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Soil microbial responses to experimental warming and clipping in a tallgrass prairie

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Abstract

Global surface temperature is predicted to increase by 1.4–5.8 °C by the end of this century. However, the impacts of this projected warming on soil C balance and the C budget of terrestrial ecosystems are not clear. One major source of uncertainty stems from warming effects on soil microbes, which exert a dominant influence on the net C balance of terrestrial ecosystems by controlling organic matter decomposition and plant nutrient availability. We, therefore, conducted an experiment in a tallgrass prairie ecosystem at the Great Plain Apiaries (near Norman, OK) to study soil microbial responses to temperature elevation of about 2 °C through artificial heating in clipped and unclipped field plots. While warming did not induce significant changes in net N mineralization, soil microbial biomass and respiration rate, it tended to reduce extractable inorganic N during the second and third warming years, likely through increasing plant uptake. In addition, microbial substrate utilization patterns and the profiles of microbial phospholipid fatty acids (PLFAs) showed that warming caused a shift in the soil microbial community structure in unclipped subplots, leading to the relative dominance of fungi as evidenced by the increased ratio of fungal to bacterial PLFAs. However, no warming effect on soil microbial community structure was found in clipped subplots where a similar scale of temperature increase occurred. Clipping also significantly reduced soil microbial biomass and respiration rate in both warmed and unwarmed plots. These results indicated that warming-led enhancement of plant growth rather than the temperature increase itself may primarily regulate soil microbial response. Our observations show that warming may increase the relative contribution of fungi to the soil microbial community, suggesting that shifts in the microbial community structure may constitute a major mechanism underlying warming acclimatization of soil respiration.

Keywords: bacteria, experimental warming, fungi, microbial activity, microbial biomass, microbial community, soil microbes, tallgrass prairie, temperature acclimatization

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Introduction

The global surface temperature has increased in the past 50 years by about 0.6 °C and is recently predicted to increase 1.4–5.8 °C within the this century (IPCC, 2001). Many field experiments have been conducted to investigate the effects of global warming on terrestrial ecosystems and potential ecosystem feedbacks (Harte & Shaw, 1995; Saleska *et al*., 1999, 2002; Giardina & Ryan, 2000; Shaver *et al*., 2000; Luo *et al*., 2001; Melillo *et al*., 2002; Liski *et al*., 2003; also see review by Rustad *et al*., 2001). Most of those experiments have mainly focused on plant community structure (Harte & Shaw, 1995), primary productivity (Chapin *et al*., 1995), exchange of greenhouse gases (Peterjohn *et al*., 1994), nutrient cycling (Liski *et al*., 2003), soil or ecosystem respiration (Luo *et al*., 2001), and soil carbon (C) dynamics (Hobbie & Chapin, 1998; Oechel *et al*., 2000; Melillo *et al*., 2002; Liski *et al*., 2003). A few studies have been specifically devoted to the potential impacts of elevated temperature on soil microbial properties (Rillig *et al*., 2002;

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Staddon *et al*., 2003), in particular the microbial community structure *in situ* (Panikov, 1999; Tscherko *et al*., 2001). A further understanding of microbial response to warming is of critical significance in predicting terrestrial ecosystem feedbacks and their C budget in a warmer climate. Soil microorganisms exert a dominant influence on the net C balance of terrestrial ecosystems by controlling soil organic matter (SOM) decomposition and plant nutrient availability (Paul & Clark, 1996; Liski *et al*., 2003). Any changes in soil microbial activities and community structure may thus affect the nutrient availability, plant growth and the C budget of terrestrial ecosystems. Soil contains nearly twice as much C as the atmosphere (~735 Gt C), and makes up about 65% of the terrestrial ecosystem C (~2060 Gt C) (Schlesinger, 1996). Therefore, the potential switch of the terrestrial biosphere from its current role as a C sink (Schimel *et al*., 2001) to a C source is critically dependent upon the long-term impacts of global warming on soil microbes (Melillo *et al*., 2002).

Climate, soil physical and chemical properties, vegetation, and substrate quantity and quality are the principal factors affecting the soil microbes (Gholz *et al*., 2000). Atmospheric warming can directly alter soil environments, particularly soil temperature and moisture (Saleska *et al*., 1999; Wan *et al*., 2002b). Alterations in temperature and moisture will induce changes in physiology and growth of some specific groups within soil microbial communities (Panikov, 1999; Avrahami *et al*., 2003; Fierer *et al*., 2003). Compared with bacteria, fungi are more resistant to water stress induced by warming (Holland & Coleman, 1987), as Gram-negative bacteria predominate in the soil at high water regimes (Fenchel *et al*., 1998). Auge (2001) reviewed previous studies on water stress on mycorrhizae and found that drought often increased root colonization of mycorrhizal fungi. In addition, warming has been reported to alter plant community composition (Harte & Shaw, 1995; Grime *et al*., 2000; Sturm *et al*., 2001; Weltzin *et al*., 2003) and growth rate (Luo *et al*., 2001; Melillo *et al*., 2002). These alterations will have profound impacts on the quality and quantity of organic substrates (Saleska *et al*., 2002), which in turn affect the community structure and activities of soil microorganisms. Moreover, warming generally stimulates plant growth in temperate areas (Shaver *et al*., 2000). This stimulation may cause nutrient limitation to soil microbes, intensifying the competition for nutrients between plant and soil microbes. Nutrient limitation, in turn, may induce changes in soil microbial communities. Therefore, it is expected that global warming may influence the soil microbial community and activities, but convincing data are still scarce.

