

AN ABSTRACT OF THE THESIS OF

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Changes in fire frequency have allowed native woody shrub species to encroach on native herbaceous tallgrass prairie in the Great Plains of North America. Although these shrub species are native to this ecosystem, their abundance and distribution have increased dramatically due to different changes in land use and land management. We examined vegetative type and microhabitat type effects on decomposition rates and soil CO₂ efflux under a representative tallgrass species, *Sorghastrum nutans* (Indian grass), and shrub species *Cornus drummondii* (rough-leaf dogwood) at the Ross Natural History Reservation in Americus, Kansas. Shrub microhabitats had greater decomposition rates than tallgrass microhabitats and thus decreased C storage. Rough-leaf dogwood litter had faster decomposition rates than Indian grass litter. Tallgrass litter contained higher levels of lignin and cellulose thus slowing their decomposition rate. Soil respiration rates were not different between grass and shrub habitats. Shrub litter and tallgrass litter had different carbon isotope values, $\delta^{13}\text{C}$; however, soil organic matter $\delta^{13}\text{C}$ was not different between habitat types. This suggests that soil organic matter under shrub habitats may be a legacy effect from previous tallgrass conditions. These results suggest that shrub encroachment in tallgrass prairie could result in less soil carbon storage in the long term.

Changes in land management, primarily the inclusion of fire, should reduce shrub encroachment while promoting tallgrass species. The promotion of tallgrass prairie species could help offset rising atmospheric CO₂ levels by increasing soil carbon storage.

Assessment of shrub encroachment on belowground carbon
dynamics in tallgrass prairie using an isotopic approach

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Preface

My thesis contains 2 chapters. The first chapter contains necessary background information for my thesis. The second chapter is written in the style dictated by The American Midland Naturalist Journal.

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Chapter 1- Background

Over the last century woody vegetation has increased in abundance and density in grass-dominated ecosystems worldwide (Bragg and Hulbert, 1976; Hobbs and Mooney, 1986; Knight et al., 1994; Archer, 1995; Wilson and Kleb, 1996; Hoch and Briggs, 1999; Roques et al., 2001; Briggs et al., 2002a; Briggs et al., 2002b). Less than 4% of the native tallgrass prairie remains compared to pre-European settlement (Sampson and Knopf, 1994). Herbaceous tallgrass species evolved concurrently with 3 – 5 year fire intervals (Wright and Bailey, 1982). Changes in land management, fire suppression and change in fire frequency have allowed litter accumulation causing a decline in grass production and allowing an increase in the abundance and distribution of woody plants (Bragg and Hulbert, 1976; Abrams et al., 1986; Briggs and Knapp, 2001). Expansion of woody plants in Kansas is, in large part, not a result of introduced woody species; instead these plants are native to prairie ecosystems and have increased in density and abundance due to changes in abiotic and biotic conditions (Van Auken, 2000; Heisler et al., 2003). Shifting from dominant herbaceous grass to woody vegetation can alter aboveground plant resources (Nepstad et al., 1994; Jackson et al., 2000) and belowground nutrient cycling and carbon storage (Trumbore, 1997; Jobbagy and Jackson, 2000).

The expansion of woody plants is currently one of the greatest threats to the tallgrass prairie ecosystem of the central United States (Heisler et al., 2004; Briggs et al., 2005). Changes in fire frequency, grazing patterns, climate change and increasing atmospheric CO₂ are important contributing factors to the shift in the tallgrass plant community (Archer et al., 2001). Changes in fire frequency have been implicated as the major factor leading to an increase in density and cover of shrubs such as *Cornus drummondii* (rough-leaf dogwood) and *Rhus glabra* (smooth sumac) within tallgrass

prairies of the central United States (Briggs et al., 1995; Briggs et al., 2002; Heisler et al., 2003; McCarron et al., 2003). Annual fire frequency has been effective in preventing woody encroachment, while 4-year and 20-year fire frequencies do little to stop woody encroachment (Heisler et al., 2003). Once woody species are established even annual fire frequency does not reduce their abundance (Heisler et al., 2003).

Nutrient cycling is predicted to change with shrub expansion due to the different morphological structures between shrubs and native tallgrass (Briggs and Knapp, 1995). The encroachment of shrubs into native tallgrass prairie may influence soil's ability to store carbon (C) (Briggs et al., 2005), possibly reducing soil C storage and increasing atmospheric CO₂ (Williams et al., 2004). Shrubs contain woody biomass, suggesting a greater capacity to store C aboveground than herbaceous grasses, but, shrub species may store less C belowground than grasses thus potentially offsetting gains in aboveground biomass C storage (Jackson et al., 2002). Herbaceous tallgrass species allocate greater than 50% of their productivity belowground (Seastedt, 1988; Seastedt and Knapp, 1993); however, the effects of shrub encroachment on belowground C storage are not fully understood.

Long term C storage is becoming increasingly important as atmospheric CO₂ concentrations continue to rise. Atmospheric CO₂ concentrations have increased dramatically since the industrial revolution and current climate models predict that CO₂ levels will continue to rise during this century (IPCC, 2007). According to the National Oceanic and Atmospheric Administration (NOAA, 2011) Mauna Loa observatory the atmospheric global annual mean CO₂ concentration for 2010 was 389 ppm. Increased atmospheric CO₂ concentrations can have positive effects on plant photosynthesis and

water usage efficiency (Morgan et al., 2008). Carbon dioxide is, however, a greenhouse gas having the potential to increase global temperatures (IPCC, 2007). Global climate models predict increases in temperature due to potential greenhouse warming (Houghton et al., 1990). Higher temperatures would increase the rate of C efflux from soils and could further increase atmospheric CO₂ (Raich and Schlesinger, 1992). Climate change models predict that some locations will receive more annual precipitation, but in the form of more extreme precipitation events (Karl and Trenberth, 2003), potentially altering soil respiration, soil temperature and soil moisture content. A more comprehensive understanding of the alteration in C storage caused by shrub encroachment is important for managing the balance of the global C budget (Van Auken, 2000).

The amount of C within the soil system is dependent on the balance between C additions and C losses from the soil. Decomposition, the process whereby organic matter is broken down into inorganic compounds that are then available for uptake by primary producers, is one of the processes that facilitates losses from the soil. Vegetative shifts from herbaceous tallgrass species to shrub species will alter the chemical composition of decomposing material (Jackson et al., 2002; McCarron et al., 2003; Lett et al., 2004). Tallgrass litter decomposes slower due to higher levels of lignin and cellulose compared to shrub litter (Sage and Monson, 1999; McCarron et al., 2003). Faster decomposition rates result in more C released into the atmosphere and less C entering the soil system thus potentially reducing soil C storage. Small changes in soil C storage can significantly impact C cycling since soils are the largest pool of terrestrial organic C on the planet, storing more C than is present in plants and in the atmosphere combined (Schlesinger, 1997). Microorganisms, such as bacteria and fungi, are the main organisms that

decompose organic material in soils, and their abundance and activity levels have been shown to be positively correlated to soil moisture content during a typical tallgrass prairie growing season (Williams et al., 2004). If annual precipitation increases, as is predicted by some climate change models (IPCC, 2007), soil microbial activity may increase potentially reducing the amount of C entering the belowground soil system. If, however, these precipitation events occur with greater intensity, albeit with less frequency (IPCC, 2007), soil microbes may not persist from one event to the next. The labile fraction of soil organic C is decomposed rapidly by microbes but other fractions are chemically and physically recalcitrant (Stevenson and Cole, 1999), possibly remaining in the soil system for hundreds and thousands of years (Luo and Zhou, 2006).

