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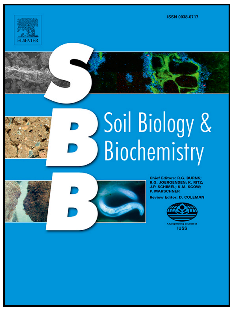

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PII: S0038-0717(22)00182-1
DOI: https://doi.org/10.1016/j.soilbio.2022.108725
Reference: SBB 108725

To appear in: *Soil Biology and Biochemistry*

Received Date: 11 August 2021
Revised Date: 26 April 2022
Accepted Date: 18 May 2022

Please cite this article as: Holguin, J., Collins, S.L., McLaren, J.R., Belowground responses to altered precipitation regimes in two semi-arid grasslands, *Soil Biology and Biochemistry* (2022), doi: https://doi.org/10.1016/j.soilbio.2022.108725.

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Belowground responses to altered precipitation regimes in two semi-arid grasslands

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Keywords: Extreme drought, precipitation seasonality, belowground net primary productivity, extracellular enzymes, microbial biomass, soil C and N

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Abstract

Predicted climate change extremes, such as severe or prolonged drought, may considerably impact carbon (C) and nitrogen (N) cycling in water-limited ecosystems. However, we lack a clear and mechanistic understanding of how extreme climate change events impact ecosystem processes belowground. This study investigates the effects of five years of reoccurring extreme growing season drought (66% reduction, extreme drought treatment) and two-month delay in monsoon precipitation (delayed monsoon treatment) on belowground productivity and biogeochemistry in two geographically adjacent semi-arid grasslands: Chihuahuan Desert grassland dominated by *Bouteloua eriopoda* and Great Plains grassland dominated by *B. gracilis.* After five years, extreme drought reduced belowground net primary productivity (BNPP) in the Chihuahuan Desert grassland but not in the Great Plains grassland. Across both grasslands, extreme drought increased soil pH and available soil nutrients nitrate and phosphate. The delayed monsoon treatment reduced BNPP in both grasslands. However, while available soil nitrate decreased in the Chihuahuan Desert grassland, the delayed monsoon treatment had little effect on soil ecosystem properties. Extreme drought and delayed monsoon treatments did not significantly impact soil microbial biomass, exoenzyme potentials, or soil C stocks relative to ambient conditions. Our study demonstrates that soil microbial biomass and exoenzyme activity in semi-arid grasslands are resistant to five years of extreme and prolonged growing season drought despite changes to soil moisture, belowground productivity, soil pH, and nutrient availability.

Introduction

Drylands are highly responsive to changes in the amount and seasonality of growing season precipitation (Knapp et al., 2008; Maurer et al., 2020) and are expected to be particularly vulnerable to climate change (Diffenbaugh et al., 2008; Hoover et al., 2020; Lian et al., 2021; Hanan et al., 2021). Climate models predict that many dryland regions will experience increasingly variable precipitation patterns, enhanced aridity, and more frequent, severe, and prolonged droughts (Cook et al., 2015; Schlaepfer et al., 2017; Bradford et al., 2020). In the Southwestern U.S., some models predict little change in total summer precipitation (Gutzler and Robbins, 2011). Other models forecast increasingly extreme and irregular rain events delivering less rain overall (Seager et al., 2007) and extended pre-monsoon hyper-arid periods (Notaro et al., 2010; Cook and Seager, 2013; Pascale et al., 2017). However, empirical evidence in these regions demonstrates that aridity is increasing (Maurer et al., 2020), and prolonged and severe droughts are already occurring (Cook et al., 2021; Zhang et al., 2021).

In aridlands, rainfall pulse size and frequency drive many ecological processes which regulate biogeochemical cycles, e.g., plant primary production and soil microbial activity (Noy-Meir

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1973; Collins et al., 2008). Thus, changes to the amount and timing of rainfall may considerably alter biogeochemical cycling, including carbon (C) cycling in drylands (Scott et al., 2009; Reed et al., 2012; Song et al., 2020). Modifications to belowground dynamics with changing climate conditions may be particularly important in drylands. Globally, drylands are estimated to store ~38% of the global belowground biomass C pool and ~44% of global organic matter C pool in surface soils (top 30cm) (Hanan et al., 2021). However, our understanding of the climatic controls that drive C dynamics belowground remains unclear (Canarini et al., 2017; Gherardi and Sala, 2020; Deng et al., 2021).

There are various ways severe and extended dry periods can impact belowground biogeochemical cycling and C storage. For instance, plants may allocate C from aboveground biomass to belowground biomass to enhance water uptake during drought (Poorter et al., 2012; Meng et al., 2022). Alternatively, as the duration and intensity of drought are critical factors in the depletion of plant C and water reserves (DaCosta and Huang, 2009; Poorter et al., 2012), extreme or prolonged drought events may create conditions that inhibit root C allocation (Arcioni et al., 1985; Volaire 1995). Chronic dry and hyper-arid periods may also negatively impact soil microbial function, e.g., by reducing plant C inputs, increasing osmotic stress, and limiting substrate availability and enzyme mobility (as reviewed by Schimel, 2018). However, in water-limited ecosystems, dew or small rain events (<1mm) can also activate nitrogen (N) and C fixation by biological soil crusts or decomposition (e.g., C and N mineralization) by soil microbes (Schwinning and Sala, 2004; Pointing and Belnap, 2012; Collins et al., 2014). Therefore, as microbes may remain biologically active at water levels below that required by

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plants, drought conditions that maintain small rainfall events may preserve soil microbial function.

Widespread concern over the effects of climate change on ecosystem structure and function has led to experimental studies and meta-analyses assessing the impacts of altered precipitation regimes on NPP and biogeochemical cycling across a variety of ecosystems (e.g., Canarini et al., 2017; Wilcox et al., 2017; Song et al., 2019; Zhang et al., 2020). Among these studies, evidence suggests that the sensitivity of belowground processes to drought in arid ecosystems differs from mesic ecosystems. For example, a meta-analysis of altered precipitation studies in grasslands suggests that belowground net primary productivity (BNPP) in arid regions is more sensitive to precipitation increases or decreases than in wetter regions (Zhang et al., 2020). In contrast, soil microbial biomass and hydrolytic C-degrading extracellular enzyme activities in aridlands appear less sensitive to rainfall reductions than in more mesic ecosystems (Ren et al., 2017). Our predictions of the effects of climate change on belowground dynamics, however, are challenged due to the broad variation in factors between studies, e.g., climates, soil, ecosystem types, experimental manipulation treatment, and duration (Canarini et al., 2017; Wilcox et al., 2017; Hoover et al., 2018; Deng et al., 2021). It is also uncertain what influences ecosystem responses to altered precipitation regimes. Thus, calls have been made for more studies to include site-level characteristics (e.g., soil texture, nutrients, soil moisture, and pH), which may help identify factors that influence the sensitivity of ecosystems to climate change (Ren et al., 2017; Wilcox et al., 2017).

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To improve our understanding of the effects of predicted climate extremes on belowground C and biogeochemical cycling in aridland ecosystems, we assessed the effects of two altered precipitation patterns – severe reductions in growing season precipitation and extended hyper-arid periods – both of which are predicted to occur in the North American southwest (Cook and Seager, 2013; Cook et al., 2015) on belowground primary productivity and biogeochemical processes in two Southwestern semi-arid grassland ecosystems. The two grasslands in this study are considered an ecotone between a Chihuahuan Desert grassland and a Great Plains grassland ecosystem. The Chihuahuan Desert grassland is dominated by *Bouteloua eriopoda* (black grama); the Great Plains grassland is dominated by *B. gracilis* (blue grama). Chihuahuan Desert grasslands are generally restricted to desert regions (Schmutz et al., 1991) but are expanding northward into the southern edge of Great Plains grassland at our study site (Knapp et al., 2015; Hoffman et al., 2020; Collins et al., 2020). For five years, each grassland experienced reoccurring extreme drought (-66% reduction in growing season rainfall; extreme drought treatment) or a two-month delay in monsoon precipitation timing (complete omission of rain occurring during the monsoon season and captured rain applied later in the season; delayed monsoon treatment).