Land management practices such as hay harvest may significantly influence the direct impacts of warming on soil environment and soil microbial responses to warming (Shaver *et al*., 2000). Aboveground biomass removal can significantly reduce C inputs to soil and lead to significant N loss, resulting in substrate limitation to microbes (Wan & Luo, 2003). Also, hay harvest, mowing or animal grazing can significantly alter aboveground community composition (Knapp *et al*., 2002). This biomass removal may offset the effect of warming on plant community structure, thereby masking the warming effects on soil microbial properties. In addition, biomass removal can alter energy the budget and water fluxes through modifying the vegetation coverage and albedo of the earth's surface (Walker *et al*., 1999). Reduced vegetation coverage through hay harvest, mowing or grazing may change the boundary layer near the soil surface, increase energy absorbed and emitted by the soil, and amplify the diurnal soil–temperature range (Wan *et al*., 2002b). Finally, plant removal can have opposite effects on evaporation and transpiration, resulting in an unpredictable net effect on soil moisture (Dahlgren & Driscoll, 1994). Therefore, climatic warming and land-use/cover change may interactively affect soil microbes under future climatic conditions.

This study was designed to characterize soil microbial responses to experimental warming under two management regimes (i.e., clipped and unclipped treatments). It is a part of a comprehensive research project in a tallgrass prairie ecosystem in the US Great Plains (Luo *et al*., 2001, Wan *et al*., 2002a, b). The clipping treatment was designed to mimic the hay harvest, a major land-use practice in many types of grassland, and was expected to enhance warming effects on microbes. The specific objectives were to (1) quantify impacts of experimental warming on microbial biomass and activities in clipped and unclipped plots; and (2) identify changes in the microbial community structure, especially the relative contribution of fungi and bacteria to total soil microbial biomass.

Materials and methods

Site description

The experimental site is located at the Great Plain Apiaries (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 20 years. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium*, *Sorghastrum nutans, and Eragrostis* spp.), and C_3 forbs (*Ambrosia psilostachyia* and *Xanthocephalum*

texanum). This grassland is temporally very dynamic with C_3 plants being dominant in the winter and spring, and C_4 being species dominant in the summer and early fall. Warming may alter plant-growing period, resulting in changes in the grass community dynamics. The mean annual temperature is 16.3 °C with January being the coldest month (3.3 °C) and July the warmest (28.2 °C). The mean annual precipitation is 914 mm. The soil is part of the Nash–Lucien complex, which is characterized as having a low permeability rate, high available water capacity and a deep, moderately penetrable root zone (USDA Soil Conservation Service and Oklahoma Agricultural Experiment Station, 1963).

Experimental design

The field experiment used a blocked split-plot design with warming as the main factor and clipping and unclipping as subplot treatments. There were five pairs of 2 m × 2 m plots (each pair formed a block). In each pair, one plot had been warmed continuously with infrared heaters since November 1999 and the other was the unwarmed control. In each warmed plot, a single 165 cm × 15 cm infrared heater (Kalglo Electronics Inc., Bethlehem, PA, USA) was suspended 1.5 m above the ground. In the unwarmed control plot, a 'dummy' heater of the same shape and size as the infrared heater was suspended 1.5 m high in order to simulate the shading effects of the heater. For each paired plot, the distance between the control and the warmed plot was approximately 5 m in order to avoid heating the control plot by infrared heater. The distances between the individual sets of paired plots varied from 20 to 60 m.

Each 2 m × 2 m plot was divided into four 1 m × 1 m subplots. Two diagonal subplots in each plot were clipped 10 cm above the ground in November 1999, July 2000, July 2001, and July 2002, with the other two subplots being the unclipped controls. Clipping removed approximately 85% of the aboveground biomass (Luo *et al*., 2001). After each clipping, plants were allowed to grow until the next clipping. Experimental warming significantly increased both the daily mean air temperature (to 1.2 °C) and daily mean soil temperatures (to 1.8 and 2.7 °C without and with clipping, respectively) (Wan *et al*., 2002b). Air and soil temperature and soil moisture over the experimental duration were continuously recorded in the field, which has recently been published (Wan *et al*., 2002b). The four treatments in the experiment were (1) unclipped unwarmed (UC), (2) unclipped warmed (UW), (3) clipped unwarmed (CC), and (4) clipped warmed (CW).

Soil sampling

Soil samples were collected from the topsoil (0–15 cm) of all the subplots in September 2001, and in May and September of 2002. Four cores (2.54 cm diameter × 15 cm deep) were taken at each subplot. The four soil cores from each subplot were mixed to get one composite sample and delivered immediately to North Carolina State University for laboratory analyses. Each composite sample was passed through a sieve (4 mm diameter), and any visible living plant material (e.g. roots) was manually removed from the sieved soil. Two subsamples of the sieved soil from each composite sample were obtained, one was kept in the refrigerator at 4 °C for routine biological analyses and the other at −20 °C, for phospholipid fatty acids (PLFAs) analysis.

