Differential responses of grassland community nonstructural carbohydrate to experimental drought along a natural aridity gradient

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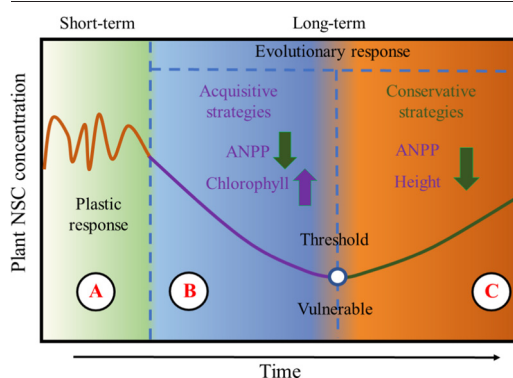[h] Department of Biology and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523, USA
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HIGHLIGHTS

- A conceptual model to evaluate drought on soluble sugar (SS) and nonstructural carbohydrates (NSC) in grassland ecosystem.
- SS and NSC were lowest at intermediate aridity, with this pattern driven primarily by species turnover.
- Plant resource strategies were more acquisitive at mesic sites, but more conservative at drier sites.

GRAPHICAL ABSTRACT

ABSTRACT

Plant nonstructural carbohydrates (NSC) can reflect community and ecosystem responses to environmental changes such as water availability. Climate change is predicted to increase aridity and the frequency of extreme drought events in grasslands, but it is unclear how community-scale NSC will respond to drought or how such responses may vary along aridity gradients. We experimentally imposed a 4-year drought in six grasslands along a natural aridity gradient and measured the community-weighted mean of leaf soluble sugar (SS_{CWM}) and total leaf NSC (NSC_{CWM}) concentrations. We observed a bell-shape relationship across this gradient, where SS_{CWM} and total NSC_{CWM} concentrations were lowest at intermediate aridity, with this pattern driven primarily by species turnover. Drought manipulation increased both SS_{CWM} and total NSC_{CWM} concentrations at one moderately arid grassland but decreased total NSC_{CWM} concentrations at one moist site. These differential responses to experimental drought depended on the relative role of species turnover and intraspecific variation in driving shifts in SS_{CWM} and total NSC_{CWM} concentrations. Specifically, the synergistic effects of species turnover and intraspecific variation drove the responses of leaf NSC concentrations to

drought, while their opposing effects diminished the effect of drought on plant SS_{CWM} and total NSC_{CWM} concentrations. Plant resource strategies were more acquisitive, via higher $chlorophyll_{CWM}$ concentration, to offset reduced NSC_{CWM} concentrations and net aboveground primary productivity (ANPP) with increasing aridity at more mesic sites, but more conservative (i.e., decreased plant $height_{CWM}$ and ANPP) to reduce NSC consumption at drier sites. The relationship between water availability and NSC_{CWM} concentrations may contribute to community drought resistance and improve plant viability and adaptation strategies to a changing climate.

1. Introduction

Grasslands cover over 40% of the earth's terrestrial surface and provide many critical ecosystem services, such as soil stability, water conservation, biodiversity, and forage for livestock (Gao et al., 2016). Climate change projections for the 21st century suggest that summer droughts will become more frequent (IPCC, 2013) and grasslands are particularly sensitive to drought (Knapp et al., 2015; Wilcox et al., 2015), given they are primarily water-limited ecosystems (Craine et al., 2012; Hsu et al., 2012; Hoover et al., 2014). Thus, understanding the physiological and community mechanisms by which grasslands respond to drought is important for determining how climate change will impact these essential ecosystems (Smith, 2011).

Many plant physiological responses to drought can be interpreted from measuring non-structural carbohydrates (NSC). Plant NSC are produced during photosynthesis and are essential for maintaining plant metabolism during all life stages (Bouma, 2005; Li et al., 2008a; Hartmann and Trumbore, 2016). Plant NSC are mainly synthesized in leaves from assimilated carbon by photosynthesis and translocated to different organs by mass flow (Du et al., 2020). Given their role in photosynthesis, leaves have higher metabolic activities than roots and stems and therefore store significant portions of the plant's total NSC content (Du et al., 2020; Martinez-Vilalta et al., 2016). Leaf NSCs are used to maintain cell turgor via osmotic adjustment as water becomes limiting (Gersony et al., 2020). When resources are plentiful, NSC production generally exceeds demand, leading plants to store the excess NSC in their vacuoles or plastids for later use (Hartmann and Trumbore, 2016; Ai et al., 2017). During drought, plants reduce stomatal conductance to improve water use efficiency (Chapin et al., 1990), and access stores of NSC to meet metabolic demands (Poorter and Kitajima, 2007; Li et al., 2018c). When stored NSC concentrations fall below a critical threshold required for plant survival, further reductions in water availability can trigger plant mortality and potential shifts in plant community composition (McDowell et al., 2008; Jin et al., 2018). Due to their central role in plant function, NSC dynamics have been used in vegetation models to represent metabolism, species habitat range shifts, plant vulnerability to climate extremes, and even species extinction risks (Rosas et al., 2013). Thus, an in-depth understanding of long-term impacts of drought on community scale NSC is potentially important for forecasting shifts in ecosystem processes and functioning during drought.

