RESEARCH ARTICLE

Key Points:
- A mesic grassland was subjected to 2 years of drought and heat waves followed by a recovery year
- Soil respiration was reduced during drought but increased following drought
- During the growing season soil respiration was more sensitive to moisture than temperature

Supporting Information:
- Supporting Information S1

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The immediate and prolonged effects of climate extremes on soil respiration in a mesic grassland

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Abstract The predicted increase in the frequency and intensity of climate extremes is expected to impact terrestrial carbon fluxes to the atmosphere, potentially changing ecosystems from carbon sinks to sources, with positive feedbacks to climate change. As the second largest terrestrial carbon flux, soil CO_2 efflux or soil respiration (R_s) is strongly influenced by soil temperature and moisture. Thus, climate extremes such as heat waves and extreme drought should have substantial impacts on R_s. We investigated the effects of such climate extremes on growing season R_s in a mesic grassland by experimentally imposing 2 years of extreme drought combined with midsummer heat waves. After this 2 year period, we continued to measure R_s during a recovery year. Two consecutive drought years reduced R_s by about 25% each growing season; however, when normal rainfall returned during the recovery year, formerly droughted plots had higher rates of R_s than control plots (up to +17%). The heat wave treatments had no effect on R_s, alone or when combined with drought, and during the growing season, soil moisture was the primary driver of R_s with little evidence for R_s temperature sensitivity. When compared to aboveground net primary production, growing season R_s was much less sensitive to drought but was more responsive postdrought. These results are consistent with the hypothesis that ecosystems become sources of CO_2 during drought because carbon inputs (production) are decreased relatively more than outputs (respiration). Moreover, stimulation of R_s postdrought may lengthen the time required for net carbon exchange to return to predrought levels.

1. Introduction

Globally, net soil CO_2 efflux, or soil respiration (R_s) is the second largest terrestrial carbon flux to the atmosphere, releasing approximately $98\,Pg\,C\,yr^{-1}$ [*Raich et al.*, 2002; *Bond-Lamberty and Thomson*, 2010], and therefore, small proportional changes in this flux could have large impacts on global carbon cycles. A predicted increase in the frequency and intensity of extreme drought and heat waves [*Intergovernmental Panel on Climate Change*, 2013] may alter the net carbon balance of terrestrial ecosystems, converting them into sources of carbon to the atmosphere [*Reichstein et al.*, 2013]. For example, during the 2003 European heat wave, gross primary production decreased by 30%, changing this region from a net sink to a source of CO_2 [*Ciais et al.*, 2005]. The impacts of such extreme events on carbon fluxes between the land surface and atmosphere may be immediate (occurring during the event) and/or prolonged (occurring after the event) [*Reichstein et al.*, 2013]. While immediate impacts of extremes on carbon fluxes have been observed [*Reichstein et al.*, 2007; *Arnone et al.*, 2008; *Schwalm et al.*, 2010], effects that extend after the climate extreme are rarely documented and may be even more important. For example, tree mortality triggered by events such as drought or major storms can result in prolonged effects on carbon fluxes, driven by losses in annual carbon uptake coupled with the decomposition of dead trees [*Reichstein et al.*, 2013]. Given the potential for extreme events to alter carbon fluxes, there is a growing need to understand both the responses and recovery dynamics of key carbon cycling processes.

Two processes contribute to R_s: autotrophic (plant roots) and heterotrophic (microbes/soil fauna) respiration, and these are generally limited by soil moisture, soil temperature, and the carbon substrate [*Luo and Zhou*, 2006]. Decreased precipitation generally reduces R_s [*Vicca et al.*, 2014], although the components of R_s may differ in their drought sensitivities; heterotrophic respiration has been shown to be more sensitive to drought stress than autotropic respiration [*Wang et al.*, 2014]. Over wide temperature ranges, R_s rates increase exponentially with soil temperature, as higher temperatures accelerate respiration-related metabolic processes [*Lloyd and Taylor*, 1994]. Therefore, climate change-associated increases in mean annual temperature or an increase in the frequency and intensity of heat waves could enhance this carbon source to the atmosphere. However, the response of R_s to soil temperature is also dependent on soil moisture [*Mielnick and Dugas*, 2000],

with drought reducing the sensitivity of R_s to soil temperature [*Wang et al.*, 2014]. For example, in a tallgrass prairie ecosystem, *Harper et al.* [2005] found that R_s was most sensitive to temperature at moist soil moisture levels compared to very dry conditions. Therefore, R_s-temperature relationships are also influenced by precipitation patterns and resultant soil moisture dynamics. In addition to climate, substrate supply is a critical factor regulating R_s, with carbon substrates taking many forms including labile carbon from root exudates, aboveground and belowground plant litter, and soil organic matter [*Wan and Luo*, 2003], which can be reduced with drought [*Wang et al.*, 2014]. For example, photosynthesis and R_s have close short-term coupling [*Bahn et al.*, 2008; *Brüggemann et al.*, 2011], and drought has been shown to reduce the amount and speed of recently assimilated carbon below ground, slowing R_s [*Hasibeder et al.*, 2015]. Therefore, the processes contributing to R_s will likely differ in their sensitivities to climate extremes and the timescale of response.