Soil organic C that is mineralized by decomposers can be released into the atmosphere as CO₂ as part of a process known as soil respiration. Soil respiration is the process by which C is released from soils by soil organism respiration, root respiration, and chemical oxidation of carbon-containing materials and is a measure of the belowground structure and function of an ecosystem (Raich and Schlesinger, 1992). Small changes in soil respiration can have a large effect on CO₂ in the atmosphere and represent a major efflux in the global carbon cycle (Raich and Schlesinger, 1992, Schlesinger and Andrews, 2000). Soil respiration rates are positively correlated with mean annual air temperature and mean annual precipitation (Raich and Schlesinger, 1992). The amount of available N in soils also affects soil respiration rates, and if insufficient N is available then decomposition will slow (Schimel, 1995). Shrub encroachment does not significantly change N availability, but has been shown to decrease soil CO₂ flux (McCarron et al., 2003; Lett et al., 2004), suggesting greater soil C

storage under shrub dominated conditions, but, decreased CO₂ flux under shrub dominated conditions may result from less plant material entering the soil system under these conditions.

One way to determine the source of C is to examine the isotopic signature of C in the litter and soil. Atmospheric CO₂ contains three naturally occurring C isotopes; ¹²C, ¹³C and ¹⁴C in the total C pool (Lambers et al., 2002). ¹⁴C is radioactive, and only contributes a very small amount (10⁻¹⁰) to the total C pool, while ¹²C and ¹³C are stable isotopes which represent 98.9% and 1.1%, respectively, of the atmospheric CO₂ (Lambers et al., 2002). The processes of C fixation during photosynthesis differ between native shrubs and dominant native tallgrasses. Shrub species engage in C₃ photosynthesis, where the enzyme rubisco captures and fixes CO₂ into a three C molecule (Taiz and Zeiger, 2006). C₃ plants are more adapted to mesic environments with CO₂ concentrations greater than 200 parts per million (Taiz and Zeiger, 2006). Dominant tallgrass species engage in C₄ photosynthesis, where the enzyme PEP carboxylase captures and fixes CO₂ into a four C molecule (Taiz and Zeiger, 2006). C₄ plants are better adapted to arid environments, with CO₂ concentrations under 200 ppm (Taiz and Zeiger, 2006). Since C₄ plants are more adapted to lower CO₂ concentrations photorespiration is minimized and the duration stomata remain open is minimized more than in C₃ shrubs, resulting in lower transpiration losses and thus better water use efficiency (Taiz and Zeiger, 2006). Increasing atmospheric CO₂ is predicted to favor photosynthesis in C₃ shrubs because they are adapted to high CO₂ environments while C₄ grasses are adapted to low CO₂ environments (Taiz and Zeiger, 2006). Due to the difference between the carboxylating enzymes in C₃ shrubs, rubisco, and C₄ tallgrasses, PEP carboxylase, the values of the

isotopic C signature vary between the two photosynthetic pathways (Lambers et al., 2002). Stable isotope ratios in plant residues can be used to determine the composition of plants using a particular type of photosynthetic pathway. ^{13}C is heavier than ^{12}C because it contains one more neutron in its nucleus (Lambers et al., 2002). During photosynthesis the lighter ^{12}C isotope will react more readily than the heavier ^{13}C isotope (Lambers et al., 2002). C_3 plants discriminate against the heavier isotope of ^{13}C more than the lighter ^{12}C during photosynthesis, and therefore the ratio between ^{13}C and ^{12}C can be used to identify the source of C in soils (Lambers et al., 2002). C_3 carbon signature ($\delta^{13}\text{C}$) has an average value of -28‰, while C_4 carbon signature ($\delta^{13}\text{C}$) has an average value of -14‰ (Wedin et al., 1995; Lambers et al., 2002). Different C signatures are used to evaluate the sources of belowground C that is being respired into the atmosphere as CO_2 . Under shrub conditions C respired could be from soil organic matter produced by tallgrass species prior to shrub encroachment.

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Chapter 2: Assessment of shrub encroachment on belowground carbon dynamics in tallgrass prairie using an isotopic approach

ABSTRACT

Changes in fire frequency have allowed native woody shrub species to encroach on native herbaceous tallgrass prairie in the Great Plains of North America. Although these shrub species are native to this ecosystem, their abundance and distribution have increased dramatically due to different changes in land use and land management. I examined vegetation type and microhabitat type effects on decomposition rates and soil CO₂ efflux under a representative tallgrass species, *Sorghastrum nutans* (Indian grass), and shrub species *Cornus drummondii* (rough-leaf dogwood) at the Ross Natural History Reservation in Americus, Kansas. Shrub microhabitats had greater decomposition rates than tallgrass microhabitats and thus decreased C storage. Shrub litter had faster decomposition rates than tallgrass litter. Tallgrass litter contained higher levels of lignin and cellulose thus slowing their decomposition rate. Soil respiration rates were not different between grass and shrub habitats. Shrub litter and tallgrass litter had different carbon isotope values, $\delta^{13}\text{C}$; however, soil organic matter $\delta^{13}\text{C}$ was not different between habitat types. This suggests that soil organic matter under shrub habitats may be a legacy effect from previous tallgrass conditions. These results suggest that shrub encroachment in tallgrass prairie could result in less soil carbon storage in the long term. Changes in land management, primarily the inclusion of fire, reduce shrub encroachment while promoting tallgrass species. The promotion of tallgrass prairie species could help offset rising atmospheric CO₂ levels by increasing soil carbon storage.

INTRODUCTION

Over the last century woody vegetation has increased in abundance and density in grass-dominated ecosystems worldwide (Bragg and Hulbert, 1976; Hobbs and Mooney, 1986; Knight et al., 1994; Archer, 1995; Wilson and Kleb, 1996; Hoch and Briggs, 1999; Roques et al., 2001; Briggs et al., 2002a; Briggs et al., 2002b). Less than 4% of the native tallgrass prairie remains compared to pre-European settlement (Sampson and Knopf, 1994). Shifting from dominant herbaceous grass to woody vegetation can alter aboveground plant resources (Nepstad et al., 1994; Jackson et al., 2000) and belowground nutrient cycling and carbon (C) storage (Trumbore, 1997; Jobbagy and Jackson, 2000). I investigated how vegetative change from a grass dominated to a shrub dominated system alters C dynamics by examining microhabitat and vegetative composition influences on decomposition rates and soil carbon dioxide (CO₂) efflux.

The expansion of woody plants is currently one of the greatest threats to the tallgrass prairie ecosystem of the central United States (Heisler et al., 2004; Briggs et al., 2005). Increases in woody vegetation are referred to as woody encroachment because these woody plants are native to this ecosystem, increasing in density and abundance as a result of changes in abiotic and biotic conditions (Van Auken, 2000; Heisler et al., 2003). Changes in fire frequency, grazing patterns, increasing atmospheric CO₂ and climate change are important contributing factors to the shift in the tallgrass plant community (Archer et al., 2001). Changes in fire frequency have been implicated as the major factor leading to an increase in density and cover of shrubs such as *Cornus drummondii* (rough-leaf dogwood), *Rhus glabra* (smooth sumac) and *Juniperus virginiana* (eastern red cedar); within tallgrass prairies of the central United States (Briggs et al., 1995; Briggs et

al., 2002; Heisler et al., 2003; McCarron et al., 2003). Herbaceous tallgrass species allocate more than half of their productivity belowground (Seastedt, 1988; Seastedt and Knapp, 1993); however, the effects of shrub encroachment on belowground C storage are not fully understood. Encroachment of shrubs into tallgrass prairie may influence soil's ability to store C (Briggs et al., 2005) possibly reducing the amount of C stored in soils and increasing atmospheric CO₂ (Williams et al., 2004).

