

Terrestrial carbon-cycle feedback to climate warming: experimental evidence on plant regulation and impacts of biofuel feedstock harvest

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Abstract

Feedback between global carbon (C) cycles and climate change is one of the major uncertainties in projecting future global warming. Coupled carbon–climate models all demonstrated a positive feedback between terrestrial C cycle and climate warming. The positive feedback results from decreased net primary production (NPP) in most models and increased respiratory C release by all the models under climate warming. Those modeling results present interesting hypotheses of future states of ecosystems and climate, which are yet to be tested against experimental results. In this study, we examined ecosystem C balance and its major components in a warming and clipping experiment in a North America tallgrass prairie. Infrared heaters have been used to elevate soil temperature by approximately 2 °C continuously since November 1999. Clipping once a year was to mimic hay or biofuel feedstock harvest. On average of data over 6 years from 2000 to 2005, estimated NPP under warming increased by 14% without clipping ($P < 0.05$) and 26% with clipping ($P < 0.05$) in comparison with that under control. Warming did not result in instantaneous increases in soil respiration in 1999 and 2000 but significantly increased it by approximately 8% without clipping ($P < 0.05$) from 2001 to 2005. Soil respiration under warming increased by 15% with clipping ($P < 0.05$) from 2000 to 2005. Warming-stimulated plant biomass production, due to enhanced C₄ dominance, extended growing seasons, and increased nitrogen uptake and use efficiency, offset increased soil respiration, leading to no change in soil C storage at our site. However, biofuel feedstock harvest by biomass removal resulted in significant soil C loss in the clipping and control plots but was carbon negative in the clipping and warming plots largely because of positive interactions of warming and clipping in stimulating root growth. Our results demonstrate that plant production processes play a critical role in regulation of ecosystem carbon-cycle feedback to climate change in both the current ambient and future warmed world.

Keywords: bioenergy, biofuel feedstock production, climate change, net ecosystem carbon balance, net ecosystem production, plant growth, primary production

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Introduction

As a consequence of anthropogenic buildup of CO₂ and other greenhouse gases in the atmosphere, the Earth's surface temperature has increased by 0.76 °C since 1850 and is expected to increase by another 1.8–4.0 °C by the end of this century (Solomon *et al.*, 2007). While

temperature influences almost all ecosystem processes (Shaver *et al.*, 2000), it is still not well understood whether or not climate warming would stimulate net ecosystem carbon (C) release, accelerate buildup of atmospheric CO₂ concentration, and then amplify climate warming (Luo, 2007). Global models that couple climate change with C cycles all predicted a positive feedback that climate warming accelerates CO₂ buildup in the atmosphere by 20–200 ppmv and amplifies climate warming by 0.1–1.5 °C (Friedlingstein *et al.*, 2006).

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The modeling predictions represent a set of interesting hypotheses on future states of climate and ecosystems, which have to be carefully examined against empirical evidence from experiments and observations (Moorcroft, 2006).

Terrestrial C-cycle feedback to climate warming is positive if warming results in net C release from ecosystems but negative if warming results in net C storage in ecosystems (Luo, 2007). Many processes can potentially affect ecosystem C balance and then regulate terrestrial C feedback to climate change. For example, modeling analysis mostly suggested that respiratory C release is highly sensitive to changes in temperature and is a key process leading to positive feedback to climate warming (Cox *et al.*, 2000; Friedlingstein *et al.*, 2006). Inspired by the modeling analysis, the research community has conducted extensive studies on temperature sensitivity of decomposition of soil organic matter (SOM) in past decades (Davidson & Janssens, 2006; Luo & Zhou, 2006). Results are diverse, sometimes controversial and contradictory (e.g., Liski *et al.*, 1999; Luo *et al.*, 2001; Fang *et al.*, 2005, 2006; Knorr *et al.*, 2005; Ågren & Wetterstedt, 2007), owing to (a) different temperature levels used in studies, (b) different components of an ecosystem examined, (c) variations in confounding environmental and edaphic factors among studies, (d) interactions of various factors in influencing temperature sensitivity, (e) different durations of studies, and (f) different interpretations of experimental data by different investigators.

More importantly, respiratory C release is tightly coupled with ecosystem C uptake (Högberg *et al.*, 2001; Wan & Luo, 2003; Larsen *et al.*, 2007). Root and mycorrhizal respiration accounts for a large fraction, mostly ranging from 30% to 70%, of soil respiration (Hanson *et al.*, 2000; Bond-Lamberty *et al.*, 2004). Any environmental fluctuation or manipulations that alter canopy photosynthetic C uptake usually influence respiratory C release a few hours or days later (Ekblad & Högberg, 2001; Bowling *et al.*, 2002; Ekblad *et al.*, 2005; Tang *et al.*, 2005). Long-term changes in plant C uptake affect not only autotrophic respiration but also substrate supply to heterotrophic respiration via litterfall and soil organic C formation (Luo & Zhou, 2006). Thus, temperature sensitivity of ecosystem respiratory C release cannot be fully evaluated in isolated soil components without plant interactions.

It has been well known that climate warming does not only affect leaf-level photosynthesis but also other processes (Luo, 2007), such as biomass growth (Saleska *et al.*, 2002; Dukes *et al.*, 2005; Wan *et al.*, 2005), nutrient availability (Emmett *et al.*, 2004), plant nutrient uptake and use efficiency (Melillo *et al.*, 2002; An *et al.*, 2005), and species composition (Harte & Shaw, 1995; Harte

et al., 2006). Any of those changes that affect plant C uptake will influence C release in intact ecosystems, feeding back to climate change. In addition, recent sensitivity analysis by global models has also shown that responses of photosynthesis and net primary production (NPP) to climate strongly regulate the climate-carbon feedback (Matthews *et al.*, 2007). Thus, it is imperative to conduct experiments that expose whole ecosystems to warming to fully evaluate feedback of terrestrial C cycling to climate warming.

Terrestrial carbon-cycle feedback to climate warming can strongly vary with land-use practices (Chapin *et al.*, 2008). Every year, human land-use activities result in net release of about 1.6 Pg of carbon to the atmosphere (Houghton, 2007), enhancing climate warming. In grasslands, major land-use activities include grazing and hay harvesting, both of which potentially influence ecosystem carbon balance (Sanderson, 2008) and feedback to climate change. As bioenergy has been proposed to be a major measure to mitigate climate change, the Southern Great Plains, where this study was conducted, is potentially to be a major region for biofuel feedstock production. It is urgent to evaluate how biofuel feedstock production impacts ecosystem carbon balance and feedback to climate warming at the present and future warmed climates.

