



Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping

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ABSTRACT

Carbon (C) and nitrogen (N) fluxes are largely controlled by the small but highly bio-reactive, labile pools of these elements in terrestrial soils, while long-term C and N storage is determined by the long-lived recalcitrant fractions. Changes in the size of these pools and redistribution among them in response to global warming may considerably affect the long-term terrestrial C and N storage. However, such changes have not been carefully examined in field warming experiments. This study used sulfuric acid hydrolysis to quantify changes in labile and recalcitrant C and N fractions of soil in a tallgrass prairie ecosystem that had been continuously warmed with or without clipping for about 2.5 years. Warming significantly increased labile C and N fractions in the unclipped plots, resulting in increments of 373 mg C kg⁻¹ dry soil and 15 mg N kg⁻¹ dry soil, over this period whilst clipping significantly decreased such concentrations in the warmed plots. Warming also significantly increased soil microbial biomass C and N in the unclipped plots, and increased ratios of soil microbial/labile C and N, indicating an increase in microbial C- and N-use efficiency. Recalcitrant and total C and N contents were not significantly affected by warming. For all measured pools, only labile and microbial biomass C fractions showed significant interactions between warming and clipping, indicating the dependence of the warming effects on clipping. Our results suggest that increased soil labile and microbial C and N fractions likely resulted indirectly from warming increases in plant biomass input, which may be larger than warming-enhanced decomposition of labile organic compounds.

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1. Introduction

Soil is an important source or sink of carbon (C) and nitrogen (N), and plays a major role in the cycling of these elements in terrestrial ecosystems. On a global scale, the soil contains 1500 Pg (1 Pg = 10¹⁵ g) of organic carbon and 300 Pg of total nitrogen in the upper soil layer up to 1 m, and these amounts are considerably larger than those in terrestrial biomass (Schlesinger, 1997; Amundson, 2001). Relatively small changes in the amounts of soil C and N may therefore bring about substantial effects on atmospheric concentrations and on global C and N cycling at large. In other words, the stability of C and N in soil organic matter (SOM) to perturbations such as global warming is critically important for the global C and N cycles.

SOM is composed of a continuum of materials of varying chemical complexity with mean turnover times from days to years and millennia (Davidson and Janssens, 2006). The broad continuum

can be conceptually divided into several discrete pools characterized by distinct turnover times in model studies, such as Century (Parton et al., 1987), ROTH-C (Jenkinson, 1990), and APSIM (McCown et al., 1996). In experimental studies, a variety of chemical, physical, and biological fractionation procedures have been developed to characterize various pools of soil C and N (Olk and Gregorich, 2006). SOM pools are chemically divided into a *labile* pool with a small size and rapid turnover and a *recalcitrant* fraction with a large size and slow turnover using acid hydrolysis procedure (McLaughlan and Hobbie, 2004), although there is considerable discrepancy between soil C pools simulated in modeling studies and SOM fractions determined in laboratory analyses.

While C and N fluxes are largely dominated by the small but highly bio-reactive labile pool, long-term C and N storage is often determined by the long-lived recalcitrant fraction (Trumbore et al., 1990). Being a direct reservoir of readily available nutrients, the labile pool is particularly important and exerts considerable control on ecosystem functioning. Through its impact on microbial biomass and activity and on the turnover and supply of nutrients to vegetation, the labile pool can alter both productivity and community structure of ecosystems (Pastor and Post, 1986). Such alterations, by

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bringing about changes in the size and distribution of inputs in labile and recalcitrant pools, not only may lead to new conditions in the short term but can also influence the long-term terrestrial net C and N storage and feedback to the atmosphere (Zak et al., 1993; Luo, 2007). Several studies have also demonstrated that the labile pool is very sensitive to alterations in soil moisture, temperature, and plant community structure resulting from climate change (Zak et al., 1993; Trumbore et al., 1996). Hence, analyzing the labile pool may provide insights into and early indications of impacts of climate change on soil C and N dynamics.

Together with the recalcitrant fraction, information on the labile pool could improve detection and prediction of changes in soil C and N dynamics that may not be readily evident with the traditional monitoring of total C and N contents or net gas exchange measurements. Specifically, analysis of total C and N does not normally permit detection of small changes because of the high background levels and natural soil variability. Net gas exchange measurements may also not provide information about the size and distribution of inputs in labile and recalcitrant pools. Fractionating SOM into labile and recalcitrant pools and quantitative analysis of these pools could be useful for better understanding C and N dynamics and their responses to global warming. However, the labile and recalcitrant fractions of C and N have not been widely used to evaluate climate change impacts under field conditions, especially in grasslands that are one of the most important ecosystems occupying nearly a quarter of the global land cover (Schimel, 1995). Furthermore, clipping – a widely practiced land use in grasslands – may affect responses of the labile and recalcitrant pools to climate change and has not been examined yet.