Determination of soil microbial biomass and activity, extractable N and net N mineralization

Soil microbial biomass C (MBC) and N (MBN) were determined using the fumigation–extraction method (48 h fumigation, Vance *et al*., 1987). Soil extractable organic C in the K_2SO_4 extracts before and after the fumigation was quantified using a total C analyzer (Shimadzu TOC-5050A, Shimadzu Co., Kyoto, Japan). Soluble N (NH_4-N, NO_3-N and some organic N) in the extracts of nonfumigated and fumigated soils was measured on a flow injection analyzer (Lachat Quickchem Systems, Milwaukee, WI, USA) after alkaline persulfate digestion (Cabrera & Beare, 1993). The differences in organic C and inorganic N between the fumigated and nonfumigated soils were assumed to be released from lysed soil microbes. The released C and N were converted to MBC and MBN, respectively, using k_{ec} – 0.33 (Sparling & West, 1988) and k_{en} – 0.45 (Jenkinson, 1988). Potential mineralizable N was quantified following soil incubation for 4 weeks (23 ± 2 °C in the dark). Extractable inorganic N (NH_4-N and NO_3-N) in unincubated controls and incubated soils were determined. Net N mineralization was estimated by the difference in extractable inorganic N between unincubated and incubated soil subsamples.

Soil microbial activity (i.e., the microbial respiration rate excluding root respiration) was estimated by determining CO_2 production during a 2-week incubation period. Respired CO_2 was captured in 5.0 mL of 0.5 M NaOH contained in a beaker suspended inside each Mason jar (Hu & van Bruggen, 1997). The NaOH solution was removed and titrated to determine the amount of CO_2 evolved. The soil microbial respiration was expressed as mg CO_2 kg^{-1} day^{-1}. Unless otherwise noted, all the results were calculated using oven-dried soil (105 °C, 24 h).

Substrate utilization patterns

Substrate utilization patterns of the soil microbial community were examined using BIOLOG microplates. Subsamples of moist soil (5.0 g dry wt equivalent) collected in September 2001 and 2002 were put in 50 mL autoclaved DI water and shaken for 30 min. The suspensions were diluted with sterile Ringer solution (final dilution 10^{-3}). Each well of BIOLOG EcoPlate (Biolog Inc., Hayward, CA, USA) was inoculated with 125 μL of the diluted soil extracts and incubated at 25 °C in the dark. The color formation at 590 nm was measured every 24 h for 7 days using an automatic plate reader (Bio-Tek Instrument Inc., Winooski, VT, USA). The data were corrected by subtracting the absorbency of the control well from the wells containing substrates. In order to minimize the effects of different inoculation densities, data from the 72 h reading were normalized through dividing the absorbency of each well by the average absorbency for the whole plate (average well color development, AWCD) (Buyer & Drinkwater, 1997).

PLFAs of soil microbes

PLFAs were extracted and quantified from 5.0 g freeze-dried soils collected in September 2001 and 2002, using a procedure previously described by Bossio *et al*. (1998). The separation and identification of extracted PLFAs were carried out according to the standard protocol of the Sherlock Microbial Identification System $V_{3.1}$ (MIDI, 1999) and a Gas Chromatograph (Hewlett Packard 5890A, Hewlett-Packpard Co., Avondale, PA, USA). Fatty acid nomenclature used in the present research was as that defined by Bossio *et al*. (1998). The following fatty acids i14 : 0, i15 : 1c, i15 : 0, a15 : 0, i16 : 1c, i16 : 0, 16 : 1ω7c, i17 : 0, 17 : 1ω6c, a17 : 0, 17 : 0cy, 18 : 1ω7c, 18 : 1ω5c and 19 : 0cy were chosen to represent the PLFAs of the bacterial group (Federle *et al*., 1986; Tunlid *et al*., 1989; Frostegård *et al*., 1993; Frostegård & Bååth, 1996). Also three fatty acids (16 : 1ω5c, 18 : 2ω6.9c and 18 : 1ω9c) were used to represent the fungal group (Federle *et al*., 1986; Olsson *et al*., 1998; Frostegård & Bååth, 1996; Mikola & Setälä, 1999).

Data from the PLFAs was presented as the percentage of the total PLFAs detected within a sample. Total percentages of PLFAs identified for each microbial group were calculated to represent their relative contributions to the total microbial biomass. In addition, the ratio of signature fungal and bacterial fatty acids (fung/bact) was also included in the data analysis. This ratio has often been used as the indicator of the change in the soil microbial community structure (Bardgett *et al*., 1998; Olsson *et al*., 1999; Zelles, 1999; Fierer *et al*., 2002).

Statistical analysis

Analysis of variance (ANOVA) for a blocked split-plot design was used to detect the effects of warming, clipping and their interactions. Multiple comparisons were also performed to permit separating of effect means using the least significant difference test at the significance level of $P = 0.05$. In addition, substrate utilization patterns (BIOLOG data) and PLFA profiles were analyzed using principal component analysis (PCA) to identify the differences in soil microbial community structure induced by warming and clipping. For ANOVA and multiple comparison statistical analyses, the SAS V. 6.11 (SAS Systems, Cary, NC, USA) software package was used. For PCA, the SPSS V.10.0 (SPSS Inc., Chicago, IL, USA) software package was used.

