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Rain use efficiency as affected by climate warming and biofuel harvest: results from a 12-year field experiment

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Abstract

The efficiency of a terrestrial ecosystem to use rainfall in production is critical in regulating the ecological functions of the earth system under global change. However, it remains unclear how rain use efficiency (RUE) will be altered by changes in climate and human activities such as biofuel harvest. In this study, we used RUE data from a long-term experiment in a tallgrass prairie to analyze the effects of warming and biofuel harvest (clipping). From 2000 to 2011, experimental warming enhanced RUE in most years, with larger positive effects in normal and wet than dry hydrological years. Clipping decreased RUE in dry and normal hydrological years, but had no impact on RUE in wet years. The observed RUE responses resulted from treatment-induced changes in both biologically ineffective (i.e., runoff and soil evaporation) and effective (i.e., transpiration) parts of precipitation. For example, litter cover was increased in warming plots, but reduced by clipping, leading to negative and positive effects on runoff and soil evaporation, respectively. The dominance of C_4 species, which usually have higher water use efficiency than C_3 species, was enhanced by warming, but reduced by clipping. Moreover, RUE was positively correlated with ratios of rainfall in the late growing season (June–August), when the growth of C_4 plants was most active, relative to that in the other seasons. Our results indicate that RUE is positively influenced by climate warming, but negatively affected by biofuel harvest in tallgrass prairie of the Great Plains. These findings highlight the important roles of plant community structure and temporal distribution of precipitation in regulating ecosystem RUE.

Keywords: biofuel harvest, C_3 species, C_4 species, clipping, Great Plains, precipitation, rain use efficiency, warming

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Introduction

Global climate change will greatly impact both carbon and hydrological cycles of terrestrial ecosystems (Luo, 2007), especially grasslands, where water is a key limiting factor to production (Huxman *et al*., 2004; Yu *et al*., 2008; Niu *et al*., 2011). However, as global air temperature continues to increase, it remains unclear whether grassland ecosystems will increase their ability to use precipitation for production under climate warming. As climate warming can stimulate grassland carbon (C) release through litter and soil organic carbon decomposition (Luo *et al*., 2009), warming-induced changes in rain use efficiency (RUE) (the ratio of aboveground net primary production (ANPP) to annual precipitation; Le Houérou, 1984) may critically affect the grassland C influx and thus the ecosystem C balance. Besides climate change, human management of the land (e.g., biofuel harvest) can profoundly affect how grassland species use precipitation (Varnamkhasti *et al*., 1995). However, it is unknown how yearly harvests of biofuels will interact with climate warming to influence the RUE of grassland ecosystems. Thus, a better understanding of RUE and the key factors controlling its response to warming and biofuel harvest are important for projecting C feedbacks to climate change.

Precipitation can be separated into two parts, including 'ineffective' (i.e., runoff and soil evaporation) and 'effective' precipitation (i.e., transpiration, Noy-Meir, 1973). Climate warming can impact grassland RUE by changing the efficiency of plants use of the effective precipitation for production or by changing the allocation between effective and ineffective precipitation. In general, warming promotes the more efficient use of rain water for production by terrestrial plants (Rustad *et al*., 2001; Lin *et al*., 2010). However, various warming effects on aboveground productivity, including positive (Luo *et al*., 2009), negative (De Boeck *et al*., 2008), and none (Dukes *et al*., 2005; Xia *et al*., 2009), have been reported

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in grasslands. It indicates that the impact of warming on RUE could vary greatly among different types of grasslands because of the complex interactive effects between temperature and water availability on ecosystem production (Fay *et al.*, 2011). Both experimental and modeling studies have shown that climate warming usually enhance evaporation and transpiration and therefore reduce plant-level water use efficiency (usually a ratio of ANPP over transpiration) (Allen *et al.*, 2003; Bell *et al.*, 2010; Niu *et al.*, 2011). However, climate warming has been shown to increase transpiration and also decrease runoff (Wetherald & Manabe, 2002), making it very difficult to predict ecosystem-level RUE under climate warming. In fact, great spatiotemporal variation in RUE has been observed in grassland ecosystems, ranging from 0.5 to 2.0 g m^{-2} of dry matter production per millimeter rainfall (Paruelo *et al.*, 1999; Bai *et al.*, 2008; Hu *et al.*, 2010; Yang *et al.*, 2010). Such large variation in RUE results from several factors, including edaphic factors (i.e., soil texture and nutrient availability; Yang *et al.*, 2010) and vegetative constraints (i.e., vegetation cover and species composition; Huxman *et al.*, 2004). Many previous studies have tried to explore the dependence of RUE on precipitation, and found both positive and negative relationships between RUE and mean annual precipitation (Bai *et al.*, 2008; Hu *et al.*, 2010). However, the dependence of RUE on temperature is less known, and the underlying mechanisms of RUE in response to climate warming have not been carefully studied (Bell *et al.*, 2010).

