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Plant nitrogen concentration, use efficiency, and contents in a tallgrass prairie ecosystem under experimental warming

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Abstract

Plant nitrogen (N) relationship has the potential to regulate plant and ecosystem responses strongly to global warming but has not been carefully examined under warmed environments. This study was conducted to examine responses of plant N relationship (i.e. leaf N concentration, N use efficiency, and plant N content in this study) to a 4-year experimental warming in a tallgrass prairie in the central Great Plains in USA. We measured mass-based N and carbon (C) concentrations of stem, green, and senescent leaves, and calculated N resorption efficiency, N use efficiency, plant N content, and C : N ratios of five dominant species (two C_4 grasses, one C_3 grass, and two C_3 forbs). The results showed that warming decreased N concentration of both green and senescent leaves, and N resorption efficiency for all species. N use efficiencies and C : N ratios were accordingly higher under warming than control. Total plant N content increased under warming because of warming-induced increases in biomass production that are larger than the warming-induced decreases in tissue N concentration. The increases in N contents in both green and senescent plant tissues suggest that warming enhanced both plant N uptake and return through litterfall in the tallgrass ecosystem. Our results also suggest that the increased N use efficiency in C_4 grasses is a primary mechanism leading to increased biomass production under warming in the grassland ecosystem.

Keywords: global warming, N concentration, N resorption efficiency, N use efficiency, tallgrass prairie

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Introduction

Global warming because of elevated atmospheric greenhouse gases has increased the Earth's surface temperature by 0.6 °C in the past century, and the temperature will continue to increase by 1.4–5.8 °C over the 21st century (IPCC, 2001). Such unprecedented changes in temperature are predicted to influence ecosystem processes and global carbon (C) cycling substantially (Cox *et al*., 2000). As the most limiting nutrient for plant growth, nitrogen (N) availability and its dynamics in ecosystems and their responses to global change will have significant impacts on the long-term C sequestration in terrestrial ecosystems (Hungate *et al*., 2003; Luo *et al*., 2004). While changes in soil N pools and dynamics under global warming have been observed in various ecosystems (Shaw & Harte, 2001; Melillo *et al*., 2002), our knowledge on responses of plant N relationships and their potential impacts on ecosystem C processes (e.g. plant growth, net primary productivity, litter quality, and decomposition) is limited (Olszyk *et al*., 2003; Lewis *et al*., 2004).

Leaf N concentration is one of the most important plant N variables that determine photosynthetic C fixation and plant productivity. Numerous studies, mostly conducted in greenhouses and chambers, have shown that warming could either increase or decrease leaf N concentrations of trees (Hobbie *et al*., 2001; Luomala *et al*., 2003) and grasses in arctic or alpine tundra (Arft *et al*., 1999; Grogan & Chapin, 2000). Responses of leaf N concentration to warming are often different among plant function groups, even in the

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same ecosystem. For example, Read & Morgan (1996) reported that leaf N concentration decreased in *Bouteloua fracilis* (C_4 grass) but increased in *Pascopyrum smithii* (C_3 grass) under warming. Different responses of plant functional groups to warming can lead to shifts in plant community structure (Lilley *et al*., 2001a), plant N uptake (Lilley *et al*., 2001b), and partitioning (Read & Morgan, 1996).

In addition to leaf N concentration, many other plant N variables may affect ecosystem C and N processes. For example, plant N resorption efficiency and its response to warming could significantly impact plant and ecosystem N use efficiency and cycling (Norby *et al*., 2000). Enhanced plant N uptake (Ineson *et al*., 1998a, b), rather than increased N use efficiency (Maranville & Madhavan, 2002), resulted in stimulation of plant C accumulation under warming. Altered C : N ratio in plant litter in response to global change affects rates of decomposition and N release from plant litter (Norby *et al*., 2000; Billings *et al*., 2003). The N amount retained in organic plant material and soil N availability, as affected by changes in net primary productivity and altered plant N concentration and litter C/N ratio may feedback to change plant growth, net primary, and ecosystem productivity (Luo *et al*., 2004). Thus, understanding the responses of plant N relationships to warming is critical for predicting ecosystem C and N cycling in future climatic scenarios.

As part of a comprehensive project investigating experimental warming in a tallgrass prairie (Luo *et al*., 2001; Wan *et al*., 2002a, b, 2005; Zhang *et al*., 2005), this study examined possible changes in plant N relationships and their potential impacts on ecosystem C and N processes under experimental warming in a tallgrass prairie. Our objectives are to examine (1) differences among species and functional types in response of leaf N concentrations to warming; (2) effects of warming on plant N use efficiency at both species and ecosystem levels; (3) responses of plant N resorption efficiency to experimental warming; and (4) potential impacts of the warming-induced changes in plant N relationships on ecosystem C and N processes.

Materials and methods

Experimental design

Plant material used in this study was collected from a warming × clipping experiment in a natural grassland ecosystem. The experimental site is located at a tallgrass prairie in the US Great Plains in McClain Country (34°58′54″N, 97°3114W), 40 km southwest from Norman campus of the University of Oklahoma. This site has not been grazed for 20 years. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans)* and C_3 forbs (*Ambrosia psilostachyia, Aster ontarionis,* and *Aster ericoides)*. The mean annual temperature is 16.0 °C, with monthly mean temperature of 3.1 °C in January and 28.0 °C in July. The mean annual precipitation is 967.2 mm (average values from 1948 to 1999, data from the Oklahoma Climatological Survey). The soil is part of the Nash–Lucien complex (sand: 32%; silt: 60%; clay: 8%), which is characterized as having a low permeability rate, high available water capacity, and deep and moderate penetrable root zone (Wan *et al*., 2005).

