



Extreme-duration drought impacts on soil CO₂ efflux are regulated by plant species composition

Chaoting Zhou · Joel A. Biederman · Hui Zhang ·
Linfeng Li · Xiaoyong Cui · Yakov Kuzyakov ·
Yanbin Hao  · Yanfen Wang

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Abstract

Aims Long-duration drought can alter ecosystem plant species composition with subsequent effects on carbon cycling. We conducted a rainfall manipulation field experiment to address the question: how does drought-induced vegetation change, specifically shrub encroachment into grasslands, regulate impacts of subsequent drought on soil CO₂ efflux (R_s) and its components (autotrophic and heterotrophic, R_a and R_h)?

Methods We conducted a two-year experiment in Inner Mongolia plateau, China, using constructed steppe communities including graminoids, shrubs and their mixture

(graminoid + shrub) to test the effects of extreme-duration drought (60-yr return time) on R_s , R_h and R_a . **Results** Our results indicated that extreme-duration drought reduced net primary production, with subsequent effects on R_s , R_h and R_a in all three vegetation communities. There was a larger relative decline in R_a (35–54%) than R_s (30–37%) and R_h (28–35%). Interestingly, we found R_s in graminoids is higher than in shrubs under extreme drought. Meanwhile, R_h declines were largest in the shrub community. Although R_a and R_h both decreased rapidly during drought treatment, R_h recovered quickly after the drought, while R_a did not, limiting the R_s recovery.

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C. Zhou · L. Li · X. Cui · Y. Hao · Y. Wang
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 10049, China

J. A. Biederman
Southwest Watershed Research Center, Agricultural Research Service, Tucson, AZ 85719, USA

H. Zhang
College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China

X. Cui · Y. Hao (✉) · Y. Wang
CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences (CAS), Beijing 100101, China
e-mail: ybhao@ucas.ac.cn

Y. Kuzyakov
Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, University of Göttingen, 37077 Göttingen, Germany

Y. Kuzyakov
Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia

Conclusions This study suggests that plant species composition regulates several aspects of soil CO₂ efflux response to climate extremes. This regulation may be limited by above- and below-ground net primary production depending on soil water availability. The results of this experiment address a critical knowledge gap in the relationship between soil respiration and plant species composition. With shrub encroachment into grasslands, total soil respiration is reduced and can partly offset the effect of reduction in productivity under drought stress.

Keywords Extreme drought · Soil CO₂ efflux · Autotrophic · Heterotrophic · Plant species composition · Net primary production

Introduction

Under ongoing global climate change, the frequency and intensity of extreme drought are expected to increase due to higher temperature and changing precipitation patterns (Dai 2012; Trenberth et al. 2013). Extreme drought can dramatically change plant community composition (Tielbörger et al. 2014; Hoover and Rogers 2016), in turn altering ecosystem carbon (C) cycling due to different physiological responses of plant species and shifts in size and activity of soil microbial populations (Metcalfé et al. 2011; Tietjen et al. 2016). Total CO₂ efflux from soil (R_s) returns the majority of photosynthetically-fixed carbon to the atmosphere, therefore, it is a critical component of the terrestrial carbon cycle (Schlesinger and Andrews 2000; Högberg and Read 2006).

In past decades, many experimental and modeling studies have addressed the effects of changes in mean climate (precipitation and temperature) on ecosystem carbon cycling (Reichstein et al. 2013; Poulter et al. 2014). Previous studies showed differing drought responses of total soil CO₂ efflux (R_s) across biomes. Drought has resulted in increased rates of total R_s predominantly in wetland habitats (Savage and Davidson 2001; Jensen et al. 2003), while in mesic or xeric habitats, drought has reduced R_s but with variable effects on its components (Selsted et al. 2012; Suseela et al. 2012; Balogh et al. 2016), as well as having little to no effect in some cases (Freeman et al. 1996;

Domínguez et al. 2017). However, there has been relatively less focus on extreme climate events such as long-duration drought. Furthermore, the relative importance of plant community composition in regulating R_s is still unknown in the context of extreme-duration drought. Understanding these relationships is pivotal because vegetation change is widespread and increasing under climate change, with pronounced impacts on the carbon sink function of terrestrial ecosystems (Putten et al. 2013).

Extreme climate events can lead to dramatic shifts in community composition (Tielbörger et al. 2014; Hoover and Rogers 2016). Vegetation change may impact R_s through altering primary production (both above and below ground) and associated substrate production (Xu et al. 2015). Prior studies suggest that the quantity of organic matter input to soil is the principal mechanism by which vegetation change may alter R_s (Metcalfé et al. 2011; Xu et al. 2015). Shifting of community composition can change the amount of photosynthetic carbon channeled below-ground, which could substantially alter R_s due to autotrophic root respiration as well as respiration by root-associated heterotrophic communities (Metcalfé et al. 2011). Generally, extreme drought will lead to alterations of species composition, with consequences for soil C storage and dynamics (Knapp et al. 2008a, b; Davis et al. 2000). Therefore, as abiotic (soil moisture and temperature) and biotic (plants and microbial activity) factors change associated with community composition under extreme drought stress, differential responses of autotrophic (R_a) and heterotrophic CO₂ respiration (R_h) to these factors are very likely to cause a shift in their contributions to R_s . However, how these shifts in species composition modification the contribution of R_a and R_h to R_s remains poorly understood.

Grassland covers approximately 80% of the Inner Mongolia Plateau and constitutes a major part of East Asian grasslands (Batima and Dagvadorj 2000). Water availability is the main control on plant productivity in this semiarid biome (Bai et al. 2008). Decreasing rainfall and increasing duration and severity of drought likely contribute to observed vegetation change from grassland to shrubland (Li et al. 2012). Here, we conducted a two-year rainfall manipulation field experiment with extreme-duration drought to address a specific question: How does plant species composition

regulate the drought response of total CO₂ efflux and its components (autotrophic CO₂ and heterotrophic CO₂) during and after extreme drought?

Material and methods

Experimental design

The study site is located in the Research Station of Animal Ecology (44°18' N, 116°45' E, 1079 m a.s.l), Maodeng Pasture of Inner Mongolia Autonomous region, China. The study area belongs to the continental temperate semi-arid climate with mean annual temperature of 3 °C and mean annual precipitation of 350 mm. Precipitation distribution is unimodal with a major peak in July and more than 80% occurring during the growing season (May–September). The experiment was carried out from early May 2012 as a two-factor random block design with extreme drought treatment and three compositions of plant species. Twenty-four 2 m × 2 m plots were grouped into four blocks with 1-m intervals between plots. Weeding was conducted periodically to maintain species composition. A metal flashing was dug into the ground 40 cm deep around each plot and extended 10 cm above ground to prevent lateral water transfer. Polyvinylchloride (PVC) collars (5 cm in height and 20 cm in internal diameter) were inserted to depths of 2–3 cm in each plot to measure total CO₂ efflux from soil.

