BIOGEOCHEMISTRY OF SOIL CARBON AND NITROGEN IN RESPONSE TO ELEVATED TEMPERATURES AND ALTERED RAINFALL REGIMES IN OAK SAVANNA: A GLOBAL CHANGE EXPERIMENT

A Dissertation

by

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ABSTRACT

Increasing concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.5 to 4.5 °C by the end of the century, and magnify the intensity and variability of seasonal precipitation distribution. The mid-latitude savannas of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons. Despite these predictions, little is known concerning the effects of these global climate change drivers or their potential interactive effects on the carbon (C) and nitrogen (N) biogeochemical cycles. In the post oak savanna region, I examined the effects of warming and rainfall manipulation on: (1) seasonal variation in root biomass and root C and N pool sizes, (2) soil organic C (SOC) and soil total N (STN), and (3) rates of N-mineralization, nitrification, and N-losses via leaching in soil subjected to experimental warming and rainfall manipulation. Research was conducted between August 2008 and April 2010 at the Texas Warming and Rainfall Manipulation (WaRM) Site in east-central Texas. Treatments included plots comprised of *Juniperus virginiana* and *Schizachyrium scoparium* monocultures and a *J. virginiana* + *S. scoparium* combination in native soil. Warming alone and rainfall redistribution alone seldom affected the response variables in this study. Species composition and time appeared to be the most consistently important main effects; however, warming and rainfall redistribution interacted with other experimental variables to influence root biomass and soil nutrient pools. N-mineralization rates were dominated by a 3-way interaction of species x rainfall x time and a warming x species composition interaction, and SOC and STN results were dominated by rainfall x species, highlighting the complexity of global change impacts. The combined results of this study suggest that soils beneath *J. virginiana* and *S. scoparium* growing alone or together behaved as carbon sinks during years 6 and 7 of this climate change experiment. These changes in root dynamics, soil C and N, and rates of soil N-transformations in response to global change drivers may have important implications for ecosystem dynamics in the oak savanna region of North America.

DEDICATION

This dissertation is dedicated to my parents Sylvia Wells Wellman and Albert Randolph Wellman who planted the seed of curiosity in me at an early age, encouraged me to stay in school, inspired me to follow my dreams, never doubted my abilities, and always believed I would achieve great things. You are the foundation of my accomplishments and I can’t thank you enough for your love and support. ~Your loving daughter

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NOMENCLATURE

ANOVA	Analysis of Variance
C	Carbon
$CaCl_2$	Calcium Chloride
CO_2	Carbon Dioxide
ºC	Degrees Celsius/Centigrade
DATA	Dissertation and Thesis Assistance
DOE	Department of Energy
D-optimal	Determinant-Optimal Statistical Design
EA	Elemental Analyzer
g	Gram
HSD	Honest Significant Difference
IPCC	Intergovernmental Panel on Climate Change
LSM	Least Square Mean
m	Meter
n	Sample Size
N	Nitrogen
N_2	Dinitrogen gas
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate

NICCR	National Institute for Climatic Change Research
p	Probability Value (p = 0.05, %95 confidence level)
pH	Molar Concentration of Hydrogen Ions in Solution
REML	Restricted Maximum Likelihood
RGR	Relative Growth Rate
S- based	Syringyl-Based Lignin Phenol
SE	Standard Error
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SRL	Specific Root Length
STN	Soil Total Nitrogen
TAMU	Texas A&M University
V- based	Vanillyl-Based Lignin Phenol
WaRM	Warming and Rainfall Manipulation

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1. Global Change

Increasing concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.5 to 4.5 °C by the end of the 21st century, and potentially magnify the intensity and variability of seasonal precipitation distribution (IPCC 2013). The mid-latitude, continental grasslands of North America are predicted to experience substantial modification in precipitation regimes (IPCC 2013; Weltzin *et al.* 2003; Melilllo *et al.* 2014) and a shift from summer to spring precipitation is anticipated to be more probable than a substantial change in mean annual precipitation amount (Easterling *et al.* 2000; Groisman & Knight 2008; Manabe & Wetherald 1986). Higher temperatures and greater evaporation coupled with a decrease in summer precipitation will intensify summer drought events (MacCracken *et al,* 2003; Pope *et al.* 2000; Wetherald & Manabe, 1995). In spite of these general predictions, little is known concerning the relative effects of these global climate change drivers or their potential interactive effects on ecosystem processes related to the Nitrogen (N)-cycle (Norby & Luo 2004; Rustad *et al.* 2001; Weltzin *et al.* 2000).

Elevated temperatures generally accelerate N-transformations and other key processes linked to N-cycling such as net primary production, soil respiration, and organic matter decay (Davidson & Janssens 2006; Rustad *et al.* 2001). However, most of these studies have been conducted in temperate, boreal, and arctic ecosystems, and it is unknown if warm-temperate, subtropical, and tropical ecosystems will respond in a comparable manner. Similarly, the timing and magnitude of rainfall events may affect a range of N-cycle processes (Yahdjian *et al.* 2006). But, much less is known concerning the combined, interactive effects of warming and altered precipitation patterns on N-cycle processes (Beier *et al.* 2004; Pendall *et al.* 2004; Rustad *et al.* 2001; Weltzin *et al,* 2000). In addition, there is a critical gap in our knowledge of the plant functional trait responses (Cornwell *et al.* 2008; DeDeyn *et al*. 2008) and species interactions (Grime *et al.* 2008; Suttle *et al.* 2007) that may underpin ecosystem responses to various global climate change scenarios. Warmer temperatures, in combination with increased cool season precipitation, could potentially lead to shifts in plant communities (species composition, relative dominance, and/or growth forms) that could alter the quantity and quality of organic matter inputs to the soil, thereby affecting nutrient storage, water holding capacity, and rates of root production (Gill & Jackson 2000; Fiala *et al*. 2009; Bai *et al*. 2010; Zhou *et al*. 2011; Zhou *et al*. 2012; Verburg *et al*. 2013).

2. Post Oak Savanna

The central US is bisected by an ecotone that separates the grasslands of the Great Plains and the deciduous forest of the eastern US. In the south, this transition zone is comprised of the post oak (*Quercus stellata* Wangenh.) savanna region located in south central Texas and eastern Oklahoma (Hatch *et al.,* 1990). Post oak is the major canopy tree dominant and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the major perennial grass dominant (Hatch *et al.* 1990). Covering about 3 million hectares, this savanna has undergone a transition from a *Q. stellata – S. scoparium* dominated savanna toward a *Q. stellata* - juniper (*Juniperus virginiana* L.) woodland over the last century (Breshears 2006; Briggs *et al.* 2005; Reich *et al.* 2001), particularly in the absence of fire (Norris *et al.* 2001b; Eggemeyer *et al.* 2006). Of particular significance to this research, succession from grassland to *Juniperus* woodland in this region results in increased soil C and N storage (McKinley & Blair 2008) and altered N-cycling processes (McKinley *et al.* 2008; Norris *et al*. 2007). Widespread encroachment by *Juniperus* throughout this oak savanna region suggests an important role for this vegetation change in the N cycle of the central US (McKinley & Blair 2008; McKinley *et al.* 2008; Norris *et al*. 2007).

3. Root Dynamics

Root biomass and nutrient concentrations may be altered due to changes in temperature (Kaspar & Bland 1992; Luo *et al.* 1995; McMichael & Burke 1998; Gill & Jackson 2000; Arai-Sanoh *et al.* 2010; Zhou *et al.* 2011) and seasonal rainfall patterns (Green *et al.* 2005; Fiala *et al.* 2009; Bai *et al.* 2010; Zhou *et al.* 2012; Verburg *et al*. 2013). Several CO_2 enrichment experiments demonstrated that root production and rates of turnover provide valuable data on biomass, and carbon (C) and N fluxes as ecosystems go through global change (Jongen *et al.* 1995; Matamala & Schlesinger 2000; Norby *et al.* 2004; Phillips *et al.* 2006). However, very few studies have investigated how differences in both temperature and rainfall (Gorissen *et al.* 2004;

Dukes *et al.* 2005; Bai *et al.* 2010; Wu *et al.* 2011; Zhou *et al.* 2012) combined with shifts in species composition affect root biomass and soil nutrient dynamics.

4. Soil Organic Carbon and Soil Total Nitrogen

Vegetation shifts from grassland to shrub or forested land can dramatically increase total root biomass (McKinley 2006) and soil C inputs from increased root exudation. However, in some cases, shrub encroachment into grasslands could lead to lower fine root production and turnover (Zhou *et al.* 2012) and an increase in antimicrobial allelochemicals (Borchardt *et al.* 2008; Eller *et al.* 2010; Post & Urban 1995). These changes in the quantity and quality of soil organic matter inputs have the potential to alter the size of the soil microbial biomass pool (Liao & Boutton 2008), as well as microbial community structure (Hollister *et al.* 2010; Yannarell *et al.* 2014), with potential implications for soil C and N storage.

Temperature and precipitation are known factors that control the size and turnover rates of SOC and soil total nitrogen (STN) pools. In fact, an increase in average temperatures by just 1ºC could lead to a 3% loss of SOC for soils at 30ºC and a 10% loss in soils at 5 ºC (Kirschbaum 1995). Because significantly more C is stored in the world's soils than in the atmosphere (Batjes 1996; Kirschbaum 2000; Post *et al.* 1982), the loss of SOC and acceleration of decomposition processes could lead to a positive feedback to climate change (Davidson *et al.* 2000). However, if primary production also increases with higher temperatures and exceeds the rate of decomposition, then a negative feedback to climate change would occur (Davidson & Janssens 2006). It is interesting to note that increases in plant C storage in response to warming have been attributed to warming induced increases in N availability from accelerated decomposition (Melillo *et al.* 2011). However, the influence of temperature on SOC and STN stores may be modified by soil moisture. For example, coupling of the RothC soil carbon model with output from a coupled climate-carbon cycle general circulation model showed that changes in soil moisture acted to oppose temperature-driven decreases in SOC and tended to increase soil carbon storage (Falloon *et al.* 2011).

An analysis of pedon and climate data from 500 rangelands and 300 cultivated lands in the U.S. Central Plains Grasslands found that SOC decreased with temperature and increased with precipitation and clay content (Burke *et al.* 1989). In later studies, Century model results (Schimel *et al.* 1994) and a global analysis on >2700 soil profiles also came to the same conclusions (Jobbágy & Jackson 2000). Interestingly, Jackson *et al.* (2002) found that following woody encroachment into grasslands, drier sites generally gained and wetter sites generally lost SOC. However, a more comprehensive analysis of the effects of woody encroachment on SOC in grasslands and other dryland ecosystems of North America showed that SOC generally increases following encroachment (Barger *et al.* 2011). In addition, woody species commonly contain significantly more syringyl (S) and vanillyl (V) based lignin phenols than grasses, which are harder to decompose (Boutton *et al.* 2009). Therefore, as woody encroachment occurs the quality of the new plant material may decrease in degradability and lead to an accumulation of terrestrial C and N. For example, McKinley and Blair (2008) reported a rapid accrual of C and N in biomass and soil as *J. virginiana* outcompeted nearby grasslands.

Soil C and N accumulation rates under different plant community types can result in changes in soil C:N ratios. Knops and Tilman (2000) demonstrated that C:N ratios of soil organic matter increased with field age and the abundance of C4 grasses, and decreased with C3 grasses and forb abundance. The soils under the C4 grasses in this study were accumulating C while there was no difference in soil N. This can be explained by the nitrogen use efficiency of the C4 photosynthetic pathway, which allows plants to produce more biomass under nitrogen limitation. Therefore, C4 plant material can be slower to degrade and lead to higher soil C accumulation rates with no effect on soil N accumulation rates. In comparing C4 grasses and C3 leguminous woody species, Archer *et al.* (2000) found very little difference in C:N ratios. Even though C4 grasses use N more efficiently, C3 legumes can fix atmospheric N (N_2) and accumulate more soil N. In addition, the higher S- and V-phenol lignin content in the woody litter inputs would accumulate more C compared to that of grass roots and shoots, thus not demonstrating a difference in soil C:N ratios between species.

It is clear that there are complex interactions of temperature, rainfall, and species compositions with SOC (Davidson *et al.* 2000), STN, and soil C:N ratios, and investigating these changes is important in understanding how climate change will affect the post oak savanna ecosystem.

5. Soil Nitrogen Transformations

In general, controls on N-cycling processes in the soil are reasonably well understood (Booth *et al.* 2005; Galloway 2005; McNeill & Unkovich 2007; Nannipieri & Eldor 2009; Robertson & Groffman 2009). Important known drivers include temperature, soil water content, substrate availability, and microbial activity. Following the Q_{10} rule for biological systems, rates of biological (microbial) processes and chemical reactions double for every 10°C increase in temperature (Myrold 2005). Previous research has examined this claim with respect to soil processes including N-transformations. In a meta-analysis of 32 experimental warming sites, Rustad *et al.* (2001) discovered significant increases in soil respiration, N-mineralization, and plant productivity rates with increasing temperature. In a later study, Agehara and Warncke (2005) observed a linear relationship with increasing N-mineralization rates and increasing temperature as well as with increasing soil moisture. However, few studies have examined the response of net N-mineralization to simultaneous modifications of both temperature and rainfall.

Temperature and Soil Moisture

Rising temperatures coupled with seasonal drought may also alter rates of N losses via soil leaching and run-off as demonstrated by Joslin and Wolfe (1993) and Lukewille and Wright (1997). This is probably due to higher rates of N-mineralization and nitrification which produce larger pools of leachable N. Periods of drought intensified by experimental rainfall reduction and elevated temperatures could lead to higher concentrations of NO_3^- in the soil because N-mineralization and nitrification will continue even after plant N-uptake processes are inhibited by drought (Booth *et al.* 2005). Consequently, when these soils are rewetted at the onset of seasonal wet periods, these large pools of NO_3^- will be susceptible to increased leaching losses. Thus, changes in temperature and rainfall patterns predicted for Texas could have important implications for N-losses via leaching from ecosystems, with potential implications for water quality in surface waters and groundwater.

Microbial Activity and Organic Matter

Increases in soil water stimulate microbial community activities as they are replenished with intracellular water. This enables them to produce extracellular enzymes capable of depolymerizing complex organic matter and ultimately mineralizing nutrients such as N. For example, a significant rise or flush of soil mineral N is generally observed as soils are dried and rewetted, though the increase in net mineralization may be due to the quality of the organic substrate released after soil disturbance induced by water rather than the effect of hydration alone (Austin *et al*. 2004; Birch 1958; Fierer & Schimel 2002; Franzluebbers *et al.* 1996; Saetre & Stark 2005). Nevertheless, ecosystems with varying substrate qualities have shown considerable increases in nutrient concentrations after water is introduce into a dried soil; therefore, the exact mechanism remains unclear.

Substrate quality and quantity clearly influences N-transformation rates (Booth *et al.* 2005). Organic matter with C:N values between 20-40 tend to encourage net N-immobilization while C:N values < 20 promote net N-mineralization (Cabrera *et al.* 2005). In an evaluation of 100 studies, Booth *et al.* (2005) concluded that soil organic matter from grasslands containing lower C:N values of ~ 5 to 25 produce more NH_4^+ compared to the soil organic matter from woodlands with higher C:N values of ~ 8 to 48. However, other studies have found that rates of N-mineralization in soils beneath woody species such as *Prosopis glandulosa* (honey mesquite) and *Juniperus virginiana* were increased relative to adjacent grassland soils (Hibbard *et al.* 2001; McCulley *et al.* 2004; McKinley *et al.* 2008).

Plant Species Composition

It seems likely that inherent species contrasts in photosynthetic pathway, root and leaf habit, tissue chemistry, and phenology provide functional contrasts that will be important in understanding the effects of elevated temperatures and altered rainfall regimes on N-transformations. Post oak savanna species composition may be especially sensitive to climate change because each of the dominant species possesses contrasting photosynthetic pathways and leaf habit (e.g., evergreen and deciduous). Deciduous *Q. stellata* and evergreen *J. virginiana* are C_3 species, while the grass *S. scoparium* is C_4. Species with the C_3 metabolic pathway require more N and may compete more effectively against plants with the more N-use efficient C_4 pathway when there is an increase in available mineral N. For example, Turner and Knapp (1996) demonstrated a significant increase in aboveground biomass with C_3 forbs compared to that of C_4 grasses due to N addition. Thus, climatically driven changes in rates of N-mineralization and N-availability may alter plant species interactions and influence plant community composition.

Native grasslands that have been invaded by woody species have been shown to shift the C:N and biochemical composition of above- and belowground litter inputs, and alter rates of decomposition and mineralization processes (Filley *et al.* 2008; Hibbard *et al.* 2001; Knapp *et al.* 2008; Liao *et al.* 2008; McCulley *et al.* 2004; Norris *et al.* 2007). In fact, preliminary analyses of leaf tissue samples taken from the WaRM Site in 2007 reveal that the C:N ratio was 23-28 for *Q. stellata*, 33-44 for *J. virginiana*, and 44-59 for *S. scoparium*. Therefore, we can predict a decrease in C:N values within litter as woody species such as *Q. stellata* and *J. virginiana* encroach into native *S. scoparium* grass communities, which could affect the rate of N-mineralization. N-mineralization rates under isolated oak species within California and Minnesota oak savannas were higher than the rates under neighboring grassland species (Dijkstra *et al.* 2006; Herman *et al.* 2003; Jackson *et al.* 1990). In contrast, N-mineralization rates showed little to no initial change as a dogwood community encroached on native grasslands (McCarron *et al.* 2003), and juniper litter decayed more slowly than big bluestem litter in Kansas (Norris *et al.* 2001b). Therefore, investigating N-transformations within the post oak savanna ecosystem under different species composition scenarios will be beneficial to the understanding of how climate and vegetation shifts will alter rates of N-cycling processes and N availability.

6. Objectives

Associated with two dominant species of the post oak savanna (*J. virginiana* and *S. scoparium*), the primary objectives of this study were to quantify the main effects of elevated temperature, modified rainfall distribution, plant species composition, and time, as well as their interactions on: root biomass and root C and N pools (Chapter II); soil organic carbon and soil total nitrogen (Chapter III); and rates of net N-mineralization, nitrification, and N losses via leaching (Chapter IV).

CHAPTER II

ROOT DYNAMICS IN RESPONSE TO ELEVATED TEMPERATURES AND ALTERED RAINFALL REGIMES IN POST OAK SAVANNA: A GLOBAL CHANGE EXPERIMENT

1. Synopsis

Increasing concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.5 to 4.5 °C by the end of the century, and potentially magnify the intensity and variability of seasonal precipitation distribution. The mid-latitude savannas of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons. Despite these predictions, little is known concerning the effects of these global climate change drivers or their potential interactive effects on root biomass dynamics. Therefore, the purpose of this study was to quantify seasonal variation in root biomass and root carbon and nitrogen pool sizes under experimental warming and rainfall manipulation. Research was conducted between August 2008 and April 2010 at the Texas Warming and Rainfall Manipulation (WaRM) Site in east-central Texas. Treatments included plots comprised of *Juniperus virginiana* and *Schizachyrium scoparium* monocultures and a *J. virginiana* + *S. scoparium* combination in native soil. Fine (<2 mm), coarse (>2 mm), and total (fine + coarse) root biomass were quantified seasonally for 22 months on soil cores taken to 10 cm depth and analyzed for their C and

N concentrations. Total root biomass ranged from 94.7 to 3,163.2 g m^{-2} where total *S. scoparium* roots ranged from 94.7 to 1,932.1 g m^{-2} and total *J. virginiana* and *J. virginiana* + *S. scoparium* roots ranged from 247.6 to 3,163.2 g m^{-2}**.** In this multifactor global change experiment, species composition and time appeared to be the most consistently important main effects that determined root biomass, C, N, and C:N ratios. Warming alone and rainfall redistribution alone seldom affected the root response variables in this experiment. However, warming and rainfall redistribution interacted with other experimental variables to influence root biomass and nutrient pools. These changes in root dynamics in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

2. Introduction

Increasing concentrations of greenhouse gases are projected to elevate global mean surface air temperatures by 1.5 to 4.5 °C by the end of the century, and potentially magnify the intensity and variability of seasonal precipitation distribution (IPCC 2013; Melillo *et al.* 2014). The mid-latitude savannas of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons (Manabe & Wetherald 1986; Easterling *et al*. 2000) and extending the length of growing seasons (Linderholm 2006; Schwartz *et al.* 2006; Tjoelker & Zhou 2007). Because changes in temperature and/or rainfall can influence primary productivity (Fay *et al.* 2003) and above- (Cleland *et al.* 2007; Bertin 2008) and belowground (Fiala *et al.* 2009; Hollister & Flaherty 2010) biomass allocation, it is important to quantify root system responses to these global change drivers. In general, inconsistent reports of root responses such as productivity, turnover, and biomass to moisture and temperature within a 186 stand database further emphasize the importance of investigating these effects at the species level (Finér *et al.* 2011).

The central US is bisected by an ecotone that separates the grasslands of the Great Plains and the deciduous forest of the eastern US. In the south, this transition zone is comprised of the post oak (*Quercus stellata* Wangenh.) savanna region located in south central Texas and eastern Oklahoma (Hatch *et al.,* 1990). Post oak is the major canopy tree dominant and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the major perennial grass dominant (Hatch *et al.* 1990). Covering about 3 million hectares, this savanna has undergone a transition from a *Q. stellata – S. scoparium* dominated savanna toward a *Q. stellata* - juniper (*Juniperus virginiana* L.) woodland over the last century (Breshears 2006; Briggs *et al.* 2005; Reich *et al.* 2001), particularly in the absence of fire (Norris *et al.* 2001b; Eggemeyer *et al.* 2006). Of particular significance to this research, succession from grassland to *Juniperus* woodland in this region results in increased soil C and N storage (McKinley & Blair 2008) and altered N-cycling processes (McKinley *et al.* 2008; Norris *et al*. 2007). Widespread encroachment by *Juniperus* throughout this oak savanna region suggests an important role for this vegetation change in the N cycle of the central US (McKinley & Blair 2008; McKinley *et al.* 2008; Norris *et al*. 2007).

Native grasslands being invaded by woody species such as *J. virginiana* and honey mesquite (*Prosopis glandulosa* Torr.) have been shown to shift the C:N and biochemical composition of above- and belowground litter inputs, and alter rates of decomposition and mineralization processes (Hibbard *et al*. 2001; McCulley *et al*. 2004; Norris *et al.* 2007; Filley *et al*. 2008; Knapp *et al*. 2008; Liao *et al*. 2008). In fact, unpublished preliminary analyses of leaf tissue samples taken from the Texas Warming and Rainfall Manipulation (WaRM) Experimental Site in College Station, TX in 2007 reveal that mean C:N was 38.6 for *J. virginiana*, 51.0 for *S. scoparium*, and 43.9 for plots containing a *J. virginiana + S. scoparium* species combination, comparable to previous studies (Coile 1937; Vivanco & Austin 2006; Tjoelker & Boutton unpublished data; see Table A.2). Based on this, we predict a decrease in C:N ratios in root biomass as woody species such as *J. virginiana* encroach into native *S. scoparium* grassland communities, which could affect microbial activity, decay rates, nutrient availability, and C storage (Rasse *et al.* 2005).

In addition, there is a critical gap in our knowledge of the plant functional trait responses (Cornwell *et al.* 2008; DeDeyn *et al.* 2008) and species interactions (Suttle *et al.* 2007; Grime *et al.* 2008) that may underpin ecosystem responses to various global climate change scenarios. Warmer temperatures, in combination with increased cool season precipitation, could potentially lead to shifts in plant communities (species composition, relative dominance, and/or growth forms) that could alter the quantity and quality of organic matter inputs to the soil, thereby affecting nutrient storage, water holding capacity, and rates of root production (Gill & Jackson 2000; Fiala *et al*. 2009; Bai *et al*. 2010; Zhou *et al*. 2011; Zhou *et al*. 2012; Verburg *et al*. 2013).

Root biomass and nutrient concentrations may be altered due to changes in temperature (Kaspar & Bland 1992; Luo *et al.* 1995; McMichael & Burke 1998; Gill & Jackson 2000; Arai-Sanoh *et al.* 2010; Zhou *et al.* 2011) and seasonal rainfall patterns (Green *et al.* 2005; Fiala *et al.* 2009; Bai *et al.* 2010; Zhou *et al.* 2012; Verburg *et al*. 2013). Several CO_2 enrichment experiments demonstrated that root production and rates of turnover provide valuable data on biomass, and C and N fluxes as ecosystems go through global change (Jongen *et al.* 1995; Matamala & Schlesinger 2000; Norby *et al.* 2004; Phillips *et al.* 2006). However, very few studies have investigated how differences in both temperature and rainfall (Gorissen *et al.* 2004; Dukes *et al.* 2005; Bai *et al.* 2010; Wu et al. 2011; Zhou *et al.* 2012) combined with shifts in species composition affect root biomass and nutrient dynamics.

Therefore, the purpose of this study was to quantify the effects of experimental warming and rainfall manipulation on seasonal patterns of root biomass and root C and N pools associated with two dominant species of the post oak savanna (*J. virginiana* and *S. scoparium*) growing alone and in combination in an established global change experiment. Four main hypotheses were tested. We predicted that (1) elevated temperatures will increase coarse, fine, and total root biomass, and increase belowground C and N pool sizes in each vegetation type due to increased aboveground growth (Volder *et al.* 2013); (2) plots receiving redistributed rainfall of 40% less rainfall during the summer months and 52% more rainfall during the fall and spring periods will have less root biomass and smaller C and N pools due to plant stress from reduced moisture availability compared to control rainfall treatments; (3) the woody plant *J. virginiana* and the grass *S. scoparium* will differ in their belowground responses to temperature and rainfall manipulation due to inherent differences in their structural and functional attributes; and (4) root C:N ratios will be lower in *J. virginiana* monocultures and *J. virginiana* + *S. scoparium* plots compared to *S. scoparium* monocultures.

3. Methods and Materials

Study Area and Experimental Design

Soils were collected for root biomass and root C and N analyses at the Texas Warming and Rainfall Manipulation (WaRM) Experimental Site between August 2008 and April 2010. The facility was located in a remnant post oak savanna in College Station, Texas (N 30°34” W 96°21”), and was constructed in March 2004 to investigate the combined effects of altered precipitation distribution and warming on tree and grass dominants of the southern oak savanna (Volder *et al.* 2010). Eight 18 x 9 x 4.5 m (L x W x H) rainout shelters were covered with clear polypropylene film while ends and sidewalls below 1.5 m were not covered to maintain ambient microclimatic conditions (Fay *et al.* 2000; Weltzin & McPherson 2003; Volder *et al.* 2010; see Figure A.6). Shade cloth was used to exclude precipitation. Sheet metal 40 cm in width was installed to 30 cm depth into the soil to penetrate the clay pan and separate each shelter from water flow above and belowground.

The shelter design preserved natural variation in a microenvironment that was similar to ambient conditions. An on-site weather station (EZ Mount Groweather, Davis Instruments, Hayward, CA) recorded precipitation, air temperature and humidity, while solar radiation (total and PAR), air temperature, and relative humidity were monitored continuously and recorded by Hobo data loggers in each shelter (Hobo U12; Onset Computer Corp., Bourne, MA, USA). Mean daily temperatures in the shelters were on average 1.5 °C higher, RH values 2% lower and PAR levels 30% lower than the unsheltered controls. The NOAA weather station at Easterwood Airport (<0.5 km from the site) reported a mean annual temperature of 20.56°C and mean annual precipitation of 1,017.5 mm based on a 50-year period (NOAA 2010; Volder et al. 2013).

Ten 2 x 2 m plots were established within each shelter in native soil, a fine sandy loam Alfisol of the Booneville series (Fine, smectitic, thermic Chromic Vertic Albaqualf) in 2003, one year before the experiment began. Each shelter included five plant species combinations with monocultures of *S. scoparium*, *Q. stellata*, and *J. virginiana*, and each of the tree species were grown with the *S. scoparium* in separate mixed species plots. An overhead irrigation system supplied reverse osmosis water from onsite water holding tanks to simulate rainfall to each shelter. Infrared lamps (Kalglo Electronics, Bethlehem, PA; model MRM-1208L) were installed to hang 1.5 m above half of the plots within each shelter and were warmed continuously with 400 W m^{-2} of radiant energy (Harte & Shaw 1995; Wan *et al.* 2002). To accommodate increased height growth of trees, lamps were raised by 0.5 m and the total wattage output to the plots was increased by an additional 400 W m^{-2} of radiant energy to maintain comparable levels of warming in February 2008. This additional warming from the heat lamps increased soil temperatures (averaged over all canopy types) by approximately 0.6 °C at 3 cm depth, and canopy leaf temperatures by about 1-1.5 °C. However, it is important to note that actual soil and canopy warming can vary with wind speed (Kimball 2005). Metal sheeting in the shape of the infrared lamps was placed above the second half of the plots to mimic cover and shading without the heat source.

