REGULAR ARTICLE

Chronic and intense droughts differentially influence grassland carbon-nutrient dynamics along a natural aridity gradient

Wentao Luo · Xiaoan Zuo · Robert J. Griffin-Nolan · Chong Xu · Jordi Sardans · Qiang Yu · Zhengwen Wang · Xingguo Han · Josep Peñuelas

Abstract

Background and aims Grasslands are expected to experience both long-term chronic reductions in precipitation as well as increased frequency of short-term intense droughts. However, few studies have assessed how these two types of drought differentially alter carbon-nutrient dynamics of grassland vegetation and soil on broad spatial and temporal scales.

Methods We conducted a two-year drought experiment in three types of grasslands along a natural aridity gradient in northern China. In each grassland, we removed ~ 50% of annual rainfall using two methods—chronic drought (66% reduction of each rainfall event for four months) and intense drought (100% removal of rainfall for two months). This allowed us to compare the effects of these drought characteristics on carbon and nutrient content of both vegetation and soil.

Results Drought largely led to decreased carbon and nutrient pools, with vegetation concentrations being less responsive than pools likely due to decreased plant biomass. These responses depended on drought type, with no clear directional pattern of intense droughts having a greater effect than chronic drought. Sensitivity of biogeochemical responses to drought treatments decreased with increased aridity, likely due to the high abundance of drought-tolerant species in more xeric grasslands. Overall, ecosystem biogeochemical responses to manipulative drought did not match trends observed along the natural aridity gradient.

Responsible Editor: Luca Bragazza.

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Conclusions The sensitivity of carbon-nutrient dynamics of plant and soil strongly depends on drought type as well as local climate and species composition. Such differential drought responses highlight the challenge of predicting ecosystem responses to climate change over large spatial scales.

Keywords Aridity · Biogeochemistry · Climate change · Drought · Grasslands · Plant-Soil interactions

Introduction

Grasslands constitute an extensive fraction of Earth's terrestrial area and provide multiple ecosystem services, including the storage and cycling of carbon (C) and various nutrients (Homyak et al. 2017; Sagar et al. 2019). Given they are highly sensitive to rainfall variability, grasslands are expected to be particularly vulnerable to predicted increases in drought severity and frequency (Chi et al. 2013; Gao et al. 2016; Hsu et al. 2012; Knapp and Smith 2001; Song et al. 2016). Drought generally reduces plant C assimilation and nutrient uptake through decreased diffusion of soil nutrients to root surfaces and/or reduced nutrient translocation from below- to above-ground tissues following stomatal closure during drought (Austin et al. 2004; Kong et al. 2017). The potential negative effects of drought on plant C and nutrient pools (i.e., total amount independent of biomass) are further enhanced when plant biomass is more reduced relative to leaf C assimilation and nutrient uptake during drought (Austin and Sala 2002; Evans and Burke 2013; Xie et al. 2014). These responses of plant and soil biogeochemistry to water limitation may relate to the 'type' of drought experienced by an ecosystem (Schaeffer et al. 2017). Grasslands are expected to experience both long-term chronic reductions in precipitation (i.e. chronic drought) as well as increased frequency of short-term intense droughts (Bradford et al. 2020; Chi et al. 2013; Hsu et al. 2012; Knapp and Smith 2001). Disentangling the effects of chronic vs. intense drought on ecosystem C and nutrient dynamics is essential to predicting grassland responses to climate.

Drought is often defined simply as 'dry' conditions that differ from long-term mean rainfall conditions (Slette et al. 2019). However, there are many defining characteristics that distinguish one drought from another, such as drought magnitude, duration and seasonality (Slette et al. 2019). Previous studies often manipulate these specific drought characteristics in a single site, yet it is unlikely that ecosystem responses to altered rainfall patterns and drought can be extrapolated to other sites (Dukes et al. 2014; Hoover et al. 2015; Peñuelas et al. 2004; Wang et al. 2017). Indeed, several studies have found that minor differences in climate and species composition can create strong site contingencies in ecosystem biogeochemical responses to altered rainfall patterns and drought (Cherwin and Knapp 2012; Griffin-Nolan et al. 2019; Grime et al. 2000; Tielbörger et al. 2014). Coordinated multi-site experiments are therefore needed to determine how ecosystems vary in their responses to different types of drought. Manipulative drought experiments and observations along natural aridity gradients are two common approaches to explore ecosystem responses to water limitation in the short and long term, respectively (Dunne et al. 2004; Yuan et al. 2017). Manipulative drought experiments allow ecologists to study the phenotypic plasticity and plant acclimation to short-term water stress, while natural aridity gradients reveal plant adaptations that have evolved under long-term water limitation (Dunne et al. 2004). Integrating these two approaches provides a means to assess both short- and long-term effects of water limitation on plant and soil biogeochemistry (Dunne et al. 2004). Previous studies have shown that soil and vegetation C and nutrient dynamics vary along aridity gradients (Luo et al. 2015, 2017; Yuan et al. 2017). Few studies, however, have compared how such patterns are affected by chronic and intense drought.