Extreme drought events

The extreme drought events were designed by statistical extremity with respect to a historical reference period (extreme value theory) independent of biological effects. Fitting a Gumbel I distribution to the ~60-year local weather data (longest available dataset, Xilin Gol League Meteorological Administration), we estimated a 60-year return-period drought of 30 days with no rainfall. This was applied during the peak growing season from mid-July to mid-August (Fig. 1a).

Extreme drought events were simulated using a 27 m² (4.5 m × 6 m) rainout shelter consisting of a steel frame supporting a transparent polyester fiber board, which permitted ca. 90% penetration of photosynthetically active radiation, with no obvious shading effects. Unwanted greenhouse effects on microclimate were minimized by starting the roof from a height of 3 m,

allowing ample near-surface air exchange (Fig. 1b). Near-surface air temperature was slightly increased by the roofs during the weather manipulation period, but differences were not significant compared with ambient conditions. After the experimental drought, the roof was removed. The ambient control plots remained without manipulation throughout the entire experiment period. After plot establishment in 2012, all plots were exposed to the same drought treatments as those described in the present study, which began in 2015.

Experimental plant communities

Experimental plots were planted with one of three plant species compositions: G (graminoid), S (shrub) and GS (graminoid + shrub) (Table 1). Four widespread plant species were chosen to represent the dominant flora of this region. These selected species were separated into functional groups of grasses and shrubs, based on their overall importance in this area, and the fact that they naturally grow on the soil similar to what was used in this experiment. Seeds were collected in the same region in order to avoid agricultural cultivars. All plants were sown on site beginning in April 2012. To eliminate the effects of plant densities, the plants were kept consistently to the species abundance patterns measured in the field at the onset of the experiment. The plots were irrigated regularly in the first three months in 2012, to avoid potential seeding fatality due to drying. No additional irrigation treatment was applied after this period. Throughout the experiment, the composition of the plant community was maintained by removing seedlings of all other species monthly during growing season.

Quantifying total CO₂ efflux from soil

Nylon mesh bags, 40 cm deep × 25 cm diameter, were used to selectively exclude only roots (1 μm mesh spacing). We removed soil cores (30 cm deep × 25 cm diameter) by hammering a metal cylinder into the ground, then placed them into mesh bags and back to their original position in the ground, to minimize disturbance. This method is considered more accurate than the root excision method, because the effects of root rot on R_s can be avoided (Kuzyakov 2006). R_s and R_h were estimated directly as the total CO₂ efflux from soil

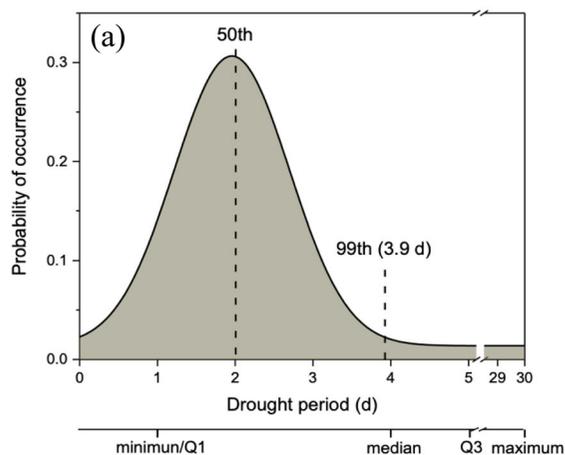


Fig. 1 The Extreme Drought Experiment was established in 2012 in a typical steppe in Inner Mongolia Plateau. **(a)** Frequency of occurrence of the duration of drought based on an estimated probability function calculated from ~60 years of growing season rainfall events. **(b)** During 2015 and 2016's growing seasons, four

27 m² shelters were applied to remove precipitation to impose drought treatment in the mid-growing stage. Three plant species composition (graminoid, shrub and graminoid +shrub) was nested within the rainfall shelters

collars with or without root exclusion, while R_a was estimated by their difference ($R_a = R_s - R_h$) (Moyano et al. 2007).

CO₂ efflux was measured in situ between 10:00 and 14:00 BJT with an infrared gas analyzer Li-Cor 8100 (LiCOR, Inc., Lincoln, NE, USA). Each soil collar was sampled two times before extreme drought treatment, once per week during drought treatment and again on the 1st, 4th, 7th, 11th, 15th and 35th days after drought (recovery period). CO₂ flux was measured continuously for 120 s after steady-state conditions were achieved (usually requiring 15–30 s before the recording interval). Increases in air temperature within the chamber during the measurement intervals were less than 0.2 °C. A soil CO₂ flux chamber attached to the infrared gas analyzer was placed on each collar for the measurements

of R_s and R_h , and then the chamber was moved to the next collar. During each measurement, soil temperature was measured with a T-type thermocouple (Li-COR, Inc., Lincoln, NE, USA), and soil moisture (volumetric water content, %) was measured with an ML2X soil moisture sensor at a depth of 10 cm (LI-COR, Inc., Lincoln, NE, USA).

Below-ground and above-ground net primary productivity

Belowground net primary productivity (BNPP) was estimated using root length (Jentsch et al. 2011) quantified with monthly minirhizotron images from July to September in 2015 and 2016 using a CI-600 (CID, Camas, Wash., USA) high-resolution color scanner

Table 1 Experimental plant communities of two vegetation types (grassland and shrub) were used at three functional diversity levels, resulting in three plant community compositions

Abbreviation	Vegetation type	Description	Species
G	Graminoid	Two species	<i>Leymus chinensis</i> , <i>Stipa grandis</i> ,
GS	Graminoid + Shrub	Four species	<i>Leymus chinensis</i> , <i>Stipa grandis</i> , <i>Caragana microphylla</i> , <i>Artemisia frigida</i>
S	Shrub	Two species	<i>Caragana microphylla</i> , <i>Artemisia frigida</i> ,

head mounted on a rotating motor. In April 2012, at the time of plant sowing, mineralized tubes were placed in the soil to ensure that the roots could be observed during experimental measurements. The scanner was inserted inside a minirhizotron tube installed at a 45° angle at known depths in each plot prior to the experiment. The upper part of the tube was covered with a black plastic cap to stop entry of water, light and heat. At each depth, the scanner head revolves 360° and records the interface between the clear tube and the soil. At each tube, three sequential images (21.6 cm × 19.6 cm) were taken at soil depths of 0–12, 12–24, and 24–36 cm. Images were analyzed using Root-Analysis imaging software (Duke University, Raleigh, N.C., USA).

