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Water stress in grasslands: dynamic responses of plants and insect herbivores

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Global climate change is altering precipitation patterns. The effect of water stress on plant–herbivore interactions is poorly understood even though this is a primary ecological interaction that will be altered by climate change. This is especially true for grasslands where water is often limiting. In this study we manipulated water inputs in open grassland plots (1 m^2) during a severe drought and assessed plant and insect herbivore responses. There were two watering treatments: ambient and supplemented. Supplemented plots received water weekly in amounts that mimicked average seasonal rainfall. For plants, we were interested in how water input affected protein and digestible carbohydrate content; previous studies predicted water stress would increase the concentration of these two nutrients. Grasshoppers are the dominant insect herbivores in grasslands and we assessed their responses to water inputs by measuring abundance and diversity. Previous studies suggested grasshoppers would prefer water-stressed plots. Protein and carbohydrate content in bulk grass and forb samples, plus plant biomass and diversity, were measured monthly (May–August). Immediately prior to harvesting plant tissue, we counted and identified individual grasshoppers in each plot. Grass biomass was reduced with water stress, but macronutrient content and species diversity were unaffected. After three months water-stressed forbs were less protein biased, and diverse, relative to watered forbs; forb biomass was indistinguishable between treatments. Grasshopper abundance and diversity were lower in water-stressed plots as the season progressed. However, grasshopper-feeding biology mattered: densities of mixed-feeders and grass-feeders, but not forb-specialists, decreased over time in water-stressed plots, but not in water supplemented plots. Our results demonstrate the importance of focusing on plant and insect herbivore functional groups and provide valuable new data that can be incorporated into models to explore the effects of global climate change in greater detail.

Global climate change is predicted to dramatically alter precipitation patterns and increase the frequency with which plants will be water stressed (Knapp et al. 2008, Dai 2010). This will likely have strong effects on associated herbivores, but despite high interest and years of study the effects of water stress on plant–herbivore interactions are still poorly understood (Huberty and Denno 2004). It has been suggested that water stress is beneficial to insect herbivores (White 1969, Mattson and Haack 1987). The proposed mechanism is that when plants are water stressed their quality to herbivores increases due to higher nutrient concentrations (White 1984, Brodbeck et al. 1987, Behmer and Joern 2012). However, water stress can constrain plant growth and decrease total nutrient content via decreased uptake of soil nutrients, decreased turgor pressure, xylem cavitation, reduced photosynthesis, senescence, and dieback of roots and shoots (Hsiao 1973). In some plants, allelochemical concentrations can also increase under water stress (Inbar et al. 2001). Many of these water-stress responses can vary as a function of plant photosynthetic pathway, growth form, species, and genotype (Chaves et al. 2002), but under prolonged or severe water stress plants eventually die as a result of carbon starvation as well as hydraulic and symplastic failure (McDowell et al. 2008).

From an herbivore's perspective, water stress induces changes in plant diversity (intraspecific variation in drought tolerance), quantity (changes in plant structure and biomass), and quality (shifts in nutrient concentration and allocation, reduced water content, increased leaf toughness, and altered defensive chemistry), which can all affect foraging and performance (White 1969, Koricheva et al. 1998, Huberty and Denno 2004). Plants differ in their tolerance of water stress (Chaves et al. 2002) and mortality of some species (McDowell et al. 2008) can lead to decreased diversity. High plant diversity might be particularly important for generalist herbivores because it provides greater opportunity to achieve a balanced nutrient intake via diet mixing (Bernays et al. 1994, Raubenheimer and Simpson 1999). When water is limiting, plant growth can be constrained (Hsiao 1973), limiting the quantity of plant food available to an herbivore. Plant macronutrient content, including protein and digestible carbohydrates (henceforth carbohydrate), is particularly critical for insect herbivores (Behmer and Joern 2012) which are known to simultaneously, and independently, regulate

their protein and carbohydrate intake (reviewed by Behmer 2009). Despite their functional importance, data describing the multidimensional nutrient landscape of plant protein and carbohydrate content across time, space, and plant taxa, let alone water gradients, are virtually non-existent.

In this study we tracked, over three months, the effects of water inputs in open field plots (1 m^2) on grassland plants and grasshoppers, the dominant grassland invertebrate herbivore. Grasslands are important ecosystems in which to study the effects of water stress because they make up ~ 40% of terrestrial landmass, experience frequent drought, and support most of the world's agriculture (Gibson 2009). Water availability can affect multiple traits in plants, but we were particularly interested in macronutrient content due to the increasing emphasis on nutrition in insect herbivore foraging decision-making (reviewed by Behmer 2009). We quantified actual levels of protein and carbohydrate in field collected plants across multiple time points in the growing season. In addition, we recorded two other potential bottom–up factors that can affect herbivore density and diversity: plant biomass and plant diversity. Each time plant data were collected we recorded the abundance of individual grasshopper species without destructive sampling. A key aspect of our study is that the open plots allowed the mobile grasshoppers to self-distribute, which allows the grasshoppers to tell us which plots are preferred. Because we identified individual grasshoppers to species, we could evaluate distributions in light of functional feeding groups (grass-specialists, forb-specialists and mixed-feeders).

