

Contrasting responses of heterotrophic and autotrophic respiration to experimental warming in a winter annual-dominated prairie

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Abstract

Understanding how soil respiration (Rs) and its source components respond to climate warming is crucial to improve model prediction of climate-carbon (C) feedback. We conducted a manipulation experiment by warming and clipping in a prairie dominated by invasive winter annual *Bromus japonicus* in Southern Great Plains, USA. Infrared radiators were used to simulate climate warming by 3 °C and clipping was used to mimic yearly hay mowing. Heterotrophic respiration (Rh) was measured inside deep collars (70 cm deep) that excluded root growth, while total soil respiration (Rs) was measured inside surface collars (2–3 cm deep). Autotrophic respiration (Ra) was calculated by subtracting Rh from Rs. During 3 years of experiment from January 2010 to December 2012, warming had no significant effect on Rs. The neutral response of Rs to warming was due to compensatory effects of warming on Rh and Ra. Warming significantly ($P < 0.05$) stimulated Rh but decreased Ra. Clipping only marginally ($P < 0.1$) increased Ra in 2010 but had no effect on Rh. There were no significant interactive effects of warming and clipping on Rs or its components. Warming stimulated annual Rh by 22.0%, but decreased annual Ra by 29.0% across the 3 years. The decreased Ra was primarily associated with the warming-induced decline of the winter annual productivity. Across the 3 years, warming increased Rh/Rs by 29.1% but clipping did not affect Rh/Rs. Our study highlights that climate warming may have contrasting effects on Rh and Ra in association with responses of plant productivity to warming.

Keywords: autotrophic respiration, climate-carbon feedback, climate warming, clipping, grasslands, heterotrophic respiration, soil respiration

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Introduction

Climate warming induced by rising concentrations of atmospheric greenhouse gases (mostly CO₂) has increased the Earth's surface temperature by 0.76 °C since 1850, and the temperature is expected to increase by another 1.1–6.4 °C by the end of 21st century (IPCC, 2007). A warming climate may impact terrestrial carbon (C) balance by altering net primary production (NPP), belowground C allocation and soil C decomposition (Luo, 2007). Soil respiration (or soil CO₂ efflux, Rs) represents the largest single source of CO₂ from terrestrial ecosystems to the atmosphere with a magnitude of about ten times greater than anthropogenic fossil fuel combustion (Metcalfe *et al.*, 2011). Rs comprises of an autotrophic component (Ra) and a heterotrophic component (Rh) (Hanson *et al.*, 2000; Kuzyakov, 2006; Subke *et al.*, 2006). Ra originates from roots and the

associated rhizosphere dependent on the supply of recent photosynthates that have very low residence time in soil. Rh involves the decomposition of soil organic matter (SOM) including litter and older soil organic matter (SOM), which has residence time from months to centuries (Hanson *et al.*, 2000; Hogberg & Read, 2006; Kuzyakov, 2006). The two components of Rs may respond differently to climate warming (Zhou *et al.*, 2007; Gaumont-Guay *et al.*, 2008; Schindlbacher *et al.*, 2009; Gomez-Casanovas *et al.*, 2012). Therefore, understanding the responses of Ra and Rh would help to better predict feedbacks of terrestrial C cycling to climate warming.

Many studies have already been conducted to investigate the responses of Rs to climate warming. According to meta analyses, warming significantly stimulates Rs (Rustad *et al.*, 2001; Wu *et al.*, 2011); but the responses of Rs to warming in individual studies vary from positive (Zhou *et al.*, 2006; Wan *et al.*, 2007; Lin *et al.*, 2011), neutral (De Boeck *et al.*, 2007; Wan *et al.*, 2007) to negative (Pajari, 1995; Saleska *et al.*, 1999;

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Verburg *et al.*, 2005; Liu *et al.*, 2009). However, the positive responses do not necessarily mean a positive feedback loop between SOM pool and the atmosphere since they could be driven by higher C inputs to soil rather than by stimulated decomposition of SOM (Bond-Lamberty & Thomson, 2010). Due to the possible differential responses of Rh and Ra to climate warming (Zhou *et al.*, 2007; Gaumont-Guay *et al.*, 2008; Schindlbacher *et al.*, 2009; Gomez-Casanovas *et al.*, 2012), increase of Rs does not necessarily mean a concurrent positive response of Rh; while neutral or even negative responses of Rs may involve increase of Rh. A positive feedback of soil C cycle to climate warming may be triggered if (i) Rh increases without corresponding increases in detritus inputs or even decrease in detritus inputs; (ii) Rh does not change but detritus inputs decrease. Since it is the difference between Rh and detritus inputs that determines the net soil C increment (Hanson *et al.*, 2000), measurements of Rs alone have limited value for evaluating whether the soils act as a source or sink of atmospheric CO₂ under warming conditions (Subke *et al.*, 2006). Nevertheless, so far only limited studies have reported responses of both components to experimental warming (Lin *et al.*, 2001; Zhou *et al.*, 2007, 2010; Schindlbacher *et al.*, 2008, 2009). Results from these studies generally show warming stimulation of both Rh and Ra, but with Rh being more (Lin *et al.*, 2001), less (Zhou *et al.*, 2007) or equally (Schindlbacher *et al.*, 2008) sensitive relative to Ra. However, Zhou *et al.* (2010) reported that both Rh and Ra were decreased by warming in a mesocosm experiment. Considering the limited number of studies, more investigations are undoubtedly needed in order to better understand the responses of the two source components.

Terrestrial C cycle feedback to climate warming can strongly vary with land-use practices (Chapin *et al.*, 2008). In grasslands, one of the major land-use activities is clipping/grazing or hay harvesting, which may significantly influence the direct impacts of warming on soil environment and soil microbial community (Wan & Luo, 2003; Zhang *et al.*, 2005). The reported effects of clipping include reduced C input to microbes via aboveground biomass removal (Wan & Luo, 2003), altered soil microclimate (Wan *et al.*, 2002; Wang *et al.*, 2012), and belowground productivity (Lin *et al.*, 2011; Xu *et al.*, 2012). Divergent results have been reported regarding the effects of clipping/grazing on Rs (Wan & Luo, 2003; Bahn *et al.*, 2006; Lin *et al.*, 2011), but very few studies quantified the responses of Rh and Ra to warming under the influence of clipping/grazing (Zhou *et al.*, 2007; Lin *et al.*, 2011). To our knowledge, the combined effects of clipping and warming on Rh and Ra have only been reported by one study (Zhou *et al.*, 2007). It remains unclear whether or not Rs

and its components respond in a similar manner to simultaneous warming and clipping under different site conditions.

