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Drought and heat wave impacts on grassland carbon cycling across hierarchical levels

Running Head: climate extremes effects on carbon cycling

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ABSTRACT

Droughts and heat waves are increasing in magnitude and frequency, altering the carbon cycle. However, understanding of the underlying response mechanisms remains poor, especially for the combination (hot drought). We conducted a 4-yr. field experiment to examine both individual and interactive effects of drought and heat wave on carbon cycling of a semiarid grassland across individual, functional group, community and ecosystem levels. Drought did not change below-ground biomass (BGB) or above-ground biomass (AGB) due to compensation effects between grass and non-grass functional groups. However, consistently decreased BGB under heat waves limited such compensation effects, resulting in reduced AGB. Ecosystem CO₂ fluxes were suppressed by droughts, attributed to stomatal closure-induced reductions in leaf photosynthesis and decreased AGB of grasses, while CO₂ fluxes were little affected by heat waves. Overall the hot drought produced the lowest leaf photosynthesis, AGB and ecosystem CO₂ fluxes although the interactions between heat wave and drought were usually not significant. Our results highlight that the functional group compensatory effects that maintain community-level AGB rely on feedback of root system responses, and that plant adjustments at the individual level, together with shifts in composition at the functional group level, co-regulate ecosystem carbon sink strength under climate extremes.

KEY WORDS: biomass, climate extremes, CO₂ flux, compensatory effect, global change, Inner Mongolia, leaf physiology, precipitation

INTRODUCTION

Globally, droughts, often in concert with heat waves (i.e. hot drought (Overpeck, 2013)), are the most widespread events affecting terrestrial ecosystem carbon cycling (Reichstein *et al.*, 2013). Hot droughts are likely to occur with increasing frequency and intensity (Easterling, 2000; Stocker, 2014). Although outcomes are highly variable, multiple lines of evidence concur that droughts and heat waves generally cause reductions in ecosystem carbon uptake and productivity (Ciais *et al.*, 2005; Doughty *et al.*, 2015; Hoover, Knapp, & Smith, 2014; Schwalm *et al.*, 2012; Zhao & Running, 2010; Zscheischler *et al.*, 2014). Despite significant efforts to understand drought and heat wave impacts, current understanding of the mechanisms behind ecosystem responses to these stressors remains insufficient in multi-model simulations of climate extremes-induced changes in the carbon cycle (Piao *et al.*, 2019). Besides, the interaction between droughts and co-occurring heat waves remains poorly understood (Reichstein *et al.*, 2013; Smith, 2011a).

Droughts and heat waves may cause widespread changes at many levels of the ecological hierarchy spanning the individual, functional group, community and ecosystem levels. Responses at lower hierarchical levels (i.e. individual or community) can have either additive, offsetting or synergistic effects and thus provide mechanisms for higher-levels responses (i.e. ecosystem). Therefore, climate extreme experiments focused on carbon cycling responses across multiple biological scales could significantly improve our understanding of mechanisms underlying carbon cycling responses to climate extremes (Corlett, 2016; Jentsch *et al.*, 2011).

At the individual level, plants may tolerate environmental stress by a variety of physiological or structural adjustments. For example, in order to prevent leaf water potential from falling below a critical level during periods of water stress, plants decrease stomatal conductance, in turn curtailing CO₂ uptake (De Boeck, Bassin,

Verlinden, Zeiter, & Hiltbrunner, 2016). Additionally, individuals can adapt to water stress by specifically allocating more photosynthate to fine root production in order to increase capacity for water and nutrient uptake, at the expense of leaf production (Burri, Sturm, Prechsl, Knohl, & Buchmann, 2014; Hasibeder, Fuchslueger, Richter, & Bahn, 2015; Jaleel *et al.*, 2009). When environmental stress reaches a tipping point beyond an individual plant's threshold tolerance, plant mortality may result (Niu *et al.*, 2014). Response to climate extremes may vary across different functional groups due to various resource-use strategies and threshold tolerances (e.g. C₄ vs. C₃ functional group in responses to extreme heating event in White, Campbell, Kemp, & Hunt, 2000; forb vs. grass functional group in responses to extreme droughts in Hoover *et al.*, 2014). As a result, asynchronous or even opposite responses of different functional groups potentially contribute to maintain community-level stability (Bai, Han, Wu, Chen, & Li, 2004). From a community perspective, such structural changes may entail profound consequences for the ecosystem carbon budget, as plant community structure regulates ecosystem-level CO₂ flux responses to climate change (Kuiper, Mooij, Bragazza, & Robroek, 2014; Ward *et al.*, 2013; Xu *et al.*, 2015). Collectively, physiological and structural processes at the individual level, compositional shifts at the functional group level, and structural changes at the community level, as well as their interactions, culminate in complex ecosystem responses to climate extremes.

