

RESEARCH ARTICLE

Limited legacy effects of extreme multiyear drought on carbon and nitrogen cycling in a mesic grassland

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The intensification of drought throughout the U.S. Great Plains has the potential to have large impacts on grassland functioning, as has been shown with dramatic losses of plant productivity annually. Yet, we have a poor understanding of how grassland functioning responds after drought ends. This study examined how belowground nutrient cycling responds after drought and whether legacy effects persist postdrought. We assessed the 2-year recovery of nutrient cycling processes following a 4-year experimental drought in a mesic grassland by comparing two different growing season drought treatments—chronic (each rainfall event reduced by 66%) and intense (all rain eliminated until 45% of annual rainfall was achieved)—to the control (ambient precipitation) treatment. At the beginning of the first growing season postdrought, we found that in situ soil CO₂ efflux and laboratory-based soil microbial respiration were reduced by 42% and 22%, respectively, in the intense drought treatment compared to the control, but both measures had recovered by midseason (July) and remained similar to the control treatment in the second postdrought year. We also found that extractable soil ammonium and total inorganic N were elevated throughout the growing season in the first year after drought in the intense treatment. However, these differences in inorganic N pools did not persist during the growing season of the second year postdrought. The remaining measures of C and N cycling in both drought treatments showed no postdrought treatment effects. Thus, although we observed short-term legacy effects following the intense drought, C and N cycling returned to levels comparable to nondroughted grassland within a single growing season regardless of whether the drought was intense or chronic in nature. Overall, these results suggest that the key aspects of C and N cycling in mesic tallgrass prairie do not exhibit persistent legacies from 4 years of experimentally induced drought.

Keywords: Postdrought period, Legacy effects, Climate extreme, Biogeochemical cycling, Grasslands

Introduction

Climate models predict that semiarid regions, such as the U.S. Great Plains grasslands, are forecast to experience more and intense and widespread drought throughout the next century (Intergovernmental Panel on Climate Change, 2014; Asadieh and Krakauer, 2015; Guinard et al., 2015; Rahmani and Harrington, 2019). This predicted increased extremity of drought has the potential to catastrophically impact Central Plains grassland production if

past droughts are any indication of future response (Cook et al., 2015; Lesk et al., 2016). For example, the widespread, extreme drought of 2012 affected 65% of the continental United States and cost the US\$30 billion in agricultural and rangeland losses (Rippey, 2015) and had large impacts on aboveground productivity (Knapp et al., 2015; Knapp et al., 2020). Further, extreme drought has been shown to deplete about 1% of vegetated land each year and consequently cause significant losses in C every year (Du et al., 2018). Thus, there is a pressing ecological, economic, and societal imperative to understand the impacts of intensifying drought on grassland and rangeland ecosystems, particularly those deemed most vulnerable to these events, such as grasslands of the Central Plains (Cook et al., 2015).

Numerous studies have shown that drought, by reducing soil moisture and water availability, can cause large reductions in aboveground production of grasslands (Hoover et al., 2014; Knapp et al., 2015; Arredondo et al., 2016; Hoover et al., 2016; Kreyling et al., 2017; Knapp et

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al., 2020) with an accompanying reordering of the dominant species (Fry et al., 2014; Hoover et al., 2014). Drought may also strongly affect the soil microbial community (Lucia et al., 2014; Naylor et al., 2017; Edwards et al., 2018; Naylor and Coleman-Derr, 2018; Xu et al., 2018; Schimel, 2018) with potential feedbacks on belowground processes (e.g., nutrient cycling, decomposition). This will cause further potential losses in grassland productivity, since plants rely heavily on these belowground processes (Jacoby et al., 2017), for essential nutrients (nitrogen [N], phosphorus [P], etc.). Further, grasslands store approximately one-third of all terrestrial C (White et al., 2000) with the potential to mediate increases in atmospheric CO₂ due to human impacts. Carbon sequestration under drought may be additionally impacted by decreases in C mineralization (Hinjosa et al., 2019) and soil microbial respiration (Hoover et al., 2016; Hinjosa et al., 2019) and decreases in stored soil organic matter (Ren et al., 2018). Belowground nutrient cycling and nutrient pool responses to drought will be key indicators of belowground functioning; thus, it will be critically important to understand these responses to mitigate potential losses in belowground grassland function.

The potential for legacy effects postdrought is a key way in which drought may inhibit recovery of biogeochemical cycling. Legacy effects are persistent positive or negative differences from predrought or control conditions after a drought ends (Cuddington, 2012). Positive legacies, as defined in our study, create conditions after drought that increase plant or microbial growth/functioning or increase nutrient availability, while negative legacies have the opposite effect. If an ecosystem is unable to recover before a new drought occurs (e.g., exhibits negative legacies), then the ecosystem could be further damaged, making understanding legacy effects highly important (Schwalm et al., 2017). Thus, both positive and negative legacy effects could have important implications for the nature and pace of recovery of ecosystem functioning following drought.

However, current research is uncertain whether legacy effects occur after drought and, if so, how they impact belowground nutrient cycling postdrought. In particular, studies have found conflicting and mixed responses postdrought. Some studies report no legacy effects after drought and high resilience to drought (Rousk et al., 2013; Hoover et al., 2014; Hofer et al., 2016; Bunting et al., 2017). Others report positive legacies, particularly in plant growth (Griffin-Nolan et al., 2018; De Long et al., 2019; Guo et al., 2020). Additional research reports recovery of all functions measured within 2 years, such as resource efficiency (Xu et al., 2017) and aboveground plant production (De Boeck et al., 2018; Griffin-Nolan et al., 2018). Other legacies include increases in soil enzyme activities (Alster et al., 2013) and N pools (Homyak et al., 2017). Negative legacy effects can also occur postdrought, including changes in microbial biomass (Hinojosa et al., 2019), shifts in plant–soil feedbacks leading to shifts in plant-community dynamics (Hassan et al., 2021), decreases in soil enzyme activities and microbial biomass (Zeglin et al., 2013; Legay et al., 2018), and decreases in

C mineralization and available soil nutrients, such as P, potassium, and soil organic matter (Evans and Wallenstein, 2012; Hawkes et al., 2017; Kreyling et al., 2017). Further, multisite studies indicate that grasslands can be the least resilient ecosystem to drought (Peng et al., 2019) or the most resilient ecosystem to drought (Li et al., 2020). Thus, research is largely inconsistent with regard to postdrought responses, whether legacies persist, or whether recovery occurs. Moreover, we have shown that although a number of studies find postdrought legacies, few of these studies have examined belowground legacies, particularly nutrient cycling legacies postdrought (Vilonen et al., in prep).