Rainfall was manipulated in 8 of the 10 shelters to simulated precipitation regimes categorized as control and redistributed, although each regime received the same total amount of annual rainfall (1018 mm) and the same number of rainfall events (Figure 2.1). Four of the 8 shelters received control rain events based on the long-term (50 year) mean reported by a local NOAA weather station in College Station, TX (NOAA 2010). The frequency and intensity of rainfall events were also simulated from the long-term precipitation record of the region. In the other 4 shelters, 40% of the precipitation during 5 summer months (May – September) was withheld from each event and evenly redistributed in the previous 2 month spring (March and April) and subsequent 2 month fall (October and November), increasing the rainfall to 52% of the normal spring and fall patterns (see Figure A.7). This redistribution pattern effectively increased the intensity of the summer drought and the amount of precipitation that occurs during the cooler spring and fall seasons. Precipitation redistribution during spring and fall increased volumetric soil water content from 21.0 to 23.1% in the top 20 cm of soil; in contrast, during the summer drought, soil water content decreased from 15.5% in the ambient to 10.7% in the redistributed treatment. Although soil warming

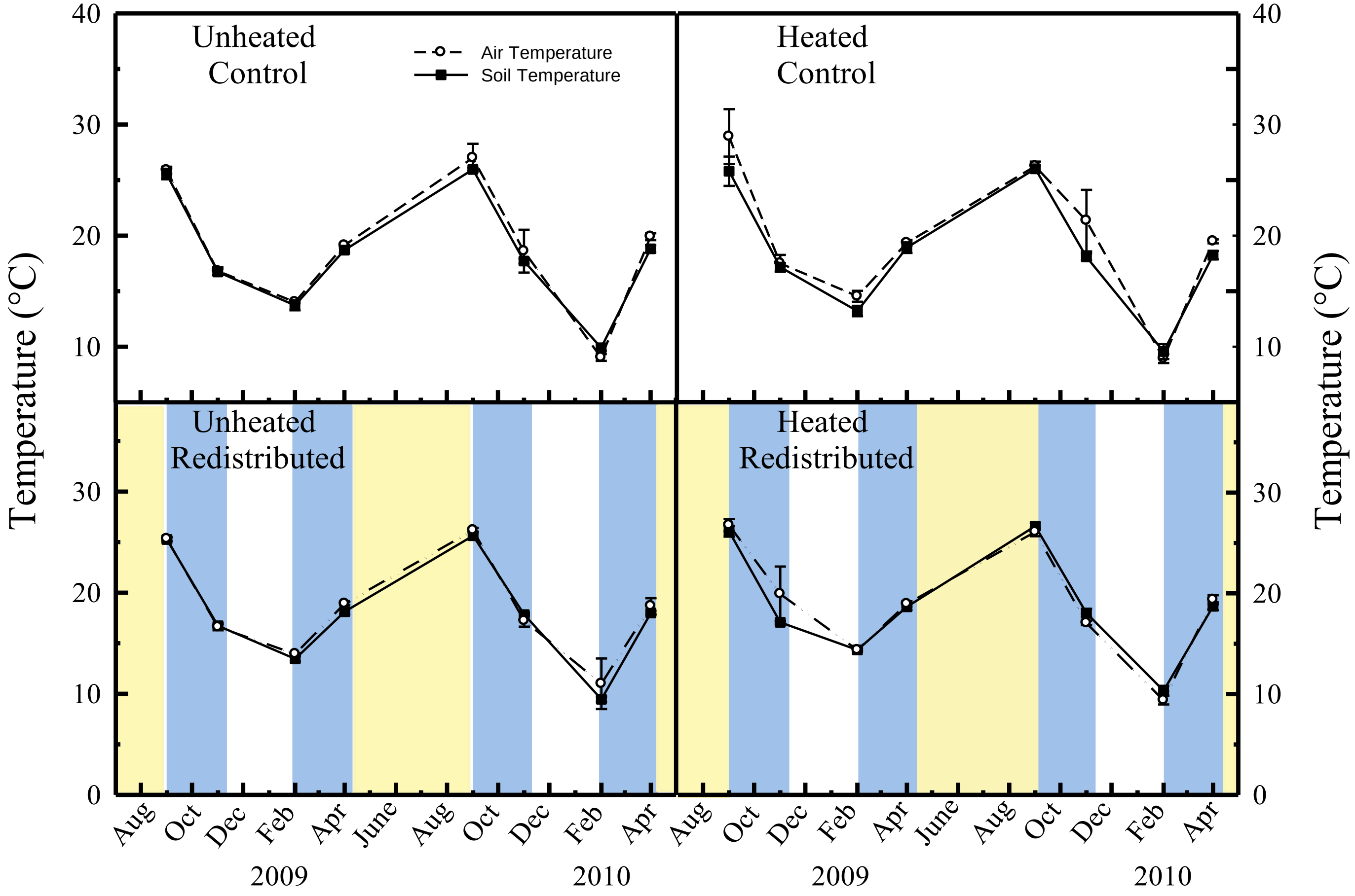

Fig. 2.1 Mean air and soil temperatures were averaged over each 30-day soil incubation period across all plots from August 2008 to April 2010. For each point, n = 4 and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

differed between canopy types, volumetric water content was generally 1% lower in warmed plots.

Field Sampling and Soil Analysis

Due to time and logistical constraints a subset of vegetation treatments containing monocultures of *J. virginiana* and *S. scoparium*, and the *J. virginiana + S. scoparium* combination were sampled from the full factorial design during years six and seven of the WaRM experiment. Both *J. virginiana* and *S. scoparium* are among the most dominant species within the post oak savanna region.

Surface litter in each sampling area was moved aside prior to soil coring. Two soil cores (5.5 cm diameter x 10 cm depth) were collected 8 times per year, at the beginning and end of the last thirty days of each precipitation change, in control and treated plots under each vegetation cover type (*J. virginiana*, *S. scoparium*, and *J. virginiana + S. scoparium*) for 22 months (August 2008 to April 2010). Each soil sample was weighed immediately after sampling to obtain whole-soil wet weight. A 30 g subsample was then dried at 105 °C for 24 hours and reweighed to determine gravimetric water content and used to compute soil bulk density. Volumetric water content was then calculated using the bulk density of each sample. Two additional aliquots of each soil sample were utilized for soil pH and texture analysis. pH was quantified by using the $CaCl_2$ method and glass electrode (McLean 1982), and texture was determined by using the hydrometer method (Bouyoucos 1926).

Fine, Coarse, and Total Root Biomass

The remaining soil was weighed and passed through a 2 mm sieve to recover coarse roots (>2 mm). An aliquot (approximately 100g) of the soil that passed through the 2mm screen was weighed and then passed through a hydropneumatic elutriation system (Gillison's Variety Farbication, Inc., Benzonia, MI) to remove fine roots (<2 mm) using a 410 µm mesh filter. Both coarse and fine roots were dried at 60 °C to a constant mass for 48 hours and weighed for root biomass. Root biomass was corrected to represent the entire volume of the original soil core. Total root biomass was calculated by adding the values of coarse and fine root biomass.

C and N Concentrations and C:N Ratios in Root Tissues

Dried coarse and fine root samples were individually pulverized and homogenized using an Angstrom TE250 ring pulverizer (Angstrom, Inc., Belleville, MI). Samples were weighed into tin capsules using a microbalance, and analyzed for C and N concentrations using a Carlo-Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ). These values were then used to calculate C:N ratios.

Statistical Analyses

The effects of rainfall, temperature, species combination, and their interactions on all response variables were analyzed by repeated measures analysis of variance (ANOVA) in JMP Pro 10.0.0 (SAS Institute, Cary NC). The experimental design was a completely randomized split-plot with four replicates per treatment combination. Whole plots consisted of the two precipitation treatments (control vs. redistributed) in factorial combination with two temperature treatments (unheated vs. heated); the subplots were the three vegetation types [two monocultures (*J. virginiana*, *S. scoparium*) and one tree-grass combination (*J. virginiana + S. scoparium*)]. Each sampling period (time) was treated as an individual repeated measure using the D-optimal split-plot design as a repeated measures approximation procedure, which allows for a robust mixed model analysis (Goos & Vandebroek 2001; SAS 2005; Jones & Nachtsheim 2009; Smucker 2011). Variances were estimated with Restricted Maximum Likelihood (REML) and the Tukey's HSD post hoc test was used to distinguish differences between the treatment combinations. The Student's t-test was used when a variable had only two treatment levels and a normal distribution (e.g. warming = heated vs. unheated; rainfall = control vs. redistributed) (Ott & Longnecker 2010).

4. Results

Microclimate

Air temperatures were averaged over each 30-day soil incubation period. The mean air temperature was 18.63 C in unheated plots and 19.25 C in heated plots; temperatures ranged from 9.2 to 27.8 C across all plots and sampling periods. Air temperatures in the heated plots averaged 1 °C higher than unheated plots, but soil temperatures were not affected by warming by the 6th year of the WaRM experiment (Volder *et al*. 2013; Figure 2.1). Heaters were raised by 0.5 m and infrared outputs were increased to 800 W in February 2008 to accommodate the height of the vegetation

(Volder *et al.* 2013), which ultimately shaded the soil surface and minimized the potential for soil warming. Rainfall events were manipulated to simulate summer drought by reducing precipitation by 40% and redistributing the rainfall to the spring and fall seasons (Figure 2.2). Soil volumetric water content decreased in the summer and increased in the spring and fall months within the redistributed rainfall plots with a significant interaction of rainfall x warming x species combination ($p < 0.001$) (Figure 2.2). Heated *S. scoparium* monocultures under control rainfall had the highest volumetric water content ($p < 0.001$) and heated *J. virginiana* monocultures under control rainfall had the lowest volumetric water content ($p < 0.001$).

Soil bulk density ranged from 0.98 to 1.85 gm^{-2}, and was affected by precipitation x time ($p = 0.004$) and precipitation x species composition ($p = 0.001$) interactions (Table 2.1; see Table A.1). The highest soil bulk density values were in March 2010 in *S. scoparium* plots with redistributed rainfall, and the lowest values were in October 2008 in *J. virginiana* plots with control rainfall. Values for soil pH ranged from 3.02 to 6.83 and were significantly affected by interactions between precipitation x species composition ($p = 0.029$), precipitation x time ($p = 0.002$), and warming x species composition ($p < 0.001$) (Table 2.1; see Table A.1). Heated *J. virginiana* monocultures were significantly ($p <0.001$) more acidic than all other warming x species combination treatments and soils were most acidic within the redistributed rainfall regime during the winter ($p = 0.002$). All treatments fell within the sandy loam soil texture class with no significant difference between shelters (Table 2.1; see Table A.1).

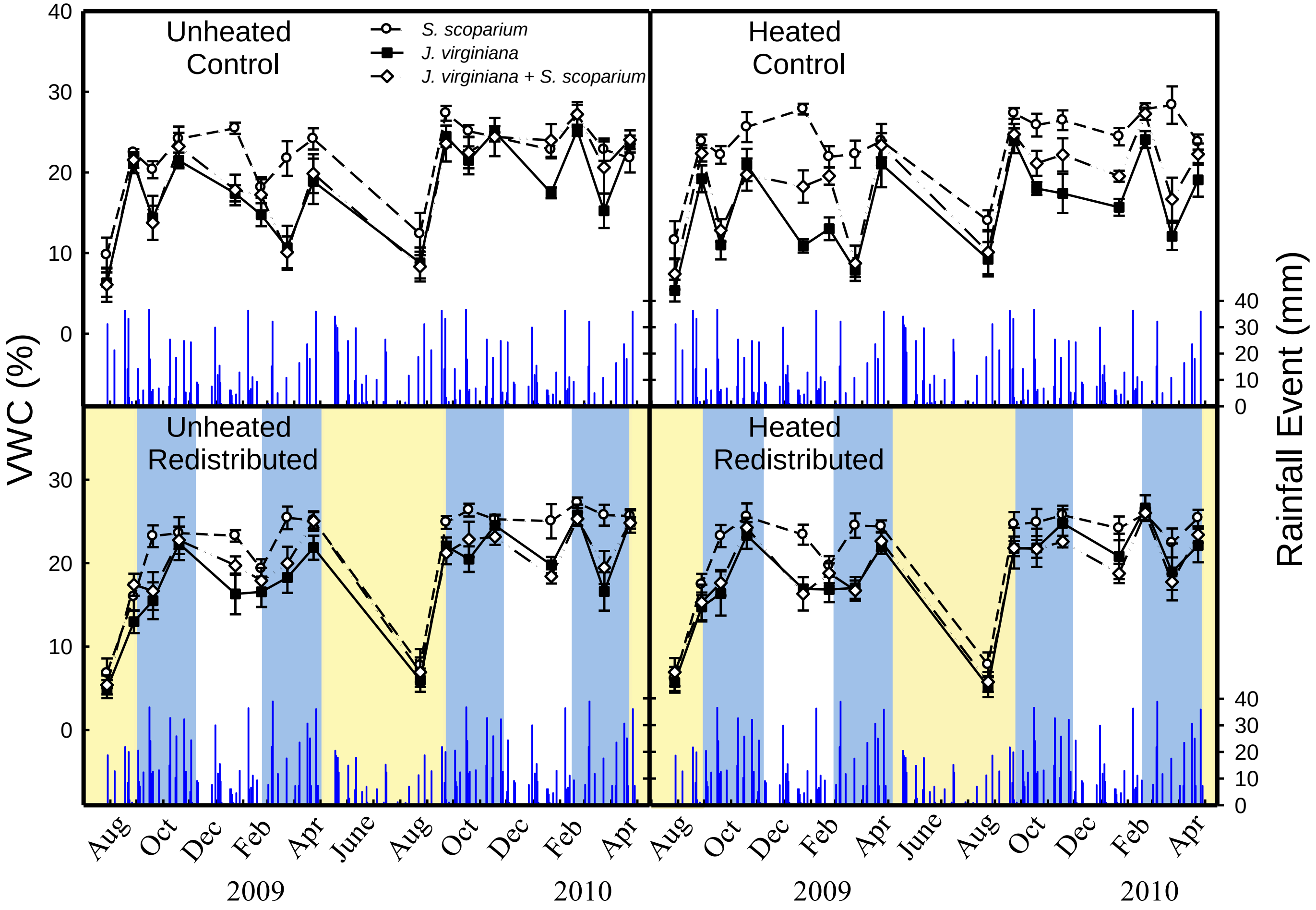

Fig. 2.2 Soil volumetric water content (VWC; 0-10cm; shown as symbols) and rainfall event size (mm; vertical bars) for the duration of the study, August 2008 to April 2010. For VWC, n = 4 for each point, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

Table 2.1 Soil properties (0-10 cm) by species (mean ± SE). Bulk density was measured during each of the 16 sample collection periods. pH was measured four times each year for two years. Soil texture analysis was measured once in August 2008.

	S. scoparium		***J. virginiana***		***J. virginiana + S. scoparium***	
	Mean	***SE***	***Mean***	***SE***	***Mean***	***SE***
Bulk Density (n = 512) g/cm^3	1.33	0.00	1.30	0.00	1.30	0.00
pH (n = 256)	5.31	0.03	4.95	0.04	5.02	0.03
Soil Texture (n = 18)						
% Sand	59.3	0.7	59.1	0.9	59.0	0.8
% Silt	30.1	0.5	30.5	0.5	30.4	0.5
% Clay	10.6	0.6	10.5	0.6	10.7	0.7

Coarse, Fine, and Total Root Biomass

Total root biomass in individual plots ranged from 94.7 to 3,163.2 g m^{-2}, and this range was influenced by plant species composition. Total root biomass in plots comprised only of *S. scoparium* ranged from 94.7 to 1,932.1 g m^{-2}, while root biomass in plots comprised of *J. virginiana* only or *J. virginiana + S. scoparium* mixtures was generally higher and ranged from 247.6 to 3,163.2 g m^{-2}**.** Total root biomass varied seasonally ($p < 0.001$) and in response to species composition ($p < 0.001$) (Table 2.2; Figure 2.3). Peak values occurred in late winter/early spring and again in late summer/fall in the *J. virginiana* and *J. virginiana + S. scoparium* plots. In contrast, root biomass in monocultures of *S. scoparium* peaked in summer and late fall seasons (Table 2.2; Figure 2.3).

Coarse root biomass was significantly highest in the winter and spring seasons and lowest during the summer and fall ($p < 0.001$). There was a significant time x precipitation interaction, with higher coarse root biomass in redistributed rainfall plots in winter 2010 and lowest in redistributed rainfall plots in Summer 2008 ($p = 0.037$). *J. virginiana* and *J. virginiana* + *S. scoparium* treatments had significantly higher amounts of coarse root biomass than the *S. scoparium* ($p < 0.001$) (Table 2.2; Figure 2.3).

Fine root biomass dominated the total root biomass pool (Figure 2.3). In contrast with the coarse roots, fine root biomass was significantly highest in the fall and summer seasons and lowest during the winter and spring seasons ($p < 0.001$). There was a significant interaction between vegetation type and warming where unheated *J. virginiana* plots had higher fine root biomass than heated *J. virginiana* plots ($p = 0.004$).

Table 2.2 Probability values (* p<.05, **p<.01, ***p<.001) using repeated measures ANOVA for changes in coarse, fine, and total root biomass, C, N, and C:N ratio from August 2008 to April 2010.

Treatment	**Coarse Root**				**Fine Root**				**Total Root**			
	Mass	**C**	**N**	**C:N**	**Mass**	**C**	**N**	**C:N**	**Mass**	**C**	**N**	**C:N**
Rainfall (R)	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*
Warming (W)	*ns*	*ns*	**	*ns*	*ns*	*	*	*ns*	*ns*	*	**	*ns*
R x W	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Species Comp. (SC)	***	***	***	*ns*	***	***	***	*ns*	***	***	***	***
R x SC	*ns*	*	*ns*	*	*ns*	*ns*	*ns*	**	*ns*	*	*ns*	*
W x SC	*ns*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*
R x W x SC	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Time (T)	***	**	***	*ns*	***	***	***	***	***	*	***	***
R x T	*	*ns*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
W x T	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
R x W x T	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
SC x T	***	*ns*	*ns*	**	*ns*	**	*	*ns*	*ns*	*ns*	*	*ns*
R x SC x T	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
W x SC x T	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
R x W x SC x T	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

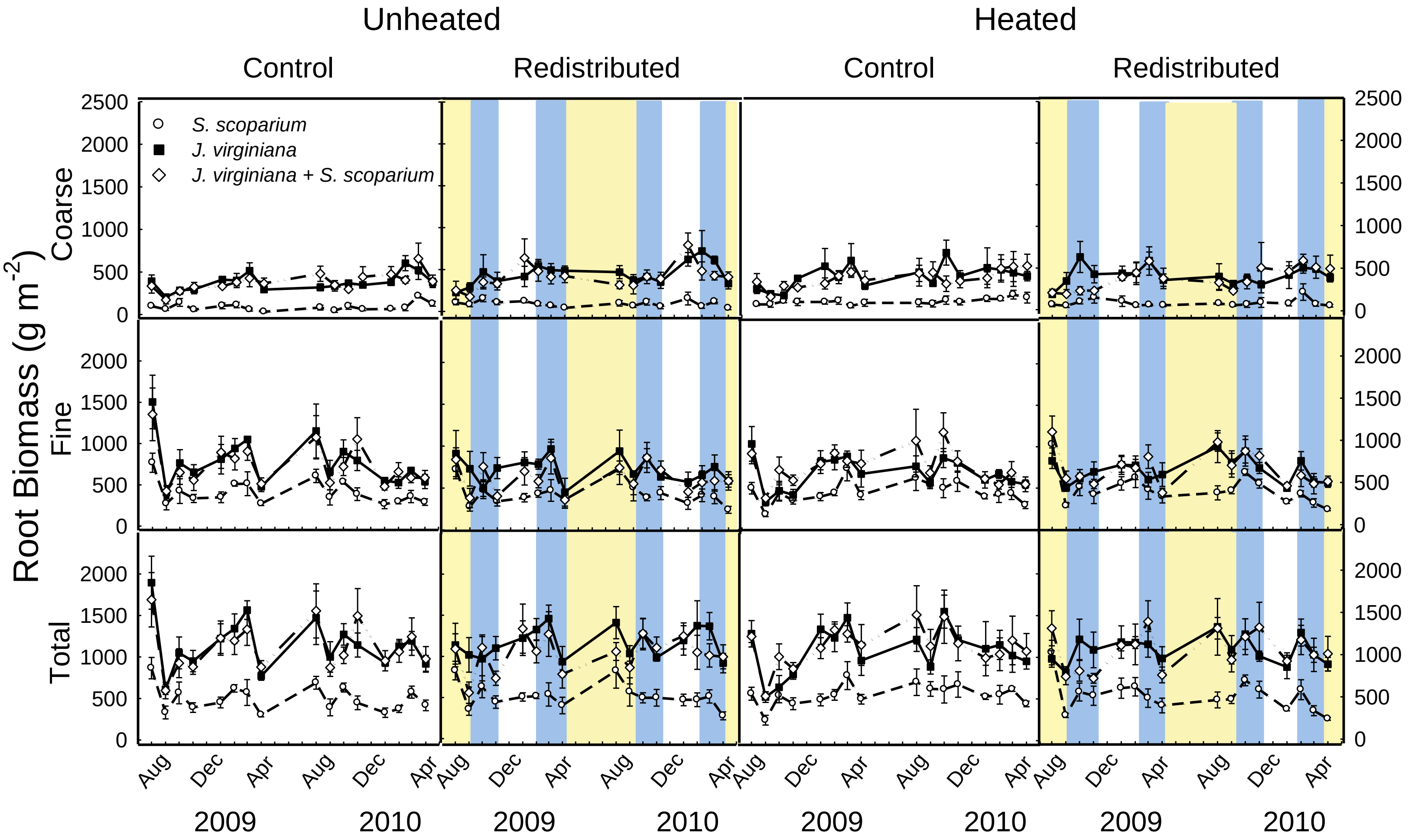

Fig. 2.3 Coarse, fine, and total root biomass (0-10 cm) in soils collected between August 2008 and April 2010. For each point, n = 4, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

However, *J. virginiana* and *J. virginiana + S. scoparium* plots still had higher amounts of fine root biomass than the *S. scoparium* monocultures ($p < 0.001$) (Table 2.2; Figure 2.3).

Root C and N Pools, and C:N Ratios

Coarse root C pools in individual plots ranged from 9.1 g m^{-2} under *S. scoparium* to 1,152.9 g m^{-2} under *J. virginiana + S. scoparium* (Figure 2.4). Carbon pools significantly increased in size over time among all treatments ($p = 0.023$). There was a significant rainfall x vegetation type interaction where coarse root C pools in *J. virginiana + S. scoparium* plots were lower under redistributed rainfall than the *J. virginiana + S. scoparium* under control rainfall ($p = 0.033$). Coarse root N pools in individual plots ranged from 0.1 g m^{-2} under *S. scoparium* to 14.0 g m^{-2} under *J. virginiana + S. scoparium*. Coarse root N was significantly reduced by warming ($p = 0.009$) (Figure 2.4, Table 2.2). Nitrogen pools varied significantly through time ($p = 0.023$), increasing from August 2008 to August 2009 but remaining relatively constant from August 2009 to April 2010. The C:N ratios of coarse root biomass ranged from 23.2 in the *S. scoparium* plots to 95.0 in the *J. virginiana + S. scoparium* plots. A significant interaction occurred between time and vegetation type where *S. scoparium* C:N ratios were higher in August 2008 and lowest among all treatments in April 2010 ($p = 0.001$). Redistributed rainfall significantly reduced coarse root C:N ratios ($p = 0.025$).

Fine root C pools in individual plots ranged from 22.5 g m^{-2} in the *S. scoparium* treatments to 640.2 g m^{-2} in the *J. virginiana* treatments (Figure 2.5). Warming

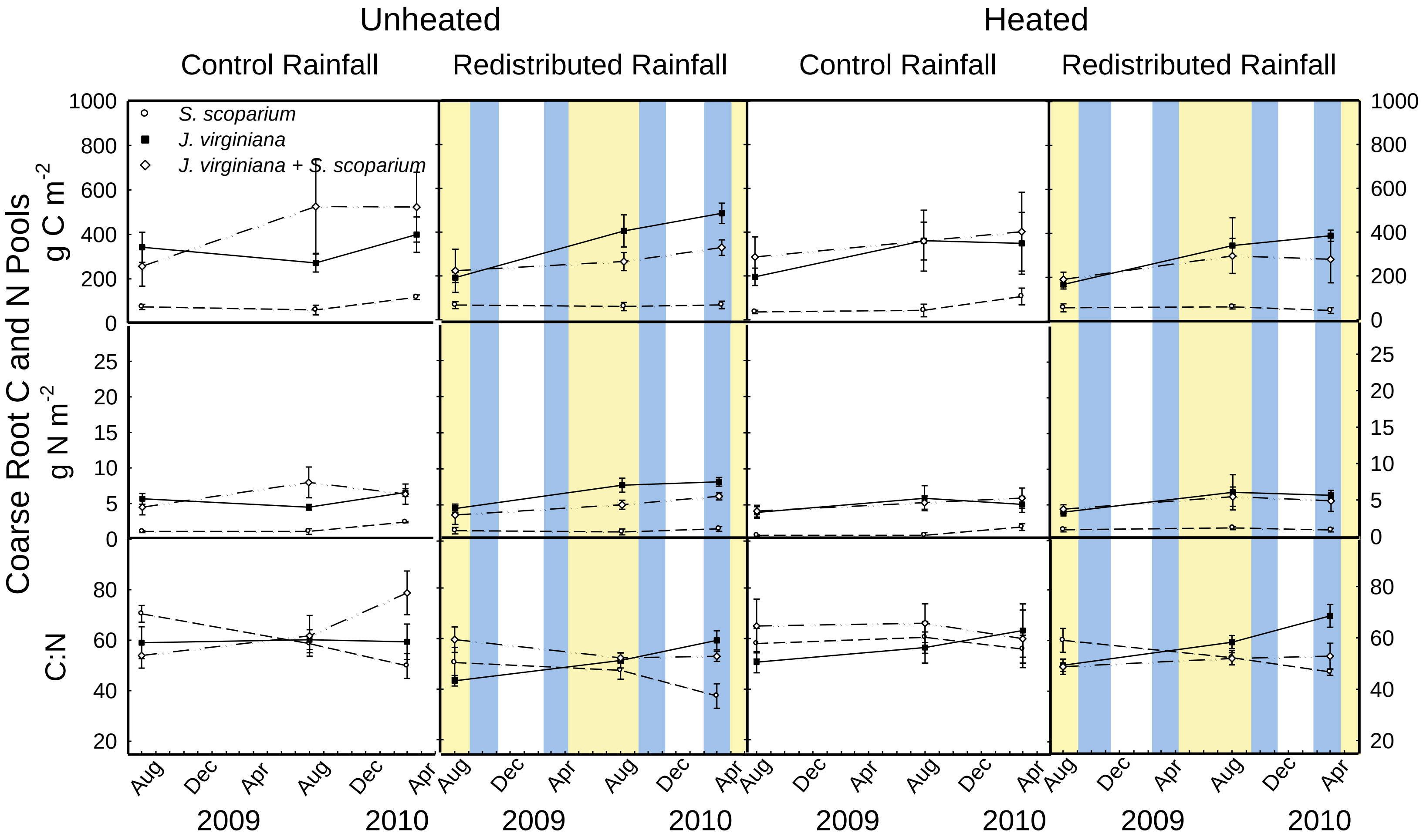

Fig. 2.4 Coarse root carbon and nitrogen pools (0-10 cm) in soils collected between August 2008 and April 2010. For each point, n = 4, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

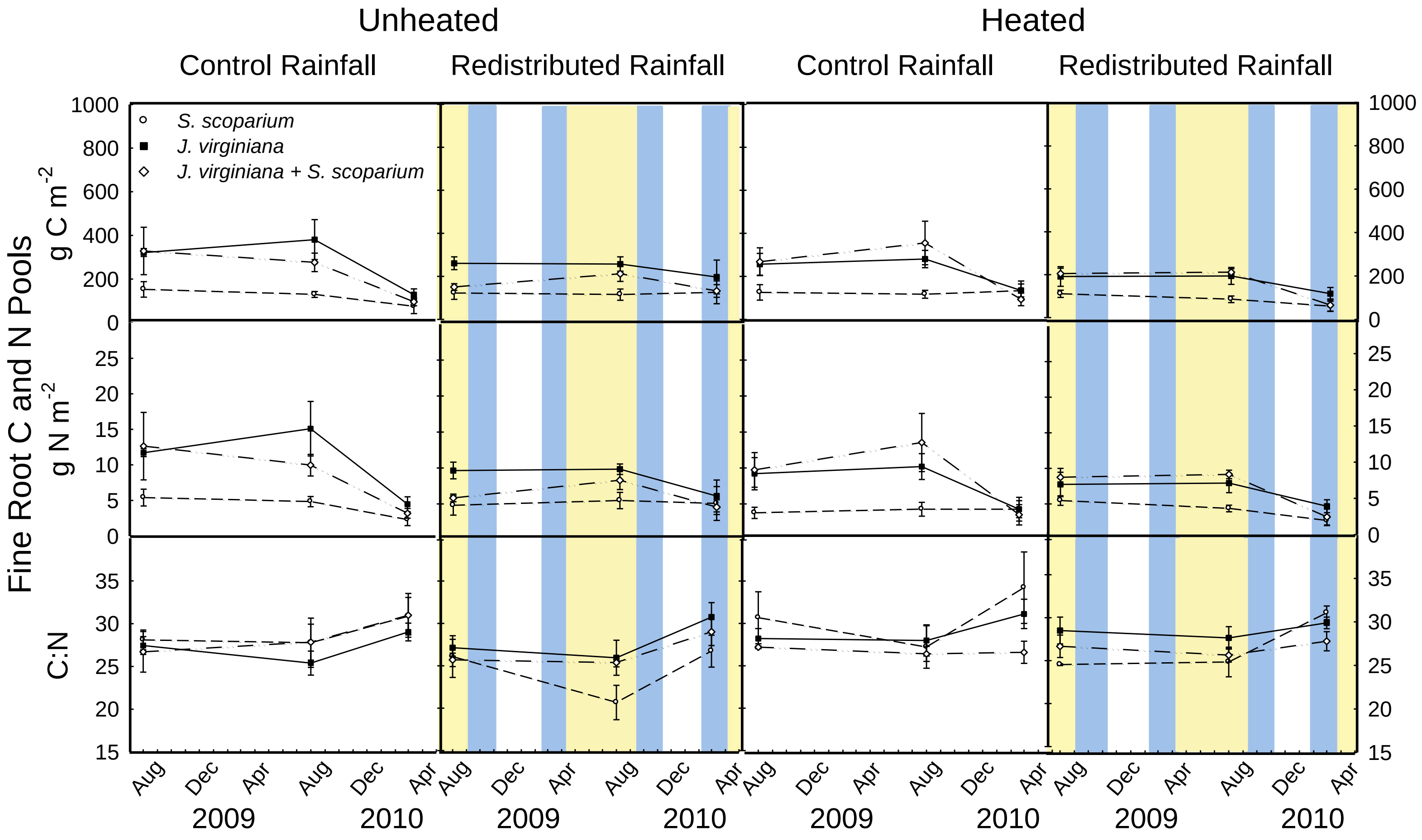

Fig 2.5 Fine root carbon and nitrogen pools (0-10 cm) in soils collected between August 2008 and April 2010. For each point, n = 4, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

significantly affected fine root C pools at the $p < 0.10$ level ($p = 0.055$), though the post hoc test did not reveal a difference. There was a significant interaction between time and precipitation in August 2008 and 2009 where fine root C pools were larger under control rainfall compared to those under redistributed rainfall ($p = 0.006$) (Table 2.2). Data also indicated a significant vegetation type x time interaction in which fine root C in both *J. virginiana* and *J. virginiana + S. scoparium* was larger in the summer months (August 2008 and August 2009) compared to spring (April 2010) ($p = 0.006$). Fine root N pools in individual plots ranged from 0.59 g m^{-2} under *S. scoparium* to 26.3 g m^{-2} under *J. virginiana*. Similar to the fine root C pools, warming significantly affected fine root N pools at the $p < 0.10$ level ($p = 0.059$), though the post hoc test did not reveal a difference. In addition, the *J. virginiana* and *J. virginiana + S. scoparium* fine root N pools were significantly larger in both August 2008 and August 2009 compared to April 2010 ($p = 0.013$) (Table 2.2; Figure 2.5). Fine root C:N ratios ranged from 17.5 in redistributed rainfall *S. scoparium* treatments to 44.8 in control rainfall *S. scoparium* treatments, which indicated a significant interaction between precipitation and vegetation type ($p = 0.005$). C:N ratios in fine roots increased through time across all species (Figure 2.5), and were significantly higher in April 2010 than in August 2008 and August 2009 ($p < 0.001$).

