

Ecological responses to heavy rainfall depend on seasonal timing and multi-year recurrence

Linfeng Li^{1,2}, Zhenzhen Zheng¹, Joel A. Biederman³ , Cong Xu¹, Zhihong Xu², Rongxiao Che⁴, Yanfen Wang^{1,5}, Xiaoyong Cui^{1,5} and Yanbin Hao^{1,5} 

¹College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China; ²Environmental Futures Research Institute, School of Environment and Science, Griffith University, Brisbane, Qld 4111, Australia; ³Southwest Watershed Research Center, Agricultural Research Service, Tucson, AZ 85719, USA; ⁴Institute of International Rivers and Eco-security, Yunnan University, Kunming, Yunnan 650091, China; ⁵CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences (CAS), Beijing 100101, China

Summary

Author for correspondence:

Yanbin Hao

Tel: +86 136 6124 1601

Email: ybhao@ucas.ac.cn

Received: 4 November 2018

Accepted: 25 March 2019

New Phytologist (2019) **223**: 647–660

doi: 10.1111/nph.15832

Key words: biomass, carbon cycle, climate extremes, CO₂ flux, EBEC-II, hierarchical level, leaf photosynthesis, precipitation.

- Heavy rainfall events are expected to increase in frequency and severity in the future. However, their effects on natural ecosystems are largely unknown, in particular with different seasonal timing of the events and recurrence over multiple years.
- We conducted a 4 yr manipulative experiment to explore grassland response to heavy rainfall imposed in either the middle of, or late in, the growing season in Inner Mongolia, China. We measured hierarchical responses at individual, community and ecosystem levels.
- Surprisingly, above-ground biomass remained stable in the face of heavy rainfall, regardless of seasonal timing, whereas heavy rainfall late in the growing season had consistent negative impacts on below-ground and total biomass. However, such negative biomass effects were not significant for heavy rainfall in the middle of the growing season. By contrast, heavy rainfall in the middle of the growing season had greater positive effects on ecosystem CO₂ exchanges, mainly reflected in the latter 2 yr of the 4 yr experiment. This two-stage response of CO₂ fluxes was regulated by increased community-level leaf area and leaf-level photosynthesis and interannual variability of natural precipitation.
- Overall, our study demonstrates that ecosystem impacts of heavy rainfall events crucially depend on the seasonal timing and multiannual recurrence. Plant physiological and morphological adjustment appeared to improve the capacity of the ecosystem to respond positively to heavy rainfall.

Introduction

Global warming affects the hydrological cycle over land, resulting in observed changes to precipitation frequency, intensity, duration and amount (Sherwood & Fu, 2014; Stocker, 2014). Although significant attention is paid to how changes in seasonal and annual precipitation sums affect ecosystems, relatively less is known about the ecological impacts of heavy rainfall events (large cumulative depths over 1 d or more), which are being observed with increasing frequency and severity and are expected to increase in the future (O’Gorman & Schneider, 2009; Stocker, 2014; Otto *et al.*, 2018).

Previous studies based on precipitation gradients across multiple sites (Knapp & Smith, 2001; Huxman *et al.*, 2004; Ponce-Campos *et al.*, 2013; Yu *et al.*, 2013; Biederman *et al.*, 2016) or a long temporal scale at a single site (Scott *et al.*, 2010, 2015; Wu *et al.*, 2013; Ren *et al.*, 2017) usually report positive relationships of precipitation with gross ecosystem CO₂ uptake or above-ground net primary productivity (ANPP). Ecosystem benefits from increased precipitation manifest in changes across hierarchical levels, including increased metabolism (e.g. leaf photosynthesis) (Niu *et al.*, 2008; Ren *et al.*, 2017), improvement of soil

nutrient availability (Lu *et al.*, 2014), and resultant changes in community composition (Knapp *et al.*, 2012). However, variability in precipitation totals over seasonal to annual scales is very different from altered incidence of heavy rainfall events, with large cumulative depths occurring over multiple consecutive days. Heavy rainfall events have unique impacts which may include soil water saturation or flooding and related oxygen depletion, surface flooding, poor nutrient transport and ethylene production in plants, which restricts growth (Chen *et al.*, 2002, 2005; Voesenek *et al.*, 2004). Heavy rainfall may reduce long-term productive capacity of soil as a result of nutrient loss (Sepulveda *et al.*, 2008; Jha *et al.*, 2015; Burt *et al.*, 2016; Marzen *et al.*, 2017). Collectively, these studies demonstrate how the temporal pattern and event size of precipitation and resulting soil moisture regime represent an important knowledge gap. Recent studies suggest that heavy rainfall caused even greater negative effects on grass reproduction and wheat yield than extreme drought (Gellesch *et al.*, 2017; Mäkinen *et al.*, 2017), which is often presumed to have the strongest and most widespread effects on terrestrial ecosystems (Reichstein *et al.*, 2013; Frank *et al.*, 2015). However, except for these limited examples, little is known about heavy rainfall impacts across multiple hierarchical levels from individual plants to the ecosystem scale.

Understanding mechanisms of ecosystem response to heavy rainfall requires measurements across multiple levels of ecological hierarchy, which may respond at different rates but often interact with one another (Ahl & Allen, 1996). Higher levels provide context for lower levels, which, in turn, provide mechanisms that explain the higher-level functional outcomes (O'Neill *et al.*, 1991). For example, individual plant photosynthesis is the fundamental mechanism underlying ecosystem-level carbon (C) uptake (Patrick *et al.*, 2007; Niu *et al.*, 2008), whereas at the intermediate community level, leaf area moderates the connection between individuals and ecosystem function (Hussain *et al.*, 2011; Liu *et al.*, 2017). Therefore a multi-hierarchy perspective facilitates a more comprehensive understanding across levels.

Ecological responses to precipitation change may vary over time and depend on the persistence or recurrence of the change (Ren *et al.*, 2017). Plants might form 'stress memory' in physiology after a stress experience (e.g. drought), which may stabilize ecosystems; that is, ecosystems tend to remain stable when faced with climate stress if the ecosystem has previously experienced a similar stress (Walter *et al.*, 2011, 2013; Backhaus *et al.*, 2014). Moreover, a plant may alter its morphological traits to survive under extreme water conditions, such as through changes in root:shoot ratio (Chen *et al.*, 2010) or root and leaf structure (Insausti *et al.*, 2001). Such an adaptation in morphology may increase an individual's tolerance to subsequent extreme conditions (Wang *et al.*, 2017). Therefore, it seems logical to hypothesize that responses of ecosystem structure and function to repeated (i.e. annually) heavy rainfall would change over time. However, to date, most heavy rainfall results are from opportunistic studies of events that occurred naturally without repetition (Smith, 2011). These studies are usually too short (1 or 2 yr) to establish a clear trajectory of change with time. As a consequence, ecological responses to recurrent heavy rainfall remain unclear. We may gain uniquely valuable information about ecosystem response to multiyear heavy rainfall with manipulative experiments, which offer the advantages of multiyear repeated treatment, precise conditions, replication and experimental control.

Another critical knowledge gap is whether ecological responses are regulated by the within-season timing of heavy rainfall. Seasonal timing of climate variability plays an essential role in affecting structure and functioning of ecosystems (Sippel *et al.*, 2016; Wolf *et al.*, 2016), yet seasonal timing remains little explored, especially with regard to heavy rainfall events. It is apparent that precipitation variability occurring in the early part or middle of the growing season could have large impacts during these periods of rapid plant growth (Zhou *et al.*, 2013; Morgan *et al.*, 2016; D'Orangeville *et al.*, 2018). By contrast, climate variability late in the growing season could have minimal impact (De Boeck *et al.*, 2011; Parton *et al.*, 2012). For example, Craine *et al.* (2012) showed that reduction in grassland productivity induced by both drought and high-intensity precipitation occurred during a 110 d period, whereas productivity was not impacted over the remainder of the year. Despite such evidence regarding the importance of the seasonal timing of drought, we lack such information for the effects of heavy rainfall at different times of the growing

season. The effects of seasonal timing are essential for predicting the fate of natural ecosystems under future increases in heavy rainfall.

In order to address these critical knowledge gaps, this study quantified and compared the effects of recurrent heavy rainfall with different seasonal timing on grassland ecosystem structure and function at individual, community and ecosystem scales. Specifically, we hypothesized that:

- (1) Heavy rainfall negatively affects grassland ecosystem biomass and C sink function because excess soil water content suppresses plant growth.
- (2) Such negative effects of heavy rainfall may weaken with annual repeated occurrence, because plants adjust physiologically and/or morphologically.
- (3) Heavy rainfall occurring in the middle of the growing season, a fast-growing stage for plants, would exert larger negative effects than heavy rainfall late in the growing season.

To test these three hypotheses, we performed a 4 yr field experiment in which heavy rainfall was imposed in the middle of as well as late in the growing season in a semiarid grassland in Inner Mongolia, China. Responses of ANPP and ecosystem CO₂ fluxes in the first 2 yr of the 4 yr experiment were reported previously (Hao *et al.*, 2017). The present study advances significantly upon the foundation of the previous work by quantifying heavy rainfall impacts across multiple levels of ecological hierarchy, including leaf photosynthesis at the individual level, stem density, leaf area index and biomass above and below ground at the community level, and CO₂ flux at the ecosystem level. This 4 yr dataset across hierarchical levels enables us to test hypotheses regarding the mechanisms underlying ecosystem response to heavy rainfall.

Materials and Methods

Study site

We conducted the study in a semiarid grassland at the Inner Mongolia Grassland Ecosystem Research Station, within the Xilin River Basin (43°20'N, 116°40'E, 1200 m asl). The site has a semiarid continental climate which is dry in spring and humid in summer. The mean annual temperature (1953–2017) is 2.5°C and the mean annual precipitation is 281 mm, of which 86% (c. 242 mm) falls during the growing season (May to September). The soil is classified as dark chestnut in Chinese soil classification or Calcis-orthic Aridisol in US Soil Taxonomy classification, with 60% sand, 21% clay and 19% silt. The field capacity is 0.29 m³ m⁻³. This grassland has been fenced off since 1979 and the plant community is mainly dominated by perennial rhizome and bunch grasses such as *Leymus chinensis*, *Stipa grandis*, *Achnatherum sibiricum* and *Agropyron cristatum* (Liu *et al.*, 2017).

