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RESPONSES OF BELOWGROUND CARBON DYNAMICS TO WARMING IN SOUTHERN GREAT PLAINS

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To my mother, Lijuan Mei.

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ABSTRACT

Compared with research on aboveground carbon (C) processes in grassland ecosystems, little is known on how belowground C processes would respond to projected global warming. In this dissertation, responses of belowground net primary productivity (BNPP) and its partitioning with respect to aboveground NPP (f_{BNPP}), soil organic C (SOC) pools, and the temperature sensitivity of SOC decomposition to warming were studied in a tallgrass prairie in the Southern Great Plains, USA. Infrared heaters were used to elevate soil temperature by approximately 2 °C since November 1999. Annual clipping was nested within warming treatment to mimic hay harvest. In the first study (BNPP, Chapter 2), I examined temporal patterns in responses of BNPP and NPP partitioning to experimental warming and annual clipping from 2005 to 2009. Over the years, warming increased BNPP by an average of 41.89% in the unclipped subplots and 66.93% in the clipped subplots, with significant increase observed in wet years. Clipping also had significant positive impact on BNPP, which was mostly found under warming. Overall, f_{BNPP}, the fraction of BNPP to NPP, increased under both warming and clipping treatments, more in dry years. Water availability (either precipitation or

soil moisture) was found to strongly dominate the interannual variability in NPP, f_{BNPP}, and their responses to warming and clipping, suggesting that water availability regulated tallgrass prairie's responses to warming and land use change, which may eventually influence the global C cycle.

In the second study (SOC pools, Chapter 3), the modified potassium permanganate ($KMnO_4$, 0.02 mol L^{-1}) procedure and combustion method were used to estimate chemically labile organic C (LOC) and SOC, respectively, from 2000 to 2008. Warming significantly decreased chemically labile organic C (LOC) by an average of 15.56%, but had little effect on SOC content. A two-stage variation in LOC responses to warming was observed, with no significant changes from 2000 to 2004 and significant decreases from 2005 to 2008. Lagged temporal dynamics of LOC (2 yrs) were asynchronously correlated with warming-increased C_4 proportion of aboveground biomass (AGB). These findings suggest that staged decreases in LOC under warming may be related to the increase in C_4 biomass through detrital inputs, making it difficult to predict the effect of warming on C cycling in this region.

In the third study (SOC decomposition, Chapter 4), the “C quality-temperature” hypothesis was tested in a 170-day laboratory experiment by incubating soil samples with changing temperature (low-high-low) at a ±5 °C step every 24 hours. I had four treatments of soil samples before lab incubation: control (C), warmed (W), field incubation (FI), and warmed plus field incubation (WFI). Results showed that SOC decomposition rate was positively influenced by LOC content under different treatments and lab incubation cycles. Field warming and field incubation increased the temperature sensitivity of SOC decomposition in the 1st two lab incubation cycles but the treatment effects diminished as decomposition proceeded. Q_{10} values of SOC decomposition increased with decreasing SOC quality. This relationship held across treatments and lab incubation cycles, regardless of whether the differences in SOC quality resulted from inherent differences in SOC chemistry or from differences in the extent of SOC decomposition. Treatment effects of field warming and field incubation on SOC quality and Q_{10} values were also negatively correlated with each other. These findings suggest that dynamics of low-quality SOC had the highest potential to impact long-term C stocks in soils, probably

resulting in a positive feedback of SOC to climate change in the future.

Keywords: warming, belowground net primary productivity (BNPP), NPP partitioning (f_{BNPP}), water availability, clipping, soil organic carbon (SOC), labile organic carbon, C_3-C_4 composition, carbon quality, SOC decomposition, temperature sensitivity, tallgrass prairie

Chapter 1

Belowground carbon dynamics: introduction

Globally, terrestrial ecosystems are considered to be a sink of atmospheric carbon (C) dioxide (CO_2) (Le Quéré et al., 2009). This is because photosynthesis assimilates 120 Pg C yr^{-1} from the atmosphere, which exceeds the 117 Pg C yr^{-1} released through autotrophic and heterotrophic respiration back to the atmosphere (Houghton, 2007). In the terrestrial environment, soil is the largest C pool (1500-2300 Pg C), approximately twice as large as the atmosphere pool (800 Pg C) and three times larger than the living plants pool (550 Pg C) (Dixon et al., 1994; Houghton, 2007). Relatively small changes in the amount of soil-stored C may therefore shift soils from being C sinks to sources of atmospheric CO_2, bringing about substantial effects on atmospheric CO_2 concentration and on global C cycling (Belay-Tedla et al., 2008).

Human activities, such as fossil fuel combustion and deforestation, have substantially altered the chemical composition of the global atmosphere. For instance, atmospheric CO_2 abundance has increased by 35% in the industrial era, with substantial implications for climate (IPCC, 2007). In response to the rising concentrations of greenhouse gases in the atmosphere, global mean temperature is predicted to increase 2-7

°C by the end of this century (Allison et al., 2009). Concerns have been raised on the role of soil as a potential C sink to buffer this greenhouse effect (Houghton, 2007; Cheng et al., 2011). It is likely that warmer temperatures may stimulate the decomposition of soil-stored C, accelerating global warming-the so called positive feedback (Bardgett, 2011). Conversely, a negative feedback may occur if the amount of C assimilated through photosynthesis exceeds the C loss through decomposition. Despite much research, a consensus has not yet been reached on whether we will have a positive feedback or not in the future, largely due to our limited knowledge on belowground C dynamics under warming.

Soil organic C (SOC) is almost exclusively derived from plants growing at a given site (Cheng et al., 2006). For plants, the belowground component represents more than half of plant productivity and plays an essential role in biogeochemical cycles (Aerts et al., 1992; McCulley et al., 2005; Gao et al., 2008). In grasslands, for instance, the proportion of belowground productivity can be as high as 86% (Hui and Jackson, 2005). Compared to the aboveground component, such as aboveground net primary productivity (ANPP) and leaf photosynthesis, however, belowground C dynamics is less studied (Milchunas and Lauenroth, 2001; Wu et al., 2011). Scientists have become aware that understanding the consequences of global warming for C cycling requires much more consideration of belowground C dynamics, such as C sequestration (e.g. Oren et al., 2001; Lal, 2004; Monson et al., 2006), the interannual variation of belowground NPP (BNPP), linkage between aboveground and belowground biota (Wardle et al., 2004; Bardgett and Wardle, 2010), and the temperature sensitivity of microbial decomposition of SOC (e.g. Fang et al., 2005; Davidson and Janssens, 2006; Craine et al., 2010). In 2004, for example, the journal Science launched a special issue entitled "Soils-The final Frontier", focusing on belowground ecological processes (e.g. Lal, 2004; Wardle et al., 2004).

Concerns about climate change and the subsequent long-term impact on the global C cycling have intensified research interest in BNPP and its fraction with respect to ANPP. BNPP could be the major input of organic matter to soil because of the periodical removal of aboveground biomass by mowing, fires, and grazing in grassland ecosystems (Briggs and Knapp, 1995; McNaughton et al., 1998). Additionally, belowground root systems play an important role in resource acquisition and biomass allocation (Gao et al., 2008), but we know little about it (Wu et al., 2011). For example, limited previous studies have shown that both the magnitude and the direction of BNPP varied in response to warming, with either increases (Piñeiro et al., 2009, Wu et al., 2011) or decreases (Lilley et al., 2001) having been reported. At the site level, factors, such as precipitation, soil moisture and temperature, and grazing may also influence the response of BNPP to warming (Sala et al., 1988; Gao et al., 2008).

Plant allocation of photosynthates between aboveground and belowground organs, usually defined as f_{BNPP}=BNPP/(ANPP+BNPP), is always represented by fixed coefficients in models (e.g. Friedlingstein et al., 1999; Wullschleger et al., 2001) because there are not enough data to model the factors controlling allocation in biogeochemical models (Hirsch et al., 2004). However, in the real world, allocation pattern vary. For instance, a study by Gao et al. (2008) in Inner Mongolia found that f_{BNPP} varied between 0.50 and 0.66 under grazing. In addition, a synthesis of field studies across the world's grasslands showed that f_{BNPP} ranged between 0.40 and 0.86 (Hui et al., 2005). The allocation is less well-understood and is still under debate (e.g. Enquist and Niklas, 2002; Shipley and Meziane, 2002), especially in relation to warming, leading to tremendous variation for either short-term forecasts or long-term projections of global NPP (Knorr and Heimann, 2001; Friedlingstein et al., 2006, Sitch et al., 2008). Therefore, empirical results from field manipulative experiments are of great importance to improve our knowledge of the direction and magnitude of BNPP and f_{BNPP} in response to warming at the community level.

Global warming is hypothesized to influence soil C through a variety of routes, both directly and indirectly (Fig. 1.1, Fissore et al., 2008; Bardgett, 2011). With respect to direct effects, many modeling studies predicted that C storage in soils may decrease because respiration is stimulated more than photosynthesis by warmer temperatures (Cox et al., 2000; Friedlingstein et al., 2006; Heimann and Reichstein, 2008). However, empirical studies have shown that the warming effect on soil-stored C varied greatly, with increases (Welker et al., 2004; Day et al., 2008; Sardans et al., 2008), no changes (Marchand et al.; 2004; Zhang et al., 2005; Luo et al., 2009; Schindlbacher et al., 2011), or decreases (Eliasson et al., 2005;

Oberbauer et a., 2007; Bradford et al., 2008; Schuur et al., 2009) being observed either in labile soil organic C (LOC) or SOC.

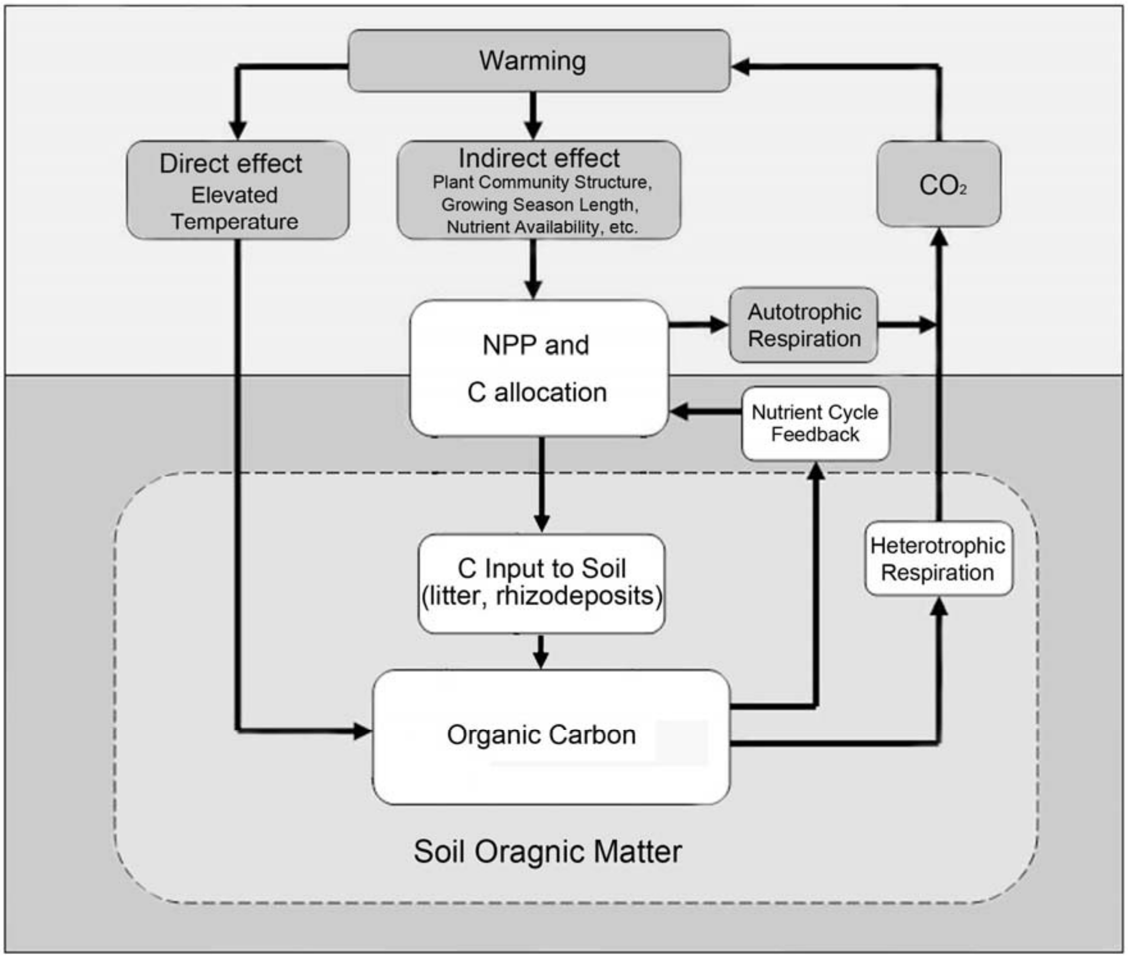

Figure 1.1 Direct and indirect warming effects on soil organic matter and on subsequent decomposition of SOC. NPP: net primary productivity. C: carbon. Adapted from Bardgett (2011).

Alternatively, the potential for global warming to have a strong indirect effect on soil-stored C is through changing plant community structure and composition, which is little studied (Fig. 1.1, Hobbie, 1996; Fissore et al., 2008). Changes in plant species composition under warming have been reported (Harte and Shaw, 1995; Walker et al., 2006; Luo et al., 2009) and Hobbie (1996) suggested that the changes are just as important as direct warming effects in altering C cycling (Hobbie, 1996). Plant species composition may alter C cycling through interspecific differences in the amount and quality of detrital inputs to soil (Day et al., 2008; Bardgett, 2011), rates of litter decomposition (Hobbie, 1996; Wynn and Bird, 2007), and nutrient release (Gosz et al., 1973; Hobbie, 1992). In general, we still have little understanding of how LOC and SOC may respond to warming and warming-induced changes in plant community structure.

Understanding the temperature sensitivity of SOC decomposition has received much interest due to its importance in the global C cycle and potential feedback to climate change (Davidson and Janssens, 2006). The temperature sensitivity of decomposition is often represented by Q_{10} values, the factor by which the respiration rate differs for a temperature interval of 10 °C (Bosatta and Ågren, 1999; Fang et al., 2005). Basic thermodynamic theory predicts that decomposition of high-quality SOC has a lower activation energy for enzymatic reactions and thus lower temperature sensitivity than that of low-quality SOC (Bosatta and Ågren, 1999). However, results from both empirical and modeling studies have paradoxically concluded that the decomposition of low-quality SOC is greater (Fierer et al., 2005; Conant et al., 2008; Wetterstedt et al., 2010; Xu et al., 2010), similar (Giardina et al., 2000; Fang et al., 2005; Conen et al., 2006), or less (Giardina and Ryan, 2000; Reichstein et al., 2000) sensitive to temperature than that of high-quality SOC. The response of SOC decomposition to current global warming is thus not clearly understood (Davidson and Janssens, 2006), preventing accurate parameterization of temperature effects on SOC decomposition (Cox et al., 2000; Friedlingstein et al., 2006) as well as quantitative prediction of the C balance between ecosystem C fixation and decomposition (Cox et al., 2000; Fierer et al., 2005; Conant et al., 2008).

The work in this dissertation addresses the effects of projected global warming on belowground C cycling, including BNPP, f_{BNPP}, LOC, SOC, and SOC decomposition, in a grassland ecosystem in the Southern Great Plains, USA. Grassland ecosystems, which accounts for approximately a quarter of global land cover (White et al., 2000; Scurlock et al., 2002) and 54% of the land cover of the conterminous USA (US Department of Agricultrue, 1972), play an important role in global C cycling. The results of this research may help scientists, politicians, farmers, and the general public to better understand the implications of global climate change on ecosystem processes, especially changes in belowground C cycling. Additionally, these results could be useful for modelers in understanding and implementing local ecosystem processes and feedbacks into regional and global scale models.

My research was conducted in a tallgrass prairie in Oklahoma, which has been subjected to continuous warming (+2 °C) since November 1999. Specifically, three objectives are explored in this research. The first objective (Chapter 2) is to explore temporal responses of BNPP and NPP partitioning to warming and clipping from 2005 to 2009. Annual clipping, a widely practiced land use in the Southern Great Plains of the USA, was a minor factor nested within experimental warming. The second objective (Chapter 3) is to examine effects of experimental warming on the labile and whole SOC pools in association with vegetation changes from 2000 to 2008. The third objective (Chapter 4) is to test the "C quality-temperature" hypothesis by conducting a 170-day laboratory incubation of soil samples with changing temperatures (low-high-low) at a ±5 °C step every 24 hours. Finally, Chapter 5 presents the conclusions of this dissertation and discussion of future research needs in global climate change. Chapter 2, 3, and 4 are developed for peer-review publication.

Chapter 2

Interannual variability in responses of belowground NPP and NPP partitioning to long-term warming and clipping in a tallgrass prairie[1]

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ABSTRACT

The dynamics of belowground net primary productivity (BNPP) is of fundamental importance in understanding carbon (C) allocation and storage in grasslands. However, our knowledge of the interannual variability in response of BNPP to ongoing global warming is limited. In this study, we explored temporal responses of BNPP and NPP partitioning to warming and clipping in a tallgrass prairie in Oklahoma, USA. Infrared heaters were used to elevate soil temperature by approximately 2 °C since November 1999. Annual clipping was to mimic hay harvest. On average from 2005 to 2009, warming increased BNPP by 41.89% in the unclipped subplots and 66.93% in the clipped subplots, with significant increase observed in wet years. Clipping also had significant positive impact on BNPP, which was mostly found under warming. Overall, f_{BNPP}, the fraction of BNPP to NPP, increased under both warming and clipping treatments, more in dry years. Water availability (either precipitation or soil moisture) was the most limiting factor for both BNPP and f_{BNPP}. It strongly dominated the interannual variability in NPP, f_{BNPP}, and their responses to warming and clipping. Our results suggest that water availability regulates tallgrass prairie's responses to warming and land use change, which may eventually influence the global C cycle. With increasing variability in future precipitation patterns, warming effects on the vegetation in this region may become less predictable.