Total root C pools in individual plots ranged from 46.0 g m^{-2} in the *S. scoparium* treatments to 1500.8 g m^{-2} in the *J. virginiana + S. scoparium* treatments (Figure 2.6). Carbon pools were significantly larger in August 2009 ($p = 0.023$) and larger in the *J. virginiana* and *J. virginiana + S. scoparium* treatments compared to the *S. scoparium*

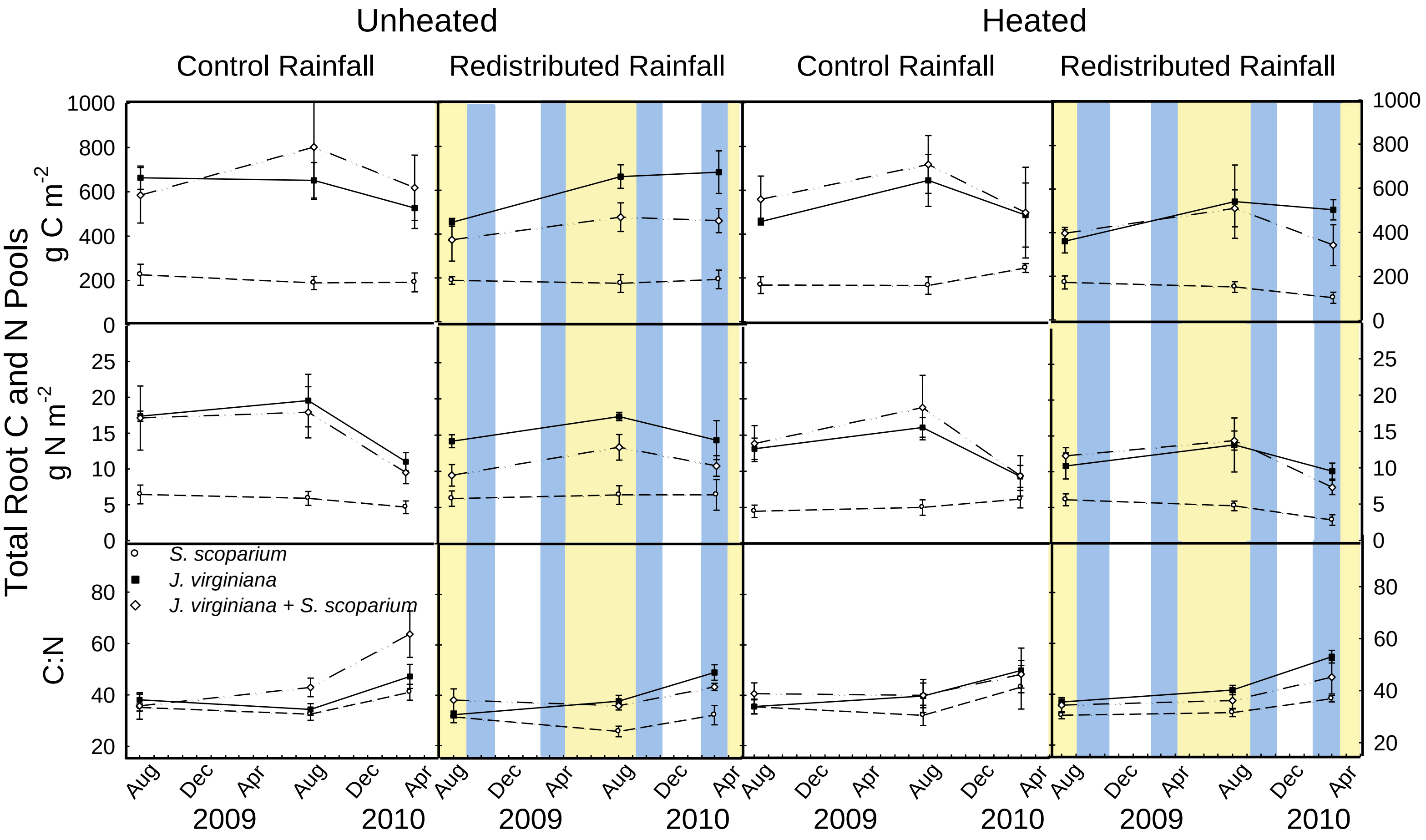

Fig. 2.6 Total root carbon and nitrogen pools (0-10 cm) in soils collected between August 2008 and April 2010. For each point, n = 4, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

treatments ($p < 0.001$) (Table 2.2). Redistributed rainfall significantly decreased root C pools in the *J. virginiana + S. scoparium* treatments (rainfall x species composition, p = 0.014) and warming significantly decreased root C pools across all treatments (p = 0.014). Total root N pools in individual plots ranged from 1.3 g m^{-2} in the *S. scoparium* treatments to 30 g m^{-2} in the *J. virginiana* and *J. virginiana + S. scoparium* treatments. Warming decreased the total root N pool size among all vegetation types (p = 0.004) (Table 2.2; Figure 2.6). There were significant interactions between time and species compostition among all *J. virginiana* and *J. virginiana + S. scoparium* treatments where total root N pools were larger in August 2009 compared to August 2008 and April 2010 (p = 0.022). Total root C:N ratios varied significantly through time ($p < 0.001$), and generally increased from August 2009 to April 2010 (Figure 2.6; Table 2.2). Total root C:N was also affected significantly by the rainfall x species interaction (p = 0.021) where *J. virginiana + S. scoparium* decreased with redistributed rainfall. *S. scoparium* treatments had significantly lower C:N ratios compared to *J. virginiana* or *J. virginiana + S. scoparium* ($p < 0.001$).

5. Discussion

In this multifactor global change experiment, species composition and time appeared to be the most consistently important main effects that determined root biomass, C, N, and C:N ratio. Warming alone and rainfall redistribution alone seldom affected the root response variables in this experiment. However, warming and rainfall redistribution interacted with other experimental variables to influence root biomass and nutrient pools. Results of this study support the emerging consensus that global change drivers are likely to have their greatest impact by mediating complex interactions with plant species composition (Brooker 2006; Gilman *et al.* 2010).

Root Biomass

Although elevated temperatures alone seldom influenced the root response variables in our experiment, there was a significant interaction between elevated temperatures and species composition. In particular, elevated temperatures significantly decreased fine and total root biomass measurements in *J. virginiana* monoculture, but did not affect coarse root biomass. This interaction did not affect root biomass in the other vegetation treatments which disproves our first hypothesis that elevated temperatures would increase root biomass in all vegetation types. Our results indicate that root responses in this setting do not follow the same pattern of increased aboveground growth to warming as previous demonstrated in this experiment (Volder *et al*. 2013). Interestingly, similar decreases in fine root growth to warming were observed under black spruce (*Picea mariana*) in a boreal forest (Bronson *et al*. 2008), under red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) trees in a temperate forest (Wan *et al.* 2004), under a mixed deciduous forest (Melillo *et al*. 2011), under herbaceous and woody perennials in a temperate steppe (Bai *et al.* 2010), and under a Yorkshire fog (*Holcus lanatus*) dominated grassland (Hartley *et al.* 2007). Negative fine root responses to higher temperatures are therefore expected (Pendall *et al.* 2004). In contrast, Wan *et al.* (2005) reported that warmer temperatures increased fine root biomass under a tallgrass prairie dominated by *S. scoparium* and yellow Indiangrass (*Sorghastrum nutans*). The effect of temperature on root growth, root biomass, and root behavior are primarily species specific and are dependent on the optimal conditions of each species (Faget *et al.* 2013).

Warming significantly reduced fine and total root biomass, even though it significantly increased aboveground biomass of *J. virginiana* in this experiment (Volder *et al.* 2013). Warming generally enhances nutrient mineralization rates and increases the availability of limiting nutrients (Bai *et al.* 2013, Rustad *et al.* 2001), resulting in a smaller root biomass compartment (Melillo *et al.* 2011). In addition, Fitter *et al.* (1999) found that higher temperatures indirectly (via radiation flux and nutrient availability) increased fine root birth and death rates of sheep fescue (*Festuca ovina* L.) and colonial bentgrass (*Agrostis capillaris* L.) in an upland grassland, thus increased fine root turnover rates. Similar results were found in a Mediterranean mixed conifer ecosystem in California where fine root turnover rates increased with increasing soil temperature and water content (Kitajima *et al.* 2010). Warmer temperatures also increased root mortality under a sugar maple (*Acer saccharum* Marsh.) forest by accelerating maintenance costs and increasing soil organism activity (Hendrick & Pregitzer 1993). It is also important to note that fine root turnover rates tend to increase with increasing N availability (Hendricks *et al.* 1993). Even though soil CO_2 efflux over the first five years of the WaRM study did not significantly differ from heated to unheated plots in any vegetation type (Cartmill 2011), N mineralization rates were significantly higher in the heated plots during the same time of our study (Wellman *et al.* unpublished data). We speculate that because nutrients may be relatively more available in response to warming, the need for an extensive fine root system in *J. virginiana* may be diminished, thereby shifting biomass allocation to aboveground structures (Volder *et al.* 2013). Miller *et al.* (1990) reported that another *Juniperus* species (*J. occidentalis*) invested larger amounts of resources to aboveground foliage compared to belowground biomass to optimize photosynthetic capacity in a semi-arid environment. Nutrient concentrations in the fine roots were also several times higher than in the foliage, indicating no need for additional fine root growth or nutrient exploration into the soil (Miller *et al.* 1990). Therefore, we suggest that the decrease in fine root biomass in *J. virginiana* under warming could be partially explained by higher nutrient turnover rates belowground, providing adequate nutrients for root acquisition, and therefore by re-distributing available resources to aboveground growth. The overall lack of the coarse root response in this case could be due to the fact that thicker roots tend to have slower turnover rates, are more resistant to decomposition compared to fine roots, and they contribute to long-term root development (Fitter 2002).

Soil water content is also a strong determinant of root biomass production and seasonal distribution patterns (Fiala *et al.* 2009). *P. glandulosa* increased fine root biomass in irrigated soils compared to control, natural, and rainout treatments during a long-term soil moisture manipulation experiment, and coarse root biomass was significantly higher in the rainout treatments receiving less water (Ansley *et al.* 2014). Spring wheat (*Triticum aestivum* cv. Tonic) also had significantly more root biomass in wetter treatments compared to dry treatments (Van Vuuren *et al.* 1997). A meta-analysis investigating below-ground responses to precipitation indicated that increases in precipitation increased belowground biomass while decreases in precipitation had no effect on belowground biomass (Wu *et al.* 2011). In the present study, rainfall manipulation, unlike warming, had no direct effect on fine, coarse, or total root biomass, contrary to our second hypothesis. However, there was a significant rainfall x time interaction. Coarse root biomass in the redistributed rainfall plots was significantly higher within all vegetation treatments during January 2009 and 2010. These January peaks likely reflect a coarse root growth response to the 52% increase in rainfall amount that occurred during September and October of each year. In addition, high rates of net N-mineralization and relatively high concentrations of NH_4^+ and NO_3^- in soil solution are observed during late summer and fall, perhaps reflecting priming of these N transformations following the end of the summer drought period (Wellman *et al.* unpublished data).

As predicted in our third hypothesis, species was a significant main effect on root biomass due to differences in plant functional types. *S. scoparium* monocultures had significantly lower biomass values in all root size classes compared to the *J. virginiana* and *J. virginiana + S. scoparium* plots. *S. scoparium* is a C_4 perennial bunchgrass with a shallow fibrous root system and little root production during winter senescence (West *et al*. 2003), whereas *J. virginiana* is a C_3 coniferous evergreen tree with a deep tap root system (Knapp *et al*. 2008). Similarly, McKinley (2006) found more than double the amount of root biomass under *J. virginiana* compared to adjacent grasses in Kansas.

Time also played a key role as a significant main effect in root biomass within each size class. Given the strong seasonal variations in both temperature and rainfall that characterize this site (NOAA 2010; Figure 2.1), we expected significant temporal variation of root biomass among all root size classes and species composition. Mean air temperatures and the distribution and amount of rainfall throughout our study were comparable to the 50 - year means for a NOAA weather station located <0.5 km from this site (NOAA 2010; Volder *et al*. 2013; Figure 2.1; Figure 2.2; see Figure A.3; Figure A.4; Figure A.5).

Fine and total root biomass across all treatment combinations were highest towards the end of each summer (August 2008 and 2009), with smaller biomass peaks occurring during the early spring of each year (March 2009 and 2010) (Figure 2.3). In contrast, fine and total root biomass was lowest during the late spring (April 2009 and 2010) of each year. Similar results were found in a native tallgrass prairie in Missouri dominated by big bluestem grass (*Andropogon gerardii* Vitman) and *S. scoparium* where root mass reached a peak in July and a minimum in April (Dahlman & Kucera 1965). In general, root activity is expected to increase with higher temperatures (Gill & Jackson 2000) and when water and nutrients are readily available (Oren & Sheriff 1995). As mentioned earlier, N mineralization rates measured within the same soil cores sampled for root biomass were highest during the warmest month (August) in each species combination (Wellman *et al.* unpublished data), and coincided with peaks in fine and coarse root biomass. However, we did not expect an increase in root biomass during the winter, regardless of the rainfall regime or vegetation composition.

In a review of fine root responses to temperature, Pregitzer *et al.* (2000) discuss the relationship of seasonality to fine root production. Root systems within various ecosystems have shown a distinct increase in root growth during early spring when temperatures begin to rise, shoot growth increases, and soils are still relatively cool (Pregitzer *et al*. 2000). López *et al.* (2001) found that root biomass was highest during the fall and winter seasons in a Mediterranean holm oak (*Quercus ilex* L.) forest in Spain where water availability is generally higher in the winter due to lower temperatures and a decrease in water stress; therefore, fine and total roots within the post oak savanna may be responding to the increase in available water during the winter months (Figure 2.2) by increasing root growth (Figure 2.3). It is not surprising that the evergreen *J. virginiana* would continue to produce new growth during these cooler months, but we did not anticipate *S. scorparium* (warm season grass) roots to increase as well. Interestingly, live tillers of *S. scorparium* have been documented to persist throughout most winters in this region, following their initiation during the previous autumn (Butler & Briske 1988; Briske & Butler 1989) and maximum tiller number per plant occurred in April in the WaRM experiment (Volder *et al.* 2013). This indicates that C_4 perennial grasses in low latitude (30 N) savannas exhibit a prolonged growing season compared to similar grasses in more temperate regions (Volder *et al.* 2013). It is plausible that *S. scorparium* in central Texas may be developing roots from the live, over wintering tillers in March, prior to a large increase in tiller growth and recruitment in April.

Coarse root biomass was highest in January or March of each year within the *J. virginiana* and *J. virginiana + S. scoparium* species composition in a time x species interaction. An increase in coarse root biomass with time regardless of rainfall pattern indicates a more resilient root behavior to shifts in rainfall in these species compositions. Coarse root biomass was lowest during August and September when temperatures, fine and total root biomass, and mineralization rates were peaking (Figure 2.1; Figure 2.2; Figure 2.3; Wellman *et al.* unpublished data). There was no difference in coarse root biomass in the *S. scoparium* monocultures even though N mineralization rates followed the same patterns. The lack of the time x species interaction with the *S. scoparium* monocultures may indicate that the warm season C_4 grass is more dependent on seasonal rainfall patterns than it is on time or N availability. This is yet another example why root biomass responses need to be evaluated at the species level. These results show an important cycle of root behavior in response to interactions with environmental factors in the post oak savanna and should be considered when studying phenological characteristics.

Root C and N Pools, and C:N Ratios

Warming significantly reduced C and N pool sizes within the total root biomass and reduced N pool sizes within the coarse root biomass across all species (Figure 2.4; Figure 2.6). This analysis is in contrast to our first hypothesis that elevated temperatures would increase root C and N pool sizes in each vegetation type due to increased aboveground growth (Volder *et al.* 2013). It is also important to note that fine root C and N pools were affected by warming at the 90% confidence level, although post hoc tests did not indicate specific treatment differences (Figure 2.5). The differences in total root

C and N pools and coarse N pool sizes are likely due to a shift in C and N allocation from root to shoot as temperatures rise and growing seasons are extended. Although each species response is dependent on specific optimal conditions (Faget *et al.* 2013), warming is often associated with increases in belowground growth. Increased temperatures may also increase evapotranspiration and reduce soil water content, which could induce water stress and suppress root activity in arid to semi-arid environments where water is often a major limiting factor (Bai *et al.* 2010). Plant root strategies may then shift nutrient balances when conditions are warmer than their optimum. Therefore, changes in environmental factors such as warming can affect the functional equilibrium within plant growth and development similar to a supply and demand response (Friend *et al.* 1994). Plants may be able to adjust growth patterns by diverting resources from one plant organ or structure to another (Brouwer 1983). In our study, species in the post oak savanna ecosystem may be shifting C and N allocation to aboveground growth when temperatures increase.

Our analysis suggests that rainfall redistribution and species composition interacted to decrease coarse and total root C pools and coarse, fine, and total C:N ratios in the *J. virginiana + S. scoparium* plots (Figure 2.4; Table 2.2). However, a reduction in C with no change in biomass or N concentration contradicts current understanding of how these factors interact. Normally, C:N ratios are controlled by biomass and N concentration, and although there was a decreasing trend in root biomass and root N concentration with redistributed rainfall, they were not significant. The decrease in coarse root C pools could be explained by a combination of small scale dynamics we cannot account for in our study. For example, data from the WaRM experiment on specific root length (SRL) indicates that *S. scoparium* roots increased in SRL by as much as 50% in the same *J. virginiana + S. scoparium* plots with redistributed rainfall and warming (Volder *et al.* unpublished). In addition, C:N ratios generally decrease as N concentrations in roots increase with decreasing diameter (Augusto *et al.* 2014; Chen *et al*. 2010; Gordan & Jackson *et al.* 2000; Guo *et al.* 2004; Hellsten *et al.* 2013; Pregitzer *et al.* 1997; Pregitzer *et al.* 2002). It is also important to consider that our coarse root analysis included root sizes >2mm (measured) and up to ~15mm (observed). Coarse root samples containing the larger root diameters (~15mm) could have had higher C:N ratios compared to samples whose root diameters were closer to 2mm. The smaller diameter roots would have higher root N pool sizes and lower C:N ratios. There might have been a larger proportion of smaller (2-5mm) coarse roots in redistributed treatments, creating a decreasing effect in both root C pool size and root C:N ratio. Therefore, it is plausible that coarse root C pools could appear to be lower with no significant effect of the root N pool if N is increasing as the roots are decreasing in diameter, thus giving an appearance of a loss of C in the coarse root pool.

Our root C pool estimates in the *J. virginiana + S. scoparium* plots include biomass from both species which have different C and N concentrations. The mean total root C:N ratios were 33.8, 40.2, and 42.0 for the *S. scoparium* monocultures, *J. virginiana* monocultures, and the *J. virginiana + S. scoparium* species composition respectively. As expected, C:N ratios were also significantly lower in the fine roots compared to the coarse roots. If the proportion of root biomass contributed by each species changed in the *J. virginiana* + *S. scoparium* plots under redistributed rainfall where *S. scoparium* SRL significantly increased by 50%, then the overall root biomass (*J. virginiana* + *S. scoparium* combined) in the plot could reflect a lower C:N ratio.

Based on this analysis, our results partially support our hypothesis that redistributed rainfall regimes of summer droughts and intensified rainfall in the spring and fall seasons would in general reduce root C and N pool sizes. Both monocultures of *J. virginiana* and *S. scoparium* were unaffected and this effect was not significant with N pools or in fine roots. With coarse root N pools unaffected, the decrease in coarse root C pools reduced coarse root C:N ratios. The difference was great enough to affect the total root C pool and total root C:N ratio. This could potentially make the belowground plant material more susceptible to decay following root senescence. Differentiating species roots and identifying differences by specific diameter ranges beyond the 2mm cutoff could eliminate this error.

As predicted in our third hypothesis, species differences in root C and N pools were strong among each vegetation composition treatment (Figure 2.4; Figure 2.5; Figure 2.6; Table 2.2). The C_3 woody plant *J. virginiana* had significantly larger root C and N pools compared to those of the C_4 grass *S. scoparium*. This was mostly attributed to the distinct differences in growth forms per individual plant and root system sizes (Schenk & Jackson 2002). All plots containing *J. virginiana* had overwhelmingly more root biomass than monocultures of *S. scoparium.* However, root C:N ratios were significantly higher in the *J. virginiana* monocultures and *J. virginiana* + *S. scoparium* plots compared to *S. scoparium* monocultures, disproving our fourth hypothesis. This is consistent with other studies that showed that *J. virginiana* root C:N ratios can be twice as high as the leaf C:N ratio of the same plant (Norris *et al.* 2001b). These higher C:N ratios in *Juniperus* roots may reflect a poorer belowground litter quality that favors the accumulation of soil organic carbon when *Juniperus* invades grasslands throughout the Great Plains region (Jessup *et al.* 2003; McKinley & Blair 2008).

Similar to the root biomass response, time was a significant main effect of coarse, fine, and total root C and N pools, and C:N ratios (Table 2.2). The four most limiting factors to primary production are light, temperature, soil water content (rainfall), and N availability. In relation to time, seasonal changes are controlled by temperature and rainfall, and can influence root and soil C and N concentrations. Fine and total root C and N pool sizes were highest during the second summer (August 2009). However, fine root C and N pools were lowest in early spring (March 2010), a direct result of the fine root biomass response which was lowest during the same time period. A smaller root biomass pool will generally constrain the amount of C and N stored in the root compartment. In contrast, coarse root biomass and C and N pool sizes were lowest during the first summer (August 2008) and highest in early spring (March 2010). We suggest that fine roots in the post oak savanna are more abundant during warmer months when N mineralization rates are high (Wellman *et al.* unpublished), while coarse roots are more abundant during cooler months when soil moisture is more available.

Time and species composition also interacted to influence root C:N ratios (Table 2.2). The same factors that control seasonal variation can also control competitive interactions between species (February *et al.* 2013; Ludwig *et al.* 2004) and their influence on nutrient cycling (Casper & Jackson 1997; Wedin & Tilman 1993). The post hoc tests in our analysis revealed that the significance in the time x species interaction only occurred with *S. scoparium*. Coarse roots in the *S. scoparium* plots had higher C:N ratios during the first summer (August 2008), lower C:N ratios in early spring (March 2010), and there was a negative trend through time. Although not significant, the inverse of this relationship is true for the other species compositions of *J. virginiana* and *J. virginiana + S. scoparium* . In these plots the lowest coarse root C:N ratios were measured during August 2008, the highest in March 2010, and a positive trend through time. This implies that the *S. scoparium* coarse roots either increased in N or that the root diameters decreased with an increase in SRL through time because there was no significant difference or noticeable trend in coarse root biomass.

Likewise, the significant interaction of rainfall x time (Table 2.2) indicated that fine root C pools were higher in summer (August 2008 and 2009) and lower in early spring (March 2010) in control rainfall plots across all species (Figure 2.5). The interaction was not significant in the redistributed rainfall plots, with fine root N pools, or with fine root biomass, though there was a similar decreasing trend from August 2009 to March 2010 in the fine root N pool (Figure 2.5). As mentioned earlier, combining the two different species in this analysis could explain why we found a difference in C and not N. However, the control rainfall plots did not receive a summer drought when temperatures and N mineralization rates were also highest (Figure 2.1; Wellman *et al.* unpublished). The combination of these factors likely increased fine root C and N pools in the *J. virginiana* and *J. virginiana + S. scoparium* species compositions with little difference in fine root C and N pools in the *S. scoparium* plots (Figure 2.5). Therefore, the redistributed rainfall pattern we used with *S. scoparium* may have reduced the significance of the fine root C pool response enough to reflect no difference, likely narrowing the root C pool range between species (Figure 2.5).

6. Conclusions

Root Behavior and Distribution in the Post Oak Savanna Under Climate Change

The analysis of this multifactoral study highlights the complexity of global change impacts on post oak ecosystem dynamics. Increases in temperature, alterations in precipitation patterns, and plant species composition produced outcomes that were not as straightforward as originally expected. Interactions of these variables produced results that indicate a a complex multilayered response to these global change drivers. Additional analysis by identifying and separating individual roots of each species in the *J. virginiana* + *S. scoparium* species compositions will probably help clarify the differences found in this study. However, it is clear that *J. virginiana* is an opportunistic species that will increase in abundance with warmer temperatures while reducing their need to produce roots belowground, particularly when competing with *S. scoparium*. The absence of proper grassland management practices in the post oak savanna could accelerate this process. *J. virginiana* will likely continue to outcompete species like *S. scoparium* even though this low latitude (30 N) C_4 grass showed signs of continued growth during the cooler months when they are typically dormant and undergoing winter senescence. Greater overall biomass accumulation by *J. virginiana* above- and belowground is likely due to their aggressive nature found in a variety of ecosystems. For example and related to this study, *J. virginiana* takes advantage of resources produced from the decaying organic matter in the diminishing grasslands of the Great Plains.

Additional investigations into above- and belowground lignin content and microbial community structure may provide further insight as to what may be driving these interactions. These changes in root dynamics in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

CHAPTER III

SOIL ORGANIC CARBON AND SOIL TOTAL NITROGEN IN OAK SAVANNA: RESPONSE TO TEMPERATURE AND RAINFALL MANIPULATION

1. Synopsis

Increasing concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.1 to 4.5 °C by the end of the century, and potentially magnify the intensity and variability of seasonal precipitation distribution. The mid-latitude grasslands of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons. Despite these predictions, little is known concerning the effects of these global climate change drivers or their potential interactive effects on soil organic carbon (SOC), soil total nitrogen (STN), and soil C:N ratios. Therefore, the purpose of this study was to quantify seasonal variation in SOC and STN in soil subjected to experimental warming and rainfall manipulation. Research was conducted at the Warming and Rainfall Manipulation (WaRM) Site in east-central Texas. Experimental treatments were initiated in 2004, and SOC and STN were assessed 3 times between 2008 and 2010. SOC ranged from 419.4 g C m^{-2} and 1,768.9 g C m^{-2} with a mean of 959.4 g C m^{-2} (n = 287) and standard error of 13.5 across all samples. STN ranged from 23.7g N m^{-2} and 126.0 g N m^{-2} with a mean of 65.0 g N m^{-2} (n = 287) and standard error of 1.0. Soil C:N ratios ranged from 9.4 to 32.5 with a mean of 15.1 (n =

287) and standard error of 0.2. In this experiment, species composition and time appeared to be the most consistently important main effects that determined SOC and STN. Warming alone and rainfall redistribution alone did not affect SOC or STN in this experiment; however, warming and rainfall redistribution interacted with other experimental variables to influence SOC and STN. The results of this study were dominated by interaction effects between species x rainfall. The results of this multifactor global change experiment highlight the complexity of global change impacts on SOC, STN, and soil C:N ratios in the semi-arid post-oak savanna ecosystem. Interactions of these global change variables produced results that indicate a multilayered response. Results of this study suggest that changes in soil carbon and nitrogen storage in response to climate change may be mediated by changes in plant species composition.

2. Introduction

Increasing concentrations of greenhouse gases are projected to elevate global mean surface air temperatures by 1.5 to 4.5 °C by the end of the century, and potentially magnify the intensity and variability of seasonal precipitation distribution (IPCC 2013). The mid-latitude savannas of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons (Manabe & Wetherald 1986; Easterling *et al*. 2000; Melillo *et al.* 2014) and extending the length of growing seasons (Linderholm 2006; Schwartz *et al.* 2006; Tjoelker & Zhou 2007). Despite these predictions, little is known concerning the effects of these global climate change drivers or their potential interactive effects on soil organic carbon (SOC), soil total nitrogen (STN), and soil carbon:nitrogen ratios (C:N).

The central US is bisected by an ecotone that separates the grasslands of the Great Plains and the deciduous forest of the eastern US. In the south, this transition zone is comprised of the post oak (*Quercus stellata* Wangenh.) savanna region located in south central Texas and eastern Oklahoma (Hatch *et al.,* 1990). Post oak is the major canopy tree dominant and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the major perennial grass dominant (Hatch *et al.* 1990). Covering about 3 million hectares, this savanna has undergone a transition from a *Q. stellata – S. scoparium* dominated savanna toward a *Q. stellata* - juniper (*Juniperus virginiana* L.) woodland over the last century (Breshears 2006; Briggs *et al.* 2005; Reich *et al.* 2001), particularly in the absence of fire (Norris *et al.* 2001b; Eggemeyer *et al.* 2006). Of particular significance to this research, succession from grassland to *Juniperus* woodland in this region results in increased soil C and N storage (McKinley & Blair 2008) and altered N-cycling processes (McKinley *et al.* 2008; Norris *et al*. 2007). Widespread encroachment by *Juniperus* throughout this oak savanna region suggests an important role for this vegetation change in the N cycle of the central US (McKinley & Blair 2008; McKinley *et al.* 2008; Norris *et al*. 2007).

In addition, there is a critical gap in our knowledge of the plant functional trait responses (Cornwell *et al.* 2008; DeDeyn *et al.* 2008) and species interactions (Suttle *et al.* 2007; Grime *et al.* 2008) that may underpin ecosystem responses to various global climate change scenarios. Warmer temperatures, in combination with increased cool season precipitation, could potentially lead to shifts in plant communities (species composition, relative dominance, and/or growth forms) that could alter the quantity and quality of organic matter inputs to the soil.

Native grasslands being invaded by woody species such as *J. virginiana* and honey mesquite (*Prosopis glandulosa* Torr.) have been shown to shift the C:N ratio and biochemical composition of above- and belowground litter inputs, and alter rates of decomposition and mineralization processes (Hibbard *et al*. 2001; Jackson *et al.* 2002; McCulley *et al*. 2004; Norris *et al.* 2007; Filley *et al*. 2008; Knapp *et al*. 2008; Liao *et al*. 2008). Vegetation shifts from grassland to shrubland or woodland can dramatically increase total root biomass (McKinley 2006; Boutton et al. 2009) and soil carbon (C) inputs from increased root exudation. However, in some cases, shrub encroachment into grasslands could lead to lower fine root production and turnover (Zhou et al. 2012) and an increase in antimicrobial allelochemicals (Borchardt et al. 2008; Eller et al. 2010; Post & Urban 1995). These changes in the quantity and quality of soil organic matter inputs have the potential to alter the size of the soil microbial biomass pool (Liao & Boutton 2008), as well as microbial community structure (Hollister et al. 2010; Yannarell et al. 2014), with potential implications for soil C and N storage.

Temperature and precipitation are known factors that control the size and turnover rates of SOC and STN pools. In fact, an increase in average temperatures by just 1ºC could lead to a 3% loss of SOC for soils at 30ºC and a 10% loss in soils at 5 ºC (Kirschbaum 1995). Because significantly more C is stored in the world's soils than in the atmosphere (Batjes 1996; Kirschbaum 2000; Post *et al.* 1982), the loss of SOC and acceleration of decomposition processes could lead to a positive feedback to climate change (Davidson *et al.* 2000). However, increases in plant C storage in response to warming have been attributed to warming induced increases in nitrogen (N) availability from accelerated decomposition (Melillo *et al.* 2011). If primary production increases with higher temperatures and exceeds the rate of decomposition, then a negative feedback to climate change would occur (Davidson & Janssens 2006).