We conducted a manipulation experiment since July 2009 to investigate the impacts of warming and clipping on ecosystem C dynamics including Rs and its source components at a *Bromus japonicus* Thunb.-dominated prairie. *B. japonicus* is an introduced species to the North America where it now can be found throughout the conterminous United States and a large portion of Canada (Fig. S1). It is a C3 grass and typically behaves as a winter annual that germinates in fall, survives the winter in a vegetative state (Baskin & Baskin, 1981). There are two biomass peaks in the *B. japonicus*-impacted prairie in Southern Great Plains, i.e., one occurs in late spring or early summer dominated by *B. japonicus*, and another occurs in fall dominated by other C3/C4 species. Although invasive plant species are well known to threaten native and managed ecosystems worldwide (Vitousek *et al.*, 1996), very limited studies have been conducted to investigate how climate warming will impact the processes and functioning in invasive species-dominated ecosystems. To our knowledge, no studies have been conducted regarding the responses of soil respiration and its components to warming in a *B. japonicus*-dominated prairie.

We hypothesized that both plant growth and soil respiration responses to warming at the *B. japonicus*-dominated prairie might be different from those observed at a nearby long-term warming manipulation experiment (long-term experiment), which started from 1999 and was conducted at a C4 species-dominated prairie (Luo *et al.*, 2001, 2009; Wan *et al.*, 2005; Zhou *et al.*, 2006, 2007). This is because photosynthesis in C3 plants is more efficient at lower temperatures relative to C4 plants (Taiz & Zeiger, 2002), so that plant growth responses to warming may be different between the *B. japonicus*- and C4 species-dominated prairie sites, which in turn may mediate the warming effects on soil respiration and its components. Therefore, the main objectives were to address: (i) How do Rs and its source components respond to warming in a *B. japonicus*-dominated prairie? (ii) Are warming effects on Rs and its components mediated by clipping?

Materials and methods

Site description

The experiments were conducted on the Kessler's Farm Field Laboratory (34°58'54"N, 97°31'14"W) in McClain County, OK. The sites have not been grazed for the past 40 years (Luo *et al.*, 2009). Mean annual precipitation (MAP) from 1948 to 2012 was 895 mm with monthly precipitation ranging from 33 mm

in January to 126 mm in May; and mean annual temperature (MAT) is 16.3 °C with mean monthly air temperature ranging from 3.5 °C in January to 28.1 °C in July (data from Oklahoma Climatological Survey).

The experimental site is dominated by C3 grass *Bromus japonicus* Thunb., C3 forbs *Ambrosia trifida*, *Apocynum* sp., *Solanum carolinense*, *Veronia baldwinii*, *Securigera varia* and C4 grass *Tridens flavus*, *Sorghum halapense*. Biomass of C3 species accounted for 72.4% before treatment. The soil type is Port-Pulaski-Keokuk complex, which is a well-drained soil that is formed in loamy sediment on flood plains (USDA, 1979). The soil texture class is loam with 51% of sand, 35% of silt and 13% of clay. The soil bulk density is 1.2 g cm⁻³. The concentrations of soil organic matter and total N are 1.9% and 0.1%, respectively.

Experimental design

The experiment used a split-plot design, with warming manipulated at the plot level and clipping manipulated at the subplot level. There were four blocks each with an area of 13 m × 15.5 m. In each block, there were two 2.5 m × 3.5 m plots, with one assigned as ambient and another one as warming (ambient plus 3.0 °C) treatment. Each plot was divided into two subplots with one clipping subplot and another unclipping subplot. Two infrared heaters were suspended 1.5 m above the ground in each warming plot to evenly distribute the heat. Two dummy heaters of the same size and shape as the heaters were suspended in the control plots. Therefore, four treatments were included, i.e., unclipping and control (ambient) temperature (UC), unclipping and warming (UW), clipping and control temperature (CC), and clipping and warming (CW). Each treatment had four replications. Experimental treatments started since July 2009.

Measurement protocols

To measure soil respiration, PVC collars (80 cm² in area) were inserted 2–3 cm (for Rs measurements) or 70 cm (for Rh measurements) into the soil permanently at the center of each subplot (Zhou *et al.*, 2007). Small living plants inside the soil collars were clipped at the soil surface at least 1 day before the measurement to eliminate aboveground plant respiration. The clipped plant material was left in the collars to decompose. Soil respiration were measured once or twice a month between 10 : 00 and 15 : 00 h (local time), using a LI-8100 portable soil CO₂ fluxes system attached to soil CO₂ flux chamber (LI-COR Inc., Lincoln, NE, USA).

The 70 cm long PVC collars cut off old plant roots and prevented new roots from growing inside the tubes, and thus soil respiration from these collars represent Rh after about 5 months of collar insertion to exclude the transient response of dead root decomposition (Zhou *et al.*, 2007). Ra is calculated as the difference between Rs and Rh.

Soil temperature was monitored by thermocouples installed at the depth of 7.5 cm in the center of the clipping and unclipping subplots in each main plot. The hourly average data were stored in an SM19 Storage Module (Campbell Scientific,

Logan, Utah, USA). Volumetric soil water content (%V) was manually measured along with soil respiration measurements using Time Domain Reflectometry (TDR) (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at 0–12 cm depth in both the subplots.