In this study, we quantified ecosystem C budget and its major components in an ecosystem-level, multiyear warming and clipping experiment with a mix of new data and synthesis of published results. We measured aboveground plant biomass three times per year and belowground biomass three times from 1999 to 2005. The peak aboveground biomass was used to estimate aboveground NPP (ANPP). From published results, we estimated a root turnover rate, which was combined with measured root biomass to estimate belowground NPP (BNPP). Monthly measured soil respiration at soil surface and with deep-insertion cores, which was reported by Zhou *et al.* (2007b) primarily on source components and interannual variability, was again used in this study to estimate total soil C release and its autotrophic and heterotrophic components. We estimated net ecosystem production (NEP) from NPP minus heterotrophic respiration, and net ecosystem C balance (NECB) from NEP minus C in biomass removal. Cumulative NECB over 6 years was compared with changes in measured soil C content three times from 1999 to 2005. A statistical linear model was used to estimate error propagation from individual measurements to derived quantities that represent ecosystem C balance. Although the clipping treatment of this study was originally motivated to mimic hay harvesting, our results are useful for evaluating impacts of biofuel feedstock removal on ecosystem C balance.

Materials and methods

Experimental site, design, and facility

The experimental site was located on the Kessler's Farm Field Laboratory (formerly Great Plains Apiaries) in McClain County, OK (34°58'54"N, 97°31'14"W), approximately 40 km from Norman campus of the University of Oklahoma. This site has not been grazed for the past 40 years. The grassland was dominated by C₄ grasses – *Sorghastrum nutans*, *Andropogon gerardii* and *Panicum virgatum*; and C₃ forbs – *Ambrosia psilotachyia*, *Solidago rigida*, *Solidago nemoralis*, and *Hemiachyris dracunculoides*. The mean annual precipitation averaged from 1948 to 1998 was 914 mm and the mean annual temperature was 16.3 °C (Oklahoma Climatological Survey, Norman, OK, USA). The soil belongs to the Nash–Lucien complex, characterized by deep and moderately penetrable root zone, high available water-holding capacity, and a neutral pH (U.S. Department of Agriculture, 1963).

The experiment used a paired factorial design with warming as the main factor nested by clipping factor. Each treatment had six replicates (i.e., six pairs). Each pair had two plots of 2 m × 2 m. One plot had been subjected to continuous warming since 21 November 1999 to the present while the other was the control with ambient temperature. A single infrared heater (165 cm × 15 cm; Kalglo Electronics, Bethlehem, PA, USA) having a radiation output of 100 W m⁻² was suspended 1.5 m above the ground in each warmed plot. Reflector surfaces of the heaters were adjusted so as to generate evenly distributed radiant input to soil surface (Kimball, 2005). As a result, temperature increments generated by the infrared heaters were relatively even over the entire areas of plots and similar at different soil depths (Wan *et al.*, 2002). The control plot had a 'dummy' heater with same dimensions as the infrared heater suspended at a similar height to mimic the shading effects of the heater. For each paired plot, the distance between warmed and control plots was approximately 5 m to avoid heating of the control plots. The distances between the paired plots varied from 20 to 60 m.

Each 2 m × 2 m plot was 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 or biofuel feedstock production while the other two subplots were unclipped. Clipped materials were taken away and not returned back to the plots. Thus, this experiment has four treatments: unclipping and control (ambient) temperature (UC), unclipping and warming (UW), clipping and control temperature (CC), and clipping and warming (CW).

Temperature, moisture, and precipitation measurement

Soil temperature was monitored by homemade thermocouples installed at the depth of 2.5 cm at the centers of one clipping and one unclipping subplot in each plot. Air temperature at the height of 25 cm above the ground was measured by thermocouples at the centers of each plot. The thermocouples were connected to a CR10 data-logger (Campbell Scientific Inc., Logan, UT, USA) and recorded air and soil temperature every 10 min. Then the hourly average was stored in an SM196 Storage Module (Campbell Scientific). In addition, soil temperature at the depth of 5 cm was measured adjacent to each PVC collar at the time of the soil respiration measurement using a thermocouple connected to a LI-6400 portable infrared gas analyzer (IRGA) (LI-COR Inc., Lincoln, NE, USA).

Soil moisture content was measured gravimetrically twice a month from September 1999 to December 2000. Soil samples from the top 5 cm were taken from one clipped and one unclipped subplot in each plot and oven-dried at 105 °C for 24 h and weighed. Soil moisture was expressed as a percentage of dry soil on a mass basis. Beginning from January 2001, volumetric soil water content (%V) was measured using manual Time Domain Reflectometry (TDR) equipment (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at the depth interval of 0–15 cm. TDR was calibrated by measured gravimetric soil moisture content. To be consistent for analysis, those gravimetric soil moisture data before December 2000 were calibrated to volumetric soil moisture according to their relationship between mass-based and measured soil moisture by TDR. Soil bulk density used for the conversion from gravimetric to volumetric soil moisture content was 1.282, 1.310, 1.398, and 1.337, respectively, in the UC, UW, CC, and CW treatment plots. The soil water content was measured once or twice a month. Precipitation was recorded at an Oklahoma Mesonet Station located about 200 m away from the experimental site.

Aboveground plant biomass and root biomass

Aboveground plant biomass (AGB) was measured using two methods: clipping to directly measure AGB and pin-contact counts to indirectly estimate AGB. Plants were clipped at 10 cm height annually from two diagonal clipping subplots at the peak biomass seasons, usually in July–August. Clipped plant biomass (including both brown and green tissues) were first separated into C₃ and C₄ plants and then oven-dried at 65 °C for 48 h. Clipping was done right after the indirect estimation of peak aboveground biomass with a pin-contact method in summer.