To date, much research has focused on assessing NSC responses to drought at the individual species level, whereas such studies are less common at the community scale, especially in grasslands. However, changes in NSC dynamics in response to drought are more relevant to community structure and ecosystem functions when assessed at the community level (Violle et al., 2012). Shifts in plant community NSC concentrations in response to drought can be due to species turnover (i.e., species with different NSC concentrations replacing others and a shift in relative abundance of each species) and/or intraspecific variation (i.e., plasticity and genetic differentiation) in NSC concentrations (Albert et al., 2010; Violle et al., 2012). If shifts in species turnover and intraspecific trait variation in response to drought act in parallel (e.g., species increase NSCs and species with inherently high NSCs become more abundant), then their effects on community NSC dynamics are enhanced (synergistic effects), whereas an opposing shift (e.g., species reduce NSC and high NSC species become more abundant) can be masked in the average community responses (opposing effects). Short-term manipulative drought experiments (i.e., months to years) may impact communities through shifts in both species turnover and intraspecific

variation, while observations along natural aridity gradients, which represent long-term exposure to water stress (i.e. decades to centuries), are likely to reveal community functional responses primarily through species turnover (Volf et al., 2016). Therefore, quantifying the relative contributions of species turnover and intraspecific variation is important for understanding the responses of community NSC concentrations to water limitation.

We established a coordinated 4-yr drought experiment at six grasslands sites spanning an aridity gradient in northern China. At each site, we measured plant NSC concentrations of all species cumulatively representing ~90% of total plant biomass to estimate community-weighted mean NSC (NSC_{CWM}) concentrations. The effect of drought on NSC_{CWM} concentrations is influenced by both species turnover (i.e., changes in species relative biomass) and intraspecific trait variability (i.e., changes in species NSC). We tested the hypothesis that experimental drought would have no effect on NSC_{CWM} concentrations due to the opposing effects of intraspecific trait responses (i.e., stomatal closure and reductions in NSC reserves) and species turnover (i.e., increased abundance of drought tolerant species with high NSC concentrations). Additionally, we predicted that NSC_{CWM} concentrations would be maximized at the extremes of the aridity gradient as xeric-adapted species accumulate NSC for osmotic regulation and more productive mesic-adapted species produce more photosynthate (Martinez-Vilalta et al., 2016). This trend would largely be driven by species turnover.

2. Material and methods

2.1. Study sites and experimental design

In 2014, we selected six sites arrayed across the east-west extent of the grassland biome in northern China (see the site names and abbreviations in Table 1). These six sites encompass the three major grassland types in China (i.e. meadow steppe, typical steppe and desert steppe) and vary in mean annual precipitation (MAP), mean annual temperature (MAT), plant species composition and edaphic properties. We extracted climatic variables (e.g., MAT, MAP, and potential evapotranspiration (PET, mm) for each site from the global Worldclim data set with a resolution of 0.0083° × 0.0083° (http://www.worldclim.org). We defined the aridity index (AI) of each site as 1 - MAP/PET (Delgado-Baquerizo et al., 2013). Among the six sites, aridity increases from east (NHG) to west (UDR) (Table 1). Soil texture also varies across the six sites from clay loams to sandy, as well as other soil characteristics (Table 1). All six sites had not been grazed by domestic herbivores for the 3 years prior to the 2015 drought. The dominant species are *Stipa baicalensis* and *Leymus chinensis* in the meadow steppe, *S. baicalensis* and *L. chinensis* in the typical steppe, and *S. breviflora* and *Peganum harmala* in the desert steppe.

We established experimental drought infrastructure at each site which remained in place from 2015 to 2018. Using a randomized complete block design, we established twelve 6 × 6 m plots per site (6 drought; 6 control) in a topographically uniform area. Plots were located at least 2 m from each other and were hydrologically isolated by trenching the perimeter to a depth of 1 m and installing 6-mm-thick plastic barriers to prevent lateral water flow. We constructed large rainout shelters to block 66% of ambient growing season precipitation from May to August in each year (Fig. S1). These rainout shelter roofs were built with transparent polyethylene panels covering 66% of the surface and were supported by a light scaffolding structure. To allow for air flow below the panels and minimize any potential greenhouse effect, we installed shelters 2 m above the ground.

Table 1
Climate, plant and soil community properties of six grasslands sites in northern China.