To fill this major knowledge gap of whether belowground legacies exist postdrought, we took advantage of an existing project to evaluate postdrought impacts on soil C and N cycling in a mesic tallgrass prairie grassland. At the study site, a 4-year, growing season drought was experimentally imposed in two ways: (1) chronically, that is, by reducing each rainfall event throughout the growing season by 66% or (2) intensely, that is, by completing excluding rainfall during the growing season until approximately 45% of mean annual precipitation (MAP) was removed. The drought decreased both aboveground net primary productivity (ANPP) and belowground net primary productivity (BNPP) throughout the 4 years of drought with stronger decreases in the intense treatment compared to the chronic treatment (Carroll et al., 2021). Changes in the plant species composition in the third and fourth years of drought were also observed (unpublished data). After 4 years, the drought shelters were removed from both treatments, and we measured key indices (representing both pools and fluxes) of belowground C and N cycling over two postdrought growing seasons. The main objectives of this study were to determine whether legacy effects occurred and persisted after the drought, whether recovery occurred in some or all measures of C and N cycling, and whether measures of one nutrient cycle recovered more quickly than the other. We hypothesized that legacy effects would occur, but primarily in the first growing season, because carryover of soil water deficits is typically less than a year in duration (Liu et al., 2018), and more significant legacy effects would occur in the N cycle compared to the C cycle, since C pools at the study site are generally large and relatively stable (Wilcox et al., 2016), and the N cycle is generally more responsive to perturbations at this site.

Methods

Study site and climate conditions

This study was conducted during the growing seasons (May–August) of 2018 and 2019 at the Konza Prairie Biological Station, a native, tallgrass prairie research site located in the Flint Hills of northeastern Kansas (39.09°N, 96.48°W). The climate consists of warm, wet summers and dry, and cold winters. MAP is approximately 835 mm with approximately 75% of rainfall occurring during the growing season (April–September). Annual precipitation for the 2 years of the study was 811 mm in 2018 and 971 mm in 2019, with approximately 64%

and 75% of the precipitation occurring during the growing season in each year, respectively (Figure S1). For this study, we utilized a large-scale, well-replicated drought experiment (the Extreme Drought in Grasslands Experiment [EDGE]) that was established in 2013 in an annually burned and ungrazed native tallgrass prairie site. The site was located on a flat, level upland with relatively deep (approximately 1 m or more), well-drained clay loam soils characterized as silty clay Mollisols.

Experimental design

The EDGE experiment imposed drought in two ways from 2014 to 2017 using large rainfall exclusion shelters ($n = 20$ total), each 6×6 m in size and hydrologically isolated to a depth of approximately 1 m (see Griffin-Nolan et al., 2019, for more details). For the chronic drought treatment, 10 shelters were covered with strips of clear corrugated polycarbonate spaced so as to reduce each growing season rainfall event by 66% (April–September). For the intense drought treatment, the remaining 10 shelters were completely covered with panels of clear corrugated polycarbonate to exclude all rainfall events with no precipitation entering the intense treatment plots until a similar amount of total growing season rainfall was excluded as the chronic treatment (May–July), resulting in a shorter, but more intense reduction in rainfall. Both drought treatments resulted in an approximately 45% reduction in annual rainfall. Shelter roofs were put in place in May each year for both drought treatments. Roofs were removed each year in early September for the chronic treatment, while they were removed after a similar amount of rainfall was reduced in the intense treatment; this was typically reached after approximately 2 months of the panels being installed (typically May–July). The control treatment plots were unsheltered ($n = 10$), but still hydrologically isolated and received ambient rainfall throughout the growing season. The three treatments were arranged in blocks, each containing a replicate of each treatment, for a total of 10 blocks ($n = 30$ plots).

To assess postdrought legacy effects on C and N cycling, we removed the shelters after the 4 years of drought treatments and allowed ambient rainfall to fall onto all of the treatments in 2018 and 2019 (the first 2 years following drought). This allowed us to measure whether legacy effects were present and whether recovery occurred.

Soil sampling

In 2018 and 2019, we collected soils monthly throughout the growing season (late May, early July, and mid-August) to measure soil C and N cycling. We homogenized four random soil core samples (15 cm depth \times 5.7 cm diameter) collected from each “destructive plot” as detailed in Griffin-Nolan et al. (2019). The samples were immediately placed on ice and sieved to 2 mm within 24 h. A subsample of these soils was kept fresh and unfrozen for laboratory-based microbial respiration measurements. The rest of the soil was transferred to a -20°C freezer until further analysis for all other non-in situ measurements.

All analyses on frozen soils were performed within a year after collection.

Soil moisture

We measured soil moisture in both the field and the lab to assess whether soil moisture exhibited any legacies as a mechanism for the responses observed. We used a hand-held TDR (time domain reflectometry) to measure in situ soil moisture to a depth of 15 cm at each time of soil sampling. We additionally dried field-collected soil (the same soil used to measure nutrients) for 48 h at 60°C to calculate moisture and soil wet soil/dry conversion factors for subsequent nutrient and enzyme analyses.

Soil nutrient fluxes and pools

To characterize legacy effects of drought on C and N cycling, we measured in situ belowground respiration, lab-based soil microbial respiration, extractable inorganic N (ammonium and nitrate), extractable total dissolved organic C (DOC) and N (DON), in situ net N mineralization, and total soil organic C and N concentrations to measure the main components of C and N cycling.

Belowground respiration was measured in situ using a Li-Cor 8100 infrared gas sampler (Lincoln, Nebraska). Two polyvinyl chloride (PVC) collars were installed in each plot to a 6 cm depth and left in the field for the duration of the growing season. All biomass and living plants were removed from the collars at the beginning of the season and prior to every measurement. We then used a Li-Cor 8100 infrared gas sampler to measure CO_2 flux from the soil over a 60-s interval. Measurements were taken midday and during sunny and nonwindy conditions to ensure uniform conditions for each measurement. Measurements were taken monthly in 2018 and weekly in 2019. More detailed methods can be found in Slette et al. (2021).

To measure soil microbial respiration in the lab, we placed 30 g of sieved, fresh soil (the fresh unfrozen subsample; extracted from the field <24 h prior) from each plot in a sealable mason-jar (8 cm wide \times 15 cm deep). We kept the soils at the same moisture from the field by sealing the soils in plastic bags and sealing the jars immediately after adding the soil. We measured microbial respiration once within 24 h of extracting soil by opening the jars to allow re-equilibration with ambient CO_2 and then resealing the jars for 1–2 h to measure accumulated headspace CO_2 . Respiration was then quantified as detailed in Zeglin and Myrold (2013).

