

DISSERTATION

EFFECTS OF DROUGHT ON THE SHOTGRASS STEPPE OF THE NORTH AMERICAN
GREAT PLAINS

Submitted by

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ABSTRACT

EFFECTS OF DROUGHT ON THE SHORTGRASS STEPPE OF THE NORTH AMERICAN GREAT PLAINS

Global climate models predict the frequency, intensity, and duration of extreme weather events, such as severe drought, will increase during the 21st century across many regions of the world, including the semi-arid grassland biomes of North America. Periods of water stress routinely occur in semi-arid grasslands, therefore, the consequences of even greater aridity on ecosystem services such as aboveground net primary production (ANPP) warrants considerable attention. Integrating complementary field studies that spanned a latitudinal gradient, this dissertation had three main objectives. The first was to assess the sensitivity of the shortgrass steppe (SGS), a semi-arid grassland that extends from northern Colorado to New Mexico and Texas, to varying degrees of drought. The second was to explore whether invasibility of the SGS increased following drought. And the third was to examine drought legacy effects on ecosystem functions of this semi-arid grassland after drought conditions subsided. Results from the study assessing drought sensitivity revealed variability in how these ecosystems responded to drought, ranging from no reductions in ANPP at the southern end of the latitudinal gradient to 51% reductions in ANPP at the northern end. I concluded that patterns of ANPP responses to drought across these grasslands were strongly related to rainfall event size. Specifically, when rainfall regimes were characterized by many small events, significant reductions in ANPP occurred whereas when rainfall regimes were dominated by fewer events that were larger in size reductions in ANPP did not occur. Results from the study exploring whether invasibility

increased during the post-drought period showed that drought can create a “window of opportunity” for plant invasions – even in invasion resistant semi-arid grasslands – if rainfall is sufficient and soil nitrogen is elevated following the drought. This study found that invasibility increased in the northern end of the latitudinal gradient but not in the central region or the southern end, thus indicating that invasions are highly context-dependent. Finally, results from the study that examined drought legacy effects revealed that impacts of drought persisted into the post-drought year at two of the three sites in the SGS biome and that these were related to decreases in plant cover and increases in soil nitrogen. Results showed that when soil N is elevated post-drought, there may be a positive legacy effect, but when soil N is not higher following drought, a negative legacy effect will likely occur. Overall, these results indicate that the semi-arid SGS grasslands I studied were generally quite resilient to drought. However, when these ecosystems displayed drought sensitivity, invasion windows and legacy effects were evident.

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DEDICATION

*To my mother, Shirley M. Cherwin-Engbretson,
and in loving memory of William C. Cherwin and Betty A. Marg.*

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CHAPTER 1: INTRODUCTION

Global climate changes resulting from anthropogenic emissions of greenhouse gases are affecting terrestrial ecosystems worldwide (Weltzin 2003; Seager 2007; IPCC 2014). One of the major impacts of the changing climate is the increased occurrence and severity of extreme weather events, including droughts (Easterling et al. 2000; Rosenzweig et al. 2001; Smith 2011). Although aridity has been intensifying in many regions of the world since the mid-1900s (Dai 2013), droughts are expected to increase even more in terms of frequency, intensity, and duration in the coming decades (Kirtman et al. 2013; Dai 2013; Cook et al. 2015). In fact, robust climate model projections suggest that during the 21st century certain areas of the world, including the semi-arid shortgrass steppe (SGS) biome of the North American Great Plains, will face drought conditions that will meet or exceed the worst droughts ever to occur in the previous millennium, such as the Dust Bowl drought that occurred in the 1930s (Cook et al. 2014). Periods of water stress are already a defining characteristic of all semi-arid grasslands (including the SGS; Lauenroth et al. 2008). Thus, given the dire predictions of escalating droughts for this region, it is important to understand how this biome will respond to increasing aridity.

It is widely recognized that precipitation, due to its effect on soil moisture availability, is the primary abiotic factor limiting ecosystem processes in many terrestrial ecosystems, including the SGS, particularly aboveground net primary productivity (ANPP; Sala et al. 1988). The amount of primary production in semi-arid grasslands influences numerous other ecosystem functions. For instance, ANPP in these ecosystems determines forage availability and in turn controls herbivore carrying capacity (Lauenroth and Sala 1992), serves as an important carbon reservoir and thus plays an important role in the carbon cycle (Burke et al. 2008; Hoover and

Rogers 2016), and mediates organic matter inputs into soil and thereby affects soil pedogenesis (Kelly et al. 2008). Since semi-arid ecosystems cover roughly 40% of Earth's land area (Austin et al. 2004; Sala et al. 2012), their ability to respond to the drying trends predicted for the future could seriously impact the global carbon cycle (Yahdjian and Sala 2006; Sala et al. 2012; Poulter et al. 2014; IPCC 2014; Ahlström et al. 2015).

The relationship between precipitation inputs and ANPP has been evaluated on both large spatial and long temporal scales (Webb et al. 1978; Lauenroth and Sala 1992; Knapp and Smith 2001; Huxman et al. 2004). However, the majority of these analyses have used either: 1) observational data from multiple sites across biomes that span natural precipitation gradients, or 2) empirical data from single sites located within biomes with the implicit assumption that responses are, at least in part, representative of the entire biome. Far fewer experimental studies exist that incorporate multiple sites within individual biomes, including the semi-arid grassland biome.

My research was conducted over three years at three study sites distributed along a latitudinal gradient that spanned much of the north to south range of the semi-arid grassland biome, located on the western edge of the North American Great Plains. The northern site was at the Central Plains Experimental Range (CPER; 40°49'N latitude, 104°46'W longitude) in north-central Colorado, ca. 24 km north of Nunn, CO. The central site was Sand Creek Massacre National Historic Site (SAND; 38°32'N latitude, 102°31'W longitude) in south-east Colorado, ca. 19 km east of Eads, CO. The southern site was at Fort Union National Monument (FOUN; 35°91'N latitude, 105°01'W longitude) in north-central New Mexico, ca. 45 km north of Las Vegas, NM. Mean annual precipitation (MAP) across the sites ranges from 342 mm in the north to 425 mm in the south and mean annual temperature (MAT) ranges from 7.2° to 9.4° C.

At each of the study sites, two levels of drought treatments (-50% and -80% ambient precipitation) were imposed over the growing seasons (May – September) of 2007 and 2008. Drought treatments were achieved by constructing rainout shelters that passively excluded precipitation.

The overall objectives of my research were to assess the effects of drought at three sites in the North American semi-arid grassland biome by experimentally reducing precipitation. I structured this dissertation around three complementary field studies that addressed these objectives. In the first study, presented in chapter 2, I assessed the sensitivity of semi-arid grasslands to a drought disturbance event and compared the degree of sensitivity across sites. Because disturbance events are generally known to enable the success of plant invasions (Hobbs and Huenneke 1992; Schrama et al. 2016), a second study, presented in chapter 3, explored whether a drought disturbance can create a “window of opportunity” for plant invasions in semi-arid grasslands following drought. And because drought disrupts ecosystem functioning more broadly (van der Molen et al. 2011), a third study, presented in chapter 4, investigated whether drought disturbances generate legacy effects that persist even after the drought subsides.

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CHAPTER 2: UNEXPECTED PATTERNS OF SENSITIVITY TO DROUGHT IN THREE SEMI-ARID GRASSLANDS¹

INTRODUCTION

Global climate models predict an increase in inter-annual variability in precipitation regimes and more intense and frequent extreme weather and climate events, including multi-year droughts (IPCC 2007; Ray et al. 2008). North American semi-arid grasslands (shortgrass steppe) which cover the western region of the US Great Plains routinely experience seasonal water stress with multi-year droughts common historically (Lauenroth et al. 2008). Thus, the extreme climate of the shortgrass steppe is projected to become even more so in the future. As in many terrestrial and most grassland ecosystems, precipitation has been identified as a primary factor limiting ecosystem processes in semi-arid grasslands, particularly aboveground net primary productivity (ANPP; Sala et al. 1988). Furthermore, these grasslands are considered to be among the most sensitive ecosystems to changes in water availability (Knapp and Smith 2001; Huxman et al. 2004). Therefore, any predicted alterations in climate that may affect ecosystem water balance, including changes in precipitation or warming temperatures, are expected to have significant impacts on ANPP and ecosystem processes in semi-arid grasslands.

The influence of precipitation on ANPP in grasslands and across biomes in North America has been assessed at both large spatial and long temporal scales (Webb et al. 1978; Sala et al. 1988; Lauenroth and Sala 1992; Knapp and Smith 2001; Huxman et al. 2004). However, most relationships developed are based on observational data, either from single sites or multiple

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sites that span natural precipitation gradients. There are far fewer experimental studies across multiple sites (e.g., Gilgen and Buchmann 2009; Heisler-White et al. 2009). A key limitation to relying on observational data is the lack of control for extrinsic and/or co-varying factors that may influence precipitation–ANPP relationships. Wet years (and sites) may differ in many ways from dry years (and sites) beyond precipitation amount (Gilgen and Buchmann 2009). For example, the average size of precipitation events in wet years is usually larger, events may be more numerous, average temperatures may be lower, cloud cover higher, etc., and previous year’s climate and production may influence current year’s responses (Webb et al. 1978; Sala et al. 1992; Oesterheld et al. 2001; Wiegand et al. 2004). At present, the role these other factors play in determining responses to drought, in addition to precipitation amount, is unclear.

Experimentally altering precipitation inputs into an ecosystem is a more direct way to assess the sensitivity of ANPP to changes in precipitation (Sala et al. 1988, 1992; Weltzin et al. 2003; Hanson and Wullschleger 2003; Gilgen and Buchmann 2009; Heisler-White et al. 2008, 2009), but such experiments are usually performed at a single site, with the implicit assumption that responses are, to some extent, representative of the biome. Indeed, because sites within biomes share broadly similar climates, plant community composition, and potential meristem limitation constraints (Lauenroth and Sala 1992; Knapp and Smith 2001; Sala et al. 1988), any variation in sensitivity in ANPP to precipitation would be expected to be much less within than across biomes. There are, however, few experimental tests of this prediction.

The objectives of my study were twofold: (1) to directly assess the sensitivity of the shortgrass steppe to experimentally imposed reductions in precipitation amount to test the inference from observational relationships of strong drought sensitivity in this biome, and (2) to evaluate patterns of within-biome drought sensitivity by repeating the experiment at three sites

that span different soil types, and a significant portion of the range in mean temperature and precipitation that occurs across this important North American grassland biome. I imposed two levels of growing season rainfall reduction (50 and 80%) for 2 years at these sites, and predicted (1) that significant reductions in ANPP would result at all sites at both levels of drought, and (2), that if differential sensitivity to drought was evident across the three sites, reductions in ANPP (sensitivity) would be greater in drier than wetter sites within the shortgrass steppe, consistent with the continental pattern observed when multiple biomes have been compared across North America (Knapp and Smith 2001; Huxman et al. 2004).

MATERIALS AND METHODS

Study sites – Research was conducted at three sites located along a latitudinal gradient that encompassed much of the north to south extent of the North American semi-arid grassland biome. Representative of the shortgrass steppe biome, the soils at all three sites were Aridic Argiustolls (Kelly et al. 2008) and approximately 60% of root biomass is found in the top 15 cm of soil (Gill et al. 1999). The northern site was located at the Central Plains Experimental Range (CPER) in north-central Colorado (40°49N, 104°46W), 61 km northeast of Fort Collins, CO. Experimental plots were located in a site on which cattle grazing had been excluded since 1999. At the CPER, the sandy loam soils (Lauenroth et al. 2008) were the coarsest among sites and the bulk carbon-to-nitrogen ratio was 13.8 (Cherwin, unpublished data). The C₄-grass dominated plant communities at the particular sites of my experiment, as well as across the CPER, were dominated by the perennial rhizomatous grass, blue grama [*Bouteloua gracilis* (HBK) Lag. ex Griffiths] with total plant canopy cover averaging 80%. In general, *B. gracilis* accounts for 70% of total canopy cover and 90% of the aboveground biomass of grasses at this site with other

grasses, forbs, shrubs, and succulents accounting for approximately 20% of total canopy cover (Table 1; Sala and Lauenroth 1982).

The central site was located at the Sand Creek Massacre National Historic Site, a unit of the National Park Service (NPS), approximately 12 miles east of Eads, CO (38°32N, 102°31W). Research plots were located in an area of the park that was used for livestock grazing for nearly 150 years until the NPS acquired the site in 2001 and excluded grazing. Soils at this site had the finest texture and were classified as clay soils with a carbon-to-nitrogen ratio of 6.7. Vegetation canopy cover varied from 65 to 80% at this site with *B. gracilis* also dominant here (85–97% of cover; Table 1).

The southern study site was located at Fort Union National Monument, a former military post also now a unit of the NPS, in northeastern New Mexico (ca. 28 miles N of Las Vegas, NM, 35°91N, 105°01W). The semi-arid ecosystem at Fort Union was used extensively in the past (1851–1956) for horse and cattle grazing. However, livestock grazing has been excluded at the site for the past 60 years. This exclusion, along with native grass seeding and soil conservation efforts beginning in the mid-1970s, has allowed the native ecosystem to recover (Stubbendieck and Willson 1987). Soils at this site were sandy clay loams and had a carbon-to-nitrogen ratio of 8.0. Total vegetation canopy cover is approximately 75% and is dominated by *B. gracilis*, which accounts for roughly 70% of the total canopy cover (Table 1).

Combined these three sites encompassed a N–S gradient in mean annual precipitation from 342 to 425 mm and a mean annual temperature range from 7.2 to 9.4°C (Table 1). Across the entire shortgrass biome precipitation varies from 300 to 600 mm and temperatures from 7.0°C in the north to 16°C in the south (Lauenroth et al. 2008); thus, the three sites, all of which were dominated by *B. gracilis* with similar levels of plant community richness (Table 1),

captured a substantial amount of climate variation from the drier, cooler portion of the range of this grassland type.

Rainout shelters – To impose precipitation reductions, 20 passive rain deflection shelters were constructed at each site based on the design of Yahdjian and Sala (2002). The shelters covered 5.6-m² plots (2.25 x 2.5 m) and had angled roofs composed of transparent Plexiglas troughs alternating with open areas. Ten shelters were constructed with eleven 2.5-m-long by 11-cm-wide troughs equally spaced and targeted to reduce ambient precipitation inputs by 50%, and 10 were constructed with 18 troughs more closely spaced to reduce precipitation by 80%. Ten control plots, without any infrastructure, received ambient amounts of precipitation. All plots were randomly located in 900-m² areas that were flat or with <1% slope and with relatively homogenous vegetation cover and no obvious signs of animal disturbance. Gutters and downspouts were installed on the downhill side of the deflection shelters to drain water away from the target plot and all adjacent plots. All plots were located >1 m from the nearest neighboring plot. Shelters were in place from early June 2007 through November 2007 and from April 2008 through November 2008 thereby creating drought for two consecutive growing seasons.

Volumetric soil moisture content was measured with Decagon soil moisture sensors (ECH₂O probes). At each site, half the plots in each treatment (5 per treatment, 15 total) had these sensors placed near the center of the plot. Soil moisture probes were inserted vertically and integrated soil moisture over the top 20 cm of the soil. Measurements were recorded on a data logger every 4 h and averaged to produce daily mean soil moisture content.