Site	NHG	EFS	IMG	XTR	DGS	UDR
Ecosystem type	Meadow steppe	Meadow steppe	Typical steppe	Typical steppe	Desert steppe	Desert steppe
Aridity index	0.17	0.25	0.40	0.42	0.49	0.51
Location	N49°21′ E120°0.6′	N50°10′ E119°22′	N43°33′ E116°40′	N43°32′ E116°33′	N41°47′ E111°53′	N41°25′ E106°58′
Dominant Species	*Stipa baicalensis, Leymus chinensis*	*Stipa baicalensis, Leymus chinensis*	*Leymus chinensis, Stipa grandis*	*Stipa grandis, Leymus chinensis*	*Leymus chinensis, Stipa krylovii*	*Stipa klemenzii*
ANPP (g m^{-2})	183.49	135.27	114.63	111.08	56.36	14.38
Soil type	Chernozems	Chernozems	Dark chestnut soil	Dark chestnut soil	Castanozems	Brown pedocals + Gray brown desert soil
MAP (mm)	380	336	331	331	257	180
MAT (°C)	−2.4	−2	0.3	0.3	3.4	5.3
SOC (g kg^{-1})	43.20	28.12	24.26	21.35	16.45	8.68
TN (g kg^{-1})	4.10	3.11	3.15	2.94	2.83	2.16
AP (g kg^{-1})	5.99	1.13	2.42	2.56	2.23	1.61
pH	6.98	7.89	7.28	8.06	7.68	9.05

Notes: NHG, National Hulunber Grassland Ecosystem Observation and Research Station, Institute of Agricultural Resources and Regional Planning; EFS, Erguna Forest-Steppe Ecotone Research Station; IMG, Inner Mongolia Grassland Ecosystem Research Station; XTR, Xilinhot Typical-Grassland Research Station; DGS, Damaoqi Grassland Station; UDR, Urat Desert-Grassland Research Station; ANPP, aboveground net primary production; MAP, mean annual precipitation; MAT, mean annual temperature; SOC, soil organic carbon; TN, soil total nitrogen; AP, soil available phosphorus.

Similar experimental infrastructure has been used in previous experiments with minimal effects on light environment (permitting nearly 90% transmission) (Yahdjian and Sala, 2002; Wilcox et al., 2015). For control plots, we established similar scaffolding structures but did not install polyethylene panels.

2.2. Sampling and measurements

At six experiment sites, each plot contained a 4 × 4 m sampling plot, which we divided into four 1 × 1 quadrats. In August 2017, we randomly selected one of these quadrats and further divided it into four 50 × 50 cm sub-quadrats. We designated two diagonal sub-quadrats for destructive measurements of plant biomass, and the other two for surveys of plant traits (Luo et al., 2019).

We estimated aboveground net primary productivity (ANPP) in the two designated sub-quadrats by clipping plant material at the ground level during peak growth. We sorted biomass by species before oven-drying (48 h at 65 °C) and weighing to estimate species-specific ANPP. We calculated species relative abundance as the species' percent contribution towards total ANPP.

In the other two diagonal sub-quadrats, we measured plant traits of the most abundant species (i.e., cumulatively representing at least 90% of the total ANPP). Rare species were not sampled unless enough leaf material was available for each trait measurement. First, we estimated plant height for three individuals of each species per plot. We then collected the youngest, fully expanded leaf from the same individuals (Perez-Harguindeguy et al., 2016) and oven-dried these leaves at 105 °C for 30 min to stop enzymatic activity before drying at 65 °C until constant weight. We estimated foliar soluble sugar (SS) and starch concentrations spectrophotometrically (ultraviolet-visible spectrophotometer 723 S, Yoke Precision Instruments Co., Ltd., Shanghai, China) at 620 nm using the Sulfuric acid-Anthrone method (Li et al., 2008b). We used the sum of starch and SS concentrations as an estimate of total NSC concentrations for each species. The concentrations of SS and total NSC have been widely used as indicators of NSC (Martinez-Vilalta et al., 2016).

In August 2018, we sampled leaves of the same species (i.e., representing 90% of biomass) to determine leaf chlorophyll concentrations in each plot of each site. We stored samples at −20 °C until foliar chlorophyll was extracted using spectra-analyzed grade N, N dimethylformamide. We measured absorbance at 663, 646 and 480 nm using a Shimadzu UV-1700 spectrophotometer (Wellburn, 1994; Chen et al., 2013) and total chlorophyll concentration was estimated according to Porra et al. (1989).

2.3. Data analysis

We used R statistical programming (R version i386 3.6.1) to run all data analyses described below. First, we used Shapiro-Wilk and Levene's tests to confirm the normality and heteroscedasticity of all trait data. Based on this confirmation, we used the original non-transformed data in all statistical analyses. We calculated community weighted mean (CWM) traits (i.e. plant height, chlorophyll concentration, SS concentration, starch concentration and total NSC concentrations) weighted by each individual species' relative contribution to biomass in each plot (Khasanova et al., 2013; Griffin-Nolan et al., 2018).

