






Plant traits and soil fertility mediate productivity losses under extreme drought in C₃ grasslands

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Abstract. Extreme drought decreases aboveground net primary production (ANPP) in most grasslands, but the magnitude of ANPP reductions varies especially in C₃-dominated grasslands. Because the mechanisms underlying such differential ecosystem responses to drought are not well resolved, we experimentally imposed an extreme 4-yr drought (2015–2018) in two C₃ grasslands that differed in aridity. These sites had similar annual precipitation and dominant grass species (*Leymus chinensis*) but different annual temperatures and thus water availability. Drought treatments differentially affected these two semiarid grasslands, with ANPP of the drier site reduced more than at the wetter site. Structural equation modeling revealed that community-weighted means for some traits modified relationships between soil moisture and ANPP, often due to intraspecific variation. Specifically, drought reduced community mean plant height at both sites, resulting in a reduction in ANPP beyond that attributable to reduced soil moisture alone. Higher community mean leaf carbon content enhanced the negative effects of drought on ANPP at the drier site, and ANPP–soil-moisture relationships were influenced by soil C:N ratio at the wetter site. Importantly, neither species richness nor functional dispersion were significantly correlated with ANPP at either site. Overall, as expected, soil moisture was a dominant, direct driver of ANPP response to drought, but differential sensitivity to drought in these two grasslands was also related to soil fertility and plant traits.

Key words: community-weighted traits; drought; functional dispersion; plant traits; productivity; soil fertility; species richness.

INTRODUCTION

The expected rise in global temperatures over the next century will increase the frequency and magnitude of climate extremes, such as drought (Trenberth 2011). Intensified drought will impact terrestrial ecosystem functioning, with many ecosystems expected to experience a reduction in aboveground net primary

productivity (ANPP; Zhao and Running 2010). Understanding the magnitude of these impacts is especially important in grassland ecosystems as they are particularly sensitive to precipitation variability (Huxman et al. 2004, Maurer et al. 2020), and harbor economically valuable ecosystem services, such as forage production and carbon storage. The negative impacts of extreme drought on grassland ANPP have been previously documented (Knapp et al. 2015); however, the underlying mechanisms of drought sensitivity remain understudied (Griffin-Nolan et al. 2019).

Species diversity is a major determinant of ecosystem function in grasslands and differences in diversity may

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also influence drought sensitivity (Tilman et al. 1997). Experimental manipulations of plant communities suggest that ANPP of species-rich communities is more resistant to climate extremes than species-poor communities (Tilman et al. 1997). However, species diversity fails to capture ecological differences or similarities among communities that might be better characterized by the functional traits of resident species (Loreau et al. 2001). Community functional composition, defined by both community-weighted mean (CWM) plant traits (i.e., the aggregated community trait mean weighted by species relative abundances) as well as the diversity of traits in a community, can reflect eco-physiological and morphological strategies and is a mechanistic approach to understand ecosystem responses to changing environmental conditions (Loreau et al. 2001, Cadotte et al. 2011).

Community functional composition can shift due to species turnover (i.e., species migration or relative abundance shift) and/or intraspecific variation (i.e., phenotypic plasticity or shifts in genotypic composition; Albert et al. 2010, Luo et al. 2018). Such changes in community functional composition can alter the sensitivity of ANPP to climate extremes through two mechanisms: selection effects and/or complementarity (Loreau et al. 2001). Selection effects may be inferred if ANPP or its response to drought are mediated by CWMs, suggesting a potential advantage related to particular trait values (Loreau and Hector 2001). Alternatively, complementarity occurs when ANPP responses to drought are mediated by functional dispersion (Fdis, the diversity of functional units in a multidimensional trait space), whereby a diversity of trait values confers “functional insurance” in response to environmental change (Loreau et al. 2001). Given that selection effects and complementarity may be manifest simultaneously, quantifying their relative contribution is necessary to understand the mechanisms of ANPP response to environmental change.

Soil fertility is another potentially important mediator of grassland ANPP drought sensitivity (Grime et al. 2000, 2008, van der Sande et al. 2007, 2018). As a colimiting resource in many grasslands, soil nutrient availability can affect ANPP dynamics directly by interacting with water availability (Grime et al. 2000, Harrison et al. 2015) or indirectly by altering species diversity and trait composition (van der Sande et al. 2007, 2018). To date, relatively few studies have focused on how differences in soil fertility alter ANPP responses to experimental drought in grasslands, especially along natural water availability gradients where drought sensitivity is expected to increase along with aridity (Knapp et al. 2015).

Grassland ecosystems may also differ in their responses to drought due to differences in the photosynthetic pathway of dominant species. Much of our understanding of grassland ecosystem drought sensitivity stems from work in C_4 -dominated grasslands (Knapp

et al. 2015, Griffin-Nolan et al. 2019), yet C_3 -dominated grasslands are important and abundant ecosystems that may respond differently to drought (Knapp et al. 2020). Physiological evidence suggests that grasslands dominated by C_3 species should be highly sensitive to drought given their generally low water-use efficiency and heat tolerance (Taylor et al. 2014). Alternatively, C_3 grasslands may be relatively insensitive to warm-season droughts given that their ANPP may peak during the cool season when soil moisture tends to be higher (Frank et al. 2007, Knapp et al. 2020). Indeed, C_3 grasses and forbs often exhibit drought escape strategies that are more beneficial during long-term droughts, leading to increased abundances of species belonging to these plant growth forms (Griffin-Nolan et al. 2019, Knapp et al. 2020). As a consequence, less is known about C_3 grasslands and the interactive role that community functional composition and soil fertility may play in ANPP sensitivity to drought.