I examined the shelter effects on the light environment by measuring transmittance of photosynthetically active radiation (PAR) with a 1-m linear quantum light meter (LI250A; Li-

COR) beneath and outside the shelters at 1200 hours MST under full sun conditions at midseason. Effects on the light environment were small, with the 50 and 80% treatments permitting 92 and 80% transmission, respectively. Similar effects on light transmittance have been documented by other studies using structures designed to manipulate ambient rainfall (Yahdjian and Sala 2002; Heisler-White et al. 2008; Fay et al. 2000). Short-term measurements of air and soil temperature indicated that these were only slightly elevated under the shelters (data now shown), also consistent with effects documented by Yahdjian and Sala (2002).

Vegetation measurements – I quantified ANPP by harvesting all aboveground biomass to just above the root crown in a 0.10-m² quadrat from each plot at the end of each growing season. Plant material was placed in a drying oven (60°C) until all biomass was dry (48–72 h), then sorted by species and weighed. Previous year's dead biomass was separated from current year's production based on color and structural changes that are visible after overwintering to allow for accurate estimates of ANPP. This estimate did not include any production by succulents or the woody portions of shrubs, both of which were minor components of total ANPP at these sites. To characterize plant species composition, percent canopy cover by species was visually estimated for four 1-m² quadrats in each plot (Daubenmire 1959; Collins 1992). Cover was estimated as the vertical projection of a polygon around each plant and projections were summed for each species within a quadrat. Species cover values from the four quadrats were then averaged to obtain the species cover value representative of the plot (see Table 1; and study site description).

Experimental design and analysis – The experimental design was a multi-site randomized complete block design with year as a repeated measure. At each site, 30 plots were organized into 10 blocks to control for any potential within-site variation. The three levels of rainfall

interception (0, 50, and 80%) were randomly assigned to plots within each block. A three-factor repeated measures analysis of variance (ANOVA) was used to test for main and interactive effects of site, year, and drought treatment on ANPP and soil moisture. ANPP was $\log_{10}$ -transformed to improve symmetry and homogeneity of variance. The model included three fixed effects and their interactions as well as random effects for blocks, nested in sites, and the interaction of treatment within blocks, nested within sites. Computations were performed using the Restricted Maximum Likelihood (REML) method of the MIXED procedure in SAS software version 9.2 (2008, SAS Institute). When main effects and interactions of these factors were detected, I used differences of least squares means (LSMEANS) to compare year and treatment effects within sites. The level of significance was set at $p < 0.05$ for all tests performed, and degrees of freedom were estimated using the Satterthwaite method.

RESULTS

Precipitation Regimes – During the course of this 2-year experiment, ambient growing season rainfall regimes varied substantially between sites and years. Growing season rainfall was 224 mm at the northern site in 2007, with rain falling on 43 days and a mean event size of 5.2 mm. In 2008, amounts and patterns were similar with 247 mm of growing season precipitation falling over 39 rain days, and a mean event size of 6.3 mm. Rainfall regimes differed more between years at the central and southern sites. Growing season precipitation was 268 mm (36 days of rain, mean event size of 7.4 mm) in 2007 at the central site but only 185 mm in 2008 (41 rain days, mean event size 4.5 mm). As expected, the southern site received the most growing season precipitation in both years (307 mm in 2007 and 400 mm in 2008). Interestingly, in both years, this site also had the fewest number of rain days (33 in 2007 and 32 in 2008) along with the largest mean event sizes (9.3 mm in 2007 and 12.5 in 2008).

Rainout shelter effects – In spite of these differing rainfall regimes, the rain deflection shelters consistently reduced soil moisture throughout the growing season at all three sites in both years. Although the 50 and 80% precipitation reductions were not expected to reduce soil moisture by 50 and 80%, differences among the treatments were maintained throughout both growing seasons during both wet and dry periods (Fig. 1; Table 2). Overall, compared to the plots receiving ambient precipitation, the 50% shelters reduced soil moisture by 21% and the 80% treatment caused a 46% reduction across the entire growing season (Fig. 2; Table 2). These patterns of soil moisture reduction among treatments were similar across sites and there were no interactions among site, year, or treatment (Figs. 1, 2; Table 2). Further, even though the same plots were subjected to these treatments for two consecutive years, soil moisture levels in the 2nd year of the drought treatments were typically not lower than in the 1st year, likely because roofs were removed in the dormant season and soil moisture recharge occurred.

Aboveground net primary productivity – Despite consistent reductions in soil moisture at all sites in both years, there was surprising variation in effects on ANPP (Fig. 3; Table 2). At the northern site, ANPP was significantly reduced ($p < 0.05$) in both drought treatments in 2007 and in the 80% treatment in 2008. At the central site, however, there was no significant reduction in ANPP in 2007, but both drought treatments reduced ANPP in 2008. Most unexpected were the lack of ANPP responses to either treatment in either year at the southern site. Thus, the northern site (driest, coolest) was the most sensitive to these imposed growing season precipitation reductions, the central site was intermediate and the southern site (wettest, warmest) was insensitive to these treatments.

DISCUSSION

My research was designed to test two predictions: (1) that the semi-arid shortgrass steppe ecosystem would display strong sensitivity (via reduced ANPP) to experimental reductions in precipitation, as inferred from observational analyses, and (2) that variation among sites within the biome would be small, but if there were differential sensitivity to drought, drier sites would be more sensitive than wetter sites, consistent with patterns observed at the cross-biome scale. Based on previous analyses of observational data (Sala et al. 1988; Lauenroth and Sala 1992; Huxman et al. 2004), the sensitivity of the shortgrass steppe to changes in precipitation [defined as the slope of the ANPP vs. mean annual precipitation (MAP) relationship by Huxman et al. 2004] varies from a maximum of approximately $0.3 \text{ g m}^{-2} \text{ mm}^{-1}$ based on continental scale data (Huxman et al. 2004) to $0.13 \text{ g m}^{-2} \text{ mm}^{-1}$ based on long-term data from the northern site in the study (Lauenroth and Sala 1992). Considering only the three cases in which ANPP did respond to experimental reductions in growing season precipitation (Fig. 3), sensitivity in my study was estimated to be $0.18 \text{ g m}^{-2} \text{ mm}^{-1}$, more similar to the estimate from Lauenroth and Sala (1992), although the maximum response quantified in my experiment ($0.32 \text{ g m}^{-2} \text{ mm}^{-1}$ at the central site) was similar to the estimate by Huxman et al. (2004). However, when all sites and years were considered, these observationally-based estimates of ANPP sensitivity to alterations in precipitation clearly overestimate drought sensitivity. With all six cases combined, the overall sensitivity of ANPP to changes in precipitation was only $0.10 \text{ g m}^{-2} \text{ mm}^{-1}$, with substantial inter-site and interannual variability (Fig. 3).

The apparent overestimate of ANPP sensitivity to precipitation derived from long-term observations may reflect the importance of other co-varying climatic factors during wet and dry years. Indeed, although the general pattern of differential sensitivity (most sensitive in the drier

sites, less so in the wetter) was consistent with larger scale patterns observed across biomes (Knapp and Smith 2001; Huxman et al. 2004), the extreme variation in sensitivity—from no response in ANPP to a 51% reduction was unexpected. Gilgen and Buchmann (2009) reported that there was no consistent grassland response to drought in Switzerland and argued that differences in site management might explain site-specific responses. In my study, however, all three grassland sites were unmanaged. One potential explanation for the overall pattern within this grassland biome would be edaphic gradients from north to south. In these semi-arid grasslands, fine textured soils would be expected to have less plant available water than coarse textured soils (Noy-Meir 1973; Lauenroth and Sala 1992) and, as a result, sites with fine textured soils would be more sensitive to reductions in precipitation. There was no support for this explanation, however, as I detected no consistent relationships between texture (or soil fertility) and drought sensitivity. An alternative explanation for this pattern, consistent with that proposed for continental-scale patterns, is that ANPP in areas with higher amounts of precipitation becomes limited by other resources, and thus such sites are less sensitive to alterations in precipitation (Huxman et al. 2004). This explanation is not supported by the strong variation in interannual ANPP observed at the southern site, however (Fig. 3). While the experimental rainfall reductions did not significantly reduce ANPP at the southern site in either year, greater growing season precipitation in 2008 significantly increased ANPP relative to 2007, suggesting that precipitation amount can and does affect ANPP even at the wettest end of the biome.

I explored a third hypothesis to explain the strong differential sensitivity observed in my experiment—one based on recently documented ANPP responses to alterations in the size of rainfall events in the shortgrass steppe. Sala et al. (1992) argued that large rainfall events, even though they were few in number, were disproportionately important in years with the highest

ANPP in the shortgrass steppe. Moreover, recent experiments in which precipitation event size and frequency were altered, but not total amount, showed that fewer but larger growing season rain events led to significantly higher levels of ANPP than precipitation regimes characterized by more frequent, small events (Heisler and Knapp 2008; Heisler-White et al. 2008). Although I did not experimentally vary rainfall event size independently of growing season precipitation amount, for each of my experimental plots I calculated a drought sensitivity index—the ratio of the response in ANPP (control–treatment) divided by the reduction in precipitation for each treatment (ANPP/mm precipitation)—and related this ratio to mean growing season event size (Fig. 4). This analysis revealed a strong inverse relationship between sensitivity to drought and event size. Rainfall patterns characterized by larger events, which have previously been shown to lead to higher levels of soil moisture and ANPP (Heisler-White et al. 2008, 2009), were associated with a lack of ANPP response (insensitivity) to the drought treatments. Mechanistically, I propose that, if rainfall events are sufficiently large, losses of precipitation to evaporation will be minimized, soil moisture will remain at levels permitting growth for extended periods, rain use efficiency will be increased, and for these inherently low production grasslands, ANPP can become uncoupled from precipitation amount (Knapp et al. 2008). Even with proportional reductions in individual event sizes by the shelters, these soil moisture thresholds may have been exceeded by large storms allowing for relatively high levels of ANPP. Overall, these results suggest that there is an important interaction between rainfall regime and sensitivity to changes in precipitation amount in this, and perhaps other, biomes (Leuzinger and Körner 2010), although more direct experimental evidence will be necessary to confirm this. I conclude that, because future climate changes are expected to alter overall ecosystem water balance, total precipitation amounts, and patterns of intra- and interannual variability, past

observational relationships between mean annual precipitation and ecosystem response may be inadequate for providing insight into future spatial and temporal dynamics of ANPP (Nippert et al. 2006; Knapp et al. 2008).

TABLES

Table 2.1 Description of three study sites along a latitudinal gradient in the semi-arid grassland biome of North America. Total canopy cover of grasses was heavily dominated by C₄ species, predominantly blue grama [*Bouteloua gracilis* (HBK) Lag. ex Griffiths]. Climate data for the northern site are 69-year means from 1939 to 2008 (USDA-ARS CPER). The central site climate data are 101-year means from 1907 to 2008 (CoAgMet). Climate data for the southern site are 157-year means from 1851 to 2008 (Fort Union National Monument).

Site	Mean annual temperature (°C)	Mean annual precipitation (mm)	Elevation (m)	Total cover of grass species (%)	Relative cover of C grasses (%)
North (USDA-ARS CPER)	7.2	342	1,650	64.9	90.5
Central (Sand Creek NHS)	8.3	385	1,219	58.1	99.4
South (Fort Union NM)	9.4	425	2,043	75.6	96.0

Table 2.2 Results from ANOVA for multi-site analysis of drought treatment x site x year for soil moisture and ANPP.

Effect	df	Soil Moisture		ANPP	
		F	P	F	P
Site	2	61.65	<0.0001	723.82	<0.0001
Year	1	90.16	<0.0001	52.29	<0.0001
Site x Year	2	6.27	0.0043	66.74	<0.0001
Trt	2	130.92	<0.0001	15.98	<0.0001
Site x Trt	4	1.70	0.1696	10.74	<0.0001
Year x Trt	2	0.64	0.5291	3.71	0.0286
Site x Year x Trt	4	0.43	0.7877	2.64	0.0380

FIGURES

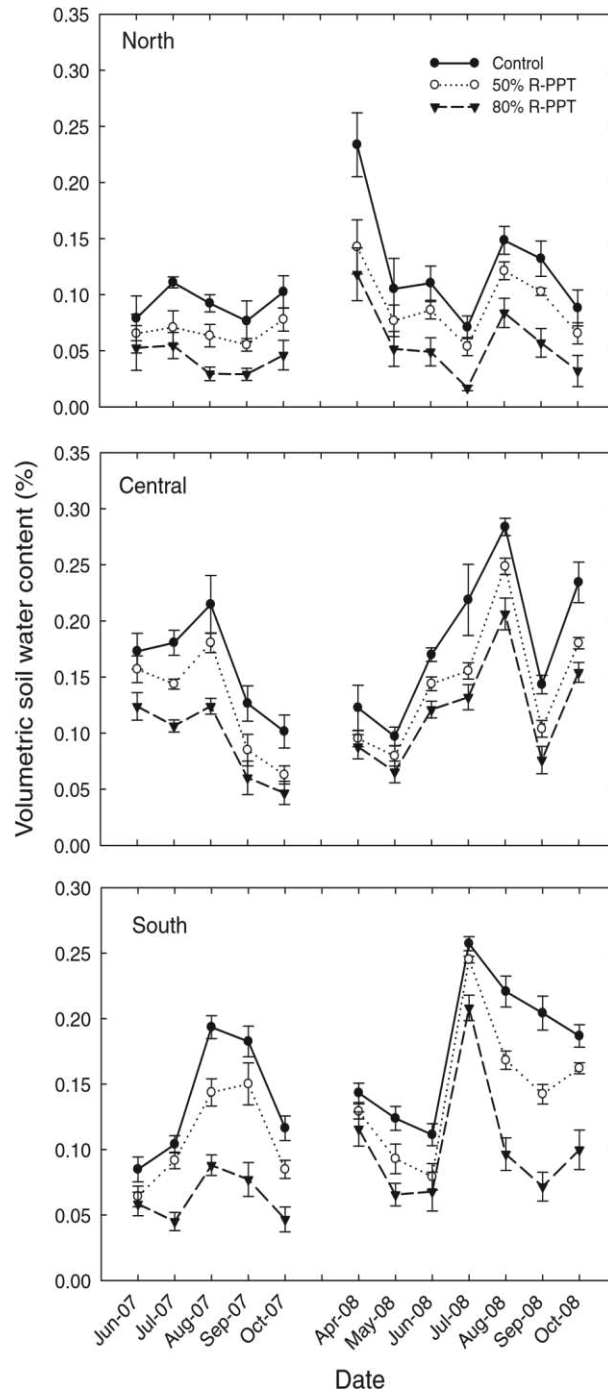


Figure 2.1 Dynamics of growing season mean soil moisture (0–20 cm) for ambient and reduced precipitation (R-PPT) experimental plots for 2007 and 2008 at three study sites. Shelters were in place for at least 7 days prior to initial soil moisture measurements. Each point represents the monthly mean soil moisture content with error bars indicating standard errors calculated from replicate plots for each treatment.