Aboveground net primary productivity (ANPP) was determined at the end of each experimental year (first week in September) by clipping all aboveground plant biomass in 0.5 m × 0.5 m quadrats within each plot. All above-ground biomass collected was sent to the laboratory immediately. The harvested materials were oven-dried at 65 °C until a constant weight and was recorded. The aboveground net primary productivity of *C. mycophylla* in the shrub plots was determined by measuring the annual increase in aboveground woody biomass (wood productivity) and annual foliage productivity. We measured the average increase of diameter and height of basal stems and used stem densities to estimate the increase in biomass (Huenneke et al. 2001). Additionally, foliage litter production was harvested in the shrub plots. Foliage and wood productivity of each stem were summed to compute an estimate of above-ground productivity. This nondestructive process was repeated for each measurement period.

Microbial biomass

Soil microbial biomass mediates biogeochemical cycling and is a sensitive indicator of soil microbial activity under the varying soil water availability of extreme drought (Meisner et al. 2013). Soil samples were collected during and after treatment from the top 10 cm of each quadrat. Three soil cores (d = 3 cm) were composited after removing plant litter and roots. Microbial biomass was determined by the fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Briefly, paired soil samples were incubated for 24 h at 4 °C. Ten grams of sample were fumigated in the dark for 24 h with ethanol-free CHCl₃, while a separate ten-gram aliquot was not fumigated. Both fumigated

and non-fumigated aliquots were extracted with 0.5 M K₂SO₄ in a shaker for 0.5 h. The extract was filtered through filter paper and analyzed using a TOC analyzer (Elementar, Germany).

Statistical analyses

Treatment differences in soil moisture were analyzed using a one-way ANOVA, with extreme drought as the explanatory variable and block as an error term. We used Linear models combined with analysis of variance (ANOVA) to test the influence of drought treatments and plant species composition on soil water content (SWC), ANPP, BNPP, net primary productivity (NPP, the sum of ANPP and BNPP), microbial biomass carbon (MBC), R_s, R_h and R_a. In order to investigate the sensitivity of the three species compositions to extreme drought treatment, we used the simple formula $(x - x_{control})/x_{control}$ to standardize R_s, R_h and R_a. We used repeated measures ANOVAs with sampling date to test the effects of seasonal variations on normalized R_s, R_h and R_a. A paired-t test was adopted to compare the annual means of SWC, ANPP, BNPP, NPP, and MBC in each year for the paired control and extreme drought treatments. Significant differences were evaluated at the level $P \leq 0.05$. Finally, we performed path analysis to quantify direct and indirect impacts of drought on R_s, R_h and R_a. We created a conceptual model of hypothetical relationships based on a priori and theoretical knowledge (Dias et al. 2010). Five major pathways were constructed to explore the effect of drought on R_s, R_h and R_a. Among them were drought-induced changes in environmental variables (soil temperature and moisture), and plant production (ANPP and BNPP). All analyses were done in R (3.4.0).

Results

Microclimate and soil water content

2015 was a climatologically normal growing season for this site, with precipitation of 230 mm as compared to the long-term mean (1953–2010) of 240 mm (Fig. 2a, b). 2016, however, was 31% drier than average, with 165 mm of precipitation. The amounts of natural rainfall excluded during the extreme drought treatments were 81 mm and 24 mm for 2015 and 2016, respectively. Mean daily air temperature during the growing season

