figure 3.2. Burned management effect on vegetation composition of mesic grassland. Arrow indicates absence of woody in burned prairie. Error bars represent standard error of means (n = 4).

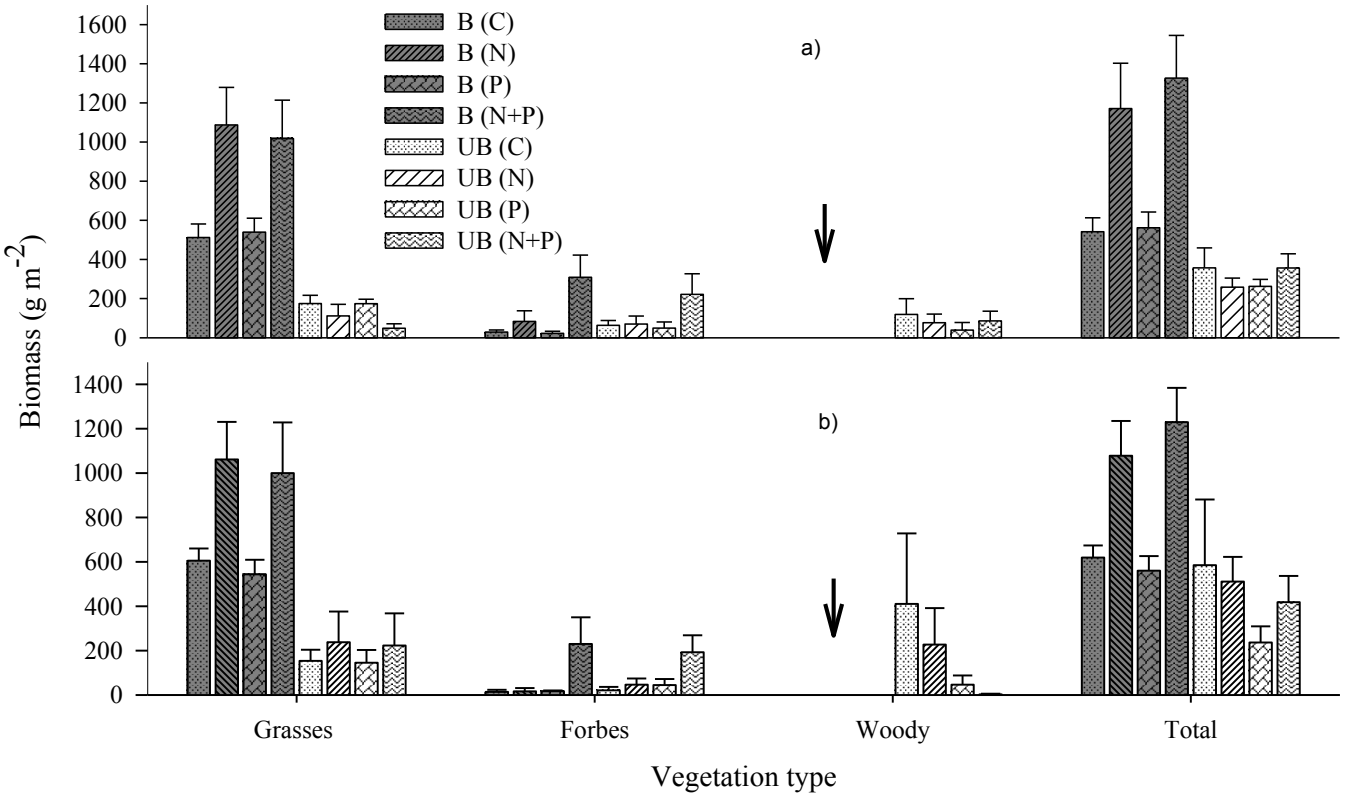

Figure 3.3. Burned and nutrient management effect on annual primary productivity and vegetation composition of a mesic grass during a) 2009 and b) 2010. Arrows indicates absence of woody in burned prairie. Error bars represent standard error of means (n = 4).

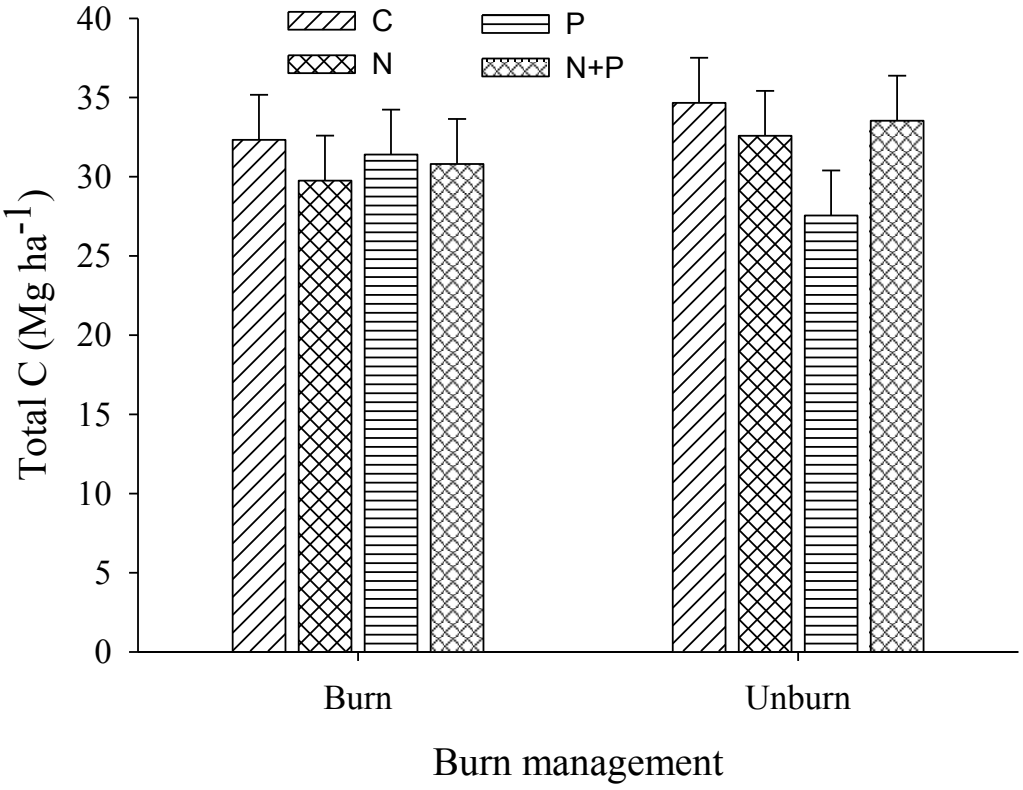

Figure 3.4. Burned management x nutrient amendment interaction means ($P<0.02$) of soil total organic C (TOC) at 15-30 cm depth. Error bars represent standard error of the mean (n=4).

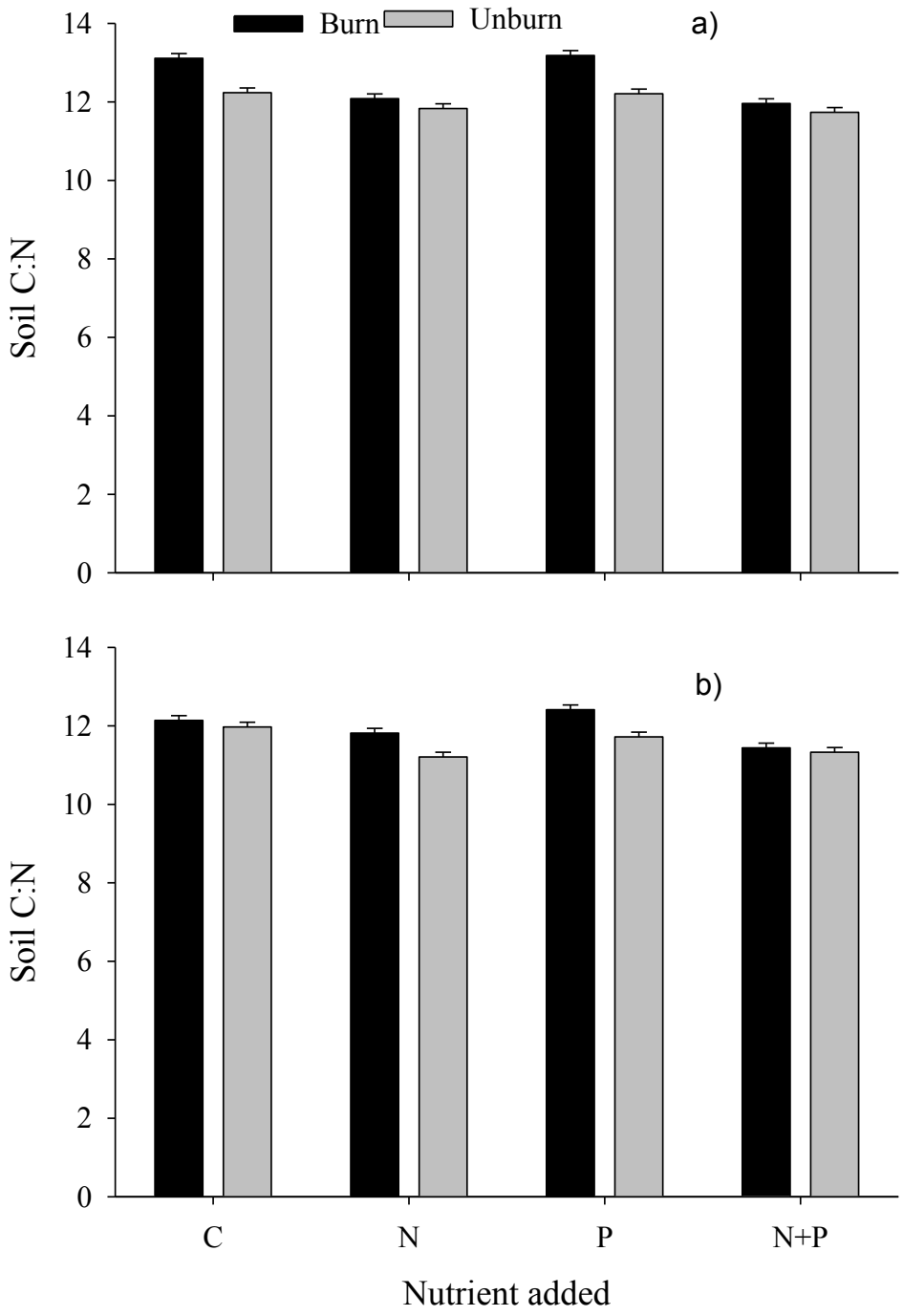

Figure 3.5. Burned management x nutrient amendment interaction means on soil C:N ratio at (a) 0-5 cm (P<0.006) and (b) 5-15 cm (P<0.008) depths. Error bars represent standard error of the mean (n=4).

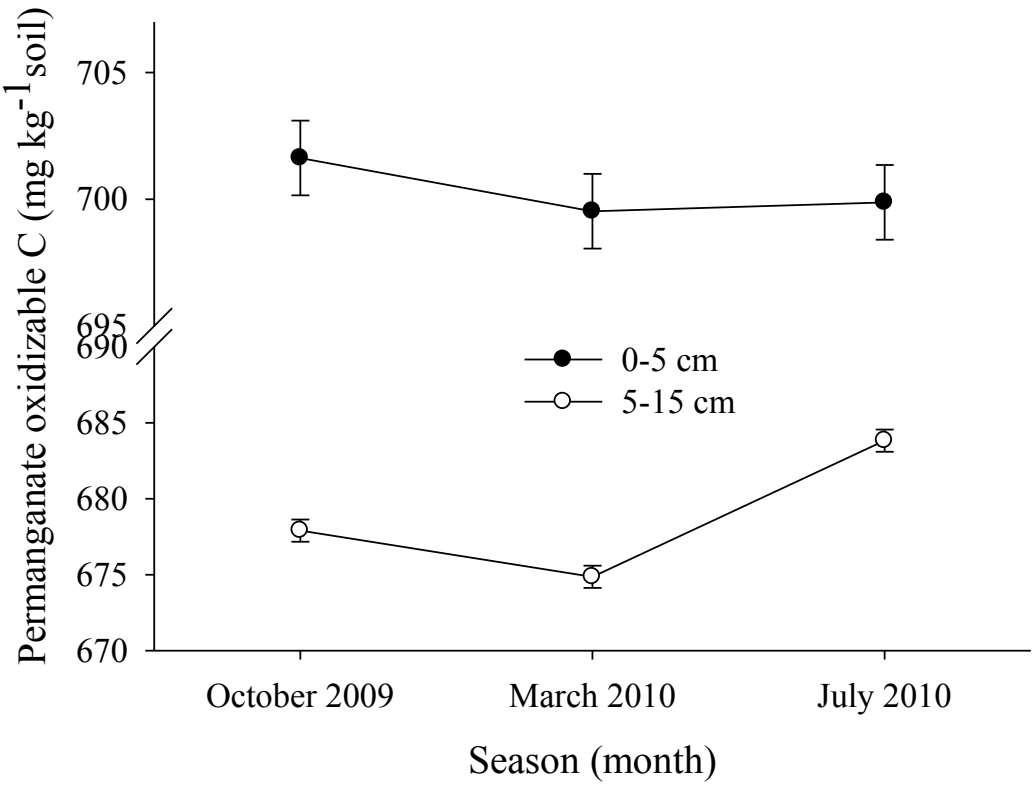

Figure 3.6. Seasonal effect means at 0-5 (P<0.2) and 5-15 (P<0.0001) cm depths of soil permanganate oxidizable C of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

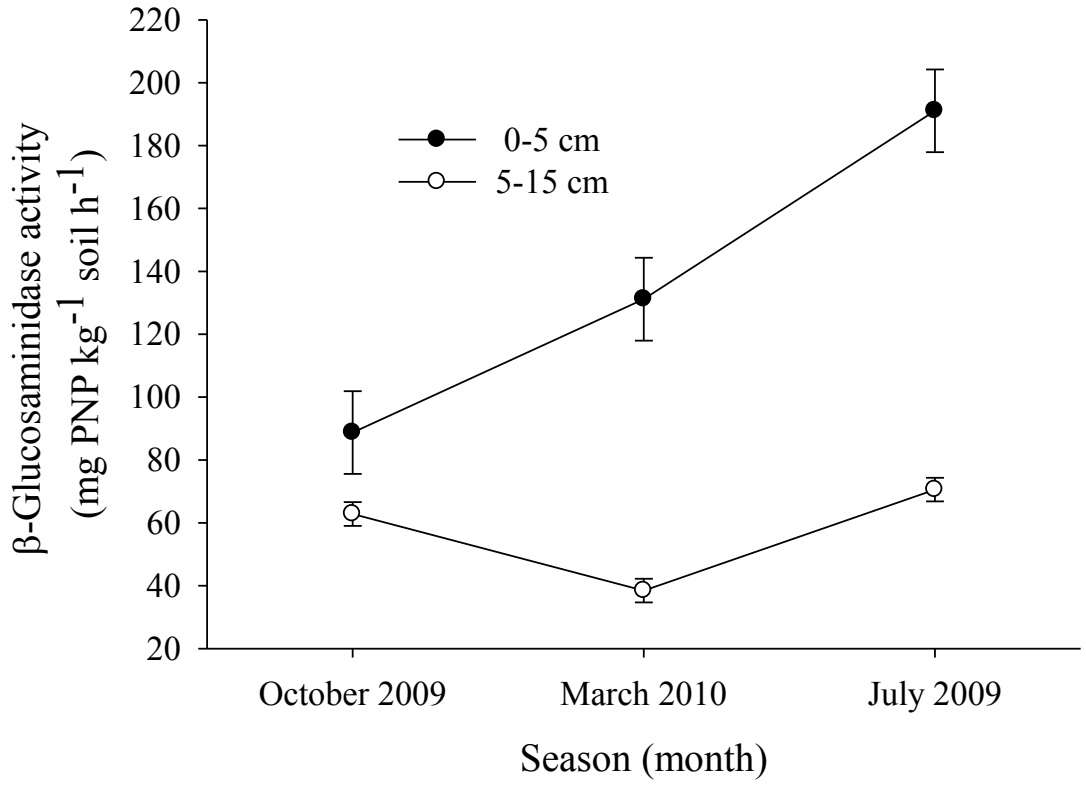

Figure 3.7. Seasonal effect means at 0-5 (P<0.0001) and 5-15 (P<0.0001) cm depths of soil β-glucosaminidase activity of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

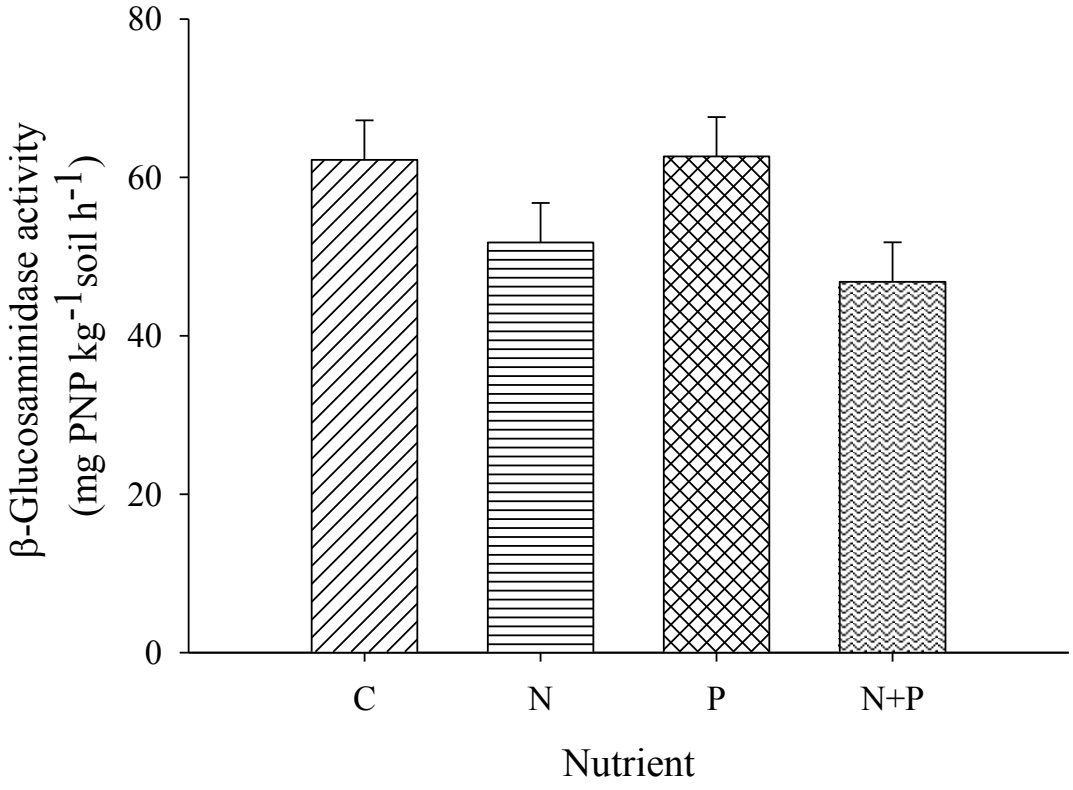

Figure 3.8. Nutrient (C, N, P, N+P) amendment effect means ($P<0.025$) of β -glucosaminidase activity of tallgrass prairie soil at 5-15 cm depth. Error bears represent standard error of means (n = 4).

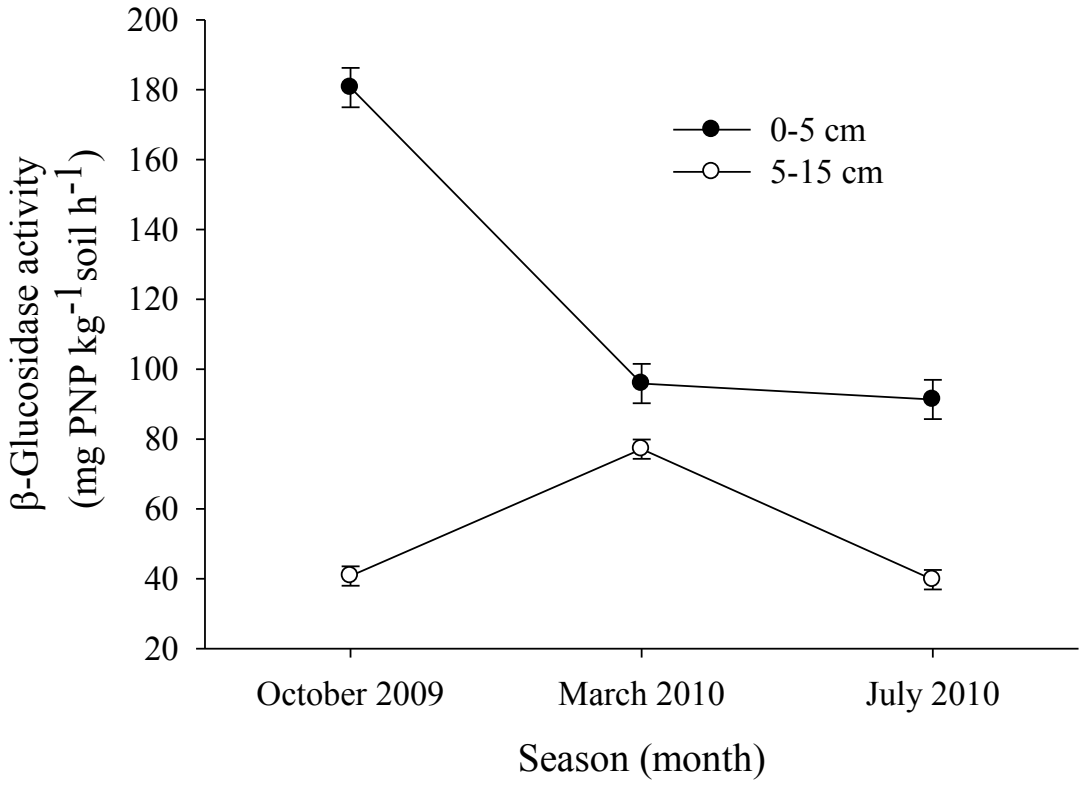

Figure 3.9. Seasonal effect means at 0-5(P<0.0001) and 5-15 (P<0.0001) cm depths of soil β-glucosidase activity of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

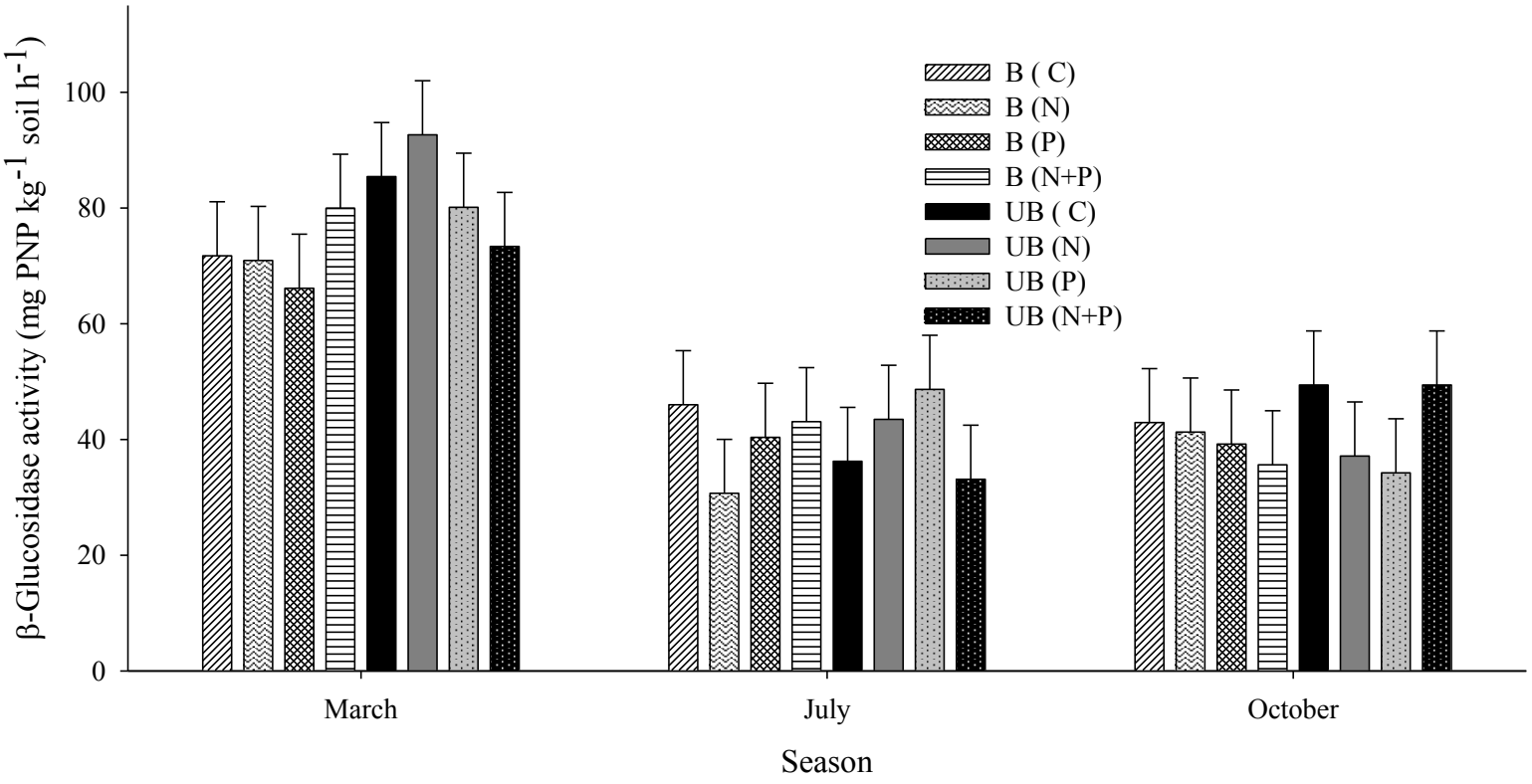

Figure 3.10. Burned management x nutrient amendment x season interaction means ($P<0.05$) of soil β-glucosidase activity at 5-15 cm depth of a tallgrass prairie soil. Error bears represent standard error of means (n = 4).

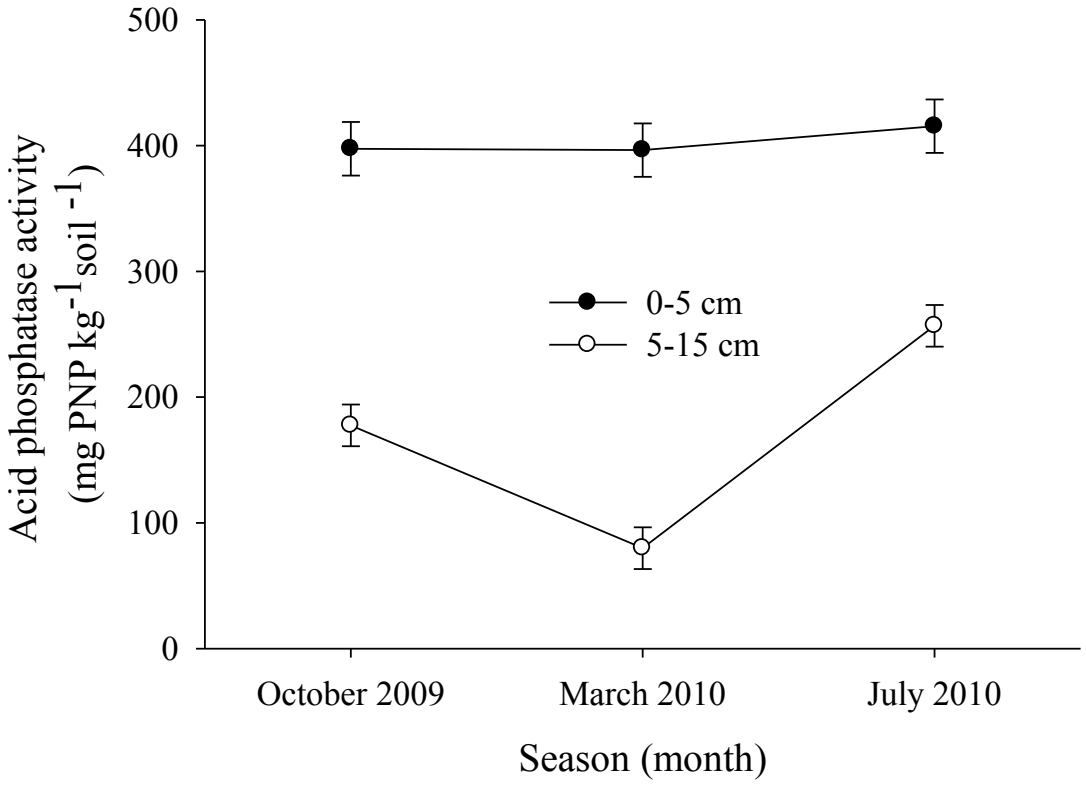

Figure 3.11. Seasonal effect means at 0-5 (P<0.8) and 5-15 (*P*<0.0001) cm depths of soil acid phosphatase activity of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

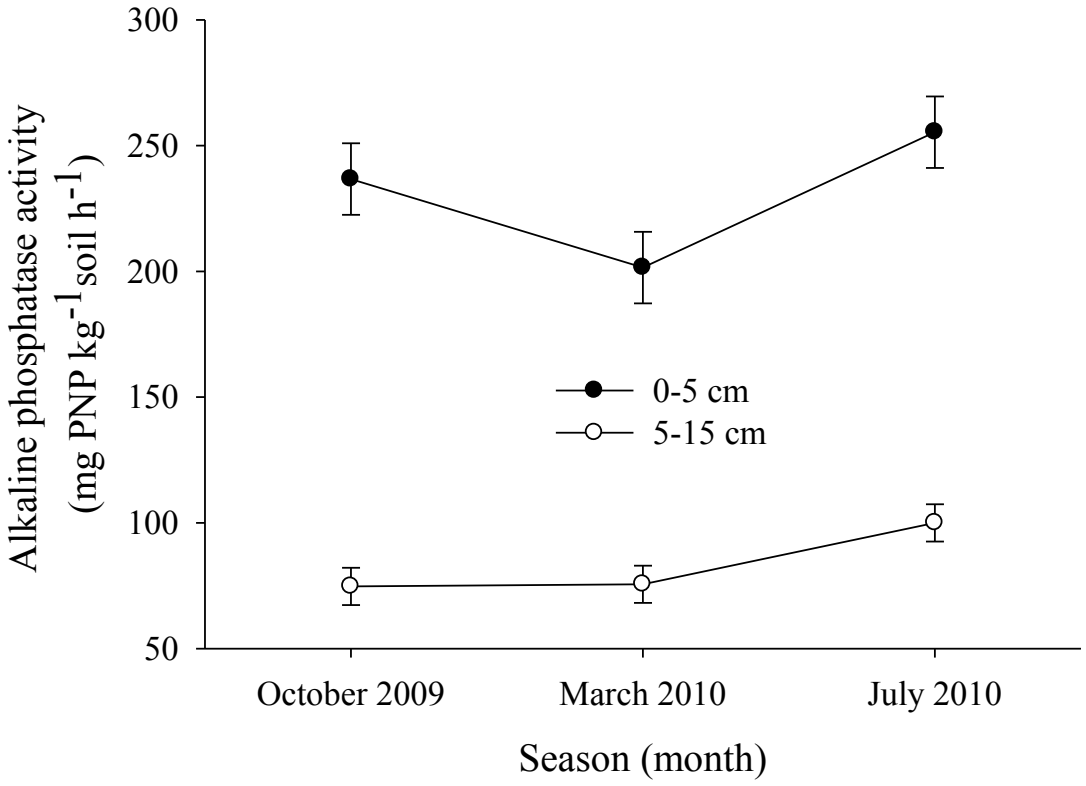

Figure 3.12. Seasonal effect means at 0-5 (P<0.01) and 5-15 (P<0.005) cm depths of soil alkaline phosphatase activity of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

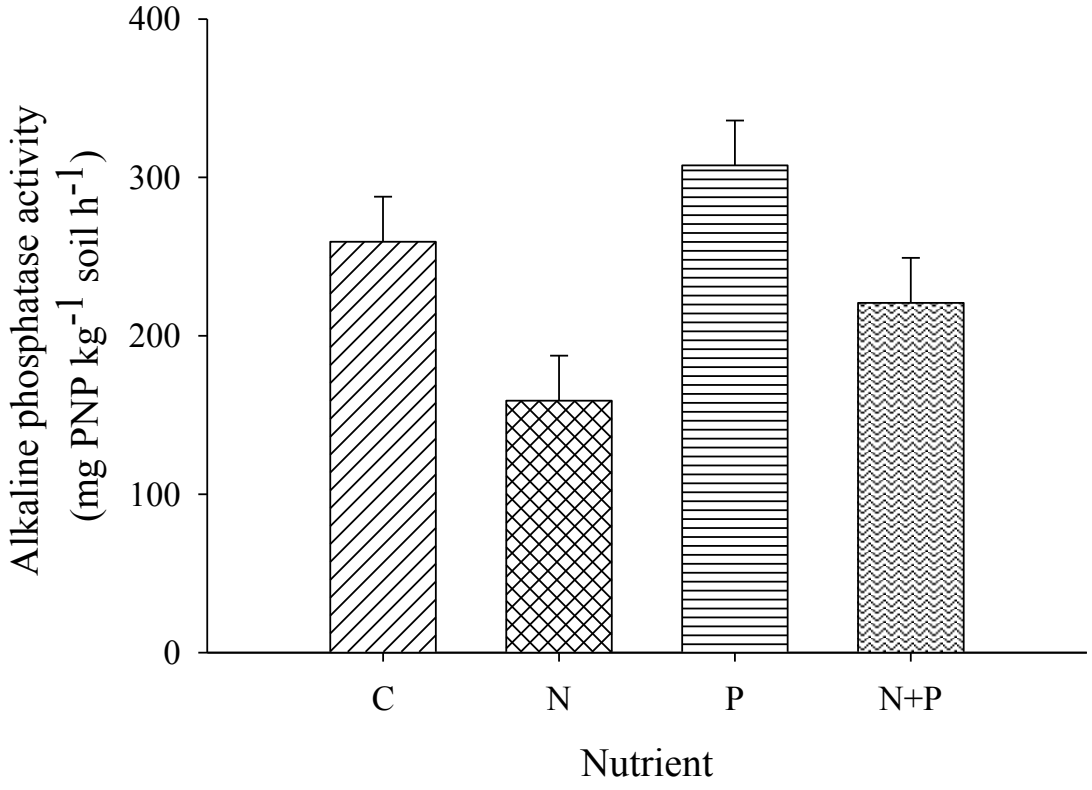

Figure 3.13. Nutrient (C, N, P, N+P) amendment effect means (P<0.0001) of alkaline phosphatase activity of tallgrass prairie soil at 0-5 cm depth. Error bears represent standard error of means (n = 4).