Increased temperature and reduced precipitation are projected to cause the Central Plains of North America to experience the most severe droughts since the medieval era [*Cook et al.*, 2015]. Grasslands in this region store vast amounts of carbon belowground [*White et al.*, 2000], and thus, the relative sensitivities of carbon sources and sinks will determine the strength and direction of carbon feedbacks to the atmosphere. Although some mesic grasslands are considered to have a carbon balance near zero [*Owensby et al.*, 2006], recent observations suggest that drought can turn grasslands into temporary carbon sources [*Zhang et al.*, 2010]. R_s and aboveground net primary production (ANPP) are two important carbon fluxes between the land surface and atmosphere governing the net carbon balance in grasslands, and both of these are susceptible to climate extremes. In many studies, ANPP responds much more negatively to drought than respiration, shifting ecosystems from being a carbon sink to a source [*Ciais et al.*, 2005; *Schwalm et al.*, 2010; *Jongen et al.*, 2011]; however, there have been exceptions [*Welp et al.*, 2007; *Jentsch et al.*, 2011]. Recent modeling experiments have found that production is more sensitive to drought than respiration in grasslands, with sensitivity of both dependent on the magnitude of long-term drought [*Shi et al.*, 2014] and interannual drought pattern [*Hoover and Rogers*, 2016].

In this study, we investigated the effects of two climate extremes, drought and heat waves, on R_s in a productive, mesic grassland. We focused on growing season R_s because in this ecosystem, the months of June–August are when maximum R_s occur and fluxes during this time are fivefold greater than in other months [*Knapp et al.*, 1998a]. Extreme drought was experimentally imposed for two growing seasons (April through September), and we continued measurements for 1 year postdrought. In addition to drought, we imposed a short-term heat wave treatment midsummer during the first 2 years to examine the independent and combined effects of drought and heat, since they naturally co-occur [*De Boeck et al.*, 2010]. We tested three hypotheses (1) that growing season drought would have greater immediate and prolonged effects on R_s than the 2 week heat wave and (2) that the sensitivity of R_s to soil temperature would be dependent on soil moisture, and (3) that R_s would be less sensitive to extreme drought than ANPP.

2. Methods

2.1. Study Site

This study was conducted at the Konza Prairie Biological Station in NE Kansas, USA (39°05'N, 96°35'W), on an intact, native tallgrass prairie ecosystem. The climate is midcontinental, with cold, dry winters and warm wet summers; mean annual temperature is 13.0°C, and mean annual precipitation is 835 mm [*Knapp et al.*, 1998b]. This region also has high interannual variability in precipitation [*Knapp et al.*, 1998b], as well as episodic heat waves and drought [*Woodhouse and Overpeck*, 1998; *Burnette and Stahle*, 2012]. The experiment took place on an annually burned lowland site with deep soils classified as Typic Argiustoll, with a silty clay loam texture (8% sand; 32% clay), and a bulk density of 1.5 g m^{-3} [*Blecker*, 2005]. Fire occurred historically in this grassland and is required today to reduce woody plant encroachment [*Knapp et al.*, 1998b]. The plant community at this study site was dominated by the C_4 grasses *Andropogon gerardii* and *Sorghastrum nutans*, and the C_3 forb *Solidago canadensis* [*Hoover et al.*, 2014b].

2.2. Experimental Design

In 2010, the Climate Extremes Experiment was established to examine the independent and combined effects of drought and short-term heat waves on the tallgrass prairie ecosystem (see Figure S1 in the supporting information for plot layout). Treatments were applied during two consecutive years (2010 and 2011),

followed by a recovery year (2012) when no treatments were imposed. Precipitation treatments (drought and control) were imposed during the growing season (1 April to 30 August), beneath four modified 6×24 m greenhouse frames, constructed in an undisturbed grassland. Drought was imposed by passively reducing ambient precipitation inputs with roofs (composed of strips of Dynaglas Plus® clear polycarbonate plastic; PALRAM Industries Ltd., Kutztown, PA, USA) that removed approximately 66% of each event (as measured by rain gauges beneath shelters). The control precipitation treatment received ambient rainfall plus supplemental irrigation to reduce water limitation during naturally dry periods (2011 and 2012). To mimic the effects of shading by the drought shelters (~10% reduction in photosynthetically active radiation, PAR), control greenhouse shelter frames were covered with deer netting (TENAX Manufacturing, Alabama, USA), which produced equivalent light reductions, but allowed rainfall to pass through. Within each rainfall shelter, ten 2×2 m plots were established and randomly assigned one of four heat wave treatments (ambient, low, medium, and high), which were imposed for 2 weeks in late July using passive warming chambers and infrared heat lamps. Transparent chambers consisted of a $2 \times 2 \times 1.5$ m polyvinyl chloride (PVC) frame, with 1 m, 6 mil polyethylene walls and polycarbonate roofs (Dynaglas Plus, Greentek, Edgerton, WI, USA). Gaps at the base and the top of the chamber allowed air to circulate between the chamber and ambient environment. To provide four levels of increased heat input, infrared heat lamps (HS/MRM 2420, 2000 W, Kalglo Electronics, Inc., Bethlehem, PA, USA) were placed within in chambers in the following combinations: control = no lamp, low heat = one lamp at one-half power ($+250$ W m^{-2}), medium heat = one lamp at full power ($+500$ W m^{-2}), and high heat = two lamps at full power ($+1000$ W m^{-2}). Heat treatments were imposed 24 h d^{-1}, during the 2 weeks of the simulated heat wave. During 2012, no precipitation or heat wave treatments were imposed. However, because 2012 was a natural drought year [*Knapp et al.*, 2015], all plots received supplemental irrigation to maintain precipitation inputs near long-term monthly averages for the site.