Previous work in these grasslands revealed that black grama is more sensitive to drought than blue grama (Knapp et al., 2015; Griffin-Nolan et al., 2019; Lagueux et al., 2020). Another earlier study found that extreme drought altered soil microbial community assembly, but soil microbial exoenzyme activity did not significantly change after two years (Ochoa-Hueso et al., 2018). Fernandes et al. (2018) found that the extreme drought and delayed monsoon treatment negatively affected cyanobacterial biological soil crusts after three years. However, the

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Chihuahuan Desert grassland experienced greater losses in cyanobacteria biomass and diversity, and the delayed monsoon treatment had weaker effects overall in this study. The results from earlier research in these grasslands lead us to predict that after five years:

1) The extreme drought treatment will have greater effects on belowground primary productivity and biogeochemistry than the delayed monsoon treatment
2) Chihuahuan Desert grassland will be more sensitive to the altered precipitation treatments than Great Plains grassland

Specifically, we hypothesized that five years of severe reductions in growing season rainfall (extreme drought treatment) would reduce belowground primary productivity and soil microbial biomass and function due to chronic water stress. We predicted responses to the delayed monsoon treatment to be less pronounced than the extreme drought treatment because this treatment does not reduce the total amount of rainfall (as captured rainfall is reapplied later in the season). Additionally, in earlier studies, this treatment has shown to be less impactful than the extreme drought treatment (Fernandes et al., 2018). Lastly, black grama has been shown to be more drought sensitive than blue grama (Knapp et al., 2015; Griffin-Nolan et al., 2019; Lagueux et al., 2020). Therefore, we expected to observe stronger responses to altered precipitation treatments in the Chihuahuan Desert grassland than in the Great Plains grassland.

2. Materials and Methods

2.1. Site description

This study takes place in the Sevilleta National Wildlife Refuge (SNWR), a Long-Term Ecological Research in central New Mexico, USA. The SNWR straddles the ecotone between

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the Colorado Great Plains and the Chihuahuan Desert (Buxbam and Vanderbilt, 2007). Thus, the two sites in our study, a black grama-dominated Chihuahuan Desert grassland and a blue grama-dominated Great Plains grassland, are ~5km apart. Soils are < 2 million years old and are classified as Typic Haplargids with a lithology of piedmont alluvium (Buxbaum and Vanderbilt, 2007). Soil texture between the two sites slightly varies. In the Chihuahuan Desert site, soils are a sandy loam mixture; soils in the Great Plains site are a mixture of sand, clay, and loam (Kröel-Dulay et al., 2004). However, soils beneath the dominant grasses are generally similar in texture and nutrient concentrations (Ladwig et al., 2021). The average annual temperature at the SNWR is 13.2°C, with an average low of 1.6°C and a high of 33.4°C in July (Collins et al., 2008). Mean annual precipitation is ~250mm, with most (~60%) falling during the summer monsoon, which typically occurs from July to September (Pennington and Collins, 2007), but rainfall is spatially quite variable and often highly localized during the monsoon season. During the year we sampled (2017), the Great Plains site received almost double the rain as the Chihuahuan Desert site during the summer monsoon (156mm vs. 83mm).

2.2. Experimental design

The two grasslands in this study are a part of the Extreme Drought in Grassland Experiment (EDGE) platform. The experiment began in the spring of 2012 (pre-treatment) at the SNWR. Each site contains three treatments (ten replicates): ambient rainfall, extreme growing season drought, and delayed monsoon. The extreme drought treatment reduces growing season rainfall (April through September) each year by 66%, which equates to a 50% reduction in annual precipitation. The extreme drought treatment was imposed using transparent polyethylene roof panels spaced apart to cover 66% of the roof's surface area (Yahdjian and Sala, 2002). The

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delayed monsoon treatment altered monsoon precipitation timing by omitting 100% of monsoon season precipitation from July to August each year. Rainfall was blocked using complete polyethylene roof panels and gutters that directed rainfall into adjacent storage tanks. Each year captured rain was applied over multiple watering events using raindrop quality sprinkler heads from September to early October. All plots are 3x4 m in size and are paired spatially into blocks with treatments assigned randomly within a block. Plots were hydrologically isolated from the surrounding soil matrix by aluminum flashing to a depth of 15cm. Drought shelters are tall, open-sided, and open-ended minimizing microclimate effects (Whitney et al., 2019). During an average rainfall year, these passive drought shelters would simulate a 1-in-100-year drought (Knapp et al., 2015) while maintaining rainfall size and frequency patterns typical of natural drought years (Knapp et al., 2017).

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2.3. Belowground net primary productivity and standing crop root biomass

During the fifth year of this experiment, BNPP was measured using root ingrowth bags (5 cm diameter by 20 cm deep), with five replicates per treatment. Three ingrowth bags (per plot) were inserted in the ground adjacent to clumps of grass in late June and removed in October after the extreme drought and delayed monsoon treatments were completed. Roots were removed from the bags by hand, washed, and then dried at 60 °C for at least 48 h and weighed to the nearest 0.0001g. For standing crop root biomass (root biomass), three samples per plot were randomly sampled from beneath a patch of either blue or black grama grass (depending on the grassland site) at a depth of 15 cm using a bucket auger. Replicate samples from each plot were combined before determining soil volume. Roots were extracted by passing soil and roots through multiple sieves with the smallest diameter sieve size of 600 µm. Roots were then washed and dried at 70º C for 48h. Root biomass was calculated as root biomass per unit soil volume (g/cm^3).

2.4. Soil sampling

During the fifth year of this experiment, we collected soil samples at three time points: pre-monsoon (mid-June), monsoon (late July), and post-monsoon (early October). During each sampling period, five soil cores were randomly sampled from each plot at a depth of 10 cm using a 1.9 cm wide soil corer. Following collection, soil samples were homogenized, passed through a 2 mm sieve, and kept at 4°C until further processing. For soil microbial exoenzyme analyses, a subsample of soil was immediately frozen at -20°C.

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2.5. Soil moisture and pH

In each plot, soil moisture was measured every 15 minutes using two Campbell Scientific CS-616 probes. One probe was buried at 45° to obtain an integrated measure of moisture at the top ~20cm of soil; a second probe was vertically inserted to integrate soil moisture from 0-30 cm depth. Soil pH was determined using a 2:1 (dH_2O: soil) slurry after stirring and then allowing it to settle for 30 minutes.

2.6. Soil carbon and nutrient stocks and pools

Available soil nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) were measured during the pre-monsoon, monsoon, and post-monsoon sampling periods, and extractable organic carbon (EOC), and extractable total nitrogen (ETN) were measured during the monsoon and post-monsoon sampling periods only. Extracts were obtained by shaking 5g of fresh soil in 0.5 M

K_2SO_4 for two hours and then filtering through glass filter paper. All nutrients were assessed using colorimetric microplate assays (BioTEK SynergH.T.HT, Winooski VT, USA). Available soil NO_3^-—N was analyzed with a modified Griess reaction (Doane and Horwáth, 2003), available soil NH_4^+-N was quantified using the Berlethot reaction protocol (Rhine et al., 1998), and PO_4^{3-}—P was measured with a malachite green assay (D'Angelo et al., 2001). EOC and ETN concentrations were determined using a Shimadzu analyzer (TOC-VCPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Total % organic C (%OC) and % total N (%N) were measured once using soils collected during the monsoon sampling period. %OC and % TN subsamples were dried at 50°C, ground and carbonates were removed with an HCl fumigation (Harris et al., 2001) and then quantified with a dry combustion C and N analyzer (ElementarPyroCube®).

2.7. Microbial Responses

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were assessed during the monsoon and post-monsoon sampling periods. Five grams of fresh soil were incubated with 2mL of ethanol-free chloroform for 24 hours at room temperature. Following incubation, soils were extracted and analyzed for EOC and ETN as above. MBC and MBN were calculated as the difference between EOC and ETN concentrations, respectively, from fumigated and non-fumigated samples.

