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Nitrogen regulation of the climate–carbon feedback: evidence from a long-term global change experiment

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Abstract. Modeling studies have shown that nitrogen (N) strongly regulates ecosystem responses and feedback to climate warming. However, it remains unclear what mechanisms underlie N regulation of ecosystem–climate interactions. To examine N regulation of ecosystem feedback to climate change, we have conducted a warming and clipping experiment since November 1999 in a tallgrass prairie of the Great Plains, USA. Infrared heaters were used to elevate soil temperature by an average of 1.96°C at a depth of 2.5 cm from 2000 to 2008. Yearly biomass clipping mimicked hay or biofuel feedstock harvest. We measured carbon (C) and N concentrations, estimated their content and C:N ratio in plant, root, litter, and soil pools. Warming significantly stimulated C storage in aboveground plant, root, and litter pools by 17%, 38%, and 29%, respectively, averaged over the nine years (all $P < 0.05$) but did not change soil C content or N content in any pool. Plant C:N ratio and nitrogen use efficiency increased in the warmed plots compared to the control plots, resulting primarily from increased dominance of C_4 plants in the community. Clipping significantly decreased C and N storage in plant and litter pools (all $P < 0.05$) but did not have interactive effects with warming on either C or N pools over the nine years. Our results suggest that increased ecosystem nitrogen use efficiency via a shift in species composition toward C_4 dominance rather than plant N uptake is a key mechanism underlying warming stimulation of plant biomass growth.

Key words: *biofuel harvest; carbon storage; climate change; C:N ratio; land use change; nitrogen use efficiency; soil carbon; warming.*

INTRODUCTION

In the past decade, scientists have made substantial progress in understanding the feedbacks between terrestrial ecosystems and climate warming (Jones and Donnelly 2004, Field et al. 2007, Luo 2007, Heimann and Reichstein 2008). Most modeling studies predict ecosystem C storage will decrease as respiration is stimulated more than photosynthesis by rising temperature, with a consequent positive feedback to climate warming (Cox et al. 2000, Friedlingstein et al. 2006, Heimann and Reichstein 2008). Nevertheless, experimental studies have shown diverse responses of C storage to climate warming, observing increases (Welker et al. 2004, Oberbauer et al. 2007, Day et al. 2008, Sardans et al. 2008), decreases (Oberbauer et al. 2007), and no changes (Marchand et al. 2004, Luo et al. 2009). These diverse experimental results may be partially due to variance in N regulation of carbon processes over time scales and across ecosystems (Shaver et al. 2000, Luo 2007). Indeed, N regulation can cause large uncertainties in projections of climate–C feedbacks (Hungate et al. 2003, Heimann and Reichstein 2008).

Interactions between C and N are important in the response of ecosystem C cycling to climate change because of the close coupling between these two elements (McGuire et al. 1992, Hungate et al. 2003, Reich et al. 2006). Some recent ecological models of climate–carbon cycle feedbacks have emphasized C–N interactions (Thornton et al. 2007, 2009, Sokolov et al. 2008), and suggested that C sequestration will be underestimated, resulting in inaccurate conclusions about terrestrial feedbacks to climate warming if terrestrial C–N interactions under climate warming are ignored (Sokolov et al. 2008). In these models, increased N mineralization (availability) due to the kinetic sensitivity of microbe activity to climate warming has been considered to be a major mechanism for the proposed increase in plant C uptake. However, no consistent results regarding N mineralization have been reported in experimental warming studies, which see increases (Hobbie 1996, Rustad et al. 2001, Shaw and Harte 2001) or no change (Verburg et al. 2009) in N mineralization with warming. Some of the studies also reported that the mineralization response to warming changed with time (Verburg and van Breemen 2000,

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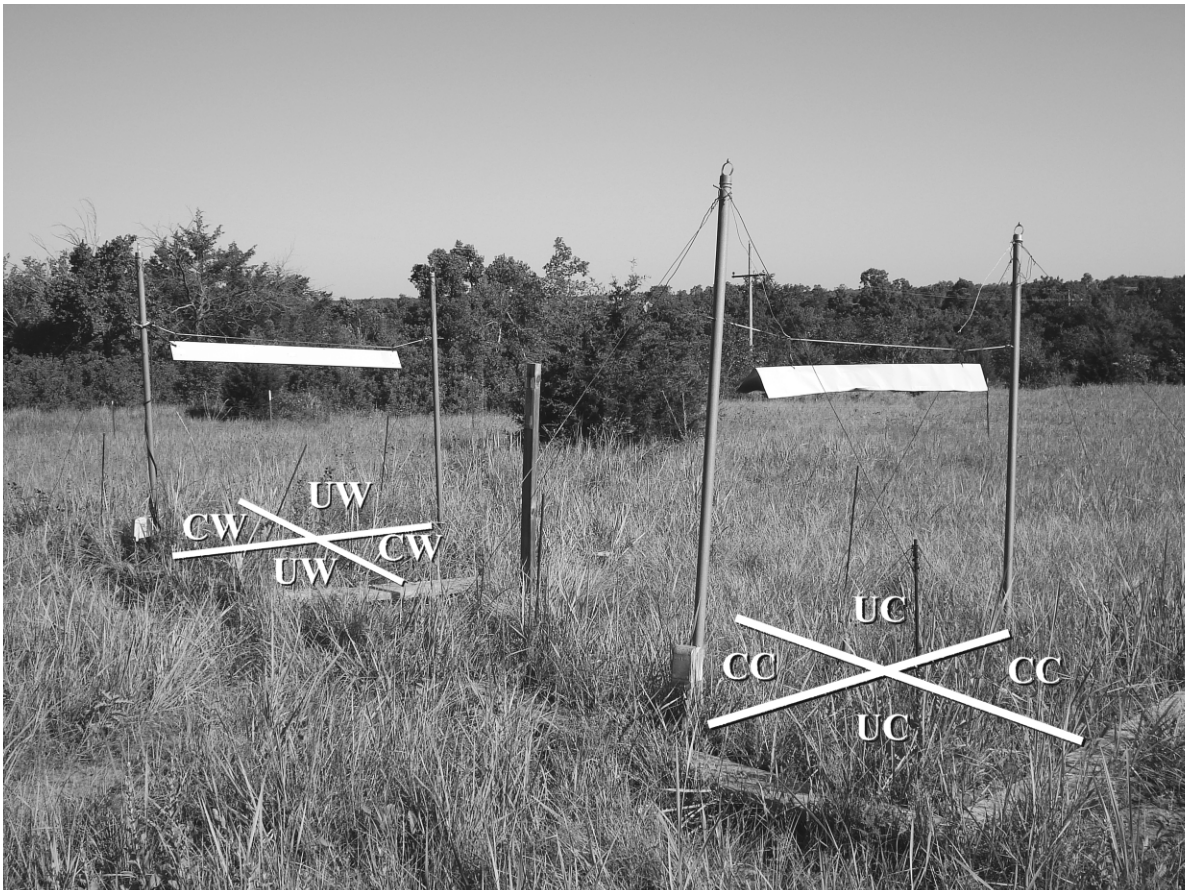

PLATE 1. Layout of the experiment plots. Key to abbreviations: UC, unclipped with control (ambient) temperature; CC, clipped with control temperature; UW, unclipped and warmed; CW, clipped and warmed. Photo credit: S. Niu.

Melillo et al. 2002, Wan et al. 2005). Therefore, we need more field evidence about how N processes regulate C storage in response to elevated temperature, which will allow us to constrain model predictions.

