THESIS

SEMI-ARID GRASSLAND ECOSYSTEM FUNCTIONAL COLLAPSE AFTER EFFECTS OF FIVE YEARS OF EXTREME DROUGHT

Submitted by

Alicia Lenners

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Master's Committee:
  Advisor: Melinda D. Smith

  Caroline Havrilla
  Troy Ocheltree

ABSTRACT

SEMI-ARID GRASSLAND ECOSYSTEM FUNCTIONAL COLLAPSE AFTER EFFECTS OF FIVE YEARS OF EXTREME DROUGHT

A key outcome of climate change is an increase in the frequency and intensity of drought events in many regions of the globe. The largest impacts on ecosystem structure and function are likely to occur in water-limited ecosystems, such as semi-arid grasslands, potentially leading to a collapse of ecosystem function. While short-term studies have been conducted on various grassland ecosystems, the goal of this study is to fill in the gap of the effect multi-year extreme droughts have on the semi-arid shortgrass steppe of the Central US by characterizing the change in structure and function of these ecosystems. The drought was conducted between 2018-2022, and I had conducted various measurements over the summer of 2022 within the USDA-Central Plains Experimental Range (CPER) of Northeastern Colorado. The experimental drought was imposed using four rainfall exclusion shelters, two of which blocked 66% of precipitation from entering, and the other two remaining uncovered (control plots). Ten plots in each of the four shelters were measured weekly for soil moisture (%), soil temperature (^{o}C) and soil respiration (CO_2 efflux); twice per season for soil nutrient availability; and at the end of the growing season for aboveground (ANPP; stems and leaves) and belowground net primary production (BNPP; roots). The extreme drought resulted in an ~40% reduction in growing season soil moisture and an average 2°C increase in soil surface temperatures. Within the 13 weeks of study, drought led to an ~50% reduction in soil respiration (CO_2 efflux). ANPP was drastically reduced (~99%) with extreme drought, while cactus surface area increased 3-fold. The extreme drought treatment also resulted in large reductions in BNPP measured from 0-30 cm (79%); however, root growth was reduced most in the shallowest soil depth (0-10cm) when compared to control plots. Lastly, there was an increase in nitrogen availability (both NH_4^+ and NO_3^-) with extreme drought by the end of the growing season. These results suggest that extreme, multi-year drought can cause an almost complete collapse in ANPP and significantly reduce BNPP particularly in the top 10 cm of the soil profile, which could have important implications for carbon sequestration. It remains unknown what impact the dramatic reduction in ecosystem productivity but accumulation of available nitrogen in the soil will have for recovery of the shortgrass steppe ecosystem post-drought, but it is likely that recovery will be prolonged despite the increase in soil resources.

ACKNOWLEDGEMENTS

I want to give thanks to my mom and sister for their constant encouragement and support in every stage of my life. To my advisor Melinda Smith for her patience, her guidance in teaching me how to be a better professional, and most of all, for spearheading the International Drought Experiment. To my committee Carrie Havrilla and Troy Ocheltree for their guidance and support. To the graduate students in the Smith and Knapp labs for setting an extraordinary example as grassland ecologists, their compassion and support, and help with species composition, biomass, and soil sampling, and to the undergraduate students in the labs who helped with data collection and data entry. A final thanks to funders who made this project possible, including: the US Department of Agriculture, the Colorado Water Center, and the Graduate Degree Program in Ecology.

DEDICATION

In loving memory of my father, Tony L. Lenners, and my grandfather, Herman A. Hofeling.

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INTRODUCTION

Since the Industrial Revolution, increases in atmospheric greenhouse gasses have resulted in both warming and altered precipitation patterns globally. Altered precipitation patterns, including reductions in amount and increases in variability, are projected to be a trend within mid-continental regions (IPCC, 2022). When paired with the additional evaporative demand of plants stemming from increased vapor pressure deficit, which is a direct result of increasing air temperatures, these alterations in precipitation will increase the risk of drought events that are more frequent, longer in duration, and more extreme (Cook et al., 2015, IPCC, 2022). Because these changes in climatic patterns will vary among regions across the Earth and ecosystem responses will be based on an ecosystem's sensitivity to change climate parameters and the speed of that change, it is important to understand how intensification of drought will impact terrestrial ecosystems, particularly those that are water-limited and potentially more vulnerable to intensified drought.

Grasslands cover between 25-45% of Earth's terrestrial surface area (Cook et al, 2015; Chen et al, 2019; Gibson & Newman, 2019), store between 10-30% of the total soil organic carbon (Wang et al., 2008; Chen et al, 2019), and are utilized extensively for grazing by domesticated cattle worldwide (Godde et al, 2019; Rojas-Downing et al, 2017). Climate change is expected to affect grasslands in many regions of the globe with more frequent and intense periods of drought (IPCC, 2022). Given that grassland ecosystems are particularly sensitive to both intra- and interannual variation in precipitation (Knapp & Smith, 2001), the expectation is that these ecosystems will experience some of the largest impacts in ecosystem functioning and services (Gibson & Newman. 2019; Reid et al, 2014). Furthermore, the effects of intensified drought are likely to be most profound for water-limited grassland ecosystems, such as arid and semi-arid (dry) grasslands, which are strongly water limited and exhibit the greatest sensitivity to interannual variation in precipitation when compared to other grassland ecosystems (Huxman et al. 2004). Sensitivity of dry grasslands to precipitation changes could have important implications for these ecologically and economically important ecosystems in which a large fraction of humanity relies upon, as well as for carbon cycling variability globally (Poulter et al. 2014).

The shortgrass steppe (SGS), which covers the western portion of the US Central Plains, is a representative of such semi-arid grassland ecosystems. By the mid-21st century, the SGS region is projected to experience a 3°C increase in average summer temperatures and decreasing soil moisture even with typical precipitation levels (Walsh et al., 2014). Lessons from the past have demonstrated how devastating multi-year extreme droughts can be. The most devastating drought that occurred in recorded US history was the Dust Bowl of the 1930s, which affected much of the Central Plains (Schubert, 2004). The Dust Bowl drought was characterized by anomalous high temperatures, low precipitation, and low soil moisture over a period of a decade, which played a major part in the creation of the disastrous dust storms throughout the Central Plains (Burnette & Stahle, 2013). While the Dust Bowl did not occur within the area of study in the SGS region of Colorado, the expectation is for climate change to produce Dust Bowl-type droughts within this century (Cook et al. 2015). Such multi-year, extreme drought events will no doubt have large ecological and economic impacts on the SGS, based on what was observed during the Dust Bowl and the more recent extreme, short-term drought of 2012, which hit the agricultural sector by a total loss of approximately 30 billion USD (Smith & Matthews, 2015; Rupp et al., 2017; IPCC, 2022), and was the fourth most widespread drought on US record since 1895 (NOAA, 2012; Knapp et al, 2015). Given this, there is a pressing need to understand what impacts intensified droughts expected in the future will have on the SGS ecosystem.

The goal of my research is to advance understanding on how extreme, multi-year droughts affect the SGS ecosystem functioning as well as how these droughts differ from the effects caused by single-year droughts. With this study, I aim to answer an overarching question: How will five years of continuous drought affect above and belowground productivity, biomass of different functional groups, particularly C_4 grasses and forbs, and soil processes, including soil respiration (CO_2 efflux) and nutrient availability? By addressing this question, I hypothesize to observe a reduction of above (ANPP) (Slette et al, 2021; Wang et al, 2019; Cherwin & Knapp, 2012), belowground net primary production (BNPP) (Slette et al 2021; Denton et al, 2017) and soil respiration (Meeran et al, 2021; Ingrisch et al 2020; Balogh et al 2016; Carbone et al, 2008) after five years of continuous drought. I also expect to see an alteration of plant functional composition in favor of C_4 graminoids and forbs (Carroll et al, 2021) and an increase in soil N availability (Ochoa-Hueso et al, 2020; Yuan et al. 2017; Sala et al, 2012).

