

# Responses of soil respiration to elevated CO<sub>2</sub>, air warming, and changing soil water availability in a model old-field grassland

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## Abstract

Responses of soil respiration to atmospheric and climatic change will have profound impacts on ecosystem and global carbon (C) cycling in the future. This study was conducted to examine effects on soil respiration of the concurrent driving factors of elevated atmospheric CO<sub>2</sub> concentration, air warming, and changing precipitation in a constructed old-field grassland in eastern Tennessee, USA. Model ecosystems of seven old-field species were established in open-top chambers and treated with factorial combinations of ambient or elevated (+300 ppm) CO<sub>2</sub> concentration, ambient or elevated (+3 °C) air temperature, and high or low soil moisture content. During the 19-month experimental period from June 2003 to December 2004, higher CO<sub>2</sub> concentration and soil water availability significantly increased mean soil respiration by 35.8% and 15.7%, respectively. The effects of air warming on soil respiration varied seasonally from small reductions to significant increases to no response, and there was no significant main effect. In the wet side of elevated CO<sub>2</sub> chambers, air warming consistently caused increases in soil respiration, whereas in the other three combinations of CO<sub>2</sub> and water treatments, warming tended to decrease soil respiration over the growing season but increase it over the winter. There were no interactive effects on soil respiration among any two or three treatment factors irrespective of time period. Treatment-induced changes in soil temperature and moisture together explained 49%, 44%, and 56% of the seasonal variations of soil respiration responses to elevated CO<sub>2</sub>, air warming, and changing precipitation, respectively. Additional indirect effects of seasonal dynamics and responses of plant growth on C substrate supply were indicated. Given the importance of indirect effects of the forcing factors and plant community dynamics on soil temperature, moisture, and C substrate, soil respiration response to climatic warming should not be represented in models as a simple temperature response function, and a more mechanistic representation including vegetation dynamics and substrate supply is needed.

*Keywords:* air warming, atmospheric CO<sub>2</sub> concentration, old-field grassland, soil respiration, soil water availability, temperature sensitivity

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## Introduction

There are concurrent changes in the driving forces of global change (i.e., increasing atmospheric CO<sub>2</sub> concen-

tration, rising temperature, and changing precipitation). How soil respiration – the second largest carbon (C) flux between the atmosphere and terrestrial biomes – responds to the above drivers will have profound impacts on global C cycling, with consequent feedbacks to the atmospheric and climatic changes (Cox *et al.*, 2000; Raich *et al.*, 2002; Ryan & Law, 2005; Trumbore, 2006). Even though the main effects on soil respiration of elevated CO<sub>2</sub> (Luo *et al.*, 1996; Craine *et al.*, 2001),

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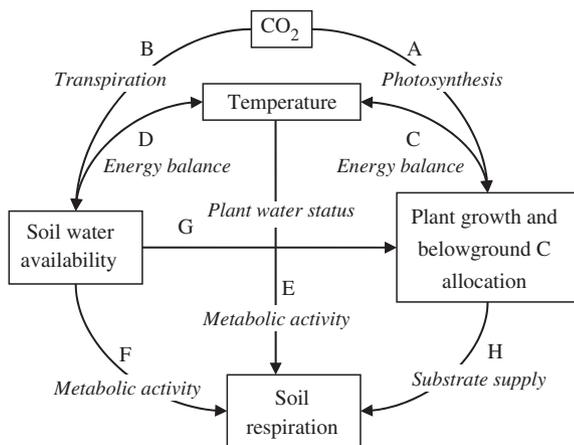
warming (Luo *et al.*, 2001; Rustad *et al.*, 2001), and changing precipitation or soil water availability (Davidson *et al.*, 1998, 2000a; Reichstein *et al.*, 2005; Borken *et al.*, 2006) have been well studied, it is still not clear how soil respiration responds to the concurrent changes in these drivers and whether and how they interact with each other to impact soil respiration, which hampers the projection of soil respiration and ecosystem/global C cycling (Norby & Luo, 2004). The scarcity of multifactor manipulative experiments and the complexity of disentangling multifactor effects (Norby & Luo, 2004; Pendall *et al.*, 2004) are primarily responsible for the limited understanding.

Soil temperature, soil moisture, and C substrate have long been identified as the controlling factors over soil respiration in terrestrial ecosystems (Raich & Tufekcioglu, 2000; Rustad *et al.*, 2000; Högberg *et al.*, 2001) and the fundamental parameters in predicting the responses of soil respiration to global change (Cox *et al.*, 2000; Reichstein *et al.*, 2005; Trumbore, 2006). To get a clear picture of the response of soil respiration, we developed a conceptual model to identify and distinguish between the complicated direct and indirect effects of simultaneous global change drivers on soil respiration (Fig. 1). Elevated CO<sub>2</sub> concentrations influence soil respiration indirectly by regulating plant growth, belowground C allocation and availability of substrate (Processes A and H) and soil water (Processes B and F). These indirect effects are usually positive. However, stimulated plant growth by CO<sub>2</sub> enrichment can increase plant cover and affect evaporation and energy balance at the soil surface, leading to lower soil temperatures (Process C). In addition, CO<sub>2</sub>-induced reductions in stomatal conductance and transpiration can increase soil water availability. Higher soil water content will dissipate more energy as latent heat and less as soil heat flux, lowering soil temperatures (Pro-

cess D). Lower soil temperatures as consequences of Processes C and D may negatively impact soil respiration, counteracting the positive effects of elevated CO<sub>2</sub> through Processes A and B. Higher temperatures can directly stimulate root and microbial activities and respiration (Process E) and indirectly impact soil respiration via changes in plant growth, belowground C allocation and substrate availability (Processes C and H), as well as soil water availability (Processes D and F). Soil water availability also has both direct (Process F) and indirect effects on soil respiration through regulation of soil temperature (Processes D and E) and plant growth and belowground C allocation (Processes G and H).

In addition to absolute changes in soil respiration, the temperature sensitivity of soil respiration is also of great concern in global change research. Temperature sensitivity of soil respiration is a critical index in quantifying and predicting the responses of ecosystem and global C cycling to climate change (Cox *et al.*, 2000; Kirschbaum, 2000; Reichstein *et al.*, 2003; Ryan & Law, 2005; Davidson & Janssens, 2006; Davidson *et al.*, 2006). The temperature acclimation of soil respiration receives substantial interest but still remains controversial (Lloyd & Taylor, 1994; Kirschbaum, 1995, 2000; Davidson *et al.*, 2000b, 2006; Giardina & Ryan, 2000; Davidson & Janssens, 2006). In addition, most research focuses on the impacts of global warming on the temperature sensitivity of soil respiration; little is known about whether elevated CO<sub>2</sub> (Liu *et al.*, 2006; Tingey *et al.*, 2006), changing precipitation or soil water availability (Reichstein *et al.*, 2005), and their possible interactions with warming affect the temperature sensitivity of soil respiration.

