Unchanged carbon balance driven by equivalent responses of production and respiration to climate change in a mixed-grass prairie

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Abstract

Responses of grassland carbon (C) cycling to climate change and land use remain a major uncertainty in model prediction of future climate. To explore the impacts of global change on ecosystem C fluxes and the consequent changes in C storage, we have conducted a field experiment with warming (+3 °C), altered precipitation (doubled and halved), and annual clipping at the end of growing seasons in a mixed-grass prairie in Oklahoma, USA, from 2009 to 2013. Results showed that although ecosystem respiration (ER) and gross primary production (GPP) negatively responded to warming, net ecosystem exchange of CO_2 (NEE) did not significantly change under warming. Doubled precipitation stimulated and halved precipitation suppressed ER and GPP equivalently, with the net outcome being unchanged in NEE. These results indicate that warming and altered precipitation do not necessarily have profound impacts on ecosystem C storage. In addition, we found that clipping enhanced NEE due to a stronger positive response of GPP compared to ER, indicating that clipping could potentially be an effective land practice that could increase C storage. No significant interactions between warming, altered precipitation, and clipping were observed. Meanwhile, we found that belowground net primary production (BNPP) in general was sensitive to climate change and land use though no significant changes were found in NPP across treatments. Moreover, negative correlations of the ER/GPP ratio with soil temperature and moisture did not differ across treatments, highlighting the roles of abiotic factors in mediating ecosystem C fluxes in this grassland. Importantly, our results suggest that belowground C cycling (e.g., BNPP) could respond to climate change with no alterations in ecosystem C storage in the same period.

Keywords: altered precipitation, clipping, ecosystem respiration, grasslands, gross primary production, net ecosystem exchange, net primary production, warming

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Introduction

Increases in temperature and changes in precipitation regimes have already been evident globally (IPCC, 2013). This unprecedented global warming and altered precipitation regimes can have substantial impacts on the terrestrial carbon (C) cycle and its feedback to global climate change (Niu *et al.*, 2013; Xu *et al.*, 2013, 2015; Shi *et al.*, 2015). Variables of particular interest to ecologists include C fluxes and storage, which affect how fast climate will change (e.g., Ciais *et al.*, 2005; Niu *et al.*, 2008; Wan *et al.*, 2009; Lu *et al.*, 2013). The

mechanism by which climate change stands to alter terrestrial C storage is by shifting the balance, net ecosystem exchange of CO_2 (NEE), between C uptake through gross primary production (GPP) and loss through ecosystem respiration (ER) (Ciais *et al.*, 2005; Oberbauer *et al.*, 2007; Piao *et al.*, 2008). Over sufficient timescales, C budgets of an ecosystem are perceived to be in steady state with ER/GPP ratio necessarily constrained at or below 1 (Anderson-Teixeira *et al.*, 2011; Niu *et al.*, 2013). However, this steady state could be disrupted by warming and altered precipitation, leading to altered patterns of C uptake or release.

Temperature is undoubtedly one of the major drivers of terrestrial ecosystem processes (e.g., Rustad *et al.*, 2001; Piao *et al.*, 2008; Lu *et al.*, 2013; Xu *et al.*, 2015). As ER and GPP often respond differently to warming

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(Anderson-Teixeira *et al.*, 2011), the impacts of warming on NEE are less clear. Some empirical studies suggest warming increases net C uptake (Oberbauer *et al.*, 2007; Day *et al.*, 2008). As a result, the ratio of ER/GPP decreases with temperature because the ecosystems tend to capture C in response to increases in temperature. This is supported by global C cycle models that predict increased terrestrial C uptake under warming by 2050 (Canadell *et al.*, 2007; Sitch *et al.*, 2008). However, in view of stronger temperature sensitivity of respiration compared to photosynthesis, ecosystem C release is assumed to be stimulated more than C uptake under warming, leading to decreases in C storage as observed in experiments and as projected in most global biogeochemical models (e.g., Illeris *et al.*, 2004; Friedlingstein *et al.*, 2006; Schuur *et al.*, 2009). No consensus has been reached because knowledge of the responses of NEE and its components (ER and GPP) to climate change are still limited (Niu *et al.*, 2013). Field manipulative experiments, which could shed light on the direction and magnitude of ecosystem C fluxes in response to warming, are thus needed.

Precipitation could profoundly impact ecosystem C fluxes as well, with consequent changes and uncertainties in C storage (e.g., Harper *et al.*, 2005; Patrick *et al.*, 2007; Xia *et al.*, 2009). While supplemental precipitation is reported to enhance ER and GPP, reduced precipitation usually suppresses them (Niu *et al.*, 2008). However, the magnitude of changes in ER and GPP may not be equal, and so the effect on NEE is less clear. For example, increases in NEE have been reported because GPP increased more than ER in a temperature steppe (Niu *et al.*, 2008). Moreover, changes in precipitation are expected to influence responses of C fluxes to warming. The interactive effects of altered precipitation and warming are of particular relevance as increased precipitation could favor the positive effects of warming on ecosystem processes and decreased precipitation could enhance water stress induced by warming (Xu *et al.*, 2013). A modeling study using four models by Luo *et al.* (2008) showed that warming combined with doubled precipitation consistently increased GPP across seven ecosystems in different climate zones. The impacts of warming and halved precipitation, however, were not consistent, with decreases and no changes in GPP projected. Current changes in temperature and precipitation may trigger complex interactive influences on ecosystem C fluxes and storage, differing greatly from single-factor responses. But few field studies, with manipulation of both precipitation and temperature, have studied dynamics of C fluxes as indicated by several meta-analyses (Rustad *et al.*, 2001; Niu *et al.*, 2008; Lin *et al.*, 2010).

