

Nitrogen addition amplifies the nonlinear drought response of grassland productivity to extended growing-season droughts

BO MENG ^{1,2} JUNQIN LI,¹ GREGORY E. MAURER,³ SHANGZHI ZHONG,⁴ YUAN YAO,¹ XUECHEN YANG,^{1,5} SCOTT L. COLLINS ² AND WEI SUN^{1,6}

¹*Institute of Grassland Science, Key Laboratory of Vegetation Ecology of the Ministry of Education, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun 130024 China*

²*Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131 USA*

³*Jornada Basin LTER Program, New Mexico State University, Las Cruces, New Mexico 88003 USA*

⁴*College of Grassland Science, Grassland Agri-Husbandry Research Center, Qingdao Agricultural University, Qingdao 255109 China*

⁵*Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081 China*

Citation: Meng, B., J. Li, G. E. Maurer, S. Zhong, Y. Yao, X. Yang, S. L. Collins, and W. Sun. 2021. Nitrogen addition amplifies the nonlinear drought response of grassland productivity to extended growing-season droughts. *Ecology* 102(11):e03483. 10.1002/ecy.3483

Abstract. Understanding the response of grassland production and carbon exchange to intra-annual variation in precipitation and nitrogen addition is critical for sustainable grassland management and ecosystem restoration. We introduced growing-season drought treatments of different lengths (15, 30, 45 and 60 d drought) by delaying growing-season precipitation in a long-term nitrogen addition experiment in a low diversity meadow steppe in northeast China. Response variables included aboveground biomass (AGB), ecosystem net carbon exchange (NEE), and leaf net carbon assimilation rate (*A*). In unfertilized plots drought decreased AGB by 13.7% after a 45-d drought and 31.7% after a 60-d drought (47.6% in fertilized plots). Progressive increases in the drought response of NEE were also observed. The effects of N addition on the drought response of productivity increased as drought duration increased, and these responses were a function of changes in AGB and biomass allocation, particularly root to shoot ratio. However, no significant effects of drought occurred in fertilized or unfertilized plots in the growing season a year after the experiment, N addition did limit the recovery of AGB from severe drought during the remainder of the current growing season. Our results imply that chronic N enrichment could exacerbate the effects of growing-season drought on grassland productivity caused by altered precipitation seasonality under climate change, but that these effects do not carry over to the next growing season.

Key words: drought period; grassland productivity; *Leymus chinensis*; net ecosystem carbon exchange; nitrogen addition; root: shoot ratio.

INTRODUCTION

Evidence is mounting that more frequent and intense drought events are occurring globally (Lei and Duan 2011, Spinoni et al. 2014), and such changes are forecast to continue or intensify, especially in arid and semiarid regions (Davidowitz 2002, Touma et al. 2015). Compounding the effects of drought, the nitrogen economies of grasslands are being altered through deposition and fertilization (Reay et al. 2008), with consequent effects on community resistance to, and recovery from, disturbances that affect primary production, species dominance, and other ecosystem processes or states (Clark et al. 2007, Collins et al. 2017).

In the absence of changes in total precipitation amount, the effect of rainfall variability on grassland ecosystem function may become more significant as within-year drought episodes increase in duration (Knapp et al. 2002). While a significant number of studies have examined the effect of interannual variability in precipitation on grassland productivity (Knapp et al. 2017, Gherardi and Sala 2019, Maurer et al. 2020), few have attempted to isolate the effect of growing-season drought duration on primary production. Indeed, vegetation in arid and semiarid grassland could exhibit greater stability under within-season drought than mesic ecosystem due to historically high precipitation variability (Davidowitz 2002, Ruppert et al. 2015), and because dominant species in these grasslands may have high physiological or morphological tolerance to drought (Craine et al. 2013). Plant responses to temporary precipitation shortages occur via multiple physiological processes, including stomatal closure, photosynthetic apparatus damage, and reductions in cell division and

Manuscript received 8 December 2020; revised 8 March 2021; accepted 6 April 2021; final version received 15 July 2021.
 Corresponding Editor: Joseph B. Yavitt.

⁶ Corresponding Author. E-mail: sunwei@nenu.edu.cn

elongation, and these responses may compound with the prolongation of drought events (Chaves et al. 2003). Thus, it may be that prolonged drought episodes induce concurrent nonlinear responses in grassland productivity, and significant post-drought effects on annual productivity (Griffin-Nolan et al. 2018).

Soil N enrichment through agricultural management and atmospheric N deposition can influence grassland community responses to precipitation variability through several possible mechanisms (Fig. 1). First, N addition may decrease in species richness (Stevens et al. 2004, Zhang et al. 2019), which may lead to declines in ecosystem stability since greater species richness is associated with stronger compensatory effects through species asynchrony (Zhang et al. 2019). Second, N addition can lead to greater aboveground biomass, which increases transpiration and consumes soil water more rapidly (Wang et al. 2007, 2018). Third, soil nutrient addition can decrease belowground biomass allocation (Poorter et al. 2012), which limits plant acquisition of soil water resources (Comas et al. 2013). However, stability mechanisms may be context dependent. For example, the diversity–stability relationship is weakened in some communities with strong dominance and low evenness (Hallett et al. 2014), which can occur under N addition. Despite the increase in experimental studies demonstrating relationships between N addition and

ecosystem response to drought, these relationships, particularly with respect to plant production, remain unclear.

The effects of drought on annual productivity not only depend on the sensitivity of grasslands but also on their ability to recover following drought (Fig. 1). Empirical data and ecosystem models predict that N addition will enhance post-drought recovery of grassland productivity (Kinugasa et al. 2012, Bharath et al. 2020). Indeed, results from drought experiments in temperate grasslands suggest that in communities with larger plant size and/or more shoots, N addition can increase seed production (Wesche and Ronnenberg 2010) and promote photosynthetic C assimilation (Chen et al. 2005), thereby speeding up post-drought recovery of productivity. However, other studies have reported nonsignificant effects of N addition on post-drought recovery (Tilman 1996, Xu et al. 2014) because N addition was associated with stronger responses by plant shoots, such as mortality or wilting, during drought periods (Southon et al. 2012) offsetting the positive effects of N addition on post-drought recovery. Wilcox et al. (2020) suggested that compensation plays a critical role in ecosystem resilience to extreme drought in savanna grasslands, and thus long-term N addition will negatively impact recovery if diversity or compensation are reduced. Hence, we still do not have a clear understanding of the effects of N