This study was conducted to examine the concurrent effects of elevated CO<sub>2</sub>, air warming, and changes in availability of soil water on soil respiration and its temperature sensitivity in a model old-field grassland ecosystem in eastern Tennessee, USA. Based on the direct and indirect effects of global change drivers in regulating soil respiration as described in the conceptual model (Fig. 1), the following hypotheses can be proposed: (1) Since elevated atmospheric CO<sub>2</sub> concentration stimulates plant growth and belowground C input, we hypothesize that elevated CO<sub>2</sub> will increase soil respiration in the old-field grassland by providing more C substrate for respiratory processes of plant roots and soil microbes. (2) Given the water limitation on plant and microbial activities, we hypothesize that greater soil moisture content will enhance soil respiration. (3) Because of the well-documented exponential relationships between respiratory rates and temperature, it is expected that air warming would stimulate soil respiration (Rustad *et al.*, 2001). However, warming-induced increases in evapotranspiration and reduction



**Fig. 1** Conceptual model of the direct and indirect effects on soil respiration of elevated atmospheric CO<sub>2</sub> concentration, air warming, and changing precipitation.

in soil water availability may negatively affect root and microbial activity and respiration. In addition, air warming has variable effects on plant production (Rustad *et al.*, 2001), belowground C allocation, and hence, root and microbial respiration with opposing positive and negative impacts on soil respiration. (4) As such, we hypothesize that elevated CO<sub>2</sub>, air warming, and changing precipitation will have complex and interactive effects on soil respiration mediated through changes in soil temperature and moisture, and aboveground and belowground plant activity.

## Methods

### Site description

The experiment was conducted at the Global Change Field Research Facility on the Oak Ridge National Environmental Research Park in Oak Ridge, Tennessee, USA (25°54'N; 84°21'W). The site was abandoned from agricultural use in 1943 and left fallow until 1964 when a managed fescue field was established. The soil, which is derived from floodplain alluvium deposited by the nearby Clinch River, is classified as Captina silt loam – fine-silty, siliceous, mesic typic fragiudult, well drained, and slightly acidic (Soil Conservation Service, 1967; Edwards & Norby, 1999). Precipitation is generally evenly distributed throughout the year with an annual mean of 1322 mm; the mean July maximum temperature is 31.2 °C, and the mean January minimum temperature is –2.7 °C.

### Plot construction and planting

The experimental plots were established during 2002. Five plots were laid out in each of three blocks. Trenches were cut 75 cm deep around each 4 m diameter plot and through the center on a north–south axis, thereby defining two 6.3 m<sup>2</sup> experimental units (split plots). The trenches were lined with insulating foam and 4 mil polyvinylchloride (PVC) film to provide a thermal and moisture barrier and backfilled with packed soil. In June 2002, vegetation was killed with an application of glyphosate herbicide. Dead plant biomass was extracted (to a depth of ~1 cm) to remove aboveground meristems along with some of the plant seed bank.

Plots were planted with seven plant species common to old-field communities in the southeastern United States and representing a variety of growth habits and functional groups. The species are: *Plantago lanceolata* L., an herbaceous, annual C<sub>3</sub> dicot; *Andropogon virginicus* L., a cespitose C<sub>4</sub> bunchgrass; *Festuca pratense* L. syn *F. elatior* L., a C<sub>3</sub> bunchgrass; *Dactylis glomerata* L., a C<sub>3</sub> bunchgrass; *Trifolium pratense* L., a C<sub>3</sub> legume; *Solidago canadensis*, a perennial forb; and *Lespedeza cuneata* (Dum.

Cours.) G. Don., a C<sub>3</sub> perennial N<sub>2</sub>-fixing shrub. Seedlings were established in a greenhouse and all seven species were transplanted into the plots in August 2002 in a predetermined grid such that seedlings were 18 cm apart and no individual neighbored a conspecific. Approximately 170 individuals were planted per plot. Plots were watered and weeded to ensure seedling establishment until treatment initiation in May 2003.

Two permanent (0.49 m<sup>2</sup>) subplots were established in each split plot to enable the collection of plant response data throughout the experiment. Each subplot contained one to three individuals of each of the seven planted species, and had identical initial species composition and layout in different chambers.

### Experimental infrastructure

The CO<sub>2</sub>, temperature, and soil moisture treatments were applied through the use of open-top chambers (OTCs) surrounding four plots in each of the three blocks (the fifth plot in each block was left unchambered). OTCs were constructed of aluminum frames (4 m in diameter, 2.2 m in height) covered with clear polyvinylchloride (PVC) panels; the double-walled panel on the lower half of each OTC was a plenum perforated on the inner wall with 2.5 cm holes (Rogers *et al.*, 1983). A fan pushed ambient air through an evaporative cooler and in-line heating coils and into the plenum. The heaters and coolers were regulated to maintain desired temperatures within the chambers as described by Norby *et al.* (1997). Pure CO<sub>2</sub> was introduced into the plenum at a constant rate 24 h day<sup>-1</sup> to achieve a daytime CO<sub>2</sub> enrichment of 300 ppm over ambient air. Whole plots received treatments of ambient or elevated [CO<sub>2</sub>] (ambient +300 ppm) in combination with ambient or elevated temperature (ambient +3 °C) in a randomized, complete-block design. An open-sided shelter was assembled over each OTC to exclude all precipitation. The 6 m × 5 m shelters were constructed of 6 mil PVC film stretched over 9 cm width pressed steel greenhouse bows attached to a steel frame. Each split plot within each whole plot was randomly assigned to one of two soil moisture treatments ('wet' or 'dry') created by differential irrigation. Rainwater was collected at the site in 10 000 L tanks and used to irrigate the plots with specified amounts of water. Hence, each 6.3 m<sup>2</sup> split plot represented a unique combination of soil moisture, [CO<sub>2</sub>], and temperature treatment within one of three blocks (*n* = 3).

### Treatment application and monitoring

Temperature and [CO<sub>2</sub>] treatments were initiated in April 2003 and maintained 24 h day<sup>-1</sup> since then. Atmospheric variables of air temperature and [CO<sub>2</sub>] were

continuously monitored. Mean air temperatures between May 13, 2003 and December 31, 2004 were  $15.9 \pm 0.1$  °C in ambient-temperature chambers and  $18.5 \pm 0.3$  °C in elevated temperature chambers; the error term is the standard deviation across the six chambers within a temperature regime over the 2-year period. The difference between chamber air temperature and outside air temperature averaged  $0.55 \pm 0.23$  and  $3.20 \pm 0.21$  °C in ambient and elevated-temperature chambers, respectively. The hourly averaged temperature differentials were within 0.5 °C of the mean 74% of the time in ambient-temperature chambers and 89% of the time in elevated-temperature chambers. CO<sub>2</sub> concentration within the chambers during daylight hours averaged  $395.6 \pm 2.8$  ppm in ambient [CO<sub>2</sub>] chambers and  $695.8 \pm 10.0$  ppm in elevated [CO<sub>2</sub>] chambers. The standard deviations represent the variation across the six chambers within a CO<sub>2</sub> treatment; the standard deviations of the hourly observations over 2 years were 29.2 and 71.5 ppm in ambient and elevated [CO<sub>2</sub>] chambers, respectively.