Biofuel harvest, as one important land use in grassland ecosystems, can influence the RUE of grassland ecosystems in many ways. For example, biofuel harvest can directly reduce RUE by removing the aboveground parts of grassland plants, which take up C from the atmosphere for production. The clipping-reduced vegetative cover can lead to a further decrease in grassland RUE by enhancing transpiration and runoff (Day & Detling, 1994). Some studies have found that biofuel harvest can also trigger changes in species composition (Moog *et al.*, 2002), and thus indirectly affect ecosystem RUE. Consequently, biofuel harvest itself is likely to reduce grassland RUE. However, it remains unclear if biofuel harvest will influence the warming impact on RUE of grassland ecosystems. For example, the positive impact of warming on transpiration could be reduced after the harvest of aboveground biomass. Biofuel harvest also can benefit the understory vegetation of grassland by reducing their radiation limitation (Day & Detling, 1994), leading to indirect influences on warming response of RUE. Thus, our understanding of grassland RUE under climate warming is strongly limited by interactive effects between warming and biofuel harvest.

Here, we analyzed the observed RUE from a long-term (2000–2011) field manipulative experiment with warming and clipping treatments in a tallgrass prairie of the Great Plains. The study attempts to address potential responses and underlying mechanisms of changing RUE under warming and biofuel harvest. The mean annual precipitation during the study period was 865 mm, but varied greatly among years. During this 12-year period, the annual precipitation covered the uppermost range (1449 mm), lowermost (556 mm), and average (840 mm) of the last 110 years of recorded climate data, providing us a unique chance to study the RUE response to warming and biofuel harvest. Specifically, we sought to address the following scientific questions: (i) how does RUE respond to warming and clipping treatments over 12 years? (ii) does clipping interacts with warming to influence RUE? and (iii) what is the role of precipitation changes on RUE responses to warming and clipping?

Material and methods

The experimental site and design

The experimental site is in a tallgrass prairie, located on the Kessler Atmospheric & Ecological Field Station (formerly Great Plains Apiaries) in McClain County (34°59′N, 97°31′W), Oklahoma, USA. Mean annual temperature and precipitation are 16.3 °C and 914 mm, respectively (Oklahoma Climatological Survey, Norman, OK, USA). The soil of the study site is part of the Nash–Lucien complex with around 37% water holding capacity, a neutral pH, and a moderately penetrable root zone (US Department of Agriculture, 1979) . The grassland, ungrazed for the past 40 years, is dominated by C_4 grasses (e.g., *Sorghastrum nutans* and *Andropogon gerardii*), and C_3 forbs (e.g., *Ambrosia psilotachyia* and *Solidago rigida*).

The experiment was established in November 1999 as a paired factorial design, with warming as the main factor nested with clipping (Luo *et al.*, 2009; Niu *et al.*, 2010). Twelve 2 × 2 m plots were arranged into six pairs (six replicates), with a distance ranging from 20 to 60 m between replicates. Each pair had two plots, with the distance between them approximately 5 m. One plot was the control with ambient temperature and the other was a warmed plot subjected to continuous warming from 21 November 1999 to the present. Each plot was divided into four 1 × 1 m subplots. Plants in two diagonal subplots were clipped at a height of 10 cm above the ground once a year to mimic hay harvesting or biofuel feedstock production while the other two subplots were unclipped. Clipped materials were taken away and not returned to the plots. Thus, this experiment had four treatments: unclipping and control (ambient) temperature (UC), unclipping and warming (UW), clipping and control temperature (CC), and clipping and warming (CW). All the warmed plots were heated continuously by a single 165 × 15 cm infrared heater (Kalglo Electronics, Bethlehem, PA, USA) suspended 1.5 m above the ground. In each control plot, one 'dummy' heater with the same shape and size as the infrared heater was suspended at 1.5 m to simulate the shading effects of the infrared heater.

Soil temperature and moisture

Soil temperature was monitored by homemade thermocouples installed at a depth of 2.5 cm in the center of one clipped and one unclipped subplot in each plot. The hourly average data were stored in an SM19 Storage Module (Campbell Scientific, Logan, UT, USA). Volumetric soil water content (%V) was measured once or twice a month using manual Time Domain Reflectomery equipment (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) at 0–15 cm depth.

Aboveground net primary productivity (ANPP) and cover assessment

Aboveground biomass in the two diagonal clipped subplots was measured directly by clipping annually at 10 cm at the time of peak biomass, usually August. The clipped plants were first separated into C_3 and C_4 plants, and then oven dried at 65 °C for 48 h. In the unclipped subplots, an indirect pin-contact method (Frank & McNaughton, 1990) was used concomitantly to estimate aboveground biomass. Information on the pin-contact method was introduced in Sherry *et al.* (2008). Because the aboveground biomass was clipped at the time of peak biomass each year, it can represent the ANPP in this ecosystem. Cover of bare ground, litter and plant material was quantitated in summer at peak biomass by the point-frame method (Sherry *et al.*, 2008). In August, green (live plant) and brown (standing litter) hits on each of 10 pins in a frame were counted at each of four directions in each plot. A linear function was first obtained from correlation between the pin hits and covers of bare ground, litter and plant material in the same calibration plots. Then, the cover information in each experimental plot was calculated from the linear function and measured pin hits.