We experimentally reduced precipitation for four years at two C_3 -dominated grasslands in Inner Mongolia, China (Table 1, Fig. 1). We used structural equation modeling (SEM) to test several hypothesized pathways by which community composition and soil fertility may mediate ANPP responses to drought. We expected the drier of the two grasslands to exhibit greater drought sensitivity due to lower species richness and functional diversity (Loreau et al. 2001) as well as greater baseline water limitation (Huxman et al. 2004). We also tested the hypothesis that reduced ANPP sensitivity to drought is conferred by (1) higher species richness and functional dispersion, (2) a greater abundance of species with conservative leaf economic traits, and (3) higher soil fertility (Tilman et al. 1997, Grime et al. 2000), and that such drought resistance mechanisms would be strongest at the site with greater water availability (Huxman et al. 2004).

MATERIAL AND METHODS

Study sites and experimental design

In 2014 (pretreatment year), we established experimental drought infrastructure at the two semiarid grassland sites in Inner Mongolia (Fig. 1). The two sites have similar mean annual precipitation (MAP) but differ in mean annual temperature and thus in their aridity index (AI; Table 1). The northern site (AI = 0.58), located at the National Hulunber Grassland Ecosystem Observation and Research Station, Hulunber (49.35° N, 120.01° E), receives 354 mm of mean annual precipitation (MAP) and has a mean annual temperature (MAT) of -1.1°C . The southern site (AI = 0.49) is located at the Inner Mongolia Grassland Ecosystem Research Station, Xilingol (116°40' E, 43°33' N) and is drier and warmer than the other site (MAP = 346 mm; MAT = 1.9°C). Large grazers were excluded from each site for more than 20 yr before the experiment began. Both sites are classified as semiarid steppe with Kastanozem soil

TABLE 1. Climate, vegetation, and soil properties (mean \pm SD) of the two study sites located within the semiarid steppe of northern China.

Properties	Hulunber site	Xilingol site
General		
Latitude	49.35° N	43°33'
Longitude	120.01° E	116°40'
Climate		
MAP (mm)	354 \pm 89	346 \pm 81
MAT (°C)	-1.1 \pm 0.21	1.9 \pm 0.18
GSP (mm)	263 \pm 72	242 \pm 68
PET (mm)	610 \pm 103	706 \pm 112
Aridity index	0.58 \pm 0.09	0.49 \pm 0.07
Vegetation		
ANPP (g/m ²)	237 \pm 46	158 \pm 52
Dominant species	<i>Leymus chinensis</i>	<i>L. chinensis</i>
Species richness	17 \pm 4.6	12 \pm 1.8
Soil		
C:N ratio	11.5 \pm 0.78	9.5 \pm 0.53

Notes: See the map in Fig. 1 for the locations of each site. Aridity index was calculated as the ratio of MAP to PET, with values closer to 0, denoting greater aridity. These climatic variables are calculated from a 45-yr record (1972–2016) for the Hulunber site, and a 33-yr record (1982–2014) for the Xilingol site. All vegetation and soil characteristics were calculated using data from the control plots of the experiment (2015–2018). C:N ratio is the ratio of soil organic carbon to soil total nitrogen. ANPP, aboveground net primary production. Species richness, the number of species, was estimated from a permanent 2 \times 2 m quadrat. AI, aridity index; GSP, growing season precipitation; MAP, mean annual precipitation; MAT, mean annual temperature; PET, potential evapotranspiration (see Materials and Methods for details).

and are dominated (>50% cover) by *Leymus chinensis*, a widespread and abundant species throughout eastern regions of the Eurasian steppe.

The drought experiment involved a completely randomized block design with six blocks, each including one control and one drought treatment. Each plot was 6 \times 6 m (n = 6 plots/treatment; 12 plots per site). Experimental infrastructure was established within a topographically flat area where species composition was representative of the region. The drought treatment consisted of a 66% reduction in growing season rainfall. To manipulate rainfall, we used passive rainfall exclusion shelters constructed with light scaffolding and covered by strips of transparent polyethylene (Beijing Plastics Research Institute, Beijing, China). The roofs of these shelters were 2.5 m high at the highest point allowing for near surface air exchange while avoiding unwanted greenhouse effects. In the year before treatment (2014), both control and treatment plots were hydrologically isolated from the surrounding soil by trenching the perimeter to a depth of 1 m and lining the trench with 6 mm thick plastic and metal flashing. In the center of each plot, 16-m² (4 \times 4 m) sampling plots were established. Soil was sampled every two weeks during the growing season (May–August) of 2015–2018 in each sampling

plot to determine soil water content (calculated as the percentage of mass loss from fresh to dry soil) and to calculate the mean soil water content during the growing season each year.

Measurements

A permanent 2 \times 2 m quadrat was established under each shelter and within each unsheltered control plot in 2015. Plant species richness (i.e., species number per 4 m²) was surveyed within this quadrat in mid-August from 2015 to 2018. An additional 1 \times 1 m quadrat was randomly located in each plot (excluding the 2 \times 2 m species composition sampling quadrat) in a specific year (2015–2018) to avoid repeated destructive harvest. Each quadrat was split into four 50 \times 50 cm sub-quadrats, two of which were designated for destructive measurements of plant biomass in mid-August of each year. After excluding the previous year's dead material, aboveground biomass of each species was harvested in the two sub-quadrats by clipping at the ground level in mid-August and oven-dried at 65°C for 48 h to estimate the per-species biomass and ANPP (i.e., total aboveground biomass per m²) every year (2015–2018).

The other two 50 \times 50 cm sub-quadrats were designated for community-scale surveys of plant traits. Trait measurements were repeated from 2015 to 2018 at the Hulunber site, but only from 2015 to 2017 at the Xilingol site. Average trait values (2015–2017) for other years combined were used as trait values in 2018 at the Xilingol site. Plant height was measured from three sun-exposed individuals of each species per sub-quadrat before three of the most recently emerged, fully expanded leaves were collected from the same individuals. In our study, the species in which traits were measured cumulatively represented at least 90% of the total ANPP in both control and drought treatment plots at each site.