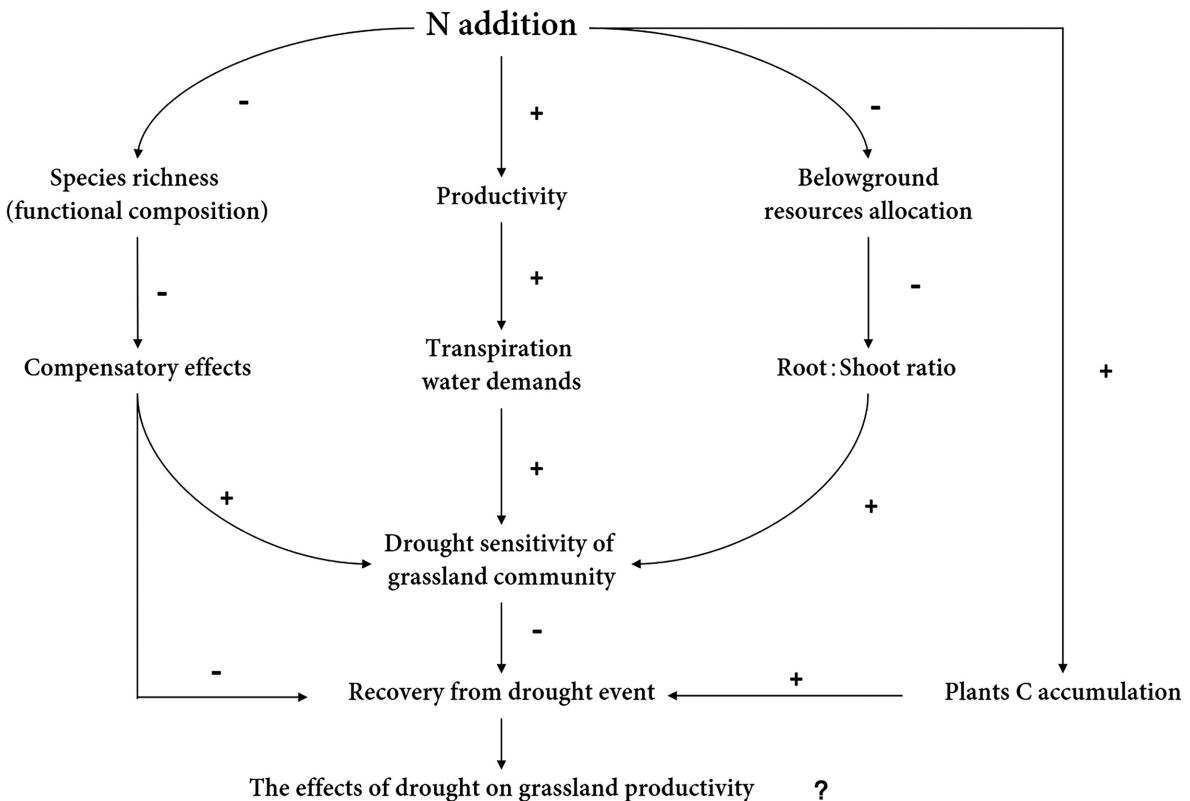


FIG. 1. Hypothesized positive (+) and negative (–) relationships between nitrogen addition, grassland productivity, and prolonged within-season drought.

addition on grassland recovery immediately after drought events or at longer time scales.

In this study, we induced growing-season droughts of varying lengths in fertilized and un-fertilized plots to observe the responses of productivity, biomass allocation, and species richness of a semiarid grassland that has historically experienced frequent drought episodes (Lei and Duan 2011, Liu et al. 2014). This manipulative experiment in a low-diversity meadow steppe (>90% dominance by a perennial grass *Leymus chinensis*) included one N addition level, five drought durations, and a post-drought irrigation to equalize annual precipitation during the growing season. We observed ecosystem responses immediately after drought, at the end of the growing season, and one year later. We hypothesized that (1) grassland productivity and C exchange will nonlinearly decrease as drought duration continues; (2) the N-induced enhancement of drought response in productivity will increase with drought duration; and (3) long-term N addition will not accelerate recovery following drought because of drought-induced injury to plant tissues.

MATERIALS AND METHODS

Study site

The study site was located at Changling Horse Breeding Farm (44°30'–44°45' N, 123°31'–123°56' E) in Western Jilin Province, Northeast China. The grassland is part of the Songnen meadow steppe, which has a semiarid continental climate with a mean annual temperature of 6.4°C (1950–2004). The average growing season lasts from mid-April to September, with an average growing season precipitation of 411 mm. The average length of prolonged drought episodes, defined as more than 10 consecutive days without significant rainfall (≤ 2 mm), during the growing season is 17.4–21.9 d (Liu et al. 2014). The 90th percentile length of a drought episode can last a month or more over this semiarid region (Lei and Duan 2011).

Vegetation in the studied grassland was dominated by the C₃ rhizomatous perennial grass *Leymus chinensis* (over 90% of plant abundance). Other accompanying perennials (*Phragmites australis* and *Kalimeris integrifolia*) and annuals, such as *Chloris virgata*, were also present at lower densities (Wang et al. 2018). The maximal rooting depth of *L. chinensis* was no more than 30 cm, and 70% of the roots were located from 0–15 cm soil depth in this study site. The main soil type of the study area is chernozem with a pH of 8.5–9.5, 0.15% total nitrogen, and 2.0% total organic carbon content. Soil field capacity is approximately 0.255 g/g.

Experimental design

Starting in 2010, a 100 × 100 m area of grassland was fenced to exclude grazing and mowing. In May 2015,

four blocks, each with an area of 10 × 20 m and similar in vegetation composition, were laid out within the fenced grassland. We divided each block into 10 2.5 × 2.5 m plots and randomly assigned a combination of ambient and N addition (10 g N·m⁻²·yr⁻¹ as granular urea) treatments crossed with drought (precipitation exclusion) as follows: unfertilized with ambient precipitation (Ambient), 15 d drought (15D), 30 d drought (30D), 45 d drought (45D), and 60 d drought (60D); fertilized with ambient precipitation (Ambient + N), 15 d drought (15D + N), 30 d drought (30D + N), 45 d drought (45D + N), and 60 d drought (60D + N). Buffer zones were 2 m wide between the adjacent plots. The N addition treatment started in May 2015. For each year, the N fertilizer was evenly divided into five parts and manually spread on the plots once per month on a rainy day (2 g N·m⁻²·month⁻¹) from May to September.