The experiment used a split-plot design, with warming as the main factor and clipping nested within warming. There were six pairs of $2 \times 2\,\mathrm{m}^2$ plots. In each pair, one plot had been warmed continuously using infrared heaters since 21 November, 1999 with the other control plots at ambient temperature. One $165 \times 15\,\mathrm{cm}^2$ infrared heater (Kalglo Electronics Inc., Bethlehem, PN, USA, radiation output of about $100\,\mathrm{W\,m^{-2}}$) was suspended 1.5 m above ground in each warmed plot. In a control plot, one 'dummy' heater with the same shape and size as the infrared heater was suspended 1.5 m high to simulate the shading effects of the heater (Fig. 1). For each pair of plots, the distance between control and warmed plots was approximately 5 m in order to prevent heating of the control plot by the infrared heater. Distances between the individual sets of paired plots varie from 20 to 60 m. The daily mean and minimum air temperatures increased by 1.1 and 2.3 °C in the heated plots, respectively, in comparison with those in control plots (Wan *et al*., 2002a). Soil temperatures at a depth of 2.5 cm increased by 2.6 °C, 2.7 °C, 2.4 °C, and 2.0 °C in 2000, 2001, 2002, and 2003, respectively, in the warmed plots compared with those in the control plots (Wan *et al*., 2002a, 2005).

Each $2 \times 2\,\mathrm{m}^2$ plot was divided into four $1 \times 1\,\mathrm{m}^2$ subplots (Fig. 1). Two diagonal subplots in each plot were clipped 10 cm above the soil every year; the other two were the unclipped controls. After clipping, plants were allowed to grow until next clipping. The four treatments in the experiment were unclipped control, unclipped warmed, clipped control, and clipped warmed. Plant materials used in this study were from clipped plots only. As the unclipped subplots were designed to have minimal disturbances over the long term, no plant materials were taken from the unclipped plots.

Tissue sample collection and chemical analyses

Plants were clipped at 10 cm above soil on 28 July, 2000, 24 July, 2001, 24 July, 2002, and 26 September, 2003, respectively, in the clipped subplots. The clipped plants

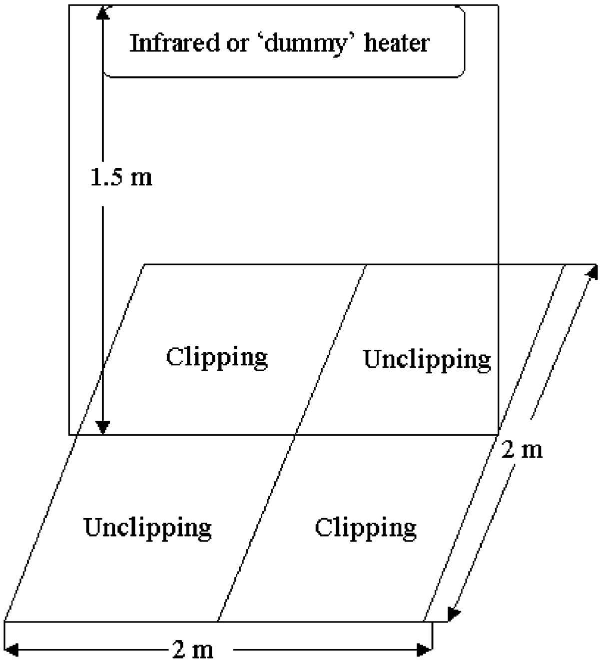

Fig. 1 Experimental layout for each warmed or control plot. Each plot ($2 \times 2\,m^2$) was divided into four $1 \times 1\,m^2$ subplots, in which two diagonal subplots were clipped and the other two were unclipped controls.

were sorted into three categories, C_3 grasses, C_3 forbs, and C_4 grasses, and were oven-dried at 65 °C for 48 h, and weighted by category. From the available tissue, green and senescent leaves from two C_4 grass species (*S. scopariums* and *Sporobolus asper*), one C_3 grass species (*Dichanthelium oligosnathes*), and two C_3 forb species (*A. psilostachya* and *A. ericoides*) were selected for analyses of N and C concentrations. The five species accounted for 85–90% of total aboveground biomass in each plot. We identified green or senescent leaves of the three C_3 species (*D. oligosnathes*, *A. psilostachya*, and *A. ericoides*) and one C_4 grass (*S. asper*) according to their distinguishable shoot characteristics. The shallow-red color of leaves was the main trait used in identification of recent senescent leaves of *S. scopariums*. We also analyzed C and N concentrations of stems for the two forb species. Thus, we had 4 years of samples for each species (except *D. oligosnathes* in September 2003), 12 replicates for each type of tissues (e.g. stem, green, and senescent leaves) from the five species at both warmed and control plots, and the total sample size was 552 (minus either green or senescent leaves of *S. asper* in 2003) in this study. The C and N concentrations were analyzed with a LECO CNS-2000 Elemental Analyzer (LECO Corp., St Joseph, MI, USA). We used LECO soil standard to verify the accuracy of the analysis instrument and duplicate samples in every 10 samples to verify the precision of measurements.

Data analyses

Concentrations of C and N in plant tissues were used to calculate the following parameters:

1. *Plant N content (PNC):* The ground-area-based aboveground plant N content was the sum of N contents in grasses and forbs, which were calculated by %N × biomass. At our experimental site, C_4 grasses accounted for more than 95% of the total aboveground biomass of grasses. Thus, we used an averaged leaf N concentration of the two C_4 grasses (NG) multiplying aboveground biomass of grasses (BG) to approximate N contents in grasses (NCG) in each plot. To estimate N contents of C_3 forbs (NCF), we separated the aboveground tissues into two categories: leaves and stems, and weighted by leaves (LW) and stems (SW) separately. We multiplied LW and SW with an averaged N concentration of both leaves and stems of the two C_3 forbs, separately, to approximate the N contents in leaves (NLF) and stems (NSF), and then summed them up for the N content of forbs in each plot. Thus, PNC = NCG + NCF in each plot. The aboveground biomass data were reported by Wan *et al*. (2005).
2. *N resporation efficiency (NRE):* Mass-based N resporation efficiency for each species was calculated by the following equation:

$$\mathrm{NRE} = \frac{N_g - N_s}{N_g} \times 100\%, \tag{1}$$

where N_g is the green leaf N concentration and N_s is the senescent leaf N concentration.