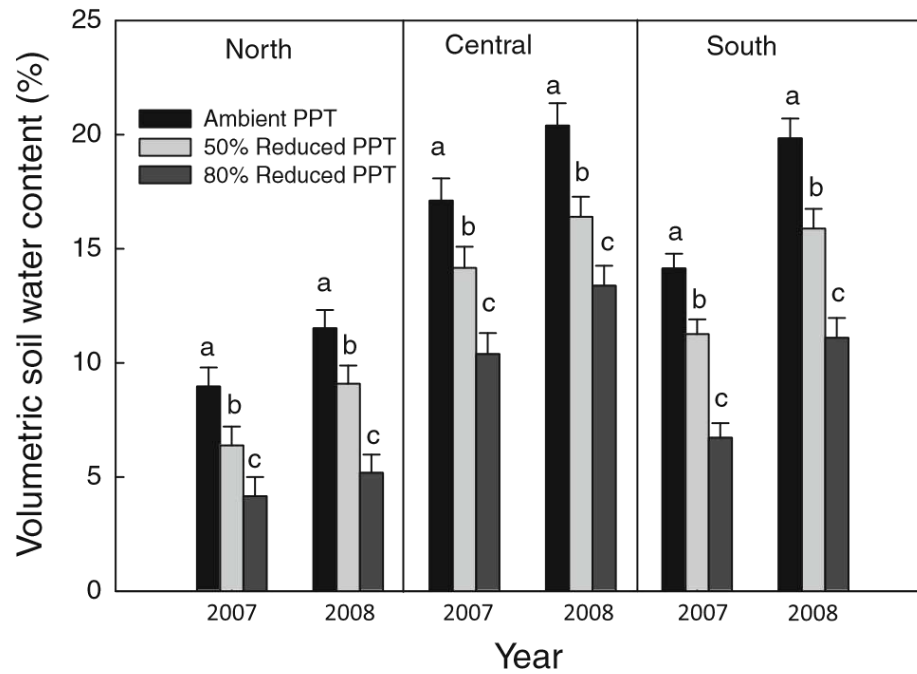


Figure 2.2 Mean growing season soil moisture (0–20 cm) for ambient and reduced precipitation (PPT) experimental plots for 2007 and 2008 at three study sites. Error bars indicate one standard error and different letters represent significant differences ($p < 0.05$) within each site by year combination. Although there was a significant site x year effect (Table 2), those comparisons are not indicated.

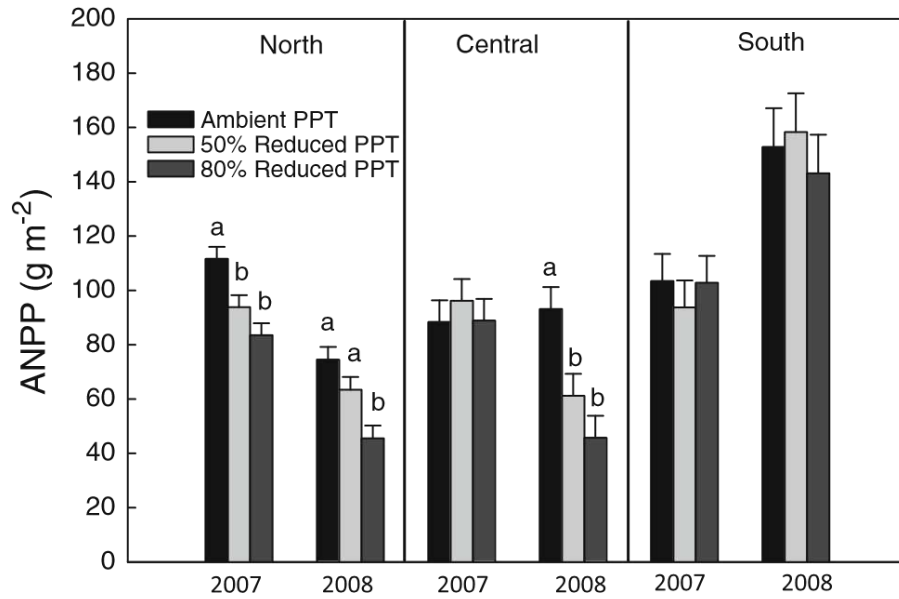


Figure 2.3 Total aboveground net primary productivity (ANPP; g·m⁻²) for ambient and reduced precipitation treatments (R-PPT) at the three shortgrass steppe sites. Within each site x year combination, significant treatment differences are represented by different letters ($p < 0.05$). See Table 2 for the overall ANOVA results. At the North site, there were significant treatment ($F = 24.29$, $df = 45$, $p < 0.0001$) and year ($F = 112.18$, $df = 45$, $p < 0.0001$) effects; at the Central site, there were also significant treatment ($F = 6.23$, $df = 36$, $p = 0.0048$) and year ($F = 16.72$, $df = 36$, $p = 0.0027$) effects; at the South site there was not a significant treatment effect ($F = 0.19$, $df = 36$, $p = \text{NS}$) but there was a significant year effect ($F = 32.03$, $df = 9$, $p = 0.0003$).

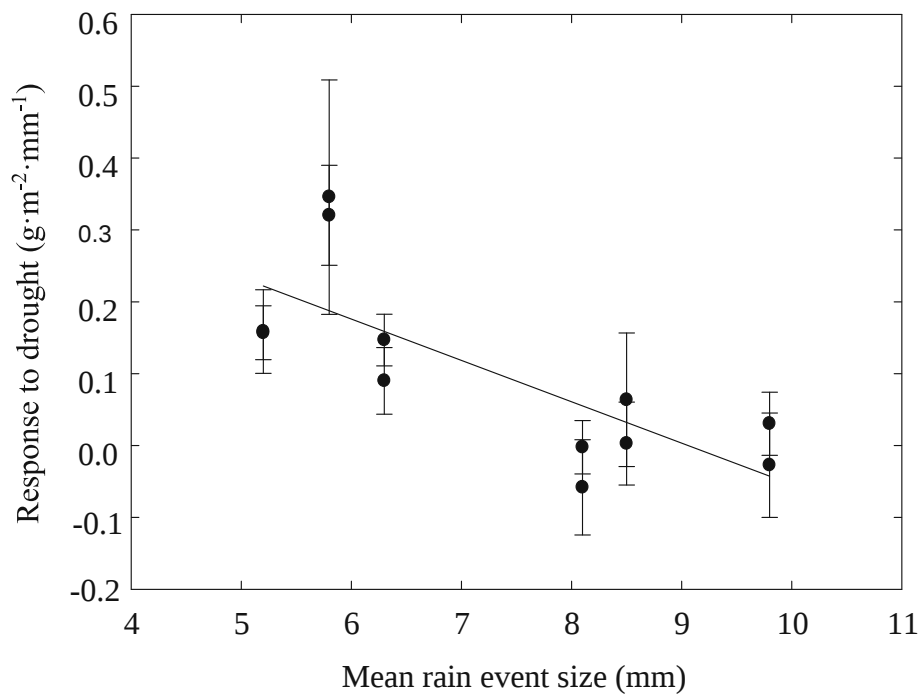


Figure 2.4 The relationship between mean rainfall event size during the growing season versus the response (or sensitivity) of ANPP to rainfall reductions ($r^2 = 0.57$, $p = 0.0045$). Sensitivity was calculated at the site level as a ratio of the reduction in ANPP (control–treatment) divided by the reduction in rainfall for each site and year. Both -50 and -80% rainfall reduction treatments are included, and error bars represent one standard error of the mean for each year x treatment combination.

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CHAPTER 3: DOES DROUGHT FACILITATE PLANT INVASIONS IN SEMI-ARID GRASSLANDS?

INTRODUCTION

It is widely recognized that exotic plant invasions of natural ecosystems can have severe ecological and economic impacts (Davis et al. 2000; Hooper et al. 2005; Catford et al. 2012; Eviner et al. 2012). Numerous mechanisms have been proposed to explain patterns of invasion including those that focus on plant traits (Rejmánek and Richardson 1996; Alpert et al. 2000; Pyšek and Richardson 2006; Van Kleunen et al. 2010), propagule pressure (Richardson and Pyšek 2006; Colautti 2006), novel weapons and/or environmental conditions (Didham et al. 2005; Cherwin et al. 2009; Sorte et al. 2013), community structure (Naeem et al. 2000; Smith and Knapp 2003), disturbance (Renne et al. 2006), and resource opportunities (Huston and DeAngelis 1994; Davis et al. 2000; Blumenthal 2009). Based on a number of attempts to synthesize these mechanisms, and the apparent stochasticity of invasion patterns, it is clear that no single mechanism predominates, and that the invasion process is inherently idiosyncratic and context-dependent (Crooks 2005; Blumenthal 2006; Seastedt and Pyšek 2011; Diez et al. 2012).

Predicting plant invasions is likely to become increasingly difficult due to climate changes that include directional shifts in temperatures and greater variability in precipitation regimes (Smith et al. 2009; IPCC 2014). Of particular importance for semi-arid grasslands in the Central Great Plains Region of the United States are the predicted increases in climate extremes such as severe and prolonged drought. In fact, over the next century the magnitude of droughts in this region is expected to exceed those observed in the past (Cook et al. 2015). This underscores

the importance of understanding how climate change will impact the invasibility of natural ecosystems, such as semi-arid grasslands.

Semi-arid grassland ecosystems can be affected by drought in many ways. In the short-term, plant physiological performance may be decreased (Signarbieux and Feller 2012) and aboveground net primary production (ANPP) reduced (Herbel et al. 1972; Gilgen and Buchmann 2009; Cherwin and Knapp 2012; Reichmann et al. 2013). In the long-term, drought can lead to a marked decrease in plant cover, diminished competitive abilities of dominant plant species, and changes in community composition (Oesterheld et al. 2001; Sala et al. 2012; Reichmann et al. 2013). Drought-induced changes in plant community structure and reduced cover may be accompanied by increases in light, space and soil nitrogen. Thus, the combined effects of changes in community structure and alterations in resource availability post-drought may facilitate ruderal plant species establishment in general and of potentially invasive species (Blumenthal 2005; Schrama and Bardgett 2016).

The fluctuating resource hypothesis (Davis et al. 2000) posits that the invasibility of an ecosystem increases during periods when unused resources are available to facilitate colonization and establishment of ruderal and potentially invasive species. Resources may fluctuate for a variety of reasons. For example, resource uptake may be strongly seasonal, resulting in time periods when resources (e.g., soil nutrients, water, and light) exceed demand. Fluctuations in resources may also occur after disturbances, and as noted above, after extreme climate events. Regardless of the cause, any period of excessive resource availability potentially presents a window of opportunity that may allow species to invade a plant community (Davis et al. 2000; Stachowicz et al. 2002; Walker et al. 2005; Schrama and Bardgett 2016).

Semi-arid grasslands are typically considered resistant to plant invasions (Lauenroth et al. 1978); partly because resource levels are chronically low and partly because dominant plant species have evolved tolerance and avoidance mechanisms for coping with the stressful environment. Yet, alterations in species composition in semi-arid grasslands can vary with the distribution and amount of annual precipitation (Hyder et al. 1975; O'Connor et al. 2001). Thus, projected increases in precipitation variability (both droughts and wet periods) may lead to more frequent pulses in resource availability and consequently facilitate invasions (Davis et al. 2001; Bradley et al. 2010).

The objective of this study was to determine if severe drought creates a window of opportunity for increased plant invasions of semi-arid grasslands. I hypothesized that following an extended drought, increased resource availability (e.g., soil nutrients and space) would result and subsequently facilitate invasion of these ecosystems. I tested this hypothesis by imposing two levels of drought, 50% reduced precipitation (R-PPT) and 80% R-PPT over two growing seasons, at three different semi-arid grassland sites. I then added seeds of locally weedy species, to assure that propagules were available for invasion in the post-drought growing season. I also added a C source to reduce and thus assess the role of post-drought N levels on grassland invasion.

I expected the size of the window of opportunity for species to invade would depend on the intensity of drought such that the 50% R-PPT would moderately increase invasibility and 80% R-PPT would greatly increase invasibility. I further hypothesized that invasibility would be related to the amount of inorganic soil nitrogen available after the drought treatments. Hence, I predicted that drought would reduce plant cover and uptake of soil nutrients, generating a pulse

in available resources post-drought when precipitation increased and this would facilitate the establishment of ruderal species.

MATERIALS AND METHODS

Study sites – Drought was experimentally imposed at three sites that spanned approximately 600 km (North – South) of the North American shortgrass steppe biome (Cherwin and Knapp 2012). Average annual precipitation across the sites ranges from 342 mm in the north to 425 mm in the south and mean annual temperature ranges from 7.2° to 9.4°C. These grasslands have high annual potential evapotranspiration (PET), which ranges from 1200 mm in the northern site to 1700 mm in the southern site (Pielke and Doesken 2008). All sites shared the same soil type (Aridic Argiustolls, Kelly et al. 2008) but soil texture varied between sites. In these semi-arid grasslands, a majority of the root biomass is located near the surface, with approximately 90% occurring in the top 20 cm of surface soil (Schimel et al. 1985). Plant communities at all three sites were typical of the shortgrass steppe, dominated by the perennial rhizomatous grass, blue grama [*Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths], which represented approximately 90% of aboveground biomass. Experimental plots at all sites were located in areas that were protected from cattle grazing.

The northern site was located in northern Colorado at the Central Plains Experimental Range (CPER), approximately 15 miles north of Nunn, CO (40°49'N latitude, 104°46'W longitude), near the northern edge of the range of the shortgrass steppe biome. The CPER is managed by the Agricultural Research Service and has coarse soil, classified as sandy loam (Lauenroth et al. 2008), with a bulk carbon-to-nitrogen (C:N) ratio of ca. 13.8 (Cherwin, unpubl. data).

The central site was located in southeastern Colorado at Sand Creek Massacre National Historic Site (SAND), approximately 12 miles east of Eads, CO (38°32'N, 102°31'W), in the north-central and eastern-most region of the shortgrass-steppe biome. SAND is managed by the National Park Service (NPS) and has the finest soil texture among the three study sites, classified as clay, with a bulk C:N ratio of ca. 6.7 (Cherwin, unpubl. data).

The southern site was located in northern New Mexico at Fort Union National Monument (FOUN), approximately eight miles north of Watrous, New Mexico (35°91'N, 105°01'W), near the central and western-most region of the shortgrass-steppe biome. FOUN is managed by the NPS and the soil texture is intermediate between the other two study sites, classified as sandy clay loam, with a C:N ratio of ca. 8.0 (Cherwin, unpubl. data).

Experimental Design – At each site, 30 plots (2.5 m x 2.25 m) were arrayed across 10 blocks to control for within-site variation in a split-plot design. Two levels of ambient rainfall reduction (50% and 80%) were randomly assigned to plots within each block to induce drought during the 2007 and 2008 growing seasons (May – Sept). Drought was imposed using shelters that passively excluded either 50 or 80% of ambient rainfall (Yahdjian and Sala 2002) erected over plots (n=10 per treatment). Ten control plots with no shelters received ambient amounts of rainfall. Volumetric soil moisture content in the upper 20 cm of soil was measured six times per day in half the plots at each site (i.e., 15 plots, 5 per treatment) using Decagon ECH₂O EC-20 probes (Decagon Devices, Inc.) soil moisture sensors (see Cherwin and Knapp 2012 for further description of drought treatments). The shelters were removed at the end of the 2008 growing season and 2009 was considered the post-drought year. In 2009, plots were divided into four subplots and four treatments (carbon addition, seed addition, carbon + seed addition, and control) were randomly assigned.

Seeds (3 grams per live seed (PLS) per m²) of five native species (*Sphaeralcea coccinea*, *Artemisia frigida*, *Helianthus annuus*, *Argemone polyanthemus*, and *Grindelia squarrosa*), obtained from Pawnee Buttes Seed, Inc. in early March 2009, were added to two subplots in mid- to late-March 2009 to increase the propagule availability of native ruderal species that are locally weedy. These ruderal species seeds were chosen to serve as benign surrogates for potentially invasive exotic species since previous research has shown that in many cases native and non-native species are similar in terms of fitness, resource acquisition, and growth related traits (Smith and Knapp 1999; Daehler 2003; Seastedt 2007; Funk and Vitousek 2007). To aid seedling recognition in the field, seeds of all species were grown in pots in the laboratory before seeds were added to study subplots. In the field, subplots were surveyed one to two weeks after seed applications at each site and again three weeks later to detect germination. Subplots were subsequently surveyed for seedlings intermittently throughout the growing season.