To determine the spatial relationships between aridity and NSC concentrations, we regressed AI against CWM of SS and total NSC concentrations using linear or curvilinear (quadratic) equations. Based on both explained variation and Akaike's Information Criterion (AIC) values, we found that the relationships between AI and both SS_{CWM} and total NSC_{CWM} concentrations were best described by a second-order polynomial, with lowest levels at a site with intermediate aridity. To determine trait-trait relationships, we used mixed linear models to relate SS_{CWM} and total NSC_{CWM} concentrations to $height_{CWM}$ and $chlorophyll_{CWM}$ concentration along the sampled aridity gradient as well as on each side of the aridity gradient (i.e., moist vs. dry regions) with blocks as random effect. In addition, to determine trait-ANPP relationships, we used mixed linear models to relate SS_{CWM}, total NSC_{CWM}, to ANPP along the sampled aridity gradient as well as on each side of the aridity gradient (i.e., moist vs. dry regions) with blocks as random effect. Here, the two sites with intermediate aridity were included as both moist and dry regions. To determine the effect of experimental drought on both SS_{CWM} and total NSC_{CWM} concentrations, we ran a mixed model analysis of variance with drought treatment and site as fixed factors and block as a random factor. As interactive effects of drought treatment and site were all significant ($P < 0.05$), mixed-effect models were applied separately for each site with drought treatment as fixed factor and block as a random factor. Additionally, the total effects of experimental drought on SS_{CWM} and total NSC_{CWM} concentrations were analyzed with mixed models with drought treatment as a fixed factor and site and block as random factors.

Shifts in plant SS_{CWM} and total NSC_{CWM} concentrations can be attributed to both species turnover (C_{Turn}, changes in species relative biomass) and intraspecific variation (C_{Intra}, changes in species NSC); thus, we isolated their relative contributions using the following equation (Jung et al., 2014; Luo et al., 2018): $C_{Turn} = N_{Dr*} - N_{Ct}$ and $C_{Intra} = N_{Dr} - N_{Dr*}$, where N_{Ct} is calculated by each species' SS and total NSC concentrations in control plots weighted by species' relative biomass in control plots, N_{Dr} is calculated by each species' SS and total NSC concentrations in drought plots weighted
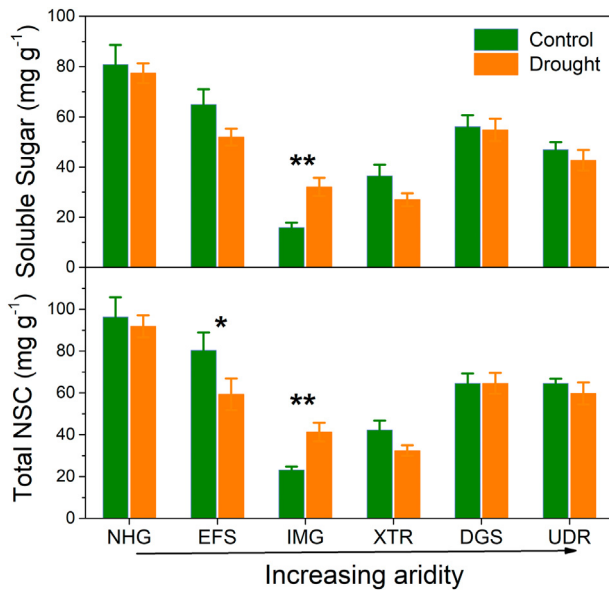
Fig. 1. Plant community-weighted mean of soluble sugar and total nonstructural carbohydrate (NSC) concentrations in plots exposed to experimental drought and control plots for six grassland sites along a natural aridity gradient in northern China. See Table 1 legend for the site abbreviation. Soluble sugar and total NSC concentrations are shown as mean $\pm$ 1SE ($n = 6$). Statistical significance between treatments is represented by asterisks: $*P < 0.05$, $**P < 0.01$.

by species' relative biomass in drought plots, and N_{Dr*} is calculated by each species' SS and total NSC concentrations in control plots but weighted by species' relative biomass in drought plots.

3. Results

Along the aridity gradient, plant SS_{CWM} and total NSC_{CWM} concentrations were lowest at the IMG site with intermediate aridity, and highest at

the driest and wettest sites (Fig. 1). In contrast, plant height$_{CWM}$ and chlorophyll$_{CWM}$ concentration were highest at the intermediate aridity site (Fig. S2). Accordingly, plant SS_{CWM} concentrations were negatively related to plant height$_{CWM}$ ($R^2 = 0.79$, $P = 0.004$) and chlorophyll$_{CWM}$ concentration ($R^2 = 0.68$, $P = 0.096$), and plant total NSC_{CWM} concentrations were negatively related to plant height$_{CWM}$ ($R^2 = 0.76$, $P = 0.003$) and chlorophyll$_{CWM}$ concentration ($R^2 = 0.64$, $P = 0.012$) along the entire natural aridity gradient (Fig. S3).