We structured our hypotheses based on the findings of previous physiological work with water stressed plants and observations of associated herbivores. Water stress tolerance varies between plant species and functional groups (Hsiao 1973, Lauenroth et al. 1978, Chaves et al. 2002, McDowell et al. 2008), so under continuous water stress we predicted plant diversity could decrease as drought intolerant species perish. Because continuously water stressed plants experience constrained growth, dieback of roots and shoots, and eventually plant mortality (Hsiao 1973), we predicted biomass could be maintained at first, but would decrease over time. Our focal variable, plant macronutrient content, may initially increase in water stressed plants because of increased soluble nutrient concentration (White 1984, Brodbeck et al. 1987, Behmer and Joern 2012), but should eventually decrease with continuous stress as photosynthesis is disrupted (Hsiao 1973, Chaves 1991, Huberty and Denno 2004). As previously stated, though, surprisingly little is known about actual variability of macronutrient content in plants. Based on earlier work with grasshoppers (White 1984, Mattson and Haack 1987, Franzke and Reinhold 2011), we hypothesized grasshopper abundance and diversity would be higher in unwatered plots early in the season due to these plants having higher nutritional value. However, because prolonged water stress might reduce plant quality, quantity, and diversity, we expected grasshopper abundance and diversity to eventually decrease as the season progressed. Our study provides the first analysis of how drought affects macronutrient content of native grassland plants, coupled with responses of grasshoppers, the key insect herbivores in our system.

Methods

Study system

This study was conducted at the Balcones Canyonlands National Wildlife Refuge (BCNWR) located northwest of Austin, Texas. The refuge covers parts of Burnet, Williamson and Travis Counties. The geology of the study site is characteristic of the Edwards plateau with limestone hills and shallow rocky soils. The BCNWR (established in 1992) is not grazed and is managed with prescribed burns on a 2–4 year cycle. The experiment utilized areas of mixed-grass prairie and oak *Quercus* sp. savannah, and was conducted between 8 May and 4 August 2011. Site locations are given in Supplementary material Appendix 1 Table A1. This grasshopper community is diverse with 56 species of grasshoppers (Orthoptera: Acrididae) and includes widespread species of the Great Plains as well as several Texas endemics (Supplementary material Appendix 1 Table A2). The grasshopper community is dominated by polyphagous mixed-feeding grasshoppers (eating both forbs and grasses) and, to a lesser extent, grass specialists. Forb specialists make up the smallest proportion of the community. Plant and grasshopper communities were similar across all sites (Supplementary material Appendix 1 Table A3, A4).

Experimental protocol

From May to August of 2011 we conducted a water manipulation experiment to examine the effects of drought on a diverse grasshopper community. This time period spans the nymphal and adult stages for most of the area's grasshopper fauna. We delimited four sites on the BCNWR with 28 1-m^2 plots each. Plots were arranged in 2–3 rows and marked with survey flags. Plots were randomly assigned to two treatments: either supplemented with water from May to August or maintained as unmanipulated ambient controls. Control plots were the drought treatment and were allowed to desiccate during the La Niña-driven severe drought (based on predictions by the National Weather Service's Climate Prediction Center, <www.cpc.ncep.noaa.gov/>). The la Niña-driven drought of 2011 was the worst single year drought in recorded Texas history (Nielsen-Gammon 2011). From September 2010 to September 2011 the BCNWR received 21.26 cm of rainfall, while the average annual rainfall since 1996 was 81.03 cm with peaks in May / June and September / October based on a weather station at the BCNWR. We supplemented watered plots at a rate intended to mimic higher rainfall in the area. Watering occurred weekly and was limited to 2.5cm of simulated rainfall per plot (25 $l\ m^{-2}$); by the end of sampling in August, watered plots had been supplemented with a total of 30 cm of simulated rainfall. Ambient rainfall during this same period was limited to 13.0 cm. The average rainfall in the area for this period from 1996–2010 was 25.7 cm with a range of 10–53.7cm. We watered during early morning to allow infiltration and avoid excessive evaporation.

Plots were spaced 2–5 m from adjacent plots to eliminate run-off effects and decrease movement of grasshoppers between experimental plots. While open plots have the potential for non-independence (Gotelli and Ellison 2012),

we mitigated this by randomizing which plots were treated, and spacing plots at variable distance from one another (Gotelli and Ellison 2012). Movement in rangeland grasshoppers is density dependent, and has been found to be only 1–3 m over 36 h even at densities higher than what was observed in this study (Narisu et al. 1999). Furthermore, it is unlikely that grasshoppers detected more preferable plant traits in the watered plots at any great distance. Previous work has found that rangeland grasshoppers move in relation to the prevailing wind or randomly (Narisu et al. 1999) and are retained by areas with preferable host plants that are encountered through close range olfactory cues or tactile contact and tasting (Mulkern et al. 1969, Blust and Hopkins 1987, White and Chapman 1990, Szentesi et al. 1996, Chen and Kang 2000, Kang and Hopkins 2004).

We took an initial sample of four control plots at each site the first week of May before watering began. After watering treatments had been established, we randomly sampled eight plots per site (four control, four watered) during the first week of June, July and August. We did not re-sample individual plots from month to month and water manipulations ceased after a plot had been sampled. When sampling we quantified the grasshopper species richness and plot density as well as plant functional group species richness, biomass and macronutrient content.