Land-use practices, such as clipping to mimic hay/biofuel harvest, could make the responses of C fluxes to climate change more complex. In the southern Great Plains of the USA, hay harvest is a widely practiced land use and hay production occupies 3.25 million acres in Oklahoma (USDA, National Agricultural Statistic Service). Clipping may substantially decrease GPP because it directly removes aboveground biomass and stimulate ER by altering allocation patterns between roots and shoots (Xu *et al.*, 2012). In addition, clipping could affect ecosystem C fluxes and ER/GPP ratio by enhancing evapotranspiration and exacerbating water stress (Niu *et al.*, 2008; Xu *et al.*, 2012). Clipping has the potential to fundamentally alter ecosystem C cycling (e.g., Niu *et al.*, 2013; Xu *et al.*, 2013). In comparison with the other global change aspects such as increased temperature and altered precipitation, the impacts of land use (clipping) on C-climate feedbacks are poorly understood.

In this study, we assessed the influence of global changes, including warming, altered precipitation, and annual clipping, in influencing ecosystem C fluxes in a mixed grassland in Oklahoma, USA. Grassland ecosystems, accounting for ca. 54% of the conterminous USA, play an essential role in climate regulation and global C cycle. They are ideal for addressing responses of ecosystem C cycling to global changes as grasslands' rapid response to them (Sherry *et al.*, 2008; Xu *et al.*, 2013). Specifically, we aimed to: (1) examine the magnitude and directions of ecosystem C fluxes (ER, GPP, and NEE) in response to global changes and (2) explore the roles of soil temperature and moisture in shaping ecosystem C balance. This provides a basic understanding of how the C balance of a mixed-grass prairie in the southern Great Plains will respond to multiple aspects of global change.

Materials and methods

Experimental site and design

The experimental site is located on the Kessler Atmospheric and Ecological Field Station in Oklahoma, USA (34°59′N, 97°31′W). The site is on an old-field prairie abandoned from field cropping 40 years ago with light grazing until 5 years ago. The soil is part of the Nash–Lucien complex with neutral pH. Mean annual temperature (MAT) is 16.3 °C, with monthly air temperature ranging from 3.3 °C in January to 28.1 °C in July. Mean annual precipitation (MAP) is 914 mm (Oklahoma climatological survey, Norman, OK, USA). The site is dominated by C_3 forbs (*Ambrosia trifida*, *Solanum carolinense*, and *Euphorbia dentate*) and C_4 grasses (*Tridens flavus*, *Sporobolus compositus*, and *Sorghum halapense*). C_3 forbs and C_4 grasses account for 70% and 30% of the total aboveground biomass, respectively.

The site uses a randomized block design to manipulate temperature and precipitation, within which is nested a clipping factor (Xu *et al.*, 2013). Each treatment is randomly repeated four times for a total of 24 plots of 2.5 × 3.5 m. The distance between adjacent plots is at least 3 m. Infrared heaters have been used since July 2009 to achieve whole ecosystem warming. In each warmed plot, two infrared heaters (165 × 15 cm; Kalglo Electronics, Bethlehem, PA, USA) are suspended approximately 1.5 m above the ground to warm the area of 2.5 × 1.75 m. The control plot has 'dummy' heaters with the same dimensions as the infrared heaters suspended at a similar height to mimic the shading effects of the heaters. We use a rainfall-collection-redistribution device to double precipitation and a rainout-shelter to halve precipitation. To minimize disturbance, we inserted fiberglass sheets into the ground to a depth of 120 cm around each plot to cut off lateral movement of soil water. The lower side of the rainout-shelters tilts toward the prevailing upwind direction. Each 2.5 × 3.5 m plot is divided into two 2.5 × 1.75 m subplots. Plots under doubled and halved precipitation treatments on average received 195.8% and 70.1% of the ambient precipitation, respectively (Xu *et al.*, 2013). Plants in one of the 2.5 × 1.75 m subplots are clipped at a height of 10 cm above the ground once a year (usually in late August at the peak biomass) to mimic the land-use practice of mowing for hay while the other subplot is unclipped. Clipped materials are taken away and not returned to the plots. The unclipped group or the clipped group each has six treatments: control (ambient) temperature and ambient precipitation (CA), control temperature and doubled precipitation (CD), control temperature and halved precipitation (CH), warming and ambient precipitation (WA), warming and doubled precipitation (WD), and warming and halved precipitation (WH).