Keywords: belowground net primary productivity, NPP partitioning, warming, water availability, clipping, tallgrass prairie

2.1 Introduction

Net primary productivity (NPP), a key variable of terrestrial ecosystems, is the primary driver of the global carbon (C) cycling (Ni, 2004; Hui and Jackson, 2005). The projected global warming of between 2 and 7 °C by the end of this century (Allison et al., 2009) is anticipated to significantly influence NPP by altering biogeochemical processes such as plant photosynthesis and microbial mineralization of soil organic matter (SOM). Therefore, understanding the sensitivity of NPP to projected global warming is of high priority for both empirical studies and future model development. However, how NPP would respond to warmer temperatures is limited by our scarcity of knowledge on belowground NPP (BNPP), which largely inhibits progress in validating global NPP models and projecting future ecosystem function under climate change (Cramer et al., 1999).

BNPP represents more than one-half of NPP in grasslands and is the major input of organic matter into soil (Briggs and Knapp, 1995; McNaughton et al., 1998; Luo et al., 2009). Understanding BNPP dynamics is fundamentally important to improve our knowledge of C allocation and storage in grasslands. However, knowledge of BNPP is still quite limited in comparison to reasonably well-understood ANPP (above-ground NPP), largely due to the methodological difficulties associated with observing and measuring root biomass (Milchunas and Lauenroth, 2001; Wu et al., 2011). Experimental estimates of the magnitude and direction of BNPP change are still few, especially from field manipulative experiments (Long et al., 1989; Scurlock et al., 2002; Wu et al., 2011). The limited available measurements of BNPP in response to projected global warming have resulted in both increases (Piñeiro et al., 2009, Wu et al., 2011) and decreases in BNPP

(Lilley et al., 2001). The lack of consistency is not surprising because the responses of BNPP to warming can be regulated by many factors at the site level, such as water availability (Li et al., 2011), and plant species composition (Gao et al., 2008). However, our understanding of temporal patterns of BNPP and its responses to climate change are much weaker compared with studies on aboveground NPP (ANPP). Therefore, examining the interannual variability of BNPP and its responses to climate change is critical to understand the driving factors in controlling the BNPP in response to future global warming.

The partitioning of BNPP with respect to ANPP, commonly defined as f_{BNPP}, is a central issue in plant ecology and evolution, which, however, remains controversial (e.g. Enquist and Niklas, 2002; Shipley and Meziane, 2002). From a physiological perspective, f_{BNPP} may reflect the cumulative responses of plants to environmental changes. Optimal partitioning theory suggests that plants allocate biomass among different organs in response to environmental changes to maximize their growth through efficient capture of resources, such as nutrients and water (Bloom et al., 1985; Chapin et al., 1987). However, how f_{BNPP} would respond to ongoing global warming is still unclear, making quantifying f_{BNPP} important for modeling studies (Hui and Jackson, 2005). In ecosystem C modeling, f_{BNPP} provides important constraints for the calibration and testing of dynamic C-cycling models (Ågren and Franklin, 2003; Hui and Jackson, 2005). However, it is often held constant in the majority of global terrestrial models when estimating root C stocks (e.g. Friedlingstein et al., 1999; Wullschleger et al., 2001) due to the lack of reliable f_{BNPP} estimates. Therefore, results from field manipulative experiments which can shed light on the direction and magnitude of f_{BNPP} in response to warming at community level are badly needed.

The responses of BNPP and f_{BNPP} to climate warming may vary strongly with land use practices (McNaughton et al., 1998; Gao et al., 2008). Clipping to mimic hay harvest, for example, a widely practiced land use in the Southern Great Plains of the USA, may further confound warming effects on BNPP and f_{BNPP}. In Oklahoma, hay production occupies 3.25 million acres, nearly as much as wheat (US Department of Agriculture, National Agricultural Statistic Service). Clipping may have considerable effects on BNPP because it directly takes aboveground biomass away from the ecosystem, influencing f_{BNPP} by breaking down inherent allocation rules. While water availability, temperature, nitrogen (N) availability, and grazing are reported to affect BNPP (e.g. Gower et al., 2001; Ni, 2004; Gao et al., 2011; Li et al., 2011), field-based BNPP data in response to haying or biofuel feedstock harvest is still lacking, making the prediction of potential ecosystem-level responses to future climate change in grasslands difficult.

Grassland ecosystems occupy approximately a quarter of the global land cover and hold 10% of the global C stocks, thus playing an important role in global C cycling (Scurlock et al., 2002). Grasslands with long-term experimental warming and clipping treatments offer an unique opportunity to address potential responses of BNPP to global warming (Hui and Jackson, 2005), because (1) most natural grasslands are potentially sensitive to climate change and land use practices (Mitchell and Csillag, 2001; Luo et al., 2009; Niu et al., 2010), and (2) belowground biomass of grasslands is easier to measure compared to that of forests (Gill et al., 2002). The specific objectives of this study were to: (1) explore the effects of warming and clipping on BNPP and f_{BNPP}; and (2) examine the driving factors in controlling temporal responses of BNPP and f_{BNPP} to warming and clipping in a tallgrass prairie on the Great Plains of the USA.

2.2 Materials and Methods

2.2.1 Experimental site and design

The experimental site is located on the Kessler Farm Field Laboratory in Oklahoma, USA (34°59•N, 97°31•W). The site has never been cultivated and has not been grazed for the past 40 years. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans*) and C_3 forbs (*Ambrosia psilostachyia*, *Solidago rigida*, and *Solidago nemoralis*). Mean annual temperature is 16.3 °C and mean annual precipitation is 914 mm (Oklahoma Climatological Survey, Norman, OK, USA). The soil is part of the Nash-Lucien complex with neutral pH, high available water holding capacity (around 37%), and a moderately penetrable root zone (US Department of Agriculture, 1979).

The experiment uses a paired factorial design with warming as the main factor nested by a clipping factor (Luo et al., 2001; Wan et al., 2005; Niu et al., 2010). Each treatment has six replicates (i.e. six pairs of plots). Each pair has two plots of 2 m × 2 m. One plot has been subjected to continuous warming since 21 November 1999 to the present while the other serves as the control with ambient temperature. Infrared heaters (165 cm × 15 cm; Kalglo Electronics, Bethlehem, PA, USA) having a radiation output of 100W m^{-2} are suspended 1.5 m above the ground in each warmed plot. The control plot has a ‘dummy’ heater with the same dimensions as the infrared heater suspended at a similar height to mimic the shading effects of the heater. Temperature increments generated by the infrared heaters are relatively even over the entire area of the plots and similar at different soil depths (Wan et al., 2002). For each pair of plots, the distance between warmed and control plots is approximately 5 m from centers to avoid heating of the control plots. The distances between the paired plots vary from 20 to 60 m.

Each 2 m × 2 m plot is divided into four 1 m × 1 m subplots. Plants in two diagonal subplots were clipped at a height of 10 cm above the ground once a year to mimic hay harvesting while the other two subplots are unclipped. Clipped materials were taken away and not returned back to the plots. Thus, this experiment has four treatments: unclipped and control (ambient) temperature (UC), unclipped and warming (UW), clipped and control temperature (CC), and clipped and warming (CW).

2.2.2 Temperature, moisture, and precipitation measurements

Air temperature and soil temperature were measured by thermocouples at the height of 25 cm above the ground and at a depth of 2.5 cm in the center of one clipped and one unclipped subplot. Volumetric soil water content (%V) in the top 15 cm was measured twice monthly using portable Time Domain Reflectometry (TDR) equipment (Soil Moisture Equipment Crop., Santa Barbara, CA, USA). Precipitation data was obtained from an Oklahoma Mesonet Station (Washington Station) located approximately 200 m away from our experimental site. Detailed information of these measurements can be found in Luo et al. (2009).

2.2.3 BNPP measurement and f_{BNPP} estimation

From 2005 to 2009, the root ingrowth-core method was applied to estimate BNPP (Derner and Briske, 1999; Gao et al., 2008). Soil cores (5.2 cm in diameter) of 0-45 cm depth were taken from the same spot in one unclipped and one clipped subplot in each plot every year since 2006. The cores were immediately filled with sieved root-free soil originating from the same depth (0-15, 15-30, 30-45 cm) outside of the plots. Separated soil cores were put into plastic bags, transported in several coolers to the Ecolab at the University of Oklahoma, Norman, and stored at -30 °C before analyzing. Root samples were carefully washed by wet sieving (0.5 mm) under gently flowing water to remove attached soil and dark-brown/black debris, oven-dried at 70 °C for 48 hr, and weighted to calculate BNPP. f_{BNPP} is calculated as: f_{BNPP}=BNPP/(ANPP+BNPP) (Hui and Jackson, 2005). ANPP datasets were obtained from Luo et al. (2009) in 2005 and from Luo et al. (unpublished data) from 2006 to 2009. A detailed description on ANPP estimation and ANPP data from 2005 to 2009 are provided in appendix A. Rain use efficiency (RUE) is defined as the slope of the relationship between BNPP and precipitation (Le Houerou, 1984).

2.2.4 Statistical analysis

Repeated-measures ANOVA were used to examine the main and interactive effects of experimental warming, clipping, and year on BNPP and f_{BNPP}. Within each year, one-way ANOVA was performed to analyze the differences of the above parameters among the four treatments. Linear regression analyses were used to evaluate the relationships of the above parameters with climatic factors. All statistical analyses were conducted using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA).

2.3 Results

2.3.1 Microclimate

Annual precipitation varied from 515 mm in 2004 to 1307 mm in 2007 with a mean of 879 mm during the 6 years (Table 2.1). Air temperature was elevated by experimental warming by an average of 1.38 °C with a two-fold variation from 0.99 °C in 2008 to 2.22 °C in 2005. Experimental warming also significantly increased soil temperature by an average of 1.45 °C in unclipped subplots and 2.49 °C in clipped subplots, respectively, from 2005 to 2009. Increase in both air and soil temperature was higher in relatively dry years (2006 and 2007) than in wet years (2008-2010). Over the years, volumetric soil moisture was lowered by an average of 1.89% in the unclipped subplots and 2.05% in the clipped subplots, respectively.

Table 2.1 Precipitation (AP, PPT*), mean air and soil temperature (T_{air}, T_{soil}), and mean soil moisture (W_{soil}) under four treatments.

Variable	Treatment	2004	2005	2006	2007	2008	2009	Average
AP (mm)		966	515	744	1307	726	1017	879
PPT* (mm)			741	630	1026	1017	872	857
T_{air}	C		16.78	17.12	16.58	16.65	16.77	16.78
(°C)	W		19.00	18.66	17.61	17.64	17.89	18.16
	W-C		2.22*	1.54*	1.03*	0.99*	1.12*	1.38*
T_{soil}	UC		16.91	17.31	16.78	16.14	16.74	16.78
(°C)	UW		18.95	19.50	18.15	17.22	17.34	18.23
	UW-UC		2.04*	2.19*	1.37*	1.08*	0.60*	1.45*
	CC		17.54	17.86	17.06	16.03	17.04	17.11
	CW		20.23	20.60	19.45	18.19	19.55	19.60
	CW-CC		2.69*	2.74*	2.39*	2.16*	2.51*	2.49*
W_{soil}	UC		24.77	23.50	29.94	26.96	26.55	26.34
(°C)	UW		21.7	21.95	28.74	25.21	24.63	24.45
	UW-UC		-3.07*	-1.55	-1.20	-1.75	-1.92*	-1.89
	CC		24.10	23.14	29.67	26.05	26.02	25.80
	CW		20.92	20.92	28.58	24.35	23.98	23.75
	CW-CC		-3.18*	-2.22*	-4.09	-1.70	-2.04*	-2.05*

Note: C: control (ambient) temperature, W: warmed; U: unclipped, C: clipped. AP: annual precipitation; PPT*: average of precipitation of the current and previous year, for example, $PPT^*_{2008} = (AP_{2008}+AP_{2007})/2$. Asterisks (*) indicate significant warming effect at $P<0.05$.

2.3.2 Warming and clipping effects on BNPP

BNPP varied from 255.06±25.57 in 2006 to 335.35±39.85 g m^{-2} in 2007 in UC from 2005 to 2009 (Fig. 2.1a). The warming effect on BNPP was significant over the years ($P<0.001$, Table 2.2) and from 2006 to 2010 (Fig. 2.1b). On average, it increased BNPP by 41.89% and 66.93% in the unclipped and clipped subplots, respectively (all $P<0.05$, Fig. 2.1a, inset). Experimental warming significantly interacted with year in influencing BNPP ($P<0.05$, Table 2.2; Fig. 2.1b), with higher stimulation in wet years than in dry years. No significant relationship was found between the warming effect on BNPP and the warming-induced changes in soil temperature and soil moisture (all $P>0.05$). Thus we cannot say that either temperature or soil moisture is more important than the other. Clipping also showed significant impacts on BNPP from 2005 to 2009 ($P=0.003$, Table 2.2) and increased BNPP by an average of 7.94% in the unwarmed plots ($P>0.05$) and 26.99% in the warmed plots ($P<0.05$, Fig. 2.1a, inset). The interactive effects of clipping and year had little impact on BNPP ($P=0.442$, Table 2.2).

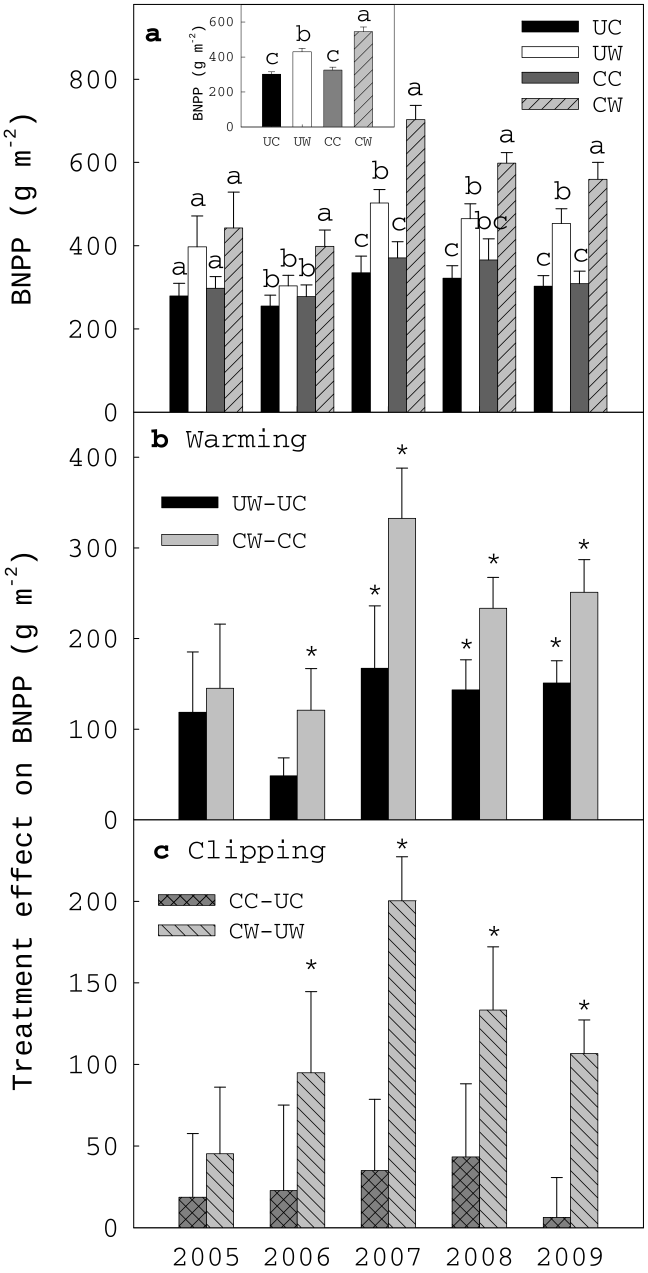

Figure 2.1 Variation in BNPP under four treatments (a) and changes in BNPP induced by warming (b) and clipping (c) from 2005 to 2009. Values are mean±SE (n=6). Inserted figure in panel a shows the average values of BNPP under four treatments over the years, values are mean±SE (n=30). Different letters indicate

statistically significant difference between treatments at *P*<0.05. Asterisks (*) represent statistically significant warming or clipping effects on BNPP at *P*<0.05.

Table 2.2 Results of repeated-measures ANOVA (*P* values) for responses of BNPP and f_{BNPP} to warming (W), clipping (CL), year (Y), and their interactions (n=6). *P* values smaller than 0.05 and 0.08 are in bold and italic, respectively.