Leaf trait measurements included area, dry mass, carbon, nitrogen, and phosphorus. Leaf area was measured for each of the three collected leaves per individual using Image J ([available online](https://imagej.nih.gov/ij/)).¹³ Leaf dry mass was measured on the same leaves after oven-drying leaves at 65°C for 48 h to calculate both SLA (area per dry mass; m²/kg) and leaf dry matter content (LDMC; dry mass per fresh mass, mg/g). Leaf carbon (LCC; mg/g) and nitrogen concentrations (LNC; mg/g) were measured using a 2400II CHN elemental analyzer (Perkin-Elmer, Waltham, MA, USA). Leaf phosphorus concentration (LPC; mg/g) was measured using inductively coupled plasma atomic emission spectrometry (OPTIMA 3000 DV, Perkin-Elmer) after H₂SO₄-H₂O₂ digestion.

After harvesting the plant biomass and removing the litter layer, five soil cores (0–10 cm depth) were randomly sampled in the two 50 \times 50 cm sub-quadrats designated for measurements of plant biomass in mid-

¹³ <https://imagej.nih.gov/ij/>

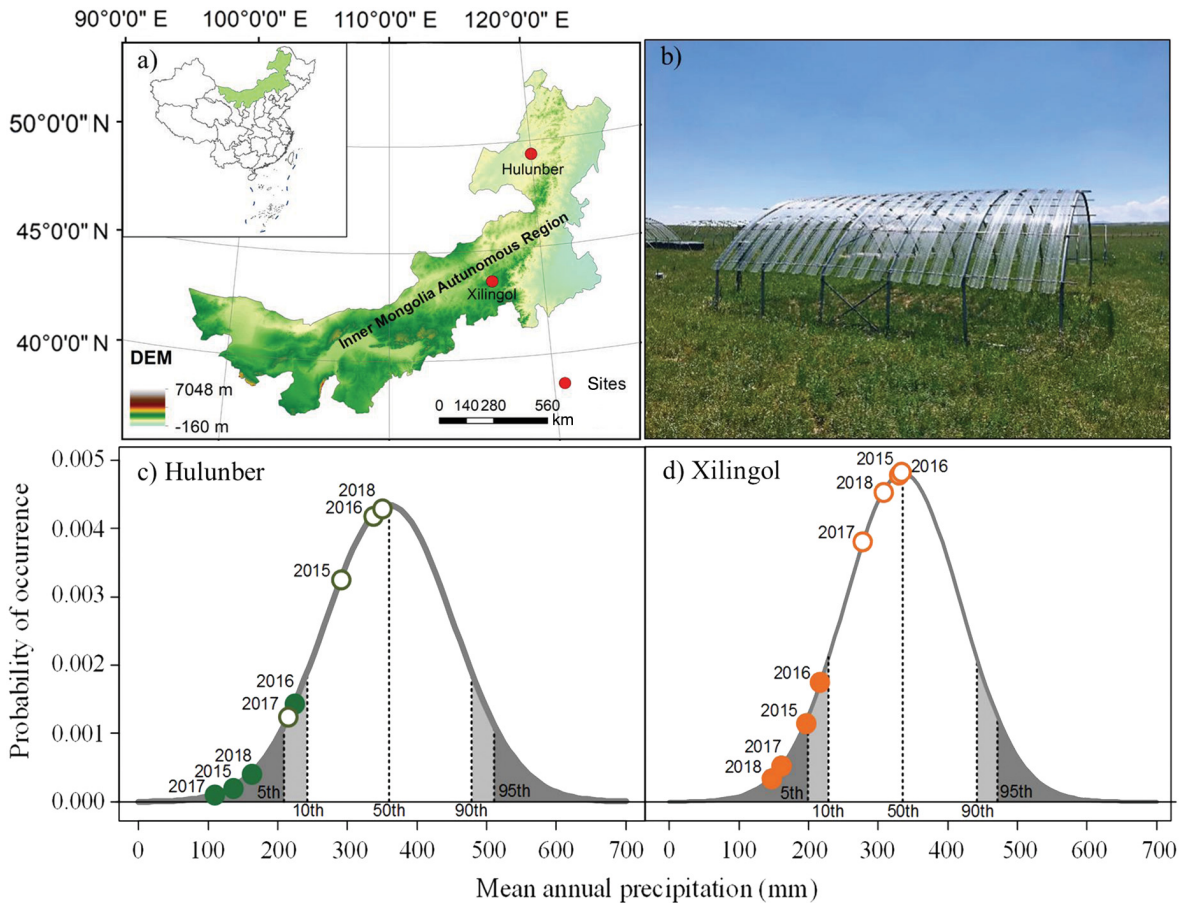


FIG. 1. (a) Map of the study site locations in the Inner Mongolia grasslands of northern China. (b) At each site, a manipulative drought experiment was conducted using rainout shelters. Probability density functions (pdfs) of mean annual precipitation amounts for the (c) Hulunber and (d) Xilingol sites. Control and drought treatments are represented by open and filled symbols, respectively. Mean annual precipitation and pdfs are based on a 45-yr data set (1972–2016) for the Hulunber site, and a 33-yr data set (1982–2014) for the Xilingol site.

August of every year (2015–2018) for each plot per site using a soil auger (2.5 cm diameter). Samples were pooled together to obtain one composite soil sample per plot. Soil samples were air-dried, ground, and passed through a 1 mm sieve (NM200, Retsch, Haan, Germany). Total organic carbon (TOC) and total nitrogen (TN) concentrations were measured using a 2400II CHN elemental analyzer (Perkin-Elmer) and the mass ratio of soil C:N was calculated.