3. *Relative response of N concentration to warming* (*RR*): The relative response of N to warming on a mass basis for each species was calculated by

$$RR = \frac{|N_c - N_w|}{N_c} \times 100\%, \tag{2}$$

where N_c is the leaf N concentration in the control plots and N_w is the leaf N concentration in the warmed plots.

4. *N use efficiency (NUE):* We based N use efficiency on total biomass produced per unit plant N (Wilkins *et al*., 1997; Maranville & Madhavan, 2002; Knops *et al*., 2002), and NUE of two plant functional groups (C_3 forbs and C_4 grasses) and of the whole ecosystem were calculated by the following equations:

$$\text{NUE of } C_4 \text{ grass} = \mathrm{BG}/(\mathrm{BG} \times \mathrm{NG}) = 1/\mathrm{NG}, \tag{3}$$

$$\text{NUE of } C_3 \text{ forbs} = (\mathrm{LW} + \mathrm{SW})/(\mathrm{NLF} + \mathrm{NSF}), \tag{4}$$

$$\text{NUE of ecosystem} = (\mathrm{BG} + \mathrm{LW} + \mathrm{SW})/\mathrm{PNC}. \tag{5}$$

We did not calculate NUE of C_3 grass because we only had one C_3 grass species in our study.

Data analysis used SAS 8.0 software to test for a significant difference between warmed and control plots.

Results

N concentrations in both green (Fig. 2a–e) and senescent (Fig. 2f–j) leaves tended to be lower in the warmed than control plots for all species. The decreases were statistically significant ($P<0.05$) in green leaves in the last 2 years (2002 and 2003), except for *S. asper* in 2003 (Fig. 2b). *D. oligosanathes* also exhibited significantly ($P<0.05$) lower N under warming than control in 2001 (Fig. 2c). Warming had no effect on stem N concentration for the two C_3 forbs in any year (Fig. 3a–b). N concentrations were significantly higher in green than in senescent leaves in warmed or control plots (Fig. 2). Among the three plant function groups, C_3 forbs (*A. psilostachya* and *A. ericoides*) had the highest

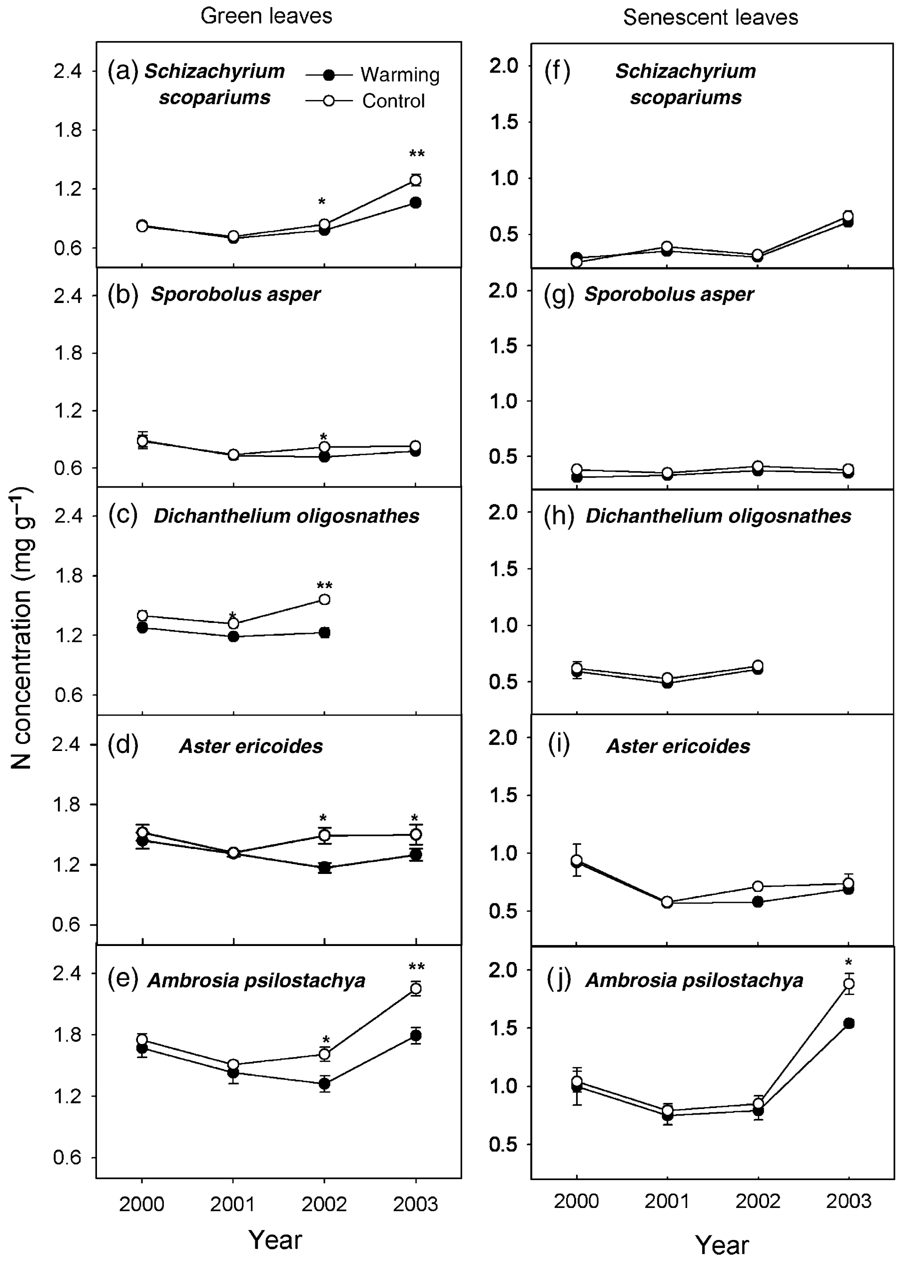

Fig. 2 N concentrations in green (a–e) and senescent (f–j) leaves of (a, f) *S chizachyrium scopariums*; (b, g) *Sporobolus asper*; (c, h) *Dichanthelium oligosnathes*; (d, i) *Aster ericodies*; and (e, j) *Ambrosia pssilostachya*. The data are shown as means and standard errors, with $n=6$. * and ** represent statistical significance at $P<0.05$ and $P<0.01$, respectively.