An analysis of pedon and climate data from 500 rangelands and 300 cultivated lands in the U.S. Central Plains Grasslands found that SOC decreased with temperature and increased with precipitation and clay content (Burke *et al.* 1989). In later studies, Century model results (Schimel *et al.* 1994) and a global analysis on >2700 soil profiles also came to the same conclusions (Jobbágy & Jackson 2000). Interestingly, Jackson *et al.* (2002) found that SOC and soil N concentrations decreased with increased precipitation and woody encroachment of grasslands in drier sites and increased in wetter sites.

As mentioned above, woody encroachment into grasslands generally results in increased SOC. Woody species commonly contain significantly more syringyl (S)- and vanillyl (V)-based lignin phenols than grasses, which are harder to decompose (Boutton *et al.* 2009). Therefore, as woody encroachment occurs the quality of the new plant material may decrease in degradability and lead to an accumulation of soil C and N. For example, McKinley and Blair (2008) reported a rapid accrual of C and N in biomass and soil as *J. virginiana* encroached into grasslands of the central Great Plains region.

Soil C and N accumulation rates under different plant community types can result in changes in soil C:N ratios (Charley & West 1975). Knops and Tilman (2000) demonstrated that C:N ratios of soil organic matter (SOM) increased with field age and the abundance of C_4 grasses, and decreased with C_3 grasses and forb abundance. The soils under the C_4 grasses in this study were accumulating C while there was no difference in soil N. This can be explained by the higher nitrogen use efficiency of C_4 plants, which allows them to produce more biomass under nitrogen limitation. Therefore, C_4 plant material can be slower to degrade and lead to higher soil C accumulation rates with no effect on soil N accumulation rates. In comparing C_4 grasses and C_3 leguminous woody species, Archer et al. (2001) found very little difference in C:N ratios. Even though C_4 grasses use N more efficiently, C_3 legumes can fix atmospheric N (N_2) and accumulate more soil N. In addition, the higher S- and V- based lignin phenol content in the woody litter inputs would accumulate more C compared to that of grass roots and shoots, thus not demonstrating a difference in soil C:N ratios between species.

It is clear that there are complex interactions of temperature, rainfall, and species composition with SOC (Davidson *et al*. 2000), STN and soil C:N ratios, and investigating these interactions is essential for understanding how climate change will affect the post oak savanna ecosystem. Therefore, the purpose of this study was to quantify the effects of experimental warming and rainfall manipulation on seasonal patterns of SOC, STN, and soil C:N ratios associated with two dominant species of the post oak savanna (*J. virginiana* and *S. scoparium*) growing alone and in combination in an established global change experiment. We predicted that (1) elevated temperatures will decrease SOC and STN pool sizes due to acceleration of organic matter decay by warming; (2) plots receiving redistributed rainfall of 40% less rainfall during the summer months and 52% more rainfall during the fall and spring periods will have smaller SOC and STN pools due to soil moisture limitations on primary production during the summer growing season; (3) plots including *J. virginiana* will have larger SOC and STN pool sizes than plots with *S. scoparium* due to more recalcitrant litter; and (4) soil C:N ratios will not demonstrate any treatment difference between soils under *J. virginiana* and *S. scoparium* monocultures and *J. virginiana - S. scoparium* within the short two year experiment.

3. Methods and Materials

Study Area and Experimental Design

Soils were collected for C and N analyses at the Texas Warming and Rainfall Manipulation (WaRM) Experimental Site between August 2008 and April 2010. The facility was located in a remnant post oak savanna in College Station, Texas (N 30°34" W 96°21"), and was constructed in March 2004 to investigate the combined effects of altered precipitation distribution and warming on tree and grass dominants of the southern oak savanna (Volder *et al.* 2010). Eight 18 x 9 x 4.5 m (L x W x H) rainout shelters were covered with clear polypropylene film while ends and sidewalls below 1.5 m were not covered to maintain ambient microclimatic conditions (Fay *et al.* 2000; Weltzin & McPherson 2003; Volder *et al.* 2010; see Figure A.6). Shade cloth was used to exclude precipitation. Sheet metal 40 cm in width was installed to 30 cm depth into the soil to penetrate the clay pan and separate each shelter from water flow above and belowground.

The shelter design preserved natural variation in a microenvironment that was similar to ambient conditions. An on-site weather station (EZ Mount Groweather, Davis Instruments, Hayward, CA) recorded precipitation, air temperature and humidity, while solar radiation (total and PAR), air temperature, and relative humidity were monitored continuously and recorded by Hobo data loggers in each shelter (Hobo U12; Onset Computer Corp., Bourne, MA, USA). Mean daily temperatures in the shelters were on average 1.5 °C higher, RH values 2% lower and PAR levels 30% lower than the unsheltered controls. The NOAA weather station at Easterwood Airport (<0.5 km from the site) reported a mean annual temperature of 20.56°C and mean annual precipitation of 1,017.5 mm based on a 50-year period (NOAA 2010; Volder *et al.* 2013).

Ten 2 x 2 m plots were established within each shelter in native soil, a fine sandy loam Alfisol of the Booneville series (Fine, smectitic, thermic Chromic Vertic Albaqualf) in 2003, one year before the experiment began. Each shelter included five plant species combinations with monocultures of *S. scoparium*, *Q. stellata*, and *J. virginiana*, and each of the tree species were grown with the *S. scoparium* in separate mixed species plots. An overhead irrigation system supplied reverse osmosis water from onsite water holding tanks to simulate rainfall to each shelter. Infrared lamps (Kalglo Electronics, Bethlehem, PA; model MRM-1208L) were installed to hang 1.5 m above half of the plots within each shelter and were warmed continuously with 400 W m^{-2} of radiant energy (Harte & Shaw 1995; Wan *et al.* 2002). To accommodate increased height growth of trees, lamps were raised by 0.5 m and the total wattage output to the plots was increased by an additional 400 W m^{-2} of radiant energy to maintain comparable levels of warming in February 2008. This additional warming from the heat lamps increased soil temperatures (averaged over all canopy types) by approximately 0.6 °C at 3 cm depth, and canopy leaf temperatures by about 1-1.5 °C. However, it is important to note that actual soil and canopy warming can vary with wind speed (Kimball 2005). Metal sheeting in the shape of the infrared lamps was placed above the second half of the plots to mimic cover and shading without the heat source.

Rainfall was manipulated in 8 of the 10 shelters to simulated precipitation regimes categorized as control and redistributed, although each regime received the same total amount of annual rainfall (1018 mm) and the same number of rainfall events (Figure 3.1). Four of the 8 shelters received control rain events based on the long-term (50 year) mean reported by a local NOAA weather station in College Station, TX (NOAA 2010). The frequency and intensity of rainfall events were also simulated from the long-term precipitation record of the region. In the other 4 shelters, 40% of the precipitation during 5 summer months (May – September) was withheld from each event and evenly redistributed in the previous 2 month spring (March and April) and subsequent 2 month fall (October and November), increasing the rainfall to 52% of the normal spring and fall patterns (see Figure A.7). This redistribution pattern effectively increased the intensity of the summer drought and the amount of precipitation that occurs during the cooler spring and fall seasons. Precipitation redistribution during

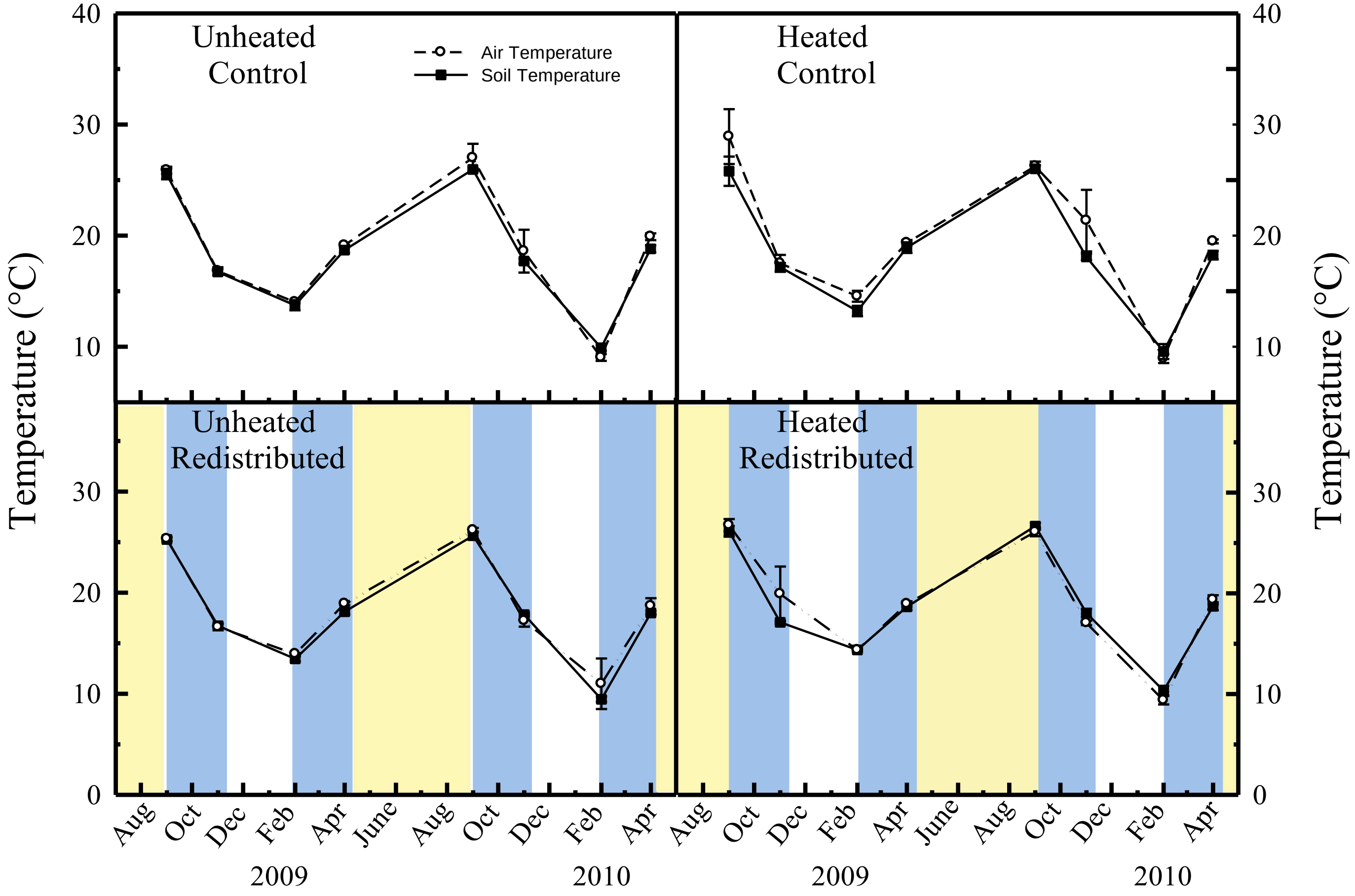

Fig. 3.1 Mean air and soil temperatures were averaged over each 30-day soil incubation period across all plots from August 2008 to April 2010. For each point, n = 4 and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

spring and fall increased volumetric soil water content from 21.0 to 23.1% in the top 20 cm of soil; in contrast, during the summer drought, soil water content decreased from 15.5% in the ambient to 10.7% in the redistributed treatment. Although soil warming differed between canopy types, volumetric water content was generally 1% lower in warmed plots.

Field Sampling and Soil Analysis

Due to time and logistical constraints a subset of vegetation treatments containing monocultures of *J. virginiana* and *S. scoparium*, and the *J. virginiana* + *S. scoparium* combination were sampled from the factorial design during years six and seven of the WaRM experiment. These three vegetation treatments provide an analog for the encroachment of *J. virginiana* into post oak savanna ecosystems where *S. scoparium* is usually the dominant grass species.

Surface litter in each sampling area was moved aside prior to collecting. Two soil cores with 5.5 cm diameter and 10 cm depth were collected 3 times (August 2008, August 2009, May 2010) in control and treated plots under each vegetation cover type (*J. virginiana, S. scoparium*, and the *J. virginiana* + *S. scoparium* combination). Samples were placed in a labeled Ziploc freezer bag and stored in a cooler until lab analysis later each day of collection.

Laboratory Analyses

Each soil sample was weighed for whole-soil wet weight then mixed thoroughly and passed through a 2 mm sieve to remove large roots. A 30 g subsample was weighed and dried at 105 °C for 24 hours to determine gravimetric water content for use in calculating soil bulk density and soil volumetric water content. Two additional aliquots of each soil sample were weighed for soil pH and texture analysis. pH was quantified by using the $CaCl_2$ method and glass electrode (McLean 1982), and texture was determined by the hydrometer method (Bouyoucos 1926). An additional 10g subsample was dried at 60 °C for 48 hours, then pulverized and homogenized using a ring pulverizer (Angstrom TE250; Angstrom, Inc., Belleville, MI). Samples were weighed into tin capsules using a microbalance, and analyzed for C and N concentrations using a Carlo-Erba EA 1108 elemental analyzer (CE Elantech, Lakewood, NJ). These values were then used to calculate C:N ratios. Soil C and N concentrations and bulk densities were used to compute g C m^{-2} and g N m^{-2} stored in the upper 10cm of the soil profile.

Statistical Analyses

The effects of rainfall, temperature, and species combination on all response variables were analyzed by repeated measures ANOVA in JMP Pro 10.0.0 (SAS Institute, Cary NC). The experimental design is a completely randomized split-plot with four replicates per treatment combination. Whole plots consisted of the two precipitation treatments (control vs. redistributed) in factorial combination with two temperature treatments (unheated vs. heated); the subplots were the three vegetation types [two monocultures (*J. virginiana*, *S. scoparium*) and one tree-grass combination (*J. virginiana* + *S. scoparium*)]. Each sampling period (time) was treated as an individual repeated measure using the D-optimal split-plot design as a repeated measures approximation procedure, which allows for a robust mixed model analysis (Goos and Vandebroek 2001; Jones and Nachtsheim 2009; SAS 2005; Smucker 2011). Variances were estimated with Restricted Maximum Likelihood (REML) and the Tukey's HSD post hoc test was used to distinguish differences between the treatment combinations. The Student's t-test was used when a variable had only two treatment levels and a normal distribution (e.g. warming = heated vs. unheated; rainfall = control vs. redistributed) (Ott & Longnecker 2010).

4. Results

Microclimate

Mean air temperatures ranged from 9.2 to 27.8 °C across all plots and sampling periods. Air temperatures in the heated plots were 1 °C higher than unheated plots, but soil temperatures were not affected by the 6th year of the WaRM experiment (Volder *et al.* 2013; Figure 3.1). Heaters were raised to accommodate the growing vegetation, which shaded the soil and minimized the potential for infrared radiation to reach the soil surface. Rainfall events were manipulated to simulate summer drought by reducing precipitation by 40% and redistributing the rainfall to the spring and fall seasons (Figure 3.2). Soil volumetric water content decreased in the summer and increased in the spring and fall months within the redistributed rainfall plots (Figure 3.2). Soil bulk density

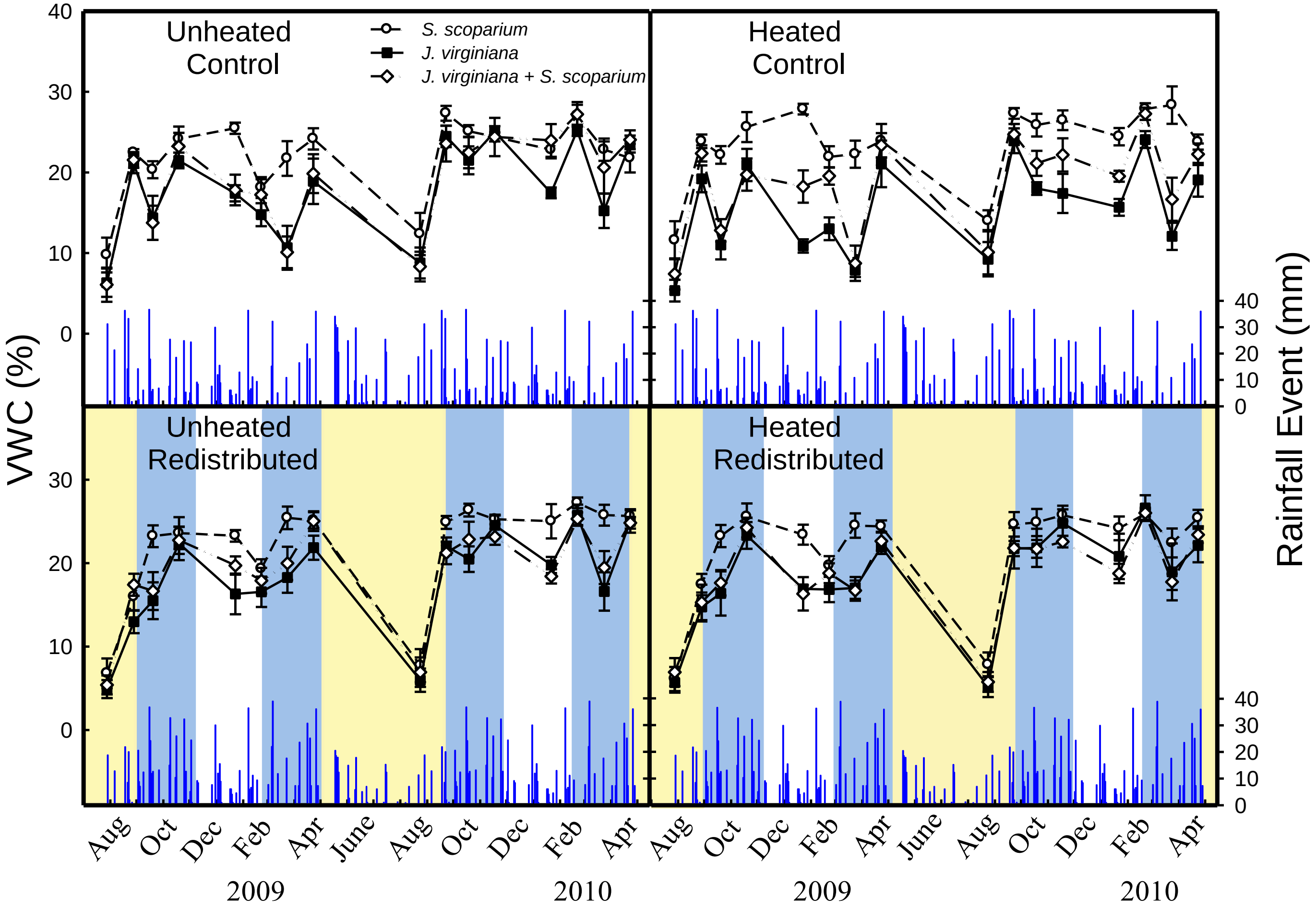

Fig. 3.2 Soil volumetric water content (VWC; 0-10cm; shown as symbols) and rainfall event size (mm; vertical bars) for the duration of the study, August 2008 to April 2010. For VWC, n = 4 for each point, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

ranged from 1.30 to 1.33 gm^{-2} with no significant difference between treatments and pH ranged from 4.9 to 5.3 (see Table A.1). Juniper monocultures were significantly ($p<.0001$) more acidic than the grass plots (Table 3.1; see Table A.1). All treatments fell within the sandy loam soil texture class with no significant textural difference between shelters (Table 3.1; see Table A.1).

Soil C and N pools, and C:N ratios

SOC in the upper 10 cm of the soil profile ranged from 419.4 g C m^{-2} to 1,768.9 g C m^{-2} with a mean of 959.4 g C m^{-2} ($n = 287$) and standard error of 13.5 across all samples (Table 3.2). Plots containing the *J. virginiana* + *S. scoparium* species composition generally had higher SOC values compared to the *J. virginiana* and *S. scoparium* monocultures and the difference of this main effect was significant ($p < 0.001$) (Table 3.3; Figure 3.3; Figure 3.4). Time was also a significant main effect ($p = 0.012$) where SOC was significantly higher in May 2010 compared to August 2008 and August 2009 (Table 3.3; Figure 3.4).There were 2 significant 2-way interactions with warming x species composition ($p = 0.007$) and rainfall x species composition ($p = 0.014$) (Table 3.3; Figure 3.3; Figure 3.4). The repeated measures ANOVA analysis and Tukey's HSD post hoc test indicated that SOC was significantly higher in the *J. virginiana* + *S. scoparium* plots compared to the *J. virginiana* and *S. scoparium* monocultures under control rainfall (Figure 3.3). This difference between species composition type was not significant under the redistributed rainfall regime. Likewise, SOC was significantly higher in the heated *J. virginiana* + *S. scoparium* plots compared

Table 3.1 Soil properties (0-10cm) by species (mean ± SE). Bulk density was measured during each of the 16 sample collection periods. pH was measured four times each year for two years. Soil texture analysis was measured once in August 2008.

	S. scoparium		***J. virginiana***		***J. virginiana + S. scoparium***	
	Mean	***SE***	***Mean***	***SE***	***Mean***	***SE***
Bulk Density (n = 512) g/cm^3	1.33	0.00	1.30	0.00	1.30	0.00
pH (n = 256)	5.31	0.03	4.95	0.04	5.02	0.03
Soil Texture (n = 18)						
% Sand	59.3	0.7	59.1	0.9	59.0	0.8
% Silt	30.1	0.5	30.5	0.5	30.4	0.5
% Clay	10.6	0.6	10.5	0.6	10.7	0.7

Table 3.2 Range, mean, and standard error of soil organic C (g C m^{-2}), soil total N (g N m^{-2}), and soil C:N ratio (n=287) from August 2008 to April 2010.

	Min	**Max**	**Mean**	**S.E.**
Soil Organic Carbon	*419.3*	*1,768.6*	*959.4*	*13.5*
Soil Total Nitrogen	*23.7*	*125.9*	*64.9*	*1.0*
C:N Ratio	*9.4*	*32.5*	*15.1*	*0.2*

Table 3.3 P values (* p<.05, **p<.01, ***p<.001) using repeated measures ANOVA for changes in soil C, soil N, and soil C:N ratio (n=4) from August 2008 to April 2010.

Treatment	**SOC** p value	**STN** p value	**Soil C:N Ratio** p value
Rainfall (R)	*ns*	*ns*	*ns*
Warming (W)	*ns*	*ns*	*ns*
R x W	*ns*	*ns*	*ns*
Species Composition (SC)	***	***	*ns*
R x SC	**	**	**
W x SC	**	*ns*	*ns*
R x W x SC	*ns*	*ns*	*
Time (T)	*	*	*
R x T	*ns*	*ns*	*ns*
W x T	*ns*	*ns*	*ns*
R x W x T	*ns*	*ns*	*ns*
SC x T	*ns*	*ns*	*ns*
R x SC x T	*ns*	*ns*	*ns*
W x SC x T	*ns*	*ns*	*ns*
R x W x SC x T	*ns*	*ns*	*ns*

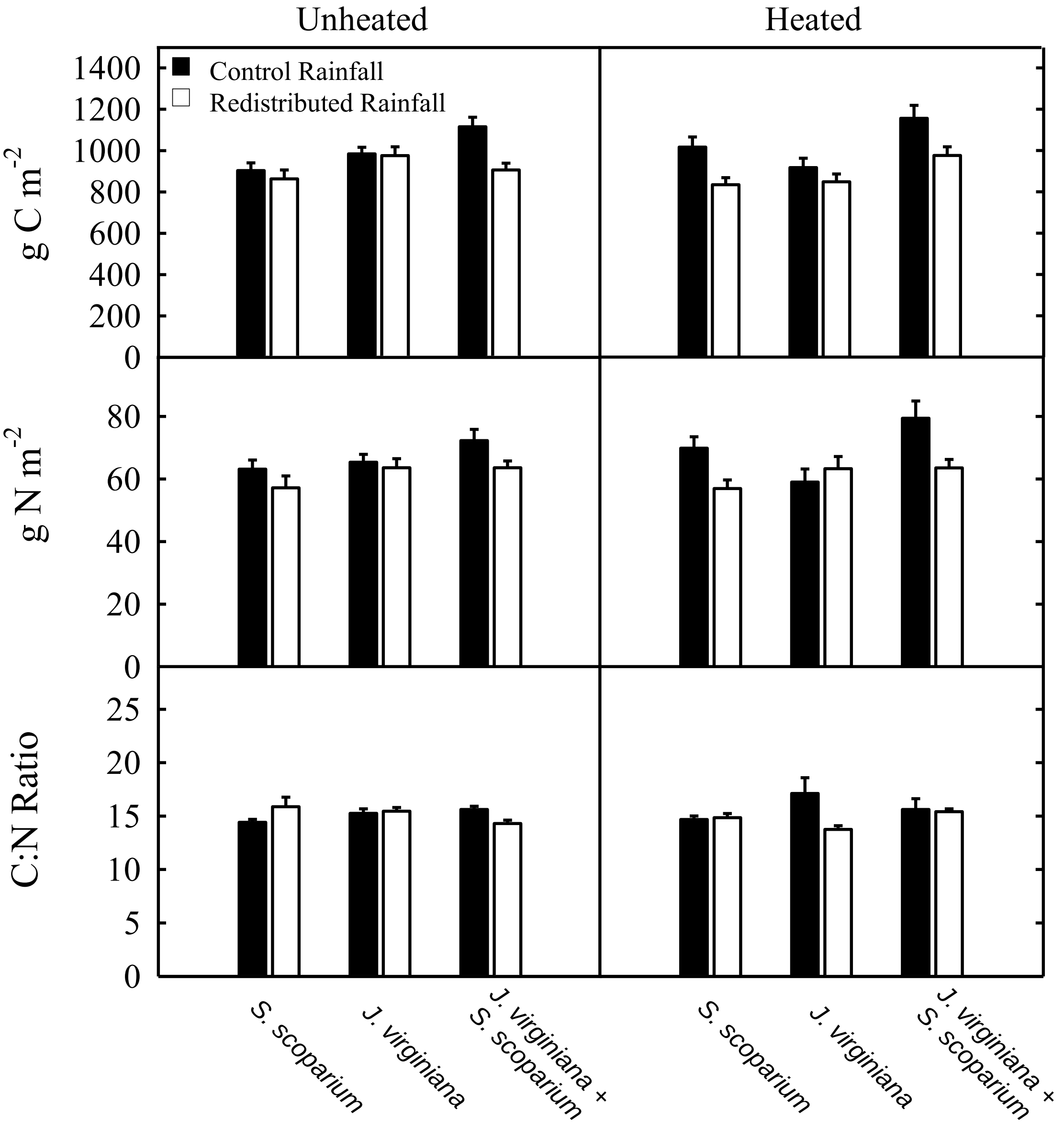

Fig. 3.3 Mean soil organic C (g C m^{-2}), soil total N (g N m^{-2}), and C:N ratio by species (n = 8) within experimental treatments from August 2008 to April 2010.

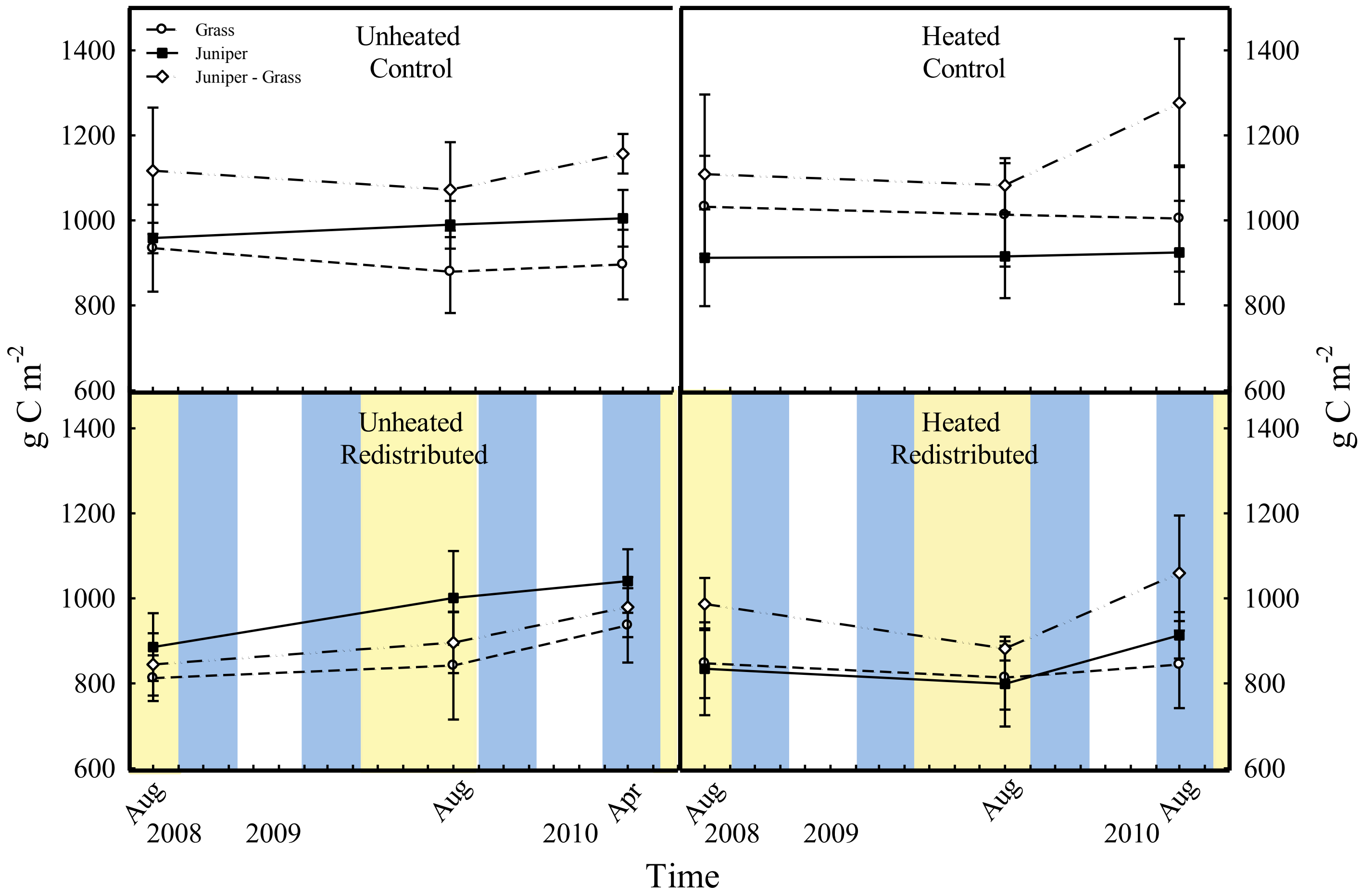

Fig. 3.4 Soil organic carbon (g C m^{-2}) from August 2008 to April 2010 (n = 4). Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

to the heated *J. virginiana* and *S. scoparium* monoculture plots. There were no species differences under unheated conditions (Figure 3.3).