Microbial biomass C and N pools represent vital components of ecosystem cycling with a turnover time from days to years (Hu et al., 1997), and serve as a source (mineralization) or a sink (immobilization) of labile nutrients. Studies have shown that microbial biomass responded quickly to change in soil perturbation by tillage (Carter, 1986) and soil moisture (Skopp et al., 1990). Laboratory incubation indicated that soil microbial biomass and activity increased in response to soil warming (Sprent, 1987; Fang et al., 2005). In field experiments, there was a lag time for microbial biomass C and N to respond to experimental warming in subarctic soils (Ruess et al., 1999). In tallgrass prairie, understanding the responses of soil microbial biomass to warming and clipping are also key to predicting future changes in carbon and nutrient cycling.

In this study, we investigated effects of warming and clipping on labile and recalcitrant C and N fractions and microbial biomass C and N of soil in a tallgrass prairie ecosystem. The site is located in the Southern Great Plains of USA and is dominated by *Schizachyrium scoparium*, *Sorghastrum nutans*, and *Eragrostis curvula* (all C₄ grasses) and *Ambrosia psilostachya* and *Xanthocephalum texanum* (C₃ forbs). The field experiment has been continuously warmed with or without clipping since its establishment on November 21, 1999. We hypothesized that both warming and clipping would decrease labile pool sizes due to enhanced oxidation of labile pools and reduced substrate input, respectively (Peterjohn et al., 1993; Wan and Luo, 2003; Niinistö et al., 2004).

2. Materials and methods

2.1. Site description

The study was conducted at the Great Plains Apiaries in McClain County, Oklahoma (34°58'54"N, 97°31'14" W), approximately 40 km southwest of the Norman campus of the University of Oklahoma, USA. This site is an old-field tallgrass prairie abandoned from agriculture 30 years ago and without grazing during the past 20 years. The grassland is dominated by three C₄ grasses:

S. scoparium (Michx.) Nash, *S. nutans* (L.) Nash, and *E. curvula* (Schrad.) Nees, and two C₃ forbs: *A. psilostachya* DC. and *X. texanum* (DC.) Shinnars. The three C₄ grasses represent approximately 75% of the total plant biomass (R. Sherry and Y. Luo, unpublished data). Mean annual temperature is 16.3 °C, with monthly air temperature ranging from 3.3 °C in January to 28.1 °C in July. Mean annual precipitation is 915 mm, with monthly precipitation ranging from 30 mm in January to 135 mm in May (average values from 1948 to 1998, Oklahoma Climatological Survey). A silt loam soil in the grassland includes 35.3% sand, 55.0% silt, and 9.7% clay (A. Subedar and Y. Luo, unpublished data). The soil belongs to part of the Nash-Lucien complex with neutral pH, high available water capacity, and a deep, moderately penetrable root zone (USDA, US Department of Agriculture, 1979).

2.2. Experimental design

The field experiment used a paired, nested design with warming as the main factor and clipping as a secondary factor. Twelve 2 × 2 m plots were divided into six pairs of control (i.e., unwarmed) and warmed plots. In each warmed plot, one 165 × 15 cm infrared heater (Kalglo Electronics Inc., Bethlehem, Pennsylvania, USA) was suspended in the middle of the plot at a height of 1.5 m above the ground. The height of 1.5 m was determined by considerations of vegetation height and radiative energy output. The heating was on year-around, 24 h per day and 365 days per year in the field since November 21, 1999. To simulate shading effects of heaters, we installed one 'dummy' heater made of metal flashing with the same shape and size as the heating device over each control plot. A previous study by Wan et al. (2002) has documented that warming increased daily mean air temperature at 25 cm above the ground by 1.1 °C and soil temperature at 2.5-cm depth by 2.0 °C, and the effects of the heater on soil temperature were spatially uniform in the warmed plots. For each paired plot, the distance between the control and the warmed plots was approximately 5 m to avoid heating the control plot by the heater. The distances between the individual sets of 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 the height of 10 cm above the ground yearly, usually in August. The other two were the unclipped controls. On average from 2000 to 2002, clipping removed 187 and 225 g m⁻² of biomass from control and warmed plots, respectively. Based on plant C and N concentrations from An et al. (2005), we calculated that clipping removed 84 and 101 g C m⁻² and 2.8 and 3.4 g N m⁻² under control and warming, respectively. Clipping in this manner effectively mimics hay mowing, a widely practiced land use in the southern Great Plains. Usually farmers and ranchers in the southern Great Plains mow grass pasture once to twice per year, depending on rainfall. Our study site is rather xeric, and thus yearly clipping was conducted to mimic hay mowing once a year. The four treatments in the experiment were unclipped control (UC), unclipped warmed (UW), clipped control (CC), and clipped warmed (CW) with 6 replicates. Further details of the study were described in Wan et al. (2002, 2005).