Based on long-term climate records (Manhattan, KS 1900–2012, National Climate Data Center's Global Historical Climatology Network, station USC00144972), the control treatments in this experiment received above average precipitation while the precipitation in the drought treatments was equivalent to a severe drought in 2010 (<10th percentile) and an extreme drought in 2011 (<5th percentile) [*Hoover et al.*, 2014b]. Combined, the 2 years of the drought treatment were drier than any 2 year period in the 1930s US Dust Bowl [*Hoover et al.*, 2014b]. The heat wave treatments imposed canopy temperatures that also ranged from average to extreme, with several heat levels exceeding the 95th percentile for maximum July temperature [*Hoover et al.*, 2014b].

2.3. Environmental Measurements

In each plot (40 total), we continuously measured soil moisture and soil temperature during the growing season. Soil moisture was measured in the top 0–15 cm of the soil using time domain reflectometry probes (model CS616, Campbell Scientific, Inc., Logan, UT, USA). This soil depth contains over 50% of the root biomass [*Knapp et al.*, 2002], and previous work has shown a strong relationship between soil moisture in the top 15 cm and the ecophysiological responses of the dominant plants in this ecosystem [*Nippert et al.*, 2009; *Hoover et al.*, 2014a]. We measured soil temperature at a depth of 5 cm using thermocouples (K-type, OMEGA Engineering Inc., Stamford, CT, USA). Data were sampled at 30 min intervals and recorded using data loggers (CR10X Datalogger, Campbell Scientific, Inc., Logan, UT, USA).

Prior to the heat wave, we measured light transmission through the canopy (photosynthetically active radiation, PAR) as a proxy for canopy structure using a 1 m ceptometer (ACCUPAR LP-80 Decagon, Pullman, WA, USA). PAR was measured midday above the canopy and at the soil surface in four locations per plot. Light penetration was expressed as the percentage of above-canopy PAR available at the soil surface.

2.4. Soil Respiration Measurements

In situ soil CO_2 flux measurements (R_s) were sampled approximately twice per month during all three growing seasons using a Li-Cor 8100 portable gas exchange system (LiCOR Inc., Lincoln, NE, USA). In each plot, two polyvinyl chloride (PVC) collars (10 cm diameter $\times$ 8 cm deep, buried 6 cm into the ground) were placed in opposite corners of each 2×2 m plot, and 25 cm from the edge. Collars were placed in the interspace between plant tillers and any living plant material and litter were carefully removed from within each collar so that we only measured R_s. Due to slight shifting of collars throughout the summer, we took monthly measurements of the interior height of each collar and adjusted the flux values to account for

changes in aboveground collar volume. Flux measurements for each collar required about 1 min and were taken midday between 1100 and 1300 CDT. During the experimental heat wave, R_s measurements were taken without moving heat chambers as we were able to access PVC collars through the 75 cm bottom gap of the heat chamber.

2.5. Aboveground Net Primary Production

End of season ANPP was estimated in the first week in September each year. Within each plot, all above-ground plant material was harvested within three $0.1 \, m^2$ quadrats (locations were changed each year to prevent resampling). Because the site was burned each spring, this biomass closely approximates ANPP [*Knapp et al.*, 2007]. Samples were oven dried at 60°C for 48 h and then weighed to the nearest 0.1 g.

2.6. Statistical Analyses

Prior to analysis, all plot level replicates were averaged including light penetration ($n = 4$ replicates per plot), R_s ($n = 2$ replicates per plot), and ANPP ($n = 3$ replicates per plot). The experimental design was a randomized split plot; block was nested within the precipitation treatment, the heat wave treatment was nested within the drought treatment, and the block x heat wave treatment interaction was a random effect. We analyzed the treatment effects of drought and heat with a repeated measures mixed-model analysis of variance (ANOVA) for each year separately. We also used a stepwise linear regression model to assess the relative effects of soil temperature and soil moisture (during 2010 and 2011) on R_s. Factors were eliminated from the model using a cutoff of $\alpha = 0.05$. The relationship of R_s with soil moisture and soil temperature over the entire growing season was analyzed at intermediate (20–40%) and low (<15%) volumetric water content during 2010 and 2011 using values from the response surface analysis of *Harper et al*. [2005], which describes the response of R_s to soil temperature and soil moisture. All analyses were conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC, USA), and significance was set a $p \leq 0.05$.