Previous studies on N regulation of C cycling under climate warming mainly considered the direct dependence of mineralization and soil N availability on temperature, but neglected changes in plant N use strategy in a warmer environment. This leads to an incongruous representation of the N cycle and its impact on C feedback to climate warming. Increased plant N use efficiency (NUE) under elevated temperature (An et al. 2005) may stimulate plant photosynthesis. In addition, shifts to species with inherently different NUE and C:N ratio can impact both plant C uptake and litter decomposition (Hobbie 1996, Cornelissen et al. 2007). Therefore, we hypothesize that changes in plant NUE associated with shifts in species composition could also play an important role in regulating C cycling and ecosystem feedbacks to climate change. However, the relative importance of the roles of soil N availability and plant N use strategy have not been well evaluated.

The responses of terrestrial C storage to climate warming may vary strongly with land use practices (Chapin et al. 2008). Land use changes can affect ecosystem carbon and N storage by altering input rates of organic matter produced by plant assimilation, changing the decomposability of organic matter in litter (Cambardella and Elliott 1992), and thus increasing or decreasing soil carbon stores (Houghton et al. 1999, Jones and Donnelly 2004). The southern Great Plains, where this study was conducted, is potentially a major production region for biofuel feedstocks (Luo et al. 2009). Taking plant biomass from the ecosystem without returning any organic matter likely decreases C storage in each pool and alters ecosystem response to climate warming.

In this study, we examined C and N interactions in a tallgrass prairie ecosystem under climate warming and biofuel harvesting for nine years. The C and N content and C:N ratio were estimated for aboveground C_3 and C_4 plants, C_3 and C_4 litter, roots, and soil pools. The objectives of this study were to examine (1) how long-term warming and biofuel harvest impact ecosystem C and N dynamics, and (2) how N processes (N availability vs. plant NUE) under climate warming regulate climate–C cycle feedbacks.

METHODS

Experimental site, design, and facility

The experimental site was located in a tallgrass prairie in the United States Great Plains in McClain County, Oklahoma (34°58′54″ N, 97°31′14″ W), ∼40 km from the Norman campus of the University of Oklahoma. This site has not been grazed for the past 40 years. The grassland is dominated by C_4 grasses (*Sorghastrum nutans*, *Schizachryum scoparium*, *Sporobolus asper*, and *Andropogon gerardii*) and C_3 forbs (*Ambrosia psilotachyia*, *Aster ericoides*, *Solidago rigida*, *Solidago nemoralis*, and *Hemiachyris dracunculoides*). The mean annual precipitation (from 1948 to 1998) was 914 mm and the mean annual temperature was 16.3°C (Oklahoma Climatological Survey, Norman, Oklahoma, USA). The soil is part of the Nash-Lucien complex with 32% sand, 60% silt, and 8% clay (A. A. Subedar, *unpublished data*) and is characterized by high available water-holding capacity, a deep and moderately penetrable root zone, and a neutral pH (USDA 1979). It is medium to high in natural fertility and organic matter content. The surface layer is a reddish brown loam 12.7 cm thick. The subsoil is a yellowish red loam to a depth of 40.6 cm. The underlying material is weakly indurated sandstone.

The experiment used a paired nested design with warming as the main factor nested by a clipping factor. There were six pairs of 2 × 2 m plots. We randomly selected one plot of each pair to serve as the warmed plot, and the other to serve as the control. The warmed plots have been treated continuously since 21 November 1999. One infrared heater (165 ×15 cm; Kalglo Electronics, Bethlehem, Pennsylvania, USA; radiation output is 100 W/m^2) was suspended 1.5 m above the ground in each warmed plot. The radiation output and heater height were sufficient to increase soil temperature by 2°C, which is within the range of the 1.8–4°C increase predicted for the end of this century, according to IPCC projections (IPCC 2007). Reflector surfaces of the heaters were adjusted so as to generate an evenly distributed radiant input to the soil surface (Kimball 2005). In control plots, a "dummy" heater with the same size and shape was suspended at the same height to mimic shading effects of the heater. The distance between warmed and control plots in each pair of plots was ∼5 m from centers, to avoid heating of control plots. The distances between paired plots varied from 20 to 60 m.

Each 2 × 2 m plot was divided into four 1 × 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 to the plots. Therefore, this experiment has four treatments: unclipped with control (ambient) temperature (UC); unclipped warmed (UW); clipped with control temperature (CC); and clipped warmed (CW) (see Plate 1).

Climate variables and soil temperature and moisture

Air temperature and precipitation were recorded at an Oklahoma Mesonet Station located about 200 m away from the experimental site. Soil temperature was monitored by homemade thermocouples installed at a depth of 2.5 cm in the center of one clipped and one unclipped subplot in each plot. The hourly average data were stored in an SM19 Storage Module (Campbell Scientific, Logan, Utah, USA). Volumetric soil water content ($\%V$) was measured once or twice a month using manual Time Domain Reflectomery (TDR) equipment (SoilMoisture Equipment Corporation, Santa Barbara, California, USA) at 0–15 cm depth.

Plant C and N content

Two diagonal subplots were clipped annually at 10 cm at the time of peak biomass, usually August, to measure aboveground biomass (AGB). Clipped plants were first separated into C_3 and C_4 plants, and then oven dried at 65°C for 48 hours. Clipping was done concomitantly with indirect estimation of peak AGB in the unclipped subplots using the pin-contact method (Frank and McNaughton 1990). The pin frame is 0.5 m long and holds 10 pins 5 cm apart at a 60° angle from the soil. Pins are 0.75 m long and could be raised within the frame up to 1m high. Every contact of pins with plant tissue was counted and used to estimate AGB using calibration equations derived from 10 calibration plots, randomly selected each year at least 5 m away from the experimental plots. Biomass in the calibration plots was clipped to the ground surface, then oven dried and correlated with the total contact number. A linear regression of total hits vs. total biomass was used as the calibration equation. Other information on the pin contact method was introduced in Luo et al. (2009).

The clipped C_3 and C_4 plant samples were used for analyzing plant C and N concentration. C_3 grass accounted for <1% of the total aboveground biomass. Therefore, we did not consider C_3 grasses in this study. Green leaves of the three dominant C_4 grass species (*Sorghastrum nutans*, *Schizachryum scoparium*, and *Sporobolus asper*) and two C_3 forb species (*Aster ericoides* and *Ambrosia psilostachya*) were selected to separately analyze C and N concentrations. The C and N concentrations were analyzed with a LECO CNS-2000 Elemental Analyzer (LECO Corporation, St. Joseph, Michigan, USA) by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory (Stillwater, Oklahoma). Because concentrations of C and N among different C_3 and C_4 species were not significantly different (An et al. 2005), we averaged the N and C concentration of the three C_4 grass species, as well as the N and C concentration of the two C_3 forb species. C and N contents of C_3 and C_4 plants in each plot were calculated by multiplying biomass by the C and N concentration in each plot. In 2008, we sampled plants from both the clipped and unclipped plots for analysis of C and N concentration, but did not find