There is emerging evidence that droughts and heat waves are often coupled in nature through soil moisture-temperature feedbacks (Ciais *et al.*, 2005; De Boeck & Verbeeck, 2011; Overpeck, 2013; Seneviratne, Luthi, Litschi, & Schar, 2006). The expectation is that the negative impacts of drought are intensified by the joint occurrence of higher temperatures (hot drought). However, past manipulative experiments showed high variation in the interactive effects between droughts and high temperatures (De Boeck *et al.*, 2016; Dreesen, De Boeck, Janssens, & Nijs, 2012; Hoover *et al.*, 2014).

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Compared with a heat wave alone, hot drought is likely to exert a greater influence on ecosystem structure and function (Ciais *et al.*, 2005; De Boeck *et al.*, 2016; De Boeck, Dreesen, Janssens, & Nijs, 2011), with the outcome related to the combined effects on soil moisture. In contrast, a soil microbial experiment suggested that drought could induce community tolerance to heat waves (Bérard, Sassi, Renault, & Gros, 2012). In some cases, heat waves combined and droughts unexpectedly triggered additive positive effects on grassland biomass (Dreesen *et al.*, 2012). These heterogeneous responses to the interaction of heat and drought and the unclear mechanisms underlying vegetation responses to these combined stresses highlight that carbon cycle impacts of combined heat waves and droughts remain poorly understood.

To understand how droughts, heat waves and hot droughts affect grassland carbon cycling, we conducted a replicated, factorial manipulative experiment over four years in a semiarid grassland of Inner Mongolia, China. We simultaneously measured key carbon cycle processes at different levels of ecosystem hierarchy to comprehensively examine mechanistic responses, including photosynthetic physiology at the individual level, above-ground biomass (AGB) of grasses, forbs and shrubs at the functional group level, community structure, AGB, below-ground biomass (BGB) and leaf area at the community level, and CO₂ fluxes at the ecosystem level. Specifically, we tested the following three hypotheses to single and combined climate extremes: 1) Climate extremes would close stomata or even damage leaves and thereby reduce leaf photosynthetic rate (*A*) and leaf area index (LAI). Consequently, community-level biomass and ecosystem-level CO₂ exchange would be curtailed. 2) Different functional groups (e.g. grass vs. forb vs. shrub) or/and different part of biomass (i.e. AGB vs. BGB) would inconsistently or oppositely respond to climate extremes, which would amplify or buffer the reduction in community-level biomass and ecosystem-level CO₂ exchange. 3) Drought would cause larger negative effects than heat waves in semiarid grasslands,

because drought reduces soil moisture directly and plants often cope with high temperature well, especially when water supply is sufficient (De Boeck *et al.*, 2011; Hoover *et al.*, 2014). Hot drought is expected to have the largest negative effects on all of these, as warming aggravates soil water stress (Ciais *et al.*, 2005; Teskey *et al.*, 2015).

MATERIALS AND METHODS

Study site

This study, as a part of the Extreme Climate Events and Biodiversity-II (ECEB-II) experiment, was conducted at the Inner Mongolia Grassland Ecosystem Research Station in the Xilin River Basin (43°20' N, 116°40' E, 1200 m a.s.l). The region is characterized by a semiarid continental climate, dry in spring and humid in summer, with a mean annual air temperature (1953 - 2017) of 2.5°C and mean annual precipitation of 281 mm, of which 86% (~242 mm) falls during the growing season from May to September. The soil type is classified as dark chestnut in the Chinese soil classification or as Calcis-orthic Aridisol under US Soil Taxonomy classification. The water content is 0.29 m³ m⁻³ at field capacity and 0.12 m³ m⁻³ at the wilting point (Hao *et al.*, 2010). In 2012, we established the ECEB-II experiment in an undisturbed, native temperate semiarid steppe which has been fenced-off to prevent grazing since 1979. The community is dominated by C3 perennial rhizome and bunch grasses, including *Leymus chinensis*, *Stipa grandis*, *Achnatherum sibiricum* and *Agropyron cristatum*, which cumulatively account for approximately 75% of total aboveground biomass.

Experimental design

In this study, we defined drought by the length of the precipitation-free period. The local precipitation regime is typically centered within the growing season. Thus, we analyzed growing-season weather data (1953-2010, longest record period) obtained from the Meteorological Station of Xilinhote city and found the longest period between two sequential rainfall events was 30 days. We therefore defined an extreme drought treatment as a rain-free period of 30 days. The drought treatments using passive rain exclusion shelters lasted between 20 July and 19 August during mid-growing season each year from 2013 to 2016. The 99th percentile of daily maximum temperature during

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the growing season is 38 °C, which was used as the cutoff temperature to define a heat wave as per De Boeck, Dreesen, Janssens, & Nijs (2010). We found just one such event in the historical record. It occurred in July 2000 and lasted 7 days, during which the difference in daily maximum temperature above the historical average was greater (+7.0 °C) than the difference in minimum nighttime temperature (+1.2 °C). Thus, a heat wave in this region is characterized by large increases in daytime temperature with minimal differences at night (Li *et al.*, 2016). Therefore, our heat wave treatment was designed to produce air temperatures of 38 °C during daytime (9:00 - 15:00 h) for a period of seven consecutive days, with no nighttime treatment. Warming started from 09:00 a.m., raised the air temperature to around 38 °C, then maintained this temperature till 15:00. Warming was applied with a transparent infrared lamp (2000 W, 220 V, 100 cm × 31.4 cm, PHILIPS) connected with a thermal resistor (CU 50, Micro Sensor Co., Ltd., China) to an intelligent temperature controller (XMT 7100, Huibang technology Co., Ltd., China).