Experimental design

This study is a part of the Extreme Climate Events and Biodiversity II (ECEB-II) experiment that began in 2012 (Supporting Information Notes S1). Since climate records began in 1953, the

longest continuous period of daily precipitation during the growing season was 20 d. We then calculated the total effective precipitation (i.e. the sum of rainfall for events ≥ 3 mm; Hao *et al.*, 2010, 2012) over all 20 d periods. The 99th percentile of total effective precipitation over any 20 d period was 282 mm. Thus, in this study, heavy rainfall (large cumulative depths over 1 d or more) was defined as 282 mm rainfall in total applied uniformly over 20 d (14.1 mm d^{-1}) (Hao *et al.*, 2017). Our experimental design was similar to those conducted in different ecosystems by Kreyling *et al.* (2008) (170 mm over 14 d) and Koide *et al.* (2010) (120 mm over 6 d). Based on observed timing of seedling establishment, growth and vegetation senescence, we divided the growing season into three periods: early (from early May to mid-June), middle (from late June to mid-August) and late (from late August to the end of September). The manipulative treatments consisted of heavy rainfall imposed in the middle of the growing season (HR-mid), heavy rainfall imposed late in the growing season (HR-late) and ambient conditions for control (ambient). The periods of HR-mid were from 17 June to 6 July in 2013 and 2014, and from 27 June to 16 July in 2015 and 2016. The periods of HR-late were from 20 August to 8 September in these 4 yr.

Quadruplicate 4 m^2 ($2 \text{ m} \times 2 \text{ m}$) plots were randomly established for each treatment (ambient, HR-mid and HR-late, 12 plots in total) in four blocks. During the experimental heavy rainfall periods, 9 m^2 rain-exclusion shelters ($3 \times 3 \text{ m}$, height 1.8 m) covered treatment plots to prevent natural rainfall. These consisted of a steel frame supporting a transparent polyester fibre-board (90% light transmission). At the beginning of the study in May 2012, we compared the air temperature (HMP45C temperature probe; Vaisala, Woburn, MA, USA) and photosynthetically active radiation (LI-190SB quantum sensor; Li-Cor Inc., Lincoln, NE, USA) measured under the shelters with the values measured in an open space close to the plots and found no significant differences (Liu *et al.*, 2017). Even with *c.* 10% light reduction under shelters, daytime conditions during the growing season remained light-saturated for the dominant plants (*c.* $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$; Li *et al.*, 2009, Li, 2010) (Table S1). Lateral surface flow to/from treatment plots was prevented by metal flashing installed from *c.* 40 cm below to 10 cm above the ground surface. The shelters were removed for the remainder of the year. Control plots remained uncovered and received ambient rainfall year-round. Heavy rainfall treatments were applied by hand using a sprinkling can of local groundwater.

Abiotic data measurement

Daily ambient precipitation data were recorded by a tipping bucket rain gauge near the plots (*c.* 50 m) at 1.5 m above ground (TE525MM; Campbell Scientific Inc., Logan, UT, USA). Soil water content (SWC) of the top 20 cm of soil was measured by time domain reflectometry (TDR 300; Spectrum Technologies Inc., Aurora, IL, USA) equipped with a pair probe (20 cm long) which was inserted vertically into the soil. Soil moisture was measured in three spots per plot and their mean was used to represent the plot SWC. SWC was measured every *c.* 5 d in 2013, 2015 and 2016 and every *c.* 10 d in 2014.

Individual-level measurements

In 2014–2016, light-saturated photosynthetic rates (P_{max}) of three dominant grasses (*Leymus chinensis*, *Stipa grandis*, *Achnatherum sibiricum* (this last species was measured in 2015 and 2016 only)) were measured by a portable photosynthesis system (GFS-3000; Heinz Walz GmbH, Effeltrich, Germany) equipped with a light source providing $1700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with CO_2 concentration, block temperature and block humidity under ambient conditions. The mean sum of relative abundance and proportion of leaf area of these three species were 42% and 51%, respectively (Fig. S1), which could largely represent responses of mutual and dominant species in this grassland. P_{max} was measured between 09:00 and 11:30 h in the morning. For the HR-mid treatment, P_{max} was measured on the last day of treatment, and on days 13, 26, 44 and 53 after treatment in 2014; on day 13 of treatment, and days 53 and 71 after treatment in 2015; and on the third to last day of treatment, and days 14 and 49 after treatment in 2016. For the HR-late treatment, P_{max} was measured on day 8 of and after the treatment in 2014; on the last day of treatment and on day 17 after treatment in 2015; and on day 13 of treatment and day 15 after treatment in 2016. These measurements covered a large range of SWC each year (Fig. S2). In each plot, two individuals per species were randomly selected for measurement on each sampling occasion, and we present their means as the P_{max} of the species in this plot.

Community-level measurements

We used the harvest method to estimate above-ground biomass (AGB). We clipped all living plants just above the soil surface in a $50 \times 50 \text{ cm}$ quadrat of each plot after heavy rainfall treatments were concluded (*c.* 15 September). Before clipping, individual stems (tillers) in the quadrat were censused to estimate stem density (stems m^{-2}). After harvest, vascular plants were sorted by species, and then fresh living leaves were separated from the plant to measure the leaf area within 4 h using a leaf area meter (LI-3000C; Li-Cor). Leaf area index (LAI) was calculated as the ratio of total leaf area to the quadrat area. Lastly, all tissues were dried and weighed. For each year, the locations of quadrats were different to prevent resampling of the same quadrat.

Root length was observed by the minirhizotron technique. In May 2012, one transparent minirhizotron tube (7 cm external diameter, 100 cm long) was installed at a 45° angle from the horizontal in each plot. A 20 cm length of each tube protruding above ground was covered with adhesive aluminium foil to block the sunshine. We used a root scanner system (CI-600 Root Growth Monitoring System; CID Inc., Vancouver, WA, USA) to take images (greyscale with 400 dpi) at vertical depths of 0–14, 14–28 and 28–42 cm at the end of each growing season in 2014–2016 (no measurements were done in 2013). The root length of each was determined using ROOTANALYSIS software (Analysis Ome Co. Ltd, Beijing, China). Plot-level root length was calculated as the sum of the three depths in each tube. Below-ground biomass (BGB) was estimated using Eqn (1) (Fischer *et al.*, 2007):

$$\text{BGB (g m}^{-2}\text{)} = \frac{L \times D}{\text{SRL} \times \text{AOI} \times \text{DOF}}, \quad \text{Eqn 1}$$

where L is the length of the root (m), D is the vertical depth sampled (m), SRL is specific root length (m g^{-1}), AOI is the measurement area of the image (m^2), and DOF is the estimated depth of field of image (m). In this study, a DOF of 0.0025 m was assumed based on the maximum root diameter. The SRL was estimated at 32 m g^{-1} based on measurements of elutriated roots (Cheng *et al.*, 2016). Estimation of root biomass based upon root length and specific root length has been used in natural multispecies communities, such as alpine meadow (Wu *et al.*, 2014), deciduous forest (Norby *et al.*, 2002) and shrub (Brown *et al.*, 2009). We have good confidence in BGB estimation by this method because it matched BGB based upon traditional soil-coring methods in an area adjacent to the experiment (Fig. S3). Additionally, similar conclusions about below-ground productivity were obtained whether we used BGB or directly measured root length and root volume (Fig. S4; Table S2). Total biomass (TB) was calculated as the sum of AGB and BGB. The root:shoot ratio is the ratio of BGB to AGB.

Ecosystem-level measurements

Ecosystem CO_2 fluxes, including net ecosystem exchange (NEE) and ecosystem respiration (ER), were measured directly every 1–2 wk by an infrared gas analyzer (LI-840A; Li-Cor) and a transparent chamber ($50 \times 50 \times 50$ cm) attached to one air pump (6262-04; Li-Cor) and two plastic pipes. The chamber was placed on a preinstalled square metal frame (50×50 cm in area and 10 cm in height with 3 cm extending above ground) in each plot to measure NEE (with sunlight) and ER (with lightproof cloth covering), respectively (Li *et al.*, 2016). Gross ecosystem production (GEP) was calculated as the difference between NEE and ER. Each measurement lasted 2 min, and CO_2 flux rates were calculated from the time-course of CO_2 concentrations measured every 1 s. Only data from the middle 100 s (deleting the first and last 10 s) were used (Chen *et al.*, 2009) to calculate the flux, as shown in Eqn 2:

$$F = \frac{V \times P \times (1000 - W)}{R \times S \times (T + 273)} \times \frac{dc}{dt}, \quad \text{Eqn 2}$$

where F is the CO_2 flux ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). Negative values indicate that the ecosystem absorbs CO_2 from the atmosphere, and positive values indicate CO_2 emission. V is the volume of chamber (m^3), P is the average pressure (kPa) during the measurement period, W is the average water mole fraction (mmol mol^{-1}) during measurement, R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), S is the surface area covered by the chamber (m^2), T is the average temperature ($^\circ\text{C}$) during the measurement, and dc/dt is the slope of least-squares linear regression of CO_2 concentration over time.

Statistical analysis

We used mixed-effects models conducted using the NLME package in R v.3.4.4 (R Core Team, 2018) to test treatment effects and temporal trends while accounting for repeated measurements following the lme function (Eqn 3):

$$\text{lme}(Y \sim (\text{HR-mid} + \text{HR-late}) * \text{Year}; \text{random} \\ = \sim 1 | \text{Plot}; \text{correlation} = \text{corAR1}(\cdot, \text{form} = \sim 1 | \text{Plot})), \quad \text{Eqn 3}$$

where Y was the response variable while HR-mid and HR-late were binary (0, not imposed; 1, imposed). In other words, heavy rainfall imposed in two periods (HR-mid and HR-late) and year were fixed effects, plot replication was included as a random effect, and a first-order autoregressive temporal covariance structure was assumed in all models, which was accounted for by repeated measurement. Some response variables showed obvious two-stage responses (the first 2 yr of the experiment showed distinctly different results from the latter 2 yr). To emphasize the different responses between the former 2 yr and the latter 2 yr, for these variables (NEE, ER, GEP, stem density and LAI), year was divided into two periods (1, 2013–2014; 2, 2015–2016). Similar analysis methods have been used in other studies with two-stage responses observed (Ren *et al.*, 2017). For the response variables with no obvious two-stage responses (AGB, BGB and TB), year was a factor with four levels (2013, 2014, 2015 and 2016). *Post hoc* tests (Duncan test) were used to compare the differences in AGB, BGB and TB across treatments in each year.

Student's t -test analysis was used to compare differences of average P_{max} of dominant species between heavy rainfall treatments and ambient controls in two periods, during and after treatment, respectively. Similarly, t -test analysis was also used to compare differences of LAI between heavy rainfall treatments and ambient controls in two periods, the former and latter 2 yr, respectively. Linear regression was used to correlate biomass (AGB, BGB and TB) with growing season precipitation (GSP). Binomial regression and linear regression were used to correlate P_{max} with SWC in 2014, and 2015 and 2016, respectively. All statistical analyses were implemented in R.