The pin-contact method (Frank & McNaughton, 1990) was used to estimate AGB in both the unclipping and clipping 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 were 0.75 m long each and could be raised within the frame to count hits up to 1 m high (hits above 1 m 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 consider the brown tissues to be dead plant materials 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 were 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 instead of 10 cm above the ground. 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. Total hits per subplot (1 m²) ranged from 73 to 771. Regression coefficients varied from 0.51 in August 2002 to 0.84 in August 2003 with an average $r^2 = 0.70$. To account for increased C4 plants in warmed plots, the clipped biomass from warmed and unwarmed clipping subplots was regressed against contact numbers separately to derive calibration equations. The estimated AGB during the peak season in summer (July or August) was considered to be ANPP since our ecosystem satisfied primary criteria of virtually no carryover of living biomass from previous years due to a distinct dormant season and negligible decomposition of biomass produced during the growing season (Knapp *et al.*, 2007). Biomass was converted to C content by a factor of 0.45.

In January 2002, October 2004, and October 2005, soil cores (5.2 cm in diameter and 45 cm in depth) were taken from one unclipped and one clipped subplots in each plot to measure root biomass. The roots were oven-dried at 65 °C for 48 h. We estimated BNPP from root biomass and root turnover rates. Root turnover was quantified in this area of our study (Marshall, 1977; Sims & Singh, 1978) and correlated with temperature according to a meta-analysis of 62 studies in temperate grasslands (Gill & Jackson, 2000). From the temperature–turnover relationship, we estimated a root turnover rate using a mean annual temperature of 16.3 °C at our site. The estimated turnover rate is slightly higher but within a range of the measured ones in Marshall (1977) and Sims & Singh (1978). Then, deviations of the 62 observed root turnover rates in the

meta-analysis database were computed from the temperature–turnover regression line as an estimate of variance for the turnover rate.

Soil respiration and soil carbon content

Zhou *et al.* (2007b) reported source components and interannual variability of soil respiration with detailed measurement methods. This study used the same dataset to compute ecosystem C balances under treatments of clipping and warming. In brief, soil respiration was measured on PVC collars (80 cm² in area and 5 cm in height) inserted 2–3 cm into the soil permanently at the center of each subplot. Living plants inside the soil collars were clipped at the soil surface at least 1 day before the measurement to eliminate aboveground plant respiration. The clipped plant materials were left in the collars. Soil respiration was measured once or twice a month between 10:00 and 15:00 hours (local time), using a LI-COR 6400 portable photosynthesis system attached to a 6400-09 soil respiration chamber (LI-COR Inc., Lincoln, NE, USA). A measurement consisted of placing the chamber on PVC collars, scrubbing the CO₂ to subambient levels, and determining soil CO₂ efflux over a short period. Data were recorded at 5-s intervals by the datalogger in LI-COR 6400 console. Each of the measurements usually took 1–3 min after placing the chamber over the collar.

To estimate annual C loss from soil, we multiplied the number of days between measurements with corrected daily flux according to Wan & Luo (2003), who made hourly measurements of soil respiration to characterize its diurnal patterns at a similar site. The hourly measurements were averaged to estimate a daily mean of soil respiration. Measured soil respiration between 10:00 and 15:00 hours was 103.63% of the daily mean based on diurnal patterns. We used this correction factor in this study to compute annual soil C release for each plot.

We also inserted long PVC tubes (80 cm² in area) to soil by 70 cm in depth to cut off existing roots and to prevent new roots from growth inside the tubes. Measured CO₂ effluxes on the tops of the inserted tubes were, after an initial period of dead root decomposition, considered to be heterotrophic respiration (Zhou *et al.*, 2007b), which was highly comparable with measured heterotrophic respiration in a similar ecosystem using clipping and shading methods (Wan & Luo, 2003). Autotrophic respiration was estimated by subtracting heterotrophic respiration from soil respiration.

We measured soil C density and soil bulk density in soil depth of 0–15 cm, which were used to estimate soil C content in 1999 (pretreatment), 2002, and 2005.

Statistical analysis and error propagation to derived variables

We conducted analysis of variance (ANOVA) of paired plot design (one pair of plots being considered a block) to examine the statistical significance of warming, clipping, plot, year, and their interactive effects on aboveground biomass, belowground biomass, soil surface litter mass, soil respiration, heterotrophic and autotrophic respiration, biomass removal (clipped biomass), and soil C content. All statistical analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, USA). Differences and interactions between the treatments were compared according to Duncan's multiple range test or Student's *t*-test at a probability level of 5%.

To assess error propagation from measurements of aboveground and belowground biomass, heterotrophic respiration, and removed biomass to derivative variables such as NPP, NEP, and NECB, we used a statistical linear model (Sahai & Ojeda, 2004). First, X_{ijkl} is to represent an observation of one variable (e.g., aboveground biomass) in a plot i , where $i = 1, 2, \dots, 6$ for six plots; under a warming treatment j , where $j = 1$ and 2 for control and warming; under a clipping treatment k , where $k = 1$ and 2 for unclipping and clipping; and in year l , where $l = 1, 2, \dots, 6$ for 6 years from 2000 to 2005. X_{ijkl} can be expressed by a statistical linear model as:

$$X_{ijkl} = \mu + \rho_i + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}, \quad (1)$$

where μ is the grand mean of all observations across all plots, treatments, and years; ρ_i is plot effects; α_j is warming effects; β_k is clipping treatment effects; γ_l is year effects; $(\alpha\beta)_{jk}$ is interactive effects of warming and clipping; $(\alpha\gamma)_{jl}$ is interactive effects of warming and year; $(\beta\gamma)_{kl}$ is interactive effects of clipping and year; $(\alpha\beta\gamma)_{jkl}$ is three-way interactions of warming, clipping, and year; and ε_{ijkl} is random error. We considered interactive effects of blocks with the other three factors as part of random error. Thus, random error can be estimated by:

$$\varepsilon_{ijkl} = X_{ijkl} - \left[\mu + \rho_i + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} \right]. \quad (2)$$

We analyzed error distributions and found that errors of all the variables followed normal distributions (data not shown). We did not find significant correlations of errors among variables. Thus, covariance between variables was negligible when we assessed error propagation from measurements to derivative variables.