METHODS

Study site and experimental protocol

My research was carried out during the 2022 growing season (May-Aug) at the USDA-ARS Central Plains Experimental Range (CPER) in northeastern Colorado. This experimental area lies within the shortgrass steppe (SGS) biome which covers ~20 million acres of the Central US (Holechek et al, 1989) and is dominated by two C_4 grass species (*Bouteloua gracilis* and *B. dactyloides*). I leveraged on an ongoing extreme drought experiment, which is part of a larger network of experiments - the International Drought Experiment (IDE). IDE is a coordinated, distributed experiment consisting of 140 sites across the globe that utilize common protocols to determine how and why terrestrial ecosystems may differ in their response to extreme drought.

For five years (2018-2022), an extreme, year-round (365 days) drought treatment was imposed on intact grassland plots at the study site. This was accomplished by passively reducing each rainfall event using four 6 x 26 m shelters (Stuppy Greenhouse, North Kansas City, MO). Two shelters equipped with roofs covered with strips of clear corrugated polycarbonate, blocking 66% of the ambient precipitation. Control plots were not covered with roofing material, allowing the full amount of each rainfall event to enter. To hydrologically isolate each shelter, the perimeter of each shelter was trenched to a depth of 0.5 m, 6 mil plastic was installed, and aluminum flashing (20 cm) was installed to a depth of 15cm to prevent overland flow from occurring. A 0.5 m buffer zone was established on each side of the long axis of each shelter, and a 3 m buffer zone was established at the end of each shelter. Within the remaining area in each shelter, 10 2 x 2 m plots were established in a checkerboard pattern (Figure A1), for a total of 40 2 x 2 m plots. Each 2 x 2 m plot was subdivided into 1 x 1 m subplots, with one subplot dedicated to non-destructive sampling of plant community composition, and the remaining three subplots dedicated to destructive sampling for ANPP and BNPP, soil sampling and measurements of soil moisture, soil nutrients and soil CO_2 efflux.

Soil moisture, temperature, and respiration

Soil moisture and temperature measurements with taken within each of the 40 plots on a weekly basis during the growing season (30 May to 27 Aug 2022). Soil moisture was measured to a depth of 12 cm using a Hydrosense II probe (Campbell Scientific; Logan, UT). Soil temperature was also measured to a depth of 12 cm with a digital probe thermometer (Taylor; Oak Brook, IL). Concurrent with the soil moisture and temperature measurements, soil CO_2 efflux measurements were taken in each plot using a PVC tube collar (10 cm long and 12 cm internal diameter), which was inserted 5 cm into the soil on 16 May. Efflux of CO_2 from the soil surface was measured using a LICOR-6400 XT Portable Photosynthesis System with a soil respiration chamber (LICOR Inc.; Lincoln, NE).

Nutrient Availability

To measure availability of soil nutrients, two sets of Plant Root Simulator (PRS, Western Ag; Saskatoon, SK, Canada) resin probes (2 cation and 2 anion) were installed in the same subplots in which biomass samples were collected. Early season probes were installed on 21 May and late season probes were installed on 9 July. Each set of probes was collected six weeks after installation (early season: 2 July, late season: 27 August). After collection, the probes were shipped to Western Ag, where they were analyzed for NO_3^-, NH_4^+, and P, as well as a suite of micronutrients, including K, S, Ca, Mg, Mn, Al, Fe, Cu, Zn, B, Pb, and Cd.

In addition, soil cores (6 cm x 30 cm) were collected from each plot on 17 September to determine available NH_4^+ and NO_3^- . Soil samples were homogenized, processed through a 2mm mesh sieve, and weighed into 15.00 g and 10.00 g subsamples. To prevent moisture loss during processing, soil samples were stored in refrigeration and were sieved within a total of 4 hours of each other and weighed within an hour. The 10g subsamples were dried at 60°C for 48 hours and reweighed after this period for the purpose of measuring the gravimetric soil water content (Yu et al, 2019). The 15g samples were mixed with 50mL 2M KCl solution in acid-washed beakers and placed on a shaker table for 90 minutes. This solution was then filtered through grade 40 Whatman paper into Nalgene bottles (one per sample), to leave only the liquid, organic N extract. These samples were then processed by EcoCore at Colorado State University. The resulting concentrations of mg/L NO_3^- of NH_4^+ were then multiplied by the volume of 2M KCl solution used to extract N from each soil sample and divided by the calculated soil weight (g) to estimate concentrations of NH_4^+ and NO_3^- on a per gram soil basis.

Above- and Belowground Net Primary Production

To assess the ANPP, two strips of biomass within one of two destructive subplots were clipped to ground level at the end of the growing season during peak growth (13 August 2022). Biomass was separated in the field into C_3 graminoid, C_4 grass, and forb functional groups (cacti were measured in situ by surface area). The biomass samples were then dried at 60°C for 48 hours. After drying, the previous year's dead biomass was separated from the current year's growth. The current year's biomass from each functional category was weighed to the nearest 0.01 grams (g). For the purposes of this study, ANPP was estimated as the sum of biomass of C_3 and C_4 graminoids and herbaceous forbs (measured in g m^{-2}). Cacti were measured separately as the area of ground cover (measured in cm^2 m^{-2}).

BNPP was estimated using the root ingrowth core method. The ingrowth cores were constructed of fiberglass mesh to create a tube of 5 cm in diameter (20 cm circumference) and 33 cm in length. The ingrowth cores were filled with soil collected adjacent to the study site that had been homogenized, removed of all roots, and sieved with a 4mm sieve and again with a 2mm sieve. The ingrowth cores were filled to the top, tapped down to create a denser filling, and filled back to the top again. The filled ingrowth cores were then inserted to a depth of 30 cm in each plot in early June 2022, where they remained for 3 months (until the end of August). At the end of this period, the root ingrowth cores were removed from each plot (note 6 cores from control plots were damaged during removal and therefore are not included in the analysis). The cores were stored at 4°C until they could be processed in the lab.

For processing, the ingrowth cores were cut into three-10cm depth sections with a boxcutter and a ruler (used to measure the sections and to segregate the soil and roots from each section from mixing with the others). After trimming all excess roots from the outside of the baskets, each section was gently run under water with a 2mm sieve attached to a pan. This allowed the soil (which was previously sieved into 2mm diameter particles) to fall to the bottom of the pan while leaving most of the root biomass on top of the sieve. After the initial wash, a wide pan was filled with a shallow amount of water (approximately halfway), and the remaining biomass was added into the water in the pan. This allowed live biomass to float to the top, and the remaining roots were individually picked with tweezers and placed into a coin envelope. All non-root and dead biomass were discarded. The collected roots were dried, soil was removed, and weighed for root biomass, which was used to quantify BNPP (g m^{-2}).

Statistical Analysis

A mixed linear effects model, using the lmer() function and the "Kenward-Roger" method (Pinheiro, Bates, & R Core Team, 2023) in R-studio (version 4.2.2 (2022-10-31 ucrt))was used to assess the effects of drought on the response variables measured and to account for the block effect of the experimental design (Appendix Figure A1). With the Kenward-Roger method, the F statistic is modified to improve the accuracy in small sample properties by scaling the F-statistic to a smaller scale (Halekoh & Højsgaard, 2014). The approximate mean and variance of the F-statistic is calculated and then moments within the F distribution are matched to obtain the denominator degrees of freedom.