Peak aboveground plant biomass (AGB) was measured at the peak biomass period using two methods: clipping to directly measure AGB in the clipped subplots and indirect point-frame method to estimate AGB in the unclipped subplots. Detailed description of the two methods were presented in our previous paper (Luo *et al.*, 2009). The indirect point-frame method was used to estimate the green (live plant) and brown (standing litter) hits on each of 10 pins in a frame facing each of four directions at both clipping and unclipping subplots (Frank & McNaughton, 1990). Clipping was done only in the clipping subplots right after the indirect estimation of peak aboveground biomass. Plants were clipped to a height of 10 cm. Clipped plant biomass (including both brown and green tissues) were first separated into C₃ and C₄ plants and then oven-dried at 65 °C for 48 h. The same subplots were clipped at the same time each year. Pin hits in the unclipping subplots were correlated with a linear function to pin hits in the clipping subplots and thus the peak AGB in the unclipping subplots were estimated. Biomass was converted to C content by a factor of 0.45.

AGB measurement was conducted only in late August in 2010 and 2011. However, we realized that sampling at this time would greatly underestimate the total AGB since there were two biomass peaks with one in late April/early May and another one in late August. Therefore, AGB was measured at both biomass peaks in 2012. Accordingly, only AGB measured in 2012 was used in this paper.

Annual soil respiration

Annual soil respiration for each treatment was estimated by summing the daily soil respiration and the number of days between samples. It was corrected further for diurnal patterns in soil respiration. Our measurements, collected between 10 : 00 and 15 : 00 h, were assumed to represent daytime averages based on diurnal patterns at a nearby site (Wan & Luo, 2003). The calculated average daily soil respiration was 96.5% of the observed daytime average. The corrected daily soil respiration was used to compute annual soil respiration (Zhou *et al.*, 2007). It has to be noted that this method may result in some biases in estimating annual soil respiration, although it has been often used (e.g., Wan & Luo, 2003; Zhou *et al.*, 2007).

Data analysis

Since the sampling dates were not exactly the same among years, monthly means of Rs, Rh, Ra, soil temperature, and soil moisture were used to keep consistent in statistical analysis and to calculate annual mean values. Repeated-measures split-plot ANOVA was used to examine the effects of warming, clipping, year, sampling date and their interactions on soil respiration (Rs, Rh, Ra and Rh/Rs), soil temperature and

moisture across the 3 years using the GLM procedure and type III sum of squares. Between-subject factors (or main factors) were warming and clipping, and within-subject factors were year and sampling date. In addition, repeated-measures split-plot ANOVA was also used to examine the effects of warming, clipping, sampling date and their interactions on soil respiration for each year with warming and clipping used as between-subject factors while sampling date as within-subject factors. 2-way ANOVA was used to examine the effects of warming, clipping, and their possible interactions on AGB in 2012 and annual soil respiration. If there were no significant interactive effects of warming and clipping, only the main effects, i.e., warming or clipping were presented. The effects were considered to be significantly different if $P < 0.05$. The statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL, USA).

Results

Microclimate

There was a clear seasonal pattern for air temperature which was higher during June–August period than

December–February period (Fig. 1a). In contrast to air temperature, there was no clear seasonal pattern for precipitation during the 3 years. MAT in 2010 (15.8 °C) was very close to the average from 1948 to 2012, but was about 1 °C greater than the average in 2011 (17.3 °C) and 2012 (17.5 °C). MAP in 2010 was 906 mm, which was similar to the average from 1948 to 2012, but MAP in the other 2 years (549 mm for 2011 and 678 mm for 2012) was much lower than the history average.

Warming significantly elevated mean annual soil temperature by 3.2 and 3.4 °C, respectively, for the unclipping and clipping subplots from 2010 to 2012 ($P < 0.001$, Fig. 1b); but clipping did not affect soil temperature significantly ($P > 0.05$). No significant interactive effect of warming and clipping was found.

Daily averaged volumetric soil moisture for UC treatment varied from 2.0 to 20.9% with an annual average of 8.9% (Fig. 1c). Consistent with annual precipitation, soil moisture was greater in 2010 than the other 2 years. The averaged soil moisture for the UC treatment in 2010, 2011, and 2012 was 11.6%, 7.6%, and 8.1%,

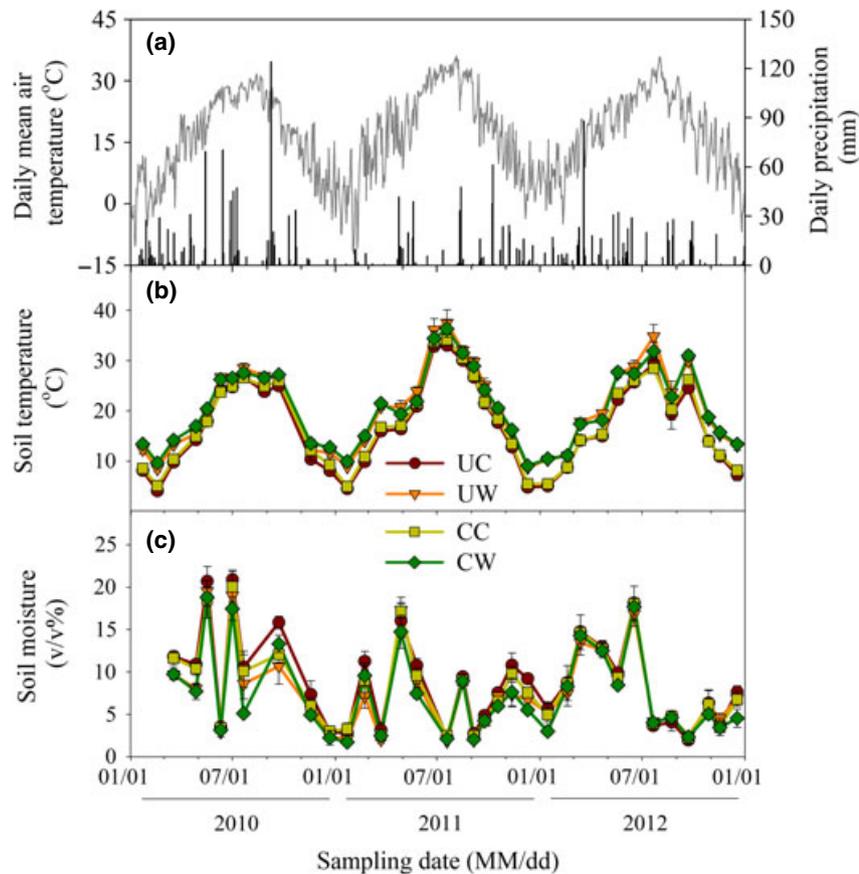


Fig. 1 Temporal variation of climate factors from January 2010 to December 2012. (a) Daily mean air temperature (gray line) and daily precipitation (black bar), (b) soil temperature at 7.5 cm, and (c) volumetric soil moisture to a depth of 12 cm (v/v%). Data of air temperature and precipitation were collected from a nearby weather station of Mesonet.

respectively. Treatment effects of warming and clipping on soil moisture were not detectable probably due to the low sampling frequency.