A 2 mm thick stainless-steel plate was placed around the drought plots (10 cm aboveground and 50 cm belowground) to prevent water from overland runoff and belowground lateral soil infiltration. All drought manipulations were simultaneously started on 22 May (day of the year [DOY] 142) in 2017. Experimental droughts were imposed for the specified periods by installing 3 × 3 m rainout shelters with transparent acrylic roofs. After each experimental drought period, the excluded rainfall was immediately irrigated onto the drought plots so that total precipitation was the same among all treatments. To avoid waterlogging, the excluded rainwater from 45- and 60-d droughts was added over multiple days in one week.

Environmental conditions

Precipitation and air temperature were measured using an RG2-M sensor (Onset Computer Corporation, Bourne, Massachusetts, USA). Gravimetric soil water content at 0–10 cm depth (SWC) was measured by oven drying 100-cm³ soil samples collected from all plots. At the end of each drought treatment, SWC measurements were made in the corresponding drought plots and in all ambient precipitation plots. Two blocks were randomly selected for continuous monitoring of soil water content at 10 cm depth in all 10 treatment plots throughout the growing season of 2017 using soil moisture sensors (S-SMC-M005, Decagon, Pullman, Washington, USA).

Vegetation sampling

Vegetation surveys were conducted four times: (1) at the beginning of the drought treatments, on or around 22 May 2017, (2) immediately after each 15-, 30-, 45-, or 60-d drought treatment ended, (3) at the end of the 2017 growing season (September 4), and (4) approximately one year later in mid-August of 2018. In each plot, we surveyed the number of plant species, and counted the number of individuals for each species in three randomly placed 0.5 × 0.5 m quadrats. For each survey, 20 live

shoots of *L. chinensis* were randomly collected from each plot to calculate the proportion of dead leaves (Meng et al. 2019).

After the vegetation survey, one of the quadrats was randomly selected for the measurement of aboveground biomass (AGB) by harvesting all aboveground live plant shoots. Harvest quadrats were not allowed to overlap. For belowground biomass (BGB), we washed the roots out of three soil cores with a diameter of 10 cm and a depth of 30 cm. The soil cores were randomly collected in the quadrat used for the harvesting of AGB. The harvested plant and root materials were oven-dried at 70°C to a constant mass (48 h). Leaf C:N ratio was determined by an elemental analyzer (vario EL cube, Elementar, Langensfeld, Germany). The root:shoot (R:S) ratio was calculated as BGB/AGB.

The densities of buds and tillering nodes were measured at the end of the 2017 growing season (14 September). A soil sample with an area of 0.25 × 0.25 m and a depth of 0.2 m was collected from each plot. The collected soil was gently crushed by hand, and the numbers of buds and tillering nodes of *L. chinensis* were counted directly in the field to avoid damage of the buds by dehydration.

Leaf carbon exchange measurements

The measurements of leaf net carbon assimilation rate (*A*) and transpiration rate (*E*) were conducted from 08:00 to 10:00 on a sunny day at the end of each drought treatment using a portable infrared gas analyzer (LI-6400, LiCor, Lincoln, Nebraska, USA). Five representative intact *L. chinensis* leaves (uppermost, fully expanded) were randomly selected in each plot for leaf gas exchange measurements. Photon flux density and CO₂ concentration in the leaf chamber were set at 1,500 μmol·m⁻²·s⁻¹ and 400 μmol/mol, respectively. Leaf chamber temperature was set to match the ambient value.

Ecosystem carbon exchange measurements

Concurrently with leaf-level measurements, we measured short-term net ecosystem CO₂ exchange (NEE) and evapotranspiration (ET) using an infrared gas analyzer (LI-6400, LiCor) attached to a transparent chamber (0.5 × 0.5 × 1 m polymethyl methacrylate). To stabilize the transparent chamber, an iron frame (0.5 × 0.5 m) was fixed in the center of each plot in early 2017. For each measurement, 11 consecutive recordings of CO₂ and H₂O concentration were logged at 10-s intervals during a 2-minute period after the chamber was mounted on top of the iron frame, then the slope of the linear regression of CO₂ and H₂O concentration was used to determine NEE and ET (Wang et al. 2018). Within the chamber, four small electric fans ran continuously to mix air during the measurement.

Statistical analysis

The response ratio of productivity to drought was calculated as the ratio of differences in AGB or NEE between the drought and ambient precipitation plots to the AGB or NEE in ambient precipitation plots

$$RR_{AGB} = \frac{\text{Mean}(AGB_D) - AGB_D}{\text{Mean}(AGB_A)} \times 100\%$$

$$RR_{NEE} = \frac{\text{Mean}(NEE_D) - NEE_D}{\text{Mean}(NEE_A)} \times 100\%$$

where RR_{AGB} and RR_{NEE} is the drought response ratio of AGB and NEE, respectively; AGB_D and AGB_A are the AGB of the drought plots and the ambient precipitation plots at the end of each drought period; NEE_D and NEE_A are the values of NEE in the drought plots and the ambient precipitation plots, respectively. The treatment effect on productivity recovery was assessed by comparing AGB and NEE with ambient precipitation plots at the end of the 2017 growing season and one year later in mid-August of 2018.

Two-way analysis of variance (ANOVA; with block as a random factor) was used to analyze the effects of drought duration, N addition, and their interaction on AGB, BGB, R:S ratio, bud density, *A*, *E*, NEE and ET. One-way ANOVAs followed by Tukey post hoc tests were used to further assess differences in the recovery of AGB, BGB, *A*, and NEE among different drought treatments under the unfertilized or fertilized conditions. Regression analysis was employed to evaluate the change of drought response ratio with length of drought. Final regression models were selected on the basis of Akaike information criterion (AIC_c) and coefficient of determination (R^2). The relationship between RR_{AGB} (or RR_{NEE}) and AGB was tested by linear mixed models (LMM) with AGB as a fixed effect, drought and N treatment as a random effect. A statistical probability of $P < 0.05$ determined significance. All statistical analyses were performed using SPSS software 22.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Environmental conditions