We examined hydrolytic and oxidative enzymes which release C, N, and phosphorous (P) at the terminal stages of organic matter decomposition during the monsoon and post-monsoon sampling periods using standard high throughput microplate protocols (Saiya-Cork et al., 2002;

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McLaren et al., 2017). Examined hydrolytic exoenzymes include cellulose-degrading β-glucosidase (β-gluc), and cellobiohydrolase (Cello), protein-degrading leucyl aminopeptidase (LAP), chitin-degrading N-acetylglucosaminidase (NAG), and acid phosphatase (Phos), and oxidative enzymes peroxidase (Perox), and phenol oxidase (Phenol), which aid in the decomposition of recalcitrant organic matter. Frozen samples were thawed immediately at room temperature before analysis. One gram of soil was blended with 125mL of modified universal buffer at pH 7 and pipetted into 96-well plates with eight analytical replicates per sample. Fluorescing, 4-methylum-belliferone (MUB) tagged substrate (β -D-glucoside, β -D-cellobioside, N-acetyl- β -D-glucosaminide and phosphatase) or 7- amino-4-methylcoumarin (MC) tagged substrate (Leucine amino peptidase) was added to each hydrolytic enzyme assay. Hydrolytic enzyme assays were incubated at room temperature for 5 hours and 25 minutes, with measurements taken every 45 minutes to ensure activity was measured in the linear range of the reaction. Background fluorescence was measured for each soil, substrate, and quenching of MUB or MC (LAP only) by soils, and we used MUB/MC standard curves to calculate the rate of substrate hydrolyzed. Oxidative enzyme analysis was performed using L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate and incubated at 10°C for 25 hours. Sample fluorescence of hydrolytic enzymes and oxidative enzyme color absorbance was measured at 360 nm excitation and 460 nm emission, respectively, using a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

2.8. Statistical analysis

Continuous measures of soil moisture data were averaged by month (April- October 2017) and were then grouped according to relevant treatment periods. Soil moisture differences between

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ambient and the extreme drought treatment plots were analyzed during the drought treatment period in which the extreme drought plots received -66% of rainfall (April-September). The delayed monsoon treatment and ambient plots were grouped into two treatment periods: delayed monsoon treatment period when rain was 100% omitted (July-August), and post-delay, when captured rainfall was applied to delayed monsoon treatment plots (September-October). All other variables were measured either once (BNPP, root biomass, %OC, and %N), twice (EOC, ETN, MBC, MBN, and all exoenzymes), or three times (soil pH and nutrients) (see Table 1).

The effects of rainfall treatments were evaluated using repeated-measures linear mixed-effects model ANOVA [LMM, R-package lme4, (Bates et al., 2015)] or when appropriate generalized linear mixed-effects models (GLMM) [glmmTMB, R package (Brooks et al., 2017)]. Our fixed effects were treatment, site, sample period (when more than one sampling occurred), and their interactions, and to account for repeated sampling, we included sampling block as a random effect. All LMM and GLMM assumptions were evaluated using the 'Diagnostics for HierArchical Regression Models' (DHARMa) package (Hartig, 2021). When LMM passed diagnostics, we used the 'anova' function on the LMM model object. When LMM violated assumptions of equal variance and normally distributed residuals (response variables: BNPP, available soil nitrate, exoenzymes: Phos, β-gluc, and NAG), we performed GLMM analyses using a Gamma distribution log-link function and to account for the zero-inflated response variables (exoenzymes: Cello and LAP), we conducted zero-inflated Tweedie distribution log-link function analyses (Brooks et al., 2017). For the oxidative enzymes, most of the values were below minimum detectable levels, and these data were not analyzed. For GLMM models, the significance of the main effects and their interaction was determined based on chi-squared tests of their fitted values, using the Type-III

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sum-of-squares ‘Anova’ function from the car R package (Fox and Weisberg, 2019). For both LMM and GLMM, we used the emmeans R package (Lenth, 2021) to conduct Tukey post hoc comparisons between estimated marginal means for all treatment effects or interactions. All statistical analyses were performed using R statistical software version 4.1.2 (R Development Core Team, 2021) and R studio (RStudio Team, 2021). Data figures were constructed using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Belowground net primary productivity and standing crop root biomass

BNPP response to altered precipitation differed between the two grasslands (site-by-treatment interaction, Table S1). In the Chihuahuan Desert grassland, both extreme drought and delayed monsoon decreased BNPP (Table 2; Table S6; Fig. 1a), whereas, in the Great Plains grassland, only the delayed monsoon treatment reduced BNPP (Table 2; Table S6; Fig. 1b). Root biomass did not differ between treatments in either grassland (Table 2; Table S1; Fig. S1a,b).

3.2. Soil moisture and pH

The extreme drought and delayed monsoon treatment altered soil moisture during each treatment period (Table 2; Table S2). During the periods when rainfall was reduced (drought period for extreme drought treatment) or 100% omitted (delay period for the delayed monsoon treatment), soil moisture was significantly lower under both altered precipitation treatments (Table 2; Table S2; Fig. 2a,b). The delayed monsoon treatment also showed a treatment-by-period interaction (Table S2). Pairwise comparisons revealed that during the post-delay treatment (when captured rainfall was applied to delayed monsoon plots), there was marginally higher soil moisture in the

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delayed monsoon treatment plots than in the ambient plots (Table S6; Fig.3b). Additionally, during the treatment periods (drought and delay periods), soil moisture was consistently higher in the Great Plains grassland than in the Chihuahuan Desert grassland across all treatments (Table S2; Table S7; Fig. S2). Extreme drought increased soil pH at both sites (Table 2; Table S3; Table S6).

3.3. Soil carbon and nutrient stocks and pools

The effects on soil carbon and nutrients were more pronounced under the extreme drought treatment, and there were few effects under the delayed monsoon treatment (Table 2; Table S1; Table S3). Extreme drought altered most available soil nutrients (Table 2; Table S3). Available soil NO_3^- had a three-way interaction with treatment, site, and sampling period (Table S3). During the monsoon sampling period, extreme drought increased available soil NO_3^- in the Great Plains grassland (Fig.3b; Table 2; Table S6) and marginally increased NO_3^- in the Chihuahuan Desert site during the post-monsoon sampling period (Fig.3a; Table 2; Table S6). At both sites, extreme drought marginally decreased available soil NH_4^+ and marginally increased PO_4^{3-} (Table 2; Fig. 3a,b; Table S3). The delayed monsoon treatment had few effects on available soil nutrients except for NO_3^- which showed a three-way interaction between treatment, site, and sampling period (Table 2; Table S3). Here, the delayed monsoon treatment reduced available soil NO_3^- in the Chihuahuan Desert site during the monsoon sampling period (Fig. 3a; Table 2; Table S6). Across both sites and sampling periods, EOC, ETN, total soil %OC, and %N did not significantly differ between altered precipitation treatments (Table S2; Table S3; Fig. 3c,d,e,f).

3.4. Microbial responses

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Compared to the ambient treatment, soil microbial responses were not significantly affected by our extreme drought or delayed monsoon treatments (Table 2). Although MBN and many enzymes displayed treatments by sampling or site interactions (Table S4; Table S5; Fig. 4), pairwise comparisons revealed no significant differences (albeit marginal differences were present between treatments, but not between controls and treatments, for MBN and some enzymes) between ambient and altered precipitation treatments (Table S6).

4. Discussion

In this study, we assessed the effects of five years of two altered precipitation regimes predicted to occur in the future (Cook and Seager, 2013; Cook et al., 2015), extreme growing season drought, and delayed monsoon timing on BNPP and root biomass and soil biogeochemistry in two semi-arid grasslands. We identified more ecosystem soil responses to extreme drought than the delayed monsoon treatment., supporting our first hypothesis. Additionally, although we observed only a few differences between sites, the Chihuahuan Desert grassland was generally more sensitive to our altered precipitation treatments, supporting our second hypothesis. Extreme drought reduced BNPP in the Chihuahuan Desert grassland and increased soil pH and soil nutrients. The delayed monsoon treatment reduced BNPP in both sites and decreased available soil NO_3^- in the Chihuahuan Desert site. Finally, despite declines in BNPP and changes to soil ecosystem properties, extreme and prolonged drought did not significantly impact soil microbial biomass and exoenzyme potentials, nor did we detect effects on soil C pools (EOC and %OC).