Factor	Warming	Clipping	Year	W×CL	W×Y	CL×Y	W×CL×Y
BNPP	**<0.001**	**0.003**	**<0.001**	**0.042**	**0.025**	0.442	0.420
f_{BNPP}	**0.038**	**0.001**	*0.076*	0.477	0.848	**0.003**	0.232

2.3.3 Warming and clipping effects on f_{BNPP}

f_{BNPP} varied from 0.52±0.02 in 2005 to 0.60±0.02 in 2009 in UC across the 2005-2009 growing seasons (Fig. 2.2a). Both warming and clipping had significant effects on f_{BNPP} (all $P<0.05$, Table 2.2). For example, warming increased f_{BNPP} from 0.58±0.02 to 0.60±0.01 ($P=0.20$) in the unclipped subplots and from 0.64±0.02 to 0.68±0.02 ($P<0.05$) in the clipped subplots (Fig. 2.2a, inset). Clipping increased f_{BNPP} from 0.58±0.02 to 0.64±0.02 and from 0.60±0.01 to 0.68±0.02 in unwarmed and warmed subplots, respectively (all $P<0.05$, Fig. 2.2a, inset). The interaction of warming and year had little effect on f_{BNPP} ($P=0.848$, Table 2.2). Clipping significantly interacted with years in influencing f_{BNPP}, with a higher increase in the dry years ($P=0.003$, Table 2.2; Fig. 2.2c).

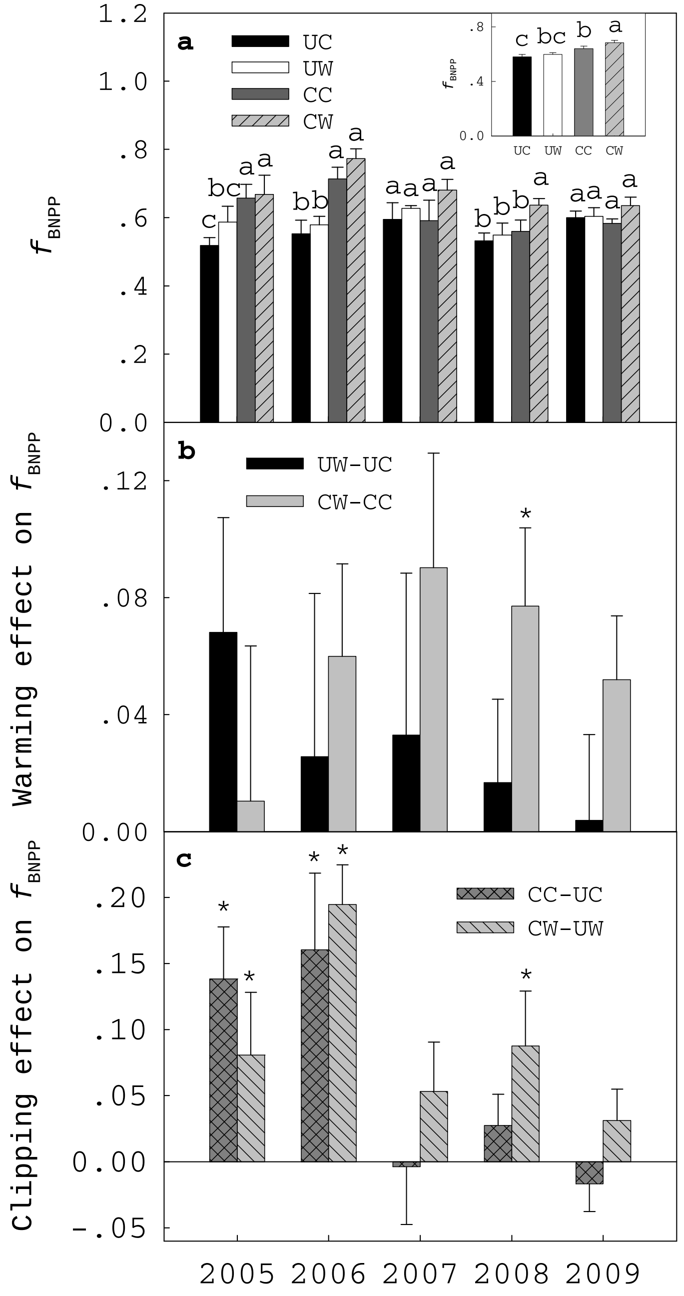

Figure 2.2 Variation in f_{BNPP} under four treatments (a) and changes in f_{BNPP} induced by warming (b) and clipping (c) from 2005 to 2009. Values are mean±SE (n=6). Inserted figure in panel a shows average values of f_{BNPP} under four treatments over the years, values are mean±SE (n=30). Different letters indicate

statistically significant difference between treatments at $P<0.05$. Asterisks (*) represent statistically significant warming or clipping effects on BNPP at $P<0.05$.

2.3.4 Relationships of BNPP and f_{BNPP} with climatic factors

BNPP significantly correlated with precipitation across the five years under each of the four treatments (all $P<0.05$, Fig. 2.3a, b). Warming increased the slopes of BNPP vs precipitation (equivalent to rain use efficiency) both with and without clipping (all $P<0.05$, Fig. 2.3c). Generally, the effects of precipitation and soil moisture on warming-induced changes in BNPP were positive while that of air temperature were negative (Fig. 2.4). For clipping-induced changes in f_{BNPP}, negative effects of precipitation and soil moisture and positive effect of air temperature were observed (Fig. 2.5). Multifactor linear regression showed that water availability, either PPT^* (average of precipitation of the current and previous year) or soil moisture, was the dominant climatic factor in controlling interannual variation in warming-induced changes in BNPP (r^2=0.53, P=0.017) and clipping-induced changes in f_{BNPP} (r^2=0.59, P=0.009). PPT*, rather than annual precipitation or the previous year's precipitation, significantly correlated with variation in BNPP, warming-induced changes in BNPP, and clipping-induced changes in f_{BNPP} (Fig. 2.3a, b, Fig. 2.4a, Fig. 2.5a), indicating previous year's precipitation had much influence on BNPP and f_{BNPP}.

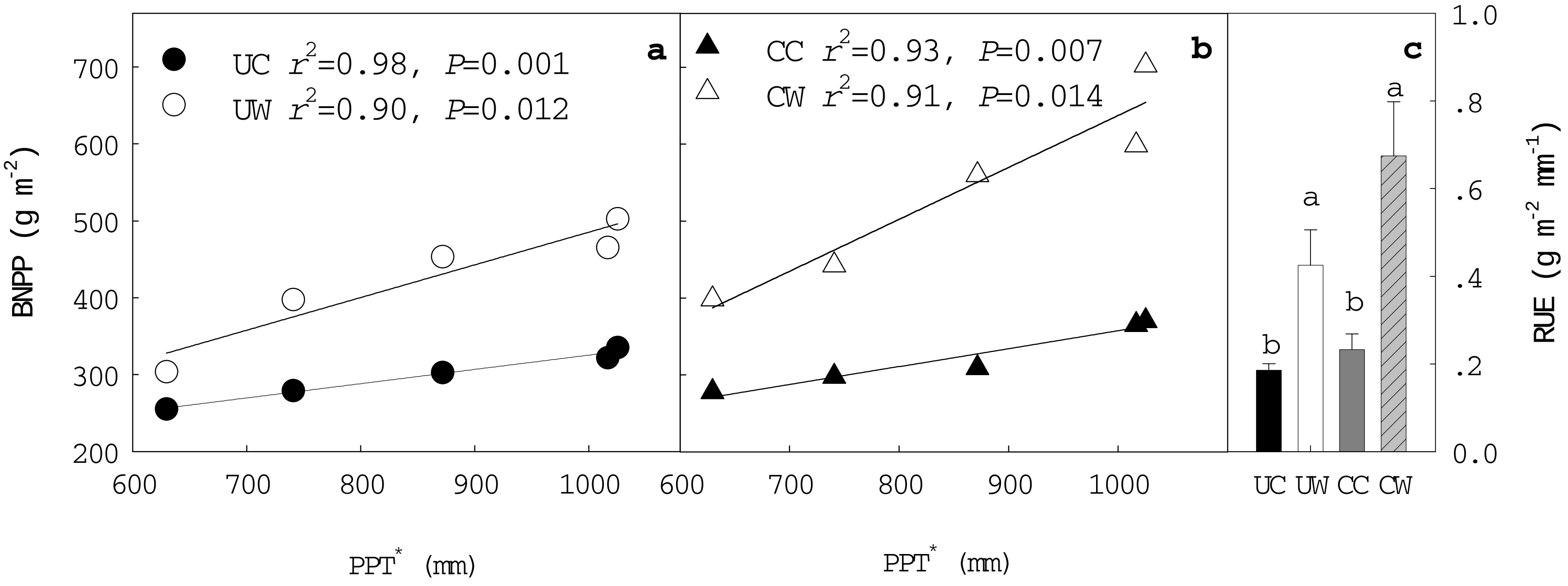

Figure 2.3 Relationships of BNPP with PPT* under four treatments (a, b) and increased rain use efficiency (RUE) under warming (c). PPT*: average of precipitation of the current year and the previous year.

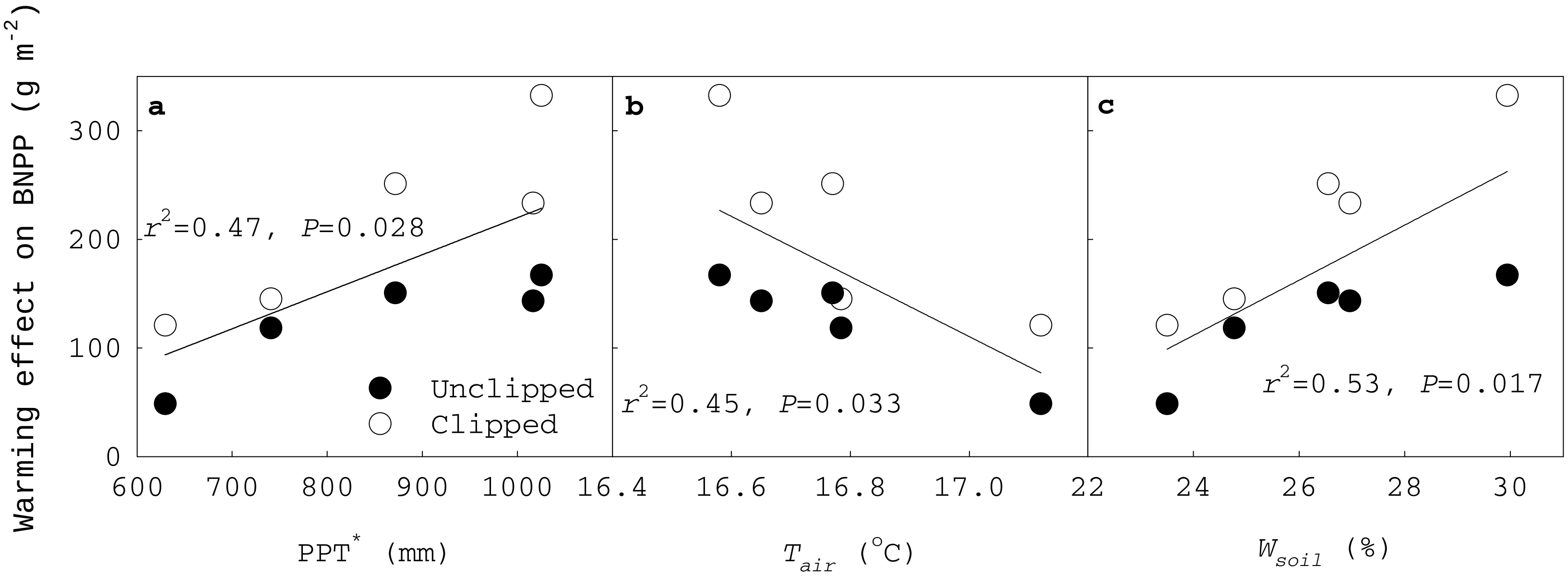

Figure 2.4 Relationships of warming-induced changes in BNPP with PPT^* (a), mean air temperature (T_{air}, b), and mean soil moisture (W_{soil}, c).

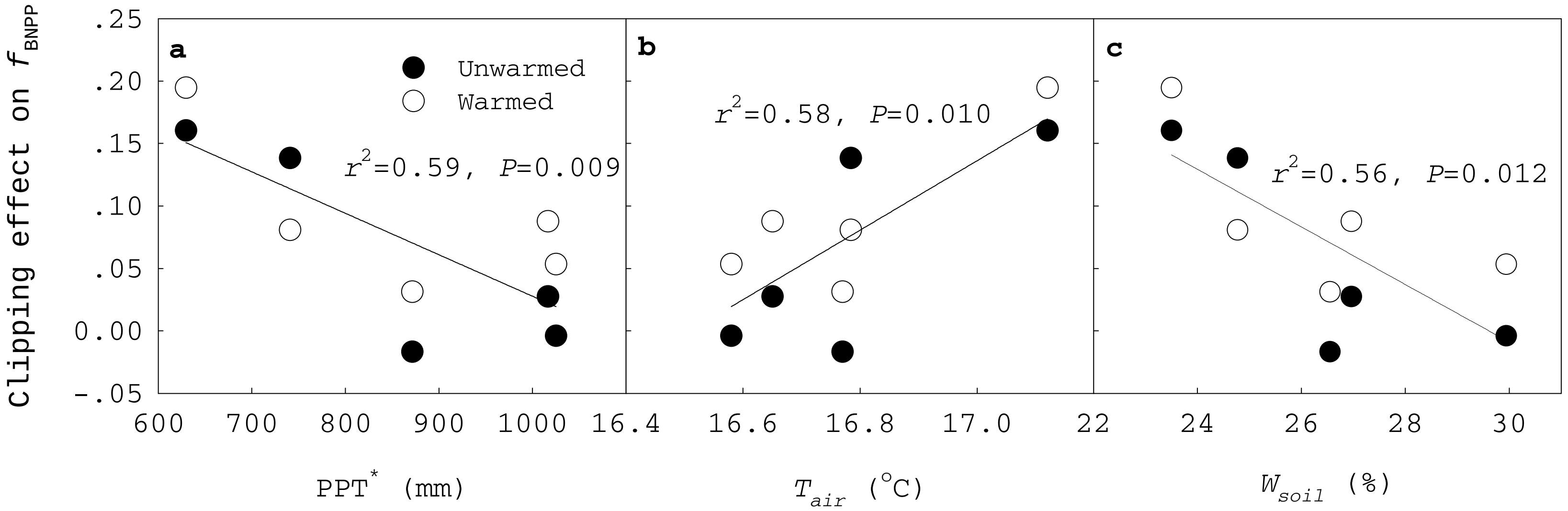

Figure 2.5 Relationships of clipping-induced changes in f_{BNPP} with PPT* (a), mean air temperature (T_{air}, b), and mean soil moisture (W_{soil}, c).

2.4 Discussion

2.4.1 Warming and clipping effects on BNPP

Temperature is one of the major factors constraining terrestrial plant productivity (Luo et al., 2009; Wu et al., 2011). The significant increase in BNPP under warming ($P<0.001$, Table 2.2; Fig. 2.1 a, b) is in line with a meta-analysis which demonstrated that experimental warming which increased soil temperature of 0.1-10.2 °C significantly increased BNPP (Wu et al., 2011). The increase in BNPP in our experiment may result from: (1) longer growing season under year-round warming (Wan et al., 2005); and (2) warming-induced increase in nutrient mineralization (Hartley et al., 1999; Rustad et al., 2001) and the consequent increase in plant nutrient uptake (Sardans et al., 2008) and plant productivity (Wan et al., 2005). Another possible reason that may account for the increase in BNPP is higher availability of photosynthates for roots, which originated from increased photosynthetic rates at higher temperatures (Rustad et al., 2001; Luo et al., 2009; Wu et al., 2011). However, a previous study at the same site showed that leaf-level photosynthesis of four dominant species did not change much under warming during the whole growing season (Zhou et al., 2007), suggesting the direct warming effect on photosynthesis is at most only a minor cause of increased BNPP.

Responses of BNPP to warming varied widely by year, with significant warming effects observed in wet years (all $P<0.05$, Fig. 2.1a, b) mainly due to water availability being one of the primary controls over plant productivity (Sala et al., 1988; Wu et al., 2011). In clipped subplots in 2007, for example, BNPP was mostly increased by warming probably due to the large amount of precipitation. Our results are similar to other studies investigating interactive effects of warming and drought on plant productivity. For example, Peñuelas et al. (2004) found that warming increased biomass in wet northern areas while it had no effect in dry southern areas at four shrubland sites. Both Peñuelas et al. (2004) and other studies (e.g. Li et al., 2011) indicated that the responses of BNPP to warming were greatly controlled by water availability.

Increased BNPP with clipping may be attributable to at least four causes. First, less standing-litter in the clipped than unclipped subplots can improve light conditions for the lower canopy leaves and thus increase leaf photosynthesis and belowground root productivity. Second, there may be positive clipping-induced compensatory growth mechanisms, similar to the grazing optimization hypothesis that increases plant productivity (McNaughton, 1983; McNaughton et al., 1998). Third, if a compensatory growth mechanism exists, belowground plant productivity may be stimulated by a clipping-induced increase in nutrient uptake due to increased sink strength (Wegener and Odasz, 1997). Fourth, clipping may change plant physiological processes and allocation patterns (Oesterheld and McNaughton, 1988), leading to the increase in belowground productivity with decreased soil moisture (Table 2.1) based on optimal partitioning theory (Bloom et al., 1985; Chapin et al., 1987) and supported by previous studies (e.g. McConnaughay and Coleman, 1999; McCarthy and Enquist, 2007). Additionally, increased nutrient availability (Rustad et al., 2001) and use efficiency (Sardans et al., 2008, Niu et al., 2010) under warming may explain that the significant clipping effect was mostly observed in warmed subplots (Fig. 2.1c).