The amount of precipitation during the 2017 growing season (415.4 mm) was similar to the average precipitation of the last 50 yr (1962–2012). The ambient plots received 130.6 mm of precipitation during the 60 d drought treatment (DOY 142–202, Appendix S1: Fig. S1). Compared to the start of the drought treatments, soil moisture rapidly decreased in the 15-d drought plots (37% in unfertilized and 40% in fertilized plots) and 30-d drought plots (59% in unfertilized and 64% in fertilized plots). In the 45- and 60-d drought treatments, soil moisture dropped significantly below the ambient

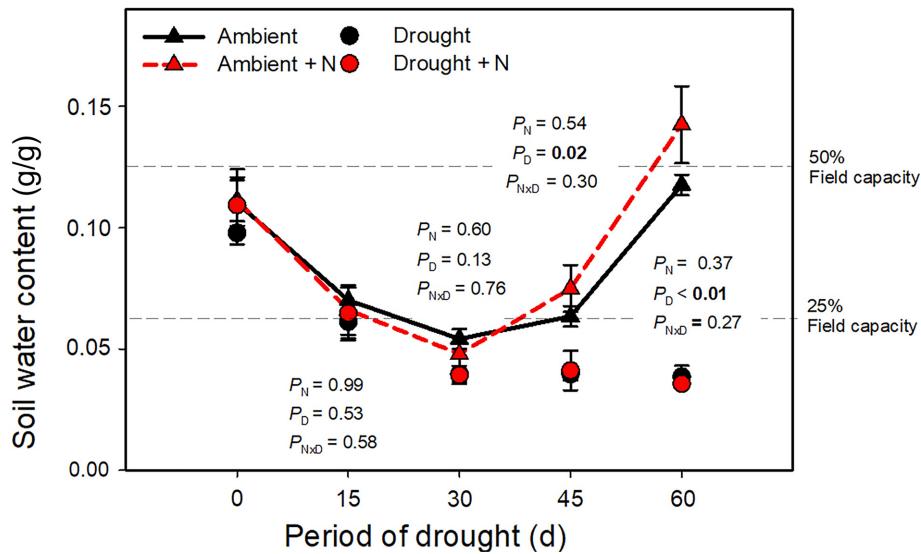


FIG. 2. Soil water content (SWC, g/g) at 0–10 cm soil depth for four treatment combinations (Ambient, Drought, Ambient + N, and Drought + N) at the start of the drought treatments and at the end of each drought period. Data are reported as mean \pm SE ($n = 4$). SWC in Ambient + N and Drought + N treatments was measured periodically during drought treatments. Values in bold-face type indicate significant treatment effects at $P < 0.05$. Full statistical results are shown in Appendix S1: Table S3.

precipitation plot values to around 0.04 g/g, approximately 15.5% of field capacity (Fig. 2). Soil moisture did not differ between fertilized and unfertilized treatments.

Vegetation and biomass

Average species richness was 4.2 and relative abundance of *L. chinensis* was 97.5% in ambient precipitation plots at the end of the 60-d experimental period. Although N addition significantly increased AGB, no significant effects of both N addition and drought treatments were detected on richness or relative abundance of *L. chinensis* (Appendix S1: Table S2). The 45-d drought treatment decreased AGB by 13.7% in the unfertilized plots and 19.5% in the fertilized plots ($F_{1,3} = 10.3$, $P = 0.05$), and the 60-d drought reduced AGB by 31.7% in the unfertilized plots and 47.6% in the fertilized plots ($F_{1,3} = 34.2$, $P = 0.01$, Fig. 3A). Average BGB was 1285.6 g/m² in ambient precipitation plots. No significant effect of N addition, drought, or their interactions on BGB were detected following the drought treatments (Appendix S1: Fig. S3). Drought treatments significantly increased the R:S ratio from 5.8 to 10.9 in the unfertilized plots and from 5.2 to 11.4 and in the fertilized plots after 60-d drought treatments ($F_{1,3} = 22.9$, $P = 0.02$). Although we observed a trend of N addition-induced decrease in R:S ratio, the R:S ratio showed a greater drought response in N addition plots (Appendix S1: Fig. S3). Moreover, we found that drought significantly decreased *L. chinensis* density in the 60-d drought plots, and a significant interaction occurred between N addition and drought on *L. chinensis* density in the 60-d drought treatment ($F_{1,3} = 10.3$, $P = 0.05$, Table 1). The

percentage of dead leaves also significantly increased in the 45-d ($F_{1,3} = 11.4$, $P = 0.04$) and 60-d drought treatments ($F_{1,3} = 12.7$, $P = 0.03$, Table 1). Fertilized plots had a lower leaf C:N ratio than unfertilized plots, but the C:N ratio gradually increased as drought continued in both fertilized and unfertilized plots (Appendix S1: Fig. S4).

At the end of the 2017 growing season, there were significant differences in AGB among the drought treatments in the fertilized plots, but no difference in the unfertilized plots (Fig. 3B). There were no significant effects of the drought treatment on the density of buds and rhizome tillering nodes at the end of the growing season in 2017 (Appendix S1: Fig. S5). In 2018, no significant drought effects on AGB were observed in either fertilized or unfertilized plots (Fig. 3C).

Leaf and ecosystem carbon exchange

Nitrogen addition tended to enhance leaf net carbon assimilation rate (A) and short-term net ecosystem CO₂ exchange (NEE) during the drought treatments, but the effects were not statistically significant (Fig. 3D, G). Significant drought-induced reductions in A were detected in the 30, 45 and 60 d drought treatments (Fig. 3D). The 45 d drought treatment decreased NEE by 19.7% in the unfertilized plots and 65.6% in the fertilized plots, and the 60-d drought treatment decreased NEE by 34.3% in the unfertilized plots and 81.0% in the fertilized plots (Fig. 3G). Significant drought effects on NEE were only detected in the 60-d drought treatments ($F_{1,3} = 280$, $P < 0.01$, Fig. 3G). No significant N