We calculated effect sizes to compare the sensitivities of ANPP and R_s to drought, which allowed us to compare the magnitude of the treatment effects on a common scale [*Nakagawa and Cuthill*, 2007]. Since the response variables were continuous and the predictor variable was categorical, we calculated effect size using Cohen's d:

$$d = \frac{m_{drt} - m_{cont}}{s_{pooled}} \tag{1}$$

[*Cohen*, 1988]

$$s_{pooled} = \sqrt{\frac{(n_{cont} - 1)s^2_{cont} + (n_{drt} - 1)s^2_{drt}}{n_{con} - n_{drt} - 2}} \tag{2}$$

[*Cohen*, 1988] where m_{drt} and m_{cont} are the treatment means of the drought and control treatments, respectively. The pooled standard deviation (s_{pooled}) was calculated using the sample size of (n) and standard deviation (s) of the control (cont) and drought (drt) treatments. Effect size values (d) above zero indicate that the experiment had a positive effect on the response variable, while values below zero indicate a negative effect. The variance around d was calculated using approximate 95% confidence intervals (CIs):

$$CI = d \pm 1.96se \tag{3}$$

[*Nakagawa and Cuthill*, 2007]

$$se = \sqrt{\left(\frac{n_{cont} + n_{drt} - 1}{n_{cont} + n_{drt} - 3}\right)\left[\left(\frac{4}{n_{cont} + n_{drt}}\right)\left(1 + \frac{d^2}{8}\right)\right]} \tag{4}$$

[*Hunter and Schmidt*, 2004]

For this analysis, if the confidence intervals overlapped zero, we considered the effect to be nonsignificant.

3. Results

During 2010 and 2011, the drought treatment significantly reduced mean soil moisture by 43% and 56%, respectively (Figure 1a). In 2012, the ambient rainfall plus supplemental irrigation applied to both control and drought treatments resulted in similar mean soil moisture (Figure 1a) and dynamics over the course of
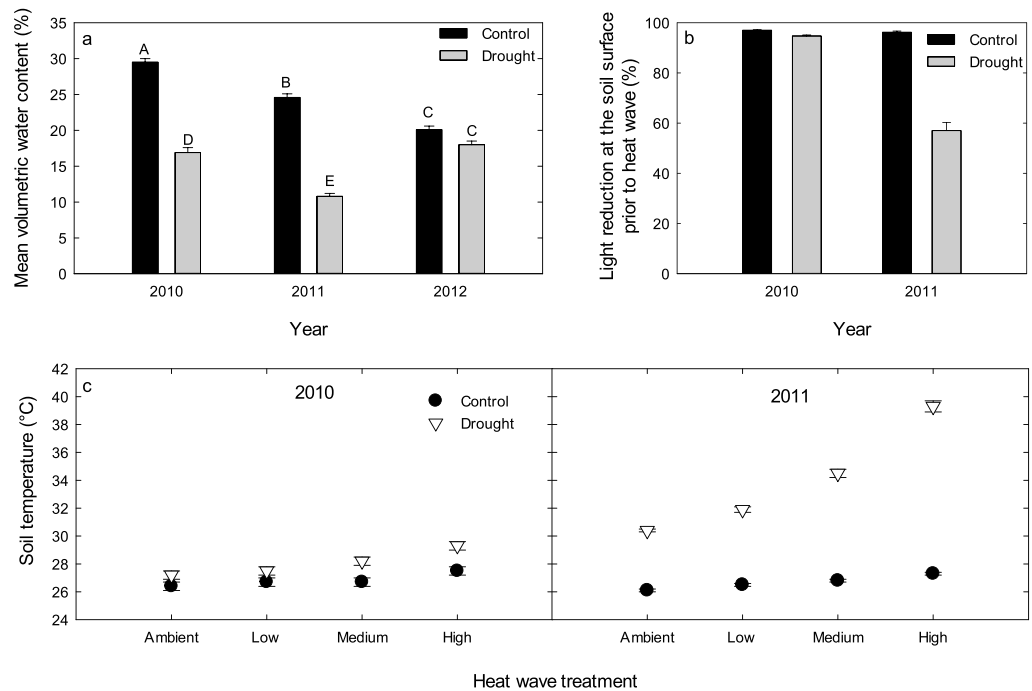
Figure 1. Environmental responses to drought and heat wave treatments. (a) Mean volumetric water content (0–15 cm; $n = 20$) for control and drought treatments during the extreme years, 2010 and 2011 (control = ambient precipitation plus supplemental irrigation; drought = 66% reduction in ambient precipitation) and recovery year, 2012 (ambient precipitation plus supplemental irrigation for both treatments). Letters denote significant difference ($p < 0.05$) across precipitation treatments and years. (b) Canopy density as determined by the percent reduction in photosynthetically active radiation at the soil surface from above the canopy (full light) prior to imposing heat wave treatments. (c) Soil temperature for each heat wave x precipitation treatment combination ($n = 5$) during the 2 week simulated heat wave in 2010 and 2011.

the growing season (Figure 2). In both years, the start of the 2 week heat wave coincided with substantial differences in soil moisture (Figure 2). Additionally, canopy structure differed between years and treatments; the canopy was well developed in both treatments in 2010 and in the control only in 2011, as indicated by light penetration (Figure 1b). The heat wave treatments had minimal impacts on soil temperature within the control treatments in both years (1°C range; Figure 1c), and in the drought treatment in 2010 (2°C range; Figure 1c). However, in 2011, the most extreme drought year, we observed a much larger range in soil temperatures across the different heat wave treatments (9.0°C; Figure 1c), despite equal thermal inputs for each level of heat wave treatments across both precipitation treatments and years. This was likely due to the sparse density of the plant canopy in the drought plots prior to the heat wave in 2011 (Figure 1b), which allowed greater thermal inputs directly to the soil surface.