Here, we report results from a two-year coordinated drought experiment across three types of grasslands spanning an aridity gradient in northern China. Drought was imposed by experimentally removing ~ 50% of annual rainfall using two methods —chronic drought (66% reduction of each rainfall event) and intense drought (100% removal of rainfall). This approach allowed us to contrast the effects of drought type, chronic vs. intense, on plant and soil C and nutrient dynamics and assess how these responses vary with aridity. We test the following hypotheses: (I) Plant and soil C and nutrient concentrations and pools will decrease in response to experimental drought and with increasing aridity, with higher sensitivity for C and nutrient pools due to likely reductions in total plant biomass. (II) The magnitude of plant and soil C and nutrient responses to drought would depend on drought type, with short-term intense drought having greater influence than chronic

drought as intense drought is more likely to push plants beyond physiological and mortality thresholds. (III) The sensitivity of plant and soil C and nutrient to manipulative drought as well as drought type would vary across grasslands, decreasing from the least to the most arid grassland. (IV) Plant and soil C and nutrients would decrease in a different manner after experimental drought than along a natural aridity gradient due to differences in the temporal scale of water limitation (short-term acclimation vs. long-term adaptation).

Materials and methods

Study sites

Experimental plots were established in 2014 at three grasslands in the arid and semiarid biome of northern China (Figure S1, Table 1). The three grasslands studied here were part of the 'Extreme Drought in Grassland Experiment' (EDGE) (http://edge.biology. colostate. edu/EDGEchina.html) designed to empirically assess the mechanisms underlying differential ecosystem sensitivity to climate change. The grasslands were arranged along a moderate aridity gradient with mean annual precipitation (MAP) ranging from 170 to 323 mm (Table 1). Inter-annual precipitation variability (assessed as coefficient of variation) ranged for 24–32

% across grasslands and increased with aridity (Figure S2). All grasslands were characterized by uniform geomorphic template and similar temperatures. Grassland communities at all sites were dominated by perennial species (90% of the total biomass) with community cover and productivity decreasing with increasing aridity from east to west. The dominant species are *Stipa grandis* and *Leymus chinensis* in the low aridity grassland, *S. grandis* and *Stellera chamaejasme* in the medium aridity grassland, and *S. glareosa* and *Peganum harmala* in the high aridity grassland. Soils types belonged to the Kastanozem soil group in the Food and Agriculture Organization (FAO) classification system. The soils derived from loess and rich in calcium mainly were silty to sandy in texture, with > 60% sand and a strong acid reaction (pH > 7.0).

Experimental design

In the 2015 growing season, eighteen 6×6 m plots were established at each grassland in randomized locations within a 900-m² area and organized into six blocks. Each plot included a 1-m external buffer to minimize edge effects associated with the experimental infrastructure and was located at least 2 m from the nearest neighboring plot. Each plot was covered by a light scaffolding capable of supporting rain-exclusion shelters (Yahdjian and Sala 2002). The plots within each

Table 1 Characteristics of the three grasslands in the arid and semiarid grasslands in northern China

	Inner Mongolia Grassland Ecosystem Research Station (IMG)	Damaoqi Grassland Station (DGS)	Urat Desert-Grassland Research Station (UDR)
	Low aridity grassland	Medium aridity grassland	High aridity grassland
General			
Latitude	43°32′N	41°47′N	41°25′N
Longitude	116°33′E	111°53′E	106°58′E
Grassland type	Typical steppe	Transition zone	Desert steppe
Climate			
MAP (mm)	346	251	175
GSP (mm)	249	183	133
MAT (°C)	1.9	4.5	5.6
Aridity index	0.40	0.25	0.17
Vegetation			
Dominant species	*S. grandis* and *L. chinensis*	*S. grandis* and *Stellera chamaejasme*	*S. glareosa* and *Peganum harmala*

Note: Climatic variables are calculated from a 32-year record (1982–2014) for the two low aridity grasslands and a 44-year record (1971–2014) for the intermediate- and high-aridity grasslands. MAP, mean annual precipitation; GSP, growing season (May-August) precipitation; MAT, mean annual temperature. Species richness was calculated as the number of species. Aridity index was calculated as the ratios of MAP to potential evapotranspiration (PET), with values closer to 0–denoting greater aridity

block were randomly assigned to one of three treatments (each with six replicates): control (no roofs on the shelters), chronic-drought (66% reduction in rainfall from May to August) and intense-drought (100% reduction in rainfall from June to July) during the growing season. Both the chronic and intense drought treatments decreased total annual rainfall by ca. 50%, albeit with a different intra-annual rainfall pattern. The roofs of these structures consisted of transparent polyethylene panels arrayed at a density for either passively reducing each rainfall event during the growing season by ~66% (chronic) or 100% (intense) (see Figure S1b). Each plot was hydrologically isolated in 2014 by trenching to a depth of 1 m around the plot; the trench was lined with plastic flashing to prevent subsurface and surface water flow between the target plots. Untreated control plots had similar light scaffolding and trenching, but the panels were not attached. The panels permitted ~90% penetration of photosynthetically active radiation (Yahdjian and Sala 2002). The roof was situated 2 m above the ground at the highest point, and the rain shelters remained open at ground level to allow the exchange of air near the surface and to avoid unwanted effects of elevated temperature (see Figure S1b). The shelters remained in place in both 2015 and 2016, thereby simulating an extreme two-year summer drought. Soil moisture content was measured with a soil moisture sensor (PG-110, Jingchuang Electronic Technology Co., Handan, China) placed at 10-cm depth in the center of each plot. Measurements were recorded on a data logger every hour and averaged to produce daily mean values (see Figure S3).