Grasshopper and plant sampling

Just prior to harvesting plant material, one of us (PAL) counted and identified all grasshopper species present in each plot by flushing them by hand. Because we only sampled a random subset at each time point, distance between plots sampled at the same time was much larger than the minimum distance between plots (2 m). When nymphs were disturbed during counting they would move less than the distance between plots. When sampling we took careful note of where flying adult grasshoppers went when flushed to make sure they were not counted in a different plot. In later months when grasshoppers became adults, densities were lower making this tracking relatively easy. Grasshoppers were not collected to avoid changing the grasshopper community at each site. Vouchers of grasshopper species collected away from experimental plots were deposited in the Texas A&M Univ. Insect Collection (TAMUIC). Focusing on grasshoppers as a taxonomic group (at the family level of Acrididae) can reveal interesting general patterns, but this approach also obscures the reality that not all acridids are biologically equivalent. During data analysis, we also grouped species into their functional diet groups: forb-, grass- and mixed-feeders (Supplementary material Appendix 1 Table A1). We measured plant species richness within a $0.25m^2$ quadrat placed within each plot. Vouchers of plant species were deposited in the Texas A&M Univ. Tracy Herbarium.

Plant biomass and nutrient content

To estimate drought-induced changes in the biomass of plant functional groups and their respective macronutrient content, we clipped a 1×0.1 m strip of vegetation across the center of each plot at ground level. We separated living forbs and grasses from each sample because rangeland grasshoppers rarely consume dead dry litter. Samples were then lypholized, weighed, and subsequently milled and homogenized using a Wiley cutting mill (size 20 mesh). Any hard stems from forbs were removed from samples prior to milling because rangeland grasshoppers generally do not eat stems. From these milled samples of forb and grass, replicated 20 mg subsamples were taken for protein and carbohydrate analysis.

Total nonstructural carbohydrates and soluble protein were analyzed using the methodology of Clissold et al. (2006). Protein was extracted from 20 mg samples with 500 µl 0.1 M NaOH by sonication for 30 min and heating at 90°C for 15 min. Samples were centrifuged (13 000 rpms for 10 min), the supernatants were removed, and the pellet washed with 300 µl of 0.1 M NaOH and centrifuged again. After removing this supernatant and combining it with the previous supernatant, the pH was neutralized using 11 µl of 5.8 M HCl. Protein was then precipitated with 90 µl of 100% trichloroacetic acid. The samples were centrifuged to form a pellet of protein that was quickly washed with 100 µl of –20°C acetone after the supernatant was removed. The acetone was allowed to evaporate and proteins were resuspended in 1 ml of 0.1 M NaOH and then diluted to ensure the concentration of NaOH were less than 0.01 M so that it did not interfere with Coomassie blue solution used by the Bradford assay. To quantify digestible protein we used the Bio-Rad micro assay based on the Bradford assay (Bradford 1976) with 0–8 µg of IgG (bovine gamma globulin) as the standard with duplicate samples read in triplicate. Total non-structural carbohydrates were extracted from 20 mg samples placed for 1 h in a boiling water bath with 1 ml 0.1 M H_2SO_4 and determined colourimetrically (0–75 mg (D +) glucose standard) using the phenol–sulphuric acid assay (Dubois et al. 1956).

Statistical analysis

The four sites used were treated as blocks in all analyses. Site, month, watering treatment and month × treatment interactions were used as explanatory variables for grasshopper plot density and species richness among all grasshoppers and for each individual feeding group (forb-, grass- and mixed-feeders) in a generalized linear model (GLM). Within the GLM a Poisson distribution was used as the count data were not normally distributed (O'Hara and Kotze 2010). Within-group contrasts, i.e. between months or treatments, were made using likelihood-ratio tests (JMP 10 Modeling and multivariate methods, SAS Inst.). Because of the importance of the protein and carbohydrate ratio in insect herbivore nutrition (Behmer 2009) we analyzed protein and carbohydrate together using MANOVA against the same explanatory variables as above with arcsine transformed data. Plant macronutrient content was quantified in terms of percent dry mass. The Roy's greatest root test statistic is reported as most of the variance occurred in terms of protein. Effects of site, month, watering treatment and month × treatment interactions on species richness and plant functional group biomass were analyzed using ANOVA for a randomized complete block design. All analyses were conducted in JMP 10 (SAS Inst.).

Results

Grass responses

Protein and carbohydrate levels in grasses were unaffected by watering treatment (Fig. 1a–c, Table 1a). The protein-carbohydrate profile of grasses, however, varied significantly over the course of the growing season and between sites (Fig. 1a–c, Table 1a). Grass protein content decreased from June to July, ($F_{1,84} = 27.86$, $p < 0.001$), but then increased from July to August (Fig. 1b; $F_{1,84} = 17.13$, $p < 0.001$). Grass protein content varied significantly between sites, with marginally significant ($0.1 > p > 0.05$) variation in carbohydrate content (Table 1a).