The drought treatments dominated the responses of growing season R_s (Table 1). During the extreme treatment years, we observed significant main effects of drought as well as drought x date interactions (Table 1). Drought treatments significantly reduced R_s relative to the control by 26% in 2010 and 25% in 2011 (Figure 3). In the recovery year (2012), both control and drought treatments were slightly lower than the controls in the previous years, yet we observed an overall increase (6%) in R_s for the formerly drought treatment relative to the control (Figure 3). The increase in R_s was most pronounced in the first half of the growing season, where R_s was up to 17% higher in the drought treatment (Figure 2). Because of the strong effect of the growing season drought, we observed no significant effects of the 2 week heat waves or any interactions with drought in either of the treatment years (Table 1). Thus, we focus below on the precipitation treatment effects and interactions with soil temperatures throughout the growing season.

To investigate the effects of soil temperature and soil moisture as drivers of responses in R_s, a stepwise regression model was constrained to the two extreme years, given the legacy effects we observed in the former drought treatment in 2012. Soil temperature was dropped from the model ($\alpha > 0.05$), while volumetric water
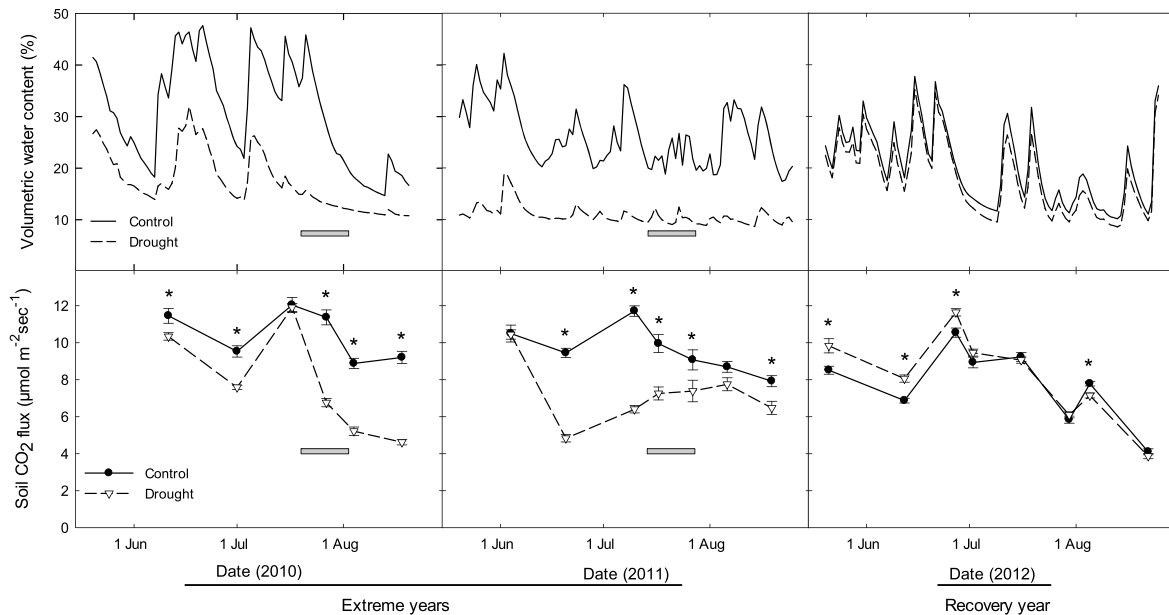
Figure 2. (Top) Mean volumetric water content for the top 15 cm of the soil and (bottom) soil CO_2 flux (R_s) during the 2010–2012 growing seasons. Grey bar indicates the timing of the 2 week heat wave. No treatments were imposed in 2012, with plots receiving ambient rainfall plus supplemental irrigation. Asterisks indicate significant differences ($p < 0.05$) on individual dates between control and drought treatments for soil CO_2 flux.

content was retained ($r^2 = 0.34$, $p < 0.001$; Figure 4a). The relationship between volumetric water content and R_s was positive throughout the range of soil moisture, with no decrease noted at the highest or lowest soil moisture levels. Porosity in these soils ranges from 47 to 53% [*Blecker*, 2005], suggesting that we did not encounter saturated soil moisture conditions when CO_2 fluxes could be limited by diffusion rates.

As noted earlier, the sensitivity of R_s to temperature may be dependent on soil moisture levels, particularly during extreme years. Therefore, we examined the relationship between soil temperature and R_s under two soil moisture conditions, low (volumetric water content < 15%) and intermediate (volumetric water content 20–40%) across the entire growing season. At both low and intermediate soil moisture levels, we observed no relationship between growing season variations in soil temperature and R_s (intermediate: $F = 0.33$, $p = 0.564$; low: $F = 1.25$, $p = 0.264$; Figure 4b).

Finally, we compared the drought sensitivities of growing season R_s and ANPP across all 3 years of the experiment. Both of these key carbon cycling processes had strong relationships with mean volumetric water content during the growing season (R_s: $r^2 = 0.93$, $p = 0.002$; ANPP: $r^2 = 0.76$, $p = 0.023$; Figure S2). However, when we examined the effect size of drought over the 3 years, their relative sensitivity differed (Figure 5).