To measure extractable inorganic N, we extracted ammonium and nitrate from the previously frozen soil subset collected monthly. We shook 11 g of thawed field-moist soil with 1-M KCl for 1 h and then filtered the samples using Whatman filters (Grade 42–2.5 μm filter). We then froze the extracts in a -20°C freezer until analysis. Extractable N was expressed on a per gram soil dry weight basis. To measure net N mineralization, a 12-cm deep PVC tube (3.81 cm diameter) with the top two centimeters above ground was pounded into the ground next to the initial soil cores taken on the same date. The PVC tubes were capped, with holes in the aboveground portion of the tubes for gas exchange and left in place for

approximately 30 days. Cores were retrieved at the end of the incubation interval, then sieved within 24 h, frozen in a -20°C freezer, and later extracted with 1-M KCl using the methods above. We used an Alpkem analyzer to measure extractable ammonium and nitrate on all KCl extracts (Saskatoon, SK). Net N mineralization was measured as the difference between extractable inorganic N in the initial and final cores. This was then divided by the days the cores were left in the field to calculate a daily rate.

To measure total DOC and DON, we extracted 20-g field-moist subsamples of the previously frozen soil with 100 mL of 0.5-M K_2SO_4 . We shook the soils for 4 h and filtered the samples using Whatman 42 filters and then froze the extracts in a -20°C freezer. We used a Shimadzu TOC-L analyzer (Kyoto, Japan) to measure DOC and DON.

To measure total C and N, we oven dried the soils at 60°C for several days until the soil was depleted of any moisture. The soils were then ground and analyzed for total C and N in an LECO TruSpec CN combustion analyzer (St. Joseph, MI) at the KSU Soil Testing Lab.

Extracellular enzyme activity

We measured the potential extracellular enzyme activities of several microbially produced enzymes as an index of nutrient limitation. We measured C-cleaving enzymes: α -Glucosidase (AG), β -Glucosidase (BG), β -D-cellulosidase (CB), and β -Xylosidase (XYL); N-cleaving enzymes: N-acetyl glucosaminidase (NAG) and leucyl aminopeptidase (LAP); and phosphorus-cleaving enzymes: phosphatase (PHOS). Substrates for each enzyme were attached to a highly fluorescent cleavage product. The substrates for AG, BG, CB, XYL, NAG, and PHOS were attached to 4-methylumbelliferyl (MUB), and the substrate for LAP was attached to 7-amino-4-methylcoumarin (MUC). We added a Tris buffer adjusted to a pH of 8 to our soils to create a soil slurry and shook our samples for 40 min. We then added our samples to a 96 well-plate and added substrates to our soil slurries with two replicates per sample. Additionally, we created MUB and MUC standard curves for each individual soil. To simulate standard soil conditions, the plates were incubated for 3 h in the dark at 25°C . Fluorescence was measured using a multiplate reader (Tecan Infinite M200 plate reader, Switzerland) with a 365-nm excitation and 460-nm emission filters. A quench control was used. More detailed methods can be found in Bell et al. (2013) and Trivedi et al. (2016). We summed the C enzymes for total C enzyme activity and the N enzymes for total N enzyme activity (Bell et al., 2013; Dove et al., 2020).

Statistical analyses

To compare treatments across each year's growing season, we calculated confidence intervals and standard error using mixed models that accounted for repeated measures over the growing season (monthly sampling). We conducted separate statistical analyses for 2018 and 2019 due to the different climatic conditions of the 2 years. Further discussion of why the 2 years were split can be found in the Results section. Our mixed model contained both fixed and random effects. Both time and treatment were fixed variables with an interaction term to account for the

repeated measures aspect of this experiment (lme4 package). As mentioned previously, our experiment had a blocked design. Blocks were treated as a random variable except for some models where we had to treat block as a fixed variable. In our 2018 enzyme analysis, we ran into a problem of overfitting due to block variance being estimated as zero in the model. To correct this overfitting, we treated block as a fixed effect and used this model to draw conclusions. Additionally, we applied a natural log conversion to all enzyme activity data due to unequal variances detected from the residual versus fitted plot of the original nontransformed models. For the belowground respiration models, we included soil moisture as a covariate, since soil moisture has strong effects on belowground respiration. Further, we calculated correlation coefficients for soil moisture and belowground respiration in both years. For all statistical analyses, we utilized R statistical software (R Core Team, 2013) and used several packages including lme4, lmerTest, pbkrtest, emmeans, and GGally. We also used R to create the graphics for this article using ggplot2 and Hmisc to create 95% confidence intervals for each graphic.

Results

Soil moisture differences between the years

The differences in timing and amount of growing season precipitation that occurred each year postdrought led to drier soils in 2018 than 2019 (**Figure 1**). A majority of the rainfall fell after the growing season in 2018, which led to a dry growing season in 2018 (Figure S1), followed by large rain events in September and October. We collected soil in the growing season, as we expected microbial activity to be low the rest of the year (Carson and Zeglin, 2018). In 2019, about 75% of the total annual rainfall fell during the growing season. However, the annual rainfall was 970.8 mm, which was well over the long-term average of 835 mm. This led to above-average soil moisture, especially since rain fell mostly in May, June, and July during the peak growing season. Therefore, we chose to assess the two postdrought years separately and compare legacy effects between a relatively dry year and a relatively wet year.

Carbon cycling

At the beginning of 2018 (May), in situ belowground respiration (root and microbial respiration) was significantly lower (approximately 50%) in the former intense drought treatment compared to the control treatment (**Figure 2a**, Table S2). Belowground respiration in the former chronic drought treatment was intermediate to the intense drought and control treatments, but not significantly different than the control treatment (**Figure 2a**). Lower belowground respiration in the intense drought treatment was accompanied by lower soil water content (measured in situ) compared to the control treatment ($P = 0.001$; **Figure 1a**, Table S1). This decrease in soil moisture was likely the cause of the lower activity we observed in the intense treatment. We found that soil moisture and belowground respiration were significantly correlated in both 2018 and 2019 ($r = .695$, $P < 0.01$; $r = .322$, $P < 0.05$,