Grass biomass was consistently higher in watered plots (Fig. 1d; ANOVA, treatment: $F_{1,1} = 4.30$, $p = 0.041$, time × treatment $F_{2,2} = 0.21$, $p = 0.814$), differed across the four sites (ANOVA, site: $F_{3,3} = 3.66$, $p = 0.015$), and decreased from May to August (ANOVA, time: $F_{2,2} = 5.07$, $p = 0.008$). Grass species richness was unaffected by the watering treatment, (Fig. 1e; ANOVA, treatment: $F_{1,1} = 0.80$, $p = 0.374$, time × treatment: $F_{2,2} = 0.48$, $p = 0.621$) and remained constant throughout the growing season (ANOVA, time: $F_{2,2} = 2.07$, $p = 0.132$).

Forb responses

Forb protein–carbohydrate content showed greater variation (Fig. 2a–c) than grasses. As the growing season progressed,

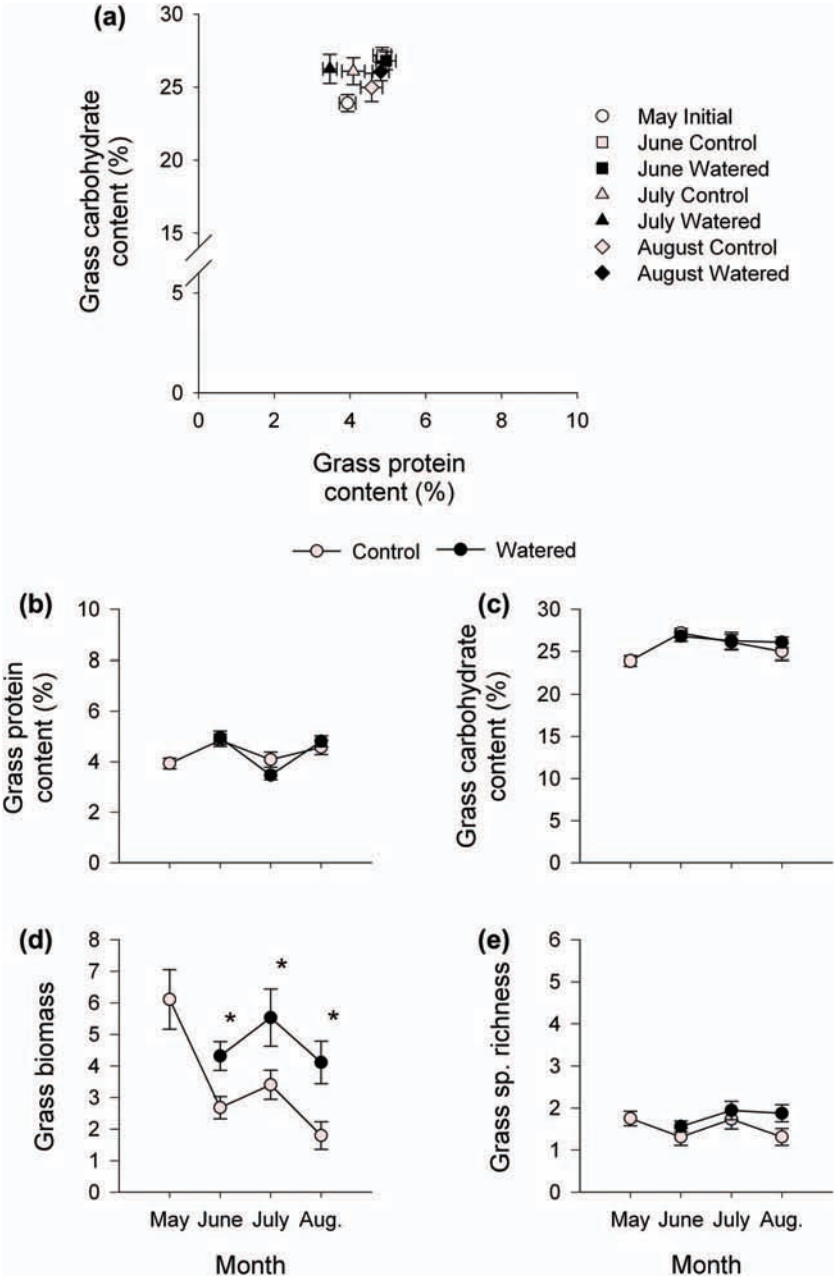

Figure 1. Grass responses to water stress, (a) protein and digestible carbohydrate biplot, (b) soluble protein content, (c) digestible carbohydrate content, (d) biomass (dry weight g 0.1 m^{-2}), and (e) species richness of grasses for control and watered plots across months (May–August). Mean and standard error bars are displayed. Asterisks denote significant $p < 0.05$.

Table 1. Results of MANOVAs for protein and carbohydrate content of (a) grass and (b) forb both combined and with univariate analyses for protein and carbohydrate separately. Approximate F for the Roy's greatest root test statistic is given for multivariate comparisons, except for Treatment effects and all univariate comparisons, which has the exact F reported. Asterisks denote significant $p < 0.05$.