Irrigation treatments initiated in June 2003 provided different amounts of water each week based on long-term mean weekly precipitation records from the nearby Oak Ridge, Tennessee, weather station, and modified by  $\pm 50\%$  to create 'wet' and 'dry' irrigation treatments. Soil volumetric water content (VWC) within each plot was monitored using time-domain reflectometry (TDR): a 15 cm long TDR probe was installed vertically into the soil at each of two arbitrary locations 1.3 m apart near the center of each plot. Soil VWC was recorded three times weekly during the growing season (March–October), and twice weekly when plants were senescent (November–February). During the 2003 growing season, VWC differed little between wet and dry treatments (unpublished data); therefore, in September 2003, we modified our irrigation protocol to weekly additions of 2 mm (dry) and 25 mm (wet).

#### *Soil respiration measurement*

Two PVC soil collars (80 cm<sup>2</sup> in area and 5 cm in height) were permanently installed 2–3 cm into the soil in each split plot for the measurement of soil respiration. In order to exclude respiration from the aboveground parts of plants, living plants inside the collars were eradicated by hand once a week and the removed plant material was left inside the collars to die. Therefore, soil respiration we measured did not include aboveground respiration from living plants. Soil respiration was measured twice a month using a LI6200 infrared gas analyzer (Li-Cor Inc., Lincoln, NE, USA) with attached chamber between 10:00 and 15:00 hours local time. The LI6200 chamber was put on the PVC collar for 2–3 min

to measure soil respiration at each subplot and then moved to the next subplot. Soil temperature at the depth of 5 cm was also monitored at the time of soil respiration measurement.

#### *Aboveground biomass and fine-root production*

Live plants were clipped in August 2003 at 2 cm above the soil surface from a 0.75 m<sup>2</sup> subplot near the center of each split plot; plant material was oven dried at 65 °C for at least 2 days to determine total aboveground biomass.

Four minirhizotron tubes, constructed of cellulose acetate butyrate (Bartz Technology Corporation, Santa Barbara, CA, USA) and measuring 185 cm in length by 5 cm in diameter were inserted into the ground at the angle of 60° from vertical to a depth of 60 cm in each chamber in March 2003. Tubes were stabilized with iron rods and wrapped above the soil surface with black foam insulation. The upper ends were sealed with rubber stoppers. Digital images were captured twice a month in the field using the I-CAP system (Bartz Technology Corp.) and analyzed with ROOTRACKER software (Duke University, Durham, NC, USA). Length and width of each root segment were measured and the incremental growth or disappearance (mortality) recorded. Fine-root production (mm) for a time period was calculated for each chamber as the total length of new roots and segments of new growth on existing roots for that date. We equated disappearance with mortality.

#### *Statistical analyses*

Repeated measures of ANOVA were used to examine treatment effects on soil respiration using factorial analysis. Between-subject effects were evaluated as treatments and within-subject effects were time-of-season. A General Linear Model (GLM) with a Duncan test was used to examine the statistical difference in the mean values of the main effects of CO<sub>2</sub>, warming, water, and their possible interactions. Simple and multiple linear and nonlinear regression analyses were used to examine relationships between soil respiration and soil temperature, soil moisture, root production, and aboveground biomass. Respiration data for the two soil collars and soil moisture data from the two TDR probes within each split plot were averaged before analysis. All statistical analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, USA).

## **Results**

#### *Microclimate*

During the 19-month time period from June 1, 2003 to December 31, 2004, elevated CO<sub>2</sub> reduced mean soil

temperature at the depth of 5 cm by 0.15 °C ( $P < 0.05$ ). Annual mean soil moisture contents were only slightly and not significantly higher in the elevated (24.8% in 2005 and 25.6% in 2006) than ambient CO<sub>2</sub> chambers (23.3% and 23.7%).

Although humidity in these chambers was not controlled, the chambers in different temperature regimes had similar absolute humidity; hence relative humidity was lower and vapor pressure deficit was higher in the elevated compared with ambient temperature chambers (Table 1). Air warming increased mean soil temperatures by 1.1 and 1.8 °C ( $P < 0.0001$ ) in the dry and wet sides, respectively. Mean soil water content in the elevated temperature chambers (21.2%) was lower ( $P < 0.01$ ) than in the ambient temperature chambers (26.7%).

Mean soil temperature in the wet side was 0.23 °C higher than that in the dry side ( $P < 0.001$ ). Soil water content differed ( $P < 0.001$ ) between the dry (21.7%) and wet (26.2%) side. However, during the first 3 months (June–August 2003) when watering amount and frequency simulated the natural precipitation regime, the watering treatment created little difference in soil water content between the dry (24.9%) and wet (25.2%) sides. No interactive effects among any two or three treatments on soil water content were found during the 19-month experimental period.

#### Season patterns of soil respiration

The seasonal patterns of soil respiration generally followed those of soil temperature and root production (i.e., greater in summer and lower in winter), but opposite to that of soil water content (Fig. 2a). An exponential function ( $r^2 = 0.93$ ,  $P < 0.0001$ ) described the relationship between soil respiration and soil temperature (Fig. 2b) across the 19-month experimental period. Further, a second-order polynomial function described the relationship between soil respiration and soil moisture over this period ( $r^2 = 0.52$ ,  $P < 0.0001$ , Fig. 2c). We combined

the two functions to examine the interactive effects of soil temperature ( $T$ ) and soil moisture ( $M$ ) on soil respiration ( $R$ )

$$R = \alpha e^{\beta T} \frac{\chi M^2 + \delta M + \varepsilon}{\chi M_0^2 + \delta M_0 + \varepsilon}, \quad (1)$$

where  $M_0$  is the soil moisture at which soil respiration is greatest. The inclusion of soil moisture in the regression model explained only an additional 1% of the seasonal variation in soil respiration. This indicates that soil temperature is more important than soil moisture in regulating the seasonal patterns of soil respiration. Soil respiration was linearly and positively correlated with root production ( $r^2 = 0.70$ ,  $P < 0.0001$ , Fig. 2d) although root production increased exponentially with soil temperature ( $r^2 = 0.75$ ,  $P < 0.0001$ ).

#### Treatment effects on soil respiration across seasons

Repeated measures of ANOVA (ANOVAR) revealed significant effects of time ( $P < 0.0001$ ), CO<sub>2</sub> ( $P < 0.0001$ ), and water ( $P < 0.05$ ), but no effects of warming on soil respiration. Elevated CO<sub>2</sub> increased soil respiration consistently on all the measuring dates across the entire experimental period (Fig. 3a). On average, soil respiration was 35.8% ( $P < 0.01$ ) greater under elevated CO<sub>2</sub> ( $3.45 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than under ambient CO<sub>2</sub> ( $2.54 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). When the entire 19-month experimental period was considered, soil respiration was 15.7% ( $P < 0.05$ ) higher on the wet than dry side. However, soil respiration did not differ ( $P > 0.05$ ) between the wet and dry sides during the first 3 months of the experimental period (i.e., June–August 2003) when soil moisture contents were little affected by the watering treatments (Fig. 3c). There were no interactive effects on soil respiration among any two or three treatments, but there were significant interactions between time and CO<sub>2</sub>, warming, and water treatments; temporal variation in soil respiration in response to these effects are discussed in the following sections.