2.4.2 Warming and clipping effects on f_{BNPP}

Our estimates of f_{BNPP} with a mean of 0.61±0.01 under four treatments are comparable with a synthesis of field studies with f_{BNPP} across world's grasslands ranging between 0.40 and 0.86 with a mean of 0.71 (Hui and Jackson, 2005). Biomass allocation is a specialized survival strategy of plants (Bloom et al., 1985). Allocation patterns among plant organs vary during growth and development (Farrar and Gunn, 1996) and the patterns can reflect the differential investment of photosynthates between the aboveground and belowground organs (Stirling et al., 1998; McConnaughay and Coleman, 1999). Experimental warming significantly increased f_{BNPP} indicating that plants were adjusting their allocation patterns to maximize their relative growth rate. According to optimal partitioning theory (Bloom et al., 1985; Chapin et al., 1987), which is supported by previous studies, such as Hui and Jackson (2005), warming-induced dry conditions increased f_{BNPP} when plants allocate proportionally more biomass to roots in response to low moisture conditions (McConnaughay and Coleman, 1999; McCarthy and Enquist, 2007).

Clipping for hay, similar to grazing in removing aboveground biomass, disturbs the equilibrium between BNPP and ANPP (Gao et al., 2008). We found that clipping significantly increased f_{BNPP} (P=0.001, Table 2.2). Plants may adjust physiological processes because the demand for water and nutrient supply by aboveground biomass decreased dramatically after clipping, leading to reallocation of more resources to roots (Oesterheld and McNaughton, 1988). Additionally, clipping may increase f_{BNPP} through decreasing soil moisture (Table 2.1) and stimulating root growth to capture water as supported by previous studies that show plants growing at low water availability exhibited stronger growth in BNPP than those under favorable water conditions (Hui and Jackson, 2005; Gao et al., 2011). Our results also showed that f_{BNPP} was significantly increased by clipping in dry years compared to non-significant changes in wet years (Fig. 2.2c). With increasing extreme rain events (IPCC, 2007), variation in f_{BNPP} in response to land use change should be considered for incorporation into global C models to accurately predict future climate change.

2.4.3 Interannual variation in BNPP and f_{BNPP} and their responses to warming and clipping

Water availability is one of the most frequent controlling factors over plant productivity (Burke et al., 1997). In our study, the interannual variability in BNPP was dependent on precipitation (Fig. 2.3a, b). This is in line with the results of a meta-analysis by Wu et al. (2011) showing that increased precipitation generally stimulated plant growth while decreased precipitation had the opposite effect. Moreover, N mineralization increases with increasing precipitation (Burke et al., 1997), resulting in increased N availability to plants, which indirectly stimulated BNPP (Burke et al., 1997; Wan et al., 2005). Precipitation is especially important among climatic factors in regulating plant productivity as pointed out by Briggs and Knapp (1995). Additionally, RUE of BNPP significantly increased under warming (Fig. 2.3c) probably due to increased C_4 species dominance (Luo et al., 2009, Niu et al., 2010) because C_4 species have higher RUE compared to that of C_3 species (Niu et al., 2003).

Water availability not only controls the interannual variability in BNPP, but also regulates the impacts of warming on BNPP. The multifactor linear regressions showed that soil moisture was the dominant controlling factor on the responses of BNPP to warming across years, leading to the great interannual variability of warming effect on BNPP. Previous field and modeling studies in grasslands both confirmed that changes in plant productivity could be dependent on soil moisture (Knapp and Smith, 2001; Weng and Luo, 2008). Soil moisture, integrating impacts of both precipitation and temperature, can play an important role in regulating variation in BNPP and function as an important index in projecting ecosystems' responses to climate change (Friedlingstein et al., 1999; Sherry et al., 2008).

The clipping effect on f_{BNPP} decreased with increasing water availability (Fig. 2.5a, c). Multifactor linear regression analyses indicated that precipitation dominated the interannual variation in f_{BNPP}. Very few studies are available that consider clipping effects on the temporal variation in f_{BNPP}. We hypothesized that in comparison to the wet conditions, clipping stimulated BNPP relatively more than ANPP under low water availability conditions. In the unwarmed subplots, for example, clipping decreased ANPP and BNPP by 37.74% and 6.68% in 2005 (dry year), respectively; while in 2007 (wet year), clipping increased ANPP by 9.82% and BNPP by 10.42%, resulting in significantly higher clipping effect on f_{BNPP} in 2005 ($P<0.05$, Fig. 2.2c). Generally, the correlations of changes in BNPP and f_{BNPP} with climatic factors suggest that water availability (either precipitation or soil moisture) regulates the interannual variation of BNPP and f_{BNPP} in response to warming and clipping. Given the projected increase in extreme rain events under climate change (IPCC, 2007) and fundamental importance of BNPP in understanding C allocation and storage, varied f_{BNPP} should be included in evaluating ecosystem responses to warming from the whole plant perspective.

In our study, lagged effects of precipitation on the temporal variation in BNPP and f_{BNPP} were evidenced by a correlation of BNPP and f_{BNPP} with the average of precipitation of the current and previous year (all $P<0.05$; Figs. 2.3a b, 2.4a, 2.5a). The relationships of annual precipitation *or* previous year's precipitation alone with variation in BNPP and f_{BNPP} were not statistically significant (all $P>0.05$). Similar phenomena have been documented in many grassland ecosystems in North America, Europe, and South Africa (e.g. O’Connor et al., 2001; Sherry et al., 2008). For example, an increase in biomass induced by carry-over effects from wet years in grasslands can last one or more years in which higher biomass is usually observed than expected (Oesterheld et al., 2001; Sherry et al., 2008). In general, one year is the most commonly reported lag time (e.g. Webb et al., 1978; O’Connor et al., 2001), as observed in our study that previous year’s precipitation substantially influenced BNPP and f_{BNPP}. Frequency of extreme weather events, such as unusual wet and dry years, is predicted to increase under global climate change (IPCC, 2007). Therefore, field manipulative experiments with different precipitation quantity, frequency, and intensity are needed to clarify mechanisms that control plant productivity in response to temporal and spatial variation in precipitation.

2.5 Conclusions

Our results from the long-term warming and clipping experiment in the tallgrass prairie in the US suggest that warming and hay-harvest will have substantial and year dependent effects on BNPP and NPP partitioning (f_{BNPP}). Across the years, warming had positive effects on BNPP likely through prolonged growing seasons and higher nutrient availability and nitrogen use efficiency. BNPP also positively responded to clipping, possibly through increased photosynthesis with less shading, compensatory growth, and changed allocation patterns. A relative increase in belowground allocation, f_{BNPP}, was observed as a consequence of the increased BNPP and dry conditions caused by warming and clipping treatments. Practical use of varied f_{BNPP} in global terrestrial models would thus improve C cycle modeling. Multifactor linear regressions showed that water availability regulated the interannual variability in BNPP, warming-induced changes in BNPP, and clipping-induced changes in f_{BNPP}. Global mean temperature and extreme rain events are projected to increase in future. Therefore, to accurately forecast ecosystem feedback to climate change, models may have to simulate responses of BNPP and f_{BNPP} to global warming and land use change as well as their consequences for ecosystem functions.

2.6 Acknowledgements

We would like to thank two anonymous reviewers for their comments and many lab members for their help with field work. This study is financially supported by the Office of Science (BER), Department of Energy, under grant DE-SC0004601; by the National Science Foundation (NSF) under grants DEB 0078325 and DEB 0743778; and through the Midwestern Regional Center of the National Institute for Climatic Change Research at Michigan Technological University, under award number DE-FC02-06ER64158.

Appendix A: Aboveground net primary productivity (ANPP)

A1 ANPP measurement

ANPP was directly measured by clipping in clipped subplots and indirectly estimated by pin-contact counts in unclipped subplots. Plants are clipped annually at 10 cm height at peak biomass (usually August) in the diagonal clipping subplots. Because we did clipping each year, the total summer biomass represents annual productivity. Clipped biomass was first separated into C_3 and C_4 plants and then oven-dried at 65 °C for 48 hours. Plots were clipped immediately after the indirect estimation of peak aboveground biomass by the pin-contact method (Frank and McNaughton, 1990).

The pin-contact method was used to estimate ANPP in both the unclipped and clipped subplots in spring, summer, and fall each year. Our pin frame is 0.5 m long and holds 10 pins 5 cm apart at 30° from vertical. Pins are 0.75 m long each and could be raised within the frame to count hits up to 1 m high (hits above 1m are negligible at this site). In each subplot, the point frame was placed four times in each of the four cardinal directions to record the contact numbers of the pins separately with green and brown plant tissues (i.e., leaves and stems). We considered the brown tissues to be dead plant litter produced in the current year. The contact numbers of both green and brown tissues were then used to estimate AGB using calibration equations derived from 10 calibration plots, which are randomly selected each season and year and located at least 5 m away from the experimental plots. Biomass in the calibration plots was clipped to the ground surface. Clipped plant materials were oven-dried and then correlated with the total contact number. A linear regression of total hits vs. total biomass was used to derive the calibration equation. To account for increased C_4 plants in warmed plots, the clipped biomass from warmed and unwarmed clipping subplots was regressed against contact numbers separately to derive calibration equations.

A2 Impacts of warming and clipping on ANPP from 2005 to 2009

ANPP varied from 199.52±12.91 in 2009 to 276.99±3.23 g m^{-2} in 2008 in UC from 2005 to 2009 (Fig. A1a). The warming effect on BNPP was significant over the years (*P*=0.009, Table A1). On average, it increased BNPP by 29.92% and 35.26% in the unclipped and clipped subplots, respectively (Fig. A1b). Experimental warming significantly interacted with year in influencing ANPP, with higher stimulation in wet years than in dry years ($P<0.001$, Table A1; Fig. A2a). Clipping showed little effect on ANPP from 2005 to 2009 ($P=0.171$, Table A1) though it decreased ANPP by an average of 12.58% in the unwarmed plots and 2.53% in the warmed plots (all $P>0.05$, Fig. A1b). The interactive effects of clipping and year had significant impact on BNPP ($P<0.001$, Table A1), which was observed in relatively dry years (Fig. A2b).

Table A1 Results of repeated-measures ANOVA (*P* values) for responses of ANPP to warming (W), clipping (CL), year (Y), and their interactions (n=6). *P* values smaller than 0.05 are in bold.

Factor	Warming	Clipping	Year	W×CL	W×Y	CL×Y	W×CL×Y
ANPP	**0.009**	0.171	**<0.001**	0.990	**<0.001**	**<0.001**	0.428

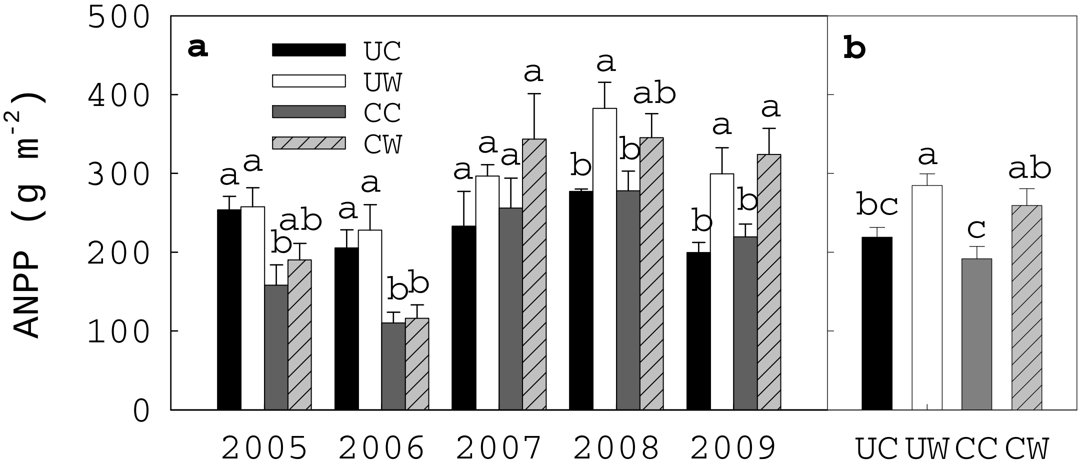

Figure A1 Variation in ANPP (a, n=6) from 2005 to 2009 and their averages (b, n=30) under four treatments. Values are mean±SE. Different letters represent statistically significant difference between treatments at *P*<0.05.

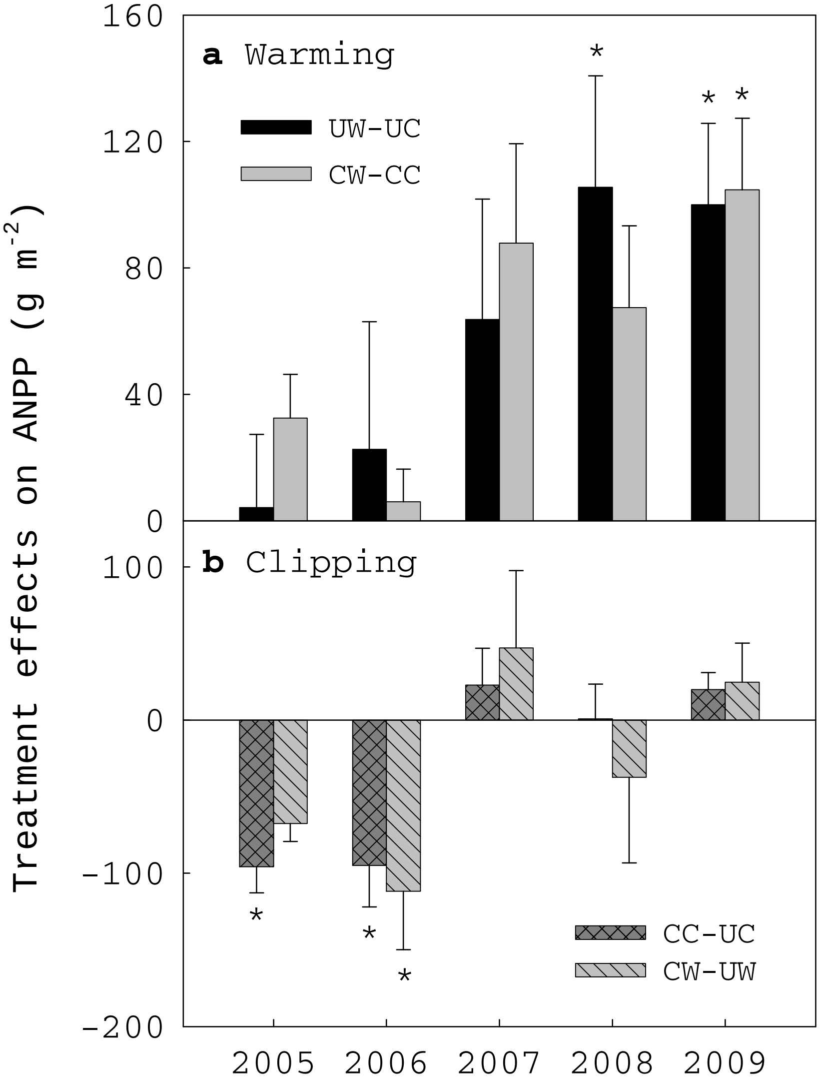

Figure A2 Warming (a) and clipping (b) effect on ANPP from 2005 to 2009. Values are mean±SE (n=6). Asterisks (*) represent statistically significant warming or clipping effect on ANPP at $P<0.05$.

Chapter 3

Long-term experimental warming decreased labile soil organic carbon in a tallgrass prairie[2]

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[2] Xu et al. (2012) Plant Soil, in revision.

ABSTRACT

Climate warming has been hypothesized to influence dynamics of soil organic carbon (SOC), especially labile SOC due to its rapid response to changes in temperature and carbon (C) supply. In this study, we examined impacts of experimental warming on the labile and whole SOC pools in association with vegetation changes from 2000 to 2008 in a tallgrass prairie in Oklahoma, USA. Infrared heaters were used to elevate soil temperature by approximately 2 °C since November 1999. The modified potassium permanganate ($KMnO_4$, 0.02 mol L^{-1}) procedure was used to estimate chemically labile organic C (LOC). Our results showed that warming significantly decreased chemically labile organic C (LOC) by an average of 15.56%, but had little effect on SOC content. The decrease in LOC was probably attributable to increased soil respiration, enhanced soil erosion, and possibly stimulated C_4 dominance under warming. A 2-stage variation in LOC response to warming was observed, with no significant changes from 2000 to 2004 and significant decreases from 2005 to 2008. Lagged temporal dynamics of LOC (2 yrs) asynchronously correlated with warming-increased C_4 proportion of aboveground biomass (AGB). Our findings suggest that staged decrease in LOC under warming may relate to the increase in C_4 biomass through detrital inputs, making it complex to predict the effect of warming on C cycling in this region.

Key words: soil organic carbon (SOC), experimental warming, labile organic carbon, C_3-C_4 composition, tallgrass prairie

3.1 Introduction

Global mean temperature is expected to increase substantially (2-7 °C) by the end of this century in response to rising concentrations of greenhouse gases in the atmosphere (Allison et al., 2009). Potential consequences of such warming for soil organic carbon (SOC) have received considerable attention because how SOC may respond to warming is still not clearly understood (Luo et al., 2001; Fang et al., 2005; Davidson and Janssens, 2006; Craine et al., 2010). Soil is the largest terrestrial carbon (C) pool, storing approximately 2 times the amount of C present in atmospheric CO_2 (Dixon et al., 1994). A slight change in SOC may greatly stimulate buildup of atmospheric CO_2 concentration due to the huge fluxes between soil and atmosphere (Marin-Spiotta et al., 2009). Though soil is considered to be an important potential C sink to retard the greenhouse effect (Cheng et al., 2011), warmer temperatures may stimulate SOC mineralization, leading to a positive feedback to future climate warming (Conant et al., 2008; Hartley and Ineson, 2008). Therefore, understanding the response of SOC decomposition to warming is particularly important because it partially determines whether we will have a positive or negative feedback to current global warming in the future (Hartley and Ineson, 2008; Craine et al., 2010).