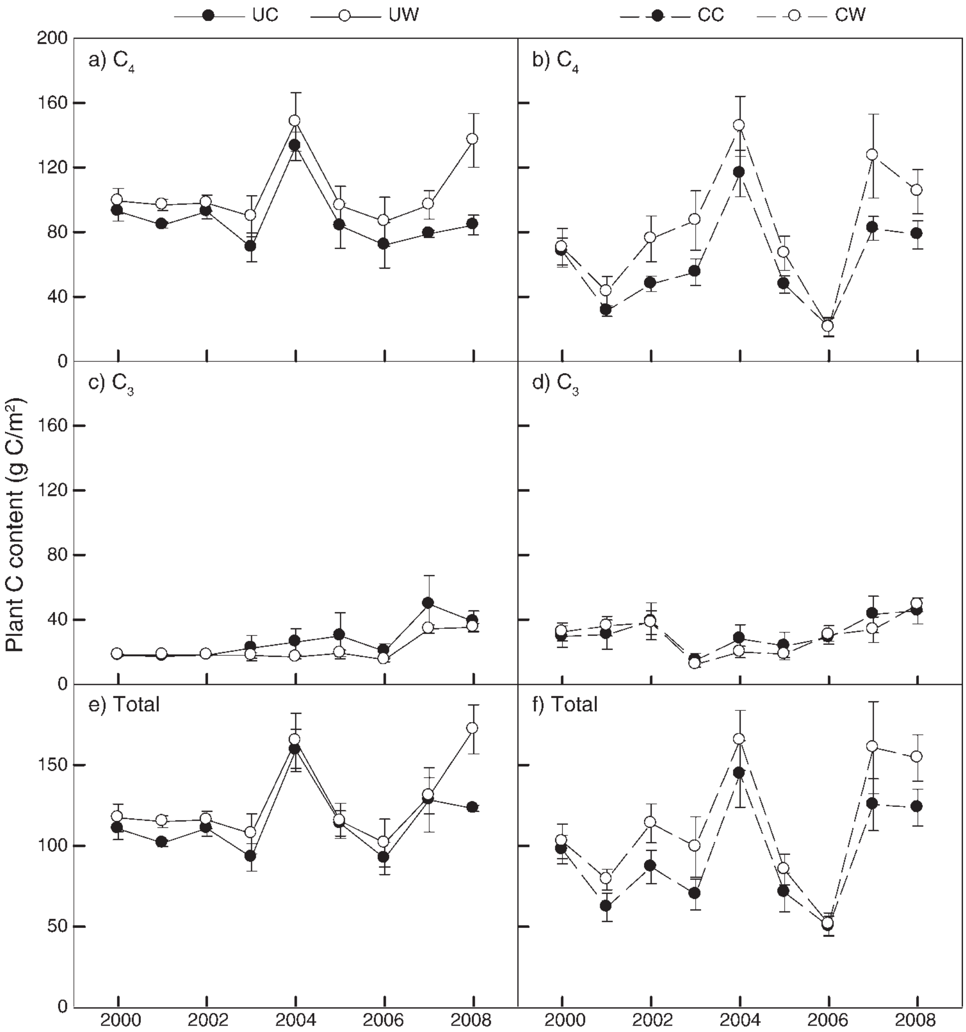

FIG. 1. Carbon (C) and nitrogen (N) contents of (a, b, g, h) aboveground C_4, (c, d, i, j) C_3, and (e, f, k, l) total plants under the four treatments, conducted since 1999 in a tallgrass prairie of the Great Plains, near Norman, Oklahoma, USA. Key to abbreviations: UC, unclipped and unwarmed; UW, unclipped and warmed; CC, clipped and unwarmed; CW, clipped and warmed. Left-hand panels show unclipped treatments, with and without warming; right-hand panels show clipped treatments, with and without warming. Data are means ± SE.

significant differences between the clipped and unclipped plots in either C or N concentration ($0.15 \leq P \leq 0.94$; Appendix A). Therefore, we used C and N concentration in the clipped plots to calculate plant C and N content for the unclipped plots. Total plant C and N storage was calculated by summing the C and N content for C_3 and C_4 plants in each subplot.

Litter and root C and N content

Surface litter was collected from one unclipped and one clipped subplot in each plot on 2 June 2003 and 4 April 2006 and separated into C_3 litter and C_4 litter. At our site, all the forbs are C_3 species. All the grasses (except one) are C_4 grasses. The C_3 grass, (*Dichanthelium oligsanthes*) is very easy to distinguish from other grasses by its short, wide leaves on small shoots. Litter was oven dried at 65°C for 48 h and weighed. After taking a 3–5 g sample for C and N concentration analysis, the litter was returned back into the subplot.

In 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 subplot in each plot to measure root biomass. Root biomass at the 0–45 cm layer accounts for >85% of the total biomass in the study site (S. Fei, *unpublished data*). After washing soil through a 0.25-mm mesh sieve, roots were oven dried at 65°C for 48 h and weighed. Root C and N concentration was measured in 2005 and litter C and N concentration in 2006 using a LECO CNS-2000 Elemental Analyzer at the OSU Soil, Water, and Forage Analytical Laboratory. Litter and root C and N content were calculated by multiplying plot litter and root mass by the plot element concentration for that pool in each year.

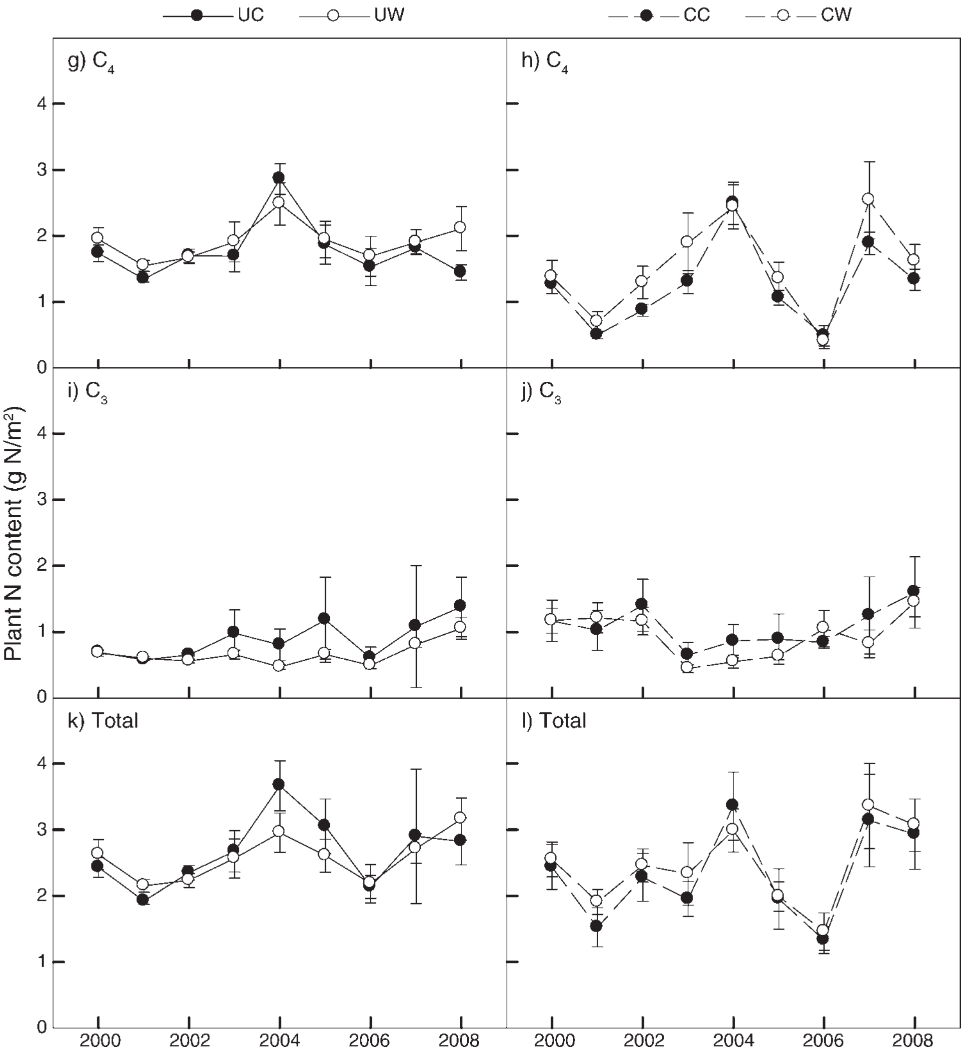

FIG. 1. Continued.