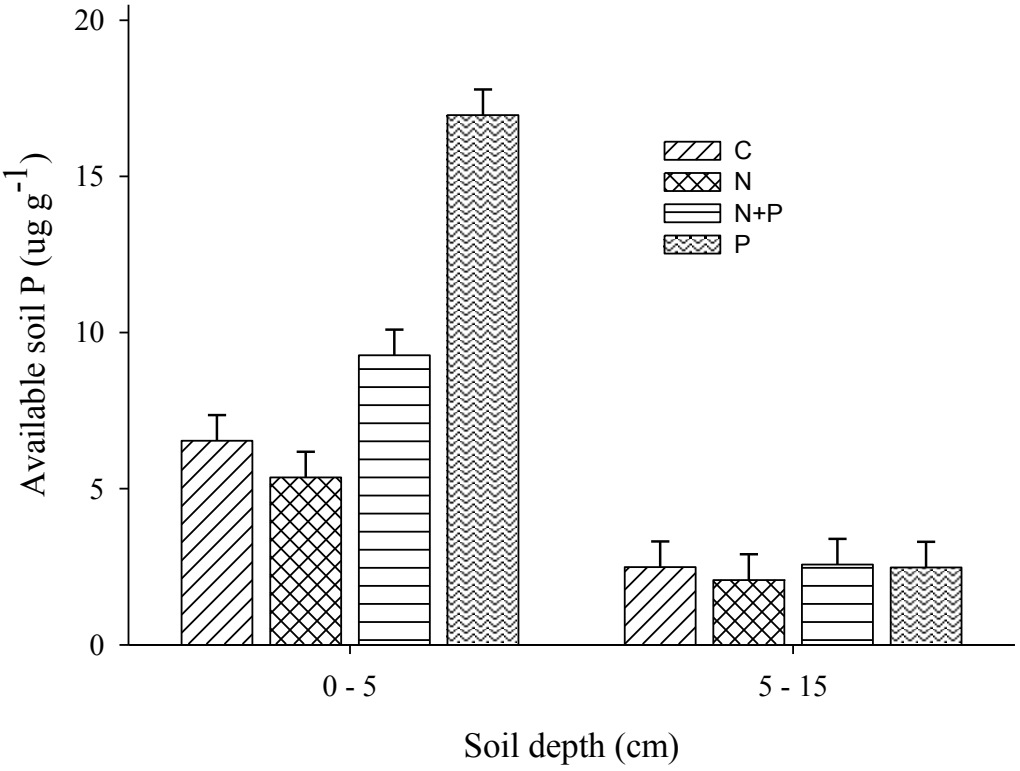

Figure 3.14. Nutrient x depth interaction effect means (P<0.0003) of Mehlich available P of tallgrass prairie soil. Error bears represent standard error of means (n = 4).

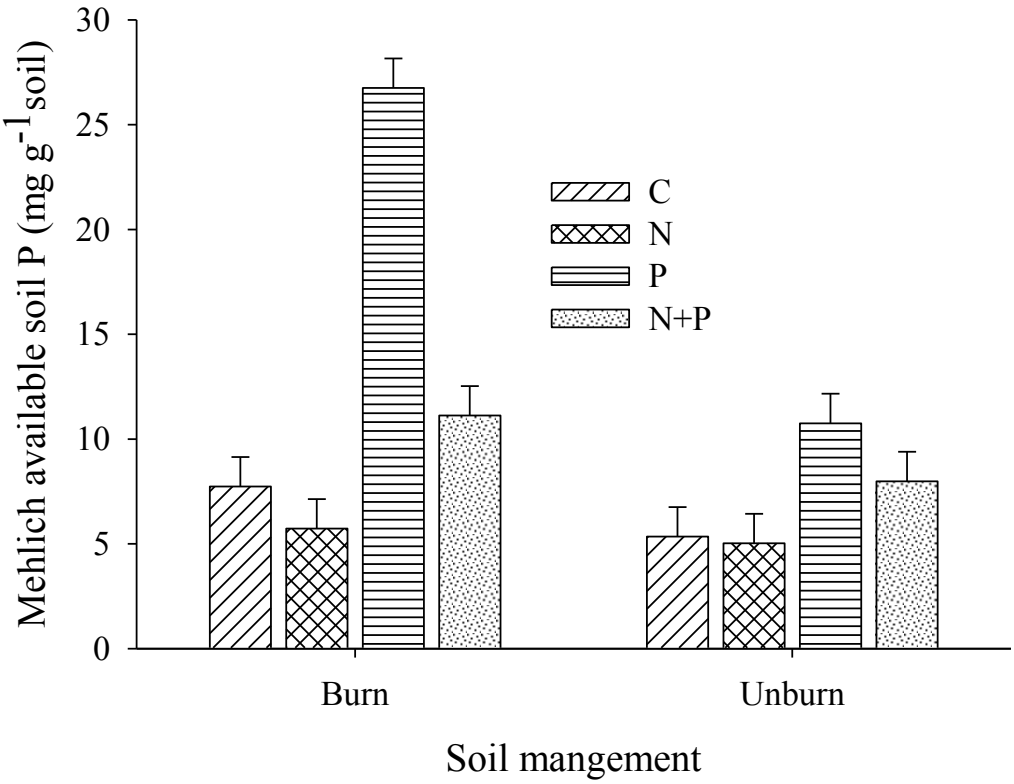

Figure 3.15. Burned management and nutrient amendment interaction means (P<0.0001) of Mehlich available P at 0-5 cm depth of a tallgrass prairie soil. Error bears represent standard error of means (n = 4).

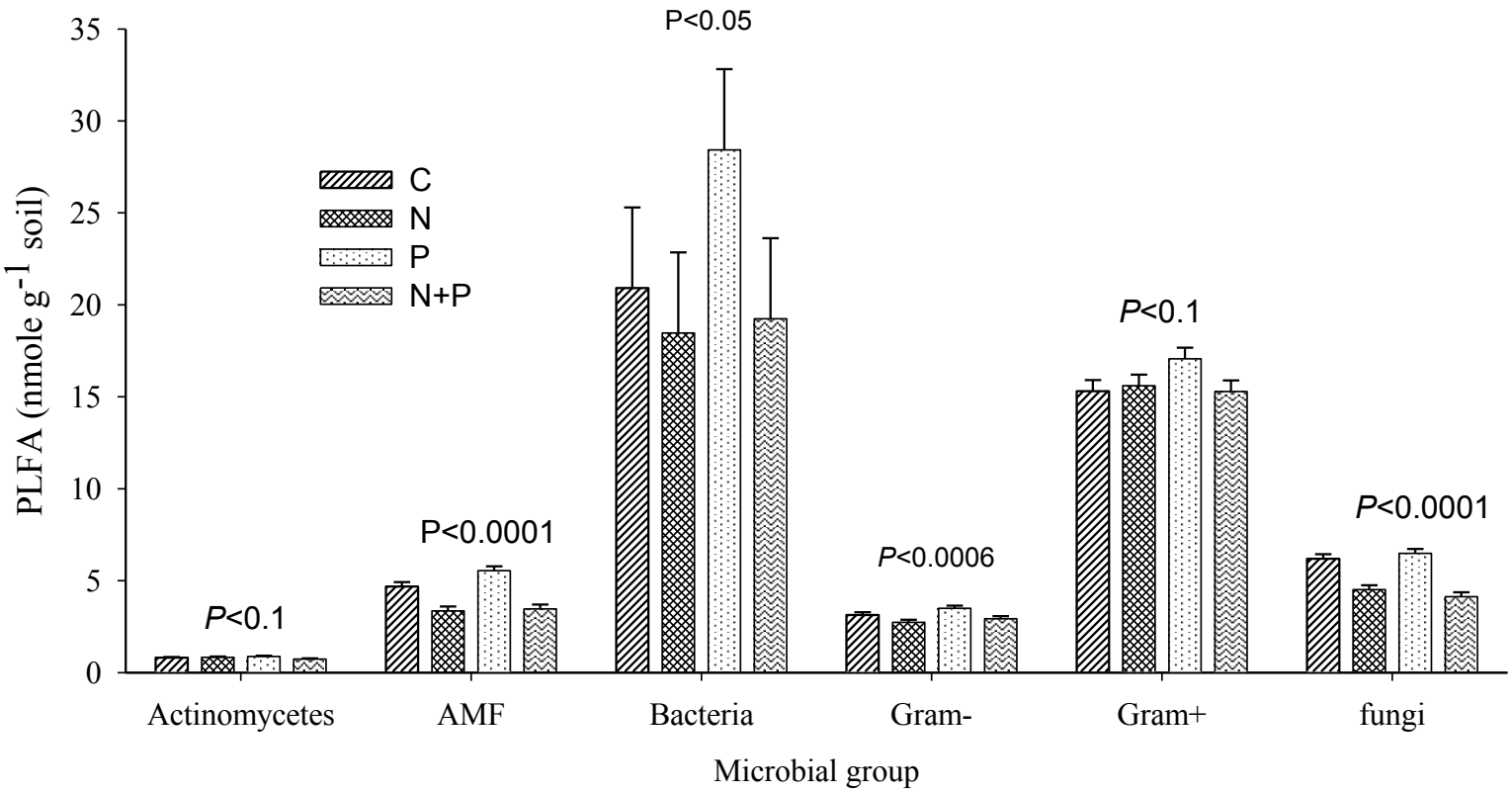

Figure 3.16. Abundance of specific phospholipid fatty acids (PLFA) due to nutrient amendment of tallgrass prairie at 0-5 cm depth. Error bars represent standard error of means (n=4).

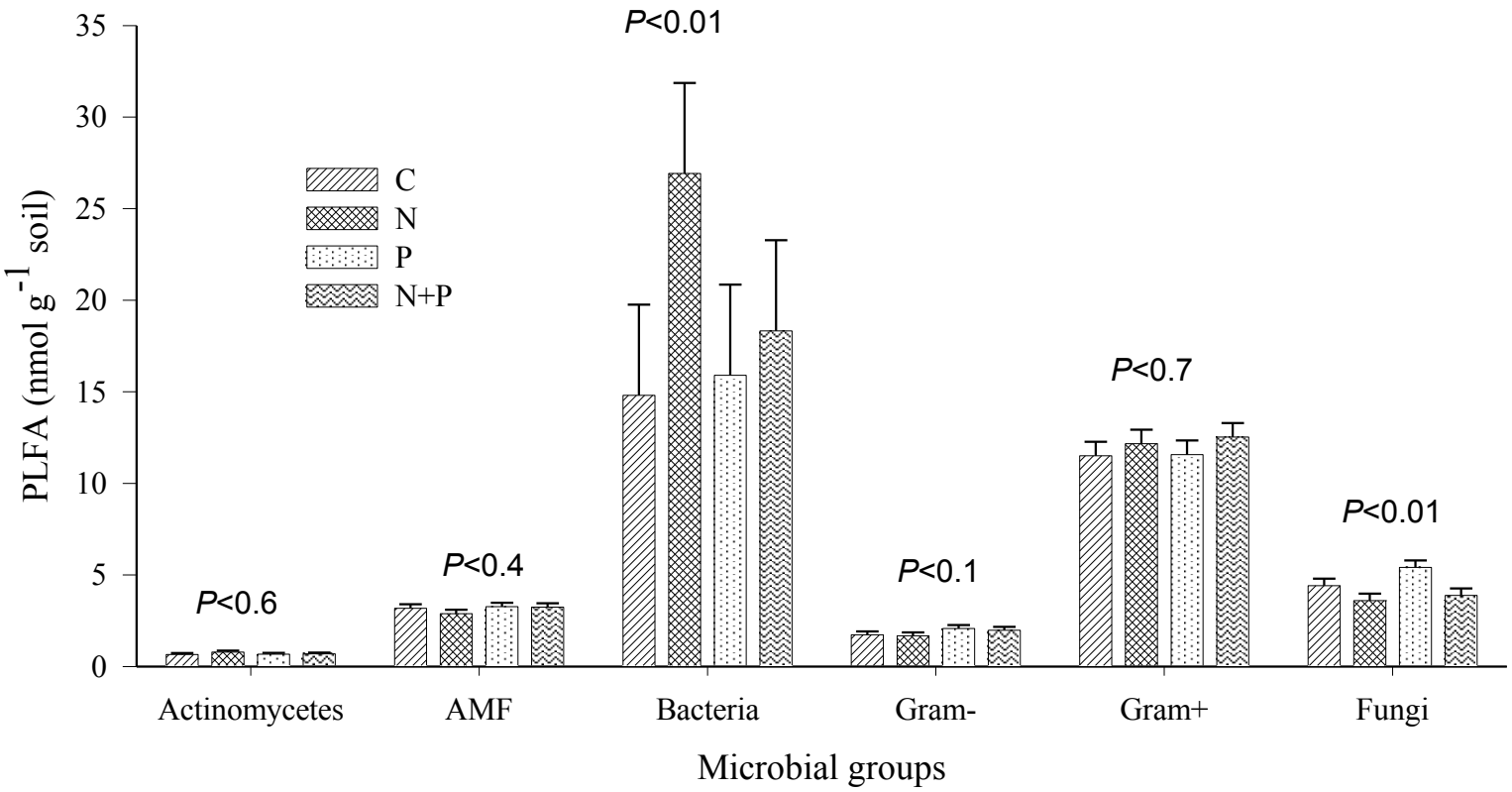

Figure 3.17. Abundance of specific phospholipid fatty acids (PLFA) due to nutrient amendment of tallgrass prairie at 5-15 cm depth. Error bars represent standard error of means (n=4).

Table 3.1. Analysis of variance (*P*-values) for total N (TN), total organic C (TOC), C:N ratio and permanganate oxidizable C (POXC) of long term burned management and nutrient addition of a tallgrass prairie soil at various depths.

		Burned (B)	Nutrient (N)	B x N	Season (S)	B x S	N x S	B x N x S
		P-values						
0-5	Total N	0.5787	0.0007	0.7114	0.2396	0.4276	0.4159	0.6025
	Total OC	0.6944	0.2496	0.3803	0.4727	0.5125	0.3787	0.545
	C/N	0.0057	<.0001	<.0001	0.2261	0.8989	0.4428	0.6093
	POXC	0.3701	0.4821	0.9468	0.2264	0.4254	0.7075	0.5977
5-15	Total N	0.0003	0.082	0.1243	0.0789	0.1199	0.8432	0.6487
	Total OC	0.0235	0.709	0.0625	0.1601	0.125	0.9327	0.6849
	C/N	0.0082	<.0001	0.0148	0.0415	0.8638	0.224	0.562
	POXC	0.7549	0.1336	0.216	<.0001	0.6343	0.4543	0.8951
15-30	Total N	0.8763	0.0283	0.0878	0.2739	0.3272	0.9119	0.5664
	Total OC	0.7755	0.0279	0.0336	0.6189	0.3132	0.8218	0.6219
	C/N	0.6774	0.0265	0.7554	0.4319	0.053	0.9946	0.9148
	POXC	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0-30	Total N	0.2915	0.1037	0.197	0.0319	0.5082	0.7509	0.1892
	Total OC	0.4482	0.377	0.1064	0.3227	0.9885	0.7081	0.2169
	C/N	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	POXC	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Significant values are $P<0.05$

Table 3.2. Effect of nutrient (N and P) amendments on total N (TN), total organic C (TOC), C:N ratio and permanganate oxidizable C (POXC) of a tallgrass prairie soil at various depths.

		0 – 5	5 - 15	15 - 30	0 - 30
		----------Depth (cm)----------			
Total N	*Control*	1.28c§	2.23	2.85a	6.39
(Mg N h^{-1})	*N*	1.38ab	2.3	2.82a	6.53
	P	1.34b	2.14	2.56b	6.05
	N+P	1.48a	2.3	2.93a	6.82
Total organic C	*Control*	16.1	26.9	33.5a	76.9
(Mg C h^{-1})	*N*	16.4	26.4	31.4ab	74.5
	P	17	25.8	29.4b	72.3
	N+P	17.4	26.6	32.1a	77.1
C:N ratio	*Control*	12.7a	12.1a	11.9a	N/A
	N	12b	11.5b	11.7ab	N/A
	P	12.7a	12.1a	11.4ab	N/A
	N+P	11.9b	11.4b	10.9b	N/A
Permanganate oxidizable C	*Control*	669	680	N/A	N/A
(mg kg^{-1})	*N*	700	678	N/A	N/A
	P	701	678	N/A	N/A
	N+P	701	679	N/A	N/A

§ Different letters represent significant differences between nutrient amendment for each variable at each soil depth (P<0.05).

Table 3.3 Seasonal variation in total N (TN) and soil C indices total organic C (TOC) and permanganate oxidizable C (POXC) at various soil depths of a tallgrass prairie.

	----------0-5cm----------			----------5-15cm----------			----------15-30----------		
	March	July	October	March	July	October	March	July	October
C:N ratio	12.27a	12.23a	12.35a	11.77ab	11.62b	11.85a	11.33a	11.13a	11.45a
POXC	699.52a	699.87a	701.63a	674.86c	683.82a	677.90b	N/A	N/A	N/A

§ Different letters represent significant differences between seasons for each variable at each soil depth ($P<0.05$).

Table 3.4. Analysis of variance (*P*-values) for enzyme activities of a tallgrass prairie soil under long term burned management and nutrient (N and P) amendment at various soil depths.

	β-glucosaminidase	β-glucosidase	Acid phosphatase	Alkaline phosphatase
	P-values			
	0-5cm depth			
Burned (B)	0.5479	0.0764	0.7605	0.5151
Nutrient (N)	0.0612	0.5657	0.4331	<.0001
B x N	0.3897	0.1598	0.1833	0.2823
Season (S)	<.0001	<.0001	0.8117	0.0138
B x S	0.5395	0.2413	0.7055	0.1025
N x S	0.1845	0.2786	0.3826	0.5553
B x N x S	0.3348	0.7457	0.4167	0.7539
	5-15cm depth			
Burned (B)	0.1513	0.6556	0.1747	0.9714
Nutrient (N)	0.0254	0.6984	0.9699	0.1623
B x N	0.8718	0.6601	0.4944	0.3567
Season (S)	<.0001	<.0001	<.0001	0.0054
B x S	0.4343	0.6084	0.2752	0.3638
N x S	0.5767	0.5071	0.2367	0.494
B x N x S	0.3199	0.0544	0.9971	0.7862

Significant values are $P<0.05$

Table 3.5. Analysis of variance (*P*-values) for major microbial community groups and indices, using PLFA, of a tallgrass prairie soil under long term burned management and nutrient (N and P) amendment at various soil depths.

	Actinomycetes	AMF	Bacteria	Gram-	Gram+	Fungi	F/B
	P-values						
	0-5						
Burned (B)	0.8043	0.5195	0.3611	0.1188	0.1115	0.908	0.4423
Nutrient (N)	0.1533	<.0001	0.0536	0.0062	0.14	<.0001	0.13
B x N	0.4461	0.9346	0.3907	0.2968	0.8507	0.5984	0.4077
	5-15						
Burned (B)	0.0162	0.0648	0.1435	0.6644	0.194	0.0743	0.6369
Nutrient (N)	0.6158	0.4845	0.0155	0.1803	0.7275	0.0151	0.0028
B x N	0.3385	0.7002	0.0141	0.2099	0.8816	0.6325	0.1175

Significant values are $P<0.05$

Table 3.6. Effect of long-term annual burned management, on specific microbial biomarkers of a tallgrass prairie soil at 0-5 and 5-15 cm depths.

		----------0-5----------		----------5-15----------	
Fatty Acid	Grouping	Burned	Unburned	Burned	Unburned
		----------nmol PLFAg-1 soil ----------			
14:0	common	10.05b	11.42a	0.86	0.72
15:0	common	0.78	0.85	0.71	0.86
16:0	common	11.35	11.85	8.99a	7.72b
17:0	Common	1.92	2.30	0.59	0.77
18:0	common	2.32	2.46	1.74	2.35
20:0	common	0.37	0.38	0.37	0.49
i15:0	Gram+	0.94b	1.11a	7.54	7.08
a15:0	Gram+	7.28b	8.54a	5.95	5.93
i16:0	Gram+	5.04	5.12	5.11a	4.15b
i17:0	Gram+	2.99	3.30	2.87a	2.38b
a17:0	Gram+	3.32	3.36	3.72a	3.15b
16:1w7c	Gram-	4.15	3.95	5.19	2.68
16:1w5c	Gram-,Fungi	4.34	4.18	2.39	1.98
cy17:0	Gram-	2.04b	2.42a	1.24	1.31
18:1w7	Gram-	0.82	0.79	0.60	0.59
cy19:0	Gram-	1.87	1.04	0.53	0.73
10Me18:0	Actinomycetes	0.80	0.82	0.79a	0.58b
18:2w6,9c	Fungi	1.28	1.32	1.04	1.09
18:1w9c	Fungi, Plants	4.04	4.02	3.65a	2.87b
18:1w9t	Fungi, Plants	3.93b	4.88a	2.35	2.37
Total		69.85b	77.80a	67.36a	53.33b

§ Different letters represent significant differences between seasons for each enzyme at each soil depth ($P<0.05$).

Table 3.7. Effect of nutrient (N and P) amendments, on specific microbial biomarkers of a tallgrass prairie soil at 0-5 cm depth.

		C	N	P	N+P
Fatty Acid	Grouping	----------nmol PLFAg-1 soil ----------			
14:0	common	0.99	0.99	1.11	1.01
15:0	common	0.78	0.78	0.85	0.87
16:0	common	11.82ab	11.13b	12.69a	10.74b
17:0	common	2.24	1.60	2.85	1.85
18:0	common	2.47ab	2.23b	2.64a	2.20b
20:0	common	0.34	0.43	0.38	0.36
i15:0	Gram+	10.26	10.58	11.49	10.61
a15:0	Gram+	7.88b	7.04b	8.87a	7.87b
i16:0	Gram+	5.05	5.02	5.58	4.68
i17:0	Gram+	3.14ab	3.03b	3.43a	2.97b
a17:0	Gram+	3.58a	3.03b	3.81a	2.95b
16:1w7c	Gram-	3.72	2.91	7.76	3.20
16:1w5c	Gram-,Fungi	4.68b	3.36c	5.54a	3.47c
cy17:0	Gram-	2.11	2.10	2.43	2.25
18:1w7	Gram-	0.97a	0.61b	1.04a	0.65b
cy19:0	Gram-	1.66	1.27	1.50	1.28
10Me18:0	Actinomycetes	0.81	0.83	0.87	0.73
18:2w6,9c	Fungi	1.67a	0.97b	1.71a	0.86b
18:1w9c	Fungi, Plants	4.52a	3.54b	4.78	3.27b
18:1w9t	Fungi, Plants	4.25	4.06	5.01	4.30
Total		73.61b	66.85b	89.40a	67.11b

§ Different letters represent significant differences between seasons for each enzyme at each soil depth ($P<0.05$).

Table 3.8. Effect of nutrient (N and P) amendments, on specific microbial biomarkers of a tallgrass prairie soil at 5-15 cm depth.

Fatty AcidP	Grouping	C	N	P	N+P
		----------nmol PLFA g-1 soil ----------			
14:0	common	0.76	0.76	0.84	0.80
15:0	common	0.76	0.68	0.90	0.81
16:0	common	8.34	8.03	8.48	8.57
17:0	common	0.55	0.72	0.58	0.88
18:0	common	1.58	1.57	3.34	1.69
20:0	common	0.33	0.33	0.71	0.37
i15:0	Gram+	6.85	7.46	6.84	8.08
a15:0	Gram+	5.43	6.17	5.45	6.71
i16:0	Gram+	4.66	4.70	4.73	4.45
i17:0	Gram+	2.59	2.67	2.62	2.62
a17:0	Gram+	3.46	3.42	3.64	3.22
16:1w7c	Gram-	2.69	7.33	3.04	3.21
16:1w5c	Gram-,Fungi	2.20	1.89	2.33	2.32
cy17:0	Gram-	1.14	1.20	1.30	1.46
18:1w7	Gram-	0.59	0.48	0.79	0.54
cy19:0	Gram-	0.58	0.65	0.60	0.68
10Me18:0	Actinomycetes	0.64	0.77	0.64	0.68
18:2w6,9c	Fungi	1.13	0.75	1.43	0.95
18:1w9c	Fungi, Plants	3.29ab	2.85b	3.97a	2.93b
18:1w9t	Fungi, Plants	2.01	2.20	2.52	2.70
Total		49.99b	71.85a	60.59ab	58.94ab

§ Different letters represent significant differences between seasons for each enzyme at each soil depth ($P<0.05$).

CHAPTER 4 - PERENNIAL AND ANNUAL PLANT MANAGEMENT EFFECTS ON C DYNAMICS AND SOIL STRUCTURE

ABSTRACT

Storing C in soil could mitigate global climate in the short term if soil organic C (SOC) is stabilized and consequently improves agricultural sustainability and environmental quality. To achieve this purpose, better soil management options have to be adopted. Options that improve soil aggregation and stability will occlude SOC from increased microbial degradation and increase soil C sequestration. The objective of this study was to assess biophysical controls on SOC dynamics as influenced by perennial and annual plant management practices. Plants included annually cultivated grain sorghum (*Sorghum bicolor*) planted either in no-tillage (NT) or continuous tillage (CT), and a perennial replanted native prairie grass, (*Andropogon gerardii*) (RP). The field experiment is located at the Konza Prairie Biological Station in Manhattan, Kansas. This split plot experiment had ecosystems as the main plot treatment and phosphorus amendment (+P) and no P (-P) as sub plots. Soil sampling was done at incremental soil depths (0-5, 5-15 and 15-30 cm) over multiple years (2007 - 2010). Above- and belowground biomass were assessed using gravimetric methods. The % root AMF colonization was estimated by staining techniques and magnified gridline method was used for scoring % root colonization. Water-stable aggregates (WSA) were separated using the wet sieving method. Total organic C, total N and aggregate-associated C and N were determined by

dry combustion. All variables assessed (TN, SOC, % root AMF colonization, above- and belowground biomass, aggregate size distribution, aggregate-associated C and N, and microbial community) varied over time. However, SOC and TN were higher in the less disturbed ecosystems, RP and NT, than the more disturbed CT. The above- and belowground plant biomass after annual burning losses in RP were similar for all the ecosystems down to 7.5 cm soil depth. The % root AMF colonization was higher in RP than NT or CT and P amendment reduced AMF colonization in the RP and NT but not CT. Soil aggregation was higher in the less disturbed RP and NT than CT. The aggregate-associated C increased in the macroaggreges for RP and NT with concurrent decreases in the micricroaggregates. The saprophytic fungi abundance was greater with less soil disturbance. Our results established that several factors could concurrently influence SOC sequestration which is higher at the surface in less disturbed soils either in annual or perennial ecosystems.

INTRODUCTION

The soil resource is increasingly recognized as important not only for food production but also in provisioning of ecosystem services. One such ecosystem service is C sequestration to mitigate global climate change (IPCC, 2007; Smith et al., 2007). Soil C sequestration is the transfer of atmospheric CO_2 into soil stabilized pools for secure storage (Lal, 2004). Additionally, soil C storage will not only sequester C to mitigate global climate in the short run but also improve soil sustainability; and belowground biodiversity (Bongiovanni and Lobartini, 2006; Rice et al., 2007) over time. However, only when the rate of C inputs (plant residues, root and root exudates) surpasses the rate of outputs (heterotrophic soil respiration, burning and volatilization, erosion and leaching of organic compounds) would C be sequestered in soils (Post and Kwon, 2000; Amundson, 2001).

Currently, soils have the potential to sequester C because a proportion of the CO_2 released to the atmosphere has been assigned to previous plowing of soils for agricultural production (Davidson and Ackerman, 1993; Lal, 1997; Rice, 2006). Hence, the SOC-depleted agricultural and transformed ecosystem soils should have the potential to sequester C (West and Post, 2002; Lobell et al., 2006). It has been estimated that SOC-depleted soils, have the capacity to sequester 20-30 Pg C during the next 50-100 yr (Paustian et al., 1997a; Smith, 2004). Such sequestration capacity could account for > 0.2 Pg C y^{-1} (Lokupitiya and Paustian, 2006; Rice, 2006). It is not surprising therefore that numerous researchers have studied the effect of soil management on SOC (Lal et al., 1994; Janzen et al., 1998; Six et al., 1998; Six et al., 1999; Follett et al., 2001; West and

Post, 2002; Fabrizzi et al., 2003; Mikha and Rice, 2004; Smith et al., 2005; Wright and Hons, 2005; Dolan et al., 2006; Machado et al., 2006; McVay et al., 2006; Blanco-Canqui et al., 2009; White and Rice, 2009; DuPont et al., 2010; Luo et al., 2010; Powlson et al., 2011; Sundermeier et al., 2011; Mikha et al., 2013).

Reported results have shown inconsistencies and variations of SOC sequestration. Less soil disturbance has been reported to increase SOC (Six et al., 1999; West and Post, 2002; Fabrizzi et al., 2003; Mikha and Rice, 2004; Blanco-Canqui et al., 2009; White and Rice, 2009; Mikha et al., 2013). However, other researchers have reported no increase in SOC resulting from reduced tillage (Angers et al., 1997; Franzluebbers et al., 1999; Puget and Lal, 2005; Sainju et al., 2006; Luo et al., 2010). Such discrepancies indicate that there is still much to be understood about how soil management interacts with other factors that influence SOC dynamics. Despite the insights gained through the above-cited studies, knowledge of the factors and interactions that enhance SOC sequestration remains limited (Neff et al., 2002; Frey et al., 2003; Six et al., 2004b; Rasse et al., 2005; Lehmann et al., 2008; Schmidt et al., 2011).

For instance although plant and soil management practices influence SOC sequestration (West and Post, 2002; Mikha and Rice, 2004; Fabrizzi et al., 2009; White and Rice, 2009), both practices contribute to soil C sequestration through dissimilar mechanisms. While plants are primarily involved in the quantity and quality of organic C entering the soil, soil management practices indirectly influence the biochemical alteration and physicochemical protection of SOC. The two major mechanisms of stabilizing SOC, hence sequestration are (bio)chemical alteration and physicochemical

protection (Jastrow et al., 2007). However plant type and soil management contribute to varying degrees towards these mechanisms.

Plant roots influence SOC mineralization by altering soil water regimes, root exudation, root residue and rooting depth. Increased SOC mineralization could result from high quality rhizodeposition stimulating microbial activity and reducing SOC. Plant roots, can entangle soil particles and root exudates act as binding agents for macroaggregate formation (Six et al., 2004a). Roots have also been shown to influence aggregation due to the residue quality and root architecture (Franzluebbers et al., 1995; Martens, 2000; Wright and Hons, 2005). Therefore, plant root architecture and associated microbes contribute to both the soil biological and physical properties regulating soil C dynamics (Jastrow and Miller, 1993; Carter et al., 1994).

Additionally, the influence of plants on SOC sequestration is also important in aboveground residue quantity and quality. Native vegetation produce high quantities of plant material resulting in high potential to accumulate and preserve SOC (Rice, 2002). Plants also influence SOC dynamics due to their associated soil microbial communities and residue input (Aerts and Chapin, 2000; Waldrop and Firestone, 2004; White and Rice, 2009; Marshall et al., 2011). Plant residues act as transient binding agents for soil aggregate formation. Although plant residue is important in supplying C to the soil, microbial activity ultimately determines the residence time in soil depending on residue quality and location in soil structure.

The microbial community composition is an important control on SOC mineralization or stabilization (Fierer et al., 2003; Balser and Firestone, 2005). Microbial

communities can be largely separated as fungal and bacterial groups. Research has indicated that bacterial decomposition pathways have high turnover rates while fungal pathways involve more complex organic C with slower turnover rates (Wardle, 2002; Williamson and Wardle, 2003). Additionally, soil microbial activities influence and are influenced by SOC (Leinweber et al., 2008; Scharroba et al., 2012). For instance, the transport of C from the rhizosphere by mycorrhizal hyphae leads to C stabilization in the soil (Treseder and Allen, 2000). Residue quality influences microbial populations. Soils with higher fungal to bacterial ratios have been associated with greater C storage and slower C turnover, although the involved mechanisms remain unclear (Thiet et al., 2006). Such an association could arise because fungi and bacteria have different biochemistries which affect their C use efficiencies (Jastrow et al., 2007) and consequently their SOC sequestration potential. Additionally, melanins, chitosan, and quinines produced by saprophytic fungi are more resistant to decomposition than bacterial metabolic products (Six et al., 2006). Therefore, decomposability of different microbial group by-products could regulate their SOC mineralization.

For soil management, controls on SOC are through the influence on the micro-environment. The SOC decomposition is a function of biological (microbial community), chemical (pH) and physical (aeration) interactions (Rice and Angle, 2004). Therefore the influence of soil management practices on SOC sequestration is not from any single factor or process, but an integration of their interactions. Soil management practices indirectly modify SOC through changes in plant type and associated microbial community structure (Frey et al., 2001; Jackson et al., 2003; Six et al., 2006; Allison et

al., 2007; Gonzalez-Chavez et al., 2010) and soil aggregate formation (Miller and Jastrow, 1990; Bossuyt et al., 2001; Rillig et al., 2003; Zhu and Miller, 2003; Mikha and Rice, 2004; White and Rice, 2009; Wilson et al., 2009).

The ultimate effect of soil management on SOC sequestration is the interaction of factors that control SOC mineralization or stabilization. One such interaction exists between plants roots, AMF and soil. The root-AMF-soil association interacts to provide unique physical protection to facilitate SOC stabilization. For example, (Jastrow and Miller, 1998) hypothesized that arbuscular mycorrhizal (AM) fungal hyphae act as a biological web, encasing soil aggregates. The C thus protected within the aggregates is occluded from microbial decomposition, thus increasing its resident time in soil, hence increase SOC sequestration. Soil management which enhances soil fungi (saprophytic and arbuscular mycorrhizal fungi), such as reduce tillage; have been reported as important in SOC sequestration through the influence on soil aggregation. The degree of SOC protection from microbial decomposition is important in SOC dynamics (Jastrow et al., 2000).

Several conceptual models have been proposed for the relationship between SOC and soil aggregation (Tisdall and Oades, 1982; Oades, 1984; Elliott, 1986; Six et al., 1998). The hierarchical nature of soil is made possible by binding agents of either transient (readily decomposable organic materials of microbial and plant derive polysaccharides) or temporary binding agents of primarily fibrous roots and hyphae with microaggregates forming within macroaggregates which are released upon macroaggregate disruption. Soil aggregation separates SOC from microbial biomass and

reduces SOC mineralization. Six et al. (2004b) identified macroaggregate turnover as a crucial process in SOC sequestration. Soil aggregate distribution is a strong indicator of C sequestration. Macroaggregates contain greater concentrations of C than microaggregates (Six et al., 2000b; Mikha and Rice, 2004; White and Rice, 2009). Soil management such as tillage which disrupts aggregates have a negative impact on soil sequestration potential. Therefore the objective of our study was to assess the biophysical controls on SOC dynamics as influenced by perennial and annual plant management practices of a silty clay loam soil.