Soil total nitrogen (STN) ranged from 23.7 g N m^{-2} and 126.0 g N m^{-2} with a mean of 65.0 g N m^{-2} (n = 287) and standard error of 1.0 across all samples (Table 3.2). The main effects of species composition ($p = 0.001$) and time ($p = 0.044$) were significant main effects (Figure 3.3; Figure 3.5). *J. virginiana* + *S. scoparium* plots generally had higher STN compared to soils under the *J. virginiana* and *S. scoparium* monocultures. Although the Tukey's post hoc test did not show a significant difference in STN between time periods, there was a trend towards higher values in May 2010 compared to August 2008 and August 2009 (Figure 3.5). Similar to the SOC results, the interaction of rainfall x species composition was significant ($p = 0.007$) (Table 3.3). The post hoc test indicated that STN was significantly higher in the *J. virginiana* + *S. scoparium* plots compared to the *J. virginiana* and *S. scoparium* monocultures under control rainfall, but the difference was not significant under redistributed rainfall (Figure 3.3; Figure 3.5).

Carbon to nitrogen ratios (C:N) were calculated using values generated from SOC and STN and analyzed using the same repeated measure ANOVA analysis. These values ranged from 9.4 to 32.5 with a mean of 15.1 (n = 287) and standard error of 0.2 (Table 3.2; Figure 3.6). There was a significant main effect of C:N ratios through time ($p = 0.049$), but with no differences in the post hoc test (Table 3.3). In addition, there was a significant 3-way interaction of rainfall x species composition x warming ($p = 0.039$) (Table 3.3). The Tukey's post hoc test showed no difference between treatments, though

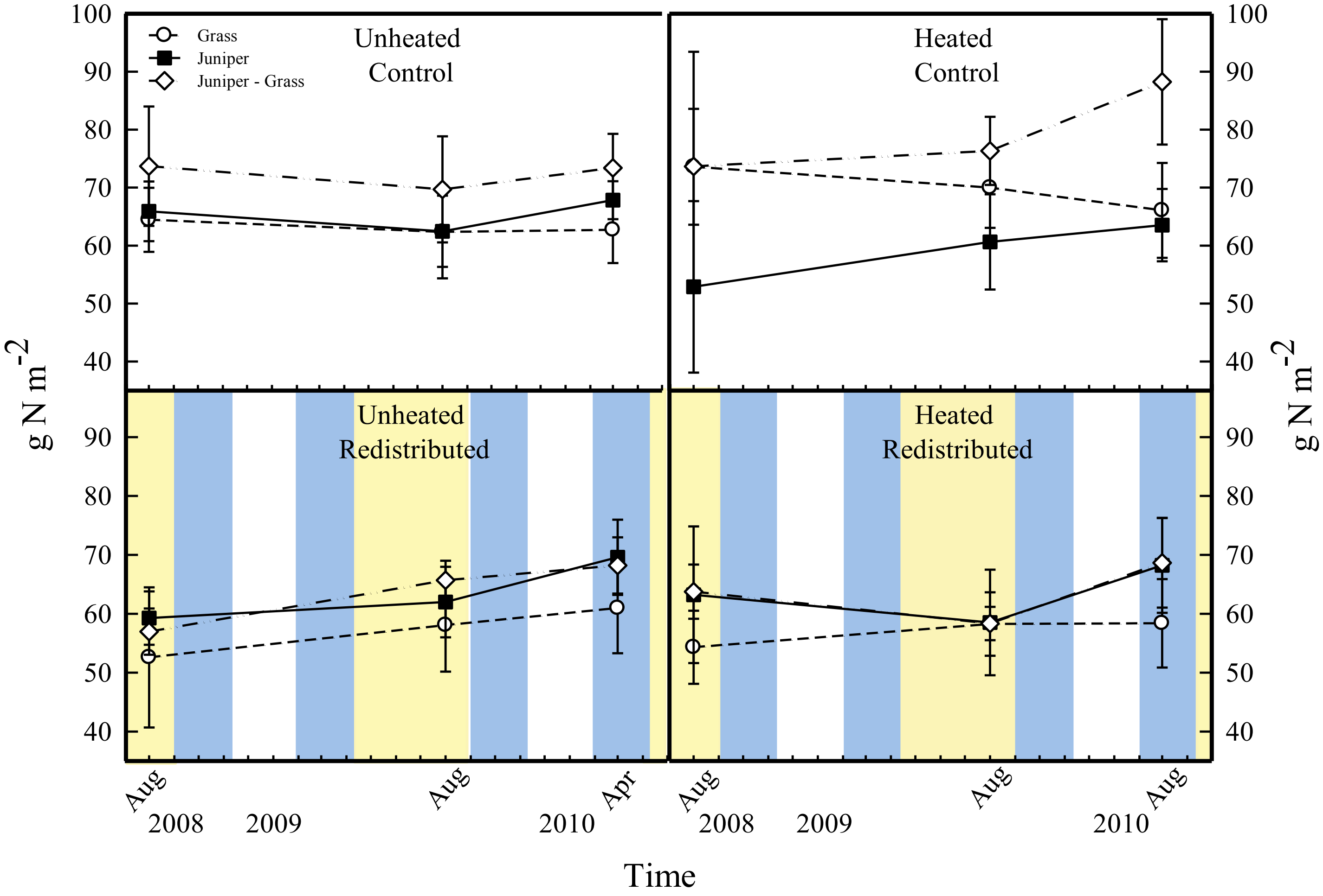

Fig. 3.5 Soil total nitrogen (g N m^{-2}) from August 2008 to April 2010 (n = 4).Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

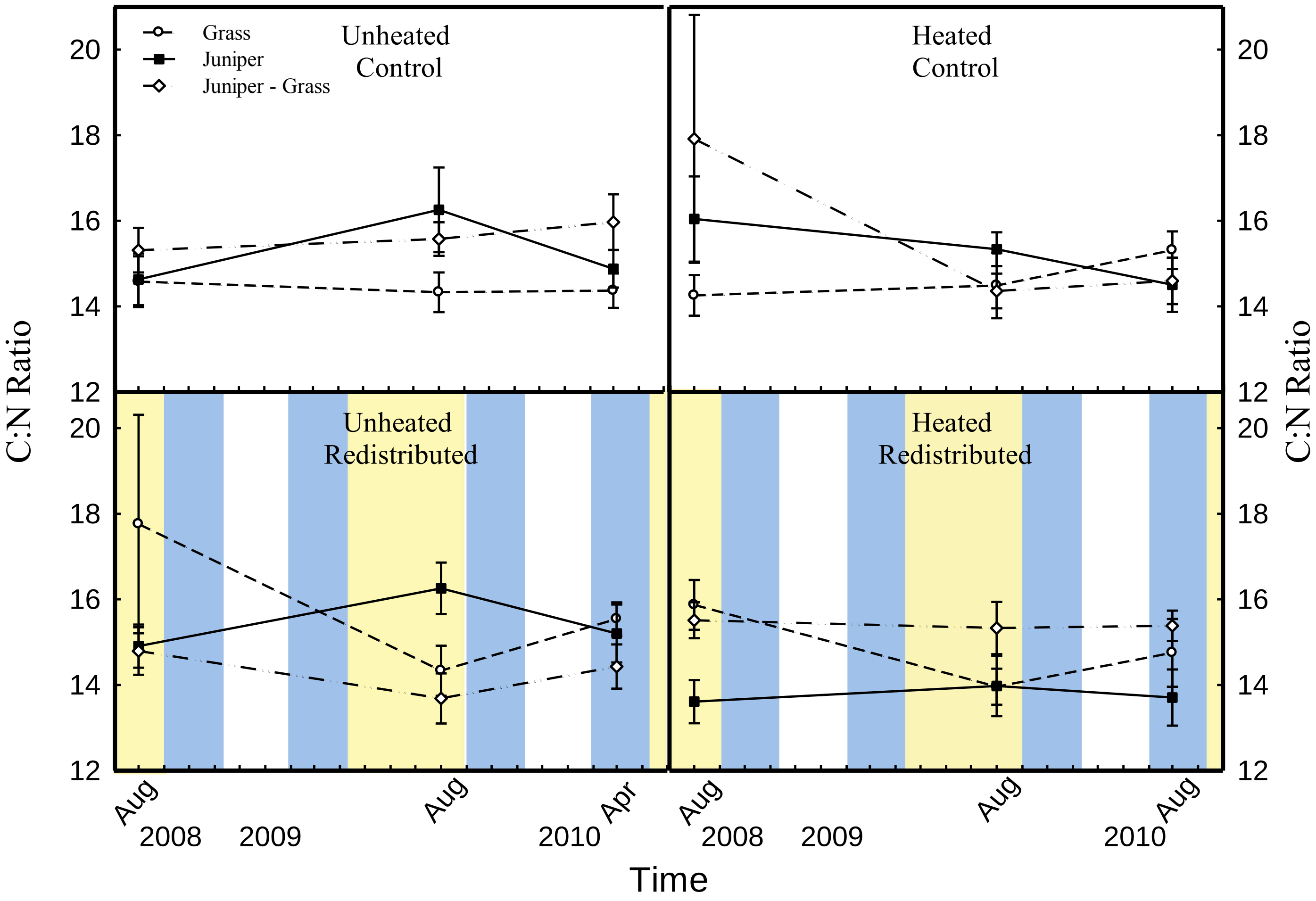

Fig. 3.6 Soil C:N ratio from August 2008 to April 2010 (n = 4 for each data point). Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

J. virginiana monocultures under control rainfall and warming (Least Square Mean (LSM) = 16.0) had higher C:N ratios compared to *J. virginiana* monocultures under redistributed rainfall and warming (LSM = 13.8) (Figure 3.3). Similarly, the 2-way interaction of rainfall x species composition (part of the significant 3-way interaction) was significant (p = 0.019) with no differences in the post hoc test (Table 3.3).

5. Discussion

In this multifactor global change experiment, species composition and time appeared to be the most consistently important main effects that determined SOC and STN. Neither warming alone nor rainfall redistribution alone affected SOC or STN in this experiment; however, warming and rainfall redistribution interacted with other experimental variables to influence SOC and STN. In particular, the interaction between species x rainfall was consistently significant (Table 3.3). The results of this study support the emerging consensus that global change drivers are likely to have their greatest impact by mediating complex interactions with plant species composition (Brooker 2006; Gilman *et al.* 2010).

The redistribution of rainfall resulting in drier summers and wetter spring and fall seasons had the most effect on the *J. virginiana* + *S. scoparium* treatment (rainfall x species composition). SOC and STN pools were significantly smaller in plots where the C_3 woody species, *J. virginiana,* competed with the C_4 grass, *S. scoparium*, under rainfall manipulation, and post hoc tests indicated that C:N ratios were not affected. In contrast, most studies have found that soil C and N concentrations increase with precipitation (Burke *et al.* 1989; Jobbágy & Jackson 2000; Schimel *et al.* 1994; Zhou *et al.* 2002).

Previous results from the WaRM site indicated that the relative growth rate (RGR) of *J. virginiana* was reduced by redistributed rainfall over a six year period, though RGR of *J. virginiana* was significantly higher when grown with *S. scoparium* compared to *J. virginiana* monocultures (Volder *et al.* 2013). Root analyses on the same soils in the present study suggests that rainfall redistribution and species composition also interacted to decrease coarse and total root C pools and coarse, fine, and total C:N ratios in the *J. virginiana* + *S. scoparium* plots (Wellman *et al.* unpublished). In addition, high N-losses via leaching of NO_2^- and NO_3^- were also observed in these soils under all treatment combinations (Wellman *et al.* unpublished). Annual increases in precipitation events during the spring and fall seasons in between a summer drought in the WaRM experiment might have induced a "Birch Effect" (Birch 1958; Jarvis *et al.* 2007; Unger *et al*. 2010; Wellman *et al.* unpublished) and influenced the vertical distribution of SOC (Jobbágy & Jackson 2000) and STN. However, this difference would likely not be observable in a short two year study.

The 2-way interaction of warming x species composition was significant where SOC pools were larger in heated than unheated *J. virginiana* + *S. scoparium* plots. No difference was found in STN pools, C:N ratios, or in other species compositions. This result is opposite to the response we predicted in our first hypothesis that elevated temperatures will decrease SOC and STN pool sizes due to acceleration of organic matter decay by warming. Fine root biomass was significantly lower in heated plots and

N mineralization rates were increased with warming during this study (Wellman *et al.* unpublished). However, *J. virginiana* significantly increased aboveground growth with warming during the first 6 years of the WaRM experiment (Volder *et al.* 2013). As mentioned earlier, increases in plant C storage in response to warming have been attributed to warming induced increases in N availability from accelerated decomposition (Melillo *et al.* 2011). If primary production increases with higher temperatures and exceeds the rate of decomposition, then a negative feedback to climate change would occur (Davidson & Janssens 2006). Warming induced accelerated metabolic processes in the form of aboveground growth and higher N mineralization rates could have reduced fine root biomass and reallocated at least some of the released C as SOC, thus increasing the SOC pool.

The largest SOC and STN pools were found in soils under *J. virginiana* + *S. scoparium* species composition in the control rainfall plots, which partially supports our third hypothesis that soils under the woody plant *J. virginiana* will have larger SOC and STN pool sizes compared to soils under the grass *S. scoparium*. Paired with this study (Chapter II), all plots containing *J. virginiana* had significantly more root biomass than monocultures of *S. scoparium* (Wellman *et al.* unpublished). Active root growth in the rhizosphere contributes a significant amount of C for microbial biomass (Grayston *et al.* 1997) and the SOC pool. In addition, root exudates play a major role in mediating plant-soil interactions and processes (Bais *et al.* 2006). Rhizodeposits from *J. virginiana* likely stimulated microbial activity and increased SOC pools under the control rainfall regime where precipitation patterns reflected a 50 year mean and a lack of a summer drought period in this study.

Relationships between temperature, rainfall, species composition, and soil C:N ratios were not clear. Post hoc tests of the significant 3-way interaction of rainfall x species composition x warming and the 2-way interaction of rainfall x species composition did not indicate specific differences. However, there was a noticeable trend in the heated *J. virginiana* monocultures. Heated *J. virginiana* monocultures under control rainfall had higher C:N ratios (Least Square Mean (LSM) = 15.96) compared to heated *J. virginiana* monocultures under redistributed rainfall (LSM = 13.76) (Figure 3.3). This implies that *J. virginiana* subjected to altered rainfall patterns and warming could reduce soil C:N ratios, which could lead to increases in SOM degradability, decomposition rates, and SOC turnover.

SOC and STN pools increased over time as a significant main effect with the highest values in May 2010 in this experiment (Figure 3.4; Figure 3.5). The main effect of time was also significant in soil C:N ratios, but there were no specific differences or noticeable temporal trends in the post hoc test. Although we did not predict how SOC, STN, or soil C:N ratios would interact with warming, rainfall, and species composition through time or seasonal variation, we tested for interactions and none were significant. These results suggest that soils beneath *J. virginiana* and *S. scoparium* growing alone or together behaved as carbon sinks during years 6 and 7 of this climate change experiment. If this pattern accurately reflects C storage patterns in the post oak savanna, this ecosystem type may potentially function as a C sink and provide a negative feedback to climate change.

6. Conclusions

Results of this multifactor study highlights the complexity of global change impacts on SOC, STN, and soil C:N ratios in the semi-arid post-oak savanna ecosystem in central Texas. Increases in temperature, alterations in precipitation patterns, and shifts in plant community structure produced outcomes that were not as straightforward as we originally expected. Interactions between these variables produced results that indicate a complex multilayered response to these global change drivers. Additional investigations into microbial community structure, lignin content, phosphorus concentrations, isotopic analysis, effects of CO_2 enrichment, and C and N concentrations in deeper soils may provide further insight as to what may be driving these interactions. It is clear that *J. virginiana* is an opportunistic species that will increase in abundance with warmer temperatures and the absence of a natural disturbance regime (i.e., frequent fire) in the post oak savanna. The species competition between *J. virginiana* and *S. scoparium* will likely result in a C sink (above- and belowground) as temperatures rise to IPCC (2013) projections. However, altered precipitation patterns may reduce this effect. These changes in SOC, STN, and soil C:N ratios in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

CHAPTER IV

SOIL NITROGEN TRANSFORMATIONS IN RESPONSE TO ELEVATED TEMPERATURES AND ALTERED RAINFALL REGIMES IN POST OAK SAVANNA: A GLOBAL CHANGE EXPERIMENT

1. Synopsis

Increasing concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.1 to 4.5°C by the end of the century, and potentially magnify the intensity and variability of seasonal precipitation distribution. The mid-latitude grasslands of North America are predicted to experience substantial modification in precipitation regimes, with a shift towards drier summers and wetter spring and fall seasons. Despite these predictions, little is known concerning the effects of these global climate change drivers or their potential interactive effects on nitrogen (N) cycling processes. Therefore, the purpose of this study was to quantify seasonal variation in rates of N-mineralization, nitrification, and N-losses via leaching in soil subjected to experimental warming and rainfall manipulation. Research was conducted at the Texas A&M Warming and Rainfall Manipulation (WaRM) Site in east-central Texas. Soil N-mineralization, nitrification, and N-losses via leaching were assessed quarterly over a period of two years using the resin core incubation method. Results were dominated by a 3-way interaction of species x rainfall x time, highlighting the complexity of global change impacts. Highest rates of net N mineralization (4.56 mg NH_4^+ /NO_2^- /NO_3^- -N/ kg soil/day) and nitrification (4.53 mg NO_2^-/NO_3^- -N/ kg soil/day)

occurred in *Schizachyrium scoparium* monocultures during summer in the redistributed rainfall plots. Lowest rates of N mineralization (-0.241 mg NH_4^+ /NO_2^- /NO_3^- -N/ kg soil/day) and nitrification (-0.225 mg NO_2^-/NO_3^- -N/ kg soil/day) occurred under *Juniperus virginiana* during fall and winter in redistributed rainfall plots. Losses of N through leaching were highest in the same treatment combinations that had high rates of nitrification. In a significant warming x species composition interaction, warmed plots containing the *J. virginiana* + *S. scoparium* species composition treatment had higher N mineralization rates than the unheated plots. Results indicate that warming and rainfall redistribution interacted strongly with other experimental treatments to influence rates of N-transformations. These changes in rates of N-transformations and leaching losses in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

2. Introduction

By the end of the 21st century concentrations of greenhouse gases are projected to elevate global surface air temperatures by 1.5 to 4.5 °C and potentially magnify the intensity and variability of seasonal precipitation distribution (IPCC 2013). Little is known of the effects of global change drivers such as elevated temperatures and shifts in rainfall patterns on nitrogen (N) cycle processes in the mid-latitude grasslands of North America (Norby & Luo 2004; Rustad *et al.* 2001; Weltzin *et al.* 2000). These grasslands are predicted to experience substantial modification in precipitation regimes (IPCC

2013; Weltzin *et al.* 2003; Melillo et al. 2014) and a shift from summer to spring precipitation is anticipated to be more probable than a substantial change in mean annual precipitation amount (Easterling *et al.* 2000; Groisman & Knight 2008; Manabe & Wetherald 1986). Higher temperatures and greater evaporation coupled with a decrease in summer precipitation will intensify summer drought events (MacCracken *et al.* 2003; Pope *et al.* 2000; Wetherald & Manabe 1995).

The central US is bisected by an ecotone that separates the grasslands of the Great Plains and the deciduous forest of the eastern US. In the south, this transition zone is comprised of the post oak (*Quercus stellata* Wangenh.) savanna region located in south central Texas and eastern Oklahoma (Hatch *et al.,* 1990). Post oak is the major canopy tree dominant and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the major perennial grass dominant (Hatch *et al.* 1990). Covering about 3 million hectares, this savanna has undergone a transition from a *Q. stellata – S. scoparium* dominated savanna toward a *Q. stellata* - juniper (*Juniperus virginiana* L.) woodland over the last century (Breshears 2006; Briggs *et al.* 2005; Reich *et al.* 2001), particularly in the absence of fire (Norris *et al.* 2001b; Eggemeyer *et al.* 2006). Of particular significance to this research, succession from grassland to *Juniperus* woodland in this region results in increased soil C and N storage (McKinley & Blair 2008) and altered N-cycling processes (McKinley *et al.* 2008; Norris *et al*. 2007). Widespread encroachment by *Juniperus* throughout this oak savanna region suggests an important role for this vegetation change in the N cycle of the central US (McKinley & Blair 2008; McKinley *et al.* 2008; Norris *et al*. 2007).

In the past decade, researchers have established links between temperature, precipitation, and soil N biogeochemistry. Elevated temperatures generally accelerate N-transformations and other key processes linked to N-cycling such as net primary production, soil respiration, and organic matter decay (Davidson & Janssens 2006; Rustad *et al.* 2001). The timing and magnitude of rainfall events may also affect a range of N-cycle processes (Yahdjian *et al.* 2006). But, much less is known concerning the combined, interactive effects of warming and altered precipitation patterns on N-cycle processes (Beier *et al.* 2004; Pendall *et al.* 2004; Rustad *et al.* 2001; Weltzin *et al.* 2000). In addition, there is a critical gap in our knowledge of the plant functional trait responses (Cornwell *et al.* 2008; DeDeyn *et al.* 2008) and species interactions (Grime *et al.* 2008; Suttle *et al.* 2007) that may underpin ecosystem responses to various global climate change scenarios. Warmer temperatures, in combination with increased cool season precipitation, could potentially lead to shifts in plant communities (species composition, relative dominance, and/or growth forms) that could alter the quantity and quality of organic matter inputs to the soil, thereby affecting rates of N-transformations and losses.

In general, controls on N-cycling processes in the soil are reasonably well understood (Booth *et al.* 2005; Galloway 2005; McNeill & Unkovich 2007; Nannipieri & Eldor 2009; Robertson & Groffman 2009). Important known drivers include temperature, soil water content, substrate availability, and microbial activity. In a meta-analysis of 32 experimental warming sites, Rustad *et al.* (2001) discovered significant increases in soil respiration, N-mineralization, and plant productivity rates with increasing temperature. In a later study, Agehara and Warncke (2005) observed a linear relationship with increasing N-mineralization rates and increasing temperature as well as with increasing soil moisture. However, no studies have examined the response of net N-mineralization to simultaneous modifications of both temperature and rainfall patterns in the North American Great Plains.

Substrate quality and quantity clearly influences N-transformation rates (Booth *et al.* 2005). Organic matter with C:N values >20 tend to encourage net N-immobilization while C:N values < 20 promote net N-mineralization (Cabrera *et al.* 2005). In an evaluation of 100 studies, Booth *et al.* (2005) concluded that soil organic matter from grasslands containing lower C:N values of ~ 5 to 25 produce more NH_4^+ compared to the soil organic matter from woodlands with higher C:N values of ~ 8 to 48. However, other studies have found that rates of N-mineralization in soils beneath woody species such as *Prosopis glandulosa* and *J. virginiana* were increased relative to adjacent grassland soils (Hibbard *et al.* 2001; McCulley *et al.* 2004; McKinley *et al.* 2008).

Seasonal drought coupled with rising temperatures may also alter rates of N losses via soil leaching and run-off as demonstrated by Joslin and Wolfe (1993) and Lukewille and Wright (1997). This is probably due to higher rates of N-mineralization and nitrification which produce larger pools of leachable N. Periods of drought intensified by experimental rainfall reduction and elevated temperatures could lead to higher concentrations of NO_3^- in the soil because N-mineralization and nitrification will continue even after plant N-uptake processes are inhibited by drought (Booth *et al.* 2005). Consequently, when these soils are rewetted at the onset of seasonal wet periods, these large pools of NO_3^- will be susceptible to increased leaching losses. Thus, changes in temperature and rainfall patterns predicted for Texas could have important implications for N-losses via leaching from ecosystems, with potential implications for water quality in surface waters and groundwater.

Native grasslands that have been invaded by woody species have been shown to shift the C:N ratio and biochemical composition of above- and belowground litter inputs, and alter rates of decomposition and mineralization processes (Filley *et al.* 2008; Hibbard *et al.* 2001; Knapp *et al.* 2008; Liao *et al.* 2008; McCulley *et al.* 2004; Norris *et al.* 2007). In fact, preliminary analyses of leaf tissue samples taken from the WaRM Site in 2007 reveal that C:N was 23-28 for *Q. stellata*, 33-44 for *J. virginiana*, and 44-59 for *S. scoparium* (Tjoelker & Boutton unpublished; see Table A.2). Therefore, we can predict a decrease in C:N values within leaf litter as woody species such as *Q. stellata* and *J. virginiana* encroach into native *S. scoparium* grass communities, which could affect the rate of N-mineralization. N-mineralization rates under isolated oak species within California and Minnesota oak savannas were higher than the rates under neighboring grassland species (Dijkstra *et al.* 2006; Herman *et al.* 2003; Jackson *et al.* 1990). In contrast, N-mineralization rates showed little to no initial change as a dogwood community encroached on native grasslands (McCarron *et al.* 2003), and juniper litter decayed more slowly than big bluestem litter in Kansas (Norris *et al.* 2001a). Therefore, investigating N-transformations within the post oak savanna ecosystem under different species composition scenarios will be beneficial to the understanding of how climate and vegetation shifts will alter rates of N-cycling processes and N availability.

The primary objective of this study was to quantify the effects of elevated temperature, modified rainfall distribution, and plant species composition on net N-mineralization, nitrification, and N-losses via leaching in an oak savanna ecosystem. The hypotheses were that (1) elevated temperatures will increase N-mineralization, nitrification, and N-losses via leaching due to the strong sensitivity of microbial activity to temperature; (2) plots receiving redistributed precipitation patterns with 40% less rainfall during summer months and 52% more rainfall during the fall period will have higher rates of N-mineralization, nitrification, and N losses via leaching compared to plots receiving normal rainfall due to microbial water stress resulting from reduction of soil moisture and subsequent mineralization of dead microbial biomass that accumulated during the prior summer drought and the rewetting of dry soil ("Birch effect"); (3) Lower C:N ratios in *J. virginiana* litter inputs will result in higher rates of net N-mineralization, nitrification, and N losses via leaching in *J. virginiana* monocultures and in *J. virginiana* + *S. scoparium* plots compared to *S. scoparium* monocultures.

3. Methods and Materials

Study Area and Experimental Design

Soils were analyzed for N-mineralization, nitrification, and N losses via leaching at the Texas Warming and Rainfall Manipulation (WaRM) Site between August 2008 and April 2010. The facility was located in a remnant post oak savanna in College Station, Texas (N 30°34" W 96°21"), and was constructed in March 2004 to investigate the combined effects of altered precipitation distribution and warming on tree and grass dominants of the southern oak savanna (Volder *et al.* 2010). Eight 18 x 9 x 4.5 m (L x W x H) rainout shelters were covered with clear polypropylene film while ends and sidewalls below 1.5 m were not covered to maintain ambient microclimatic conditions (Fay *et al.* 2000; Weltzin & McPherson 2003; Volder *et al.* 2010; see Figure A.6). Shade cloth was used to exclude precipitation. Sheet metal 40 cm in width was installed to 30 cm depth into the soil to penetrate the clay pan and separate each shelter from water flow above and belowground.

The shelter design preserved natural variation in a microenvironment that was similar to ambient conditions. An on-site weather station (EZ Mount Groweather, Davis Instruments, Hayward, CA) recorded precipitation, air temperature and humidity, while solar radiation (total and PAR), air temperature, and relative humidity were monitored continuously and recorded by Hobo data loggers in each shelter (Hobo U12; Onset Computer Corp., Bourne, MA, USA). Mean daily temperatures in the shelters were on average 1.5 °C higher, RH values 2% lower and PAR levels 30% lower than the unsheltered controls. The NOAA weather station at Easterwood Airport (<0.5 km from the site) reported a mean annual temperature of 20.56°C and mean annual precipitation of 1,017.5 mm based on a 50-year period (NOAA 2010; Volder *et al.* 2013).

Ten 2 x 2 m plots were established within each shelter in native soil, a fine sandy loam Alfisol of the Booneville series (Fine, smectitic, thermic Chromic Vertic Albaqualf) in 2003, one year before the experiment began. Each shelter included five plant species combinations with monocultures of *S. scoparium*, *Q. stellata*, and *J. virginiana*, and each of the tree species were grown with the *S. scoparium* in separate mixed species plots. An overhead irrigation system supplied reverse osmosis water from onsite water holding tanks to simulate rainfall to each shelter. Infrared lamps (Kalglo Electronics, Bethlehem, PA; model MRM-1208L) were installed to hang 1.5 m above half of the plots within each shelter and were warmed continuously with 400 W m^{-2} of radiant energy (Harte & Shaw 1995; Wan *et al.* 2002). To accommodate increased height growth of trees, lamps were raised by 0.5 m and the total wattage output to the plots was increased by an additional 400 W m^{-2} of radiant energy to maintain comparable levels of warming in February 2008. This additional warming from the heat lamps increased soil temperatures (averaged over all canopy types) by approximately 0.6 °C at 3 cm depth, and canopy leaf temperatures by about 1-1.5 °C. However, it is important to note that actual soil and canopy warming can vary with wind speed (Kimball 2005). Metal sheeting in the shape of the infrared lamps was placed above the second half of the plots to mimic cover and shading without the heat source.