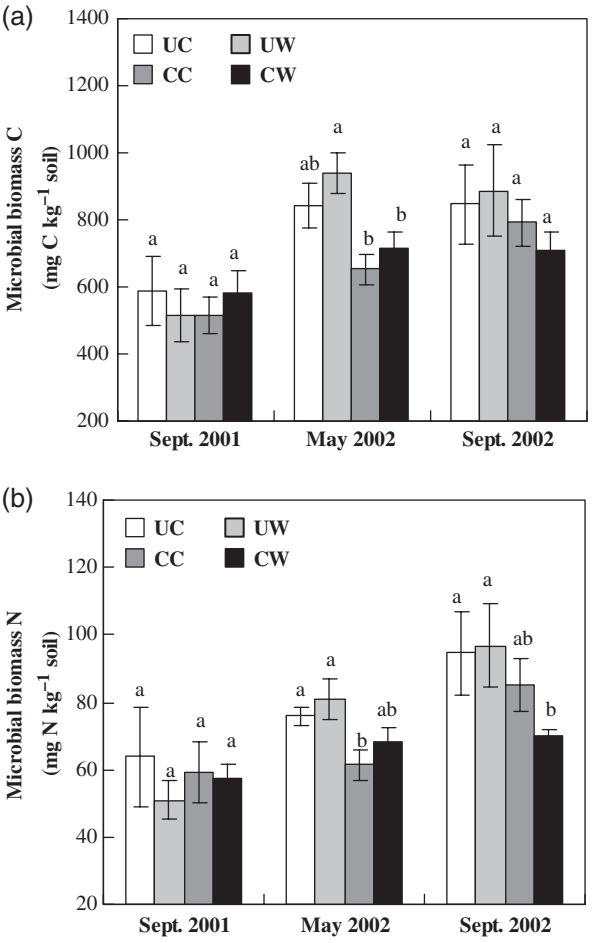

Fig. 1 Soil microbial biomass C (a) and N (b) in unclipped control (UC), unclipped warmed (UW), clipped control (CC) and clipped warmed (CW) subplots. Values are means ± 1 SE with the sample size $n = 3$ in 2001 and $n = 5$ in 2002. Values followed by a different letter are significantly different within each sampling date ($P = 0.05$).

Results

Soil MBC and MBN

MBC and MBN showed a similar response to the treatments among the sampling dates (Fig. 1). Warming did not cause significant differences in microbial biomass in either the unclipped or clipped subplots, but clipping significantly reduced MBC and MBN under both the unwarmed control and the warmed treatments in 2002 (Table 1). In May 2002, clipping reduced MBC by 23% and 24%, and MBN by 19% and 16% in unwarmed and warmed plots, respectively. No significant effects of warming × clipping interactions were found either in 2001 or 2002 (Table 1).

Soil microbial respiration and metabolic potential

Microbial activity, measured as respiration rate, was significantly higher in unclipped than in clipped subplots in both warmed and umwarmed plots in September 2001 and May 2002, but not in September 2002 (Fig. 2a, Table 1). Clipping reduced the respiration rate by 26% and 18% in unwarmed and warmed plots in September 2001, and 24% and 8% in unwarmed and warmed plots, respectively, in May 2002. However, there were no significant effects of warming and warming × clipping interaction on the respiration rate during the 2 years.

Metabolic potential of soil microbes, measured as the average absorbency for the whole BIOLOG ECO plate (AWCD), showed significant warming × clipping interactions in September 2002 (Fig. 2b, Table 1). Warming reduced the metabolic potential by 26% and 29% in unclipped subplots in September 2001 and 2002, respectively, but did not have significant effect in clipped subplots. In addition, clipping reduced metabolic potential by 34% and 29% in unwarmed subplots in September 2001 and 2002, respectively, but did not have significant effects in warmed subplots.

Extractable inorganic N and net N mineralization

Warming caused a consistent decrease in soil extractable inorganic N in unclipped subplots during the 2

Table 1 Significance values and degrees of freedom of ANOVA analysis

Source	df	MBC	MBN	RR	MP	ETN	Min-N	Fungi	Bac	F/B
September 2001 sampling										
Block	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Warming	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Residuals 1	2									
Clipping	1	ns	ns	*	ns	ns	ns	ns	ns	ns
Warming × clipping	1	ns	ns	ns	ns	ns	ns	*	ns	*
Residuals 2	4									
May 2002 sampling										
Block	4	ns	ns	ns	–	ns	ns	–	–	–
Warming	1	ns	ns	ns	–	ns	ns	–	–	–
Residuals 1	4				–					
Clipping	1	*	*	*	–	ns	ns	–	–	–
Warming × clipping	1	ns	ns	ns	–	ns	ns	–	–	–
Residuals 2	8									
September 2002 sampling										
Block	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
Warming	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Residuals 1	4									
Clipping	1	ns	*	ns	ns	ns	ns	ns	ns	*
Warming × clipping	1	ns	ns	ns	*	ns	ns	ns	**	*
Residuals 2	8									

The sample sizes were 3 and 5 in 2001 and 2002, respectively.
RR, microbial respiration rate; MP, microbial metabolic potential; ETN, extractable inorganic N; Min-N, net mineralization N; fungi, contribution of fungal PLFAs; Bac, contribution of bacterial PLFAs; F/B, the ratio of fungal to bacterial PLFAs; PLFA, phospholipid fatty acid; MBC, microbial biomass C; MBN, microbial biomass N.
ns, not significant at the level of $P = 0.05$.
*Significant at the level of $P = 0.05$.
**Significant at the level of $P = 0.01$.
–, data are not available.