4.1. Effects of extreme drought and delayed monsoon timing on soil moisture

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Aridlands, such as these grasslands, are pulse-driven ecosystems, whereby the timing and magnitude of rain pulses drive many ecological processes and are punctuated by periods of aridity and low biological activity between rain events (Noy-Meir, 1973; Austin et al., 2004; Loik et al., 2004; Collins et al., 2014). Our extreme drought and delayed monsoon treatments effectively decreased soil moisture during the rain reduction and omission periods, a factor that should be essential in driving changes in belowground structure and function. However, despite the grassland sites being geographically adjacent (separated by ~5km), soil moisture in the Chihuahuan Desert grassland was lower than in the Great Plains grassland across all treatments. During the year we sampled, the Great Plains site received almost double the rain as the Chihuahuan Desert site during the summer monsoon (156mm vs. 83mm). Thus, rainfall and soil moisture differences may explain the few responses (particularly BNPP and NO_3^-) to treatments that differed between sites.

4.2. Belowground net primary production and biomass

Relative to aboveground measures, studies of BNPP response to extreme drought and shifts in seasonal precipitation timing are scarce (Wilcox et al., 2017; Sun et al., 2021). Nevertheless, our findings are consistent with a four-year extreme growing season drought that reported declines in BNPP across four Great Plains grasslands spanning a 309- 825 mm precipitation gradient (Carroll et al., 2021). However, the extreme drought treatment did not consistently reduce BNPP across both sites in our study. While blue and black grama are drought tolerant grasses (Smith et al., 2004; Hoffman et al., 2020), as predicted by our second hypothesis, the differential sensitivity of BNPP to extreme drought suggests that the Great Plains blue grama-dominated grassland may be more drought tolerant than the Chihuahuan Desert black grama-dominated

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grassland. Still, it is important to note the difference in total rainfall between the sites that likely contributed to this difference.

Other studies show black grama is particularly sensitive to drought. For example, in semi-arid grasslands, drought strongly and rapidly reduced black grama cover and ANPP relative to blue grama (Báez et al., 2013; Munson et al., 2013; Knapp et al., 2015). Previous research in our experimental sites also found that extreme drought reduced black grama survival, biomass, and genetic variability in surviving plants (Whitney et al., 2019), while blue grama exhibited greater leaf-level drought tolerance than black grama (Griffin-Nolan et al., 2019). Additionally, during the year we sampled, although vegetation cover did not differ between delayed monsoon plots and ambient plots in either grassland, extreme drought more dramatically reduced the % cover of black grama in the Chihuahuan Desert grassland compared to blue grama in Great Plains grassland (Loydi and Collins, 2021). Thus, the differential sensitivity of the above-described aboveground responses and BNPP (our study) indicates the extreme drought treatment in the Chihuahuan Desert site and the delayed monsoon treatment in both sites pushed these grasslands beyond their water stress thresholds. This is particularly relevant in the delayed monsoon treatments where total precipitation was the same as ambient, emphasizing the importance of prolonged dry periods and precipitation seasonality.

In addition to rainfall differences between the two grasslands, the structural and physiological characteristics of the plants, and soil texture, may have influenced the soil water retained or lost from the rooting zone via transpiration or evaporation (Austin et al., 2004; Huxman et al., 2004; Loik et al., 2004). In our study, lower water inputs (differences in rainfall between the two

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grasslands and the complete omission of rainfall during the delayed monsoon treatment period), differences in plant cover, and slight differences in soil texture (i.e., sandy loam mixture in the Chihuahuan Desert site and sand, clay, and loam soil in the Great Plains site; Kröel-Dulay et al., 2004), may have led to more drastic declines in soil moisture and consequently BNPP. Soil surface cover is an essential factor influencing soil water availability (Breshears and Barnes, 1999; Loik et al., 2004). Lower soil temperatures under plant canopies can lead to lower soil evaporation rates (Breshears et al., 1998; Breshears and Barnes, 1999), where soil temperatures under grasses have been shown to be cooler than bare soils, presumably due to reduced solar radiation (Popiel et al., 2001). Additionally, while the inverse texture hypothesis suggests evaporation rates may decrease in coarser textured soil due to greater water infiltration ability (Noy-Meir et al., 1973), smaller rainfall events under our extreme drought treatment may favor the finer-textured soil in the Great Plains grassland, which tends to have a higher water holding capacity than coarser textured soils (Hook and Burke, 2000; Austin et al., 2004). Thus, under extreme drought conditions, relatively higher water inputs coupled with sustained water availability may have led to higher ANPP, which allowed BNPP to persist in the Great Plains grassland.

Alternatively, water availability can control the interannual variability of BNPP (Xu et al., 2012; Byrne et al., 2013). Therefore, our single year of measurement may not represent the overall response of BNPP to extreme drought in the Great Plains grassland. For instance, although BNPP was sensitive to three years of drought in a shortgrass steppe, variable results between years led to a lack of a robust linear relationship between BNPP and precipitation (Byrne et al., 2013). Additionally, experimental rainfall reductions can alter the vertical root distribution of

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BNPP by increasing root production at deeper soil layers (10–30 cm) (Zhang et al., 2019). Although most root biomass is located within the top 20 cm of soil at our site (Kurc and Small, 2007), black grama roots can reach depths of 45cm (Gibbens and Lenz, 2001). However, aboveground plant measures from our experimental plots suggest that black grama is particularly drought-sensitive (Griffin-Nolan et al., 2019; Whitney et al., 2019; Loydi and Collins, 2021). Therefore, it is also likely that the extreme drought treatment led to a stronger response of BNPP in the Chihuahuan Desert site.

Contrary to BNPP, we did not detect changes to standing crop biomass (live + dead). Our findings differ from other studies that reported root biomass decreased in response to drought in grasslands (e.g., Fiala et al., 2009; de Vries et al., 2016) but coincide with a recent meta-analysis that found that drought consistently reduced root biomass in forests and shrublands but showed no relationship across grasslands (Deng et al., 2021). Nevertheless, differences in root biomass may not have been detectable during the time frame of our study. In a Patagonian steppe (Berenstecher et al., 2021) and a semi-arid northern grassland (McLaren and Turkington, 2010), the rate of root decomposition belowground was much slower than leaf tissue decomposed aboveground, which indicates that the turnover of belowground tissues is relatively slow in semi-arid ecosystems. Also, it is difficult to separate living and recently dead roots (Ostertag and Hobbie, 1999). Therefore, it may be challenging to detect recent differences in root production between our treatments with belowground root biomass measurements.

4.3. Soil carbon and nutrient stocks and pools

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After five years, despite changes to BNPP under both treatments, most changes to soil ecosystem properties occurred under the extreme drought treatment and primarily only affected soil nutrient pools, particularly soil NO_3^-. Extreme drought increased soil pH, available soil NO_3^-, and marginally increased available soil PO_4^{3-}. Our findings coincide with other drought studies that report the accumulation of NO_3^- in semi-arid steppe ecosystems (White et al., 2004; Yahdjian et al., 2006; Evans and Burke, 2013) and other arid ecosystems such as a semi-arid pinon-juniper woodland (Cregger et al., 2014). Additionally, while we did not observe higher concentrations of ETN, our results partially agree with a recent meta-analysis that found higher mineral and extractable organic N in response to drought across grasslands globally (Deng et al., 2021).