Rainfall was manipulated in 8 of the 10 shelters to simulated precipitation regimes categorized as control and redistributed, although each regime received the same total amount of annual rainfall (1018 mm) and the same number of rainfall events (Figure 4.1). Four of the 8 shelters received control rain events based on the long-term (50 year) mean reported by a local NOAA weather station in College Station, TX (NOAA 2010). The frequency and intensity of rainfall events were also simulated from the long-term precipitation record of the region. In the other 4 shelters, 40% of the precipitation during 5 summer months (May – September) was withheld from each event

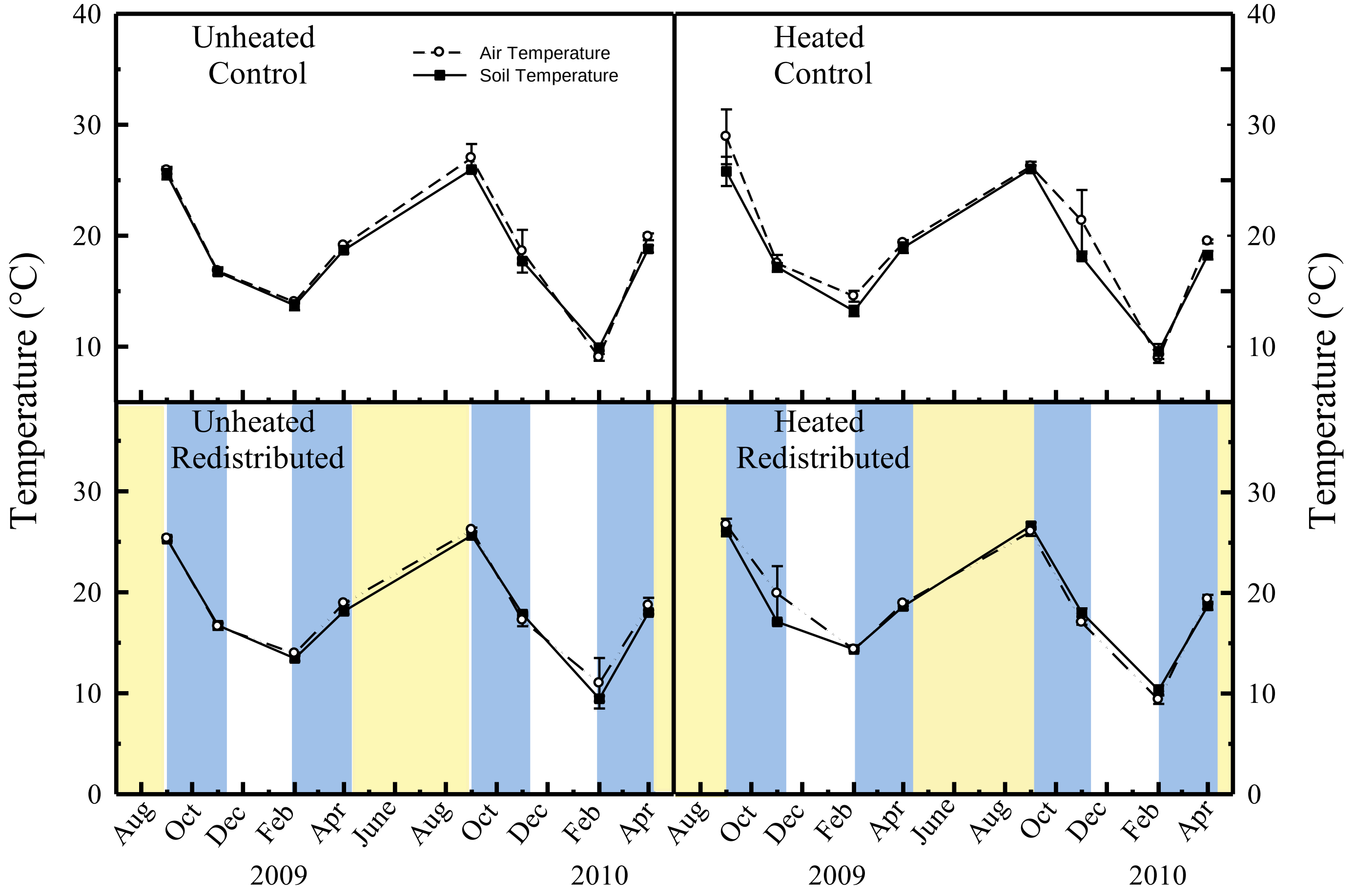

Fig. 4.1 Mean air and soil temperatures were averaged over each 30-day soil incubation period across all plots from August 2008 to April 2010. For each point, n = 4 and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

and evenly redistributed in the previous 2 month spring (March and April) and subsequent 2 month fall (October and November), increasing the rainfall to 52% of the normal spring and fall patterns (see Figure A.7). This redistribution pattern effectively increased the intensity of the summer drought and the amount of precipitation that occurs during the cooler spring and fall seasons. Precipitation redistribution during spring and fall increased volumetric soil water content from 21.0 to 23.1% in the top 20 cm of soil; in contrast, during the summer drought, soil water content decreased from 15.5% in the ambient to 10.7% in the redistributed treatment. Although soil warming differed between canopy types, volumetric water content was generally 1% lower in warmed plots.

Field Sampling and Soil Analysis

Due to time and logistical constraints a subset of vegetation treatments containing monocultures of *J. virginiana* and *S. scoparium*, and the *J. virginiana* + *S. scoparium* combination were sampled from the factorial design during years six and seven of the WaRM experiment. These three vegetation treatments represent the encroachment of *J. virginiana* as it rapidly and aggressively increased in dominance in post oak savanna ecosystems. *S. scoparium* typically remains the dominant grass species and was also included in the sampling scheme.

N-mineralization and nitrification were assessed quarterly over a period of two years using the resin core soil incubation method (Brye *et al.* 2002). The advantages of the resin core method are that it allowed the soil to be incubated in the field and under natural hydrologic conditions, and that it allowed leaching losses to be quantified. Incubations occurred during the last 30 days of each redistributed precipitation shift (four per year); thus, a total of eight time points were analyzed. Field incubations were conducted in two different locations (A and B) within each plot during each sample period. Sample locations were recorded to prevent resampling of the same locations.

Surface litter in each sampling area was moved aside prior to soil coring. Two soil cores (5.5 cm diameter x 10 cm deep) were collected 8 times a year, at the beginning (Time 0) and end of the last thirty days (Time 30) of each precipitation change, in control and treated plots under each vegetation cover type (*J. virginiana, S. scoparium*, and the *J. virginiana* + *S. scoparium* combination) for 22 months (August 2008 to April 2010). Time 0 samples were placed in a labeled Ziplock freezer bag and stored in a cooler until lab analysis later each day of collection. Incubation cores were constructed from PVC pipe (5.5 cm internal diameter x 15 cm deep) (see Figure A.9; Figure A.11). Four 0.5 cm holes were also drilled and evenly spaced above the top 1.5 cm mark of the pipe to facilitate drainage and core removal. Each Time 30 PVC core (A and B) was installed approximately 5 cm from each Time 0 sample (A and B) location using a rubber mallet and wooden plank. The 1.5 cm mark located on the top portion of the core was aligned with the surface of the soil.

To maintain the soil bulk density of each core during removal, two S-hooks attached to a T-post puller tool by rope and chain were effectively used to thread through the 0.5 cm holes, allowing for a careful upward motion to remove each core from the ground (see Figure A.12). A #11 laboratory rubber stopper snuggly fitted into the top 1.5

cm of the core served to hold the sampled soil inside while the cores were turned upside down (see Figure A.11). The bottom 3.5 cm of soil was removed from the core to enable installation of the resin bag (see Figure A.10).

We used 30 g of mixed bed ion exchange resin beads (JT Baker Mixed Bed Exchange Resin IONAC NM-60) placed in a nylon mesh along with a circular Tygon plastic tube to ensure a flush fit to the interior surface of the core (see Figure A.10). Similarly, a separate nylon mesh was filled with 30 cm^3 of silica sand and knotted. A resin bag was placed inside the PVC incubating core, the exposed soil surface flush with the pipe interior wall. The purpose of the resin bag was to capture any NO_2^-, NO_3^-, and NH_4^+ that might be leached out of the soil core during the incubation period. To provide a buffer from external soil surfaces, 1 silica sand bag was then added to the bottom of the core and held securely in the pipe by wrapping a strip of 2 mm mesh fiberglass screen (35 mm x 2 mm) around the exterior (see Figure A.11). The strap wrapped around most of the pipe, leaving the top open for experimental exposure. Finally, the PVC incubation chamber was turned right side up, and placed in its original position in the soil, and the rubber stopper removed. Two cores, each holding the top 10 cm of soil, were incubated for a 30-day period in each plot.

At the end of the 30 day incubation period, each core was removed using the same S-hook and T-post puller device. The entire PVC core, resin bag, sand bag, and fiberglass screen strap was placed into a Ziploc freezer bag and then into a cooler for laboratory analysis later that same day. All holes resulting from soil coring were refilled immediately with pre-sifted soil of the same fine sandy loam textural class.

Laboratory Analyses

Each soil sample at Time 0 and Time 30 was weighed for whole-soil wet weight. A 30 g subsample from each core was weighed and dried at 105 °C for 24 hours to determine gravimetric water content. Volumetric water content was then calculated using the bulk density of each sample. Two additional aliquots of each soil sample were used for soil pH and texture analysis. Soil pH was quantified by using the $CaCl_2$ method and glass electrode (McLean 1982), and texture was determined by using the hydrometer method (Bouyoucos 1926).

To determine concentrations of NO_2^-, NO_3^-, and NH_4^+ a 30 g subsample of the sieved soil was shaken in a 250 mL bottle with 100 mL of 2 M KCl for 1 hour. Resin bags from the incubated Time 30 cores were cut open, placed in 250 mL bottles, and shaken for one hour in 100 mL of 2 M KCl. Soil and resin bead extracts were filtered using Fisher Scientific Q-5 filter paper (preleached with 50 mL of 2 M KCl prior to extracting each sample). Filtered extracts were stored frozen prior to colorimetric analysis. Blanks were analyzed for both soil and resin bead extraction and filtration procedures. Soil not used for determination of NH_4^+ and NO_3^- was stored at 4°C. NH_4^+ and NO_3^- concentrations in KCl extracts of soils and resin beads were measured using a Seal AQ2 discrete chemistry analyzer (Seal Analytical, Mequon, WI). NH_4^+ concentrations were measured using the indophenol-blue method, and NO_3^- and NO_2^- were measured as NO_2^- using the cadmium reduction method (Seal Analytical 2006;

Seal Analytical 2008). Values for Time 0, Time 30, and resin bags were used to calculate net N-mineralization, net nitrification, and net N-losses via leaching as follows:

$$\text{Net N-mineralization} = [(NH_4^+\text{-}N + NO_3^-\text{- }N)_{SOIL} + (NH_4^+\text{-}N + NO_3^-\text{- }N)_{RESIN}]_{\text{Time 30}} - [(NH_4^+\text{-}N + NO_3^-\text{- }N)_{SOIL}]_{\text{Time 0}}$$

$$\text{Net nitrification} = [NO_3^-\text{- }N_{SOIL} + NO_3^-\text{- }N_{RESIN}]_{\text{Time30}} - [NO_3^-\text{- }N_{SOIL}]_{\text{Time 0}}$$

$$\text{Net N-Losses via Leaching} = [(NH_4^+\text{-}N + NO_3^-\text{- }N)_{RESIN}]_{\text{Time30}}$$

Statistical Analyses

The effects of rainfall, temperature, and species combination on all response variables were analyzed by repeated measures ANOVA in JMP Pro 10.0.0 (SAS Institute, Cary NC). The experimental design is a completely randomized split-plot with four replicates per treatment combination. Whole plots consisted of the two precipitation treatments (control vs. redistributed) in factorial combination with two temperature treatments (unheated vs. heated); the subplots were the three vegetation types [two monocultures (*J. virginiana*, *S. scoparium*) and one tree-grass combination (*J. virginiana* + *S. scoparium*)]. Each sampling period (time) was treated as an individual repeated measure using the D-optimal split-plot design as a repeated measures approximation procedure (SAS 2005), which allows for a robust mixed model analysis (Goos & Vandebroek 2001; Jones & Nachtsheim 2009; SAS 2005; Smucker 2011).

Variances were estimated with Restricted Maximum Likelihood (REML) and the Tukey's HSD post hoc test was used to distinguish differences between the treatment combinations. The student's t-test was used when a variable had only two treatment levels and a normal distribution (e.g. warming = heated vs. unheated; rainfall = control vs. redistributed) (Ott & Longnecker 2010).

4. Results

Microclimate

Air temperatures were averaged over each 30-day soil incubation period. The mean air temperature was 18.63 C in unheated plots and 19.25 C in heated plots; temperatures ranged from 9.2 to 27.8 C across all plots and sampling periods. Air temperatures in the heated plots averaged 1 °C higher than unheated plots, but soil temperatures were not affected by warming by the 6th year of the WaRM experiment (Volder *et al*. 2013; Figure 4.1). Heaters were raised by 0.5 m and infrared outputs were increased to 800 W in February 2008 to accommodate the height of the vegetation (Volder *et al.* 2013), which ultimately shaded the soil surface and minimized the potential for soil warming. Rainfall events were manipulated to simulate summer drought by reducing precipitation by 40% and redistributing the rainfall to the spring and fall seasons (Figure 4.2). Soil volumetric water content decreased in the summer and increased in the spring and fall months within the redistributed rainfall plots with a significant interaction of rainfall x warming x species combination ($p < 0.001$) (Figure 4.2). Heated *S. scoparium* monocultures under control rainfall had the highest

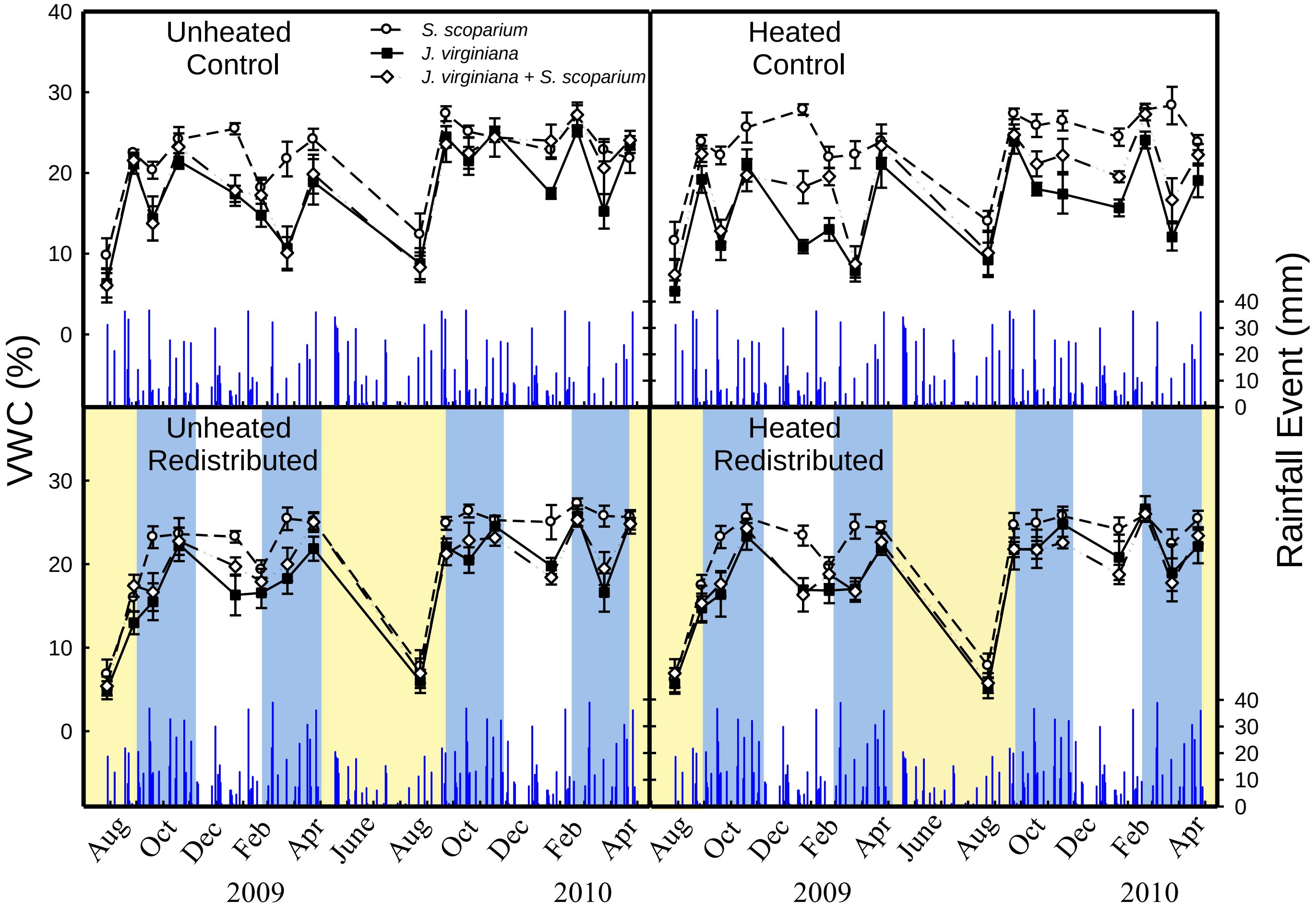

Fig. 4.2 Soil volumetric water content (VWC; 0-10cm; shown as symbols) and rainfall event size (mm; vertical bars) for the duration of the study, August 2008 to April 2010. For VWC, n = 4 for each point, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

volumetric water content ($p < 0.001$) and heated *J. virginiana* monocultures under control rainfall had the lowest volumetric water content ($p < 0.001$).

Soil bulk density (Table 4.1) ranged from 0.98 to 1.85 gm^{-2}, and was affected by precipitation x time ($p = 0.004$) and precipitation x species composition ($p = 0.001$) interactions. The highest soil bulk density values were in March 2010 in *S. scoparium* plots with redistributed rainfall, and the lowest values were in October 2008 in *J. virginiana* plots with control rainfall. Values for soil pH ranged from 3.02 to 6.83 and were significantly affected by interactions between precipitation x species composition ($p = 0.029$), precipitation x time ($p = 0.002$), and warming x species composition ($p < 0.001$). Heated *J. virginiana* monocultures were significantly ($p <0.001$) more acidic than all other warming x species combination treatments and soils were most acidic within the redistributed rainfall regime during the winter ($p = 0.002$). All treatments fell within the sandy loam soil texture class with no significant difference between shelters (Table 4.1).

N Mineralization, Nitrification, and N Losses via Leaching

Overall, soil nitrogen transformation rates during the fifth and sixth years of the WaRM experiment were strongly affected by the availability of NH_4^+ (not taken up by plants or microorganisms) and the rapid conversion to NO_3^- (nitrification) and subsequent leaching losses from the top 10 cm of the soil profile. The majority of N within the calculated daily net N mineralization rates (mg $NH_4^+/NO_2^-/NO_3^-$ -N /kg soil/day) came from daily net nitrification (mg NO_2^-/NO_3^- -N /kg soil/day) observed in

Table 4.1 Soil properties (0-10cm) by species (mean ± SE). Bulk density was measured during each of the 16 sample collection periods. pH was measured four times each year for two years. Soil texture analysis was measured once in August 2008.

	S. scoparium		***J. virginiana***		***J. virginiana + S. scoparium***	
	Mean	***SE***	***Mean***	***SE***	***Mean***	***SE***
Bulk Density (n = 512) g/cm^3	1.33	0.00	1.30	0.00	1.30	0.00
pH (n = 256)	5.31	0.03	4.95	0.04	5.02	0.03
Soil Texture (n = 18)						
% Sand	59.3	0.7	59.1	0.9	59.0	0.8
% Silt	30.1	0.5	30.5	0.5	30.4	0.5
% Clay	10.6	0.6	10.5	0.6	10.7	0.7

the resin bags below the treated and sampled soil. Therefore, the results for each measurement of daily net N mineralization and daily net nitrification were nearly identical.

N mineralization ranged from -0.24 to 4.56 mg N/kg soil/day with a mean of 0.328 (n = 765) and standard error of 0.017 (Table 4.2) across all samples. There was a significant 3-way interaction with rainfall x species composition x time (p = 0.009), with the two significant 2-way interactions of rainfall x species composition (p = 0.004) and species composition x time (p < .0001) (Table 4.3; Figure 4.4). The ANOVA analysis and Tukey's HSD post hoc test indicated that N mineralization rates were highest within plots containing *S. scoparium* monocultures and the redistributed rainfall regime during the summer sampling periods (Figure 4.4). Rates were also consistently higher in the summer months for all other species composition treatments compared to the winter, spring, and fall seasons. In addition, a 2-way interaction of warming x species composition (p = 0.001) was observed (Table 4.3 and Figure 4.3). Warmed plots containing the *J. virginiana* + *S. scoparium* species composition treatment had higher N mineralization rates than the unheated plots. In contrast, warmed *J. virginiana* monocultures had the lowest rates (Figure 4.3).

Net nitrification rates (mg NO_2^-/NO_3^- -N /kg soil/day) ranged from -0.225 to 4.53 mg N/kg soil/day with a mean of 0.295 (n = 765) and standard error of 0.016 (Table 4.2) across all samples. Similar to net N mineralization rates, there was a significant 3-way interaction with rainfall x species composition x time (p = 0.005), with the two significant 2-way interactions of rainfall x species composition (p = 0.005) and species

Table 4.2 Range, mean, and standard error of N transformation and leaching rates (mg N/Kg soil/day) from August 2008 to April 2010 (n=765)

	Min	**Max**	**Mean**	**S.E.**
N mineralization	*-0.241*	*4.56*	*0.328*	*0.017*
Nitrification	*-0.225*	*4.53*	*0.295*	*0.016*
N Leaching	*0.001*	*4.88*	*0.300*	*0.018*

Table 4.3 P values (* p<.05, **p<.01, ***p<.001) using repeated measures ANOVA for changes in N mineralization, nitrification, and N losses via leaching (n=4) from August 2008 to April 2010.

Treatment	**N mineralization** p value	**Nitrification** p value	**Leaching** p value
Rainfall (R)	*ns*	*ns*	*ns*
Warming (W)	*	*	*ns*
R x W	*ns*	*ns*	*ns*
Species Composition (SC)	***	***	***
R x SC	**	**	**
W x SC	***	***	***
R x W x SC	*ns*	*ns*	*ns*
Time (T)	***	***	***
R x T	*ns*	*ns*	*ns*
W x T	*ns*	*ns*	*ns*
R x W x T	*ns*	*ns*	*ns*
SC x T	***	***	***
R x SC x T	**	**	**
W x SC x T	*ns*	*ns*	*ns*
R x W x SC x T	*ns*	*ns*	*ns*

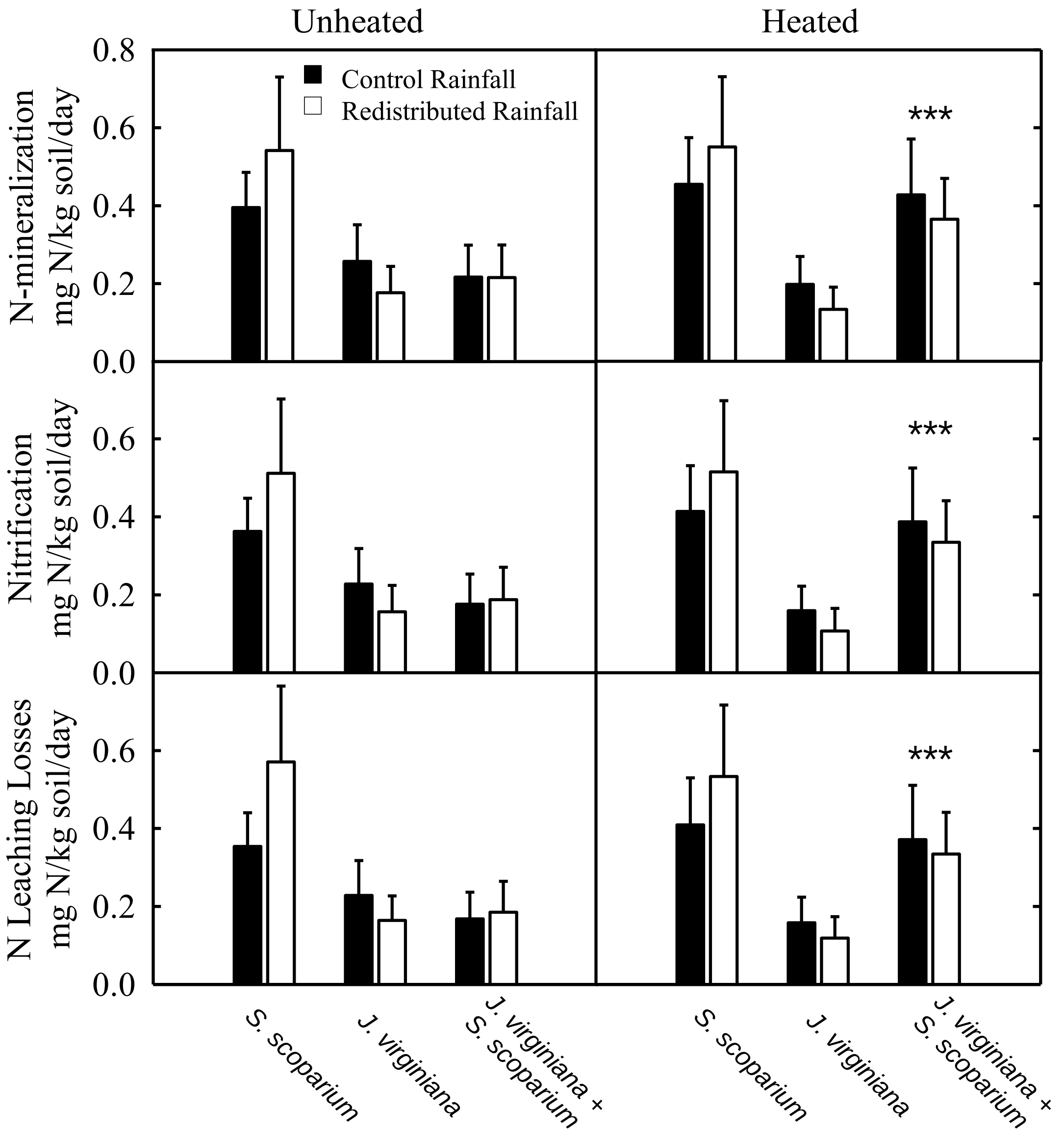

Fig. 4.3 Mean net rates of N mineralization ($NH_4^+/NO_2^-/NO_3^-$), nitrification (NO_2^-/NO_3^-), and N losses from leaching (resin only) (n=8) within experimental treatments from August 2008 to April 2010 (***p<.001).

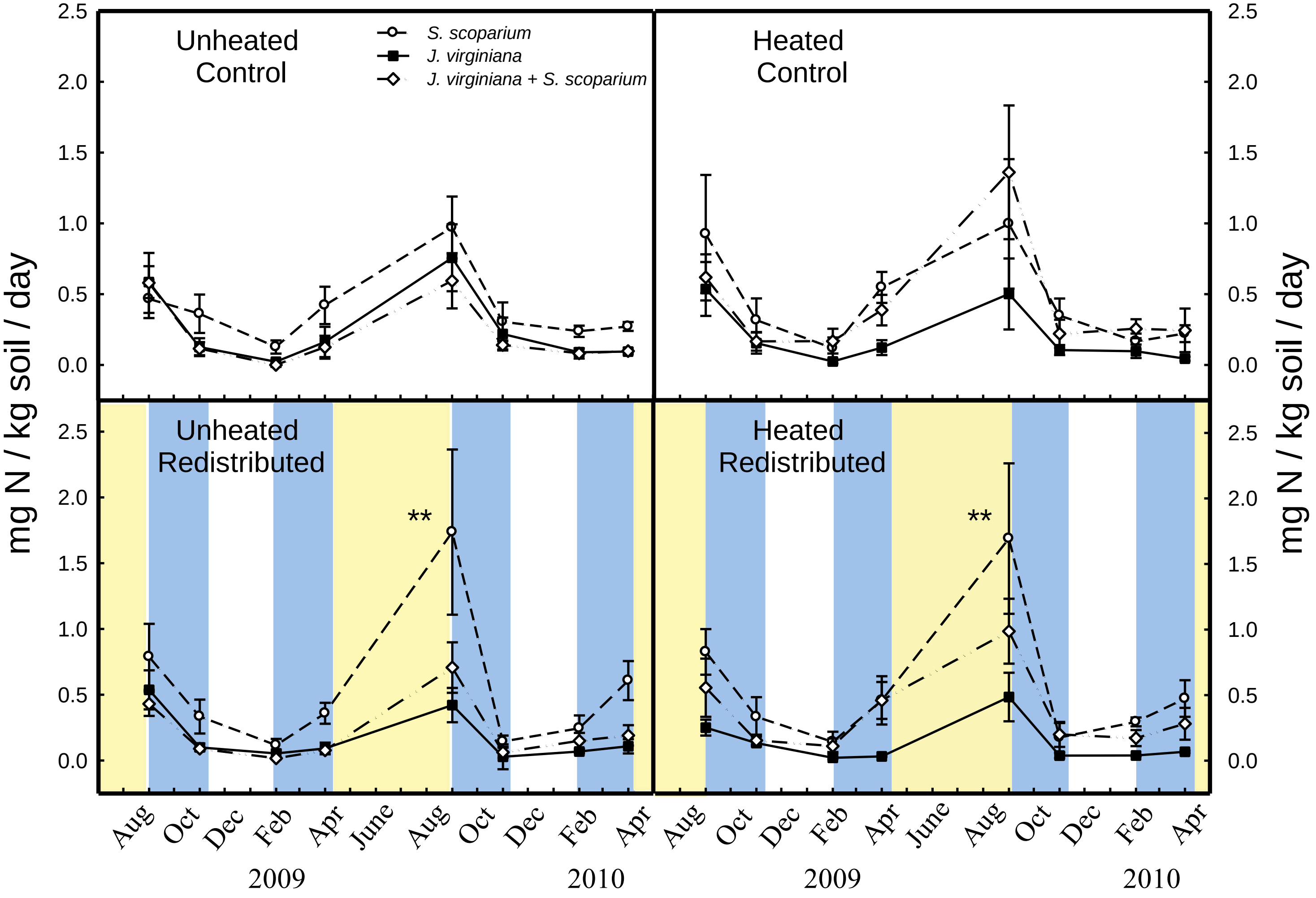

Fig. 4.4 Mean daily net N mineralization (mg $NH_4^+/NO_2^-/NO_3^-$ -N /kg soil/day) with standard error bars (n=4) from August 2008 to April 2010 (**p<.01). Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

composition x time ($p < .0001$) (Table 4.3). Post hoc analysis indicated that nitrification rates were highest within plots containing *S. scoparium* monocultures and the redistributed rainfall regime during the summer sampling periods. Rates were also consistently higher in the summer months for all other species composition treatments compared to the winter, spring, and fall seasons. In addition, a 2-way interaction of warming x species composition ($p = 0.001$) was observed (Table 4.3; Figure 4.3). Warmed plots containing the *J. virginiana* + *S. scoparium* species composition treatment had higher N mineralization rates than the unheated plots. In contrast, warmed *J. virginiana* monocultures had the lowest rates (Figure 4.3).

Nitrogen losses from leaching ranged from 0.001 to 4.88 mg N/kg soil/day with a mean of 0.300 ($n = 765$) and standard error of 0.018 (Table 4.2) across all samples. Similar to net N mineralization and nitrification rates, there was a significant 3-way interaction with rainfall x species composition x time ($p = 0.021$), with the two significant 2-way interactions of rainfall x species composition ($p = 0.001$) and species composition x time ($p < .0001$) (Table 4.3). Leaching rates were highest within plots containing *S. scoparium* monocultures and the redistributed rainfall regime during the summer sampling periods. Rates were also consistently higher in the summer months for all other species composition treatments compared to the winter, spring, and fall seasons. In addition, a 2-way interaction of warming x species composition ($p = 0.001$) was observed (Table 4.3; Figure 4.3). Warmed plots containing the *J. virginiana* + *S. scoparium* species composition treatment had higher N mineralization and leaching rates than the unheated plots. In contrast, warmed *J. virginiana* monocultures had the lowest rates (Figure 4.3).

5. Discussion

The results of this study were dominated by interaction effects between species x rainfall x time, highlighting the complexity of global change impacts. These 3-way interactions can be explained by separating them into two 2-way interactions of rainfall x species and species x time for each N mineralization, nitrification, and N-losses via leaching response variable (Table 4.3). Warming alone and rainfall redistribution alone rarely affected soil N transformation rates in this experiment; however, warming and rainfall redistribution interacted with other experimental variables to influence N-cycling processes. The analysis of this study supports the emerging consensus that global change drivers are likely to have their greatest impact by mediating complex interactions with plant species composition (Brooker 2006; Gilman *et al.* 2010).