Soil respiration, moisture, and temperature were measured weekly; thus, the week of data collection was included as a repeated measure in the mixed effects linear model. ANPP and BNPP, as well as available soil N as determined by KCl extractions, were collected only once during the season; thus, time period was not included in the model. Finally, nutrient availability, as captured by PRS probes, were collected over two separate six-week periods. In this case, a model representing each collection time period was run separately to assess drought effects on the availability of anions and cations for each sample period.

RESULTS

Soil moisture, temperature, and respiration

As expected, soil moisture differed significantly between the control and drought treatments. Soil moisture to a depth of 10 cm was 40.5% lower in the drought as compared to the control plots (Figure 1a; Table A1). In contrast, soil temperature was on average 6.2% higher in the drought vs. control plots, but this difference was not significant (Figure 1b).

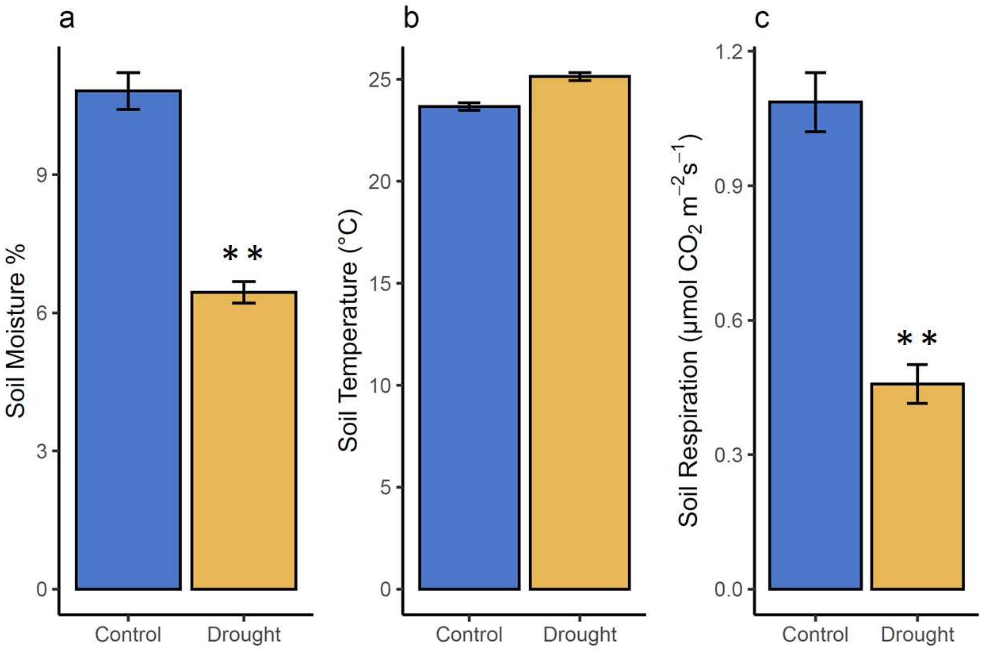

*Figure 1. Effect of extreme drought on growing season percent soil moisture (a) and soil temperature (b) to a depth of 12 cm. Bars represent mean with standard error bars. Asterisk indicates significant difference (**= $p < 0.05$) between the control and drought treatments.*

Similar to soil moisture, soil respiration (CO_2 efflux) was significantly reduced on average by 57.9% with extreme drought (Figure 1c), and this suppression of soil CO_2 efflux with the extreme drought was observed at each timepoint during the growing season (Figure A2).

Furthermore, soil CO_2 efflux was positively correlated with soil moisture (Figure A3), suggesting that the drought affected soil respiration through its influence of soil moisture.

Nutrient Availability

Drought had a significant effect on plant available nitrogen (NH_4^+ and NO_3^-), as measured with PRS probes, but the magnitude and direction of this effect differed between early vs. late season measurements. In the early season, NH_4^+ was ~40% lower in drought plots than the control plots, while NO_3^- was ~36% lower with drought (Figure 2a). In contrast, in the late season, NH_4^+ and NO_3^- were 42.5% and 117.8% (Figure 2b) higher in the drought plots when compared to control plots, respectively.

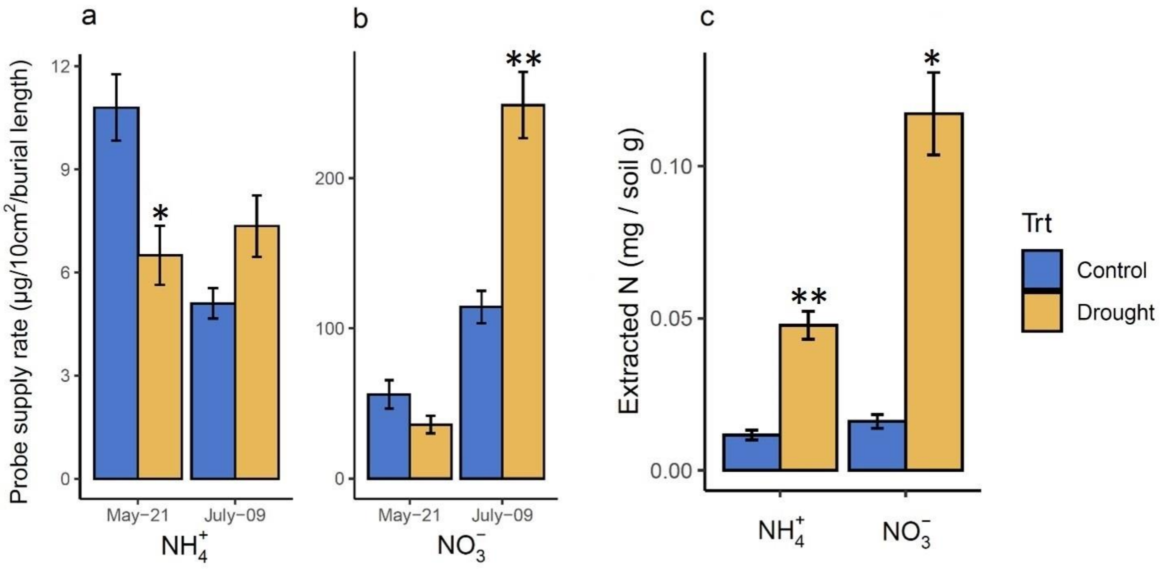

*Figure 2. Mean of NH_4^+ and NO_3^- availability in control and drought soils between May 21 – July 02 and July 09 – August 27 (a) and mean of available NH_4^+ and NO_3^- in extracted in 2M KCl solution. Bars represent mean with standard error bars. Asterisk indicates significant difference (**= $p < 0.05$, *= $p < 0.1$) between the control and drought treatments.*

The higher availability of plant available N was also observed in soil samples collected at the end of the growing season and analyzed for extractable N (NH_4^+, NO_3^-). Both available NH_4^+

and NO_3^- were significantly higher in the drought vs. control plots, with a calculated increase of 308% and 625% relatively (Figure 2c).

Other nutrients that were measured with the PRS probes and whose abundances were significantly affected by the extreme drought treatment include phosphorus (P), iron (Fe), manganese (Mn), and lead (Pb). Both treatments had similar nutrient levels of Fe, Mn, and Pb in the drought plots as the control plots early season, but these three elements increased substantially in the drought plots late season. Over the two collection periods, P experienced a 51.5% increase in the control treatment and a 209.5% increase in the drought treatment. Fe experienced a 25.2% increase in the control treatment and 183.6% in the drought treatment, Mn experienced a 11.0% increase in the control treatment and 231.8% in the drought treatment, and Pb experienced a 203.3% increase in the control treatment and 668.6% in the drought treatment (Figure A4).