Results

Growing season precipitation and soil water content

The former 2 yr (2013–2014) were humid years with natural GSP values of 288 and 256 mm, respectively, and the latter 2 yr (2015–2016) were relatively dry with natural GSP values of 242 and 182 mm, respectively (Fig. 1). Heavy rainfall treatments received nearly double the ambient GSP in each year. The GSP of HR-mid treatments were 521, 481, 487 and 425 mm in 2013–2016. HR-late had the largest GSP, with 513, 495, 501 and 439 mm in these 4 yr.

We observed ponded water at the soil surface (at least over several hours) after each irrigation during heavy rainfall treatments. Flooding and presumably anaerobic soil conditions were created,

especially in the HR-mid treatment in 2013 and 2014 and in the HR-late treatment in 2013; during these treatments, average SWC slightly exceeded field capacity (29%). After treatments, SWC gradually returned to ambient values (Fig. 1).

Individual-level responses

Overall, three dominant species showed consistent responses of light-saturated leaf-level photosynthesis (P_{\max}) to heavy rainfall (Figs S5, S6). However, P_{\max} responses to heavy rainfall depended on the seasonal timing and the progression through repeated treatments over the 3 yr during which P_{\max} was measured. P_{\max} was significantly suppressed during heavy rainfall treatments regardless of seasonal timing in 2014 ($P=0.02$; Table 1; Fig. 2a,b). However, P_{\max} was significantly enhanced during the HR-mid treatment in the latter 2 yr ($P<0.01$ for both years; Table 1; Fig. 2c,e) and during the HR-late treatment in 2015 ($P<0.01$; Table 1; Fig. 2d). P_{\max} was increased in HR-mid following treatment in all three measurement years ($P=0.04, 0.04$ and <0.01 for 2014, 2015 and 2016, respectively) whereas there were no significant effects of HR-late (Table 1; Fig. 2b,d,f). In 2014, a quadratic relationship suggested that P_{\max} reached a peak value at SWC of *c.* 29%, with greater SWC decreasing photosynthesis (Fig. 3a). By contrast, 2015 and 2016 showed monotonically increasing relationships across all values of SWC (Fig. 3b).

Community-level responses

We found a significant reduction of total BGB and TB in the HR-late treatment ($P=0.03$ and 0.02 for BGB and TB,

respectively; Table 2). By contrast, the HR-late treatment had no significant effects on BGB and TB ($P=0.89$ and 0.75 for BGB and TB, respectively; Table 2) although BGB and TB in that treatment were consistently lower than those of the ambient control from 2014 to 2016 (Fig. 4). Overall, BGB in all three soil depths (0–14, 14–28 and 28–42 cm) showed similar responses to total BGB (Fig. S7; Table S3). Meanwhile, we found no significant effect on AGB for any year or either seasonal timing of treatments ($P>0.10$ for all; Table 2), although there was an apparently increasing pattern from ambient to HR-mid to HR-late treatment in 2013 and 2015 (Fig. 4). We found a significant negative linear relationship between BGB or TB and GSP, whereas AGB had no significant relationship with GSP (Fig. 5).

Heavy rainfall affected LAI largely depending upon seasonal timing ($P=0.05$ for HR-mid \times period interaction and $P=0.08$ for HR-late \times period interaction; Table 3). LAI did not appear to be affected by the heavy rainfall during the first 2 yr ($P=0.83$ and 0.90 for HR-mid and HR-late, respectively; Fig. 6a,c), but LAI showed significant increases in HR-mid and HR-late treatments in the latter 2 yr ($P=0.01$ and 0.04 for HR-mid and HR-late, respectively; Fig. 6a,c). Community abundance showed a similarity to LAI in that effects appeared to develop during the latter 2 yr, but these were not significant ($P>0.10$ for all; Fig. 6b, d; Table 3).

Ecosystem-level responses

Overall, net ecosystem CO_2 uptake ($-1 \times \text{NEE}$) was suppressed during the heavy rainfall treatments and subsequently stimulated after treatments regardless of timing and year

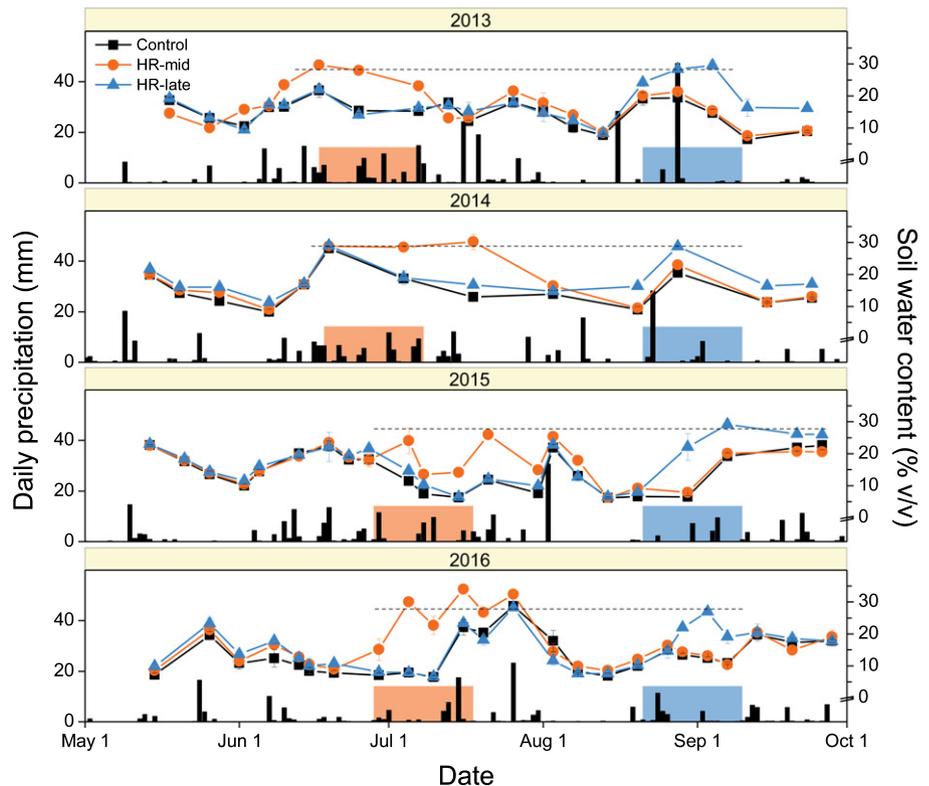


Fig. 1 Daily precipitation (bars) and soil water content (SWC, lines) during the growing season (from 1 May to 30 September) in 2013, 2014, 2015 and 2016 over a semiarid grassland. The dashed line indicates field capacity (29%). The orange and blue shaded regions indicate the periods of the HR-mid (heavy rain imposed in middle of the growing season, orange line) and HR-late (heavy rainfall imposed late in the growing season, blue line) treatments, respectively. The height of the shaded regions indicates the added rainfall event size (14.1 mm). 'Control' refers to ambient control (black lines). Error bars show one standard error of the mean.

Table 1 Results of *t*-test analyses of HR-mid (heavy rainfall imposed in the middle of the growing season) and HR-late (heavy rainfall imposed late in the growing season) treatment effects on light-saturated photosynthesis in two treatment periods (during and after treatment) for each year.

Year	Treatment period	Control vs HR-mid			Control vs HR-late		
		<i>t</i>	df	<i>P</i>	<i>t</i>	df	<i>P</i>
2014	During treatment	3.92	3.78	0.02	6.61	2.01	0.02
	Post-treatment	-3.15	3.74	0.04	2.29	3.35	0.10
2015	During treatment	-12.60	3.92	< 0.01	-6.39	3.18	< 0.01
	Post-treatment	-3.23	3.24	0.04	0.39	3.30	0.72
2016	During treatment	-6.10	3.51	< 0.01	0.20	2.74	0.86
	Post-treatment	-6.28	3.08	< 0.01	1.38	4.00	0.24

P-values in bold are statistically significant to $\alpha = 0.05$.

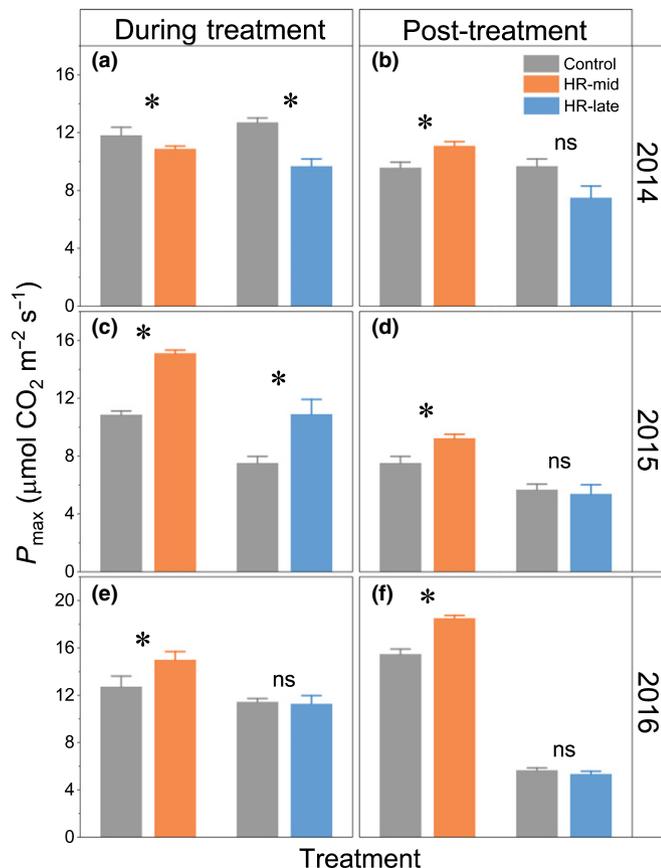


Fig. 2 Responses of leaf photosynthesis (P_{\max}) of dominant grass species, *Leymus chinensis*, *Stipa grandis* and *Achnatherum sibiricum*, to heavy rainfall treatments during and after treatment for 2014–2016 (not measured in 2013). Data used were means of three species in each period. Control, ambient; HR-mid, heavy rainfall imposed in the middle of the growing season; and HR-late, heavy rainfall imposed late in the growing season. Asterisks and 'ns' above bars indicate significant and nonsignificant differences in mean photosynthesis between heavy rainfall and ambient control treatments, respectively. Error bars show one standard error of the mean.