Undoubtedly, increasing temperature has prompted many studies investigating the response of SOC to warming (e.g. Luo et al., 2001; Fang et al., 2005; Craine et al., 2010). However, understanding responses of SOC to warming can be difficult because SOC is composed of a continuum of materials with varying chemical complexity (Marin-Spiotta et al., 2009) and minor changes are difficult to detect against the high background of SOC in soil (Wei et al., 2003). Fast-cycling labile soil organic C (LOC) has captured much more attention because of its fast response to warming (Zhang et al., 2005; Bradford et al., 2008; Carrillo et al., 2011) and its control over CO_2 fluxes between terrestrial ecosystems and the atmosphere (Trumbore et al., 1990). In the long term, LOC can influence terrestrial C storage through its impact on nutrient supply to plants (Zhang et al., 2005; Luo, 2009). Despite much research, however, the effect of warming on SOC remains unclear. For example, warming may have little effect on SOC (Marchand et al., 2004; Luo et al., 2009; Cheng et al., 2011) and LOC as measured by microbial biomass C (Zhang et al., 2005; Schindlbacher et al., 2011) or, it may increase SOC through great increase in plant biomass inputs (Day et al., 2008; Sardans et al., 2008; Carrillo et al., 2011). Conversely, other studies show that LOC and SOC may decrease in response to continuous warming (Fontaine et al., 2004; Eliasson et al., 2005; Bradford et al., 2008). An accurate quantitative prediction of C storage in soil is highly dependent on the assumed temperature sensitivity of SOC decomposition (Cox et al., 2000; Conant et al., 2008), but debate continues regarding whether LOC decomposition is more (Giardina and Ryan, 2000; Reichstein et al., 2000), similar (Fang et al. 2005; Conen et al. 2006), or less (Conant et al., 2008; Wetterstedt et al., 2010) sensitive to that of recalcitrant organic C. The lack of consensus is not surprising given that the various responses of SOC to warming depend on many factors, such as soil moisture, precipitation, microbial activity, and plant species composition (Sollins et al., 1996; Fissore et al., 2008; Schmidt et al., 2011).

One way that changes in plant species composition under warming may alter LOC and SOC is through altering the quantity and quality of detrital inputs to soil (Day et al., 2008; Fissore et al., 2008). Because SOC is almost exclusively derived from detrital inputs of plants growing on the site (Cheng et al., 2006), the magnitude of the warming effect on soil-stored C may largely depend on the quantity and quality of litter inputs. In grasslands, for example, warming significantly increases recalcitrant litter derived from C_4 species (Luo et al., 2009; Cheng et al., 2011), which may increase SOC storage. Alternatively, increases in the relative abundance of recalcitrant C compounds input to soil is expected to regulate responses of LOC and SOC to warming by altering soil chemical and physical processes (Fissore et al., 2008). Warmer temperatures may accelerate microbial utilization of labile compounds during litter decomposition (Hobbie, 1996; Prescott et al., 2000), leaving behind SOC characterized by low C quality (Conant et al., 2008; Hartley and Ineson, 2008). Although shifts in plant species composition under warming have been reported (Harte and Shaw, 1995; Wan et al., 2005; Walker et al., 2006; Luo et al., 2009), we still have little understanding of how LOC and SOC may respond to changes in plant community structure, particularly under warming. The amount of soil-stored C is likely to change under global warming, especially with alteration in plant community structure and composition (Hobbie, 1996; Cheng et al., 2011).

In a tallgrass prairie in central Oklahoma, we have conducted a warming experiment since November 1999 (Luo et al., 2001). The experimental site is dominated by C_4 grasses and C_3 forbs. Previous studies at this site have shown that warming significantly increases aboveground biomass through warming-enhanced C_4 dominance (Wan et al., 2005; Luo et al., 2009). Warming also increased C_4 species litter (low quality, high recalcitrance) inputs to soil by 47.20% and decreased C_3 species litter input to soil by 19.10% (Cheng et al., 2011). Long-term experimental warming and simultaneously shifting C_3-C_4 composition thus provide us with an ideal opportunity to evaluate variation in soil-stored C and SOC decomposition. The specific objectives of this study were to evaluate warming effects on LOC and SOC from 2000 to 2008 and to examine the potential impacts of observed shifting of C_3-C_4 species composition on variations in LOC and SOC.

3.2 Materials and Methods

3.2.1 Experimental site and design

The experimental site is located on the Kessler Farm Field Laboratory in Oklahoma, USA (34°59•N, 97°31•W). The site has never been cultivated and has been ungrazed for the past 40 years. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans*) and C_3 forbs (*Ambrosia psilostachyia, Solidago rigida,* and *Solidago nemoralis*). Mean annual temperature is 16.3 °C and mean annual precipitation is 914 mm (Oklahoma Climatological Survey, Norman, OK, USA). The soil is part of the Nash-Lucien complex with neutral pH, high water holding capacity (around 37%), and a moderately penetrable root zone (US Department of Agricultrue 1979).

The experiment uses a paired factorial design with warming as the main factor and clipping as a nested factor (Luo et al., 2001; Wan et al., 2005). Each treatment has six replicates (i.e. six pairs of plots). Each pair has two plots of 2 m × 2 m. One plot has been subjected to continuous warming from 21 November 1999 to the present while the other serves as the control with ambient temperature. Infrared heaters (165 cm × 15 cm; Kalglo Electronics, Bethlehem, PA, USA) having a radiation output of 100W m^{-2} are suspended 1.5 m above the ground in each warmed plot. The control plot has a 'dummy' heater with same dimensions as the infrared heater suspended at a similar height to mimic the shading effects of the heater. Temperature increments generated by the infrared heaters are relatively even over the entire area of the plots and similar at different soil depths (Wan et al., 2002). For each pair of plots, the distance between warmed and control plots is approximately 5 m from centers to avoid heating of the control plots. The distances between the paired plots vary from 20 to 60 m. Detailed information can be found in Luo et al. (2009). Each 2 m × 2 m plot is divided into four 1 m × 1 m subplots. Plants in two diagonal subplots are clipped at a height of 10 cm above the ground once a year while the other two subplots are unclipped. The soil experiment described in this paper was carried out only in the unclipped subplots, giving two treatments: control (C) and warmed (W).

3.2.2 Microclimate and aboveground biomass measurement

Soil temperature was measured by thermocouples installed 2.5 cm deep in the soil at the center of one unclipped subplot in each plot. Hourly average data were stored in a SM192 Storage Module (Campbell Scientific, Logan, Utah, USA). Volumetric soil water content (%V) was measured once or twice a month using Time Domain Reflectometry (TDR) equipment (Soil Moisture Equipment Crop., Santa Barbara, CA, USA) at a soil depth of 0-15 cm. Precipitation data were obtained from an Oklahoma Mesonet Station (Washington Station) located approximately 200 m away from our experimental site (Oklahoma Climatological Survey). Total aboveground biomass (AGB), and C_3 and C_4 biomass was indirectly estimated by the pin-contact method (Frank and McNaughton, 1990). A detailed description of the biomass estimation technique was provided by Sherry et al. (2008). In 2000, 2001, and 2002, the amount of C_3 biomass is obtained based on the correlation between C_3/C_4 cover ratio and C_3 biomass in clipped subplots each year (all $P<0.01$, n=12) because we did not separate AGB into C_3 and C_4 when measuring AGB during those two years. C_4 biomass is calculated as the difference between AGB and C_3.

3.2.3 Soil sampling

From 2000 to 2008 (except 2006), one soil core (4 cm in diameter, 0-15 cm in depth) was taken once a year from one unclipped subplot in each plot. Soil samples were packed in polyethylene bags, immediately stored in coolers, and transported to the Ecolab at the University of Oklahoma, Norman. The soil samples were sieved (<2 mm) to remove soil fauna, rocks, and fine roots, then air-dried, further inspected to remove rocks and fine roots, finely ground, and sieved (<0.25 mm) to measure SOC and LOC. In 2006, we did not archive soil samples.

3.2.4 LOC and SOC analyses

Given that only limited air-dried soil samples were archived, the modified potassium permanganate ($KMnO_4$, 0.02 mol L^{-1}) procedure (Weil et al., 2003; Mirsky et al., 2008) was used to measure LOC of the soil samples. Slightly alkaline $KMnO_4$ has been demonstrated to oxidize simple and active forms of soil C, such as carbohydrates, amino acids, and amine/amide sugars (Stanford, 1978; Loginow et al., 1987). $KMnO_4$ solution (0.02 mol L^{-1} $KMnO_4$ and 0.1 mol L^{-1} $CaCl_2$) and standard $KMnO_4$ solutions ranging from 0 to 0.02 mol L^{-1} were prepared. Soil samples (5 g) were added to 50 ml plastic screw-top centrifuge tubes. Then 20 ml $KMnO_4$ solution was added to each tube to react with soil samples. The soil suspension was shaken on a reciprocating shaker for 10 min at 180 rpm and then allowed to settle for 10 min. A 1 ml aliquot of the solution supernatant was diluted to 50 ml and absorbance was measured on a Spectronic Genesys 5 spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) at 550 nm. The amount of C oxidized after digestion was determined by the change in the concentration of the $KMnO_4$ solution. It was assumed that 9 g C was oxidized by 1 mol L^{-1} $KMnO_4$ changing from Mn^{7+} to Mn^{2+}. SOC was measured at Oklahoma State University, Stillwater.

3.2.5 Statistical analysis

Before ANOVA analysis, the datasets were tested for normality to meet the assumptions for statistical analysis. Repeated-measures ANOVAs were used to examine the effects of experimental warming, year, and their interactions on LOC, SOC, and the proportion of LOC to SOC. Paired t-tests were performed to analyze warming effects on AGB and the parameters mentioned above in each year. Linear regression analyses were used to evaluate the relationships of warming-induced changes in LOC with C_3-C_4 composition in the current year, 1 year later, and 2 years later. All statistical analyses were conducted using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA).

3.3 Results

3.3.1 Microclimate

Warming significantly increased soil temperature every year from 2000 to 2008 (Table 3.1, $P<0.05$). On average, it elevated soil temperature by 1.60 °C. Warming also lowered volumetric soil moisture by an average of 1.47%, but this effect was not significant (Table 3.1, $P>0.05$). Annual precipitation (AP) varied from 515 mm in 2005 to 1307 mm in 2007 with a mean of 870 mm from 2000 to 2008 (Table 3.1).

Table 3.1 Precipitation (AP) and changes (W-C) in soil temperature and moisture (T_{soil}, W_{soil}) under warming from 2000 to 2008. W: warming; C: control. Asterisks (*) represent significant warming effect, *: $P<0.05$.

Variable	Warming effect	2000	2001	2002	2003	2004	2005	2007	2008	Avg.
AP (mm)	n/a	995	883	890	647	991	522	1307	726	870
T_{soil} (°C)	W-C	2.05*	1.73*	1.33*	1.47*	1.72*	2.05*	1.37*	1.08*	1.60*
W_{soil} (%)	W-C	-3.77	-1.00	-0.78	-1.01	-0.1	-2.18*	-1.20	-1.75	1.47

3.3.2 Soil C pool and AGB

Warming significantly influenced LOC and its proportion to SOC, but had little effect on SOC from 2000 to 2008 (Table 3.2, Fig. 3.1a-f). Overall, warming decreased LOC, and the proportion of LOC to SOC by an average of 15.56% ($P<0.05$, Fig. 3.1d) and 14.94% ($P<0.01$, Fig. 3.1f), respectively. Warming did not induce an instantaneous decrease in LOC. For example, the warming effect on LOC was not significant in the first several years of treatment (2000-2004), but warming decreased LOC from 2005 to 2008 (Fig. 3.1a). Similarly, experimental warming increased AGB (Fig. 3.2a) and the warming effect on C_4 biomass was significant in the second stage (2007-2008, Fig. 3.2b).

Table 3.2 Results of repeated-measures ANOVA (*P* values) for the responses of LOC, SOC, and the proportion of LOC to SOC (n=6) to warming (W) and year (Y).

	LOC	SOC	LOC/SOC
Warming	*0.061*	0.869	**0.002**
Year	**0.006**	0.397	**0.024**
W×Y	**0.014**	0.634	**0.026**

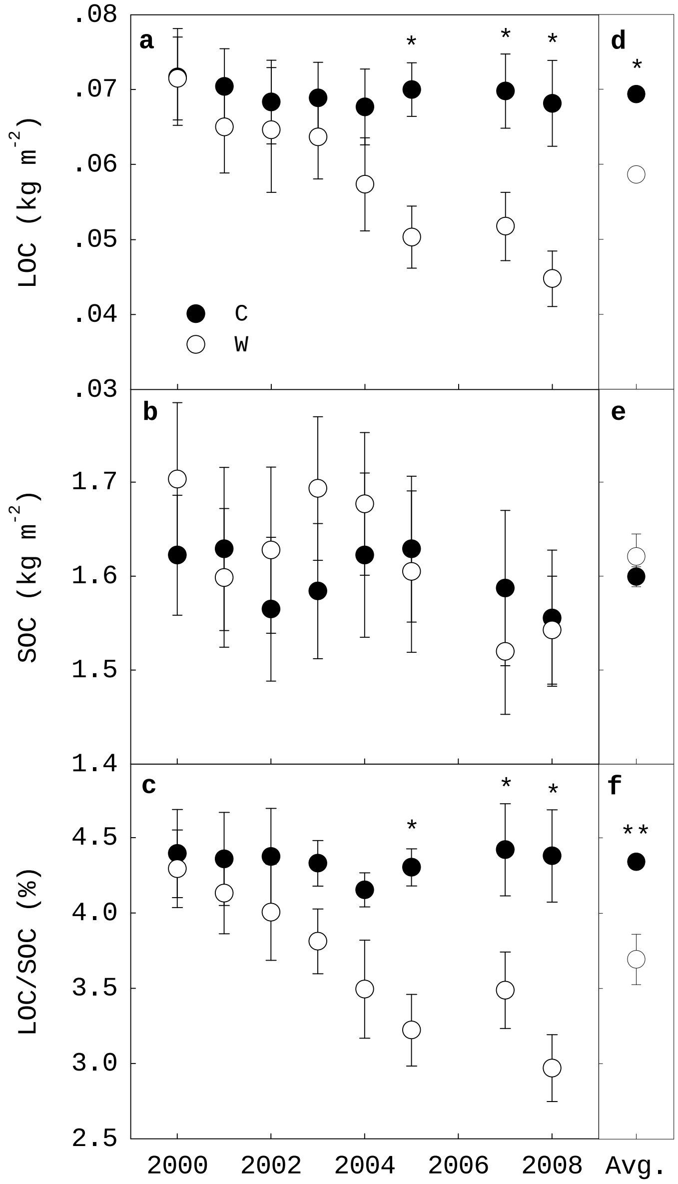

Figure 3.1 LOC (a), SOC (b), and proportion of LOC to SOC (c) from 2000 to 2008 and their averages (d, e, f) under control (C) and warming (W) treatments. LOC: chemically labile soil organic carbon; SOC: soil organic carbon. Values are mean ± SE (n=6). Asterisks (*) represent significant warming effect, *: $P<0.05$; **: $P<0.01$.

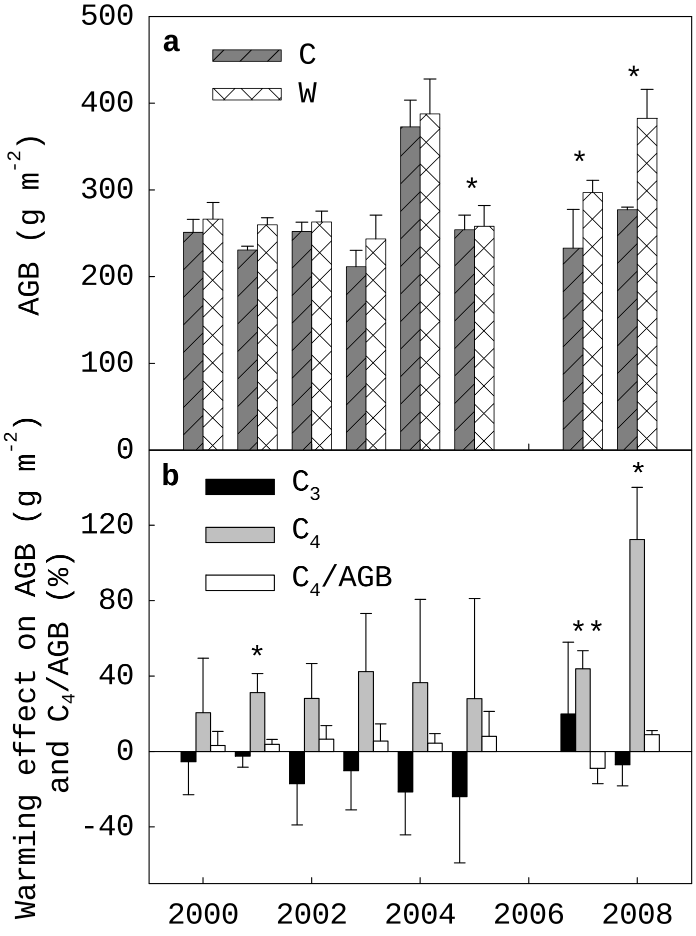

Figure 3.2 Variation in AGB under control (C) and warming (W) treatments (a) and warming-induced changes in AGB (C_3 and C_4, b) from 2000 to 2008. AGB: aboveground biomass. Values are mean ± SE (n=6). Asterisks (*) represent significant warming effect, *: $P<0.05$; **: $P<0.01$. In order to be consistent, we did not plot the biomass data in 2006 because soil samples in 2006 were not archived.