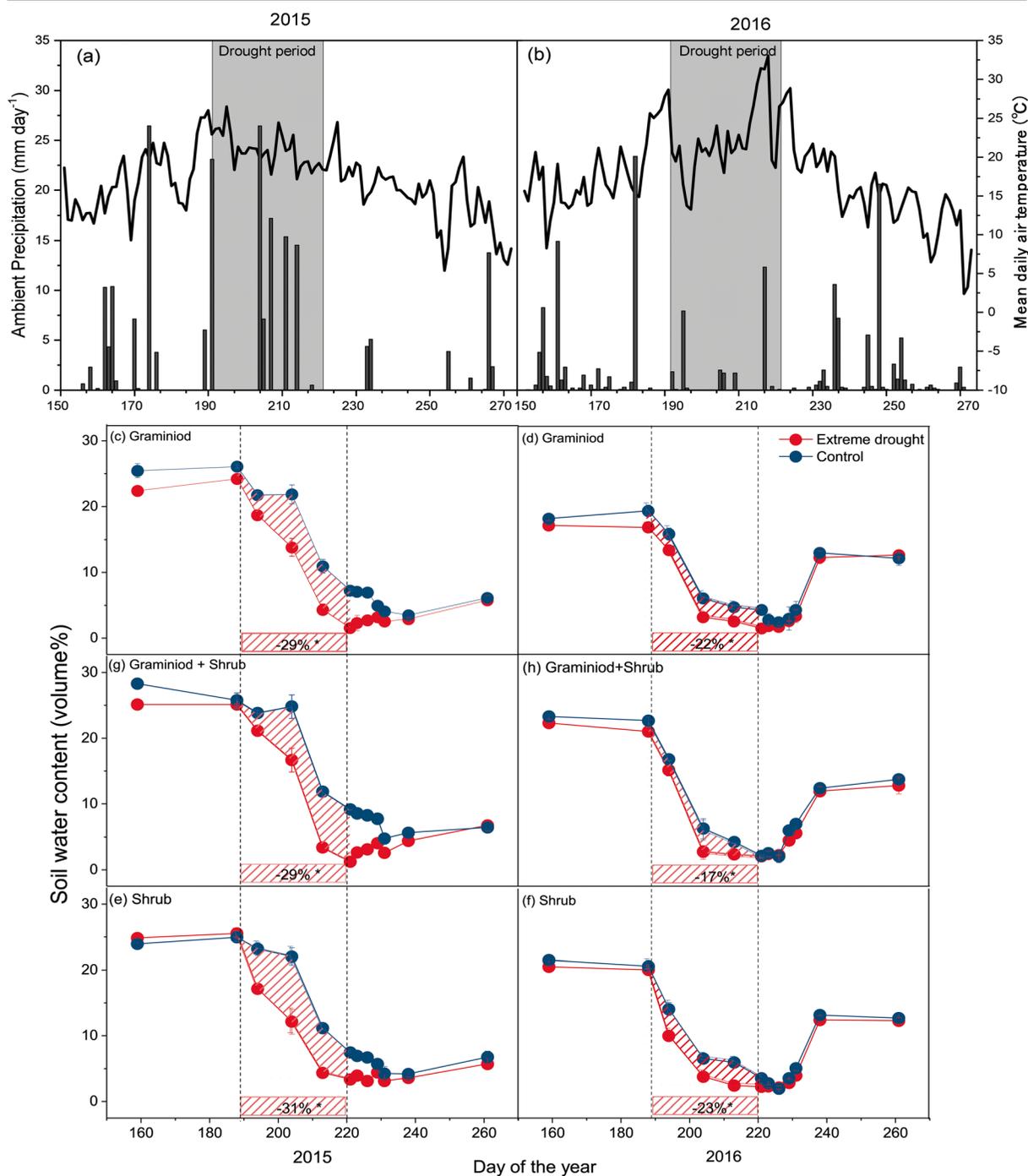


Fig. 2 (a, b) Seasonal changes in daily precipitation (mm) (bar) and mean daily air temperature (°C) (line) during the growing seasons of 2015 and 2016. Seasonal change in soil water content (volume %) at 0–10 cm soil depth in (c, d) the graminoid plots (G), (e, f) shrub plots (S) and (g, h) graminoid + shrub plots (GS) in 2015 and 2016 growing seasons, respectively. The red and dashed

rectangles indicate the period of extreme drought treatments (without 30 days' rainfall interval). The number in the dashed rectangles indicates the decrease of soil moisture during the extreme drought treatment. The annual averages of each species composition are shown in the bar charts. * means significant difference at $P = 0.05$ level. The data are mean \pm 1 SE

was 18.9 °C and 21.2 °C, and the maximal mean daily temperature was 25.9 °C on 15 July 2015 and 33.1 °C on 6 August 2016. Drought treatments were effective in reducing soil water content (SWC) of the top 10 cm of soil, which began to decline immediately after the start of experimental drought (Fig. 2c-h) with significant reductions of average SWC for all treatments in both 2015 and 2016 ($P=0.02$ in 2015; $P=0.02$ in 2016). However, no significant SWC differences were observed among the three different plant compositions (Table 2). In 2015, the drought treatment reduced SWC by 29% (G), 31% (S) and 29% (GS) compared to their controls, respectively. In the drier, warmer year 2016, SWC was reduced by 22% (G), 23% (S) and 17% (GS), respectively (Fig. 2).

Response of ANPP and BNPP to extreme drought

The extreme drought significantly decreased ANPP, BNPP and NPP in all three species compositions in 2015 and 2016. (Figure 3 and Table 2). In 2015, compared with the control group, the extreme drought ANPP decreased by 53%, 19% and 31% in the G, S and GS species compositions, respectively. In the dry year 2016, similar reductions of 52%, 20% and 26% on G, S and GS were found. Moreover, we also found a significant difference in the NPP (both ANPP and BNPP) among the three different species compositions (Table 2), with NPP of grasses higher than shrub and the mixed graminoid-shrub composition (Fig. 3 a, b).

Finally, interaction between the drought and species significantly affected NPP in 2016 (Table 2).

Response of soil respiration to extreme drought

Soil respiration (R_s), soil heterotrophic respiration (R_h) and soil autotrophic respiration (R_a) were significantly depressed by the drought treatment with immediate reductions after the start of the experimental drought. These reductions were consistently observed across two growing seasons in all species compositions: G, S and GS (Fig. 4 and Fig. S1). However, drought reduced R_s , R_h and R_a to a lesser extent in the dry year 2016 than in the average year 2015. In 2015, R_s , R_h and R_a were decreased by 31%, 33%, 54% and for G plots, 37%, 34% and 35% for S plots, 30%, 28% and 45% for GS plots compared with their control treatments, respectively.

There was different sensitivity of R_s and its components to extreme drought in three species compositions, and significant seasonal variations were observed during two growing seasons (Fig. 5; Table 3). Under extreme drought stress, R_s and R_a were more sensitive (larger reductions) in the G and S plots than in the GS plots, with maximum reduction in the G plot. However, R_h showed an opposite response to drought for G and GS plots, while the minimum reduction was observed in the S plots in both years. It was very interesting that during the period of treatment, across all three species compositions, average decreases in R_s , R_a and R_h varied

Table 2 Results of variance analysis: effects of drought, species composition and their interactive effects on soil water content (SWC), aboveground net primary productivity (ANPP), below-

ground net primary productivity (BNPP), net primary productivity (NPP), microbial biomass carbon (MBC), soil respiration (R_s), heterotrophic respiration (R_h) and autotrophic respiration (R_a)

Effect	SWC	ANPP	BNPP	NPP	MBC	R_s	R_h	R_a
2015								
Drought	0.021*	<0.001***	<0.001***	<0.001***	NA	0.004**	<0.001***	<0.001***
Species	NA	0.008**	0.035*	0.039*	NA	0.028*	0.003**	<0.001***
Drought×Species	NA	NA	NA	NA	NA	NA	0.033*	NA
2016								
Drought	0.024*	0.003**	<0.001***	<0.001***	NA	<0.001***	<0.001***	<0.001***
Species	NA	0.017*	NA	0.013*	NA	0.015*	<0.001***	<0.001***
Drought×Species	NA	0.011*	0.032*	0.041*	NA	0.013*	0.044*	0.027*