Labile carbon (sucrose, or table sugar, which consists of approximately 40% carbon) additions were applied to two randomly assigned subplots to reduce the amount of plant-available soil nitrogen (Morgan 1994; Reever Morghan and Seastedt 1999; Paschke et al. 2000; Blumenthal 2003). Sugar was evenly distributed in subplots four times throughout the 2009 growing season; 300 g was first added in mid- to late-March, followed by 150 g in late-May to early-June, again in early-July, and once again in mid-August.

The Post-Drought Environment – To measure the effect of drought and carbon additions on soil N, total inorganic nitrogen (NH₄-N, NO₃-N) was measured by collecting soil cores (5 cm diameter, 10 cm depth) from two subplots (those with no-carbon/no-seed and with carbon/no-seed) from each plot on three sampling dates. The sampling times were: 1) immediately after the drought period in the fall of 2008, to measure levels of N following the drought treatments; 2) in

the spring of 2009, to determine if accumulated nitrogen from the drought persisted over the winter; and 3) in the fall of 2009 at the end of the post-drought recovery year, to determine levels of N at the end of the growing season and to assess the effect of the labile carbon additions. Levels of inorganic N from soil cores were estimated by potassium chloride (KCl) extraction followed by colorimetric analysis on a Lachat QuickChem 8500 flow injection analyzer.

I estimated ANPP at the end of each growing season by clipping all aboveground biomass above the root crown in a 0.10-m² quadrat from each subplot. All plant biomass was dried at 60°C, then sorted and weighed by species. To estimate ANPP, current year's biomass was separated from previous year's dead biomass, which was easily distinguished after overwintering due to visible color and structural changes. Biomass from woody and succulent species was a minor component in these plots and was not included in estimates of ANPP.

Plant species composition and cover were characterized by visually estimating percent canopy cover in each of the four 1-m² subplots of each plot. Vertical projections of a polygon around each plant were summed for each species within a quadrat to obtain cover values (Daubenmire 1959; Collins 1992; Cherwin and Knapp 2012). Percent of bare ground was also estimated visually as the portion of ground having no plant, rock, or litter cover. Richness was assessed as the number of species per 1-m² subplot and Shannon's index was calculated to quantify species diversity.

Statistical analyses – Combined data from the three sites were subjected to a split-split-plot analysis with site as the whole-plot factor, blocks nested within sites, rainfall reduction as the subplot factor and carbon and seed addition jointly as the sub-subplot factors. The four factors and their interactions were fixed effects; block and the interaction of rainfall with block were random effects.

For measurements that preceded carbon and seed addition, multiple-site data analyses for a single year were based on a split plot analysis in which sites were treated as a whole plot factor, blocks were nested within site, and rainfall reduction was the subplot treatment. Multiple-site analysis for soil nitrogen for fall of 2008 and spring of 2009 (prior to carbon and seed additions) was analyzed as a split-plot analysis with year as a repeated measure.

Soil nitrogen and ANPP were $\log_{10}$ transformed to improve symmetry and homogeneity of variances. Percent canopy cover was transformed using arcsine square root. Computations were performed using the Restricted Maximum Likelihood (REML) method of the MIXED procedure in SAS software version 9.3 (SAS Institute, 2008). When interactions of site and rainfall treatments were detected, I used differences of least squares means (LSMEANS) to compare rainfall treatments within sites. When seed addition interactions with other factors were not detected, seeded and non-seeded sites were averaged when assessing the effects of the other factors. The level of significance was set at $p < 0.05$, and degrees of freedom were estimated using the Satterthwaite method.

RESULTS

Soil moisture and precipitation – The 50% and 80% R-PPT treatments significantly reduced soil moisture content by 21 and 46% respectively across all sites in the first two years of the study. Specifically, at the north, central, and southern sites, soil moisture was reduced by 25 and 54%, 18 and 37%, and 20 and 48%, respectively in the 50% and 80% R-PPT treatments (Cherwin and Knapp 2012). Soil moisture did not differ significantly among treatments in 2009 (data not shown). Growing season (May – September) precipitation in 2009 at the northern site was approximately 20% above the long-term average. The central and southern sites were 22 and 25% below the average growing season precipitation, respectively. Given that the long-term

interannual coefficient of variation of MAP for these sites is 28%, 2009 was not considered an unusually wet or dry year at any of the sites.

Soil nitrogen responses to drought – Levels of inorganic soil nitrogen ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$), differed 2-fold among sites, even in ambient plots (Table 1, Fig. 1). Drought plots at the northern site consistently had the highest levels of inorganic nitrogen, indicating that reduced rainfall increased the amount of plant available soil nitrogen at the northern site, and these high levels persisted over winter to the following spring. In contrast, drought treatments did not impact soil N at the central site and in fact, there was little variance between plots and years at this site. Finally, there was no effect of drought on soil N in the southern site immediately after the shelters were removed. Although there were differences among plots in 2009, these were not consistent with the drought treatments imposed (Fig. 1).

Vegetation responses – Despite adding seeds to experimental plots, germination success was extremely low and occurred in only a few plots at the northern site (the only species to germinate were *S. coccinea*, *A. frigida*, and *H. annuus*). There was a significant effect of the seed treatment for vegetative cover ($p = 0.0016$), small interactions of seed addition with carbon ($p = 0.040$), and a four-way interaction ($p=0.044$) (Table 2). There was also a significant effect of seed addition on percent bare ground and a significant interaction with site ($p=0.043$). Because the interactions with seed were only marginally significant and very small relative to the other effects in the model, the remaining results are presented with this treatment averaged with the others (Figs. 3 & 4).

As expected, plots exposed to severe drought (80% R-PPT) for two years had significantly reduced total plant cover (14%) in 2008 at all three sites; however there were no significant differences in bare ground (Fig. 2) due to increased amounts of litter. In contrast, the

response of ANPP to two consecutive years of reduced growing season precipitation varied among sites (Fig. 2). After the second year of growing season drought, both the 50% and 80% drought treatments had decreased ANPP at the northern site, but only the 80% drought led to reduced ANPP at the central site. Surprisingly, there were no significant differences among rainfall treatments at the southern site (Cherwin and Knapp 2012).

In 2009, my primary focus was on how drought and soil N impacted ruderal species, as surrogates for potentially invasive exotic species. Since germination of the seeds I added was so low, I classified a new subset of resident plant species to represent ruderals, defined as nonnative species adapted for colonization and rapid growth following a disturbance event (Grime and Hunt 1975; Fenner 1978). See Appendix for a complete list of species for each site. Carbon treatments significantly reduced inorganic soil nitrogen levels at all sites ($p < 0.0001$, Table 2). This, in turn, significantly affected the vegetation community by decreasing total plant canopy cover and increasing bare ground.

At the northern site, total ANPP was highest in the plots having previous rainfall reductions of 80% and no carbon additions, but over half the amount of total ANPP was comprised of ruderal species. Carbon additions significantly reduced total biomass and biomass of non-ruderal (resident) species in ambient plots, carbon had no significant effect in the 50% drought plots, while carbon addition significantly reduced total biomass, biomass of resident species, and biomass of ruderal species in the 80% drought plots (Table 2). The central site showed very little sensitivity to carbon treatments with significant reductions in total biomass only occurring in the ambient plots. At the southern site, carbon effectively reduced total biomass in all plots and significantly reduced biomass of ruderal species in the ambient plots.

The lasting negative effects of drought on cover were present in 2009 at each of the sites (Table 2, Fig. 3). In terms of bare ground, the 80% drought plots had significantly more bare ground than the other treatments at the northern and central sites. Plots that experienced drought had lower total plant cover in the central and southern sites, however total plant cover was not lower in the northern site due to the large amount of cover by ruderal species. Carbon addition had little effect on cover, only showing significant differences at the northern site. This could be due to the timing of the percent cover data collection. Cover data was collected in late-June, at a time when only two carbon additions had been added (Table 2). There were no significant differences in richness or diversity in any of the plots at any of the sites. However, the northern site had the highest species richness and diversity; the central site had extremely low richness and diversity; and the southern site was intermediate in richness and diversity (data not shown).

I regressed soil N with the cover and ANPP of ruderal species for all sites in 2009. The gradient of total plant-available soil nitrogen levels accounted for nearly 50% of variation in cover of ruderal plant species across all sites and explained nearly 30% of the variation in ANPP of these species (Fig. 4).

DISCUSSION

This study indicates that the degree to which drought creates a “window of opportunity” for invasive plant establishment in semi-arid grasslands is highly context-dependent but may be related to the impact drought has on soil N availability. I hypothesized that invasibility of semi-arid grasslands would be increased following drought. I expected that drought would decrease plant canopy cover and ANPP, and therefore increase the availability of space, light, and soil water and nutrients. I further suspected that ruderal invasive species, which typically display

opportunistic life history traits, would take advantage of the pool of unused resources that resulted from the drought treatments.

The differences in invasibility I observed between the sites were surprising as I expected to see similar patterns of invasion at all three sites. I predicted the identical experimental treatments at three sites within the same ecosystem type would yield comparable outcomes. However, my results reveal inconsistent effects of drought (Cherwin and Knapp 2012) and this, combined with the variability in post-drought weather, led to increased invasibility at only one of three sites, despite all three sites being exposed to identical manipulations (Table 2, Fig. 3). My results corroborate conclusions made by others that plant invasions are idiosyncratic by nature (Renne et al. 2006) and that predictions are difficult to make as to when an ecosystem will be invaded. Nonetheless, I have gained insight into the mechanisms that may influence the invasibility of semi-arid grassland ecosystems.

As expected, I found that drought effectively reduced total plant canopy cover (Fig. 2, left panels) although this did not lead to significant increases in bare ground. Furthermore, ANPP was not always significantly reduced as a result of drought treatments (Fig. 2, right panels). In terms of inorganic soil nitrogen, drought significantly increased levels at the northern site but did not affect levels in the same way at the central or southern sites (although, the 50% R-PPT treatment did show significantly less soil N in the Spring of 2009).

The unforeseen patterns of invasibility I found in this study are perhaps a reflection of the unexpected patterns of drought sensitivity I observed at these sites after two years of drought (see Cherwin and Knapp 2012). Hence, drought may increase inorganic soil N and reduce plant cover, and therefore create a window of opportunity for the establishment of invasive species. However, invasions are not always more prevalent following droughts. If there is sufficient

rainfall and inorganic soil N is high post-drought, then invasions may increase. But, if there is not sufficient rainfall and/or if inorganic soil N is low, invasibility may not be greater.

In summary, my results show that the invasibility of semi-arid grassland ecosystems is generally quite low but related to N availability generated by the drought-soil interaction. Plant invasions can occur in these ecosystems if a disturbance event, such as drought, is extreme enough and if drought creates a pool of unused resources. Thus, extreme drought may be necessary but not sufficient to create a window of opportunity for invasive species to be successful. Given that the Great Plains region of the United States, including the semi-arid grasslands on its western edge, is predicted to face more severe droughts in the next 100 years than it has ever seen in recorded history (Cook et al. 2015), such invasion windows may increase in frequency in the future.

TABLES

Table 3.1 ANOVA results for post-drought conditions of vegetation and plant-available soil nitrogen at the end of a severe two-year drought (growing seasons of 2007 & 2008). The percent of total plant canopy cover and completely bare ground (i.e., no litter, rocks, etc.) values were estimated mid-growing season (end of June in 2008). Total ANPP and plant-available soil nitrogen (NH₄-N + NO₃-N) values were collected at the end of the 2008 growing season (fall 2008) and soil nitrogen was again measured in spring 2009, prior to carbon and/or seed additions in 2009. Bolded values are significant (p<0.05).

Effect	<i>df</i>	Total plant cover		Bare ground		Total ANPP		Soil nitrogen	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Site	2	3.53	0.0436	1.84	0.1779	80.07	<0.0001	145.67	<0.0001
R-PPT	2	18.54	<0.0001	1.5	0.2322	15.51	<0.0001	14.62	<0.0001
Site x R-PPT	4	1.22	0.3146	0.76	0.5528	3.39	0.0151	6.36	0.0003
Year	1	-	-	-	-	-	-	51.43	<0.0001
Site x Year	2	-	-	-	-	-	-	3.1	0.0505
Year x R-PPT	2	-	-	-	-	-	-	0.01	0.9922
Site x Year x R-PPT	4	-	-	-	-	-	-	1.78	0.1402

Table 3.2 ANOVA results for 2009, the post-drought year when no rainfall reductions were imposed. Soil nitrogen (NH₄-N + NO₃-N), total ANPP and ruderal ANPP values were from the end of the growing season; also, these three models were log transformed. The percent of total plant cover and cover of bare ground values were obtained in late-June; also, I performed an arcsine transformation on percent cover values. All transformations were based on residuals. Bolded values are significant (p<0.05).

Effect	df	<u>Soil nitrogen</u>		<u>Total plant cover</u>		<u>% Bare ground</u>		<u>Total ANPP</u>		<u>Ruderal % cover</u>		<u>Ruderal ANPP</u>	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Site	2	26.72	<0.0001	32.15	<0.0001	17.71	<0.0001	56.39	<0.0001	140.91	<0.0001	92.23	<0.0001
R-PPT	2	3.27	0.0469	29.77	<0.0001	12.78	<0.0001	0.10	0.9016	4.98	0.0104	3.05	0.0554
Site x R-PPT	4	1.66	0.1758	1.92	0.1204	2.36	0.0644	1.13	0.3541	5.59	0.0008	3.30	0.0172
Seed	1	-	-	10.14	0.0016	6.59	0.0109	0.04	0.8408	0.01	0.9120	0.00	0.9926
Site x Seed	2	-	-	2.30	0.1028	3.19	0.0429	0.64	0.5295	0.25	0.7771	1.58	0.2071
R-PPT x Seed	2	-	-	0.40	0.6705	0.30	0.7407	0.27	0.7619	0.46	0.6348	0.48	0.6169
Site x R-PPT x Seed	4	-	-	1.13	0.3419	1.77	0.1360	0.44	0.7783	0.95	0.4343	0.51	0.7261
Carbon	1	200.82	<0.0001	16.64	<0.0001	14.08	0.0002	32.83	<0.0001	20.12	<0.0001	5.37	0.0213
Site x Carbon	2	13.07	<0.0001	10.23	<0.0001	11.71	<0.0001	4.87	0.0084	12.83	<0.0001	2.72	0.0679
R-PPT x Carbon	2	0.23	0.7981	0.65	0.5252	0.26	0.7731	1.15	0.3169	1.36	0.2592	0.37	0.6898
Site x R-PPT x Carbon	4	1.16	0.3327	1.17	0.3266	1.38	0.2418	1.25	0.2909	3.47	0.0089	2.44	0.0473
Carbon x Seed	1	-	-	4.28	0.0396	0.16	0.6897	0.76	0.3835	0.00	0.9795	0.83	0.3622
Site x Carbon x Seed	2	-	-	1.10	0.3340	0.54	0.5815	0.20	0.8224	1.05	0.3529	0.19	0.8241
R-PPT x Carbon x Seed	2	-	-	2.07	0.1286	0.50	0.6075	1.00	0.3686	0.24	0.7902	0.90	0.4085
Site x R-PPT x Carbon x Seed	4	-	-	2.48	0.0444	1.17	0.3237	0.33	0.8592	0.46	0.7614	1.17	0.3231