3.3.3 Relationships of changes in LOC with C_3-C_4 composition

Linear regression showed that C_3-C_4 composition, as expressed by C_4/AGB, was significantly related to 2-year-lagged changes in LOC ($P<0.05$, Fig. 3.3a). Temporal variation in LOC was asynchronous with changes in the proportion of C_4 to aboveground biomass (C_4/AGB), lagging the biomass changes by 2 years (Fig. 3.3b).

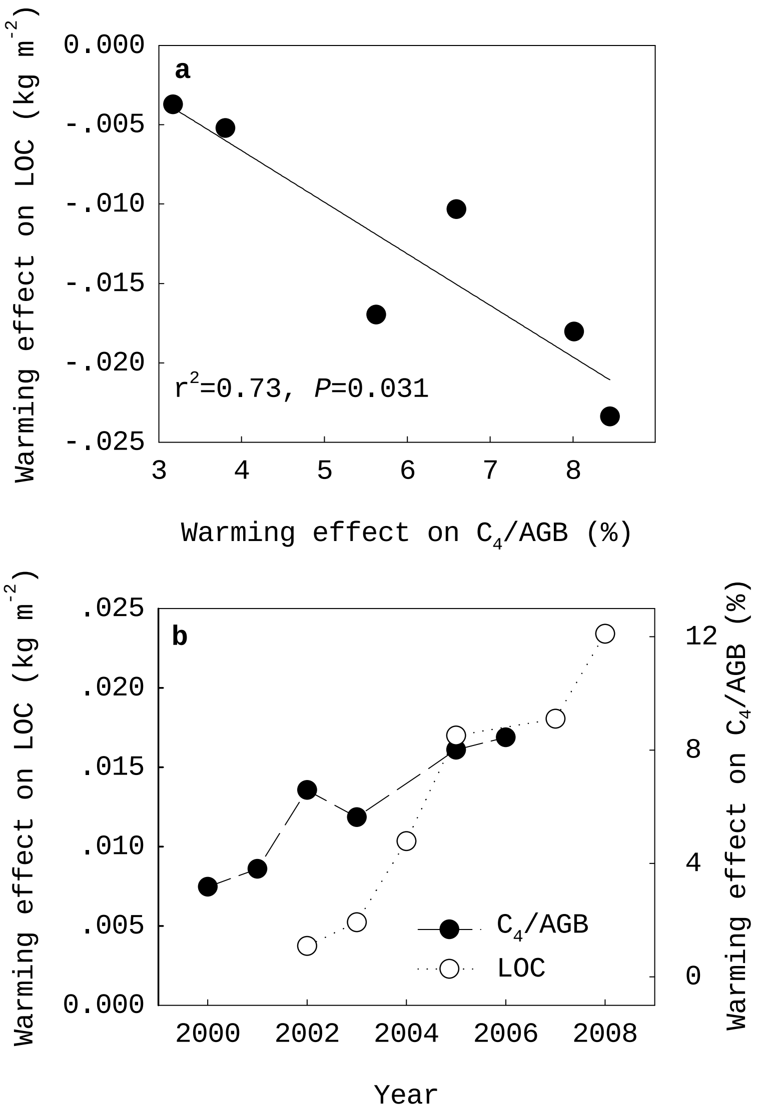

Figure 3.3 Relationship of warming-induced changes in C_4/AGB (from 2000 to 2006) with 2-years lagged changes in LOC (from 2002 to 2008, a) and asynchronous variation of LOC and C_4/AGB Under warming (b). LOC data showed in panel b was transformed by timing -1. AGB: aboveground biomass.

3.4 Discussion

3.4.1 Warming effect on LOC

Variation in LOC is substantially influenced by changes in non-biological and biological environmental conditions (Garten Jr. et al., 1999; Fissore et al., 2008). Our results showed that warming significantly decreased LOC (Table 3.2, Fig. 3.1a, d), probably due to accelerated decomposition of LOC and the warming-increased soil erosion (Xue et al., 2010). At the same site, for example, both soil respiration and heterotrophic respiration significantly increased under warming (Wan et al., 2005; Zhou et al., 2007; Luo et al., 2009),which largely originates from LOC decomposition. The reduction in LOC is in line with a previous study that long-term soil warming significantly decreased the fast-cycling LOC, as measured by mineralizable C, substrate-induced respiration (SIR), and microbial biomass C (MBC) (Bradford et al., 2008). Also, increased soil erosion under warming at the same site led to preferential removal of labile SOC from the soil, both in the form of dissolved organic carbon (DOC) and in particulate organic carbon (POC) (Ludwig and Probst, 1999; Xue et al., 2010). Though different labile SOC pools were measured and reported, they all represent a fast-cycling proportion of SOC and positive correlations of chemically LOC with SIR, MBC, and POC were found (Weil et al., 2003, Mirsky et al., 2005). Moreover, both model studies and laboratory incubation studies have suggested a faster depletion of LOC under higher temperatures (e.g. Eliasson et al., 2005; Conant et al., 2008; Hartley and Ineson, 2008).

Additionally, a warming-driven community shift from C_3 to C_4 species at our site (Wan et al., 2005; Luo et al., 2009) may be a possible factor in regulating the decrease in LOC because LOC responds rapidly to changes in C supply (Zhang et al., 2006). Hobbie (1996) suggested that changes in plant species composition are just as important as direct warming effects in determining SOC decomposition. At our site, for example, a SOC study based on ^{13}C showed that warming increased C_4-derived C input to soil by 18.77 % after 9 years' warming (Cheng et al., 2011). Compared to C_3 species, C_4 species have lower quality litter that eventually incorporates into soil with important consequences for biogeochemical processes and reduced SOC quality (Cheng et al., 2006; Fissore et al., 2008). LOC originating from C_4 species was found to decompose faster than whole SOC in mixed C_3/C_4 soils

(Wynn and Bird, 2007). Therefore, enhanced recalcitrant C_4-derived litter input to soil under warming may eventually stimulate LOC decomposition, lowering LOC content and its proportion to SOC in spite of the fact that warmed soils received more detrital inputs (Fig. 3.2).

3.4.2 Warming effect on SOC

One unexpected result from our study was that despite higher inputs due to stimulated plant productivity over the years (Fig. 3.1b, Wan et al., 2005; Luo et al., 2009), SOC content appeared to be constant under warming (Table 3.2, Fig. 3.1b, e). SOC storage is mainly determined by the balance between detrital inputs and respiratory loss. The non-significant changes in SOC may arise from the great loss of fresh C back into the atmosphere as CO_2 through warming-induced increase in soil respiration observed at the same site (Luo et al., 2009; Cheng et al., 2011). Additionally, higher plant litter inputs to soil may be offset by warming increased SOC loss through soil erosion at a rate of 69.6 g m^{-2} yr^{-1}, three times higher than that of the control at 22.5 g m^{-2} yr^{-1} (Xue et al. 2010). No changes in C storage under warming were also reported in other studies (e.g. Marchand et al., 2004). Changes in the amount of SOC are particularly hard to detect (Kirschbaum, 2006) due to the high background C levels in soil and to the small fraction of SOC that responds to warming over a long time period. It may take many centuries to observe the very slow changes in SOC (Kirschbaum, 1993). However, it is important to note that the composition of SOC has changed, as evidenced by the significant decrease in the proportion of LOC to SOC (Fig. 3.1c). Moreover, ^{13}C-SOC signatures showed that relative abundance of C_4-derived fraction in SOC significantly increased (Cheng et al., 2011), which lowered SOC quality and may impact the decomposition of SOC in response to warming.

3.4.3 Impact of C_3-C_4 composition on a two-phase variation in LOC

We observed a 2-stage variation in the response of LOC to warming. In the first stage, from 2000 to 2004, the warming effect on LOC was not significant, but warming did significantly decrease LOC in the second stage from 2005 to 2008 (Fig. 3.1a). Plant litter input and root exudates are the major sources of LOC (Catovsky et al., 2002). Therefore, unlike laboratory incubation of soil samples with no continuous fresh C input (e.g. Fang et al., 2005; Craine et al., 2010), it may take years to detect *in situ* decrease in LOC. Interestingly, we also observed a similar warming effect on C_4 biomass as in LOC, with non-significant differences from 2000 to 2005 and significant differences from 2007 to 2008 (Fig. 3.2b). Moreover, the 2-year lagged decrease in LOC under warming significantly correlated with the increase in C_4 biomass (Fig. 3.3a). Previous studies have suggested that warming may alter C cycling by changing plant community structure and species composition (Johnson, 1992; Chapin et al., 1995). The staged variation in LOC over the years may thus be partly attributable to indirect warming stimulation via alterations in C_4 litter inputs and perhaps rhizodeposition. ^{13}C-SOC signatures showed that warming increased C_4 species litter by 47.20% and increased C_4-derived litter incorporated into soil by 11.87% in 2008 (Cheng et al., 2011), which undoubtedly intensified C_4 species' control on LOC variation. Plant species influence LOC content via control over litter decomposition (Hobbie, 1996), with byproducts eventually incorporated into soil. The variation in LOC and in plant species composition (C_4/AGB) was asynchronous (Fig. 3.3b), lagging the biomass change by 2 years (Fig. 3.3b). It is reasonable that, given the processes by which plants affect LOC content through litter dynamics and rhizodeposition, SOC decomposition may take years.

3.5 Conclusions

Our results from a long-term warming experiment in the tallgrass prairie in the US suggest that warming will have substantial and staged effect on LOC. Across the years, warming significantly decreased LOC through enhanced decomposition, increased soil erosion, and possibly shifting C_3-C_4 plant community composition. SOC content under warming was not significantly different from that under control conditions, likely due to the balance between increased C input and increased C loss. It is important to note that the proportion of LOC to SOC was much lower under the warming treatment, which may influence soil C quality and decomposition in the long term. Warming may decrease LOC and simultaneously increase C_4 biomass. The 2-year lagged changes in LOC and C_4 biomass seem to relate to each other through litter inputs and rhizodeposition, making predictions of warming effects on C cycling in grasslands more complex.

3.6 Acknowledgments

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Chapter 4

Carbon quality and the temperature sensitivity of soil organic decomposition in a tallgrass prairie[3]

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[3] Xu et al. (2012) Soil Biology and Biochemistry, doi: 10.1016/j.soilbio.2012.03.007.

ABSTRACT

The temperature sensitivity of soil organic carbon (SOC) decomposition will influence the accuracy of the quantitative prediction of carbon (C) balance between ecosystem C fixation and decomposition in a warmer world. However, a consensus has not yet been reached on the temperature sensitivity of SOC decomposition with respect to SOC quality. The fundamental principles of enzyme kinetics suggest that temperature sensitivity of decomposition is inversely related to the C quality of the SOC. This "C quality-temperature" hypothesis was tested in a 170-day laboratory experiment by incubating soil samples with changing temperature (low-high-low) at a ±5 °C step every 24 hours. Soil samples were collected from a long-term warming experiment in a tallgrass prairie. There were four treatments of soil samples before lab incubation: control (C), warmed (W), field incubation (FI, litter exclusion), and warmed plus field incubation (WFI). Results showed that SOC decomposition rates were influenced by labile organic C (LOC) content, which were low in the soils under field incubation and decreased with increasing lab incubation time. Field warming and field incubation increased the temperature sensitivity of SOC decomposition in the 1st two lab incubation cycles but the treatment effects diminished as decomposition proceeded, probably due to increased contribution of recalcitrant C. In line with the hypothesis, we found that the lower the SOC quality, the higher the Q_{10} values. This relationship held across treatments and lab incubation cycles, regardless of whether the differences in SOC quality resulted from inherent differences in SOC chemistry or from differences in the extent of SOC decomposition. Treatment effects of field warming and field incubation on SOC quality and Q_{10} values also negatively correlated with each other. Our results suggest that dynamics of low-quality SOC have the highest potential to impact long-term C stocks in soils. Potential decreases in SOC quality in response to warming and consequent shifting species composition may result in a positive feedback of SOC to climate change in the future.

Keywords: soil organic carbon, temperature sensitivity, decomposition, carbon quality, global warming, tallgrass prairie

4.1 Introduction

In response to rising concentrations of greenhouse gases in the atmosphere, global mean temperature is predicted to increase 2-7 °C by the end of this century (Allison et al., 2009). Rising concerns about global warming has led to increased emphasis on understanding the role of soil as a potential carbon (C) sink to buffer the greenhouse effect (Cheng et al., 2011). Because of large C stocks in soil (Schlesinger, 1995), warmer temperatures may increase atmospheric CO_2 concentration by accelerating soil organic C (SOC) decomposition, resulting in a positive feedback to future climate warming (Hartley and Ineson, 2008; Craine et al., 2010). Predictions from coupled climate-C models differed substantially in magnitude and in the direction of the potential response of stored soil-C to warming (Cox et al., 2000; Friedlingstein et al., 2006). A negative feedback may occur if the amount of plant-derived C incorporated into soil exceeds the C loss through decomposition. So far, the temperature sensitivity of SOC decomposition remains one of the major uncertainties in predicting climate-C cycle feedback (Lenton and Huntingford, 2003; Conant et al., 2011).

The accuracy of the quantitative prediction of the C balance between ecosystem C fixation and decomposition is highly dependent on the assumed temperature sensitivity of SOC decomposition (Cox et al., 2000; Conant et al., 2008). Much research has thus addressed the responses of SOC decomposition to warmer temperatures in the last few decades (e.g. Kirschbaum et al., 1995; Fang et al., 2005; Friedlingstein et al., 2006; Xu et al., 2010) using the temperature coefficient (Q_{10}) to measure the temperature sensitivity of SOC decomposition. In modeling studies, it is in general for simplicity assumed that all types of SOC respond equally to climate warming (i.e. constant Q_{10}), independent of the differences in the C quality of SOC (Cox et al., 2000; Ågren and Bosatta, 2002; Burke et al., 2003). In empirical studies, on the other hand, the temperature sensitivity of SOC decomposition varies greatly depending on the type of SOC and the extent of SOC decomposition. Such studies have reported increases (Fierer et al., 2005; Conant et al., 2008; Wetterstedt et al., 2010), no changes (Fang et al., 2005; Conen et al., 2006), and decreases (Giardina and Ryan, 2000; Reichstein et al., 2000) in the temperature sensitivity of SOC decomposition with decreasing C quality. Despite much research, information about how the contradictory Q_{10} values and how SOC decomposition will respond to changes in temperatures is still limited. To accurately predict feedbacks of C dynamics to future climate change, we need to better understand the role of C quality in influencing SOC decomposition.

The fundamental principles of enzyme kinetics suggest that temperature sensitivity of decomposition at any specific point is controlled by the C quality of the substrates being consumed by microbes (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). Bosatta and Ågren (1999) suggested that more enzymatic steps (as expressed by activation energy) are required to release CO_2 from low-quality C substrates in comparison with that of high-quality C substrates. Therefore, temperature sensitivity of SOC decomposition should be inversely related to C quality, commonly referred to as the “C quality-temperature” hypothesis (Bosatta and Ågren, 1999; Mikan et al., 2002; Craine et al., 2010). Dozens of studies have tested this hypothesis using laboratory incubations. However, the majority have suffered from at least one of the following problems: (1) the samples were subjected to incubation/treatment for too short a time (e.g. several months) for the microbes to deplete high-quality C substrates, obscuring the temperature responses of different components of SOC; (2) a single constant incubation temperature could not well mimic the natural temperature changes in field conditions. Constant incubation temperatures may have caused microbial adaptation to different temperatures by producing new enzymes or changing membrane fatty acids (Mikan et al., 2002; Wetterstedt et al., 2010), leading to contradictory results about the temperature sensitivity of SOC decomposition (Davidson and Janssens, 2006).

To avoid those potential problems when testing the “C quality-temperature” hypothesis, we incubated soil samples from a tallgrass prairie with changing temperatures (low-high-low) at a ± 5 °C step every 24 hours. Soil samples had previously been subjected to continuous experimental warming for 10 years and field incubation (litter exclusion) for 9 years. The field incubation treatment should have depleted the original high-quality C substrate in the soil samples and changing lab incubation temperatures to mimic diurnal/seasonal temperature changes in the field should prevent microbial thermal adaptation during the whole incubation period. By changing incubation temperatures, we could mimic what happens in the field as well as focus on the relationship between substrate quality and the temperature sensitivity caused by substrate properties rather than by the properties of the decomposers (Bradford et al. 2008; Wetterstedt et al., 2010).

Grassland ecosystems play an important role in the global C cycling because they occupy approximately a quarter of the global land cover and contain 10 % of the global C stock (Scurlock et al., 2002). Soils from grasslands with warming and field incubation treatments offer us a unique opportunity to address the “C quality-temperature” hypothesis and the potential responses of SOC decomposition to projected global warming. There were four treatments: control (ambient) temperature and normal litter input (C), field warming and normal litter input (W), control temperature and field incubation (FI, 9 years’ litter exclusion), and field warming and field incubation (WFI). The specific questions addressed in this study were: (1) Are Q_{10} values of SOC decomposition relatively high with low C quality under different treatments? The four treatments represent a declining C quality that is hypothesized to be reflected by the Q_{10} of SOC decomposition. (2) Does temperature sensitivity of SOC decomposition differ during the initial and following stages as decomposition proceeds? (3) Does C quality regulate the temperature sensitivity of SOC decomposition under different treatments and under different incubation cycles??