Soil microclimate and plant production

Soil temperature was monitored by thermocouples at a depth of 7.5 cm in the centers of one clipped and one unclipped subplots in each plot. Hourly data were recorded by a CR10X data logger (Campbell Scientific, Logan, Utah, USA). Volumetric soil water content in the top 12 cm was manually measured once or twice a month using portable Time Domain Reflectometry equipment (Soil Moisture Equipment Crop., Santa Barbara, CA, USA). Aboveground net primary production (ANPP) was directly measured by annual clipping at peak biomass (usually August) in the clipped subplots and indirectly estimated by pin-contact method (Frank & Mcnaughton, 1990) in the unclipped subplots. A detailed description of biomass estimation is provided by Sherry *et al.* (2008). The root ingrowth-core method was applied to estimate belowground net primary production (BNPP) (Xu *et al.*, 2013). Briefly, soil cores (5.2 cm in diameter, 90 cm in length) were taken once a year in October at an angle of 90° from the same spots in one unclipped and one clipped subplots of each plot. Root samples were carefully washed by wet sieving (0.5 mm), oven-dried at 70 °C for 48 h, and weighted to calculate BNPP. NPP was estimated as the sum of ANPP and BNPP.

Ecosystem C fluxes measurements

We measured ecosystem C fluxes monthly in one clipped and one unclipped subplot in each plot between 10 : 30 and 15 : 00 (local time) from August 2009 to December 2013. In each subplot, one square aluminum frame (0.5 × 0.5 m) was permanently installed into soil at 3 cm depth. Each side of the frame is 3 cm wide and provides a flat base between the soil surface and the CO_2 sampling chamber. We measured ecosystem C exchange with a LI-COR 6400 (LI-COR. Inc., Lincoln, NE, USA) attached to a transparent chamber (0.5 × 0.5 × 0.6 m), which covered all the vegetation appeared within the aluminum frame. The radiation is reduced by 8% within the chamber, which was determined by a LI-COR 2003S (LI-COR. Inc.). One small electric fan was running continuously to mix the air within the chamber during the measurement. For each measurement, nine consecutive recordings of CO_2 and water vapor concentration were taken at 10-s intervals during a 90-s period after a steady state condition was achieved within the chamber. During the measurement, CO_2 concentration was allowed to build up or draw down over time, from which flux rates were determined and to calculate NEE. Increases in air temperature within the chamber during the measurement were about 0.2 °C. Details about these static-chamber flux calculations can be found in the soil-flux calculation procedure in the LI-COR 6400 manual. Following NEE measurement, the chamber was vented, replaced on the frame, and covered with an opaque cloth. We repeated the CO_2 exchange measurement to calculate ER. The difference between NEE and ER was considered to represent instantaneous GPP for the vegetation within the chamber. It is important to point out that GPP is not independent of NEE and can only be estimated from NEE and ER. All GPP results might be affected by this dependence.

Statistical analysis

We performed repeated-measures split-plot analysis of variance (ANOVA) to examine the main and interactive effects of experimental warming, altered precipitation, clipping, and sampling time on soil temperature and moisture, annual production (ANPP, BNPP, and NPP), and ecosystem C fluxes, including ER, GPP, and NEE. Specifically, warming, altered precipitation, and clipping were treated as the between-subject factors with block as a random factor. To test the warming effect on NEE around March and September, one-way ANOVA was used. Univariate general linear model was used to examine differences in the relationships of ER/GPP ratios with soil temperature and moisture among the twelve treatments. Responses of C fluxes and ER/GPP ratios to soil temperature and moisture were constructed based on 1 or 2 °C temperature bins and 1% moisture bins, respectively, across all treatments (Huxman *et al.*, 2003; Yuan *et al.*, 2011; Niu *et al.*, 2013). Data in months where low temperatures limited plant growth were excluded when analyzing the relationship between soil temperature and ER/GPP ratios. Within each treatment, ER/GPP ratios are binned into 3 °C intervals for soil temperature and 2% for soil moisture. All statistical analyses were

conducted using SPSS 17.0 for windows (SPSS Inc., Chicago, IL, USA).

Results

Soil microclimate and plant production

Soil temperature was significantly increased by warming by approximately 2.75 °C across the years ($P < 0.01$, Figs S1 and 1a). The impacts of altered precipitation and clipping on soil temperature were small (all $P > 0.05$, Table 1). Volumetric soil moisture was significantly affected by warming, altered precipitation, and clipping (all $P < 0.01$, Table 1). It was on average lowered by 6.34%, 3.43%, and 3.56% by the treatments of warming, halved precipitation, and clipping, respectively, and increased by an average of 7.32% under doubled precipitation (Fig. 1b–d). Warming, altered precipitation, and clipping all interacted with sampling time to influencing the seasonal dynamics of soil moisture (all $P < 0.01$, Table 1, Fig. S2). Warming and clipping decreased soil moisture during growing and nongrowing (after clipping) seasons, respectively, due to enhanced evaporation. Doubled and halved precipitation increased and decreased soil moisture, respectively, in spring. No significant relationship between soil temperature and moisture was found ($P > 0.05$). Additionally, warming and clipping significantly increased BNPP (all $P < 0.05$), but did not alter ANPP or NPP much (Table 1, Fig. 2). While altered precipitation had no effect on ANPP and BNPP ($P = 0.10$ and 0.11, respectively, Table 1, Fig. 2), doubled precipitation increased NPP and halved precipitation decreased NPP ($P < 0.05$, Table 1, Fig. 2).