These relationships indicate a strong tradeoff between these two functional traits and plant NSC concentrations. However, the strength of the tradeoff differed by region (Fig. 2). Specifically, in the moist region, plant SS_{CWM} concentration and plant total NSC_{CWM} concentrations were more strongly related to chlorophyll$_{CWM}$ concentration ($R^2 = 0.69$ and 0.68, respectively; $P < 0.001$) than plant height$_{CWM}$ ($R^2 = 0.15$ and 0.15, respectively; $P < 0.01$). In the dry region, however, plant SS_{CWM} concentration and plant total NSC_{CWM} concentration were more strongly correlated with plant height$_{CWM}$ ($R^2 = 0.52$ and 0.67, respectively; $P < 0.001$) than chlorophyll$_{CWM}$ concentration ($R^2 = 0.09$ and 0.24, respectively; $P < 0.05$) (Fig. 2). In the moist region, ANPP was positively correlated with plant SS_{CWM} concentration ($R^2 = 0.24$; $P < 0.001$; Fig. S4) and plant total NSC_{CWM} concentrations ($R^2 = 0.25$; $P < 0.001$; Fig. S4). In the dry region, however, ANPP were negatively correlated with plant SS_{CWM} ($R^2 = 0.33$; $P < 0.001$; Fig. S4) and plant total NSC_{CWM} concentrations ($R^2 = 0.44$; $P < 0.001$; Fig. S4).

The treatment $\times$ site interactions were significant for SS_{CWM} and total NSC_{CWM} concentrations, suggesting that drought effect on these variables depended on the site characteristics (Table 2). Experimental drought led to an increase in SS_{CWM} concentration at the IMG site but showed negligible effects at the other sites (Fig. 1). Plant total NSC_{CWM} concentrations significantly increased with experimental drought at the IMG site, decreased at the EFS site and remained unchanged at the remaining sites (Fig. 1). The overall effect of experimental drought on plant SS_{CWM} concentration and total NSC_{CWM} concentrations was non-significant with site as a random effect (Fig. S5a). However, when IMG site is removed from the analysis, there is a significant decrease in plant SS_{CWM} and total NSC_{CWM} concentrations with drought treatment (Fig. S5b). Plant total starch$_{CWM}$ concentration significantly decreased with experimental drought at the EFS site and remained unchanged at the remaining sites (Fig. S6).
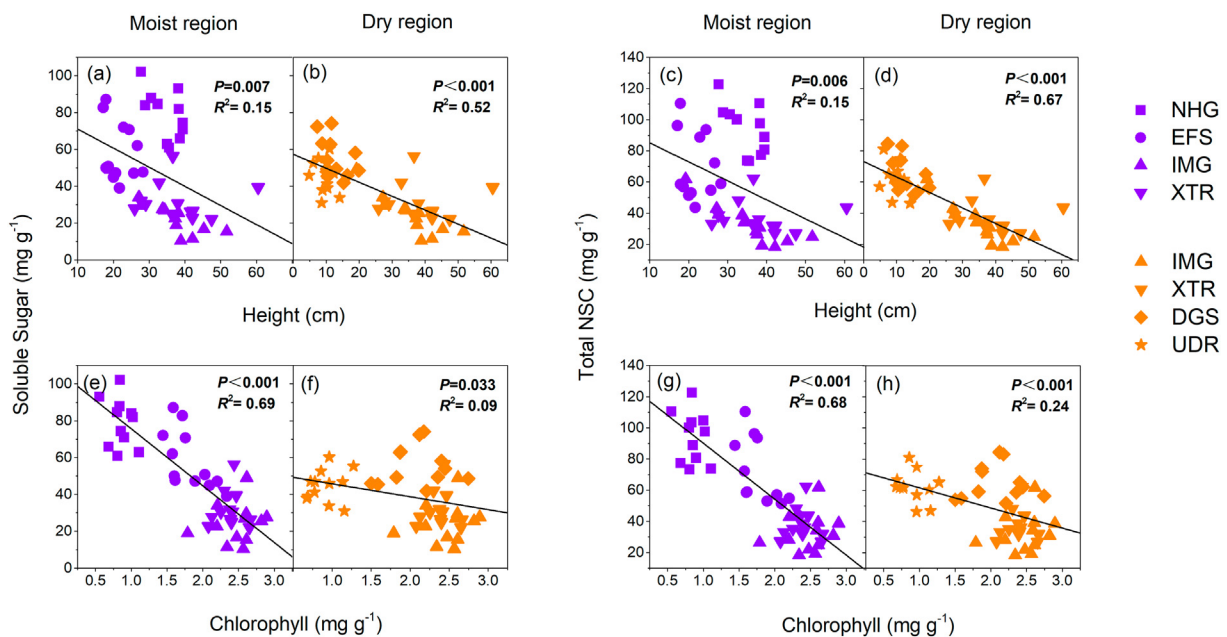