Many factors can drive nutrient accumulation and losses under dry conditions, e.g., reduced leaching (Jalali, 2009; Muhr et al., 2010; Cregger et al., 2014), lower biological soil crust N fixation (Barger et al., 2016), increased volatilization of ammonia with small rain events (Schlesinger and Peterjohn, 1991), and reduced uptake by plants (He and Dijkstra, 2014; Deng et al., 2021). However, N retention in soils remains high at our study site even under frequent small rain events (Kwiecinski et al., 2020). Consequently, nutrient leaching losses in our extreme drought plots are unlikely. Additionally, N-fixing cyanobacteria are rare in soil crusts in our study sites (Fernandes et al., 2018). Therefore, we suggest that extreme drought conditions may have led to soil N and P accumulation due to the reduced uptake of soil nutrients by plants and possibly continued activity of soil microbes. While extreme drought did not consistently reduce BNPP at both sites, this treatment consistently decreased aboveground plant cover (Loydi and Collins, 2021). Therefore, the plot-level reduction in root biomass would likely result in reduced plant nutrient uptake (Austin et al., 2004; Homyak et al., 2017; Deng et al., 2021). Additionally,

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although potential exoenzyme activity may continue even if microbial biomass and respiration decline (Geisseler et al., 2011), relative to ambient conditions, we found no changes to MBC, MBN, and exoenzyme activity, suggesting sustained activity by soil microbes under drought was likely.

Unlike extreme drought, the delayed monsoon treatment altered very few soil ecosystem properties, supporting our first hypothesis. The delayed monsoon treatment reduced available soil NO_3^- during the mid-monsoon sampling period in the Chihuahuan Desert grassland. The decline in NO_3^- during the mid-monsoon sampling period and differences in responses between the two grasslands may be due to the difference in soil moisture during the sampling period and between sites. Soil NO_3^- was lower during the sampling period when rainfall was 100% omitted, and this reduction only occurred at the Chihuahuan Desert grassland site, which had lower soil moisture than the Great Plains site across all treatments. Although some grasslands show negligible declines in N mineralization to drought (Deng et al., 2021), lower soil NO_3^- may suggest declines in N mineralization, which can be strongly influenced by moisture fluctuations triggered by rain events (Austin et al., 2004; Manzoni et al., 2012; Risch et al., 2019). Dry soil conditions may also limit soil exoenzyme mobility and substrate availability (Geisseler et al., 2011; Manzoni et al., 2012; Schimel et al., 2018; Asensio et al., 2021). However, our study found no significant effect on ETN, %N, or potential exoenzyme activity.

4.4. Microbial biomass and exoenzyme potentials

Rainfall reductions have been shown to impact soil microbial biomass and activities across the globe (Ren et al., 2017; Deng et al., 2021). However, despite apparent changes in BNPP and soil

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N pools, our study found no difference in soil microbial biomass and potential exoenzyme activity with either precipitation treatment. These results, however, are consistent with increasing evidence that microbial biomass generally has lower sensitivity to drought in aridlands, as reported in a recent metanalysis that found undetectable changes to microbial biomass in longer-term (>2-3 years) studies (Ren et al., 2017). Further, multiple studies have shown sustained exoenzyme activities under dry soil conditions (Stursova et al., 2006; Geisseler et al., 2011; Ochoa-Hueso et al., 2018). Although factors that influence the resistance or resilience of soil microbial communities under extreme climate conditions remain poorly understood, multiple mechanisms have been suggested to influence their survival and continued activity (Bardgett and Caruso, 2020). For example, soil microbial communities may shift toward drought-tolerant taxa (Ochoa- Hueso et al., 2018). At our study sites, but after only three years of extreme drought, Ochoa-Hueso et al. (2018) found that drought reduced fungal and bacterial richness in soils in the Chihuahuan Desert site but did not affect potential exoenzyme activity (Ochoa-Hueso et al., 2018). Another study revealed that extreme drought and, to a lesser extent, delayed monsoon treatment negatively affected cyanobacteria-dominated soil crusts after three years, and these effects were more pronounced in the Chihuahuan Desert grassland than in the Great Plains grassland (Fernandes et al., 2018). Therefore, despite changes to soil and biocrust communities, BNPP, and other soil properties, our study reveals that soil microbial biomass and potential exoenzyme activities were preserved after five years of extreme precipitation manipulations.

Few studies have explored the influence of soil properties on soil microbial resistance and resilience to climate extremes; however, soil resource availability has been suggested to play an essential role (Bardgett and Caruso, 2020). As our treatments occurred during the period when

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dew formation is exceptionally rare (Agam and Berliner, 2006), atmospheric moisture may not be an essential source of soil moisture for soil microbial activity and C cycling during rain-free periods at our site (Agam and Berliner, 2006; McHugh et al., 2015). In our study, we found no detectable changes to other measures of belowground C, including soil %OC or EOC to either treatment, while soil nutrients generally increased. Thus, we propose that soil microbial functional resistance in our study may be associated with sustained C, N, and water availability. However, few altered precipitation studies occur over the long term (>10 years) and combine water reductions with high temperatures, which can increase evaporative demands (Hoover et al., 2018; Deng et al., 2021). Thus, as resources are depleted over time, or water deficits intensify, aridland soil microbial response remains uncertain.

5. Conclusions

The Chihuahuan Desert grassland is expanding northward and replacing Great Plains grassland in response to changing climatic drivers and increasing aridity (Rudgers et al., 2018; Collins et al., 2020). This ongoing transition has important implications for ecosystem C dynamics in this semi-arid region, given that ANPP (Knapp et al., 2015) and BNPP (this study) of Chihuahuan Desert grasslands are more sensitive to drought than Great Plains grasslands. Additionally, water and N availability are the two most limiting factors regulating ecosystem function in aridlands (Hooper and Johnson, 1999). Changes to nutrient availability, such as N, may alter plant community growth and structure (Ladwig et al., 2012) and soil microbial community structure and function (Treseder, 2008; Ramirez et al., 2012) during periods when water is no longer limiting. Lastly, although drought experiments and meta-analyses consistently predict negative impacts of drought on the diversity and abundance of soil microbial communities (Wu et al.,

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2011; Ochoa- Hueso et al., 2018), we found an overall lack of change in soil microbial biomass and exoenzyme activity and ultimately in soil C stocks, which suggests that soil microbial processes are relatively resistant to changes in rainfall regimes over the short-term. In the long-term, however, it remains unclear if chronic drought and changes in precipitation seasonality, especially if they co-occur, will eventually impact soil microbial function with significant consequences for dryland C and nutrient cycling.

Acknowledgments

We would like to thank the Sevilleta LTER field crew, interns, and staff who maintained treatments and data, especially Stephanie Baker, Renée F. Brown, and Nathan Gehres. We are also grateful for the suggestions and comments from Associate Chief Editor Dr. Christina Kaiser and the two anonymous reviewers, which greatly improved the quality of this paper. Support for this research was provided by the NSF Macrosystems Ecology award [EF-1137363], REU Sites award [DBI-1659441], and LTER and LTREB awards [DEB-1655499; DEB-1856383].

Declaration of competing interest

The authors declare no conflicts of interest.

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Variables	**Sampling periods**	**Total times measured**
BNPP	Post-treatments (October)	1
Root biomass	Monsoon	1
Soil moisture	April- October	Continuous
Soil pH	Pre-monsoon, monsoon, and post-monsoon	3

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Available nutrients	Pre-monsoon, monsoon, and post-monsoon	3
EOC	Monsoon and post-monsoon	2
ETN	Monsoon and post-monsoon	2
%OC	Monsoon	1
%N	Monsoon	1
MBC	Monsoon and post-monsoon	2
MBN	Monsoon and post-monsoon	2
Exoenzymes	Monsoon and post-monsoon	2

Table 1. Sampling scheme summary. Belowground net primary productivity (BNPP), extractable organic carbon (EOC), extractable total nitrogen (ETN), total organic carbon (%OC), total nitrogen (%N), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN).