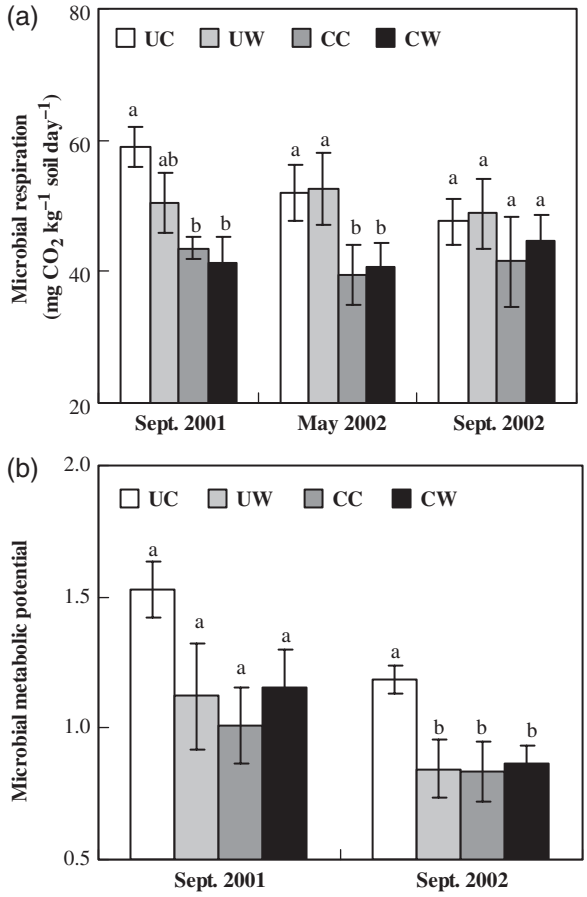

Fig. 2 Soil microbial respiration rate (a) and substrate metabolic potential (b) as influenced by warming and clipping. See Fig. 1 for abbreviations. Values are means ± 1 SE with the sample size $n = 3$ in 2001 and $n = 5$ in 2002. Values followed by a different letter are significantly different within each sampling date ($P = 0.05$).

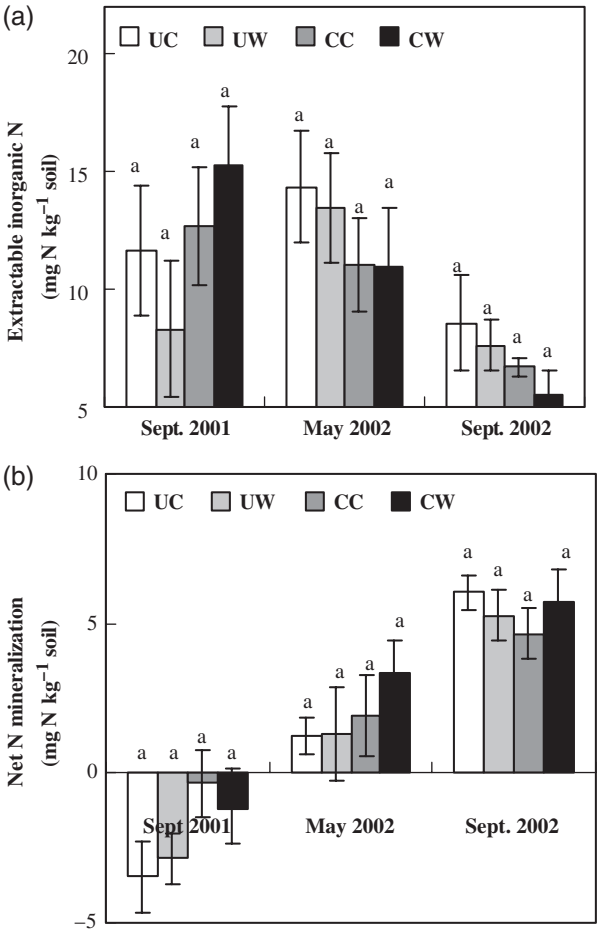

Fig. 3 Soil extractable inorganic N (a) and net N mineralization (b). See Fig. 1 for abbreviations. Values are means ± 1 SE with the sample size $n = 3$ in 2001 and $n = 5$ in 2002. Values followed by a different letter are significantly different within each sampling date ($P = 0.05$).

years, although the effects either of warming, or clipping, or their interaction were not at significant levels (Fig. 3a, Table 1). Warming reduced extractable inorganic N by 29%, 6% and 11% in unclipped plots in September 2001, May and September 2002, respectively, but did not induce consistent effects in clipped subplots. However, in the incubation experiments with the absence of plants, no consistent changes in net N mineralization were found in either unclipped or clipped subplots (Fig. 3b).

Relative contributions of fungi and bacteria to total microbial biomass

There were significant effects of warming × clipping interaction on the relative contribution of specific microbial groups to the total microbial community, measured as the PLFA ratio of fungi to bacteria (Fig. 4c, Table 1). Warming without clipping enhanced signature fungal PLFA by 28% and 13% (Fig. 4a), and reduced signature bacterial PLFA by 13% and 6% (Fig. 4b) in September 2001 and 2002, respectively. Consequently, warming resulted in an increase in the ratio of fungi to bacteria by 63% and 22% in the two sampling dates, respectively (Fig. 4c). Clipping impacted the relative contribution of fungal and bacterial fatty acids only in the warming plots (Fig. 4a and b), reducing fungal contribution by 32% and 16%, but increasing bacterial contribution by 6% and 7% in September 2001 and 2002, respectively. Consequently, clipping reduced the ratio of fungi to bacteria by 36% and 21% in the two sampling dates, respectively (Fig. 4c).