Table 1. ANOVA Results[a]

Effect	2010		2011		2012	
	F Value	*p* Value	*F* Value	*p* Value	*F* Value	*p* Value
Drought	**73.8**	**<0.001**	**75.4**	**<0.001**	**8.7**	**0.006**
Heat	0.5	0.656	0.8	0.498	2.7	0.061
Drought x Heat	0.6	0.626	0.3	0.829	0.8	0.508
Date	**185.9**	**<0.001**	**22.7**	**<0.001**	**257.7**	**<0.001**
Drought x Date	**39.6**	**<0.001**	**16.4**	**<0.001**	**7.1**	**<0.001**
Heat x Date	1.2	0.270	1.6	0.056	0.6	0.885
Drought x Heat x Date	1.3	0.226	0.8	0.728	1.2	0.302

[a]Effects of drought and heat wave treatments on growing season soil CO_2 flux (R_s) during the two consecutive extreme years (2010 and 2011) followed by a recovery year (2012). During the extreme years, drought was imposed during the growing season, while the heat wave was imposed for 2 weeks in midsummer. In the recovery year, no drought or heat wave treatments were imposed. *F* statistics and *p* values from mixed-model repeated measures ANOVAs for each year separately are reported. Bold text indicates significance at $p < 0.05$.
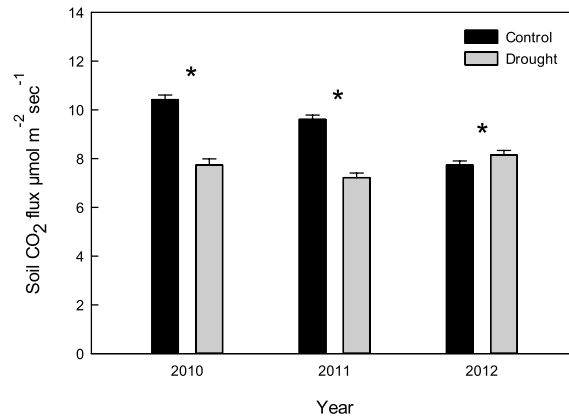
Figure 3. Mean soil CO_2 flux (R_s) during the 2010–2012 growing seasons. Asterisks indicate significant differences ($p < 0.05$) between control and drought treatments for soil CO_2 flux in a given year.

In 2010, both R_s and ANPP had similar relative reductions due to drought, but in the second and more extreme drought year, there was a much greater effect of drought on ANPP than R_s, with the latter having equivalent decreases with drought each year (Figure 5). During the recovery year (2012), the effect sizes of ANPP and R_s did not differ from zero (Figure 5).

4. Discussion

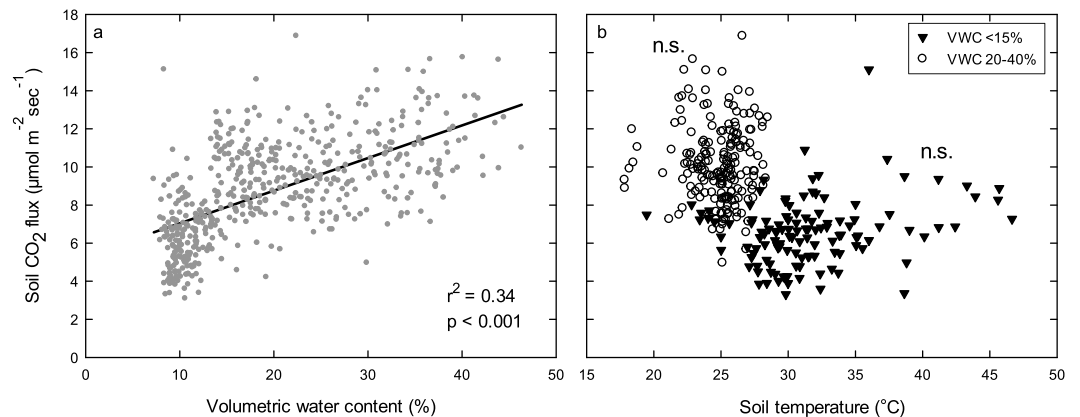We subjected a native, intact mesic grassland ecosystem to 2 years of climate extremes, followed by a recovery year to examine the impacts on R_s, a key carbon flux to the atmosphere. In addition to drought, we exposed the ecosystem to short-term heat waves at varying intensities. We observed strong effects of drought on growing season R_s with significant reductions in R_s during the drought and increases in R_s during the recovery year. In contrast, there were no direct effects of the short-term heat waves on R_s or interactive effects with drought. While this lack of sensitivity to heat waves may have been a result of the muted effects of the heat wave treatments on soil temperature, we also observed no strong effect of growing season soil temperature, even when controlling for soil moisture levels. This was likely because when soil moisture was high, the range of soil temperatures was relatively small (5–7°C), whereas when soil temperatures varied substantially (>15°C), soils were dry and R_s was very low. Finally, while both R_s and ANPP decreased during the drought, ANPP was much more sensitive to drought during the second and more extreme year than R_s.