Because we assume ANPP equals peak aboveground biomass (AGB), thus variance (V) of ANPP is:

$$V(\text{ANPP}) = V(\text{AGB}). \quad (3)$$

Standard error (SE) of ANPP is

$$\text{SE}_{\text{ANPP}} = \sqrt{\frac{V(\text{ANPP})}{n_1}}, \quad (4)$$

where n_1 is the sample size for shoot biomass measurement, equaling 72 for each of treatments (i.e., UC, UW, CC, and CW). BNPP is estimated from standing root biomass (B_r) times root turnover (T), thus its variance is calculated by:

$$V(\text{BNPP}) = B_r^2 V(T) + T^2 V(B_r). \quad (5)$$

At 16.3°C of our site, root turnover rate T was estimated to be 0.5959 year⁻¹ from a regression line of root turnover of 62 studies in grassland ecosystems with temperature (Gill & Jackson, 2000). $V(T)$ was estimated from deviation of observed root turnover from the regression line to be 0.0808. The SE of BNPP is:

$$\text{SE}_{\text{BNPP}} = \sqrt{\frac{V(\text{BNPP})}{n_2}}, \quad (6)$$

where n_2 is the sample size for measurements of root biomass, equaling 18 for each of clipping and warming treatments. Because NPP = ANPP + BNPP, its variance is:

$$V(\text{NPP}) = V(\text{ANPP}) + V(\text{BNPP}) \quad (7)$$

and SE is

$$\text{SE}_{\text{NPP}} = \sqrt{\frac{V(\text{ANPP})}{n_1} + \frac{V(\text{BNPP})}{n_2}}. \quad (8)$$

Similarly, because NEP = NPP - R_h = ANPP + BNPP - R_h , thus SE of NEP is estimated by:

$$\text{SE}_{\text{NEP}} = \sqrt{\frac{V(\text{ANPP})}{n_1} + \frac{V(\text{BNPP})}{n_2} + \frac{V(R_h)}{n_3}}, \quad (9)$$

where n_3 is the sample size for heterotrophic respiration measurement, equaling 24 for each of warming and clipping treatments. After C loss from clipping plots with biomass removal (L_b), NECB is NECB = NEP - L_b = ANPP + BNPP - R_h - L_b and its SE is estimated by:

$$\text{SE}_{\text{NECB}} = \sqrt{\frac{V(\text{ANPP})}{n_1} + \frac{V(\text{BNPP})}{n_2} + \frac{V(R_h)}{n_3} + \frac{V(L_b)}{n_4}}, \quad (10)$$

where n_4 is the sample size for clipped biomass measurements, equaling 72 for each of warming and control treatments.

We used Eqns (3)–(10) to quantify error propagation from measurements to derived variables.

Results

Microclimate

Annual precipitation varied from 522 mm in 2005 to 995 mm in 2000 with a mean of 830 mm during the 6 years (Table 1). Experimental warming elevated air temperature by an average of 1.27 °C with variation from 0.79 °C in 2002 to 2.15 °C in 2005. Increased air temperature in our study largely resulted from a placement of the thermocouples within canopy, which can reach 1 m tall in contrast with that in ecosystems with low-stature plants (Saleska *et al.*, 2002). Soil temperature increased by 1.71 °C without clipping and 2.18 °C with clipping in the warmed plots in comparison with that in the control plots from 2000 to 2005 (Table 1). Soil moisture was lowered by an average of 1.60% and 2.07% volumetrically without and with clipping, respectively, under warming in comparison with those without warming. Warming effects on soil moisture were statistically significant only in 2005 either with or without clipping but not in other years (Table 1). Frequent rainfall events in the region recharged soil moisture and periodically erased warming effects.

Biomass clipping increased soil temperature by 0.43 °C without warming and 0.90 °C with warming in comparison with that without clipping. Soil moisture was lower in clipped than unclipped plots by 0.67% without warming and 1.14% with warming.

Biomass growth and NPP

Experimental warming stimulated plant biomass growth and NPP in the tallgrass prairie (Fig. 1, Tables 2 and 3). Clipped aboveground biomass at the peak season in the clipping treatment plots was significantly higher under warming than that under the control in all experimental years except 2000 (Fig. 1a). We separated the total aboveground biomass into C₃ and C₄ plant biomass. Biomass of C₃ plants was slightly higher in 2000 and 2001 but became lower during 2002–2005 in the warmed plots than in the control plots (Fig. 1b). However, clipped biomass of C₄ plants under warming significantly increased by an average of approximately 40% ($P < 0.05$) from 2001 to 2005 in comparison with that under the control.

Indirectly estimated biomass in spring, summer, and fall each year using the pin-contact method was generally higher in warming than control treatments either with or without clipping (Table 2). Warming significantly increased spring aboveground biomass in 2001, 2002, and 2004 without clipping; and in 2000, 2001, and

Table 1 Measured precipitation (Precip), air and soil temperature (T_{air} and T_{soil} , respectively), and soil water content (W_{soil}) under four treatments

Variable	Treatment	1999†	2000	2001	2002	2003	2004	2005	Average
Precip		881	995	883	890	647	991	522	830
T_{air}	C	5.62	16.28	16.18	15.71	16.19	16.21	15.88	16.10
	W	6.17	17.45	17.52	16.51	17.27	17.33	18.02	17.37
	W–C	0.55	1.17	1.34	0.79	1.08	1.12	2.15	1.27
T_{soil}	UC		17.94	19.39	16.42	17.07	16.69	16.01	17.05
	UW		19.99	19.13	17.75	18.54	18.41	18.06	18.76
	UW–UC		2.05*	1.73*	7.33*	1.47*	1.72*	2.05*	1.71
	CC		18.84	17.88	16.67	17.18	17.00	16.60	17.48
	CW		21.31	20.47	17.98	19.23	19.04	19.27	19.66
	CW–CC		2.47*	2.59*	1.32*	2.05*	2.04*	2.67*	2.18
W_{soil}	UC	20.59	22.94	29.84	27.27	22.04	27.57	24.67	25.37
	UW	20.06	19.17	28.84	26.49	21.03	27.47	22.49	23.77
	UW–UC	–0.53	–3.77	–1.00	–0.78	–1.01	–0.09	–2.18*	–1.60
	CC	18.42	21.59	28.76	27.06	22.30	27.47	24.12	24.70
	CW	17.54	17.37	28.41	26.30	20.16	26.34	20.53	22.63
	CW–CC	–0.88	–4.21	–0.35	–0.75	–2.14	–1.27	–3.59*	–2.07

C, control plot with ambient temperature; W, warming; U, unclipping; C, clipping.

*The statistical differences of significance test for temperature and soil moisture between warming and unwarmed treatments at a level of $P < 0.05$.

†All values of measured variables were for a period from 21 November to 31 December in 1999 except precipitation, which was a yearly value.