Responses of soil respiration and its components to warming and clipping

R_s was not significantly affected by warming but was significantly increased by clipping ($P = 0.039$) across the 3 years (Fig. 2a and b; Table S1). No significant interactive effect of warming and clipping on R_s was observed. Analysis of yearly data indicated that clipping significantly increased R_s only in 2010 (Fig. 2b). Although there was significant variation in R_s among sampling dates or years, no interactive effects of warming \times date (or year) or clipping \times date (or year) were found (Table S1).

Warming significantly increased R_h ($P = 0.01$) across the 3 years (Fig. 2c). Analysis of yearly data revealed that the warming effect on R_h was not significant in 2010, but significant in 2011 and 2012 (Fig. 2c). Warming stimulated R_h by 22.0% across the 3 years and by 27.9% and 39.5%, respectively, in 2011 and 2012. Clipping did not significantly affect R_h across the 3 years or

in individual years (Fig. 2d). Marginally, ($P < 0.1$) interactive effect of warming and clipping on R_h was only found in 2011 (Table S2). Similar to R_s , there was significant variation in R_h among sampling dates ($P < 0.0001$) or years ($P < 0.0001$) (Table S1). R_h was greater in 2010 than the other years.

In contrast to R_h , warming significantly decreased R_a ($P = 0.001$) across the 3 years (Fig. 2e). For individual years, warming effect was not significant in 2010, but significant in 2011 and 2012 (Fig. 2e). Warming decreased R_a by 29.0% across the 3 years and by 35.8% and 29.9%, respectively, in 2011 and 2012. Clipping marginally increased R_a in 2010 and across the 3 years (Fig. 2f). Marginally, significant interactive effect of warming and clipping on R_a was only found in 2010 (Table S2). R_a varied significantly among dates, but not significantly among years.

Annual soil respiration and its source components

The average annual R_s , R_h , and R_a for the UC treatment over the 3 years were 1122.2 ± 58.2 , 559.1 ± 39.1 , and 563.1 ± 26.6 $\text{g C m}^{-2} \text{yr}^{-1}$, respectively. Annual R_h was greater in 2010 than in other 2 years

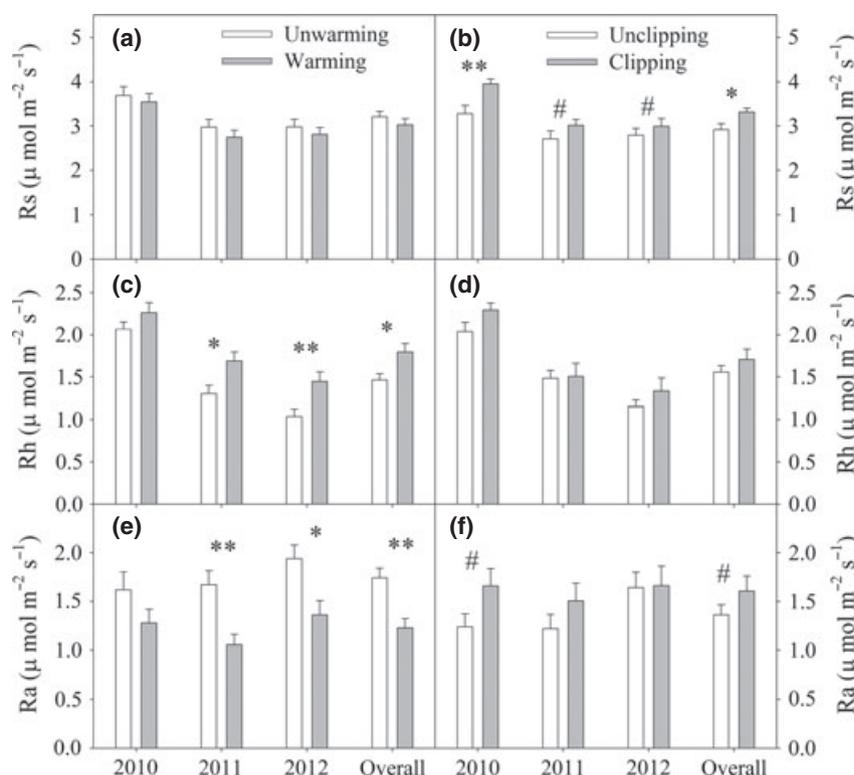


Fig. 2 Main effects of warming and clipping on total soil respiration (a, b), heterotrophic respiration (c, d) and autotrophic respiration (e, f) in each year and across the 3 years. The left three panels show warming effects and the right three panels show clipping effects. The unwarming subplots are UC + CC subplots; the warming subplots are UW + CW; the unclipping subplots are UC + UW and the clipping subplots are CC + CW. Bars represent mean \pm SE ($n = 8$). Results from the repeated-measures split-plot ANOVA are presented in Table S1 and S2. # $0.5 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$.

($P < 0.0001$) for all treatments, but annual Ra did not vary significantly among years (Fig. 3a and b). This resulted in higher Rs in 2010 than in the other 2 years. Across the 3 years, warming stimulated annual Rh by 22.0%, but decreased annual Ra by 29.0%, respectively.

Annual Rh contributed 36.6–62.3% (49.9% on average) to annual Rs across the 3 years for the UC treatment. Rh/Rs decreased significantly from 2010 to 2012 for this treatment. Clipping did not affect Rh/Rs, but warming significantly increased Rh/Rs (Fig. 3c and d). There was no interactive effect of warming and clipping on Rh/Rs (Table S1, S2). Rh/Rs was increased by warming by 12.7%, 38.8% and 48.3% and 29.1%, respectively, in 2010, 2011, 2012 and across the 3 years.