C input and storage within the soil system is dependent on the balance between C additions to and losses from the soil. Decomposition the process whereby organic matter is broken down into inorganic compounds that are then available for uptake by primary producers strongly influences C losses from the soil. Decomposition reduces C input into the soil because C in litter is respired into atmosphere as CO₂ and reduces size, and increases surface area, of organic matter in litter. Vegetative shifts from herbaceous tallgrass species to shrub species change the input of organic matter because of shifts in chemical composition of decomposing material (Jackson et al., 2002; McCarron et al., 2003; Lett et al., 2004). Tallgrass litter decomposes slower due to higher levels of lignin and cellulose as compared to shrub litter (Sage and Monson, 1999; McCarron et al., 2003). Faster decomposition rates result in more C released into the atmosphere and less C entering the soil system thus potentially reducing soil C storage. Small changes in soil C storage can significantly impact C cycling since soils are the largest pool of terrestrial organic C, storing more C than is present in plants and in the atmosphere combined (Schlesinger, 1997). Microorganisms, such as bacteria and fungi, are the main organisms that decompose organic material in soils and their abundance and activity levels have

been shown to be positively correlated to soil moisture content during a typical tallgrass prairie growing season (Williams et al., 2004).

Soil organic C that is mineralized by decomposers is released into the atmosphere as CO₂ as part of a process known as soil respiration (Raich and Schlesinger, 1992). Soil respiration results in a loss of soil C by soil microbial respiration, root respiration, and chemical oxidation of C-containing materials and is a measure of the belowground structure and function of an ecosystem (Raich and Schlesinger, 1992). Soil respiration rates are positively correlated with mean annual air temperature and mean annual precipitation (Raich and Schlesinger, 1992). The amount of available N in soils also affects soil respiration rates, and if insufficient N is available then decomposition rates will decline and C levels will increase (Schimel, 1995). Shrub encroachment does not significantly change N availability, but it does decrease soil CO₂ efflux (McCarron et al., 2003; Lett et al., 2004). The use of fire increases soil temperature, decreases soil moisture and increases soil CO₂ efflux (Knapp et al., 1998), suggesting greater soil C storage under unburned shrub dominated conditions. However, decreased CO₂ flux under shrub dominated conditions may result from less organic matter entering the soil system.

Soil C should be reflective of aboveground inputs into the soil system. One way to determine the source of C in the soil is to examine the isotopic signature of C litter and soil organic matter. Isotopic ratios differ between C₄ species and C₃ species due to differences in photosynthetic pathways. In the tallgrass prairie ecosystem dominate grass species use C₄ photosynthesis while encroaching shrubs use C₃ photosynthesis. Isotopic C signatures, $\delta^{13}\text{C}$, differ between C₃ and C₄ species because of differences in photosynthetic enzyme discrimination of the lighter C isotope, ¹²C, and heavier C

isotope, ^{13}C (Lambers et al., 2002). C_4 plants $\delta^{13}\text{C}$ averages -14‰ (parts per thousand), while C_3 plants $\delta^{13}\text{C}$ averages -28‰ (Wedin et al., 1995; Lambers et al., 2002). Soil isotope signatures will reflect the source of organic matter input into the soil pool (Ehleringer et al., 2000). $\delta^{13}\text{C}$ analysis can be used to determine the contribution that C_3 and C_4 plants have on soil organic matter (Tieszen et al., 1997; Ehleringer et al., 2000).

I investigated how shrub encroachment in tallgrass prairie will alter soil C dynamics. The objectives of my study were: (1) examine how microhabitat type influences decomposition rates in shrub and grass habitats, (2) examine how vegetative type influences decomposition rates using shrub and grass litter, (3) compare soil respiration rates in shrub and grass habitats, and (4) examine how fire influences soil respiration rates in shrub and grass habitats. I hypothesized that: (1) shrub habitats would allow for faster decomposition rates due to increased soil moisture content brought on by increased shading, (2) shrub litter would decomposed faster than grass litter due to reduced lignin and cellulose content in shrub litter, (3) grass habitats would have increased soil respiration rates due to increased aboveground inputs into the soil system, and (4) the use of fire would increase soil respiration rates under both habitat types due to removal of litter resulting in increased soil-atmosphere interactions.

METHODS

Site Description

Research was conducted at the F. B. and Rena G. Ross Natural History Reservation (“Ross” hereafter) an 81 ha research site in the Flint Hills of western Lyon County, Kansas, USA (Harrell, 2007). Previous land use history consisted of grazing and row-

crop agriculture prior to its establishment as a natural history reservation in 1958 (Spencer, 1979). The Ross experiences a typical Midwestern climate with warm, wet summers and cold, dry winters, with mean annual air temperature (30 year average) of 12.8 C, and average annual precipitation of 940 mm (NOAA, 2011). During the past four decades woody vegetation has increased, and management practices were altered to include fire to prevent further woody encroachment (Harrell et al., 2007).

In 2007, the northeast portion of the Ross was divided into 72 experimental plots (30 m X 30 m) to evaluate the impact different management practices have on shrub encroachment. These experimental plots have not been used as cropland. Plots were divided into shrub dominated and grass dominated plots determined by mean shrub stem densities and are separated by a 2 meter fire guard (Jensen, 2007). The dominant tallgrass vegetation consists of big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*) and Indian grass (*Sorghastrum nutans*), while the woody vegetation consists of rough-leaf dogwood (*Cornus drummondii*), smooth sumac (*Rhus glabra*), aromatic sumac (*Rhus aromatica*), wild plum (*Prunus americana*) and buckbrush (*Symphoricarpos orbiculatus*). Treatments included: burning season (spring or fall), burning frequencies (annually or triennially), mowing (one time in summer of 2009 or never) and undisturbed control. Undisturbed control plots, referred to as unburned, and annually burned (both spring and fall) plots were used for this study.

I examined the effects of burn presence or absence and burn season on soil respiration, soil temperature and soil moisture content. The first fall burn occurred in October/November 2008 while the first spring burn occurred in April 2009. In addition to the 72 experimental plots, three additional unburned control plots were established in the

northwest section for this project. This was done for two reasons; first, one shrub unburned plot did not meet the required shrub height of 1 m, even though mean stem density classified the plot as shrub dominated; second, this created two additional unburned plots (one grass dominated and one shrub dominated) for the project.

Soil Characterization

Soil samples were used to characterize soil texture, soil pH, total C, total N, C:N and $\delta^{13}\text{C}$. From each of the 16 un-mowed annually-burned plots (8 fall and 8 spring) and 10 unburned plots, four 30-cm deep soil cores were collected from September to November 2008. Sampling locations were randomly selected within each plot, and recorded using a GPS. At each sampling location 4 cores were collected 1 m apart in the 4 cardinal directions. The soil cores were separated into 0-10 cm and 10-30 cm, or bedrock which ever came first, sections. Samples were oven dried at 105°C for 48 hours and ground to pass through a 2 mm sieve. Sub-samples from 0-10 cm depth were sent to Kansas State University stable isotope laboratory to determine total C, total nitrogen (N) and $\delta^{13}\text{C}$. Soil pH was determined using a pH meter on samples containing 10 g of soil and 25 mL of deionized water. Soil particle size analysis was determined with the Bouyoucos hydrometer method on 50 g soil samples shaken 1 hour with 50 g/L of sodium metaphosphate.

Litter Characterization

Litter samples were used to characterize litter composition, total C, total N, C:N and $\delta^{13}\text{C}$. The chemical composition of vegetative litter was determined following the acid detergent fiber (ADF) method adapted from Van Soest and Wine, (1967). Vegetation samples were dried, weighed and placed in dry fritted glass crucibles then 100 ml Cetyl

trimethylammonium bromide was added and refluxed over heat for 1 hour. Crucibles were vacuumed dried and oven-dried for 24 hours. Changes in crucible weight represented the amount of ADF present. ADF represents the amount of lignin and cellulose in the litter, both of which decompose very slowly. Litter sub-samples were sent to Kansas State University stable isotope laboratory to determine total C, total N and $\delta^{13}\text{C}$ of each litter type.

