Climate legacies and restoration history as drivers of tallgrass prairie carbon and nitrogen cycling

by

Caitlin M. Broderick

B.S., University of Notre Dame, 2017

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Climate change is expected to alter precipitation amounts and distributions, resulting in longer, more frequent periods of wet and dry conditions in the North American Central Plains. Grasslands in this region are often limited by water availability, so novel rainfall patterns will likely affect ecosystem functioning. The rates of two key carbon (C) fluxes, aboveground net primary productivity (ANPP) and soil respiration, are tightly linked to water availability in these grasslands. Moreover, the cycling of nitrogen (N), a co-limiting nutrient, is tied to soil moisture through microbially-mediated processes such as N mineralization, microbial immobilization, and nitrification. Decomposition unites these two cycles—controlling the rate of C sequestration and N release—and can be slowed by both droughted and saturated soils. There is a growing understanding that sufficiently long and/or intense precipitation anomalies (e.g., extended wet or dry periods) can affect ecosystem processes even after the climate event ceases, resulting in climate "legacy effects". Tallgrass prairies, at the eastern and wetter end of the Central Plains grasslands, are both sensitive and highly resilient to short-term climate variability but the extent to which this climate sensitivity and resilience is shaped by previous climate history is largely unknown. If altered climate patterns cause changes in key ecosystem properties such as plant communities, microbial community functioning, or soil attributes, these climate changes may exert legacies on rates of prairie C and N cycling. Finally, while the relationship between climate and intact grassland ecosystem functioning has been relatively well-studied, less than five percent of North American tallgrass prairie remains intact. As a result, the persistence of tallgrass prairies and their associated ecosystem services relies heavily on the successful restoration of functioning prairies; yet future restorations will likely occur under a more hostile climate. It is

therefore important to assess how climate sensitivity and resilience develops as restored prairies mature.

In this dissertation, I assessed how past and current climate conditions interact to affect C fluxes, N transformations, and decomposition rates in native tallgrass prairie. I used a long-term experiment at Konza Prairie, KS, in which rainfall was supplemented by irrigation water to release tallgrass prairies from water stress for ~25 years. In 2017, I switched the irrigation and ambient treatments in a subset of plots and added new drought treatments across both historic treatments, allowing me to assess (i) how short- and long-term climate patterns differ in their effects on prairie ecosystems, (ii) whether previous climate patterns continue to shape current prairie functioning via climate legacies, and (iii) whether previous climate altered the sensitivity of prairie C and N cycling to drought conditions. In a separate project, I imposed an experimental drought across restored prairies ranging from 4 to 22 years old and measured how the sensitivity of prairie structure and function to water stress varied with restoration age. I found that a historically wetter climate increased ANPP and soil respiration on a magnitude comparable to current wet conditions, and that a history of irrigation conferred greater drought resistance to key ecosystem processes lasting up to three years. A history of irrigation also increased net N mineralization rates and nitrification rates, and microbial C/N ratios and extracellular enzyme investment suggested reduced N limitation of belowground N cycling. This legacy of increased N supply with a history of irrigation may support the higher-than-expected rates of C fluxes after ceasing irrigation. In contrast, root decomposition rates were slowest with long-term irrigation, suggesting that the increased rates of C and N mineralization may be more due to legacy effects on SOM processing than litter decay. Notably, legacy effects across response variables were most often found in lowland prairie, suggesting that topoedaphic factors are important for determining the strength of biogeochemical climate legacies. Finally, I found that restored prairie plant communities, ANPP, soil respiration, and labile N pools were surprisingly resistant to drought across all restoration ages, offering hope that restoration efforts may not be significantly hindered by future climate variability.

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Approved by:

Major Professor
Dr. John Blair

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Chapter 1 – Introduction

The earth's climate is changing at a rapid pace and is expected to negatively impact human well-being as well as ecosystems across the globe (IPCC 2018). While realized and projected changes in temperature are much discussed, anthropogenic climate change is likely already contributing to more extreme precipitation patterns (IPCC 2018). Changes in rainfall regimes can alter soil water content and the timing of water availability for critical ecosystem processes. For example, carbon (C) cycling rates depend in large part on water availability (Raich and Schlesinger 1992; Wu et al. 2011; Song et al. 2019), so altered precipitation patterns are likely to affect rates and patterns of net C uptake by ecosystems. Moreover, changes in ecosystem C source-sink dynamics associated with climate change can feed back on climate change by impacting atmospheric CO_2 concentrations (Melillo et al. 2017). Therefore, it is important to understand how climate fluctuations impact ecosystem functioning, and the implications of that for global C cycling, in order to improve the accuracy of ecosystem models and climate change mitigation efforts.