Overall, N transformations in this experiment were strongly affected by the availability of NH_4^+ (not taken up by plants or microorganisms) and the rapid conversion to NO_3^- (nitrification) and subsequent leaching losses from the top 10 cm of the soil profile as is often observed in arid and semi-arid ecosystems (Norton 2008; Figure 4.3; Table 4.2) and this relationship was strongest during the summer (Figure 4.4). Nitrifying bacteria mediate the oxidation of NH_4^+ to NO_3^-; therefore, rates of nitrification are strongly dependent on rates of ammonification. Net nitrification rates are also controlled by environmental factors such as temperature and water availability. These rates can increase as soil water content decreases due to a decrease in heterotrophic assimilation of NO_3^- or a decrease in microbial biomass (Booth *et al.* 2003), though we did not measure this parameter. In addition, annual plant senescence in late spring/early summer would supply N-rich substrate to nitrifying bacteria often found in dryer climates (Norton *et al.* 2008). A root analysis conducted during the same time of this study and on the same soil showed that there was a significant decrease in root biomass in late spring/early summer indicating the presence of root death/senescence in each species composition (Wellman *et al.* unpublished), which could have provided the substrate needed for increased ammonification and thus an increase in nitrification. The majority of the NO_2^- and NO_3^- in this study was found in the resin bags below the 10cm soil core, implying N loss from the surface soil. Any climate-driven changes or species shifts that increase nitrification could potentially lead to an increase in N loss from the surface soil via leaching in the post oak savanna.

More specifically, warming significantly increased N transformations in *J. virginiana* + *S. scoparium* plots across all time periods in a warming x species composition interaction. This result partially supports our first hypothesis that elevated temperatures would increase N mineralization, nitrification, and N-losses via leaching. The relationship was not significant in the *J. virginiana* and *S. scoparium* monoculture species composition treatments, which could imply that species competition played a major role in N cycling processes with warming. In addition, microbial community composition is known to shift with increases in both temperature and substrate availability (Zogg *et al.* 1997). Likewise, changes in plant species composition such as the woody encroachment of *J. virginiana* into *S. scoparium* dominated grasslands are likely changing microbial activity and community structure and affecting nutrient cycling (Hobbie 1992; Hobbie 1996). For example, *J. virginiana* reduced litter quality and increased arbuscular mycorrhizal (AM) fungi when comparing belowground dynamics with *Q. stellata* in Oklahoma (Williams *et al.* 2013). Similar results were found in Illinois while investigating shrub encroachment into hill prairies (Yannarell *et al.* 2014). N transformations were not affected in either of these experiments, but they did not include warming as a variable. The interaction of warming and species composition increased N transformation rates in this study. This interaction indicates that increased temperatures combined with plant community shifts may have a greater influence on nutrient cycling than on species dynamics or warming alone.

A more complex relationship was significant with redistributed rainfall x species composition as part of the 3-way interaction of species x rainfall x time. N mineralization and nitrification rates were consistently highest in the *S. scoparium* monocultures and significantly lowest in the *J. virginiana* monocultures. Plots containing the *J. virginiana* + *S. scoparium* species composition had rates that fell between the highest and lowest and were also significantly different. Each species composition effect was significant with redistributed rainfall where plots received 40% less rainfall in the summer and 52% more in the spring and fall months, but not in the control rainfall plots. This in part confirms our second hypothesis that changes in rainfall patterns in the post oak savanna would accelerate soil N transformation rates. Rates only increased under *S. scoparium* monocultures. This relationship was not significant with the other species compositions tested (*J. virginiana* and *J. virginiana* + *S. scoparium*).

The intensified summer drought in between two strong seasonal rainfall pulses during the growing season may induce a “Birch effect” or increase in respiration and mineralization rates (Birch 1958; Jarvis *et al.* 2007; Unger *et al*. 2010) and can be influenced by substrate availability (Lado-Monserrat *et al.* 2014). Increases in soil water stimulate microbial community activities as they are replenished with intracellular water. This enables them to produce extracellular enzymes capable of depolymerizing complex organic matter and ultimately mineralizing nutrients such as N. For example, a significant rise or flush of soil mineral N is generally observed as soils are dried and rewetted, though the increase in net mineralization may be due to the quality of the organic substrate released after soil disturbance induced by water rather than the effect of hydration alone (Austin *et al.* 2004; Birch 1958; Fierer & Schimel 2002; Saetre & Stark 2005).

The elemental analyses of roots conducted during this study showed that *S. scoparium* had significantly lower C:N ratios compared to the other species treatments (Wellman *et al.* unpublished), similar to what Norris *et al.* (2001a) found when comparing *J. virginiana* with big bluestem (*Andropogon gerardii* Vitman). Lower C:N ratios in grasslands can result in higher decay and decomposition rates compared to forests (Norris *et al.* 2001a) and an increase in soil N availability (Norris et al. 2007). In addition, C_4 grasses like *S. scoparium* tend to have higher N use efficiencies and would not need as much N from soil solution as a C_3 plant like *J. virginiana*, leaving a surplus in the soil under *S. scoparium*. Nevertheless, ecosystems with varying substrate qualities have shown considerable increases in nutrient concentrations after water is introduced into a dried soil; therefore, the exact mechanism remains unclear. High soil N availability can lead to high N-losses via leaching of NO_2^- and NO_3^- which were observed under all treatment combinations (Table 4.2; Figure 4.3). Increases of these N-losses with redistributed rainfall under *S. scoparium* could lead to redistribution of these biologically available forms of N in the soil profile and alter plant and microbial community structure.

Although we did not predict differences in N transformations through time or seasonal variation, we found a significant interaction with species x time in the 3-way interaction of rainfall x species x time. N mineralization, nitrification, and N-losses via leaching in the *S. scoparium* and *J. virginiana* + *S. scoparium* species compositions were significantly highest during the summer. The differences in N transformations found in the *S. scoparium* response correspond with the rainfall x species interaction where rainfall is reduced by 40% during the summer months, simulating a summer drought period when temperatures are at their annual highest (NOAA 2010; Figure 4.1; Figure 4.2). The combined effects of summer drought and the high susceptibility to decay in plots containing *S. scoparium* resulted in an increase in N mineralization rates.

N transformations in the *J. virginiana* - *S. scoparium* mixtures also increased during this time, but this relationship was not significantly different in the redistributed rainfall treatments. C_3 *J. virginiana* trees outcompeted the C_4 *S. scoparium* grass in the *J. virginiana* + *S. scoparium* plots above- and belowground after 5 years of the WaRM experiment (Volder *et al.* 2013; Wellman *et al.* unpublished data; see Figure A.8). It is likely that N transformation rates in the *J. virginiana* + *S. scoparium* plots also receiving redistributed rainfall were similar to the *S. scoparium* monocultures in the same treatment. However, *J. virginiana* could have been taking advantage of the decaying *S. scoparium* and used the available N before leaving the surface soil.

In contrast to our third hypothesis, that lower C:N ratios in *J. virginiana* litter inputs will result in higher rates of N transformation rates in *J. virginiana* monocultures and in *J. virginiana* + *S. scoparium* plots compared to *S. scoparium* monocultures, soil N transformation rates were generally higher in plots that included *S. scoparium* (Figure 4.3; Figure 4.4). This implies that *S. scoparium* inputs were relatively more labile than *J. virginiana* inputs, and suggests that grass communities may heavily influence rates of N transformations in savanna ecosystems, similar to Lata *et al.* (2004). Additional root analysis on the the same samples support this claim (Wellman *et al.* unpublished). The C_3 woody plant *J. virginiana* had significantly larger belowground C and N pools compared to that of the C_4 grass *S. scoparium*, and root C:N ratios were significantly higher in the *J. virginiana* monocultures and *J. virginiana* + *S. scoparium* plots compared to *S. scoparium* monocultures. Previous unpublished analysis of leaf litter from the study site indicated that *S. scoparium* had a higher C:N ratio (51.04) compared to *J. virginiana* (38.62) (Tjoelker & Boutton unpublished; see Table A.2). Interestingly, an analysis of litter dynamics in eastern Kansas demonstrated that *J. virginiana* root C:N ratios can be twice as high as the leaf C:N of the same plant (Norris *et al.* 2001a). These woody species are likely allocating more N to aboveground growth relative to their belowground growth, similar to a supply and demand response (Friend *et al.* 1994), and could explain the low C:N ratio in leaf litter and high C:N ratio in root litter. The lower C:N ratios in the *S. scoparium* plots may then be facilitating a faster turnover rate and an increase in N transformation rates relative to the *J. virginiana* plots.

6. Conclusions

This multifactorial study highlights the complexity of global change impacts on N cycle processes in soils beneath two of the most dominant species in the post oak savanna ecosystem type. Increases in temperature, alterations in precipitation patterns, and shifts in plant community structure produced outcomes that were not as straightforward as we originally expected. Interactions of these variables produced results that indicate a complex multilayered response to these global change drivers. Additional investigations into microbial community structure may provide further insight as to what may be driving these interactions. However, it is clear that *J. virginiana* is an opportunistic species that will increase in abundance with warmer temperatures and the absence of proper grassland management practices in the post oak savanna. It will continue to outcompete species like *S. scoparium* and take advantage of the mineralized N produced from the decaying organic matter in the diminishing grasslands. These changes in rates of N-transformations and leaching losses in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

CHAPTER V

SUMMARY AND CONCLUSIONS

The mid-latitude, continental grasslands of North America are predicted to experience substantial modification in precipitation regimes (IPCC 2013; Weltzin *et al.* 2003; Melillo *et al.* 2014) and a shift from summer to spring precipitation is anticipated to be more probable than a substantial change in mean annual precipitation amount (Easterling *et al.* 2000; Groisman & Knight 2008; Manabe & Wetherald 1986). Higher temperatures and greater evaporation coupled with a decrease in summer precipitation will intensify summer drought events (MacCracken *et al*. 2003; Pope *et al.* 2000; Wetherald & Manabe, 1995). In spite of these general predictions, little is known concerning the relative effects of these global climate change drivers or their potential interactive effects on ecosystem processes related to C and N biogeochemical cycles (Norby & Luo 2004; Rustad *et al.* 2001; Weltzin *et al.* 2000).

In addition, there is a critical gap in our knowledge of the plant functional trait responses (Cornwell *et al.* 2008; DeDeyn *et al*. 2008) and species interactions (Grime *et al.* 2008; Suttle *et al.* 2007) that may underpin ecosystem responses to various global climate change scenarios. Warmer temperatures, in combination with increased cool season precipitation, could potentially lead to shifts in plant communities (species composition, relative dominance, and/or growth forms) that could alter the quantity and quality of organic matter inputs to the soil, thereby affecting nutrient storage, water holding capacity, and rates of root production (Gill & Jackson 2000; Fiala *et al*. 2009; Bai *et al*. 2010; Zhou *et al*. 2011; Zhou *et al*. 2012; Verburg *et al*. 2013).

The central US is bisected by an ecotone that separates the grasslands of the Great Plains and the deciduous forest of the eastern US. In the south, this transition zone is comprised of the post oak (*Quercus stellata* Wangenh.) savanna region located in south central Texas and eastern Oklahoma (Hatch *et al.,* 1990). Post oak is the major canopy tree dominant and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the major perennial grass dominant (Hatch *et al.* 1990). Covering about 3 million hectares, this savanna has undergone a transition from a *Q. stellata – S. scoparium* dominated savanna toward a *Q. stellata* - juniper (*Juniperus virginiana* L.) woodland over the last century (Breshears 2006; Briggs *et al.* 2005; Reich *et al.* 2001), particularly in the absence of fire (Norris *et al.* 2001b; Eggemeyer *et al.* 2006). Of particular significance to this research, succession from grassland to *Juniperus* woodland in this region results in increased soil C and N storage (McKinley & Blair 2008) and altered N-cycling processes (McKinley *et al.* 2008; Norris *et al*. 2007). Widespread encroachment by *Juniperus* throughout this oak savanna region suggests an important role for this vegetation change in the N cycle of the central US (McKinley & Blair 2008; McKinley *et al.* 2008; Norris *et al*. 2007).

Vegetation shifts from grassland to shrubland or woodland can dramatically increase total root biomass (McKinley 2006; Boutton *et al.* 2009) and soil carbon (C) inputs from increased root exudation. However, in some cases, shrub encroachment into grasslands could lead to lower fine root production and turnover (Zhou *et al.* 2012) and an increase in antimicrobial allelochemicals (Borchardt *et al.* 2008; Eller *et al.* 2010; Post & Urban 1995). These changes in the quantity and quality of soil organic matter inputs have the potential to alter the size of the soil microbial biomass pool (McCulley *et al.* 2004; Liao & Boutton 2008), as well as microbial community structure (Hollister *et al.* 2010; Yannarell *et al.* 2014), with potential implications for soil C and N storage and turnover.

Therefore, the purpose of this study was to quantify seasonal variation in root biomass and root carbon and nitrogen pool sizes (Chapter II), soil organic carbon (SOC) and soil total nitrogen (STN) (Chapter III), and rates of nitrogen (N)-mineralization, nitrification, and N-losses via leaching (Chapter IV) in soil subjected to experimental warming and rainfall manipulation. Research was conducted between August 2008 and April 2010 at the Texas Warming and Rainfall Manipulation (WaRM) Site in east-central Texas. Treatments included plots comprised of *J. virginiana* and *Schizachyrium scoparium* monocultures and a *J. virginiana* + *S. scoparium* combination in native soil. These two species are among the most dominant components of the post oak savanna flora.

Fine (<2 mm), coarse (>2 mm), and total (fine + coarse) root biomass were quantified seasonally for 22 months on soil cores taken to 10 cm depth and analyzed for their C and N concentrations. Total root biomass ranged from 94.7 to 3,163.2 g m^{-2} where total *S. scoparium* roots ranged from 94.7 to 1,932.1 g m^{-2} and total *J. virginiana* and *J. virginiana + S. scoparium* roots ranged from 247.6 to 3,163.2 g m^{-2}**.** Species composition and time appeared to be the most consistently important main effects that determined root biomass, C, N, and C:N ratios. Warming alone and rainfall redistribution alone seldom affected the root response variables in this experiment. However, warming and rainfall redistribution interacted with other experimental variables to influence root biomass and nutrient pools.

SOC and STN were assessed 3 times between 2008 and 2010. SOC ranged from 419.4 g C m^{-2} and 1,768.9 g C m^{-2} with a mean of 959.4 g C m^{-2} ($n = 287$) and standard error of 13.5 across all samples. STN ranged from 23.7g N m^{-2} and 126.0 g N m^{-2} with a mean of 65.0 g N m^{-2} ($n = 287$) and standard error of 1.0. Soil C:N ratios ranged from 9.4 to 32.5 with a mean of 15.1 ($n = 287$) and standard error of 0.2. In this experiment, species composition and time appeared to be the most consistently important main effects that determined SOC and STN. Warming alone and rainfall redistribution alone did not affect SOC or STN in this experiment; however, warming and rainfall redistribution interacted with other experimental variables to influence SOC and STN. The results of this study were dominated by interaction effects between species x rainfall.

Soil N-mineralization, nitrification, and N-losses via leaching were assessed quarterly over a period of two years using the resin core incubation method. Results were dominated by a 3-way interaction of species x rainfall x time, highlighting the complexity of global change impacts. Highest rates of net N mineralization (4.56 mg NH_4^+ /NO_2^- /NO_3^- -N/ kg soil/day) and nitrification (4.53 mg NO_2^-/NO_3^- -N/ kg soil/day) occurred in *S. scoparium* monocultures during summer in the redistributed rainfall plots. Lowest rates of N mineralization (-0.241 mg NH_4^+ /NO_2^- /NO_3^- -N/ kg soil/day) and nitrification (-0.225 mg NO_2^-/NO_3^- -N/ kg soil/day) occurred under *J. virginiana* during fall and winter in redistributed rainfall plots. Losses of N through leaching were highest in the same treatment combinations that had high rates of nitrification. In a significant warming x species composition interaction, warmed plots containing the *J. virginiana* + *S. scoparium* species composition treatment had higher N mineralization rates than the unheated plots. Results indicate that warming and rainfall redistribution interacted strongly with other experimental treatments to influence rates of N-transformations.

These results suggest that soils beneath *J. virginiana* and *S. scoparium* growing alone or together behaved as carbon sinks during years 6 and 7 of this climate change experiment. If this pattern accurately reflects C storage patterns in the post oak savanna, this ecosystem type may potentially function as a C sink and provide a negative feedback to climate change. However, this interpretation should be regarded with caution because these soils may still be recovering C and N following the disturbance involved with plot establishment 7 years earlier. In addition, woodlands dominated by *J. virginiana* are likely allocating more N to aboveground growth relative to their belowground growth, similar to a supply and demand response (Friend *et al.* 1994), and could explain the low C:N ratio in leaf litter reported in a previous unpublished analysis (Tjoelker & Boutton unpublished; see Table A.2) and high C:N ratio in root litter in this study. The lower C:N ratios in the *S. scoparium* plots may then be facilitating a faster turnover rate and an increase in N transformation rates relative to the *J. virginiana* plots.

The analysis of this multifactorial study highlights the complexity of global change impacts on post oak ecosystem dynamics. Increases in temperature, alterations in precipitation patterns, and plant species composition produced outcomes that were not as straightforward as originally expected. Interactions of these variables produced results that indicate a complex multilayered response to these global change drivers. However, it is clear that *J. virginiana* is an opportunistic species that will increase in abundance with warmer temperatures and the absence of a natural disturbance regime (i.e., frequent fire) while reducing their need to produce roots belowground, particularly when competing with *S. scoparium* in the post oak savanna. The species competition between *J. virginiana* and *S. scoparium* will likely result in a C sink (above- and belowground) as temperatures rise to IPCC (2013) projections. *J. virginiana* will likely continue to outcompete species like *S. scoparium* even though this low latitude (30 N) C_4 grass showed signs of continued growth during the cooler months when they are typically dormant and undergoing winter senescence (Volder *et al.* 2013), and take advantage of the mineralized N produced from the decaying organic matter in the diminishing grasslands. However, altered precipitation patterns may reduce these effects. Greater overall biomass accumulation by *J. virginiana* above- and belowground is likely due to their aggressive nature found in a variety of ecosystems. For example and related to this study, *J. virginiana* takes advantage of resources produced from the decaying organic matter in the diminishing grasslands of the Great Plains.

Additional analysis by identifying and separating individual roots of each species in the *J. virginiana* + *S. scoparium* species compositions, investigations in microbial community structure, lignin content, phosphorus concentrations, isotopic analysis, effects of CO_2 enrichment, and C and N concentrations in deeper soils will probably help clarify the differences found in this study. These changes in root dynamics, SOC, STN, and N mineralization rates in response to global change drivers may have important implications for net primary production, soil fertility, carbon storage, trace gas fluxes, water quality, interspecific interactions, and vegetation dynamics in the oak savanna region of North America.

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APPENDIX

Table A.1 WaRM Soil and Environmental Properties repeated measures ANOVA

	df	Bulk Density p-value	%VWC p-value	df	pH p-value	df	Texture p-value	df	Air Temp p-value	Soil Temp p-value
Precipitation (P)	1	-	-	1	-	1	-			
Time (T)	**15**	**<.001**	**<.001**	**7**	**<.001**	-	-	**7**	**<.001**	**<.001**
Warming (W)	1	-	-	**1**	**0.003**	1	-	1	-	-
Sp. Combination (SC)	**2**	**<.001**	**<.001**	**2**	**<.001**	2	-			
P x T	**15**	**0.004**	**<.001**	**7**	**0.002**	-	-			
P x W	1	-	-	1	-	1	-			
W x T	15	-	-	7	-	-	-	7	-	-
P x W x T	15	-	-	7	-	-	-			
P x SC	**2**	**0.001**	<.001	**2**	0.029	2	-			
SC x T	**30**	-	<.001	14	-	-	-			
SC x W	2	-	0.005	**2**	**<.001**	2	-			
P x SC x T	30	-	-	14	-	-	-			
P x SC x W	**2**	-	**<.001**	2	-	2	-			
W x SC x T	30	-	-	14	-	-	-			
P x SC x W x T	30	-	-	14	-	-	-			

Table A.2 WaRM leaf carbon (%) nitrogen (%), and C:N ratio from samples collected in 2007 (n=16). (Courtesy of Mark Tjoelker and Tom Boutton, *Unpublished data*)

	S. scoparium		***J. virginiana***		***J. virginiana-S. scoparium***	
	Mean	***SE***	***Mean***	***SE***	***Mean***	***SE***
Leaf Carbon (%)	48.56	0.46	47.06	0.36	48.09	0.31
Leaf Nitrogen (%)	0.98	0.04	1.29	0.07	1.14	0.04
C:N Ratio	51.04	2.44	38.62	2.41	43.90	1.67

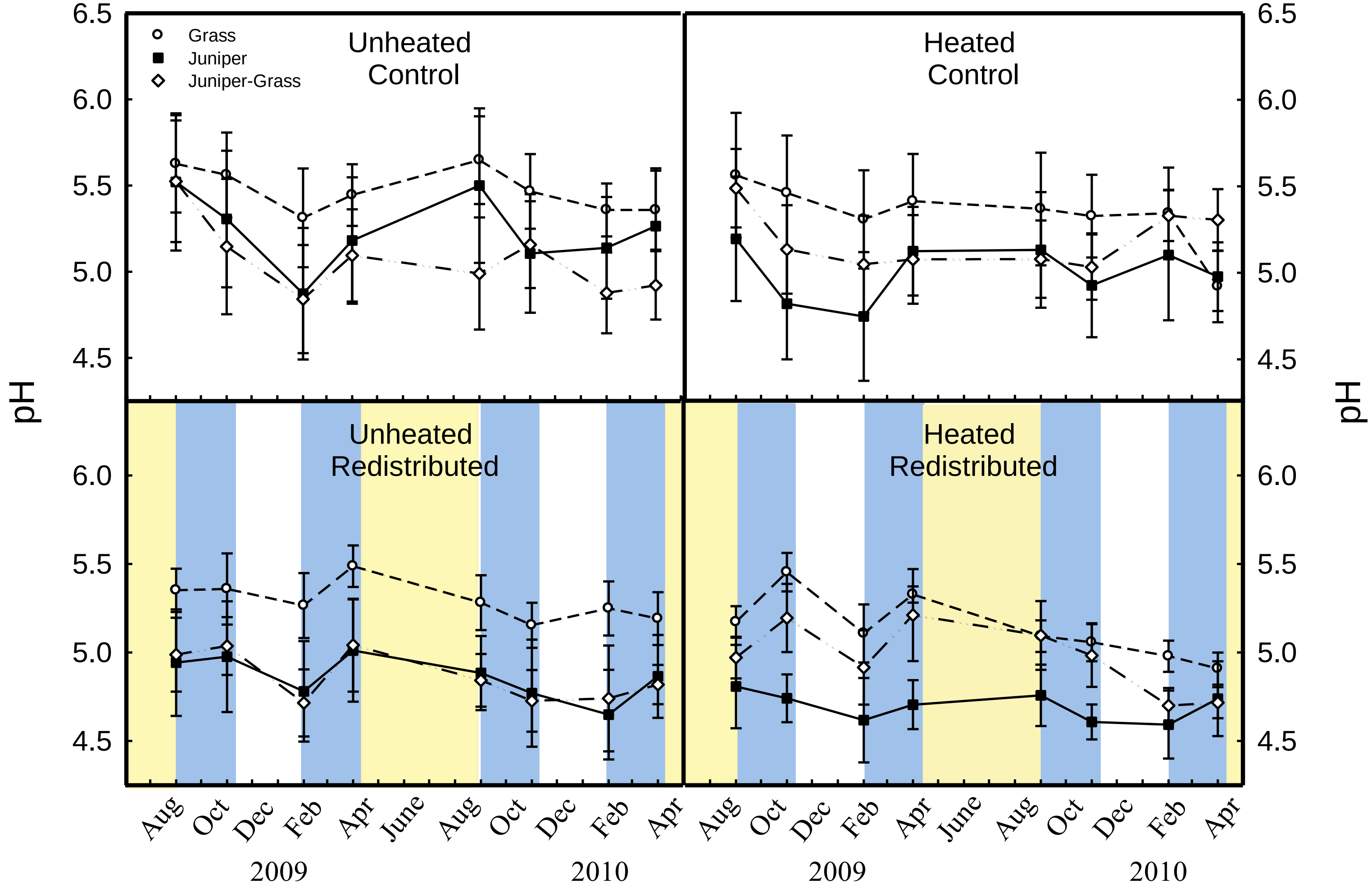

Fig. A.1 Mean pH across all plots from August 2008 to April 2010. For each point, n = 4, and error bars are ± SE. Yellow decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

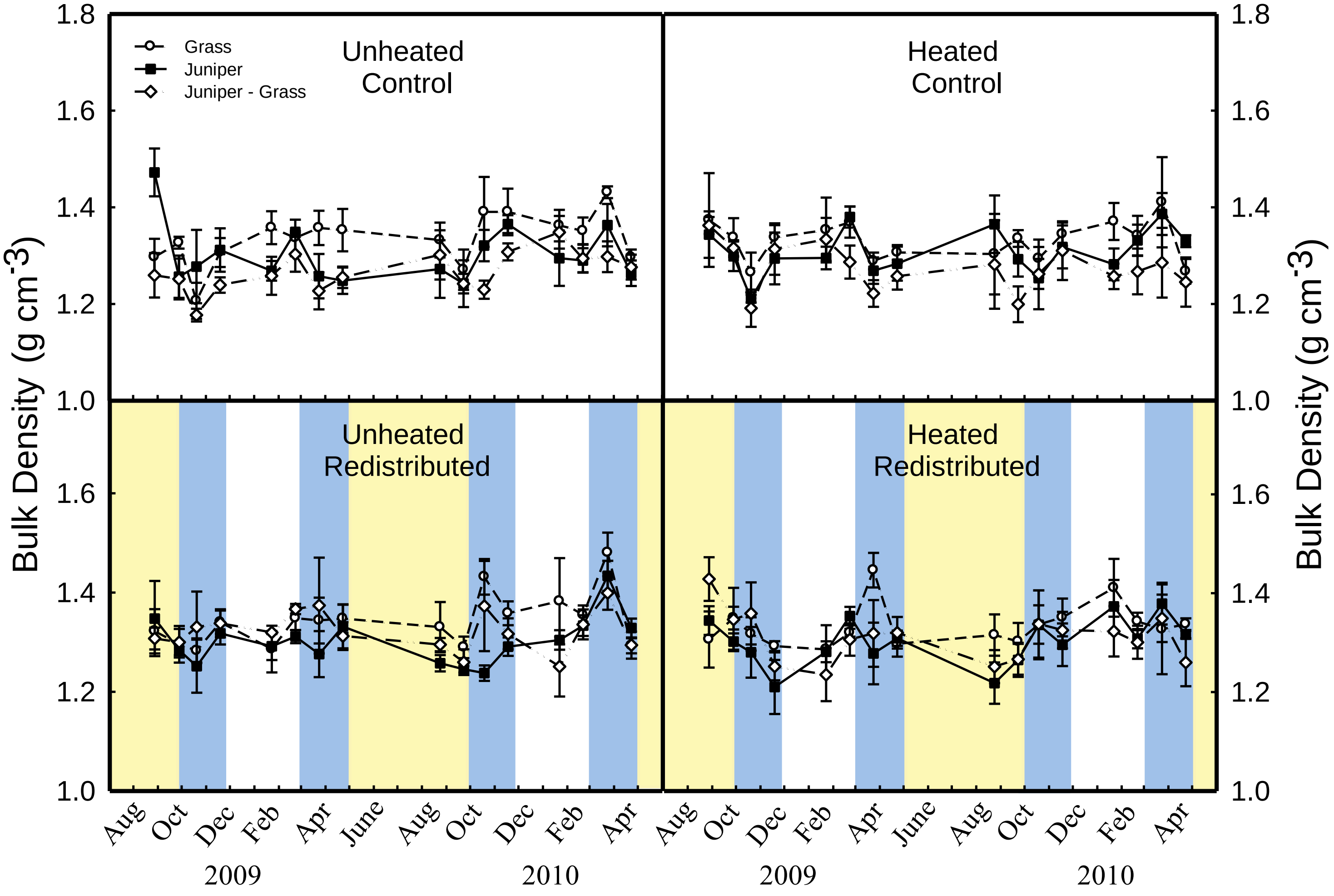

Fig. A.2 Mean bulk density across all plots from August 2008 to April 2010. For each point, n = 4, and error bars are ± SE. Yellow = decrease in rainfall (-40%). Blue = increase in rainfall (+52%). White = normal rainfall based on 50 year mean.

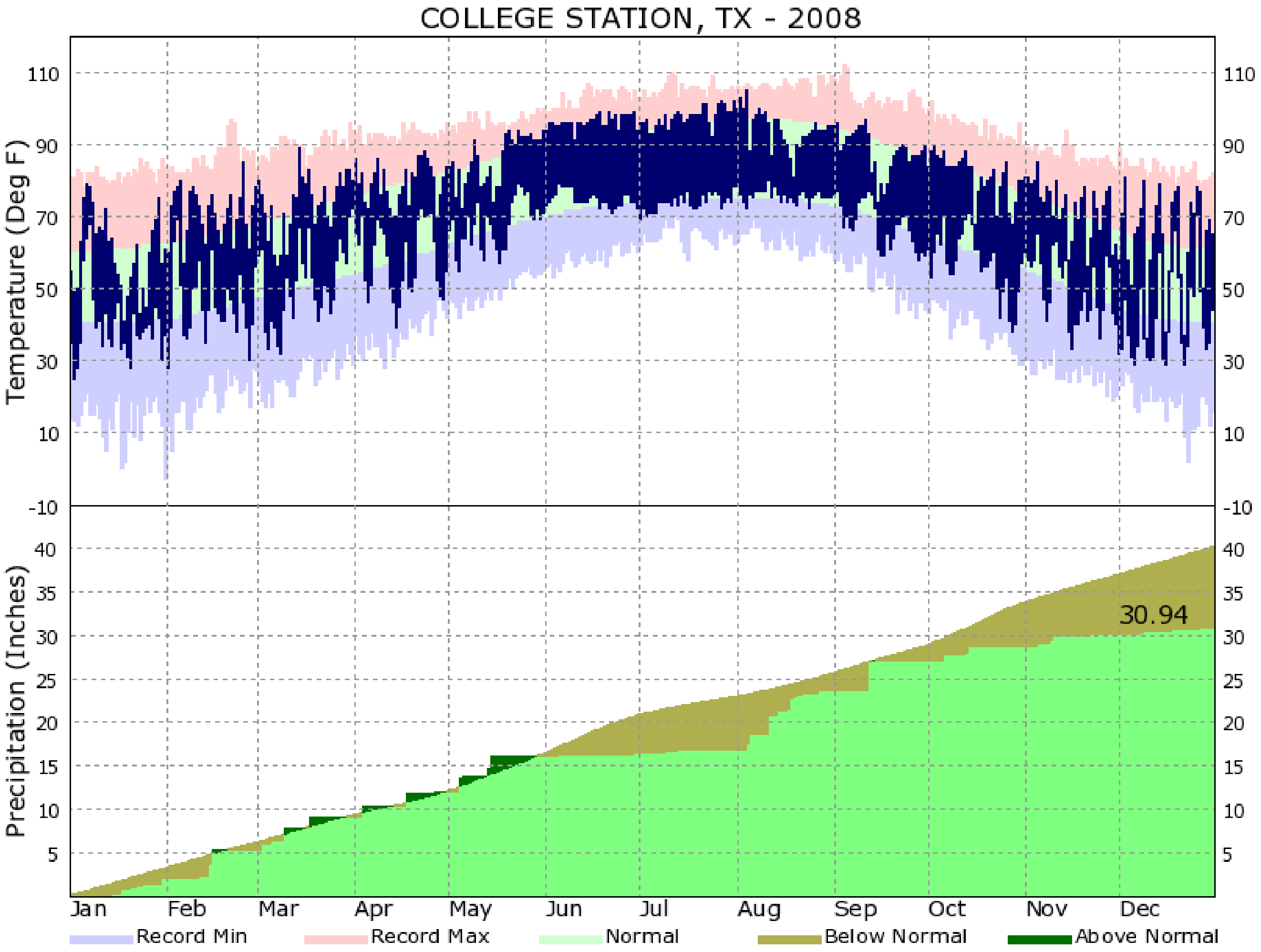

Fig. A.3 2008 air temperatures (°F in dark blue) and precipitation events (in inches) during our study (August 2008 – May 2010). Data taken from Easterwood Airport, a NOAA weather station located <0.5 km from the WaRM site (NOAA 2010).