FIGURES

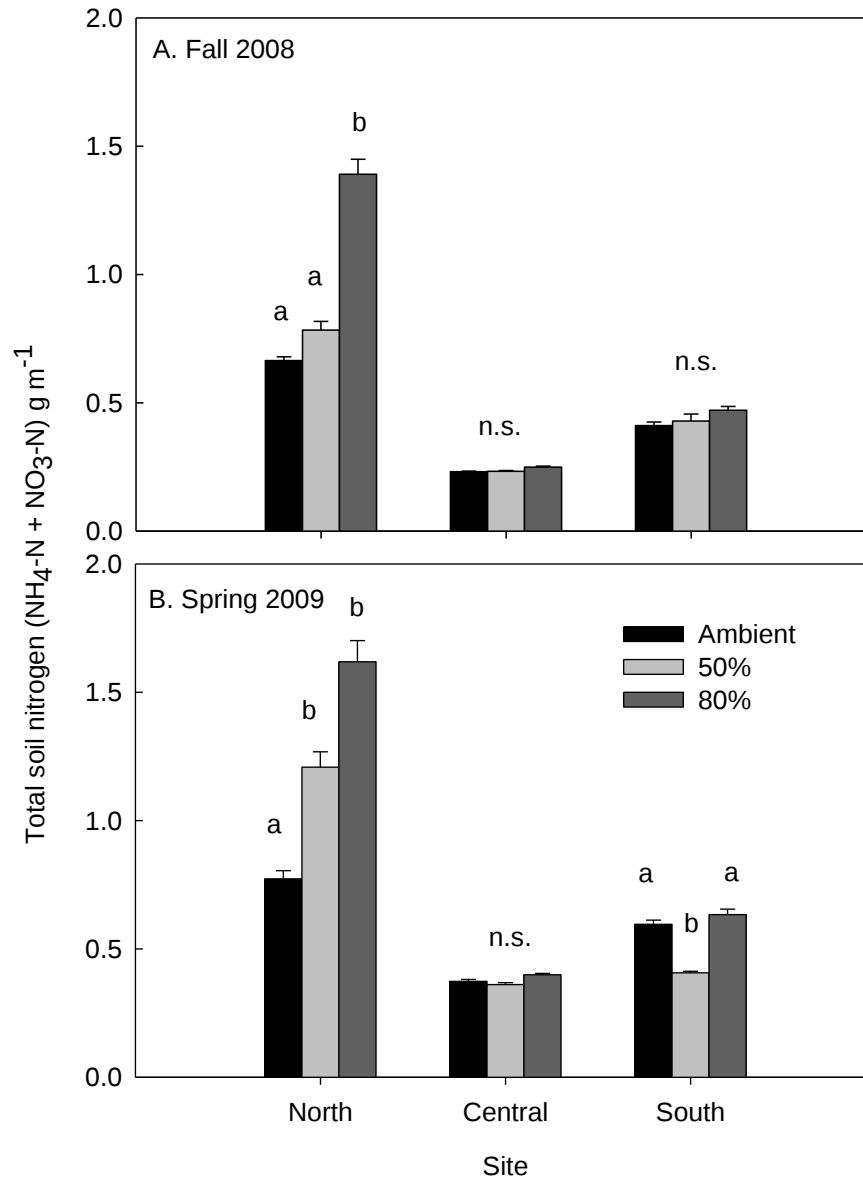


Figure 3.1 Total inorganic soil nitrogen (NH₄-N + NO₃-N) values obtained from soil cores (10 cm depth) collected in September 2008 (A), immediately following the experimentally imposed drought that lasted for two growing seasons (May – Sep), and in March 2009 (B), the start of the post-drought growing season prior to carbon and seed additions. Error bars indicate the standard error of each mean and different letters represent significant differences (p < 0.05) between rainfall treatments within each site. See Table 1 for complete ANOVA results.

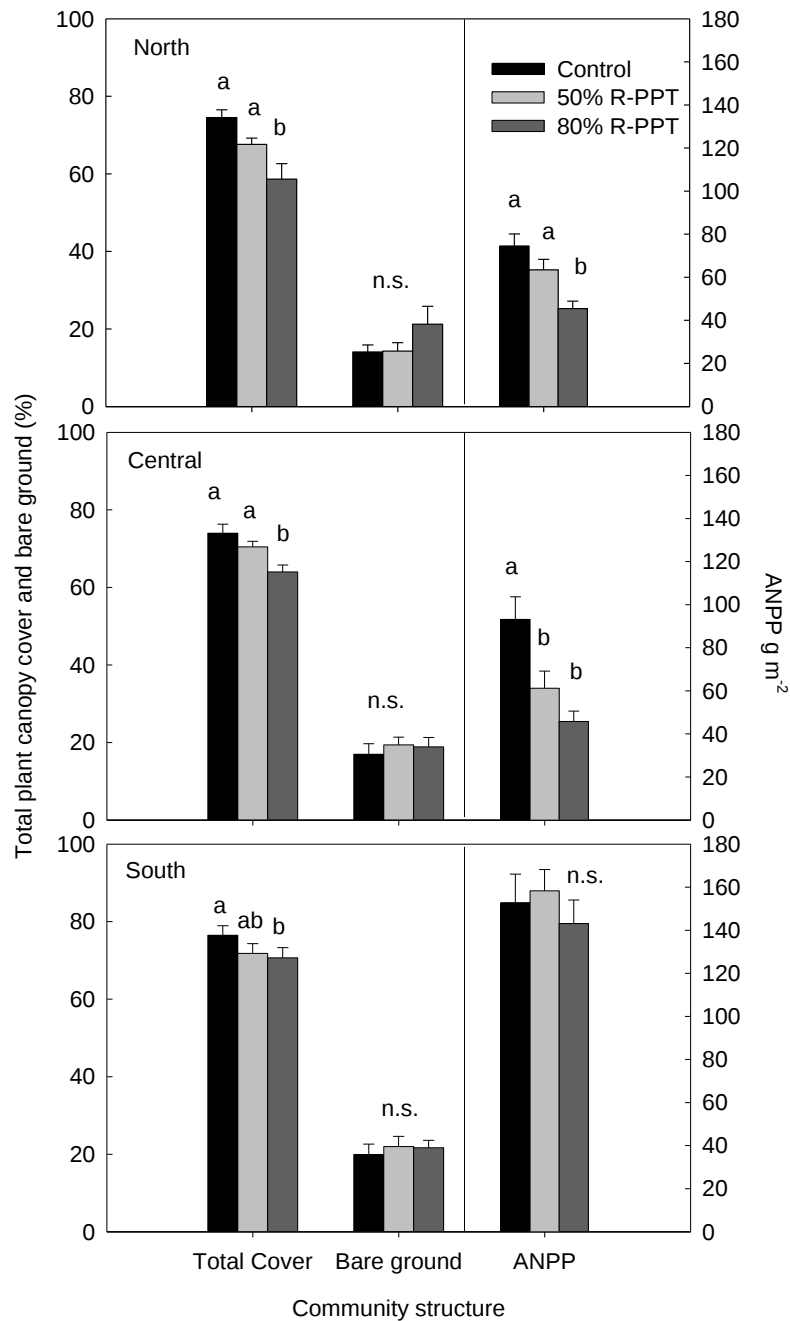


Figure 3.2 Vegetation community structure after two years of severe drought treatments. Left panel represents percent of total plant canopy cover and bare ground estimated in July 2008 while shelters were still in place. Right panel represents total ANPP (g m^{-2}) measured in September 2008 at the end of the growing season. Error bars represent the standard error of each mean and significance is noted by different letters ($p < 0.05$).

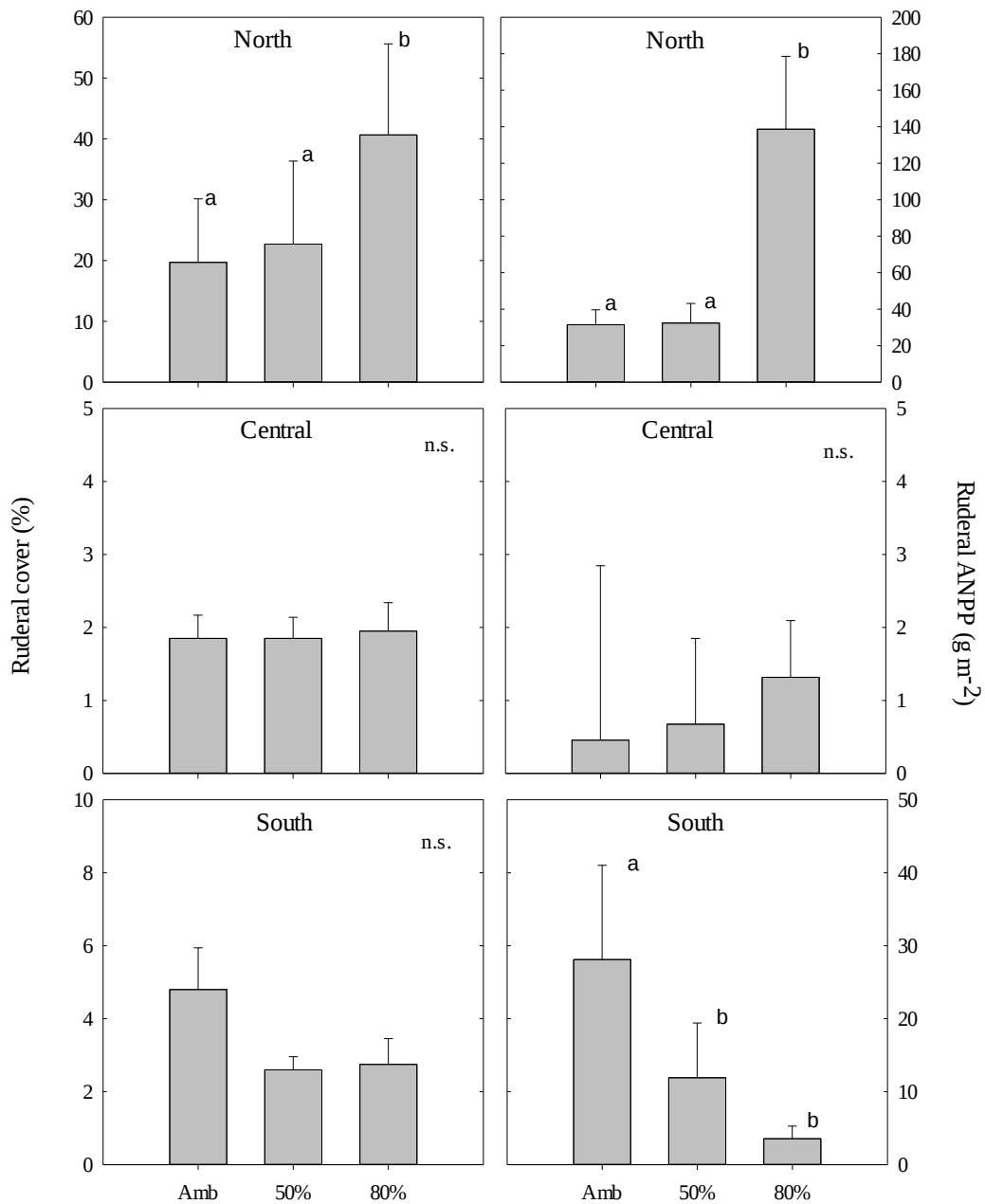


Figure 3.3 Percent canopy cover of ruderal species (left column) and ANPP of ruderal species (right column) in 2009 in treatment plots receiving ambient rainfall (Amb) or one of two levels of drought (50% or 80% R-PPT). Note the differences in the y-axis scales. Error bars represent the standard error of each mean and significant differences are represented by different letters ($p < 0.05$). See Table 2 for the overall ANOVA results. At the North site, percent canopy cover and ANPP of ruderal species significantly increased in plots receiving 20% of ambient rainfall. However, drought treatments did not significantly increase ruderal cover or ANPP at the Central or South sites.

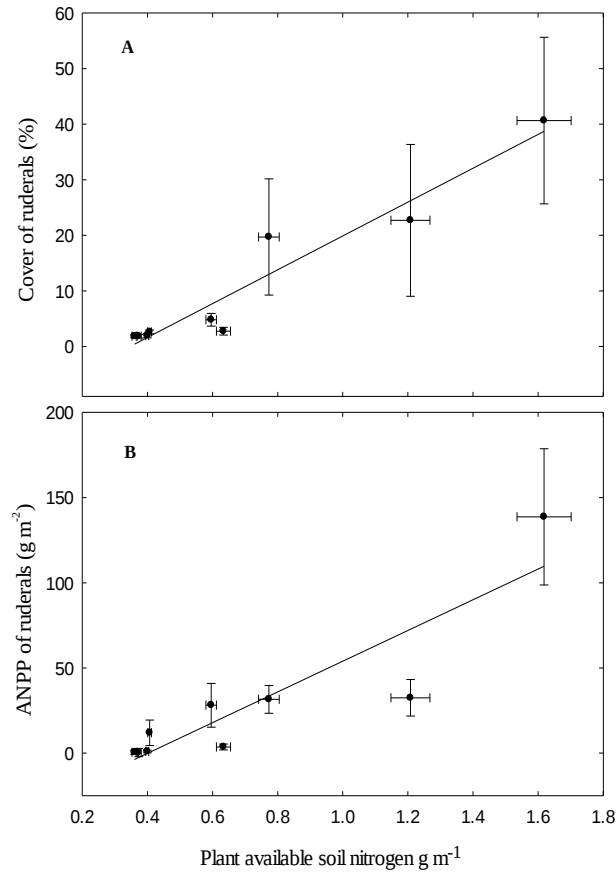


Figure 3.4 Total canopy cover (%) (A) and ANPP (g m⁻²) (B) of ruderal plant species in 2009 regressed on total plant-available soil nitrogen (NH₄-N + NO₃-N; g m⁻¹). Soil N values are from the spring 2009 sampling date (also see Fig. 1). Bi-directional error bars represent the standard error of the means. Means are used here for ease of illustration. With all points plotted the relationship between ruderal canopy cover and soil n (A) has $r^2 = 0.48$, $p < 0.001$, $F = 78.78$, and the relationship between ANPP and soil n (B) has an $r^2 = 0.28$, $p < 0.001$, $F = 33.66$.

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CHAPTER 4: VARIABLE LEGACY EFFECTS OF DROUGHT ON ABOVEGROUND PRODUCTIVITY IN A SEMI-ARID GRASSLAND BIOME

INTRODUCTION

There is consensus among global climate models that the intensity, frequency, and duration of droughts in the semi-arid grassland region of the US Central Plains will increase in coming decades (Seager et al. 2007; Dai 2013; Kirtman et al. 2013; IPCC 2014; Cook et al. 2015). For instance, Cook et al. (2015) produced statistically robust climate model projections suggesting that over the next century drought conditions in the Central Plains will equal or exceed the harshest droughts of the previous millennium, including the devastating Dust Bowl drought of the 1930s (Cook et al. 2014). Furthermore, climate models used in the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5) predict the frequency of droughts in arid regions will likely increase during the 21st century, especially if greenhouse gas (GHG) emissions continue to rise (IPCC 2014). Finally, Dai et al. (2011) presented a synthesis of climate model forecasts and asserted that certain regions of the world, including the mid-continental region of the United States, may experience persistent and severe drought conditions in the next few decades. Since water deficits are already a common occurrence in the shortgrass steppe (SGS) biome (Pielke and Doesken 2008), the impacts of even greater aridity on important ecosystem functions in this region warrants closer attention.