***indicates significant difference at $P \leq 0.001$

**indicates significant difference at $P \leq 0.01$

*indicates significant difference at $P \leq 0.05$

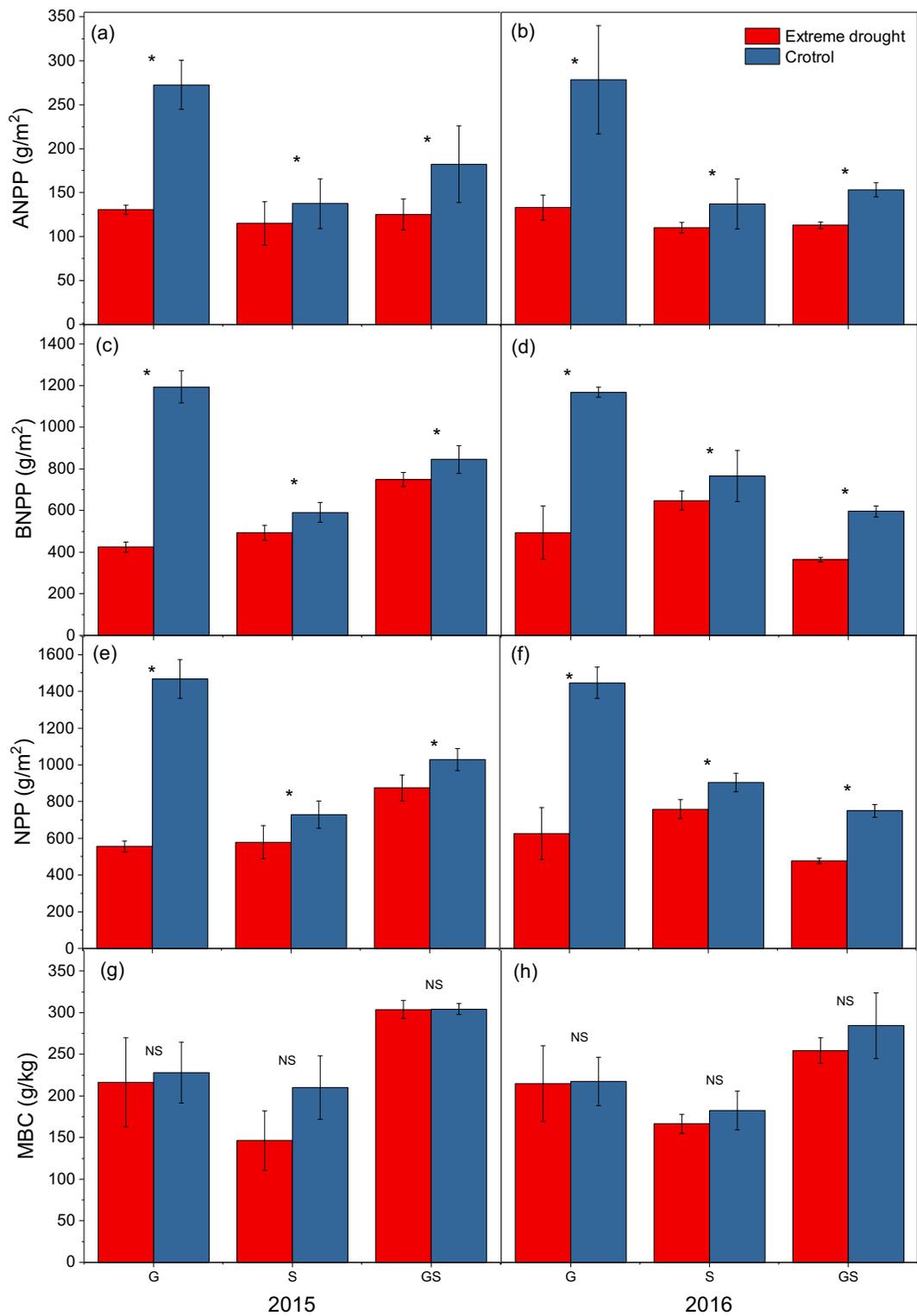


Fig. 3 (a, b) Response of aboveground net productivity (ANPP), (c, d) belowground net primary productivity (BNPP), (e, f) and total net primary productivity (NPP) to extreme drought treatment

imposed in 2015 and 2016's growing seasons. * means significant difference at $P = 0.05$ level. The data are mean \pm 1SE