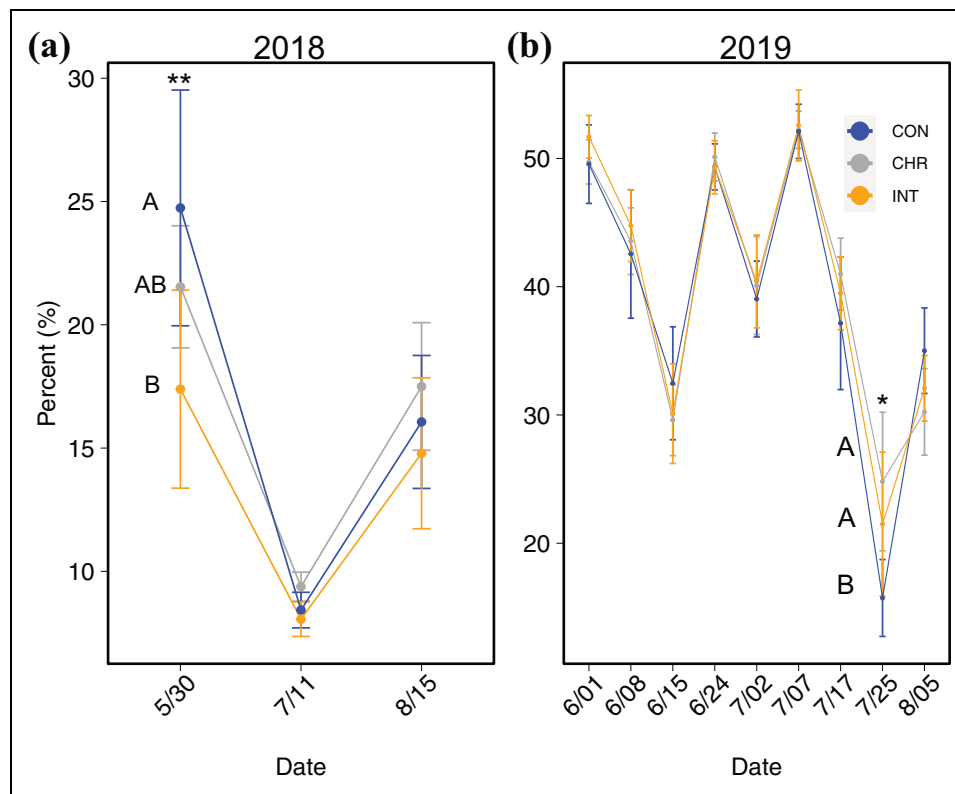


Figure 1. Soil moisture in 2018 (a) and 2019 (b). The control treatment (CON) is shown in blue, chronic (CHR) treatment in gray, and intense (INT) treatment in orange. The circles represent the average soil moisture for each treatment. The error bars represent the 95% confidence intervals. The asterisk represents significant differences among the treatments at $\alpha = .05$. Double asterisk represents significant differences at $\alpha = .01$. DOI: <https://doi.org/10.1525/elementa.2021.000093.f1>

respectively). By July and August 2018, there were no differences in belowground respiration among the treatments. Furthermore, we saw no statistical differences or changes in belowground respiration across treatments in the second growing season postdrought (**Figure 2b**, Table S2). Belowground respiration in general was significantly higher (more than double) in 2019 than in 2018, mirroring higher soil moisture in 2019. Soil moisture in 2019 (Table S1) was the same across all treatments except for on July 25, when soil moisture was lower for the control treatment compared to the intense treatment. This was also at a point when the soil moisture was low for all treatments compared to the other time points (**Figure 1b**).

We also measured soil microbial respiration in the laboratory (after removing roots) and found similar trends to the in situ belowground respiration data. Microbial respiration was significantly lower in May 2018 in the chronic drought compared to the control treatment ($P = 0.0075$; **Figure 2c**, Table S2). By July 2018, we found no differences in microbial respiration among treatments, but in August 2018, we found that both the chronic ($P = 0.014$) and intense ($P = 0.066$) drought treatment had lower microbial respiration than the control treatment (**Figure 2c**). In 2019, there were no significant differences in microbial respiration among any of the treatments (**Figure 2d**, Table S2).

There were no significant differences among treatments in total or individual C enzyme activities in 2018

(Figure S2a, Table S2) or 2019 (Figure S2b, Table S2, Figure S3). Although, C enzyme activities were highest in the chronic drought treatment throughout 2018, once log-transformed to account for unequal variances, the differences were nonsignificant. Additionally, we divided the \ln C enzyme activity by the \ln N enzyme activity to see whether relative C and N limitations varied based on the ratios of the two activities. We found no significant differences either in 2018 or 2019 (Figure S4, Table S2).

We found no differences in DOC in either 2018 or 2019. There was large variability in the data in July of both years that could have obscured any differences among treatments (Figure S5a and b, Table S2). However, total soil organic C was significantly greater in the chronic drought treatment in May ($P = 0.0070$) and July ($P = 0.0120$) of 2018 (**Figure 3a**, Table S2). In 2019, total soil C was lower in the intense drought treatment ($P = 0.0333$) and only in July (**Figure 3c**, Table S2).

Nitrogen cycling

In 2018, levels of extractable ammonium were generally higher across the entire growing season in both the chronic and intense drought treatments compared to the control treatment, but these differences were not significant (**Figure 4a**, Table S3). Further, levels of nitrate were higher across the entire growing season in the chronic and intense drought treatments, but only the intense