2005 with clipping. Estimated summer aboveground biomass was significantly higher under warming than control in 2001 in the unclipped plots and 2001–2003 and

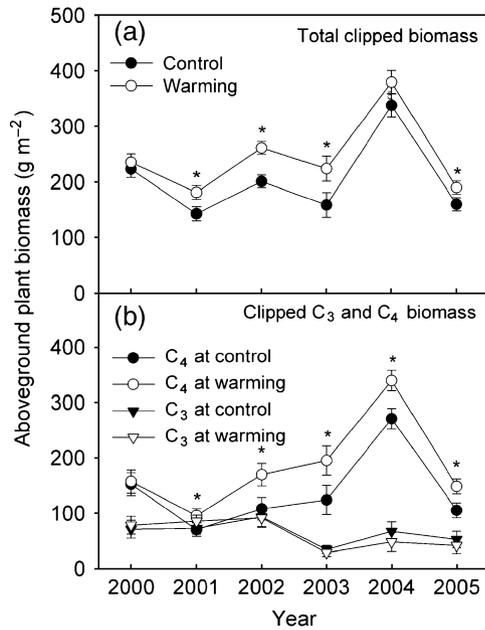


Fig. 1 Aboveground biomass under warming and/or clipping. Panels (a) and (b) represent respective total, and C₃ and C₄ plant aboveground biomass with and without warming in clipped plots. An asterisk (*) represents the statistical significance of warming effect at $P < 0.05$ and ξ at $P < 0.07$.

2005 in clipped plots (Table 4). Warming significantly increased aboveground biomass in fall only in 2001 without clipping and in 2005 with clipping. Clipping significantly reduced standing biomass in fall and spring in all years but only in 2002 and 2005 in summer.

Estimated ANPP from the summer peak biomass was 9% without clipping ($P < 0.05$) and 21% with clipping ($P < 0.05$) higher under warming than control on average of data over 6 years (Table 3). BNPP estimated from biomass measurements with soil coring and root turnover were significantly higher under warming than that under control by 20% ($P < 0.05$) without clipping and 31% ($P < 0.05$) with clipping. Warming stimulated total NPP, which is sum of ANPP and BNPP, by 14% without clipping ($P < 0.05$) and 26% with clipping ($P < 0.05$) over the 6 years. Clipping resulted in decreases in ANPP by 21% without warming ($P < 0.05$) and 12% with warming ($P < 0.05$) but nonsignificant increases in BNPP by 3% and 12% without and with warming, respectively. As a consequence, there were no significant differences in total NPP between clipped and unclipped plots regardless of warming treatments.

Soil respiration and its components

Experimental warming at our site did not result in instantaneous increases in soil respiration in 1999 and 2000 without clipping (Luo *et al.*, 2001; Fig. 2a). Soil respiration under warming significantly increased by

Table 2 Indirect estimates of plant aboveground biomass (mean \pm SE) by pin-contact methods under four treatments (UC, unclipping and control temperature; UW, unclipping and warming; CC, clipping and control temperature; and CW, clipping and warming) in spring, summer, and fall from 2000 to 2005

Year	Season	UC	UW	CC	CW
2000	Spring	149.7 \pm 8.4 a	153.3 \pm 11.0 a	119.5 \pm 8.3 b	162.4 \pm 19.9 a
	Summer	247.3 \pm 13.7 a	242.8 \pm 14.0 a	211.2 \pm 16.1 b	222.7 \pm 19.6 ab
	Fall	170.8 \pm 5.3 a	174.7 \pm 8.3 a	37.3 \pm 5.5 b	37.7 \pm 7.8 b
2001	Spring	153.7 \pm 6.4 b	190.0 \pm 8.8 a	103.5 \pm 8.2 c	157.6 \pm 22.0 b
	Summer	218.4 \pm 5.5 b	248.4 \pm 15.7 a	135.0 \pm 13.1 d	171.5 \pm 10.8 c
	Fall	177.2 \pm 10.4 b	215.7 \pm 8.7 a	100.8 \pm 11.8 c	106.5 \pm 19.1 c
2002	Spring	176.4 \pm 14.4 b	207.6 \pm 14.6 a	121.2 \pm 10.0 c	146.2 \pm 23.3 bc
	Summer	227.1 \pm 4.3 a	231.6 \pm 3.5 a	190.3 \pm 16.4 b	247.1 \pm 17.9 a
	Fall	174.7 \pm 8.3 a	188.9 \pm 9.4 a	72.1 \pm 9.5 b	84.6 \pm 16.0 b
2003	Spring	205.7 \pm 10.0 a	208.5 \pm 11.0 a	181.6 \pm 8.3 b	194.1 \pm 10.3 ab
	Summer	195.0 \pm 16.9 a	207.2 \pm 25.5 a	118.2 \pm 12.5 b	179.0 \pm 26.7 a
	Fall	148.9 \pm 10.4 a	155.3 \pm 13.8 a	93.3 \pm 11.2 b	103.8 \pm 23.2 b
2004	Spring	270.3 \pm 13.9 b	318.2 \pm 26.6 a	191.7 \pm 9.4 c	206.1 \pm 16.9 c
	Summer	356.0 \pm 19.8 a	351.7 \pm 24.9 a	320.1 \pm 32.5 a	359.1 \pm 29.1 a
	Fall	445.3 \pm 31.0 a	431.7 \pm 33.0 a	188.7 \pm 7.0 b	191.6 \pm 10.5 b
2005	Spring	209.9 \pm 11.9 a	223.8 \pm 15.0 a	107.4 \pm 3.0 b	121.9 \pm 8.0 c
	Summer	224.9 \pm 16.1 a	226.7 \pm 27.2 a	149.3 \pm 17.0 c	179.9 \pm 14.4 b
	Fall	272.1 \pm 9.7 a	272.7 \pm 12.9 a	78.9 \pm 12.4 b	115.1 \pm 20.2 c

Different letters indicate statistical differences at $P < 0.05$ among the four treatments at each measurement date.