The precipitation variables

The precipitation data at the study site during the experimental period (2000–2011) and the long-term climate data (1900–2010) were obtained from the Oklahoma Climate Survey (http://climate.ok.gov/cgi-bin/public/climate.timeseries.one.cgi). Two variables were calculated: precipitation amount over a year and over a growing season. Growing season precipitation was defined as that falling between March 1st and August 31st, early season precipitation included all rainfall from March 1st to May 30th, and late season precipitation from June 1st to August 31st. The ending date of the growing season (31 August) was determined by the harvest of ANPP in this study. A rainfall ratio was calculated by the ratio of precipitation in the late growing season to the amount in the other seasons. After dividing the 110 years into three groups (dry years, normal years, and wet years) according to the amount of annual precipitation, we calculated the means of dry/wet years by the average precipitation in the driest/wettest 30th percentile of these years and the means of the normal years by the rest of the years (Michaelsen *et al.*, 1987). In addition, we calculated the extremely dry/wet threshold by the average precipitation in the driest/wettest 10th percentile of the years.

The data analyses

RUE in this study is hydrological RUE, which is calculated for each treatment using the following equation:

$$\mathrm{RUE} = \mathrm{ANPP}/\mathrm{PPT}$$

where RUE is rain use efficiency (g m^{-2} mm^{-1}), ANPP is aboveground net primary productivity (g m^{-2}), and PPT is annual precipitation falling from 1 September in the former year to 31 August (mm).

Repeated Measures ANOVAs were used to analyze warming and clipping effects on RUE, soil temperature and moisture, species composition (C_4; and C_3 biomass and their ratio), and cover (plant, bare ground, and litter cover) over the 12 years. Between-subject effects were estimated as warming or clipping treatment and within-subject effects were year. If there is a significant interannual variability (year effect $P < 0.05$), two-way ANOVAs were used to examine the effects of treatments within each year. The warming effect on RUE was calculated by the differences between RUE in the warmed plots and in the unwarmed plots, whereas the effect of clipping was calculated by the differences between the clipped plots and unclipped plots.

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

The long-term precipitation regime over the past 110 years in central Oklahoma

The long-term annual mean precipitation in central Oklahoma during 1900–2010 was 866 mm, with the normal range from 681 to 1074 mm. The extreme range of annual precipitation was lower than 597 mm or higher than 1212 mm. 540 mm of precipitation (about 60%) fell during the growing season (March–August), 43% in the early- (March–May) and 57% in the late- (June–August) growing season. Annual precipitation had a significant increasing trend over the 110 years (Fig. 1a; $r^2 = 0.19$; $P < 0.05$). During the last 30 years, a greater rainfall ratio showed that more precipitation was distributed in the late growing season (Fig. 1a; $r^2 = 0.84$, $P < 0.01$).

Microclimate during the experimental period

During the study period, significant temporal variability in precipitation was found at the experimental site. Annual precipitation varied from 556 mm in 2006 to 1449 mm in 2007, with a mean value of 840 mm over

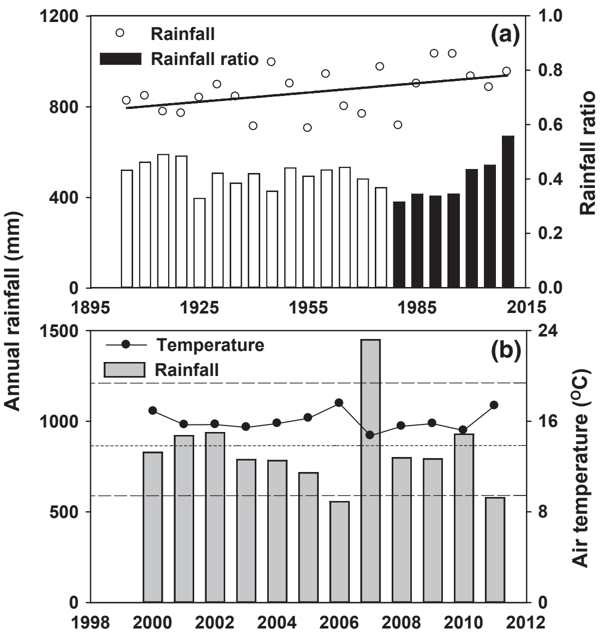

Fig. 1 (a) Five-year averages of annual rainfall (open circle) and the ratio of precipitation in the late growing season to the amount in the other seasons (rainfall ratio, bar) from 1900 to 2010 in central Oklahoma, and (b) the temporal variations of annual rainfall (gray bar) and mean air temperature (solid line) in the study site from 2000 to 2011. The lines showed the historical average rainfall (dotted line) plus extremely dry and wet threshold (long-dashed line) in panel b. The thresholds for extremely dry and wet years are obtained from the driest and wettest 10% of annual precipitation during 1900–2010 in central Oklahoma.