Above- and Belowground Net Primary Production

The most drastic effect the five-year drought treatment had on ecosystem functioning was on net primary production (NPP). By mid-August, total ANPP was on average 99.2% lower in the drought treatment when compared to the control treatment (Figure 3a). Additionally, it should be noted that plots that were droughted displayed minimal growth, with only 2 out of 20 droughted plots having enough aboveground biomass to be collected and weighed. The greatest amount of biomass was observed for C_4 graminoids in the control plots, whereas the highest amount of growth was observed for forbs with extreme drought, although this biomass was 98.3% lower than the control plots. Notably, C_3 graminoids were eliminated from the drought plots, and C_4 graminoid biomass was reduced by 99.6% with the drought treatment (Figure 3b). In contrast, although productivity of cactus could not be quantified, abundance of cacti was observed to be 3-fold greater in the drought treatment compared to the control treatment (Figure 3c).

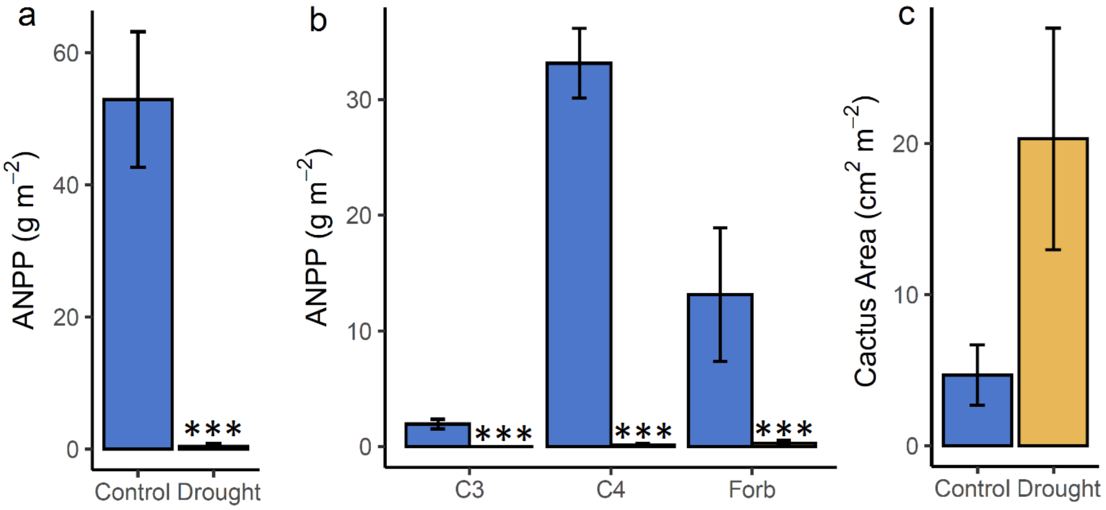

*Figure 3. Average ANPP broken down by functional type (a) along with average total ANPP (total forbs and graminoids) and cactus area in plant communities growing in control and drought conditions. Asterisk indicates significant difference (***= $p < 0.01$, **= $p < 0.05$) between the control and drought treatments.*

While ANPP in drought conditions were reduced to near zero levels, BNPP was not reduced by as much with drought. When compared to the control treatment, BNPP from 0-30 cm was significantly reduced by 67.5% with drought, and consequently the total NPP was reduced by 93.0% (Figure 4a, Table A3).

The greatest amount of reduction in BNPP occurred in the top 10 cm of the soil profile, where BNPP was reduced by 78.6% with drought compared to the control treatment (Figure 4b). BNPP was also reduced with drought for the 10-20 cm and 20-30 cm depths, but to a lesser extent (61.8% and 36.9%, respectively). As a consequence, the distribution of biomass in the 0-30 cm soil profile was more even with extreme drought when compared to control conditions.

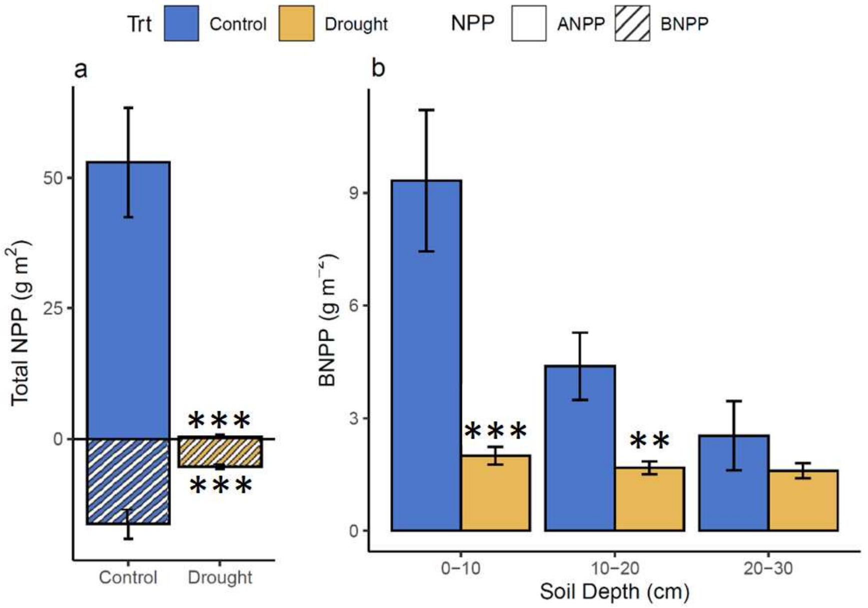

*Figure 4. Average total (a) of ANPP (measured in shoot biomass of forbs and graminoids) and BNPP (measured in root biomass of forbs, graminoids, and cactus) along with average BNPP broken down by soil depth (b) in plant communities. Bars represent mean with standard error bars. Asterisk indicates significant difference (***= $p < 0.01$, **= $p < 0.05$) between the control and drought treatments.*

The use of the three metrics of soil respiration, nutrient availability and net primary production allowed us to assess the above- and belowground responses that the shortgrass steppe in northeastern Colorado had to an extreme, long-term drought. Five years of extreme drought had reduced soil respiration by more than ~50%, increased total (KCl extracted) N availability by ~500%, and reduced total NPP by over ~90% when compared to data collected in control conditions, all of which will contribute to the legacy effects during the following recovery period, extending the temporal length of the drought's effects (Griffan-Nolan et al, 2018; Rondeau et al, 2018; Moran et al, 2014).

DISCUSSION

Soil moisture, temperature, and respiration

In this study, my primary goal was to assess changes to semi-arid grassland structure and function, and one method I used to accomplish this was to quantify CO_2 efflux response, along with soil moisture and soil temperature, to 5 years of drought within the SGS in the US Central Plains. Past studies have established a clear link between soil respiration with soil moisture and soil temperature with observed CO_2 efflux rates increase with increasing temperatures and decrease with decreasing soil moisture (Denton et al, 2017; Fay et al, 2003), Both these factors account for 40-80% of the variability in soil respiration (Fóti et al, 2014; Balogh et al., 2011; Konda et al., 2010; Fang et al., 2009; Mäkiranta et al., 2008; Kobziar and Stephens, 2006). I had hypothesized that the rate CO_2 efflux would reduce underneath the two droughted shelters, and the collected results support that hypothesis.

Soil respiration was reduced by more than 50% after five years of extreme drought, with this reduction strongly related to the low soil moisture levels. This reduction in efflux of CO_2 from the soil is consistent with similar semi-arid grassland studies, where CO_2 efflux affected by experimental droughts were reduced between 50-60% (Meeran et al, 2021; Ingrisch et al, 2020; and Balogh et al, 2016). When viewed over 13 weeks, CO_2 flux rates under drought conditions displayed a consistently lower pattern than the control measurements (FigureA2. This pattern becomes clear when CO_2 efflux is plotted against the soil moisture readings, which were taken simultaneously, a pattern formed that soil moisture had a direct link with soil respiration (Figure A3Rafalska et al, 2023; Balogh et al, 2016; Hagedorn & Joos, 2014; Fóti et al, 2014). While data from previous research indicates that warmer soil temperatures hasa short-term positive influence on soil respiration (Meeran et al, 2021; Ingrisch et al, 2020; Denton et al, 2017; Balogh et al, 2016), the higher soil temperature measured within the drought treatment was not enough to counter the negative influence that decreased soil moisture had on soil respiration.