Functional composition

After determining each species' average trait in each plot, community-weighted mean (CWM) trait values were calculated as: $CWM = \sum_{i=1}^S p_i x_i$, where p_i is the relative abundance (percent relative biomass) of species i in the two 50×50 cm sub-quadrats, x_i is the average trait value of species i from the sub-quadrat, and S is total number of species per the two sub-quadrats. Using the above-mentioned approach, CWM traits of just subordinate species (i.e., excluding dominant species) were also

calculated in each plot. The community-unweighted mean (CM) for each trait in each plot was also calculated as the mean of all collected species based on presence-absence data, including all dominant and subordinate species.

Fdis is a multidimensional index of functional diversity measured from multiple traits and $Fdis_{CWM}$ is calculated as

$$Fdis = \frac{\sum a_j z_j}{\sum a_j}$$

where a_j is the relative abundance (percent relative biomass) of species j and z_j is the distance of species j to the weighted centroid c . The centroid c is calculated as

$$c = \frac{\sum a_j x_{ij}}{\sum a_j}$$

where c is the weighted centroid in the i -dimensional space, a_j is the abundance of species j and x_{ij} is the

attribute of species j for trait i . $Fdis_{CM}$ in each plot was calculated, where all species are weighted equally.

Data analysis

All data met the assumptions of normality and homogeneity of variance following Shapiro-Wilk and Levene's test, respectively. At each site separately, we analyzed treatment effects on ANPP using a mixed-model analysis of variance with drought treatment as a fixed factor, with block as a random factor within year, and with year as a random factor across years. Similar mixed models were used to assess treatment differences in soil water content, soil C:N ratio and each index of community composition (e.g., species richness, CWM and CM traits and $Fdis$).

The relative contributions (C) of species turnover (C_{Turn}) and intraspecific trait variability (C_{Intra}) on CWM trait composition were assessed as $C_{Turn} = T_{Dr*} - T_{Ct}$ and $C_{Intra} = T_{Dr} - T_{Dr*}$, where T_{Ct} and T_{Dr} are the CWM traits in the control and drought plots respectively, and T_{Dr*} is the CWM traits in the drought-treated plots, recalculated using species relative abundance in drought-treated plots but trait values measured in control plots (Luo et al. 2018).

At each state, we explored bivariate relationships between ANPP and soil water content using linear mixed effects models with block as a random factor within year and year as a random factor across years. We then conducted indicator variables regression to identify differences in the slopes of the regression lines between sites across years. Steeper slopes indicate greater sensitivity of ANPP to soil water content in that site. We also examined bivariate relationships between ANPP and each metric of community composition (i.e., species richness, CWM traits and $Fdis$) as well as soil C:N ratio using linear mixed effects models with block as a random factor within year and year as a random factor. Bivariate relationships between ANPP and each metric of community-unweighted trait composition (i.e., those based on presence-absence data) and CWM traits of only subordinate species were assessed using linear mixed effects models with block and year as random factors within each site across years.

Lastly, we constructed a piecewise SEM to examine the causal pathways through which declines in soil water content influence ANPP, where both direct and indirect (*via* changing various aspects of community composition and soil C:N ratio) pathways were considered. Knowledge from the literature was used to develop a priori piecewise SEM with hypothesized causal relationships between variables and to test preliminary models (Appendix S1: Fig. S1). In each site, the sampling size was 48 (i.e., $n = 6$ replicates \times 2 treatments \times 4 yr = 48). We had multiple candidate variables for CWM traits, we therefore included one or two CWM traits in the initial model, which resulted in $C_6^1 + C_6^2 = 21$ possible models (van der Sande et al. 2017, 2018). We selected the best-fitting model ($P > 0.05$) with the highest explained variation of ANPP

from these models. The modified models were developed by removing direct and indirect pathways with low path coefficients, when the initial model did not produce an adequate fit (Grace et al. 2010). Although there were correlations among plant traits (Appendix S1: Table S1), variance inflation factors were all lower than 3.0 (Appendix S1: Table S2), indicating that there was no serious multicollinearity among traits (O'Brien 2007). We included block and year as random factors in each piecewise SEM and fitted the component models of the piecewise SEM as linear models. We reported the standardized coefficient for each path from each component model and focused on the conditional R^2 , which include random effects, rather than the marginal R^2 for the fixed effects only. Overall fit of the piecewise SEM was evaluated using Shipley's test of d-separation: Fisher's C statistic (if $P > 0.05$, then no paths are missing and the model is a good fit) and AIC in the R package piecewiseSEM (*available online*).¹⁴

All statistical analyses were performed in R 3.5.3 (R Core Team 2019).

RESULTS

Ambient MAP varied at each site over the 4-yr study period with many years falling below the long-term mean (approximately the 50th percentile of historic amounts) (Fig. 1c,d). Thus, an unavoidable caveat of our experimental design is that we are comparing dry years (ambient) to extremely dry years (drought plots), which can make comparison across sites more complicated (Hoover et al. 2018). Nonetheless, the drought treatment reduced rainfall close to the 5th percentile in each experiment at both sites (Fig. 1c,d) and significantly reduced average soil water content during the 2015–2018 growing seasons at each site, corresponding to a 37% relative reduction in soil water content at the Hulunber site and 22% at the Xilingol site (Fig. 2).

Experimental drought significantly reduced ANPP at the drier Xilingol site by the second year of drought, whereas significant reductions in ANPP occurred only after four years of drought at the more mesic Hulunber site (Appendix S1: Fig. S2). Averaged across years, ANPP was significantly reduced at both sites during the 2015–2018 growing seasons, with a greater reduction at the Xilingol site (50%) when compared to the Hulunber site (12%) (Fig. 2).