2.3. Soil sampling

Soil samples were collected from the topsoil (0–20 cm) of all the subplots (excluding surface litter) in May, September, and December of 2002 and in June 2003. Two or more soil cores (2.54 cm diameter × 20 cm deep) were taken from each subplot, and samples from the same treatment were pooled together, packed in polyethylene bags, and immediately stored in an ice chest until they were transported to the laboratory. The composite samples were then passed through a sieve (<2 mm diameter), and

any visible plant materials were manually removed from the sieved soil. One-half of each processed sample was stored at 4 °C for a day or two and subsequently used for microbial biomass C and N measurements. The remaining soil was air-dried, finely ground and sieved (<180 µm) for chemical analysis.

2.4. Determination of labile and recalcitrant C and N pools

Acid hydrolysis is a commonly used technique to isolate and quantify labile and recalcitrant fractions of SOM. The procedure has been applied in several SOM fractionation and modeling studies (Oades et al., 1970; Leavitt et al., 1996; Paul et al., 2001; Shirato and Yokozawa, 2005; Olk and Gregorich, 2006; Rovira and Vallejo, 2007) and found to be useful in obtaining reasonably accurate estimates of the different SOM pools (Xu et al., 1997; Paul et al., 2001). It is easy to perform and not as time consuming as the physical or biological separation methods. Moreover, the acid hydrolysis procedure can readily be applied to large number of samples and this makes the technique more convenient and appealing to most ecological researches (Rovira and Vallejo, 2007).

In most acid hydrolysis fractionation studies, hydrochloric acid (HCl) is the conventionally used extractant. Hydrolysis with sulfuric acid (H₂SO₄) has also been used widely and reportedly provides better characterization of SOM quality (Oades et al., 1970; Rovira and Vallejo, 2000, 2002, 2007; Shirato and Yokozawa, 2005; Paul et al., 2006). The two-step acid hydrolysis with 5 N H₂SO₄ in the first step and 26 N H₂SO₄ concentrations in the second step has in particular been found to give more realistic numerical SOM quality indices than the HCl hydrolysis procedure (Oades et al., 1970; Rovira and Vallejo, 2000).

In the present study, we used the two-step acid hydrolysis procedure with H₂SO₄ as the extractant to determine labile and recalcitrant C and N pools. Details of the procedure are found in Oades et al. (1970) and Rovira and Vallejo (2002). Briefly, 20 mL of 5 N H₂SO₄ was added to 500 mg soil, and the sample was hydrolyzed for 30 min at 105 °C in sealed Pyrex tubes, after which the hydrolysate was recovered by centrifugation and decantation. The residue was washed with 20 mL of de-ionized water and the washing added to the hydrolysate. This hydrolysate was taken as labile pool I (LPI) and analyzed for labile pool I carbon (LPI-C) and nitrogen (LPI-N). The remaining residue was hydrolyzed with 2 mL of 26 N H₂SO₄ overnight at room temperature under continuous shaking. The concentration of the acid was then brought down to 2 N by dilution with de-ionized water and the sample was hydrolyzed for 3 h at 105 °C with occasional shaking. The hydrolysate was recovered in the same manner as for the LPI. This second hydrolysate was taken as labile pool II (LPII) and analyzed for labile pool II carbon (LPII-C) and nitrogen (LPII-N). The labile pool I is known to predominantly contain polysaccharides which are of both plant origin (such as hemi-cellulose and starch) and microbial origin (mostly microbial cell walls) whereas the labile pool II is largely cellulose in composition (Oades et al., 1970; Rovira and Vallejo, 2007).

We also measured total organic C (TOC) and total N (TN) in soils. In this study, carbon was analyzed with a Shimadzu TC analyzer (Shimadzu Corporation, Kyoto, Japan) and nitrogen was analyzed with a Carlo Erba elemental analyzer (Carlo-Erba, Milan, Italy). Recalcitrant C (RP-C) and N (RP-N) pools were calculated as the difference between the total concentration of the elements (i.e., TOC or TN) and the labile pools (LPI and LPII summed together).

2.5. Determination of microbial biomass C and N contents

In December 2002 and June 2003, soil microbial biomass C (SMB-C) and N (SMB-N) were determined using the fumigation-extraction method (48 h fumigation, Vance et al., 1987). Soil

extractable C and N in fumigated and non-fumigated soil samples were extracted by 0.5 M K₂SO₄ and analyzed using the method mentioned above for C and N. The differences in extractable C and N between the fumigated and non-fumigated soils were assumed to be released from lysed soil microbes. The released C and N were converted to SMB-C and SMB-N using extraction factors of 2.22 and 0.54, respectively (Brookes et al., 1985; Wu et al., 1990).