Ecosystem C fluxes

Warming and altered precipitation significantly influenced ER and GPP (all $P < 0.01$, Table 1), but not NEE (all $P > 0.05$, Table 1). Overall, warming and halved precipitation decreased ER and GPP and had small impacts on NEE (Fig. 3a–f). Doubled precipitation increased ER and GPP and affected NEE little (Fig. 3d–f). Clipping significantly increased ER, GPP, and NEE (all $P < 0.01$, Table 1, Fig. 3g–i). Greater ecosystem C uptake (GPP) than release (ER) led to a net sink (NEE) under clipping treatment. No significant interactive impacts of among warming, altered precipitation, and clipping were found on C fluxes (all $P > 0.05$, Table 1). Additionally, we observed strong seasonal variation in the responses of NEE to warming ($P = 0.02$, Table 1, Fig. 4) and of C fluxes to clipping (all $P < 0.01$, Table 1, Fig. S3). Warming usually suppressed NEE around March and early September, but did not greatly alter it at the other times (Fig. 4). Clipping in general stimulated C fluxes across the sampling times (Fig. S3). Generally, we did not observe interannual variation in C fluxes (Fig. S4).

Relationships of C fluxes with soil temperature and moisture

Responses of C fluxes to soil temperature increased with increasing temperatures, peaking, and declining at

Table 1 Results (P values) of repeated-measures split-plot ANOVA for responses of soil temperature and moisture (T_{soil}, W_{soil}), aboveground and belowground net primary production (ANPP and BNPP), NPP (the sum of ANPP and BNPP), ecosystem respiration (ER), gross primary production (GPP), and net ecosystem exchange (NEE) to warming (W), altered precipitation (PPT), clipping (C), sampling time (T, date for C fluxes/year for NPPs), and their interactions from 2009 to 2013. P values smaller than 0.05 are in bold

	df	T_{soil}	W_{soil}	ANPP	BNPP	NPP	ER	GPP	NEE
W	1	**<0.001**	**<0.001**	0.57	**0.02**	0.18	**<0.001**	**<0.001**	0.18
PPT	2	0.75	**0.001**	0.10	0.11	**0.03**	**<0.001**	**0.001**	0.33
C	1	0.59	**0.008**	0.40	**0.01**	0.22	**<0.001**	**<0.001**	**<0.001**
W × PPT	2	0.96	0.08	0.14	0.32	0.20	0.05	0.11	0.55
W × C	1	0.89	0.92	0.69	0.30	0.73	0.99	0.80	0.73
PPT × C	2	0.87	0.15	0.10	0.53	0.17	0.21	0.14	0.09
W × PPT × C	2	0.94	0.15	0.68	0.53	0.44	0.79	0.69	0.75
T	48/4	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
W × T	48/4	0.93	**<0.001**	0.41	0.28	0.23	0.19	0.46	**0.02**
PPT × T	96/8	1.00	**<0.001**	0.10	0.20	0.52	0.45	0.09	0.07
C × T	96/4	0.99	**<0.001**	**0.03**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
W × PPT × T	96/8	1.00	0.31	0.73	0.41	0.40	0.15	0.07	0.11
W × C × T	96/4	1.00	0.78	0.57	0.78	0.61	0.77	0.39	0.08
PPT × C × T	96/8	1.00	0.88	0.87	0.96	0.93	0.15	0.34	0.68
W × PPT × C × T	96/8	1.00	0.87	0.83	0.90	0.94	0.29	0.30	0.26
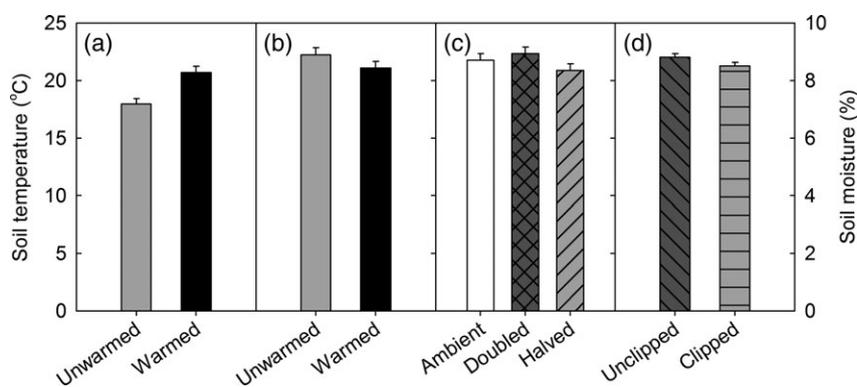
Fig. 1 The main effects of warming on soil temperature (a) and moisture (b) and of altered precipitation (c) and clipping (d) on soil moisture.
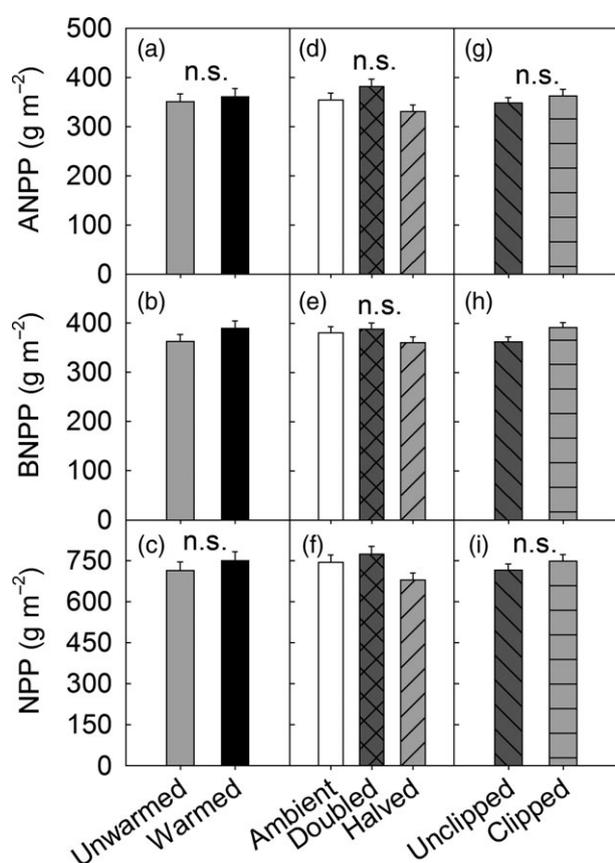
Fig. 2 The main effects of warming (a–c), altered precipitation (d–f), and clipping (g–i) on ANPP (a, d, and g), BNPP (b, e, and h), and NPP (e, f, and i). ANPP and BNPP, aboveground and belowground net primary production.