the 12 years (Fig. 1b). According to the range of extreme annual precipitation (<597 mm or >1212 mm) quantitated by the long-term precipitation record, 2006 (556 mm) and 2011 (578 mm) were two extremely dry years and 2007 (1449 mm) was an extremely wet year. Annual temperature varied from 14.7 °C in 2007 to 17.6 °C in 2006, with a mean value of 16.0 °C from 2000 to 2011 (Fig. 1b). Mean soil temperature and volumetric moisture in the 12 years was 16.8 °C and 28.1%V, respectively. The warming treatment increased soil temperature by 1.91 °C ($P < 0.05$) and reduced soil moisture by an average of 1.38% measured volumetrically ($P < 0.1$; Table 1). There was no significant difference in soil temperature or moisture between the clipped and unclipped subplots (all $P > 0.05$; Table 1).

Warming and clipping effects on the RUE

Across the 12 years, the mean RUE in the control plots was 0.27 ± 0.01 g m^{-2} mm^{-1} (Fig. 2b). Due to precipitation changes, great interannual variability in RUE was observed (year effect $P < 0.001$, Table 1; Fig. 2a); it ranged from 0.16 in 2007 to 0.48 in 2004. Across the 12 years, warming significantly increased RUE by 13.6% ($P < 0.05$), while clipping decreased it by 21.4% ($P < 0.1$; Table 1; Fig. 2b). Warming and clipping significantly interacted with year to impact RUE ($P < 0.001$; Table 1). For example, positive effects of warming on RUE were significant in 2001–2003 and 2007–2010, with the greatest effect in 2010 (+92%, $P < 0.001$). Negative effects of clipping were significant in 2000–2003 and 2005–2006 (Fig. 3a). After dividing the 12 years into three groups (dry, normal, and wet years), we found that the positive effect of warming RUE increased with precipitation, but the negative clipping effect decreased with precipitation (Fig. 3b). There was no interactive effect between warming and clipping on RUE (all $P > 0.05$; Table 1), and their additive effect on RUE did not vary with year (all $P > 0.05$; Table 1).

Dependences of RUE on abiotic and biotic factors

Although a decreasing trend in RUE was observed with the greater annual precipitation in normal years, an initial increase and a subsequent decrease in RUE was showed across the 12 years (Fig. 4a). It means that RUE was lower in extremely dry or wet years. For example, the average RUE in the control plots was 0.23 ± 0.01 and 0.19 ± 0.01 g m^{-2} mm^{-1} in the extremely dry and wet years, respectively, lower than that in normal years (0.30 ± 0.01 g m^{-2} mm^{-1}). In the control plots, RUE reached its maximum (0.34 g m^{-2} mm^{-1}) at 595 mm of annual precipitation (Fig. 4a). 595 mm can be defined as the threshold of annual precipitation, which switches the dependence of RUE on annual precipitation from positive to negative. The patterns of RUE under the other treatments were similar, but warming significantly increased the maximum RUE to 0.38 g m^{-2} mm^{-1} and the threshold to 685 mm (Fig. 4a). Clipping decreased the maximum of RUE to 0.22 g m^{-2} mm^{-1}, but increased the precipitation threshold to 745 mm (Fig. 4b). In addition, we found a significant positive relationship between RUE and the late-growing season rainfall ratio across the 12 years and four treatments (Fig. 5a and b). The C_4 biomass also showed positive linear correlations with the rainfall ratio across all years in each treatment (Fig. 5c and d).

The effects of warming and clipping on RUE were positively correlated with their impacts on C_4 biomass ($r^2 = 0.89$, $P < 0.001$) across the 12 years (Fig. 6). During the 12 years, warming significantly increased the C_4 biomass by 38% ($P < 0.001$), while clipping decreased it by 16% ($P < 0.05$; Table 1). Both warming and clipping had no significant effect on C_3 biomass and on the ratio of C_4 to C_3 (all $P > 0.05$; Table 1).

Table 1 Statistical results (*F*-values) of the effects of year (Y), warming (W), clipping (C), and their interactions on rain use efficiency (RUE), soil temperature and moisture (ST and SM), C_3 aboveground biomass (C_3), C_4 aboveground biomass (C_3), and the ratio of C_4 to C_3 aboveground biomass ($C_4 : C_3$), cover of plant, bare ground, and litter (plant, bare ground, and litter)

	RUE	ST	SM	C_4	C_3	$C_4 : C_3$	Plant	Bare ground	Litter
Y	13.03***	4.48***	61.87***	81.56***	19.99***	7.89***	70.02***	52.82***	150.67***
W	9.85*	5.74*	3.68†	15.74***	0.16	0.00	1.57	0.52	4.94*
C	3.86†	0.00	0.77	5.28*	0.75	2.37	39.73***	22.81***	165.17***
W×C	0.1	1.32	0.03	0.04	0.14	0.70	0.29	0.01	0.16
Y×W	0.73***	0.61	0.34	2.55*	5.26***	4.22**	1.06	1.6	0.76
Y×C	1.64***	2.24†	0.64	8.73***	2.73*	4.57**	6.72***	10.17***	5.91***
Y×W×C	0.09	1.13	0.03	0.93	0.29	2.77*	0.57	0.39	0.75

***, **, * and † indicate the significance at the level of $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P < 0.1$, respectively.