Both influxes and effluxes of C to ecosystems are shaped by climate. Net primary productivity is a measure of C capture by ecosystems through photosynthesis. Primary productivity generally increases with annual precipitation within and across ecosystems (Knapp and Smith 2001). However, the relationship between annual precipitation and aboveground net primary productivity (ANPP) across space is much stronger than the temporal relationship of between-year precipitation and ANPP within a site (Sala et al. 2012). This suggests that the effects of precipitation on ANPP can vary with temporal scales, making it difficult to predict the effects of long-term climate change. Indeed, longer-term dry periods can have stronger effects on ANPP than single-year droughts (Hoover et al. 2014), and extended wet periods can cause community and/or soil and biogeochemical changes that can increase responses of ANPP to high precipitation conditions over time (Knapp et al. 2012). Soil respiration is a major C efflux from ecosystems that varies with water availability at short temporal scales (Harper et al. 2005; Song et al. 2019). However, the CO_2 efflux from soils is also driven by factors that change in response to long-term climate, such as the amount and quality of litter inputs (Zhang et al. 2020; Bréchet et al. 2017) and the size and composition of soil microbial communities (Hawkes et al. 2017; Zhou et al. 2018). Therefore, to predict C dynamics under a future climate, it is important to investigate how the effects of long-term and persistent changes in climate differ from those of short-term climate variability.

The effects of precipitation regimes on ecosystems can be complex because water availability affects both C and nitrogen (N) cycling, and these cycles are tightly linked (Finzi et al. 2011). Plant and microbial processes are often limited by N availability (Schimel and Weintraub 2003; Xia and Wan 2008, LeBauer and Treseder 2008), and in turn, key N supply processes are closely linked to precipitation and soil water availability. Both net N mineralization and nitrification rates tend to increase with soil moisture in the short term (Wang et al. 2006; Risch et al. 2019). However, these fluxes do not vary systematically with annual precipitation across natural precipitation gradients (McCulley et al. 2009), suggesting that long-term effects of climate may differ from those based on shorter-term water availability. Increased water availability also increases N mobility in soils (Marschner and Rengel 2012). In the short term, this can increase N availability, but this N could be taken up by plants to support higher ANPP, immobilized by microbial communities, and/or lost to gaseous fluxes—leading to exacerbated N limitation (Ren et al. 2017). Because of the complex responses of N cycling processes to water availability, it is important to compare how the short- and long-term precipitation regimes impact N availability, and how these N responses to climate can indirectly alter C cycling processes.

An emerging aspect of the effects of climate on ecosystem function is the potential for climate legacy effects. Evidence is mounting that past climate patterns can continue to shape C cycling in the present (Sala et al. 2012, Delgado-Baquerizo et al. 2017; Song et al. 2019). Previous dry conditions, for example, can stress or kill plant organs so that growth is hindered in subsequent years even with adequate precipitation (Reichmann et al. 2013); in contrast, enhanced growth from a previous wet year may support high ANPP in subsequent years (Sala et al. 2012). In ecosystems that are limited by both water and N, a dry year may allow accumulation of N that supports future higher growth (Seastedt and Knapp 1993); in contrast, a wet year may cause N leaching that exacerbates N limitation and lowers C fluxes in subsequent years (Ren et al. 2017). While climate legacy effects are well-documented in arid systems, they can be less apparent in mesic systems (Sala et al. 2012). Moreover, while it is common to assess legacies as a function of the previous year's precipitation effects on ecosystem functioning, longer-term precipitation anomalies may cause stronger, more persistent climate legacies. The effect of precipitation history on current biogeochemical cycling is not incorporated into biogeochemical models (Averill et al. 2016), hindering predictions of the effects of climate change. There is a need to untangle where climate legacies occur, under what conditions, and how these legacies will impact the responses of ecosystems to climate change.