Decomposition Rates

Rough-leaf dogwood litter and Indian grass litter were collected in November 2008 and approximately 30 g of air dried biomass was placed inside each decomposition bag (30 cm X 30 cm) constructed of 2-mm nylon mesh. A 2-mm mesh opening was used to allow micro and meso- fauna decomposers while excluding macro-fauna decomposers that would translocate plant material (Bradford et al., 2002). Five sub-samples of rough-leaf dogwood litter and Indian tallgrass litter were dried at 70°C for 24 hours to correct for initial moisture content. Decomposition bags were placed in the 10 unburned plots, to prevent the bags from burning, and removed at 3 month intervals for two years. In each unburned plot 11 decomposition bags of each vegetation type (22 total bags per plot) were placed in direct contact with the soil surface 30 cm apart in a straight line transect in alternate positions. Each vegetative type, shrub and grass litter, were placed in each habitat type, shrub and grass habitats, to examine the effects of microhabitat type and vegetative type on decomposition rates. Bags were removed from the field and dried at 70°C for 24 hours and then weighed to determine percent mass loss within each bag, decomposition bags were also re-weighed to correct for possible change in weight over

time. A sub-sample of the plant material was ashed in a muffle oven at 500°C for 5 hours to correct for potential soil addition.

Soil Respiration

Soil respiration was measured using a modified static chamber technique adapted from Raich et al., (1990). Soil respiration chambers were installed in unburned, fall annually-burned and spring-annually burned plots in June 2009. The chambers were made of polyvinyl chloride (PVC) pipe with a volume of 785 cm³ and cylindrical surface area of 471 cm². All vegetation was removed from within the chambers prior to measurements being taken. Chambers were left uncapped when not in use. During CO₂ efflux capture, a PVC cap and o-ring were placed over the chambers and left for 24 hours. The caps contained a septum for extraction of CO₂. From each chamber 10-mL gas samples were collected using a 10-mL syringe and stored in a 2-mL vial. Of the 10-mL sample 8-mL were used to purge the sample vial and the final 2-mL were stored. Vials were refrigerated until analysis with a Varian Saturn gas chromatograph (GC) mass spectrometer (MS) (Varian Saturn 3900 GC, Varian Saturn 2100T MS, Varian Saturn CP 8410 auto injector, Varian FactorFour VF-5ms capillary column, Agilent Technologies, Inc, Santa Clara, CA, USA) (Koerner et al. 2011). Soil respiration samples were collected every 2 weeks from March to September, and every 6 weeks from October to February. Soil temperature (5 cm depth) and gravimetric soil moisture (0 – 10 cm) were collected concurrently with soil respiration samples.

Statistical Analysis

A repeated measures ANOVA was used to test for differences in mean mass loss for decomposition bags, using a least-significant means separation tests with a Tukey

adjustment. A two-way ANOVA was used to test for interactions on decomposition rates between microhabitat type and vegetation type. A three-way ANOVA and regression was used to test for interactions on soil respiration rates between soil temperature, soil moisture content and season of burning. A t-test was used to examine for differences in mean: % clay, % sand, % silt, soil total C, soil total N, soil C:N, soil pH, soil $\delta^{13}\text{C}$, ADF, litter total C, litter total N, litter C:N, and litter $\delta^{13}\text{C}$. A significance level of $\alpha = 0.05$ was used for all statistical tests. All tests were performed in SAS 9.2 (SAS Institute Inc., Cary, NC) using an $\alpha=0.05$.

RESULTS

Soil Characterization

Soil texture was similar between grass-dominated and shrub-dominated habitats ranging from classes of clay, silty clay, clay loam and silty clay loam. Soil samples taken from 0-10 cm depth did not differ in mean % clay, % sand or % silt (Table 1). Shrub-dominated habitats possessed higher mean total N and mean total C than grass-dominated habitats (Table 1). Although both mean total N and mean total C were higher in shrub-dominated habitats, C:N ratio and mean soil pH were not significantly different than grass-dominated habitats (Table 1). Isotopic $\delta^{13}\text{C}$ values of soil organic matter were not significantly different between grass and shrub-dominated habitats (Table 1).

Soil temperature was not different between grass-dominated and shrub-dominated habitats over the duration of the study ($F= 1.11$, $P=0.83$; Figure 4). Differences in soil temperature were more apparent in fall burn and spring burn habitats midway through the growing season (May – August; Figure 4). Soil moisture was not significantly different

between grass and shrub-dominated habitats over the duration of the study ($F= 1.12$, $P=0.81$; Figure 5).

Litter Characterization

Tallgrass litter with 54% mean ADF contained 1.5 times more than shrub litter with 36.5% mean ADF (Table 2). Shrub litter contained 3 times more total N than did tallgrass litter (Table 2), however, there was no significant difference in total C between shrub and tallgrass litter (Table 2). Tallgrass litter had a C:N ratio 3 times greater than shrub litter (Table 2). Shrub litter mean isotopic $\delta^{13}\text{C}$, -29 ‰, was significantly different than grass litter mean isotopic $\delta^{13}\text{C}$, -12 ‰ (Table 2).

Decomposition Rates

Microhabitat type did have a significant impact on decomposition rates over the duration of the study with shrub habitats allowing faster decomposition rates compared to grass habitats ($F=20.72$, $P<0.01$; Figure 1). Litter decomposed faster in shrub habitats than grass habitats at various times over the duration of the study (Figure 1).

Vegetation type did have a significant impact on decomposition rates over the duration of the study with shrub litter decomposing faster than grass litter ($F=107.48$, $P<0.01$; Figure 2). Shrub litter decomposed faster than tallgrass litter after 9 months of decomposition and continuing for the remainder of the study (Figure 2). After 24 months of decomposition shrub litter had almost twice as much mass loss as tallgrass litter (Figure 2).

There was no significant interaction on decomposition rates between microhabitat type and vegetative type ($F=0.34$, $P=0.06$). There was a significant positive interaction between increasing length of time that decomposition bags were in the field with

decomposition rates ($F=59.08$, $P<0.01$) and vegetative type ($F=7.2$, $P<0.01$). There was no significant interaction on decomposition rates between increasing length of time that decomposition bags were in the field and microhabitat type ($F=1.93$, $P=0.9$).

Soil Respiration

Over the duration of the study, soil respiration rates were not significantly different between tallgrass-dominated and shrub-dominated habitats ($F=0.3$, $P=0.8$). There were, however, times when respiration rates did differ between tallgrass and shrub-dominated habitats (Figure 3). In unburned conditions shrub habitats had greater soil respiration rates in August 2009 (Figure 3). In fall-burned conditions, shrub habitats had greater soil respiration rates in May 2010, while tallgrass habitats had greater soil respiration rates in June 2009 and June 2010 (Figure 3). In spring-burned conditions, tallgrass habitats had greater soil respiration rates in April 2010, June 2010 and July 2010 (Figure 3). I examined if soil temperature, soil moisture content or a combination of both would help explain soil respiration rates. Soil temperature explained none of the variability in soil respiration in grass ($r^2=0.00$, $P=0.95$) and shrub ($r^2=0.00$, $P=0.89$) habitats. Soil moisture content significantly explained only 5% and 7% of the variability in soil respiration in grass ($r^2=0.05$, $P=0.07$) and shrub ($r^2=0.07$, $P=0.04$) habitats, respectively. Soil temperature and soil moisture content together explained 7% and 8% of the variability in soil respiration in grass ($r^2=0.07$, $P=0.14$) and shrub ($r^2=0.08$, $P=0.08$) habitats, respectively.