(Fig. S8). At the annual scale, there were marginally significant effects of heavy rainfall on NEE ($P=0.08$ and 0.07 , respectively; Table 3) regardless of timing, and there was a clear interaction between HR-mid and year of the experiment, with the HR-mid treatment decreasing net ecosystem CO_2

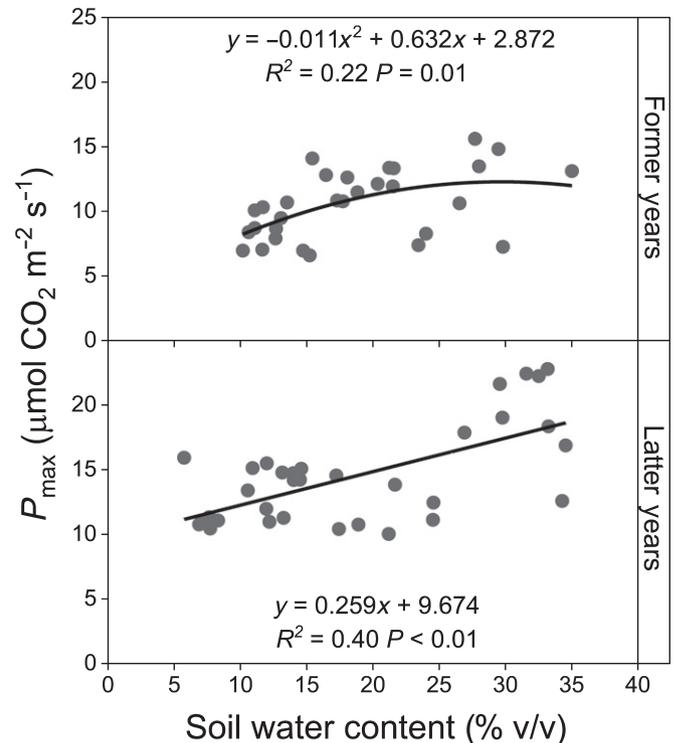


Fig. 3 Relationships between soil water content and light-saturated photosynthetic rate (P_{\max}) of three dominant species (*Leymus chinensis*, *Stipa grandis* and *Achnatherum sibiricum*) for ambient control (control) and HR-mid (heavy rainfall imposed in the middle of the growing season) treatments in 2014 (a), and 2015 and 2016 (b). Data are means of three species on each measurement date.

uptake in the former 2 yr and increasing it in the latter 2 yr (Fig. 7a,d). We found significant effects of HR-mid and interaction between HR-mid and period on GEP and ER ($P=0.03$ and 0.02 , respectively; Table 3). The HR-mid treatment slightly increased ER in former 2 yr, but largely stimulated ER in the latter 2 yr (Fig. 7b,e). Additionally, GEP was slightly depressed in the former 2 yr but largely enhanced in the following 2 yr in the HR-mid treatment (Fig. 7c,f). By contrast, such notable ecosystem flux responses were not found in heavy rainfall imposed late in the growing season ($P=0.07$, 0.18 and 0.16 for NEE, ER and GEP, respectively, and $P>0.10$ for all interactions; Fig. 7; Table 3).

Table 2 Results of mixed-effect model analyses of HR-mid (heavy rainfall imposed in the middle of the growing season) and HR-late (heavy rainfall imposed late in the growing season) treatment effects on above-ground biomass (AGB), below-ground biomass (BGB) and total biomass (TB).

Fixed effect	df		AGB		df		BGB		TB	
	Num	Den	F	P	Num	Den	F	P	F	P
HR-mid	1	6	0.12	0.74	1	6	0.02	0.89	0.09	0.77
HR-late	1	6	2.95	0.14	1	6	8.57	0.03	9.91	0.02
Year	3	18	21.88	< 0.01	2	12	1.51	0.26	3.05	0.08
HR-mid × year	3	18	0.32	0.81	2	12	1.93	0.19	1.00	0.39
HR-late × year	3	18	0.79	0.52	2	12	2.37	0.14	0.17	0.84

P-values in bold are statistically significant to $\alpha = 0.05$.

Den df, denominator degrees of freedom; Num df, numerator degrees of freedom.

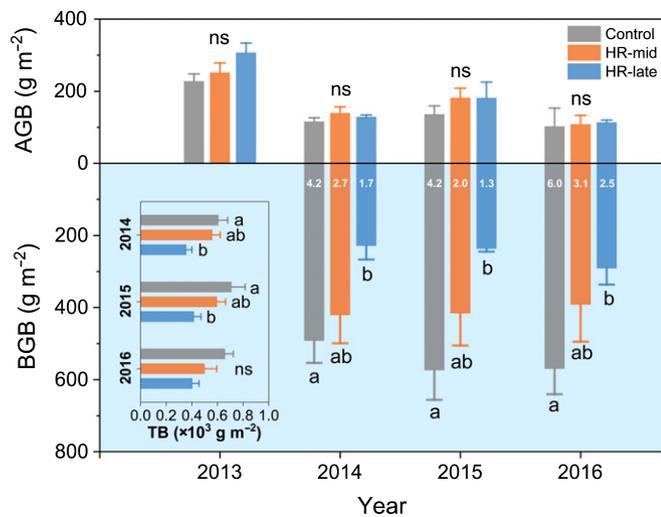


Fig. 4 Responses of above-ground biomass (AGB), below-ground biomass (BGB) and total biomass (TB, inset) to heavy rainfall treatments. Letters above each bar (a, b) indicate significant difference at $P \leq 0.05$, and 'ns' indicates nonsignificant differences. Control, ambient; HR-mid, heavy rainfall imposed in the middle of the growing season; HR-late, heavy rainfall imposed late in the growing season. Data for BGB are root : shoot ratio, or BGB : AGB. Error bars show one standard error of the mean.

Discussion

To our knowledge, our study is the first manipulative experiment to investigate the effects of seasonal timing of heavy rainfall (large cumulative depths over 1 d or more). Similar to the response in the former 2 yr (Hao *et al.*, 2017), AGB remained stable in the face of heavy rainfall over all 4 yr, regardless of the seasonal timing (Fig. 4). By contrast, BGB and the resultant TB both declined surprisingly, with the degree of negative response modulated by seasonal timing (Table 2). Late-season heavy rainfall significantly reduced BGB and TB, whereas the HR-mid treatment did not show any significant impacts (Fig. 4). Ecosystem-level CO₂ fluxes were little affected by either of the two heavy rainfall treatments in the former 2 yr (Hao *et al.*, 2017). In the latter 2 yr, however, the HR-mid treatment largely stimulated ecosystem CO₂ fluxes (Fig. 7). In the following, we discuss the disparate responses of CO₂ uptake and biomass in terms of measurements over all 4 yr across

multiple hierarchies, and address potential mechanisms explaining the observed effects of seasonal timing and multiyear recurrence of heavy rainfall.

Negligible responses of above-ground biomass to heavy rainfall over 4 yr

Although our results support our first hypothesis of negative effects of heavy rainfall on biomass, the response was seen below ground in BGB. Meanwhile, AGB remained stable, in contrast to the positive precipitation–productivity relationships usually reported in cases of chronic precipitation increases (Huxman *et al.*, 2004; Wu *et al.*, 2011; Biederman *et al.*, 2017). The inability of the ecosystem to increase AGB under heavy rainfall is notable, despite a near-doubling of the average growing season precipitation and increased SWC, often in excess of field capacity (Fig. 1). Although ponded water at the soil surface was observed (at least over several hours) after each day's irrigation during treatment periods, unchanged AGB also contrasts with previous studies showing that soil saturation and flooding usually reduce AGB (Chen *et al.*, 2002, 2005; Voisenek *et al.*, 2004). The lack of AGB response in this study was consistent with a heavy rainfall experiment conducted on assembled grass communities in Europe (Kreyling *et al.*, 2008), where 170 mm rainfall was simulated over 14 d during the peak growing season, causing saturated soil but resulting in no significant changes in AGB. Together, these two studies suggest that experimental heavy rainfall does not significantly change AGB. This could be because soil water saturation is beyond the optimum range in the root zone, even if the above-ground parts are not constantly submerged for a long period. In other words, the positive effects of increased precipitation, mainly reduction of soil water stress (Niu *et al.*, 2008; Ren *et al.*, 2017), may be cancelled by the negative effects of root-zone saturation, such as oxygen deprivation (Chen *et al.*, 2002, 2005; Voisenek *et al.*, 2004).

In our study, the lack of negative impacts of heavy rainfall on AGB may also be related to the fact that this community has been fenced for 40 yr and is therefore relatively undisturbed, late-succession and grass-dominated with high diversity. It has been suggested that higher-diversity communities are less negatively affected by floods and that some grasses are unaffected by floods regardless of plant diversity (Wright *et al.*, 2017). Furthermore,

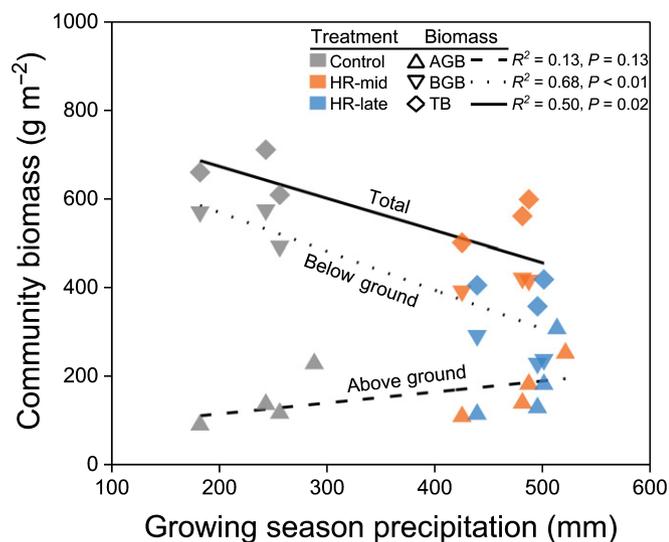


Fig. 5 Relationships between growing season precipitation and above-ground biomass (AGB), below-ground biomass (BGB) and total biomass (TB) across treatments and years, fitted with linear models. Different colours and symbols refer to different treatments (grey, orange and blue refer to ambient control, HR-mid (heavy rainfall imposed in middle of the growing season) and HR-late (heavy rainfall imposed late in the growing season), respectively) and biomasses (up triangles, down triangles and diamonds indicate AGB, BGB and TB, respectively).

mature plants may tolerate flooding better than young plants (Gattringer *et al.*, 2017).