Experimental drought had variable effects on species richness, CWM traits, functional dispersion, and soil C:N ratio (Appendix S1: Fig. S2). When averaged across years, plant height_{CWM} declined and LCC_{CWM} increased with drought across years at the Xilingol site (Fig. 2), and LPC_{CWM} was reduced with drought at the Hulunber site (Fig. 2). The remaining metrics of CWM trait composition and soil C:N ratio were unaffected by the drought treatment at either site across years (Fig. 2).

¹⁴ <https://github.com/jslefche/piecewiseSEM>

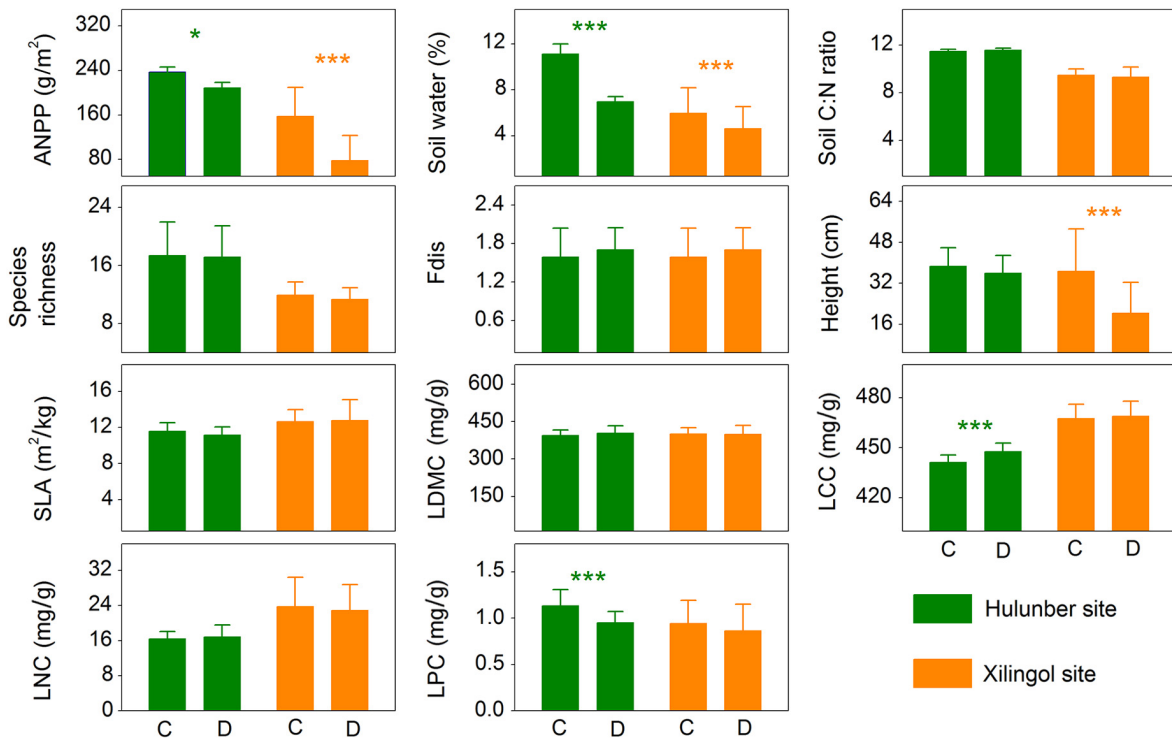


FIG. 2. Experimental drought effects on aboveground net primary production (ANPP), soil resource availability, and indices of community composition averaged across plots and years (2015–2018) for the Hulunber and Xilingol sites. Values are mean and SE. C, control; D, drought. * $P < 0.05$, *** $P < 0.001$. For detailed statistical data, see Appendix S1: Table S3. Fdis, functional dispersion of all six traits. SLA, specific leaf area; LDMC, leaf dry matter content; LCC, leaf carbon concentration; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration.

Both species turnover and intraspecific trait variation played a key role in driving changes in CWM trait composition (Appendix S1: Fig. S3). For F_{disCWM} , species turnover had a more important role than intraspecific trait variability at the more mesic Hulunber site, while intraspecific trait variability played a more important role at the more arid Xilingol site (Appendix S1: Fig. S3). For the remaining metrics of CWM trait composition, intraspecific trait variability had a more important role than species turnover at both sites (Appendix S1: Fig. S3). For instance, intraspecific trait variability accounted for 97% and 71% of the variation in plant height_{CWM} in response to drought at the Hulunber and Xilingol sites, respectively (Appendix S1: Fig. S3). Intraspecific trait variability explained 76% and 71% of total observed variance in LCC_{CWM} in response to drought at the Hulunber and Xilingol sites, respectively (Appendix S1: Fig. S3).

The strength of the relationships between soil water content and ANPP increased as drought progressed from 2015 to 2018 for both sites (Appendix S1: Fig. S4). ANPP was significantly and positively correlated with soil water content across years at both the Hulunber and Xilingol sites (Appendix S1: Fig. S5). The slope of the linear regression between soil water content and ANPP was significantly steeper for the Xilingol site compared

to the Hulunber site, as indicated by a significant interaction term ($F_{2,93} = 29.74$, $P < 0.001$, Appendix S1: Fig. S5). ANPP was not correlated with soil C:N ratio at either site across years (Appendix S1: Fig. S5).