Sampling and analysis

Plant and soil C and nutrient status was sampled in early August 2016 during peak plant growth and after the end of the 2-year drought. One main quadrat (1 × 1 m) was set up in each plot, and four sub-quadrats (50 × 50 cm) were placed within the main quadrat. Aboveground biomass of each species was harvested from two sub-quadrats by clipping at the ground level. All living plants were oven-dried at 85 °C for 48 h and weighed. Species abundance was calculated as the percent contribution towards total biomass.

Foliar samples were then collected from the most abundant species (cumulatively representing 90% of community biomass) in the other two sub-quadrats. The youngest fully expanded leaf was sampled from three individuals per plot. The plant samples were immediately dried at 105 °C for 30 min in an oven to minimize respiration and decomposition and were later completely oven-dried at 80 °C for 48 h. Five soil cores (0–10 cm) were collected from each main quadrat, following removal of litter, using a soil corer (2.5 cm diameter). The soil samples were homogenized by hand and stored in cloth bags at room temperature for later chemical analyses. Air-dried soils were passed through a 2-mm sieve, and roots and rocks were removed.

Plant [C] and [N] as well as soil organic [C] and total [N] were measured using a PE 2400 Series II CHN Elemental Analyzer (Perkin-Elmer, Norwalk, CT, USA). Plant [P] and soil Olsen [P] were measured using the molybdenum blue/ascorbic acid method (Olsen, 1954). Plant [K] and soil exchangeable [K] was measured using atomic absorption spectrometry (AA6800, Shimadzu, Tokyo, Japan).

Data analysis

General mixed effects models were used to assess the effect of drought and grassland type on chemical composition of plant species vegetation (e.g., [C], [N], [P] and [K]) and their stoichiometric ratios (C:N, C:P, C:K, N:P and P:K). In this model, drought treatment and grassland type were used as fixed effects with block and species as random effects. The mixed-model analysis of variance was also applied separately for each grassland, with drought treatment as fixed effect with block and species as random effects.

Plant community vegetation C, N, P and K pools were defined as the sum of [C], [N], [P] and [K] measured across all species weighted by their biomass in each plot. Plant community nutrient concentrations ([C], [N], [P] and [K]) were calculated as the ratio of each plant nutrient pool to the total biomass in each plot. Then, plant community C, N, P and K concentrations, pools and their ratios (C:N, C:P, C:K, N:P and P:K) were analyzed using general mixed effects models with drought treatment and grassland type as fixed factors and block as random factor. Again, the mixed-model analysis of variance was applied separately for each grassland with drought treatment as fixed effect and block as a random effect.

Similarly, the effects of drought and grassland type on soil organic [C], total [N], Olsen [P] and exchangeable [K] were assessed using general mixed effects models. In this model, drought treatment and grassland

type were used as fixed effects with block as random effects. This mixed-model analysis of variance was then applied separately for each grassland, with drought treatment as fixed effect and block as a random effect. Lastly, general discriminant analysis (GDA) was conducted to detect the ecosystem biogeochemical discrimination among drought treatments as well as among different grassland types.

Soil moisture was not included as a co-variate in any of the above-mentioned general mixed effects models as the differences between treatments and sites are represented by those fixed effects. Data were tested for model assumptions (i.e. normality and heteroscedasticity), and original data were used as these assumptions were met. In this study, general mixed models were conducted out using the *lme* function in the *nlme* package of R-project (R i386 3.1.1) and GDA was performed using the Statistica 8.0 software (Statsoft, Inc., Tulsa, USA).

Results

The impact of drought on species C and nutrient concentrations was specific to grassland type and drought type. Chronic drought led to increased [C] and [N] across species at the medium aridity grassland, DGS (Table S2). At the low aridity grassland, IMG, chronic drought led to increased species [N] while intense drought increased species [P] (Table S2). Additionally, species [N] was higher following chronic drought compared to the intense drought treatment at IMG (Table S2). Finally, at the most arid grassland, species C and nutrient concentrations unaffected by either drought treatment (Table S2).

At the low aridity grassland, IMG, intense drought led to increased C:N, C:P and C:K ratios across species, while chronic drought led to increased species C:P, C:K, N:P and N:K ratios (Table S2). Intense drought exerted greater impacts on species C:N ratio, while chronic drought exerted greater impacts on species N:P and N:K ratios at the low aridity grassland (Table S2). At DGS with medium aridity, species N:P ratio increased with chronic drought, while species N:K ratio increased with chronic and intense drought (Table S2). Chronic drought exerted greater impacts on species N:P ratio, while other biochemical ratios did not change with drought type at the medium aridity grassland (Table S2). Finally, species C:N, C:P, C:K, N:P, N:K

were unaffected by drought at the most arid grassland, UDR (Table S2).

At the community level (i.e. weighted by species abundance), vegetation C and nutrient responses to drought were much more variable. Experimental drought led to increased plant community [C] at the intermediate aridity grassland (DGS), with chronic drought having a stronger effect than intense drought (Fig. 1). At the other two grasslands, community [C] did not change with either drought treatment (Fig. 1). Drought impacts on plant community [N] were quite variable with elevated [N] in the two most arid grasslands (UDR and DGS) and decreased [N] in the wettest grassland (IMG) (Fig. 1; Table 2). The type of drought strongly influenced the sensitivity of plant community [N] with chronic drought leading to significant, albeit opposite, responses at DGS and IMG, while intense drought significantly altered plant community [N] at UDR (Fig. 1).