MATERIALS AND METHODS

Site description and characteristics

The field experiment was located at the Konza Prairie Biological Station (KPBS) near Manhattan, KS (39° 6'28.44"N, 96°36'31.28"W). The soil was a silty clay loam described as Fine, smectitic, mesic aquertic Argiudolls. The climate is continental and characterized by warm, wet summers and dry, cold winters. Mean annual precipitation is 830 mm per year. Mean monthly air temperature varies from -3^{o}C in January to 27^{o}C in July. Mean monthly soil temperature to 5 cm depth varies from 1.6^{o}C in January to 29.3 ^{o}C in July (Jangid et al., 2010).

The field was in agricultural production of winter wheat (*Triticum aestivum*), a C_3 plant, for a minimum of 20 years prior to the establishment of this experimental field in 2004. The C_4 plants for this experiment were: grain sorghum (*Sorghum bicolor*) planted either in continuous tillage (CT), or no-tillage (NT), and replanted prairie grass,

Andropogon gerardii (RP). The field was tilled and fertilized yearly as part of normal agricultural production prior to the establishment of the research plots. At commencement of the research study and 6 y after establishment, soil chemical properties were assessed (Table 4.1). The prairie grass was planted in 2004 after initial tillage of the whole experimental field. Grain sorghum (*Sorghastrum bicolor*, Pioneer 84G62) was planted annually at a density of 60,000-70,000 seeds ha^{-1}. Initially, fertilizer N (urea) was applied annually at a rate of 120 kg N ha^{-1} to all the treatments. However due to a management glitch, fertilizer N was not applied in 2008. Additionally, N application was discontinued in the replanted prairie in 2010 to reflect the natural condition of this ecosystem. Herbicide (glyphosate) was applied annually prior to planting to suppress weeds in the sorghum plots, and care was taken to avoid the herbicide application to the prairie grass. The CT plots were chisel plowed each fall and disk plowed each spring prior to planting. The RP plots were burned annually in the spring.

Field experiment design

The field experiment was a split-plot design, organized as a randomized complete bock with main plots being the ecosystems (CT, NT and RP). As subplots, half of each plot was amended with phosphorus at 90 kg P ha^{-1} (+P) applied annually in the spring while the other half, control, did not receive P (-P). Each treatment had 4 replicates. The 90 kg ha^{-1} P application rate had been reported to repress AM fungal colonization and activity in greenhouse studies (Hartnett et al., 1993). The size of each plot was 6 x 6 m (36 m^2). However it should also be noted that due the same management glitch as earlier stated, P was not applied in 2008.

Above and belowground plant C input

Carbon inputs into the ecosystem were assessed from 2008 through 2010, by quantifying above- and belowground plant biomass. Plant biomass samples were collected at the end of October or early November following plant senescence, and prior to fall chisel plow. Above ground biomass was estimated by harvesting and quantifying aboveground production from 6 m of row constituting two middle rows of sorghum planted in CT or NT. The biomass in RP was estimated by randomly placing a 0.25 m^2 quadrant in three different areas in each plot and clipping the vegetation at ground level. Aboveground material was weighed and a sub-sample dried at 60°C for 48 hr. Following above ground biomass harvest, root samples were collected using a 7.5-cm diameter x 7.5-cm height (deep) corer. Cores were taken from three locations within each plot. In the NT and CT plots one root core was taken directly on top of a cut sorghum plant, another in the middle of the row and a third half the distance between cut plant and row. In the RP plots one root core was taken directly on top of a clipped prairie grass bunch then a second on an adjacent plant-less area then a third half the distance back to the clipped prairie grass bunch. Soil cores were kept at 4^{o}C until roots were separated from the soils. Soil was removed from roots by washing in water; roots from each plot were combined, weighted and a subsample reserved for estimation of % root length AMF colonization. The remainder of the roots were dried at 60°C for two days and weighed for estimation of belowground biomass. Corrections were included in calculations to account for sub-samples when estimating total root biomass.

Percent root AMF colonization

The % root AMF colonization was assessed from 2004 through 2010. Subsample of roots were used to determine % root length AM fungi colonization by staining the subsample of roots with trypan blue using the method of (Koske and Gemma, 1989). Scoring for percentage root length colonization by AMF was accomplished using the magnified gridline intersect method (Johnson et al., 2003).

Soil sampling and analysis

Thirty random samples were collected from each plot using a 2-cm soil auger reaching 30 cm depth. Each 30 cm soil core was cut into depth increments of 0-5, 5-15 and 15-30 cm lengths and each depth composited for each plot. Soil samples were transported to the laboratory immediately. Soil (10g) was placed in diluvials (Fisher Scientific, Pittsburgh, PA-USA) and frozen for PLFA analysis while the remainder was stored in cooler (4° C) until analysis.

Soil organic C and total N

Soil organic C (SOC) and total N (TN) was assessed for 2007 through 2010. A subsample (10 g) was air-dried, ground to a fine powder, and analyzed for SOC and TN by dry combustion using a gas chromatograph with thermal conductivity detection (GC-TCD) (Thermo Finnegan Flash EA1112, Milan, Italy.

Aggregate size distribution and aggregate associated C and N

Water-stable aggregate size distribution was assessed for 2007 through 2010 by wet sieving two replicates of 50 g air-dried soil through 20, 53, 250 and 2000 μm sieves

with a Yoder-type apparatus as described by (Mikha and Rice, 2004). Air dried soil samples were placed over stacked 250 and 2000 μm sieves and submersed in water for 10 min (slaking phase) and then subjected to 10 min of 4 cm length oscillations at a frequency of 0.5 Hz. The soil remaining on the sieves was collected and allowed to settle prior to drying. The soil that passed both sieves was filtered through the 53 and 20 μm sieves. One of each replicated soil was dried at either 60^{o}C or 105^{o}C for 2d. The dried soil was weighted and used for estimating % aggregate size fraction of soil and later used for sand correction determination. The soil from each fraction was corrected for sand content by dispersing the organic matter and sand using sodium hexametaphosphate. Sub-samples dried at 60^{o}C were ground with mortar and pestle, and analyzed for aggregate-associated C and N.

Microbial community dynamics (Phospholipid fatty acid analysis)

Changes in soil microbial community were assessed for 2009 and 2010 by measuring phospholipid and neutral lipid fatty acids (PLFA and NLFA) of soil samples. The method of (Bligh and Dyer, 1959) as modified by (White and Ringlberg, 1998) was used to extract soil total lipids. The lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution for 3h from 5g of ground lyophilized soil. The total lipids extracts were then separated into neutral lipids (NLFA), polar lipids (PLFA) and glycolipids by silicic acid chromagraphy using pre-conditioned disposable silica gel columns (J.T. Baker, Phillipsburg, NJ, USA). The neutral and polar lipids were then cleaved by alkaline methanolyis (KOH saponification and methylation) which cleaves the fatly acids from the glycerol back bone replacing it with methyl groups to

form fatty acid methyl esters (FAMES) (Allison, 2005). The resulting FAMES were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer with a DB5-MS column (30 m × 250 μm i.d. × 0.25 μm film thickness). Helium was the carrier gas (1.0 mL min^{-1} constant flow). The temperature program was: 50 to 170°C at 20°C per min^{-1}; from 170 to 270°C at 2°C min^{-1}. The injector temperature was 220°C. Analysis was conducted in the electron impact (70 eV) mode and mass spectrometer scanning m/z^{+} was from 200 to 400. Bacterial acid methyl esters mix (BAME; Matreya 1114) was used to identify peaks. Tentative assignments of methyl ester peaks not present in the BAME mix were made by mass spectral interpretation. The internal standard methyl nonadecanoate was used to quantify the data. Sample peaks were quantified based on comparison of the abundance with an internal standard - nonadecanoic acid methyl ester (19:0). The abundance was expressed as nmol g^{-1} dry soil but converted to mole % before statistical analysis. Nomenclature of identified fatty acids (FA) was as described by Bossio and Scow, 1998, whereby FA were designated ***a:b***, where ***a*** is the total number of carbons and ***b*** the number of double bonds. An ***ω*** indicates the position of a double bond from the aliphatic end of the FA. The prefixes ***a*** and ***i*** refer to ***anteiso*** and ***iso*** branching, while the suffixes ***c*** and ***t*** refers to ***cis*** and ***trans*** isomers (conformations). Presence of methyl groups are indicated by ***aMe***, where ***a*** indicates the position of the methyl group. Fatty acids were grouped based on criteria by (McKinley et al., 2005) whereby Gram positive bacteria were i15:0, a15:0, 10Me16:0, i17:0 and a17:0. Gram negative bacteria as 18:1ω7c and cy19:0, while actinomycetes is 10Me18:0 and 10Me17:0, and fungi 18:2 ω 6,9c and 18:1 ω 9c.

Statistical analysis

The experiment was analyzed as a split plot in a complete randomized block design with ecosystem (CT, NT, and RP) as the main plots, and P amendment (+P and –P) as the subplot. The analysis of variance was conducted using the PROC MIXED procedure in SAS version 9.3 (Cary, N.C.) to assess differences in SOC and TN of soil, % AMF root colonization, plant above ground biomass, root biomass, mass of soil aggregate fractions, C and N concentration in aggregate fractions. Analysis was performed by soil depth. Means were considered statistically significant at $P<0.05$ except indicated otherwise. Means were compared using LSD. A correlation analysis was performed using SAS PROC CORR (SAS Institute) to determine the correlations between amount of soil aggregate and mass of SOC for each ecosystem.

RESULTS

Above- and belowground plant biomass

A significant ($P<0.0001$) ecosystem x time interaction was noted for the above ground plant biomass (Table 4.2) The aboveground biomass in 2008 for RP, was higher than NT which was similar to CT. Biomass for 2010 was higher in CT than RP and NT which were similar (Fig. 4.1). Reduced biomass in the annual systems were due to lack of N fertilizer applications. Precipitation decreased through the period (Fig. C-4.1), reducing production of the perennial grass in 2009 and 2010.

Root biomass was significantly ($P<0.0026$) greater for RP than NT or CT which were similar (Table 4.3). Root biomass was also significantly ($P<0.0001$) higher in 2010

than 2008 or 2009 which were similar (Table 4.3). The greater root biomass for prairie grass than sorghum, was expected, because the roots of perennial plants have indeterminate growth while the roots of annual sorghum requires time to establish and growth is restricted to a few months of the year.

The total biomass (aboveground and root biomass-up to 7.5 cm) was significantly ($P<0.0001$) influenced by an ecosystem x time interaction. Total biomass was greater in RP than NT or CT for all years (Fig. 4.2). However, in 2008 both NT and CT had similar biomasses while in 2009, biomass was higher in NT than CT, but in 2010 biomass was higher in CT than NT (Fig. 4.2).

When estimating plant biomass returned to soil after burning losses from RP were considered, there was no significant ($P<0.62$) ecosystem effect. This result may indicate that burning losses in RP were compensated for by root biomass, resulting in similar total biomass entering soil for both the perennial and annual ecosystems up to 7.5 cm soil depth. These results may suppose that in the annual systems, NT could be more stable than CT with regards to nutrient conservation. Reduced precipitation might have resulted in the reduced aboveground biomass in RP.

Soil organic C and total N

Soil organic C (SOC) at 0-5 soil depth was significantly ($P<0.0002$) greater in RP and NT than CT. (Table 4.5). Soil OC increased at a rate of 0.91 Mg ha^{-1} y^{-1} for the perennial ecosystem (RP), 0.8 Mg ha^{-1} y^{-1} for NT and 0.37 Mg ha^{-1} y^{-1} for CT (Fig. 4.3). The similar rate of SOC increase between NT and RP indicates that less soil disturbance

resulted in better SOC stabilization. At 5-15 cm depth, P addition significantly ($P<0.02$) reduced SOC (Table 4.6).

Similar to SOC, total N (TN) was significantly ($P<0.0001$) greater in RP and NT than CT at the 0-5 cm depth (Table 4.9). At 15-15 cm depth, sorghum planted in NT and CT had significantly ($P<0.03$) greater TN than the prairie grass (Table 4.10).

Percent root length AMF colonization

The % root length AMF colonization significantly ($P<0.04$) responded to an ecosystem x P interaction (Table 4.13). The P addition reduced % root AMF in the less disturbed NT and RP, but not in the more disturbed CT (Fig. 4.4). This indicates that tillage caused root breakage and hyphal disruption resulting in lack of P amendment effect in CT.

A significant ($P<0.018$) ecosystem x time interaction indicated that % root colonization for RP was consistently higher over time than in the agricultural systems (Fig. 4.5). A depression in % colonization in 2007 and 2008 was observed for all ecosystems in 2007 than 2008 (Fig. 4.5).

The % root AMF was also significantly ($P<0.0005$) influenced by a P x time interaction. The addition of P reduced % root AMF colonization between 2005 and 2008. However, in 2004 and post 2008 there was no effect of P addition (Fig. 4.6).

These results confirm the mycotropic nature of the prairie grass, with high dependence on AMF association. However the results revealed that reduced disturbance may enhance AMF root association in sorghum. The lack of significant effect on % AMF

colonization with P addition in CT may suggest that soil mixing could have disrupted AMF hyphae and roots in CT.

Sand-free water stable aggregate fractions

At 0-5 cm depth, the macroaggregate >2000 µm was significantly (P<0.0558) influenced by an ecosystem x time interaction (Table 4.14). Macroaggregate increased over time for all ecosystems; however macroaggregates were significantly higher in RP than the agricultural ecosystems. At the 5-15 cm, the >2000 µm aggregate was independently influenced by a significant (P<0.0087) ecosystem and significant (P<0.0001) time effects (Table 4.15). The >2000 µm aggregate was greater in RP than the agricultural ecosystems (Table 4.15)

Additionally, the macroaggreges >2000 µm responded significantly (P<0.0048) to a P x time interaction at 5-15 cm (Table 4.15) which indicated no effect of P addition prior to 2009, but a decrease with P addition in 2010 (Fig. 4.9).

At the 0-5 cm depth, macroaggregates >250 µm indicated a significant (P<0.0016) ecosystem x time interaction (Table 4.14). The aggregate amounts for RP and NT increased with time while CT was stable (Fig. 4.10a). At the 5-15 cm depth, the >250 µm was influenced by a significant (P<0.0087) ecosystem and (P<0.0001) time effects (Table 4.15). Aggregate >250 were significantly higher in RP (Table 4.15).

At the 0-5 cm, the microaggregate <250 µm size was significantly (P<0.0001) influenced by an ecosystem x time interaction (Table 4.14). A decreasing trend in aggregate amounts was observed for all the ecosystems; however aggregate amounts were significantly lower with less soil disturbace (Fig. 4.7a). At 5-15 cm, aggregate <250

μm was significantly (P<0.0001) influenced by an ecosystem x time interaction (Table 4.15). Microaggregates in RP and NT decreased with time, while CT increased (Fig. 4.7b).

Concentrations of C and N in each aggregate size fraction

The changes in C and N concentrations of aggregates are reported in pairs of microaggregates (20-53 or 53-250 μm) and the corresponding macroaggregate (250-2000 or >2000 μm) at each soil depth. The aggregate associated-C or N for 20-53 μm is paired with 250-2000 μm, while the 53 μm is paired with >2000 μm.

At the 0-5 cm depth (Table 4.16), the C in 20-53 μm significantly (P<0.0058) reduced over time (Fig. 4.11a), while the C in 250-2000 significantly (P<0.01) increased in RP and NT but reduced in CT (Fig. 4.11b). The C in 53-250 μm significantly (P<0.01) reduced for all ecosystems, while C in >2000 μm was significantly (P<0.02) lower in 2007(Table 4.16).

At the 5-15 cm depth (Table 4.17), the C in 20-53 μm significantly (P<0.0001) reduced (Fig. 4.13a) The C in 53-250 μm significantly (P<0.0001) increased (Fig. 4.14a), while C in >2000 μm was significantly (P<0.01) higher in 2007 for RP than NT or CT and lower in CT than RP or NT in 2009 with less soil disturbance (Fig. 4.14b).

At the 0-5 cm (Table 4.18), N in 20-53 μm significantly (P<0.0001) reduced (Fig. 4.15a); but the N in 250-2000 μm significantly (P<0.0001) increased with time (Fig. 4.15b). The N concentration significantly increased with time in both the 53-250 μm (P<0.0001) (Fig. 4.16a) and >2000 μm (P<0.0001) (Fig. 4.16b).

At the 5-15 cm (Table 19), the N concentration in 20-53 µm significantly ($P<0.0001$) reduce with time (Fig. 4.17a), but N concentration in 250-2000 µm significantly ($P<0.0048$) increased for all ecosystems; however, N concentration was higher in NT than either CT or RP in 2010 (Fig. 4.17b).

In addition, at the 5-15 cm the N concentration in 250-2000 µm indicated a significant ($P<0.028$) ecosystem x P x time interaction (Table 4.19). In 2010, P addition reduced N concentrations in CT, increased in NT with no effect in RP (Fig. 4. 19).

Microbial community structure

The specific abundance of PLFA showed significant ($P<0.05$) ecosystem x time interaction effects for all microbial groups assessed except Gram- bacteria at 0-5 cm depth (Table 4.20). The AMF was highest for RP in 2010 (Fig. 4.20c) which was higher than both NT (Fig. 4.20b) and CT (Fig. 4.20a). The RP had highest total fungal biomass; NT may be more stable than CT.

The relative PLFA abundance was significantly influenced by an ecosystem effect except for actinomycetes at 0-5 cm depth (Table 4.21). The trend for relative fungal and total fungal abundance was RP>NT>CT (Fig. 4.21b). The relative abundance of AMF was higher with less soil disturbance. The Gram- bacteria was relatively more abundant in NT than CT and RP which were similar. Relative abundance of Gram+ bacteria was highest in the agricultural systems (Fig. 4.21b).

A significant ($P<0.027$) ecosystem x fertilizer interaction effect on specific AMF abundance at 5-15 (Table 4.22) indicated that P addition decreased AMF abundance in CT which was increased in RP but no effect in NT (Fig. 4.22). A significant ($P<0.05$)

interaction of ecosystem x fertilizer effect on total PLFA at 5-15 (Table 4.22) was such that, P addition reduced PLFA in RP but no effect on sorghum either in CT or NT (Fig. 4.23). This result may indicate the significant contribution of fungal biomass to total soil microbial biomass in the native ecosystem and also the influence that P amendment could have on the fungal biomass. Overall, given the high variability in specific PLFA abundances, results indicated no significant difference between ecosystems for various microbial groups at 5-15 cm (Fig. 4.24a). Relative AMF, fungi and total fungal abundances at 5-15 cm was significant ($P<0.05$) influenced by ecosystem (Table 4.23) such that they were all highest in the native ecosystem than agricultural ecosystems (Fig. 4.24b).

Relationship between total C and macroaggregate

The relationship between SOC and macroaggregates (>250 μm) for the different ecosystems is shown in Fig. 4.25. The macroaggregate was strongly correlated with SOC in RP and explaining 86 % of total variation. However, in the cultivated ecosystems macroaggregate explained 42 % of total variation in SOC for NT but the correlation was poor in CT. These results demonstrate that macroaggregate protected SOC in less disturbed ecosystems (RP and NT) increasing C sequestration relative to CT

DISCUSSION

The less disturbed RP and NT had higher SOC (Table 4.5) and TN (Table 4.9) than the more disturbed CT. Other researchers have reported similar results (Six et al., 1999; West and Post, 2002; Fabrizzi et al., 2003; Mikha and Rice, 2004; White and Rice,

2009). Some studies however, did not find differences in SOC between tillage practices of cultivated ecosystems (Angers et al., 1997; Dolan et al., 2006; Machado et al., 2006; Powlson et al., 2011). The differences in observed results could be attributed to inherent soil properties and environmental conditions that may influence factors that control SOC such as clay content or precipitation that affect primary productivity. Major factors controlling SOC in addition to residue returned to soil and clay content, include physical protection from microbial activities (Rice and Angle, 2004) and soil disturbance.

After 7 y, the rate of SOC increase for NT was at the higher end of the range 0.3 - 1 Mg C $ha^{-1}y^{-1}$ reported in the literature for croplands (Follett et al., 2010; Sundermeier et al., 2010). Higher sequestration rates are expected when annual croplands are converted to perennial grass for conservation purposes or pastures (Follett, 2001b) which could range from (0.3 to 1.4 Mg C ha^{-1} y^{-1}) (Derner and Schman, 2007). The RP rate of SOC sequestration was within the estimated range for perennial grasses, however, the similar sequestration rate between NT and RP was not expected. This could imply that under current global food security concerns, cultivation in NT may provide similar C benefits as taking land out of food production for conservation purposes.

Carbon sequestration in soil was influenced by soil disturbance and crop residue quantity and quality. Our above- and belowground biomass results indicated differences in ecosystem responses to changes in environmental conditions. Our results indicated an initial aboveground biomass of 830 g m^{-2} for RP similar to 700 g m^{-2} reported by Wilson et al. (2009) with N addition, which was about 3 times the biomass of the sorghum either in NT (274 g m^{-2}) or CT (279 g m^{-2}). However, when N application for RP was

discontinued, productivity for RP reduced to within 200 - 400 g m^{-2} similar to less than 400 g m^{-2} reported for a restored prairie without N addition (Baer et al., 2002). In the agricultural systems however, biomass in NT remained stable while the biomass for CT fluctuated. NT may be more N-conservative and stable than CT. If this be the case, then, mineralization of organic matter by microbes for their N requirements may have contributed to lower C in CT than NT.

The mean total biomass for all the ecosystems entering our soil up to 7.5 cm depth was not significantly different between ecosystems (Table 4.13). Therefore other factors other than residue quantity or quality probably influence SOC stabilization to account for the higher but similar SOC in the less disturbed RP and NT relative to CT. Additionally, root-C which has been reported to have a longer soil residence time (Rasse et al., 2005), due to higher shoot tissue decomposability (Aerts and Chapin, 2000; De Deyn et al., 2008) could not explain the similar SOC between NT and RP. Similarly, NT had higher SOC relative to CT despite similar residue type and amounts.

The ecosystem response to the influence of P addition on root AMF colonization indicated differences within the agricultural systems and similarities between NT and RP. The P addition reduced AMF root length colonized in both RP and NT, but not CT (Fig. 4.4). Similarly, changes in % root length colonized by AMF over time indicated that colonization was higher in NT than CT (Fig. 4.5). The AMF root colonization has been reported to influence soil aggregate formation (Wilson et al., 2009), which was similar between RP and NT than CT. This could indicate that tillage breaks roots and disrupt AMF hyphae which is supposed to improve soil structure that protects SOC. Tillage has

been reported to disrupt roots and AMF hyphae (Kabir et al., 1998; Jansa et al., 2003; Jansa and Frossard, 2006). The proportion of macroaggregate >2000 μm (25 g) for RP after 7 y was comparable to 21.8 g reported by Wilson et al., (2009) for a native prairie close to the experimental site.

It has been widely reported that microbial biomass can be altered by tillage and higher under NT than CT (Follett and Schimel, 1989; Frey et al., 1999; Watson and Rice, 2004; Fabrizzi, 2006; Helgason et al., 2010). Total microbial biomass estimated as PLFA was greater in the RP than under the cultivated NT and CT (Table 4.21). However, the RP also had highest total fungal biomass and relative fungal abundance at 0-5 cm which was lowest in CT (Fig 4.17a). Higher fungal biomass in NT relative to CT is attributed to better hyphal establishment with reduced hyphal breakage and accumulation of surface residue which requires hyphal proliferation to establish contact with residue (Beare et al., 1992; Frey et al., 1999). Although increased fungal biomass has been reported to increase macroaggregate formation (Miller and Jastrow, 1990; Jastrow et al., 2007), the fungal biomass was not consistent through the years, hence difficulties in correlating fungal biomass and aggregation in our study.

Contrary to our expectation, the Gram- bacterial biomass was not different between ecosystems and relative abundance of Gram+ bacteria was higher in the cultivated NT and CT than RP (Fig. 4.17b). The CT was expected to be dominated by bacteria because tilling mixes the litter into soil creating direct contact which favors bacterial growth (Beare et al., 1992). Similar findings of bacterial dominance in CT and NT but higher absolute fungal biomass in NT have been reported (Frey et al., 1999). The

shifts in microbial communities of the various ecosystems resulting from soil management could have implications on soil C stabilization and aggregate formation.

Increase in macroaggregate in NT has been reported (Beare et al., 1994; Mikha and Rice, 2004; Wright and Hons, 2005). The two largest aggregate sizes exhibited the greatest SOC storage in RP and NT at 0-5 cm. The stabilization of SOC in aggregates is an important mechanism which has been recognized to influence SOC sequestration in different management practices. Our results indicated that amount of macroaggregates >250 μm increased with less soil disturbance (Fig. 4.7) with concurrent decrease in the <250 μm size fraction (Fig 4.6).

When comparing the less disturbed systems, the macroaggregates was higher in the native RP than NT. Similar results have been reported (Fabrizzi et al., 2009). The difference has been related to higher fungal biomass in native RP than cultivated NT which was reported in our study. The relative total fungal abundance was higher in RP relative to NT. Such differences can also be due to differences in residue quality (Franzluebbers et al., 1995; Martens, 2000; Wright and Hons, 2005) and also root architecture (Carter et al., 1994) and biomass which was reportedly higher in RP than NT. When the cultivated ecosystems were compared, NT had higher macroaggregates than CT. Similar results have been reported in literature (Mikha and Rice, 2004; Six et al., 1999; McVay et al., 2006; Fabrizzi et al., 2009). The higher amount of macroaggregate in NT certainly contributed to higher SOC in NT relative to CT because macroaggregates > 250 μm were better correlated with SOC in RP and NT than in CT (Fig 4.25).

The location of SOC in the soil structure has been shown to control SOC dynamics (Oades, 1993; Six et al., 2004a; Jastrow et al., 2007). The higher macroaggregates in the less disturbed RP and NT may have contributed to higher SOC in these ecosystems relative to CT. Tillage breaks up soil aggregates, mixing up crop residue with soil microbes and facilitating SOC mineralization; processes that reduce SOC. Also in CT the continuous plowing progressively exposes aggregates to rainfall action, freeze and thaw cycles which further renders protected SOC in aggregates to greater C oxidation and biodegradation (Six et al., 1998; Balesdent et al., 2000). In addition, under NT, soil is protected by surface residue which reduces the rate of aggregate disruption thus increasing SOC sequestration rates comparable to RP and higher than in CT. Tillage also affects soil microbes by fragmenting plant roots which also contribute to soil aggregate formation and stabilization. Additionally, heat flux which is lower in NT than CT, causes cooler soils under NT (Fabrizzi et al., 2005), which could reduce potential enzymatic activity in NT that is responsible for SOC mineralization (Drury et al., 2004).

We also reported higher C concentrations at 0-5 cm for the macroaggregates between 53-250 μm (Fig. 4.11b) and 250-2000 μm (Fig 4.12 b) in RP and NT over time which reduced in CT. These results illustrate that C accumulation in aggregates could explain higher sequestration rates with less soil disturbance. Similar results for aggregate-associated C have been reported (Mikha and Rice, 2004; Fabrizzi et al., 2009). This trends in aggregate C change supports the aggregate hierarchy theory proposed by (Six et al., 2000a).

Therefore our study has confirmed the fact that less soil disturbance can significantly increase SOC though SOC stabilization in macraggregates thus increasing macroaggregate-associated C relative to the more disturbed CT. Continuous tillage disrupts aggregates and aggregate forming components such as roots and fungal hyphae, releasing microaggregates and exposing previously occluded SOC to microbial attack thus reducing sequestered SOC.

CONCLUSION

Soil OC was significantly higher with less soil disturbance in RP and NT than CT. The rates of SOC sequestration were similar between RP and NT which was about 3x higher than for CT. Additionally, NT proved more nutrient conservative than CT. The macroaggregate >2000μm in RP after merely 7 yr was comparable to macroaggregate in a native prairie in the same area. Additionally, fungi and AMF were higher with less soil disturbance, implying that less soil disturbance increased fungi and AMF while disturbance severed fungal hyphae, thus reducing macroaggregate formation. The macroaggregate increased SOC by occlusion; while SOC was released upon aggregate disruption in CT, manifested as increase in microaggregate. Overall, better SOC sequestration resulted from increased soil macroaggregate due to increased fungal biomass resulting from less soil disturbance. Therefore implementation of NT can be important both as a practice to ensure global food security but also environmental conservation. It would be interesting to continue examination of the aggregate and SOC dynamic in these soils to assess how long it could take for SOC to reach equilibrium in

the different ecosystems. It would also be important to assess the mineralization kinetics of aggregate-associated SOC in the macroaggregate sizes of the various ecosystems. This could prove informative as to the rate of mineralization of occluded C especially between NT and RP whose residue quality should be different.

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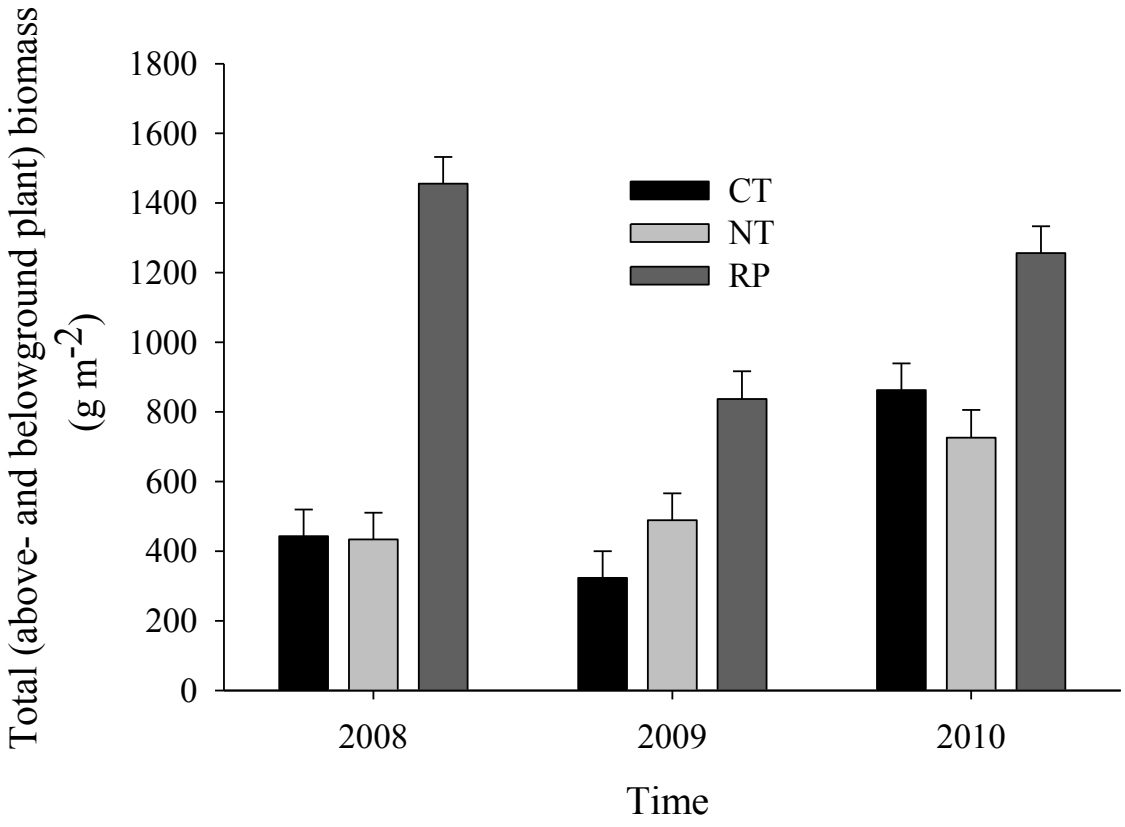

Figure 4.1. Ecosystem x time interaction (P<0.0001) means for above-ground plant biomass of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*) over time.

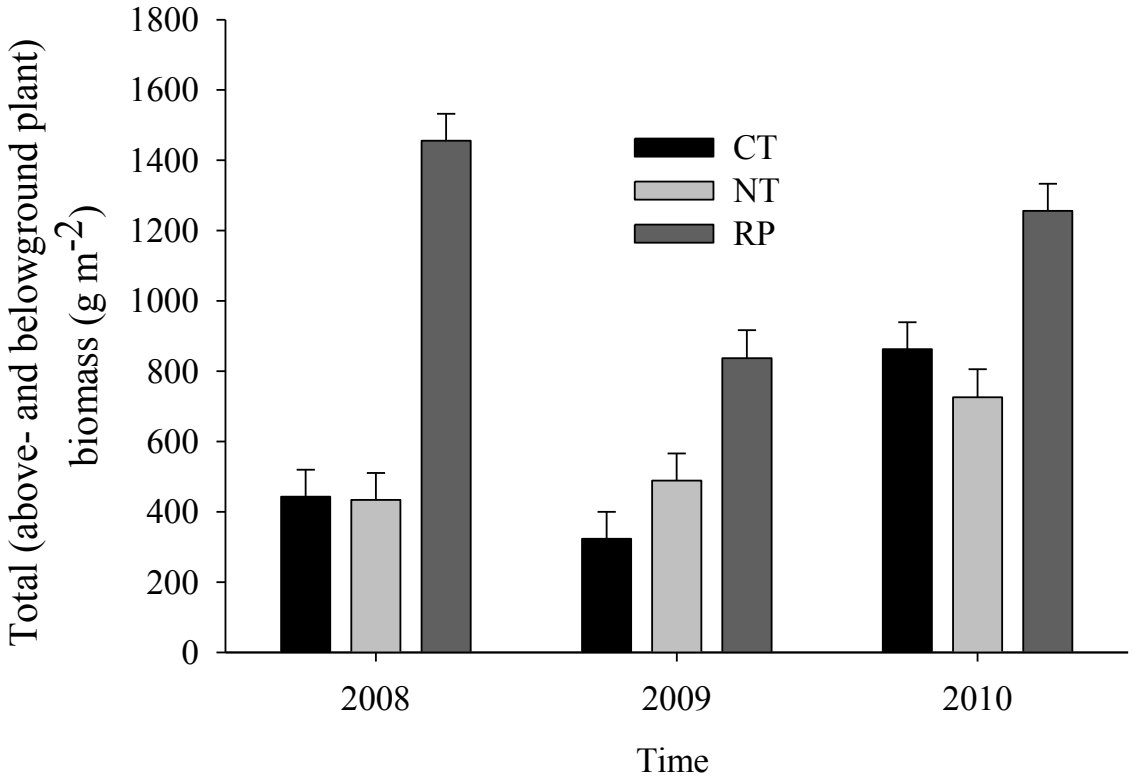

Figure 4.2. Ecosystem x time interaction (P<0.0001) means for total biomass (above- and belowground) of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (RP) (*Andropogon gerardii*) for soil depth of 0-7.5 cm.