The soil microbial community structure

Analysis of the BIOLOG data using PCA showed that warming-induced changes in substrate utilization

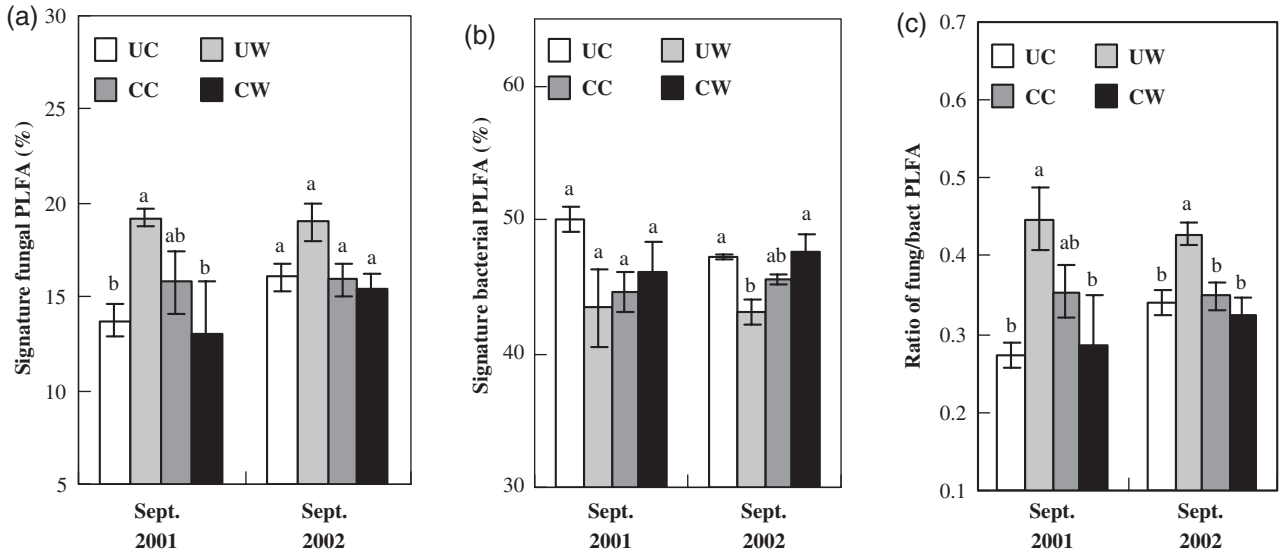

Fig. 4 Percentages of signature fungal (a) and bacterial phospholipid fatty acids (PLFAs) (b) to the total biomass PLFAs, and ratio of fungal to bacterial PLFAs (c) as influenced by warming and clipping. See Fig. 1 for abbreviations. Values are means ± 1 SE with the sample size $n = 3$ in 2001 and $n = 5$ in 2002. Values followed by a different letter are significantly different within each sampling date ($P = 0.05$).

patterns of microbial communities in unclipped subplots (Fig. 5), but not in clipped subplots (data not shown). The first two principal components accounted for 62% and 59% of the total variance in September 2001 and 2002, respectively ($PC_1 = 36\%$ and $PC_2 = 26\%$ in 2001, $PC_1 = 41\%$ and $PC_2 = 18\%$ in 2002). PC_2 clearly separated the microbial communities in the warming from those in the control plots without clipping.

The PLFA fingerprints showed similar results from the analysis of BIOLOG data, indicating that warming caused significant dissimilarities in the soil microbial community structure in unclipped subplots (Fig. 6), but not in clipped subplots (data not shown). The first two principal components explained 87% and 65% of total variance in September 2001 and 2002, respectively ($PC_1 = 66\%$ and $PC_2 = 21\%$ in 2001, $PC_1 = 45\%$ and $PC_2 = 20\%$ in 2002). The PLFA profiles of the control and warming plots were distinctly separated by PC_1 in 2001 and by PC_2 in 2002.

Discussion

Climatic warming and the soil microbial community structure

Microbial responses to changes in C inputs and temperature and moisture regimes caused by climatic warming may have the potential to significantly impact terrestrial ecosystem productivity and C balance. Climatic warming studies have so far been largely focused on quantifying the alterations of microbial processes (e.g., respiration and N mineralization) (Jonasson *et al*., 1999) and little is known about potential changes in the microbial community structure (Ruess *et al*., 1999). This negligence might be built on the assumption that microbes are physiologically versatile and can adjust to soil temperature changes of 1–2 °C induced by air warming without altering the community composition. Results from our experiment showed that the microbial community structure had changed significantly following the 3 years of experimental warming in unclipped fields in this tallgrass prairie (Figs 5 and 6). In addition, warming without clipping also significantly reduced microbial metabolic potential (as determined by using BIOLOG Eco-plates), although it did not significantly alter MBC and respiration rate. The reduced microbial metabolic potential may also indicate a shift of microbial community structure. Because fungi have lower growth rates than bacteria on BIOLOG plates, higher fungal dominance may have lower color development rate, resulting in lower absorbency. Aboveground biomass removal via clipping masked the warming effects on soil microbial communities through significantly reducing soil microbial biomass and activities (Figs 1 and 2). Also, our *in vitro* measurements of metabolic potential and respiration rates showed similar patterns with *in situ* soil respiration (Luo *et al*., 2001). These results, plus the significant higher ratio of fungal to bacterial PLFA (Fig. 4), indicate that warming-induced shifts in the soil microbial community structure in the unclipped grassland.