Soil C and N content

From 2000 to 2008 (except in 2006), one soil core (15 cm in depth and 4 cm in diameter) was collected from one clipped and one unclipped subplot in each plot once a year. Roots and organic debris were removed by hand. The soil samples were sieved through a 2-mm mesh, air-dried, ground, and sieved through a 0.25-mm mesh to measure soil total C and total N concentration. Analyses of soil samples for total C and N contents used a Finnigan DELTA plus Advantage gas isotope-ratio mass spectrometer (Thermo Finnigan MAT GmbH, Bremen, Germany), which was configured through the CONFLO III for automated continuous-flow analysis of solid inorganic/organic samples using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) at the OSU Soil, Water, and Forage Analytical Laboratory. We also measured soil bulk density at a depth of 0–15 cm in each subplot, which we used to estimate soil C and N content.

Soil samples in 2007 were also used to measure initial NH_4-N and NO_3-N concentrations. After sieving through a 0.2-mm mesh, a 10-g soil sample from each soil core was taken, and 50 mL of 2 mol/L KCl solution added. The mixture of soil and extractant was shaken for 1 h on a reciprocal shaker. After shaking, the soil suspension was filtered (Whatman No. 1 filter paper, 12.5 cm in diameter; Whatman, Florham Park, New Jersey, USA). Soil solutions were stored frozen until analysis for NH_4-N and NO_3-N on a FIAstar 5000 Analyzer (Foss Tecator, Hillerød, Denmark).

Data analysis

We calculated nitrogen use efficiency (NUE) based on total biomass produced per unit plant N (An et al. 2005). NUE of the two plant functional groups (C_3 forbs and C_4 grasses) and of the whole ecosystem were calculated with the following equations:

TABLE 1. Statistical results (P values) of the effects of year (Y), clipping (Cl), warming (W), and their interactions on carbon (C) and nitrogen (N) storage in plants (P) or litter (L) of C_4 and C_3 plants, in total plant biomass (TP), and in roots (R), and soil (S), in a Great Plains tallgrass prairie near Norman, Oklahoma, USA.

Variable	C_4PC	C_3PC	TPC	C_4PN	C_3PN	TPN	C_4LC	C_3LC	C_4LN	C_3LN	RC	RN	SC	SN
Year	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.25	0.00	0.34	0.02	0.03	0.37	0.00
Clipping	0.00	0.00	0.01	0.00	0.02	0.07	0.00	0.00	0.00	0.00	0.55	0.65	0.87	0.18
Warming	0.00	0.62	0.00	0.01	0.08	0.84	0.02	0.74	0.25	0.42	0.02	0.26	0.68	0.95
Year × clipping	0.00	0.00	0.00	0.00	0.33	0.28	0.00	0.33	0.00	0.42	0.74	0.65	1.00	0.94
Year × warming	0.02	0.05	0.00	0.65	0.93	0.88	0.80	0.40	0.87	0.55	0.49	0.54	0.99	1.00
Clipping × warming	0.92	0.11	0.44	0.42	0.67	0.38	0.43	0.55	0.88	0.43	0.67	0.81	0.56	0.86
Year × clipping × warming	0.11	0.07	0.99	0.87	0.99	0.99	0.33	0.46	0.49	0.57	0.96	0.85	1.00	0.72

Notes: Total plant carbon content (TPC) = C_4 plant carbon content (C_4PC) + C_3 plant carbon content (C_3PC). Total plant nitrogen content (TPN) = C_4 plant nitrogen content (C_4PN) + C_3 plant nitrogen content (C_3PN).

$$\text{NUE of } C_4 \text{ grass or } C_3 \text{ forbs} = \text{AGB}/(\text{AGB} \times \text{N}) = 1/\text{N}$$
$$\text{NUE of ecosystem} = (C_3 \text{ biomass } + C_4 \text{ biomass})/\text{TPN}$$

where AGB is aboveground biomass, N is N concentration, and TPN is total plant nitrogen.

The warming induced an increment of plant C content (ΔC) = C content in the warmed plots − C content in the unwarmed plots. The ΔC due to warming-induced changes in plant N content (ΔC_N) = warming-induced changes in plant N content (ΔTPN) × plant C:N ratio. The ΔC due to warming-induced changes in NUE (ΔC_{NUE}) = warming-induced changes in ecosystem NUE (ΔNUE) × TPN × C concentration.

Three-way ANOVAs were used to analyze the main and interactive effects of year, warming, and clipping on soil temperature, soil moisture, C and N content of plants, litter, roots, and soil. Within each year, the warming and clipping effects on the above-mentioned parameters were analyzed by two-way ANOVAs. Two-way ANOVAs were also used to analyze the main and interactive effects of year and warming on C:N ratio and NUE of C_3 and C_4 plants and ecosystem NUE. Linear regression analyses were used to evaluate relationships between plant C and N content or NUE, and between warming-induced changes in plant C and N content. The effects were considered to be significantly different if $P < 0.05$. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, Illinois, USA).

RESULTS

Microclimate

Annual precipitation varied from 522 mm in 2005 to 1307 mm in 2007, with a mean of 856 mm over the nine years. Annual temperature varied from 7.8°C in 2007 to 17.4°C in 2006, with a mean of 14.8°C from 2000 to 2008 (Appendix B). The warming treatment increased soil temperature by 1.96°C ($P < 0.001$) at the depth of 2.5 cm from 2000 to 2008 (Appendix B). Soil moisture was reduced by an average of 1.37% measured volumetrically ($P = 0.02$) in the warmed plots. Clipping increased soil temperature by 0.68°C (P = 0.03) compared to the unclipped subplots. There was a nonsignificant difference ($P = 0.36$) in soil moisture between the clipped and unclipped subplots (Appendix B).

Aboveground plant C and N dynamics

There was great interannual variability in aboveground plant C and N content (Fig. 1), primarily due to precipitation changes. Greater production in 2004 and 2007 was primarily caused by the higher summer precipitation in those years, whereas lower summer precipitation in 2001 and 2006 led to lower plant C and N content (Fig. 1; Appendix B). Over the nine years, warming on average increased C_4-C storage by 26.0% and total aboveground C storage by 17.2% (all $P < 0.01$;

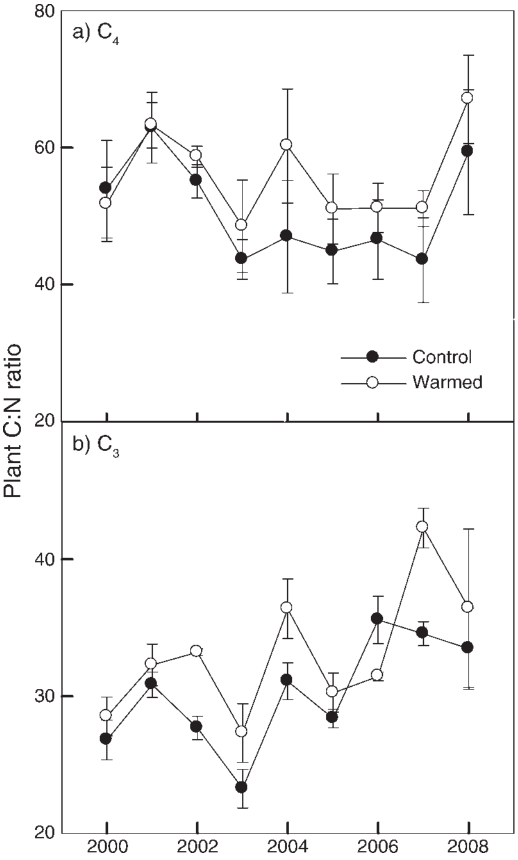

FIG. 2. Mean C:N ratios of (a) C_4 and (b) C_3 plants under the control and warmed treatments. See Fig. 1 for abbreviations. Error bars show ±SE.