These two climate extreme factors, drought and heat wave, were crossed to form a fully factorial experiment including four treatments: (1) ambient conditions for control (control), extreme drought (drought), heat wave (heat wave) and drought in combination with heat wave (hot drought). There were three replicates for each treatment and twelve plots (2.0 m × 2.0 m each) randomly located in four blocks. Water exchange across plot boundaries was prevented by a metal flashing installed to a depth of 40 cm and extending 10 cm above ground. For detailed information about the rainout shelter design and heat wave treatments, see Li *et al.*, (2016).

Due to limited electrical supply at the research site, asynchronous warming was used for heat wave and hot drought treatments. Infrared lamps were moved from heat wave treatment plots to hot drought treatment plots as soon as a heat wave treatment heating finished (for heating periods see Table S1). Ambient environmental conditions during

the two heating periods were similar each year, as indicated by air and soil temperatures, wind speed and photosynthetically active radiation (Fig. S1). Accordingly, ecosystem CO₂ fluxes of the Control treatment were also similar during these two adjacent periods (Fig. S3). Compared with the dynamics observed during the whole vegetative period (about 5 months), these adjacent 7-day periods were quite similar in weather and ecosystem state; therefore, we do not expect ecosystem structure and function responses to climate extremes to be biased by the asynchronous warming.

Micro-climate

Daily precipitation data were obtained from a nearby (50 m approximately) tipping bucket rain gauge at 1.5 m above ground (TE525MM, Campbell Scientific Inc.). During the heating period hourly from 9:00 to 15:00, soil temperature at the depth of 10 cm and canopy temperature at the height of 40-60 cm were measured by soil thermometers (TL-883, Tonglixing technology Co., Ltd., China) and plant canopy thermometers (ST-2955, Shanghai Sintek International Trade Co., Ltd., China), respectively. Over the growing season from 2013 to 2016, soil water content (SWC) of the top 20 cm was measured in each plot with time domain reflectometry probes (TDR 300, Spectrum Technologies, Inc. CST, USA) vertically inserted into soil. SWC was measured about every 5 days in 2013, 2015 and 2016 and approximately every 10 days in 2014. Soil temperature (Ts) at the depth of 10 cm and air temperature (Ta) at 50 cm above the ground were measured manually with thermometers during CO₂ flux measurements (see Ecosystem CO₂ flux section). To account for any spatial variation (Fig. S2), canopy temperature and SWC were measured in three different locations per plot each measurement, and their mean was used to represent the plot value.

Plant leaf physiology

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We made plant leaf physiological measurements (light-saturated photosynthetic rate (A), stomatal conductance (g_s) and chlorophyll fluorescence (F_v/F_m)) and leaf temperature (T_{leaf}) of the dominant grasses *Leymus chinensis*, *Stipa grandis* and *Achnatherum sibiricum* (this latter species was only measured in the latter two years) during and after experimental treatments from 2013 to 2016 (seven, four, four, and four times, respectively). Among the measurements, A , g_s and T_{leaf} were measured using a portable photosynthesis system (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany) with a 4-cm² leaf cuvette between 9:00 and 11:30 h in the morning, when ecosystem CO₂ fluxes were also measured (see below). One or more fully expanded leaves (paralleled, but not overlapped) per plant per species in each plot was/were placed into the cuvette. Note that curly leaves of *S. grandis* were directly measured without flattening. Flattening is difficult to perform repeatedly and is unnecessary given our focus on the relative change across treatments. Measurements were performed under saturated light (1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$), while CO₂ concentration, block temperature and block humidity were set to ambient conditions. Chlorophyll fluorescence (F_v/F_m , the maximum quantum efficiency of photosystem II (PS II)), is a widely-used stress indicator, because decreases in dark-adapted F_v/F_m reflect damage to PS II. F_v/F_m was recorded using a pulse-amplitude-modulated photosynthesis yield analyzer (Dual-PAM, Heinz Waltz, Effeltrich, Germany) with a leaf clip holder. We tested fluorescence values of fully expanded leaves for each species between 20:00 and 23:00, allowing adequate dark adaptation on the same days when other leaf physiological measurements were made.