Our ability to forecast the multitudinous effects of drought on grassland ecosystems is limited, in part, by our incomplete understanding of the relationship between climate events, including precipitation (PPT) deficits, and specific ecosystem responses, such as aboveground net primary production (ANPP). Increasing our knowledge of the underlying mechanisms that

govern grassland recovery from drought, and how these mechanisms vary on both spatial and temporal scales, is therefore essential for our ability to predict consequences of climate change in general, and drought in particular. The effects of drought on grassland ecosystems have long been of interest, beginning with studies focusing on the degree of grassland recovery from the great Dust Bowl drought (e.g., Weaver et al. 1935; Albertson and Weaver 1944), and in more recent years, research concentrating on grassland responses within the context of global climate change (e.g., Lauenroth and Sala 1992; Oesterheld 2001; Yahdjian and Sala 2006; Cherwin and Knapp 2012; Sala et al. 2012; Hoover et al. 2014; Concilio et al. 2016). Many of these more recent studies have revealed time lags in grassland recovery from severe drought, suggesting that the combined effects of deficits in water availability and reductions in tiller density may create drought legacies that give rise to recovery lags (Sala et al. 2012; Reichmann et al. 2013). For example, Lauenroth and Sala (1992) conducted a time-series analysis addressing the relationship between ANPP and PPT in the shortgrass steppe and found that after each two year period of severe drought, ANPP exhibited a lag in recovery (Oesterheld et al. 2001). Using the same 52-year data set as Lauenroth and Sala (1992), Oesterheld et al. (2001) further explored lags in recovery following severe drought, and determined that ANPP in the current-year is positively related to ANPP from the previous-year, implying that previous-year PPT helps explain interannual variability in ANPP. According to Sala et al. (2012), lags in recovery of ANPP result from legacies of PPT, whereby ecosystems that experience drought in the previous year(s) relative to the current year will have lower ANPP than predicted based on PPT inputs during the current-year alone. An experimental study conducted by Reichmann et al. (2013) confirmed that drought legacies can influence current-year ANPP in a desert grassland. Furthermore, drought

legacies are proportional to the differences between previous-year and current-year PPT inputs (Reichmann et al. 2013).

Precipitation, through its influence on soil water availability, is the abiotic factor that most strongly limits (ANPP) in semi-arid grassland ecosystems (Noy-Meir 1973; Lauenroth et al. 1978; Le Houérou 1984; Knapp and Smith 2001; Schwinning 2004; Reichmann 2013). The amount of primary production in these systems has several key implications. For example, ANPP determines forage availability and ultimately constrains herbivore carrying capacity of these ecosystems (Lauenroth and Sala 1992; Yahdjian and Sala 2006). ANPP also mediates the carbon cycle of semi-arid grasslands, serving as a reservoir for carbon in stored biomass (Lauenroth et al. 2008; Burke et al. 2008; Hoover and Rogers 2016). Semi-arid grasslands account for a significant portion of terrestrial ecosystems worldwide (Reynolds et al. 2007; Sala et al. 2012), and their ability to recover from drought could have serious implications for global C cycles under the drying trends predicted for the near-term future (Yahdjian and Sala 2006; Sala et al. 2012; Kirtman et al. 2013; Poulter et al. 2014; Ahlström et al. 2015). Thus, it is imperative to establish a fundamental understanding of how semi-arid grasslands will recover from severe drought in order to predict the ways in which important ecosystem services will be impacted.

The ratio of ANPP to PPT, often called rain use efficiency (RUE), is a useful tool for assessing the recovery of semi-arid grassland ecosystems following drought because it relates primary production directly to PPT inputs (Le Houérou 1984, 1988; Paruelo et al. 1999; Bai et al. 2008). There is a lack of agreement as to how ecosystem RUE will respond to increasing aridity. For instance, Le Houérou (1984) suggested that RUE tends to decrease along a spatial gradient towards drier conditions. Accordingly, Bai et al. (2008) reported that RUE in the steppe region of Inner Mongolia decreased as mean annual precipitation (MAP) decreased. On the other

hand, Huxman et al. (2004) found that RUE increased when moving from wetter biomes (e.g., mesic grasslands) to more arid biomes (e.g., arid grasslands). Many studies that have assessed RUE in semi-arid grasslands have used long-term data sets across large spatial PPT gradients. Much less is known about how RUE responds to altered PPT on shorter time scales, and at multiple sites within single biomes.

The goal of this study was to determine whether droughts lasting two years would create drought legacies in shortgrass steppe ecosystems resulting in impacts on ANPP and RUE after drought conditions subsided. To do this, I assessed ANPP at three sites in the shortgrass steppe biome after I had experimentally imposed varying degrees of drought for the previous two growing seasons. In 2007 and 2008, I imposed drought treatments reducing ambient growing season PPT by 50% and 80% using rainout shelters, and paired these with control plots receiving ambient PPT. In 2009, all plots received ambient PPT. I concurrently measured post-drought responses in plant community composition, cover and inorganic soil nitrogen (N) to elucidate whether there were lasting effects of drought on ecological attributes that influenced ANPP and RUE.

The specific objectives of this study were: (1) to analyze whether an experimentally-imposed two-year drought would create a drought legacy, whereby measurable effects of drought on ANPP and RUE would persist after the drought subsided; and (2) to determine if legacy effects of drought occurred at multiple sites within the shortgrass steppe biome of the United States.

Accordingly, I explored two hypotheses to address these objectives. First, I hypothesized that I would detect negative drought legacy effects on ANPP and RUE. This expectation was based on the previously mentioned long-term data analysis performed by Lauenroth and Sala

(1992) that found ANPP in the year following a severe, two year drought at the shortgrass-steppe always exhibited a recovery lag, with less ANPP than in pre-drought years. Potential mechanisms for negative drought legacies included reduced canopy cover and decreased storage of carbohydrate reserves after drought. Thus, I expected to see reduced ANPP and RUE in formerly droughted plots relative to control plots in 2009, the post-drought year.

Second, I hypothesized that drought legacy effects will be related to drought severity, such that severe drought treatments would create a larger legacy than the moderate drought treatments. This hypothesis is based on Reichmann et al. (2013) that found the magnitude of drought legacies in ANPP were proportional to the changes in PPT between drought and post-drought years. Therefore, I predicted the legacy of drought at my study sites would depend upon the level of drought imposed. Hence, I expected to detect larger drought legacy effects in the severely droughted plots (i.e., 80% reduced PPT) than in the moderately droughted plots (i.e., 50% reduced PPT).

MATERIALS AND METHODS

Study sites – This study was conducted at three sites distributed across a large portion of the north to south range of the shortgrass steppe biome, located on the western edge of the Central Plains region of North America (Cherwin and Knapp 2012). The study sites are representative of the core shortgrass prairie biome, with a mid-continental, semi-arid climate (Hochstrasser et al. 2002). This region is distinct from the mixed-grass and tallgrass prairies of the United States in terms of MAP (342 – 425 mm) and MAT (7.2° – 9.4°C), and also differs in dominant plant species composition. Aridic Argiustoll soils are consistently present across all three sites (Kelly et al. 2008); however, variations in soil texture exist between sites. Ninety percent of root biomass is held in the top 20 cm of soil (Schimel et al. 1985), 60% of which is

contained in the upper 15 cm of soil (Gill et al. 1999). At each site, *Bouteloua* is overwhelmingly the most abundant genus, and *Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths (blue grama) is by far the most dominant species, concerning biomass and ground cover.

The northern study site was at the Central Plains Experimental Range (CPER), situated in north-central Colorado (40°49'N latitude, 104°46'W longitude) in Weld County, roughly 18 km south of the Wyoming state border. Sandy loam soils with coarse texture predominated the CPER (Lauenroth and Burke 2008, Cherwin and Knapp 2012) and the bulk carbon-to-nitrogen ratio (C:N) was 13.8 (Cherwin, unpublished data). The central study site was at Sand Creek Massacre National Historic Site (SAND), located in south-east Colorado (38°32'N latitude, 102°31'W longitude) in Kiowa County, approximately 41 km west of the Kansas state border. Clay soils with fine texture prevailed at SAND (Cherwin and Knapp 2012) and the bulk C:N was 6.7. The southern study site was at Fort Union National Monument (FOUN), in north-central New Mexico (35°91'N latitude, 105°01'W longitude) in Mora County, about 120 km south of the Colorado state border. Sand clay loam soils with intermediate texture between the northern and central study sites were present at FOUN (Cherwin and Knapp 2012) and the bulk C:N was 8.0.

Experimental design and analysis – At each of the study sites, I established 30 plots measuring 2.25 m x 2.5 m (5.625 m²). Study plots were arranged into 10 blocks to control for variation within each site (n=10). In the first two years (2007 and 2008), I imposed two levels of drought by reducing ambient growing season precipitation (GSP; May - September) by 50% and 80%. Drought treatments were randomly assigned to plots in each block, and were induced by installing rainout shelters that intercepted either 50% or 80% of ambient PPT (Yahdjian and Sala 2002; Cherwin and Knapp 2012). In addition, each block contained 10 control plots with no

reductions in ambient precipitation. Thus, each block contained three treatments (i.e., plots), ambient PPT (i.e., control), 50% reduced precipitation (R-PPT), and 80% R-PPT. Following two years of growing season drought, all plots received ambient PPT inputs, which were within the normal range of variability at all three sites (Table 4.1). To determine the efficacy of the R-PPT treatments, volumetric soil moisture measurements were obtained for the top 20 cm of soil six times per day in half of the plots at each site (5 per treatment for a total of 15 per site) by employing Decagon ECH₂O EC-20 probes (Decagon Devices, Inc.) soil moisture sensors. Cherwin and Knapp (2012) describe the design of the field experiment in further detail.

Statistical computations were performed using the MIXED model procedure with the Restricted Maximum Likelihood (REML) method in SAS version 9.4 (SAS Institute 2013). I used a 3-factor repeated measures analysis of variance (ANOVA) to test for main and interactive effects of site, year, and R-PPT treatments on all response variables. Differences of least squares means (LSMeans) were used to compare treatment effects within sites when interactions of site and drought treatments were identified. In order to improve the symmetry and homogeneity of variance, I log₁₀-transformed the ANPP and soil N data for analysis. The Satterthwaite method was used to estimate degrees of freedom, and the significance level was set at $p < 0.05$ for all statistical analyses performed.

Response variables:

Vegetation characteristics – I measured ANPP by harvesting all end-of-growing-season aboveground biomass above the root crown from a 0.10 m² quadrat in each subplot. ANPP was clipped by species in the field, then placed into a drying oven for 2–3 days until all materials were completely dry. The biomass was then double-sorted and weighed by species in most cases, or by genus in rare cases. Previous year's dead biomass was differentiated and separated from

current year's biomass based on variations in color and structure. Biomass from woody and succulent plant species were not included in ANPP measurements because they comprised a very small portion of ANPP in these ecosystems. To determine plant canopy cover by species I conducted visual estimates in each plot (1 m² quadrats) and summed the vertical polygon projections to the ground around each plant (Daubenmire 1959; Collins 1992).

Rain use efficiency (RUE) – I calculated RUE by dividing ANPP by PPT (i.e., ANPP [g·m⁻²]/PPT [mm] = RUE [g·m⁻²·mm⁻¹]). For each individual year (2008 and 2009) I performed within-site comparisons of RUE between treatments. For 2008, the PPT inputs varied according to treatments, but in 2009 all plots received ambient PPT (Table 4.1).

Inorganic soil nitrogen – I measured total soil inorganic nitrogen (NH₄-N + NO₃-N) from soil cores (5 cm diameter, 10 cm depth) collected in the spring of 2009 to determine the legacy effect of drought on plant-available soil N. Levels of soil N were estimated by performing potassium chloride (KCl) extractions and then analyzing the extractions on a Lachat QuickChem 8500 flow injection analyzer.

Drought sensitivity and drought legacy calculations – Drought sensitivity was estimated as the percent difference between the control and drought treatments at each site at the end of the 2008 growing season when the cumulative effects of the two-year precipitation reductions were most apparent. Drought legacies were computed relative to control ANPP as: (Treatment – Control)/Control at each site at the end of the 2009 growing season. To assess the relationship between drought legacy in 2009 and drought sensitivity in 2008, I regressed legacy on sensitivity.

RESULTS

Precipitation – Ambient PPT differed considerably between sites and years of this study (Table 4.1). Given the long-term historical climate records for the three study sites, the 50% R-PPT and 80% R-PPT treatments represented moderate and severe drought conditions, respectively. In fact, when averaged across sites, the shelters designed to reduce 50% of ambient GSP actually reduced GSP by 50.3% compared to the historical mean growing season PPT (MGSP), and the shelters designed to reduce 80% of ambient PPT, reduced GSP by 80.1% relative to historical MGSP. Since the shelters were designed to reduce ambient GSP, it was an unintended consequence that they also reduced historical MGSP by the same amounts, when averaged across sites.

For the 2009 growing season, PPT was above average at the northern and central study sites with 282.45 (17.6% above MGSP) and 319.02 mm (21.7% above MGSP), respectively. At the southern site, GSP was 265.18 mm (16.7% below MGSP). Since the coefficient of variation (CV) for interannual MGSP is 34.08, 31.32, and 31.37 for the northern, central, and southern sites, respectively, GSP was well within the normal range of variability across all sites (Table 4.1).

Aboveground net primary production (ANPP) – Although identical experimental infrastructure was used and soil moisture was effectively reduced at all three study sites during the growing seasons of 2007 and 2008, the sensitivity of ANPP to drought treatments differed between sites (Cherwin and Knapp 2012). Variability in ANPP was likewise observed in 2009 between sites, and was comparable to the patterns of sensitivity observed by Cherwin and Knapp (2012). In 2008, when the cumulative effects of the two-year experimental drought were most evident, ANPP at the northern site was significantly reduced by the severe drought treatments

(80% R-PPT), but not by the moderate drought treatments (50% R-PPT). At the central site, both moderate and severe drought treatments significantly reduced ANPP compared to controls, but the difference between moderate and severe drought treatments was not significant. At the southern site, neither drought treatment had a significant effect on ANPP.

In 2009, the year after the two-year experimental drought (i.e., the post-drought recovery year), ANPP was significantly greater in the severe drought plots than in the control and moderate drought plots (Figure 1A) at the northern site, indicating the legacy effects of drought at this site were positive. In this case, the magnitude of the drought legacy was greater in the severe drought plots than in the moderate drought plots (Figure 1A insets). At the central site, ANPP was significantly lower in the severe drought plots than in the control treatment plots, indicating the legacy effects of drought at this site were negative. In this case, the magnitude of the drought legacy was not significantly different between the severe and moderate drought plots. Similar to trends observed in 2008, ANPP in previously droughted plots at the southern site were not significantly different from control plots in 2009. When compared across all sites, there was no significant difference in legacy effects between the moderate and severe drought plots (data not shown).

I found that legacy effects of drought on ANPP existed at two of the three study sites, but these effects varied between the two sites. As noted, the legacy was positive (i.e., greater ANPP) at the northern site and negative (i.e., lower ANPP) at the central site. To explore the relationships between legacies in ANPP and the various response variables, I performed simple linear regressions for each variable (Figure 4.2). There were no significant relationships between legacy and either drought sensitivity or difference in PPT between the current (2009) and

previous (2008) years (Figure 4.2 A-B). However, there were significant relationships between legacy and both total soil inorganic N and plant canopy cover (Figure 4.2 C-D).