2.6. Statistical analysis

Three-way analysis of variance (ANOVA) for a blocked split-plot design was used to examine the effects of warming, clipping, sampling date, and their interactions for labile and recalcitrant C and N pools, total C and N, soil microbial biomass C and N, and ratios of microbial biomass/labile pools. For specific sampling date, a three-way ANOVA was used to examine the effects of clipping, warming, and their interaction. The effects were considered to be significantly different if $p < 0.05$. All statistical analyses were performed using SPSS 13.0 for windows (SPSS Inc., Chicago, 2004).

3. Results

3.1. Labile and recalcitrant pools of soil C and N

Warming significantly increased LPI-C, LPII-C, and LPI-N but not LPII-N, while clipping significantly decreased all of them (Table 1). On average in three seasons, warming increased LPI-C, LPII-C, and LPI-N by 14.5, 16.5, and 17.5%, respectively, in the unclipped plots (Fig. 1, Table 1). Clipping decreased LPI-C, LPII-C, LPI-N, and LPII-N by 11.4, 20.7, 15.4, and 13.5%, respectively, in the warmed plots (Fig. 1, Table 1). The interactive effects of clipping and warming were significant on LPI-C ($p < 0.05$) and marginally significant on LPII-C ($p = 0.08$) but not on labile N (Table 1). When labile I and labile II pools were summed together, warming increases of 373 mg C kg⁻¹ dry soil (15.8%) and 15 mg N kg⁻¹ dry soil (11.4%) in the unclipped plots on labile C and N fractions, respectively. Although labile C and N contents were significantly different over three seasons, the statistical analyses show that the effects of warming, clipping, and their interaction did not depend on the seasons (i.e., no interaction with sampling date, Table 1).

The recalcitrant C and N pools contributed to a large amount of total C and N (~86%) compared to labile C and N fractions (~13%, Table 2). Warming and clipping did not significantly affect the recalcitrant and total C and N contents except clipping effects on recalcitrant N and total N (Fig. 2a, Table 2). The interactive effects of clipping, warming, and sampling date on recalcitrant C and N pools and total C and N were also not statistically significant (Table 2). In addition, warming with clipping did not significantly affect the percentages of total C and N for any measured pools (Table 2).

3.2. Soil microbial biomass C and N

Warming significantly increased both soil microbial biomass C (SMB-C) and N (SMB-N), while clipping marginally significantly decreased SMB-N but not SMB-C (Fig. 2b,c, Table 1). In winter 2002, SMB-C in the unclipped plots ranged from 347 mg C kg⁻¹ in the control to 615 mg C kg⁻¹ in the warmed plots, resulting in a significant increase of 268 mg C kg⁻¹ (77.2%) due to warming (Fig. 2b). Differences in SMB-C in spring 2003 among the treatments were smaller but exhibited a similar trend. The interaction of warming and clipping was significant on SMB-C, and depended on sampling date, which showed a marginal significance for warming × clipping × time (Table 1). Although both warming and clipping affected SMB-N, their interaction was not significant (Table 1). On average, the microbial biomass accounted for 2.3% of the total and 15% of the labile pool (Table 2).

Table 1

Results of three-way ANOVA showing the *P* values for responses of labile C and N pools I and II (LPI-C, LPI-N, LPII-C, and LPII-N), recalcitrant C and N (RP-C and RP-N), total C and N (TC and TN), and soil microbial biomass C and N (SMB-C and SMB-N) to warmed (W), clipped (CL) treatments, and sampling dates (D).

Factor	LPI-C	LPII-C	RP-C	TC	SMB-C ^a	LPI-N	LPII-N	RP-N	TN	SMB-N ^a
Warming	0.01	0.02	0.82	0.72	<0.001	0.01	0.35	0.11	0.10	0.001
Clipping	0.03	0.001	0.17	0.12	0.22	0.01	0.04	0.03	0.03	0.06
Date	<0.001	0.003	0.17	0.12	<0.001	<0.001	<0.001	0.13	0.02	0.78
W × CL	0.04	0.08	0.74	0.66	0.03	0.10	0.30	0.70	0.63	0.69
W × D	0.46	0.80	0.70	0.68	0.65	0.58	0.85	0.47	0.51	0.58
CL × D	0.30	0.51	0.44	0.42	0.60	0.97	0.72	0.65	0.67	0.58
W × CL × D	0.29	0.82	0.88	0.88	0.08	0.59	0.31	0.91	0.89	0.75

^a Soil microbial biomass C and N (SMB-C and SMB-N) were measured twice (i.e., December 19, 2002 and June 27, 2003), while others were measured three times (i.e., May 16, September 24, and December 19, 2002). *p* Values smaller than 0.05 are bold and the *italic* indicates marginal significance.