DISCUSSION

My study examined whether native shrub encroachment into herbaceous tallgrass prairie alters C cycling through changes in vegetative type and microhabitat conditions. Changes in land management, primarily changes in fire frequency, have allowed woody vegetation to encroach into the tallgrass prairie ecosystem (Briggs et al., 2002), and is one of the greatest threats to the tallgrass prairie of the central United States (Briggs et al., 2005). Nutrient cycling is predicted to change due to the different morphological structures between shrubs and native tallgrass species (Briggs and Knapp, 1995). Our results show vegetative shifts in tallgrass prairie change C cycling through both vegetative and microhabitat type effects on decomposition rates, but these impacts do not carry over to soil respiration rates. I also found differences in soil C levels, but isotopic analysis revealed that these differences likely existed prior to the vegetative shift in this ecosystem, indicating that shrub expansion may be limited to C-rich sites.

Soil dynamics are influenced by decomposition rates because decomposition reduces C input into the soil C pool. Environmental conditions such as soil moisture and soil temperature can exert strong controls on decomposition rates and vegetation change can influence the conditions under which decomposition occurs. In my study decomposition rates were faster under shrub habitats regardless of litter type (Figure 1), supporting my hypothesis. My results of increased decomposition rates under shrub habitats suggest a decrease in C input into the soil system leading to a reduction in long term soil C storage associated with shrub encroachment.

Decomposition rates can also vary according to the composition of litter. Shrub litter decomposed faster than grass litter regardless of habitat type (Figure 2), supporting

my hypothesis. Possible reasons for faster decomposition rates of shrub litter are lower levels of lignin and cellulose, and/or differences in litter total N and C:N. Shrub litter contained a higher level of total N while possessing lower levels of lignin, cellulose and C:N ratio than grass litter. Lignin and C:N concentrations have been shown to be good predictors of decomposition rates (Meentemeyer, 1978; Melillo et al., 1982). Our results agree with Carrera et al., (2000) and Billings, (2006) who found that shrub and forest habitats had increased litter N concentrations compared to grassland sites, and with Melillo et al., (1982) who found that decomposition rates are positively correlated with N concentration while being negatively correlated with lignin content. Increased total N in shrub litter reduced the C:N ratio likely allowing for faster decomposition rates of shrub litter, because N is often the limiting factor in microbial activity (Schimel, 1995). Increased decomposition rates of shrub litter results in a greater release of C into the atmosphere and less C entering the soil system for long term C storage under shrub expansion.

Litter that is not decomposed can be incorporated into soil organic matter where C can be sequestered for tens of thousands of years (Aber et al., 1998). The relatively large difference in $\delta^{13}\text{C}$ values between C_3 and C_4 litter should be observed in soil organic matter $\delta^{13}\text{C}$ values (Kelly et al., 1993; Boutton, 1996), although, soil organic matter $\delta^{13}\text{C}$ may increase due to recalcitrant soil organic matter fraction (Ehleringer et al., 2000). In our study litter $\delta^{13}\text{C}$ values were significantly different between C_3 shrub and C_4 grass species; however, soil organic matter $\delta^{13}\text{C}$ did not differ between shrub (-19.0‰) and grass (-17.6‰) habitats. Average soil organic matter $\delta^{13}\text{C}$ values were found to be within the enriched C_4 predicted range according to O'Leary, (1988). Similar soil organic matter

$\delta^{13}\text{C}$ values between shrub and grass habitats suggest that shrub litter is not being incorporated into the soil system. However, Buchmann et al., (1998); Sinton et al., (2000) and Ehleringer et al., (2002) all note that $\delta^{13}\text{C}$ vary temporally and spatially, potentially requiring decades for soil organic matter to equalize to different $\delta^{13}\text{C}$ inputs. Shrub habitats possessed increased soil total C, however, isotopic analysis revealed that soil organic matter $\delta^{13}\text{C}$ are not different between shrub and grass habitats. These results suggest that differences in soil C may have existed prior to shrub establishment, indicating that shrub expansion may be limited to C-rich sites. Our results, in addition to greater decomposition rates, suggest that less shrub litter is incorporated into the soil system resulting in less C entering the soil for long term storage under shrub encroachment.

C that is incorporated into soil organic matter may be respired into the atmosphere as CO_2 by soil microbes, root respiration and chemical oxidation of C-containing materials (Raich and Schlesinger, 1992). In my study soil respiration rates did not differ between grass and shrub habitats indicating that vegetation change at this location is not changing C loss from soils, in opposition of my hypothesis. My results contrast with McCarron et al., (2003) and Lett et al., (2004) who found that soil CO_2 efflux was reduced under shrub habitats at Konza Prairie Biological Station. Possible explanations why soil respiration rates were not different in my study are: (1) shrub habitats have not been established long enough to result in an observable difference in soil respiration rates, (2) differences might be site specific and not ubiquitous across all tallgrass prairie remnants. Shrub encroachment has occurred for approximately fifty years on this study site (Harrel et al., 2007) which may not be long enough to see a change in soil respiration

and soil C storage. My results contrast with Raich and Schlesinger, (1992) and McCarron et al., (2003) who found that soil temperature and soil moisture are usually the best predictors of soil respiration, however in this study, neither soil temperature nor soil moisture were indicative of soil respiration rates. My results show that shrub encroachment has not affected the amount of C respired from the soil system at the Ross.

In addition to potential change in environmental conditions driven by vegetation change; land management practices also have the potential to influence soil C fluxes. Fire did not affect soil temperature, soil moisture content, or soil respiration rates in either habitat type, in opposition of my hypothesis. The limited number of fire treatments on the study site may explain why differences were not observed, burning had occurred only twice on the experimental plots. Measurements of soil respiration began less than one year following the initiation of the burn treatments on this site. My results contrast with Knapp et al., (1998) who found that fire increased soil temperature and soil respiration, while decreasing soil moisture. My results show that fire had no influence on soil respiration thus not affecting soil C storage, however, fire reduces aboveground litter inputs into the soil system potentially reducing the amount of C entering the soil system.

Tallgrass species evolved concurrent with fire intervals of once every 3 – 5 years (Wright and Bailey, 1982). Fire frequency of 3 – 5 years may, however, not be enough to repress shrub encroachment. Heisler et al., (2003) found that 4 year fire frequency did not stop shrub encroachment, and even annual fire frequency did not eliminate established shrub islands. Annual fire used before the established of shrub islands can be used to prevent shrub encroachment (Heisler et al., 2003). Land management practices that prevent, reduce and eliminate shrub encroachment in the tallgrass prairie have the

potential to help off-set increasing atmospheric CO₂ concentrations by increasing the amount of C that enters the soil system for potential long term C storage.

Shrub encroachment into the tallgrass prairie has the potential to alter nutrient cycling and change soil C storage. Aboveground biomass C storage is predicted to increase with shrub encroachment, but reductions in belowground soil C storage could offset aboveground biomass C gains (Jackson et al., 2002; Lett et al., 2004). Jackson et al., (2002) found a decline in soil organic carbon after woody encroachment. My results show more soil organic carbon in shrub dominated sites, but, isotopic analysis revealed that higher soil organic carbon levels likely existed prior to shrub encroachment. In my study shrub encroachment resulted in faster decomposition leading to less C entering the soil C pool and potentially reducing soil C storage.

CONCLUSION

Shrub encroachment has historically been constrained by the use of fire; however, changes in land management have allowed shrubs to increase in abundance and density. Our study suggests that shrub encroachment results in less C entering the soil system due to increased decomposition rates under shrub habitats. Increased soil total C observed under shrub habitats is likely a legacy effect resulting from when the landscape was grass dominated. Land management practice that promote tallgrass species growth and limit or reverse shrub encroachment could increase soil C storage, potentially helping off-set increasing atmospheric CO₂ concentrations.

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Table 1- Soil characteristics for all 26 plots used within the Ross Natural History Reservation, Americus, Kansas. Table is divided into grass-dominated and shrub-dominated habitats determined by mean stem densities. Values are means \pm SE, grass dominated plots n=13, shrub dominated plots n=13. Significant differences ($P < 0.05$) are represented by different lowercase letters.