In previous studies that had observed the effect single-season droughts have on soil respiration on similar semi-arid grassland systems, soil respiration had recovered within a couple of weeks after the soil is rewetted to the levels of the control conditions (Ingrisch et al, 2020; Meeran et al, 2020). That said, because the effects of long-term droughts on grassland CO_2 efflux have not been studied, it is relatively unknown how soil respiration will respond during the following year's recovery period. During drought conditions, microbial activity also decreases, leading to mid- to long-term reductions in soil carbon content (Knapp et al. 2020; Ochoa-Hueso et al, 2020), as soil microbes are expected to use stored carbon for respiration, reducing the overall soil available carbon for future growth (Balogh et al, 2016; van der Heijden et al., 2008). On a larger scale, the depletion of carbon from semi-arid grassland soils may have an impact on the potential recovery of plant communities. With carbon stores being consumed by soil microbes and little plant biomass providing new carbon in the form of plant litter, the following recovery years can be expected to be unproductive.

A note to be made is that one limitation to the collar insertion technique used during this study is that this technique can underestimate the rate of soil respiration. Because the process of inserting the collars a few centimeters in the soil can sever fine surface roots, the autotrophic component of CO_2 is often reduced by 15% or as high as 50%; fortunately for this study, shallow collar installation has shown to have little effect on grassland study sites (Heinemeyer et al, 2011).*Nutrient availability*

The next aspect of belowground processes that was studied was nutrient availability, the goal of which was to quantify the mid-season and end-of-season availability of N, P, and micronutrients in response to 5 years of drought within the SGS in the US Central Plains. N and P are two of the most critical elements for the functioning of all organisms (Maxwell et al, 2021; Hu et al, 2020). The processes that regulate fluxes of nutrients in the soil, such as nitrogen (N) and phosphorus (P) are altered during periods of drought, which are projected to increasing in duration and intensity due to climate change (Maxwell et al, 2021; Zaehle, 2013).

Many studies have reported increases in N availability after experimentally simulating drought (Yuan et al. 2017; Ochoa-Hueso et al, 2020). In normal conditions, N is fixed into soil storage when microbes mineralize N accumulated from plant litter into ammonium (NH_4^+; Schaeffer et al, 2017) and N_2 from the atmosphere into nitrate (NO_3^-; van Sundert et al, 2020). In drought conditions, these processes continue to accumulate mineralized N slowly, as microbe activity also slows down, but as plant nitrogen-use is also reduced, this accumulated N will build up to higher levels than what would be observed in normal precipitation conditions (Homyak et al., 2017; van Sundert et al, 2020). I had hypothesized that soil available NH_4^+, NO_3^-, and P would accumulate underneath the two droughted shelters, of which the late-season data collected for NH_4^+ and NO_3^- supports.

We used two methods to test for N content that would be available for plant accumulation: PRS probes and KCl extractions. The results acquired from the PRS probes demonstrated how N builds up when fewer plants are present to utilize it. Ammonium (NH_4^+) increased over the duration of the growing season in drought plots while decreasing in the control plots, and while NO_3^- doubled within the control treatment (Figure 4 & 5), that same form of N increased by 593% within the drought treatment. This increase in soil available NH_4^+ and NO_3^- may be the result of a lack of plant growth to utilize available forms of N. While much NH_4^+ is reintroduced into the soil from decaying plant litter from the previous year's growth, the amount that is present in the soil is not being utilized by new growth.

Results acquired from PRS probes tell a different story for the availability of P at the end of the 5-year drought period. The probes detected P at lower rates within the drought plots than within the control plots. During the early season, detection rates of P were 66% lower within drought plots and 33% lower during the late season (Figure 5). Literature suggests that this decrease in P availability may come from a reduced diffusion of P within the soil, affecting the ability of the PRS probes to detect P ions during the early-season collection (Dijkstra et al, 2012; Meisser et al, 2019, Zhang et al, 2020). However, the closing gap in P between the two treatments could suggest that P slowly accumulates throughout, as P is not being actively utilized by primary producers (plants) or other organisms within the soil. This difference could also be the result of higher diffusion rates in the P ions due to larger precipitation events during the late-season. This is confirmed within short-term drought studies, that indicate in times of drought the diffusion rate of P ions declines, which in turn leads to a reduced availability of P in the soil for roots, even if the overall amount of P presence remains the same (Dijkstra et al, 2012; Meisser et al, 2019, Zhang et al, 2020).

As a side note, three micronutrients of note were identified, and while N and P are considered the most limiting elements required for plant development and growth, abiotic stressors, like drought, can also affect other trace elements, creating legacy effects for future recovery. From ions present in the soil, three trace elements were identified as having an especially significant presence, as observed the two periods of PRS readings: iron (Fe), manganese (Mn), and lead (Pb). Though important for plant function, these trace elements can likewise hinder plant function, both in deficient and excess amounts. In plants, Fe holds an essential role in electron transportation, which carries out processes like respiration, photosynthesis, and the development of chloroplasts and chlorophyll (Kim and Guerinot, 2007; Tripathi et al, 2018). Mn plays an essential role in plant function in photosynthesis and respiration, but it is also involved in nitrogen assimilation, pollen germination, pollen tube growth, root cell elongation, and resistance to root pathogens (Millaleo et al, 2010). Finally, lead (Pb), when present in higher concentrations, can have negative effects on plant processes: slowing down enzyme activities (Brown and Bates, 1972; Collin et al, 2022) and inhibiting photosynthesis (Nas & Ali, 2018).

While not much is known about the fate of the accumulated soil N after long-term doroughts within grasslands, such as with this study, studies that had experimentally increased soil available N before the recovery period after short-term droughts displayed evidence that the short-term recovery more quickly (Schaeffer et al, 2017). However, displayed by decreasing carbon availability from lost biomass litter, caused by a near-complete reduction in ANPP, it is unlikely that this temporary increase in soil available N will support the following years recovery period and may be eroded with a lack of plant cover (Yang et al, 2023; Du et al, 2019).

Above and Belowground Primary Production

Since the IDE at the SGS site took effect in 2018, the primary goal of that original study was to quantify the response of ANPP and to measure the shift of plant community and structure to an extreme long-term drought. I had hypothesized that after 5 years of a 66% reduction in precipitation, ANPP would go through a significantz reduction in biomass and the plant community structure would shift in favor of a higher proportion of warm-season, C_4 graminoids; however, the grassland plots that had been growing.

underneath the droughted shelters had gone through a functional collapse, producing little to no graminoids or herbaceous forbs aboveground.

Over the growing season of 2022, there were two main changes observed in ANPP: graminoids and forbs from biomass collection areas were near absent from the drought plots, seeing a reduction of 99.2% (Figure 7), and cactus quadrupled in drought plots, seeing an increase of 344.2%. In previous studies, a significant drop in ANPP is common, but none have had the extreme reaction as what was displayed during this study. In 2012, the SGS site was exposed to a 3-year drought experiment and displayed a variation of ANPP responses between no response to drought to a 51% reduction (Cherwin & Knapp, 2012). One explanation of this near complete collapse of ANPP function could be attributed not just to the 5 years of experimental extreme drought, but the compounding year of ambient drought in 2022. Because the populations of graminoids and forbs had decreased to nearly zero in the drought treatment plots, not much commentary can be made on the functional shift of these plant populations, however the shift favoring cactus may indicate that a drought resulting in a 66% reduction in precipitation can lead to potential desertification of the shortgrass steppe (Xu et al, 2021).