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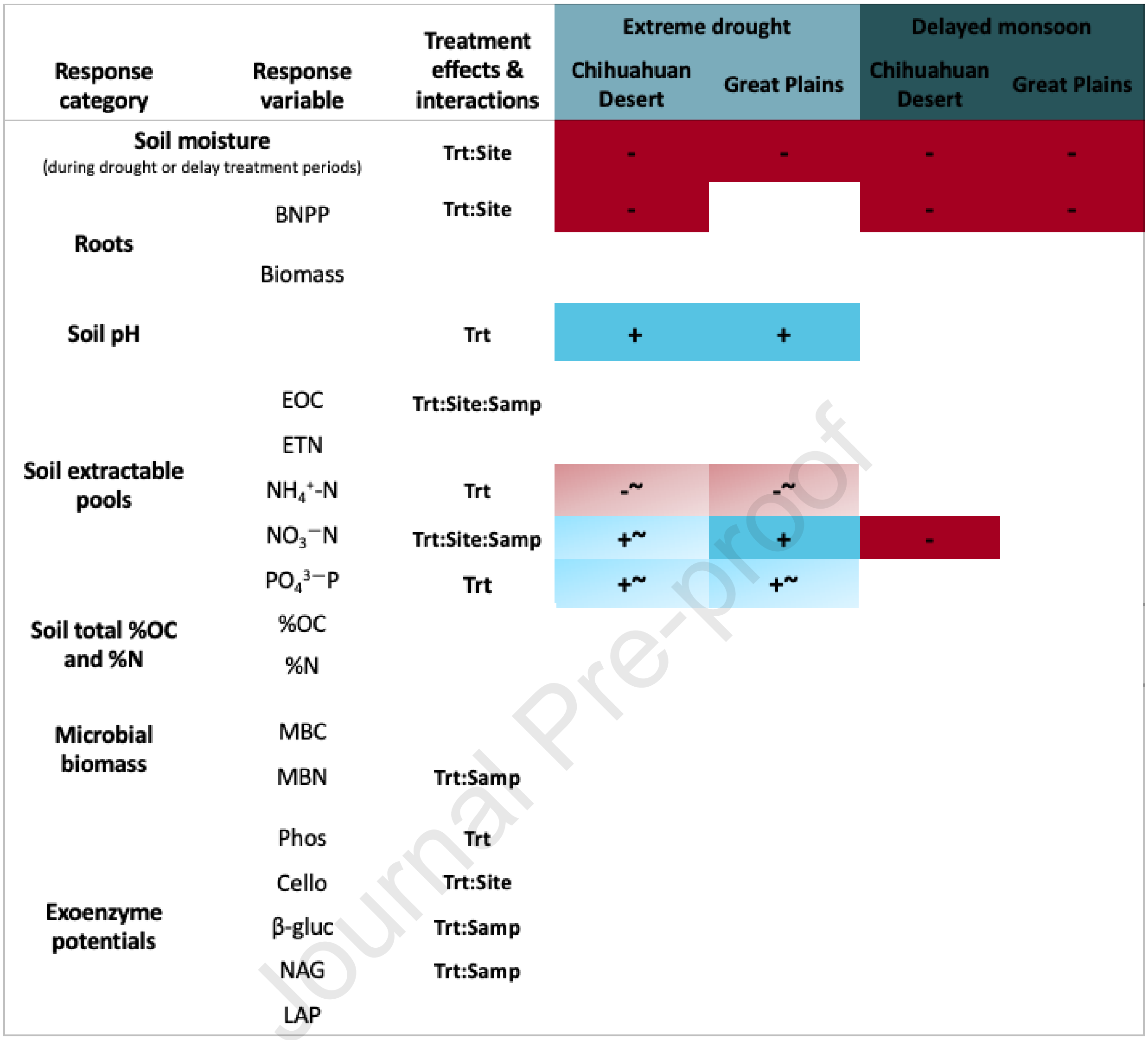

Response category	Response variable	Treatment effects & interactions	Extreme drought		Delayed monsoon	
			Chihuahuan Desert	Great Plains	Chihuahuan Desert	Great Plains
Soil moisture (during drought or delay treatment periods)		Trt:Site	-	-	-	-
Roots	BNPP	Trt:Site	-		-	-
	Biomass					
Soil pH		Trt	+	+		
Soil extractable pools	EOC	Trt:Site:Samp				
	ETN					
	NH_4^+-N	Trt	-~	-~		
	NO_3^-N	Trt:Site:Samp	+~	+	-	
	PO_4^{3-}P	Trt	+~	+~		
Soil total %OC and %N	%OC					
	%N					
Microbial biomass	MBC					
	MBN	Trt:Samp				
Exoenzyme potentials	Phos	Trt				
	Cello	Trt:Site				
	β-gluc	Trt:Samp				
	NAG	Trt:Samp				
	LAP					

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Table 2. Summary of treatment effects in a precipitation manipulation experiment in an ecotone between a black grama-dominated Chihuahuan Desert grassland and blue grama-dominated Great Plains grassland. Treatment effects and interactions are based on repeated measures ANOVA with the main factors precipitation treatments (Trt), site, and sampling periods (Samp). Treatment level differences (filled cells) are based on estimated marginal means contrasts between ambient vs. extreme drought and ambient vs. delayed monsoon treatments. Cells are filled for belowground net primary productivity (BNPP), root biomass, extractable organic carbon (EOC), extractable total nitrogen (ETN), ammonium (NH_4^+), nitrate (NO_3^-), phosphate

(PO_4^{3-}), total % organic carbon (%OC) and % total nitrogen (%N), microbial biomass carbon, (MBC), microbial biomass nitrogen (MBN), exoenzymes phosphatase (Phos), cellobiohydrolase (Cello), β-glucosidase (β-gluc), N-acetylglucosaminidase (NAG), and leucyl aminopeptidase (LAP) responses. Red cells (-) indicate negative effects (relative to ambient). Blue cells (+) indicate positive effects (relative to ambient). Darker hues (- or +) indicate significant effects ($p<0.05$) and lighter hues (-~ or +~) represent marginally significant effects ($0.1<p<0.05$). Non-significant effects between ambient and altered precipitation treatments contain no characters or color. Two and three-way interactions have complex results and are described in more detail in text and figures.

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Graphical abstract. Figure illustrating the effects of 5 years of extreme growing season drought (b) and delayed monsoon timing (c) on belowground productivity and biogeochemistry in two geographically adjacent semi-arid grasslands. Panel (a) represents ambient conditions and the two grassland sites within all panels; the blue grama (left side) represents the Great Plains grassland site, and the black grama (right side) represents the Chihuahuan Desert grassland site. In general, we found that, relative to the ambient treatment, the extreme drought and delayed monsoon treatments had few effects on soil microbial variables, including soil microbial biomass and exoenzyme potentials, and had no effect on soil C stocks (no effect= brown boxes). The lack of effects on microbial variables is despite reductions in BNPP (negative effects = red boxes with ″ - ″) and impacts on biogeochemical variables, such as increased soil pH and available soil nutrients (positive effects = blue boxes with ″+″).

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Fig. 1. Boxplot of belowground primary productivity (BNPP) in a precipitation manipulation experiment in an ecotone between a black grama-dominated Chihuahuan Desert grassland (a) and blue grama-dominated Great Plains grassland (b). The three precipitation treatments include ambient, extreme drought, and delayed monsoon treatments. Letters denote significant differences between treatments (estimated marginal means, $p<0.05$).

Fig. 2. Boxplot of average volumetric water content (%) in a precipitation manipulation experiment in an ecotone between a black grama-dominated Chihuahuan Desert grassland (left side a,b) and blue grama-dominated Great Plains grassland (right side a,b). The precipitation treatments include ambient, extreme drought, and delayed monsoon treatments. Ambient and extreme drought treatments are compared during the drought treatment period (-66% reduction of ambient growing season rainfall in extreme drought plots) (a). Ambient and delayed monsoon treatments are compared during the delay period (100% omission of rainfall in delayed monsoon plots); post-delay treatment period (captured rain is applied to delayed monsoon plots) (b). Letters denote significant differences between treatments (estimated marginal means, $p<0.05$), and ″ * ″ represents significant differences between ambient and delayed monsoon treatments within a single treatment period.