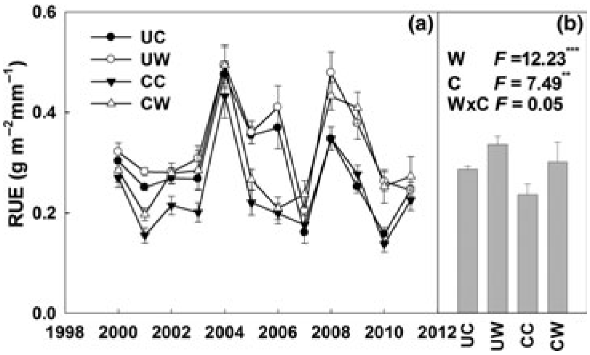

Fig. 2 Rain use efficiency (RUE) under the four treatments from 2000 to 2011 (a), and their means crossing the 12 years (b). The significance of the treatment effects was shown by *F*-values. UC, unclipped and unwarmed (filled circle); UW, unclipped and warmed (open circle); CC (clipping and control), clipped and unwarmed (filled triangle); CW (clipping and warming), clipped and warmed (open triangle). *** and ** in panel b indicate the significance at the statistical significance of treatment effects at $P < 0.001$ and $P < 0.01$, respectively.

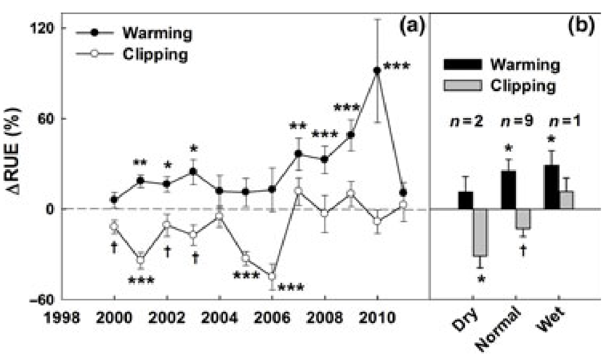

Fig. 3 The temporal variations (a) of warming and clipping effects on rain use efficiency (ΔRUE) during the study period, and (b) their mean effects in the extremely dry, normal, and extremely wet hydrological years. ***, **, * and † in the panel indicate the significance at the level of $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P < 0.1$ for effects of treatments, respectively.

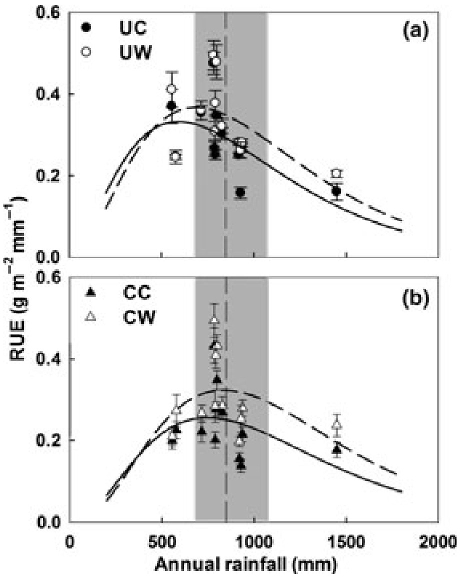

Fig. 4 Patterns of rain use efficiency (RUE) with increasing precipitation during the study period under the four treatments with the coefficient (r^2) and the significance (*P*-value). The unimodal trend in RUE has been proved by an analysis from the Tibetan Plateau (Yang *et al.*, 2010). The equations are as follows: UC = $0.0000252x^{1.762}/e^{0.00297x}$ ($r^2 = 0.34$, $P = 0.16$; solid line in panel a), UW = $0.0000274x^{2.134}/e^{0.00311x}$ ($r^2 = 0.31$, $P = 0.19$; dotted line in panel a), CC = $0.000000561x^{2.321}/e^{0.00311x}$ ($r^2 = 0.15$, $P = 0.16$; solid line in panel b), UC = $0.0000000536x^{2.727}/e^{0.00327x}$ ($r^2 = 0.13$, $P = 0.19$; dotted line in panel b), respectively. The gray area represents the normal range of precipitation in central Oklahoma (681–1074 mm). See Fig. 2 for the abbreviations.