Mechanisms by which heavy rainfall negatively influences below-ground biomass

It is noteworthy that BGB was largely reduced, even as AGB remained stable (Fig. 4). These results again show an important difference between heavy rainfall and chronic precipitation increase; previous studies in this area showed that BGB was increased by supplemental irrigation when it was distributed throughout the growing season (Bai *et al.*, 2010; Li *et al.*, 2010; Gao *et al.*, 2011). Furthermore, a meta-analysis suggested that AGB was more sensitive than BGB to experimental precipitation

addition (Wilcox *et al.*, 2017), highlighting a key difference between chronic and extreme-event precipitation changes.

Negative effects of heavy rainfall on BGB observed in the present study, especially in the HR-late treatment (Fig. 4), are generally in agreement with previous flood studies showing saturated soil moisture conditions, and the ethylene produced may limit root development or even lead to root damage and death (Kozłowski, 1984, 1997; Chen *et al.*, 2002). High turnover of roots under high GSP and SWC is another possible reason why BGB was lower in heavy rainfall treatments compared with the ambient control. Root turnover showed a significant positive relationship with annual precipitation (Yuan & Chen, 2010; Finér *et al.*, 2011) and SWC in arid climates (Kitajima *et al.*, 2010) because of accelerated decomposition and short-lived roots (Huang & Nobel, 1992). Bai *et al.* (2010) suggested that root turnover rates were stimulated 10% by an increase of 30% in mean annual precipitation in a temperate steppe near our study site.

Decreased BGB and root:shoot ratio, coupled with unchanged AGB (Fig. 4), reflects plant plasticity in C allocation, prioritizing shoot growth in order to tolerate saturated conditions (Rubio & Lavado, 1999; Voesenek *et al.*, 2004). Previous manipulative studies conducted at an adjacent site indicated that a smaller proportion of fixed C would be allocated below ground when high SWC is maintained (24%) by irrigation (Li *et al.*, 2010). Across a regional precipitation gradient of sites, the ratio of below-ground biomass to total biomass (BGB:TB) increased when annual precipitation increased from 100 to 300 mm, reached a peak at *c.* 300 mm, and then declined, with precipitation increasing from 300 to 400 mm based on a 1900 km transect across Inner Mongolia (Fan *et al.*, 2009). This C allocation strategy is possibly related to improving oxygen diffusion from the aerial parts to root systems (de Oliveira & Joly, 2009). However, heavy rainfall-driven changes in C allocation, resulting in root-poor plant communities, may decrease community stability by reducing subsequent capacity for gaining soil resources (e.g. water, nutrients) in this shallow-rooted ecosystem. Similar C allocation responses have been detected in a 2010 drought in Amazonia, with subsequent increases in tree mortality ascribed to decreased root allocation (Dougherty *et al.*, 2015).

Table 3 Results of mixed-effect model analyses of HR-mid (heavy rainfall imposed in the middle of the growing season) and HR-late (heavy rainfall imposed late in the growing season) treatment effects on net ecosystem exchange (NEE), gross primary production (GEP), ecosystem respiration (ER), leaf area index (LAI) and stem density.

Fixed effect	df		NEE		GEP		ER		LAI		Stem density	
	Num	Den	F	P	F	P	F	P	F	P	F	P
HR-mid	1	6	4.48	0.08	8.44	0.03	8.82	0.02	0.84	0.39	0.18	0.68
HR-late	1	6	4.95	0.07	2.36	0.18	2.59	0.16	0.72	0.43	1.23	0.31
Period	1	24	22.23	< 0.01	22.96	< 0.01	32.12	< 0.01	12.61	< 0.01	1.06	0.31
HR-mid × period	1	24	6.02	0.02	14.25	< 0.01	10.17	< 0.01	4.47	0.05	0.04	0.85
HR-late × period	1	24	0.18	0.68	2.54	0.12	1.63	0.21	3.38	0.08	1.22	0.28

Period was a factor with two levels, with one level indicating 2013–2014 and the other level indicating 2015–2016. *P*-values in bold are statistically significant to $\alpha = 0.05$.

Den df, denominator degrees of freedom; Num df, numerator degrees of freedom.

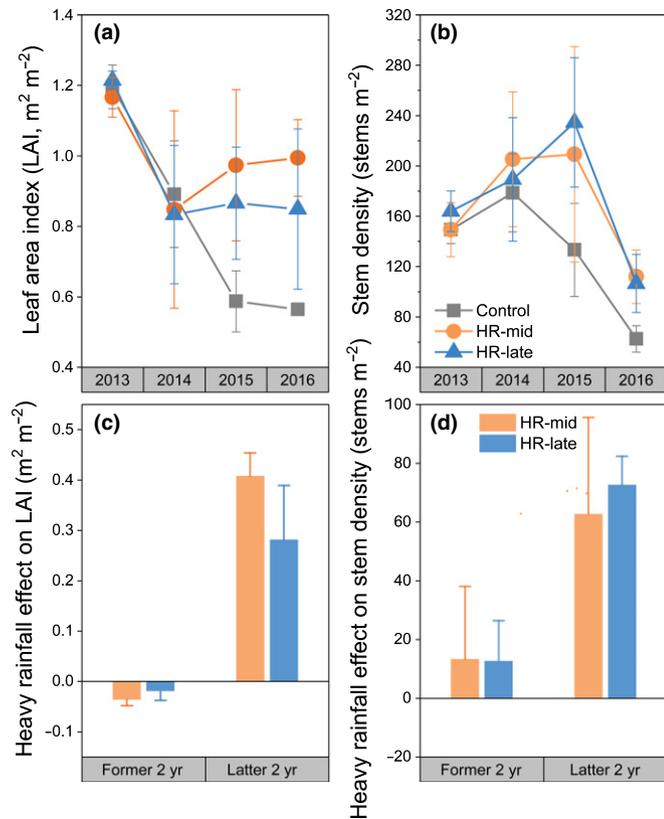


Fig. 6 Responses of leaf area index (LAI) (a) and stem density (b) to heavy rainfall treatments. The relative effects of heavy rainfall on leaf area index (c) and stem density (d) during the former 2 yr (2013–2014) and latter 2 yr (2015–2016) of the study. The relative effects were calculated as the difference between the value in the heavy rainfall treatment and the value of the ambient control. Control, ambient; HR-mid, heavy rainfall imposed in the middle of the growing season; and HR-late, heavy rainfall imposed late in the growing season. Error bars show one standard error of the mean.

Relationships of growing season precipitation and biomass under heavy rainfall

There was no significant relationship between AGB and GSP across treatments and years (Fig. 5), contrasting with the expectation of increasing annual precipitation (AP)–AGB relationships (Sala *et al.*, 2012; Ponce Campos *et al.*, 2013) or even a recently revised nonlinear ‘double asymmetry’ model, including both positive and negative asymmetry under conditions of extreme precipitation years (Knapp *et al.*, 2017). This may be a result of decreased AGB sensitivity to total AP under extreme precipitation patterns, causing reduced rain-use efficiency (Zhang *et al.*, 2013). It is possible that our results fit the observed hump-shaped (unimodal) AP–AGB relationships at sufficiently large scales temporally (e.g. decades, Zhu *et al.*, 2016) or spatially (e.g. global, Yang *et al.*, 2008). A binomial relationship between AGB and mean annual precipitation (MAP) indicates that AGB seems reach a peak at a MAP of *c.* 400 mm in Inner Mongolia (Fan *et al.*, 2009). Because our study did not contain years of GSP in the range 300–400 mm, we are unable to evaluate our results in the context of the tipping point of Fan *et al.* (2009). However, it

is plausible that our ambient and HR treatments represent similar AGB values on the rising and falling limbs of a unimodal relationship. By contrast with AGB, BGB showed a sharp negative relationship with GSP (Fig. 5), demonstrating an unexpected biomass response to heavy rainfall events. It should be noted that BGB dominated community biomass responses (TB) to heavy rainfall, indicating that commonly studied AP–AGB relationships might not capture important relationships between community biomass and precipitation. BGB is particularly important in grasslands and other ecosystems where below-ground productivity and biomass exceed those found above ground (Fan *et al.*, 2009; Liu *et al.*, 2018).

Mechanisms by which heavy rainfall influences ecosystem CO₂ fluxes over multiple years

Negative responses of CO₂ uptake (NEE) during heavy rainfall treatments regardless of timing and year (Fig. S8) support for our first hypothesis that heavy rainfall negatively affect grassland C sink function. Such a C sink reduction is consistent with well-known findings that plant photosynthesis is inhibited by flooding, mainly as a result of stomatal closure (Chen *et al.*, 2005; Zhang *et al.*, 2017; Ziegler *et al.*, 2017). In addition to stomatal factors, ecosystem-level photosynthesis reduction may result from either chlorosis and premature leaf senescence caused by ethylene production under flooding stress or a decrease in water and N uptake as a result of reduced root production (Chen *et al.*, 2002). Here, although BGB was also reduced by heavy rainfall treatments (Fig. 4), we suggest that suppressed ecosystem CO₂ uptake was the consequence of transient stomatal closure under excess water moisture, because CO₂ uptake was promoted following treatments as soon as soil moisture declined below saturated values (Figs 2, S8).

Importantly, we found that annual-scale CO₂ uptake was reduced and enhanced by the HR-mid treatment in the former and latter 2 yr, respectively (Fig. 7), supporting our second hypothesis that negative effects of heavy rainfall would be diminished over time with annual extreme event recurrence. This is because, in HR-mid treatment, the inhibiting effects on ecosystem CO₂ uptake during the treatment period were weakened, whereas the facilitating effects on ecosystem CO₂ uptake after the treatment period were enhanced in the latter 2 yr (Fig. S8).