Fig. 3. Boxplot of soil carbon and nutrient stocks and pools: nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) (a,b), extractable organic carbon (EOC), and extractable total nitrogen (ETN) (c,d), total organic carbon (%OC) and total nitrogen (%N) (e,f) in a precipitation manipulation experiment in an ecotone between a black grama-dominated, Chihuahuan Desert grassland (left side), and blue grama-dominated Great Plains grassland (right side). The three precipitation

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treatments include ambient, extreme drought, and delayed monsoon treatments. Letters denote significant differences between treatments (estimated marginal means, $p<0.05$).

Fig. 4. Boxplot of microbial biomass and exoenzyme potentials: microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) (a,b), exoenzymes: β-gluc, LAP, and Phos (c,d), and Cello and NAG (e,f), in a precipitation manipulation experiment in an ecotone between a black grama-dominated, Chihuahuan Desert grassland (left side), and blue grama-dominated Great Plains grassland (right side). Boxplots a,c,d,e, and f represent values during the monsoon period, and b represents values during the post-monsoon period. The three precipitation treatments include ambient, extreme drought, and delayed monsoon treatments. Letters denote significant differences between treatments (estimated marginal means, $p<0.05$).

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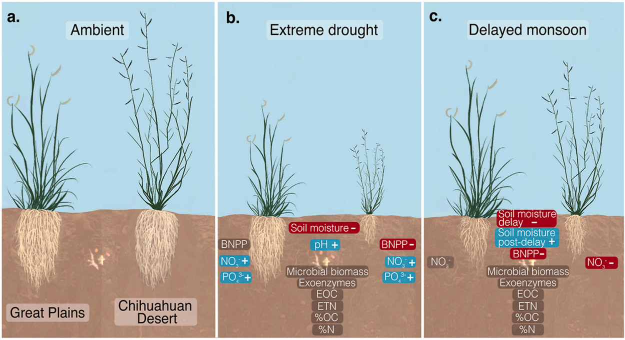
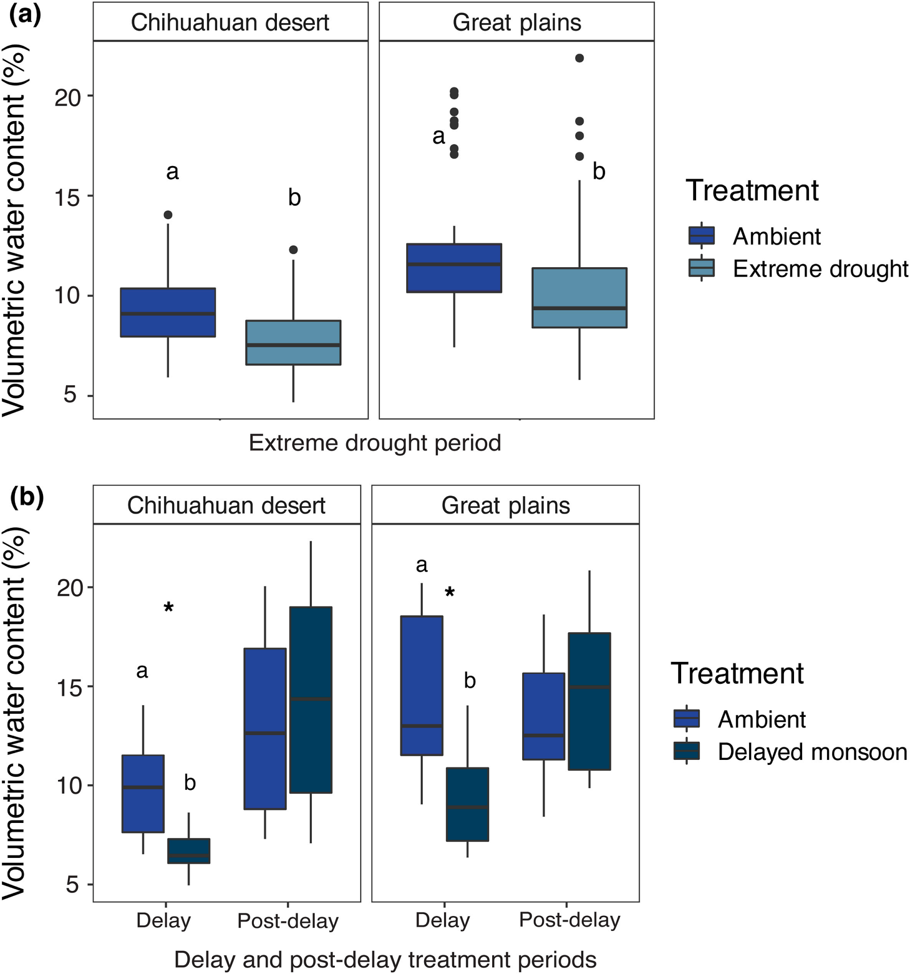
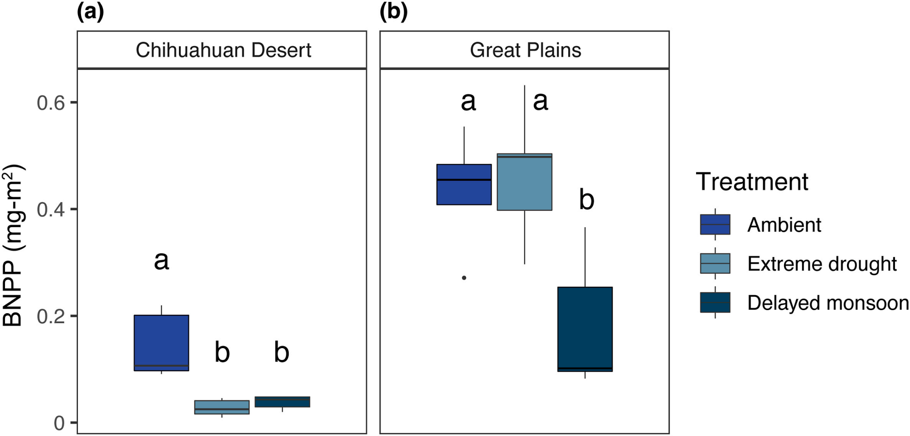
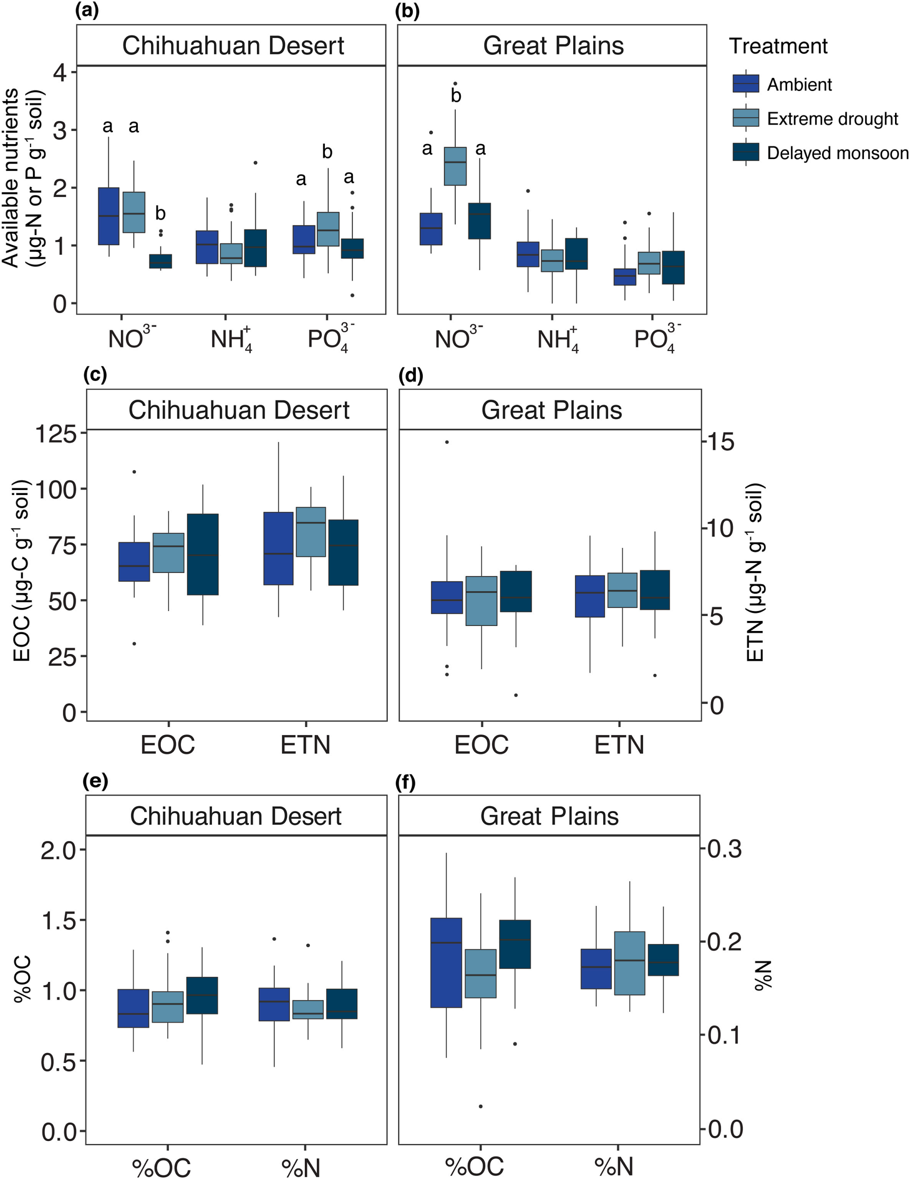
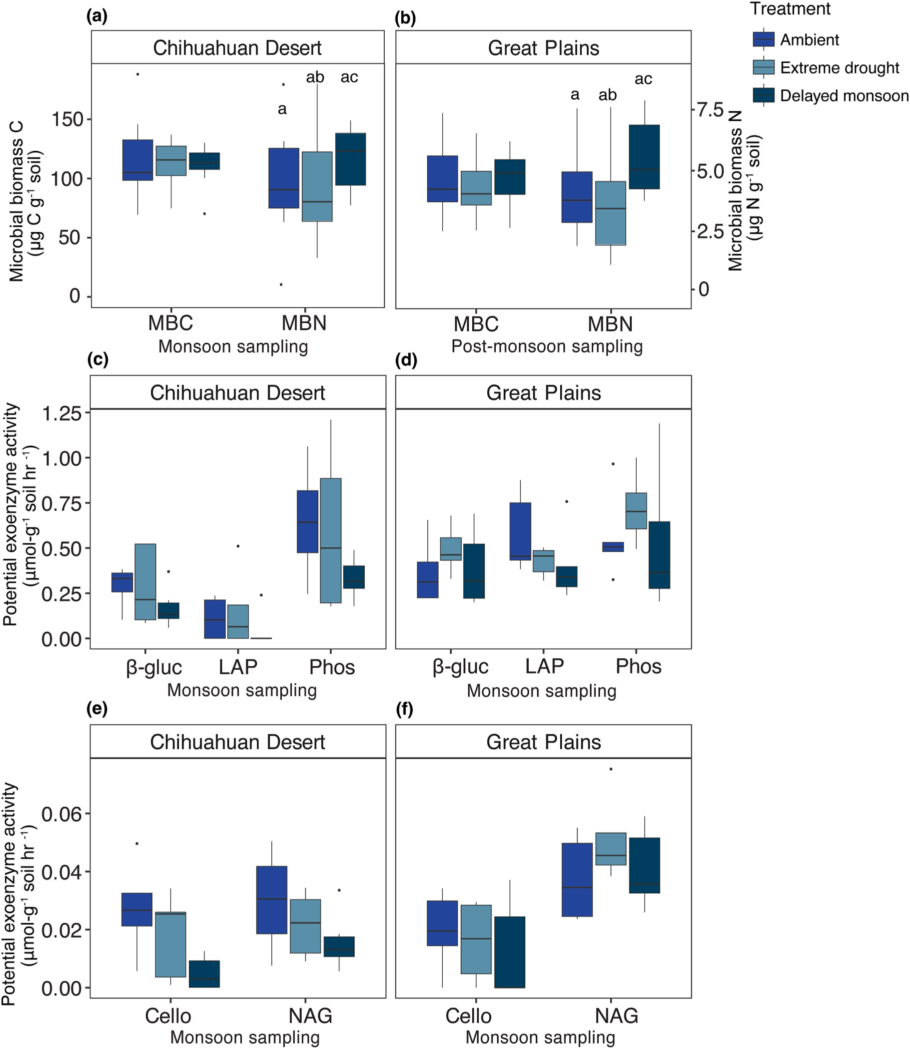