#### Warming effects on soil respiration varied with season, CO<sub>2</sub> and water treatments

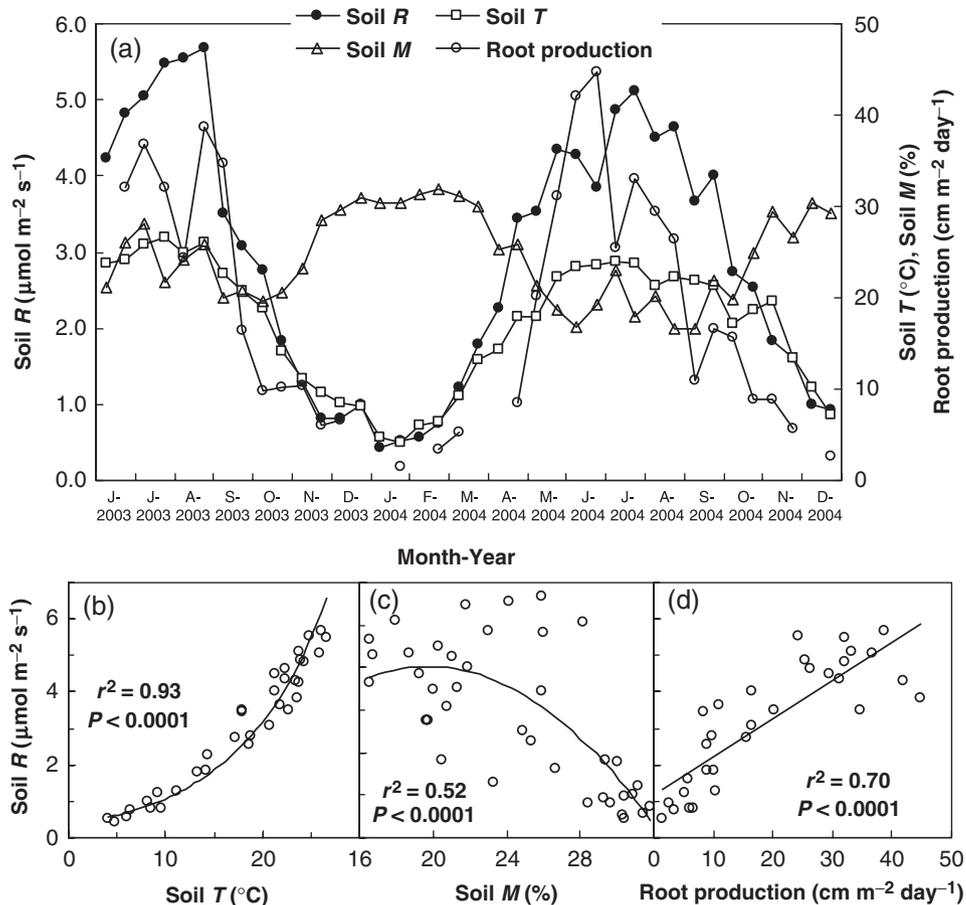
Warming-induced changes in soil respiration varied with season (Fig. 3b). From June to early November 2003, warming marginally reduced mean soil respiration by 11.7% ( $P < 0.10$ ). In contrast, soil respiration was greater (12.4%,  $P < 0.05$ ) in the elevated ( $0.95 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than ambient temperature chambers ( $0.85 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) throughout the first winter (late November 2003 to early March 2004). During the remaining 9 months of the experimental period (late

**Table 1** Microclimate in the ambient and elevated temperature chambers

	Ambient chambers	Elevated chambers
Air $T$ (°C)	15.7 ± 0.61	18.3 ± 0.57
Soil $T$ (dry side, °C)	15.4 ± 0.32	16.5 ± 0.31
(wet side, °C)	15.0 ± 0.38	16.8 ± 0.43
RH (%)	80.3 ± 0.33	67.8 ± 0.53
AH ( $\text{g m}^{-3}$ )	11.9 ± 0.04	11.7 ± 0.03
VPD (kPa)	0.48 ± 0.008	0.86 ± 0.012

Data are averages from June 1, 2003 to December 31, 2004 measured continuously using datalogger.

RH, relative humidity; AH, absolute humidity; VPD, vapor pressure deficit.



**Fig. 2** (a) Seasonal variations of soil respiration, temperature, moisture, and root production (length per unit minirhizotron window per day). (b) Exponential correlation of soil respiration with soil temperature ( $\text{Soil } R = 0.4938 \times e^{0.0934T_{\text{soil}}}$ ). (c) A second-order polynomial function ( $\text{Soil } R = -0.0245 \times M_{\text{soil}} \times M_{\text{soil}} + 0.9645 \times M_{\text{soil}} - 5.473$ ) described the relationship of soil respiration with soil moisture. (d) Soil respiration showed a positive linear correlation with fine-root production ( $\text{Soil } R = 10.471 \times \text{Root}_{\text{prod}} + 1.1749$ ). All the data are averages in 24 subplots where root productions were measured using minirhizotrons. Soil temperature (at the depth of 5 cm) was measured at the same time with soil respiration measurement.

March to December 2004), warming did not affect soil respiration.

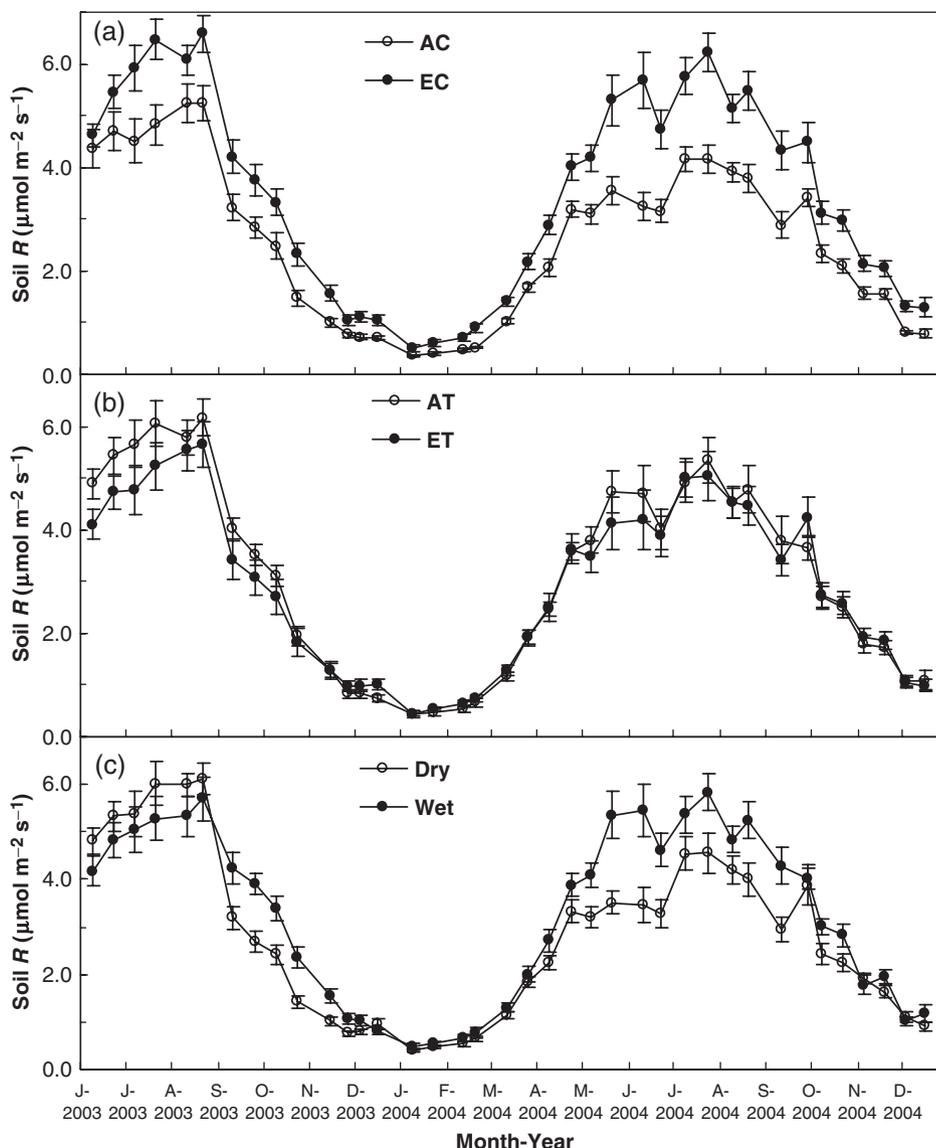
Effects of warming on soil respiration changed not only with the season, but also with  $\text{CO}_2$  and water treatments. In the dry side of both ambient and elevated  $\text{CO}_2$  chambers and in the wet side of ambient  $\text{CO}_2$  chambers, warming reduced soil respiration by 18.4%, 9.2%, and 7.6%, respectively. However, in the wet side of elevated  $\text{CO}_2$  chambers, warming increased soil respiration by 9.9% (Fig. 4).