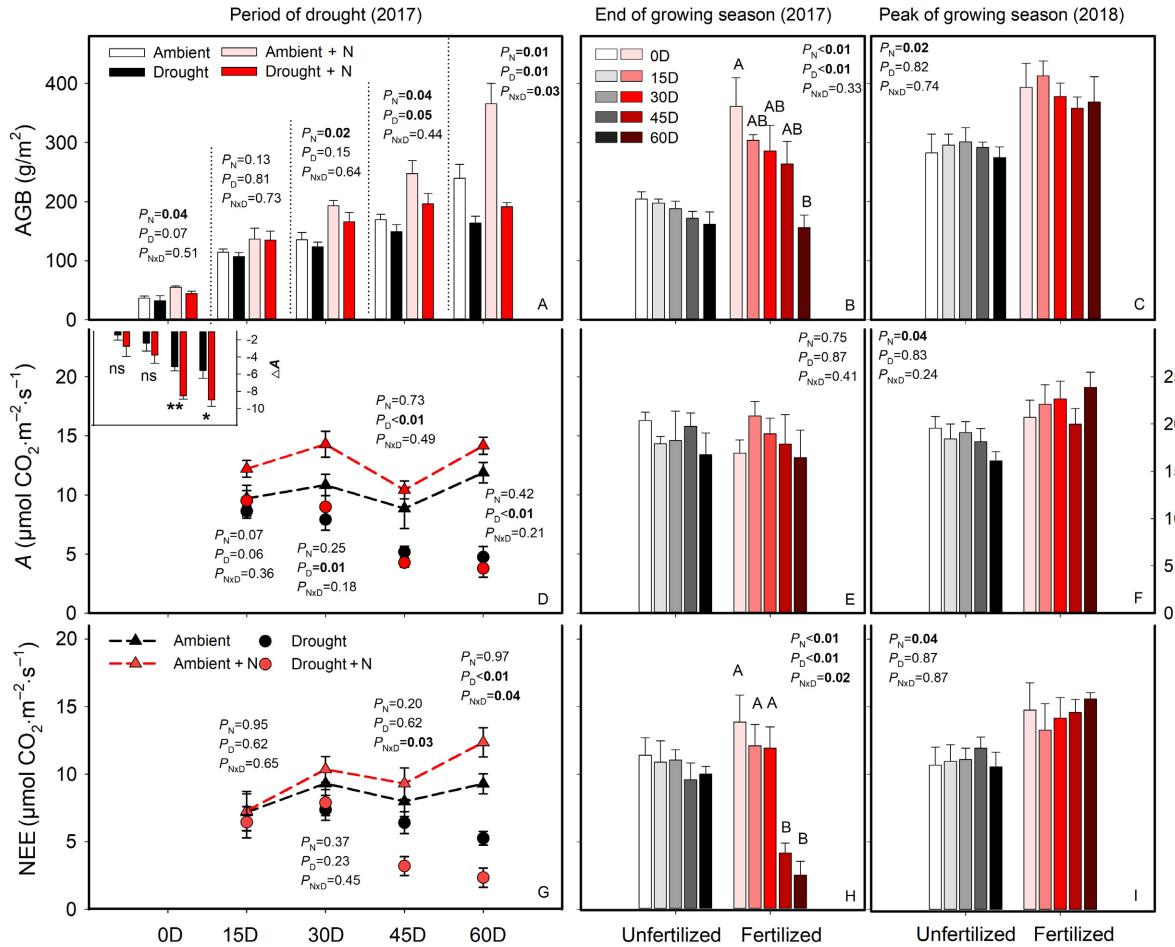


FIG. 3. Responses of (A, B, C) aboveground biomass (AGB), (D, E, F) leaf net carbon assimilation rate (*A*), and (G, H, I) short-term net ecosystem C change (NEE) to the four treatment combinations (Ambient, Drought, Ambient + N, and Drought + N) during and after drought treatments in 2017, and at peak AGB in the following growing season (2018). Data are reported as mean ± SE (*n* = 4). Different letters and values in boldface type denote significant differences between treatments (*P* < 0.05). Full statistical results are shown in Appendix S1: Tables S3, S4.

addition treatment effects on ET or *E* were observed during drought treatments (Appendix S1: Fig. S6). At the end of the 2017 growing season, no significant effects of the drought treatment on *A* were observed (Fig. 3E), but we detected significant reductions in NEE in the 45- and 60-d plots in N addition treatments (Fig. 3H). A year later, in the 2018 growing season, there were no significant effects of the drought treatments on NEE in either fertilized or unfertilized plots (Fig. 3I).

Drought response ratio

We found that RR_{AGB} increased nonlinearly (quadratic function) with the duration of the drought treatments, and that the increase of RR_{AGB} was greater in the fertilized than the unfertilized treatment when drought duration was greater than 30 d (Fig. 4A). Although RR_{NEE} also increased as drought continued, the trend was more linear than that of RR_{AGB} (Fig. 4B).

Both RR_{AGB} and RR_{NEE} were positively correlated with the AGB of the ambient precipitation plots (Fig. 4C, D).

DISCUSSION

Our results provide experimental evidence that enhanced soil nitrogen availability leads to greater drought-induced declines in grassland productivity and CO₂ exchange, and that this effect increases with duration of growing-season drought. Nitrogen addition prolonged the recovery period of AGB and NEE, which implies that soil N enrichment may increase grassland sensitivity to growing-season drought and, potentially, intra-annual precipitation variation.

Effects of nitrogen on the drought response of productivity

Relative to ambient precipitation plots, AGB declined progressively under prolonged drought (Fig. 3A). Plants

TABLE 1. Response of *L. chinensis* density and percentage of dead-leaves to combinations of drought and N addition (A, ambient precipitation, unfertilized; D, drought, unfertilized; A + N, ambient precipitation, fertilized; D + N, drought, fertilized).

Factor	Drought period			
	15D	30D	45D	60D
<i>L. chinensis</i> density (no./m ²)				
Treatments				
A	753 ± 47	690 ± 37	692 ± 32	670 ± 32
D	693 ± 55	684 ± 60	662 ± 27	579 ± 52
A + N	890 ± 50	811 ± 67	857 ± 99	799 ± 40
D + N	798 ± 64	750 ± 61	694 ± 57	459 ± 47
<i>F</i>				
<i>N</i>				
<i>D</i>				<i>F</i>_{1,3} = 49.2**
<i>N</i> × <i>D</i>				<i>F</i>_{1,3} = 10.3*
Percentage of dead-leaves (%)				
Treatments				
A	9.82 ± 2.1	19.3 ± 2.3	24.1 ± 1.4	30.9 ± 1.7
D	9.36 ± 1.6	22.5 ± 1.9	27.1 ± 1.0	36.1 ± 1.5
A + N	10.8 ± 1.4	21.8 ± 1.4	24.3 ± 1.7	29.9 ± 1.4
D + N	11.6 ± 1.5	22.7 ± 1.2	28.9 ± 1.9	41.1 ± 1.8
<i>F</i>				
<i>N</i>				
<i>D</i>			<i>F</i>_{1,3} = 11.4*	<i>F</i>_{1,3} = 17.2*
<i>N</i> × <i>D</i>				

Notes: Treatments are unfertilized with ambient precipitation (Ambient, A); nitrogen addition (N); and drought (D). Drought periods are 15 d drought (15D), 30 d drought (30D), 45 d drought (45D), and 60 d drought (60D). Data are reported as mean ± SE (*n* = 4). *F* statistics for two-way ANOVA (with block as a random factor) are provided. Values in boldface type indicate significant treatment effect.