	Grass Habitat Type	Shrub Habitat Type
Clay (%)	46.0 \pm 1.4	41.5 \pm 2.0
Sand (%)	9.6 \pm 1.6	12.5 \pm 3.3
Silt (%)	44.2 \pm 2.0	46.2 \pm 2.0
Total N (g/kg)	3.0 \pm 0.1 ^a	4.1 \pm 0.2 ^b
Total C (g/kg)	39.7 \pm 1.9 ^a	57.0 \pm 2.4 ^b
C:N	13.1 \pm 0.3	14.0 \pm 0.4
Soil pH	5.8 \pm 0.1	6.3 \pm 0.2
$\delta^{13}\text{C}$	-17.6 \pm 1.1	-19.0 \pm 0.7

Table 2 – Chemical composition of litter used during field study at the Ross Natural History Reservation, Americus, Kansas. Acid detergent fiber (ADF) values represent the percent of lignin and cellulose contained within litter samples. Total C, Total N and C:N represent shrub and grass vegetation samples obtained from grass-dominated and shrub-dominated plots. Values are means \pm SE. Significant differences ($P < 0.05$) are represented by different lowercase letters.

	Grass Litter Type	Shrub Litter Type
ADF	54.1 \pm 0.8 ^a	36.5 \pm 3.4 ^b
Total N (g/kg)	4.8 \pm 0.3 ^a	14.6 \pm 0.9 ^b
Total C (g/kg)	441.8 \pm 5.3	446.3 \pm 5.1
C:N	93.0 \pm 6.9 ^a	31.0 \pm 17.7 ^b
$\delta^{13}\text{C}$	-12.2 \pm 1.0 ^a	-29.0 \pm 0.2 ^b

Figure 1- Microhabitat type examines the % mass loss of (a) shrub litter and (b) tallgrass litter that were lost in shrub habitats (open circles and dashed lines) compared to tallgrass habitats (closed triangles and solid lines) located at the Ross Natural History Reservation, Americus, Kansas. Decomposition bags were placed in the field in November 2008 and removed at 3 month intervals for 2 years. Points are means \pm SE, n=10. Significant differences between microhabitat type effect are designated with an asterisk ($P < 0.05$).

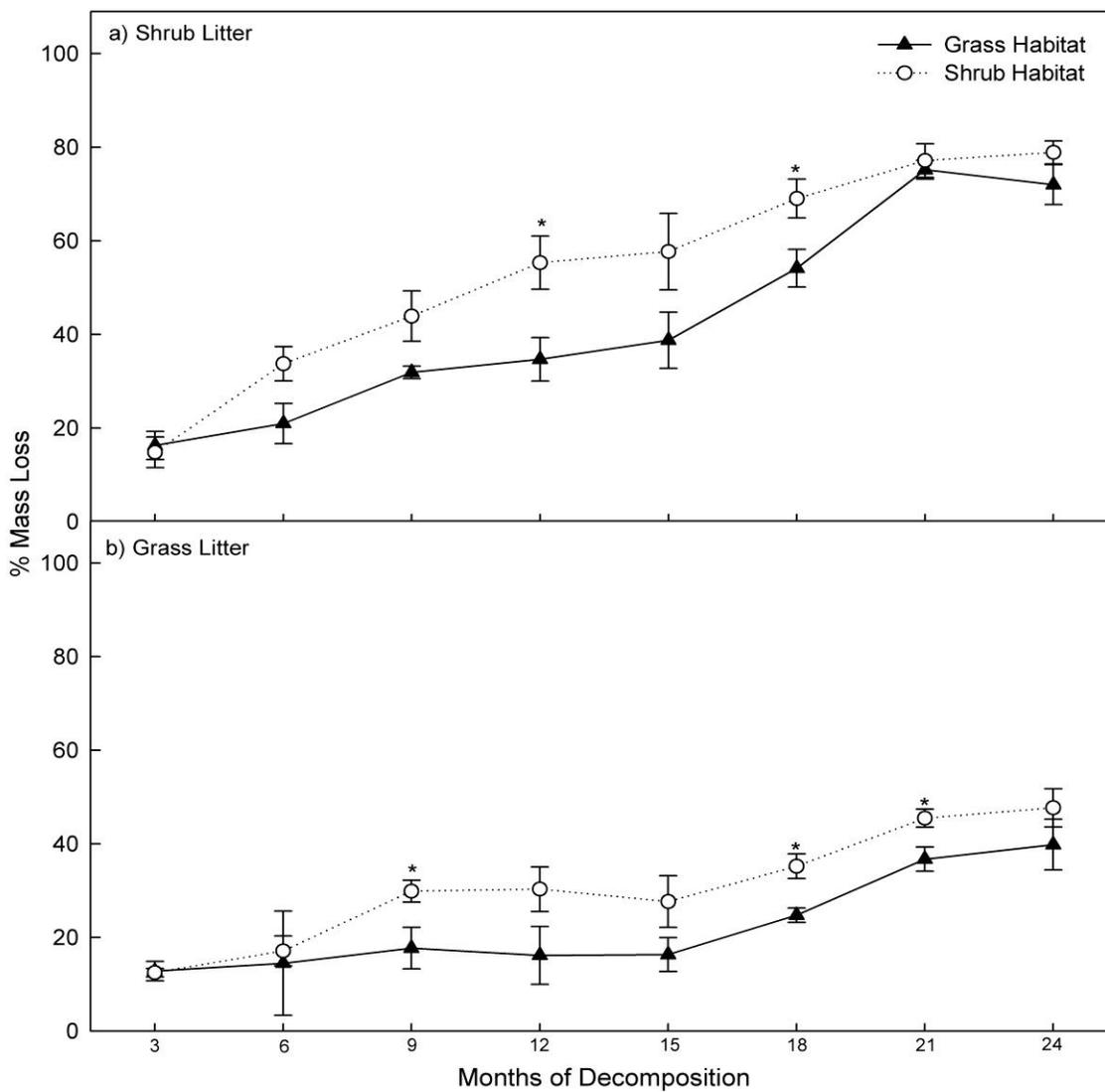


Figure 2- Vegetation type examines the % mass loss in (a) shrub habitat and (b) tallgrass habitat that contained shrub litter (open circles and dashed lines) compared to tallgrass litter (closed triangles and solid lines) located at the Ross Natural History Reservation, Americus, Kansas. Decomposition bags were placed in the field in November 2008 and removed at 3 month intervals for 2 years. Points are means \pm SE, n=10. Significant differences between vegetation type effect are designated with an asterisk ($P < 0.05$).