4.2 Materials and Methods

4.2.1 Experimental site and design

The experimental site is located on the Kessler Farm Field Laboratory in central Oklahoma, USA (34°59•N, 97°31•W). The site has never been cultivated and has been ungrazed for the past 40 years. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans*) and C_3 forbs (*Ambrosia psilostachyia, Solidago rigida,* and *Solidago nemoralis*). Mean annual temperature is 16.3 °C and mean annual precipitation is 914 mm (Oklahoma Climatological Survey, Norman, OK, USA). The soil is part of the Nash-Lucien complex with neutral pH, high available water holding capacity (around 37 %), and a moderately penetrable root zone (US Department of Agriculture, 1979).

The experiment uses a split-plot paired factorial design with warming as the main factor and clipping as the nested or split factor. Each treatment has six replicates (i.e. six pairs of plots). Each pair has two plots of 2 m × 2 m. One plot has been subjected to continuous warming since 21 November 1999 to the present while the other serves as the control with ambient temperature. Infrared heaters (165 cm × 15 cm; Kalglo Electronics, Bethlehem, PA, USA) having a radiation output of 100 W m^{-2} are suspended 1.5 m above the ground in each warmed plot. The control plots have a ‘dummy’ heater with same dimensions as the infrared heater suspended at a similar height to mimic the shading effects of the heater. Temperature increments generated by the infrared heaters are relatively even over the entire area of the plots and similar at different soil depths (Wan et al., 2002). For each pair of plots, the distance between warmed and control plots is approximately 5 m from centers to avoid heating of the control plots. The distances between the paired plots vary from 20 to 60 m.

Each 2 m × 2 m plot is divided into four 1 m × 1 m subplots. Plants in two diagonal subplots are clipped at a height of 10 cm above the ground once a year while the other two subplots are unclipped. In each plot, PVC tubes (10 cm in diameter, 70 cm in length) were permanently installed 67-68 cm into soil in two adjacent subplots (one clipped and one unclipped) in October

2001. The tubes cut off old plant roots and prevented new roots from growing into the tubes. Litter that fell into the tubes was manually removed once or twice a month. For incubation, soil samples were taken from two unclipped diagonal subplots. In the subplot with deep tube installation, soils samples were taken from the deep tube. Clipping treatment was not considered in this experiment. Thus, we have four treatments in total: control (ambient) temperature and normal litter input (C), field warming and normal litter input (W), control temperature and field incubation (FI, 9 years' litter exclusion), and field warming and field incubation (WFI).

4.2.2 Microclimate

Soil temperature was measured by thermocouples installed 2.5 cm deep in the soil at the center of one unclipped subplot in each plot. The hourly average data was stored in a SM192 Storage Module (Campbell Scientific, Logan, Utah, USA). Volumetric soil water content (%V) was measured once or twice a month using Time Domain Reflectometry (TDR) equipment (Soil Moisture Equipment Crop., Santa Barbara, CA, USA) at soil depths of 0-15 cm.

4.2.3 Soil sampling and C pool measurements

On October 4th, 2010, we took two soil cores from the subplot with no deep tube and one soil core from deep tube in the other subplot in each plot. Soil cores were 5 cm in diameter and 0-20 cm in depth. The holes in subplots without deep tubes were immediately filled with root-free soils originated from the same depth outside of the plots. In deep tubes, we filled the holes by installing PVC pipes (same size as soil cores) full of soils. Soil samples were packed in polyethylene bags, immediately stored in coolers, and transported to the Ecolab at the University of Oklahoma, Norman. The soil samples were sieved (<2 mm) to remove soil fauna, rocks, and fine roots and kept fresh at 5 °C before incubation. A small proportion of soil samples were air-dried, finely ground, and sieved (<0.25 mm) to measure soil organic carbon (SOC) and chemically labile SOC (LOC). SOC and total nitrogen (TN) were measured at the Environmental and Agricultural Testing Service at North Carolina State University. LOC content before and after lab incubation was estimated using the modified potassium permanganate ($KMnO_4$, 0.02 mol L^{-1}) procedure (Weil et al., 2003; Mirsky et al., 2008).

4.2.4 Laboratory incubation

During the 170 d incubation, fresh soil samples (100 g) were incubated in quart jars in a low temperature incubator (Model 2020, Sheldon Manufacturing Inc. Oregon, USA) after a 20-day pre-incubation at 20 °C starting on October 5th, 2010. We had 5 single incubation cycles in total and during each single cycle, temperature was continuously increased by 5 °C of each 24 hr period, beginning at 5 °C up to 35 °C (increasing temperature period, ITP) and then, at 35 °C down to 5 °C (decreasing temperature period, DTP). Six controls, with no soil, were prepared and incubated at the same time. After each cycle, soils were kept at 20 °C for 10, 20, and 30 days, respectively. Small vials (30 ml, with lids removed) containing 5 ml of 1 mol L^{-1} NaOH solution were placed in each Mason jar to trap respired CO_2 (Liu and Zou, 2002). Samples were taken every 24 hr by removing the NaOH vials. The amount of CO_2 was determined by titration of the NaOH with 1 mol L^{-1} HCl to pH 8.3 in the presence of $BaCl_2$. Quart jars were flushed with compressed air to allow replenishment of O_2 after each interval and deionized water was added to maintain moisture at 60 % of water holding capacity. No measurements were carried out at 5 °C because of low respiratory rates.

4.2.5 Statistical analysis

To describe the relationship between decomposition rates of SOC across the temperature range (10-35 °C), Eq. 1 was used (Fig. 4.1, Mikan et al., 2002; Fierer et al., 2005):

$$R_T = Ae^{kT} \qquad (1)$$

where R_T is the decomposition rate (ug CO_2-C g^{-1}soil d^{-1}) at a given temperature *T* (°C), *A* and *k* are the exponential fit parameters.

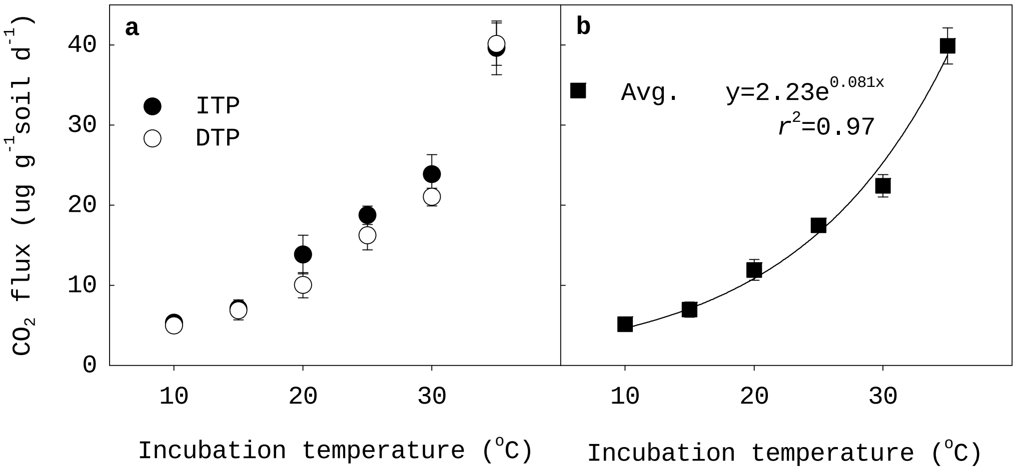

Figure 4.1 An example of Q_{10} value calculation of SOC decomposition by curve fitting. No statistically significant differences were found at any specific incubation temperature between increasing temperature period (ITP) and decreasing

temperature period (DTP) (a), we thus averaged the respiration values at each temperature when fitting the curve to get Q_{10} values (b, Fang et al., 2005). Values are mean±SE (n=6) from the first incubation cycle under control treatment. Q_{10} values based on the fitted curves with respiration rates from ITP, DTP, and averaged values were not significantly different from each other (all $P>0.05$).

Throughout this paper, Q_{10} is used to describe the temperature sensitivity of SOC decomposition, calculated as:

$$Q_{10} = e^{10k} \tag{2}$$

As in other studies (Bosatta and Ågren, 1999; Mikan et al., 2002; Fierer et al., 2005), we hypothesized that C quality (the availability and lability) of SOC equals *A* in Eq. 1, which relates decomposition rate to temperature. The parameter *A* is considered to be a simple index of the overall C quality of SOC that is being utilized by microbes at a specific time point.

Repeated-measures ANOVA were used to examine the effects of field warming, field incubation (litter exclusion), incubation cycle, and their interactions on CO_2 release, Q_{10} values, and SOC quality. One-way ANOVA was performed to test the significance of the amount of CO_2 respired and Q_{10} values between increasing and decreasing temperature periods. A *t*-test method was used to examine the significance of regression coefficients *A* and *k* in Eq. 1 between different treatments. Detailed description of the *t*-test method can be found in Toutenburg (2002) and Zhou et al. (2006). Regression analyses were used to evaluate the relationships between substrate quality (*A*) and Q_{10} values and the relationships of changes in SOC quality with changes in Q_{10} values under different treatments and under different incubation cycles. All statistical analyses were conducted using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA).

4.3 Results

4.3.1 Microclimate and soil properties

Field experimental warming elevated soil temperature by an average of 1.70 °C and lowered volumetric soil moisture in 2010 (Table 3.1). The amount of LOC was significantly lower in the soils subjected to field incubation in comparison to that in the control both before and after lab incubation. Warming or field incubation alone had little effects on SOC and TN, but their interactions significantly decreased SOC and TN (Table 3.1). C:N ratios of the soils were little affected by either field warming or field incubation.

Table 4.1 Microclimate and soil properties under four treatments in field conditions.

Treatment	T_{soil} (°C)	W_{soil} (%)	SOC (mg g^{-1})	TN (mg g^{-1})	C:N	LOC$_{b}$ (mg g^{-1})	LOC$_{a}$ (mg g^{-1})
C	16.30±0.18^{b}	29.78±1.15^{a}	12.42±1.65^{a}	1.13±0.14^{a}	10.95±0.47^{a}	0.30±0.03^{a}	0.08±0.01^{a}
W	18.00±0.15^{a}	28.62±1.31^{a}	9.72±1.07^{ab}	0.90±0.10^{ab}	10.84±0.52^{a}	0.22±0.04^{ab}	0.06±0.01^{ab}
FI	16.30±0.18^{b}	33.59±4.11^{a}	10.50±2.14^{ab}	0.98±0.16^{ab}	10.61±1.27^{a}	0.16±0.01^{bc}	0.05±0.01^{b}
WFI	18.00±0.15^{a}	27.30±1.38^{a}	7.47±0.93^{b}	0.72±0.09^{b}	10.54±0.70^{a}	0.11±0.03^{c}	0.04±0.01^{b}

Note: C: control (ambient) temperature and normal litter input; W: warming and normal litter input; FI: control temperature and field incubation (9 yrs' litter exclusion); and WFI: field warming and field incubation. T_{soil}: soil temperature; W_{soil}: volumetric soil moisture; LOC$_{b}$: labile soil organic carbon content before lab incubation; LOC$_{a}$: labile soil organic carbon content after lab incubation. The base of mg g^{-1} is soil mass (mg/g soil). Values are mean±SE (n=6). For T_{soil} and W_{soil}, values are mean±SE from 2010. Different letters indicate statistical significance at $P<0.05$.

4.3.2 SOC decomposition rates

During the whole laboratory incubation, field incubation treatment (litter exclusion) and lab incubation cycles significantly influenced SOC decomposition (all $P<0.05$, Table 4.2). The decomposition rates of SOC of soils subjected to 9 years' field incubation were significantly lower than those of control and warmed soils (Fig. 4.2a), indicating the depletion of LOC with no fresh C input under litter exclusion treatment. SOC decomposition decreased with increasing lab incubation cycles under four treatments. For example, the respired CO_2 decreased from 20.75±0.66 mg g^{-1} in the 1st cycle to 10.95±0.46 mg g^{-1} in the 5th cycle under control treatment (Fig. 4.2a). The decomposition of SOC followed a two-phase variation with a rapid decrease in the 1st two cycles and leveling off in the last three cycles (Fig. 4.2a). LOC content in the soils under the four treatments were positively correlated with the amount of CO_2 released ($P=0.006$, Fig. 4.2c insert).

Table 4.2 Results of repeated-measures ANOVA (*P* values) for the response of CO_2 release and Q_{10} values to field warming (W), field incubation (FI), each single incubation cycle (cycle), and their interactions (n=6). A single incubation cycle is composed of an increasing temperature period (ITP) and a decreasing temperature period (DTP). *P* values smaller than 0.05 are in bold.

Factor	W	FI	cycle	W×FI	W×cycle	FI×cycle	W×FI×cycle
CO_2	0.767	<**0.001**	<**0.001**	0.843	**0.041**	<**0.001**	0.727
Q_{10}	0.300	0.227	**0.006**	0.881	0.793	0.381	0.509
SOC quality	0.192	**0.001**	**<0.001**	0.247	0.082	**<0.001**	0.961

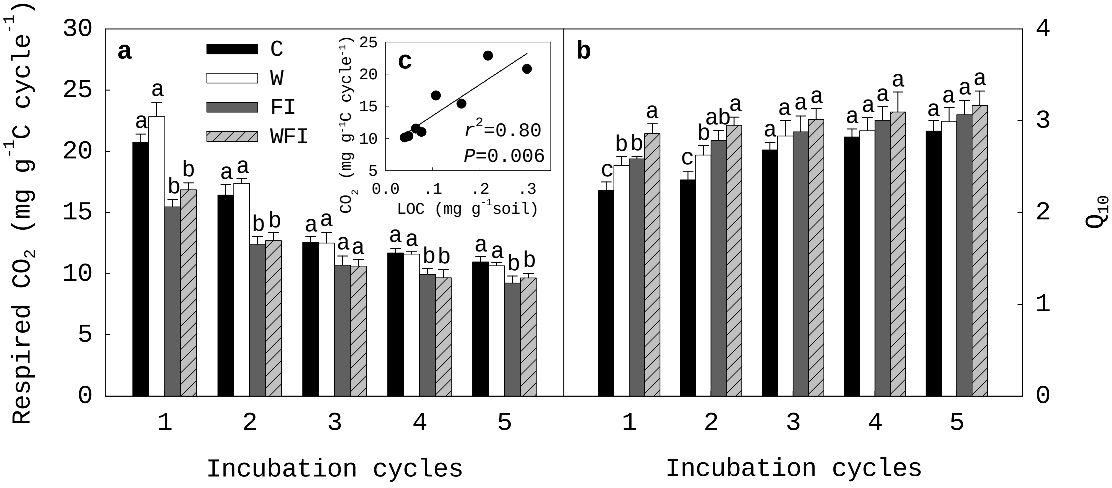

Figure 4.2 Variation in SOC decomposition (expressed as respired CO_2, a) and Q_{10} values (b) over lab incubation cycles under four treatments. The insert panel (c) shows a correlation between LOC content (before and after lab incubation) and respired CO_2 in

the 1st and 5th incubation cycles (n=8). C: control (ambient) temperature and normal litter input; W: field warming and normal litter input; FI: control temperature and field incubation (9 yrs' litter exclusion); WFI: field warming and field incubation.

4.3.3 Q_{10} of SOC decomposition

The temperature sensitivity of SOC decomposition increased with increasing lab incubation cycles (*P*=0.006, Table 4.2). Under the control treatment, for example, the estimated mean Q_{10} values based on respired CO_2 for SOC decomposition were significantly higher in the 5th cycle than that in the 1st cycle (2.89±0.12 vs. 2.24±0.09, Fig. 4.2b). The trend in Q_{10} values was the same among different treatments during the whole lab incubation cycle (Fig. 4.2b). Within each lab incubation cycle, soils with low LOC content under the four treatments exhibited high sensitivity to changes in temperature (Table 4.1, Fig. 4.2b). Significant treatment effects of field warming or field incubation on Q_{10} values were only observed in the 1st two incubation cycles, probably due to the progressively increased contribution of recalcitrant C and closer similarity in SOC quality among incubated soils under different treatments.

4.3.4 SOC quality and its relationship with Q_{10}

Both field incubation and lab incubation cycles had significant influence on SOC quality (all $P<0.01$, Table 4.2). After the 2nd, 3rd, 4th, and 5th cycle of lab incubation, there was a 30.59%, 62.94%, 70.62%, and 74.01% decrease, respectively, in SOC quality (A) in comparison to that of the 1st cycle under the control treatment. SOC quality was negatively correlated with Q_{10} values both under different treatments over all incubation cycles (Fig. 4.3a, b) and under different incubation cycles over all treatments (Fig. 4.3c), regardless of whether the differences in SOC quality resulted from inherent differences in SOC chemistry or from differences in the extent of SOC decomposition. Changes in the temperature sensitivity of SOC decomposition (Q_{10}) under field warming, field incubation, and different laboratory incubation cycles were largely regulated by the changes in SOC quality induced by field warming, field incubation, and lab incubation cycles, respectively (Fig. 4.4). Larger decreases in SOC quality led to higher increases in Q_{10} values.