Plant community [P] decreased following chronic drought at the wettest grassland, IMG (Fig. 1), yet no drought effects on plant community [P] were observed at the most arid grasslands (DGS and UDR). Lastly, plant community [K] largely decreased following experimental drought (Fig. 1). The intense drought treatment decreased plant community [K] in the two wettest (DGS and IMG) grasslands with no effect on [K] at the driest grassland (UDR), while the chronic drought led to significant declines in plant community [K] at IMG and increased [K] at UDR.

Experimental drought led to decreased plant community C and nutrient pools across all three grasslands (Fig. 2; Table 2). At the more arid two grasslands (IMG and UDR), both the chronic and intense drought led to decreased plant community pools of C and nutrients (Fig. 2). However, the intense drought led to significantly greater declines in plant community C and nutrient pools at DGS compared to the chronic drought (Fig. 2). While drought led to significant declines in plant community C and nutrient pools at the wettest grassland, there were no significant differences between chronic and intense drought treatments (Fig. 2). In the more arid grassland (UDR), both intense and chronic drought decreased C pools, whereas only chronic drought decreased N, P and K pools (Fig. 2).

Plant community biochemical ratios were most sensitive to the drought treatments at the wettest grassland, IMG (Fig. 3). Plant community C:N ratio increased following chronic drought but not intense drought at
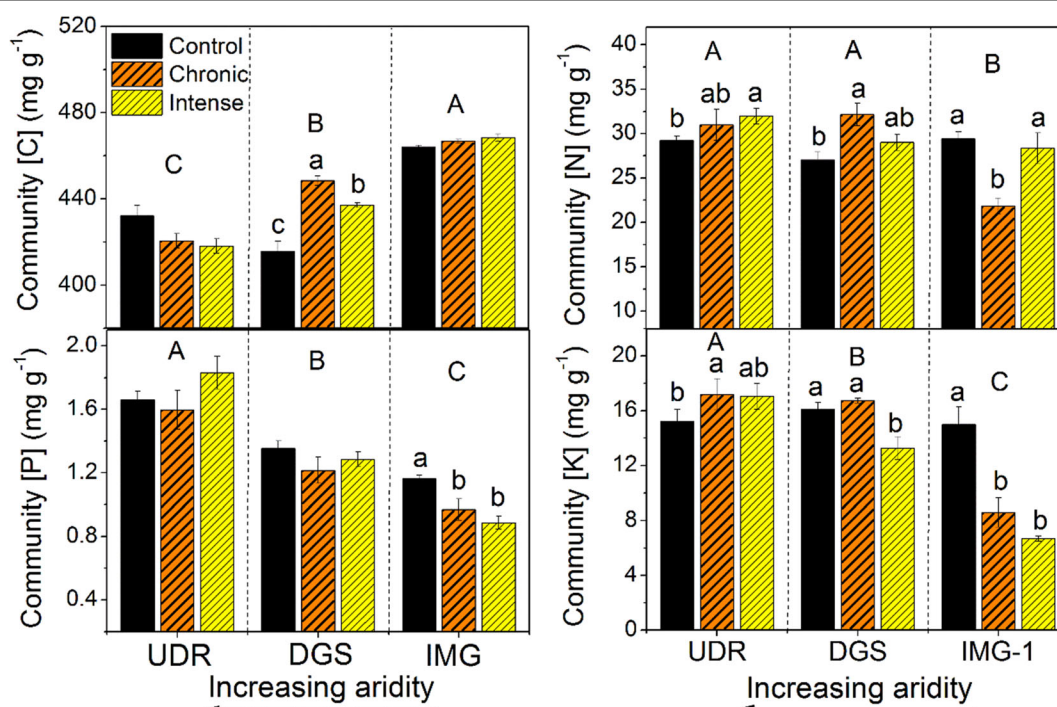
Fig. 1 Effects of chronic and intense droughts on plant community C, N, P and K concentrations for the three grasslands along the aridity gradient. Variables are shown as mean ± SE (n = 6). Lowercase letters indicate significant differences among the treatments at each grassland. Capital letters indicate significant differences among grasslands with different aridity. IMG = Inner Mongolia Grassland Ecosystem Research Station; DGS = Damaoqi Grassland Station; UDR = Urat Desert-Grassland Research Station

IMG (Fig. 3). Both chronic and intense drought led to increased plant community C:K ratio at IMG only, and intense drought led to elevated N:K ratio at both DGS and IMG (Fig. 3). Plant community C:P ratio increased following chronic drought at IMG (Fig. 3). Chronic drought led to increased plant community N:P ratio at DGS while significantly higher N:P ratio was observed at IMG only following intense drought (Fig. 3). Finally, plant community P:K ratio was significantly higher for both drought treatments at IMG and increased following intense drought at DGS (Fig. 3).

Drought treatment significantly altered soil C and N, with effects being specific to each grassland type (Table 2). Neither the chronic nor intense drought treatment significantly affected soil organic [C] or total [N] relative to the control at the high-aridity grassland, UDR (Fig. 4). Soil organic [C] and total [N] were generally lower with both chronic and intense drought at DGS, with the intense treatment having a greater affect (Fig. 4). Both soil Olsen [P] and extractable [K] were higher, albeit not significantly with both chronic and intense drought at all grasslands (Fig. 4).