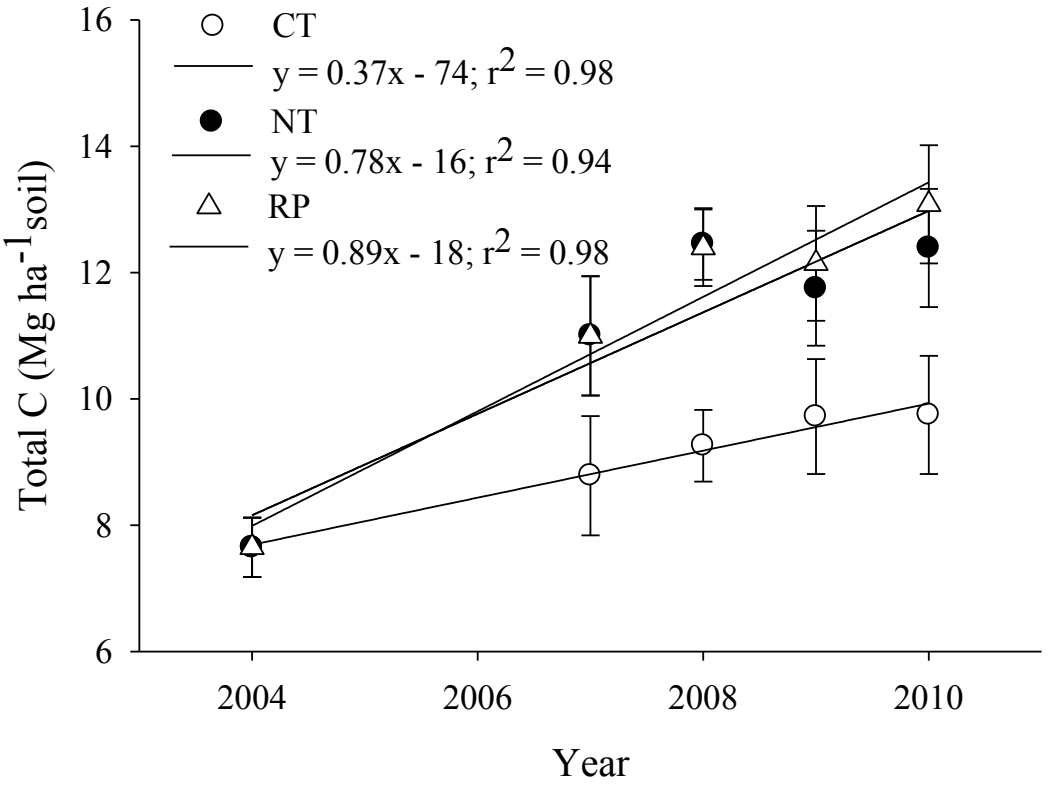

Figure 4.3. Change in soil organic carbon SOC) over time at 0-5 cm depth of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*).

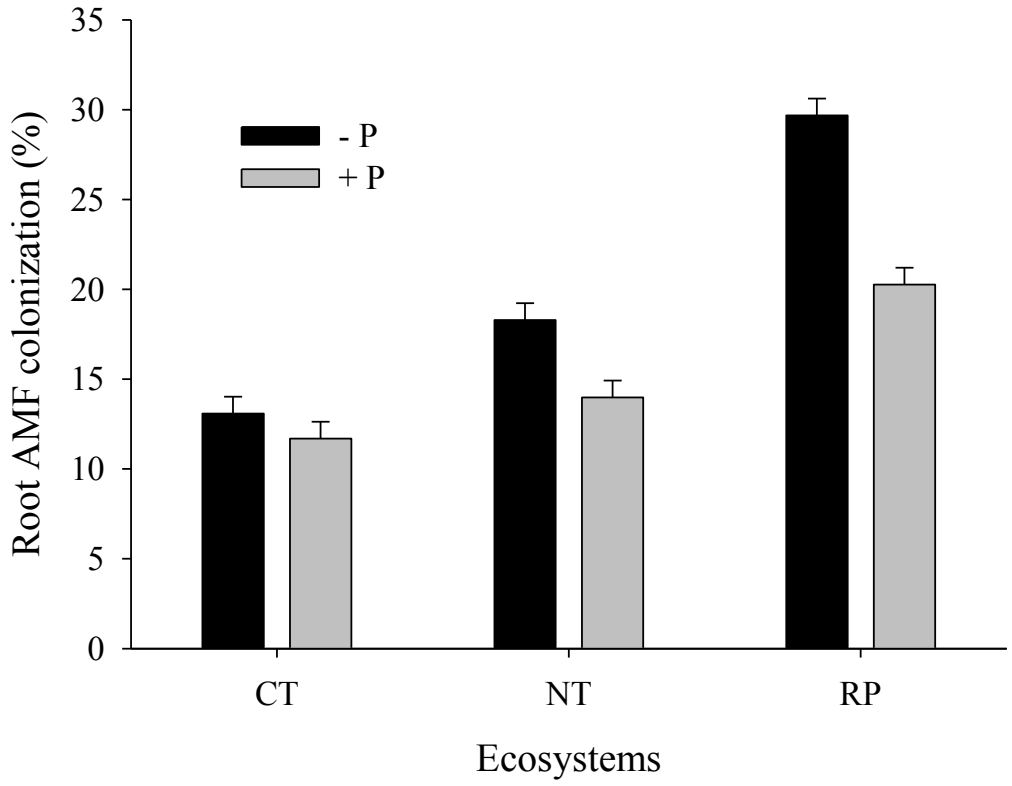

Figure 4.4. Ecosystem x P interaction (P<0.0488) means for percent root arbuscular mycorrhizae fungi (AMF) colonization at 0-7.5 cm depth for soil amended with P and planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*).

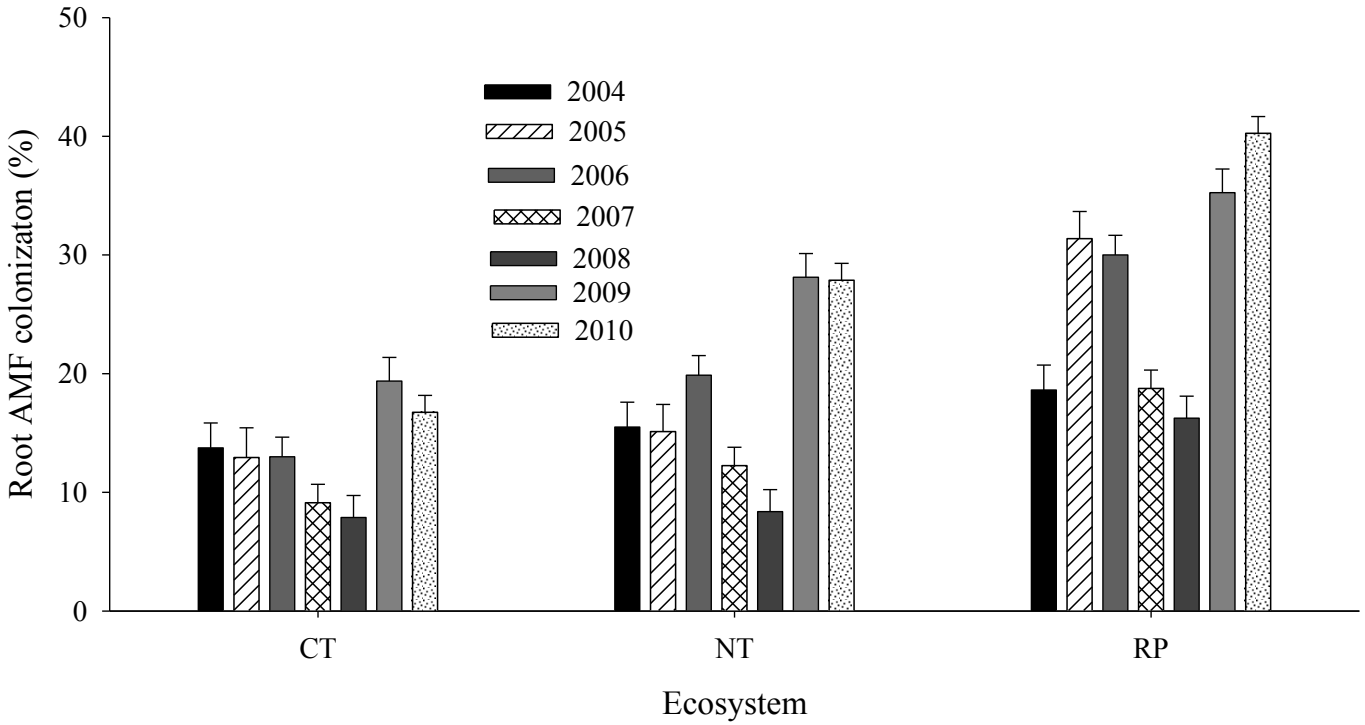

Figure 4.5. Ecosystem x time interaction means (P<0.0184) on % root arbuscular mycorrhizal fungi colonization of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*), planted in a silty clay soil.

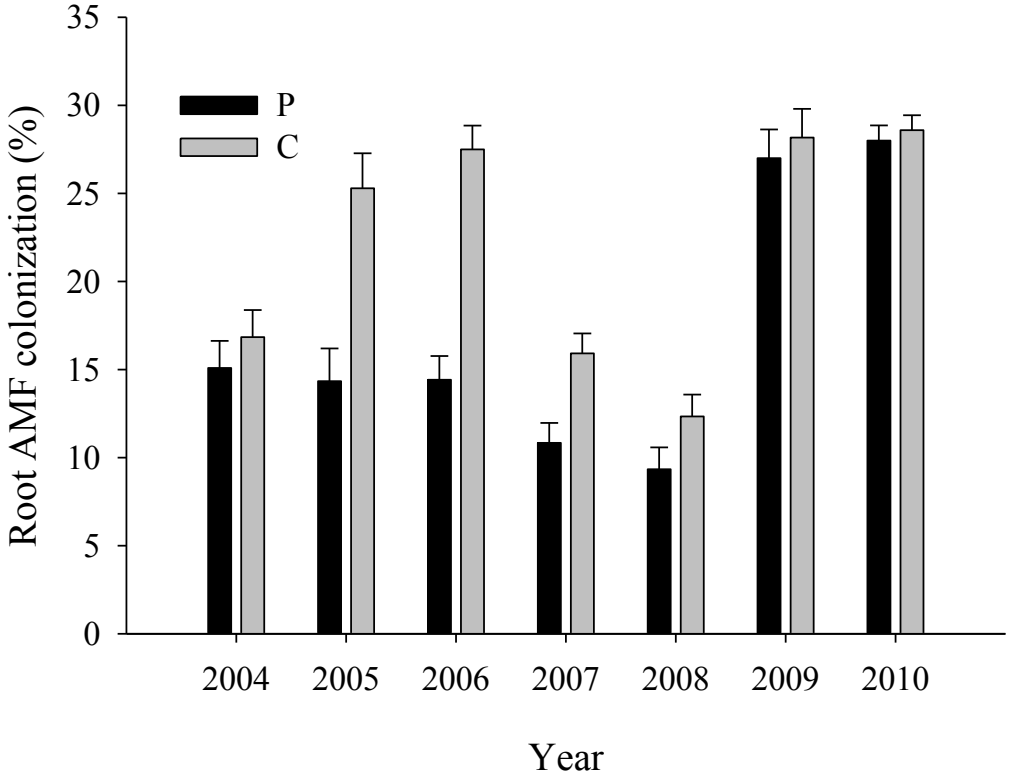

Figure 4.6. Fertilizer P x time interaction means (P<0.0005) of % root arbuscular mycorrhizal fungi colonization for sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*), planted in a silty clay soil.

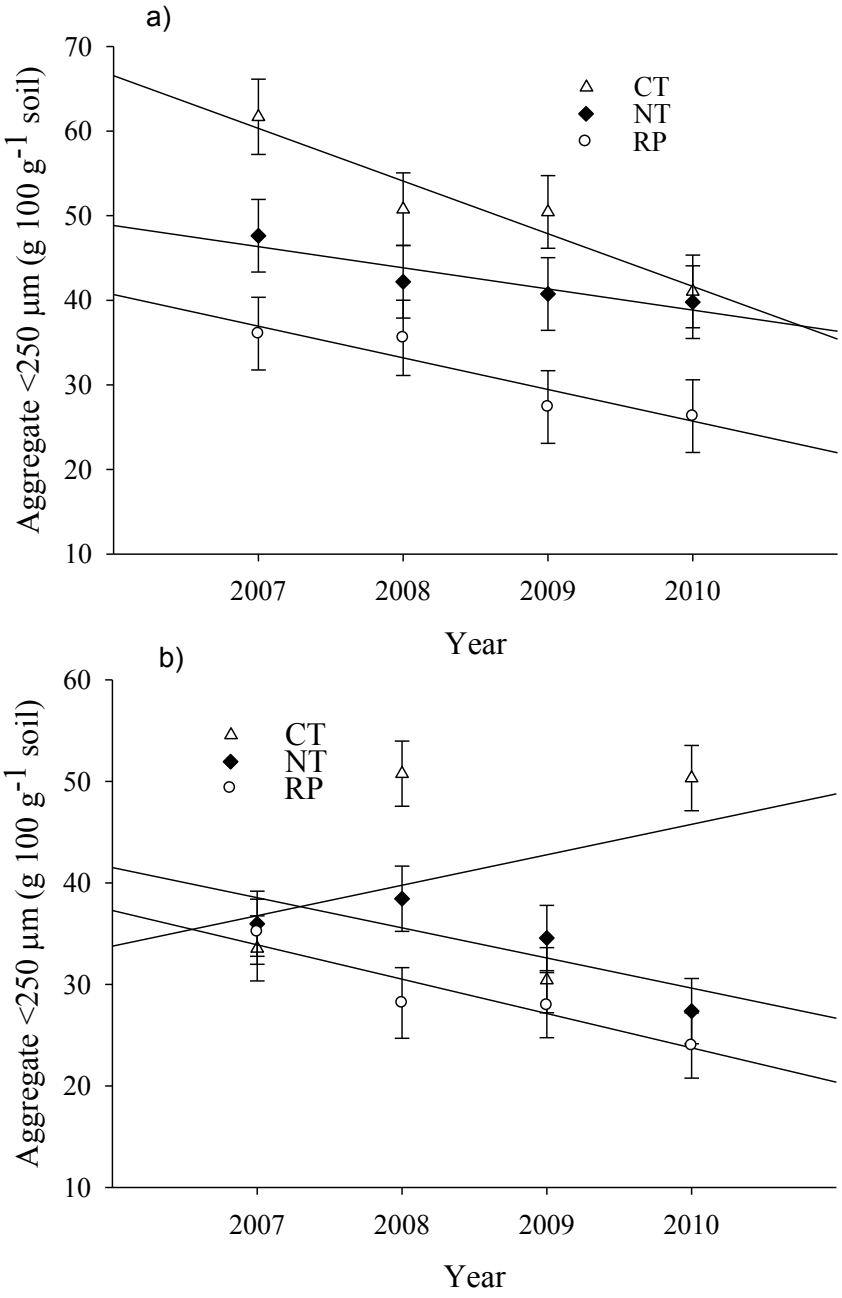

Figure 4.7. Regression analysis for sand-free water stable aggregates (WSA) < 250 µm for (a) 0-5 and (b) 5-15 cm depths in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*).

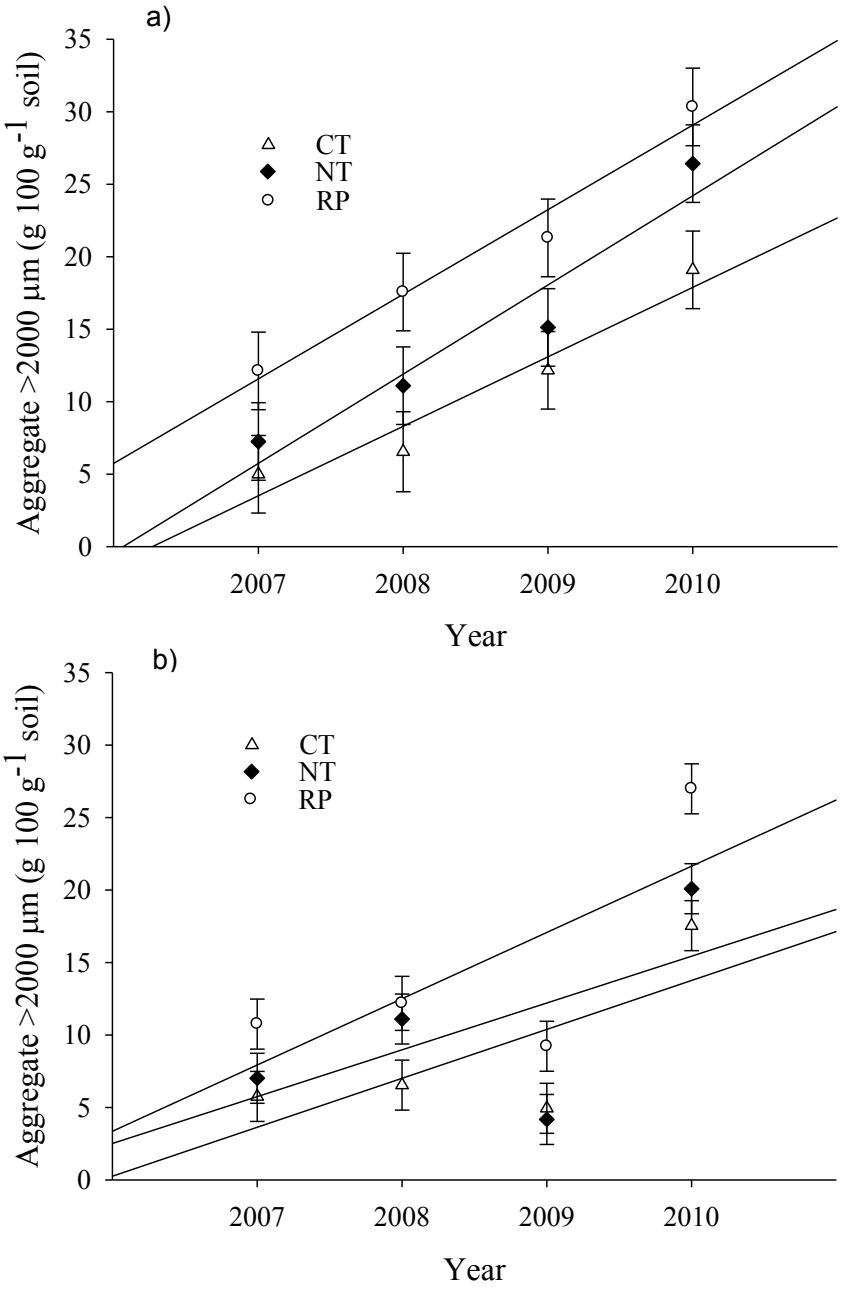

Figure 4.8. Regression analysis for sand-free water stable aggregates (WSA) > 2000 µm for (a) 0-5 and (b) 5-15 cm depths in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

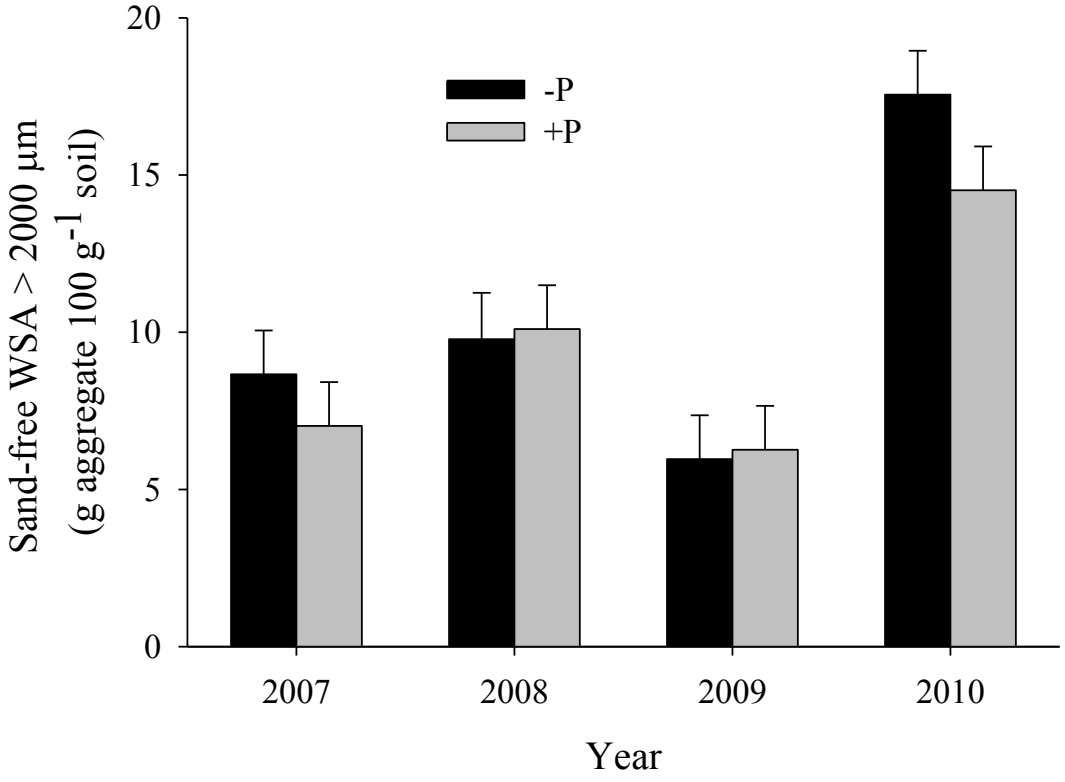

Figure 4.9. Effect of time x P interaction (P<0.0048) means for sand-free water stable aggregates (WSA) >2000 µm at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*).

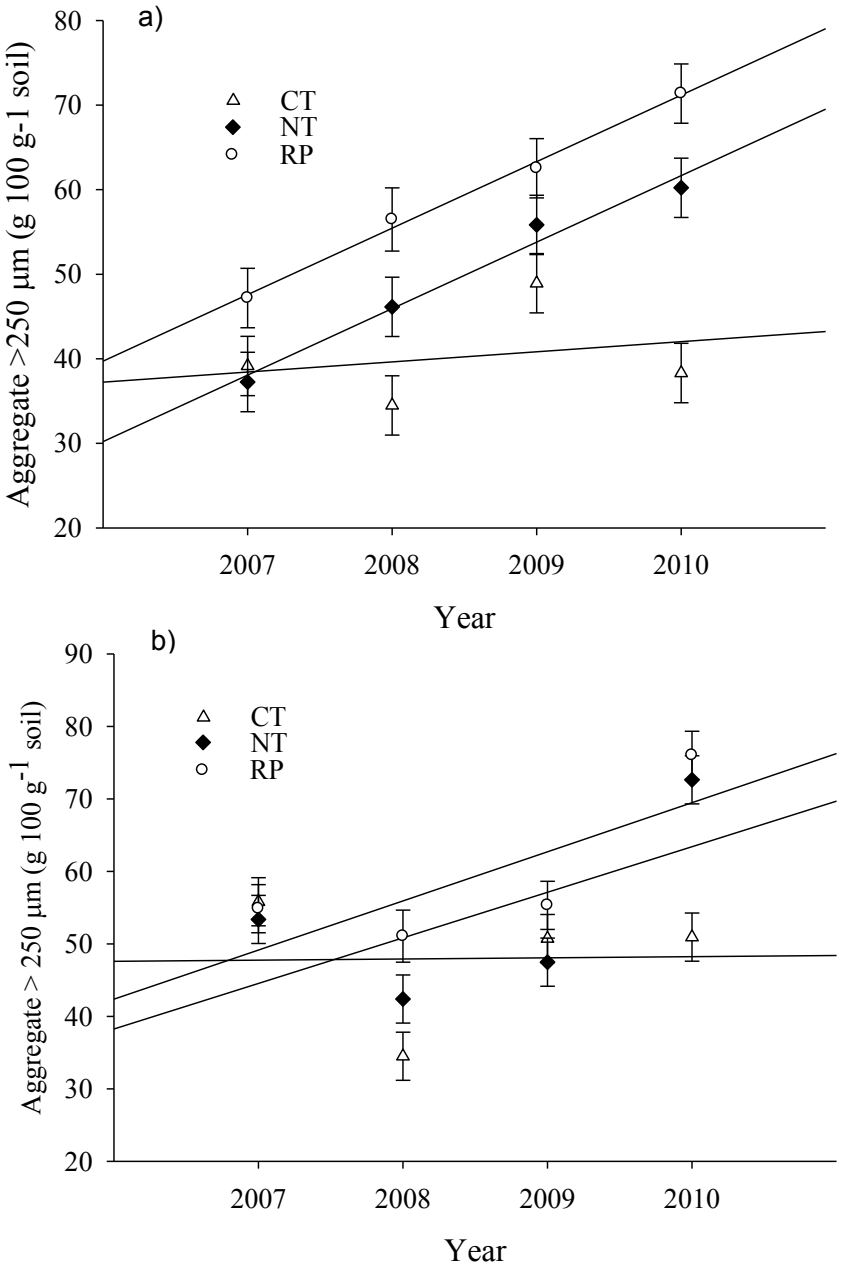

Figure 4.10. Regression analysis for sand-free water stable aggregates (WSA) >250 µm for (a) 0-5 and (b) 5-15 cm depths soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

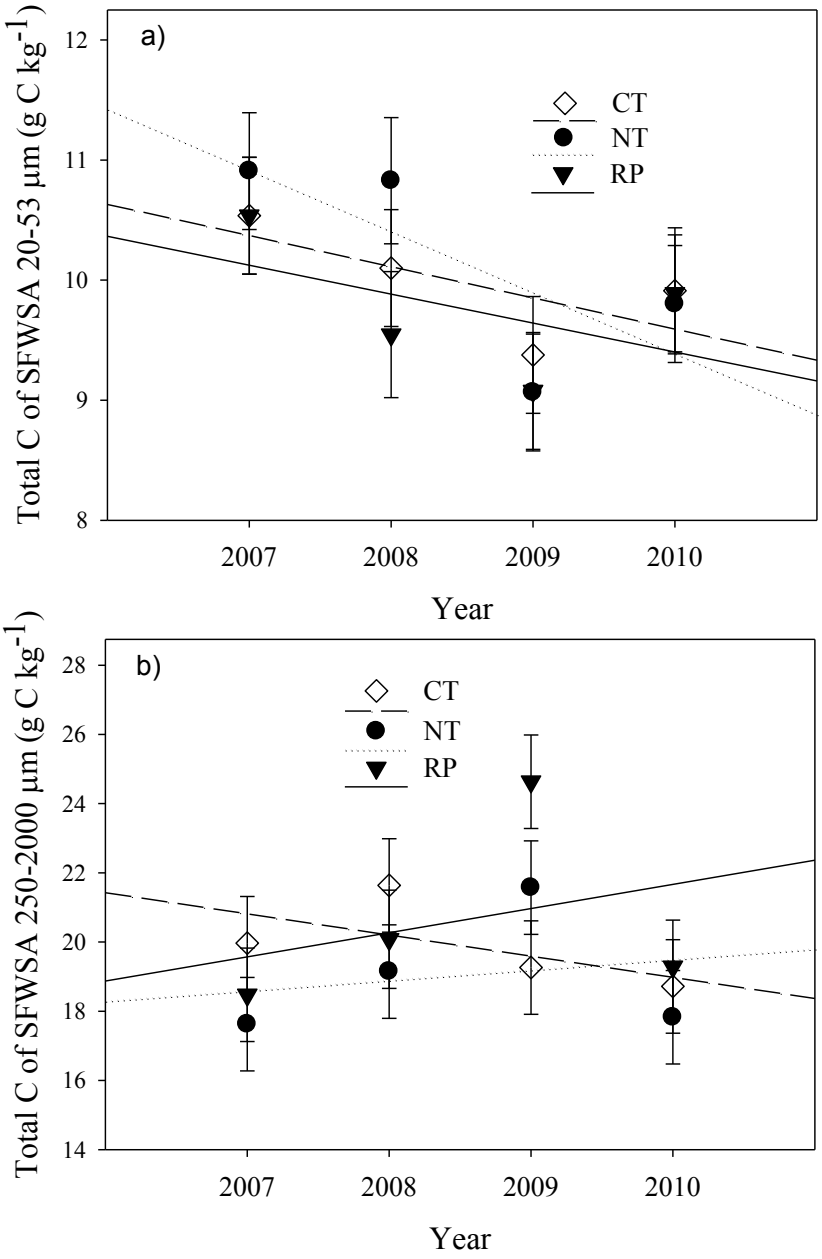

Figure 4.11. Regression analysis for sand-free water stable aggregates (SFWSA) for (a) 20-53 um fraction and (b) 250-2000 µm fraction at 0-5 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

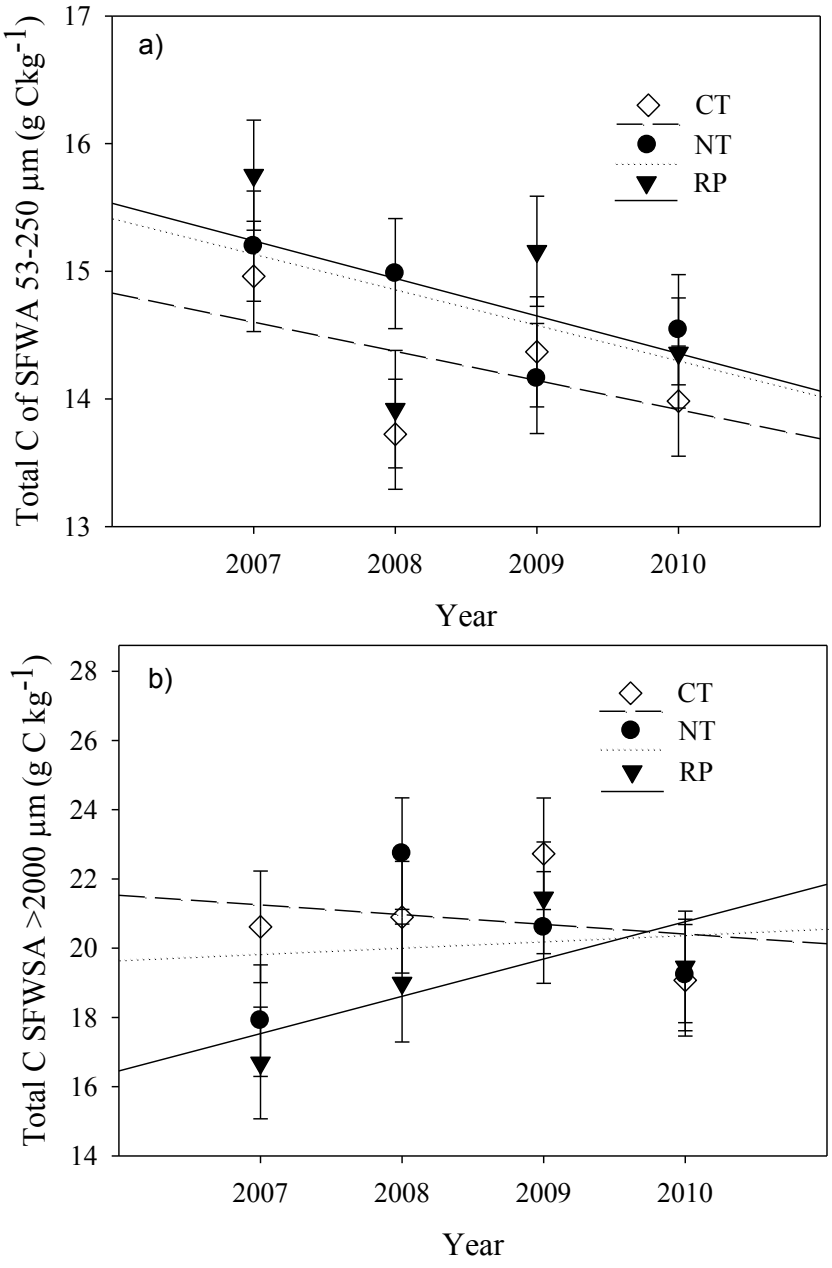

Figure 4.12. Regression analysis for sand-free water stable aggregates (SFWSA) for (a) 53-250 um fraction and (b) >2000 µm fractions at 0-5 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

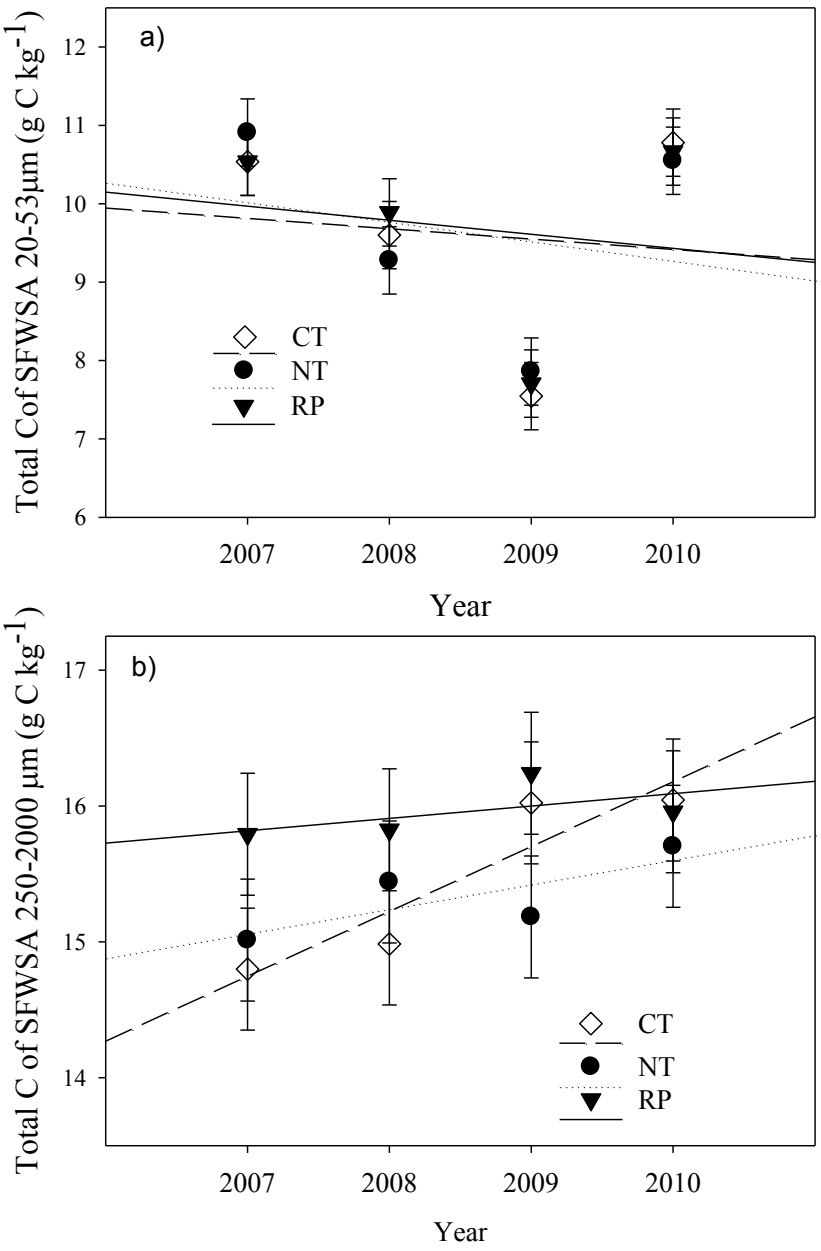

Figure 4.13. Regression analysis for total C of sand-free water stable aggregates (SFWSA) for (a) 20-53 um fraction and (b) 250-2000 µm at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