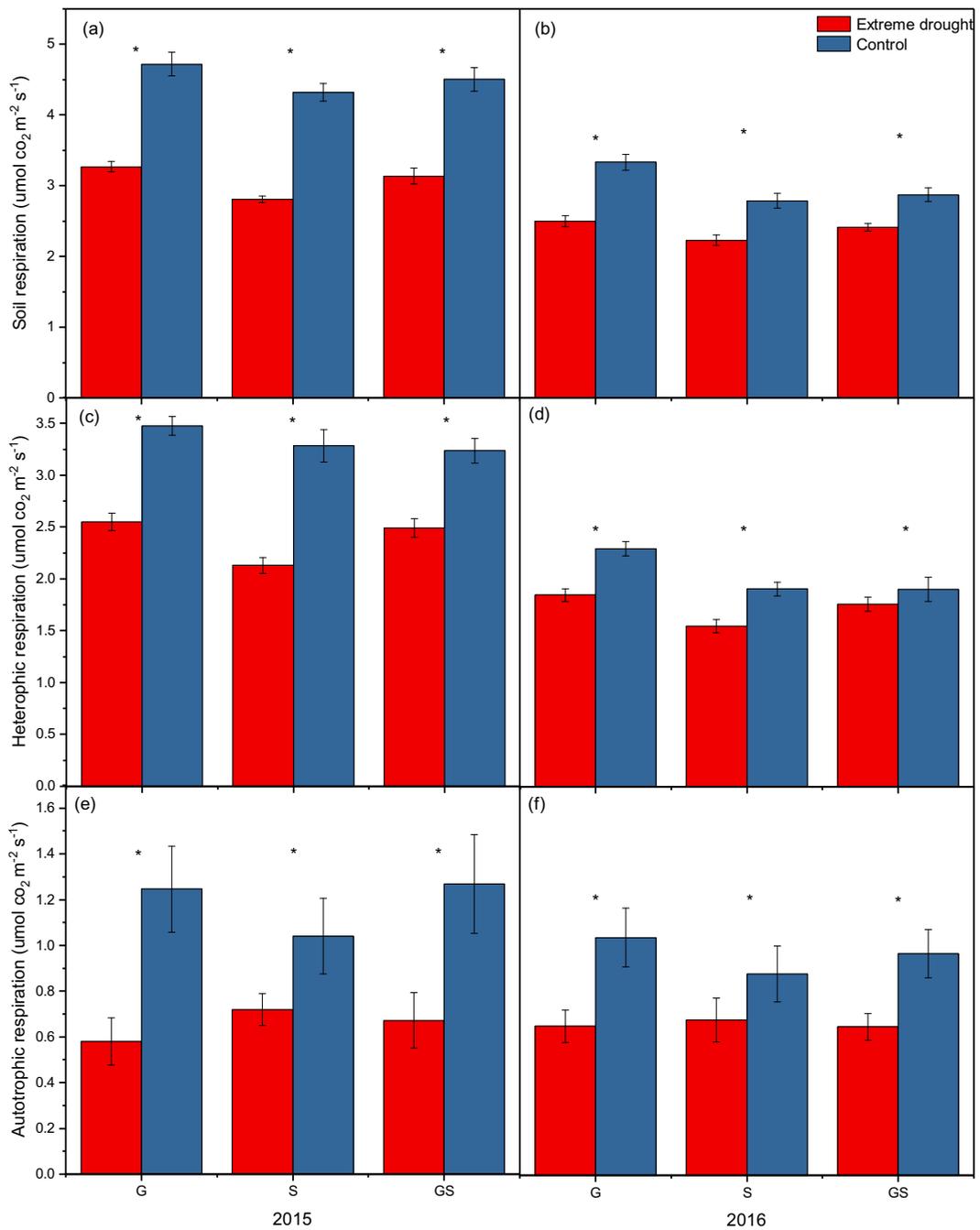


Fig. 4 Annual mean variations of ecosystem total CO_2 efflux from soil (R_s , **a**, **b**), heterotrophic respiration (R_h , **c**, **d**) and autotrophic respiration (R_a , **e**, **f**) under the extreme drought treatments

in the whole 2015 and 2016's growing seasons. Data are mean \pm 1SE. * means significant difference at $P = 0.05$ level

by 48%, 36% and 64%, respectively. After the drought, R_h recovered the original rate of release while R_s and R_a decreased on average to 8% and 31% compared with the controls (Fig. 5).

Extreme drought treatment increased the relative contribution of R_h and decreased that of R_a to the total R_s in all species compositions (Fig. 6). R_a and R_h accounted for 16–36%, and 64–84% of R_s , respectively.

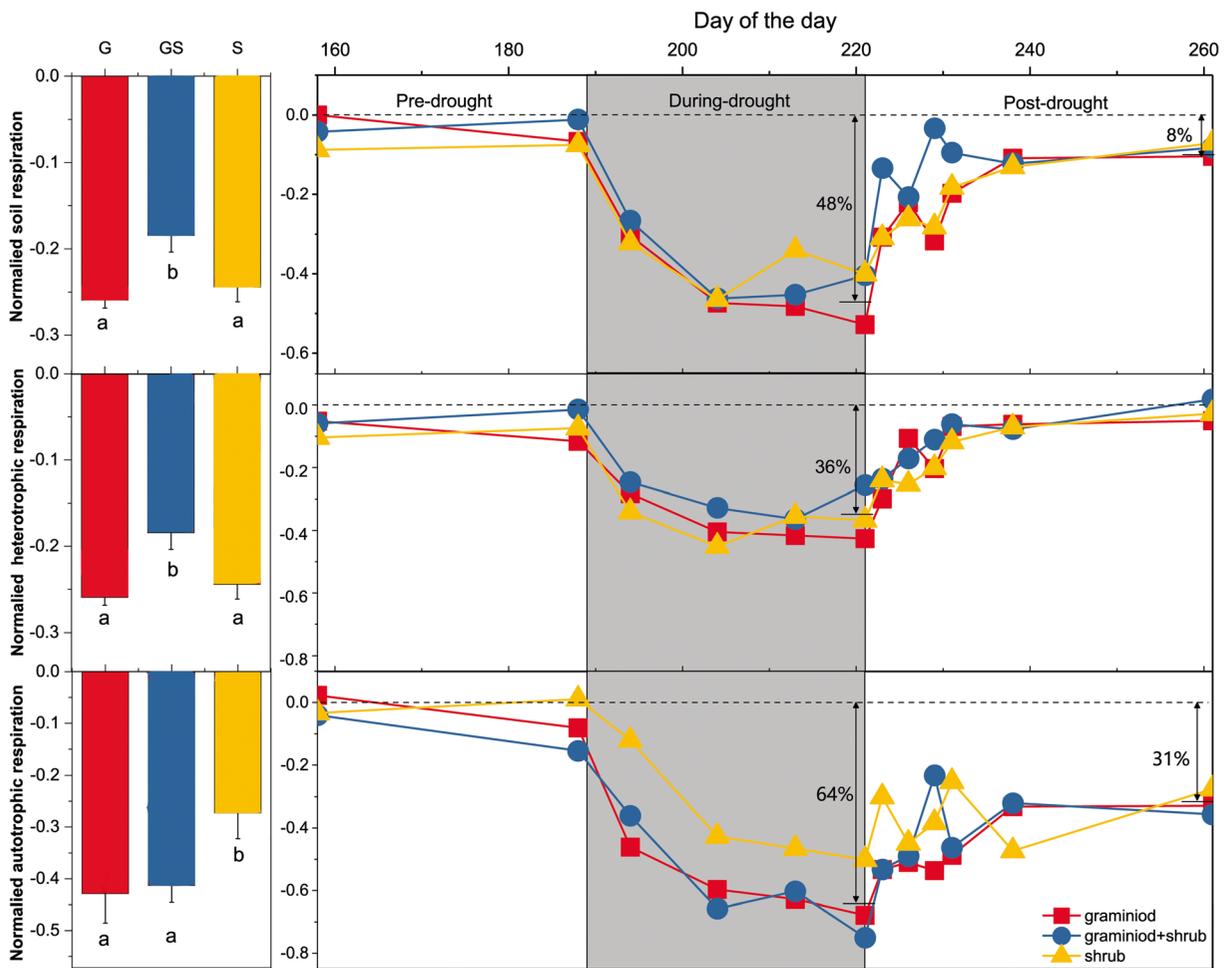


Fig. 5 Mean normalized total CO₂ efflux from soil (R_s , a), heterotrophic respiration (R_h , c), autotrophic respiration (R_a , e) as a function of whole study periods. Mean normalized soil respiration (R_s , b), heterotrophic respiration (R_h , d) and autotrophic

respiration (R_a , f) of drought treatment and control during growing seasons of 2015 and 2016. Values were normalized by formula $(x - x_{control})/x_{control}$. Same letters mean no significant difference at $P = 0.05$ level

(Figure 6 and Fig. S1 & S2). Average contributions of autotrophic respiration (R_a/R_s) decreased from 31%, 30%, 35% in the pre-drought period to 16%, 21%,

18% in post-drought period in G, S and GS species compositions, respectively (Fig. 6). Accordingly, the relative heterotrophic contributions of R_h/R_s increased

Table 3 Results of repeated-measures ANOVA for the effects of plant species composition on normalized soil respiration, soil heterotrophic respiration and autotrophic respiration in 2015 and 2016