* *P* < 0.05; ** *P* < 0.01.

in semiarid areas may already be adapted to tolerate moderate drought stress, such as the 15- to 30-d droughts that commonly occur in our study region (Liu et al. 2014), but they may be vulnerable to prolonged growing-season drought. We detected that leaf *A* responded to drought before AGB (Fig. 3D). However, contrary to our expectation, significant drought impacts on NEE were detected only for the 60-d drought treatment, and we found a linear increase in RR_{NEE}. Given that NEE is co-regulated by photosynthesis and effective canopy area, this result may be partially explained by lower ambient precipitation early in the drought treatments and lower AGB in the first half of the growing season.

Despite the fact that N addition increased AGB, it can also increase grassland vulnerability to extreme climate events, especially drought (Bharath et al. 2020). As expected, N addition increased the sensitivity of AGB and NEE to drought, and N-induced enhancement of the drought response increased as drought duration increased, and there were significant interactions between these two effects (Fig. 3A, D, G). Regression analyses suggested that the quadratic drought response of grassland productivity is amplified by N addition (Fig. 4A, B). This pattern explains other observations that the effects of drought and N on grassland productivity may be non-additive (Niu et al. 2009, Shi et al. 2018).

Mechanisms explaining the effects of N addition on drought sensitivity

In line with Wang et al. (2007) who concluded that a low biomass community exhibited greater resistance to drought stress, positive effects of AGB on RR_{AGB} and RR_{NEE} were also observed in this (Fig. 4) and previous studies in this system (Wang et al. 2018). Interestingly, we observed no significant increases in evapotranspiration in the fertilized plots, which we attribute to lower leaf transpiration in the nitrogen plus drought treatment (Appendix S1: Fig. S6). Further, Huxman et al. (2004) proposed that limited soil resources, such as nitrogen, may enhance the response of productivity to precipitation due to greater water use efficiency. In our study, nitrogen addition induced faster growth and led to greater biomass in ambient precipitation plots. Our results indicated that the increased productive potential may have led to greater sensitivity to drought (Fig. 4C, D), and may explain the positive effects of N addition on productivity responses to drought we observed. These results also highlight the importance of productive potential, which is altered by N addition, and consequences for drought response.

Our four-year N addition treatment had few effects on species richness or diversity (Shannon-Weiner index), or

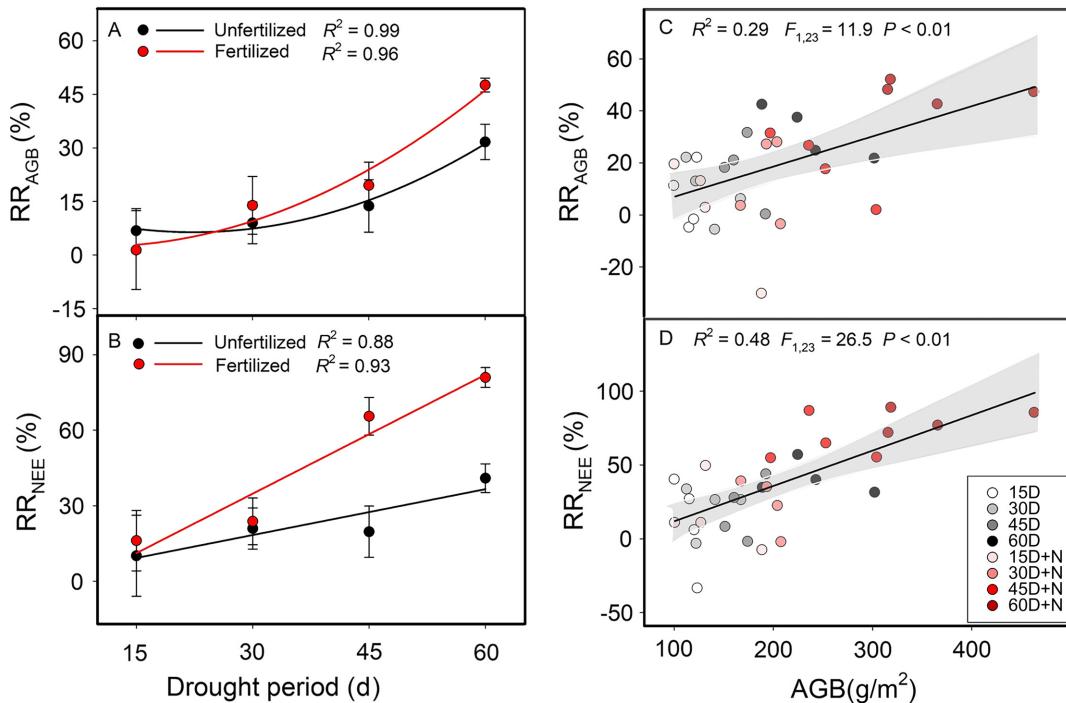


FIG. 4. Regression analysis of (A) the drought response ratio of AGB (RR_{AGB}) and (B) the drought response ratio of NEE (RR_{NEE}) during the experimental drought period. The relationships between (C) drought response ratio of AGB (RR_{AGB}) and AGB in ambient precipitation treatments, as well as (D) drought response ratio of NEE (RR_{NEE}) and AGB. Black solid lines are mixed-effects model fits and gray shading represents their respective 95% confidence intervals in panel C and D. Full regression results are shown in Appendix S1: Table S5.

the proportion of dominant species (Appendix S1: Table S1). The lack of observed diversity effects likely resulted from the strong dominance by a single perennial grass (*L. chinensis*), which may be well adapted to the frequent droughts that occur in this semiarid system. This result implies that, relative to richness, the effect of N addition on the drought tolerance of the dominant species governs the stability of low-diversity semiarid ecosystems. As expected, a decrease in R:S ratio was detected in the fertilized plots (Appendix S1: Fig. S3). Interestingly, the difference in mean R:S ratio between the fertilized and unfertilized treatments diminished as the drought treatments lengthened, which is inconsistent with N driven enhancement of drought response. This attenuation of the N effects on biomass allocation might be explained by shifts in limiting resources. On one hand, drought stress directly promotes allocation to belowground biomass (Poorter et al. 2012). On the other hand, N accumulation in plants declined with the prolongation of drought (Appendix S1: Fig. S4). Although these results indicate that the decrease in R:S ratio partially explained greater drought sensitivity in the fertilized plots, rapid adaptive adjustments in biomass allocation stimulated by drought can also act as a counterbalance that enhances ecosystem stability. This phenomenon should be further tested.