Discussions

RUE and its interannual fluctuation

The mean RUE (0.27 ± 0.01 g m^{-2} mm^{-1}) of our experiment in a tallgrass prairie is within the range of RUE (0.19–0.55 g m^{-2} mm^{-1}) reported by Yang *et al.* (2010)

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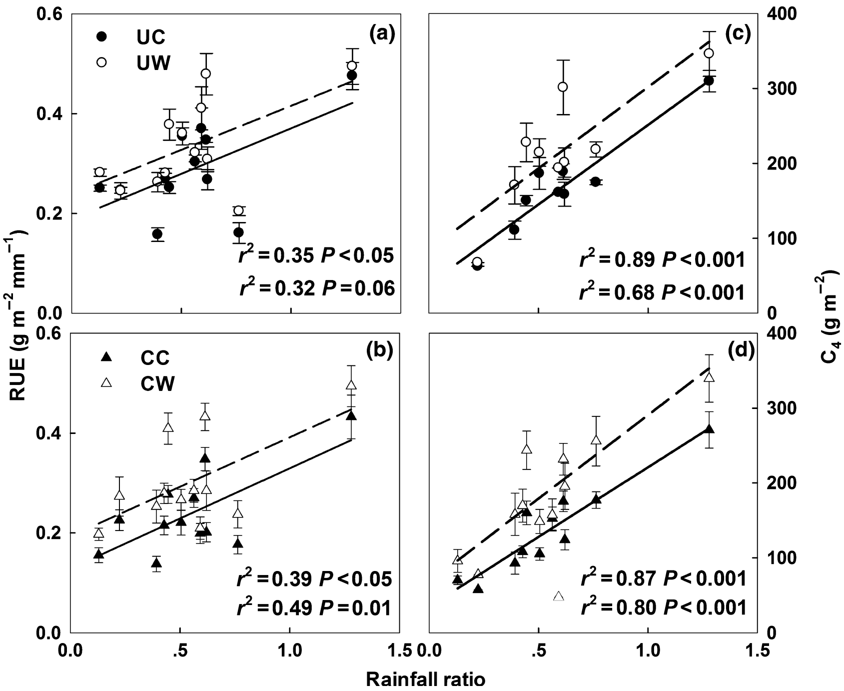

Fig. 5 The dependence of rain use efficiency (RUE; a and b) and C_4 aboveground biomass (C_4; c and d) on the ratio of precipitation in the late growing season (June–August) to the amount in the other seasons (rainfall ratio) under the four treatments with the coefficient (r^2) and the significance (P-value). The gray up-triangles represent the data from an extremely dry year (2006), and were not included in the regression analyses. See Fig. 2 for the abbreviations.

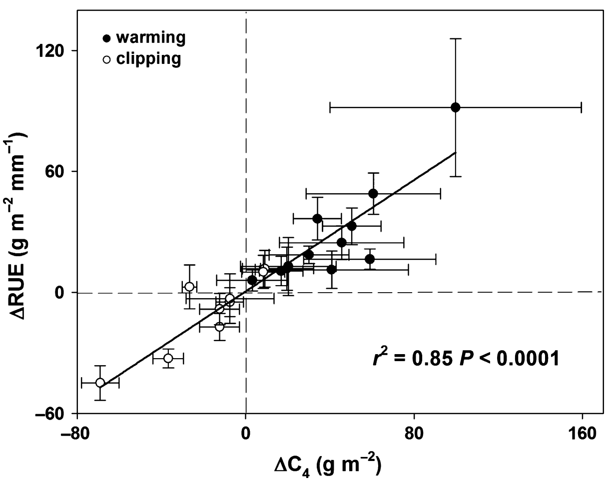

Fig. 6 The relationship of warming- and clipping-induced effects on rain use efficiency (ΔRUE) and on C_4 aboveground biomass (ΔC_4) in the 12 years with the coefficient (r^2) and the significance (P-value).

for global grassland ecosystems. However, this value is lower than the spatial mean RUE (0.73–0.82 g m^{-2} mm^{-1}) or the adjusted RUE (0.49–0.54 g m^{-2} mm^{-1}) for the North American grassland (Epstein *et al.*, 1996; Burke *et al.*, 1997; Lauenroth *et al.*, 2000). The spatial RUE is defined by the sensitivity of ANPP to changes in annual precipitation, which is based on the assumption that RUE is highest in the driest year and lowest in the wettest year. In the long-term temporal data, we observed significant interannual variation in RUE in the 12 years (0.16–0.48 g m^{-2} mm^{-1}) of this study, but RUE was lower in both extremely dry and wet years in comparison with that in normal years. Therefore, the assumption that RUE decreases with greater annual precipitation at the spatial scale is not correct at the temporal scale. The reason could be that community structure and biogeochemical processes are diverse along the spatial precipitation gradient, and have adapted to the local climate (Huxman *et al.*, 2004; Bai *et al.*, 2008; Hu *et al.*, 2010). Thus, our results suggest that temporal patterns should be taken into consideration in future studies of grassland RUE dynamics.