Overall, the literature on the belowground portion of NPP is more limited than it is for ANPP. Within semi-arid grasslands, the bulk of biomass, which includes both live and dead biomass, is stored in BNPP, including underground stem systems, like rhizomes and stem crowns, representing 20-26% of the belowground biomass (Singh et al, 1975; Lauenroth et al, 2008). BNPP can have a variety of responses to drought from having no response to drought treatments, shifting the ratio of BNPP to ANPP in favor of belowground growth (Sindhøj et al. 2000; Gill et al. 2002; Byrne et al. 2013) to having a decline in growth (Frank, 2007; Wu et al, 2011; Evans & Burke, 2013; Kang et al, 2013). When we look at root biomass distribution within the soil layers, BNPP steadily decreases in biomass when within the control treatment. 57% of that growth occurred in the top 10cm of the soil profile (50-65% growth often occurs within the top 15 cm; Denton et al, 2017; Wilcox et al, 2014; Lauenroth et al, 2008), 27% of BNPP growth occurred between 10-20, and 16% of BNPP growth occurred between 20-30cm.That growth distribution looked much different in the drought treatment. While BNPP was lower in the drought treatment than in the control treatment, the distribution was relatively even. 38% of BNPP growth occurred in the top 10cm of the soil profile, 32% of BNPP growth occurred between 10-20, and 30% of BNPP growth occurred between 20-30cm (Figure 8).

In times of drought, past grassland studies have suggested that NPP can shift in the short-term so that the bulk of plant growth will be allocated to the roots (BNPP; Carroll et al, 2021; Arndal et al. 2018; Xu et al, 2015). After 5 years of continuous drought within the SGS, I had observed a shift in the SGS drought treatment plots in that nearly all annual growth (not including cactus) occurred belowground. In addition, biomass at different depths were impacted differently, with the greatest reductions occurring within shallower depths, which is consistent with similar studies (Slette et al, 2023; Carroll et al, 2021). Within the control plots, the biomass of roots grown within a single season occurred in the top 10 cm, steadily declining as we observed deeper into the soil profile but given that drought had affected BNPP the most within the more shallow layers, it is likely that deeper root penetration increases water uptake and drought resistance (Slette et al, 2023; Fort et al. 2017; Nippert and Holdo 2015).

While past studies have indicated that in times of drought, NPP can be allocated to BNPP during drought years, it should be noted that because cacti were not included as part of the weighted ANPP biomass – as ANPP was measured as one year of growth and we do not know the age of the cacti growth – we do not know how cacti roots have affected the dry biomass measurement of

BNPP. It would be beneficial for future studies to separate cacti roots from graminoid and herbaceous roots to account for this difference.

However, what had been observed after this study, if the SGS region of the Central Plains were to face droughts similar to this 5-year study, we could reasonably expect Dust Bowl-like conditions to form within the region. While the dust storms of the 1930s Dust Bowl were partially the result of plant removal from human-led “sodbusters” (Burnette & Stahle, 2013)– intended to remove the top soil layer and grassland communities – if the Central Plains were to experience a loss of ANPP and decreased BNPP, we could expect wind-based erosion, causing similar dust storms (Schubert et al, 2004).

CONCLUSION

I found that five years of extreme, year-long drought had severely altered both above and belowground grassland functions in the shortgrass steppe in northeastern Colorado. While both soil respiration and BNPP experienced reductions of more than 50%, the largest loss in function was observed with ANPP. Aboveground productivity was reduced to near-zero levels, while cacti increased 3-fold in surface area. The greatest reduction in root growth was observed at the shallowest depth (0-10 cm), likely due to the near complete loss of graminoids from the system. The large reduction in overall NPP was accompanied by available N (both NH_4^+ and NO_3^-) accumulating in the soil, due to little N being incorporated into plant biomass. While this accumulation of available N may aid in the plant regrowth post-drought, previous research indicates that this boost in available N will quickly be depleted. And while the soil experienced an accumulation of available nitrogen, it remains unknown what impact the dramatic reduction in ecosystem productivity will have for post-drought recovery of the SGS ecosystem. These findings demonstrate that extreme, multi-year drought – the type of drought forecast for the future - can lead to a near collapse of ecosystem productivity, which has important implications for carbon storage and forage production. Given that the SGS ecosystem is one of the largest remaining grassland ecosystems in the Central US and is heavily utilized for livestock production, results from this study suggest that intensified droughts that forecast for the future could have large and long-lasting ecological and economic impacts on the SGS ecosystem.

FUTURE DIRECTIONS

The natural next step in the extreme drought experiment will be to remove the roofs from the drought shelters and monitor the progress of recovery over the next few years. Presently, a new experiment will carry on where the IDE left off. All four shelter blocks will receive the same amount of water based on the long-term average precipitation (LTA, ~320mm), and each block will receive five differing amounts (LTA, LTA +25%, +50%, +75%, and +100%) with LTA acting as the control. And much like how this experiment was based on an interconnected drought experiment that was conducted on an international scale, it would be interesting to monitor how different biomes recover after an extreme, multi-year drought.

During this study, CO_2 efflux was analyzed once a week around the same time in the day (late morning to early afternoon), but this only captures a short timeframe of soil activity. Plants, bacteria, and other organisms tend to have fluctuating levels of activity during different times of the day. If we were to increase CO_2 efflux measurements twofold (3-4 days a week during early morning, mid-day, and evening), a better understanding of investigation as to whether the time periods of highest soil activity changed due to drought. In addition, one limitation of this study was that I was unable to investigate how long after a precipitation event would a change to CO_2 efflux occur.

Another aspect that could be investigated is the relationship between ANPP and BNPP during drought conditions. We were able to observe that while the ANPP of herbaceous forbs and graminoids were reduced to near-zero levels after being exposed to extreme drought conditions, but even though BNPP was also reduced in biomass, there was still noticeable growth as well as an alteration in its growth pattern. In future studies, identifying the species, especially those of cacti, of the roots at different depth levels (0-10 cm, 10-20 cm, and 20-30 cm) would be useful in predicting which species are more likely to recover post-drought and understanding how different grassland species allocate resources in times of drought.

Finally, one measurement of this study that I was unable to investigate was the C:N ratio difference within soils from control and drought treated plots. When analyzed by the IRMS, the ratio of C and N were nearly identical. I believe that this is due to the fact that the 30 cm-depth soil samples collected from the experimental plots were homogenized and not divided into depth subcategories. In future studies, I would recommend collecting samples based on depth in 10 cm increments. This would provide a more accurate picture as to how C and N are distributed within the soil and whether this changes in times of drought.