Source	Test	DF	Prob> F
(A) Protein and carbohydrate content of grass			
Site	6.12	3,84	0.001*
Time	15.55	2,84	<0.001*
Treatment	0.08	2,83	0.922
Time × Treatment	2.28	2,84	0.109
Protein content of grass			
Site	6.06	3,84	0.001*
Time	15.54	2,84	<0.001*
Treatment	0.02	2,83	0.887
Time × Treatment	2.24	2,84	0.112
Carbohydrate content of grass			
Site	2.62	3,84	0.056
Time	1.74	2,84	0.182
Treatment	0.13	2,83	0.722
Time × Treatment	0.48	2,84	0.618
(B) Protein and carbohydrate content of forb			
Site	6.14	3,84	<0.001*
Time	14.16	2,84	<0.001*
Treatment	1.15	2,83	0.322
Time × Treatment	3.45	2,84	0.036*
Protein content of forb			
Site	1.38	3,84	0.254
Time	14.15	2,84	<0.001*
Treatment	0.12	1,84	0.727
Time × Treatment	1.73	2,84	0.184
Carbohydrate content of forb			
Site	5.33	3,84	0.002*
Time	0.36	2,84	0.699
Treatment	2.03	1,84	0.158
Time × Treatment	1.34	2,84	0.268

forbs in watered plots developed more protein-biased macronutrient profiles than unwatered forbs (Fig. 2a–b, Table 1b). In August the average protein:carbohydrate (p:c) ratio of watered plots was 1:3, compared to 1:3.6 in unwatered plots. Protein–carbohydrate content also differed between sites and months (Table 1). Despite the change in p:c ratio, univariate tests found that, independently, neither protein or carbohydrate content was affected by watering, nor was there a significant time-by-treatment interaction (Table 1b). Protein content, but not carbohydrate content, varied between months (Table 1b); protein content dropped from June to July ($F_{1,84} = 20.76$, $p < 0.001$) and then increased in August ($F_{1,84} = 21.36$, $p < 0.001$). Carbohydrate content varied between sites (Table 1b).

Forb biomass was unaffected by the watering treatment (ANOVA, treatment: $F_{1,1} = 0.14$, $p = 0.713$, time × treatment: $F_{2,2} = 1.11$, $p = 0.336$) and decreased steadily over the course of the summer (Fig. 2d; ANOVA, time: $F_{2,2} = 5.62$, $p = 0.005$). The number of forbs species in any given plot decreased over the summer (Fig. 2e; ANOVA, time: $F_{2,2} = 12.09$, $p < 0.001$), but the rate of decline was significantly higher in unwatered plots (ANOVA, time × treatment: $F_{2,2} = 3.54$, $p = 0.033$).

Grasshopper responses

Total grasshopper density declined in all field plots during the growing season, but this decline was dramatically faster in unwatered plots (Fig. 3a, Table 2). By the end of the experiment, grasshopper abundance in control plots was almost three times lower than in watered plots ($\chi^2 = 20.27$, $DF = 1$, $p < 0.001$). Grasshopper species richness followed the same pattern (Table 2, Fig. 3b) with significant differences between treatments evident in August ($\chi^2 = 7.91$, $DF = 1$, $p = 0.005$).

Grass-feeding grasshoppers responded positively to watering supplementation in the final month of sampling (Fig. 3c–d, Table 2). Between May and July the density of grass-feeding grasshoppers varied little. However, in August density in watered plots nearly doubled while the density in unwatered control plots decreased ($\chi^2 = 12.80$, $DF = 1$, $p < 0.001$). The species richness of grass-feeders also increased in watered plots, but decreased in control plots in late summer (Fig. 3d); this pattern was only marginally significant (Table 2). Forb-feeding grasshoppers, on the other hand, were unaffected by the watering treatment (Table 2, Fig. 3e–f). The abundance of mixed-feeding grasshoppers declined over the course of the growing season, but was higher in watered plots by the end of sampling (Fig. 3g–h, Table 2). From May to June mixed-feeder density did not change, although, control plots in June had a marginally significant trend towards higher density ($\chi^2 = 3.47$, $DF = 1$, $p = 0.063$). Between June and July, densities in both control and water-treated plots declined. However, in July there were marginally more mixed-feeding grasshoppers in watered plots than control plots ($\chi^2 = 3.73$, $DF = 1$, $p = 0.053$), but in August this difference was significant ($\chi^2 = 8.15$, $DF = 1$, $p = 0.004$). Over the course of the summer the species richness of these mixed-feeding grasshoppers declined, with a marginally significant trend for more species in watered plots compared to control plots in later months (Fig. 3h, Table 2).

Discussion

Previous studies exploring the effects of water stress on plant nutritional quality have focused primarily on nitrogen (N), amino acids, and/or protein (White 1984, Franzke and Reinhold 2011). However, water stress influences multiple plant primary metabolites (Mattson and Haack 1987). Furthermore, because insect herbivore performance is determined by both the amounts and ratios of multiple nutrients, especially protein and digestible carbohydrates (Raubenheimer and Simpson 1999, Behmer 2009), working in a single nutritional dimension (e.g. N) fails to adequately capture how water-stress impacts plants as nutritional resources for insect herbivores. Our results indicate that water-stress eventually affects the p:c ratio of forbs, but not grasses over the course of a growing season. A key aspect of our approach was to also track grasshopper responses to water inputs over time, and generally we found more grasshoppers (particularly grass- and mixed-feeding grasshoppers) in water-supplemented plots at the end of the summer. By combining plant and grasshopper responses to water stress over a growing season, our study