Effect	Soil respiration			Heterotrophic respiration			Autotrophic respiration		
	d.f.	f	p	d.f.	f	p	d.f.	f	p
2015									
species	2	9.76	0.0009*	2	5.10	0.0147*	2	5.94	0.0087*
2016									
species	2	5.30	0.0007*	2	3.59	0.0406*	2	6.69	0.0059*

*indicates significant difference at $P \leq 0.05$

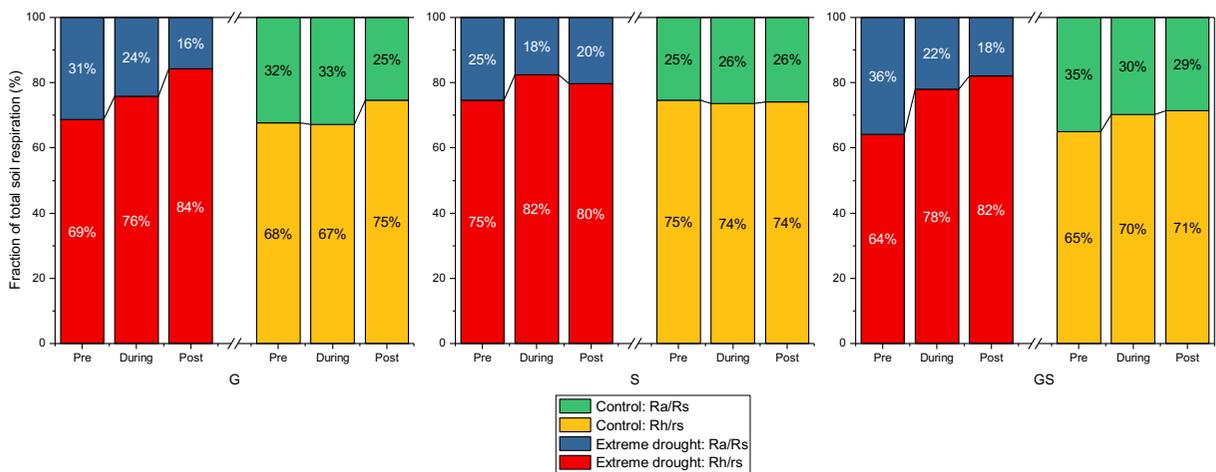


Fig. 6 Heterotrophic respiration (R_h) and autotrophic respiration (R_a) contribution to total CO_2 efflux from soil (R_s) for three species compositions in the pre-, during- and post-drought treatments.

Data are means \pm ISE. Same letters mean no significant difference at $P = 0.05$ level

during and after the drought treatments across all species compositions. It is noteworthy that the ratio of R_a/R_s or R_h/R_s fluctuated only slightly in control (no drought) plots of all species compositions (Fig. 6).

The influence of abiotic and biotic factors on CO_2 efflux from soil

R_s and its components increased with SWC across all species composition plots (Fig. S3) and explained 61%, 55% and 84% of the R_s , R_h and R_a , respectively (Fig. 7). The direct positive relationships between the biotic factors ANPP and BNPP with R_s and its components were

quantified by path coefficients of 0.17–0.67, showing that the direct effects of ANPP and BNPP on soil respiration are greater than those of other factors. The relationships of ANPP and BNPP with R_a were stronger than with R_s and R_h . Soil temperature (T_{soil}) was negatively related to R_s , R_a and R_h with path coefficients of -0.44 – -0.28 .

As mentioned above, water availability is the dominant control on R_s and its components. Soil water content explained ca. 50% of the seasonal variation in R_s , R_h , and R_a (Fig. S3). The sensitivity to water availability showed a tendency of approximately two-fold reduction in order of R_s , R_h and R_a across three species

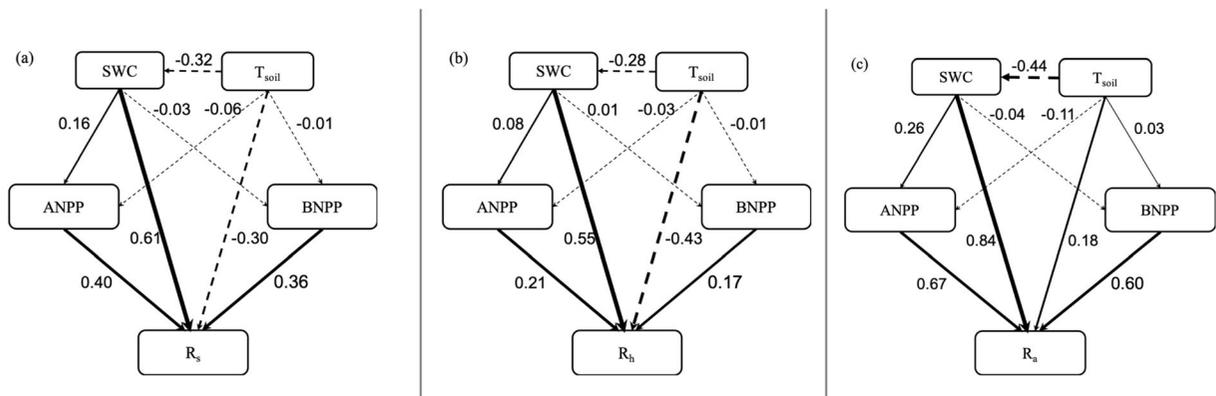


Fig. 7 Path analysis of the effects of extreme drought changes in abiotic and biotic factors on extreme drought changes in (a) total CO_2 efflux from soil (R_s) (b) heterotrophic respiration (R_h) and (c) autotrophic respiration (R_a). Solid and dashed arrows represent

significant ($P \leq 0.05$) and non-significant ($P > 0.05$). T_{soil} : soil temperature; SWC: soil water content; ANPP & BNPP: aboveground and belowground net primary production. Growing season data of 2015 and 2016 are used to perform the path analysis

composition plots (gradually reduced slope). However, there was no significant difference (i.e. slope) among the three species compositions in their response of R_s , R_h and R_a to soil water content was observed (P value for differences in the slope and intercept) (Fig.S3).