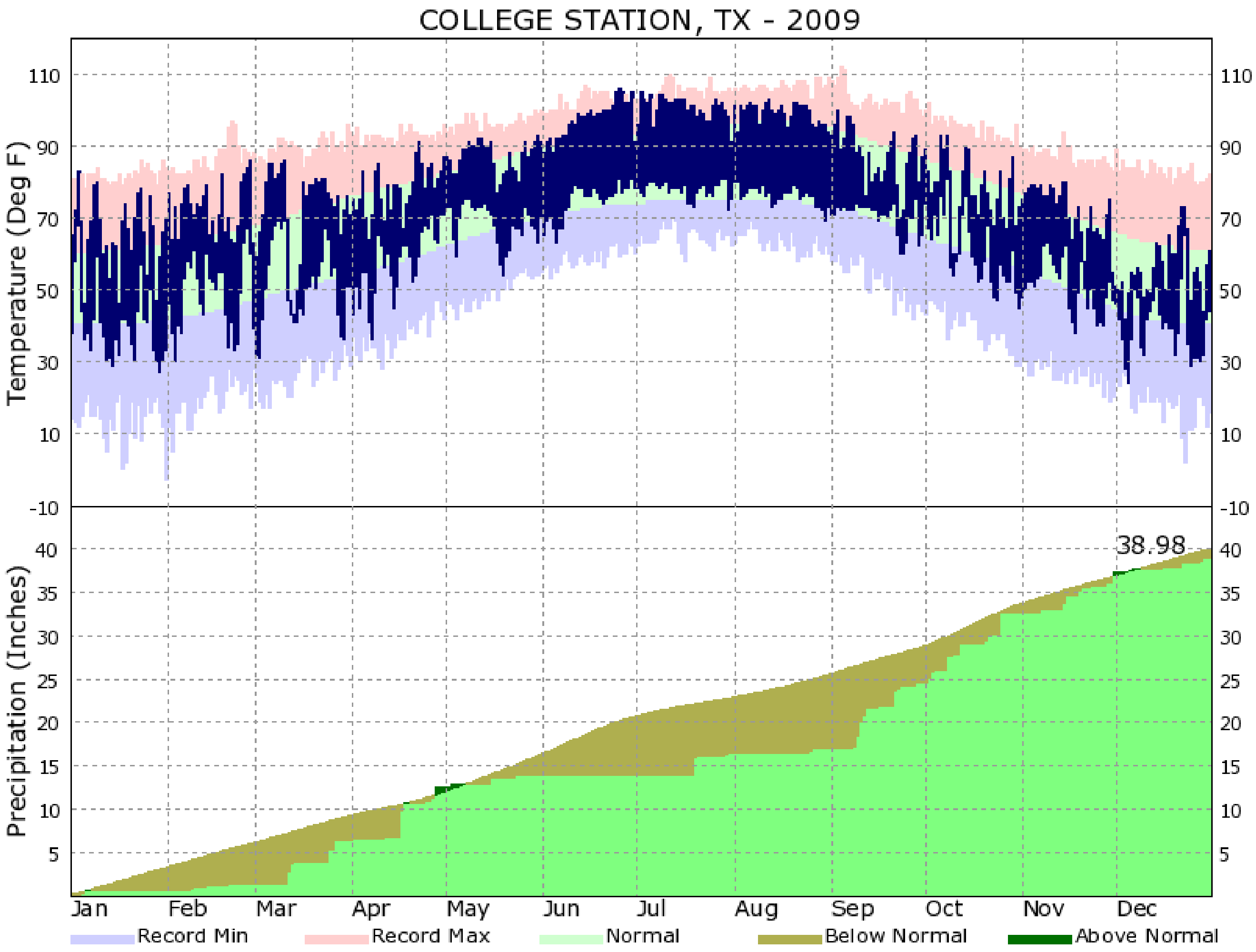

Fig. A.4 2009 air temperatures (°F in dark blue) and precipitation events (in inches) during our study (August 2008 – May 2010). Data taken from Easterwood Airport, a NOAA weather station located <0.5 km from the WaRM site (NOAA 2010).

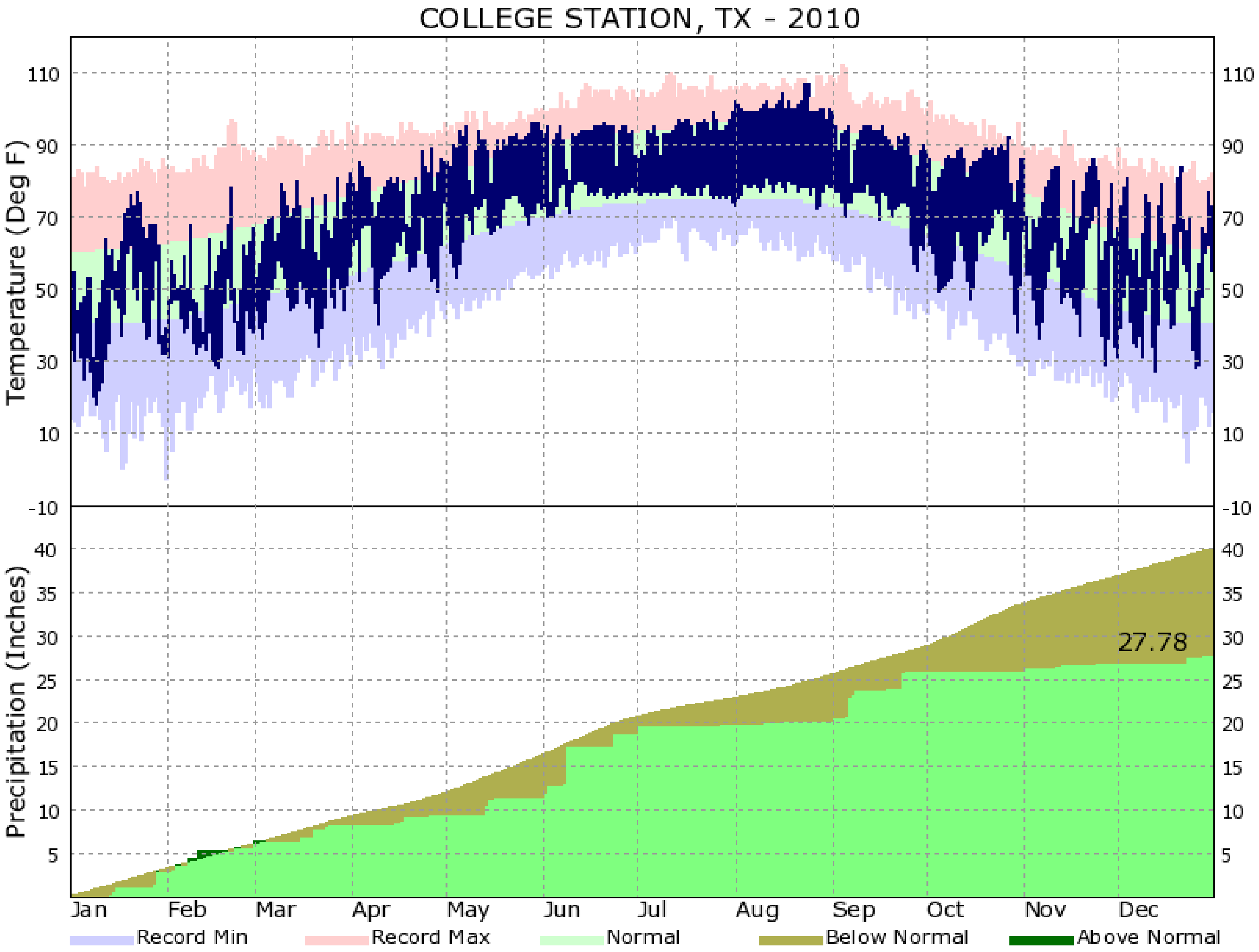

Fig. A.5 2010 Air temperatures (°F in dark blue) and precipitation events (in inches) during our study (August 2008 – May 2010). Data taken from Easterwood Airport, a NOAA weather station located <0.5 km from the WaRM site (NOAA 2010).

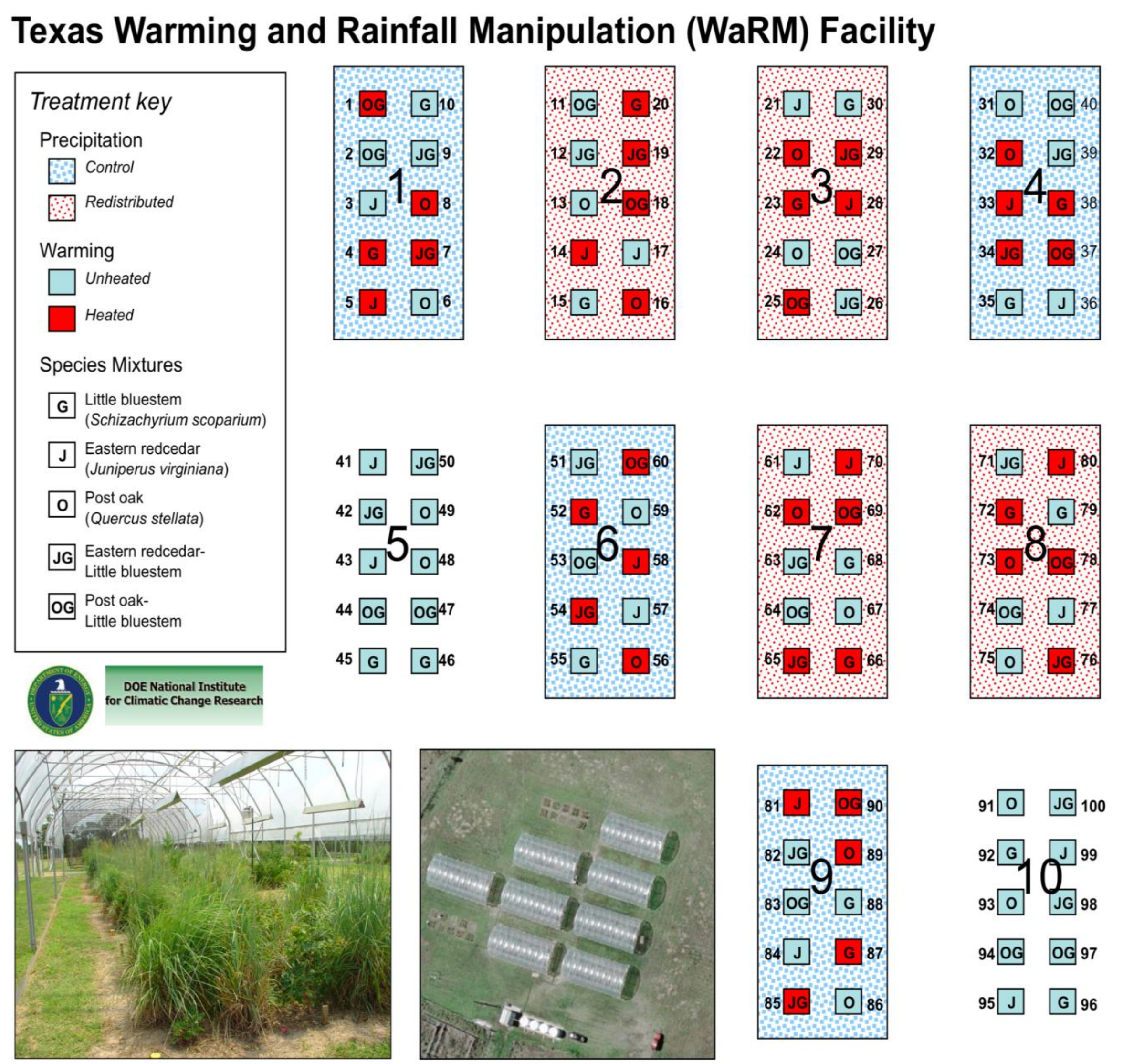

Fig. A.6 Layout of the DOE NICCR funded Texas A&M Warming and Rainfall Manipulation (WaRM) Site in College Station where eight 9x18m rainout shelters and two unsheltered controls were established in post oak savanna in 2003. Replicate (*n* = 4) annual rainfall redistribution treatments were applied at the shelter level (long term mean vs. 40% of summer redistributed to fall and spring with same annual total). Warming treatments (ambient vs. 24-hr IR canopy warming of 1-3°C) were applied to planted monocultures of the dominant tree (post oak and juniper) and grass (little bluestem) species and the two tree-grass combinations within each shelter in native soil. (Courtesy of Mark Tjoelker, Astrid Volder, and David Briske, used with permission)

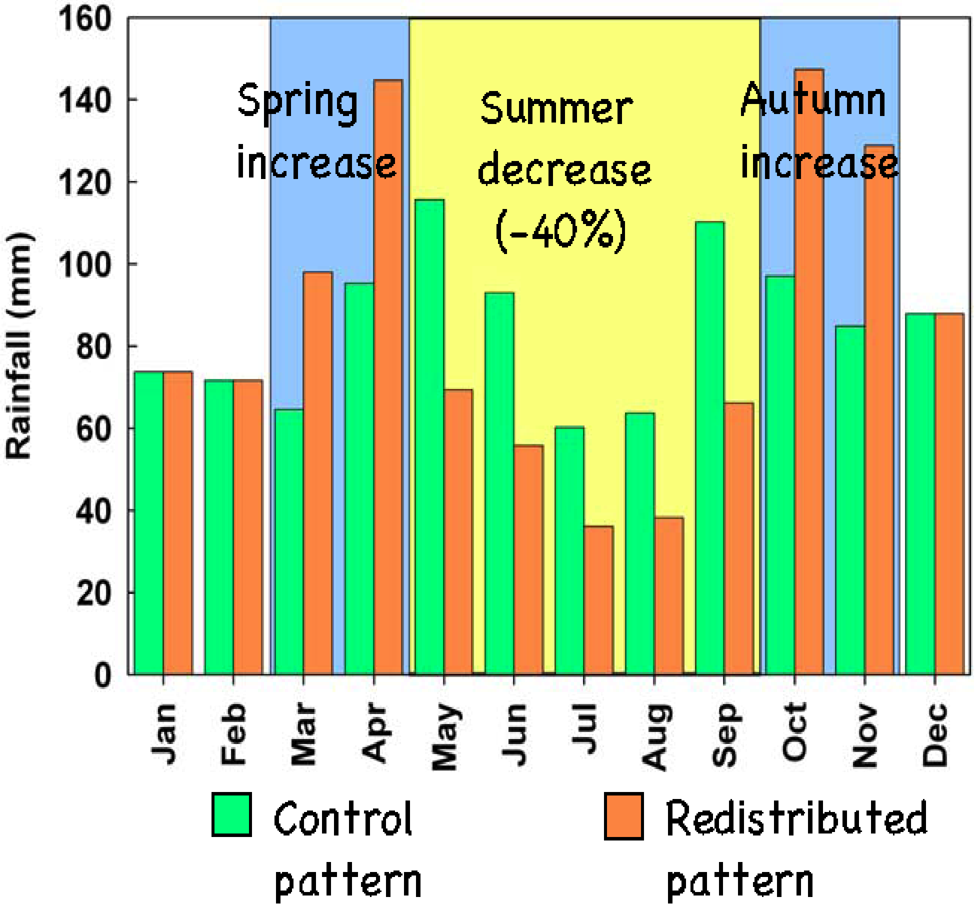

Fig. A.7 Monthly irrigation schedule in control (based on 50 year mean) and redistributed rainfall patterns in plots. Redistributed plots received 40% less in the summer (yellow), 52% more in spring and fall (blue), and normal rainfall during winter. (Courtesy of Mark Tjoelker, Astrid Volder, and David Briske, used with permission)

Fig. A.8 A view from within a shelter under the control rainfall regime after 5 years of growth at the Texas A&M WaRM research site, August 2008.

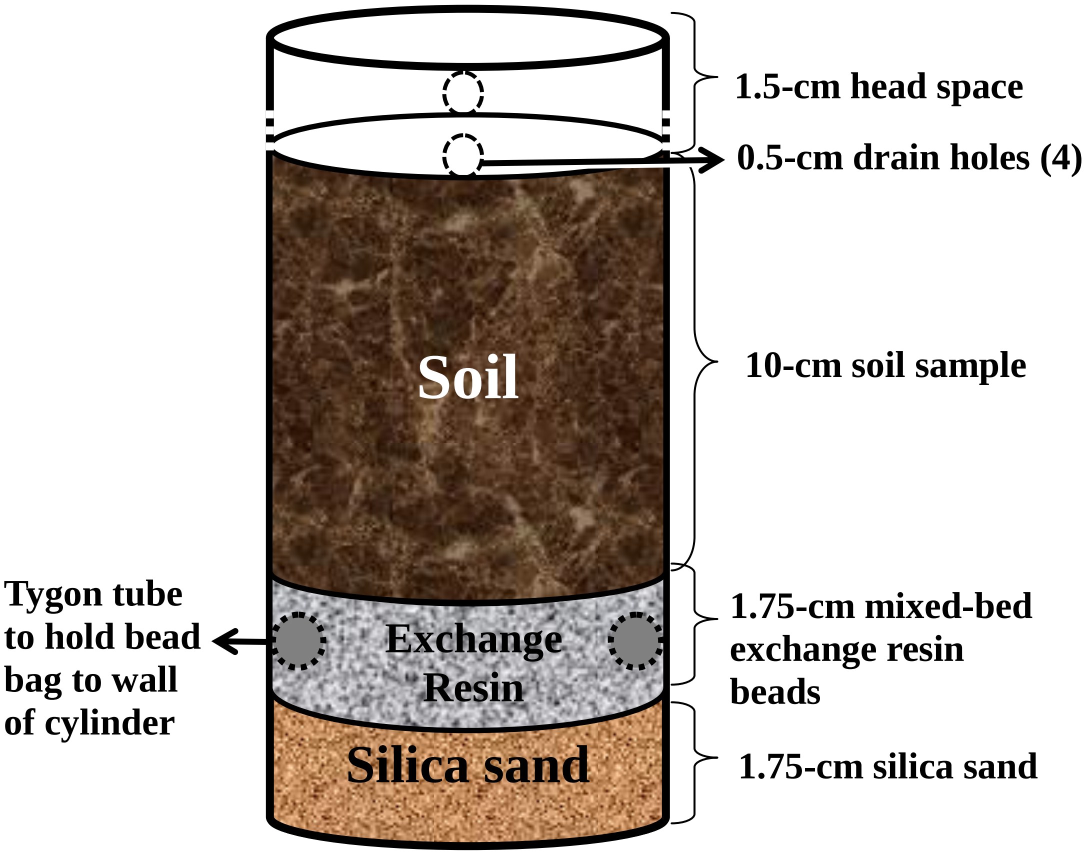

Fig. A.9 Diagram of the soil core incubation apparatus used to measure N mineralization and leaching as part of the WaRM research project at Texas A&M (drawing not to scale).

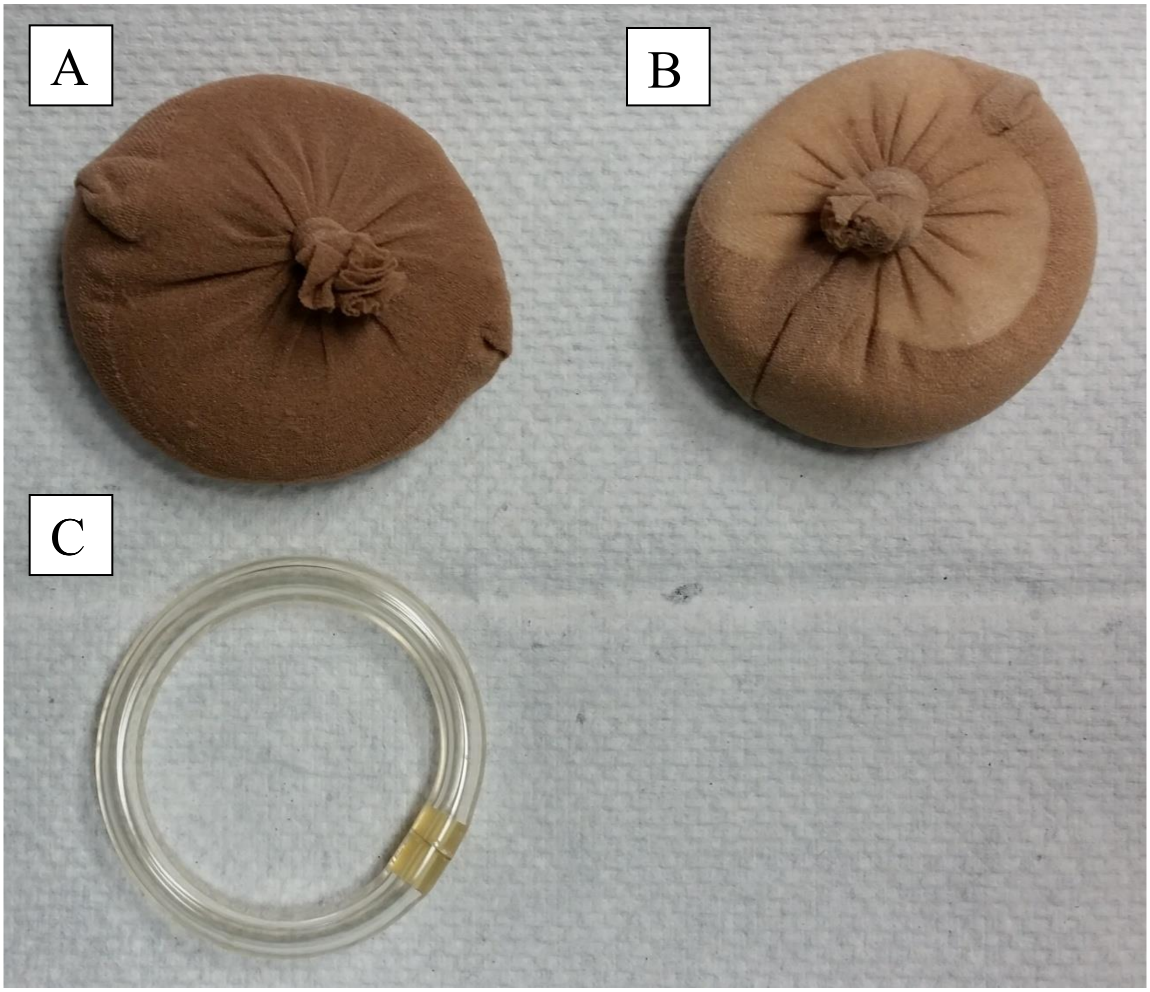

Fig. A.10 Materials used for determining N mineralization in soil cores incubated in situ (A) 30 g of mixed bed ion exchange resin beads (JT Baker Mixed Bed Exchange Resin IONAC NM-60) placed in a nylon mesh along with a circular Tygon plastic tube, (B) nylon mesh filled with 30 cm^3 of silica sand and knotted. (C) circular Tygon plastic tube to hold the resin bag against the wall of the core cylinder.

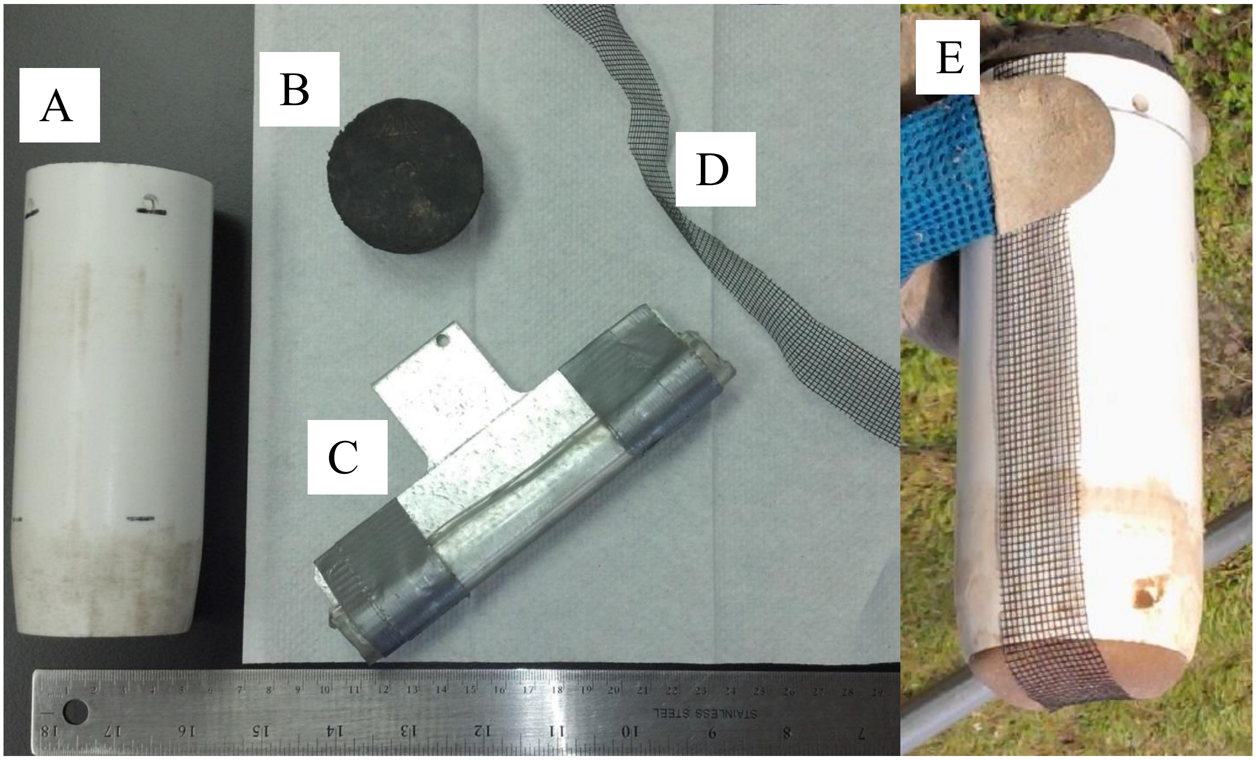

Fig. A.11 Apparatus for determining N mineralization using intact soil core incubated in situ within the shelter plots; (A) beveled PVC pipe (5.5 cm diameter x 10 cm) with drainage/retrieval holes, (B) #11 rubber stopper used to hold sample in PVC pipe while we installed ion exchange resin bead and silica sand bags at the bottom of the intact soil core, (C) metal and plastic tool created to dig out 3.5 cm of soil from the bottom of PVC pipe for resin and sand bag installation, (D) strip of fiberglass screen used to hold resin and sand bags in place while PVC core was placed back in its original position in the ground, and (E) a finished N mineralization incubation core ready for installation.

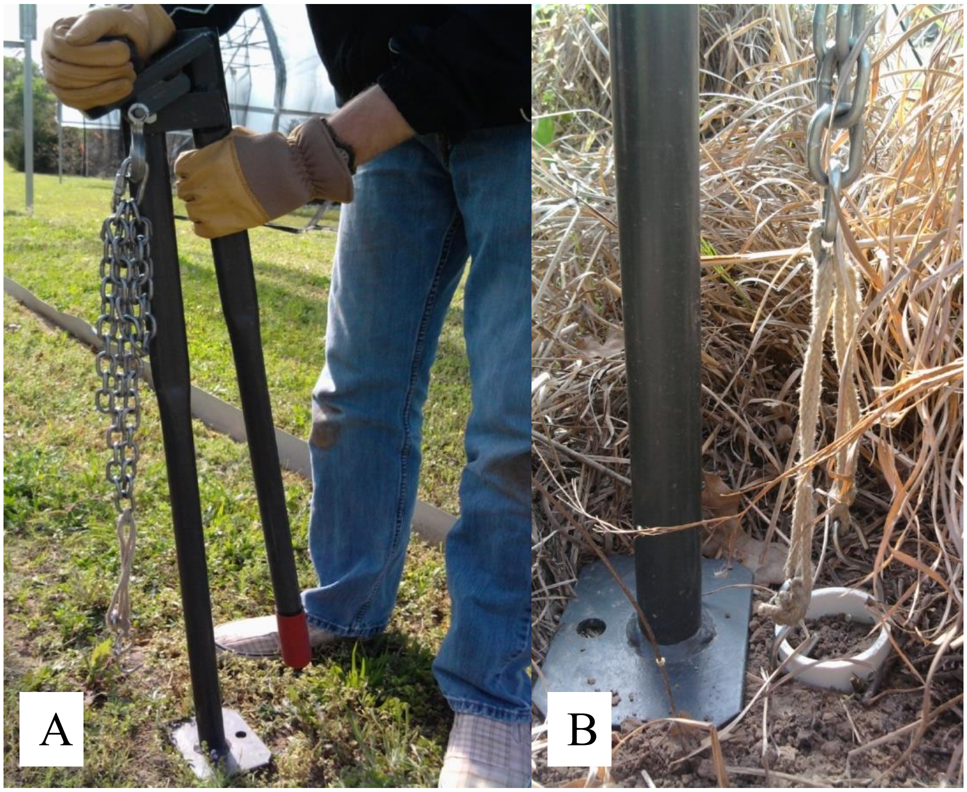

Fig. A.12 Tools used to install N mineralization incubation core; (A) T-post puller with rope, chain, and s-hooks designed to lift the apparatus (B) PVC cores from the ground without disturbing the sample or surrounding soil.