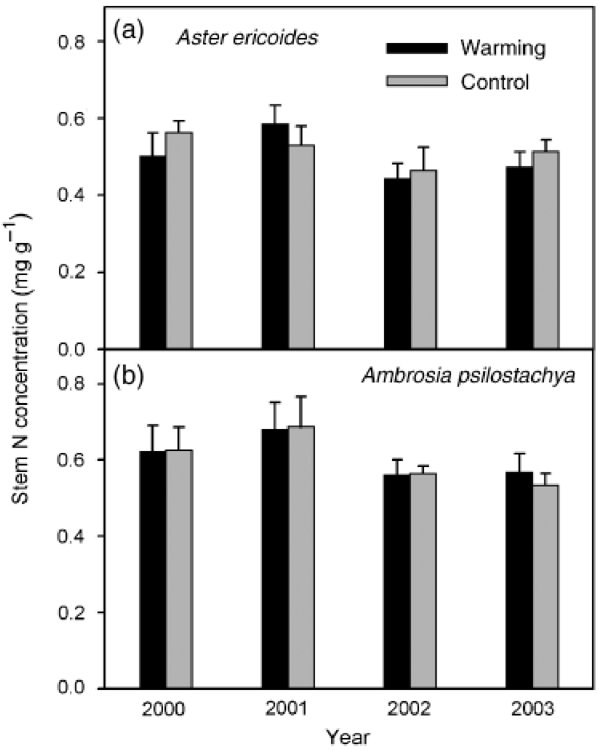

Fig. 3 N concentrations of the stem for two C_3 forbs: (a) *Aster ericodies*; (b) *Ambrosia pssilostachya*. The data are shown as means and standard errors, with $n = 6$.

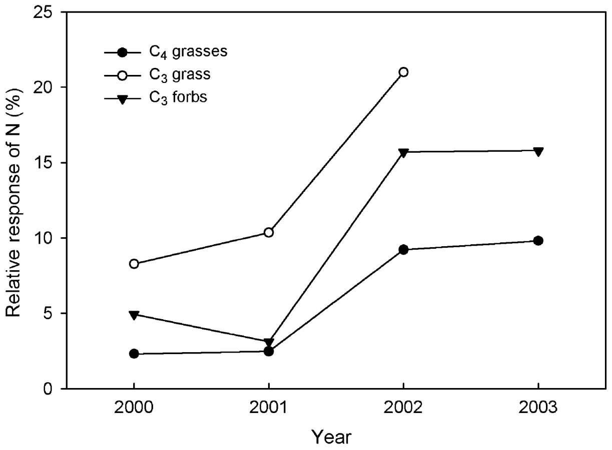

Fig. 4 Relative response of leaf N concentrations to warming in plant functional groups (C_4 grasses, C_3 grass, and C_3 forbs). Values are averages of functional groups, with $n = 12$ in C_4 grasses and C_3 forbs, and $n = 6$ in C_3 grass.

N concentrations in green and senescent leaves while C_4 grasses (*S. scopariums* and *S. asper*) had the lowest N concentrations. The differences between C_3 and C_4 species were statistically significant ($P<0.05$), but no significant difference ($P>0.05$) was found among the three C_3 species in the warmed or control plots (Fig. 2). C_3 grass was the most responsive to warming in terms of the relative N concentration whereas C_4 grasses were the least responsive (Fig. 4).

Although warming reduced leaf N concentrations, N contents of green and senescent plant tissues were greater in the warmed than control plots (Fig. 5a–b) because of the substantial increases in biomass growth (Wan *et al.*, 2005). Wan *et al.* (2005) observed that aboveground biomass was significantly ($P<0.05$) increased by 36.7%, 57.1%, and 57.4% in C_4 grasses and by 27.0%, 29.8%, and 41.3% in ecosystem (C_4 grasses plus C_3 species), but there was no significant change in C_3 forbs in 2001–2003, respectively, under warming compared with those under control in our experimental site. Warming only significantly ($P<0.05$) increased N contents in senescent plants by 29.75% and 32.65% in 2001 and 2002, respectively.

Warming tended to decrease N resorption efficiency in most of the 4 years (Fig. 6). But the decreases were not statistically significant ($P>0.05$), except for *D. oligosanathesin* in 2002 (Fig. 6d). Reduced N resorption efficiency resulted from greater N decrease in green than in senescent leaves. There were weak ($r=-0.41$ and 0.50) but statistically significant ($P<0.05$) relationships between green leaf N concentration and resorption efficiency in two C_4 grasses under warming (Fig. 7a–b). No significant correlations ($P<0.1$) were found between green leaf N concentration and resorption efficiency in either C_4 grasses in the control plots (Fig. 7a–b) or C_3 species in both the warmed and control plots (Fig. 7c–e).

Warming significantly increased N use efficiencies of C_4 grasses and the whole ecosystem in 2002 and 2003, but had no consistent effect on C_3 forbs (Fig. 8). C_4 grasses had higher N use efficiency than C_3 forbs in both warmed and control plots.

There were no significant effects ($P>0.05$) of warming on either C concentrations in both green and senescent leaves or C : N ratios in senescent leaves (Fig. 9f–j). However, C : N ratios in green leaves were significantly higher ($P<0.05$) in the warmed than control plots in the last 2 years of study (2002 and 2003) for almost all species, except for *S. asper* in 2003 (Fig. 9b) and A. *psilostachya* (Fig. 9e).