Four mechanisms may explain the observed changes over time in the response of CO₂ fluxes to the HR-mid treatment. First, precipitation variation across years may contribute to the two-stage responses of ecosystem CO₂ fluxes (Fig. 1). Natural GSP in the former 2 yr was larger than that in the latter 2 yr, especially in 2016, when only 182 mm of ambient precipitation fell (Fig. 1). By contrast, SWC was already near field capacity before the HR-mid treatment started in the former 2 yr (26% and 28% in 2013 and 2014, respectively). Thus, the imposed heavy rainfall treatment served mainly to prolong a period in which soil moisture was at field capacity (average SWC was 29% during the HR-mid treatment in both years). By contrast, in the latter 2 yr, the SWC immediately before the HR-mid treatment was lower (18% and 7% in 2015 and 2016, respectively). Ambient SWC during the

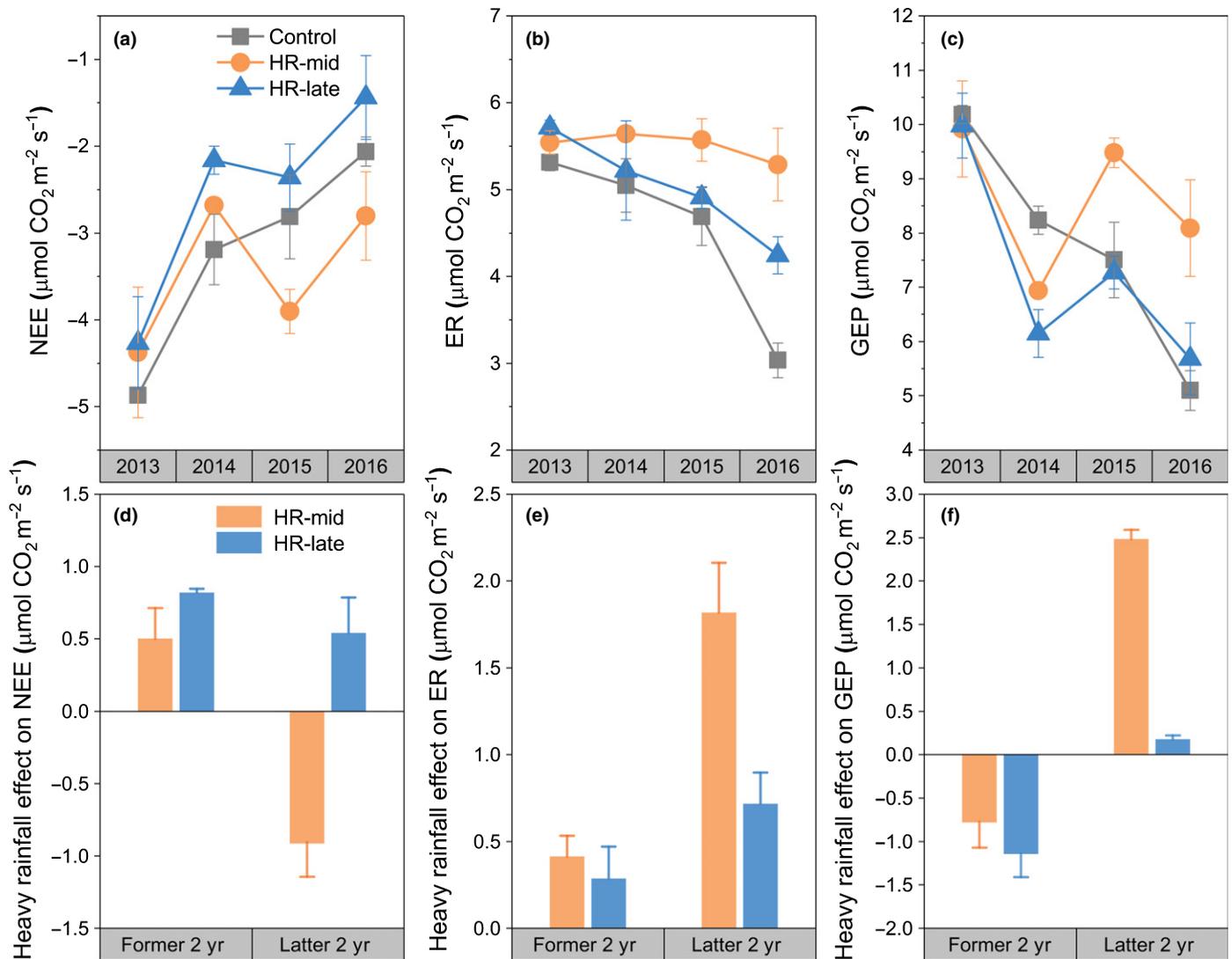


Fig. 7 (a–c) Responses of net ecosystem exchange (NEE) (a), ecosystem respiration (ER) (b) and gross ecosystem production (GEP) (c) to heavy rainfall treatments. (d–f) Relative effects of heavy rainfall on NEE (d), ER (e) and GEP (f) during the former 2 yr (2013–2014) and latter 2 yr (2015–2016) of the study. Data in (a)–(c) were means over the growing season in each year. The relative effects were calculated as the differences between the heavy rainfall treatment and ambient control. Control, ambient; HR-mid, heavy rainfall imposed in the middle of the growing season; and HR-late, heavy rainfall imposed late in the growing season. Error bars show one standard error of the mean.

HR-mid treatment in the latter 2 yr (11% and 7% in 2015 and 2016, respectively) was also lower than ambient SWC in the former 2 yr (17% and 23% in 2013 and 2014, respectively), reflecting the drier conditions in the latter 2 yr, which could buffer soil saturation stress induced by heavy rainfall in the former 2 yr (average SWC values during treatment were 17% and 22% in 2015 and 2016, respectively).

A second potential mechanism explaining the two-stage response is the development of stress memory to recurrent extreme conditions (Walter *et al.*, 2013; Backhaus *et al.*, 2014). In this study, the P_{\max} of dominant species was suppressed during the HR-mid treatment in 2014. However, these same species showed higher P_{\max} during the HR-mid treatment in 2015 and 2016 (Fig. 2), consistent with adaptation to heavy rainfall in the latter 2 yr, which strongly supports part of our second hypothesis. Furthermore, in 2014, the first year of

photosynthesis measurements, P_{\max} reached a peak when SWC was *c.* 29% and then declined at wetter values, whereas in 2015–2016, P_{\max} increased monotonically, even as SWC exceeded 30% (Fig. 3). This change in photosynthesis response to the highest SWC values suggests a beneficial adaptation in the latter 2 yr. Wang *et al.* (2017) reported that soil inundation could improve the relative growth of survivors subsequently subjected to similar conditions. Plants have been shown to tolerate flooding by regulating stomatal conductance, transpiration and thus photosynthesis (Insausti *et al.*, 2001). Such memory may last over the entire lifetime of a plant, even after harvest and resprouting (Walter *et al.*, 2011).

A third mechanism potentially underlying the two-stage response is an altered C allocation pattern. As discussed earlier, the fraction of C allocated above ground (especially in leaves) as compared with below ground (AGB : BGB) increased following

heavy rainfall (i.e. decreased root : shoot ratio; Fig. 4). Furthermore, compared with the former 2 yr, LAI greatly increased in the latter 2 yr under both heavy rainfall treatments in this study, although total AGB was not changed (Fig. 6a,c). Increasing leaf area has been reported for various herbaceous species growing in periodically flooded habitats as a result of more and longer parenchymatous cells and ethylene production (Van der Sman *et al.*, 1991; Grimoldi *et al.*, 1999; Insausti *et al.*, 2001). Large leaf area would enhance stomatal conductance at the community level, increase oxygen diffusion (de Oliveira & Joly, 2009), facilitate transpiration (Lai, 2015) and thus improve nutrition transportation and oxygen supply, which would benefit plant photosynthesis and respiration simultaneously. Additionally, increases in leaf area can improve water-use efficiency for ecosystem CO₂ exchange and thus increase ecosystem CO₂ fluxes (Hu *et al.*, 2008; Scott & Biederman, 2017). The idea that increased leaf area promoted ecosystem C fluxes was supported by previous studies at this site (Liu *et al.*, 2017; Hu *et al.*, 2018). Such morphology adaptation allowing plants to tolerate heavy rainfall strongly supports our second hypothesis.

The final potential mechanism of the two-stage response is alteration of ecosystem structure. Here, after 2 yr of heavy rainfall treatments, the ecosystem structure adapted, with increased abundance in the latter 2 yr (Fig. 6b,d), which may contribute to the increase in leaf area along with greater CO₂ fluxes.

Responses of ecosystem CO₂ fluxes to heavy rainfall depend on seasonal timing

Interestingly, the ecosystem CO₂ uptake (magnitude of NEE) in the HR-late treatment was consistently lower than the ambient control at the annual scale (Fig. 7a). This contrasting response of the late-season treatment could result from the fact that already-senescent leaves under low air temperature (Fig. S9) were unable to use the abundant water for C uptake, despite large leaf area, which partly supports our last hypothesis that heavy rainfall occurring in middle of the growing season would have a greater effect than heavy rainfall late in the growing season.

Implications for grassland response to heavy rainfall

In conclusion, projected increases in the occurrence and magnitude of heavy rainfall necessitate a deeper understanding of its effects on ecosystem structure and function. Our study provides a new perspective related to the seasonal timing and multiyear recurrence of these extreme events and their potential impacts on ecosystem services. We found that heavy rainfall late in the growing season had negative effects on community biomass largely because of a reduction in BGB, while AGB remained stable. In order to better assess precipitation–productivity relationships, which are highly relevant as benchmarks for global models of terrestrial C cycling, we encourage the implementation of heavy rainfall studies considering both event magnitude and timing and the responses both below and above ground. We also found discrepant ecosystem CO₂ flux response patterns between heavy rainfall occurring in different seasonal periods over the 4 yr,

highlighting that the differences in short- and mid-term responses to climate extremes may be timing-dependent, and that plant physiological and morphological adjustment, as well as interannual precipitation variability, regulated the effects of heavy rainfall on C cycling.

Acknowledgements

We thank five anonymous reviewers for their thoughtful comments, which helped us make better use of our dataset and reframe the study in the context of mechanistic hypotheses. This project was funded by the CAS Strategic Priority Research Programme (A) (grant nos. XDA20050103, XDA19030202) and the funds for International Cooperation and Exchange of National Natural Science Foundation of China (grant nos. 31761123001, 31761143018). We are grateful for the help from the Joint Research Station of Animal Ecology and the Inner Mongolia Grassland Ecosystem Research Station.

Author contributions

YH designed this research; LL, ZZ, CX and RC performed the research; LL analyzed the data; and LL, JAB, ZX, YW and XC wrote the paper.