4.1. Soil Respiration During Climate Extremes

Drought was the dominant factor affecting growing season R_s compared to the heat wave treatments, in part, because of the differences in the duration and/or magnitude of these extreme treatments. Consistent with natural heat waves, our experimental heat wave was short term (2 weeks), while the imposed drought lasted the entire growing season, when 75% of the precipitation occurs [*Knapp et al.*, 1998b]. The magnitude of the

Figure 4. Soil CO_2 flux (R_s) versus soil moisture and temperature. (a) The relationship between volumetric water content and soil CO_2 flux (R_s) for all dates, precipitation, and heat wave treatments combined (in 2010 and 2011). Results are from a stepwise linear regression that included volumetric water content and soil temperature as independent variables and soil CO_2 flux as the dependent variable. Soil temperature was removed from the model ($p > 0.05$), but volumetric water content was retained ($p < 0.05$), suggesting that soil moisture was the dominant variable controlling soil CO_2 flux. Statistics from the regression are reported in the figure. (b) Sensitivity of soil CO_2 flux (R_s) to soil temperature at low (volumetric water content < 15%) and intermediate (volumetric water content 20–40%) moisture levels. Linear regressions were calculated for each soil moisture level independently; however, neither resulted in a significant relationship (n.s. = nonsignificant).
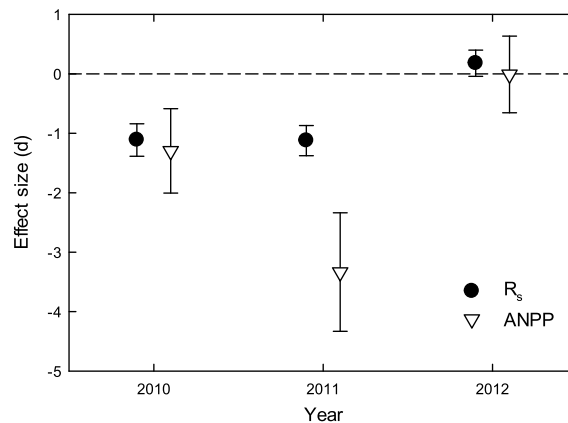
Figure 5. Drought response effect sizes (based on Cohen's d) and 95% confidence intervals for soil CO_2 flux (R_s) and aboveground net primary production (ANPP) for both treatment years (2010 and 2011) and the recovery year (2012). Negative effect size indicates a decrease in R_s or ANPP under drought relative to the control in each year. Error bars overlapping reference line at zero (dashed) indicate the effect is not significant.

drought treatment was extreme, exceeding the 10th and 5th percentiles for growing season precipitation in 2010 and 2011, respectively [*Hoover et al.*, 2014b]. In contrast, the heat wave treatments only had a significant impact on soil temperature in 2011 (drought treatments; Figure 1a), which also corresponded with a large reduction in canopy density due to the extreme drought (Figure 1b). It is likely that the reduction in canopy leaf area due to the extreme drought in 2011 increased the transmission of solar radiation and long-wave radiation from the heat lamps to the soil. This is similar to the change in radiation balance of the soil postfire when the plant canopy is removed in this ecosystem [*Hulbert*, 1969; *Knapp and Seastedt*, 1986]. However, it is important to note that even when soil temperatures were increased due to the heat wave treatments in 2011, we observed no effect of the heat wave treatments on R_s, which is likely due to the very dry soils and low R_s during this time.

Greater sensitivity of R_s to soil moisture than temperature was also evident when soil moisture and soil temperature were examined over the growing season using regression-based approaches. The stepwise regression results suggest that soil moisture is a strong predictor of R_s (Figure 4a), which supports the previous ANOVA results with significant effects of drought treatments. The lack of a direct response of R_s to soil temperature was surprising, given that it can be an important driver of R_s in grasslands [*Lloyd and Taylor*, 1994; *Mielnick and Dugas*, 2000] and specifically in this tallgrass prairie [*Knapp et al.*, 1998a]. Recently, *Poll et al.* [2013] noted that the sensitivity of R_s to soil temperature was dependent on soil moisture. Thus, we examined the response of R_s to soil temperature both under relatively moist and dry conditions. These soil moisture levels were based on a response surface analysis by *Harper et al.* [2005], which found that R_s was sensitive to temperature under intermediate but not dry conditions (our experiment was conducted adjacent to the study reported in *Harper et al.* [2005]). Even in this analysis, soil temperature did not appear to be driving dynamics of R_s at either soil moisture level (Figure 4b). As noted earlier, this may be due to the limited range in temperatures experienced during the growing season when soil moisture was high. Empirical studies on the nonlinear relationship between soil temperature and R_s are typically based on the range in soil temperatures encountered on an annual basis, including the cold winter months, not just the growing season [*Lloyd and Taylor*, 1994; *Knapp et al.*, 1998a; *Mielnick and Dugas*, 2000; *Harper et al.*, 2005]. As a result, when drought is most likely to impact carbon cycling processes in this mesic grassland, soil moisture is the dominant driver of R_s, not soil temperature.

4.2. Soil Respiration After Climate Extremes

In addition to strong impacts on R_s during extreme drought, we also observed prolonged effects of drought in the recovery year. Despite receiving the same precipitation inputs and with similar mean soil moisture during the growing season in 2012 (Figure 1a), R_s was significantly increased in the formerly droughted plots (Table 1 and Figures 2 and 3). This occurred primarily in the early growing season, when the drought plots were up to 17% higher than controls, but this difference diminished by mid-July (Figure 2). This postdrought R_s pulse may have been driven by carbon and nitrogen accumulating during the drought, which "primed" the system once soil moisture levels increased in 2012. During the drought, the carbon substrate pool may have increased as a result of high root mortality and lower decomposition during the two drought years [*Wang et al.*, 2014]. This increase in substrate combined with high soil moisture and warm temperatures in the spring of 2012 may have resulted in a transient early season R_s pulse driven by heterotrophic respiration.