Rain use efficiency – Across all sites, drought treatments significantly increased RUE in 2008 (Figure 4.1B) and these increases were proportional to the magnitude of drought. In other words, I observed that RUE increased with decreased PPT during a two-year growing season drought. In the post-drought year, RUE varied between sites and treatments. Moderate drought plots did not differ significantly from controls at any of the sites. Severe drought plots differed significantly at only the northern and central sites, but not the southern site. The differences detected in the severe drought plots at the northern and central sites were opposite of one another, such that plots that received only 20% of ambient growing season PPT (i.e., 80% R-PPT) for two years had significantly higher RUE at the northern site and significantly lower RUE at the central site. These differences are attributed to the varying proportions of plant functional groups (PFGs). At the northern site, total ANPP in the severe drought treatments had a greater proportion of forbs compared to grasses. In particular, there was a high abundance of weedy annual forbs in the 80% R-PPT plots, especially *Salsola tragus* L. (Russian thistle; Family: Chenopodiaceae). However, when comparing only the grasses, the 80% drought plots significantly reduced RUE at the northern site. In contrast, the severe drought plots at the central site consisted of nearly all grasses, accounting for the lower RUE values.

Inorganic soil nitrogen – Total plant-available soil N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) was greatest at the northern site, including in the ambient PPT treatments (Table 4.2). Drought treatments significantly increased levels of soil N at the northern site, and this effect was present in the spring of 2009, at the beginning of the growing season. At the central site, there were only small differences in soil N between treatments, and these were not significant. At the southern site, the

50% R-PPT treatments had significantly less soil N, however these differences were inconsistent with the drought treatments, particularly because soil N did not differ between treatments immediately following drought in the fall of 2008 (see chapter 2 of this dissertation for greater detail on the effects of drought on soil N).

DISCUSSION

In this study, I induced moderate (50% R-PPT) and severe (80% R-PPT) levels of drought for two consecutive growing seasons (2007 and 2008) at three sites across the semi-arid grassland biome of North America. I then assessed the status of ANPP and RUE in the subsequent year (2009) following the drought conditions. My objectives were to determine whether experimental reductions in precipitation would create drought legacy effects that were proportional to the magnitude of drought. I calculated drought legacy within each site as the percent difference between the treatment and control plots in 2009.

I hypothesized that I would detect drought legacy effects on ANPP and RUE, and that the legacy effects would be negative. I have shown that drought legacy effects were sometimes but not always present in the year following the experimental two-year drought, and that these effects were not consistently negative. The southern site was insensitive to drought treatments over the course of the two-year reduced PPT treatments (Cherwin and Knapp 2012), and as expected, there was no legacy of drought at this site. The northern site had a significant positive drought legacy where the severe drought treatments resulted in increased ANPP in the post-drought year. The central site had a significant negative drought legacy where the severe drought treatments resulted in decreased ANPP in the post-drought year. This discrepancy in the direction (+/-) of the legacy effects was unexpected as both theory suggests and studies have

shown that legacy effects of drought are typically negative. For example, analyses that have used long-term data sets (e.g., Lauenroth and Sala 1992; Sala et al. 2012), have shown that legacies reduce ANPP in the year following dry years. In addition, an experimental study conducted by Reichmann et al. (2013) revealed that negative legacy effects of drought on ANPP were a consequence of dry conditions in the previous year. Thus, contrary to my prediction that drought legacy effects would be negative across all sites, I only found this to be true at one of the three sites. The difference in the directions of the legacy effects between the northern and central sites was largely accounted for by shifts in the relative abundance of different plant functional groups (PFGs). Forbs, particularly weedy annual species, outperformed the grasses in drought treatments at the northern site whereas grasses accounted for an overwhelming proportion of ANPP at the central site. These findings corroborate conclusions made by others that shifts in climate (e.g., increasing aridity) are important determinants for altering plant community composition of different PFGs (Gherardi and Sala 2015; Concilio et al. 2016).

I also hypothesized that drought legacy effects would be related to drought severity, such that severe drought treatments (80% R-PPT) would create a larger legacy than moderate drought treatments (50% R-PPT). In support of this hypothesis, I found that when a drought legacy existed it was greater in the severe drought plot compared to the moderate drought plot. At the northern site, the legacy effect of drought in the severe drought plots was significantly larger than in the moderate drought plots. At the central site, the legacy effect of drought was also larger in the severe drought plots than in the moderate drought plots, but the difference was not significant.

A pervasive conclusion among studies to date is that ANPP is lower in years preceded by dry years with low productivity (Lauenroth and Sala 1992; Yahdjian and Sala 2006; Sala et al.

2012; Reichmann et al. 2013). In other words, much evidence supports that drought legacy effects will result in lower than expected ANPP in post-drought years when sufficient wet conditions are present. Furthermore, Sala et al. (2012) proposed that this drought legacy phenomenon persists across all grassland biomes. I found evidence of drought legacies at two of my study sites in the shortgrass steppe biome, but ANPP was lower in the post-drought year at only one of those sites. Therefore, it is important to note that drought legacies will not necessarily arise following drought, and when they do arise, they may not always manifest as reductions in ANPP. As evidenced by the northern site in this study, drought legacies can sometimes result in increases in ANPP, particularly when shifts in the relative abundance PFGs and elevated soil N occur. Thus, although drought legacies may certainly influence ecosystems in the shortgrass steppe biome, it cannot be assumed that they will always occur following drought. Nor can it be assumed that drought legacies will always give rise to immediate recovery lags in ANPP, as it remains unseen whether the appearance of drought legacy effects will arise in subsequent years.

In this study, results from the central site corroborate the conclusion that drought legacy effects will negatively affect ANPP, as plots that were previously droughted at this site showed significantly lower ANPP in the post-drought year than plots that had always received ambient precipitation (i.e., control plots). Previous researchers showed that negative drought legacy effects on ANPP in the post-drought year related to reductions in ANPP during drought year(s) and/or differences in PPT between drought year and post-drought year (Lauenroth and Sala 1992; Sala et al. 2012; Reichmann et al. 2013). However, after performing simple linear regressions relating drought legacy effects of ANPP in 2009 to reductions in ANPP in 2008 (i.e., drought sensitivity) and the difference in PPT between 2008 and 2009, I found no significant

relationships (Figure 4.2 A-B). It has also been suggested that soil N may be related to drought legacy effects on ANPP. After regressing drought legacy effects of ANPP in 2009 on total soil inorganic N, I found that soil N explained 15% of the variability in the drought legacy effects on ANPP. Another variable shown to influence drought legacy effects on ANPP is vegetation structure. For example, Reichmann et al. (2013) concluded that reduced tiller density was related to negative drought legacy effects on ANPP. After relating regressing legacy effects of ANPP in 2009 to legacy effects of total plant canopy cover in 2009, I found cover explained 20% of the variability in the drought legacy of ANPP (Figure 4.2 D). Therefore, mechanistically, I propose that both biogeochemical factors (i.e., soil N) and vegetation structure (i.e., plant canopy cover and community composition) offer the basic explanation for why drought legacy effects were present in two of the sites in this study.

In conclusion, results of this study indicate that drought legacy effects varied between sites within the shortgrass steppe biome of the US Central Plains region. The northern site showed a positive legacy effect, whereby ANPP increased in formerly droughted plots during the year that immediately followed the precipitation reductions. The increased ANPP post-drought was related to elevated soil N, reduced plant canopy cover, and shifts in the relative abundance of PFGs. In particular, a weedy annual forb outcompeted the normally dominant grasses in terms of RUE. The central site displayed a negative legacy effect, whereby ANPP decreased in formerly droughted plots. The decreased ANPP post-drought was linked to reduced plant canopy cover.

The magnitude of the drought legacy was significantly greater in the plots that were severely droughted than plots that were moderately droughted at the northern site, but this was due to the large positive drought legacy effect. Thus, I propose that when drought results in

elevated soil N and reduced plant canopy cover, positive drought legacy effects may influence ANPP, whereas when soil N is not elevated post-drought, negative legacy effects may impact ANPP.

Predicting drought legacy effects in the shortgrass steppe biome will be an increasing challenge in the coming years given the hysteresis of drought recovery patterns that will likely be evident during extreme weather events, such as droughts. Therefore, the ominous predictions for increased drought prevalence and severity in the near-term future (Kirtman et al. 2013; Dai et al. 2013; Cook et al. 2015) make further research exploring the impacts of drought legacies vital.

TABLES

Table 4.1 Growing season precipitation (GSP; May-Sep; mm) for three study sites in the semi-arid grassland biome of North America for the years of this study, 2007 and 2008. Two drought treatments, 50% and 80% reduced ambient (Amb) precipitation (R-PPT), were imposed for two consecutive growing seasons, along with control treatments that received Amb PPT. No R-PPT treatments were imposed in 2009. Percent differences from historical MGSP are noted in parentheses below GSP values for 2008 and 2009. The percent change in GSP (Δ GSP; %) between study years was calculated as the percent difference between 2009 and 2008 GSP. Historical MGSP values were based on 77, 125, and 142 years for the northern, central, and southern sites, respectively. The coefficient of variation (CV) for each site represents the interannual variation of the historical MGSP.

Site	GSP of Study Years (mm)				Δ GSP Between Study Years (% diff.)			Historical GSP	
	2008			2009				MGSP (mm)	CV
	Amb	50% R-PPT	80% R-PPT	Amb	Amb	50% R-PPT	80% R-PPT		
Northern (Central Plains Exp. Range)	246.89 (+2.8%)	123.45 (-48.6%)	49.38 (-79.4%)	282.45 (+17.6%)	12.6	56.3	82.5	240.15	34.08
Central (Sand Creek Nat'l Hist. Site)	182.63 (-30.3%)	91.32 (-65.2%)	36.53 (-86.1%)	319.02 (+21.7%)	42.8	71.4	88.5	262.23	31.32
Southern (Fort Union Nat'l Mon.)	400.81 (+25.9%)	200.41 (-37.0%)	80.16 (-74.8%)	265.18 (-16.7%)	-51.1	24.4	69.8	318.30	31.37

Table 4.2 Total soil inorganic nitrogen ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$; g m^{-2}) collected in spring 2009.

	2009 Soil N (g m^{-2})		
	Ambient	50% R-PPT	80% R-PPT
Northern (Central Plains Exp. Range)	0.77	1.21	1.62
Central (Sand Creek Nat'l Hist. Site)	0.37	0.36	0.40
Southern (Fort Union Nat'l Mon.)	0.60	0.41	0.63

FIGURES

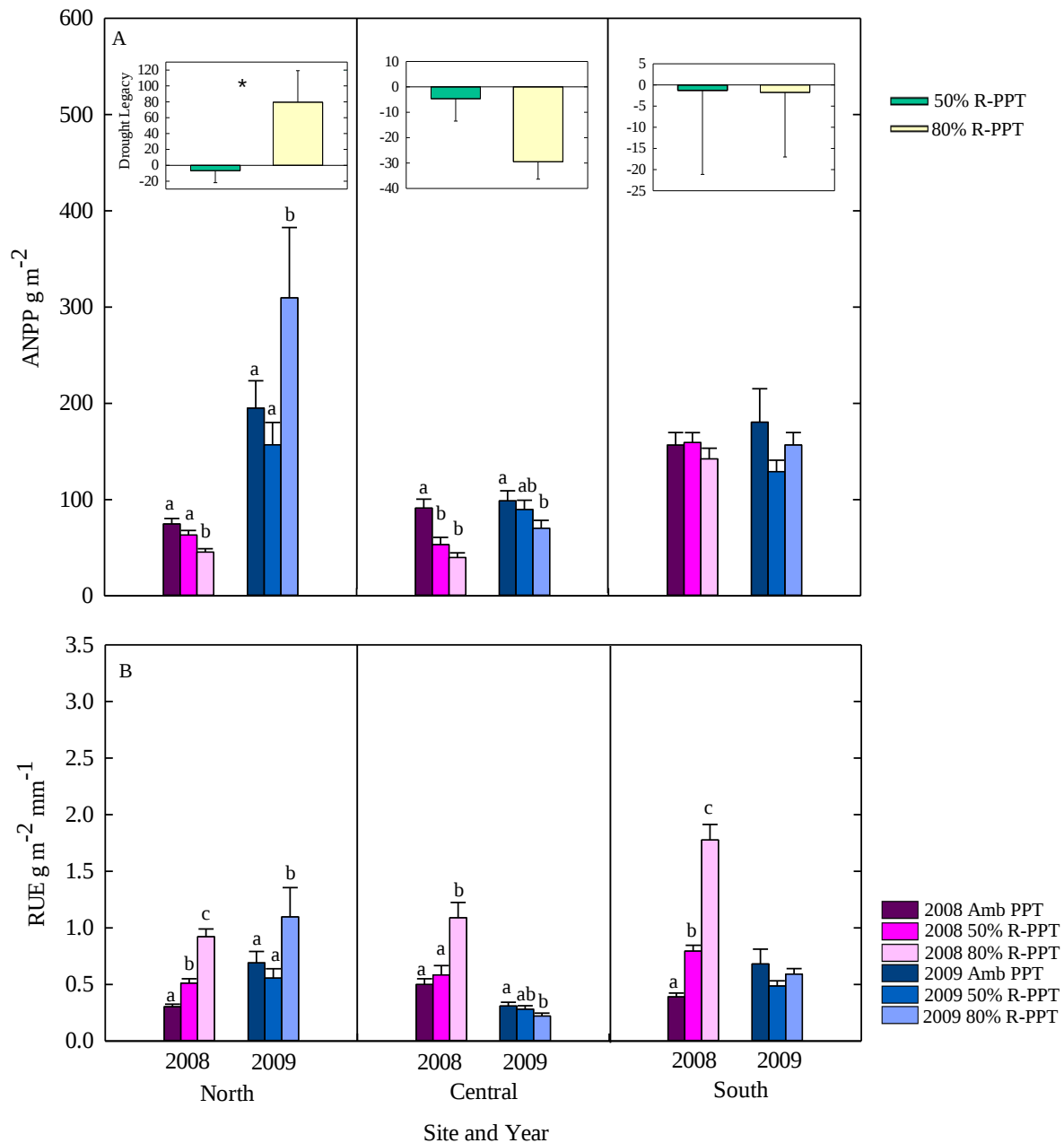


Figure 4.1 Total aboveground net primary productivity [A], drought legacy [A insets], and rain use efficiency [B] for ambient PPT (control) and reduced precipitation (R-PPT) treatments at the three semi-arid grassland study sites. [A] Total aboveground net primary productivity (ANPP; g m^{-2}) collected at the end of the 2008 growing season (pink shades) represents the sensitivity response to the experimentally-induced two-year (2007 & 2008) growing season drought. Total ANPP collected at the end of the 2009 growing season (blue shades) reveals the legacy response to the drought treatments. In 2008, significant effects of drought treatments on ANPP were

detected in 2008 at the northern and central sites, but not at the southern site. In 2009, significant effects of drought on ANPP were observed in the severe drought plots (80% reduced precipitation; R-PPT) at the northern and central sites. As expected, there significant differences were not observed at the southern site. Within each site by year combination, significant treatment differences are represented by different letters ($p < 0.05$). [A Insets] Legacies, calculated as the percent difference between treatments and controls within each site, were observed in the severe drought plots at the northern and central sites, but the legacy effects were uneven. At the northern site, there was a positive legacy effect, and at the central site there was a negative legacy effect. In 2009, the severe drought plots at the northern site consisted of a much larger proportion of forbs than grasses, accounting for the positive legacy effect observed at this site. At the central site, all plots were nearly entirely composed of grasses. Within each site, significant treatment differences are represented by an asterisk (*; $p < 0.05$). Note that the y-axes differ in the A insets. [B] Rain use efficiency (RUE; $\text{g m}^{-2} \text{mm}^{-1}$) in 2008 (pink shades) and in 2009 (blue shades). In 2008, there was a significant treatment effect on RUE at all sites, such that RUE increased with decreased PPT inputs. In 2009, there were significant treatment effects in the severe drought plots (80% R-PPT) at the northern and central sites, but no significant treatment effects at the southern site. These responses mirror the patterns in ANPP because RUE is a function of ANPP. Within each site by year combination, significant differences are represented by different letters ($p < 0.05$).