Discussion

Responses of soil CO_2 efflux soil and its components to extreme drought

As the two major abiotic factors controlling plant growth and soil microbial activities, soil temperature and water availability play critical roles in regulating spatial and temporal variations of soil respiration during the growing season (Zhou et al. 2007). However, our study shows that soil CO_2 flux and its components were strongly correlated with soil water content but not with soil temperature in the growing season. The results strongly suggest that water availability is more important than temperature in affecting variability of soil respiration in this semiarid grassland ecosystem, consistent with prior work (Liu et al. 2009; Correia et al. 2012). Soil moisture is a main driver of net primary productivity and thus strongly affects the cycling of soil carbon (Huxman et al. 2004). Previous studies have shown that drought decreased NPP, with subsequent effects on autotrophic respiration (Bond-Lamberty et al. 2004; Ciais et al. 2005; Wang et al. 2014). The same results can be found in our study. Our study provides further information, R_a decreased during drought due to decreasing of BNPP. Because R_a is known to scale with root biomass (Martin and Bolstad 2009), it is expected that R_a decreased due to decreasing of BNPP during drought, which is associated with root death (Hayes and Seastedt 1987). What's more, we observed that drought lead to autotrophic respiration decline which persisted after drought has ended, while R_h recovered quickly after the drought (Fig. 5). We speculate that extreme drought stress limited R_h by reducing microbial activity, because water is essential for all intra and extra-cellular reactions that support life and for the movement of solutes and cells (Sanaullah et al. 2014). The microorganism can recover its activity when the soil rewets, which leads to the recovery of soil microbial activity.

The average contribution of R_a to R_s was variable between 16 and 36%. This is consistent with studies

reporting the average contribution of R_a to R_s to be between 17 and 40% in the grassland (Raich and Tufekciogul 2000). Furthermore, our results suggest that under extreme drought, R_a was more sensitive than R_h (Fig. 6), in contrast with prior studies showing steeper declines of R_h than R_a with decreasing SWC (Zhao et al. 2016). We suggest this is because the response differs between the moderate drought more commonly studied and extreme drought of the type imposed here (Preece and Peñuelas 2016). Our results associating lower R_a with reduced BNPP (Fig. 5b, c; Table 2) support the idea that under extreme drought, R_a is more sensitive than R_h due to reduced biomass (Zhang et al. 2013; Balogh et al. 2016). Furthermore, root death represents a source of labile C in soil, supporting R_h (Jones et al. 2009; Frey et al. 2013).

Plant species composition regulation of soil CO_2 flux

Soil respiration and its components may be altered by changes in plant community structure and species composition because plants are the primary pathways through which carbon enters soil (Metcalf et al. 2011; Moyano et al. 2013). In our study, the total CO_2 efflux from soil of different plant species composition responds differently to extreme drought. This is in line with a previous grassland microcosm study that found consistent differences in R_s were driven by plant functional types (Johnson et al. 2008). Interestingly, we found that the graminoid communities were associated with the highest R_s , while the lowest R_s were observed in the shrub communities under extreme drought. Given the consistently positive relationship observed between R_s and plant production, it is not surprising that R_s of graminoid was higher than shrubs due to greater ANPP of graminoid than shrubs under the extreme drought treatment. Differences in plant species composition may also regulate drought response of R_s by controlling litter decomposition rates (Metcalf et al. 2011). Physical and chemical properties of plant litter vary greatly among the different plant communities, which underlay large differences in decomposition rates between different plant functional types, and decomposition of graminoid litter is faster than that of woody shrubs (Comwell et al. 2008), consistent with our results of greater R_s of graminoid than shrubs under extreme drought. Our results support the idea that plant species composition mediates responses of R_s to extreme drought, possibly due to control on the quantity and

quality of organic matter (litter) input to soil and the amount of photosynthate allocated belowground as BNPP.

We further observed that R_h in the mixed plots (GS) have lower sensitivity to extreme drought than either of the single-functional group plots, resulting in less-severe reduction of R_s (Fig. 5b). Lower sensitivity in GS plots may result because higher plant diversity increases rhizosphere carbon inputs into the microbial community, resulting in both increased microbial activity and carbon storage (Lange et al. 2015). Moreover, diversity is associated with increased activity of the enzymes involved in N/C cycling (Sanaullah et al. 2011). As for R_a , shrub (S) showed the lowest sensitivity to extreme drought as compared with graminoid (G) and their mixtures (GS). Shrubs are known to be drought tolerant because deep rooting enables them to reach water resources unavailable to herbaceous species (Hester et al. 1991; Le Houérou 2000) and are less responsive to water changes (Golluscio et al. 1998). Hence, the roots of shrubs are more tolerant to drought, leading to a lower sensitivity of R_a to extreme drought. Coarser roots are usually longer-lived with low respiratory rates, are better physically defended from herbivores, and decompose more slowly once dead (in the order of years to decades), which would collectively serve to suppress root contributions to R_a . In contrast, grasses often produce finer roots with higher respiratory rates and of higher substrate quality which turns over within weeks to years (Gill and Jackson 2000), resulting in root litter which is preferentially targeted by herbivores and decomposes relatively rapidly (Silver and Miya 2001). This also could help to explain why R_s in graminoid is higher than in shrub under extreme drought, not only determined by net primary production, but also by plant root properties. From this conclusion, we can infer that the impacts of species composition on R_s may be particularly large where they involve species that account for most plant biomass in the system, as well as species that have very different traits.

Implications for carbon sequestration under extreme drought

In semiarid regions, the frequency of severe drought has increased and is expected to grow through the end of this century (Hessl et al. 2018). An important consequence of increased severe drought is expected to be woody plant encroachment into grasslands (Kieft et al.

1998; Knapp et al. 2008a, b). Such a dramatic change in community composition will strongly change patterns of ecosystem carbon cycling and have important implications for ecosystem services. Our study found that under extreme drought treatment, the lowest soil respiration was observed in the shrub communities compared with other two species communities. The reduction of total soil respiration can partly offset the effect of reduction in ecosystem photosynthesis under extreme drought stress. However, it should not be ignored that increased cover by shrubs with high leaf area index could increase evapotranspiration at the ecosystem scale (Breshears et al. 2005), implying a positive feedback of water scarcity and increasing competitive pressure on remaining grasses.

Conclusions

To test how plant species community composition regulates the effects of extreme-duration drought on R_s , R_h and R_a , a two-year experiment was conducted in constructed steppe communities including graminoid, shrub and their mixture (graminoid + shrub) in the Inner Mongolia plateau, China. Our findings strongly imply that plant species composition regulates several aspects of soil CO₂ efflux response to climate extremes. This regulation may be limited by above- and below-ground net primary production depending on soil water availability. Changes in plant species composition are just as important as soil water content in determining soil respiration. In addition, autotrophic respiration (R_a) is more sensitive than heterotrophic respiration (R_h) under extreme drought.

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