Table 1), but had little effect on C_3-C storage ($P = 0.62$) across the clipped and unclipped plots. N storage in C_4 plants on average increased by 13.3% over the nine years ($P = 0.01$), while that in C_3 plants decreased by 17.9% ($P = 0.08$) in response to warming. As a consequence, total aboveground plant N storage changed little under warming treatments ($P = 0.55$, Fig. 1k, l). Warming significantly interacted with year to impact plant C but not N content (Table 1). For example, warming effects on plant C storage were significant in 2000, 2001, 2002, 2007, and 2008, but insignificant in other years (Fig. 1a, b). Warming-induced changes in plant C and N content were primarily due to the warming-induced changes in biomass but not to the warming-induced changes in C and N concentrations (Appendix C). Biofuel harvest by clipping significantly decreased C and N storage of C_4 plants by 34.9% and 33.1% but increased those of C_3 plants by 25.9% and 20.9%, respectively, over the nine years (all $P < 0.05$; Table 1, Fig. 1). No significant interactions between clipping and warming were detected on either plant C or N content (Table 1).

The warming-induced percentage increases in plant C contents were larger than the increases in plant N contents (Fig. 1), resulting in significant increases in C:N ratios in all the plant pools (Fig. 2). Averaged C:N ratios in C_4 and C_3 plants over the entire experimental period increased by 10.1% ($P < 0.001$) and 9.7% ($P = 0.002$), respectively, at the elevated temperature in comparison with those at ambient temperature (Fig. 2). Climate warming significantly stimulated NUE of the C_4 plants by10.0% ($P < 0.001$), C_3 plants by 9.8% ($P = 0.002$), and whole-ecosystem NUE by 12.0% ($P < 0.001$) over the nine years (Fig. 3).

Across the six subplots and nine years, aboveground plant C content positively correlated with plant N content under each treatment (all $P < 0.001$; Fig. 4a). The slopes of plant C content vs. N content were significantly higher in the warmed than the control plots ($P < 0.01$), whereas clipping did not change the slopes (Fig. 4a). Warming-induced changes in plant C content also showed positive linear correlations with warming-induced changes in plant N content across all years and subplots (all $P < 0.01$; Fig. 4b).

Litter C and N storage

Both C and N storage in litter changed greatly with year and plant type. For example, C storage of C_4 litter was 135 g C/m^2 in 2003 and 209 g C/m^2 in 2006. Over the two years, warming increased C_4 litter C storage by 17.5% ($P = 0.02$; Fig. 5a), but did not change C_4 litter N storage or C_3 litter C or N storage (Table 1, Fig. 5b–d). The main effects of clipping were also significant for litter C and N storage, with 35.1% and 33.0% decrease for C_3 litter and 39.3% and 38.1% reductions for C_4 litter, respectively (all $P < 0.01$; Fig. 5). C:N ratio in litter was 20% ($P = 0.04$) and 18% ($P = 0.02$) higher in warmed than in control plots for C_3 and C_4 litter, respectively (Fig. 5e, f).

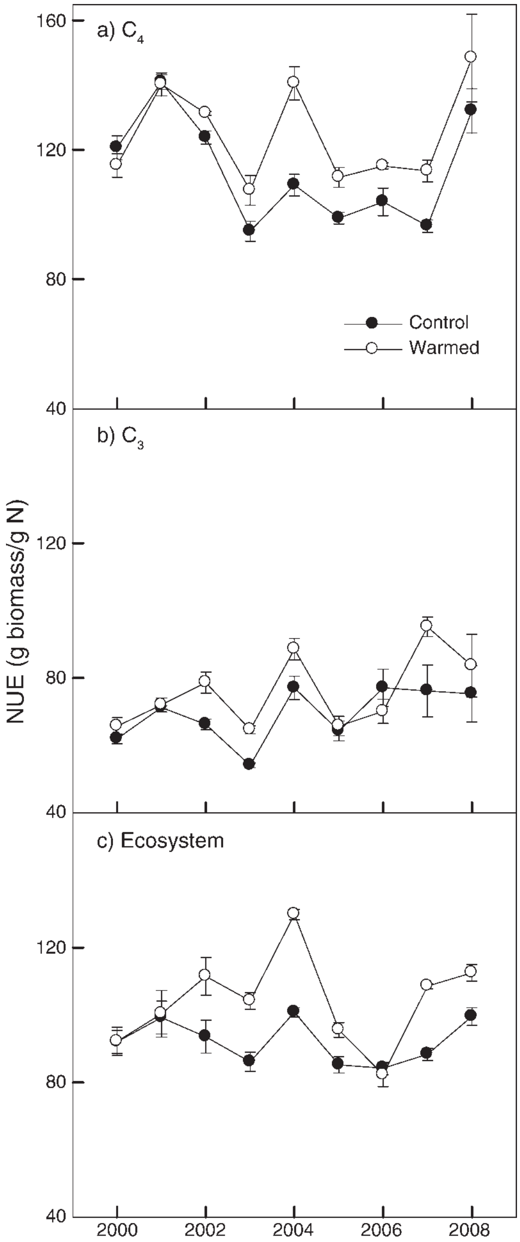

FIG. 3. Mean nitrogen use efficiency (NUE) of (a) C_4 and (b) C_3 plants and (c) the whole ecosystem under the control and warmed treatments. Error bars show ±SE.

Root C and N storage

In the control plots, root C and N storage was 92.9 g C/m^2 and 1.5 g N/m^2 in 2004, and 61.9 g C/m^2 and 0.9 g N/m^2 in 2005, respectively (Fig. 6a, b). Over the two years, warming increased root C storage on average by 29.1% ($P = 0.02$), but did not change N storage ($P = 0.26$; Table 1, Fig. 6a, b). Warming effects on root C