Grasslands cover 30-40% of earth's terrestrial surface (Dixon et al. 2014) and play an important role in the global C cycle (Scurlock and Hall 1998). Grassland soils store an enormous amount of C (Lorenz and Lal 2018) and support a suite of ecosystem services, including forage production and high biodiversity. Tallgrass prairies, in particular, have greater stocks of C per volume than those in many forests (Blair et al. 1998). Across the Central Plains, precipitation regimes are expected to become more variable, with longer, more intense periods of wet and dry conditions (IPCC 2018). Grasslands tend to be water-limited (Lauenroth et al. 1993), so changes in precipitation regimes associated with climate change are expected to profoundly affect ecosystem processes. But tallgrass prairies also are often co-limited by both water and N (Seastedt and Knapp 1993), making it difficult to predict long-term effects of climate change on C and N cycling from short-term experiments. Long-term climate manipulation experiments are therefore needed to assess how both N availability and C fluxes will respond to persistent changes in future precipitation regimes.

While legacy effects occur in drier grasslands (Sala et al. 2012; Reichmann et al. 2013), effects of previous-year precipitation may be less pronounced in tallgrass prairie, perhaps due to high drought tolerance of the dominant grasses and the tight cycling of N that minimizes losses under wet conditions. However, persistent changes in precipitation regimes may lead to shifts in the plant community (Smith et al. 2009; Knapp et al. 2012) that alter the sensitivity of ANPP to precipitation patterns, or to changes in the functionality of microbial communities that alter rates of C and N mineralization, nitrification, and other processes (Averill et al. 2016; Hawkes et al. 2017). These changes under extended wet and dry conditions could produce legacy effects in ANPP, soil respiration, and N transformations that would not be apparent in short-term experiments or through analysis of the historical precipitation record. One important pathway for legacy effects in tallgrass prairie might be changes in root biomass or decomposition. In frequently burned tallgrass prairies, litter inputs occur primarily via roots and rhizomes, so the sensitivity of belowground decomposition rates to altered precipitation regimes can have profound effects on both C and N cycling. However, root decomposition rates vary less predictably with precipitation than surface litter (Silver and Miya 2001; See et al. 2019), and both high and low soil moisture conditions can slow decomposition in tallgrass prairies (Hayes and Seastedt 1987; von Haden and Dornbush 2014). Moreover, if longer-term changes in soil moisture patterns alter the size and composition of the microbial community or the processing of soil organic matter (SOM), climate legacy effects may shape rates of mass loss and nutrient release during decomposition. In order to document the occurrence, strength, and direction of climate legacies in tallgrass prairie, it is necessary to assess C fluxes, N transformations, and decomposition dynamics as interrelated components of biogeochemical cycling.

When considering the effects of climate change on tallgrass prairie, it also is important to note that less than five percent of this ecosystem remains as intact, native tallgrass prairie due to conversion to agricultural and urban land uses (Samson and Knopf 1994). The survival of tallgrass prairie species, communities, and ecosystems in the future will be highly dependent on successful restoration efforts. However, restored prairies may function differently than native prairies, and it remains unclear how drought sensitivity changes as restored prairies mature, and to what extent future precipitation variability may jeopardize restoration efforts. Some ecosystem traits in restored prairies, such as cover of native grasses and the size of the microbial community, can recover to levels of native prairie relatively quickly (Baer et al. 2002; Scott et al. 2017), but others such as soil C pools may take centuries to recover (Baer et al. 2002; McLauchlan et al. 2006; DeLuca and Zabinski 2011; Rosenzweig et al. 2016). Due to these differences in structural recovery along prairie chronosequences, it remains unclear how the sensitivity of C fluxes to climate variability changes as restored prairies age.

In this dissertation, I asked: how do current and previous precipitation regimes affect C and N cycling in tallgrass prairie? Do C and N fluxes respond in coupled ways to long- and short-term climate treatments? Does climate sensitivity vary through time as prairies mature? To answer these questions, I used a series of climate experiments in tallgrass prairies to assess patterns of climate sensitivity in native and restored tallgrass prairies.