Warming effects on RUE

As precipitation can be separated into effective (transpiration) and ineffective precipitation (runoff and soil evaporation) (Noy-Meir, 1973), any factor influencing this partition will directly or indirectly impact grassland RUE. Soil water holding capacity, which represents the capacity of soil to retain water for plant growth, is important in preserving rainwater from loss as runoff. Le Houérou (1984) reported that soil organic carbon content (SOC) was a critical variable in determining water holding capacity. Nevertheless, SOC might not be the reason for the change in RUE under warming, because previous studies in the same experiment have observed that warming had no effect on SOC during the long-term study period (Xu *et al.*, 2012b). However, we found that plant and litter cover, which can contribute to the reduction in runoff (O'Connor *et al.*, 2001), was significantly stimulated by warming (Table 1). In addition, greater biomass allocation to roots, especially roots at the surface soil, can allow more water penetrate to the soil (Lauenroth *et al.*, 2000). The significant positive effect of warming on belowground net primary productivity, reported by Xu *et al.* (2012a) in this grassland ecosystem, confirmed that warming might increase 'effective precipitation' in ecosystems by decreasing runoff. For soil evaporation, we assumed that although the increasing temperature under warming had a potential positive effect, decreasing bare ground might offset this loss (Table 1). Similar to our expectation, a recent study from a nearby site (about 500 m from this study) has found that warming can increase the ratio between transpiration and evapotranspiration by reducing evaporation (Wang *et al.*, 2013). All these results indicate that warming can significantly stimulate RUE by decreasing 'ineffective precipitation' loss from runoff and soil evaporation.

As the RUE is the ratio of the ANPP to annual precipitation, any factor influencing aboveground plant growth will have impacts on RUE. It is well known that ANPP in grassland ecosystems are usually limited or colimited by N availability, which can be greatly improved by warming-induced increase in soil N mineralization (Rustad *et al.*, 2001; Melillo *et al.*, 2002). However, data from this experiment showed no significant changes in N mineralization after 8 years of warming treatment (Sherry RA., unpublished results), except in the first year (Wan *et al.*, 2005). As traits of dominant plant species and functional groups may affect the 'effective precipitation' use efficiency (Hooper & Vitousek, 1997; Paruelo *et al.*, 1999; Bai *et al.*, 2002), shift in species composition from low to high nitrogen use efficiency could change ecosystem RUE. In this study, shift in species composition from C_3 to C_4 is a reason for the high nitrogen use efficiency of whole plant community (Niu *et al.*, 2010), and may be a potential cause of positive responses of RUE under warming (Table 1; Fig. 3a). Previous evidence has shown that C_4 species have 1.5–4 times of the photosynthetic water use efficiency of the C_3 species under similar conditions (Larcher, 2003; Vogan *et al.*, 2007; Kocacinar *et al.*, 2008), although this advantage of C_4 species would diminish under drought (Taylor *et al.*, 2011). The relationship between the responses of RUE and C_4 aboveground biomass to warming could be autocorrelated in this study (Fig. 6), because RUE is the ratio of total aboveground biomass to precipitation. However, this can also indicate that changes in RUE are mainly generated by the response of C_4 plants. Therefore, the significant increase in C_4 plant production under warming suggests that warming increased grassland RUE by increasing 'effective precipitation' use efficiency through shifting species composition. Thus, the combination of reduced 'ineffective precipitation' loss and increased 'effective precipitation' use efficiency leads to stimulated RUE under warming in this ecosystem.

Clipping effects on RUE

Complete removal of aboveground biomass, by clipping in this study, was expected to decrease RUE through its direct negative effects on vegetation and litter cover (Milchunas & Lauenroth, 1989). This expectation is consistent with the results of the clipping treatment in our study (Fig. 2; Table 1). As the aboveground biomass was removed, clipping can cause a reduction in litter cover and an increase in bare ground, increasing water loss by runoff and soil evaporation (Table 1; Castillo *et al.*, 1997). Therefore, 'ineffective' precipitation loss will be stimulated by the clipping treatment. As little change in soil C content was observed even after 9 years of clipping in this experiment (Niu *et al.*, 2010), biofuel harvest would not affect soil water holding capacity by changing soil C cycling (Bakker *et al.*, 2002). However, more root biomass, especially at the soil surface, in the clipped plot (Xu *et al.*, 2012a) will benefit soil water penetration and thus decrease runoff loss in this study. In addition, the greater plant cover under clipping in this study also has a negative effect on water loss through increasing rainfall interception (Stocking, 1994; Table 1). Thus, the combination of increasing surface soil root density and plant cover under clipping may partially offset its negative impact on RUE via reducing litter cover.