The relationships between community composition (species richness, functional trait means, and dispersion) and ANPP did not strengthen throughout the experimental period at either site (Appendix S1: Fig. S4). A positive relationship between ANPP and plant height_{CWM} was observed across years for both the Hulunber and Xilingol sites (Appendix S1: Fig. S5). ANPP was negatively correlated with LCC_{CWM} for the Xilingol site but was not correlated at the Hulunber site across years (Appendix S1: Fig. S5). These trait–ANPP relationships remained largely consistent after excluding dominant species from the calculations (Appendix S1: Fig. S6). A positive relationship between ANPP and plant height_{CWM} of subordinate species was observed for both sites (Appendix S1: Fig. S6). ANPP positively correlated with LDMC_{CM} of subordinate species at the more mesic Hulunber site, while negatively correlated with LCC_{CWM}, LNC_{CWM}, and LPC_{CWM} at the Xilingol site (Appendix S1: Fig. S6). The effects of CM trait composition (i.e., unweighted trait means and dispersion) on ANPP dynamics were similar to that of CWM trait composition at both sites across years (Appendix S1: Fig. S7).

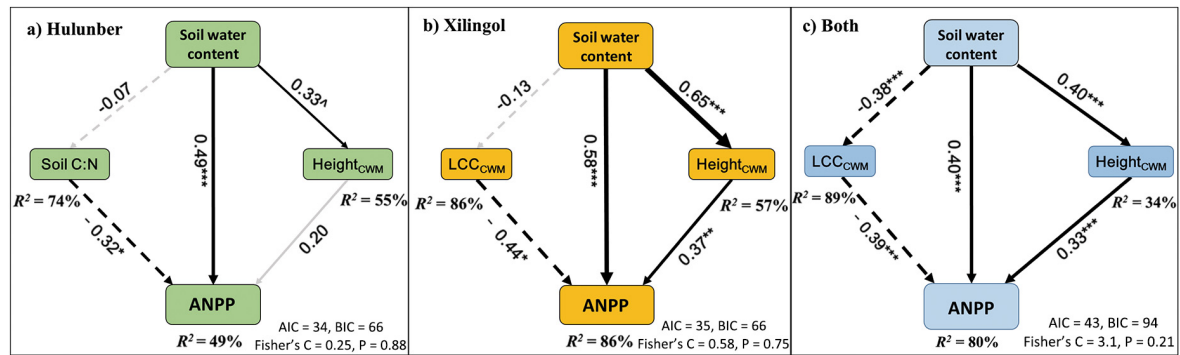


Fig. 3. Best-fit structural equation modeling relating ANPP to soil resource availability and several indices of community composition for (a) the Hulunber and (b) Xilingol sites as well as (c) both sites together in Northern China. Numbers adjacent to arrows are standardized path coefficients. Continuous and dashed arrows indicate significant ($^{\wedge}P < 0.1$; $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) positive and negative pathways, respectively. Black and gray arrows indicated significant and nonsignificant pathways, respectively. Conditional R^2 denotes the proportion of variance explained. For detailed statistical data, see Appendix S1: Table S4. For abbreviations, see Fig. 2.

When considering multivariate causal relationships with piecewise SEM, we observed a total effect of soil water content on ANPP at both sites across years (path = 0.58 and 0.88 for the Hulunber and Xilingol sites, respectively), which included direct effects (path = 0.49 and 0.58 for the Hulunber and Xilingol sites, respectively) and indirect effects (indirect effects = $-0.07 \times -0.32 + 0.33 \times 0.20 = 0.09$ at the Hulunber site; indirect effects = $-0.13 \times -0.44 + 0.65 \times 0.37 = 0.30$ at the Xilingol site; Fig. 3). Indirect effects of soil water content on ANPP were site specific, with some similarities. At both sites, decreasing soil water content indirectly reduced ANPP by reducing plant height_{CWM} (path = $0.33 \times 0.20 = 0.07$ for the Hulunber site; path = $0.65 \times 0.37 = 0.24$ for the Xilingol site; Fig. 3). We also observed a direct effect of soil C:N ratio on ANPP alongside soil water content (path = -0.32 ; Fig. 3a), with soil water content indirectly influencing ANPP via a weak effect on soil C:N ratio (path = -0.07 ; Fig. 3a) at the Hulunber site. LCC_{CWM} had a negative effect on ANPP at the Xilingol site (path = -0.44 ; Fig. 3b). The slight negative effect of soil water content on LCC_{CWM} thus led to a positive indirect effect on ANPP at this site (Fig. 3b). Combining both sites together, soil moisture was a dominant, direct driver of ANPP responses to the drought treatment, with an indirect effect of soil moisture on ANPP via plant traits (i.e., plant height_{CWM} and LCC_{CWM}) (Fig. 3c). When using the CM trait composition (i.e., trait means and dispersion) in the SEMs, we found that the effects of community composition (i.e., species richness, functional means and dispersion) and soil C:N ratio were comparable to that of CWM trait composition at each site (Appendix S1: Fig. S8).

DISCUSSION

Four years of experimental drought significantly reduced soil water content with the magnitude of direct

negative impacts on ANPP increasing through time (Appendix S1: Fig. S2). Consistent with our hypothesis, the responses of ANPP to decreasing soil water content with drought over time differed between these two C₃-dominated grasslands, with greater sensitivity at the drier site, Xilingol (Fig. 2; Appendix S1: Fig. S2). This is consistent with previous work suggesting that primary productivity is more sensitive to precipitation with increasing site aridity (Huxman et al. 2004, De Boeck et al. 2016, Maurer et al. 2020). However, contrary to our hypothesis, both SEM and univariate analyses demonstrated that plant species richness and dispersion of functional traits were not associated with ANPP responses to drought across years at either site (Fig. 3; Appendix S1: Fig. S5). At both sites, community trait means, rather than species richness and Fdis, played a more important role in mediating ANPP responses to reduced soil moisture (Fig. 3). As dominant species have a large impact on ecosystem function (Avolio et al. 2019), we expected such ANPP responses to be driven primarily by the functional traits of the dominant species and to be relatively insensitive to changes in the abundance/presence of rare and subordinate species in a community (Grime 1998, Loreau and Hector 2001). However, ANPP was similarly correlated with CWM traits of dominant and subordinate plant species in our study (Appendix S1: Fig. S6). These results imply that traits favoring higher ANPP across drought conditions were consistent for both dominant and subordinate species.