Table 3 Major components of ecosystem carbon balance under four treatments: unclipping and control (ambient) temperature (UC), unclipping and warming (UW), clipping and control temperature (CC), and clipping and warming (CW)

Variable	UC	UW	CC	CW
Aboveground net primary production (ANPP) ($\text{g C m}^{-2} \text{yr}^{-1}$)	247 ± 7.8 b	269 ± 8.4 a	196 ± 7.0 c	237 ± 8.0 b
Belowground net primary production (BNPP) ($\text{g C m}^{-2} \text{yr}^{-1}$)	239 ± 33.1 b	287 ± 37.7 ab	245 ± 30.6 b	320 ± 39.2 a
Net primary production (NPP = ANPP + BNPP) ($\text{g C m}^{-2} \text{yr}^{-1}$)	487 ± 34.0 ab	556 ± 37.8 a	442 ± 31.4 b	558 ± 40.0 a
Soil respiration (R_s) ($\text{g C m}^{-2} \text{yr}^{-1}$)	794 ± 15.6 b	853 ± 17.6 a	732 ± 11.2 c	842 ± 15.4 a
Heterotrophic respiration (R_h) ($\text{g C m}^{-2} \text{yr}^{-1}$)	513 ± 15.4 b	568 ± 18.6 a	448 ± 14.0 c	488 ± 16.9 b
Autotrophic root respiration ($R_a = R_s - R_h$) ($\text{g C m}^{-2} \text{yr}^{-1}$)	283 ± 35.9 b	287 ± 30.9 b	285 ± 19.5 b	345 ± 25.7 a
Net ecosystem production (NEP = NPP - R_h) ($\text{g C m}^{-2} \text{yr}^{-1}$)	-26.1 ± 37.3 b	-12.1 ± 42.9 b	-6.5 ± 34.4 b	69.8 ± 43.4 a
Biomass removal (L_b) ($\text{g C m}^{-2} \text{yr}^{-1}$)	0	0	91.0 ± 2.1 b	109.8 ± 2.5 a
Net ecosystem C balance (NECB = NEP - L_b) ($\text{g C m}^{-2} \text{yr}^{-1}$)	-26.1 ± 37.3 ab	-12.1 ± 42.9 a	-97.5 ± 34.4 b	-40.0 ± 43.5 ab
Change in soil C content from 1999 to 2005 [$\text{g C m}^{-2} (6 \text{ yr})^{-1}$]	-15.8 ± 196 a	-48.8 ± 126 a	-683 ± 258 b	-343 ± 173 ab

Data are expressed as mean ± 1 SE. Letters a, b, c, and d following the mean indicate statistical significance at $P < 0.05$ among the four treatments.

Table 4 Statistical significance (i.e., P values) of treatment effects on aboveground biomass (AGB), belowground biomass (BGB), soil surface litter mass (LM), soil respiration (R_s), heterotrophic respiration (R_h), autotrophic respiration (R_a), biomass removal (L_b), and soil carbon content (SC)

Source	AGB	BGB	LM	R_s	R_h	R_a	L_b	SC
Sample size (n)*	288	72	36	144	96	96	144	72
Warming	<0.001	0.004	0.005	<0.001	<0.001	0.031	<0.001	0.545
Clipping	<0.001	0.257	<0.001	0.001	<0.001	0.044		0.006
Plot	<0.001	0.001	0.008	<0.001	<0.001	<0.001	<0.001	<0.001
Year	<0.001	<0.001	<0.001	<0.001	0.013	0.056	<0.001	0.028
Clipping × Warming	0.034	0.421	0.108	0.013	0.300	0.057		0.171
Plot × Warming	<0.001	0.206	0.125	<0.001	0.301	<0.001	0.128	0.016
Plot × Clipping	<0.001	0.118	0.099	<0.001	0.003	<0.001		0.126
Year × Warming	0.062	0.729	0.382	0.028	0.339	0.134	0.394	0.690
Year × Clipping	<0.001	0.665	0.001	0.095	0.540	0.637		0.035
Year × Plot	0.001	0.147	0.162	0.010	0.020	0.023	0.062	0.224
Plot × Clipping × Warming	0.228	0.840	0.233	0.027	0.195	0.003		0.240
Year × Clipping × Warming	0.580	0.846	0.124	0.462	0.315	0.400		0.580
Year × Plot × Warming	0.024	0.862	0.306	0.022	0.289	0.155		0.324
Year × Plot × Clipping	0.044	0.312	0.263	0.155	0.031	0.028		0.330

* $n = 288$ from 6 plots × 6 years × 2 clipping × 2 warming × 2 repeated measurements for AGB; $n = 72$ from 6 plots × 3 years × 2 clipping × 2 warming for BGB; $n = 36$ from 6 plots × 2 years × 2 clipping × 2 warming for LM; $n = 144$ from 6 plots × 6 years × 2 clipping × 2 warming for R_s ; $n = 96$ from 6 plots × 4 years × 2 clipping × 2 warming each for R_h and R_a ; $n = 144$ from 6 plots × 6 years × 2 clipping × 2 repeated measurements for L_b ; and $n = 72$ from 6 plots × 3 years × 2 clipping × 2 warming for SC.

approximately 8% without clipping ($P < 0.05$) from 2001 to 2005 and 15% with clipping ($P < 0.05$) from 2000 to 2005 (Fig. 2a). Clipping did not affect soil respiration until 2003 without warming and until 2004 with warming in comparison with that without clipping. Soil respiration was about 10% lower in clipped than unclipped plots from 2003 to 2005 without warming and approximately 7% in 2004 and 2005 with warming.

Warming stimulated heterotrophic respiration by 11% without clipping ($P < 0.05$) and 9% with clipping ($P < 0.05$) (Table 3). Clipping significantly decreased heterotrophic respiration by 13% and 14%, respectively,

without and with warming. Autotrophic respiration did not change with warming without clipping but significantly increased by 20% with clipping ($P < 0.05$).

NEP and soil carbon content

NEP (= NPP - heterotrophic respiration) was not significantly affected by either warming or clipping alone ($P > 0.05$) but significantly larger than zero under the clipping and warming treatment ($P < 0.05$), primarily due to their interactive stimulation of root growth (Table 3). NECB (= NEP - biomass removal), averaged over the 6