The ratios of SMB and LP for both C and N contents were also influenced by warming and clipping treatments (Fig. 3). Warming significantly increased the SMB-C:LPC ratios in the unclipped plots (42%, $p < 0.01$), and increased the SMB-N:LPN ratios in the clipped plots (42%, $p < 0.01$). Clipping and its interactive effects with warming on ratios of SMB-C:LPC and SMB-N:LPN were not statistically significant ($p > 0.1$).

4. Discussion

Predictions of climate change largely depend on effects of warming on SOM decomposition and understanding of the temperature sensitivity of different SOM fractions (Davidson and Janssens, 2006; Luo, 2007). In the present study, experimental warming significantly increased labile C and N contents in the unclipped plots (Figs. 1 and 2). This contradicted other reports and our hypothesis that labile pool sizes of SOM may decrease under warming as a result of increased soil respiration and enhanced decomposition of organic matter (Peterjohn et al., 1993; Niinistö et al., 2004). We found that the labile C and N pools (LPI+II) showed positive linear correlations ($p < 0.01$ and $p < 0.001$, respectively) with total aboveground biomass in the same year (2002) across different subplots (Fig. 4). Furthermore, warming also

significantly increased below-ground biomass by 11.6% (Wan et al., 2005), while annual soil respiration only increased by 5.0% from 2000 to 2002 (Luo et al., 2001; Zhou et al., 2007). Greater above- and below-ground biomass production and low stimulation in soil respiration in the warmed treatments resulted in higher substrate inputs (Wan et al., 2005), and hence the increased labile C and N concentrations in these plots. The observed increases in labile C and N pool sizes were thus attributable to indirect warming stimulation via alterations in inputs and outputs rather than direct warming effects through decomposition of the recalcitrant pools. Several long-term studies have demonstrated that labile C and N levels were high in high substrate input systems and low in those with low substrate input (Cambardella and Elliott, 1992; Janzen et al., 1992). Improvements in aggregate formation due to earthworm activity could also have been a factor in the observed increments in labile C and N pools. Physical protection through aggregation is known to reduce accessibility of organic materials to microorganisms and enzymes (Tan et al., 2004).

Clipping significantly decreased labile C and N fractions, especially in the warmed plots, that could largely be a result of decreased soil moisture that might have restricted the decomposition of the recalcitrant pools or reduced litterfall (Skopp et al., 1990; Wan et al., 2002, 2005). In the unwarmed plots, however, the

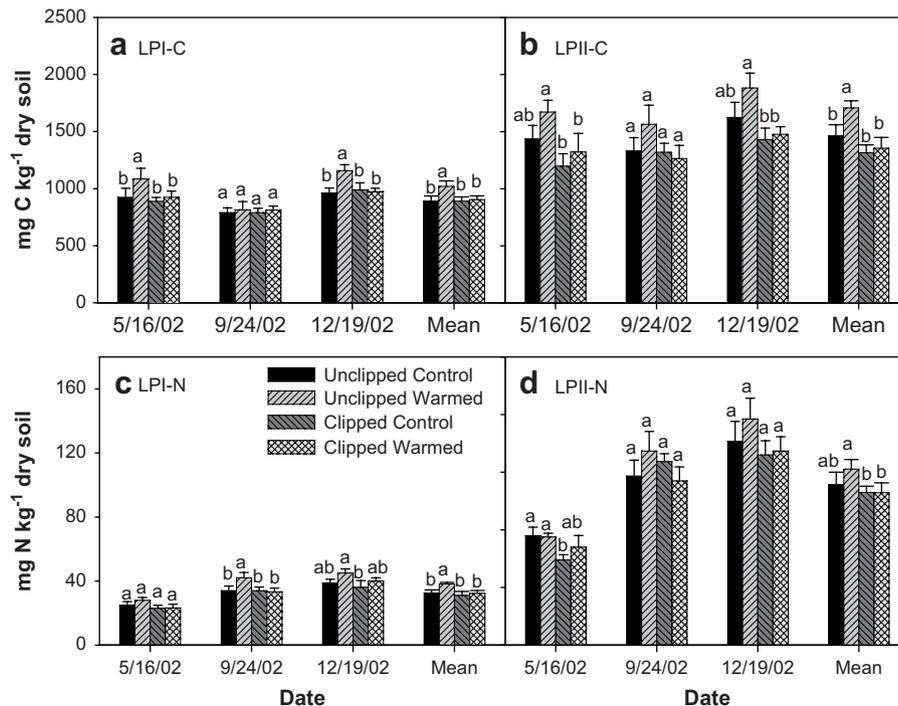


Fig. 1. Effects of warming and clipping on labile C pool I (LPI-C, a), labile C pool II (LPII-C, b), labile N pool I (LPI-N, c), and labile N pool II (LPII-N, d) in a tallgrass prairie soil. Mean represents average in three seasons (i.e., May 16, September 24, and December 19, 2002). The same letter on top of bars indicates statistically non-significance.