Traits that act as reliable indicators of community shifts in response to climate change (i.e., “response traits”) are needed to accurately utilize the response-effect trait framework outlined by Lavorel and Garnier (2002). The relationship between response traits and traits related to ecosystem function (i.e., “effect traits”) can then be used to predict the response of ecosystem functions to climate change (Suding et al. 2008). Our results show that such traits can be identified if

measured at the community scale in an appropriate environmental context (Griffin-Nolan et al. 2018). Given that semiarid grasslands are water-limited systems, we predicted that functional traits associated with water conservation strategies (i.e., low SLA and high LDMC) would buffer ANPP against the negative effects of drought (Reich 2014). On the contrary, site-specific SEM more often included functional traits linked to leaf carbon economics and plant growth ability rather than water-use efficiency (Fig. 3). At both sites, soil water content had an indirect negative effect on ANPP by decreasing plant height_{CWM}, a trait often associated with ecological strategies and ANPP (Moles et al. 2009). As expected, lower soil water content led to shorter plants due to more limited growth within species (intraspecific variability explained >70% of plant height_{CWM} variance, Appendix S1: Fig. S3), but also selection for inherently shorter species with potentially higher drought tolerance (~30% variability explained by species turnover, Appendix S1: Fig. S3), both of which led to lower ANPP.

In addition to plant community mean height, the ANPP drought response at the Xilingol site was further intensified by primarily intraspecific variability in LCC (Fig. 3). Under stressful conditions, plant relative growth rate is often more restricted than photosynthetic rate, leading to an accumulation of carbon in plant tissues relative to dry mass (Tarvainen and Nasholm 2017). Much of this excess carbon is allocated toward defensive structures (e.g., lignin or tannins) rather than investments in higher growth rate (e.g., high SLA). This is a more likely explanation for the negative relationship between LCC and ANPP (Appendix S1: Fig. S5) than one in which drought acted as an environmental filter selecting for species with higher LCC (intraspecific variability explained 71% of LCC_{CWM} variance whereas species turnover explained only 29%, Appendix S1: Fig. S3). It is noteworthy that LCC_{CWM} significantly influenced ANPP at the more arid Xilingol site but not at the Hulunber site (Fig. 3), the site that experienced the greatest increase in LCC_{CWM} following drought (Fig. 2); however, the negative relationship between LCC_{CWM} and ANPP is strong and consistent across sites (Fig. 3C). Thus, the effect of LCC on ANPP is likely generalizable but more apparent at the drier site where the community encompassed a much wider range of LCC values (Appendix S1: Fig. S5).

Concurrent with changes in community mean plant traits, SEM identified strong direct effects of the soil C:N ratio on ANPP in addition to the influence of soil moisture at the more mesic Hulunber site (Fig. 3a). The indirect effect of soil moisture on ANPP through modifications of soil fertility was weak yet consistent with previous studies noting that ANPP responsiveness to soil water content is influenced by soil nutrient availability (Grime et al. 2000, Harrison et al. 2015). That such soil fertility effects were only observed at the more mesic site (Hulunber) is likely due to the combined effect of soil water availability on nutrient mineralization rates and

plant nutrient uptake; similar studies across multiple sites are needed to confirm this hypothesis. Interestingly, the experimental drought did not have cumulative effects on community composition or soil fertility (Appendix S1: Fig. S2). Accordingly, the strength of the relationships between ANPP and these variables did not increase with prolonged treatment time (Appendix S1: Fig. S4). These results indicate that the effect of community composition and soil fertility on ANPP during drought did not change with drought duration.

In our study, we examined the mechanisms of ANPP responses to drought across two C₃-dominated grasslands. Such responses are known to vary dramatically among different grasslands (Knapp et al. 2015). Thus, multi-site manipulative drought experiments are needed to confirm our findings before generalizing across the grassland biome. Unfortunately, our analyses did not include root traits (e.g., root mass fraction or specific root length), which are critical for understanding plant responses to drought stress due to their essential roles in water and nutrient acquisition and transportation (Marksteijn and Poorter 2009). Such traits are rarely measured yet should be included in future studies on ecosystem responses to drought.

In summary, our study examined the mechanisms by which extreme drought alters ANPP in C₃-dominated semiarid grasslands both directly and indirectly through its effect on community composition and soil fertility. Importantly, our results show very little influence of species richness and dispersion of plant traits on the responses of ANPP to drought in these grasslands. Instead, ANPP was indirectly influenced by drought-induced changes in community mean plant traits and directly by soil nutrients at the moister site. These results emphasize the important role of plant traits and soil fertility in addition to the direct effects of soil moisture for determining the responses of C₃-dominated semiarid grassland to extreme drought.