In Chapter 2, I measured the responses of C fluxes and pools to current and historic precipitation manipulations in grassland long-term experiment at Konza Prairie Biological Station, Kansas, USA. In this irrigation experiment, supplemental water was added to minimize water stress and mimic a wetter climate for 25 years; a reversal of irrigation and ambient precipitation treatments and the addition of new reduced rainfall treatments in 2017 allowed me to explore how both previous and current climate treatments affected some key tallgrass prairie ecosystem processes. I found that a history of irrigation increased ANPP and soil respiration fluxes on a magnitude similar to current irrigation, and that these legacies of a wetter climate persisted up to 3 years. Previous irrigation also buffered the responses of ANPP and soil respiration to the reduced rainfall treatment. These legacies in ecosystem fluxes appear to be driven in part by changes in plant functional composition and changes in the size and activity of the microbial community.

In Chapter 3, I assessed how short- and long-term changes in precipitation regimes shape tallgrass prairie N cycling. Using the same experimental infrastructure as in Chapter 2, I assessed how past and current rainfall regimes affected N transformations in tallgrass prairie. A history of irrigation increased rates of net N mineralization and nitrification, providing evidence that increased N availability under a historically wetter climate may support the legacy effects in plant and microbial functioning outlined in Chapter 2. Moreover, changes in plant and microbial C/N ratios, as well as microbial investment in N-acquiring enzymes, suggests that while short-term irrigation may increase N-limitation, long-term wetter conditions accelerate N cycling and can lead to biogeochemical climate legacies.

In Chapter 4, I followed up my research on C and N fluxes in the irrigation study with a root decomposition experiment, in which I investigated the effects of previous and current precipitation treatments on root litter mass loss and nutrient release over two years. Root decomposition rates and N release were surprisingly resistant to both current and historic precipitation regimes, suggesting that observed increases in soil respiration and N cycling rates with long-term release from water stress are driven primarily by SOM pools rather than root litter decomposition.

In Chapter 5, I expanded my work assessing climate sensitivity of tallgrass prairie to include restored prairies of differing ages. I set up a drought experiment across a chronosequence of prairies ranging from 4 to 22 years old to assess how prairie plant communities and ecosystem functioning change as prairies mature, as well as how the sensitivity of prairie structure and function to drought varies with restoration age. I found that, despite changes in plant communities across the chronosequence, restored prairies had rates of ANPP and soil respiration similar to native prairie, and these fluxes were similarly resistant to a 2-year drought in both native and restored prairies. This may be due to the rapid establishment of drought-tolerant dominant grasses across the chronosequence, and it offers hope that restored prairies can establish and thrive under a more variable climate.

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Chapter 2 – Climate legacies determine grassland responses to future rainfall regimes

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2.1 Introduction

Chronic and episodic water shortages limit primary production and other ecosystem processes across much of the globe (Wu et al. 2011, Hoover and Rogers 2016). As a result, climate changes that alter the frequency and duration of water stress will likely affect carbon (C) and nitrogen (N) cycling in ways that are hard to predict on longer timescales. Changes in precipitation patterns over years or decades can alter C cycling by shifting plant community composition (Smith et al. 2009; Jones et al. 2016), affecting nutrient limitation (Luo et al. 2004; Ren et al. 2017), or changing litter inputs and quality (Murphy et al. 2002). Moreover, processes that control C inputs and outputs may be differentially sensitive to climate perturbations (e.g., Hoover et al. 2016) and, in ecosystems limited by both water and N, climate effects on N availability may further modulate ecosystem sensitivity to water shortages (Evans and Burke 2013). In addition, the responses of different ecosystem processes to climate shifts may be decoupled in time, resulting in altered ecosystem functioning even after periods of water stress cease (Asner et al. 1997; Evans and Burke 2013). Therefore, historic climate patterns—by altering ecosystem attributes or community structure—may influence the sensitivity of ecosystems to future climate changes.