Discussion

This study demonstrated a clear pattern that long-term experimental warming significantly decreased green leaf N concentrations in all five dominant species in the tallgrass prairie ecosystem. Even with the decreased leaf N concentrations, whole ecosystem plant N content still increased under warming in comparison with that

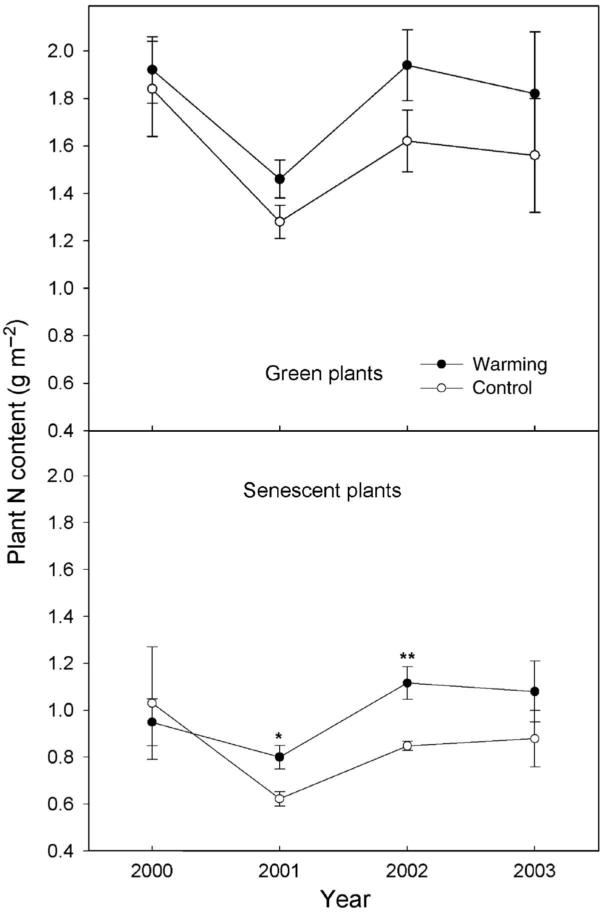

Fig. 5 Plant N contents in green (a) and senescent tissues (b). The data are shown as means and standard errors, with $n = 12$. * and ** represent statistical significance at $P < 0.05$ and $P < 0.01$, respectively.

under control because of large increases in plant biomass production (Wan *et al.*, 2005). Accordingly, N use efficiency increased under warming at the ecosystem scale and for C_4 grasses. In addition, warming-induced decreases in N concentration were much lower for the senescent leaves than the green leaves, leading to decreased N resorption efficiency.

Green leaf N concentration and N use efficiency

This study observed decreases in green leaf N concentrations for all species in the warmed than control plots. Our results are consistent with observations from some of the previous studies (Tjoelker *et al.*, 1999; Weih & Karlsson, 2001; Link *et al.*, 2003). However, other warming studies, mostly in cold regions, showed that elevated temperature increased leaf N concentrations (Nijs *et al.*, 1996; Soussana *et al.*, 1996; Luomala *et al.*, 2003). In the cold regions, elevated temperature substantially enhanced soil N mineralization (Anke & Wright, 1997; Shaw & Harte, 2001) and thus, increased plant N uptake, resulting in higher leaf N concentrations (Tingey *et al.*, 2003). In our study, N mineralization was stimulated in the first year but depressed in the second year of the warming experiment (Wan *et al.*, 2005), presumably resulting in progressively larger decreases in green leaf N concentrations over time (Fig. 2).

Warming effects on green N leaf concentrations also varied with plant functional groups (Fig. 4). At the ambient temperature, C_4 grasses have higher N use efficiency (Wedin & Tilman, 1993; Sage & Kubien, 2003), higher water use efficiency (Sage & Kubien, 2003), and more tolerance to heat stress (White *et al.*, 2000) than C_3 species. Under warming, C_4 grasses showed less decrease in N than C_3 grass and forbs (Fig. 4), probably because of less flexibility in leaf N relationships (Fig. 5). C_3 grass was the most responsive to warming among the three plant functional groups in term of adjusting leaf N concentration. Nevertheless, the lower N requirement of C_4 grasses combined with other heat-tolerant traits enhanced the competitive edge of C_4 grasses over C_3 species under warming as reflected by enhanced biomass growth (Wan *et al.*, 2005).

Our results showed that warming significantly increased N use efficiency (NUE) of C_4 grasses, but had no significant effects on NUE of C_3 forbs (Fig. 8a–b). Maranville & Madhavan (2002) summarized the results of NUE in the literature into three cases wherein improved NUE will occur: (1) maintaining constant biomass yield while reducing plant or leaf N; (2) increasing biomass yield at a constant level of plant or leaf N; and (3) reducing levels of plant or leaf N per unit of increase in biomass yield. In our study, increased NUE of C_4 grasses resulted from both an increase in biomass production and a decrease in leaf N concentration. Although their green leaf N concentrations significantly decreased (Fig. 2d–e), neither stem N concentrations (Fig. 3) nor biomass growth of C_3 forbs (Wan *et al.*, 2005) significantly changed under warming, leading to no change in NUE of C_3 forbs (Fig. 8b). Because C_4 plants accounted for the majority of ecosystem plant biomass (Wan *et al.*, 2005), the significant increase in NUE in C_4 grasses contributed to the significant increases of NUE at the ecosystem level.

Senescent leaf N concentration and N resorption efficiency

We observed consistently lower N concentrations in senescent leaves under warming than control for all species, although most of the changes were statistically not significant (Fig. 2f–j). Other studies observed lower senescent leaf N concentrations in coldest summers