Soil and vegetation C and nutrient content varied along the natural aridity gradient and did not necessarily mirror short-term responses to experimental drought. Across grasslands, species [C] decreased whereas species [N], [P] and [K] increased with increasing aridity (Table S2), and species C:N, C:P, C:K, N:P, N:K and P:K ratios were higher in the dry than wet grasslands (Table S2). Similar trends in C and nutrient concentrations were observed at the community level (Figs. 1 and 3; Table 2). Additionally, species C:N, C:P, C:K, N:P, N:K and P:K ratios were all higher with increased aridity (Table S2). Plant community C and nutrient pools decreased with increasing aridity (Fig. 2; Table 2). Soil organic [C] and total [N], Olsen [P] and extractable [K] generally decreased with increasing aridity (Fig. 4).

The GDA results showed significant differences in C and nutrient compositions of both vegetation and soil among the three grasslands with separation by treatment evident within each grassland (Fig. 5). The magnitude of the differences between control and drought treatment varied across three grasslands, being most pronounced at the wettest grassland, IMG, and little difference

Table 2 Results of the mixed-model analysis of variance for the variables of plant and soil carbon (C) and nutrients (N, P and K) concentrations and pools at the plot level. Drought treatment and grassland type are used as fixed factors, with block as a random factor

	Grassland		Treatment		Grassland × Treatment	
	F	P	F	P	F	P
Plant						
[C]	162.38	<0.001	6.98	<0.01	14.90	<0.001
[N]	18.34	<0.001	6.62	<0.01	3.52	<0.05
[P]	83.35	<0.001	3.23	<0.05	1.10	0.367
[K]	50.08	<0.001	2.53	0.092	3.54	<0.05
C pool	21.67	<0.001	61.82	<0.001	9.37	<0.001