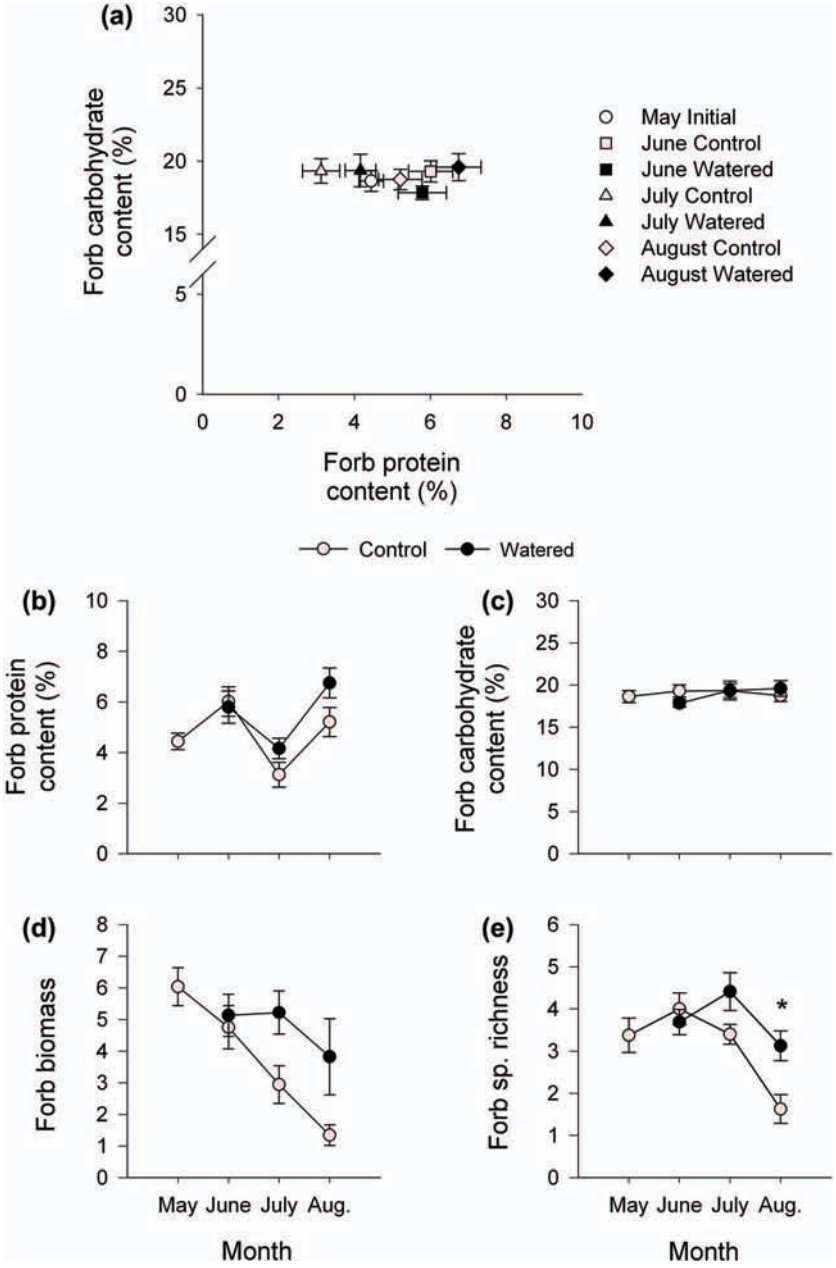

Figure 2. Forb responses to water stress. (a) protein and digestible carbohydrate biplot, (b) soluble protein content, (c) digestible carbohydrate content, (d) biomass (dry weight g 0.1 m^{-2}), and (e) species richness of forbs for control and watered plots across months (May–August). Mean and standard error bars are displayed. Note: when protein–carbohydrate content was analyzed using MANOVA (Table 1b), a significant difference between the control and watered plots was detected for August (the grey and black diamonds, respectively). Asterisks denote significant $p < 0.05$.

provides novel insights into how water stress can affect plant–insect herbivore interactions.

Grassland food webs are complex. In our case, we had many grasshopper species of variable diet breadth (Supplementary material Appendix 1 Table A2), and many plant species (Supplementary material Appendix 1 Table A4) that vary in their suitability as food. We reduced this complexity to the functional groups of grasses and forbs with the understanding that individual plant species may have responded differently than the average of each plant functional group. Furthermore, because insect herbivore foraging decisions can be highly nuanced (Mulkern et al. 1969, Bernays and Chapman 1994, Behmer 2009), these functional plant groups are a relatively coarse estimation to understand the greater grasshopper community's response to water-stressed plants. Despite these caveats, our results are important because they provide the first quantification of the plant protein–carbohydrate landscape available to insect herbivores in a grassland ecosystem. More significantly, they document the dynamic nature of protein and carbohydrate content of native grasses and forbs across a growing season, across different fields, and between watered and unwatered plots. Our data reveal that