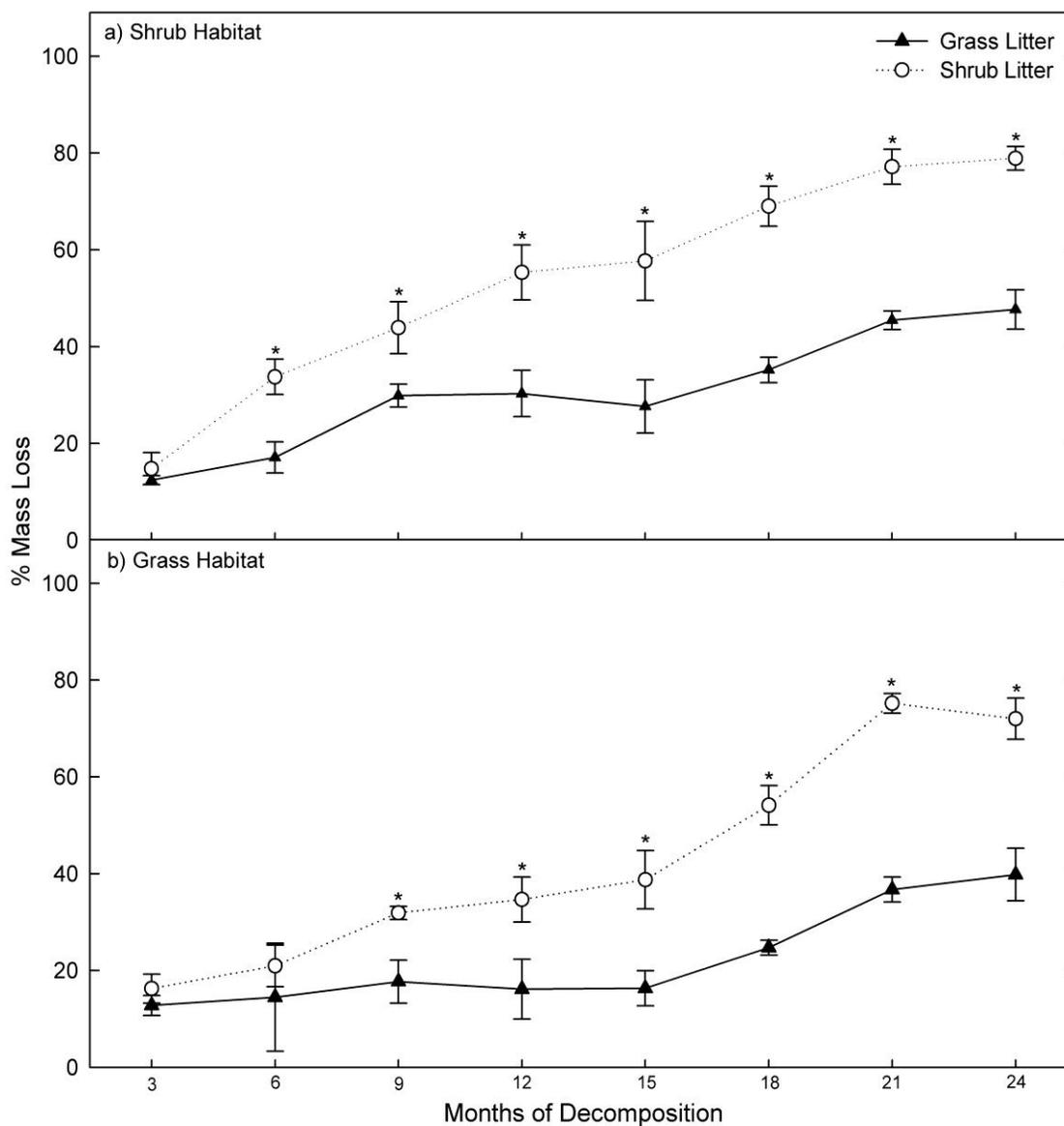


Figure 3- Soil CO₂ efflux under the canopy of a representative tallgrass species, *Sorghastrum nutans*, and shrub species *Cornus drummondii* at the Ross Natural History Reservation, Americus, Kansas. Points are means \pm SE; (a) unburned n=10, (b) fall burn n=8 and (c) spring burn n=8. Open circles and dashed lines represent shrub habitat while closed triangles and solid lines represent tallgrass habitat. Significant differences between shrub and grass plots are designated with an asterisk (P<0.05).

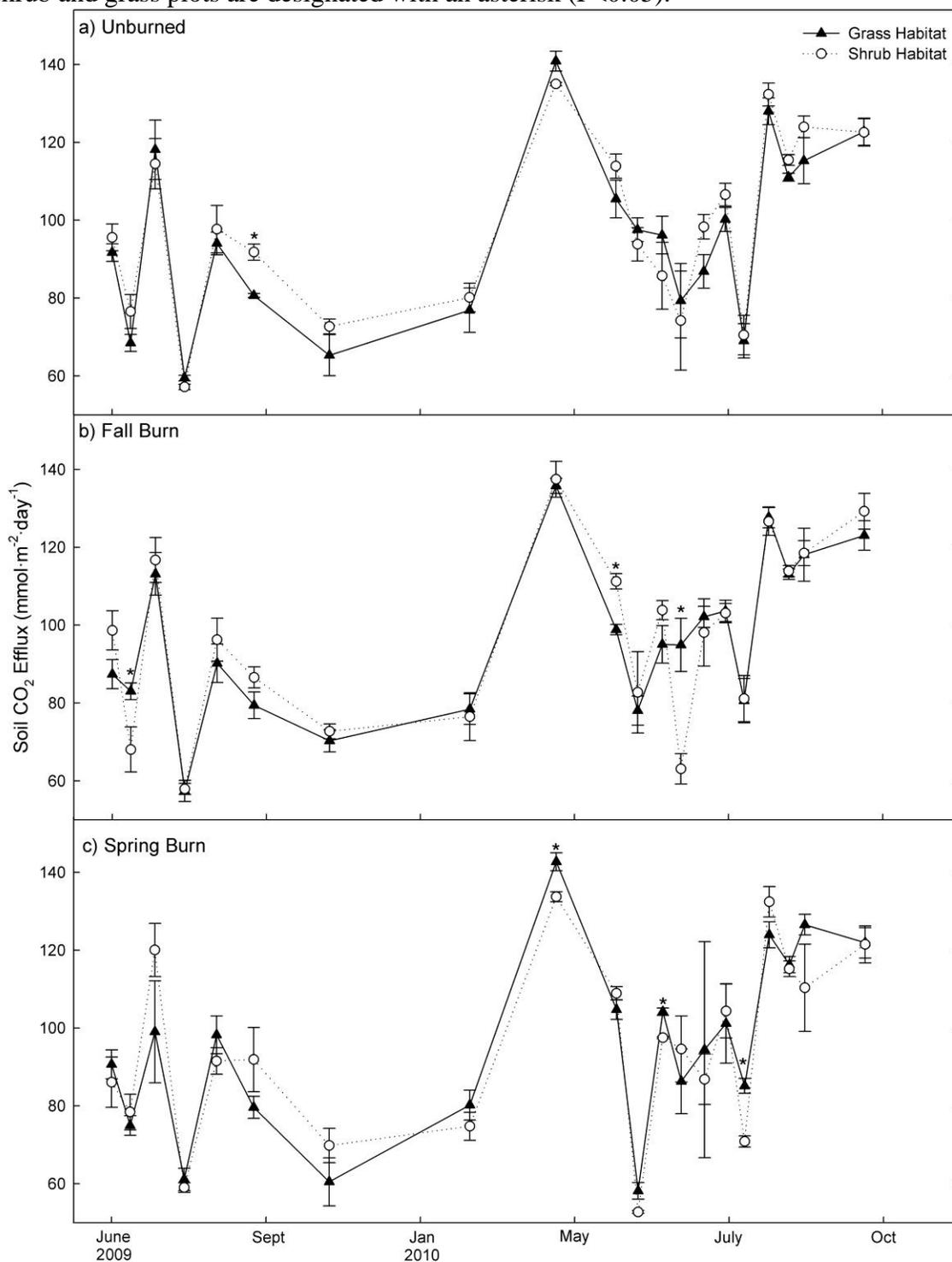


Figure 4- Soil temperature at 5 cm depth in shrub plots, open circle and dashed line, and grass plots, closed triangle and solid line, (a) unburned, (b) fall burn and (c) spring burn plots located at the Ross Natural History Reservation, Americus, Kansas. Soil temperatures were taken approximately every 2 weeks from June to October and every 6 weeks thereafter. Points are means \pm SE, n=16. Significant differences between soil temperatures are designated with an asterisk (P<0.05).