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LITERATURE CITED

- Albert, C. H., W. Thuiller, N. G. Yoccoz, R. Douzet, S. Aubert, and S. Lavorel. 2010. A multi-trait approach reveals the structure and the relative importance of intra- vs. inter-specific variability in plant traits. *Functional Ecology* 24:1192–1201.
- Avolio, M. L., E. J. Forrester, C. C. Chang, K. J. La Pierre, K. T. Burghardt, and M. D. Smith. 2019. Demystifying dominant species. *New Phytologist* 223:1106–1126.
- Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: functional diversity and the maintenance of

- ecological processes and services. *Journal of Applied Ecology* 48:1079–1087.
- De Boeck, H. J., S. Bassin, M. Verlinden, M. Zeiter, and E. Hiltbrunner. 2016. Simulated heat waves affected alpine grassland only in combination with drought. *New Phytologist* 209:531–541.
- Frank, D. A. 2007. Drought effects on above-and belowground production of a grazed temperate grassland ecosystem. *Oecologia* 152:131–139.
- Grace, J. B., T. M. Anderson, H. Olff, and S. M. Scheiner. 2010. On the specification of structural equation models for ecological systems. *Ecological Monographs* 80:67–87.
- Griffin-Nolan, R. J., et al. 2018. Trait selection and community weighting are key to understanding ecosystem responses to changing precipitation regimes. *Functional Ecology* 32:1746–1756.
- Griffin-Nolan, R. J., D. M. Blumenthal, S. L. Collins, T. E. Farkas, A. M. Hoffman, K. E. Mueller, T. W. Ocheltree, M. D. Smith, K. D. Whitney, and A. K. Knapp. 2019. Shifts in plant functional composition following long-term drought in grasslands. *Journal of Ecology* 107:2133–2148.
- Grime, J. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* 86:902–910.
- Grime, J. P., V. K. Brown, K. Thompson, G. J. Masters, S. H. Hillier, I. P. Clarke, A. P. Askew, D. Corker, and J. P. Kiely. 2000. The response of two contrasting limestone grasslands to simulated climate change. *Science* 289:762–765.
- Grime, J. P., J. D. Fridley, A. P. Askew, K. Thompson, J. G. Hodgson, and C. R. Bennett. 2008. Long-term resistance to simulated climate change in an infertile grassland. *Proceedings of the National Academy of Sciences USA* 105:10028–10032.
- Harrison, S., E. Damschen, B. Fernandez-Going, A. Eskelinen, and S. Copeland. 2015. Plant communities on infertile soils are less sensitive to climate change. *Annals of Botany* 116:1017–1022.
- Hoover, D. L., K. R. Wilcox, and K. E. Young. 2018. Experimental droughts with rainout shelters: a methodological review. *Ecosphere* 9:e02088.
- Huxman, T. E., et al. 2004. Convergence across biomes to a common rain-use efficiency. *Nature* 429:651–654.
- Knapp, A. K., et al. 2020. Resolving the Dust Bowl paradox of grassland responses to extreme drought. *Proceedings of the National Academy of Sciences USA* 117:22249–22255.
- Knapp, A. K., C. J. W. Carroll, E. M. Denton, K. J. La Pierre, S. L. Collins, and M. D. Smith. 2015. Differential sensitivity to regional-scale drought in six central US grasslands. *Oecologia* 177:949–957.
- Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* 16:545–556.
- Loreau, M., et al. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804–808.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- Luo, W., et al. 2018. Differential responses of canopy nutrients to experimental drought along a natural aridity gradient. *Ecology* 99:2230–2239.
- Luo, W., et al. 2021. Plant traits and soil fertility mediate productivity losses under extreme drought in C₃ grasslands. Dryad, data set. <https://doi.org/10.5061/dryad.djh9w0w09>
- Markestijn, L., and L. Poorter. 2009. Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *Journal of Ecology* 97:311–325.
- Maurer, G. E., A. Hallmark, R. F. Brown, O. E. Sala, and S. L. Collins. 2020. Sensitivity of primary production to precipitation across the conterminous United States. *Ecology Letters* 23:527–536.
- Moles, A. T., D. I. Warton, L. Warman, N. G. Swenson, S. W. Laffan, A. E. Zanne, A. Pitman, F. A. Hemmings, and M. R. Leishman. 2009. Global patterns in plant height. *Journal of Ecology* 97:923–932.
- O'Brien, R. M. 2007. A caution regarding rules of thumb for variance inflation factors. *Quality and Quantity* 41:673–690.
- R Core Team. 2019. R 3.5.3. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org
- Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* 102:275–301.
- Suding, K. N., S. Lavorel, F. S. Chapin III, J. H. C. Cornelissen, S. Diaz, E. Garnier, D. Goldberg, D. U. Hooper, S. T. Jackson, and M. L. Navas. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology* 14:1125–1140.
- Tarvainen, L., and T. Nasholm. 2017. Can adjustments in foliar nitrogen-use efficiency reduce drought stress impacts on boreal trees? *Tree Physiology* 37:415–417.
- Taylor, S. H., B. S. Ripley, T. Martin, L. A. De-Wet, F. I. Woodward, and C. P. Osborne. 2014. Physiological advantages of C₄ grasses in the field: a comparative experiment demonstrating the importance of drought. *Global Change Biology* 20:1992–2003.
- Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The Influence of functional diversity and composition on ecosystem processes. *Science* 277:1300–1302.
- Trenberth, K. E. 2011. Changes in precipitation with climate change. *Climate Research* 47:123–138.
- van der Sande, M. T., E. J. M. M. Arets, M. Peña-Claros, M. R. Hoosbeek, Y. Cáceres-Siani, P. van der Hout, and L. Poorter. 2018. Soil fertility and species traits, but not diversity, drive productivity and biomass stocks in a Guyanese tropical rainforest. *Functional Ecology* 32:461–474.
- van der Sande, M. T., M. Peña-Claros, N. Ascarrunz, E. J. M. M. Arets, J. C. Licona, M. Toledo, and L. Poorter. 2017. Abiotic and biotic drivers of biomass change in a Neotropical forest. *Journal of Ecology* 105:1223–1234.
- Zhao, M. S., and S. W. Running. 2010. Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science* 329:940–943.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3465/supinfo>

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Data (Luo et al. 2021) are available on the Dryad digital repository: <https://doi.org/10.5061/dryad.djh9w0w09>.