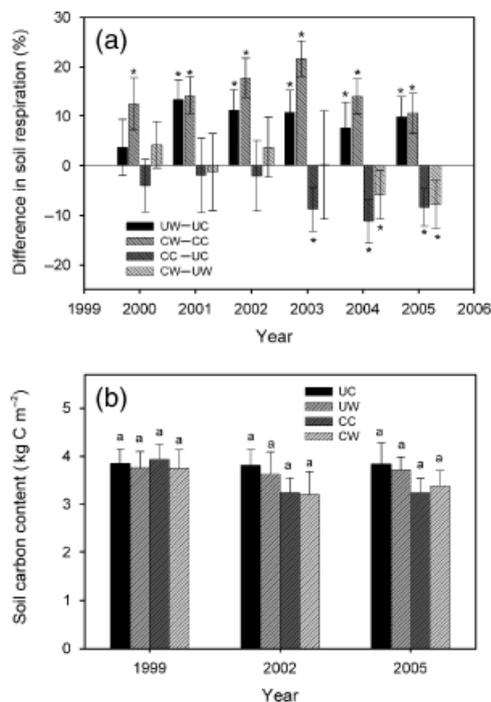


Fig. 2 Warming- and clipping-induced changes in soil respiration and soil C content. Panel (a) indicates differences of annual mean soil respiration between the warming and control treatments without (UW–UC) and with clipping (CW–CC), and between unclipping and clipping treatments under control (CC–UC) and warming (CW–UW) from 2000 to 2005. Panel (b) shows soil C content at the top 15 cm soil in 1999, 2002, and 2005 (mean \pm 1 SE). An asterisk (*) represents the statistical significance of warming and clipping effects at $P < 0.05$ in panel (a). Although clipping and warming did not have statistically significant effects on soil C content between treatments in panel B, soil C content significantly decreased in clipping plots from 1999 to 2005 when all data were lumped for ANOVA (see Table 3).

years, showed that clipping resulted in a significant carbon loss by $97.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($P < 0.05$). Cumulative NECB over 6 years were -157 , 73 , -585 , and $-240 \text{ g C m}^{-2} (6 \text{ yr})^{-1}$ under respective UC, UW, CC, and CW treatments.

Changes in soil C content over 6 years from the pre-treatment measurement in 1999–2005 was not significant from zero between the unwarmed and warmed plots without clipping (Fig. 2b). Soil C content declined by $683 \text{ g C m}^{-2} (6 \text{ yr})^{-1}$ in the clipping plots without warming ($P < 0.05$) and $360 \text{ g C m}^{-2} (6 \text{ yr})^{-1}$ with warming ($P < 0.05$).

Discussion

NPP and its responses to climate warming

Our study showed that experimental warming stimulated NPP by 14% without clipping and 26% with

clipping. Increased plant biomass production was also observed in other warming experiments. A meta-analysis of 20 warming experiments demonstrated an average of 19% increases in aboveground plant productivity under warming in comparison with that under control (Rustad *et al.*, 2001). In a transect experiment at six sites along a climatic and latitudinal gradient in Europe, warming by approximately 1°C resulted in increases in plant aboveground biomass production at sites in the UK, the Netherlands, and Hungary, but caused no change in other sites in comparison with that in control (Peñuelas *et al.*, 2007). Stem-wood growth of trees was 50% higher in heated than control plots with soil warming in a Norway spruce forest at Flakaliden in northern Sweden after 5 years (Bergh & Linder, 1999; Jarvis & Linder, 2000).

Warming stimulation of NPP in our experiment resulted from at least three mechanisms (Luo, 2007). First, warming enhanced dominance of C_4 plants by 12.2% in term of biomass in comparison with that in the control by 2005. Peak season rates of C_4 leaf photosynthesis were approximately 50% higher than that of C_3 plants (Zhou *et al.*, 2007a). Thus, warming-enhanced C_4 dominance increased canopy photosynthetic C uptake. Second, warming advanced phenological events in spring and delayed some of them in fall (Sherry *et al.*, 2007), leading to extended growing seasons (Wan *et al.*, 2005). Extension of the growing seasons, especially in early spring, can lead to substantial increases in photosynthesis and biomass production (Nemani *et al.*, 2003). Third, other studies showed that warming stimulated mineralization (Rustad *et al.*, 2001; Melillo *et al.*, 2002) and increased plant nutrient uptake and use efficiency (Sardans *et al.*, 2008). Similarly, observed increased mineralization and plant nutrient uptake are likely mechanisms leading to stimulated NPP in this study (An *et al.*, 2005; Wan *et al.*, 2005). On the other hand, our measurement showed that leaf-level photosynthesis of four dominant species increased significantly in spring, decreased in early fall, and did not change in summer and late fall under warming in comparison with that in control (Zhou *et al.*, 2007a). Thus, direct warming effects on photosynthesis were a minor cause of the stimulated NPP at our site.

Warming effects on ecosystem carbon cycling processes

Increased biomass growth under warming led to more accumulation of litter mass at the soil surface by 25% without clipping and 58% with clipping ($P < 0.01$) than that without warming (Cheng *et al.*, in review). A litter decomposition study for 2 years at the experimental site indicated that warming had no effect on litter decomposition of a dominant C_3 species (*Ambrosia*

psilostachya) but marginally inhibited litter decomposition of a dominant C₄ species (*Schizachyrium scoparium*) due to soil drying at the surface (Su, 2005). In addition, decomposition rates of C₄ litter were much lower than C₃ litter because C₄ litter had lower initial nitrogen concentration, lower C:N, and higher lignin:N than C₃ litter (Su, 2005). Increased litter production, especially C₄ litter production with lower quality, in combination of marginal direct inhibition of litter decomposition by warming resulted in substantial increases in litter mass at soil surface in the warming plots in comparison with that in the control plots. Similarly, changes in litter quality of the bulk litter due to shifts in species composition was more important in determining rates of litter decomposition than the direct effect of warming in a Colorado subalpine meadow-sagebrush steppe ecotone (Shaw & Harte, 2001). Increased C₄ litter also caused shifts in microbial community structure toward fungi abundance in soil under warming (Zhang *et al.*, 2005).

We also observed significant increases in labile C in soil (Belay-Tedla, 2004; Belay-Tedla *et al.*, submitted), opposing to a common speculation in the literature that labile pool sizes of SOM decrease under warming as a result of increased soil respiration and enhanced oxidation of organic matter (Peterjohn *et al.*, 1993; Niinistö *et al.*, 2004). At our site, warming significantly increased labile C content by 373 mg C kg⁻¹ dry soil in the unclipped and warmed plots in comparison with that in the unclipped and control plots. Clipping, however, significantly decreased the labile C content in the clipped and warmed plots in comparison with that in the unclipped and warmed plots. Without change in C input, warming-induced increases in SOM decomposition shall be accompanied with decreases in labile C. Warming-induced increases in plant biomass growth, litter input, and possibly root exudation likely led to increases in C input to the labile C pools in soil, which may be larger than warming-enhanced decomposition of labile organic compounds.