a.
Ambient
Great Plains
Chihuahuan Desert
b.
Extreme drought
Soil moisture −
BNPP
pH +
BNPP −
NO_3^- +
NO_3^- +
PO_4^{3-} +
PO_4^{3-} +
Microbial biomass
Exoenzymes
EOC
ETN
%OC
%N
c.
Delayed monsoon
Soil moisture delay −
Soil moisture post-delay +
BNPP −
NO_3^-
NO_3^- −
Microbial biomass
Exoenzymes
EOC
ETN
%OC
%N


(a)
Chihuahuan desert
Great plains
Volumetric water content (%)
20
15
10
5
a
b
a
b
Extreme drought period
Treatment
Ambient
Extreme drought
(b)
Chihuahuan desert
Great plains
Volumetric water content (%)
20
15
10
5
*
a
b
a
*
b
Delay
Post-delay
Delay
Post-delay
Delay and post-delay treatment periods
Treatment
Ambient
Delayed monsoon


(a)
Chihuahuan Desert
(b)
Great Plains
BNPP (mg-m^2)
0.6
0.4
0.2
0
a
b
b
a
a
b
Treatment
Ambient
Extreme drought
Delayed monsoon


(a)
Chihuahuan Desert
(b)
Great Plains
Treatment
Ambient
Extreme drought
Delayed monsoon
Available nutrients (µg-N or P g^{-1} soil)
0
1
2
3
4
a a b
a b a
a b a
NO^{3-}
NH$_4^+$
PO$_4^{3-}$
(c)
Chihuahuan Desert
(d)
Great Plains
EOC (µg-C g^{-1} soil)
0
25
50
75
100
125
ETN (µg-N g^{-1} soil)
0
5
10
15
EOC
ETN
(e)
Chihuahuan Desert
(f)
Great Plains
%OC
0.0
0.5
1.0
1.5
2.0
%N
0.0
0.1
0.2
0.3
%OC
%N


(a)
Chihuahuan Desert
Microbial biomass C
(µg C g-1 soil)
150
100
50
0
a
ab
ac
MBC
MBN
Monsoon sampling
(b)
Great Plains
a
ab
ac
7.5
5.0
2.5
0
Microbial biomass N
(µg N g-1 soil)
MBC
MBN
Post-monsoon sampling
Treatment
Ambient
Extreme drought
Delayed monsoon
(c)
Chihuahuan Desert
Potential exoenzyme activity
(µmol-g-1 soil hr-1)
1.25
1.00
0.75
0.50
0.25
0.00
β-gluc
LAP
Phos
Monsoon sampling
(d)
Great Plains
β-gluc
LAP
Phos
Monsoon sampling
(e)
Chihuahuan Desert
Potential exoenzyme activity
(µmol-g-1 soil hr-1)
0.06
0.04
0.02
0.00
Cello
NAG
Monsoon sampling
(f)
Great Plains
Cello
NAG
Monsoon sampling

Highlights:

- Extreme and prolonged growing season drought reduces belowground primary productivity
- Microbial biomass and enzymes unaltered by severe or prolonged growing season drought
- Belowground properties are more sensitive to extreme drought than seasonality changes

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Declaration of competing interest

The authors declare no conflicts of interest.

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