Table 2
The percentages of total C and N (TC and TN, mg kg⁻¹) for labile C and N pools I and II (LPI-C, LPI-N, LPII-C, and LPII-N), recalcitrant C and N (RP-C and RP-N), and soil microbial biomass C and N (SMB-C and SMB-N).

	TC (mg kg ⁻¹)	LPI-C (%)	LPII-C (%)	RP-C (%)	SMB-C (%)
Unclipped Control	18667 (2925)	4.77 (0.23)	7.84 (0.51)	87.38 (15.55)	1.66 (0.17)c
Unclipped Warmed	19878 (2178)	5.13 (0.24)	8.59 (0.32)	86.28 (10.71)	2.58 (0.12)a
Clipped Control	16931 (2496)	5.25 (0.22)	7.77 (0.41)	86.98 (14.90)	2.00 (0.16)ac
Clipped Warmed	16810 (2235)	5.37 (0.19)	8.05 (0.57)	86.57 (13.24)	2.45 (0.12)ab
	TN (mg kg ⁻¹)	LPI-N (%)	LPII-N (%)	RP-N (%)	SMB-N (%)
Unclipped Control	1067 (81)ab	3.05 (0.18)	10.46 (0.80)	86.49 (6.82)	2.39 (0.11)
Unclipped Warmed	1189 (111)a	3.21 (0.08)	10.27 (0.55)	86.51 (8.86)	2.74 (0.29)
Clipped Control	964 (53)c	3.22 (0.26)	11.01 (0.45)	85.78 (4.93)	2.12 (0.17)
Clipped Warmed	1030 (95)bc	3.12 (0.17)	10.25 (0.67)	86.63 (8.58)	2.84 (0.24)

Standard error of the mean is in parentheses. Different letters for SMB-C indicate significantly different means (ANOVA).

clipping effects on labile C and N fractions due to biomass removal may be offset by increased below-ground biomass (through such processes as root exudation and root decay) and decreased soil respiration (Wan et al., 2005; Zhou et al., 2007). The interactions of warming and clipping were significant for labile C but not for labile N contents (Table 1), suggesting that the warming effects on labile C fractions were negated by clipping. The profound influences of land management practices such as grazing on labile C and N levels are well documented (Janzen et al., 1992; Rühlmann, 1999). Our results suggest that the increases in labile C and N fractions due to enhanced biomass from warming may be adversely impacted by clipping.

Besides plant biomass, soil temperature and moisture are two other factors that might have impacted the observed responses of the labile C and N pools to warming and clipping treatments. First, higher soil temperature in warmed and clipped plots (Wan et al., 2002; Zhou et al., 2007) may stimulate decomposition of the recalcitrant pools (Knorr et al., 2005), which could increase labile C

and N levels. Second, decreased soil moisture under warming and clipping (Wan et al., 2002) may restrict decomposition of the labile and recalcitrant pools (Skopp et al., 1990). However, the two opposite effects may be counterbalanced, and hence their overall effects may be relatively small compared to the increases in substrate inputs, which are yet to be assessed.

Seasonally, labile C contents were lowest in summer whereas labile N fractions were lowest in spring (Fig. 2). The months of July and August are the hottest and generally dry in the area, and substrate input during this period could be presumably low, thus leading to lower labile C levels in subsequent months. The clipping treatment in this experiment has been applied in August each year and likely also lowered labile C levels in the clipped plots. The relatively low labile N contents in May coincided with the peak growing season of C₃ species, suggesting increased uptake of, and greater demand for N by the plants (Sprent, 1987).

Recalcitrant and total C and N contents may be potentially decomposed faster under warming than in control due to increased soil temperature. However, we did not detect such changes in those large pools after two and half years warming in the present study (Tables 1 and 2). Due to plant biomass removal in the clipped plots, clipping significantly decreased recalcitrant and total N contents but not recalcitrant and total C (Fig. 2a, Table 1). Note that percent decreases in recalcitrant and total N contents by clipping were similar to those for recalcitrant and total C contents. The statistical significance for the decreased recalcitrant and total N was largely due to their small variances across the six replicate plots (Table 2). In contrast, variances of recalcitrant and total C were relatively large. Thus, the different statistical results for responses of recalcitrant and total C and N to clipping may have minor ecological meanings.