N pool	21.67	<0.001	61.82	<0.001	9.37	<0.001
P pool	11.98	<0.001	73.75	<0.001	7.25	<0.001
K pool	7.99	<0.01	85.59	<0.001	11.61	<0.001
C:N ratio	39.78	<0.001	4.79	<0.05	4.25	<0.01
C:P ratio	144.01	<0.001	8.36	<0.001	1.70	0.169
C:K ratio	53.50	<0.001	4.29	<0.05	4.85	<0.01
N:P ratio	27.70	<0.001	17.52	<0.01	3.19	<0.05
N:K ratio	18.14	<0.001	4.35	<0.05	4.28	<0.01
P:K ratio	14.13	<0.001	4.14	<0.05	3.87	<0.01
Soil						
Organic [C]	235.24	<0.001	0.53	0.589	2.84	<0.05
Total [N]	105.19	<0.001	2.18	0.126	10.60	<0.001
Olsen [P]	4.99	<0.05	5.43	<0.01	0.31	0.865
Extractable [K]	15.23	<0.001	2.74	0.076	1.41	0.245
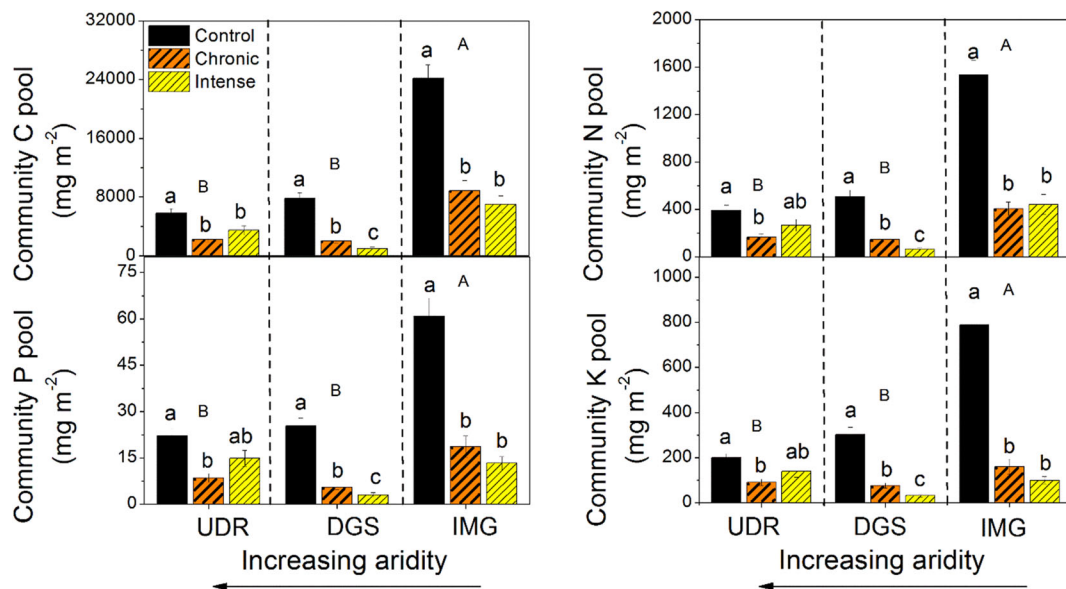
Fig. 2 Effects of chronic and intense droughts on plant community C, N, P and K pools for the three grasslands along the aridity gradient. Variables are shown as mean ± SE (n = 6). Lowercase letters indicate significant differences among the treatments at each grassland. Capital letters indicate significant differences among grasslands with different aridity. IMG = Inner Mongolia Grassland Ecosystem Research Station; DGS = Damaoqi Grassland Station; UDR = Urat Desert-Grassland Research Station
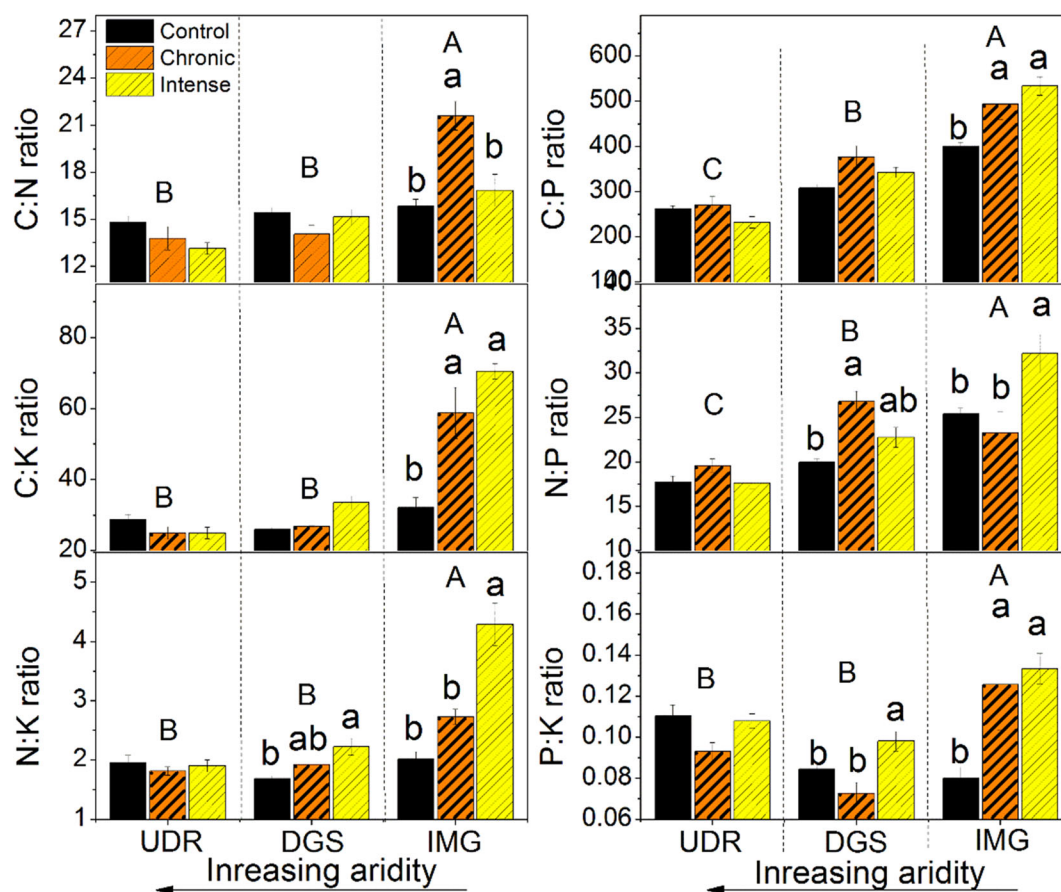
Fig. 3 Effects of chronic and intense droughts on plant community biogeochemical ratios for the three grasslands along the aridity gradient. Variables are shown as mean ± SE (n = 6). Lowercase letters indicate significant differences among the treatments observed at each grassland. Capital letters indicate significant differences among grasslands with different aridity. IMG = Inner Mongolia Grassland Ecosystem Research Station; DGS = Damaoqi Grassland Station; UDR = Urat Desert-Grassland Research Station