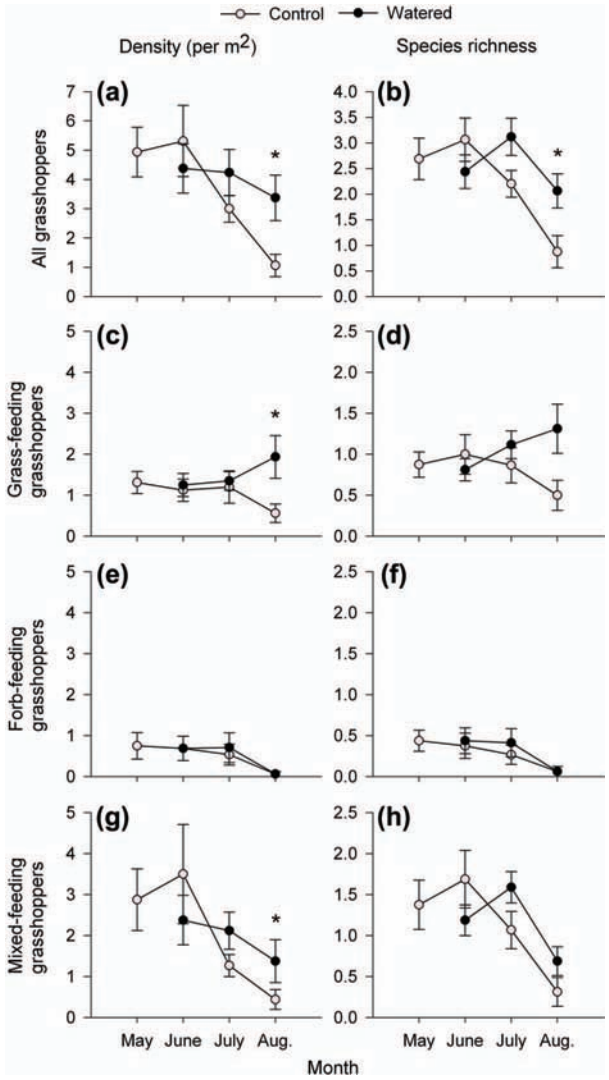

Figure 3. Grasshopper density and species richness (mean ± SE) of control and watered plots across months (May–August) for (a, b) all grasshopper species combined as well as the three functional grasshopper feeding groups separately: (c, d) grass specialists, (e,f) forb specialists, and (g ,h) mixed-feeding grasshoppers. Asterisks denote significant $p < 0.05$.

grasses and forbs responded differently to the water inputs, but neither followed the prediction that total digestible protein and carbohydrate content would increase under water stress. Our predictions of eventual decreases in species richness and diversity were partially supported, with different responses from the plant functional groups.

Grasses displayed similar macronutrient profiles on both watered and ambient controlled plots and this occurred despite several months of the worst drought in recorded Texas history (Nielsen-Gammon 2011). Grass biomass increased in watered plots immediately, possibly due to increased uptake of limiting nutrients (N and P) locked in the soil (Lambers et al. 2008), but the grasses maintained the same species richness (1–2 species) as well as average foliar protein and carbohydrate content. Previous water stress studies have found mixed effects of drought on protein content in grasses. Among C-3 grasses water stress has been reported to cause both increases and decreases in protein (Franzke and Reinhold 2011, Walter et al. 2012). In C-4 grasses, Barnett and Naylor (1966) found that soluble protein decreased with water stress. These three studies utilized greenhouse-grown plants. In contrast, our study utilized established, drought acclimated, perennial C-4 bunchgrasses (Supplementary material Appendix 1 Table A4), which use water more efficiently than C-3 grasses (Ghannoum 2009). That our grasses were drought hardened in the field, not young greenhouse reared plants, may explain the lack of a macronutrient or diversity shift in our study.

In forbs, protein and carbohydrate concentration did not shift in a significant fashion individually, but we did observe a significant shift in the average p:c ratio. Specifically, forbs in watered plots became more protein biased over the course of the growing season. Surprisingly, it took weeks of continuous drought to observe a shift in forb p:c ratios (Fig. 2a). This resilience could be due to a number of drought resistance traits such as modification of root structure, osmotic adjustments, reduced stomatal conductance, increased transpiration efficiency and high temperature tolerance (Ludlow and Muchow 1990). The eventual increases in protein content in water-supplemented forbs may reflect better uptake of soil N, leading to protein synthesis, which could be invested in growth and reproductive structures (Lambers et al. 2008). Unlike grasses, we could not detect a significant effect of water stress on forb biomass, but forb species richness was significantly decreased in water stressed plots by August, most likely due to mortality of less drought tolerant species (Chaves et al. 2002, McDowell et al. 2008).

Contrary to our predictions total grasshopper density and species richness increased on watered plots by the end of the experiment. We believe that the plant community indirectly mediated water treatment effects on grasshoppers. By August, water supplementation had established patches with more grass biomass, greater forb diversity, and forbs with higher p:c ratios. Due to the scale of the experiment (1-m^2 plots) and the open plot design, density changes were likely due to grasshoppers aggregating in watered plots. However, it is important to note that our approach precludes any inferences concerning survival or reproduction. During drought, insect herbivore populations may become more patchily distributed on surviving vegetation, for example in mesic habitat. In some instances, high densities of insect herbivores on remaining vegetation patches (during a drought) may give an impression that a given species has undergone an 'outbreak'(Mattson and Haack 1987). In the case of Orthoptera species that exhibit phase polyphenism (none occurred in our plots) drought induced patchiness can lead to outbreaks brought on by crowding (Despland et al. 2000).