Additionally, limited uptake and immobilization of nitrogen may have led to a pulse in postdrought nitrogen availability at the start of the growing season [*Yahdjian et al.*, 2006; *Evans and Burke*, 2013]. An increase in nitrogen may have stimulated autotrophic and heterotrophic activities, leading to higher respiration rates.

In the recovery year of this study (2012), there was a large shift in plant community composition in formerly droughted plots, with grasses dramatically increasing in abundance and ANPP while forbs decreased [*Hoover et al.*, 2014b]. This was primarily driven by an increase in the dominant grass *Andropogon gerardii* that compensated for the loss of the dominant forb *Solidago canadensis* and allowed full recovery in ANPP [*Hoover et al.*, 2014b]. It is possible that these dominant species differed in one or more key plant traits, and therefore, this change in plant community composition may have contributed to elevated R_s. The composition of plant communities are becoming recognized as important regulators of R_s as key traits of plant functional types or individual species [*Metcalfe et al.*, 2011]. For example, *Johnson et al.* [2008] found differences in R_s in a grassland microcosm based on which plant functional type (sedge or forb) was dominant in the community. Plant traits that control the quantity and quality of carbon input into the soil may be most influential on R_s including: photosynthetic rate, belowground allocation, root structure and chemistry, and mycorrhizal affiliation [*Metcalfe et al.*, 2011]. Therefore, community shifts that alter the abundance of species differing in such traits are likely to have a large impact on R_s.

4.3. Differential Sensitivities of Soil Respiration and ANPP to Extreme Drought

In order to forecast the effects of drought on carbon storage, it is important to examine the drought sensitivities of two key atmospheric carbon fluxes, R_s (CO_2 source) and ANPP (CO_2 sink). Both of these key carbon cycling processes had strong relationships with mean volumetric water content during the growing season (Figure S2) and had similar responses to drought in 2010 and 2012 (Figure 5). However, in the more extreme drought year (2011), there was a threefold decrease in ANPP relative to the first year (Figure 5) [*Hoover et al.*, 2014b], while R_s had similar responses to drought in 2010 and 2011 (Figure 5). These results suggest that during extreme drought, this ecosystem can become a carbon source driven by differential sensitivities of ANPP and R_s, a mechanism consistent with two independent modeling experiments [*Shi et al.*, 2014; *Hoover and Rogers*, 2016].

This differential sensitivity may be due to different response times of production and respiration to soil moisture limitations. ANPP is strongly controlled by precipitation inputs [*Sala et al.*, 1988; *Knapp and Smith*, 2001], and therefore, drought can cause immediate reductions in plant production as well as legacy effects [*Sala et al.*, 2012]. We observed little evidence for legacy effects on ANPP in the year following drought since control and drought treatments did not differ in production [*Hoover et al.*, 2014b]. While R_s was also reduced by low soil moisture in this experiment (Figures 2 and 3), *Shi et al.* [2014] suggest that carbon substrate supplies are more stable during drought and therefore R_s may be buffered against short-term precipitation variation relative to plant production. In addition, while the heterotrophic component of R_s may decrease or cease during prolonged drought, a baseline of R_s may be sustained through maintenance root respiration [*Wang et al.*, 2014]. As previously mentioned, we observed increased R_s in formerly droughted plots during the recovery year, which contrasted with the full recovery in ANPP. Combined, the net effect of these differences in carbon uptake and efflux dynamics during and after extreme drought will be to reduce net carbon storage for at least one additional growing season postdrought in this grassland.

4.4. Implications

A future with more frequent and intense climate extremes, will impact terrestrial carbon cycles, with potential feedbacks to global climate change [*Reichstein et al.*, 2013]. Our study has several important implications for the effects of extreme drought and heat waves on carbon fluxes in mesic grassland ecosystems. First, to understand the net effect of climate extremes on carbon cycles, it is important to examine both immediate responses and prolonged effects. For example, increased growing season R_s during the recovery year in formerly droughted plots may have partially offset the decreased fluxes during the 2 years of drought. Such prolonged effects of short-term climate anomalies on R_s have been documented in this ecosystem previously (yearlong warming [*Arnone et al.*, 2008]). Second, short-term heat waves had virtually no effect on R_s in this study; instead, soil moisture effects dominated. As hypothesized, we did not expect to see a strong effect of soil temperature under droughted conditions due to soil moisture limitations; however, when we specifically examined responses at higher soil moisture levels, there also was no effect of soil temperature.

These results suggest that during the growing season, R_s in this ecosystem is primarily driven by changes in soil moisture, and thus, a future with more frequent and intense droughts should impact this important carbon flux to the atmosphere more than higher temperatures. Finally, given that grasslands are important carbon sinks [*White et al.*, 2000], the differential sensitivity to drought of R_s and ANPP has important consequences for the carbon balance of this system. Extreme drought may reduce the capacity of this ecosystem to sequester carbon if ANPP is more sensitive than R_s during extreme drought, and if respiration rates increase following drought.

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