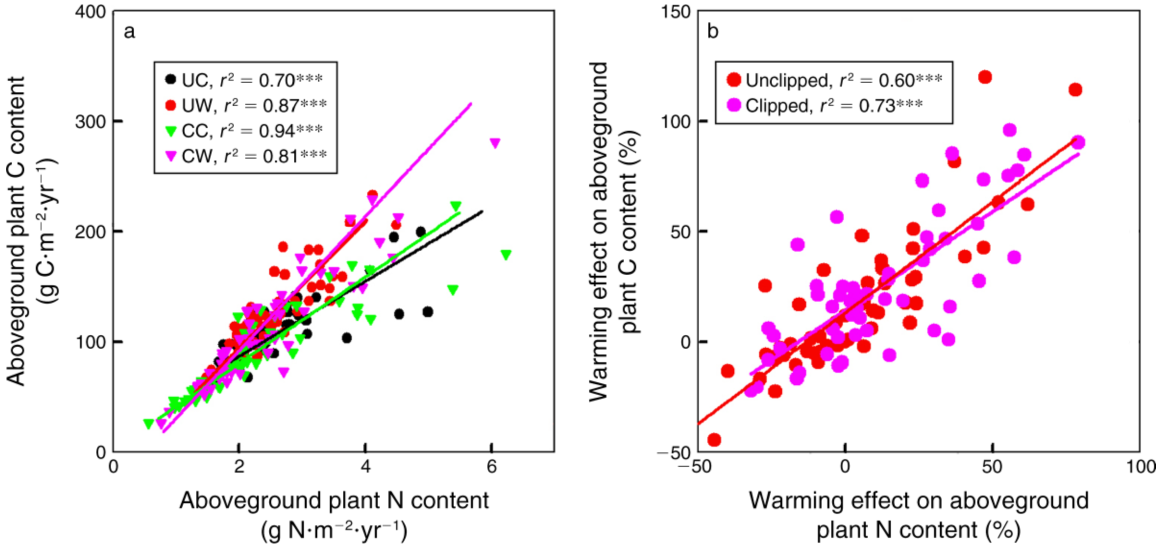

FIG. 4. (a) Correlations between plant C and N content under the four treatments, and (b) correlations between the warming-induced changes in C content and N content in the unclipped and clipped plots. In panel (a), slopes of the regressions were 34, 57, 39, and 61 for UC, UW, CC, and CW treatments, respectively. See Fig. 1 for abbreviations.
*** $P < 0.001$.

storage depended on year, with a 45.4% ($P = 0.04$) increase in 2005 and a nonsignificant change ($P = 0.19$) in 2004 (Fig. 6a). Warming did not significantly change root N storage in either 2004 ($P = 0.78$) or 2005 ($P = 0.18$; Fig. 6b). C:N ratio of roots was little changed under the warming treatment ($P = 0.19$, Fig. 6c). Clipping did not change either C ($P = 0.55$) or N storage ($P = 0.65$) in roots (Table 1).

Soil C and N content

C and N storage in the top 15 cm of soil changed little among years, with an average of 4.2 kg C/m^2 and 0.24 kg N/m^2, respectively, over the nine years (Table 1, Fig. 7). Neither warming nor clipping had significant effects on soil C or N storage or C:N ratio over the experimental period (Table 1). Soil inorganic N concentration (NH_4^+, NO_3^-, or the total inorganic N) measured in 2007 showed no significant response to either warming or clipping ($P > 0.05$). Carbon content in each pool (litter, root, and soil pools) all showed significantly positive correlations with N content in the corresponding pool (all $P < 0.05$).

Contributions of plant N uptake and NUE to plant C storage

On average over the nine years, warming increased plant C content by 14.6 g C·m^{-2}·yr^{-1} in the unclipped subplots and 20.8 g C·m^{-2}·yr^{-1} in the clipped subplots (Fig. 8c, d). The warming-induced ΔC was largely due to the increased ecosystem NUE rather than plant N uptake. For instance, increased NUE contributed 13.9 g C·m^{-2}·yr^{-1}, but changes in plant N uptake only contributed 5.1 g C·m^{-2}·yr^{-1} to the total ΔC of plant C storage under warming on average over the nine years in the clipped subplots (Fig. 8d).

DISCUSSION

Nitrogen regulation in climate–carbon cycle feedback

This study showed no changes in soil C stocks in the tallgrass prairie after nine years of warming treatments (Fig. 7). Our result is consistent with that observed in high-arctic tundra (Marchand et al. 2004), but does not support model predictions that warming will most likely reduce net ecosystem C storage (Cox et al. 2000, Friedlingstein et al. 2006, Heimann and Reichstein 2008) and thus create a positive feedback to climate warming. The discrepancy between our study and the models is at least partially due to the models' overemphasis on kinetic sensitivity, but also to ignorance of ecosystem-level regulatory processes such as shift in species composition and C–N interactions (Luo 2007). An increase in decomposition, for example, leads to an increase in N availability for plant uptake to stimulate plant growth, thus leading to enhanced C sequestration (a negative feedback) as simulated by coupled nitrogen–carbon climate models (Sokolov et al. 2008, Thornton et al. 2009) rather than a positive feedback as predicted by models without C–N interactions.

Our experimental results indeed showed higher plant C accumulation under the elevated as compared with the ambient temperature (Fig. 1), which is consistent with the results in other grassland ecosystems (Rustad et al. 2001). This increased plant C storage, especially in C_4 plants, may offset the stimulation of soil respiration under warming (Zhou et al. 2007), leading to no changes

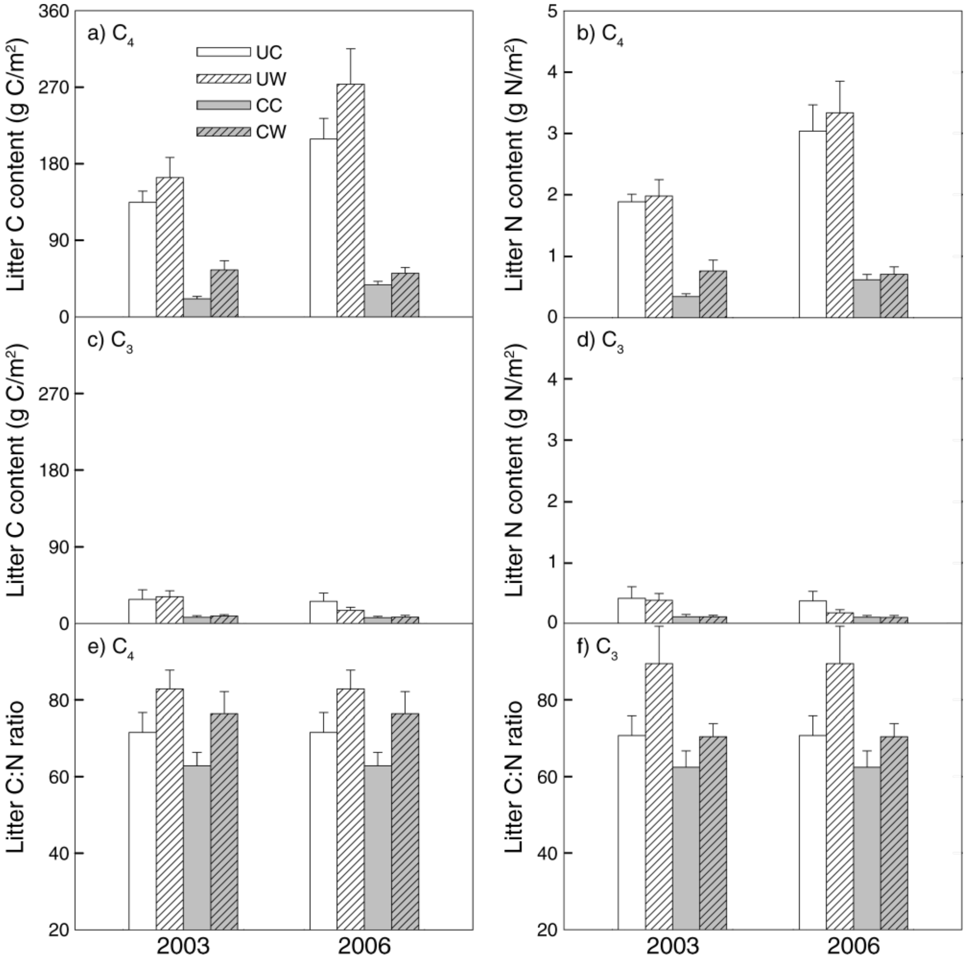

FIG. 5. Mean carbon and nitrogen storages of (a, b) C_4 and (c, d) C_3 litters in 2003 and 2006, and (e, f) their C:N ratios under the four treatments. See Fig. 1 for abbreviations. Error bars show +SE.

in soil C stocks in response to warming (Fig. 7a) (Luo et al. 2009). However, the key N mechanism underlying the increased plant C accumulation under a warming regime is increased plant NUE instead of altered soil N availability for plant N uptake.