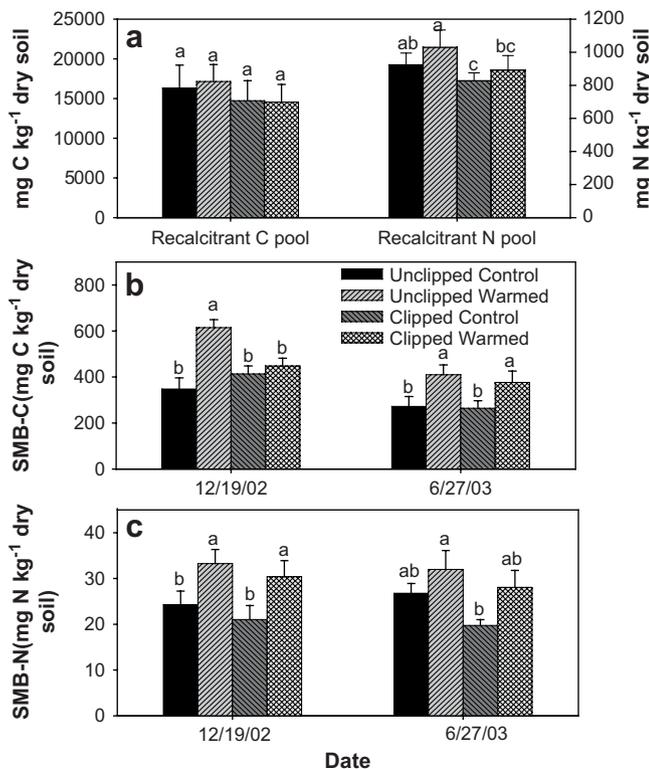


Fig. 2. Effects of warming and clipping on recalcitrant C and N pools (a) and soil microbial biomass C (SMB-C, b) and N (SMB-N, c) on December 2002 and June 2003 (mean \pm SE).

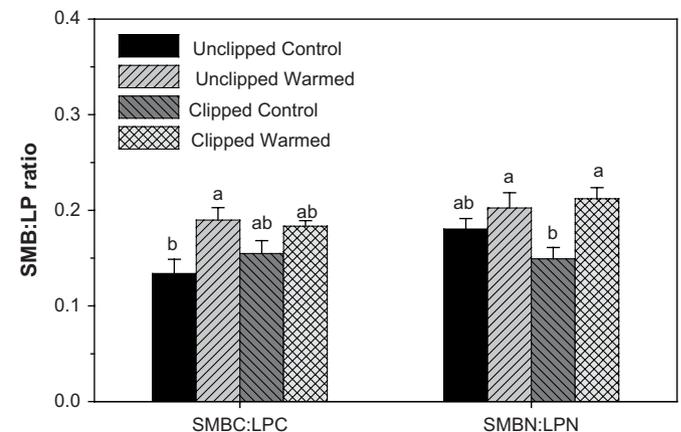


Fig. 3. Soil microbial biomass to labile pool ratio (SMB-C:LPC and SMB-N:LPN) under experimental warming and clipping in a tallgrass prairie soil (mean \pm SE).

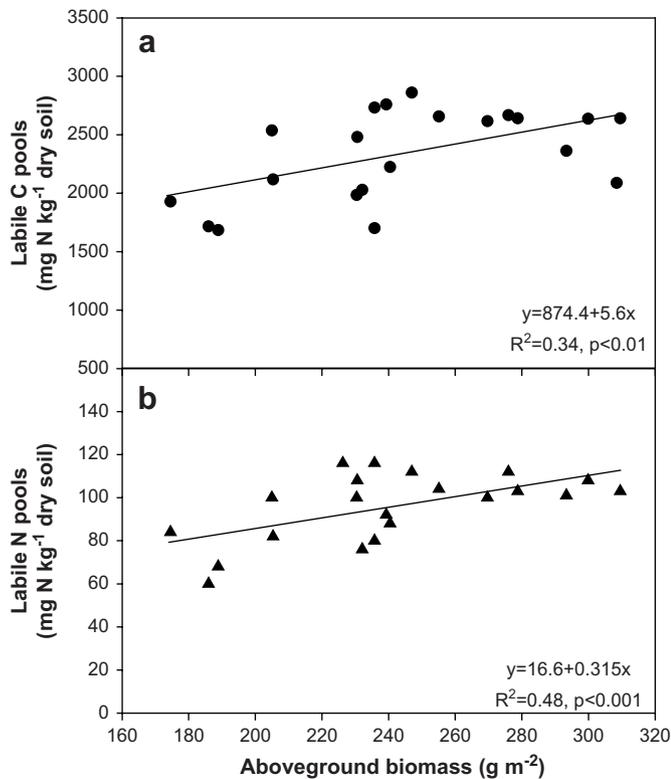


Fig. 4. Linear correlations between aboveground biomass (AGB) and mean labile C (a) and N (b) pools in 2002. AGB data were from Wan et al. (2005).