Abiotic and biotic controls on soil respiration and its components

No significant relationship was found between soil temperature and Rs or its components. Significant relationships were found between volumetric soil moisture and Rs, Rh or Ra (Fig. 4). However, the patterns of the relationship depended on treatments and source components. Quadratic functions were found to be better to fit the relationship between soil moisture and Rs or Ra, but Rh increased linearly with soil moisture.

Since AGB at the two biomass peaks was measured only in 2012, therefore only AGB data in 2012 were analyzed. AGB was not significantly affected by clipping. Peak AGB in late April accounted for 69.0% and 55.7% of annual total for the unwarming and warming treatments. Warming significantly ($P < 0.01$) suppressed

peak AGB in late April, but had no obvious effect on peak AGB in late August. Overall, warming significantly ($P = 0.032$) decreased annual AGB (Fig. 5a). Regression analysis demonstrated that annual Rs and Rh were not significantly related to AGB, but annual Ra was marginally ($P < 0.1$) related to annual AGB (Fig. 5b). However, further analysis revealed that annual Ra was positively related to AGB harvested in late April ($P < 0.01$) but not to AGB harvested in late August (Fig. 5b).

Discussion

Soil respiration and its partitioning

The mean annual Rs for the UC treatment was about $350 \text{ g C m}^{-2} \text{ yr}^{-1}$ greater than that measured at a nearby site (Luo *et al.*, 2009), but was within the range reported for tallgrass prairies of North America ($70\text{--}2100 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Bremer & Ham, 2002; Wan & Luo, 2003; Harper *et al.*, 2005; Zhou *et al.*, 2007) or reported for European grasslands ($58\text{--}1988 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Bahn *et al.*, 2008). The contribution of Rh to Rs (Rh/Rs for UC) was also within the range (25–90%) reported for grasslands (Subke *et al.*, 2006).

In the current study, root exclusion method was used to distinguish Rh and Ra. This method is among the three primary methods which have been widely used to partition soil respiration (Hanson *et al.*, 2000). The advantage of this method is simple and easy to deploy. With this method, Ra is calculated by the difference between collars with and without living roots. Our

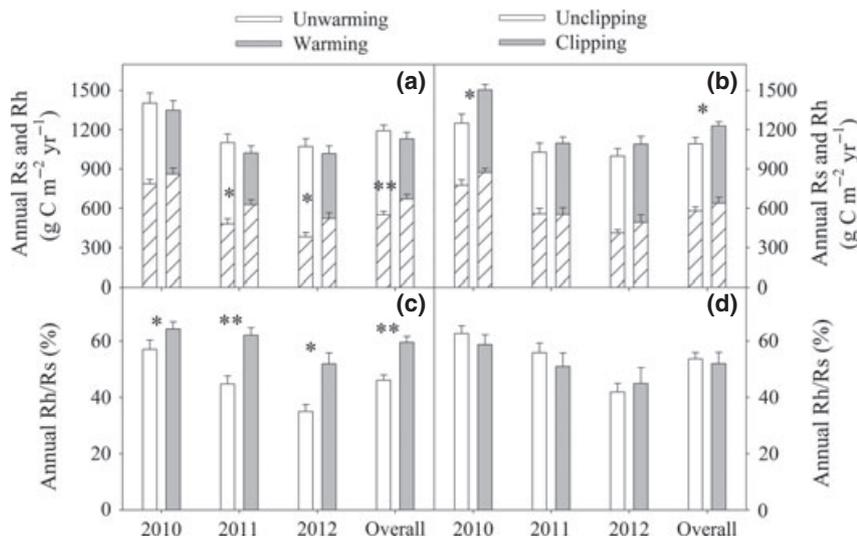


Fig. 3 (a) Annual soil respiration (Rs) and heterotrophic respiration (Rh) and (b) contribution of annual Rh to Rs under the main effect treatments (see Fig. 2) for each year and across the 3 years. The left two panels show warming effects and the right two panels show clipping effects. Bars represent mean \pm SE ($n = 8$). The hatched columns in panel (a) and (b) represent annual Rh. * $P < 0.05$; ** $P < 0.01$.