*Treatment-induced changes in soil respiration varied with soil temperature, soil moisture, and their responses to the three respective treatments*

Effects on soil respiration of elevated  $\text{CO}_2$ , air warming, and changing precipitation depended not only on soil temperature and soil moisture in the ambient condi-

tions, but also on the treatment-induced changes in soil temperature and soil moisture. Across the 19-month experimental period,  $\text{CO}_2$ -induced changes in soil respiration increased linearly with soil temperature ( $r^2 = 0.58$ ,  $P < 0.0001$ , Fig. 5a), but declined with soil moisture ( $r^2 = 0.58$ ,  $P < 0.0001$ ) in the ambient  $\text{CO}_2$  chambers (Fig. 5b). In addition, responses of soil respiration to elevated  $\text{CO}_2$  were negatively correlated with soil temperature differences ( $r^2 = 0.31$ ,  $P < 0.0001$ , Fig. 5c) and positively correlated with soil moisture differences ( $r^2 = 0.37$ ,  $P < 0.0001$ , Fig. 5d) between the ambient and elevated  $\text{CO}_2$  chambers. Multiple regression analysis showed that combination of the changes in soil temperature (partial  $r^2 = 0.12$ ) and moisture (partial  $r^2 = 0.37$ ) explained 49% of the seasonal dynamics of  $\text{CO}_2$ -induced responses of soil respiration.

Responses of soil respiration to air warming varied linearly with soil temperature (negative,  $r^2 = 0.41$ ,



**Fig. 3** Main effects on soil respiration (Mean  $\pm$  1 SE) of elevated CO<sub>2</sub> (a), air warming (b), and changing soil water availability (c) over the 19-month period from June 2003 to December 2004. AC, ambient CO<sub>2</sub>; EC, elevated CO<sub>2</sub>; AT, ambient temperature; ET, elevated temperature.

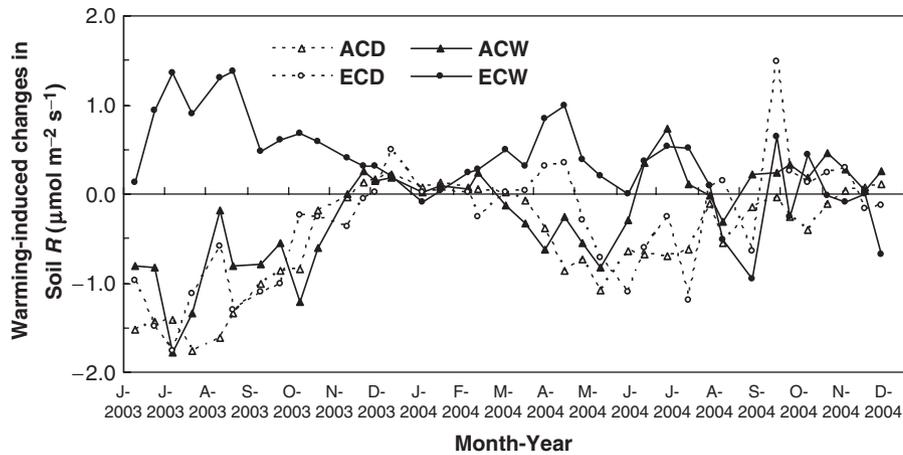
$P < 0.0001$ , Fig. 5e) and moisture (positive,  $r^2 = 0.19$ ,  $P < 0.01$ , Fig. 5f) in the ambient temperature chambers. Warming-induced changes in soil respiration linearly increased with the changes in both soil temperature ( $r^2 = 0.21$ ,  $P < 0.01$ , Fig. 5g) and moisture ( $r^2 = 0.25$ ,  $P < 0.01$ , Fig. 5h). Changes in soil temperature ( $\partial r^2 = 0.19$ ) and moisture ( $\partial r^2 = 0.25$ ) together explained 44% of the seasonal responses of soil respiration to air warming.

Differences in soil respiration between the wet and dry sides of the plots (i.e., wet–dry) linearly decreased ( $r^2 = 0.54$ ,  $P < 0.0001$ , Fig. 5j) with soil moisture and tended to increase with soil temperature in the dry side (Fig. 5i). Watering-induced changes in soil respiration were negatively correlated with soil temperature differ-

ences ( $r^2 = 0.50$ ,  $P < 0.0001$ , Fig. 5k) and positively correlated with soil moisture differences ( $r^2 = 0.40$ ,  $P < 0.0001$ , Fig. 5l) between the dry and wet sides. Results of multiple regression showed that the changes in soil temperature ( $\partial r^2 = 0.50$ ) and moisture ( $\partial r^2 = 0.06$ ) together explained 56% of the seasonal dynamics of the watering-induced changes in soil respiration.

#### *Treatment effects on base value, temperature sensitivity ( $Q_{10}$ ), and optimum soil moisture of soil respiration*

To examine whether global change forcings influence the sensitivity of soil respiration to soil temperature and moisture, we calculated base respiration and tempera-



**Fig. 4** Warming-induced changes in soil respiration (Mean $\pm$ 1SE) across different combinations of CO<sub>2</sub> and soil water availability. ACD, dry side in ambient CO<sub>2</sub> chambers; ACW, wet side in ambient CO<sub>2</sub> chambers; ECD, dry side in elevated CO<sub>2</sub> chambers; ECW, wet side in elevated CO<sub>2</sub> chambers.

ture sensitivity ( $Q_{10}$ ) of soil respiration for the 24 split plots using the exponential function (Soil  $R = \alpha \times e^{\beta t}$ ,  $\alpha$  is the base respiration,  $Q_{10} = e^{10\beta}$ ) based on data during the entire experimental period. The optimum soil moisture [ $M_0$  in Eqn (1)] was the soil moisture content at which soil respiration was greatest; it represents the moisture threshold below which soil respiration increases with soil moisture and above which soil respiration decreases with soil moisture.