Aboveground biomass and community structure

Aboveground biomass (AGB) and community structure were estimated once a year by harvesting all aboveground plant material in one 0.25-m² quadrat located within each 4-m² plot around 15th September. Before clipping, the number of individuals was

recorded by species to assess community structure, including richness (number of species in 0.25-m² quadrat), abundance (number of stem per m²) and Shannon's diversity (H'). All species were also categorized into three ecological functional groups: grass, forb and shrub. Within four hours of clipping, fresh leaves were separated from the plant and flattened to measure leaf area using a leaf area meter (LI-3000C, LI-COR Inc., Lincoln, NE, USA) and calculate leaf area index (LAI). Then all plant tissues were oven dried at 65 °C for 48 hours and weighed. For each year, the locations of quadrats were varied to prevent resampling of the same quadrat.

Belowground biomass

Belowground biomass (BGB) was estimated by specific root length (SRL) and root length (Fischer *et al.*, 2007). SRL at this site was estimated at 36 m g⁻¹ (Cheng *et al.*, 2016) and root lengths were measured by the minirhizotron technique. In each plot, one transparent minirhizotron tube (7-cm external diameter, 100-cm length) was installed in the soil at a 45° angle from the horizontal to a depth of 42 cm during May 2012. Holes were made with a soil corer of the same diameter as the tubes, which ensured tight contact with surrounding soil. Portions of the tubes (20-cm length) exposed at the surface were covered with adhesive aluminum foil, and the ends were capped to prevent entry of water, light, and heat. To quantify root length, images were taken at vertical depths of 0–14 cm, 14–28 cm and 28–42 cm, using a root scanner system (CI-600 Root Growth Monitoring System, CID Inc., Vancouver, WA, USA). Images, taken in grayscale at 400 dpi, were analyzed separately using the program RootAnalysis (Analysis One Co. Ltd, Beijing, China) to calculate root length. This method has been widely used in many natural multiple-species ecosystems to assess root biomass and we have increased confidence in BGB estimation by this method because it matched BGB based upon traditional soil coring methods in an area adjacent to the experiment

(Li *et al.*, 2019). We summed AGB and BGB to calculate total biomass (TB).

Ecosystem CO₂ flux

In May 2012, one square stainless steel frame (50 cm × 50 cm, 10 cm high with 3 cm extending aboveground) was inserted in each plot. We used an infrared gas analyzer (LI-840A, LI-COR Inc., Lincoln, NE, USA) and a transparent chamber (50 × 50 × 50 cm), attached to one air pump (6262-04, LI-COR Inc.) and two plastic pipes, to measure CO₂ fluxes between 9:00 - 11:30 h on sunny days (measurement data see Fig. S2). The chamber was successively used without and with a lightproof covering to measure net ecosystem exchange (NEE) and ecosystem respiration (ER), respectively. Each measurement lasted 90 seconds and there was an interval of at least 10 s. between two consecutive recordings to replace the air in the chamber. CO₂ flux rates were determined from the time-course of CO₂ concentrations (Chen, Lin, Huang, & Jenerette, 2009). Gross ecosystem production (GEP) was calculated as the difference between NEE and ER. Positive and negative CO₂ flux values indicate net ecosystem CO₂ emission and uptake, respectively.

Statistical analyses

We used a repeated measures ANOVA to test for the effects of heat waves, drought, year, and their interactive effects on community biomass (AGB, BGB and TB), community structure (LAI, abundance, richness and Shannon's diversity), annual averaged ecosystem CO₂ fluxes (NEE, ER and GEP), and annual averaged plant physiology (A , g_s and F_v/F_m) for each species. A *post-hoc* Duncan test was used to test for mean differences of the above variables across the four treatments each year. We also used a paired t-test for heat wave effects on soil temperature and vegetation surface temperature during the heating period. T-tests were also used to determine the

differences in treatment effects on relative abundance of grass, as well as AGB of grass and non-grass (forb plus shrub) between control and drought/ heat wave/ hot drought. Treatment effects were considered to be statistically significant at $P \leq 0.05$ and marginally significant at $P \leq 0.10$ given the small number of treatment replicates ($n = 3$). All statistical analyses were performed with R v.3.4.4, R (R Core Team, 2018).

We used structural equation modeling (SEM) to quantify the direct and indirect impacts of heat waves and drought on A per species and ecosystem-level net CO_2 uptake (-NEE). Drought and heat wave-induced changes in leaf temperature (T_{leaf}), SWC, g_s and F_v/F_m were included in the SEM to explore the effects of climate extremes on A . Similarly, several major pathways were constructed to explore the effects of climate extremes on -NEE, including environmental variables (SWC, T_a), A , and LAI, abundance of grass and non-grass, and biomass (AGB of grass and non-grass as well as BGB). Data were fitted to the model using the maximum likelihood estimation method. Adequate model fit was indicated by a non-significant chi-squared test ($P > 0.05$). SEM analysis was performed using the AMOS 25 software (IBM, SPSS, Armonk, NY, USA). Before SEM analyses were conducted, we first tested for individual relationships between each variable pairing to identify significant and linear relationships (Fig. S3 and S4).