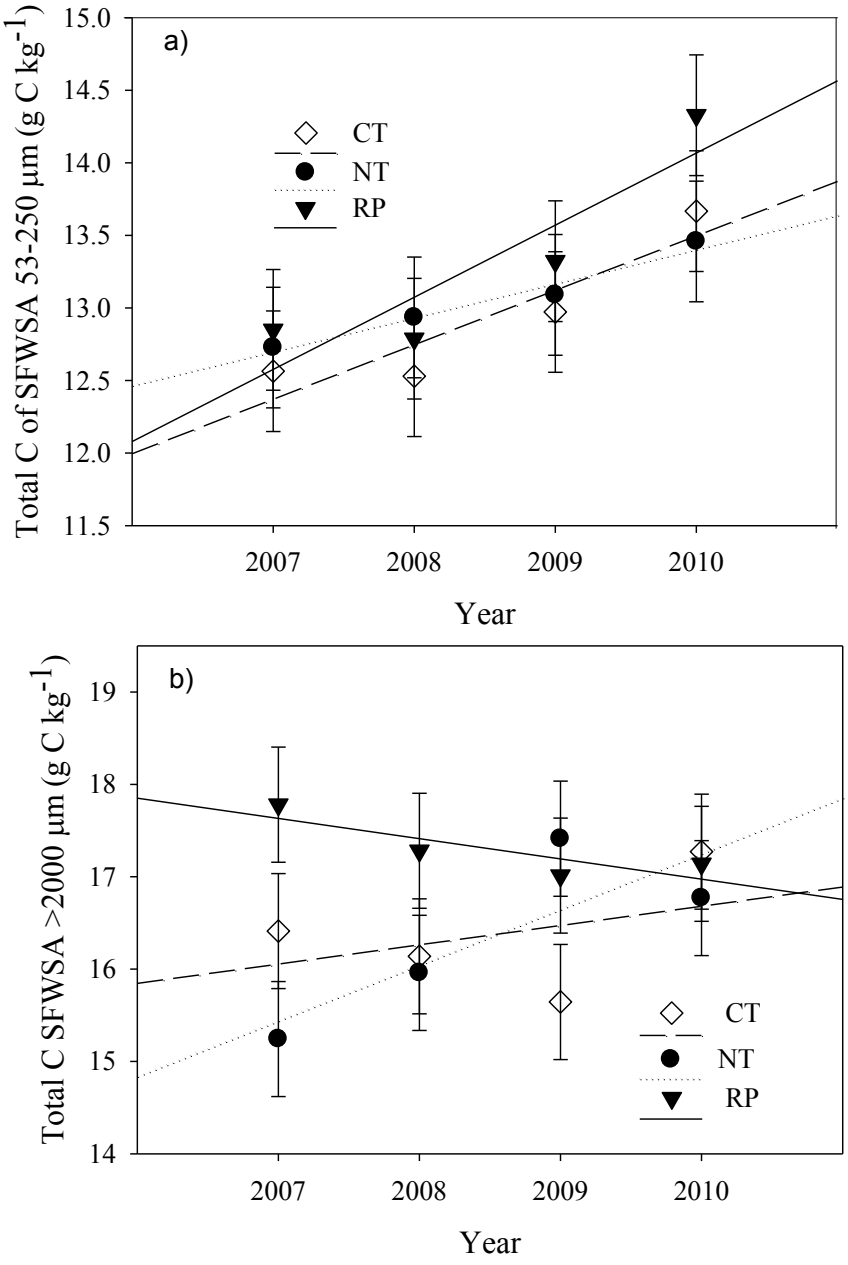

Figure 4.14. Regression analysis for sand-free water stable aggregates (SFWSA) for (a) 53-250 um fraction and (b) >2000 µm at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

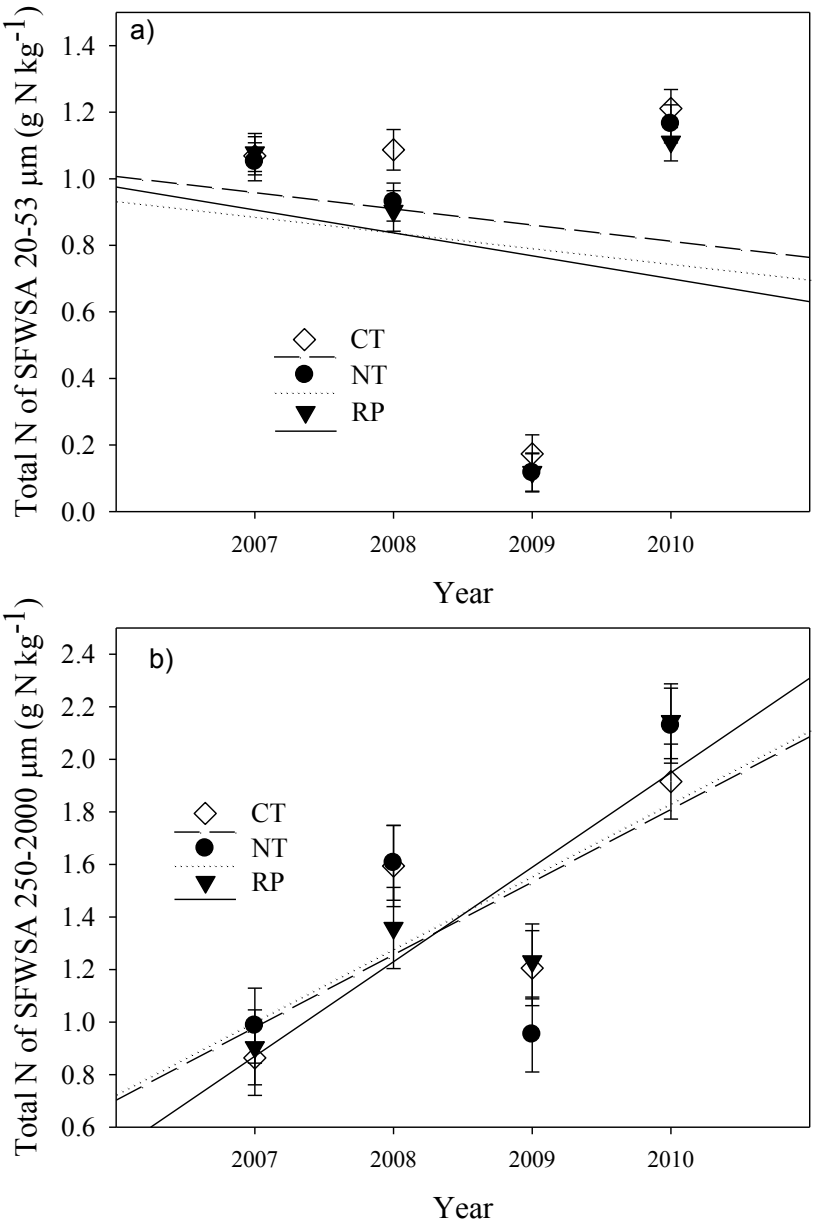

Figure 4.15 Regression analysis for total N of sand-free water stable aggregates (SFWSA) over time for (a) 20-53 um fraction and (b) 250-2000 µm fraction at 0-5 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

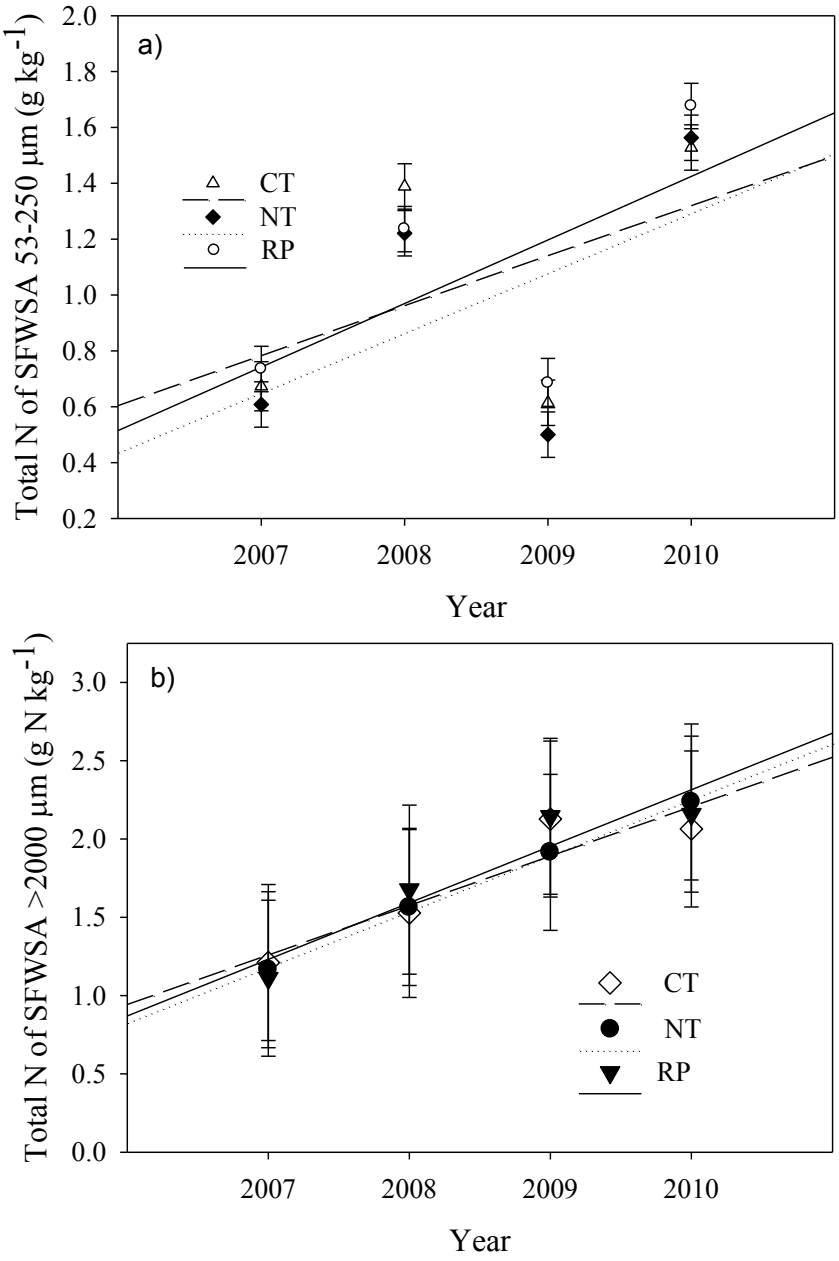

Figure 4.16. Regression analysis for total N of sand-free water stable aggregates (SFWSA) for (a) 53-250 um fraction and (b) >2000 µm at 0-5 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

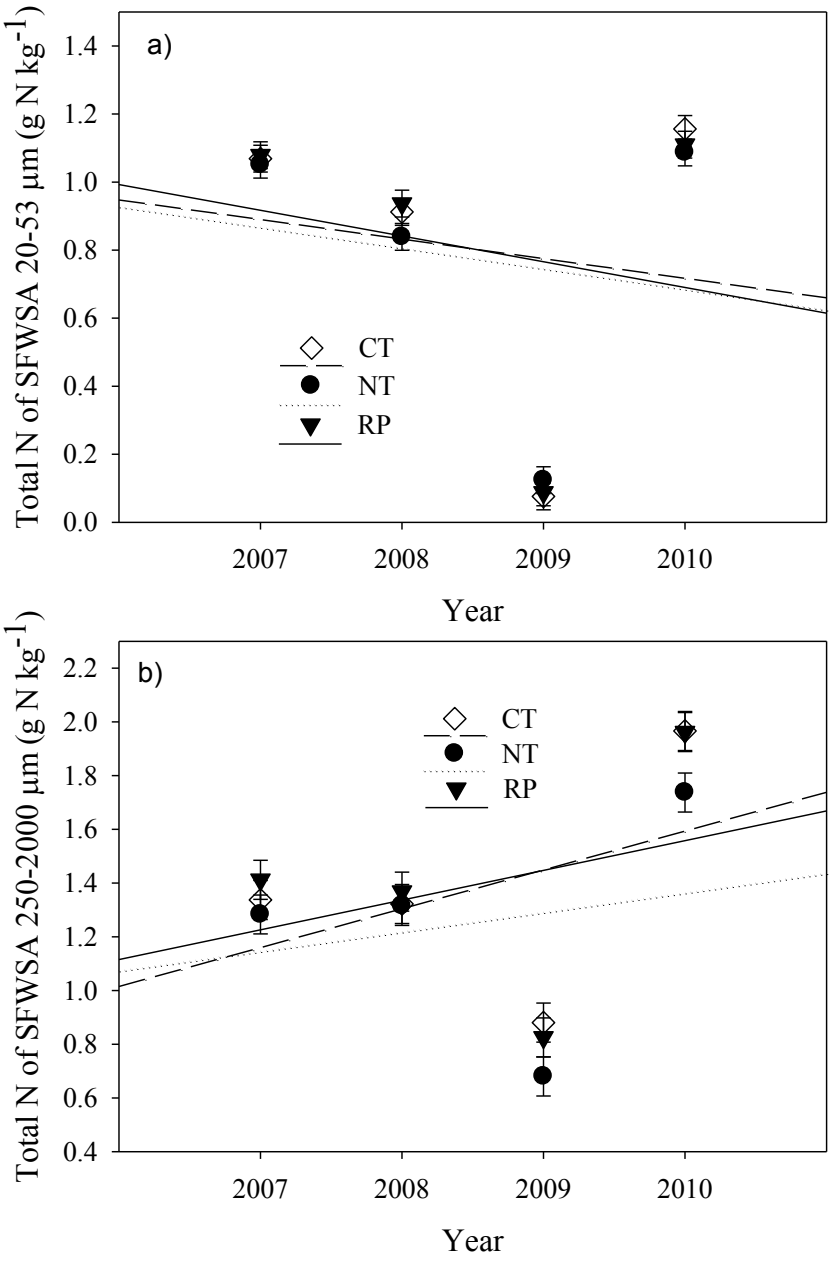

Figure 4.17. Regression analysis for total N of sand-free water stable aggregates (SFWSA) for (a) 20-53 um fraction and (b) 250-2000µm fraction µm at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

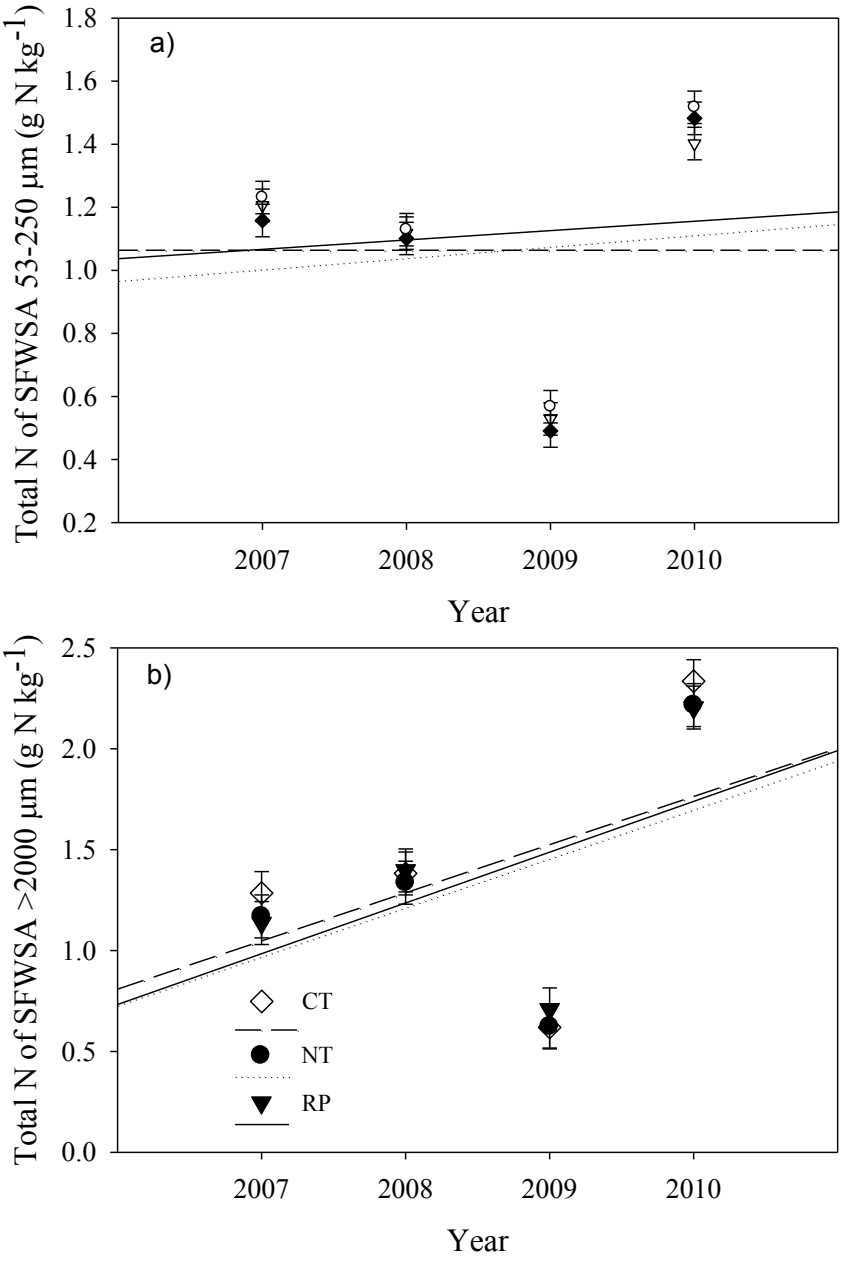

Figure 4.18. Regression analysis for total N of sand-free water stable aggregates (SFWSA) for (a) 53-250 um fraction and (b) >2000 µm fraction at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*).

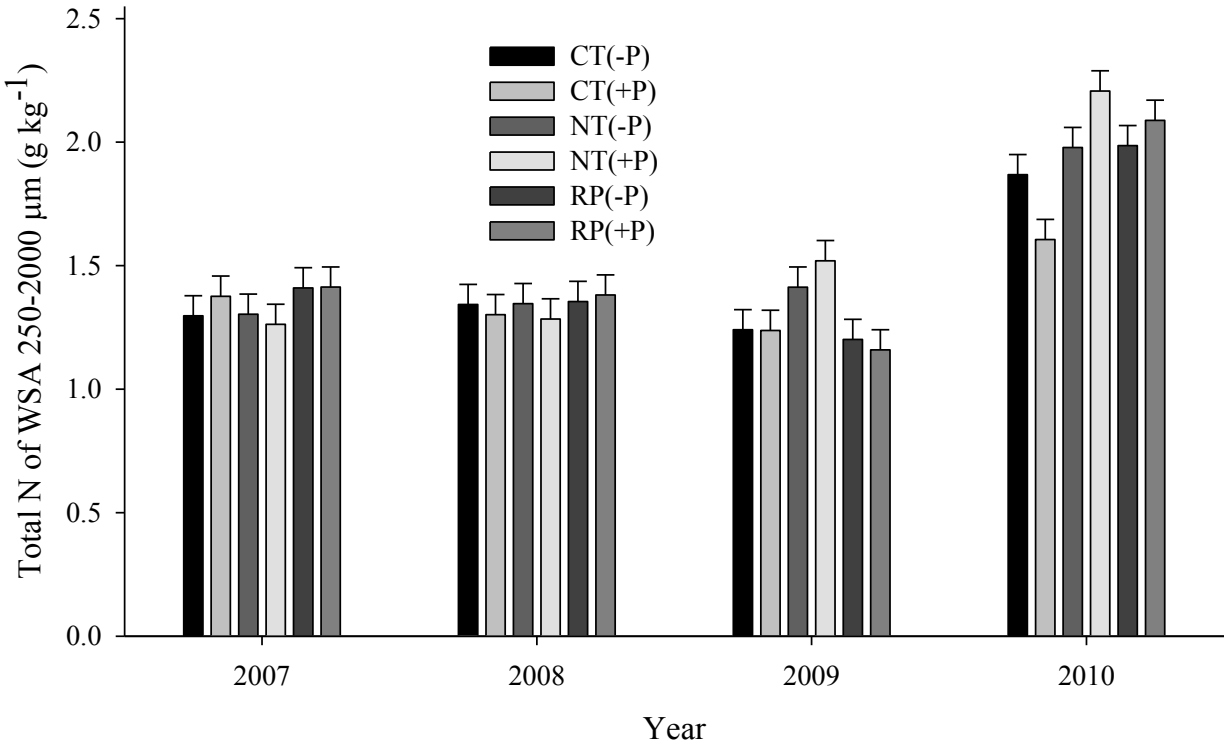

Figure 4.19. Ecosystem x P x time interaction (P<0.0288) means for aggregate-associated N in sand-free water stable aggregates (WSA) 250-2000 µm at 5-15 cm in soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*).

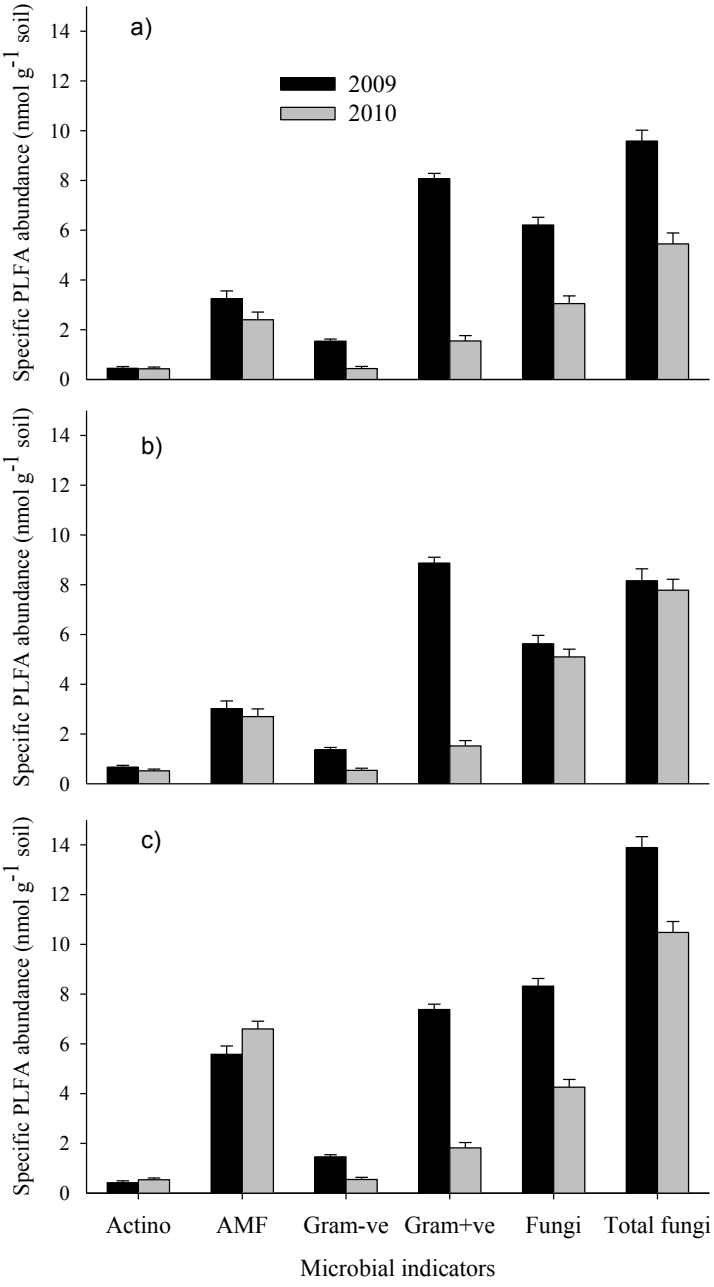

Figure 4.20. Ecosystem x time interaction on abundance of specific phospholipid fatty acids (PLFA) (a) CT (b) NT (c) RP of soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 0-5 cm depth. Microbial groups with asterisks are significantly different at (P<0.05)

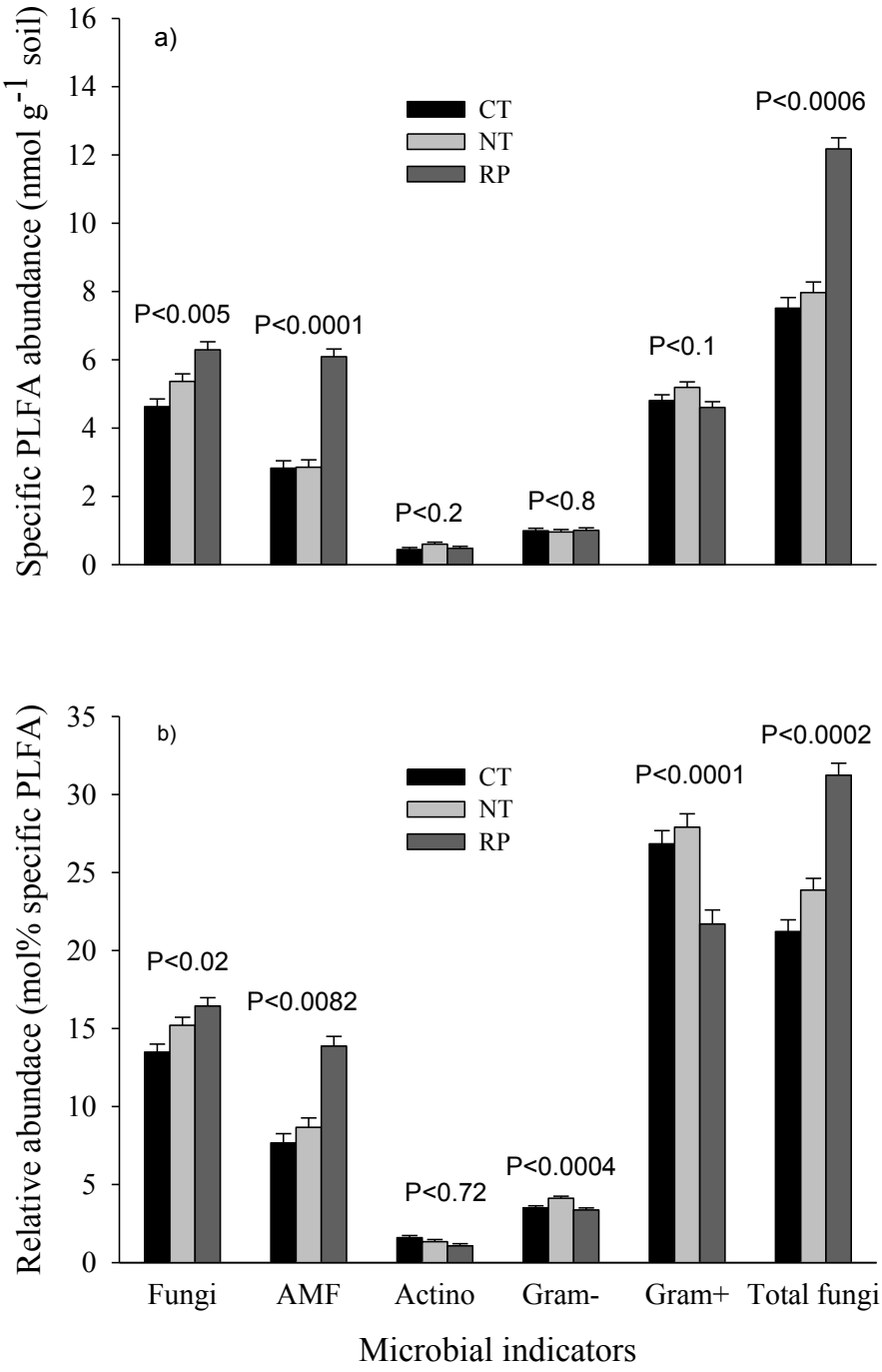

Figure 4.21. Effect ecosystems on (a) specific phospholipid fatty acids (PLFA) abundance and (b) relative abundance of PLFA of a silty clay soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

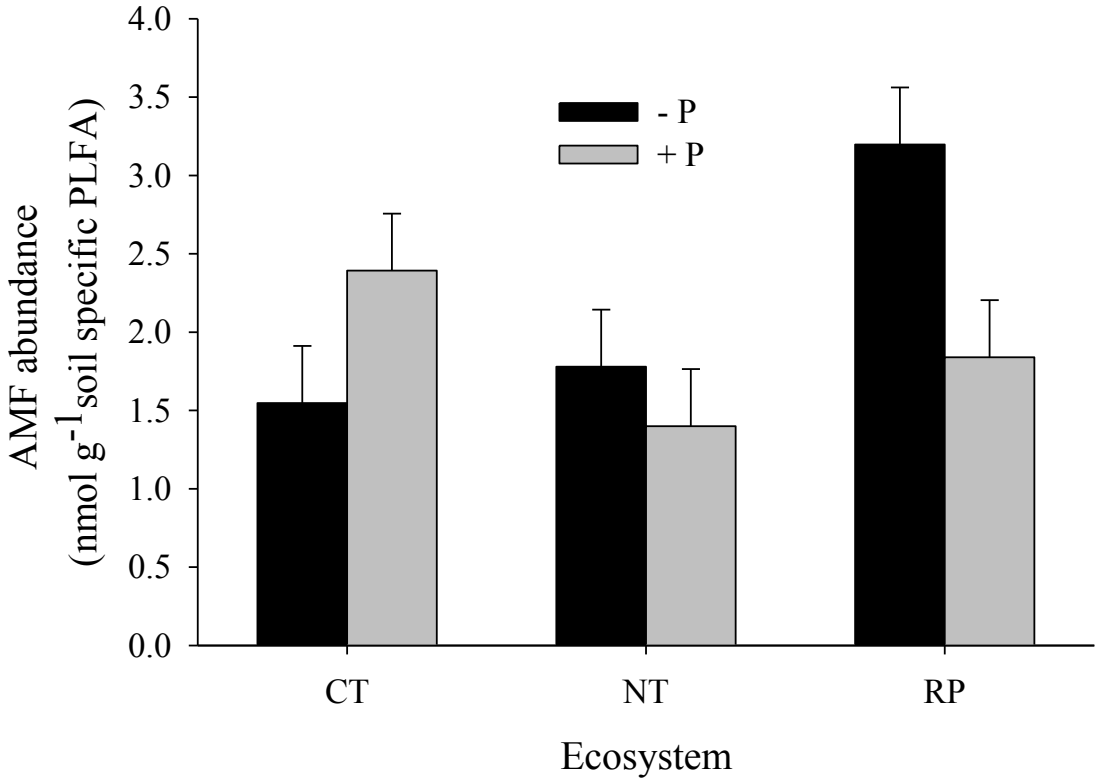

Figure 4.22. Ecosystem x fertilizer interaction means (P<0.05) of relative AMF abundance of a silty clay soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big bluestem (*Andropogon gerardii*) at 5-15 depth.

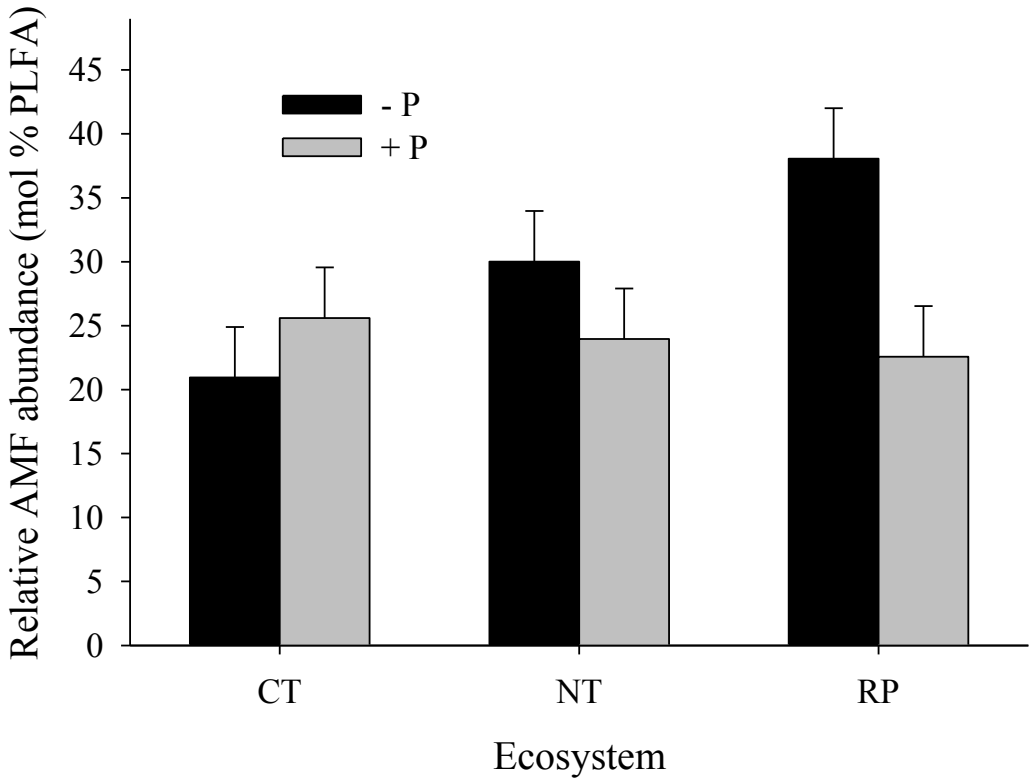

Figure 4.23. Ecosystem x P amendment interaction means (P<0.05)on total phospholipid fatty acids (PLFA) abundance of of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT), and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 5-15 cm depth.