Fig. 3 The main effects of warming (a–c), altered precipitation (d–f), and clipping (g–i) on ER (a, d, and g), GPP (b, e, and h), and NEE (e, f, and i). ER, ecosystem respiration; GPP, gross primary production; NEE, net ecosystem carbon exchange.

an optimal temperature ca. 15 °C (Fig. 5a). But responses of C fluxes to soil moisture showed a different pattern with a small peak around 7% of soil moisture and a larger one around 16% of soil moisture (Fig. 5b). NEE was nearly 0 when soil moisture was

approaching 13%. The ratio of ER/GPP significantly increased with increasing soil temperature and moisture across all treatments (Figs 6 and 7). The relationships were all significant except for the one ($P = 0.065$)
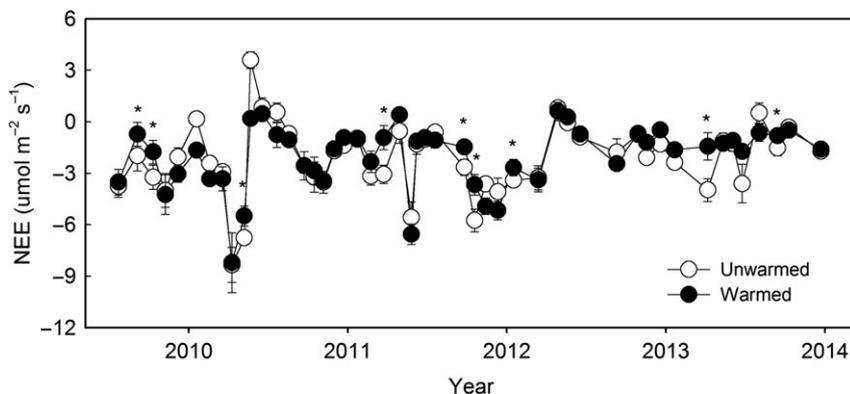
Fig. 4 Effects of warming on seasonal dynamics of NEE. Stars (*) indicate significantly decreases in NEE under warming around March and September. See Fig. 3 for abbreviations.
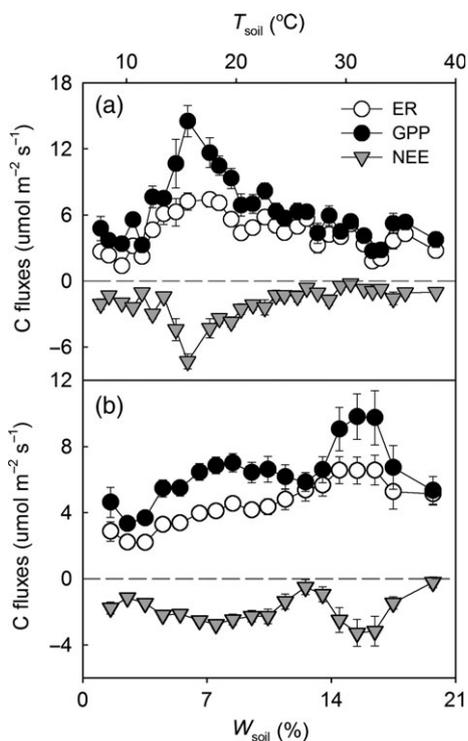
Fig. 5 Ecosystem carbon fluxes (ER, GPP, and NEE) over the years as a function of soil temperature (a) and moisture (b) across all treatments. See Fig. 3 for abbreviations.

between soil temperature and ER/GPP under WD treatment in clipped plots (Table S1). No treatment effects were found between the ratio-response curves to soil temperature and moisture (all $P > 0.05$).