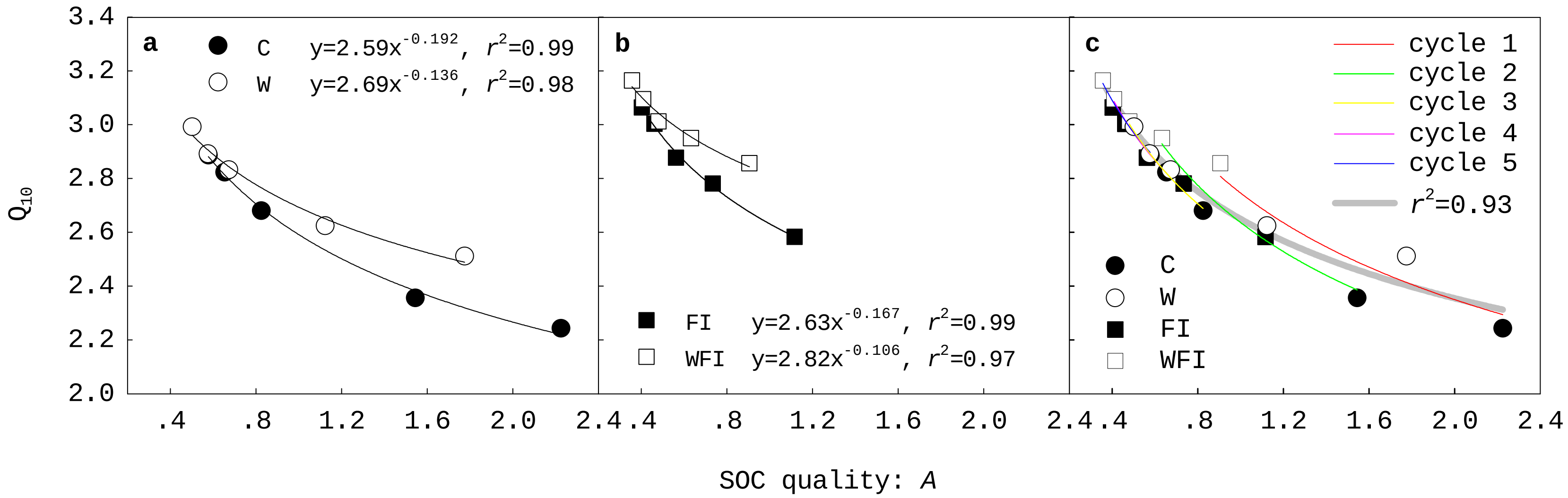

Figure 4.3 The negative relationships between SOC quality (*A*) and the Q_{10} of SOC decomposition under different treatments over all incubation cycles (a, b) and under different incubation cycles over all treatments (c). Different color lines in panel (c) represent curves fit within each lab incubation cycles (n=4). The grey line shows an overall relationship between SOC quality and the Q_{10} values across all treatments and incubation cycles (n=20). Data were fit using the two-parameter power equation: $Q_{10} = a \times A^k$. See Figure 4.2 for acronyms (C, W, FI, and WFI).

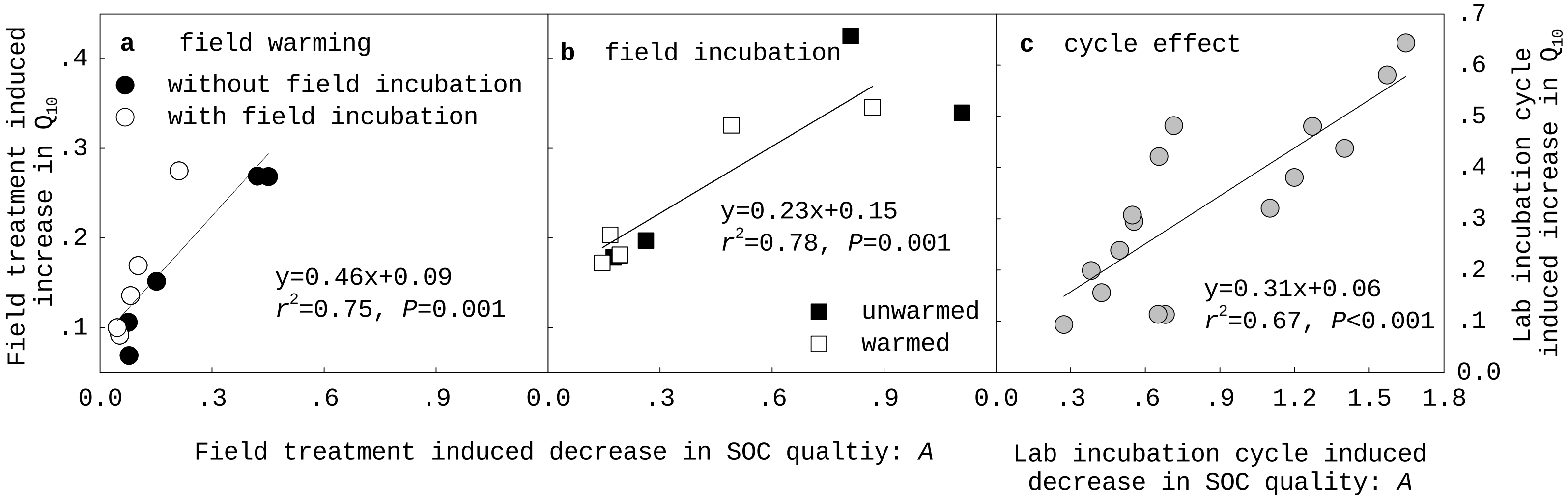

Figure 4.4 Relationships of the changes in SOC quality (*A*) and Q_{10} values under field warming (a, n=10), field incubation (b, n=10), and different laboratory incubation cycles across all treatments (c, n=16). Incubation-cycle effects in panel c represent the differences of the 1st incubation cycle with the following four (2nd - 5th) cycles.

4.4 Discussion

4.4.1 Comparisons of SOC decomposition rates

Overall, the rates of SOC decomposition of the soils subjected to 9 years’ field incubation were significantly lower in comparison to those without field incubation (Fig. 4.2a). This probably resulted from low LOC content in the soils under field incubation with no fresh C input (Table 4.1). Low-quality SOC is generally accepted to limit the availability of energy for soil microbes, leading to low rates of SOC decomposition (Paul and Clark, 1989). In contrast, soils with a plant litter supply received many labile C compounds that accelerated SOC decomposition by stimulating microbial activity (Fontaine et al., 2004).

Additionally, we observed a two-phase pattern for SOC decomposition under different treatments, with decomposition rates dropping substantially in the 1st two incubation cycles and remaining relatively low and constant in the last three cycles (Fig. 4.2a). A decline in SOC decomposition rates was widely observed with increasing laboratory incubation time (e.g. Fang et al., 2005; Conant et al., 2008; Wetterstedt et al., 2010).

The rapid drop in the 1st phase may be attributable to the depletion of LOC of soils being incubated (Eliasson et al., 2005). Microbes decomposed LOC relatively quickly under controlled lab conditions due to the cutting off of fresh C supply from plants. With the progressive increase in the contribution of recalcitrant compounds, SOC decomposition leveled off in the 2nd phase (Vanhala et al., 2007; Xu et al., 2010). Though differences in microbial composition may exist in the soils under different treatments (Zhang et al., 2005), changes in LOC or SOC quality were believed to be the major factors influencing the decline in SOC decomposition (Fig. 4.2c, Kirschbaum, 2006; Conant et al., 2008).

4.4.2 Comparisons of Q_{10} estimates

How the sensitivity of SOC decomposition responds to temperature changes has received considerable interest due to its importance in projecting future climate change (e.g. Fang et al., 2005; Davidson and Janssens, 2006; Contant et al., 2008; Craine et al., 2010). In line with many previous incubation studies (e.g. Fierer et al., 2005; Contant et al., 2008; Wetterstedt et al., 2009; Xu et al., 2010), our results showed that soils subjected to field warming or field incubation had higher temperature sensitivity than the control soils (1st two cycles, Fig. 4.2b). Since soil samples were incubated under controlled moisture and temperature conditions, this may arise from the low LOC content (low SOC quality) in warmed or field incubated soils (Table 4.1). In grasslands, warming has been reported to largely increase C_4-derived litter input to soil organic matter and decreased SOC quality without influencing SOC content (Cheng et al., 2011), suggesting SOC decomposition was quality-dependent. It is a reasonable supposition given high temperature sensitivity and low C quality are usually coupled across multiple scales and soil types (Craine et al., 2010).

The differences in the response of SOC decomposition to temperature indicated a shift to the decay of biochemically recalcitrant C, especially in the soils with the 9-year field incubation treatment. As decomposition progressed over time with increasing contribution of recalcitrant C, however, differences in Q_{10} values of different soils became much smaller (the last two cycles, Fig. 4.2b). Due to the high temperature sensitivity of the large amount of recalcitrant C stored in soil (50-90%, Trumbore, 1997), climate warming may stimulate loss of soil-stored C and cause a positive feedback to climate change.

4.4.3 Relationship between SOC quality and Q_{10}

We observed strong negative relationships between SOC quality and Q_{10} values across soils under different treatments and incubation cycles (Fig. 4.3). Many laboratory studies also found that temperature sensitivity of SOC decomposition increases with decreasing substrate quality (e.g. Mikan et al., 2002; Fierer et al., 2005; Vanhala et al., 2007; Wetterstedt et al., 2009). These results support the enzyme kinetics that temperature sensitivity of SOC decomposition is inversely proportional to the complexity of the C substrates being decomposed by microbes (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). With increasing incubation time, the quality of the decomposed C substrates decreased, as evidenced by the decrease in the *A* values (Fig. 4.3). In contrast to our results, a previous study suggests that low- and high-quality organic matter responds to temperature changes in a similar way based on the averaged Q_{10} values of the decomposition of intact and root-free soil samples from different layers (0-10, 20-30 cm)

(Fang et al., 2005). However, the dynamics of SOC decomposition are likely to be quite different between intact and root-free soils from different layers with factors such as substrate availability (Davidson and Janssens, 2006) and physical protection (Six et al., 2002), preventing us from directly comparing the results. Though temperature sensitivity of SOC decomposition is regulated by several factors, such as substrate availability (Davidson and Janssens, 2006), inhibition of microbial activity (Balser and Firestone, 2005), and physico-chemical protection (Oades, 1988; Six et al., 2002), the intrinsic C quality responds most to changes in temperature (Craine et al., 2010).

4.4.4 Relationship of treatment- or incubation cycle-effects between SOC quality and Q_{10}

Since the degree of treatment and lab incubation-cycle effects on Q_{10} values largely depended on the changes in SOC quality (Fig. 4.4), it becomes clear that accurate estimates of C storage and turnover will require an understanding of the factors controlling SOC quality under global climate change. Warming is hypothesized to influence SOC quality through directly affecting organic C composition or indirectly influencing plant community structure (Fissore et al., 2008; Cheng et al., 2011). Warming may affect the composition of SOC by accelerating LOC decomposition, which was found in forest and grassland ecosystems subjected to long-term warming (Bradford et al. 2008; Xu et al., unpublished data). In addition, changes in plant community structure under warming are widely reported, such as in tundra ecosystem and in grassland ecosystem (e.g. Chapin et al., 1995; Luo et al., 2009). Species composition in determining the magnitude of changes in SOC quality is as important as direct warming effects (Hobbie, 1996) because SOC is almost exclusively derived from detrital input of plants growing on the site (Cheng et al., 2011). For example, warming increased low-quality C_4 species litter input to soils in grasslands, resulting in substantial consequences for biogeochemical processes in reducing SOC quality (Table 4.2, Fissore et al. 2008; Cheng et al., 2011). SOC quality is likely to decrease in response to global warming (Allison et al., 2009) and shifting species composition (Luo et al., 2009). Given the high temperature sensitivity of large-amount, low-quality SOC, changes in its dynamics have the highest potential to impact long-term C stocks. Moreover, how C quality influences SOC decomposition in relation to warming may potentially become a prominent determinant of whether we will have a positive or negative feedback of SOC to current global warming in the future.

4.5 Conclusions

Our laboratory incubation of soils subjected to different field treatments in the tallgrass prairie suggests that C quality of SOC largely influences the temperature sensitivity of SOC decomposition. Field warming, field incubation, and increasing laboratory incubation time all increased Q_{10} of SOC decomposition, possibly due to the decreased contribution of LOC under the three conditions. SOC quality was lower in the soils under field warming and field incubation treatments and decreased with increasing incubation time across all treatments. Negative correlations were found between SOC quality and the temperature sensitivity of SOC decomposition and of treatment effects of field warming or field incubation on SOC quality and Q_{10}, suggesting a potentially positive feedback of SOC to future global warming in response to the decrease in SOC quality resulted directly from warmer temperatures or indirectly from warming-induced changes in plant community structure.

4.6 Acknowledgements

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Chapter 5

Conclusions and Implications

5.1 Conclusions

Global change experiments (i.e. warming) are important for isolating ecosystem responses to climate change. The analyses presented in this dissertation lead to several important conclusions regarding the responses of grassland ecosystems to projected global warming. As illustrated in Chapter 1, there is a relatively large amount of data on how global climate change impacts aboveground C dynamics. However, there is little known about belowground C cycling in response to global warming. Understanding these responses is exceedingly important for developing a conceptual framework of possible future environmental change. In this dissertation, a direct investigation of global warming was done on understanding the responses of belowground C cycling. Some of the major findings are below:

In the BNPP study, we found that experimental warming and annual clipping had substantial and yearly dependent effects on BNPP and NPP partitioning (f_{BNPP}). Across years, both warming and clipping positively increased BNPP. A relative increase in belowground allocation, f_{BNPP}, was observed as a consequence of the increased BNPP and dry conditions under warming and clipping treatments. Multifactor linear regressions showed that water availability regulated the temporal variability in BNPP, warming-induced changes in BNPP, and clipping-induced changes in f_{BNPP}. Practical use of varied f_{BNPP} in global terrestrial models would thus improve C cycle modeling. To accurately forecast ecosystem feedback to climate change, models may have to simulate responses of BNPP and f_{BNPP} to global warming and land use change as well as their consequences for ecosystem functions.

The labile C study suggested that warming may have substantial impact on both LOC and SOC in long-term. Across years, warming significantly decreased LOC. Though SOC content was not significantly influenced by warming, the proportion of LOC to SOC was much lower under the warming treatment, which may influence soil C quality and biogeochemical processes in the future. The two-year lagged decreases in LOC and warming-increased C_4 biomass seem to relate to each other, making predictions of warming effects on C cycling in grasslands more complicated.

Laboratory incubation is an effective way to study the temperature sensitivity of SOC decomposition. Compared to the natural field conditions, it suffers much less from the confounding field conditions and provides a reliable estimate of the temperature dependence of organic matter decomposition (Kirschbaum, 2006). Results from our incubation showed that C quality of SOC negatively correlated with the temperature sensitivity of SOC decomposition. This negative correlation held true across treatments (field warming and field incubation) and lab incubation cycles, regardless of whether the differences in SOC quality resulted from inherent differences in SOC chemistry or from differences in the extent of SOC decomposition, potentially suggesting a positive feedback of SOC to future global warming.

5.2 Implications

The frequency of extreme weather events, such as unusual wet and dry years, is predicted to increase under global climate change (IPCC, 2007). The BNPP study demonstrated that water availability largely influenced the interannual variability of BNPP and f_{BNPP} under warming and clipping. These results suggest that more precipitation manipulation experiments should be conducted to understand the potential responses of ecosystem processes under global warming (Wu et al., 2011). Ideally, results from such manipulation experiments will be important for understanding the availability of water resources and quantifying plant-atmosphere water feedbacks that drive weather and may inform future climate modeling.

An integrative evaluation of NPP (both ANPP and BNPP) across sites has yet to be performed due to the difficulty of the measurements. ANPP is commonly used to represent the response of plant growth to climate change. However, inclusion of BNPP would be important and more accurate in understanding whole plant response to climate change and give further indication of C allocation and sequestration. Plant allocation schemes are one of the most important C parameters in ecosystem C cycle modeling, and determines where the C goes and the turnover time in the system. Therefore, estimates of BNPP from field manipulative experiments which can shed light on the direction and magnitude of f_{BNPP} in response to climate change at community level are badly needed.

Shifts in plant species composition under climate change have been observed (Harte and Shaw, 1995; Walker et al., 2006; Luo et al., 2009) and reported to determine C cycling (Hobbie, 1996), but little is known about the effects of shifting species on soil biogeochemical process. In the labile C study, the effects of warming and warming-induced changes in plant community composition on soil C pools are confounded, thus making it difficult separate them. Thus, more research is needed to study the impact of species shifts on soil-stored C and possible feedbacks to climate change. Additionally, attention should be paid to the changes in the composition of different organic C pools in soil, as well as various C component impacts on nutrient availability, plant and microbial growth, C decomposition and sequestration.

It seems we still have a long way to go before we understand the temperature sensitivity of soil organic matter decomposition. The fundamental principles of soil biogeochemistry have been challenged by new insights gathered from different disciplines (marine science, microbiology, and material science). For example, new observations have shown that the perceived importance of recalcitrance of input biomass and humic substances were only marginally important for organic matter cycling (Marschner et al., 2008; Kleber and Johnson, 2010). Understanding of the processes related to soil C cycling are emerging, such as rhizosphere inputs, physical disconnection, and freezing/thawing (Shmidt et al., 2011). Research on the persistence of soil organic matter is badly needed for understanding why some organic matter decomposes readily, while other components persist for millennia. Without this understanding, it will be difficult to predict how soils will respond to climate change.

Because of the wide range of variability and potential unpredictability of future climate change, more research is needed on various climate scenarios and gradients to understand ecosystem responses and potential tipping points. The information will be highly valuable for validating future ecosystem models, making predictions of future climate, and guiding policy decisions.

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