Treating all grasshopper species as a single taxonomic group may fail to account for potentially important biological differences associated with functional feeding groups. For example, previous studies have shown that herbivores in different feeding guilds (e.g. leaf chewers, phloem feeders, etc.) respond differently to water-stressed plants (Schowalter et al. 1999, Huberty and Denno 2004). Our findings show that even within a family of physiologically similar leaf-chewing insects (Acrididae), response to a drought-stressed plant community differed. We suspect that these differences

Table 2. The effects of time and treatment on density and species richness for all grasshoppers combined, as well as forb, grass, and mixed-feeding grasshoppers separately. Results are based on analysis with generalized linear models (Poisson distribution). Asterisks denote significant $p < 0.05$.

		Density			Species richness		
Feeding group	Source	DF	χ^2	$p>\chi^2$	DF	χ^2	$p>\chi^2$
All grasshoppers	Site	3	60.97	<0.001*	3	13.30	0.004*
	Time	2	39.61	<0.001*	2	17.69	<0.001*
	Treatment	1	1.45	0.228	1	1.14	0.286
	Time × Treatment	2	19.95	<0.001*	2	8.90	0.012*
Grass-feeding	Site	3	29.82	<0.001*	3	16.99	0.001*
	Time	2	0.69	0.707	2	0.52	0.769
	Treatment	1	0.11	0.746	1	0.31	0.577
	Time × Treatment	2	7.70	0.021*	2	4.77	0.092
Forb-feeding	Site	3	33.28	<0.001*	3	16.59	0.001*
	Time	2	21.99	<0.001*	2	9.67	0.008*
	Treatment	1	0.00	1.000	1	0.08	0.781
	Time × Treatment	2	0.35	0.840	2	0.22	0.896
Mixed-feeding	Site	3	51.17	<0.001*	3	4.21	0.240
	Time	2	39.27	<0.001*	2	18.01	<0.001*
	Treatment	1	3.47	0.062	1	1.40	0.237
	Time × Treatment	2	14.65	0.001*	2	4.95	0.084

reflect each functional feeding group's host plant response. Previous work has shown that different grasshopper functional groups also show differential responses to weather, fire, and bison grazing (Jonas and Joern 2007).

Grasshoppers specializing on grasses showed a strong response to watering in the final month of the experiment; more of these grasshoppers were counted in watered plots relative to ambient plots. Grass-feeding grasshopper may have been responding to grass biomass, as numbers did track with changes in grass biomass; responses to other grass traits were not observed. With respect to forb-feeding grasshoppers, density and species richness were unaffected by the watering treatment despite water treatment effects on forb p:c ratio and diversity. Although forb diversity increased with water, forb-feeding species are functionally different from other generalist grasshoppers in that they specialize on only a few, related species of plants that share common similar defensive chemistry (Traxler and Joern 1999, Pfadt 2002). That forb-feeding grasshoppers did not track changes in forb p:c ratio suggests that subtle shifts in plant nutrient content are less important than secondary plant compounds, which identify plants as suitable food plants (Bernays and Chapman 1994). Finally, the response of mixed-feeder grasshoppers is likely a result of their diet-mixing feeding ecology. Mixed-feeder abundance declined at a slower rate as the drought progressed on watered plots, and this change was associated with higher grass biomass, a more protein-biased forb macronutrient profile and higher forb species richness. Although mixed-feeders utilize both grass and forbs, most of these species mainly feed on forbs (Joern 1985). Mixed-feeders tightly regulate macronutrient intake via diet mixing (Behmer and Joern 2008), so a higher forb species richness would allow generalist grasshoppers greater flexibility with respect to choices related to diet mixing. This can lead to better nutrient intake and dilutes any one plant's allelochemical defenses (Hagele and Rowell-Rahier 1999, Behmer et al. 2002, Singer et al. 2002).

Our approach reveals novel insights concerning how water inputs can affect plants and associated herbivores over a growing season, but how can these results be extended more broadly? A key challenge for ecologists and modelers of climate change is the need to understand and incorporate biotic interactions in models of future climactic conditions (Van der Putten et al. 2010). Our study provides valuable quantitative data that contrasts how different plants (C-4 grasses and C-3 forbs) and insect herbivores (grass-specialists, forb-specialists and mixed-feeding grasshoppers) respond to a key environmental variable (water availability) during the course of a growing season. Our study also demonstrates that caution must be taken to not over simplify the biology of the study organisms. In many instances the devil really is in the details, and parameterizing these devilish details into nutritional ecology models (Raubenheimer et al. 2009, Kearney et al. 2010, Simpson et al. 2010) may help us better understand global climate change. Specifically, by combining advancements in agent-based models, state-space models of nutrition and multi-scaling modeling of landscape ecology, it might be possible to predict how individual herbivores will forage in a variable landscape of plant quality and quantity. In turn, this could be scaled up to investigate how changes in plant quality, biomass, and diversity affect insect herbivore populations and communities, as well as inform ecosystem dynamics. The use of remote sensing to assess changes in plant nutrient content (Foley et al. 1998, Zengeya et al. 2013) and other plant characteristics such as water content (Ullah et al. 2012b), biomass (Ullah et al. 2012a), and possibly even plant diversity (Gould 2000), could facilitate model validation and real world application at landscape and regional scales.

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Supplementary material (available as Appendix oik.01370 at <www.oikosjournal.org/readers/appendix>). Appendix 1