Fig. 2. Relationships between plant soluble sugar and total nonstructural carbohydrate (NSC) concentrations and plant maximum height and chlorophyll concentration at the community-scale in moist (purple symbols) vs. dry (orange symbols) regions in grasslands in northern China. Different symbols indicate different sites. See Table 1 legend for the site abbreviation. Values of R^2 and P are shown for significant relationships ($P < 0.05$).

Table 2

Results of the mixed-effect model analysis of plant community-weighted mean of soluble sugar and total nonstructural carbohydrate (NSC) concentrations. Drought treatment (drought vs. control) and site (six grassland sites in northern China) were used as fixed factors and block as a random factor. Both F- and P-values are shown for fixed effects and their interactions; $n = 6$.

	Soluble Sugar		Total NSC	
	F	P	F	P
Treatment	0.466	0.498	0.894	0.349
Site	43.568	<0.001	41.481	<0.001
Treatment × Site	2.937	0.020	3.474	0.009

Shifts in plant SS_{CWM} and total NSC_{CWM} along the aridity gradient were primarily caused by species turnover, which explained more than 90% of the variance (data not shown). In contrast, changes in plant SS_{CWM} and total NSC_{CWM} concentrations caused by experimental drought were due to both intraspecific variation and species turnover (Fig. 3). The effects of species turnover and intraspecific variation were synergistic for plant SS_{CWM} concentration at the IMG and NHG sites and for total NSC_{CWM} concentrations at the IMG and EFS sites (Fig. 3). In contrast, the effects were opposite for plant SS_{CWM} and total NSC_{CWM} concentrations for the remaining grassland sites (Fig. 3).

4. Discussion

In our study, SS_{CWM} and total NSC_{CWM} concentrations were lowest in the site with intermediate aridity and highest in sites at the opposing extremes of the gradient (Fig. 1). This U-shaped relationship is perhaps due to differences in resource-use tradeoffs exhibited by plants on opposing extremes of the gradient. We document a clear tradeoff between plant investment in acquisitive growth-related traits (e.g., height and chlorophyll) and conservative resource storage traits (e.g., NSC). It is well established that water limitation leads to stomatal closure at the expense of photosynthesis

and NSC production (Casson and Gray, 2008). In extreme environments, plants often exhibit resource conservative strategies to decrease metabolism and consumption of stored NSC (Gao et al., 2015; Yue et al., 2019). This was demonstrated by reduced plant height$_{CWM}$ (Fig. 2) and ANPP with increasing aridity in the drier region of our study area (Figs. S4). Similarly, previous studies have shown a negative relationship between plant growth and plant NSC concentrations under low-temperature and water limitation (Fajardo et al., 2012; Piper and Fajardo, 2011). In more mesic sites, however, plants adopted a resource acquisitive strategy as evidenced by increased plant chlorophyll concentration to offset decreased NSC (Fig. 2) and ANPP in response to increased aridity (Fig. S4). Taken together, these results confirm previous findings that plants adopt opposing resource-use strategies on either extreme of a resource gradient (Chaves et al., 2002; Claeys and Inze, 2013) and that there is an intermediate aridity threshold where this shift in resource-use strategy occurs, resulting in lower NSC_{CWM} concentrations, but perhaps higher mortality (Sandel et al., 2010; Wei et al., 2016).