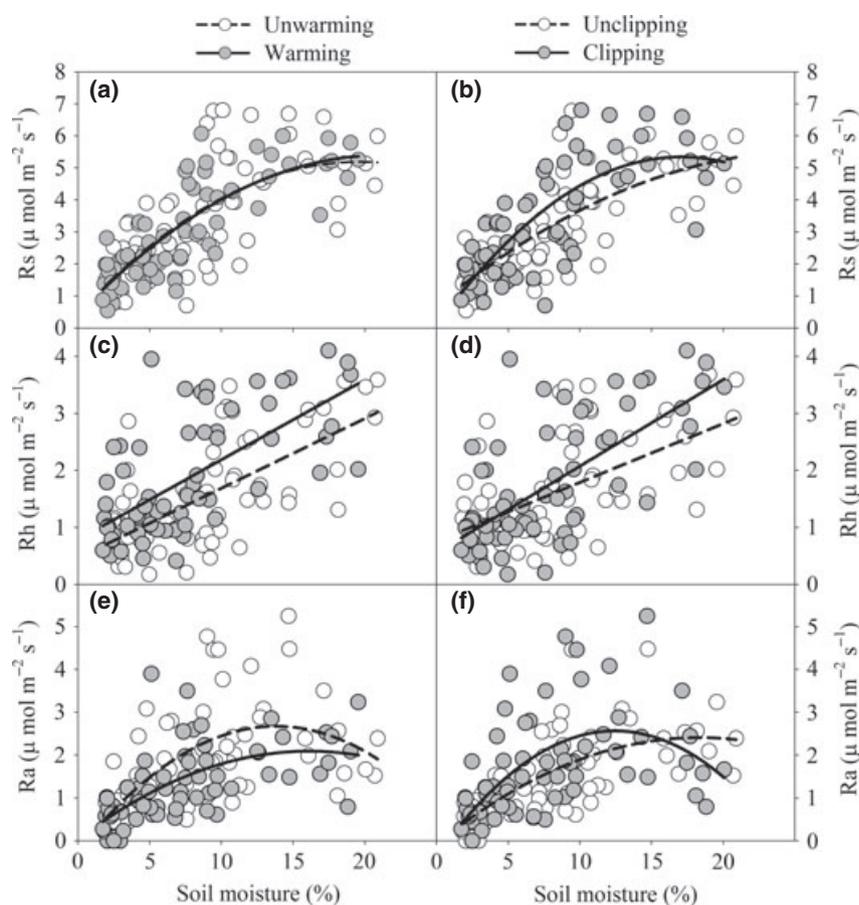


Fig. 4 Relationship between volumetric soil moisture and R_s (Unwarming: $R^2 = 0.50$, $P < 0.0001$; warming: $R^2 = 0.68$, $P < 0.0001$; unclipping: $R^2 = 0.59$, $P < 0.0001$; clipping: $R^2 = 0.62$, $P < 0.0001$, $n = 74$), R_h (Unwarming: $R^2 = 0.45$, $P < 0.0001$; warming: $R^2 = 0.43$, $P < 0.0001$; unclipping: $R^2 = 0.34$, $P < 0.0001$; clipping: $R^2 = 0.48$, $P < 0.0001$, $n = 74$), or R_a (Unwarming: $R^2 = 0.34$, $P < 0.0001$; warming: $R^2 = 0.38$, $P < 0.0001$; unclipping: $R^2 = 0.45$, $P < 0.0001$; clipping: $R^2 = 0.33$, $P < 0.0001$, $n = 74$). The left three panels are for unclipping/warming subplots, and the right three panels are for unclipping/clipping subplots. Each point is the mean of four replications.

previous study demonstrated that the 70 cm deep collars were long enough to exclude most living roots (Zhou *et al.*, 2007). It has to be noted that there are some potential problems involved in using this method, including soil moisture change, decomposition of dead roots, and soil microbial community changes (Hanson *et al.*, 2000; Diaz-Pines *et al.*, 2010). Soil moisture and soil microbial community composition in the deep collars (70 cm) were not measured in our study. According to a previous study, soil microbial biomass and community structure was not altered by root exclusion over two growing seasons (Diaz-Pines *et al.*, 2010). Root exclusion methods generally result in a transient increase of soil respiration due to decomposition of dead roots and disturbance (Hanson *et al.*, 2000; Zhou *et al.*, 2007). Our previous study showed that the transient increase of soil respiration lasted about 5 months after collar insertion (Zhou *et al.*, 2007). In the current study, collars were inserted into soil approximately 4 months before the treatment started in July 2009.

Only data after January 2010 are presented in this paper, so the effect of dead root decomposition on measured R_h should have been minimized. However, since detritus inputs through dead root and root exudates are excluded, root exclusion methods may underestimate R_h , and in turn overestimate R_a .

Responses of R_s and its source components to warming and clipping

In the present study, experimental warming had opposite effects on R_h and R_a , with R_h being stimulated while R_a suppressed (Figs 2 and 6). The net effect of the opposite responses of the two components resulted in a neutral response of R_s to warming (Figs 2 and 6). R_s has generally been found to increase under warming conditions as revealed by two meta-analyses (Rustad *et al.*, 2001; Wu *et al.*, 2011). But neutral or negative warming effects have also been reported especially by studies conducted at arid or semiarid conditions

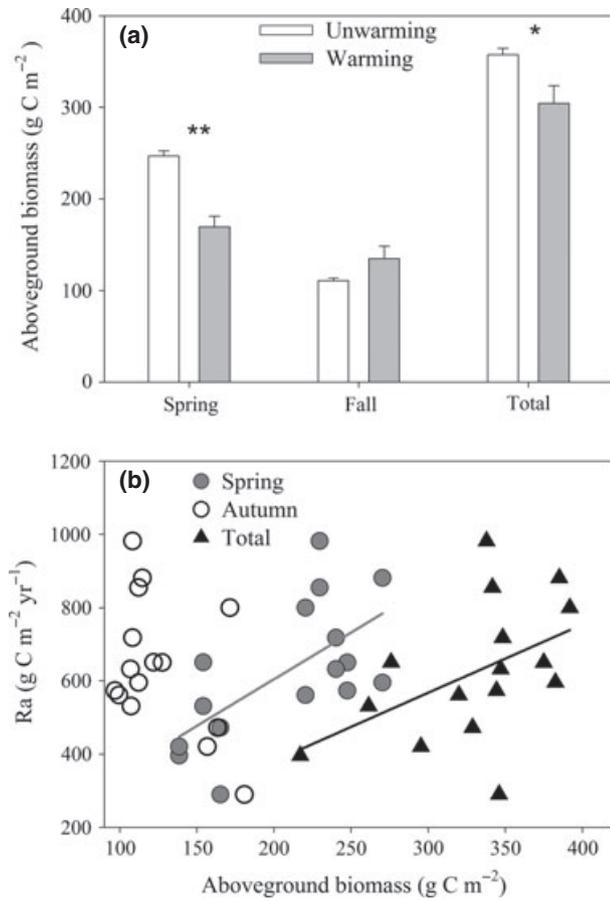


Fig. 5 (a) Main effect of warming on peak aboveground biomass (AGB, mean \pm SE, $n = 8$) in spring (late April), fall (late August), and annual total in 2012; (b) dependence of Ra on AGB in spring ($R^2 = 0.41$, $P = 0.008$, $n = 16$), AGB in fall ($R^2 = 0.23$, $P = 0.36$, $n = 16$), and annual total AGB ($R^2 = 0.23$, $P = 0.06$, $n = 16$). Asterisk denotes significant difference (** $P < 0.01$; * $P < 0.05$) for AGB between warming and unwarming treatments.