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APPENDIX

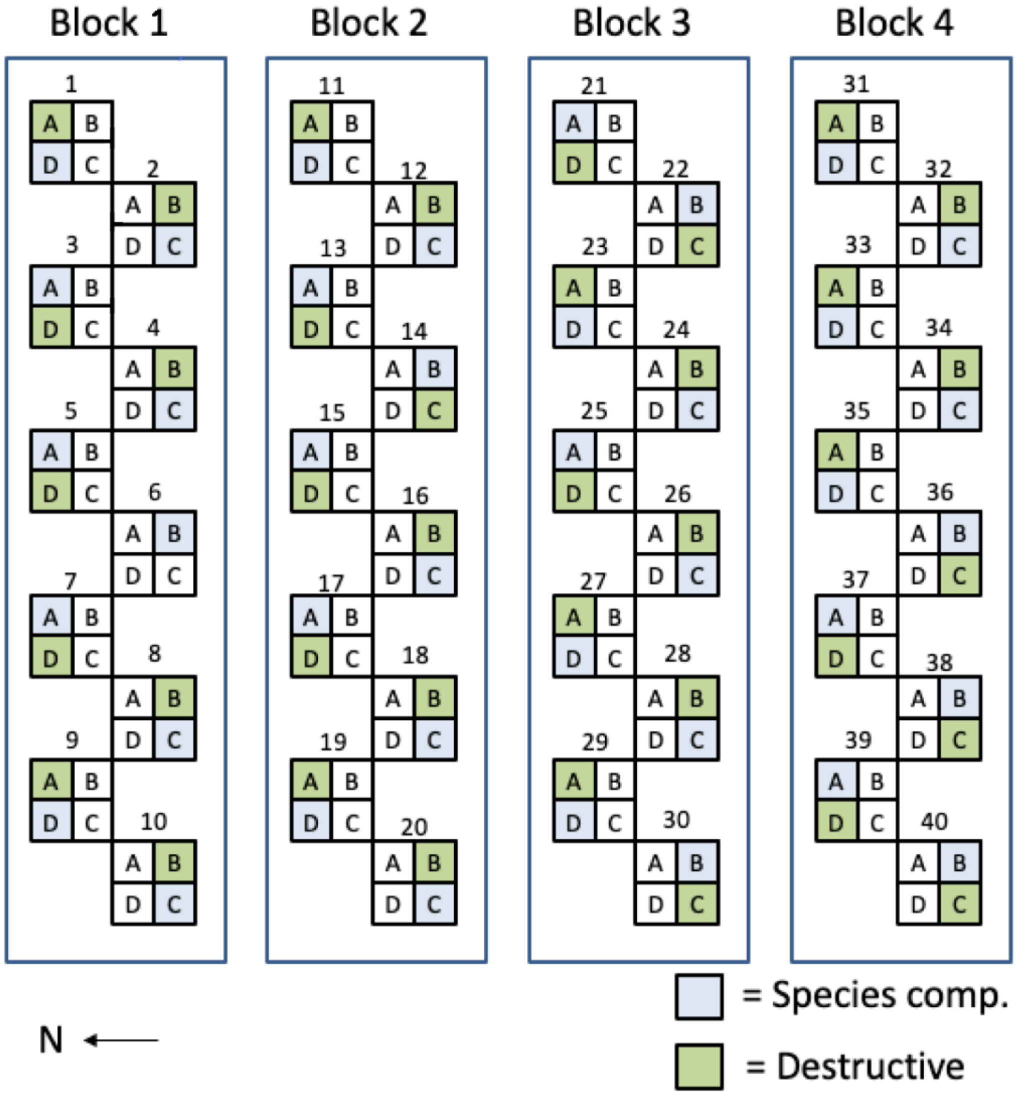

Figure A1. Map of experimental setup at the SGS-IDE site. Blocks 1 & 2 represent the drought treatment and blocks 2 & 3 represent the control treatments.

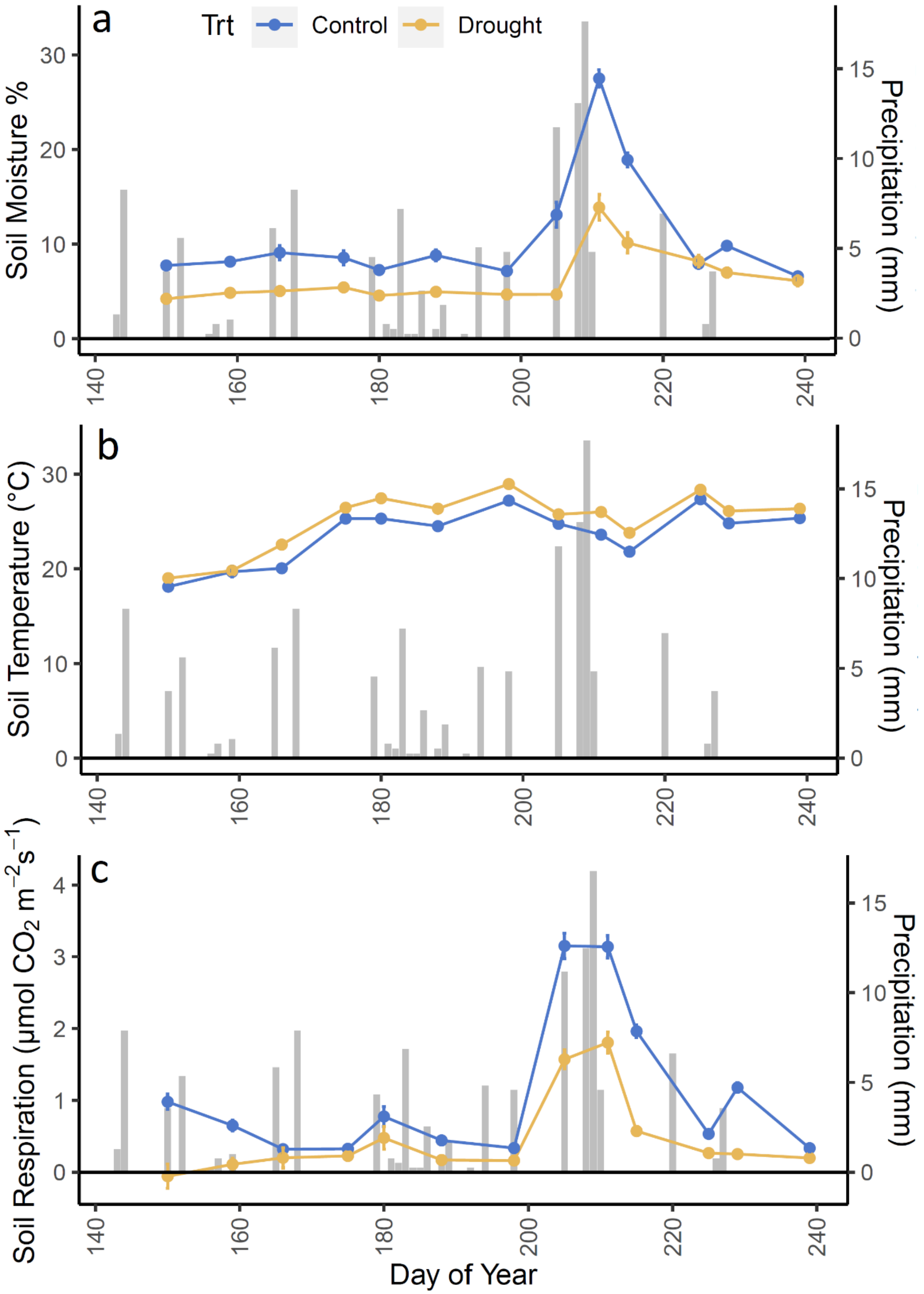

Figure A2. Timeline of weekly soil moisture (a), soil temperature (b), and soil respiration (c) plotted on the primary y-axis against precipitation events (secondary y-axis), measured in mm. Precipitation data was provided by USDA-CPER SCAN sensors.