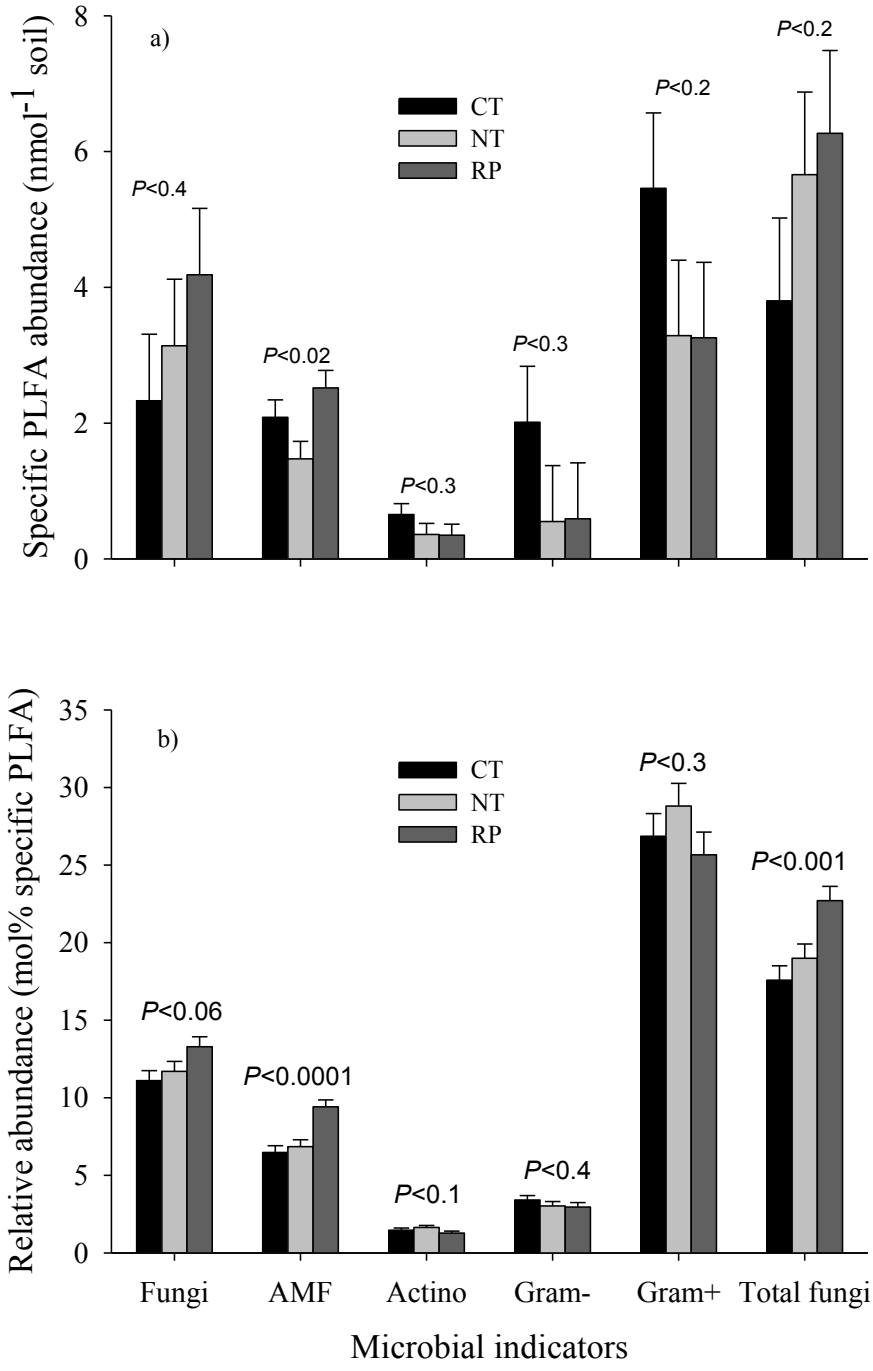

Figure 4.24. Abundance of specific phospholipid fatty acids (PLFA) (a) and relative abundance (b) of a silty clay soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 5-15 cm depth.

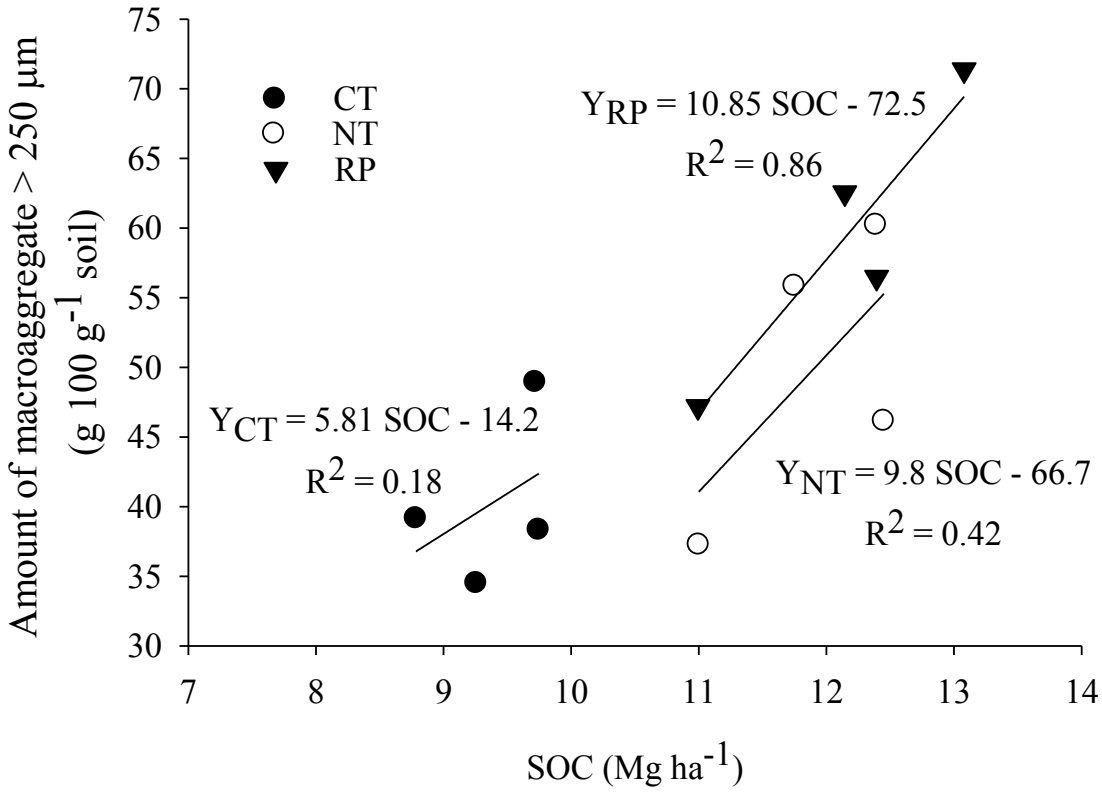

Figure 4.25. Relationship between the soil organic C (SOC) and the amount of macroaggregates > 250 um for a silty clay soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

Table 4.1. Changes in selected soil chemical properties in field experiment since initiation.

	pH (1:1)	C	N	Bray-1P	Olsen P	Mehlich P	Ca	K	Mg
	Soil:water	----------g kg^{-1}----------		---------- mg kg^{-1}----------					
2004	8 (0.3)	16.3 (0.1)	1.7 (0.01)	25 (2)	13 (1)	-	2748 (329)	382 (25)	270 (13)
2010	6.4 (0.4)	19 (1.2)	1.7 (0.1)	-	-	24 (8)	3127 (421)	392 (74)	269 (35)

Mean (n=12) values in brackets are standard deviations

Table 4.2. Analysis of variance and main effect means of above ground plant biomass of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (RP) (*Andropogon gerardii*).

	Above ground plant biomass		
	---------- *P*-values ----------		
Ecosystem (E)	0.0155		
Fertilizer (F)	0.7994		
E x F	0.2645		
Time (T)	<.0001		
E x T	<.0001		
F x T	0.4705		
E x F x T	0.082		
	---------- g m^{-2} ----------		
CT	303 b‡		
NT	316 b		
RP	500 a		
	2008	2009	2010
	471 a†	257 c	391 b

‡ Ecosystem means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.3. Analysis of variance and main effect means of root biomass at 0-7.5 cm depth of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (RP) (*Andropogon gerardii*).

	Root biomass at 0-7.5 cm		
	---------- *P*-values----------		
Ecosystems (E)	0.0026		
Fertilizer (F)	0.2026		
E x F	0.31		
Time (T)	<.0001		
E x T	0.0794		
F x T	0.974		
E x F x T	0.2741		
	---------- g m^{-2} ----------		
CT (mean)	240 b‡		
NT (mean)	237 b		
RP (mean)	655 a		
	2008	2009	2010
Time (mean)	294 b†	277 b	560 a

‡ Ecosystem means followed by different letters are not significantly different at (*P*<0.05).

† Year means followed by different letters are not significantly different at (*P*<0.05).

Table 4.4. Analysis of variance and main effect means of total above- and belowground (0-7.5 cm depth) biomass of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) (*Andropogon gerardii*).

	Total above- plus below-ground (0-7.5 cm depth) biomass		
	---------- *P*-values ----------		
Ecosystem (E)	0.0007		
Fertilizer (F)	0.3263		
E x F	0.7774		
Time (T)	<.0001		
E x T	<.0001		
F x T	0.8442		
E x F x T	0.0642		
	---------- g m^{-2} ----------		
CT (mean)	543 b‡		
NT (mean)	550 b		
RP (mean)	1183 a		
	2008	2009	2010
Time (mean)	778 b†	550 c	948 a

‡ Ecosystem means followed by different letters are not significantly different at (*P*<0.05).

† Year means followed by different letters are not significantly different at (*P*<0.05).

Table 4.5. Analysis of variance and main effect means for soil organic C (SOC) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

	Soil organic C			
	----------*P*-values----------			
Ecosystem (E)	0.0002			
Fertilizer (F)	0.2447			
E x F	0.1395			
Time (T)	<.0001			
E x T	0.204			
F x T	0.9751			
E x F x T	0.4223			
	---------- Mg C ha^{-1} soil ----------			
CT (means)	9.4 b‡			
NT (means)	11.9 a			
RP (means)	12.1 a			
	2007	2008	2009	2010
Year (Means)	10.7 c†	11.3 b	11.2 b	11.7 a

‡ Ecosystem means followed by different letters are not significantly different at (*P*<0.05).

† Year means followed by different letters are not significantly different at (*P*<0.05).

Table 4.6. Analysis of variance and main effect means for soil organic C (SOC) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 5-15 cm depth.

	Soil organic C			
	----------*P*-values----------			
Ecosystem (E)	0.1383			
Fertilizer (F)	0.0205			
E x F	0.6904			
Time (T)	<.0001			
E x T	0.9466			
F x T	0.443			
E x F x T	0.9354			
	---------- Mg C ha^{-1} soil ----------			
-P (Means)	21.3 a‡			
+P (Means)	20.9 b			
	2007	2008	2009	2010
Year (Means)	20.5 c†	21bc	20.7 b	22.2 a

‡ Fertilizer means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.7. Analysis of variance and main effect means for soil organic C (SOC) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 15-30 cm depth.

	Soil organic C			
	----------*P*-values----------			
Ecosystem (E)	0.6645			
Fertilizer (F)	0.275			
E x F	0.557			
Time (T)	0.0029			
E x T	0.7888			
F x T	0.9683			
E x F x T	0.986			
	2007	2008	2009	2010
	---------- Mg C ha^{-1} soil ----------			
Year (Means)	27.9 c‡	28.9 bc	29.5 ab	30.7 a

‡ Year means followed by different letters are not significantly different at (P<0.05).

Table 4.8. Analysis of variance and main effect means for soil organic (SOC) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) in at 0-30 cm depth.

	Soil organic C			
	----------*P*-values----------			
Ecosystem (E)	0.3107			
Fertilizer (F)	0.1981			
E x F	0.4666			
Time (T)	<.0001			
E x T	0.9271			
F x T	0.9935			
E x F x T	0.9781			
	2007	2008	2009	2010
	---------- Mg C ha^{-1} soil ----------			
Year (Means)	59.9 c‡	59.7 c	61.8 b	64.6 a

‡ Year means followed by different letters are not significantly different at (P<0.05).

Table 4.9. Analysis of variance and main effect means soil total N (TN) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

	Soil total N			
	----------*P*-values----------			
Ecosystem (E)	<.0001			
Fertilizer (F)	0.9595			
E x F	0.0957			
Time (T)	<.0001			
E x T	0.2602			
F x T	0.9169			
E x F x T	0.8087			
	---------- Mg N ha^{-1} soil ----------			
CT (means)	0.86 b‡			
NT (means)	1.08 a			
RP (means)	1.06 a			
	2007	2008	2009	2010
Year (Means)	0.92 d†	0.97 c	1.06 a	1.04 b

‡ Ecosystem means followed by different letters are not significantly different at (*P*<0.05).

† Year means followed by difference letters are not significantly different at (*P*<0.05).

Table 4.10. Analysis of variance and main effect means for soil total N (TN) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 5-15 cm depth.

	Soil total N			
	----------*P*-values----------			
Ecosystem (E)	0.0311			
Fertilizer (F)	0.2693			
E x F	0.7146			
Time (T)	<.0001			
E x T	0.2749			
F x T	0.9179			
E x F x T	0.9752			
	---------- Mg N ha^{-1} soil ----------			
CT (means)	1.98 ab ‡			
NT (means)	2.03 a			
RP (means)	1.89 b			
	2007	2008	2009	2010
Year (Means)	1.87 b†	1.80 b	2.13 a	2.07 a

‡ Ecosystem means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.11. Analysis of variance and main effect means for soil total N (TN) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 15-30 cm depth.

	Soil total N			
	----------*P*-values----------			
Ecosystem (E)	0.4287			
Fertilizer (F)	0.2669			
E x F	0.1569			
Time (T)	<.0001			
E x T	0.8118			
F x T	0.9389			
E x F x T	0.9983			
	---------- Mg N ha^{-1} soil ----------			
	2007	2008	2009	2010
Year (Means)	2.63 b‡	2.24 c	3.06 a	2.65 b

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.12. Analysis of variance and main effect means for soil total N (TN) of soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and big blue stem (*Andropogon gerardii*) at 0-30 cm depth.

	Soil total N			
	----------*P*-values----------			
Ecosystem (E)	0.1817			
Fertilizer (F)	0.8147			
E x F	0.1251			
Time (T)	<.0001			
E x T	0.6403			
F x T	0.9554			
E x F x T	0.9753			
	---------- Mg N ha^{-1} soil ----------			
	2007	2008	2009	2010
Year (Means)	5.42 c‡	5.01 d	6.25 a	5.77 b

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.13. Analysis of variance and main effect means for arbuscular mycorrhizal fungi (AMF) root colonization of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) (*Andropogon gerardii*).

	Root AMF colonization						
	P- values						
Ecosystem (E)	<.0001						
Fertilizer (F)	<.0001						
E x F	0.0488						
Time (T)	<.0001						
E x T	0.0184						
F x T	0.0005						
E x F x T	0.0708						
	% root colonization						
	2004	2005	2006	2007	2008	**2009**	**2010**
Year (Means)	15 c‡	16.8 bc	18.4 b	12.2 d	10.2 e	26.2 a	26.4 a
			CT	12.4 c†			
Ecosystem (Means)			NT	16 b			
			RP	24.5 a			
			C	19.2 a*			
Fertilizer (Means)			P	14.9b			

‡Year means followed by different letters are not significantly different at (P<0.05).

† Ecosystem means followed by different letters are not significantly different at (P<0.05).

*Fertilizer P means followed by different letters are not significantly different at (P<0.05).

Table 4.14. Analysis of variance and main effect means for aggregate size distribution of soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

	Aggregate size fractions at 0-5 cm depth		
	Aggregate sizes (µm)		
	<250	>250	>2000
	P-values		
Ecosystem (E)	0.024	0.0038	0.0681
Fertilizer (F)	0.9627	0.692	0.5407
E x F	0.7253	0.3151	0.1216
Time (T)	0.0056	<.0001	<.0001
E x T	<.0001	0.0016	0.0558
F x T	0.2669	0.6347	0.4635
E x F x T	0.0702	0.5709	0.2707
	g 100 g^{-1} soil		
CT (means)	51 a‡	40.2 c	10.7
NT (means)	42.6 ab	49.9 b	15
RP (means)	31.3 b	59.4 a	20.3

‡ Ecosystem means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.15. Analysis of variance and main effect means for aggregate size distribution of soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 5-15 cm depth.

	Aggregate size fractions at 5-15 cm depth		
	Aggregate sizes (µm)		
	<250	>250	>2000
	P-values		
Ecosystem (E)	<.0001	0.0107	0.0087
Fertilizer (F)	0.7502	0.5556	0.0824
E x F	0.9758	0.9991	0.8385
Time (T)	0.0286	<.0001	<.0001
E x T	<.0001	0.0009	0.4749
F x T	0.9109	0.8718	0.0048
E x F x T	0.7247	0.4995	0.596
	g 100^{-1} g soil		
CT (means)	41.3 a‡	48 b	8.7 b
NT (means)	34.1 b	54 ab	10.5 b
RP (means)	28.8 c	59.3 a	14.8 a

‡ Ecosystem means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.16. Analysis of variance and main effect means for soil aggregate-associated C in aggregate size fractions of a soil planted with sorghum (*Sorghum bicolor*) to continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

	Total C mass in aggregate fractions at 0-5 cm depth			
	Aggregate size fraction µm			
	20-53	53-250	250-2000	>2000
	P-value			
Ecosystem (E)	0.7666	0.3991	0.66	0.6888
Fertilizer (F)	0.8471	0.4379	0.9688	0.7008
E x F	0.355	0.6002	0.3065	0.0656
Time	0.0058	<.0001	0.0003	0.0224
E x T	0.5768	0.0578	0.0142	0.1568
F x T	0.9069	0.4425	0.3997	0.2529
E x F x T	0.7889	0.2657	0.5799	0.1819
	g C kg^{-1} aggregate fraction			
2007 (means)	10.4 a‡	15.5 a	18.7 b	18.4 b
2008 (means)	10.2 a	14.2 b	20.2 a	20.9 a
2009 (means)	9.2 b	14.5 b	21 a	20.8 a
2010 (means)	9.9 ab	14.3 b	18.6 b	19.3 ab

‡ Ecosystem means followed by different letters are not significantly different at (P<0.05).

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.17. Analysis of variance and main effect means for aggregate-associated C in aggregate size fractions of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

	Total C mass in aggregate fractions at 5-15 cm depth			
	----------Aggregate size fraction µm----------			
	20-53	53-250	250-2000	>2000
	---------- *P*-value----------			
Ecosystem (E)	0.4856	0.7393	0.5036	0.2458
Fertilizer (F)	0.6146	0.7591	0.467	0.5704
E x F	0.7333	0.7805	0.1297	0.5442
Time	<.0001	<.0001	0.0125	0.0198
E x T	0.8632	0.6228	0.2078	0.0121
F x T	0.7412	0.6781	0.6256	0.5865
E x F x T	0.0336	0.1123	0.4342	0.9221
	---------- g C kg^{-1} aggregate fraction ----------			
2007 (means)	10.3 a‡	12.7 c	15.2 c	16.5 b
2008 (means)	9.6 b	12.8 bc	15.4 bc	16.3 b
2009 (means)	7.7 c	13.1 b	15.8 ab	16.2 b
2010 (means)	10.7 a	13.8 a	15.9 a	17.1 a

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.18. Analysis of variance and main effect means for aggregate-associated- N in aggregate size fractions of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

	Total N mass in aggregate fractions at 0-5 cm depth			
	----------Aggregate size fraction µm----------			
	20-53	53-250	250-2000	>2000
	---------- *P*-value----------			
Ecosystem (E)	0.7148	0.0119	0.9602	0.5938
Fertilizer (F)	0.5503	0.086	0.4344	0.9201
E x F	0.3724	0.5888	0.9085	0.0689
Time	<.0001	<.0001	<.0001	<.0001
E x T	0.5366	0.1549	0.3731	0.481
F x T	0.4701	0.2297	0.3983	0.3907
E x F x T	0.1196	0.5778	0.621	0.4694
	---------- g N kg^{-1} aggregate fraction ----------			
2007	1.1 b‡	0.7 c	0.9 d	1 c
2008	0.9 c	1.2 b	1.6 b	1.7 b
2009	0.1 d	0.6 c	1.1 c	1 c
2010	1.14a	1.5 a	2.0 a	2.2 a

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.19. Analysis of variance and main effect means for aggregated-N in aggregate size fractions of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

	Total N mass in aggregate fractions at 5-15 cm depth			
	Aggregate size fraction µm			
	20-53	53-250	250-2000	>2000
	P-value			
Ecosystem (E)	0.5216	0.5607	0.1358	0.6479
Fertilizer (F)	0.4657	0.8049	0.8763	0.55
E x F	0.8447	0.8586	0.0036	0.86
Time (T)	<.0001	<.0001	<.0001	<.0001
E x T	0.4963	0.7265	0.0048	0.9363
F x T	0.8645	0.9207	0.931	0.3834
E x F x T	0.3771	0.9442	0.0288	0.9938
	g N kg^{-1} aggregate fraction			
2007 (means)	1.1 b‡	1.2 b	1.3 b	1.2 c
2008 (means)	0.9 c	1.1 c	1.3 b	1.4 b
2009 (means)	0.9 c	0.9 d	1.1 c	1 d
2010 (means)	1.11 a	1.5 a	2 a	2.3 a

† Year means followed by different letters are not significantly different at (P<0.05).

Table 4.20. Analysis of variance and main effect means of soil microbial groups estimated as phospholipid fatty acids (PLFA) for a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

	Microbial biomass (PLFA) at 0-5 cm depth						
	Actino	AMF	Gram-	Gram+	Fungi	Total fungi	Total PLFA
	P-value						
Ecosystem (E)	0.2143	<.0001	0.8846	0.1074	0.0057	<.0001	0.0006
Fertilizer (F)	0.4551	0.2041	0.6339	0.9476	0.2311	0.1672	0.6344
E x F	0.4742	0.3473	0.9626	0.4998	0.4162	0.0876	0.1653
Time (T)	0.7233	0.8149	<.0001	<.0001	<.0001	<.0001	0.0076
E x T	0.0339	0.0166	0.0643	0.0007	<.0001	0.0004	0.0536
F x T	0.1238	0.2706	0.2289	0.9236	0.351	0.2319	0.7274
E x F x T	0.2896	0.3013	0.5619	0.2667	0.1866	0.079	0.1601
	nmol PLFA g^{-1} soil						
CT (means)	0.44	2.82 b	0.95	4.81	4.63 b	7.51 b	33.77 b
NT (means)	0.6	2.85 b	0.99	4.6	5.37 b	7.97 b	37.47 b
RP (means)	0.48	6.09 a	1	5.19	6.29 a	12.18 a	42.07 a

‡ Ecosystem means for each microbial group followed by different letters are not significantly different at (P<0.05).

Table 4.21. Analysis of variance and main effect means for soil microbial groups estimated as phospholipid fatty acids (PLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

	Microbial (PLFA) biomass at 0-5 cm depth					
	Actino	AMF	Gram-	Gram+	fungi	Total fungi
	mol % PLFA					
2009 (means)	1.23	9.5	3.46 b‡	20.18 b	16.14 a	26.28 a
2010 (means)	1.44	10.64	3.88 a	30.78 a	13.95 b	24.59 b
	P-values					
Ecosystem (E)	0.0798	0.0008	0.0004	<.0001	0.0209	0.0002
Fertilizer (F)	0.9559	0.2425	0.5778	0.3564	0.5841	0.1742
E x F	0.8655	0.7783	0.7699	0.8741	0.5795	0.2595
Time (T)	0.0792	0.0742	0.0074	<.0001	0.0006	0.0293
E x T	0.4337	0.0271	0.4224	0.654	0.08	0.0638
F x T	0.5031	0.6225	0.9794	0.9623	0.2124	0.5677
E x F x T	0.6581	0.983	0.2886	0.6417	0.4303	0.199

‡ Different letters represent significant differences within columns (microbial groups).

Table 4.22. Analysis of variance and main effect means for soil microbial groups estimated as phospholipid fatty acids (PLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

	Microbial biomass (PLFA) at 5-15 cm depth						
	Actino	AMF	Gram-	Gram+	Fungi	Total fungi	Total PLFA
	nmol PLFA g^{-1} soil						
2009 (means)	0.55	1.98	1.74	7.09 a‡	4.49 a	6.47	31.75 a
2010 (means)	0.36	2.07	1.37	4.9 b	2.95 b	4.02	22 b
	P-values						
Ecosystem (E)	0.3359	0.0244	0.3706	0.2892	0.4546	0.3407	0.2193
Fertilizer (F)	0.4815	0.5083	0.389	0.4749	0.5408	0.7238	0.0906
E x F	0.2337	0.0277	0.3245	0.2626	0.2169	0.1528	0.0516
Time (T)	0.3096	0.7811	0.1598	<.0001	0.0333	0.0907	0.0047
E x T	0.4374	0.7769	0.3905	0.3297	0.5169	0.571	0.4573
F x T	0.3286	0.7599	0.36	0.4208	0.4777	0.5243	0.2686
E x F x T	0.3294	0.1268	0.3522	0.2723	0.1974	0.1707	0.327

‡ Different letters represent significant differences within columns (microbial groups).

Table 4.23. Analysis of variance and main effect means for soil microbial groups estimated as phospholipid fatty acids (PLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

	Microbial biomass (PLFA) at 5-15 cm depth					
	Actino	AMF	Gram-	Gram+	fungi	Total fungi
	mol % PLFA					
2009 (means)	1.33	5.86 b‡	3.04	21.41 b	11.86 b	17.71 b
2010 (means)	1.60	9.3 a	3.23	32.81 a	12.21 a	21.81 a
	P-values					
Ecosystem (E)	0.1712	<.0001	0.4935	0.32	0.0598	0.0013
Fertilizer (F)	0.7548	0.167	0.938	0.3545	0.8028	0.5686
E x F	0.2422	0.3521	0.587	0.2427	0.2758	0.7655
Time (T)	0.0794	<.0001	0.5776	<.0001	0.6323	0.0005
E x T	0.9112	0.2099	0.6468	0.5311	0.4379	0.5559
F x T	0.3893	0.5495	0.3867	0.757	0.728	0.7523
E x F x T	0.4811	0.3452	0.4899	0.3736	0.9613	0.4725

‡ Different letters represent significant differences within columns (microbial groups).

CHAPTER 5 - GENERAL SUMMARY

Global surface temperatures have increased due to increases in atmospheric concentrations of greenhouse gases (GHGs), resulting in global climate change. In addition, global climate change will potentially alter global precipitation patterns which affect soil water content that is important to soil C dynamics.

In order to find long term solutions to climate change mitigation, climate models need to accurately predict the effect of temperature change on SOC dynamics. The relationship between SOC mineralization, temperature and soil water content is complex and the role of soil microbes which undertake SOC mineralization is still a black box. In addition, most studies that have evaluated temperature effects on SOC mineralization, have not considered the role of water in SOC mineralization. This could be the reason why a consensus has not been reached between scientists on the feedback of CO_2 on climate change. Also, there are no common grounds with regards to response of different SOC pools to temperature change. This information is important to evaluate the different soil pool contribution towards CO_2.

Our results suggest a strong influence of soil water content on temperature response of SOC mineralization. Therefore, the effect of water should be included in models to appropriately predict climate change feedbacks on soil C dynamics. The microbial biomass that mineralizes SOC, decreased with increasing soil water content. The substrate availability for microbial utilization expressed as PLFA stress ratios were influenced by temperature. This result may imply that temperature effect on microbial biomass may be indirect. The mineralizable C was higher at higher temperatures, but the

change in mineralizable C was larger when temperature changed at lower temperature ranges and under drier conditions. Therefore cooler soils under wet conditions may hold more C, but as temperature increases and become drier, these soils may release more CO_2. Additionally, our $\delta^{13}C$-CO_2 results suggest that the contribution of CO_2 from either younger or older SOC will dependent on the magnitude of temperature shift, soil water content and duration of environmental conditions. Results indicated that optimal SOC mineralization in this soil was between 25 and 35 ^{o}C under drier soil conditions. We speculate that mineralization of labile pool would not contribute much towards a positive feedback on climate change because it acts more like a revolving door. On the other hand, older C mineralization could be important in accelerating climate change because of the larger pool size, whereby even small changes over long time span may contribute significantly to climate feedback.

Agricultural and native soils can be sources or sinks of CO_2 that could influence the climate feedback from soils. Therefore, an evaluation of the changes in SOC due to land use change and a comparison of agricultural management practices are important for regional assessment and estimates of C sequestration potentials of soils, which could be informative to both land managers and policy makers in their decision making processes. The native vegetation such as tallgrass prairie produces high belowground biomass in the form of roots which can contribute to storing SOC.. Therefore it is important to assess various management practices of the tallgrass prairie to inform decision making with regards to environmental sustainability. The management practices of agricultural ecosystems too need evaluation with regards to soil management. Soil tillage has been recognized as detrimental to environmental sustainability.

Our results indicated that, despite shifts in vegetation, total annual net primary productivity (ANPP) was similar between burned and unburned prairie. There was no significant difference in the SOC content at the 0-30 cm soil depth in both burned and unburned tallgrass prairie. We also report that, 24 y of long term burning did not affect SOC relative to the unburned tallgrass prairie. Therefore this ecosystem is stable with regards to SOC and burning is an appropriate management practice to preserve the tallgrass prairie without loss in SOC. The burning loses of aboveground vegetation could have been compensated by higher belowground productivity reported in burned tallgrass prairie. The burned and unburned tallgrass prairie, however differed in the depth distribution of SOC and TN at 5-15 and 15-30 cm depths. This could indicate differences in the internal processes that cycle OC and nutrients in these systems as indicated by depth-related differences in the microbial communities, enzyme activities and labile C.

The microbial community was different between the burned and unburned prairie and influenced differently by different nutrient addition. The MB was higher at 0-5 cm in the unburned tallgrass prairie, but higher for burned at 5-15 cm depth. In addition, while the MB at 0-5 accumulated P, at 5-15 cm depth it was N that accumulated in MB. This indicates differences in the processes that operate at different soil depths in these ecosystems in spite of the similar SOC. These results suggest differences in nutrient cycling, but similar mechanisms of SOC stabilization.

Our results indicated higher SOC in the less disturbed RP and NT ecosystems than the disturbed CT, in spite of similar amounts of total biomass returned to soil for all ecosystems at 0-7.5 cm depth. Also, the rate of SOC sequestration was similar between RP and NT which was about 3x higher than CT. We also report greater fungi and AMF

biomass with less soil disturbance. The macroaggregate increased with less soil disturbance with current decrease in the microaggregates. Also, the aggregate-associated C was greater in RP and NT than CT. These results indicate that, less soil disturbance is important in SOC stabilization and was the driving factor for the higher SOC in RP and NT than CT.

Therefore our study has confirmed that less soil disturbance significantly increase SOC. The important mechanism for increasing soil C involves soil management practices that do not break fungal and AMF hyphae, and roots. The management practices that reduce soil disturbance, increase soil macroaggregates and will occlude SOC. Additionally, increasing soil macroaggregates has the additional benefits of improving soil structure, better, aeration and nutrient retention, and better temperature regulation. A buildup of soil macroaggregates will increase the associated C and N protected within the macroaggregate, separating OC from microbes and reducing SOC mineralization. Therefore implementation of NT can be important both as a practice to ensure global food security but also environmental conservation.

Future research is required to continue examination of the aggregate and SOC dynamic in these soils in order to assess how long it could take for SOC to reach equilibrium in the different ecosystems. It would also be important to assess the mineralization kinetics of aggregate-associated SOC in the macroaggregate sizes of the various ecosystems. This could prove informative as to the rate of mineralization of occluded C especially between NT and RP with supposedly different residue quality.

Appendix A - Chapter 2

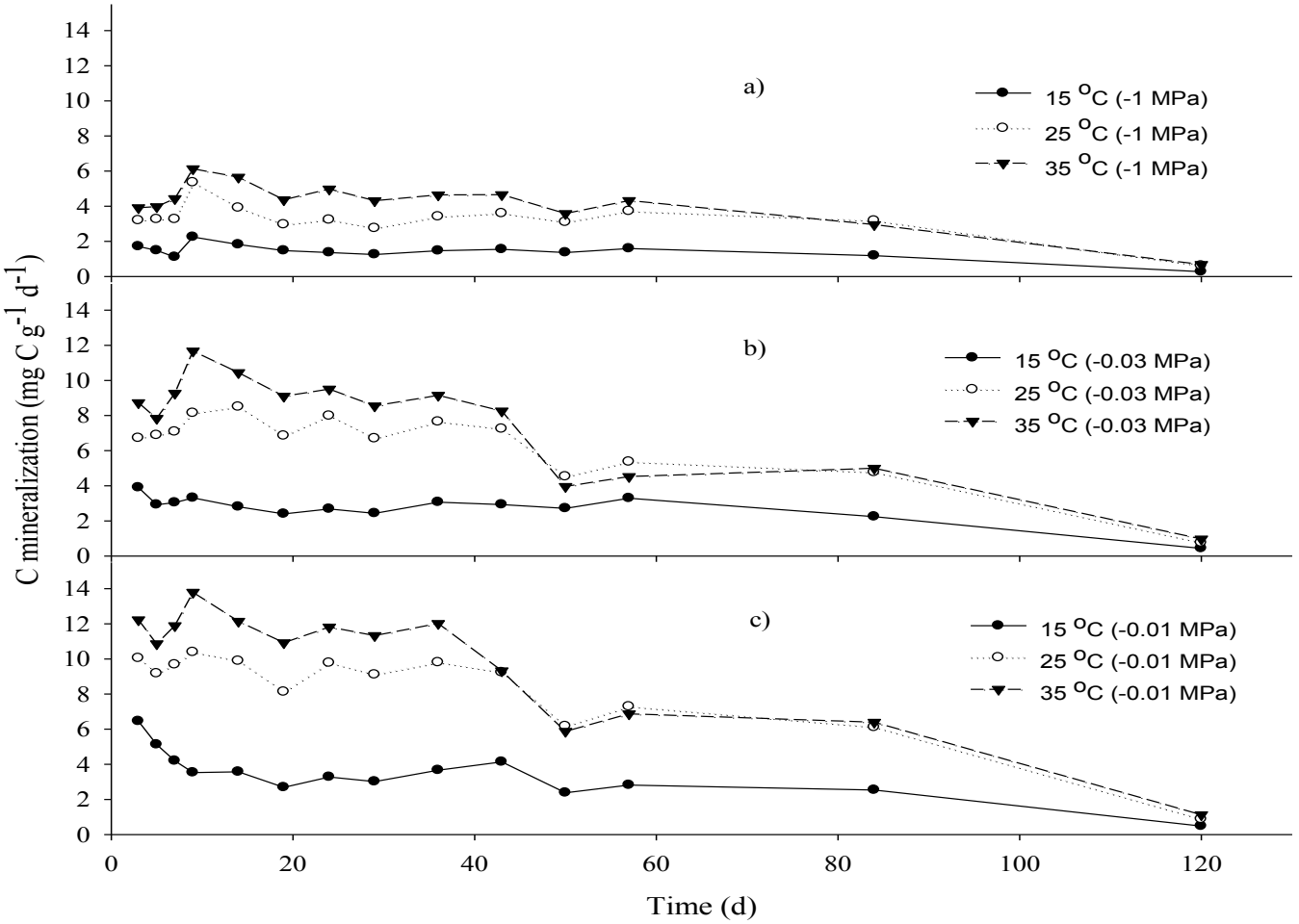

Figure A-2. 1. Dynamics of SOC mineralization rate of soils incubated at 15, 25 and 35 ^{o}C at a) -1 b) -0.03 and c) -0.01MPa for 120 d.