(Saleska *et al.*, 1999; De Boeck *et al.*, 2007; Liu *et al.*, 2009). Very limited studies have simultaneously measured the warming effects on Rh and Ra in the warming manipulation experiments. To our knowledge, most of the available studies have reported positive effect of warming on both Rh and Ra (Zhou *et al.*, 2007; Schindlbacher *et al.*, 2008; Bond-Lamberty & Thomson, 2010). But warming-induced decrease of both Rh and Ra was also reported in a mesocosm study, in which Rs was partitioned into Rh and Ra using deconvolution analysis (Zhou *et al.*, 2010). It should be noted that mesocosm study may change environmental conditions and hence the results may be different from those observed under field conditions. For example, the soil properties and plant community were similar in two warming manipulation experiments conducted as mesocosm (Arnone *et al.*, 2008; Zhou *et al.*, 2010) or under

field conditions (long-term warming experiment) (Zhou *et al.*, 2007), but warming decreased both Rh and Ra according to deconvolution analysis of the measured Rs in the mesocosm experiment (Zhou *et al.*, 2010), while increased both Rh and Ra under field conditions (Zhou *et al.*, 2007). The current study was conducted at a site near (<1 km) the long-term warming experiment (Wan *et al.*, 2005; Zhou *et al.*, 2006, 2007; Luo *et al.*, 2009), but the plant communities were different as aforementioned. The results indicated that the warming effects on Rs and its components were different between the two sites, probably implying that plant community composition played an important role in soil respiration responses to warming.

Ra is related to the activities of roots and their symbionts (Hanson *et al.*, 2000; Kuzyakov, 2006), which heavily depend on the import of new C from the canopy during the growing season (Fig. 6). It has been shown that for herbaceous plants, photosynthesis and Ra are directly coupled on timescales ranging from minutes to a few days (Craine *et al.*, 1999; Pregitzer *et al.*, 2000; Bahn *et al.*, 2009; Kuzyakov & Gavrichkova, 2010). Field studies demonstrated that Ra was significantly related to AGB (a proxy of plant productivity) (Hogberg *et al.*, 2001; Flanagan & Johnson, 2005; Yan *et al.*, 2010), while many empirical models estimate Ra according to root or plant productivity (e.g., GPP, NPP and root biomass) (Pregitzer *et al.*, 2000; Piao *et al.*, 2010; Zhou *et al.*, 2010). Our study also showed that annual Ra was significantly related to AGB, supporting that the decreased Ra was caused by the warming-induced decline in plant productivity. Consistent with the current study, increases of Ra were accompanied with warming-stimulated plant production at the nearby experiment (Zhou *et al.*, 2007; Luo *et al.*, 2009; Xu *et al.*, 2012), in which both AGB and belowground biomass were stimulated by warming. It appears that whether warming increases or decreases Ra is dependent on how warming affects plant productivity.

Rh depends on substrate quality, quantity, and other factors (Hanson *et al.*, 2000). The largest substrate source of Rh was proposed to originate from the decay of young organic matter inputs of new above- and belowground detritus into the soil that are linked to photosynthate supply (Bond-Lamberty *et al.*, 2004). Because of the strong dependence of Rh on new C inputs, Rh showed strong seasonal patterns because of the seasonality in C supply even under constant temperature and moisture conditions (Verburg *et al.*, 2004). Positive relationship has been reported between Rh and total detritus input or aboveground litterfall (Bond-Lamberty *et al.*, 2004). In the present study, significant relationship was not found between Rh and aboveground litterfall (which was assumed to be

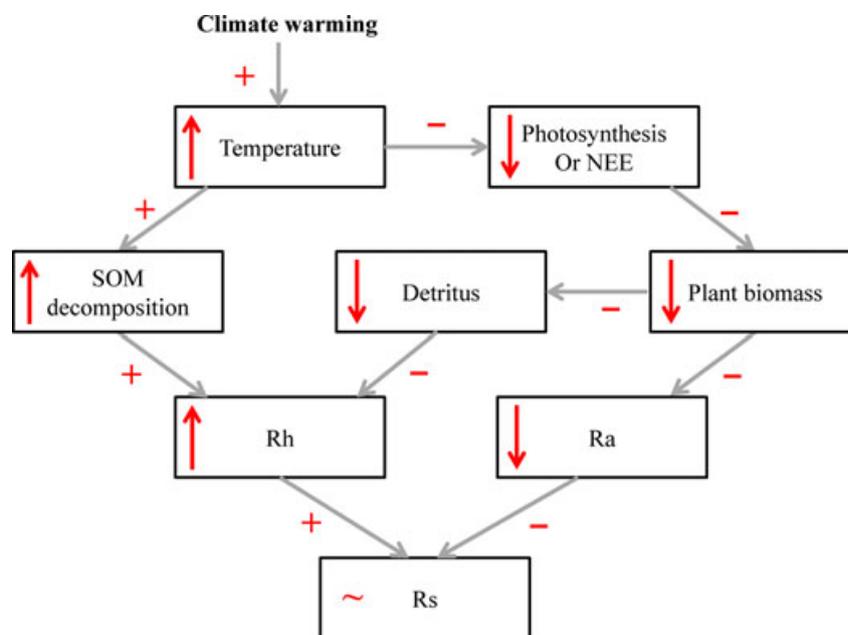


Fig. 6 A diagram showing the mechanisms and processes that regulate heterotrophic respiration (Rh), autotrophic respiration (Ra), and total respiration (Rs) under warming conditions in the winter annual-dominated prairie. +, – and ~ represent positive, negative, and neutral effects. Upward and downward arrows stand for increase or decrease of the corresponding variables, respectively. NEE denotes net ecosystem exchange.

equivalent to annual AGB for the annual grassland) partly due to the differential warming effects on Rh and litterfall (or AGB), i.e., warming-stimulated Rh, but suppressed aboveground litterfall. This pattern could not be explained by a detritus-dominated dynamics of Rh, since Rh should have been reduced by warming along with the decline of detritus input (Fig. 6). The opposite responses of Rh and detritus input likely entailed changes of decomposer community composition which stimulated the decomposition of SOM under warming (Fig. 6). According to a study conducted at a nearby site (Wan & Luo, 2003), respiration from decomposition of SOM and dead roots is three times that from decomposition of aboveground litter with the former contributing 56% and the latter 14% to total soil respiration. Therefore, warming effect on Rh from decomposition of SOM and dead roots is likely much more important in determining the overall responses of Rh to warming (Fig. 6). Further studies are needed to investigate how the microbial community has been changed which results in the enhanced SOM decomposition.