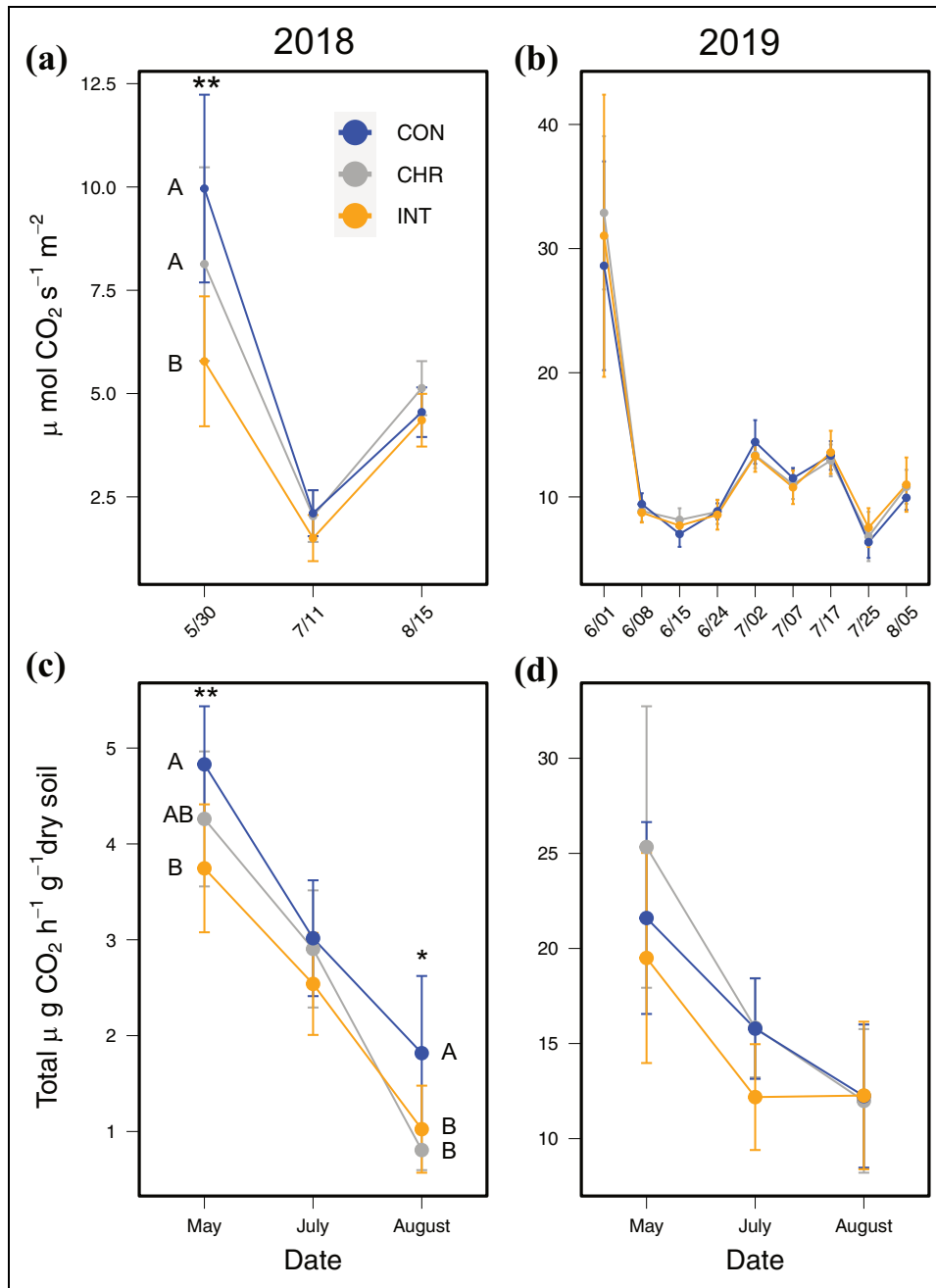


Figure 2. Belowground respiration measured in situ (a and b) and soil microbial respiration measured in the lab (c and d) in 2018 (left) and 2019 (right). The control treatment is shown in blue, chronic treatment in gray, and intense treatment in orange. The circles represent the average belowground respiration for each treatment. The bars represent the 95% confidence intervals. The asterisk represents significant differences among the treatments at $\alpha = .05$. Double asterisk represents significance at $\alpha = .01$. Note the difference in scales between 2018 and 2019. DOI: <https://doi.org/10.1525/elementa.2021.000093.f2>

treatment was statistically different than the control in July and August (Figure 4b, Table S3). We also saw higher levels of total inorganic N in the chronic and intense drought treatments across the growing season of 2018, but only the intense treatment was statistically greater than the control in July and August (Figure 4c, Table S3). These differences in total inorganic N were driven by the increases in nitrate. In 2019, these differences in inorganic N largely disappeared. In May 2019, the control treatment had statistically higher levels of ammonium

compared to the intense treatment and had greater non-statistically significant levels than the chronic treatment (Figure 4d; analysis of variance table in Table S18). There were no statistical differences among treatments in nitrate in 2019 (Figure 4e, Table S3). We did see statistically higher concentrations of total inorganic N in May 2019 in the control treatment compared to the intense treatment. Inorganic N in the chronic drought treatment was lower than the control treatment, but the difference was not statistically significant (Figure 4f, Table S3). Between