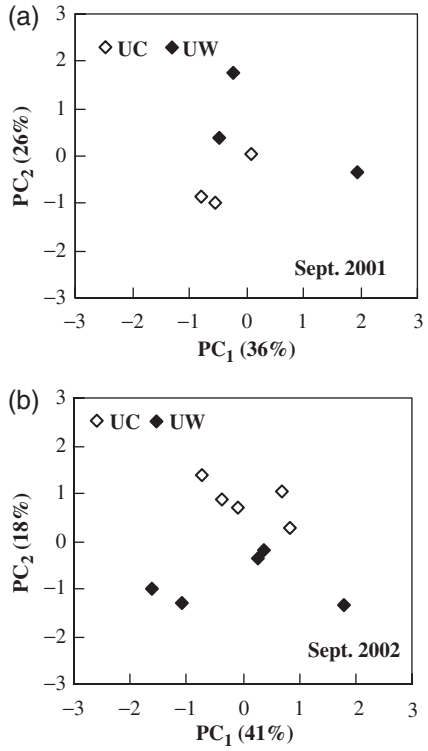

Fig. 5 Principal component analysis (PCA) plots of BIOLOG data in September 2001 (a) and September 2002 (b) in unclipped control (UC) and unclipped warmed (UW) subplots. PC_1 and PC_2 explain 36% and 26% in 2001, and 41% and 18% in 2002, respectively. The sample size is 3 and 5 in 2001 and 2002, respectively.

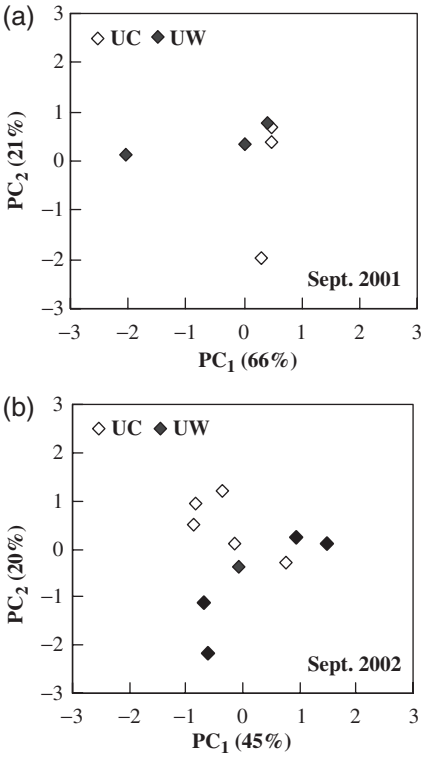

Fig. 6 Principal component analysis (PCA) plots of all phospholipid fatty acids signatures detected in September 2001 (a) and September 2002 (b) in unclipped control (UC) and unclipped warmed (UW) subplots. PC_1 and PC_2 explain 66% and 21% in 2001, and 45% and 20% in 2002, respectively. The sample size is 3 and 5 in 2001 and 2002, respectively.

Various mechanisms may have contributed to the observed shift of the microbial community structure showing an increasing contribution of fungi. Firstly, warming-induced increases in plant growth and decrease in soil N availability may benefit fungi over bacteria. Experimental air warming increased plant productivity by more than 13–30% in the present tallgrass prairie in the second and third year (S. Wan *et al.*, unpublished results). Experimental soil warming also resulted in significant increases in stem–wood growth of trees on heated plots relative to the controls after a 5 years heating period in a mid-latitude hardwood forest (Jarvis & Linder, 2000; Melillo *et al.*, 2002). Warming enhancement of plant growth, on the one hand, may significantly increase C inputs to the soil as plant litter, root exudation and fine root turnover increase, benefiting soil microbial growth. On the other hand, it may accelerate nutrient transfer to plants and reduce N availability in soil, intensifying nutrient competition between plant and soil microbes and resulting in nutrient limitation of microbes (Hu *et al.*, 2001). The decreased N availability was evident by the relatively lower extractable inorganic N (Fig. 3a). Changes in the relative availability of C and N may have differential effects on soil microbes. Fungi can relocate nutrients because of their filamentous nature and recycle limited nutrients (especially N) via cytoplasm translocation. This feature may give fungi competitive advantages over bacteria for exploitation of available C and nutrients (Hu & van Bruggen, 1997). Consequently, an increase in C inputs and a decrease in N availability to soil microbes likely contribute to the enhancement of fungal dominance in the whole soil microbial community (Bardgett *et al.*, 1999; Smith *et al.*, 2003).

Secondly, soil fungi likely benefit from the changes in the plant community composition induced by the elevated temperature. Three years warming significantly enhanced aboveground biomass of C_4 plants by about 37–57% but did not cause any change in the aboveground biomass of C_3 plants (S. Wan *et al.*, unpublished results). This difference in the stimulated biomass growth between C_3 and C_4 plants resulted in a decrease in the quality of C inputs to the soil, because

C_4 plants have higher C : N ratio as compared with C_3 plants. Sturm *et al*. (2001) also reported a widespread increase in shrub abundance induced by the warming in the Alaskan Arctic over the past 50 years, leading to an increase in quantity and a decrease in quality of plant litter inputs to the soil. The increased plant organic inputs with high C : N ratios likely benefit fungi growth over bacteria in soils. The fact that warming did not have significant effects on the microbial community in clipped plots re-enforces the role of enhanced litter inputs (rather than the temperature itself) in mediating microbial community response to temperature elevation. In those plots, warming did effectively promote plant growth and increase soil temperature, but removal of up to 80% of plant litter (Wan *et al*., 2002b), combined with depletion of labile C following warming (Luo *et al*., 2001; Melillo *et al*., 2002), likely led to energy and nutrient limitation for the soil microbes and constrained microbial responses.