Mechanisms of nitrogen regulation

The main mechanisms that have been incorporated into the coupled nitrogen–carbon climate models are warming-induced increases in N mineralization and availability for plant uptake to stimulate plant growth and C accumulation (Sokolov et al. 2008, Thornton et al. 2009). Many warming experiments also showed that warming caused faster microbial decomposition and increased N mineralization and availability (Rustad et al. 2001, Melillo et al. 2002), thus stimulating plant N uptake (Rustad et al. 2001, Welker et al. 2004, Wan et al. 2005). For example, in a meta-analysis of 12 ecosystem warming experiments, net N mineralization showed a 46% increase with warming (Rustad et al. 2001). Nevertheless, in our study, although changes in plant N content partly contributed to the increment of plant C content under climate warming (Fig. 4b), the insignificant changes in soil N availability and plant N uptake suggest that warming-stimulated N mineralization may not be a key mechanism regulating ecosystem responses to climate warming. Indeed, plant N uptake averaged over the nine years of this experiment was lower in the warmed plots than in control plots without clipping, making no direct contribution to plant C uptake (Fig. 8b). The increment of plant C storage due to changes in plant N uptake only accounted for 24% of the total increment in plant C storage in the clipped plots (Fig. 8d). In the same experiment, warming increased net N mineralization rate only in the first year, but not in the second (Wan et al. 2005) and eighth years (R. Sherry, *unpublished data*), partly supporting the results reported in this paper. This transient increase of net N mineralization may lead to increasing plant N uptake in the short term (An et al. 2005) but not over the long term (Fig. 1). The great increase of soil temperature (Appendix B) in the warmed plots was strong enough to

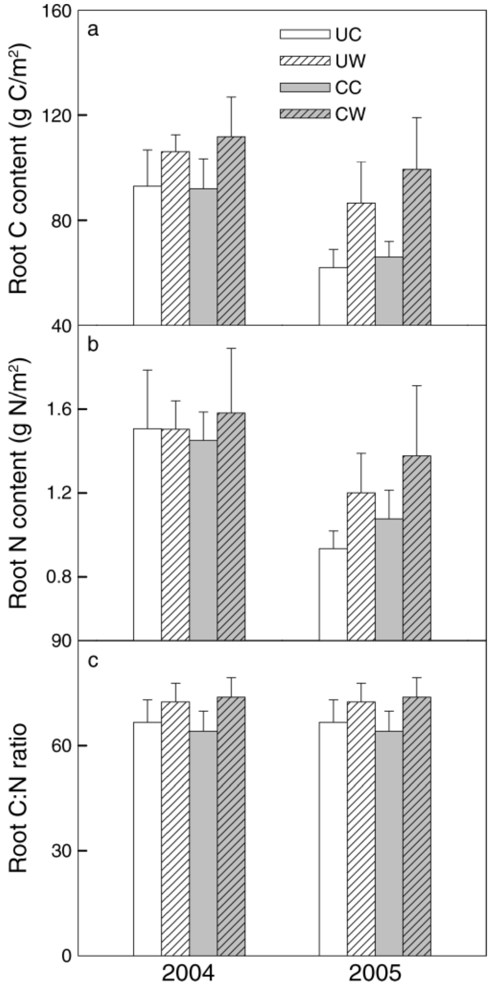

FIG. 6. Mean (a) root carbon, (b) nitrogen storage, and (c) C:N ratios in 2004 and 2005 under the four treatments. See Fig. 1 for abbreviations. Error bars show +SE.

stimulate mineralization, according to Rustad's study (Rustad et al. 2001). The insignificant changes in N mineralization may be due to a reduction in soil moisture in the warmed plots, which was consistent with other studies in tallgrass prairie ecosystems (Wan et al. 2005, Verburg et al. 2009).

Shifted C_4 dominance and the C–N relationship

Although the total amount of plant N uptake did not significantly change under warming, the increases in C_4 plant N content and the decreases in C_3 plant N content (Fig. 1) suggest N redistribution between the two plant functional groups under climate warming. C_4 plants accumulate much more C than C_3 plants when consuming the same amount of N (Fig. 3; Sage and Pearcy 1987). Therefore, with more N redistributed to C_4 plants under warming, the efficiency of ecosystem-scale N use increased so as to facilitate greater plant canopy CO_2 uptake in the warmed than in the control plots. Warming-induced changes in C_4 plant N content can explain >90% of warming-induced changes in C_4 plant C content, which accounts for 78% of total plant C storage. Thus, stimulation of plant C storage under warming is primarily due to N redistribution between plant functional types. The mechanism that warming favors C_4 plants can be due to the higher optimal temperature for photosynthesis and the higher energy requirement in C_4 compared to C_3 plants (Sage 2003). Shifts of species composition toward a community with higher resource use efficiency could facilitate C assimilation and thus have important implications for ecosystem C balance under climate warming (Luo 2007, Day et al. 2008).

In addition to N redistribution between the two functional types, C_3 and C_4 plants both increased NUE at the plant level under warming in comparison to the control (Fig. 3). Increased NUE may be a kind of physiological adjustment to alleviate presumed N limitation under climate warming (Luo 2007). Our results were consistent with those from a previous study at the same site with a three-year data set (An et al. 2005). However, Sardans et al. (2008) reported no change in NUE under climate warming in a mediterranean shrubland ecosystem.

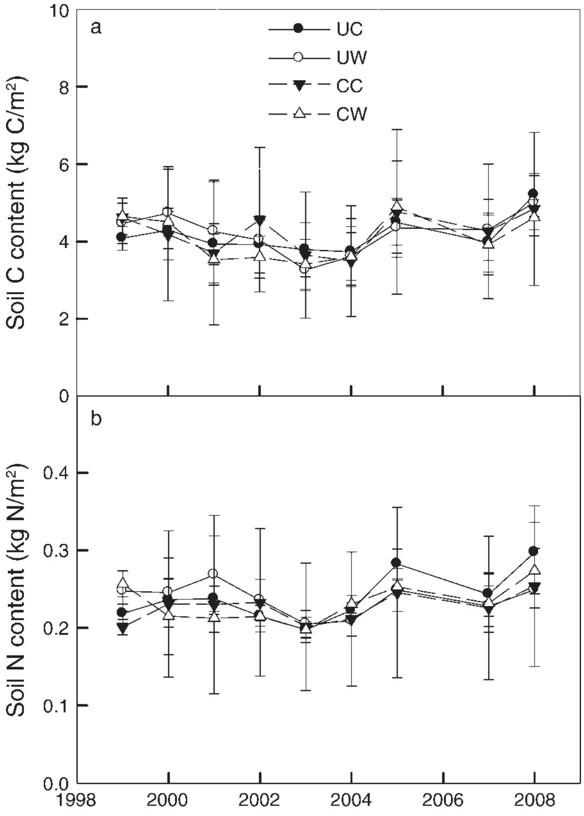

FIG. 7. Mean (a) soil carbon and (b) soil nitrogen content under the four treatments. See Fig. 1 for abbreviations. Error bars show ±SE.