Kinetic sensitivity of soil respiration to climate warming has been considered to be one major mechanism underlying climate-warming C-cycle feedback (Davidson *et al.*, 2006). If climate warming primarily stimulates soil respiration with an assumed increase in turnover rates in models by $Q_{10} = 2$, warming by 1.71 °C without clipping and 2.18 °C with clipping would result in total C loss via soil respiration by 601 and 731 g m⁻², respectively, after 6 years of the experiment at our site. Soil C in the warming plus clipping plots would decrease by 1316 g C m⁻² (6 yr)⁻¹ due to the both warming and clipping effects on soil loss. However, soil respiration at our experimental site did not directly respond to warming in 1999 and 2000 (Luo *et al.*, 2001) and was

stimulated by warming from 2001 to 2005 without clipping (Fig. 2a). The lagged responses of soil respiration were coincided with warming effects on plant biomass growth, which was not significant in 2000 but in most years afterwards. The synchronized responses of soil respiration and plant biomass growth suggest that warming-stimulated respiratory C release may be driven primarily by increased C input from plant production although mechanisms underlying the lagged responses are yet to be examined.

Our results also challenge the notion that respiration is more sensitive than primary production to climate warming (Cox *et al.*, 2000; Friedlingstein *et al.*, 2006). In our study, NPP under warming increased by 14% without clipping and 26% with clipping. In comparison, soil respiration under warming increased by 8% without clipping and 15% with clipping. Warming stimulated heterotrophic respiration by 11% without clipping and 9% with clipping. Although photosynthetic sensitivity to temperature is smaller than respiration sensitivity (Pearcy & Ehleringer, 1984; Atkin *et al.*, 2000; Luo, 2007), NPP is regulated by many other processes, rendering a possibility that NPP is more sensitive than soil respiration to climate warming.

Impacts of biofuel feedstock harvest on ecosystem carbon balance

Bioenergy as alternative energy was proposed to mitigate climate change. Evaluation of the mitigation potential requires knowledge on how biofuel feedstock production is, on one hand, affected by climate warming and, on the other hand, influences ecosystem C balance (Fargione *et al.*, 2008; Searchinger *et al.*, 2008) and feedback to climate warming. Our results showed that biomass removal by clipping for biofuel feedstock production alone significantly reduced ANPP but did not affect BNPP (Table 3). Reduced ANPP plus yearly removal of biomass from the clipped plots decreased C input to soil, leading to decreased SOM. The amount of soil C loss from the clipped plots was equivalent to the C content in the removed biomass at ambient temperature. Thus, biofuel feedstock production in the US Great Plains may not be C-negative or help offset atmospheric C buildup due to anthropogenic activities.

Clipping positively interacted with warming in influencing BNPP, probably due to over-compensatory responses of plant physiological processes to clipping (Wan *et al.*, 2005; Derner *et al.*, 2006; Owensby *et al.*, 2006). As a result, NEP was positive and the amount of soil C loss was less than the amount of C in the removed biomass in the warming and clipping treatment. Our result suggested that biofuel feedstock production could be C-negative only under the scenario of climate

warming, which we attempt to avoid. Thus, it does not appear that biofuel production is a win–win proposal even in marginal lands in Southern Great Plains.

Carbon-cycle feedback to climate warming

Increases in C loss by soil respiration (Fig. 2a) were roughly balanced by stimulated NPP under warming in our experiment (Table 3). Experimental warming accelerated ecosystem C cycling via stimulation of both C uptake and release without much impact on net ecosystem C storage. Other studies also showed an increase or no change in NEP under experimental warming (Johnson *et al.*, 2000; Marchand *et al.*, 2004; Luo, 2007), providing no support to the positive terrestrial feedback to climate warming as demonstrated by global models (Cox *et al.*, 2000; Friedlingstein *et al.*, 2006).

Coupled carbon-cycle–climate change models are an effective tool to project future states of climate and ecosystems. Accuracy of the model projections, however, depends on how well the models represent feedback mechanisms in the real world. Most of the coupled carbon–climate models are primarily based on kinetic sensitivity of photosynthesis and respiration processes to temperature. Minor direct responses of photosynthesis and respiration to experimental warming as observed in this and other studies indicate that kinetic sensitivity to temperature is less critical than other plant and ecosystem processes, such as shifted plant and microbial species composition (Peñuelas *et al.*, 2004, 2007; Zhang *et al.*, 2005; Harte *et al.*, 2006), changes in phenology and extension of growing seasons (Nemani *et al.*, 2003; Cleland *et al.*, 2007; Sherry *et al.*, 2007), and altered nitrogen uptake and use efficiency (Rustad *et al.*, 2001; Melillo *et al.*, 2002; An *et al.*, 2005), in regulating terrestrial feedback to climate change. Although some of the coupled carbon–climate models have accounted for changes in phenological events and length of growing seasons, leading positive biomass growth in the middle latitude regions under the future warmed world, representing species- and/or nitrogen-mediated feedbacks in climate models is still challenging. Realistic projections of future states of climate and ecosystems, nonetheless, have to consider regulatory mechanisms via changes in plant and ecosystem processes in addition to photosynthetic and respiratory kinetics (Luo, 2007).

Summary

Results from the warming and clipping experiment in Oklahoma, USA, suggested that warming extended growing seasons, enhanced C₄ plant dominance in the plant community, and increased plant nitrogen uptake

and use efficiency. As a consequence, NPP increased under warming in comparison with that in control. Increased NPP in combination of decreased quality and decomposition rates of bulk litter, primarily due to increased dominance of C₄ plants, led to increases in litter production and soil surface litter mass accumulation under warming in comparison with that in control. Increased root biomass growth, litter mass, and labile soil C resulted in increases in soil respiration, which were roughly balanced by increased C uptake via NPP, leading to little change in soil C storage under warming while warming accelerated rate processes of both C uptake and release.

Biomass removal by clipping for biofuel feedstock production at ambient temperature significantly reduced ANPP. Reduced ANPP and yearly removal of biomass from the clipped plots resulted in decreased C input to soil and SOM. The amount of soil C loss from the clipped plots was equivalent to the C content in the harvested biomass. Clipping positively interacted with warming in stimulating BNPP and C input to soil. As a consequence, the amount of soil C loss was less than the amount of C in the harvested biomass in the warming and clipping treatment. Thus, biofuel feedstock production could be C-negative only under the scenario of climate warming. The latter is what we attempt to avoid.

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