Thirdly, warming-induced changes in plant growth, nutrient and moisture availability may significantly increase mycorrhizal colonization of many grasses. Auge (2001) reviewed the effects of soil water stress on mycorrhizae and found that soil drought mostly increased root colonization of mycorrhizal fungi. Rillig *et al*. (2002) have recently showed that compared with its unwarmed control, air warming increased hyphal length of arbuscular mycorrhizal fungi and mycorrhizal root colonization in a grassland. Staddon *et al*. (2003) also reported both winter warming and summer drought increased the proportion of root length colonized and reduced the density of external mycorrhizal hyphal. They summarized that much of the response of mycorrhizal fungi to climate change could be attributed to climate-induced changes in the vegetation, especially plant species relative abundance.

Finally, warming may enhance fungal contribution to the microbial community through altering soil temperature and moisture conditions. Luo *et al*. (2001) demonstrated that the present experimental warming increased the mean soil temperature by 2.0 °C and decreased soil moisture by 6.4% in unclipped subplots. These stresses of soil temperature and moisture likely facilitate fungi to survive better, because soil fungi rely on more aerobic conditions (Šantrůčková *et al*., 2003) and are more tolerant to higher soil temperature and drying because of their filamentous nature (Holland & Coleman, 1987). However, these effects may be of less importance as compared with plant growth effects, because greater changes in soil temperature and moisture induced by air warming in clipped plots did not lead to corresponding changes in the microbial community, which is against our expectation. Warming-induced higher soil temperature (2.7 °C on average) and lower water availability (a decrease in soil moisture more than 11%) in clipped than in unclipped subplots (Wan *et al*., 2002b). If warming-induced changes in soil temperature and moisture were dominantly regulating the composition of microbial community, we should have observed a significant change in microbial community structure following warming in the clipped plots.

Implications of warming-induced alteration in microbial composition and activities

Warming acclimatization – decreases in the temperature sensitivity of soil respiration as the warming treatment progresses – has been recently reported for soil respiration in tallgrass prairies (Fitter *et al*., 1998; Luo *et al*., 2001) and forest soils (Bryla *et al*., 2001; Melillo *et al*., 2002), but the underlying mechanisms are not altogether understood (Burton & Pregitzer, 2003). Because substrate quality and quantity are the main factors regulating the responses of soil respiration to temperature (Cheng *et al*., 1996; Giardina & Ryan, 2000), most reports suggest that this acclimatization is because of the depletion of labile C (McHale *et al*., 1998; Rustad & Fernandez, 1998; Oechel *et al*., 2000; Luo *et al*., 2001). Our data indicate that warming acclimatization of soil respiration also likely results from the shift in the soil microbial community structure. The increase in substrate quantity and the decrease in C quality and N availability induced by experimental warming benefited soil fungal growth over bacterial growth resulting in an increase in fungal contribution to the total soil microbial community (Figs 4–6). Other results from laboratory incubation experiments also have shown that shifts in the microbial community structure occurred under elevated temperature conditions (Zogg *et al*., 1997). Dalias *et al*. (2001) reported that in an incubation experiment, initial temperature responses of microbial communities were different from those observed after a long period of incubation, suggesting a change in the community structure. An increase in fungal contribution may shift the decomposition process from bacterial- to fungal-based channels, which may have some long-term implications (Moore & Hunt, 1988). Fungi have greater C assimilation efficiency as compared with bacteria (Adu & Oades, 1978; Sakamoto & Oba, 1994), leading to relatively lower CO_2 release. In addition, compared with bacteria, fungal cell walls contain more polymers such as melanin and chitin, which can persist in soil for years and account for significant pool of SOM (Bailey *et al*., 2002). Hence, the greater C utilization efficiency in fungi may lead to more organic C being transformed into more recalcitrant humic materials. Furthermore, fungal hyphae

have long been recognized to enmesh microaggregates (<250 μm) into macroaggregates (>250 μm) (Jastrow *et al*., 1998; Bossuyt *et al*., 2001), which contributes to organic C protection through facilitating the formation and stabilization of soil aggregates. Warming-induced changes in the microbial community structure may, therefore, bear important implications for soil C dynamics in a warmer world and deserve further attention.

Changes in the microbial community structure may partially contribute to the observed differential response of soil respiration and N mineralization to experimental warming. In our current experiment, net N mineralization increased significantly in the first year, although soil respiration (including microbial and root respiration) increased only in a few weeks (Luo *et al*., 2001). Reduced extractable N under warming in the second and third years (Fig. 3a) is likely because of increased plant uptake rather than reduced N mineralization, as net N mineralization did not change in the incubation experiments when plants were absent (Fig. 3b). A similar phenomenon was observed in a hardwood forest at Harvard Forest in central Massachusetts (Melillo *et al*., 2002). In that experiment, stimulation of N mineralization continued into the eighth warming year although the enhancement of soil respiration essentially ceased by the seventh warming year (Melillo *et al*., 2002). Because fungi require relatively low N per unit of biomass, a fungal-dominant community might release relatively more N for plant growth. Enhanced mycorrhizal colonization in other experiments (Rillig *et al*., 2002; Staddon *et al*., 2003) also suggests that plants may improve their nutrient acquisition through mycorrhizally mediated nutrient uptake under a warmer climate, particularly in forest systems.

In summary, results from our experiment indicate that 3-year continuous warming without clipping has led to significant changes in the microbial community structure, resulting in an increase in fungal to bacterial ratios. However, clipping significantly masked the warming effects on soil microbes. These findings suggest that belowground microbes may exert important feedback controls over the long-term ecosystem response or feedbacks to climatic warming and management regimes may impact these microbial processes.

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