As the clipping treatment in our study was conducted at the peak biomass in August, it may decrease RUE through exporting nitrogen from the ecosystem before plant senescence and recycle nutrients to soil. However, this mechanism could not happen because soil nitrogen pools have not been changed in this study (Niu *et al.*, 2010). As suggested by Moog *et al.* (2002), biofuel harvest can significantly alter aboveground community composition. In this study, clipping the peak aboveground biomass each year did not change the biomass of C_3 plants, but significantly decreased that of C_4 plants. According to the difference between the water use efficiency of C_3 and C_4 species, the clipping-induced shift of plant community structure will result in a significant decrease in the 'effective precipitation' use efficiency. Thus, vegetation dynamics may have an important role in mediating the negative effect of clipping on RUE in the tallgrass Prairie in North America.

During the 12 years of this study, no interactive effect on RUE was found between warming and clipping treatments (Table 1). That may be because the negative effect of warming on the 'ineffective' precipitation loss was offset by the positive effect of clipping. Or the stimulation of the dominance of C_4 plants due to warming was dampened by the clipping treatment. However, both global warming and clipping would cause nonlinear responses of ecohydrological processes (Zhou *et al.*, 2008; Ruppert *et al.*, 2012). Therefore, to evaluate how RUE responds to global changes in a real world, it will be useful to consider the importance of multilevel design in field manipulative experiments. Furthermore, appropriate human management in grasslands under global warming can help avoid ecosystem C and water loss.

Precipitation regimes and its potential influences on RUE

RUE is quantitated as a ratio of ANPP to rainfall that is influenced by variation in precipitation regimes, including both total annual precipitation amount and its seasonal distribution (Lauenroth & Sala, 1992; Swemmer *et al.*, 2007). In this study, the long-term analyses of historical precipitation record showed an increasing trend in the annual precipitation amount and greater distribution into late growing season in the recent years in the Central Oklahoma (Fig. 1). These results suggest that precipitation regime shifts have occurred and should be already influencing RUE in tallgrass prairies of the Great Plains.

We found a decreasing trend of RUE with increasing annual precipitation across the normal years (681–1074 mm). It is consistent with the temporal patterns reported in temperate steppe in Eurasian grassland (Bai *et al.*, 2008). When precipitation increases, the most limiting factor for plant growth and ecosystem production would shift from water to other resources, such as nitrogen (Xia *et al.*, 2009). However, when the extremely dry and wet years were included, we found a unimodal pattern of RUE with increasing annual precipitation (Fig. 4). Although these relationships were not statistically significant because of the small sample size (only 12-year data; Ruppert *et al.*, 2012), they still can provide important information on ecosystem responses to climate change. For example, the fitted curve showed that RUE peaked at 595 mm of precipitation in the control plots (0.33 g m^{-2} mm^{-1}), which is comparable to previous analyses from global precipitation gradient (415 mm and 0.48 g m^{-2} mm^{-1}; Yang *et al.*, 2010) and a North American steppe (475 mm and 0.64–0.77 g m^{-2} mm^{-1}; Paruelo *et al.*, 1999). Similar patterns, but higher corresponding precipitation threshold under warming (685 mm and 0.38 g m^{-2} mm^{-1}) and clipping (745 mm and 0.22 g m^{-2} mm^{-1}), suggest that both warming and clipping may increase the precipitation demand for optimal RUE in this ecosystem.

Ecosystem responses to climate change are not only accounted by the magnitude of climate variability but also by its timing (Craine *et al.*, 2012). In the recent years in central Oklahoma, we found that more precipitation was falling in the late growing season (Fig. 1a). It is known that wet summer is beneficial to C_4 expansion, whereas dry summers are favorable for C_3 abundance (Paruelo & Lauenroth, 1996; Niu & Wan, 2008). Although no trend in seasonal distribution of precipitation was observed in the 12 experimental years in this study, our data showed significant relationship between the late-growing season rainfall ratio and the C_4 biomass. As a consequence, the more precipitation in late season that occupies later summer season can promote the species composition shift from C_3 to C_4 (Fig. 5c and d). Such a shift in precipitation regime may enhance the positive impact of warming and partially cancel the negative effect of biofuel harvest on RUE.

Conclusions

In this study, we found a positive effect of warming, but negative effect of clipping on RUE in a tallgrass prairie of the Great Plains, USA. Both warming and clipping effects were significantly promoted by increasing precipitation. Positive relationships between the responses of RUE and C_4 biomass to warming and clipping indicate that the RUE changes under warming and clipping primarily resulted from their impacts on the C_4 biomass in this ecosystem, because of its higher efficiency on water use in comparison with that of C_3 species. During the study period, RUE exhibited a unimodal trend with the increasing annual precipitation, peaking at 595 mm in the control plots, but at 685 mm and 745 mm under the warming and clipping treatment, respectively. Increasing annual precipitation in the future may decrease the RUE of this ecosystem, but greater distribution into the late growing season could increase RUE and positively influence RUE under climate warming and biofuel harvest. Our findings indicate that species composition change is critical in regulating grassland RUE under future climate change and human managements. Future projection of grassland production should take climate change trends, human management regimes, and vegetation dynamics into consideration. More information about precipitation partitions and their response to climate change and human activities are useful to improve our understanding on water–carbon coupling in grassland ecosystem.

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