Post-drought recovery of productivity

Although our results are consistent with studies showing drought sensitivity to be co-regulated by N and water (Xu et al. 2014, Bharath et al. 2020), we did not observe any positive effects, as hypothesized, on post-drought recovery in N addition plots. Although leaf photosynthesis entirely recovered in both fertilized and unfertilized plots (Fig. 3E), fertilized plots had significantly lower AGB in the 60-d drought treatment, and significantly lower NEE in the 45- and 60-d drought treatments at the end of the growing season (2017, Fig. 3B, H). The legacy effects of each drought treatment in the unfertilized plots, however, were not statistically significant. Therefore, although our observations in the unfertilized plots differed from previous studies on intra-annual precipitation variability (Knapp et al. 2002, Fay et al. 2003), they do show that N addition could amplify the negative effect of seasonal reductions in precipitation on annual productivity.

Further, our results support the hypothesis that greater drought response resulted in greater productivity losses and more serious physiological damage in severe drought events. N addition and drought interacted to alter (reduce) the density of the dominant species, and

there were greater proportions of dead leaves in the drought-N treatment than drought-only treatment after 60 d of drought (Table 1). Thus, drought-induced plant mortality or senescence constrains productivity recovery in the post-drought period of the same year (Yahdjian and Sala 2006, Southon et al. 2012). Limitations on post-drought recovery probably also occur because N addition can decrease energy distribution to belowground organs (Poorter et al. 2012). Altogether, our results suggest that N addition probably has a negative effect on the recovery of plant productivity immediately following severe growing-season drought.

In line with previous research in different systems, substantial recovery of grassland productivity occurred after drought (Hoover et al. 2014, Stampfli et al. 2018). We observed no legacy effects of our drought treatments on community productivity in the next year (2018, Fig. 3 C), even when imposing an historically unprecedented 60-d growing-season drought. Given that a large proportion of legacy effects result from changes in previous-year tiller and bud density in semiarid and arid ecosystems (Reichmann et al. 2013, Reichmann and Sala 2014), this strong recovery could be attributed to rapid recruitment of belowground plant meristems at the end of the growing season when precipitation alleviated the water deficit (Appendix S1: Fig. S2, S5). The rapid recruitment from the underground bud bank of the dominant species ensures the longer-term stability of ecosystem function.

CONCLUSIONS

Grasslands are known to be prone to drought-induced declines in primary production. This study highlights significant interactions between N enrichment and drought duration as grasslands respond to growing season drought. In this monodominant grassland, N addition significantly increased productivity, but also increased sensitivity to drought and hindered recovery from drought during the growing season, particularly as experimental droughts lengthened. Nitrogen impacts on drought sensitivity and recovery appear to be a consequence of the simultaneous increases in productive potential and declines in belowground biomass allocation that we observed. Though this is a decidedly low-diversity ecosystem, it is possible that similar mismatches between aboveground and belowground processes are a mechanism for the destabilizing effect of N enrichment even in more diverse ecosystems. For many grasslands, both climate and N cycling are changing in tandem, which has important implications for estimating future grassland productivity and carbon balance. Sustainable management of grassland ecosystems will thereby require a nuanced, mechanistic understanding of aboveground and belowground interactions between N enrichment, drought response, and drought recovery.

ACKNOWLEDGEMENTS

This study was financially supported by National Natural Science Foundation of China (31570470, 31870456) and the Program of Introducing Talents of Discipline to Universities (B16011). B. Meng's stay at UNM was supported by China Scholarship Council (CSC). S. L. Collins was partially supported by NSF LTREB award DEB-1856383. The authors declare no conflict of interest.

LITERATURE CITED

- Bharath, S., E. T. Borer, L. A. Biederman, D. M. Blumenthal, P. A. Fay, L. A. Gherardi, J. M. H. Knops, A. D. B. Leakey, L. Yahdjian, and E. W. Seabloom. 2020. Nutrient addition increases grassland sensitivity to droughts. *Ecology* 101: e02981.
- Chaves, M. M., J. P. Maroco, and J. S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. *Functional Plant Biology* 30:239–264.
- Chen, S., Y. Bai, L. Zhang, and X. Han. 2005. Comparing physiological responses of two dominant grass species to nitrogen addition in Xilin River Basin of China. *Environmental And Experimental Botany* 53:65–75.
- Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Fargione, L. Gough, K. L. Gross, S. C. Pennings, K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters* 10:596–607.
- Collins, S. L., L. M. Ladwig, M. D. Petrie, S. K. Jones, J. M. Mulhouse, J. R. Thibault, and W. T. Pockman. 2017. Press–pulse interactions: effects of warming, N deposition, altered winter precipitation, and fire on desert grassland community structure and dynamics. *Global Change Biology* 23:1095–1108.
- Comas, L. H., S. R. Becker, V. M. Cruz, P. F. Byrne, and D. A. Dierig. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science* 4:442.
- Craine, J. M., T. W. Ocheltree, J. B. Nippert, E. G. Towne, A. M. Skibbe, S. W. Kembel, and J. E. Fargione. 2013. Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change* 3:63–67.
- Davidowitz, G. 2002. Does precipitation variability increase from mesic to xeric biomes? *Global Ecology and Biogeography* 11:143–154.
- Fay, P. A., J. D. Carlisle, A. K. Knapp, J. M. Blair, and S. L. Collins. 2003. Productivity responses to altered rainfall patterns in a C₄-dominated grassland. *Oecologia* 137:245–251.
- Gherardi, L. A., and O. E. Sala. 2019. Effect of interannual precipitation variability on dryland productivity: A global synthesis. *Global Change Biology* 25:269–276.
- Griffin-Nolan, R. J., C. J. W. Carroll, E. M. Denton, M. K. Johnston, S. L. Collins, M. D. Smith, and A. K. Knapp. 2018. Legacy effects of a regional drought on aboveground net primary production in six central US grasslands. *Plant Ecology* 219:505–515.
- Hallett, L. M., et al. 2014. Biotic mechanisms of community stability shift along a precipitation gradient. *Ecology* 95:1693–1700.
- Hoover, D. L., A. K. Knapp, and M. D. Smith. 2014. Resistance and resilience of a grassland ecosystem to climate extremes. *Ecology* 95:2646–2656.
- Huxman, T. E., et al. 2004. Convergence across biomes to a common rain-use efficiency. *Nature* 429:651–654.
- Kinugasa, T., A. Tsunekawa, and M. Shinoda. 2012. Increasing nitrogen deposition enhances post-drought recovery of