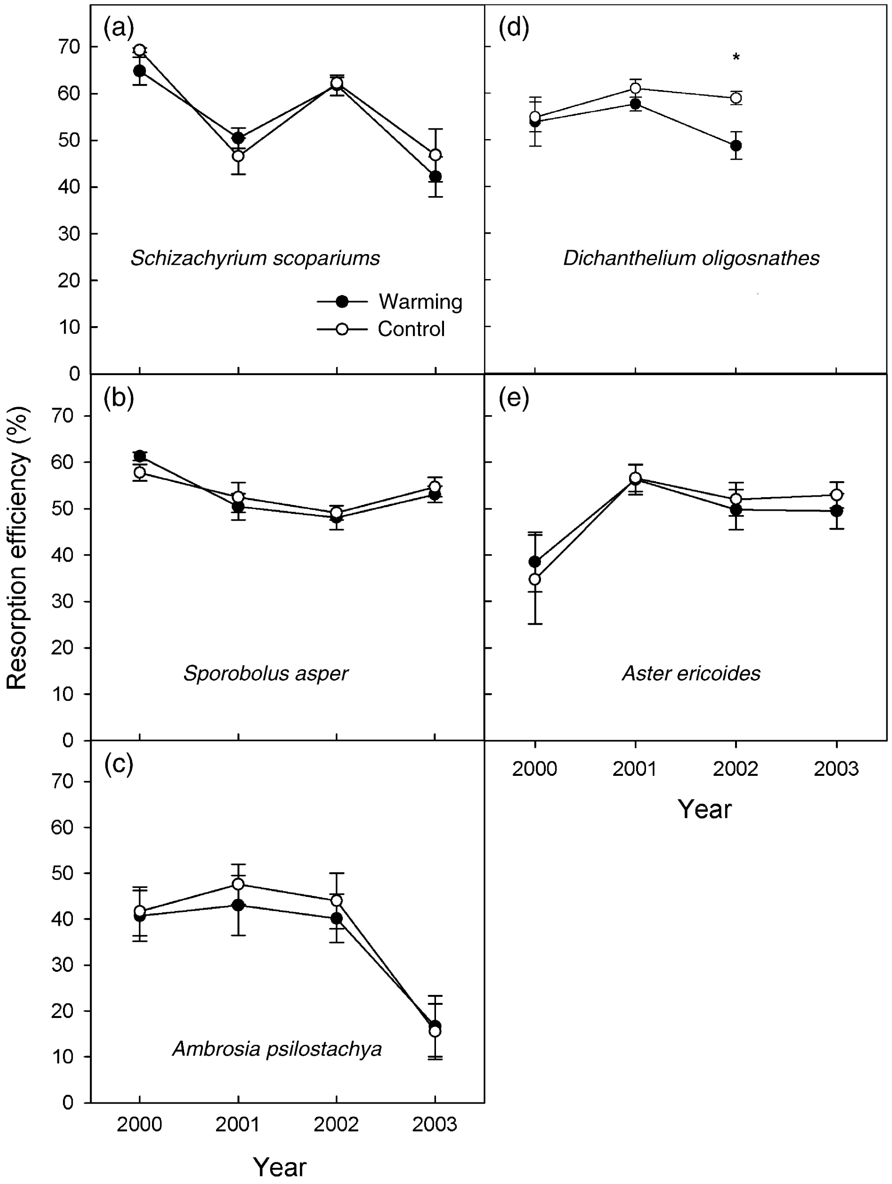

Fig. 6 N resorption efficiency of (a) *S chizachyrium scopariums*; (b) *S porobolus asper*; (c) *Dichanthelium oligosnathes*; (d) *Aster ericodies*; and (e) *Ambrosia pssilostachya*. The data are means and standard errors, with $n = 6$. * represents statistical significance at $P < 0.05$.

than other summers for mountain birth trees [*Betula Pubescens ssp tortuosa (Ledeb.) Nyman*] (Nordell & Karlsson, 1995) or in the control than warmed plots for *Acer rubrum* trees (Norby *et al.*, 2000). Warming-induced decrease in N concentration was much less in senescent leaves than green leaves. As a consequence, N resorption efficiencies generally decreased under warming in comparison with those in control, consistent with observations of other studies (Nordell & Karlsson, 1995; Norby *et al.*, 2000). The ratio of leaf soluble to structural N is an important determinant of resorption efficiency (Pugnarie & Chapin, 1993; Rien, 1996). In our study, a higher percent of leaf N may be converted to structural N to meet a large increase in biomass growth in warmed plots (Wan *et al.*, 2005), resulting in decreased N resorption efficiency. The decreased N resorption means that less N was transported from senescent to alive leaves before leaves died in the warmed than control plots. In addition, lower soil moisture in the warmed plots may also contribute to a decrease of N resorption efficiency (Del *et al.*, 1991; Escudero *et al.*, 1992).

Although warming decreased N concentration in senescent leaves, N contents in senescent plant increased significantly (Fig. 5b) under warming in comparison with that in the control, largely because