Alterations in the quantity and quality of plant biomass inputs in response to warming could exert substantial impacts on soil microbes. In the present study, our results have shown significant warming increases in soil microbial biomass C and N after three-year manipulation (Fig. 2), which was consistent with the increases in labile C and N contents (Fig. 1). Warming may directly promote nutrient cycling by increasing soil and litter decomposition rates and net N mineralization (Hobbie, 1996; Ruess et al., 1999). Additionally, changes in plant community composition (Zhang et al., 2005), increased above- and below-ground biomass, (Figs. 1 and 2, Wan et al., 2005), and increased population density (Ruess et al., 1999) under warming may also enhance microbial activity. Therefore, rising temperature would lead to higher physiological activities of soil microorganisms, and in turn to higher decomposition rates and soil respiration (Hobbie, 1996; Zhou et al., 2007). A long-term study in a grass and dwarf shrub ecosystem in Denmark has shown that microbial biomass C and N could be significantly enhanced with increases in labile C input into the soil (Michelsen et al., 1999). In two other studies in New Zealand and the United Kingdom, soil microbial biomass was stimulated by higher organic inputs (Sparling, 1992; Degens, 1998). In June 2003, differences in microbial biomass C and N among the treatments exhibited a similar trend but were less in magnitude compared to those in December 2002, probably owing to the competition for nutrients between plants and microbes during the active growing season. Clipping significantly decreased soil microbial biomass N contents but not microbial biomass C (Fig. 2, Table 1), reflecting higher sensitivity of the former to plant biomass removal. Soil microbial biomass C was significantly impacted by the interactive effects of warming and clipping. The effects of warming on soil microbial biomass C largely depended on sampling date, thus indicating that microbial activity might have been affected by seasonal variations in temperature and moisture.

Although Zhang et al. (2005) found no warming-associated increases of microbial biomass C and N by September 2002,

increases had occurred by December 2002 (Fig. 2). This suggests a lag time of about three years for response from the beginning of the trial. A similar long-term study in subarctic soils near Abisko, northern Swedish Lapland also showed a lag time of about six years for responses of microbial biomass C to warming (Ruess et al., 1999). The difference in the lag time between the two studies may be attributed to the differences in temperature increments, which was about 2 °C in our study compared to 0.9–1.9 °C in the subarctic soils (Ruess et al., 1999; Wan et al., 2002).

The SMB/LP ratio, which is essentially the amount of C or N converted to microbial biomass per unit labile C or N, can be considered as a measure of nutrient use efficiency by the microbes and be used to infer information about the available soil substrates. The increases in SMB/LP ratios observed in the present study (Fig. 3) show that warming, by altering the quality as well as quantity of substrates returning to the soil, can bring about changes in the microbes' nutrient use efficiency. The latter is an indication of a possible shift in soil microbial community composition, most likely due to dominance by fungi since fungi have higher nutrient use efficiency than bacteria (Zak et al., 1996). Dominance by fungi could favor soil C storage in ecosystems (Holland and Coleman, 1987). Irrespective of treatments, the SMB-N/LPN ratio was proportionally greater than the SMB-C/LPC ratio suggesting relatively higher N use efficiency by the microbes. Enhanced N use efficiency by microbes can potentially minimize the competition between microbes and plants, resulting in greater nutrient availability for plants, higher plant growth and a continued supply of organic inputs into the soil (Hu et al., 2001).

5. Conclusions

The study has demonstrated significant increases in both labile C and N (including microbial biomass) pools in response to experimental warming. An alteration in C and N use efficiency by microbes was also observed, indicating a possible shift to a fungi-dominated microbial community. Such a shift could favor soil C storage. The warming increases in labile and microbial biomass C and N pools largely resulted from increased above- and below-ground biomass. The warming effects on labile C and soil microbial biomass C fractions were largely negated by clipping. In addition, our results also provided evidence that warming increased the percentage of total N for microbial biomass N, suggesting warming impacts not only the pool sizes but also its distribution. These changes, especially the enhanced N use efficiency, may be conducive for a continued supply of organic inputs, thus favouring long-term N retention and C accumulation in soils, leading to negative feedbacks of terrestrial ecosystems to climate warming. Such negative feedbacks could however be adversely affected by management practices such as grazing. Thus, land use management may considerably influence response to global warming.

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