RESULTS

Micro-climate

During the four years of the experiment (2013-2016), rain shelters excluded 49 mm, 37 mm, 52 mm and 42 mm of ambient precipitation during 30 days of drought treatments, which is equal to 17%, 15%, 22% and 22% of growing season precipitation (GSP) (Fig. 1a-d). The soil water content (SWC) declined clearly during drought periods and recovered rapidly with the first precipitation after drought, while the heat wave treatment did not significantly change SWC (Fig. 1a-d). Heat waves significantly increased the mean canopy temperature by 3.2 °C and 3.5 °C during the heating period in heat wave and hot drought treatments, respectively ($P < 0.01$ for both, Fig. 1e-h). Meanwhile, soil temperature was significantly raised by 0.4 °C and 0.5 °C in the heat wave and hot drought treatment compared with the ambient control, respectively ($P < 0.01$ for both, Fig. 1e-h).

Plant physiological responses

Both drought treatments (drought and hot drought) decreased stomatal conductance (g_s) and light-saturated photosynthetic rate (A) over the last three years (but not in 2013), regardless of species and heat wave treatment (Fig. 2 and Table S2). The F_v/F_m values of the three species were ~ 0.8 in all treatments across the four years, even though they were significantly and marginally significantly reduced by drought for *Leymus chinensis* and *Stipe grandis*, respectively (Figs. 2b, e, h and k, and Table S2). No clear significant heat wave effects were recorded for g_s , A and F_v/F_m over the four years. There were no significant interaction effects between drought and heat wave for these plant physiology parameters in any years, except for the drought and heat wave interaction for F_v/F_m of *Stipe grandis* (Fig. 2 and Table S2).

Results of SEM suggest that SWC had significant positive effects on g_s which further

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had a strongly positive effect on A for all three species. Similarly, SWC had a significant positive effect on F_v/F_m , but F_v/F_m had significant positive effects on A only for *Achnatherum sibiricum*. T_{leaf} had significant or marginally significant negative relationships with SWC and G_s . T_{leaf} significantly increased F_v/F_m for *Leymus chinensis* and *Stipe grandis* but not *Achnatherum sibiricum*. SWC significantly increased A for *Stipe grandis* and *Achnatherum sibiricum*, but not *Leymus chinensis*. A was significantly increased by T_{leaf} and F_v/F_m only for *Achnatherum sibiricum* (Fig. 3).

Functional group-level responses

At the functional group-level, we found a decreasing trend of relative abundance of grass in response to all of the climate extreme treatments across the four years, which are evident under the heat wave treatment in year 2014 and under the drought treatment in years 2015 and 2016, except for the hot drought treatment in the first year. Similarly, AGB of grass consistently declined under the heat wave or drought treatments over the first three years, while AGB of non-grass increased. Both AGB of grass and non-grass decreased under the hot drought treatment across the four years and under the separate heat wave and drought treatments in the last year (Fig. 4 and Table S4).

Community-level responses

At the whole community level, heat waves significantly reduced AGB, BGB and thereby TB, while droughts did not significantly change any component of biomass (Fig. 5 and Table 1). There was a marginally significant two-way interaction between heat wave and year on TB with larger reductions in 2016 than in the other two years, as AGB reduction was mainly reflected in 2016. Interactive effects of drought and heat wave on biomass were not significant. However, the lowest AGB was usually found in the hot drought treatment (Fig. 5 and Table 1). Overall, climate extremes had little

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effects on the whole community-level structure (LAI, abundance, richness and Shannon's diversity (H'), Fig. S5 and Table S3).

Ecosystem CO₂ fluxes

Droughts significantly suppressed growing season average ecosystem CO₂ fluxes (NEE, ER and GEP) across the four years, and such effect was smaller in 2015 than the other three years (drought $\times$ year interaction, $P = 0.03$ and 0.08 for NEE and GEP, respectively; Fig. 6, Fig. S6 and Table 1). Heat waves had no significant effects on any growing season average CO₂ fluxes, whereas there was a marginally significant positive effect on NEE ($P = 0.07$, Fig. 6, Fig. S2 and Table 1). Across all CO₂ fluxes, there were no significant two-way interactions between drought and heat wave, nor three-way interactions among drought, heat wave and year; however, the magnitude of CO₂ fluxes were generally the lowest in the hot drought treatment (Fig. 6).

Results of SEM suggest that SWC had positive effects on A , LAI and grass abundance. LAI and grass abundance further had positive effects on AGB of grass. A and AGB of grass had positive effects on ecosystem CO₂ uptake. Air temperature (T_a) had no significant effects on other variables (Fig. 7).