Discussion

Ecosystem C processes in response to warming, altered precipitation, and clipping vary in direction and magnitude (e.g., Xia *et al.*, 2009; Anderson-Teixeira *et al.*,
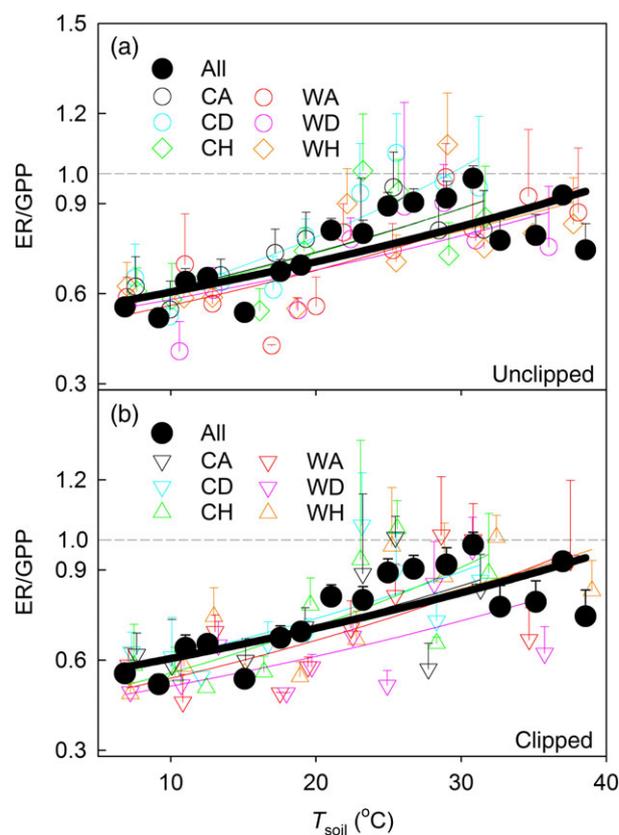
Fig. 6 Relative advantage of respiration over production (ER/GPP) as a function of soil temperature in unclipped subplots (a) and clipped subplots (b) across the years. Colored lines represent significant ($P < 0.05$) within-treatment relationships (except for WD in panel b where $P = 0.065$), and black lines represent the general response across the 12 treatments ($P < 0.01$). See Fig. 3 for ER, GPP, and NEE. CA, control (ambient) temperature and ambient precipitation; CD, control temperature and doubled precipitation; CH, control temperature and halved precipitation; WA, warming and ambient precipitation; WD, warming and doubled precipitation; and WH, warming and halved precipitation.

2011; Abdalla *et al.*, 2013; Niu *et al.*, 2013). Climate change does not necessarily lead to increased C storage or release in terrestrial ecosystems. In this study, we found that significant responses of ER and GPP to warming and altered precipitation largely offset, leading to consequently unchanged NEE. GPP increased more than ER under clipping with increases in NEE observed. BNPP generally responded more than ANPP to climate change and land use although no changes either in NEE or NPP were found. Additionally, relative advantage of ER over GPP (the ER/GPP ratio) consistently increased with increasing soil temperature and moisture across the treatments.