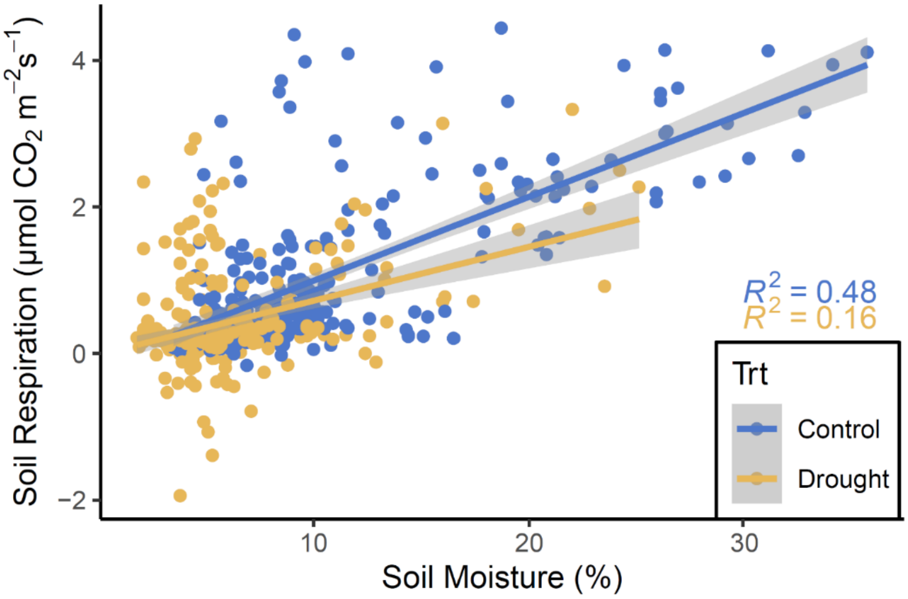

Figure A3. Linear regression of soil moisture (x-axis) on soil respiration (y-axis); Control R^2 = 0.48, Drought R^2 = 0.16.

*Table A1. Mixed model results testing the main and interactive time effects of drought treatment on soil respiration, soil moisture, and soil temperature. Asterisks represent significance (***= p < 0.01, **= p < 0.05)*

	Predictor	**F-value**	**df**	**Pr(>F)**
Efflux	Moisture_per	4.43	1	0.036**
	Trt	5.24	1	0.146
	Week	94.20	12	<0.001***
	Trt:Week	15.38	12	<0.001***
Moisture	Trt	95.36	1	0.010**
	Week	113.48	12	<0.001***
	Trt:Week	22.03	12	<0.001***
Temperature	Trt	2.46	1	0.257
	Week	472.89	12	<0.001***
	Trt:Week	6.57	12	<0.001***

Table A2. Mixed model results testing the main and interactive time effects of drought treatment on PRS collected available ions and KCl extracted available NH_4^+ *and* NO_3^-*. Asterisks represent significance (**= p < 0.05, * = p < 0.1)*

	21 May - 02 July		**09 July - 27 Aug**		**KCl Extracted**	
Ion	**F value**	**Pr(>F)**	**F value**	**Pr(>F)**	**F value**	**Pr(>F)**
Al	1.52	0.342	18.81	0.049**		
B	0.47	0.563	0.31	0.633		
Ca	34.77	0.027**	0.003	0.961		
Cu	2.05	0.288	0.23	0.676		
Fe	1.64	0.328	14.38	0.063*		
K	2.79	0.237	0.25	0.665		
Mg	65.21	0.014**	1.19	0.389		
Mn	2.65	0.245	52.35	0.018**		
NH_4^+	**11.04**	**0.079***	**3.18**	**0.216**	**40.55**	**0.024****
NO_3^-	**1.02**	**0.418**	**30.07**	**0.031****	**10.99**	**0.080***
P	**14.93**	**0.060***	**1.28**	**0.375**		
Pb	0.33	0.624	9.26	0.093*		
S	13.28	0.067*	6.52	0.125		
Zn	4.11	0.18	3.05	0.222		

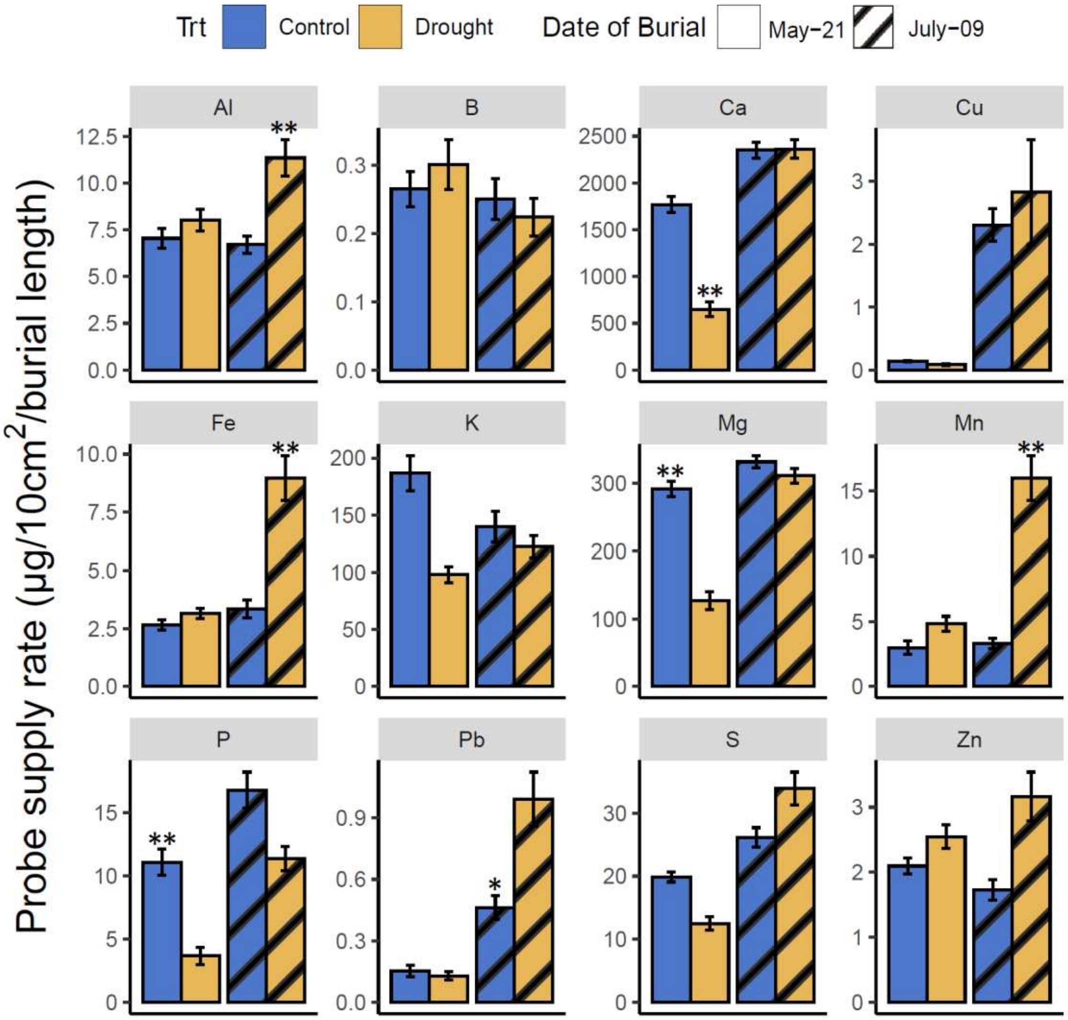

Figure A4. Average nutrient availability in control and drought soils between May 21 – July 02 and July 09 – August 27.

*Table A3. Mixed model results testing the main and interactive time effects of drought treatment on primary production. Asterisks represent significance (**= p < 0.1)*

ANPP			**BNPP**				
Depth	**W**	**P-value**	**Depth**	**W**	**P-value**	**F value**	**Pr(>F)**
Total	400	>0.001***	Total (30 cm)	244	>0.001***	17.44	0.070**
C3	1360	>0.001***	*0-10 cm*	262	>0.001***	7.80	0.115
C4	1600	>0.001***	*10-20 cm*	225.5	0.003**	10.82	0.105
Forbs	1045.5	>0.001***	*20-30 cm*	114	0.372	1.20	0.408
Cacti	701	0.175					