DISCUSSION

It has long been recognized that carbon cycling processes in semiarid grasslands are highly sensitive to climate change (Christensen, Coughenour, Ellis, & Chen, 2004; Biederman *et al.*, 2017). Here we conducted a full factorial manipulative experiment to test single and especially interaction of climate extremes, which is more meaningful and powerful in predicting global change effects than single-factor studies (Luo *et al.*, 2008; Zhu, Chiariello, Tobeck, Fukami, & Field, 2016). Importantly, we measured physical and biological responses across multiple levels of ecological hierarchy simultaneously, allowing us to identify comprehensive mechanisms of grassland carbon cycle response to heat wave and drought.

Drought impacts on grassland carbon cycling across hierarchical levels

Overall, drought reduced stomatal conductance and leaf photosynthetic rate of all three dominant grass species, *Leymus chinensis*, *Stipe grandis* and *Achnatherum sibiricum* (Table S2), partly supporting our first hypothesis that reduced soil water content would have a negative effect on stomatal conductance, reducing leaf photosynthetic rate (Fig. 2). Our results are in accordance with other drought studies showing that plants may respond to water deficit by closing their stomata to prevent water loss, consequently curtailing leaf photosynthetic rate due to reductions of CO₂ diffusion from air into leaf (Asensio, Penuelas, Ogaya, & Llusia, 2007; Erice *et al.*, 2011). However, drought did not reduce chlorophyll fluorescence (around 0.8, Fig. 2), the optimal value of which is about 0.83 for most non-stressed plant species (Björkman & Demmig, 1987). Our results suggest that immediate but transient stomatal closure allowed plants to tolerate the water stress without permanent leaf damage.

Decreased leaf photosynthesis of grasses led to reductions in AGB of grasses functional group under drought. Additionally, relative abundance of grass was generally reduced

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in the face of drought (Fig. 4b). It means grass functional group is vulnerable to drought in this semiarid grassland. However, community-level AGB was not significantly reduced by drought (Fig. 5a), which was attributed to the fact that AGB and relative abundance of non-grass functional group were increased at the same time (Fig. 4b). This may be because many shrub and forb species in this grassland, such as *Artemisia frigida*, *Potentilla acaulis* and *Carex*, are characterized by drought tolerance or/and strong colonization ability (Y. Gao *et al.*, 2009; Y. Z. Gao *et al.*, 2007). Such compensatory effects among major components at the functional group levels buffered the effects of climate inter-annual and seasonal variability on community-level structure and biomass in this mature grassland with high species richness and community biodiversity (Bai *et al.*, 2004; Liu *et al.*, 2017).

In addition to biomass, whole community-level structure was stable in the face of drought (Fig. S5 b, c, and d). Taken together, our results suggest aboveground components of this semiarid grassland were resistant to the drought of ~60-year recurrence interval. The minor changes in community structure and AGB under droughts in this study might also partly result from feedbacks of root system response. There was a slight non-significant increasing trend of BGB in the drought treatment (Fig. 5), possibly due to plant structural adaptive strategies in response to water stress; that is, drought stimulated translocation of newly assimilated carbon from shoot to root (Burri *et al.*, 2014; Huang & Fu, 2000; Sanaullah, Chabbi, Rumpel, & Kuzyakov, 2012) to increase the potential for water and nutrient uptake (van der Molen *et al.*, 2011). Stronger root systems in turn promote resistance of aboveground parts to climate stress.

SEM results suggest that decreases in leaf photosynthesis and AGB of grass via LAI and grass abundance under drought made large contribution to reduced net carbon uptake at the ecosystem level in this semiarid ecosystem (Fig. 6 and 7). As we hypothesized, ecosystem CO₂ uptake in response to water stress was strongly regulated

by photosynthesis at the individual level (Fig. 7), as CO₂ uptake directly achieved by this metabolic process. Individual photosynthesis was mainly controlled by stomatal conductance (Fig. 3), indicating stomatal conductance is a powerful proxy for ecosystem CO₂ uptake.

Prior manipulative experiments in this grassland have demonstrated that LAI has a positive relationship with carbon uptake in the face of precipitation changes at the ecosystem scale (Liu *et al.*, 2017). A multiple-model study at this site suggested that inter-annual variability of GEP was attributed to responses of A and LAI to soil water (Hu *et al.*, 2018). Our SEM results provide an insight into positive effects of LAI on ecosystem CO₂ uptake and suggest that LAI regulated CO₂ uptake through AGB of grasses, but not of forbs and shrubs (Fig. 7). Besides, grass abundance could also exert positive effects on ecosystem CO₂ uptake via AGB of grass, while non-grass abundance had no significant effects. It highlights the key and important role of grass in controlling ecosystem CO₂ uptake, consistent with vegetation removal experiments showing that the greatest decrease in CO₂ sink strength after warming when grasses were present in peatlands (Ward *et al.*, 2013) and that grass removal lead to larger losses in CO₂ sink than shrub removal under drought (Kuiper *et al.*, 2014).