Elevated CO<sub>2</sub> stimulated base respiration by 54% from  $0.441 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the ambient CO<sub>2</sub> chambers to  $0.680 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the elevated CO<sub>2</sub> chambers ( $P < 0.01$ , Fig. 6a). Base respiration was 85% ( $P < 0.001$ ) greater in the wet side ( $0.729 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than in the dry side ( $0.393 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). However, warming did not affect base respiration ( $P > 0.05$ ). CO<sub>2</sub> treatment had no effects on  $Q_{10}$  of soil respiration ( $P > 0.05$ ), and warming treatment caused a marginal reduction ( $P = 0.081$ ) in  $Q_{10}$  from 2.746 in the ambient temperature chambers to 2.460 in the elevated temperature chambers (Fig. 6b).  $Q_{10}$  of soil respiration in the wet side (2.294) was lower ( $P < 0.01$ ) than in the dry side (2.913). Neither CO<sub>2</sub> nor water treatments affected the optimum soil moisture of soil respiration. In contrast, warming reduced ( $P < 0.05$ ) the optimum soil moisture of soil respiration from 42.9% in the ambient chambers to 37.7% in the elevated temperature chambers (Fig. 6c). No interactive effects of the treatments were observed for base respiration,  $Q_{10}$ , or  $M_0$ .

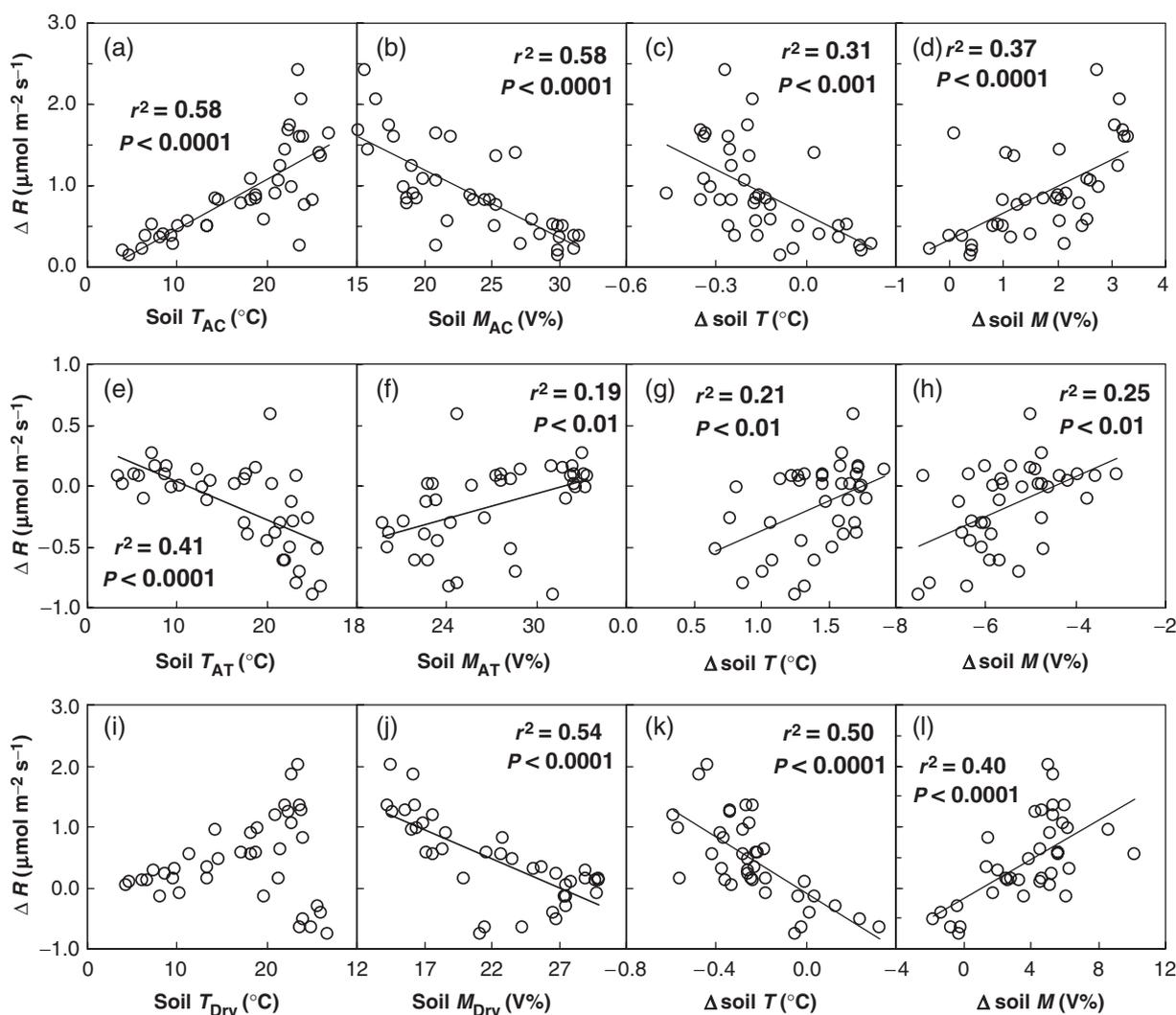
## Discussion

### Control factors over soil respiration

It has well been documented that C substrate, temperature, and water availability are the major factors con-

trolling soil respiration (Raich & Schlesinger, 1992; Lloyd & Taylor, 1994; Raich & Tufekcioglu, 2000; Rustad *et al.*, 2000; Högberg *et al.*, 2001; Wan & Luo, 2003; Scott-Denton *et al.*, 2006). Since soil respiration is a process of transferring organic C into inorganic C, the rate of soil respiration is ultimately controlled by the supply of C substrate. Soil temperature and water availability can directly affect soil respiration by altering activities of plant roots and soil microbes and indirectly by changing plant growth and substrate supply (Fig. 1). Distinguishing between the different and interactive impacts of the above three factors on soil respiration will not only improve our understanding of the dynamics and patterns of soil respiration in terrestrial ecosystems, but also facilitate the projections of the responses of soil respiration under global change.