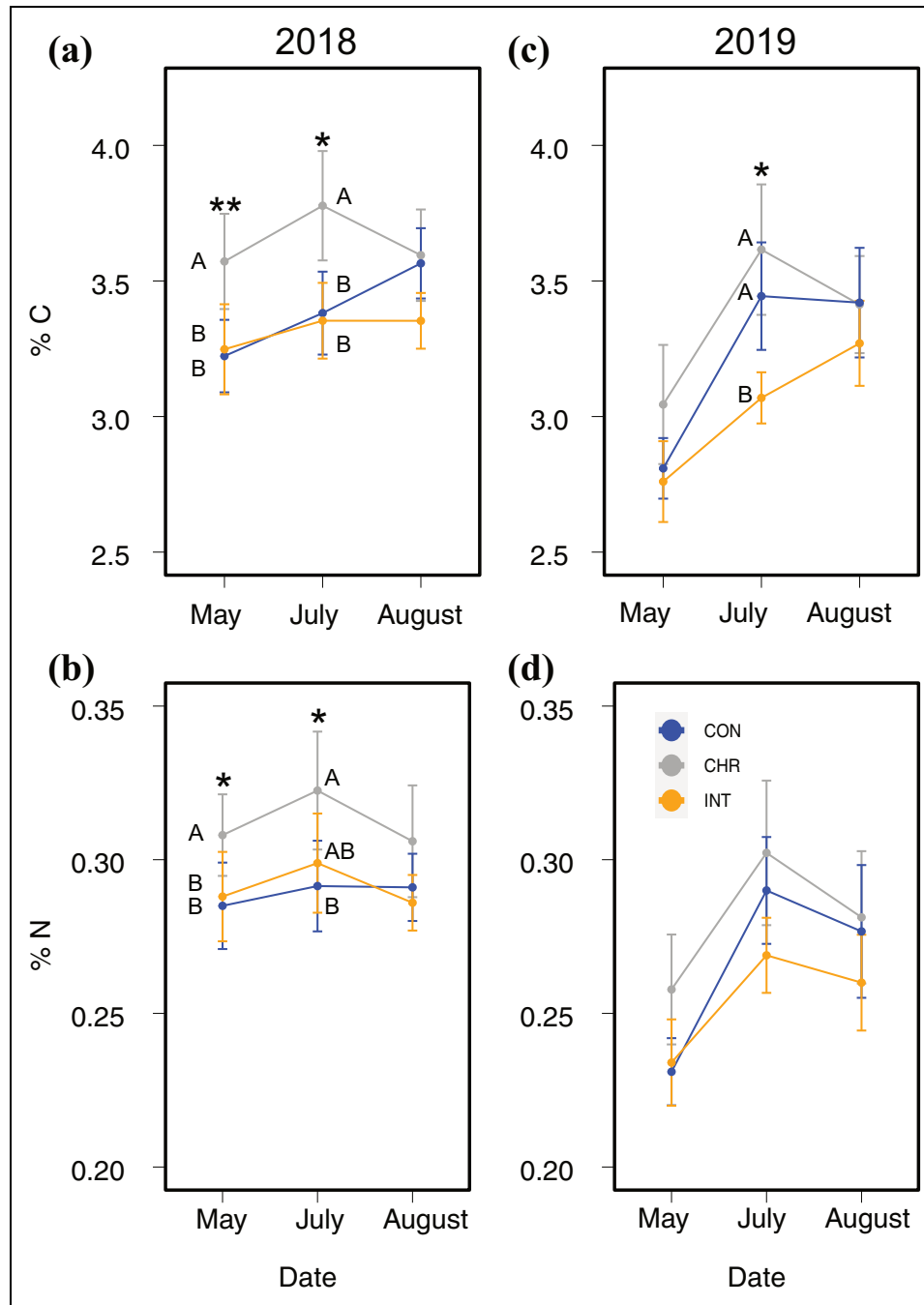


Figure 3. Percent soil organic C (a and c) and total soil N (b and d) in 2018 (left) and 2019 (right). The control treatment is shown in blue, chronic treatment in gray, and intense treatment in orange. The circles represent the average values for each treatment. The error bars represent the 95% confidence intervals. The asterisk represents significant differences among the treatments at $\alpha = .05$. Double asterisk represents significance at $\alpha = .01$. DOI: <https://doi.org/10.1525/elementa.2021.000093.f3>

years, ammonium and total inorganic N were much higher across all treatments in 2019 compared to 2018, but we found no differences among treatments in net N mineralization across 2018 or 2019. Notably, N mineralization values were mostly negative in 2019 (Figure S6, Table S3).

Total soil N in the chronic drought treatment was significantly higher in May ($P = 0.0216$) and July ($P = 0.0216$) of 2018 (Figure 3b, Table S3). In 2019, there were no significant differences among treatments throughout the entire year (Figure 3d, Table S3). We additionally

found no significant differences in total and individual N enzyme activities among treatments in either 2018 or 2019 (Figure S7, Table S3). Finally, there were no significant differences among treatments for DON in 2018 or 2019 (Figure S5c and d, Table S3).

Discussion

We aimed to understand whether legacy effects in C and N cycling occurred and whether they persisted postdrought in a mesic grassland following two prolonged drought

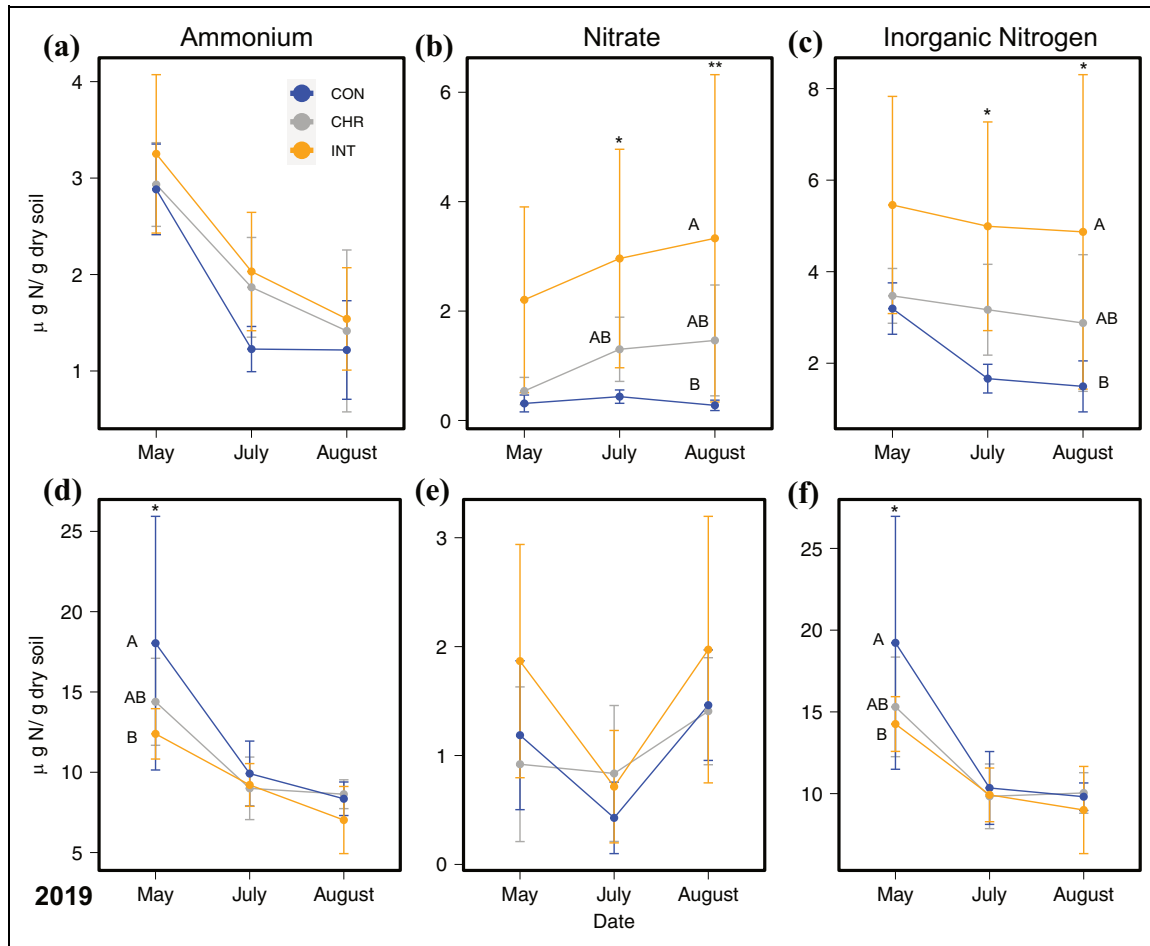


Figure 4. Extractable ammonium (a and d), nitrate (b and e), and total inorganic N (ammonium + nitrate; c and f) over the growing season in 2018 (top row) and 2019 (bottom row). The control treatment is shown in blue, chronic treatment in gray, and intense treatment in orange. The circles represent the average value for each treatment. The error bars represent the 95% confidence intervals. The asterisk represents significant differences among the treatments at $\alpha = .05$. Double asterisk represents significance at $\alpha = .01$. DOI: <https://doi.org/10.1525/elementa.2021.000093.f4>

treatments: chronic and intense drought. We predicted that legacies in C and N cycling will be short-lived and most prevalent in the first growing season due to short-term grassland drought memory (Liu et al., 2018). Given differences in rainfall in the 2 postdrought years, we further expected that any differences in the strength of legacies in the first year compared to the second year would be reinforced by lower levels of water availability inhibiting recovery in 2018. We also expected to see more significant legacy effects in the fluxes and pools of the N cycle compared to the C cycle, since C pools at the study site are generally large and relatively stable (Wilcox et al., 2016). Conversely, the N cycle in this grassland is generally more responsive to perturbations such as fire (Blair, 1997) or grazing (Johnson and Matchett, 2001), and N is often limiting at this site (Seastedt et al., 1991). Therefore, we expected to see available soil N build up over the drought period due to the lack of uptake and leaching and decreased general belowground activity. By 2019, we expected these legacy effects to disappear due to short-term water memory (Liu et al., 2018) and short life cycles of belowground organisms. More specific predictions

regarding C and N pools and processes are provided in the following paragraphs.