Interestingly, this intermediate aridity site experienced the greatest increase in NSC_{CWM} concentrations following experimental drought (Fig. 1), which suggest the co-occurrence of species with divergent resource-use strategies manifests as a higher capacity for community NSC adjustment following drought. These results were consistent with the positive covariation observed between species turnover and intraspecific variation at this site (Fig. 3), which implies that experimental drought acted as an environmental filter selecting both species with inherently higher NSC_{CWM} concentrations and those with a greater potential to increase NSC_{CWM} concentrations in response to drought (Jung et al., 2014). Consistent with our expectation, plant total NSC_{CWM} concentrations significantly reduced with drought treatment at the EFS site, which was caused by the reduced starch$_{CWM}$ concentration with drought but not reduced SS_{CWM} concentration. This result is in line with the combined effects of species turnover and intraspecific variation in leaf total NSC_{CWM} concentrations and suggests experimental drought selected for plant species with lower starch concentration but similar SS concentration at this grassland site (Jung et al., 2014).
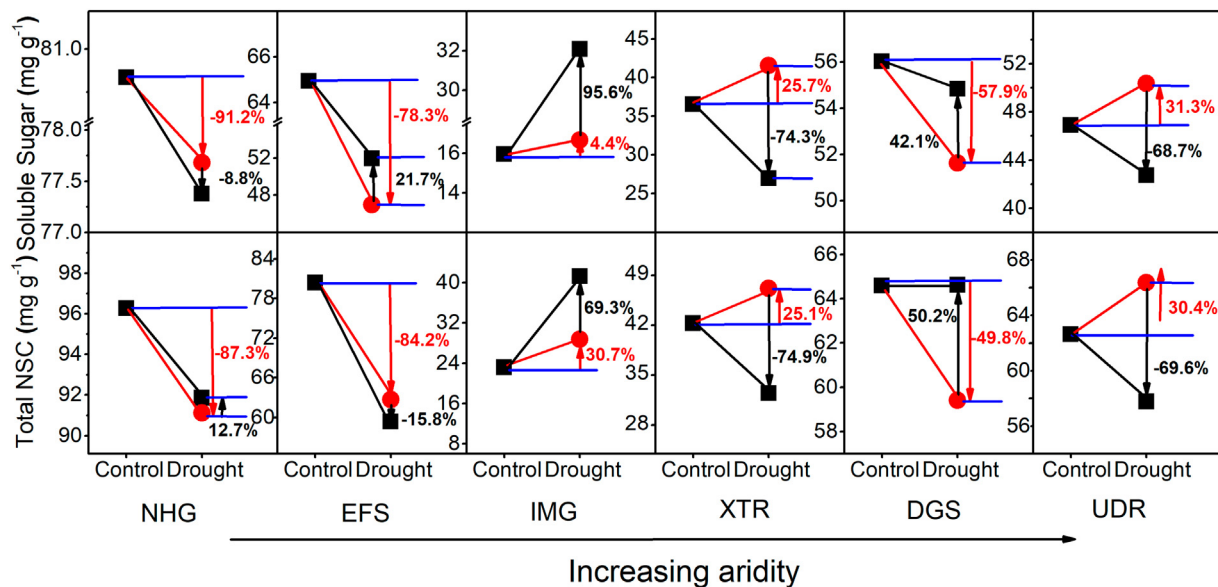

Fig. 3. Changes in plant community-weighted mean of soluble sugar and total nonstructural carbohydrate (NSC) concentrations in response to drought in grasslands of northern China due to species turnover (changes in species relative biomass) and intraspecific variation (changes in species NSC) or species turnover only for each site. Black squares correspond to community-weighted mean of soluble sugar and total NSC concentrations in control and drought plots; Red circles are calculated by each species' SS and total NSC concentrations in control plots but weighted consistent with species' relative biomass in drought plots. Arrows indicate the contributions of species turnover (red arrows, changes in species relative biomass) and intraspecific variation (black arrows, changes in species NSC) to the change in community-weighted mean of soluble sugar and total NSC concentrations. Species turnover and intraspecific variation are expressed as percentages of their cumulative magnitude. A parallel direction of shift in species turnover and intraspecific variation effects indicates positive (synergistic) covariation, whereas an opposite direction of shift in species turnover and intraspecific variation indicates negative (opposing) covariation. See Fig. 1 legend for the site abbreviation. Community-weighted mean of soluble sugar and total NSC concentrations are shown as mean ± SE ($n = 6$).

In contrast, plant NSC$_{CWM}$ concentrations did not respond significantly to experimental drought at all other experimental sites. This result is in line with the opposing effects of species turnover and intraspecific variation in leaf NSC concentrations at more arid sites. For example, at the XTR and UDR sites, mean NSC concentrations of all species were higher in control plots compared to drought plots (intraspecific variation), while species with high-NSC concentrations gradually replaced those with low-NSC concentrations (species turnover) in response to experimental drought (Figs. 3 and S7). The opposing effects of species turnover and intraspecific trait variability have also been observed for plant nutrient responses to drought (Luo et al., 2018). These results indicated that species turnover and intraspecific trait variability interact to stabilize NSC$_{CWM}$ concentrations in response to extreme drought (Leps et al., 2011). The influences of both intraspecific trait variability and species turnover should be considered simultaneously when evaluating the function of community trait structure (Volf et al., 2016).