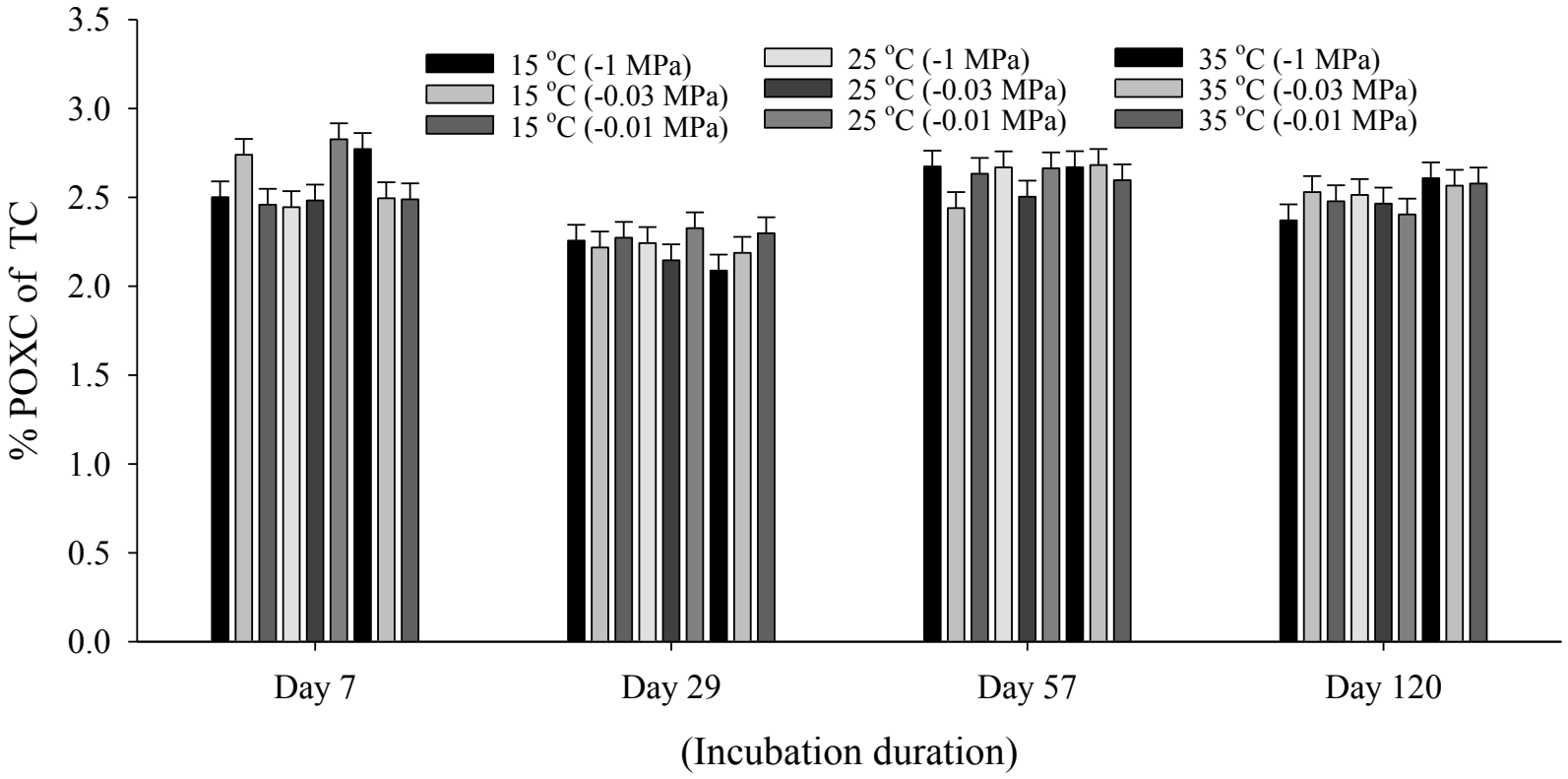

Figure A-2. 2. Permanganate oxidizable C (POCX) of soil incubated at 15, 25 and 35 °C under varying soil water contents (-1, -0.03, -0.01 MPa) for samples collected on days 7, 29, 57 and 120 d. Error bars represent standard error of the mean (n = 4).

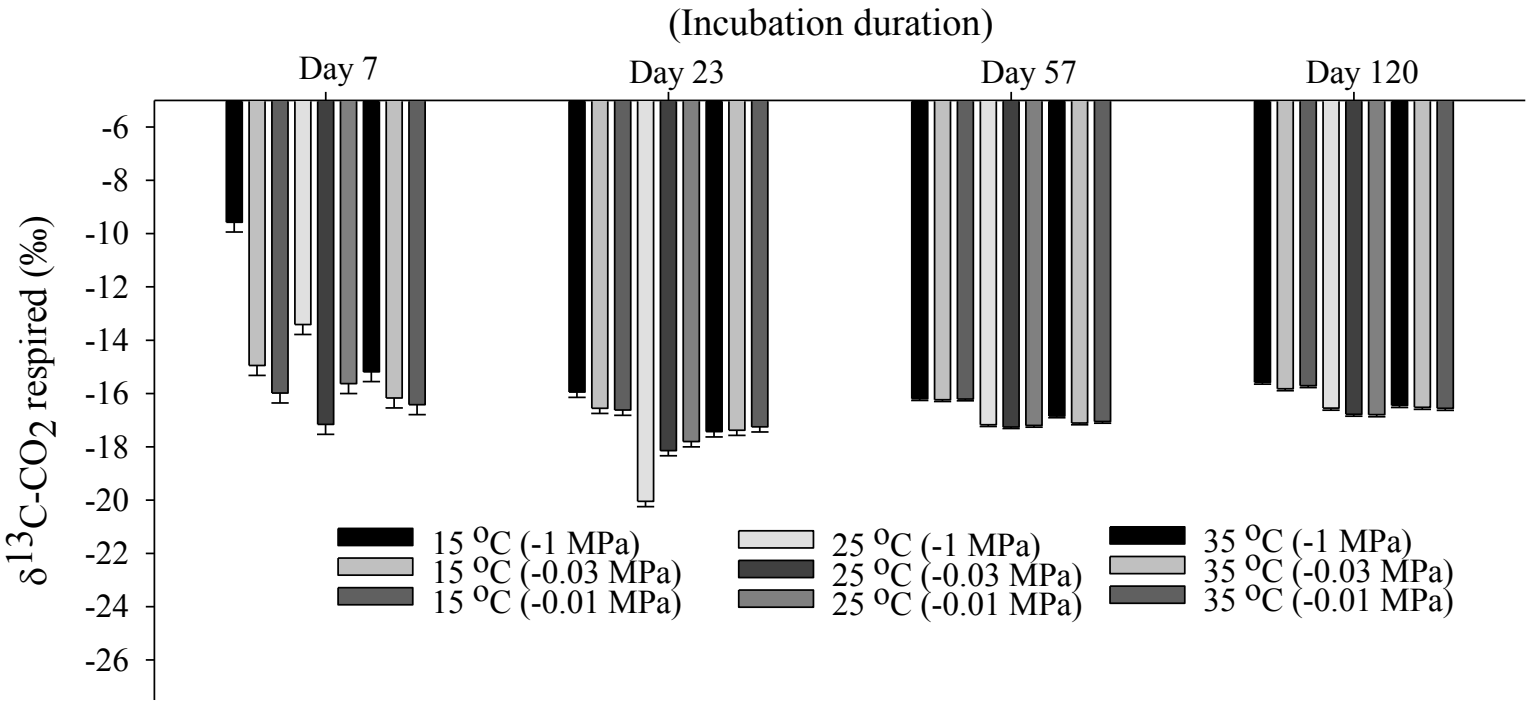

Figure A-2. 3. The δ13C C (POCX) of soil incubated at 15, 25 and 35 ^{o}C under varying soil water contents (-1, -0.03, -0.01 MPa) for samples collected on days 7, 29, 57 and 120 d. Error bars represent standard error of the mean (n = 4).

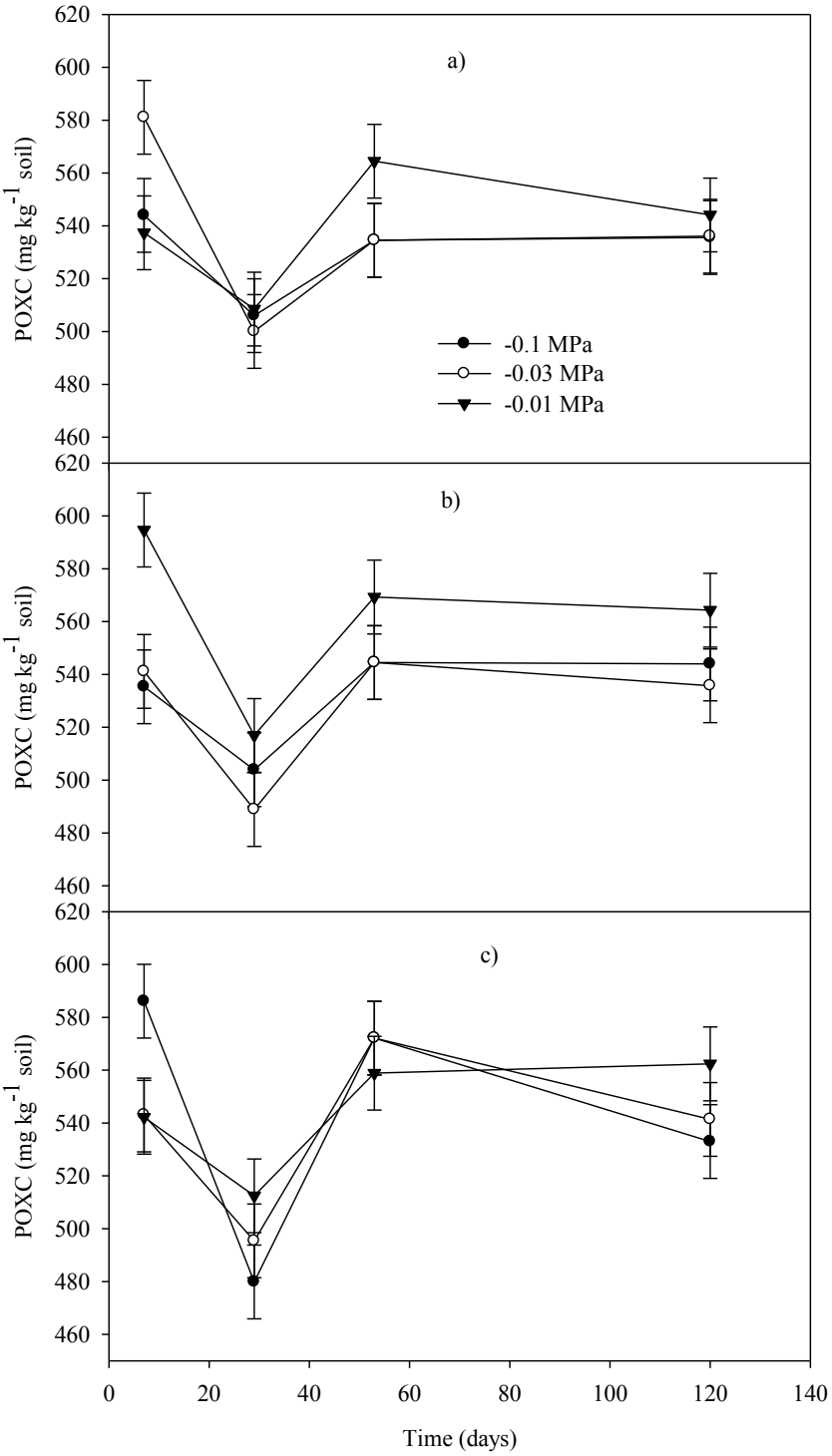

Figure A-2. 4. Permanganate oxidizable C (POXC) dynamics of soil incubated at 15, 25 and 35 ^{o}C under varying soil water potentials (-1, -0.03, -0.01 MPa) for 120 d. Error bars represent standard error of the mean (n = 4).

Appendix B - Chapter 3

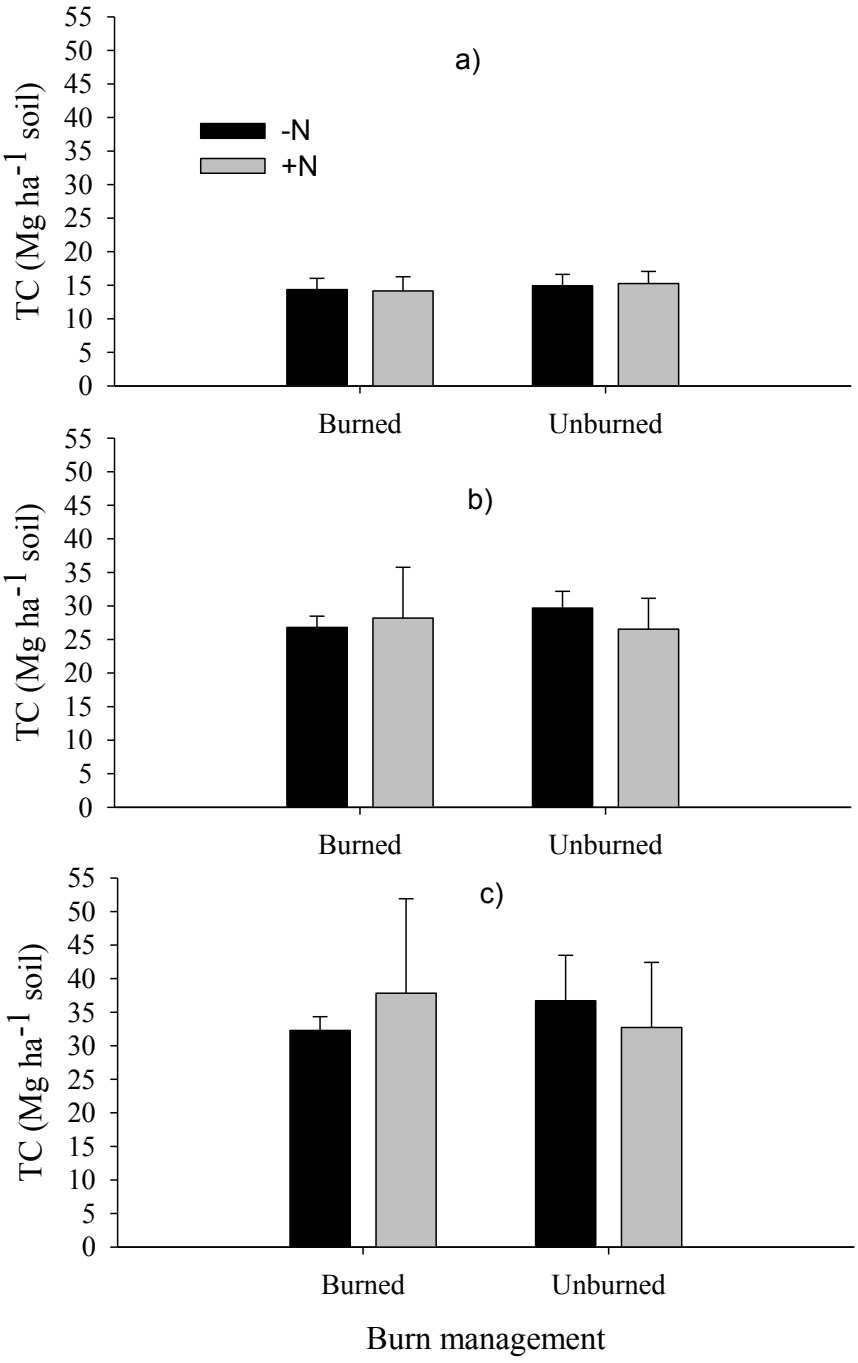

Figure B-3. 1. Burn and nutrient N amendment effect on TC at (a) 0-5 (B) 5-15 and (c) 15-30 cm depth of a mesic tallgrass prairie at the Konza Prairie Biological Station in 1989.

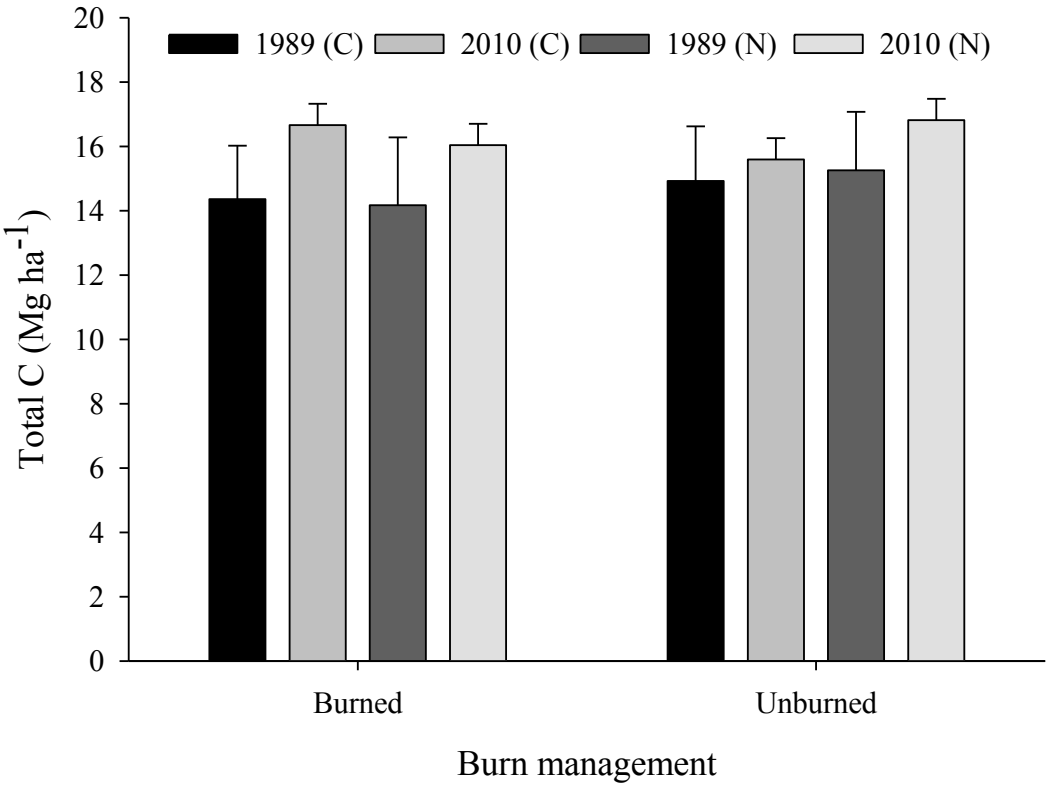

Figure B-3. 2. Comparison of total C for 1989 and 2010 of a tallgrass prairie soil under long term burned management and nutrient (N) amendment at various 0-5 cm depth. N:B C in legend denotes control.

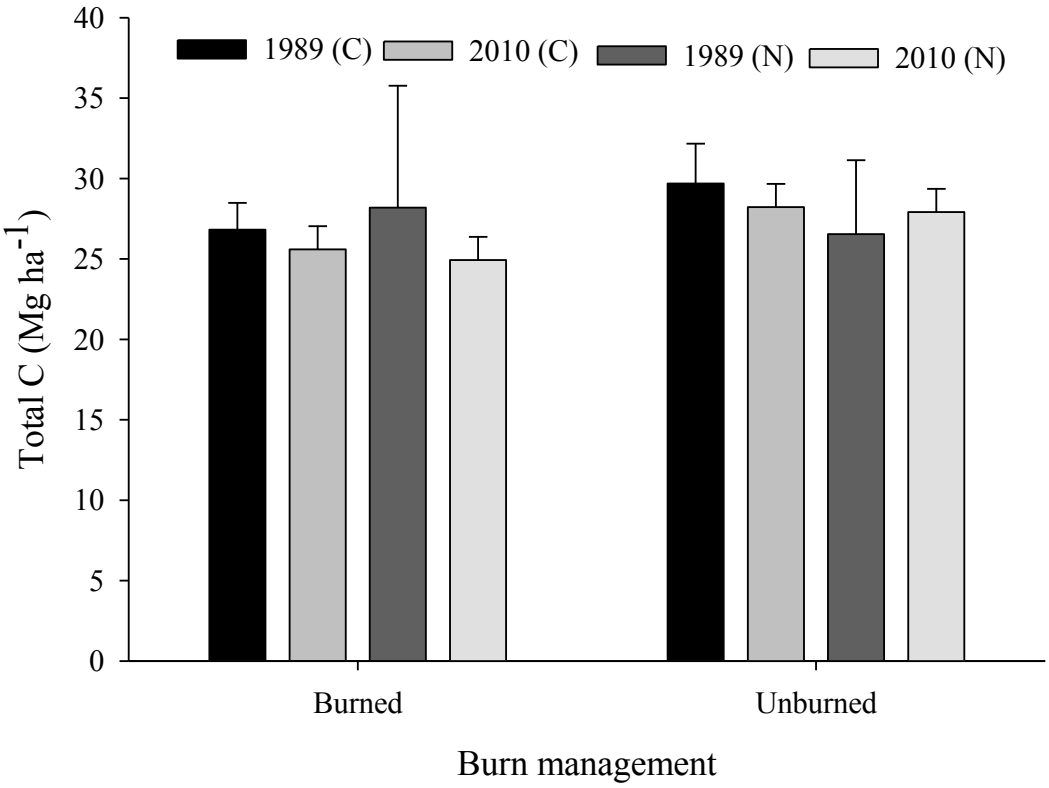

Figure B-3. 3. Comparison of total C for 1989 and 2010 of a tallgrass prairie soil under long term burned management and nutrient (N) amendment at various 5-15 cm depth. N:B C in legend denotes control.

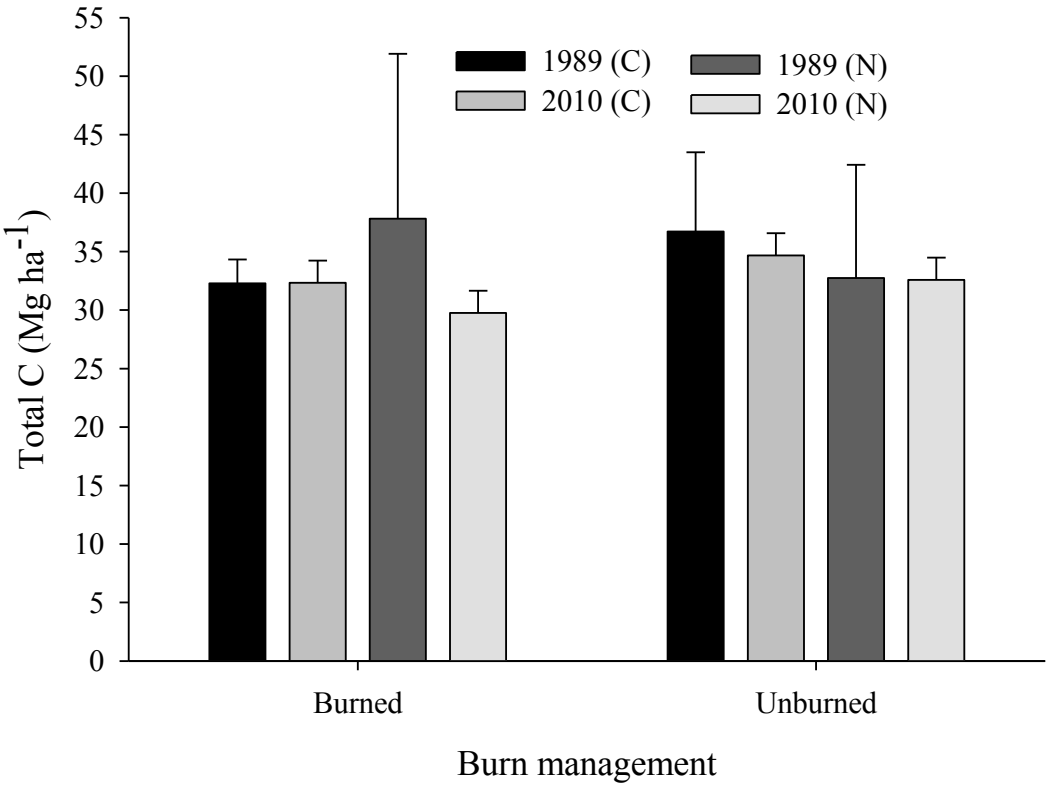

Figure B-3. 4. Comparison of total C for 1989 and 2010 of a tallgrass prairie soil under long term burned management and nutrient (N) amendment at various 15-30 cm depth. N:B C in legend denotes control.

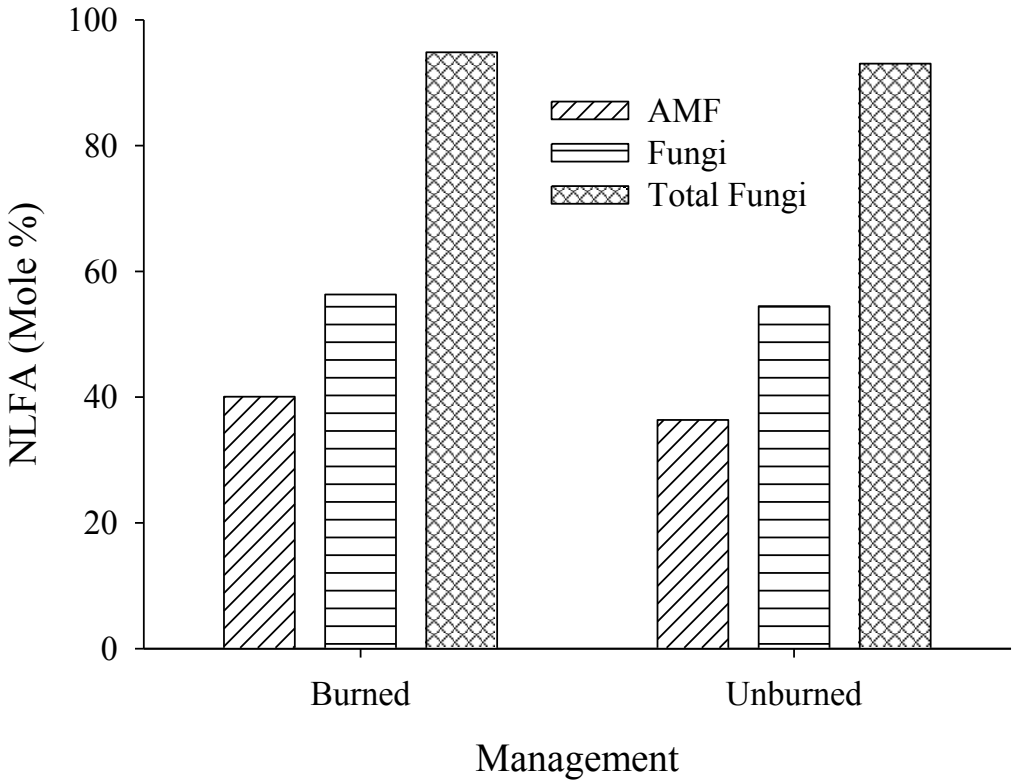

Figure B-3. 5. Abundance of specific neutral lipid fatty acids (NLFA) due to burn management of tallgrass prairie at 0-5 cm depth. Error bars represent standard error of means (n-4).

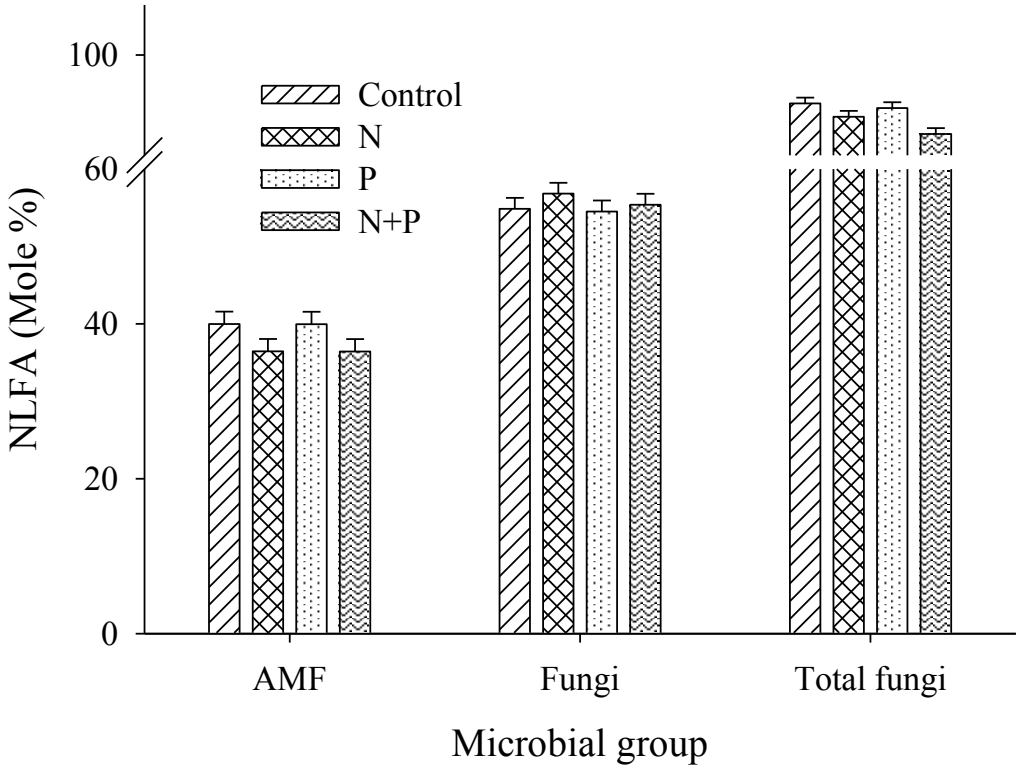

Figure B-3. 6. Abundance of specific neutral lipid fatty acids (NLFA) due to nutrient amendment of tallgrass prairie at 0-5 cm depth. Error bars represent standard error of means (n-4).

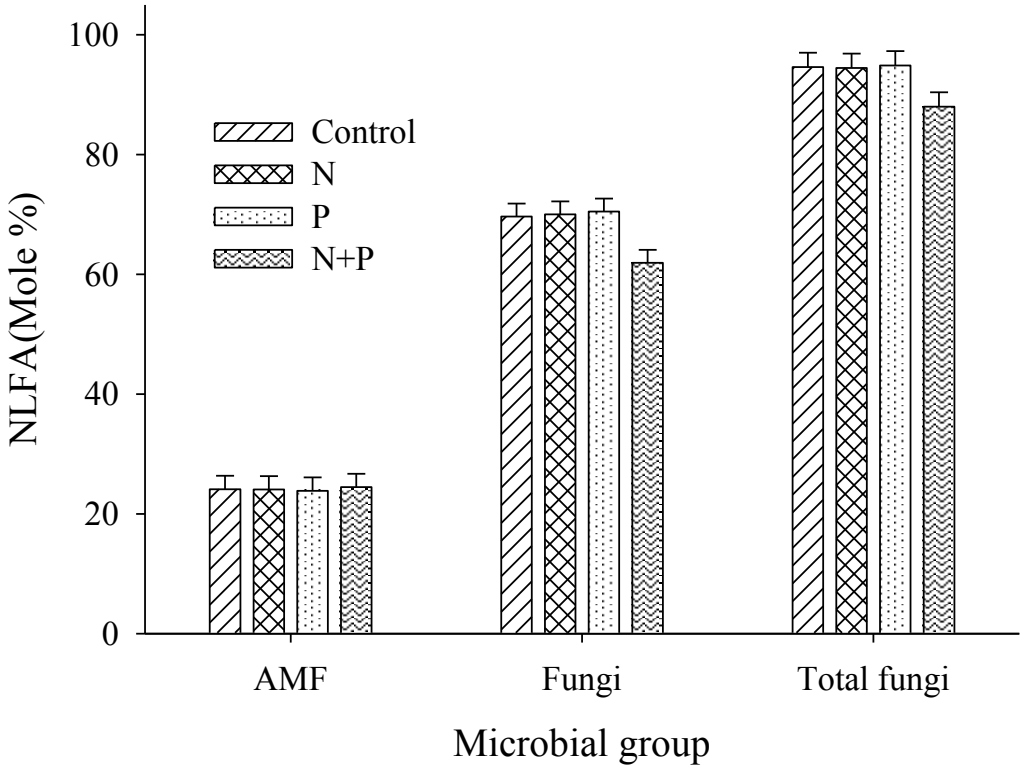

Figure B-3. 7. Abundance of specific neutral lipid fatty acids (NLFA) due to nutrient amendment of tallgrass prairie at 5-15 cm depth. Error bars represent standard error of means (n=4).

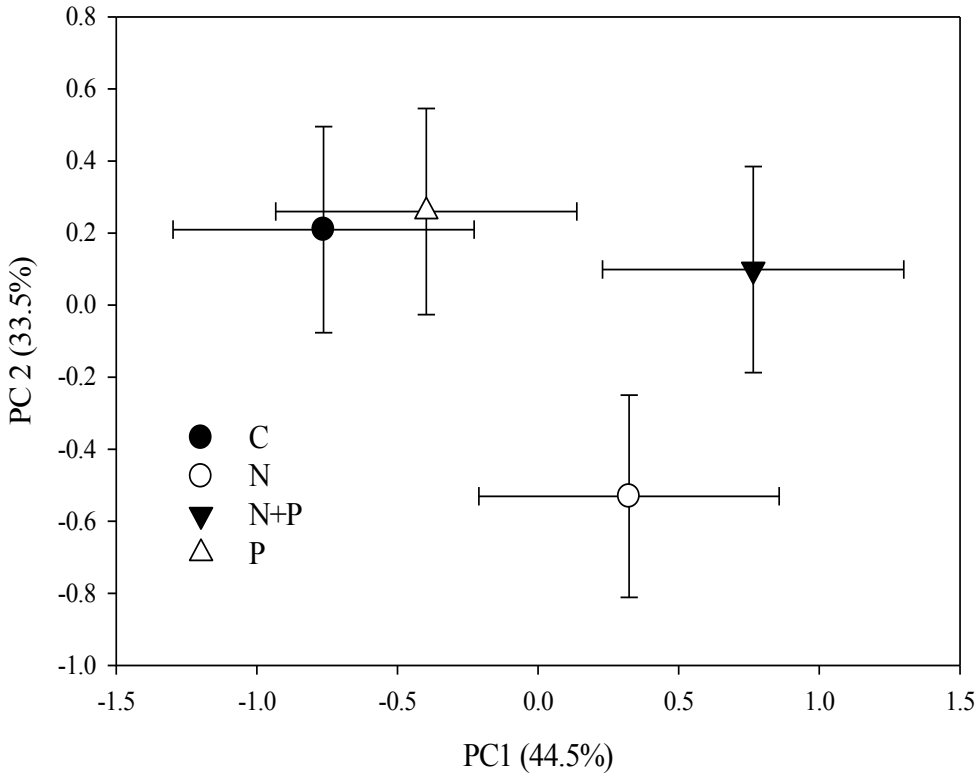

Figure B-3. 8. Soil total N (TN) represented by the first and second principal components of TN ($Mgha^{-1}$ soil) at 0-5 cm depth. While there was no difference at the first principal component, nutrient N addition separated from control, P and N+P along principal component (PC2) axis. Nitrogen accumulation in soil was altered relative to control by N amendment. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n = 12).