Legacies in C cycling

We expected that C cycling fluxes would be reduced in the drought treatments relative to control treatment, particularly in parts of the belowground C cycle that are more affected by reductions in soil moisture, which include soil and microbial respiration, while C pools would remain unchanged. Specifically, we hypothesized based on previous research that soil and microbial respiration in previously droughted treatments would be lower (Hoover et al., 2016), C enzyme activity would be higher (Ochoa-Hueso et al., 2018), and C pools would remain relatively stable (Wilcox et al., 2016; Canarini et al., 2018). Our results mostly matched our hypotheses, but shifts in the C cycle were limited to soil/microbial respiration and only in the first year, with recovery happening much quicker than we expected. Belowground respiration (Figure 2a and c) was reduced relative to the control following 4 years of drought, but only in the first month of 2018 and only in the intense drought treatment, while microbial

respiration was reduced in the first month of 2018 and August of 2018 in the intense treatment. These differences disappeared for the rest of the growing season and no differences in soil or microbial respiration was detected in 2019. There was a legacy of decreased soil moisture in May 2018 in the intense drought treatment only (**Figure 1a**). This decrease in soil moisture was likely the cause of the lower activity we observed in the intense treatment due to the correlation we found in soil moisture and belowground respiration. Several possible reasons exist for this legacy of decreased soil moisture, such as changes in the water holding capacity (WHC) of the soil during the drought, which could be caused losses in organic matter or other changes in the soil that could lead to reduced WHC. Unfortunately, we did not measure WHC and therefore cannot confirm this as a mechanism. Although the decrease in belowground respiration was likely due to the legacy of lower soil moisture, other possible reasons for this decrease exist such as decreased microbial biomass or decreased root production, both of which were not measured in this study. Further, this decrease could have been due to changes in microbial community composition that led to differences in microbial C use efficiency.

We found no differences in DOC throughout our study (Figure S5), which was consistent with our original hypothesis of stable C pools. Unexpectedly, we found higher percent total organic C in the former chronic drought treatment in 2018 relative to the control and intense drought treatment (**Figure 3a**). We expected organic C pools to remain unchanged due to the stability of C pools at our site (Wilcox et al., 2016) or decrease due to a decrease in plant contribution to the C cycle from reduced aboveground biomass (Hoover et al., 2014; Knapp et al., 2015; Hoover et al., 2016; Kreyling et al., 2017; Knapp et al., 2020). Other studies have reported decreases in soil organic C during drought in grasslands, including a meta-analysis of 148 publications (Deng et al., 2021), which is contradictory to what we found. On the other hand, drought has been shown to not limit root exudation, which could lead to an accumulation of C allowing for quick microbial recovery postdrought (Karlowsky et al., 2018). The increase in organic C we observed after drought in the chronic treatment could help explain quick recovery in key aspects of the C cycle. However, the increase in organic C disappeared by 2019 and thus appeared to be a short-term response postdrought, which could further indicate that microbial communities used the additional C leading to a lack of lasting legacy effects in the chronic treatment.

We also found no difference in C-releasing enzymes between the control and either of the drought treatments indicating no difference in the levels of relative C limitation among treatments (Figures S2 and S4). Our results are contrary to other studies of drought in this grassland system. For example, Ochoa-Hueso et al. (2018) found increases in all enzyme activities throughout drought at this site, while another study showed a decrease in all enzyme activities after seasonal drought (Zeglin et al., 2013). Our data indicate that for this measure of C cycling,

the system recovered quickly postdrought and exhibited resilience to drought.

Interestingly, we only saw decreases in soil/microbial respiration and soil moisture in the intense drought treatment and observed no statistically significant differences between the chronic drought and control treatments. We expected to see changes in both drought treatments, since the chronic drought reduced similar amounts of rainfall, although we still expected to see more profound responses from the intense treatment due to complete exclusion of rainfall early in the growing season when plants are actively growing. Carroll et al. (2021) studied the 4 years of drought at our site and found decreases in ANPP and BNPP throughout the 4 years of drought. Particularly, they found that the intense treatment led to greater decreases compared to the chronic treatment, although the decreases in ANPP and BNPP were still significant for the chronic treatment. This may partially explain why we only saw decreases in activity in the intense treatment, while the chronic treatment exhibited no legacy effects other than an increase in total organic C. The intense treatment also eliminated precipitation from entering the plots during the beginning of the growing season, which was likely much more detrimental than simply reducing each rainfall event and allowing some precipitation to enter the plots. Overall, although we saw some legacies in the C cycle, these legacies were relatively weak and did not persist. The C cycle was not strongly affected by 4 years of growing season drought and had few legacy effects.

Measures of N cycling

For the N cycle, we predicted that we would find greater pools of inorganic N following drought due to decreased plant uptake but also decreases in postdrought N cycling fluxes due to a decrease in microbial activity. Results were partially consistent with predictions. Although we observed no postdrought differences in the N cycle fluxes, we found higher levels of soil inorganic N in the former intense drought treatment and higher total soil organic N in the former chronic drought treatment.

The increase in total inorganic N and ammonium in the intense treatment (**Figure 4**) in the first year after drought (2018) was likely due to a decrease in plant uptake or microbial immobilization, which led to an accumulation of inorganic N in the soil. It is unclear whether this difference was due to the accumulation of N during the drought due to reduced plant uptake or whether plants were still unable to recover to predrought growth levels and subsequently took up less N postdrought. Preliminary findings of ANPP postdrought indicate that ANPP fully recovers 1 year after drought (unpublished data); thus, it is likely that the former is true. Other studies also have found increases in inorganic N throughout drought events (Dijkstra et al., 2015; Canarini et al., 2016). In one study, uptake of nitrate by plants was shown to be sensitive to drought and less N was taken up during drought (Dijkstra et al., 2015).