Evidence is accumulating that climate history is important for understanding and predicting ecosystem responses to climate change (Song et al. 2019). Drought years, for example, can negatively impact aboveground net primary productivity (ANPP) in subsequent years (i.e., negative legacies) by altering attributes such as plant density (Yahdjian and Sala 2006) and bud bank dynamics (Carter et al. 2012; Ott et al. 2019; Reichmann and Sala 2014). Dry years can also have positive legacies on ecosystem function if, for example, they decrease N immobilization relative to mineralization, increasing available N in the following year when water is less limiting (Sala et al. 2012; Seastedt and Knapp 1993). Across much longer temporal scales (e.g., millennia), climate legacies exert large controls over global soil C stocks (Delgado-Baquerizo et al. 2017). Current efforts to model the impacts of climate change generally do not account for the effects of climate history on ecosystem C cycling (Wieder et al. 2013; Hawkes et al. 2017), but including lagged effects of past precipitation regimes may improve our ability to predict how ecosystem structure and function will respond to future changes in precipitation (Sala et al. 2012; Paruelo et al. 1999). A challenge to these efforts is the marked variation in the presence, strength, and direction of documented legacy effects, and the need for additional studies of how spatial and temporal variability modulate climate sensitivity. For example, Strickland and others (2015) found that climate history explained as much variation in microbial CO_2 mineralization rates as did current abiotic conditions across a broad climatic gradient, while other studies report little effect of climate history on microbial decomposition (Baker et al. 2018). Short-term experimental climate manipulations and studies across natural precipitation gradients can identify and characterize climate legacies, but longer-term manipulative climate experiments are required to more robustly assess how climate change and climate legacies over decades shape ecosystem functioning and sensitivity to novel climate perturbations (e.g., drought).

Grasslands cover 30-40% of the earth's terrestrial surface and are often water limited (Pendall et al. 2018). The sensitivity of these ecosystems to inter- and intra-annual climate variability will play a major role in future global C cycling (Beer et al. 2009). Climate variability in grasslands affects the composition of plant (Cleland et al. 2013; Jones et al. 2016) and microbial communities (Zeglin et al. 2013; Bell et al. 2008), primary productivity (Wilcox et al. 2017), and soil CO_2 efflux (Harper et al. 2005; Miao et al. 2017). The largest and most important C fluxes in these ecosystems are soil respiration and net primary production (NPP), which are often similar in magnitude (Longdoz et al. 2000; Raich and Schlesinger 1992). Assessing the relative sensitivity of these two processes to altered climate conditions is key to projecting the effects of climate change on ecosystem C balance (Lui et al. 2009). Moreover, most biomass in grasslands is below ground (Rice et al. 1998), so the sensitivity of root biomass, depth distribution, and chemistry to climate and potential effects on the quantity and quality of root inputs are important controls on C storage and flux in these ecosystems (Carrillo et al. 2014, Parton et al. 2007, Bardgett et al. 2014; Silver and Miya 2001).

The tallgrass prairies at the eastern extent of the North American Central Plains provide ecosystem services such as forage production, carbon sequestration, and maintenance of biodiversity (Zhou et al. 2019; Hoover et al. 2014; Collins et al. 1998), and the processes driving these services are closely tied to inter- and intra-annual variation in precipitation (Briggs and Knapp 1995; Knapp and Smith 2001; Harper et al. 2005). These systems are characteristically resilient to short-term drought (Hoover et al. 2014), but the unprecedented increases in temperatures and rainfall variability projected for this region (IPCC 2018) may cause persistent changes in ecosystem functioning that alter how ecosystem processes respond to subsequent climate variation. Positive legacies of past precipitation on current functioning can occur after both wet and dry periods—e.g., higher ANPP following community shifts to high-producing species during a wet period, or buildup of limiting nutrients during a dry period supporting higher ANPP. Negative legacies can also occur, such as when dry periods result in mortality or plant community shifts toward more drought-tolerant, slow-growing species (Huxman et al. 2004), or when wet periods increase leaching or gaseous losses of limiting nutrients (Shen et al. 2016; Evans and Burke 2013). In tallgrass prairie, natural droughts can cause higher-than expected ANPP in the subsequent year (Griffin-Nolan et al. 2018). However, longer experimental droughts in tallgrass prairies can result in community-mediated negative legacies on ANPP (Sherry et al. 2008) and decreased drought sensitivity of heterotrophic soil respiration (Veach and Zeglin 2020). In contrast, long-term experimental increases in precipitation in a temperate Mongolian grassland ultimately led to N losses and caused decreased sensitivity of carbon cycling to wet conditions (Ren et al. 2017), which has important implications for ecosystems co-limited by N and water such as tallgrass prairie (Knapp et al. 1998a). These results suggest that prolonged climate anomalies may have distinct legacy effects that manifest slowly over time through mechanisms such as community shifts or indirect effects on co-limiting resources. Moreover, since the effects of both long-term climate trends (Knapp et al. 2001) and climate variability (Briggs and Knapp 1995) on carbon cycling vary dramatically across topography in these grasslands, it is imperative to assess how the strength and direction of climate legacies differ across the landscape.