Unchanged C balance under climate change

Warming affects ecosystem C fluxes and C balance by altering plant photosynthesis and plant and heterotrophic respiration (Oberbauer *et al.*, 2007; Niu *et al.*, 2013). In line with previous findings (Niu *et al.*, 2008, 2013), our results showed that warming significantly decreased ER and GPP equally across the years. The negative effects of warming on C fluxes resulted partly because warming stimulated evapotranspiration, reduced soil moisture, and exacerbated water stress (Xia *et al.*, 2009). Although warming could stimulate heterotrophic respiration (Rustad *et al.*, 2001), increased water stress may suppress and balance the stimulation. Additionally, the plant community is mainly composed of C_3 species, warming is likely to suppress their photosynthesis and respiration simultaneously due to their low optimal temperatures (Sitch *et al.*, 2008; Niu *et al.*, 2013). As a result, ecosystem C balance (NEE) did not change much under warming, which is supported by unchanged NPP and possibly unchanged heterotrophic respiration discussed above. Across the sampling times, we found that NEE was suppressed around March and early September. This resulted because warming favored respiration more especially at the beginning of growing seasons when temperature is low (in March) and because of the senescence of plants at the end of growing seasons (in September). Decreases in ER and GPP in our study are inconsistent with previous findings of positive responses of ER and GPP to warming in tundra (Oberbauer *et al.*, 2007) and are also inconsistent with findings of no changes in ER and GPP under warming in a temperate steppe (Xia *et al.*, 2009). Warming could stimulate plant growth and respiration in these systems by breaking the low-temperature limit on plant physiology at some point (MAT: ca. −12.0 °C for the tundra and 2.1 °C for the steppe). In the steppe with a MAP of 383 mm, however, the increases in ER and GPP is likely to be offset by the decreases resulted from warming-enhanced water stress. These discrepancies
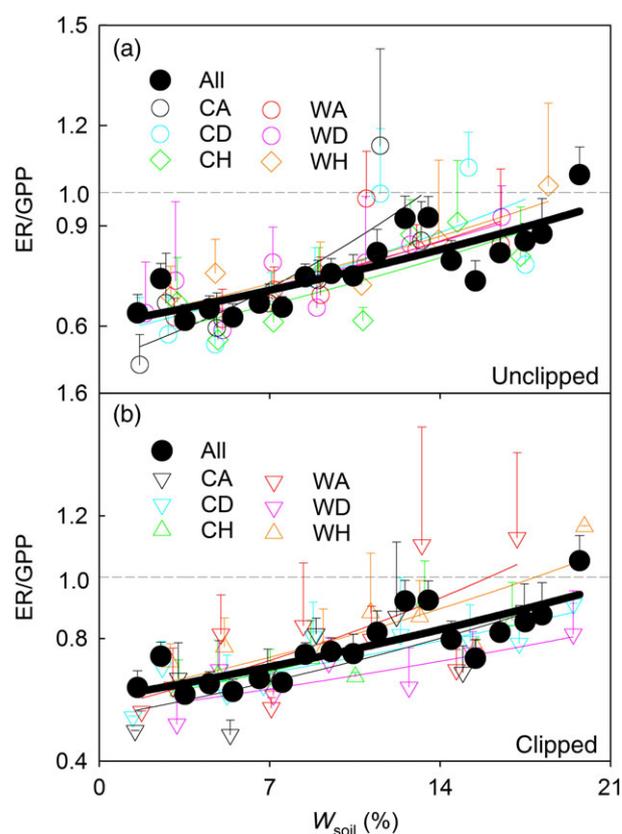
Fig. 7 Relative advantage of respiration over production (ER/GPP) as a function of soil moisture in unclipped subplots (a) and clipped subplots (b) across the years. Colored lines represent significant within-treatment relationships (all $P < 0.05$), and black lines represent the general response across the 12 treatments ($P < 0.01$). See Fig. 6 for abbreviations.

highlight ecosystem-specific responses of ER and GPP to warming depending on initial conditions (Shaver *et al.*, 2000). As generally reported in site-level studies (Xia *et al.*, 2009; Niu *et al.*, 2013) and in synthesis studies (Niu *et al.*, 2008; Yuan *et al.*, 2009), NEE is relatively stable due to the offsetting of responses of ER and GPP to warming.

Ecosystem C fluxes are sensitive to changes in precipitation (e.g., Niu *et al.*, 2008; Xia *et al.*, 2009). In accordance with precious findings, we found that doubled precipitation stimulated ER and GPP, whereas halved precipitation suppressed ER and GPP (Niu *et al.*, 2008). Signals of altered precipitation were modified into differential biological activity, impacting performance of the grassland (Hamerlynck *et al.*, 2002). In terms of heterotrophic respiration and NPP, doubled and halved precipitation is likely to increase and decrease them as well, consistent with the findings on C fluxes. These results indicate critical roles of water in regulating ecosystem C fluxes in this mixed-grass prairie in Oklahoma. In view of

treatment interactions, our results in general showed that none of the two-way interactions between warming and altered precipitation greatly affected plant growth and respiration. These results raise the question whether the treatments of warming x altered precipitation are of sufficient magnitude to create a strong response, which may be a great contribution to synthetic efforts examining treatment magnitude vs. response magnitude. Although doubled precipitation could ameliorate the effect of warming on C fluxes and halved precipitation could enhance the effect of warming on them, low statistical power limited the strength of these conclusions. However, these suggest that results from single-factor experiments are useful to informing us of potential ecosystem C storage in response to multifactor global change. Interestingly, we found that BNPP generally responded more than ANPP to climate change with no significant changes in NEE observed in the same period. Belowground C processes (e.g., BNPP) may change without alterations in ecosystem C storage.