In 2019, these differences disappeared for the most part. Ammonium was highest in the control treatment

in 2019 and also significantly higher in 2019 than 2018. Nitrate was relatively lower in 2019 than 2018 (**Figure 4**). The large amount of precipitation in 2019 likely led to a large amount of losses from the system via leaching or denitrification and/or increased plant uptake and microbial immobilization. Ammonium is generally less mobile in clay soils, while nitrate is more readily leached (Cameron et al., 2013).

Importantly, these differences among treatments we observed in inorganic N were not present in measures of organic N (DON and TON; Figure S5 and **Figure 3**). Organic forms of N are much more stable (Kaye et al., 2002) than inorganic pools. Interestingly though, we did see a higher percentage of total soil organic N in the chronic treatment (**Figure 3**). Generally, N tracks total C, which could indicate that the chronic treatment changed the balance of belowground productivity and decomposition, allowing C and N to accumulate during the drought period. Deng et al. (2021) conducted a meta-analysis across 148 publications and found a positive effect size and increase in organic N throughout drought as well. This increase in organic N was likely due to decreased mineralization of the organic forms throughout drought. However, our assays showed that net N mineralization recovered quickly after drought, which is likely why we did not detect any differences in inorganic N in the chronic treatment. Preliminary data on aboveground productivity at this site have shown that the aboveground community recovers quickly after drought in the chronic treatment, which could further explain why inorganic N in the chronic treatment was comparable to the control treatment.

Further, we found no differences among treatments in any N-releasing enzymes (Figures S7 and S8). An increase in N-releasing enzymes would indicate that soil microorganisms were experiencing higher N limitation (Schimel and Weintraub, 2003). We saw no difference between the control and either drought treatment for the N-releasing enzymes indicating that there were no significant differences in relative N limitation amount in the treatments. This is supported by the increase in available N that we noted earlier. We also observed no changes in mineralization of N, indicating that the fluxes of the N cycle were unchanged by drought. Notably, mineralization was negative in 2019, which was likely due to the water-logged soils and potential denitrification or net N immobilization due to higher soil moisture and more favorable conditions for microbial growth and immobilization. In summary, N cycling was mostly resilient to drought, although there were increases in nitrate and total inorganic N throughout the first year postdrought.

Lack of legacies of nutrient cycling postdrought

Our general findings indicate that only a few key measures of soil C and N cycling processes showed legacy effects of extreme, multiyear drought. The majority of C and N cycling measures showed no legacies (no statistical difference from the control plots), and of the few legacy effects observed, almost none persisted into 2019, which was comparably much wetter than 2018. Further, the intense

treatment produced more legacies than the chronic treatment. This is likely due to the severity of the drought limiting plant and microbial activity. On the other hand, the chronic treatment seemed to recover immediately after drought or alternatively the chronic treatment did not significantly impact C and N cycling processes. But given the reductions in plant and root growth that occurred in this treatment throughout drought (Carroll et al., 2021) and the shift in plant species composition in the third and fourth years of drought (unpublished data), a lack of effect on belowground processes is probably unlikely. Other studies have found that legacy effects often are not as prevalent as expected. Wu et al. (2017) found that postdrought legacies only lasted 1 year in grasslands, while finding longer legacies in forest and shrubland systems. Rousk et al. (2013) worked across 5 shrubland ecosystems and found no legacy effects to drought resulting from short-term warming and drought. A study at Konza Prairie found that grassland production (ANPP) fully recovered 1 year postdrought, through increases in the dominant grasses compensating for the loss of biomass from less abundant species (Hoover et al., 2014). Another study found that after drought, recovery occurred quickly in a grassland system and normal levels of productivity were achieved (Hofer et al., 2016).

There are 2 possible hypotheses for why we observed some legacies in 2018 but not in 2019. One is that postdrought legacies were short-lived and the indices of nutrient cycling we examined had all recovered to control levels by 2019. Support for this hypothesis is shown by all legacies disappearing in 2019. Quick recovery has been seen at other studies at this site, which found full recovery in plant production in the first growing season after drought (Hoover et al., 2014; Griffin-Nolan et al., 2018). The second hypothesis is that when water is abundant and not limiting growth, drought legacies are obscured or overcome more rapidly. As support for the latter hypothesis, both precipitation and soil moisture in 2019 were above average, which likely made water a nonlimiting resource. In contrast, precipitation in 2018 was below average and there was a shift in seasonality, which led to lower soil moisture levels that could have limited both above- and belowground processes. This suggests that postdrought climate conditions that impact soil moisture availability could play an important role in the nature and pace of recovery and the potential for prolonged legacy effects. Future field studies would benefit from excluding all rainfall and manually adding back precipitation even to the control plots to control for natural variability in rainfall.

In summary, our study suggests that belowground processes in the mesic grassland studied are able to recover quickly after drought, similar to the observations of rapid recovery of aboveground productivity (Hoover et al., 2014; Griffin-Nolan et al., 2018). It is plausible that this may be the case more generally for grasslands. Indeed, rapid recovery (or high resilience) after extreme drought appears to be a common feature of grasslands globally with aboveground productivity (Stuart-Haentjens et al., 2018). Our findings were mostly consistent with other studies,

although we did not find the positive legacies that have been reported in some other (Griffin-Nolan et al., 2018; De Long et al., 2019; Guo et al., 2020). This may be due to the longer duration of our experimental drought.

However, additional research is needed to assess the role that drought magnitude and duration separately and together play in eliciting legacy effects on nutrient cycling postdrought. Specifically, we suggest extending the duration of studies looking at legacies postdrought to better understand how long the legacies last and allow for more year-to-year climate variability. We also suggest studying across a larger set of sites to account for variability in MAP and mean annual temperature to better find general patterns of drought legacy effects beyond one specific site. Further, within our study, it was challenging to determine the mechanisms for the changes we saw. Thus, we suggest to measure a broader scope of soil characteristics and microbial measurements, such as WHC, soil pH, and so on, and microbial biomass and community composition. Finally, we suggest that studies use greenhouse or field studies to measure across a range of soil moistures or rainfall amounts postdrought to determine whether soil moisture levels postdrought are a factor in determining whether legacies occur or do not occur after drought ends.

Data accessibility statement

The data that support the findings of this study are openly available at <https://doi.org/10.5061/dryad.d7wm37q28>.

Supplemental files

The supplemental files for this article can be found as follows:

Figures S1–S8. Tables S1–S3. Docx

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Competing interests

There are no competing interests.

Author contributions

Contributed to conception and design: LLV, MS.

Contributed to acquisition of data: LLV, LZ, PT.

Contributed to analysis and interpretation of data: LLV, JB, PT, MS.

Drafted and/or revised this article: LLV, JB, PT, LZ, MS.
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