ORCID

Joel A. Biederman  <https://orcid.org/0000-0002-1831-461X>
Yanbin Hao  <https://orcid.org/0000-0001-6821-0395>

References

- Ahl V, Allen TF. 1996. *Hierarchy theory: a vision, vocabulary, and epistemology*. New York, NY, USA: Columbia University Press.
- Backhaus S, Kreyling J, Grant K, Beierkuhnlein C, Walter J, Jentsch A. 2014. Recurrent mild drought events increase resistance toward extreme drought stress. *Ecosystems* 17: 1068–1081.
- Bai W, Wan S, Niu S, Liu W, Chen Q, Wang Q, Zhang W, Han X, Li L. 2010. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biology* 16: 1306–1316.
- Biederman JA, Scott RL, Bell TW, Bowling DR, Dore S, Garatuza-Payan J, Kolb TE, Krishnan P, Krofcheck DJ, Litvak ME. 2017. CO₂ exchange and evapotranspiration across dryland ecosystems of southwestern North America. *Global Change Biology* 23: 4204–4221.
- Biederman JA, Scott RL, Goulden ML, Vargas R, Litvak ME, Kolb TE, Yezzer EA, Oechel WC, Blanken PD, Bell TW. 2016. Terrestrial carbon balance in a drier world: the effects of water availability in southwestern North America. *Global Change Biology* 22: 1867–1879.
- Brown AL, Day FP, Stover DB. 2009. Fine root biomass estimates from minirhizotron imagery in a shrub ecosystem exposed to elevated CO₂. *Plant and Soil* 317: 145–153.
- Burt T, Boardman J, Foster I, Howden N. 2016. More rain, less soil: long-term changes in rainfall intensity with climate change. *Earth Surface Processes and Landforms* 41: 563–566.
- Chen H, Qualls RG, Blank RR. 2005. Effect of soil flooding on photosynthesis, carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*. *Aquatic Botany* 82: 250–268.
- Chen H, Qualls RG, Miller GC. 2002. Adaptive responses of *Lepidium latifolium* to soil flooding: biomass allocation, adventitious rooting,

- aerenchyma formation and ethylene production. *Environmental and Experimental Botany* 48: 119–128.
- Chen S, Lin G, Huang J, Jenerette GD. 2009. Dependence of carbon sequestration on the differential responses of ecosystem photosynthesis and respiration to rain pulses in a semiarid steppe. *Global Change Biology* 15: 2450–2461.
- Cheng J, Chu P, Chen D, Bai Y, Niu S. 2016. Functional correlations between specific leaf area and specific root length along a regional environmental gradient in Inner Mongolia grasslands. *Functional Ecology* 30: 985–997.
- Craine JM, Nippert JB, Elmore AJ, Skibbe AM, Hutchinson SL, Brunsell NA. 2012. Timing of climate variability and grassland productivity. *Proceedings of the National Academy of Sciences, USA* 109: 3401–3405.
- De Boeck HJ, Dreesen FE, Janssens IA, Nijs I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. *New Phytologist* 189: 806–817.
- D'Orangeville L, Maxwell J, Kneeshaw D, Pederson N, Duchesne L, Logan T, Houle D, Arseneault D, Beier CM, Bishop DA *et al.* 2018. Drought timing and local climate determine the sensitivity of eastern temperate forests to drought. *Global Change Biology* 24: 2339–2351.
- Doughty CE, Metcalfe DB, Girardin CA, Amezcua FF, Cabrera DG, Huasco WH, Silva-Espejo JE, Araujo-Murakami A, da Costa MC, Rocha W *et al.* 2015. Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature* 519: 78–82.
- Fan JW, Wang K, Harris W, Zhong HP, Hu ZM, Han B, Zhang WY, Wang JB. 2009. Allocation of vegetation biomass across a climate-related gradient in the grasslands of Inner Mongolia. *Journal of Arid Environments* 73: 521–528.
- Finér L, Ohashi M, Noguchi K, Hirano Y. 2011. Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. *Forest Ecology and Management* 262: 2008–2023.
- Fischer DG, Hart SC, LeRoy CJ, Whitham TG. 2007. Variation in below-ground carbon fluxes along a *Populus* hybridization gradient. *New Phytologist* 176: 415–425.
- Frank D, Reichstein M, Bahn M, Thonicke K, Frank D, Mahecha MD, Smith P, van der Velde M, Vicca S, Babst F *et al.* 2015. Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. *Global Change Biology* 21: 2861–2880.
- Gao YZ, Chen Q, Lin S, Giese M, Brueck H. 2011. Resource manipulation effects on net primary production, biomass allocation and rain-use efficiency of two semiarid grassland sites in Inner Mongolia, China. *Oecologia* 165: 855–864.
- Gattringer JP, Donath TW, Eckstein RL, Ludewig K, Otte A, Harvolk-Schoning S. 2017. Flooding tolerance of four floodplain meadow species depends on age. *PLoS ONE* 12: e0176869.
- Gellesch E, Arfin Khan MAS, Kreyling J, Jentsch A, Beierkuhnlein C. 2017. Grassland experiments under climatic extremes: reproductive fitness versus biomass. *Environmental and Experimental Botany* 144: 68–75.
- Grimoldi A, Insausti P, Roitman G, Soriano A. 1999. Responses to flooding intensity in *Leontodon taraxacoides*. *New Phytologist* 141: 119–128.
- Hao YB, Kang XM, Cui XY, Ding K, Wang YF, Zhou XQ. 2012. Verification of a threshold concept of ecologically effective precipitation pulse: from plant individuals to ecosystem. *Ecological Informatics* 12: 23–30.
- Hao YB, Wang YF, Mei XR, Cui XY. 2010. The response of ecosystem CO₂ exchange to small precipitation pulses over a temperate steppe. *Plant Ecology* 209: 335–347.
- Hao YB, Zhou CT, Liu WJ, Li LF, Kang XM, Jiang LL, Cui XY, Wang YF, Zhou XQ, Xu CY. 2017. Aboveground net primary productivity and carbon balance remain stable under extreme precipitation events in a semiarid steppe ecosystem. *Agricultural and Forest Meteorology* 240–241: 1–9.
- Hu Z, Shi H, Cheng K, Wang YP, Piao S, Li Y, Zhang L, Xia J, Zhou L, Yuan W *et al.* 2018. Joint structural and physiological control on the interannual variation in productivity in a temperate grassland: a data-model comparison. *Global Change Biology* 24: 2965–2979.
- Hu Z, Yu G, Fu Y, Sun X, Li Y, Shi P, Wang Y, Zheng Z. 2008. Effects of vegetation control on ecosystem water use efficiency within and among four grassland ecosystems in China. *Global Change Biology* 14: 1609–1619.
- Huang B, Nobel PS. 1992. Hydraulic conductivity and anatomy for lateral roots of *Agave deserti* during root growth and drought-induced abscission. *Journal of Experimental Botany* 43: 1441–1449.
- Hussain MZ, Grunwald T, Tenhunen JD, Li YL, Mirzae H, Bernhofer C, Otieno D, Dinh NQ, Schmidt M, Wartinger M *et al.* 2011. Summer drought influence on CO₂ and water fluxes of extensively managed grassland in Germany. *Agriculture and Ecosystems & Environment* 141: 67–76.
- Huxman TE, Smith MD, Fay PA, Knapp AK, Shaw MR, Loik ME, Smith SD, Tissue DT, Zak JC, Weltzin JF. 2004. Convergence across biomes to a common rain-use efficiency. *Nature* 429: 651.
- Insausti P, Grimoldi A, Chaneton E, Vasellati V. 2001. Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. *New Phytologist* 152: 291–299.
- Jha A, Schkade U, Kirchner G. 2015. Estimating short-term soil erosion rates after single and multiple rainfall events by modelling the vertical distribution of cosmogenic ⁷Be in soils. *Geoderma* 243: 149–156.
- Kitajima K, Anderson KE, Allen MF. 2010. Effect of soil temperature and soil water content on fine root turnover rate in a California mixed conifer ecosystem. *Journal of Geophysical Research: Biogeosciences* 115: G04032.
- Knapp AK, Briggs JM, Smith MD, Fox C. 2012. Community stability does not preclude ecosystem sensitivity to chronic resource alteration. *Functional Ecology* 26: 1231–1233.
- Knapp AK, Ciais P, Smith MD. 2017. Reconciling inconsistencies in precipitation–productivity relationships: implications for climate change. *New Phytologist* 214: 41–47.
- Knapp AK, Smith MD. 2001. Variation among biomes in temporal dynamics of aboveground primary production. *Science* 291: 481–484.
- Koide T, Saito H, Shiota T, Iwahana G, Lopez c ML, Maximov TC, Hasegawa S, Hatano R. 2010. Effects of changes in the soil environment associated with heavy precipitation on soil greenhouse gas fluxes in a Siberian larch forest near Yakutsk. *Soil Science and Plant Nutrition* 56: 645–662.
- Kozłowski T. 1984. Plant responses to flooding of soil. *BioScience* 34: 162–167.
- Kozłowski T. 1997. Responses of woody plants to flooding and salinity. *Tree Physiology* 17: 490.
- Kreyling J, Wenigmann M, Beierkuhnlein C, Jentsch A. 2008. Effects of extreme weather events on plant productivity and tissue die-back are modified by community composition. *Ecosystems* 11: 752–763.
- Lai H-Y. 2015. Effects of leaf area and transpiration rate on accumulation and compartmentalization of cadmium in *impatiens walleriana*. *Water, Air, & Soil Pollution* 226: 2246.
- Li J. 2010. Photosynthetic parameters of major grass species in Inner Mongolia China plain. *Chinese Agricultural Science Bulletin* 1: 050.
- Li J, Lin S, Taube F, Pan Q, Dittler K. 2010. Above and belowground net primary productivity of grassland influenced by supplemental water and nitrogen in Inner Mongolia. *Plant and Soil* 340: 253–264.
- Li L, Fan W, Kang X, Wang Y, Cui X, Xu C, Griffin KL, Hao Y. 2016. Responses of greenhouse gas fluxes to climate extremes in a semiarid grassland. *Atmospheric Environment* 142: 32–42.
- Li L, Zhang D, Xin X, Yan Y, Yang G, Li J, Wang X. 2009. Photosynthetic characteristics of *Leymus chinensis* under different soil moisture grades in Hulunber prairie. *Acta Ecologica Sinica* 29: 5271–5279.
- Liu H, Mi Z, Lin L, Wang Y, Zhang Z, Zhang F, Wang H, Liu L, Zhu B, Cao G *et al.* 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proceedings of the National Academy of Sciences, USA* 115: 4051–4056.
- Liu WJ, Li LF, Biederman JA, Hao YB, Zhang H, Kang XM, Cui XY, Wang YF, Li MW, Xu ZH *et al.* 2017. Repackaging precipitation into fewer, larger storms reduces ecosystem exchanges of CO₂ and H₂O in a semiarid steppe. *Agricultural and Forest Meteorology* 247: 356–364.
- Lu XT, Dijkstra FA, Kong DL, Wang ZW, Han XG. 2014. Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland. *Scientific Report* 4: 4817.
- Mäkinen H, Kaseva J, Trnka M, Balek J, Kersebaum KC, Nendel C, Gobin A, Olesen JE, Bindi M, Ferrise R *et al.* 2017. Sensitivity of European wheat to extreme weather. *Field Crops Research* 222: 209–217.