observed at UDR, the most arid grassland (Fig. 5). The magnitude of the differences between chronic and intense drought was also smallest at the driest grassland, UDR (Fig. 5).

Discussion

We experimentally imposed an extreme drought (~50% reduction in total annual precipitation over two-years) across three grasslands in northern China using two approaches – chronic drought (~66% reduction of each growing season rainfall event) and intense drought (100% removal of rainfall for a given time) – to reflect two separate predictions of increased water limitation in grasslands with climate change. These grasslands, which range from desert to typical steppe, are situated along an aridity gradient which allowed us to characterize the response of grassland soil and vegetation biogeochemistry to different types of drought (chronic vs. intense) as well as water limitation on both short- and long temporal scales.

Consistent with our hypothesis, our results indicate that plant community C and nutrient pools largely decreased following two years of drought, however, C and nutrient concentrations were more variable (Fig. 2). Drought has been shown to negatively affect plant nutrient concentrations due to decreased mass flow of nutrients in soils driven by reduced stomatal conductance during drought (Austin et al. 2004; Fierer and Schimel 2002; Lin et al. 2010). However, drought also reduces plant growth which may lead to enriched tissue nutrient concentrations relative to pre-drought conditions (Peñuelas et al. 2019; Yue et al. 2019). These
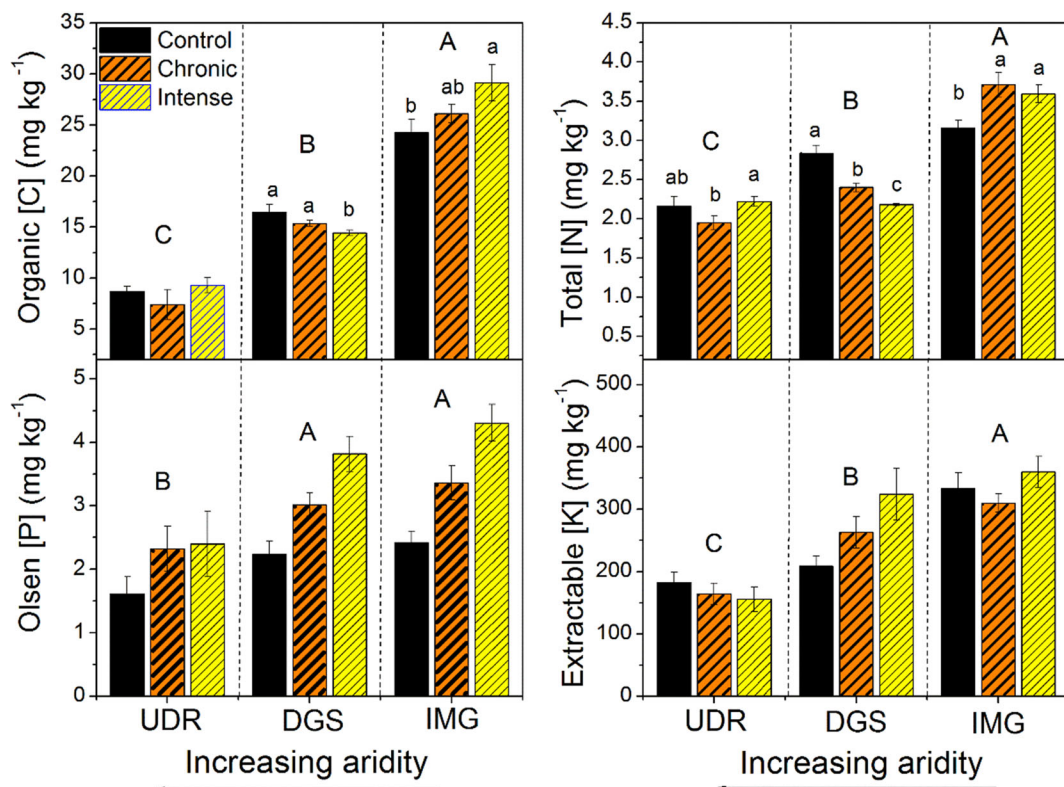
Fig. 4 Effects of chronic and intense droughts on soil nutrient variables for the three grasslands along the aridity gradient. Variables are shown as mean ± SE (n = 6). Lowercase letters indicate significant differences among the treatments at each grassland.

Capital letters indicate significant differences among grasslands with different aridity. IMG = Inner Mongolia Grassland Ecosystem Research Station; DGS = Damaoqi Grassland Station; UDR = Urat Desert-Grassland Research Station

two opposing processes might explain the variable responses to drought observed here. It is worth noting that previously reported negative/positive effects of drought stress on plant C and nutrient concentrations may not necessarily indicate decreases/increases in the total amount of ecosystem C and nutrients, as drought stress often reduces plant biomass (Peñuelas et al. 2004; Yue et al. 2019). However, plant C and nutrient concentrations and stoichiometry provide a wealth of information pertaining to plant strategies for coping with environmental change (Liang et al. 2013; Peñuelas et al. 2019; Wang et al. 2019). We therefore recommend measuring both pools and concentrations of plant C and nutrients when assessing ecosystem sensitivity to climate change, as both measures provide important and complementary information pertaining to ecosystem function.

Our results indicate that drought type (i.e. chronic vs. intense) can differentially influence ecosystem responses to drought depending on site characteristics. For instance, intense drought caused a greater reduction in plant community C and nutrient pools as well as soil

organic [C] and total [N] compared to chronic drought at the medium aridity grassland. These specific results support our hypothesis and are consistent with previous work suggesting different types of drought influence semi-arid grassland dynamics (Hoover et al. 2015). It is worth noting, however, that there was no clear directional pattern of intense droughts having a greater effect on soil and vegetation C-nutrient dynamics than chronic drought across sites. These results do suggest that the effect of drought on ecosystem processes are not simply related to MAP or drought severity of the studied site (Huxman et al. 2004; Maurer et al. 2020), but also related to drought characteristics (Hoover et al. 2015).