Heat wave impacts on grassland carbon cycling across hierarchical levels

Heat wave did not affect chlorophyll fluorescence while slightly decreased leaf photosynthetic rates for all the species (Fig. 2), consistent with the negative relationship between leaf temperature and stomatal conductance (Fig. 3). Our results suggest that photosynthesis may remain resistant to temperatures as high as 38 °C in the dominant species of this long-term fenced semiarid grassland.

Similar to drought, functional group compensation, decreasing grass and increasing non-grass biomass, was also found under heat wave during the first three years (Fig.

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4a), maintaining the community-level AGB largely unchanged. However, a negative AGB response was measured in the fourth year, when AGB of grass and non-grass declined together (Fig. 5a). We suggest the AGB compensatory effects disappeared because of the cumulative negative effects of four years of heat waves on root production, limiting nutrient and water uptake and ultimately aboveground growth (Fig. 5b). Reduced AGB in the fourth year advances understanding from prior, shorter-term manipulative heat wave experiments showing AGB is little affected after 1-2 years heat wave treatment, especially when soil moisture remains sufficient (De Boeck *et al.*, 2011; De Boeck *et al.*, 2016; Dreesen *et al.*, 2012; Hoover *et al.*, 2014). It is unknown whether reductions in BGB was prevalent in these prior heat wave studies, because it was not measured. It is also unclear whether the observed unchanged AGB in prior short-term heat wave experiments would have shown declines after additional years.

Our results that community-level biomass both aboveground and belowground were significantly reduced in response to heat wave (Fig. 5), in agreement with another heat wave experiment suggesting that a 7-day heat wave of +12 °C above ambient decreased leaf, stem and root biomass (Bauweraerts *et al.*, 2014). However, a recent meta-analysis suggested that experimental warming stimulates grassland aboveground and belowground primary productivity (Wang *et al.*, 2019). Large reductions in BGB dominated the negative response of total biomass (TB), also contrasting with past findings that long-term climate warming shifted TB allocation belowground (Liu *et al.*, 2018). These results highlight discrepant effects of long-term “press” warming and short-term “pulse” heat waves on grassland biomass.

Combined drought and heat wave impacts on grassland carbon cycling across hierarchical levels

Contrary to our third hypothesis, the interactions of drought and heat wave on

ecosystem carbon cycling were not significant at any hierarchical level, regardless of timing of hot drought treatment (Table 1, S5 and S6). A potential limitation with respect to our examination of interactions was asynchronous warming for heat wave and hot drought treatments due to limited electrical supply at the research site. Fortunately, in the absence of large fluctuation in ambient environmental conditions during the two heating periods each year made the four treatments comparable to a large extent. Additionally, direct comparison of drought with hot drought treatment showed key states and fluxes of the hot drought treatment (e.g. A , AGB, NEE, ER, and GEP), just non-significantly lower than those of the drought treatment alone. Therefore, we are confident that there were not significant interactions of drought and heat wave in this study.

In contrast to the single drought or heat wave treatment, we did not find compensatory effects between grass and non-grass functional groups under the hot drought treatment. Instead, AGB of both grass and non-grass were consistently reduced across the four years (Fig. 4c). Hot drought plots had the lowest AGB of all treatments in each year, although the interactions of heat wave and drought were not significant (Fig. 5a). It is because root production was reduced, similar to the heat wave treatment (Fig. 5b), curtailing capacity for water and nutrition uptake as mentioned above. Importantly, soil water content was lower under the hot drought treatment than the heat wave treatment alone, further limiting transportation of water and nutrition to aboveground plant parts. Consequently, the compensatory effects of plant functional groups which initially maintained AGB under the hot drought treatment disappeared after the first year.

Although the interactions of drought and heat wave on ecosystem CO_2 fluxes were not significant, the hot drought treatment had the lowest magnitudes of NEE, ER and GEP in each year (Fig. 6), suggesting apparent additive negative effects. This non-

significance of interaction may be because the heat wave did not cause significant effects on soil moisture as compared with the drought alone (Fig. 1). The additive negative effects of drought and heat wave on ecosystem CO₂ exchange could result from negative effects of leaf temperature on stomatal conductance and thereby leaf photosynthetic rates (Fig. 1e-h and 3) as well as larger reduction in AGB of grass (Fig. 5c).

This study has direct implications for understanding of grassland structure and functions amid future climate changes. By connecting responses across multiple hierarchical levels, we can provide a more comprehensive mechanism behind carbon cycle response to climate extremes than past “patch” studies. Our findings indicate that individual physiological adjustments controlled photosynthesis responses, and then drove corresponding species biomass responses, ultimately co-regulating ecosystem carbon exchange. Meanwhile, individual structural adjustments and compensation effects between functional groups co-determined total biomass response.

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Conflict of Interest statement

There is no conflict of interest to declare.