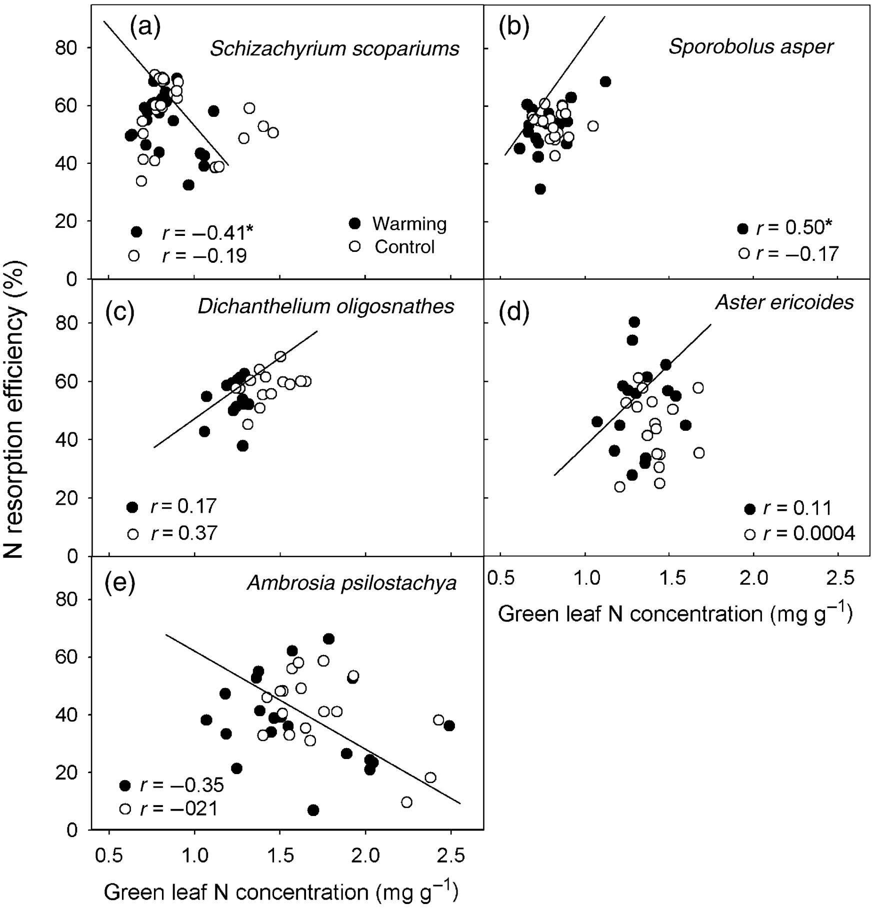

Fig. 7 Relationship between green leaf N concentration and resorption efficiency under warming and control for (a) *Schizachyrium scopariums*; (b) *Sporobolus asper*; (c) *Dichanthelium oligosnathes*; (d) *Aster ericodies*; and (e) *Ambrosia pssilostachya*. Each data point is from one plot in the control (open symbols), and warming (closed symbols). r is the correlation coefficient; * represents statistical significance at $P<0.05$.

of warming-induced increase in aboveground biomass (Wan *et al.*, 2005). Increased senescent plant N content suggests that N accumulation in litter likely increased in the warmed in comparison with control plots.

Among the three plant functional groups, warming did not change the relationships between leaf N concentration and resorption efficiency in C_3 species (Fig. 7c–e), which supports a view that resorption efficiency is not related to plant N status (Rien, 1996; Norby *et al.*, 2000). However, the relationships were statistically significant ($P<0.05$) for C_4 grasses under warming, suggesting that changes in leaf N concentrations of C_4 grasses under warming altered N resorption efficiency.

Plant N content and ecosystem implications

At this experimental site, we observed that the percent increases in aboveground biomass (Wan *et al.*, 2005) were higher than the percent decreases in leaf N concentrations under warming. As a consequence, plant N contents were 4.55%, 13.77%, 19.58%, and 16.24% greater under warming than control in 2000–2003, respectively (Fig. 5a). Warming-induced increases in biomass in the cold climate region partly resulted from enhanced N uptake from soil (Ineson *et al.*, 1998a, b; Rustad *et al.*, 2001). Although warming consistently stimulated N uptake across the 4 years of the experiment in the tallgrass prairie ecosystem, the increases in plant N content were not statistically significant ($P>0.05$). Our results suggest that increased biomass growth in our experimental site for the prairie ecosystems (Wan *et al.*, 2005) primarily resulted from direct, stimulatory effects of temperature on plant growth of C_4 species rather than from indirect effects through N uptake as reflected by significant increases in NUE of C_4 grasses.

The warming-induced changes in plant N contents and other relationships potentially alter ecosystem N cycling. The increased plant N contents in this grassland ecosystem with seasonal growth and death of aboveground tissues must be accompanied by increases

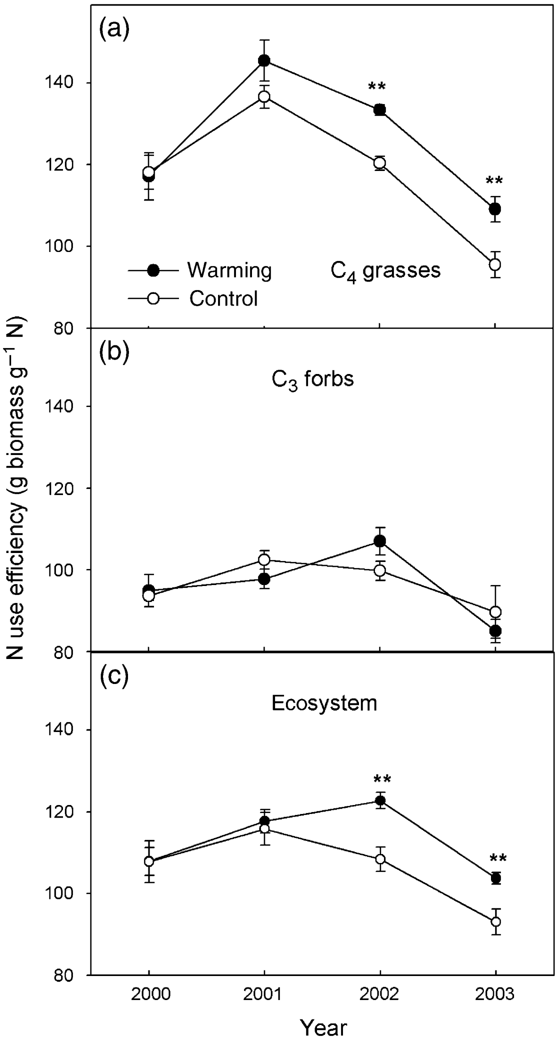

Fig. 8 N-use efficiencies in plant functional groups of (a) C_3 forbs, (b) C_4 grasses, and (c) the ecosystem. The data are shown as means and standard errors, with $n = 12$. * and ** represent statistical significance at $P < 0.05$ and $P < 0.01$, respectively.

in plant N uptake from soil available N pools. On the other hand, increased senescent plant N contents (Fig. 5b) transfer more N back to soil-N pools through litterfall in the warmed than control plots. Thus, warming increased annual N flux rates through soil N uptake and litterfall N back to soil.