Soil temperature and root productivity both contributed to the seasonal dynamics of soil respiration in the old-field grassland. Even though the correlation coefficient of soil respiration with soil temperature is much greater than that with root productivity, it could not be concluded that soil temperature is more important than root productivity in determining the seasonal dynamics of soil respiration, because both soil temperature and root productivity showed the concomitant temporal patterns (one peak during the middle of the growing season). When the temporal dynamic of plant growth is not synchronous with that of temperature (such as for winter wheat or in Mediterranean regions), soil respiration rates vary seasonally with plant phenology and leaf area index (Pendall *et al.*, 2001) and soil water availability (Luo *et al.*, 1996). Other studies also observed influences of leaf area index (Curiel Yuste *et al.*, 2004) and net primary production-weighted greenness index (Verburg *et al.*, 2005) on soil respiration. Moreover, even



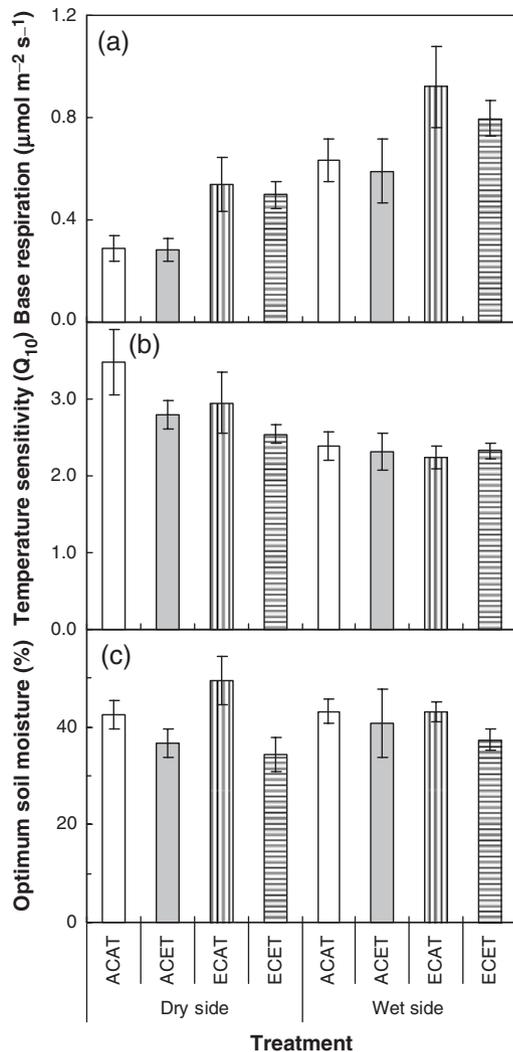
**Fig. 5** Effects on soil respiration of  $\text{CO}_2$ - (a–d), warming- (e–h), and water-treatments (i–l) depended on soil moisture, temperature, and the treatment-induced changes in soil temperature and moisture.

under constant temperature and moisture condition, soil  $\text{CO}_2$  efflux still showed strong seasonal dynamics in a grassland (Verburg *et al.*, 2004), which could be attributed to the seasonal dynamics of plant growth. The positive correlation of soil respiration with root production (Fig. 2d) and aboveground biomass (data not shown) at our experimental site also supported the above conclusions. These results suggest critical roles of plant growth in regulating the seasonal dynamics of soil respiration.

#### *CO<sub>2</sub> effect on soil respiration*

Stimulation of soil respiration by elevated  $\text{CO}_2$  concentration has been observed in different grassland ecosystems (Luo *et al.*, 1996; Zak *et al.*, 2000; Craine *et al.*, 2001). In the old-field grassland,  $\text{CO}_2$  enrichment increased

both base values (Fig. 6a) and seasonal averages (Fig. 3a) of soil respiration, supporting our first hypothesis. Several mechanisms could explain the stimulation of soil respiration by elevated atmospheric  $\text{CO}_2$  concentrations (Fig. 1). Elevated  $\text{CO}_2$  can increase plant photosynthesis, growth, and belowground C input and substrate, leading to greater root and microbial activities and respiration (Edwards & Norby, 1999; Zak *et al.*, 2000; Anderson *et al.*, 2001). In addition, higher soil moisture content resulting from reduced stomatal conductance and transpiration of plants under elevated  $\text{CO}_2$  (Hungate *et al.*, 2002; Morgan *et al.*, 2004) will also enhance root and microbial activities and respiration. Increased soil respiration under elevated atmospheric  $\text{CO}_2$  concentrations suggests accelerated soil and ecosystem C release and cycling and limited ecosystem C sequestration (Hungate *et al.*, 1997).



**Fig. 6** Effects of CO<sub>2</sub>, warming, and water treatments on the base values (a), temperature sensitivity (b), and optimum soil moisture contents (c) of soil respiration (mean ± 1 SE). ACAT, ambient CO<sub>2</sub> and ambient temperature; ACET, ambient CO<sub>2</sub> and elevated temperature; ECAT, elevated CO<sub>2</sub> and ambient temperature; ECET, elevated CO<sub>2</sub> and elevated temperature.

Previous studies (Craine *et al.*, 2001; Pendall *et al.*, 2003) reported variation of soil respiration responses to elevated CO<sub>2</sub> with soil water availability. For example, Pendall *et al.* (2003) reported that elevated CO<sub>2</sub> increased soil respiration by about 25% in a moist growing season and by about 85% in a dry season in shortgrass steppe in North America. Because of the higher water-use efficiency of plant under CO<sub>2</sub> enrichment, it is generally assumed that plant responses to CO<sub>2</sub> are greater under drier than wetter conditions (Owensby *et al.*, 1999). Therefore, it might be expected that the CO<sub>2</sub> effect on soil respiration would be greater in the dry than wet side in the old-field grassland. However, across the 19-month

experimental period, CO<sub>2</sub>-induced stimulation of mean soil respiration did not differ between the dry side (33%) and the wet side (38%). Excluding the first 3 months of the experiment from the analysis did not alter this result. Our findings are unexpected and the underlying mechanisms need further study.

Observations of the positive correlation of CO<sub>2</sub>-enhanced soil respiration with temporal variation in soil temperature and the negative correlation with soil moisture (Fig. 5a and b) supported our fourth hypothesis. However, the results do not necessarily support the above speculations that CO<sub>2</sub> enhancement of soil respiration is greater under drier conditions. The observed correlations in our study could be primarily attributable to plant activities associated with seasonal dynamics soil temperature and moisture. During the growing season when soil temperature was higher and soil moisture was lower than in winter, plants were more active; thus, CO<sub>2</sub> enrichment apparently resulted in greater stimulation of plant growth, root respiration, and total soil respiration in summer than in winter.

The negative correlation of CO<sub>2</sub>-stimulated soil respiration with temperature changes (Fig. 5c) and the positive correlation with moisture changes (Fig. 5d) supported the presumptions of the indirect CO<sub>2</sub> effect on soil respiration via altering soil temperature and moisture (Fig. 1). It is also consistent with our argument in the previous paragraph that CO<sub>2</sub> effects on soil respiration vary with plant activity. Greater CO<sub>2</sub> enhancement on plant growth and root respiration during the growing season, with attendant effects on plant growth, and canopy cover apparently led to greater differences in soil temperature and moisture between the elevated and ambient CO<sub>2</sub> chambers.

The CO<sub>2</sub> enhancement of soil respiration was greater under elevated temperature than ambient temperature. Given that air warming reduced soil moisture, higher soil moisture content under elevated CO<sub>2</sub> may ameliorate warming-induced water stress on root and microbial activities, leading to greater stimulation of soil respiration by CO<sub>2</sub> enrichment in the elevated than ambient temperature chambers. Moreover, CO<sub>2</sub>-enrichment can increase the temperature optimum for plant photosynthesis and growth (Farquhar *et al.*, 1980; Long, 1991), relieve the heat stress under warming, and thus enhance belowground C allocation and root and microbial respiration (Lilley *et al.*, 2001).