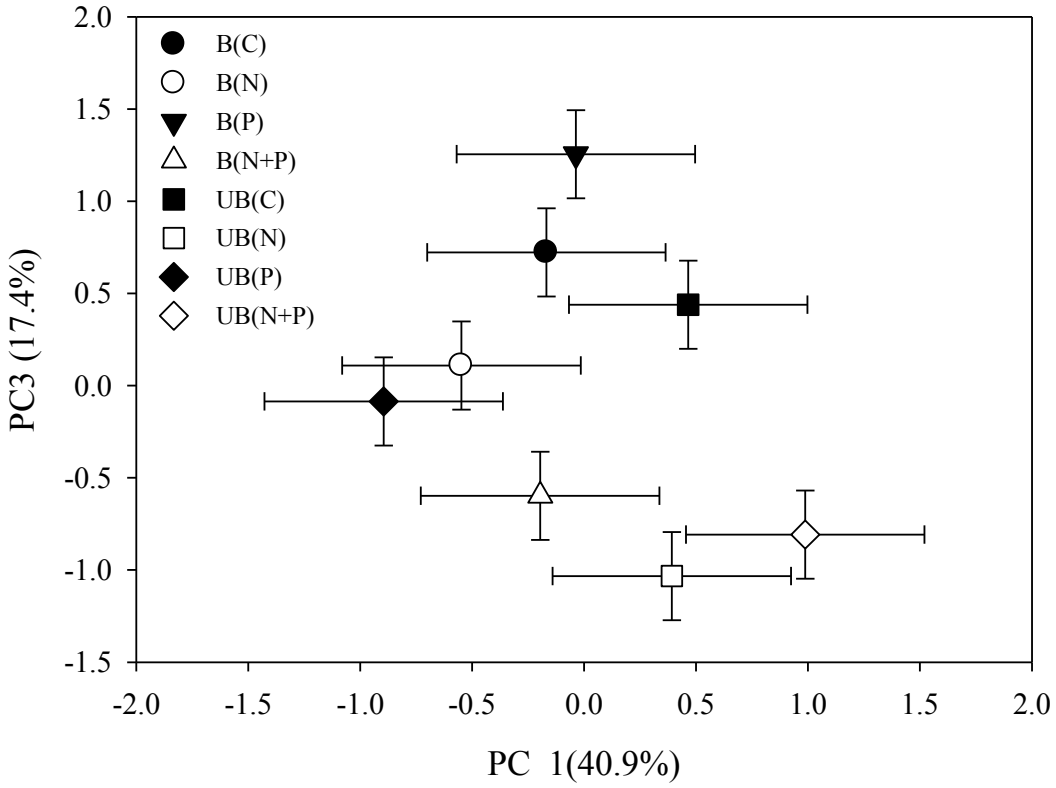

Figure B-3. 9. Soil total organic C accumulation represented by the first and third principal components TOC (Mgha-1 soil) at 5-15 cm depth. While there was no difference at the first principal component, B(N) and UB(P) separated on from B(N+P),UB(N+P) and (UB(N) which were also separated from B(P), (BC) and UB(C) along Principal Component (PC3) axis. Carbon accumulation in soil at 5-15 cm depth was altered by nutrient amendment. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n = 12).

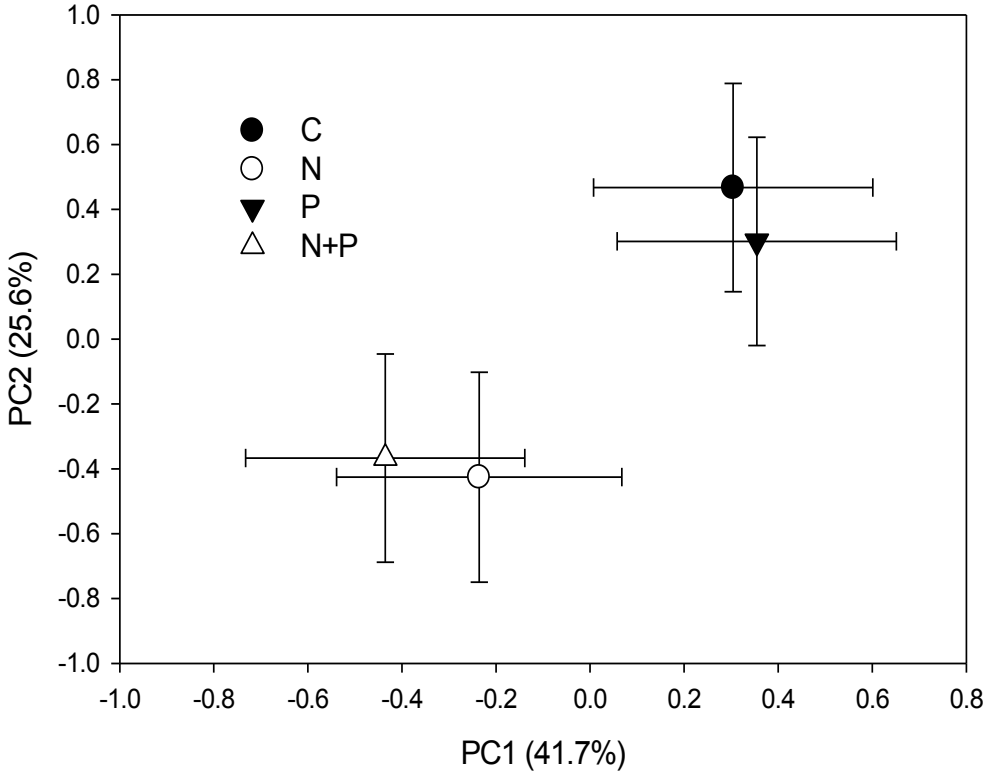

Figure B-3. 10. Soil enzyme (metabolic capacity) represented by the first and second principal components of enzyme activities (µg PNP g^{-1} soil h^{-1}) at 5-15 cm depth. Control and P against N and N+P differed along Principal Component (PC2) axis. Metabolic capacity of the soil was altered relative to control by N amendment. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n = 12).

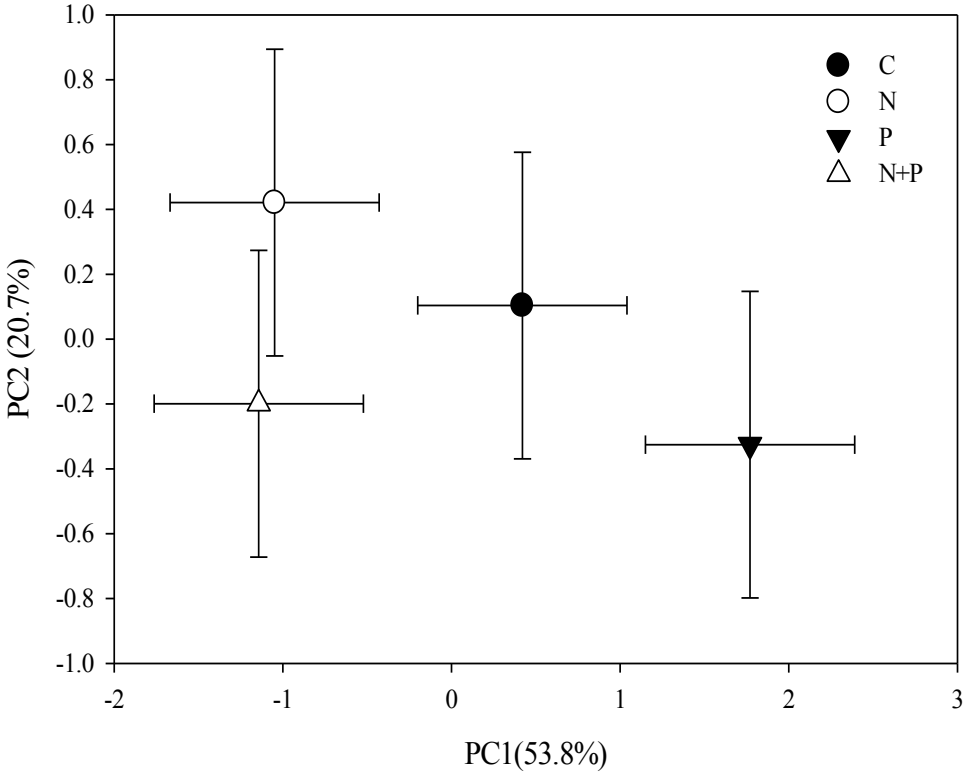

Figure B-3. 11. Soil microbial community represented by the first and second principal components of soil phospholipid fatty acids (PLFA) estimated as nmol PLFA g^{-1} soil at 0-5 cm depth. While there was no difference at the second principal component, nutrients N+P and N amendment separated from control, which as different from P amendment along Principal Component (PC1) axis. Phosphorus amendment increased microbial biomass while N either reduced it alone or increased it when added together with P. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n = 4).

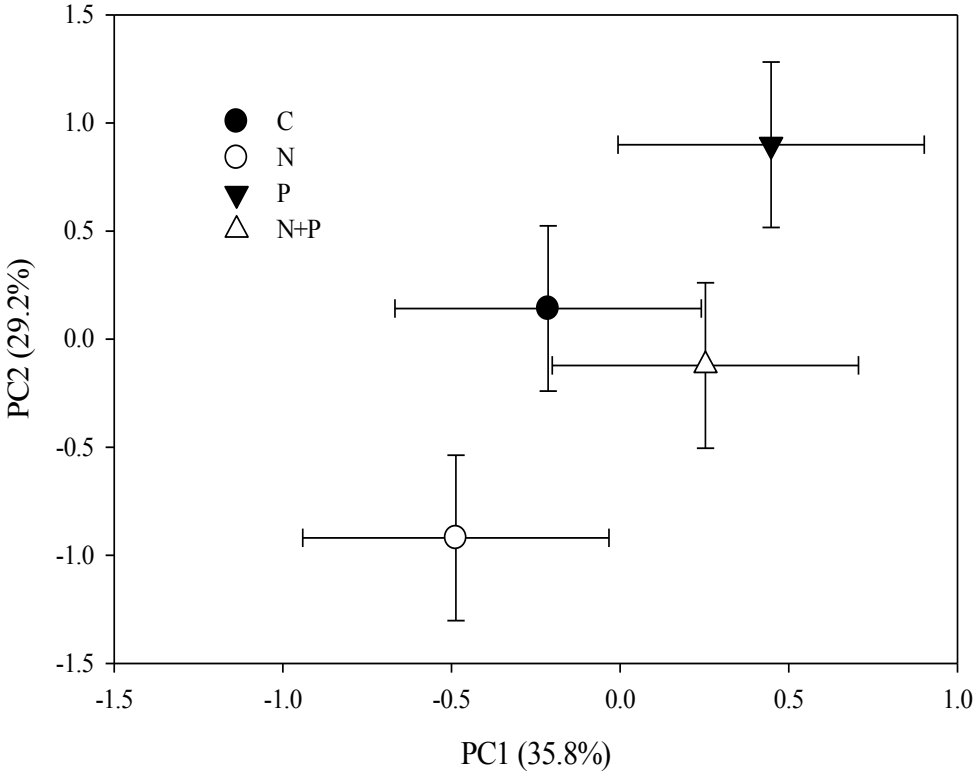

Figure B-3. 12. Soil microbial community represented by the first and second principal components of soil phospholipid fatty acids (PLFA) estimated as nmol PLFA g-1 soil at 5-15 cm depth. There was no difference at the first principal component. At the second principal component, nutrients N separated from the control which was similar to N+P and also separated from P amendment. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n = 4).

Table B-3. 1. Analysis of variance (*P*-values) for total N (TN), total organic C (TOC), C/N and permanganate oxidizable C (POXC) responses to long term burned management and nutrient addition of a tallgrass prairie soil at various depths during different seasons.

	Total nitrogen	Total organic C	C/N	Permanganate
	---------- *P*-values ----------			
Burned (B)	0.0943	0.5173	0.0048	0.5197
Nutrient (N)	0.0005	0.5966	<.0001	0.5884
B x N	0.1182	0.0474	0.0002	0.5008
Depth (D)	<.0001	<.0001	<.0001	<.0001
B x D	0.038	0.0444	0.1079	0.2904
N x D	0.2512	0.2779	0.5957	0.1793
B x N x D	0.5568	0.5711	0.0029	0.7338
Season (S)	0.1893	0.496	0.0103	<.0001
B x S	0.2821	0.3965	0.9203	0.5446
N x S	0.7872	0.7059	0.1813	0.6647
B x N x S	0.3335	0.3159	0.5719	0.7368
D x S	0.0832	0.1725	0.5283	<.0001
B x D x S	0.1455	0.1873	0.8335	0.504
N x D x S	0.5802	0.559	0.4169	0.6421
B x N x S	0.9569	0.9364	0.576	0.7806

Significant values are $P<0.05$

Table B-3. 2. Analysis of variance (*P*-values) for enzyme activities of a tallgrass prairie soil, under long term burned management and nutrient (N and P) amendments at various soil depths during different seasons.

	β-glucosaminidase	β-glucosidase	Acid phosphatase	Alkaline phosphatase
	P-values			
Burned (B)	0.3878	0.1611	0.3306	0.6944
Nutrient (N)	0.0099	0.985	0.6684	<.0001
B x N	0.6825	0.1749	0.1012	0.1285
Depth (D)	<.0001	<.0001	<.0001	<.0001
B x D	0.0097	0.0895	0.0692	0.2114
N x D	0.453	0.4306	0.6293	0.0015
B x N x D	0.6732	0.8464	0.7599	0.7992
Season (S)	<.0001	<.0001	<.0001	0.0003
B x S	0.4175	0.7289	0.5921	0.2239
N x S	0.4274	0.5856	0.7757	0.7006
B x N x S	0.1915	0.0873	0.5854	0.6035
D x S	<.0001	<.0001	<.0001	0.2138
B x D x S	0.7261	0.2686	0.4025	0.2312
N x D x S	0.5094	0.4012	0.1102	0.4001
B x N x S	0.8411	0.8562	0.8528	0.9437

Significant values are $P<0.05$

Table B-3. 3. Analysis of variance (*P*-values) for major microbial community groups and indices, using PLFA, of a tallgrass prairie soil under long term burned management and nutrient (N and P) amendment at various soil depths.

	Actinomycetes	AMF	Bacteria	Gram-	Gram+	Fungi	F/B
	P-values						
Burned (B)	0.0397	0.0857	0.3966	0.0864	0.7499	0.1613	0.9245
Nutrient (N)	0.4055	<.0001	0.211	0.0061	0.8876	<.0001	0.0011
B x N	0.2309	0.9458	0.0127	0.1007	0.8771	0.5336	0.0816
Depth	0.0138	<.0001	0.0943	<.0001	<.0001	0.0001	0.8734
B x D	0.0228	0.4367	0.0056	0.2396	0.0226	0.1299	0.3803
N x D	0.5092	0.0001	0.0025	0.3884	0.3676	0.1822	0.0207
B x N x D	0.5738	0.6156	0.2047	0.4163	0.76	0.8141	0.2827

Significant values are $P<0.05$

Table B-3. 4. Analysis of variance (*P*-values) for fungi, using mole of % NLFA(neutral lipid fatty acids) for a tallgrass prairie soil under long term burned management and nutrient (N and P) amendment at various soil depths.

	AMF	Fungi	Total
		P-values	
		0-5	
Burned (B)	0.0338	0.3678	0.008
Nutrient (N)	0.2244	0.633	0.0103
B x N	0.6881	0.6858	0.6873
		5-15	
Burned (B)	0.2665	0.8735	0.3087
Nutrient (N)	0.9985	0.0397	0.288
B x N	0.3779	0.9896	0.4808

Significant values are $P<0.05$

Table B-3. 5. Enzyme activity ratios for different enzyme involved in C mineralization and nutrient N cycling at 0-5cm depth.

		Enzyme activity ratios		
β-Glucosidase		N	P	N+P
	Burn	0.99	1.01	1.02
	Unburn	1.04	1.02	1.02
β-Glucosidase		March	July	October
	Burn	1	0.98	0.99
	Unburn	0.97	1.03	1.05
		March	July	October
β-Glucosaminidase		1	0.95	1.06

Potential β-glucosidase activity > 1 indicates substrate availability for C acquisition, while activity ratio < 1 indicate low C substrate. Activity ratios of β-glucosaminidase> 1 indicate nutrient limitation i.e. higher microbial investment for nutrient acquisition. Potential β-glucosaminidase enzyme activity ratios < 1 indicate sufficiency.

Table B-3. 6. Enzyme activity ratios for different enzyme involved in C mineralization and nutrient N and P cycling at 5-15cm depth.

	Enzyme activity ratios					
β-Glucosidase		B(N)	B(P)	B(N+P)	UB(N)	UB(P)	UB(N+P)
	March	0.99	0.98	0.95	0.93	0.91	1.00
	July	0.89	0.97	0.98	1.05	1.08	0.98
	October	0.97	1.01	1.01	1.02	0.99	0.95
β-Glucosaminidase				N	P	N+P	
				0.947	1.0136	0.9326	
Acid phosphatase	Burn			0.98			
	Unburn			1.00			
Acid phosphatase				March	July	October	
				0.9609	1.0212	0.9793	
				March	July	October	
Alkaline phosphatase	Burn			0.98	0.98	0.94	
	Unburn			0.90	1.02	0.96	
Alkaline phosphatase	March			N	P	N+P	
	July			0.94	0.94	0.94	
	October			0.99	1.05	0.96	
				0.88	1.03	0.94	

Potential β-glu activity > 1 indicates substrate availability for C acquisition, while activity ratio < 1 indicate low C substrate. On the contrary, activity ratios of β-glsm and phosphatases > 1 indicate nutrient limitation i.e. higher microbial investment for nutrient acquisition of the specific nutrients. Potential β-glsm and phosphatases enzyme activity ratios < 1 indicate sufficiency.

Appendix C - Chapter 4

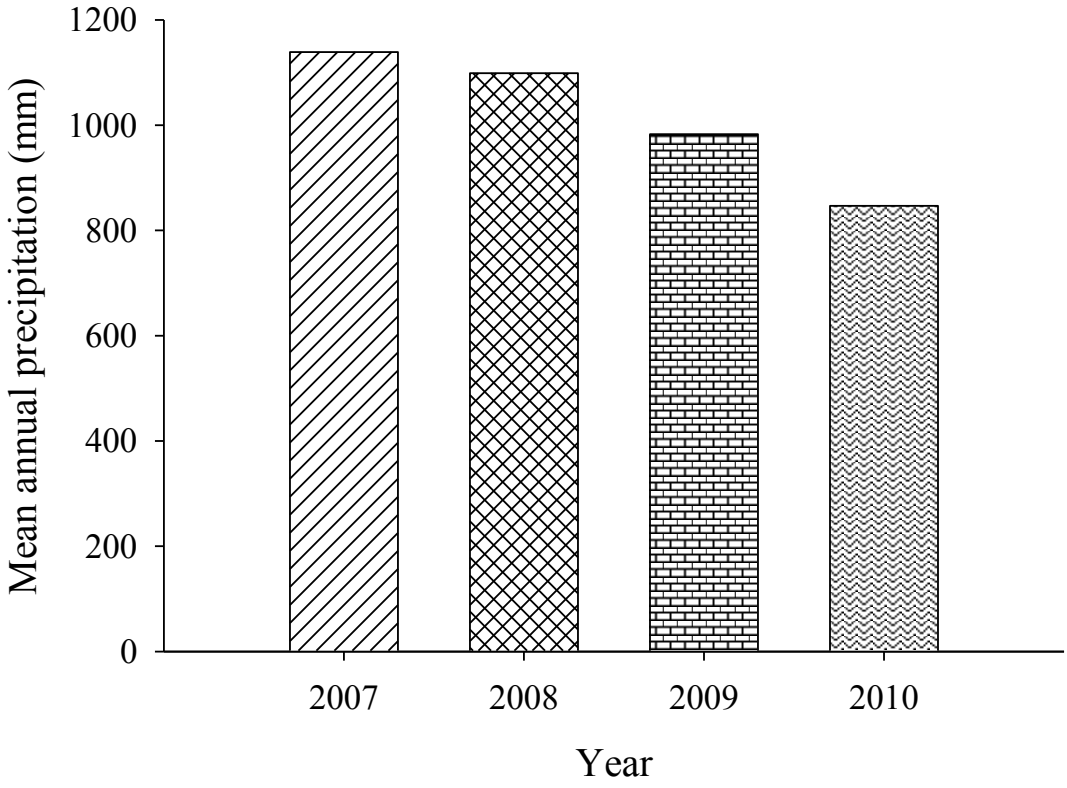

Figure C-4. 1. Mean annual precipitation for Manhattan from 2007 thru 2010

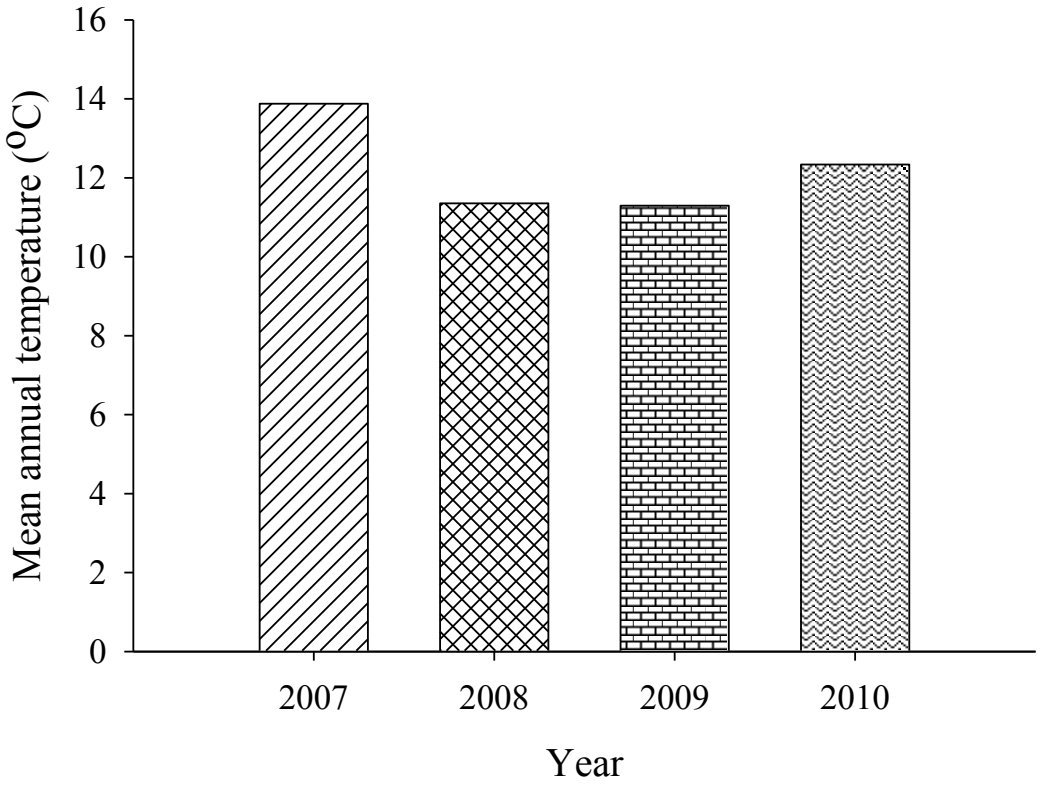

Figure C-4. 2. Mean annual temperature for Manhattan from 2007 thru 2010

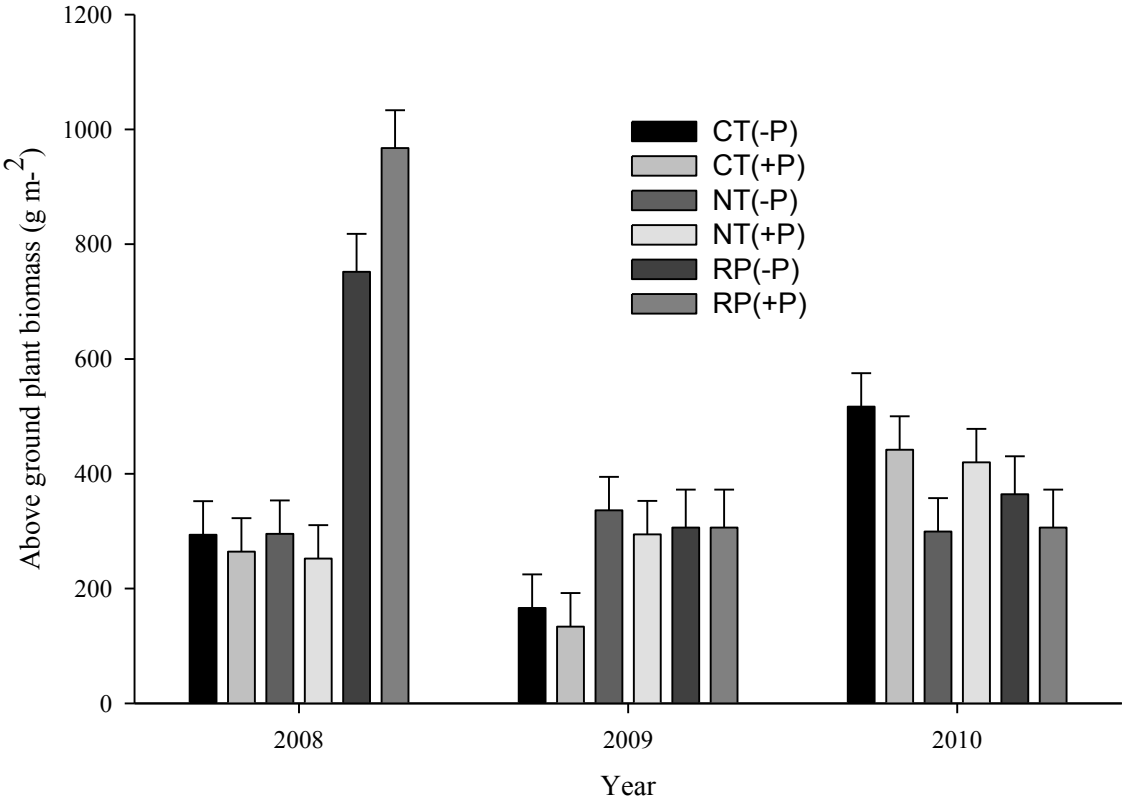

Figure C-4. 3. Ecosystem x fertilizer x time interaction (P<0.082) for aboveground plant biomass of sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*), planted in a silty clay soil.

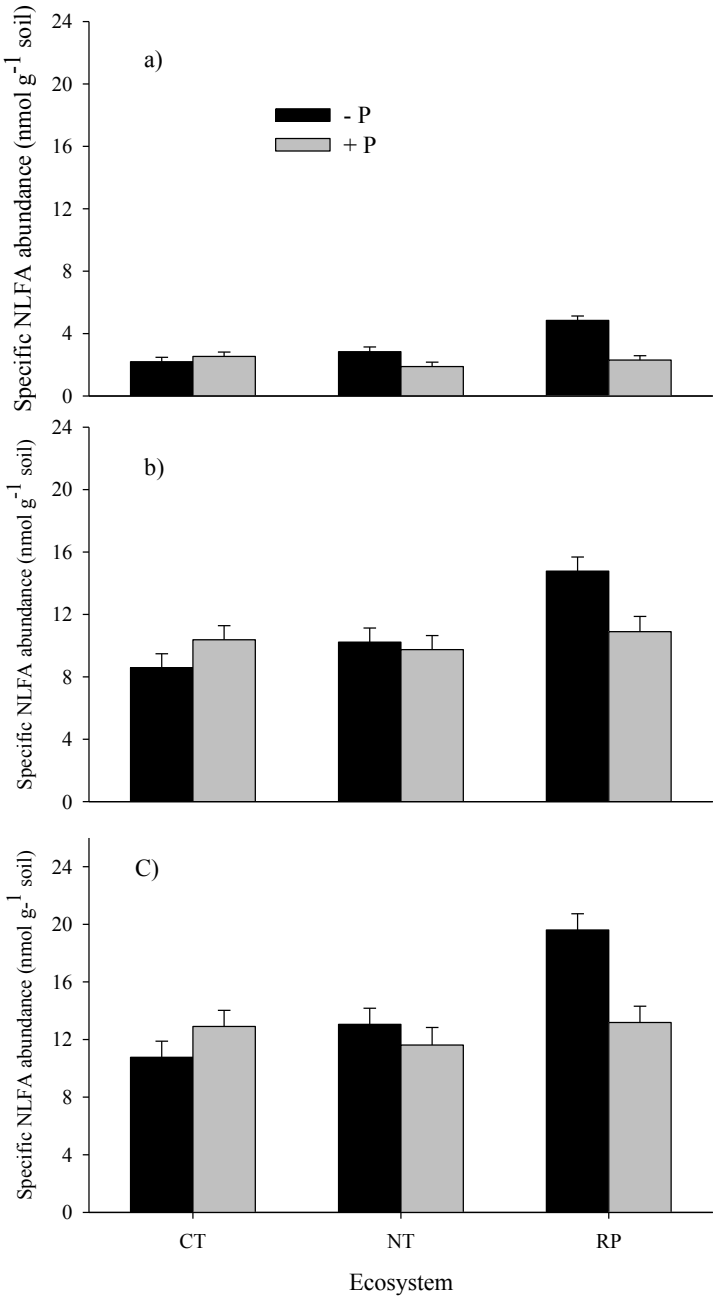

Figure C-4. 4. Ecosystem x P interaction on specific neutral lipid fatty acids (NLFA) abundance for a) arbuscular mycorhizhae (AMF) b) fungi and c) total fungi of a silty clay soil planted to sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT) and replanted prairie grass (RP) big blue stem (*Andropogon gerardii*) at 0-5 cm depth.

Table C-4. 1. Soil microbial groups estimated as phospholipid fatty acids (NLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

	Microbial biomass (NLFA) at 0-5 cm depth		
	AMF	Fungi	Total fungi
	---------- nmol g^{-1} soil ----------		
RP (means)	3.57 a‡	12.49 a	16.06 a
NT (means)	2.37 b	10.05 b	12.42 b
CT (means)	2.36 b	9.73 b	12.09 b
2009 (means)	3.8a†	13.66 a	17.51 a
2010 (means)	1.69 b	7.86 b	9.54 b
	---------- *P*-values----------		
Ecosystem (E)	0.0001	0.009	0.002
Fertilizer (F)	0.083	0.4826	0.3146
E x F	<.0001	0.002	0.0002
Time (T)	<.0001	<.0001	<.0001
E x T	0.8028	0.7539	0.8569
F x T	0.0942	0.0821	0.0692
E x F x T	0.0628	0.1602	0.063

‡ Different letters represent significant with columns (microbial group).

† Different letters represent significant between years for each microbial group.

Table C-4. 2. Soil microbial groups estimated as phospholipid fatty acids (NLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 0-5 cm depth.

		Microbial biomass (NLFA) at 0-5 cm depth		
		AMF	Fungi	Total fungi
		----------mol%----------		
CT	2009	7 c‡	26 cd	33
	2010	30 b	35 a	65
NT	2009	5 c	23 d	29
	2010	31 b	34 ab	64
RP	2009	9 c	28 c	37
	2010	40 a	30 bc	70
		----------*P*-values----------		
Ecosystem (E)		<.0001	0.3833	0.0116
Fertilizer (F)		0.2171	0.3751	0.9168
E x F		0.4343	0.7786	0.5487
Time (T)		<.0001	<.0001	<.0001
E x T		0.016	0.0171	0.6782
F x T		0.663	0.7282	0.9718
E x F x T		0.4847	0.759	0.861

‡ Different letters means differences within columns.

Table C-4. 3. Soil microbial groups estimated as phospholipid fatty acids (NLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

	Microbial biomass (NLFA) at 5-15 cm depth		
	AMF	Fungi	Total fungi
	---------- nmol g^{-1} soil ----------		
CT (means)	20.07	19.34 c†	39.41
NT (means)	21.49	24.37 b	45.85
RP (means)	34.37	29.58 a	63.95
2009 (means)	29.76 a‡	33.3 a	63.06 a
2010 (means)	12 b	17.31 b	36.41 b
	----------*P*-values----------		
Ecosystem (E)	0.2045	0.0357	0.1187
Fertilizer (F)	0.1378	0.2416	0.161
E x F	0.6609	0.7512	0.7052
Time (T)	<.0001	<.0001	<.0001
E x T	0.1471	0.092	0.1114
F x T	0.1868	0.2761	0.2057
E x F x T	0.4042	0.3847	0.3852

‡ Different letters means differences between years for each microbial group.

† Different letters means differences between ecosystems for each microbial group

Table C-4. 4. Soil microbial groups estimated as phospholipid fatty acids (NLFA) of a soil planted with sorghum (*Sorghum bicolor*) under continuous tillage (CT) and no-till (NT); and replanted prairie grass (*Andropogon gerardii*) at 5-15 cm depth.

		Microbial biomass (NLFA) at 5-15 cm depth		
		AMF	Fungi	Total fungi
		----------mol%----------		
CT	2009	4 c‡	20 c	25
	2010	25 b	33 a	60
NT	2009	4 c	20 c	24
	2010	33 b	33 a	59
RP	2009	4 c	21 c	25
	2010	24 a	29 b	62
		----------*P*-values----------		
Ecosystem (E)		0.006	0.3117	0.7089
Fertilizer (F)		0.2879	0.9011	0.2192
E x F		0.4333	0.3487	0.3432
Time (T)		<.0001	<.0001	<.0001
E x T		0.0144	0.0323	0.6113
F x T		0.7819	0.5518	0.9795
E x F x T		0.7232	0.1792	0.3152

‡ Different letters means differences within columns.