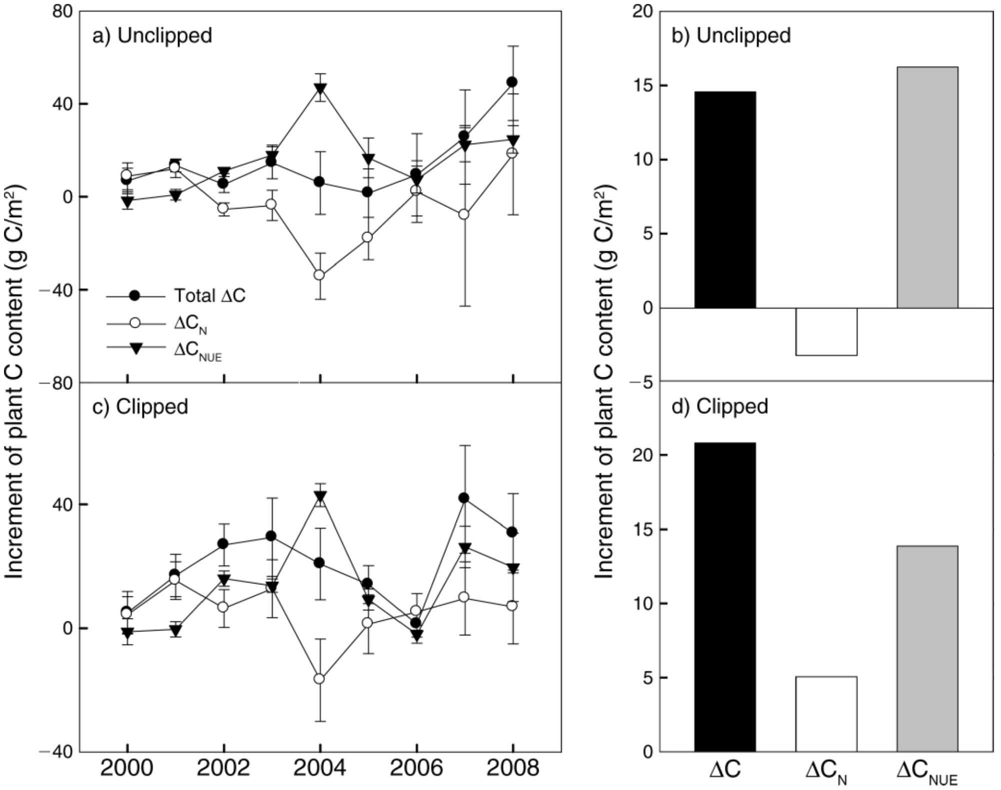

FIG. 8. Total warming-induced increment in aboveground plant carbon content (ΔC) and the contributions of plant N content (ΔC_N) and ecosystem N use efficiency (ΔC_{NUE}) in (a, c) each year, and (b, d) the averages over nine years. Error bars show ±SE.

Increased C_4 dominance also changed litter quality and decomposition. Greater production of C_4 biomass that has inherently higher C:N ratio than C_3 biomass (Figs. 1 and 2), together with the enhancement of C:N ratios in both C_3 and C_4 plants (Fig. 2), lead to lower quality litter under warming (Fig. 5). This could slow down decomposition and potentially offset any direct warming stimulation of respiratory C loss (Rustad et al. 2001, Luo and Zhou 2006). Increases of C:N ratio with warming were also reported in other studies (Welker et al. 2004, Day et al. 2008, Sardans et al. 2008). Increased plant C uptake and decreased decomposition potential due to higher C:N ratio resulted in negative C feedback to warming in this study.

Biofuel harvest effects

Biofuel harvesting is an important land use in grasslands that is projected to influence ecosystem C balance (Houghton et al. 1999, Fargione et al. 2008, Searchinger et al. 2008). In this study, the complete removal of aboveground biomass to mimic biofuel harvest leads to lower C and N storage in plants and litter (Figs. 1 and 5). Although aboveground biomass was harvested, the belowground biomass that forms the main source of soil organic C and N was little changed (Fig. 6) (Luo et al. 2009), which in combination with decreased C release (Zhou et al. 2007), leads to little change in soil C and N even after nine years of clipping (Fig. 7).

Houghton et al. (1999) used ecosystem C losses of 13.6 kg C/m^2 in land use change models for the boreal biome, which put the total theoretical reduction in boreal C at 40–95 Pg (10^{15}g). Our average soil C loss of 10.4 g C·m^{-2}·yr^{-1} from biofuel harvesting is much lower than Houghton's estimate for the boreal biome. As the soil pool stores large amounts of C (2975 g C/m^2), removal of aboveground biomass harvest did not cause significant C loss in the total ecosystem C stock in the first nine years. However, long-term impacts of the destructive harvest on ecosystem C and N dynamics are yet to be examined. One effective way to reduce biofuel harvest impact on ecosystem C and N cycling may be harvesting at a later time. There is a growing body of literature suggesting that grasses harvested for biofuel should be harvested after plant senescence to maximize nutrient cycling, even though biomass may be slightly lower at that time of year (Heaton et al. 2009). If clipping is conducted after the plants have senesced, C and N loss will be reduced, and will have less impact on soil C and N stocks.

CONCLUSIONS

It has been clearly demonstrated by coupled nitrogen–carbon climate models that N can considerably regulate C cycle feedback to climate warming. Most of those coupled models simulated warming-enhanced N mineralization and availability for plant uptake to increase biomass growth and C accumulation in ecosystems. In contrast, this field study showed little change in plant N uptake or soil N pools. Rather, a shift in species composition with associated redistribution of plant N pools from C_3 to C_4 plants was found to be the major mechanism regulating ecosystem response to climate warming. Experimental warming at our site stimulated C_4 plant growth but decreased C_3 plant growth. C_4 plants have higher NUE than C_3 plants. With increased C_4 dominance, a similar amount of N in plant pools supported more biomass growth and C assimilation under climate warming than in controls. Increased litter production in combination with decreased litter quality (higher C:N ratio) resulted in higher litter accumulation at the soil surface in warmed plots than in controls. Increased inputs of more recalcitrant material into soil counterbalanced any direct warming stimulation of C release, leading to little change in soil C stock and no apparent feedback to climate warming. Our results suggest increased NUE plays a more important role than soil N availability in regulating ecosystem C cycling in this ecosystem. To accurately forecast the ecosystem feedback to climate change, ecological models may have to simulate responses of species composition to climate change and their consequences for ecosystem functions.

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APPENDIX A

Nitrogen and carbon concentration (%) of C_3 and C_4 plants in the clipped and unclipped plots in 2008 (*Ecological Archives* E091-230-A1).

APPENDIX B

Summer precipitation (June–August), air temperature, soil temperature at a depth of 2.5 cm, and volumetric soil moisture ($\% V$) at a depth of 0–15 cm under the four treatments (*Ecological Archives* E091-230-A2).

APPENDIX C

R^2 and P values of the relationship between the warming-induced changes in plant carbon (ΔC) and nitrogen (ΔN) content and the warming-induced changes in biomass or carbon ([C]) and nitrogen ([N]) concentration across nine years (2000–2008) (*Ecological Archives* E091-230-A3).