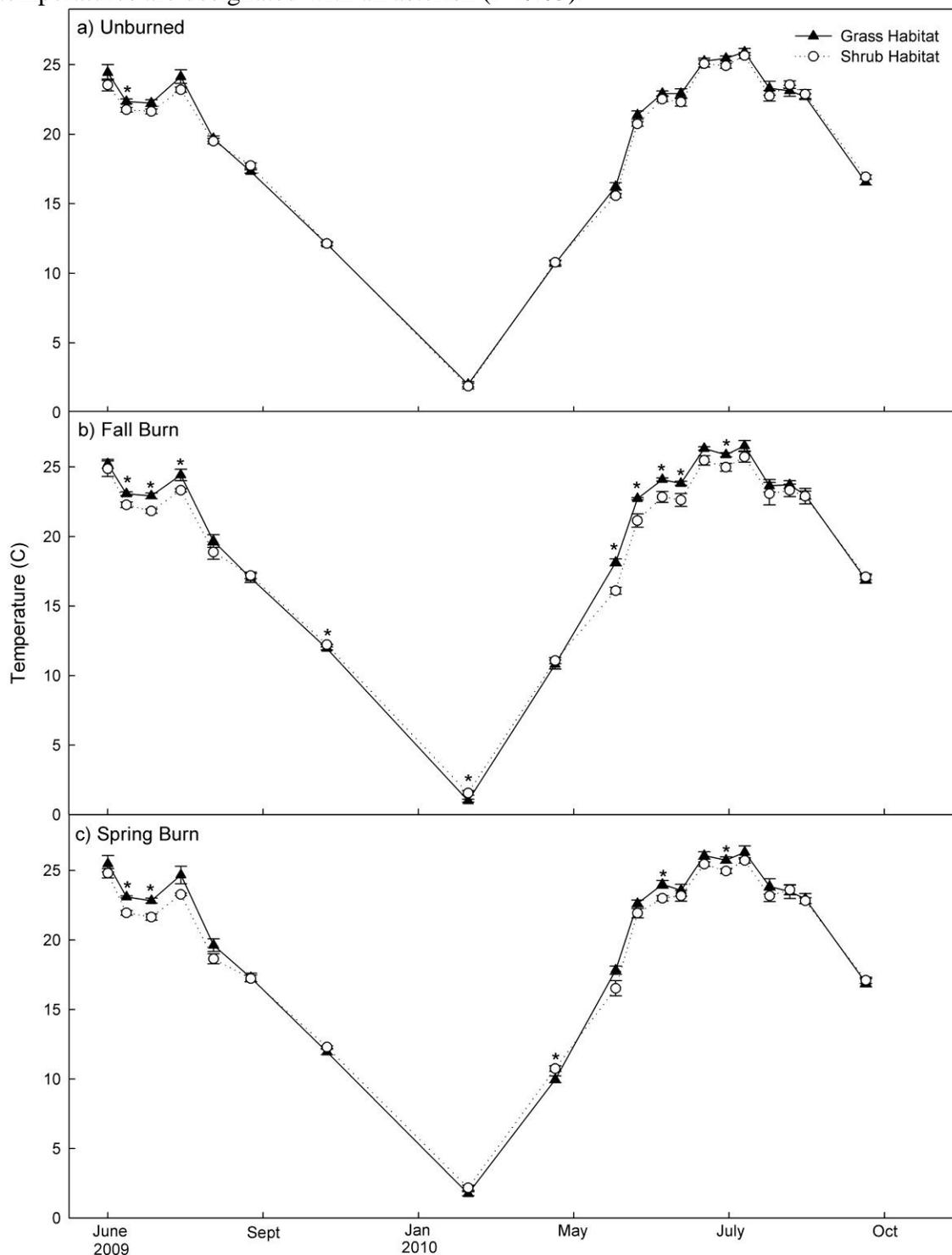
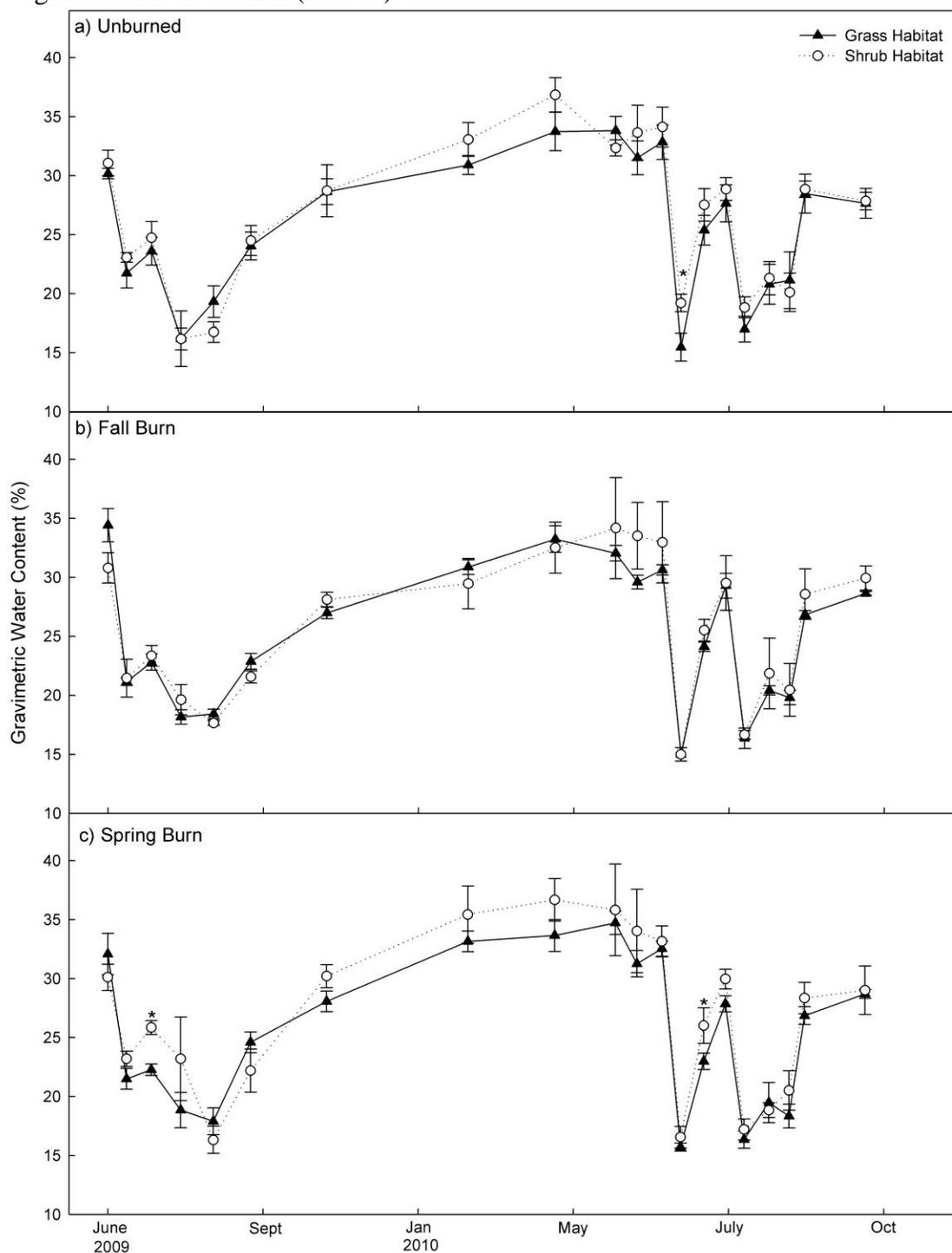


Figure 5- Percent water content within top 10 cm of the soil in shrub-dominated habitats (open circle and dashed lines) and tallgrass dominated habitats (closed triangle and solid line) (a) unburned (b) fall burn and (c) spring burn plots located at the Ross Natural History Reservation, Americus, Kansas. Water content samples were taken approximately every 2 weeks from June to October and every 6 weeks thereafter. Points are means \pm SE, n=16. Significant differences between percent water content are designated with an asterisk (P<0.05).



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