N release from litter pools to soil-available N pools is regulated by litter decomposition rates. At the ecosystem scale, we observed that C_4 litter significantly increased whereas C_3 plant litter showed no change under warming in comparison with control (Wan *et al.*, 2005), resulting in decreased quality of bulk litter because of a higher C : N ratio in C_4 grasses than in C_3 forbs (Fig. 9). Thus, an increased litter amount and decreased litter quality presumably resulted in N accumulation and/or immobilization in litter pools, and slowed N releases from litter pools to soil available N pools in the warmed compared with control plots. As a consequence, an imbalance likely occurred between the demand for more plant N uptake and less available N supply because of slowed litter decomposition in the warmed than control plots. Indeed, studies on soil N dynamics at the same site support the imbalance notion. Wan *et al.* (2005) showed that soil N mineralization rates substantially decreased from years 1 to 2 of the experiment. The total soil N content decreased by 13.7% in the warmed plots compared with that in the control plots without clipping in May 2003 (A. Subedar & Y. Luo, in manuscript). The 13.7% decrease in soil N content may result partly from the redistribution of N between plant and soil pools and partly from N losses under warming. Moreover, N isotope (^{15}N) data suggest that the experimental warming accelerated N cycling in the ecosystem (A. Subedar & Y. Luo, in manuscript). The imbalance possibly leads to progressive nitrogen limitation (Luo *et al.*, 2004), as reflected by warming-induced decreases in leaf N concentrations that were becoming more substantial over time (Figs 2 and 4).

The growing imbalance between N demand and supply in our warming experiment had not yet significantly limited biomass production in the tallgrass ecosystem by 2003, after 4 years of warming as warming induced significant increases in N use efficiency for both C_4 grasses and whole ecosystem. As of 2003, the warming-induced increases in N use efficiency appeared to have helped sustain higher aboveground biomass production (Wan *et al.*, 2005) under warming than control. However, Knops *et al.* (2002) argued that the increased N use efficiency could not support long-term increases in productivity at ecosystem scales. Therefore, it is important to observe whether or not the imbalance will continue and would eventually limit aboveground biomass production in the warming experiment in the future.

Conclusions

Nitrogen is one of the most important nutrients that determines plant productivity and rates of carbon cycling through the ecosystem, thus regulating their responses to global warming. Therefore, it is important to understand plant N relationships in warmed environments. Our observations indicate that long-term experimental warming significantly decreased N concentrations of both green and senescent leaves of all five species that we studied in the tallgrass prairie ecosystem. Warming also considerably stimulated growth of C_4 grasses and significantly enhanced biomass production in the

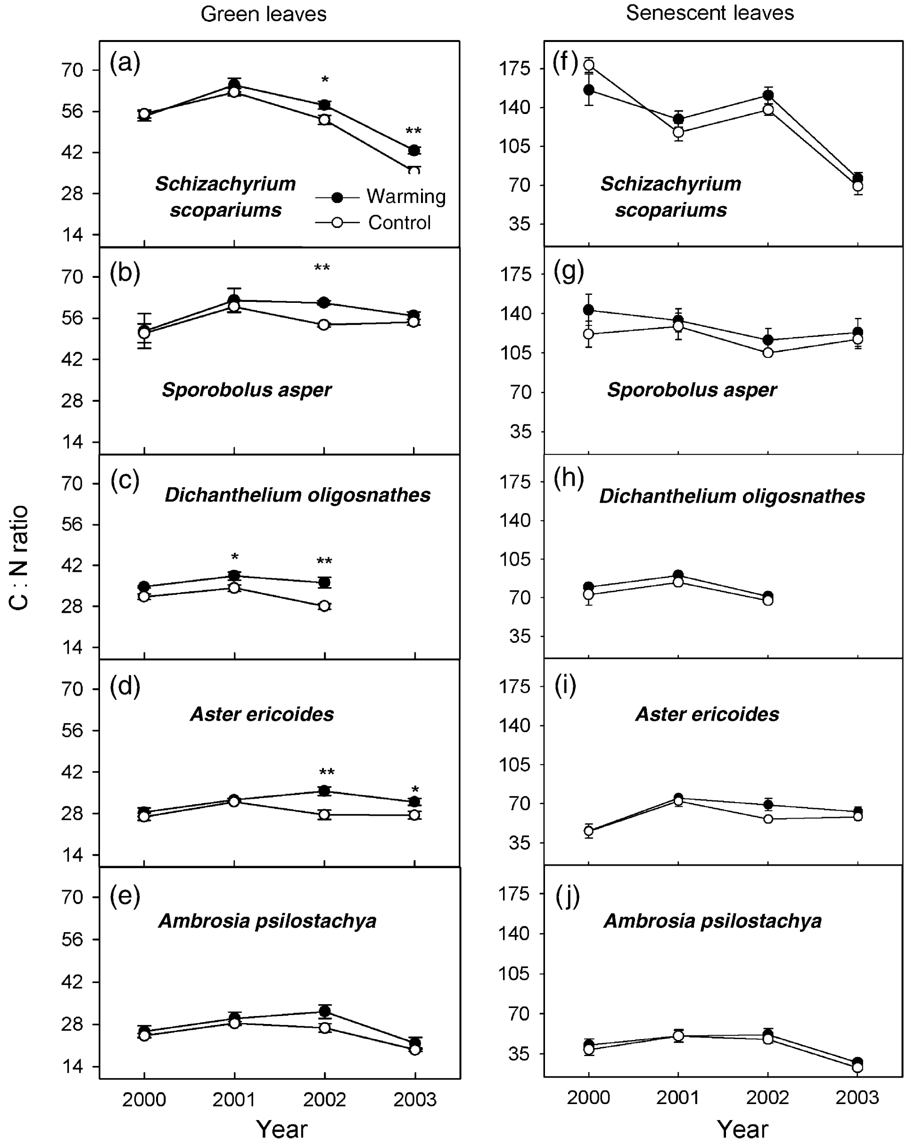

Fig. 9 C : N ratios of green (a–e) and senescent leaves (f–j) of (a, f) *Schizachyrium scopariums*; (b, g) *Sporobolus asper*; (c, h) *Dichanthelium oligosnathes*; (d, i) *Aster ericodies*; and (e, j) *Ambrosia pssilostachya*. The data are shown as means and standard errors, with $n = 6$. * and **represent statistical significance at $P < 0.05$ and $P < 0.01$, respectively.

ecosystem. Increased biomass production in this ecosystem (Wan *et al.*, 2005) under warming was associated with increased N use efficiency in C_4 grasses. Increases in growth and death of aboveground biomass over seasons are likely accompanied by increases in N uptake from and N return to soil. The long-term implications of the altered plant N uptake and return in ecosystem N and C cycles are yet to be evaluated in the future.

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