- grassland productivity in the Mongolian steppe. *Oecologia* 170:857–865.
- Knapp, A. K., P. Ciais, and M. D. Smith. 2017. Reconciling inconsistencies in precipitation-productivity relationships: implications for climate change. *New Phytologist* 214:41–47.
- Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–2205.
- Lei, Y., and A. Duan. 2011. Prolonged dry episodes and drought over China. *International Journal of Climatology* 31:1831–1840.
- Liu, X., B. Liu, M. Henderson, M. Xu, and D. Zhou. 2014. Observed changes in dry day frequency and prolonged dry episodes in Northeast China. *International Journal of Climatology* 35:196–214.
- Maurer, G. E., A. J. Hallmark, R. F. Brown, O. E. Sala, and S. L. Collins. 2020. Sensitivity of primary production to precipitation across the United States. *Ecology Letters* 23:527–536.
- Meng, B. 2021. Nitrogen addition amplifies the nonlinear drought response of grassland productivity to extended growing-season droughts. Dryad data set. <https://doi.org/10.5061/dryad.wwpzgmjsb>
- Meng, B., B. Shi, S. Zhong, H. Chai, S. Li, Y. Wang, H. A. L. Henry, J.-Y. Ma, and W. Sun. 2019. Drought sensitivity of aboveground productivity in *Leymus chinensis* meadow steppe depends on drought timing. *Oecologia* 191:685–696.
- Niu, S., H. Yang, Z. Zhang, M. Wu, Q. Lu, L. Li, X. Han, and S. Wan. 2009. Non-additive effects of water and nitrogen addition on ecosystem carbon exchange in a temperate steppe. *Ecosystems* 12:915–926.
- Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* 193:30–50.
- Reay, D. S., F. Dentener, P. Smith, J. Grace, and R. A. Feely. 2008. Global nitrogen deposition and carbon sinks. *Nature Geoscience* 1:430–437.
- Reichmann, L. G., and O. E. Sala. 2014. Differential sensitivities of grassland structural components to changes in precipitation mediate productivity response in a desert ecosystem. *Functional Ecology* 28:1292–1298.
- Reichmann, L. G., O. E. Sala, and D. P. C. Peters. 2013. Precipitation legacies in desert grassland primary production occur through previous-year tiller density. *Ecology* 94:435–443.
- Ruppert, J. C., K. Harms, Z. Henkin, H. A. Snyman, M. Sternberg, W. Willms, and A. Linstädter. 2015. Quantifying drylands' drought resistance and recovery: the importance of drought intensity, dominant life history and grazing regime. *Global Change Biology* 21:1258–1270.
- Shi, B., Y. Wang, B. Meng, S. Zhong, and W. Sun. 2018. Effects of nitrogen addition on the drought susceptibility of the *Leymus chinensis* meadow ecosystem vary with drought duration. *Frontiers in Plant Science* 9:254.
- Southon, G. E., E. R. Green, A. G. Jones, C. G. Barker, and S. A. Power. 2012. Long-term nitrogen additions increase likelihood of climate stress and affect recovery from wildfire in a lowland heath. *Global Change Biology* 18:2824–2837.
- Spinoni, J., G. Naumann, H. Carrao, P. Barbosa, and J. Vogt. 2014. World drought frequency, duration, and severity for 1951–2010. *International Journal of Climatology* 34:2792–2804.
- Stampfli, A., J. M. G. Bloor, M. Fischer, and M. Zeiter. 2018. High land-use intensity exacerbates shifts in grassland vegetation composition after severe experimental drought. *Global Change Biology* 24:2021–2034.
- Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.
- Tilman, D. 1996. Biodiversity: population versus ecosystem stability. *Ecology* 77:350–363.
- Touma, D., M. Ashfaq, M. A. Nayak, S.-C. Kao, and N. S. Diefenbaugh. 2015. A multi-model and multi-index evaluation of drought characteristics in the 21st century. *Journal of Hydrology* 526:196–207.
- Wang, Y., B. Meng, S. Zhong, D. Wang, J. Ma, and W. Sun. 2018. Aboveground biomass and root/shoot ratio regulated drought susceptibility of ecosystem carbon exchange in a meadow steppe. *Plant and Soil* 432:259–272.
- Wang, Y., S. Yu, and J. Wang. 2007. Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecology Letters* 10:401–410.
- Wesche, K., and K. Ronnenberg. 2010. Effects of NPK fertilisation in arid southern Mongolian desert steppes. *Plant Ecology* 207:93–105.
- Wilcox, K. R., et al. 2020. Rapid recovery of ecosystem function following extreme drought in a South African savanna grassland. *Ecology* 101:e02983.
- Xu, Z., H. Ren, J. Cai, R. Wang, M.-H. Li, S. Wan, X. Han, B. J. Lewis, and Y. Jiang. 2014. Effects of experimentally-enhanced precipitation and nitrogen on resistance, recovery and resilience of a semi-arid grassland after drought. *Oecologia* 176:1187–1197.
- Yahdjian, L., and O. E. Sala. 2006. Vegetation structure constrains primary production response to water availability in the patagonian steppe. *Ecology* 87:952–962.
- Zhang, Y., J. Feng, M. Loreau, N. He, X. Han, and L. Jiang. 2019. Nitrogen addition does not reduce the role of spatial asynchrony in stabilising grassland communities. *Ecology Letters* 22:563–571.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3483/supinfo>

OPEN RESEARCH

The data (Meng 2021) used for this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wwpzgmjsb>