- Marzen M, Iserloh T, de Lima JL, Fister W, Ries JB. 2017. Impact of severe rain storms on soil erosion: experimental evaluation of wind-driven rain and its implications for natural hazard management. *Science of the Total Environment* 590: 502–513.
- Morgan JA, Parton W, Derner JD, Gilmanov TG, Smith DP. 2016. Importance of early season conditions and grazing on carbon dioxide fluxes in Colorado shortgrass steppe. *Rangeland Ecology & Management* 69: 342–350.
- Niu S, Wu M, Han Y, Xia J, Li L, Wan S. 2008. Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. *New Phytologist* 177: 209–219.
- Norby RJ, Hanson PJ, O'Neill EG, Tschaplinski TJ, Weltzin JF, Hansen RA, Cheng W, Wullschlegel SD, Gunderson CA, Edwards NT. 2002. Net primary productivity of a CO₂-enriched deciduous forest and the implications for carbon storage. *Ecological Applications* 12: 1261–1266.
- O'Gorman PA, Schneider T. 2009. The physical basis for increases in precipitation extremes in simulations of 21st-century climate change. *Proceedings of the National Academy of Sciences, USA* 106: 14773–14777.
- de Oliveira VC, Joly CA. 2009. Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses. *Trees* 24: 185–193.
- O'Neill E, O'Neill R, Norby R. 1991. Hierarchy theory as a guide to mycorrhizal research on large-scale problems. *Environmental Pollution* 73: 271–284.
- Otto FEL, van der Wiel K, van Oldenborgh GJ, Philip S, Kew SF, Uhe P, Cullen H. 2018. Climate change increases the probability of heavy rains in Northern England/Southern Scotland like those of storm Desmond - a real-time event attribution revisited. *Environmental Research Letters* 13: 024006.
- Parton W, Morgan J, Smith D, Del Grosso S, Prihodko L, LeCain D, Kelly R, Lutz S. 2012. Impact of precipitation dynamics on net ecosystem productivity. *Global Change Biology* 18: 915–927.
- Patrick L, Cable J, Potts D, Ignace D, Barron-Gafford G, Griffith A, Alpert H, Van Gestel N, Robertson T, Huxman TE *et al.* 2007. Effects of an increase in summer precipitation on leaf, soil, and ecosystem fluxes of CO₂ and H₂O in a sotol grassland in Big Bend National Park, Texas. *Oecologia* 151: 704–718.
- Ponce Campos GE, Moran MS, Huete A, Zhang Y, Bresloff C, Huxman TE, Eamus D, Bosch DD, Buda AR, Gunter SA *et al.* 2013. Ecosystem resilience despite large-scale altered hydroclimatic conditions. *Nature* 494: 349–352.
- Reichstein M, Bahn M, Ciais P, Frank D, Mahecha MD, Seneviratne SI, Zscheischler J, Beer C, Buchmann N, Frank DC *et al.* 2013. Climate extremes and the carbon cycle. *Nature* 500: 287–295.
- Ren H, Xu Z, Isbell F, Huang J, Han X, Wan S, Chen S, Wang R, Zeng DH, Jiang Y. 2017. Exacerbated nitrogen limitation ends transient stimulation of grassland productivity by increased precipitation. *Ecological Monographs* 87: 457–469.
- Rubio G, Lavado RS. 1999. Acquisition and allocation of resources in two waterlogging-tolerant grasses. *New Phytologist* 143: 539–546.
- Sala OE, Gherardi LA, Reichmann L, Jobbagy E, Peters D. 2012. Legacies of precipitation fluctuations on primary production: theory and data synthesis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 367: 3135–3144.
- Scott RL, Biederman JA. 2017. Partitioning evapotranspiration using long-term carbon dioxide and water vapor fluxes. *Geophysical Research Letters* 44: 6833–6840.
- Scott RL, Biederman JA, Hamerlync EP, Barron-Gafford GA. 2015. The carbon balance pivot point of southwestern US semiarid ecosystems: insights from the 21st century drought. *Journal of Geophysical Research: Biogeosciences* 120: 2612–2624.
- Scott RL, Hamerlync EP, Jenerette GD, Moran MS, Barron-Gafford GA. 2010. Carbon dioxide exchange in a semidesert grassland through drought-induced vegetation change. *Journal of Geophysical Research-Biogeosciences* 115: G03026.
- Sepulveda A, Schuller P, Walling DE, Castillo A. 2008. Use of ⁷Be to document soil erosion associated with a short period of extreme rainfall. *Journal of Environmental Radioactivity* 99: 35–49.
- Sherwood S, Fu Q. 2014. A drier future? *Science* 343: 737–739.
- Sippel S, Zscheischler J, Reichstein M. 2016. Ecosystem impacts of climate extremes crucially depend on the timing. *Proceedings of the National Academy of Sciences, USA* 113: 5768–5770.
- Smith MD. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology* 99: 656–663.
- Stocker T. 2014. *Climate change 2013: the physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
- R Core Team. 2018. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL <https://www.r-project.org/>.
- Van der Sman A, Voisenek L, Blom C, Harren F, Reuss J. 1991. The role of ethylene in shoot elongation with respect to survival and seed output of flooded *Rumex maritimus* L. plants. *Functional Ecology* 5: 304–313.
- Voisenek L, Rijnders J, Peeters A, Van de Steeg H, De Kroon H. 2004. Plant hormones regulate fast shoot elongation under water: from genes to communities. *Ecology* 85: 16–27.
- Walter J, Jentsch A, Beierkuhnlein C, Kreyling J. 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany* 94: 3–8.
- Walter J, Nagy L, Hein R, Rascher U, Beierkuhnlein C, Willner E, Jentsch A. 2011. Do plants remember drought? Hints towards a drought-memory in grasses. *Environmental and Experimental Botany* 71: 34–40.
- Wang S, Callaway RM, Zhou D-W, Weiner J, Cahill J. 2017. Experience of inundation or drought alters the responses of plants to subsequent water conditions. *Journal of Ecology* 105: 176–187.
- Wilcox KR, Shi Z, Gherardi LA, Lemoine NP, Koerner SE, Hoover DL, Bork E, Byrne KM, Cahill J Jr, Collins SL *et al.* 2017. Asymmetric responses of primary productivity to precipitation extremes: a synthesis of grassland precipitation manipulation experiments. *Global Change Biology* 23: 4376–4385.
- Wolf S, Keenan TF, Fisher JB, Baldocchi DD, Desai AR, Richardson AD, Scott RL, Law BE, Litvak ME, Brunsell NA. 2016. Warm spring reduced carbon cycle impact of the 2012 US summer drought. *Proceedings of the National Academy of Sciences, USA* 113: 5880–5885.
- Wright AJ, de Kroon H, Visser EJ, Buchmann T, Ebeling A, Eisenhauer N, Fischer C, Hildebrandt A, Ravenek J, Roscher C *et al.* 2017. Plants are less negatively affected by flooding when growing in species-rich plant communities. *New Phytologist* 213: 645–656.
- Wu Y, Che R, Ma S, Deng Y, Zhu M, Cui X. 2014. Estimation of root production and turnover in an alpine meadow: comparison of three measurement methods. *Acta Ecologica Sinica* 34: 3529–3537.
- Wu F, Deng X, Yin F, Yuan Y. 2013. Projected changes of grassland productivity along the representative concentration pathways during 2010–2050 in China. *Advances in Meteorology* 2013: 1–9.
- Wu Z, Dijkstra P, Koch GW, Penuelas J, Hungate BA. 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology* 17: 927–942.
- Yang Y, Fang J, Ma W, Wang W. 2008. Relationship between variability in aboveground net primary production and precipitation in global grasslands. *Geophysical Research Letters* 35: L23710.
- Yu GR, Zhu XJ, Fu YL, He HL, Wang QF, Wen XF, Li XR, Zhang LM, Zhang L, Su W *et al.* 2013. Spatial patterns and climate drivers of carbon fluxes in terrestrial ecosystems of China. *Global Change Biology* 19: 798–810.
- Yuan Z, Chen HY. 2010. Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: literature review and meta-analyses. *Critical Reviews in Plant Sciences* 29: 204–221.
- Zhang Q, Huber H, Beljaars SJM, Birnbaum D, de Best S, de Kroon H, Visser EJW. 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. *Annals of Botany* 120: 171–180.
- Zhang Y, Susan Moran M, Nearing MA, Ponce Campos GE, Huete AR, Buda AR, Bosch DD, Gunter SA, Kitchen SG, Henry McNab W *et al.* 2013. Extreme precipitation patterns and reductions of terrestrial ecosystem production across biomes. *Journal of Geophysical Research: Biogeosciences* 118: 148–157.
- Zhou J, Zhang Z, Sun G, Fang X, Zha T, McNulty S, Chen J, Jin Y, Noormets A. 2013. Response of ecosystem carbon fluxes to drought events in a poplar plantation in Northern China. *Forest Ecology and Management* 300: 33–42.

- Zhu K, Chiariello NR, Tobeck T, Fukami T, Field CB. 2016. Nonlinear, interacting responses to climate limit grassland production under global change. *Proceedings of the National Academy of Sciences, USA* 113: 10589–10594.
- Ziegler VH, Ploschuk E, Weibel A, Insausti P. 2017. Short-term responses to flooding stress of three *Prunus* rootstocks. *Scientia Horticulturae* 224: 135–141.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Relative abundance and proportion of leaf area of three dominant species.

Fig. S2 Soil water content on P_{\max} measurement days.

Fig. S3 Comparison of BGB in the ambient control treatment between the experimental platform and a location in the vicinity of the experimental platform.

Fig. S4 Responses of BNPP, root length and root volume to heavy rainfall events.

Fig. S5 Responses of P_{\max} of three dominant grass species to the HR-mid treatment during and after treatment.

Fig. S6 Responses of P_{\max} of three dominant grass species to the HR-late treatment during and after treatment.

Fig. S7 Responses of BGB at three soil depths to heavy rainfall treatments.

Fig. S8 Temporal dynamics of NEE, ER, and GEP in response to heavy rainfall treatments.

Fig. S9 Mean daily temperature during the growing season in 2013–2016.

Notes S1 Introduction to the Extreme Climate Events and Biodiversity II (ECEB-II) experiment.

Table S1 Mean of ambient photosynthetically available radiation between 9:00 and 11:00 h on ecosystem CO_2 measurement days during heavy rainfall treatments.

Table S2 Results of mixed-effect model analyses of HR-mid and HR-late treatment effects on BGB, root length and root volume.

Table S3 Results of mixed-effect model analyses of HR-mid and HR-late treatment effects on BGB at three soil depths.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.



About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**