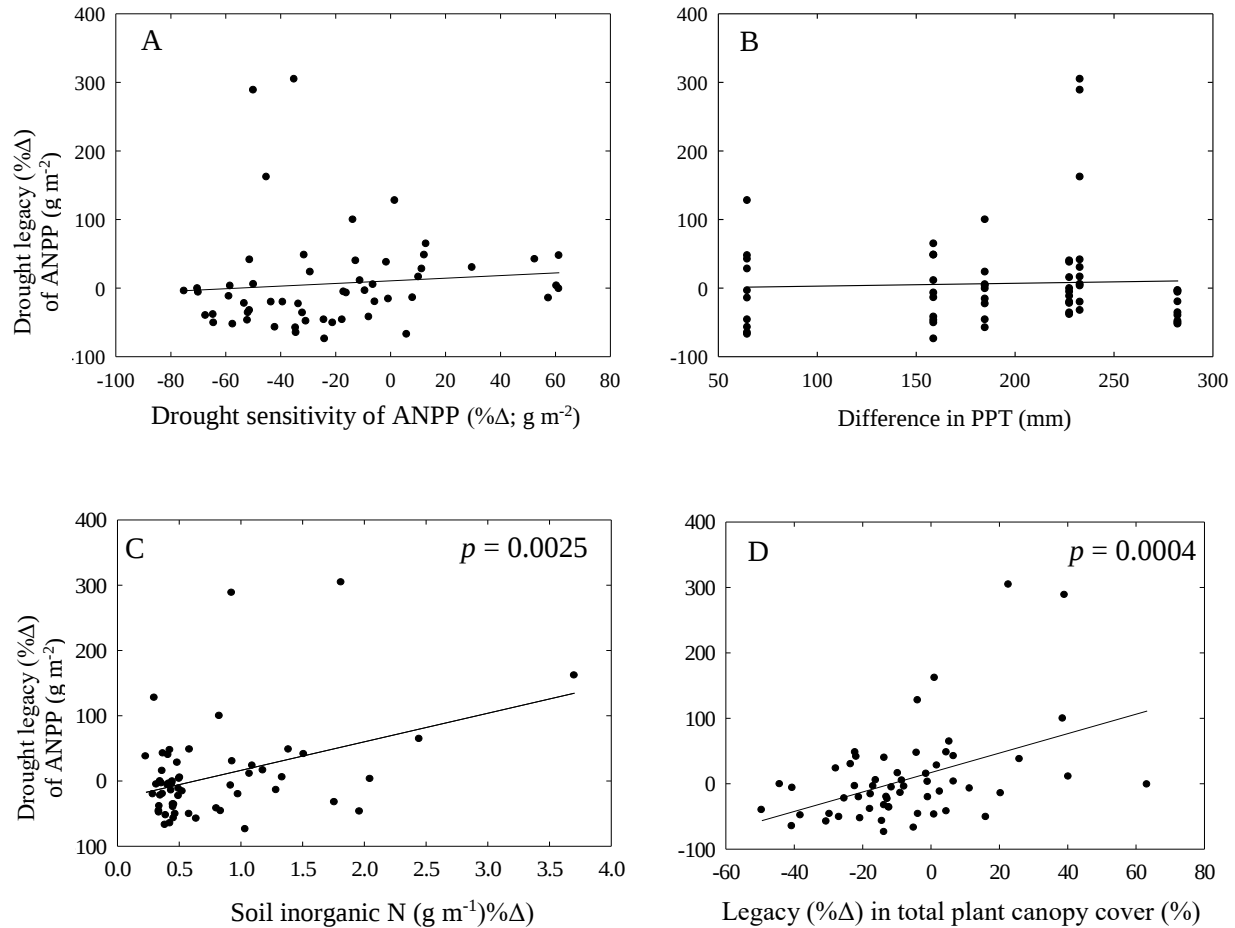


Figure 4.2 Relationships (simple linear regressions) between drought legacy effects of total aboveground net primary productivity (ANPP; %Δ g m⁻², relative to controls) and [A] drought sensitivity (%Δ g m⁻², relative to controls), [B] difference (Δ) in precipitation (PPT; mm) between 2009 and 2008, [C] total soil inorganic N (NH₄-N + NO₃-N; g m⁻¹), and [D] legacy of total plant canopy cover (%Δ, relative to controls). Drought legacy of ANPP was calculated as the percent difference (%Δ) between drought treatments and controls within sites for 2009. [A] Drought sensitivity was calculated as the percent difference (%Δ) of ANPP between drought treatments and controls for 2008. No significant relationship was found between drought legacy and drought sensitivity. [B] No significant relationship was found between drought legacy and difference in PPT. [C] Soil nitrogen values were obtained from soil cores collected in the spring of 2009. For the legacy of soil N effect, $R^2 = 0.15$, $F = 10.2$, and $P = 0.0025$. Data points represent values from individual drought treatment plots. [D] Legacy in total plant canopy cover was calculated as the % difference between drought treatments and controls, (T-C)/C, within sites for 2009. For the legacy of plant canopy cover effect, $R^2 = 0.20$, $F = 14.1$, and $P = 0.0004$. Data points represent values from individual drought treatment plots.

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CHAPTER 5: CONCLUSIONS

The overarching goals of this study were to investigate drought sensitivity of semi-arid grasslands and, in turn, determine whether sensitivity patterns influence plant invasion potential and legacy effects following drought. To address these goals, I experimentally induced two levels of rainfall reductions (i.e., drought treatments) that decreased growing season precipitation by 50% and 80% for two consecutive years (2007 & 2008), with the following year (2009) serving as the post-drought year.

In the first study, presented in chapter 2, I assessed the sensitivity of three semi-arid grassland ecosystems to experimentally induced drought conditions. I predicted all three ecosystems would exhibit strong sensitivity to drought in terms of reduced aboveground net primary productivity (ANPP), and that all sites would respond similarly to drought. Contrary to my predictions, results showed that sensitivity varied between sites – from no reduction in ANPP to a 51% decrease. To help elucidate the unexpected variability between sites, I formulated a drought sensitivity index – the ratio between the response of ANPP (control – treatment) and the reduction in precipitation (PPT) for each drought treatment. I then compared drought sensitivity to differences in rainfall regimes and detected a strong inverse relationship between drought sensitivity and rainfall event size. I concluded that when rainfall events are sufficiently large, less precipitation is lost to evaporation, which therefore maintains soil moisture for longer periods and increases rain use efficiency (RUE). Thus, this study indicated there is an important interaction between rainfall regime and drought sensitivity in semi-arid grasslands.

In the second study, presented in chapter 3, I explored whether drought facilitates plant invasions in semi-arid grasslands by creating pools of unused resources that can be exploited

post-drought by weedy plants with opportunistic growth strategies. Specifically, I expected plant-available soil nitrogen (N) to increase and total plant cover to decrease due to drought, and that these fluctuations in resources (e.g., soil N, space, light) would increase invasibility of these ecosystems (Davis et al. 2001). Results of this study revealed that the abundance of opportunistic weeds increased at only one site, and this increase coincided with elevated soil N levels. Hence, drought can sometimes create a “window of opportunity” for the successful establishment of invasive plants in semi-arid grasslands, however invasions are not always more prevalent as a result of drought. Thus, this study indicated that semi-arid grasslands are generally quite resistant to plant invasions, however, invasibility may increase if a disturbance event, such as severe drought, generates a pool of unused resources.

In the third study, presented in chapter 4, I analyzed drought legacy effects on ecosystem functioning in semi-arid grasslands. I predicted that experimental precipitation reductions would result in negative legacy effects, whereby ANPP would be lower than expected in the post-drought year (Lauenroth and Sala 1992; Oesterheld et al. 2001; Sala et al. 2012). I also predicted that the legacy effects would be proportional to drought magnitude (Reichmann et al. 2013), such that severe drought treatments would create larger legacy effects than moderate drought treatments. Results of this study showed that when a semi-arid grassland ecosystem displayed sensitivity to drought, drought legacy effects were present after the drought subsided, but the direction of the legacy (+/-) was somewhat less predictable. At one site, I observed a highly positive legacy effect resulting from the interaction between increased soil N and changes in species composition. At another site, I detected a modest negative legacy effect. And at the final site, there was no drought legacy due to drought insensitivity.

Given the large consensus among global climate model projections that droughts will become more frequent and severe over the North American semi-arid grassland biome in the near-term future (Dai 2013; Kirtman et al. 2013; IPCC 2014; Cook et al. 2015), this research has several important implications for these ecosystems. First, there is an important interaction between rainfall regime and sensitivity to altered precipitation amounts. Second, invasion windows may become more common in the future. Third, legacy effects that influence ground cover and soil nutrient resources may give rise to lags in the recovery of ANPP following drought. And finally, it is clear that formulating predictions for how semi-arid grasslands will respond to extreme weather events such as drought will become an increasing challenge for stakeholders.

In closing, the research presented in this dissertation highlights the variability in ecological responses to increased aridity in the shortgrass steppe biome of the US Central Plains region. Future research is needed to gain a better understanding of how climate change will impact these ecosystems. For example, more direct experiments that manipulate rainfall event size are needed to test the interaction between precipitation regimes and ecosystem functions such as carbon and nitrogen cycling. In addition, to build on the results from the invasion study in this dissertation, studies that create larger soil nutrient gradients and/or generate greater disturbances to vegetation structure along with precipitation treatments may reveal whether invasibility of these ecosystems will increase when faced with more intense weather fluctuations. Also, given the unexpected patterns of responses to drought within the shortgrass steppe biome, it may be necessary to perform studies at multiple sites within biomes in order to gain a more definitive description of biome-level ecological responses to climate change. Furthermore, a greater number of long-term drought studies, which are ideally replicated within biomes, will

provide better insight into impacts of increased aridity on these ecosystems, including drought legacy effects. For example, examining the temporal dynamics of drought recovery will be important given the projections for increased frequency and duration of drought events. Finally, exploring the impacts of increasing aridity in shortgrass ecosystems that lie near ecotones may help reveal ecological thresholds and novel ecosystem states that result from drought.

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APPENDIX

Table A1-1. List of plant species and plant functional group (PFG) classifications for each study site. Because seed germination was so low, we classified a subset of resident plant species as ruderals. Ruderals were defined as nonnatives that are well adapted at colonizing disturbed habitats as well as species considered to be locally weedy. Asterisks (*) denote species that were seeded.

Site	Species Name	PFG
Central Plains	<i>Artemisia frigida</i> Willd.*	forb
Experimental	<i>Euphorbia revoluta</i> Engelm.	forb
Range	<i>Eriogonum annuum</i> Nutt.	forb
(CPR)	<i>Eriogonum effusum</i> Nutt.	forb
	<i>Heterotheca villosa</i> (Pursh) Shinnars	forb
	<i>Ipomopsis laxiflora</i> (J.M. Coult.) V.E. Grant	forb
	<i>Lepidium densiflorum</i> Schrad.	forb
	<i>Mirabilis linearis</i> (Pursh) Heimerl	forb
	<i>Oenothera albicaulis</i> Pursh	forb
	<i>Oenothera coronopifolia</i> Torr. & A. Gray	forb
	<i>Plantago patagonica</i> Jacq.	forb
	<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.*	forb
	<i>Aristida purpea</i> Nutt.	graminoid
	<i>Bouteloua gracilis</i> (Willd. Ex. H.B.K.) Lag. ex. Steud.	graminoid
	<i>Carex filifolia</i> Nutt.	graminoid
	<i>Elymus junceus</i> Fisch.	graminoid
	<i>Lycurus phleoides</i> Kunth	graminoid
	<i>Sporobolus cryptandrus</i> (Torr.) Gray	graminoid
	<i>Astragalus mollissimus</i> Torr.	ruderal forb
	<i>Chenopodium leptophyllum</i> (Moq.) Nutt. ex. Wats.	ruderal forb
	<i>Helianthus annuus</i> L.*	ruderal forb
	<i>Salsola tragus</i> L.	ruderal forb
	<i>Cirsium arvense</i> (L.) Scop.	ruderal forb
	<i>Vulpia octoflora</i> (Walt.) Rydb.	ruderal graminoid
Sand Creek	<i>Artemisia frigida</i> Willd.*	forb
Massacre	<i>Eriogonum effusum</i> Nutt.	forb
National	<i>Eriogonum jamesii</i> Benth.	forb
Historic Site	<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.*	forb
(SAND)	<i>Psoraleidium tenuiflorum</i> (Pursh) Rydb.	forb
	<i>Aristida purpea</i> Nutt.	graminoid
	<i>Bouteloua gracilis</i> (Willd. ex. H.B.K.) Lag. ex. Steud.	graminoid
	<i>Lycurus phleoides</i> Kunth	graminoid
	<i>Sporobolus cryptandrus</i> (Torr.) Gray	graminoid
	<i>Astragalus mollissimus</i> Torr.	ruderal forb
	<i>Gaura coccinea</i> L.	ruderal forb
	<i>Salsola tragus</i> L.	ruderal forb

Fort Union National Monument (FOUN)	<i>Allium cernuum</i> Roth.	forb
	<i>Artemisia frigida</i> Willd.*	forb
	<i>Eriogonum annuum</i> Nutt.	forb
	<i>Eriogonum effusum</i> Nutt.	forb
	<i>Erysimum capitatum</i> (Douglas ex. Hook.) Greene	forb
	<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.*	forb
	<i>Gutierrezia sarothrae</i> (Pursh) Britton & Rusby	forb
	<i>Heterotheca villosa</i> (Pursh) Shinnery	forb
	<i>Hymenopappus filifolius</i> Hook.	forb
	<i>Ipomopsis laxiflora</i> (J.M. Coulter.) V.E. Grant	forb
	<i>Machaeranthera tanacetifolia</i> (Kunth) Nees	forb
	<i>Machaeranthera canescens</i> (Pursh) A. Gray	forb
	<i>Mirabilis linearis</i> (Pursh) Heimerl	forb
	<i>Psoralea tenuiflorum</i> (Pursh) Rydb.	forb
	<i>Aristida purpurea</i> Nutt.	graminoid
	<i>Bouteloua gracilis</i> (Willd. ex. H.B.K.) Lag. ex. Steud.	graminoid
	<i>Elymus junceus</i> Fisch.	graminoid
	<i>Lycurus phleoides</i> Kunth	graminoid
	<i>Sporobolus cryptandrus</i> (Torr.) Gray	graminoid
	<i>Gaura coccinea</i> L.	ruderal forb
	<i>Salsola tragus</i> L.	ruderal forb
	<i>Cirsium arvense</i> (L.) Scop.	ruderal forb