Clipping can affect Rs and its components through several possible mechanisms, including increased soil temperature (Wan *et al.*, 2002; Bahn *et al.*, 2006), enhanced root productivity (Lin *et al.*, 2011; Xu *et al.*, 2012), reduced aboveground litter inputs, and temporarily reduced supply of current photosynthates to roots and symbionts (Craine *et al.*, 1999; Zhou *et al.*,

2006). The reported effects of clipping on Rs and its components are contradictory and depend on the combined effects of the above factors. In the current study, clipping only marginally stimulated Ra in 2010 but had no effect on Rh, and significantly interactive effects of warming and clipping were not observed. Therefore, warming effects on Rs and its components were not likely mediated by clipping according to the current study.

Temporal variability of Rs and its source components

Temporal variability of soil respiration may be caused by substrate availability, temperature, and soil moisture, which have been recognized as the major factors controlling soil respiration (Luo & Zhou, 2006). 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 (Wan *et al.*, 2007). Both diurnal and seasonal variation of soil respiration have been reported to follow patterns similar to soil temperature dynamics (Luo & Zhou, 2006), i.e., with one peak of soil respiration during the middle of the growing season. But this pattern of soil respiration variation is frequently affected by soil moisture with soil respiration decreased during drought events (Zhou *et al.*, 2007). In the current study, there were many drops in soil

respiration and its components, especially R_a , so that a major peak of soil respiration during the growing season was masked, particularly in 2011 and 2012 and for R_a (Fig. S2). This could be caused by two reasons. First, soil respiration is not only controlled by soil temperature but also by plant productivity. If both soil temperature and plant productivity show the concomitant temporal patterns, leading to one peak during the middle of the growing season, strong relationship between soil respiration and soil temperature have been frequently found (Wan *et al.*, 2007; Zhou *et al.*, 2007). However, when the temporal dynamics of plant productivity is not synchronous with that of temperature, such as for winter wheat or in Mediterranean regions, soil respiration vary seasonally with plant phenology, leaf area index (Pendall *et al.*, 2001) and soil water availability (Luo *et al.*, 1996). Similarly, the current study shows that the temporal dynamics of winter annual productivity is not synchronous with that of temperature, and soil respiration was strongly related to soil moisture but not soil temperature. Second, soil respiration was strongly affected by frequent drought events, especially in historically drought years of 2011 and 2012. This was demonstrated by the seasonal pattern of R_h , which was less affected by root productivity. R_h decreased during drought events, so a strong relationship between R_h and soil temperature could have been masked.

Inter-annual variability of R_s and its components have been frequently observed in both grassland and other ecosystems (Lin *et al.*, 2001; Zhou *et al.*, 2007; Concilio *et al.*, 2009; Liu *et al.*, 2009). In the present study, annual R_s was lower in 2011 and 2012 than in 2010 mainly due to the decline of annual R_h . The lower annual R_h in 2011 and 2012 was likely caused by concurrent decrease of precipitation and in turn soil moisture (Fig. 1). The low water availability in 2011 and 2012 likely limited soil microbial activity and SOM decomposition. Another possibility was that labile SOM fraction within the deep collars gradually became depleted due to decreased inputs of belowground detritus.

Whether the warming effect on SOM decomposition lasts or not have caused much attention. Several studies show that plants and soil processes respond to climate warming in a transient way (Lin *et al.*, 2001; Rustad *et al.*, 2001; Melillo *et al.*, 2002). For example, Lin *et al.* (2001) reported that R_s increased significantly under warming in a two-year experiment, but the enhancement was much less in the second year for both R_s and SOM decomposition. In the current study, the warming effects on both R_h (positive) and R_a (negative) were amplified in 2011 and 2012 relative to those in 2010. Since precipitation in these 2 years were extremely low, we were curious whether the warming effects were

amplified due to drought, which needs to be explored by further studies.

Implications for soil C balance in winter annual-dominated prairies

The warming-induced decrease of winter annual biomass resulted in the decrease of annual AGB. The C3 species *B. japonicas* was almost the sole species for the spring biomass peak (Fig. S3). It is not clear whether or how the warming-induced decrease of winter annual biomass was caused by interactions of drought and warming. De Boeck *et al.* (2007) reported that warming benefitted plant activity of several perennial C3 species in fall and winter, but not in late spring and summer, when photosynthesis was suppressed under warming due to low soil moisture (De Boeck *et al.*, 2007). As for our study, we observed significant warming-induced decrease of R_a and increase of R_h for the two drought years, but not in 2010 when precipitation was at a normal level. If belowground biomass (BGB) was also decreased by warming as parallel effects of warming on AGB and BGB in a nearby experiment (Luo *et al.*, 2009), soil C storage may decrease over time due to lower detritus inputs and increased R_h under warming. Based on our results, it seems that the *B. japonicas*-dominated prairie may be a net C source under warming and drought conditions, which is projected to occur for most parts of the temperate America in the future (Dai, 2013).

In summary, results from the winter annual-dominated prairie revealed contrasting effects of warming on heterotrophic respiration (R_h) and autotrophic respiration (R_a), with R_h stimulated while R_a decreased. The net effect of the opposite responses of R_h and R_a resulted in no change in total soil respiration (R_s) under experimental warming. Clipping had no significant effects on R_h and R_a . R_s was significantly increased by clipping only in 2010. There were no interactive effects of warming and clipping on R_s and its components, implying that the warming effects on R_s or its components were not mediated by clipping. The decline of R_a was attributable to warming-induced decrease of the winter annual productivity which led to an overall decline of annual plant production. The warming effects on both R_h and R_a have found to persist in the experimental period of 3 years.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

- Figure S1.** Distribution of *B. japonicus* over North America.
- Figure S2.** Temporal variability of soil respiration and its components.
- Figure S3.** Photos showing *B. japonicas* dominance in spring biomass peak.
- Table S1.** *P*-values of repeated measures ANOVA across the 3 years.
- Table S2.** *P*-values of repeated measures ANOVA for each year.