#### Warming effect on soil respiration

Soil respiration generally shows positive exponential correlations with temperature. Thus, it is often assumed that global warming will stimulate soil respiration and lead to a positive feedback loop between atmospheric

CO<sub>2</sub> and air temperatures (Cox *et al.*, 2000). However, our results showed that experimental warming does not necessarily lead to greater soil respiration, at least in this old-field grassland. Responses of soil respiration to air warming depended on season (Fig. 3b), CO<sub>2</sub> concentration, and soil water availability (Fig. 4), in agreement with our Hypotheses 3 and 4.

During the first growing season, soil respiration showed negative responses to air warming. While keeping similar absolute humidity in the ambient and elevated temperature chambers, air warming caused significant reductions in relative humidity and increases in vapor pressure deficit, leading to greater evapotranspiration and lower soil moisture. Soil water stress would not only directly suppress microbial and root activities and respiration, but also indirectly decrease soil respiration via inhibition of plant growth, belowground C allocation, and substrate availability. The positive linear correlation of the warming-induced changes in soil respiration with soil moisture reductions (Fig. 5h) supports this conclusion. Greater vapor pressure deficit under air warming would also cause stomatal closure and reduce stomatal conductance (Lemmens *et al.*, 2006), plant photosynthesis and growth, and root, as well as total soil respiration.

During the first winter, plants were dormant and microbial respiration accounted for the majority of total soil respiration. Moreover, soil moisture was higher and no longer a limiting factor. Therefore, air warming led to greater soil respiration during the winter. The lack of response of soil respiration to warming during the second growing season might have been related to changes in species composition. Old-field grasslands are usually mowed or grazed. However, mowing and grazing were excluded in the established old-field community inside the OTCs in our experiment, which allowed a perennial nitrogen-fixing shrub species (*L. cuneata*) to become the dominant species in the community. Differences in the physiology of *L. cuneata* and other species might have contributed to the insignificant responses of soil respiration in the second growing season.

Season-specific responses of soil respiration to air warming were also reported in a tallgrass prairie with negative effects during the growing season and no response during the nongrowing season (Verburg *et al.*, 2005). Negative impacts of warming on soil respiration were observed in an alpine meadow (Saleska *et al.*, 1999). Seasonal variability of soil respiration responses to warming has been ascribed to the presence or absence of soil moisture limitations (Janssens & Pilegaard, 2003), but covariation between soil temperature and moisture often limits the ability to separate temperature from moisture effects (Davidson *et al.*, 1998). Since root respiration comprises much of total

soil respiration, the seasonal variability of warming impacts on the plant growth would be primarily responsible for the season-specific warming responses of soil respiration observed in this and previous studies (Nijs *et al.*, 1996; Verburg *et al.*, 2005).

#### *Effects of soil water availability on soil respiration*

Soil water availability may constrain root and microbial activities and respiration at high and low water contents (Liu *et al.*, 2002; Borken *et al.*, 2006; Scott-Denton *et al.*, 2006). During the 19-month experimental period, no severe drought occurred, and soil moisture contents were near or above the optimum point in the second-order polynomial function between soil respiration and moisture (Fig. 2c). In addition, the effect of soil water treatment on soil respiration was confounded by seasonal variations of soil temperature and plant growth. Therefore, soil moisture had little effect on the seasonal dynamics of soil respiration in this study. Excluding the first 3 months of the experiment from the analysis did not alter this result. However, the significant linear correlations of soil respiration with soil moisture across different subplots (data not shown) and the greater soil respiration in the wet than dry sides (Fig. 3c) suggest the important role of soil moisture in regulating spatial variations of soil respiration in the old field grassland.

Soil water availability can directly influence plant root and microbial respiration and indirectly by affecting plant growth, belowground C allocation and substrate availability (Fig. 1). The response of soil respiration to soil moisture treatment declined as the difference in soil temperature between the dry and wet sides declined (Fig. 5k), suggesting that there were indirect effects of soil water availability on soil respiration via changes in soil temperature, as described in the conceptual model (Fig. 1). In addition, soil moisture has been found to indirectly affect soil respiration by limiting the diffusion of soluble substrates at low water content and the diffusion of oxygen at higher water content (Skopp *et al.*, 1990). Owing to the above direct and indirect effects, soil water availability can modulate the impacts of other global change driving factors (i.e., elevated atmospheric CO<sub>2</sub> and temperature) on soil respiration (Pendall *et al.*, 2003). The correlations of soil respiration differences between the wet and dry sides with soil temperature and soil moisture could also be ascribed to the seasonal growth activities of plants (as described above).

#### *Temperature sensitivity of soil respiration*

Atmospheric and climate change could affect basal respiration and/or temperature sensitivity ( $Q_{10}$ ) of soil

respiration. Increased base and total soil respiration under elevated CO<sub>2</sub> suggest greater C release and cycling, offsetting the CO<sub>2</sub>-induced C uptake and weakening the C sequestration potential in terrestrial ecosystems. Irrespective of the stimulation of base respiration and total soil respiration by CO<sub>2</sub> enrichment, no responses of Q<sub>10</sub> of soil respiration were observed under CO<sub>2</sub> treatment (Fig. 6b). Our results were inconsistent with those reported in a previous study with tree seedlings (Tingey *et al.*, 2006), in which elevated CO<sub>2</sub> did not stimulate soil respiration but increased the Q<sub>10</sub> of soil respiration.

There were no changes in base and mean total soil respiration under warming in the old-field grassland, which is inconsistent with observations in other ecosystems (Rustad *et al.*, 2001). By contrast, reductions in the Q<sub>10</sub> of soil respiration under higher temperature supported those observed in a tallgrass prairie (Luo *et al.*, 2001), suggesting acclimation of soil respiration to climate warming via physiological downregulation and/or alteration of substrate supply (Atkin & Tjoelker, 2003; Davidson & Janssens, 2006; Davidson *et al.*, 2006).

Lower temperature sensitivity of soil respiration has been reported in previous studies during drying periods, which may have resulted largely from substrate limitation caused by limited diffusion of solute in thin water films (Janssens & Pilegaard, 2003; Curiel Yuste *et al.*, 2004; Reichstein *et al.*, 2005; Davidson *et al.*, 2006). However, we found higher temperature sensitivity and lower base respiration in the dry side in the old-field grassland. Across different split plots, Q<sub>10</sub> was negatively correlated ( $r^2 = 0.63$ ,  $P < 0.001$ ) with base respiration. Since base respiration and Q<sub>10</sub> calculated from parameters in the exponential function of soil respiration and temperature are interdependent and covarying with each other, our results do not refute the conclusions in previous studies.

In conclusion, elevated CO<sub>2</sub> and higher soil water availability stimulated soil respiration in the old-field grassland as expected. However, the effects of warming on soil respiration varied with season and other treatments. The well-known relationship between soil respiration and temperature explained seasonal variation in soil respiration, but did not explain responses to our experimental climate change treatments. Simple temperature-response functions should not be used to represent future soil respiration responses. Rather, this study demonstrates the importance of both direct and indirect effects on the biotic (plant and microbial activities via substrate regulation) and abiotic (soil temperature and water availability) mechanisms associated with the concurrent driving factors of atmospheric and climatic change.

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