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Table 1. Results of repeated ANOVA for responses of aboveground biomass (AGB), belowground biomass (BGB), total biomass (TB), net ecosystem exchange (NEE), ecosystem respiration (ER), and gross ecosystem production (GEP) to drought, heat wave, year, and their interactions.

	AGB	BGB	TB	NEE	ER	GEP
Heat wave	0.05	0.01	<0.01	0.07	0.81	0.13
Drought	0.31	0.34	0.40	<0.01	<0.01	<0.01
Year	<0.01	0.68	0.17	<0.01	<0.01	<0.01
Heat wave $\times$ Drought	0.48	0.71	0.68	0.73	0.99	0.79
Heat wave $\times$ Year	0.68	0.06	0.07	0.91	0.84	0.89
Drought $\times$ Year	0.79	0.80	0.70	0.03	0.59	0.08
Heat wave $\times$ Drought $\times$ Year	0.56	0.34	0.37	0.93	0.42	0.93

P-values in bold are statistically significant to an alpha value of 0.05.

Figures Legends

Fig. 1. Precipitation and soil water content during the growing season (a-d) and heating effects on soil and canopy temperature during the heating periods (e-h). Δ Temperature is the difference between heating treatments and control treatment. The blue, orange and green shaded stripes in a-d indicate periods of drought treatment, and periods of heating in heat wave and hot drought treatments, respectively. Error bars indicate 1 SE.

Fig. 2. Climate extremes effects on leaf physiologies of the three dominant species. *gs*: stomatal conductance; *Fv/Fm*: chlorophyll fluorescence; and *A*: leaf photosynthetic rate. Error bars indicate 1 SE. Different letters indicate significant differences ($P \leq 0.05$) among treatments.

Fig. 3. Structural equation model showing the effects of abiotic factors and biotic factors on plant leaf photosynthesis. T_{leaf} : leaf surface temperature, SWC: soil water content, *gs*: stomatal conductance, *Fv/Fm*: chlorophyll fluorescence, and *A*: leaf photosynthetic rate. Solid black and solid grey arrows represent significant ($P \leq 0.05$) or marginally significant ($0.05 < P < 0.10$) paths for all there species, for only two species, respectively, while grey dashed arrows represent significant ($P \leq 0.05$) paths for only one species. Standardized path coefficients are listed on each path (three numbers for *Leymus chinensis*, *Stipe grandis* and *Achnatherum sibiricum* successively), in which, black indicates significant, grey indicates marginally significant while ns indicate non-significant ($P \geq 0.10$). r^2 values represent the proportion of variance explained for each variable. Column graph in below panel showing the standardized total effects (sum of direct and indirect effects) derived from the structural equation model.

Fig. 4. Climate extremes effects on aboveground biomass of grass and non-grass and relative abundance of grass. Δ is the difference of treatment and control. Error bars indicate 1 SE. Asterisks are significant at different levels between treatments and

control: * $P < 0.10$, ** $P < 0.05$.

Fig. 5. Climate extremes effects on community biomass. AGB: aboveground biomass; BGB: belowground biomass; and TB: total biomass. Error bars indicate 1 SE. Different letters indicate significant differences ($P \leq 0.05$) among treatments while ns indicates no significant difference among treatments.

Fig. 6. Climate extremes effects on ecosystem CO₂ fluxes. Solid column and empty column in upper half represent ecosystem respiration (ER) and gross ecosystem production (GEP), respectively, while solid column in bottom half represents net ecosystem exchange (NEE). Negative values indicate the ecosystem absorbs CO₂ from the atmosphere (net sink), while positive values indicate the ecosystem emits CO₂ into the atmosphere (net source). Error bars indicate 1 SE. Different letters indicate significant differences ($P \leq 0.05$) among treatment.

Fig. 7. Structural equation model showing the effects of abiotic factors and biotic factors on net ecosystem CO₂ uptake. SWC: soil water content, Ta: air temperature, A: leaf photosynthetic rate; LAI: leaf area index. Abundance_{grass} and Abundance_{non-grass} indicate abundance of grass and non-grass (forb plus shrub), respectively. AGB_{grass} and AGB_{non-grass} indicate aboveground biomass of grass and non-grass, respectively. BGB: belowground biomass. Ecosystem CO₂ uptake is absolute value of net ecosystem exchange (NEE). r^2 values represent the proportion of variance explained for each variable. Solid and dashed arrows represent significant ($P \leq 0.05$) and non-significant ($P > 0.05$) paths, respectively. Standardized path coefficients are listed on each path. Column graph in left bottom showing the standardized total effects (sum of direct and indirect effects) derived from the structural equation model.

- It is indispensable to explore the mechanisms and impacts of climate extremes on the carbon cycle given they are increasing in magnitude and frequency.
- Aboveground biomass responses to climate extremes rely on the functional group compensatory effects and feedbacks of root system responses.
- Plant physiological adjustments and shifts in community composition co-regulate ecosystem CO₂ fluxes under climate extremes.