The sensitivity of ecosystem C and nutrient pools/concentrations to drought varied across grasslands, with the driest grassland being least sensitive. This can be partly explained by the greater contribution of intraspecific variation to plant biochemical variables at the drier grassland compared to the other grasslands. The observed changes to vegetation C and nutrient content across grasslands were driven by different mechanisms.
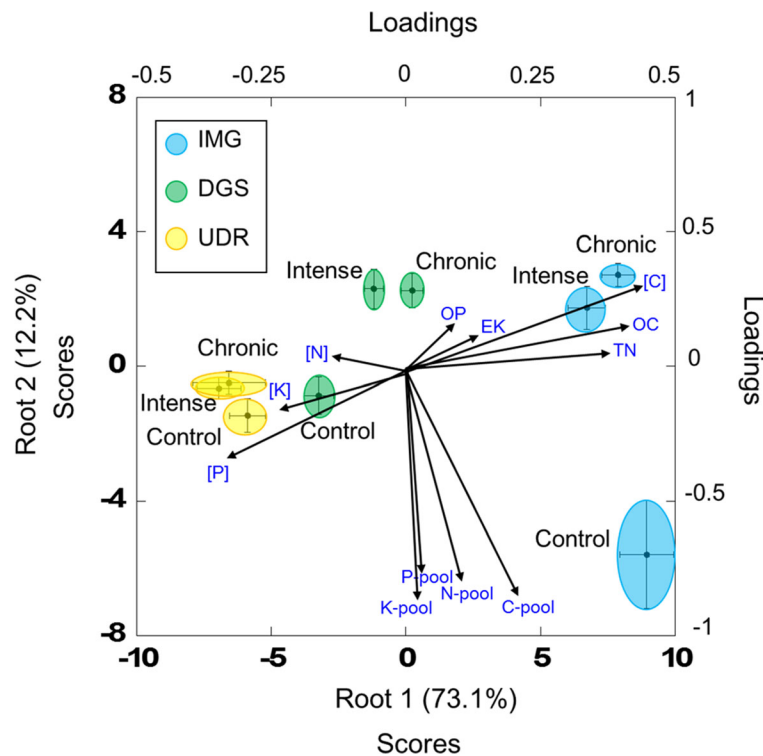
Fig. 5 Representation of the first two roots of plant and soil biogeochemical variables among the three grasslands along aridity gradients with chronic and intense drought treatments. The draw color areas represent the area of the sample scores cases by the 95% of interval of confidence around the centroids that represents the mean of the scores distribution along the two axis. IMG = Inner Mongolia Grassland Ecosystem Research Station; DGS = Damaoqi Grassland Station; UDR = Urat Desert-Grassland Research Station. OC = soil organic carbon concentration, OP = soil Olsen-P concentration, EK = soil extractable K concentration, TN = soil N total concentration, [C] = plant community C concentration, [N] = plant community N concentration, [P] = plant community P concentration, [K] = plant community K concentration, C-pool = plant community C pool, N-pool = plant community N pool, P-pool = plant community P pool, and K-pool = plant community K pool

For example, at the driest grassland, vegetation C and nutrient alterations were caused by reordering of 1–2 dominant species, whereas dominant species were replaced by several subordinate species at the wetter grasslands (Table S1). Plant C and nutrient stoichiometries were less sensitive to manipulative drought at the drier vs. wetter grassland, regardless of drought type. These results support conclusions from previous studies suggesting that arid ecosystems are less sensitive to drought due to the high abundance of stress tolerating species in drier regions compared to wetter regions (Grime et al. 2000; Tielbörger et al. 2014). Plant C and nutrient stoichiometry are intrinsically linked to plant physiological and metabolic processes that occur during drought (Sterner and Elser 2002; Peñuelas et al. 2019). The dominant species in these grassland communities generally have invariant nutrient stoichiometries in their tissues to better withstand drought perturbations (Yu et al. 2010, 2015). At the driest grassland, the invariant plant C and nutrient stoichiometries may have reduced this ecosystem's sensitivity to drought (Sterner and Elser 2002; Peñuelas et al. 2019). Such mechanisms related to C and nutrients have the potential to decrease a plant's sensitivity to environmental change.

In line with our hypothesis, plant and soil biogeochemical responses to short-term manipulative drought at each grassland were largely different from trends observed along the aridity gradient. Observational studies along natural gradients are used to identify the long-term evolutionary changes in ecosystem functioning (perhaps over centuries or millions of years) (Luo et al. 2015; Yuan et al. 2017). Optimization theory suggests evolutionary forces have selected for plant communities with ideal traits for coping with abiotic and biotic stressors of their local environment (Wright et al. 2001). Indeed, species composition gradually changed, and plant biomass decreased significantly from the least to the most arid grassland along the aridity gradient

(Table S1). Accordingly, effects of species turnover on plant community C and nutrient composition were relatively strong, while the intraspecific effects were relatively weak along the aridity gradient.

Plant nutrient concentrations are generally mediated by the soil nutrient regimes in terrestrial ecosystems (Luo et al. 2015, 2018; Sardans et al. 2008). However, our experiments failed to show similar responses of plant and soil nutrients to manipulative drought or increased aridity. The mismatch between plant and soil nutrients can be caused by severe water limitation, which restricts nutrient mass flow within soils and nutrient uptake by plant roots (Luo et al. 2015, 2018). These results suggest that intrinsic factors were more important in affecting nutrient composition under long-term stress conditions (Luo et al. 2015, 2018). Moreover, we found that the ratios of C and nutrients generally decreased with increasing aridity along the gradient. This result indicates that plant species preferred to conserve more nutrients in biomass under long-term water stress conditions, which is decoupled with C assimilation process (Luo et al. 2017). Additionally, the lower plant C:P and N:P ratios and higher plant K:P ratios apparent at the drier grasslands suggests that plant species have evolved a stronger P-conservation mechanism than C, N and K in the xeric grassland biomes (Peñuelas et al. 2004, 2019).

Conclusions

Our study compared the impacts of both chronic and extreme drought on C and nutrient dynamics of grassland vegetation and soil and determined the ecosystem biogeochemical responses to manipulative drought (short-term water limitation) vs. natural aridity (long-term water limitation). Ecosystem biogeochemistry responded variably to drought with responses depending on both the type of grassland and drought imposed. The plant and soil biogeochemical responses to short-term manipulative drought did not match the long-term trends observed along the natural aridity gradient. These findings highlight the inherent complexity in predicting the responses of terrestrial biochemical dynamics to novel climatic conditions, even within a single biome.

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