While plant NSC$_{CWM}$ concentrations changed along the aridity gradient (Fig. 1), it did not respond strongly to experimental drought treatments (Figs. 1 and S5). The mismatch between experimental drought and aridity trends is not surprising and can be explained by differences in the temporal scale of aridity. Manipulative experiments reveal the initial plastic responses of plant communities and ecosystem functioning following extreme drought (Smith, 2011; Yuan et al., 2017). In contrast, natural aridity gradients allow for observations of the long-term responses of plant communities to water-limitation as well as shifts towards optimal function for a given aridity level (Smith, 2011; Yuan et al., 2017). Thus, manipulative and gradient experiments measure the plastic and evolutionary responses to drought, respectively. Ecosystem models should thus incorporate both experimental and gradient data to properly assess the responses of plant community structure and functioning to climate change such as increased drought frequency and intensity.

From these observed trends, we propose a conceptual model to evaluate the effects of short- and long-term water limitation on patterns of SS$_{CWM}$ and total NSC$_{CWM}$ concentrations in grassland ecosystem (Fig. 4). We propose a three-phase hypothetical process by which plant NSC$_{CWM}$ concentrations respond to aridity. Initially (phase A), there is a short-term plastic response during which a community shows stochastic fluctuation over time around some equilibrium NSC concentrations. Over time (phase B and C; long-term evolutionary response), plant NSC$_{CWM}$ concentrations

showed a U-shape relationship with increasing aridity, with an aridity threshold at intermediate levels of long-term aridity. In areas more mesic than this threshold (Phase B), plants adopt an acquisitive strategy (e.g., increasing leaf chlorophyll) to enhance photosynthetic capacity to offset the reduction in NSC concentrations and ANPP due to increased aridity. However, the resource acquisitive strategies cannot overcome the detrimental effects of reduced stomatal conductance on NSC concentrations under long-term aridity. At this critical aridity threshold, minimum NSC concentrations are reached to maintain the communities, but with potential high plant mortality. Plant NSC$_{CWM}$ concentrations are most vulnerable to short-term droughts such as those imposed here at this intermediate aridity threshold. Once the aridity threshold is passed over time, plants favor a conservative strategy (e.g., decreasing plant height) to reduce the NSC consumption in areas above this threshold. In this conceptual model, we substitute space for time, which has inherent caveats. In addition, our analyses did not include root NSC, which are critical for understanding plant responses to drought stress due to their essential roles in water and energic accumulation, transportation and supply (Du et al., 2020). Such root traits are rarely measured yet should be included in future studies on ecosystem responses to drought and to test the validity of the conceptual model described here.

CRediT authorship contribution statement

W.L. conceived the research; L.S., X.Z., Q.Y., W.M., and J.C. performed experiments; L.S., and W.L. analyzed data and drafted the manuscript, and all authors, especially Z.W., R.J.G., M.H.L., S.L.C., M.D.S., and H.H. contributed to further revising of the text. All authors read and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
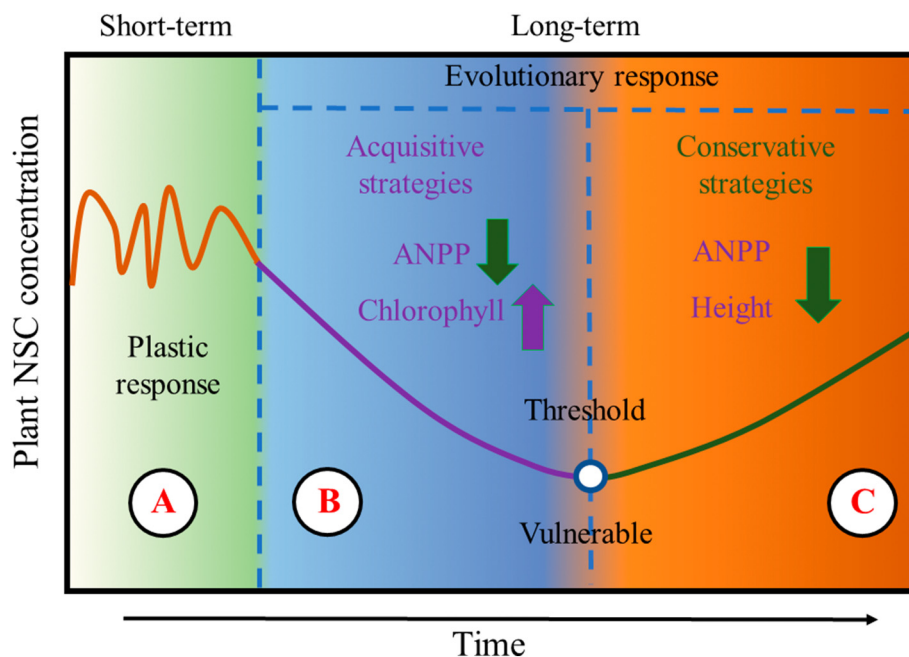
Acknowledgments

Fig. 4. A three-phase process conceptual model to evaluate the effects of short- and long-term drought on the patterns of community nonstructural carbohydrates in grassland ecosystems.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153589.

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