Impact of clipping on C fluxes

Clipping can substantially alter ecosystem C fluxes (Rogiers *et al.*, 2005; Searchinger *et al.*, 2008; Niu *et al.*, 2010). Our study showed that clipping significantly stimulated ER, GPP, and NEE, consistent with the findings in a temperature steppe (Niu *et al.*, 2010). Possible reasons accounting for the positive effects of clipping on C fluxes include but not limited to: (1) increased seed germination at the beginning of growing seasons because clipping decreased litter accumulation and could facilitate germination (Ruprecht & Szabo, 2012); (2) increased photosynthesis under improved light conditions (less shading) by removal of standing litter (Niu *et al.*, 2010; Xu *et al.*, 2012); and (3) clipping favored the growth of the winter annual, *Bromus japonicas* Thunb., which highly dominated the site in winter and early spring (Li *et al.*, 2013). Unchanged NPP under clipping is not contradictory to the increases in NEE because heterotrophic respiration is likely to decrease with little fresh C input. Interestingly, clipping did not result in sharp decreases in GPP and NEE. This is because clipping in our study is usually carried out at the end of growing seasons when plants are senescent. The discrepancy between our study and the study by Rogiers *et al.* (2005), which finds that clipping in the middle of growing season (June) greatly decreased ecosystem C uptake, can be reasonably explained. In addition, clipping did not significantly interact with warming and altered precipitation to influencing ecosystem C fluxes,

indicating small additive effects of land use and climate change. Our results suggest that use of prairie for hay/biofuel production does not necessary increase greenhouse gases emission. The timing of clipping is of importance for C sequestration.

Regulation of soil temperature and moisture on C fluxes

The single peak curves of the responses of ecosystem C fluxes to temperature are widely reported (e.g., Anderson-Teixeira *et al.*, 2011; Niu *et al.*, 2013). Consistently, similar curves were observed in our study and we found an optimal temperature of ca. 15 °C for the C fluxes. It is relatively low because our site is dominated by C_3 species (Sitch *et al.*, 2008). In terms of soil moisture, surprisingly, we found two peaks for the moisture response curves of C fluxes. This resulted probably because the winter annual dominated the site in winter and early spring. The two peaks in GPP and NEE as well as the valley between the two peaks, when soil moisture was ca. 7%, 16%, and 13%, respectively, were concurrent with the two peak biomass periods of *B. japonicas* and the ecosystem and the senescence of *B. japonicas*.

On the other hand, production and respiration responded differently to soil temperature and moisture. Respiration responded more strongly to temperature than did photosynthesis, indicated by the positive relationship between temperature and the ratio of ER/GPP. As a result, the system tends to release C with increasing temperature (Oechel *et al.*, 1993; Rustad *et al.*, 2001; Illeris *et al.*, 2004; Atkin *et al.*, 2007). The ratio of ER/GPP reached ca. 1 around 31 °C. This must be resulted from the senescence of the winter annual in late spring as well as the end of growing seasons in September. Additionally, lower ER/GPP ratios found in the temperature range between 32 and 40 °C resulted because plants grew well and reached the peak biomass in summer when temperature was high. C_3 species (shrub and forb) usually concentrate their roots deeper to 30 cm than C_4 grasses do to 10 cm (Nippert & Knapp, 2007; Xu *et al.*, 2014), which may ameliorate the negative impact of high temperature on their growth. We also found that the ratio of ER/GPP increased with increasing soil moisture, resulting from the following: (1) respiration was favored more than photosynthesis by higher soil moisture possibly through stimulated microbial activity. In general, soil moisture was low at this site, and therefore, microbial activity was not likely inhibited by 'high' soil moisture; (2) the two fast growth periods of the winter annual and the peak biomass were associated with relatively low soil moisture (<13%). Oklahoma usually has wet springs and dry summers: rain and heat are not in the same period. The

observed low soil moisture during the fast growth of *B. japonicas* resulted because of the low temperature in spring. With the increasing soil moisture (>13%), plants of the peak biomass period began to grow and sequester C.

To conclude, our results from the 5-year time frame global change experiment in the mixed-grass prairie suggest that belowground C cycling could be sensitive to climate change with unchanged ecosystem C storage. Additionally, hay/biofuel harvest (clipping) at the end of growing seasons could be an effective land practice that does not necessarily reduce C storage. Whether the observed responses of C fluxes to global changes persist in the long-term will be tested with further monitoring of the plots.

Author contribution

XX, ZS, and YL (Yiqi Luo) conceived the idea. XX analyzed the data and wrote the article with input from all authors. YL (Yiqi Luo) designed the experiment. XX, XC, SN, LJ, and RL conducted the measurements.

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Supporting Information

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

Figure S1. Seasonal dynamics of soil temperature (T_{soil}) in unclipped subplots (a) and clipped subplots (b) across the years.
Figure S2. Effects of warming (a), altered precipitation (b), and clipping (c) on seasonal dynamics of soil moisture.
Figure S3. Effects of clipping on seasonal dynamics of ER (a), GPP (b), and NEE (c).
Figure S4. Response ratios of carbon (C) fluxes associated with warming (a, d, g), altered precipitation (b, e, h), and clipping (c, f, i). Values are average ratios of each year of the C fluxes between warmed and unwarmed (a, d, g), between doubled (black) or halved (grey) precipitation and ambient precipitation (b, e, h), and between clipped and unclipped (c, f, i). High ratios of ER (c) and GPP (f) in 2009 resulted probably because measurements began in August 2009 and clipping significantly increased photosynthesis and respiration of the winter annuals. ER, ecosystem respiration; GPP, gross primary production.
Table S1. Relationships of ER/GPP with soil temperature and moisture across treatments.