In this study, we used a long-term irrigation experiment that simulated a wetter climate starting in 1991, and in 2017 we implemented new treatments in plots with contrasting precipitation legacies to assess how historic and current precipitation regimes shape carbon cycling in a tallgrass prairie landscape. Irrigation in this experiment increased annual precipitation (1991-2016) by an average of ~32% across a topoedaphic gradient that spans upland and lowland annually burned prairie (Caplan et al. 2019). While initial functional responses (e.g., ANPP) to added water were modest, after ~10 years a species reordering within the C_4 grass functional group was associated with a 64% increase in ANPP (Knapp et al. 2012; Collins et al. 2012). Belowground C and N cycling appeared to be more resistant to changes in water availability in an earlier study done in uplands only (Wilcox et al. 2016), but the most prominent plant compositional and functional changes manifested in the lowlands, suggesting that treatment effects on carbon cycling might vary across the landscape. In 2017, 26 years after the initiation of the irrigation treatments, we (1) switched the irrigated and control treatments in a subset of experimental plots in both upland and lowland topographic positions and (2) imposed an experimental drought (66% reduction in ambient rainfall) in both historically irrigated and control treatments. This new treatment design enabled us to assess how a multi-decade legacy of contrasting climate conditions (i.e., long-term irrigation vs. ambient precipitation) affected ecosystem responses to a range of new precipitation conditions (i.e., reduced, ambient and increased water availability). This combination of ongoing and new precipitation manipulations offered a rare opportunity to compare ecosystem functional responses to altered climates in one ecosystem at a single site with distinct climate histories. We assessed how precipitation legacies and current precipitation treatments independently and interactively affected tallgrass prairie C cycling, and how these responses varied across years and landscape position. Our hypotheses included: (1) positive legacies of irrigation on ANPP, especially in lowlands where previous studies had documented concurrent changes in plant species composition and ANPP, (2) negative legacies of irrigation on soil respiration, if decades of supplemental irrigation increased sensitivity to water stress and drought (3) persistence of increased extractable, microbial, and total soil C pools in the long-term irrigation treatment even after treatment reversal as a result of enhanced C inputs with long-term irrigation, (4) persistent effects of long-term irrigation on root biomass and nutrient concentration (i.e., higher biomass with lower N content in irrigated vs. control plots), and (5) more pronounced legacy effects in dry years than in wet years, and in lowlands than in uplands, due to greater effects of water limitation in dry vs. wet years and in drier upland vs. wetter lowland sites.

2.2 Methods

Study site—This study leveraged the ongoing Irrigation Transect Experiment (Knapp et al. 2001) in an annually burned, ungrazed grassland site at the Konza Prairie Biological Station (KPBS, 39°05'N, 96°35'W). Mean annual temperature for KPBS is 12.8 °C and mean annual precipitation (1987-2016) is 851 mm (range = 483–1674 mm), with ~75% of that precipitation falling during the growing season (April-September). Intra-annual and inter-annual variation in precipitation is high (Hayden 1998), with frequent water deficits during the growing season (Wilcox et al. 2016). Soils at KPBS are generally silty clay Mollisols, but vary in depth, texture, and average soil water content with landscape position. At this specific site, upland soils belong to the Clime-Sogn complex (fine, mixed mesic Udic Haplustolls), with soil texture (0–10 cm) of 15% sand, 58% silt and 27% clay; while lowland soils are Irwin silty clay loams (fine, mixed mesic Pachic Argiustolls), with a soil texture (0–10 cm) of 15% sand, 51% silt and 34% clay. Uplands have shallower, rockier soils that experience more frequent and severe water deficits than deeper-soil lowlands, though the difference in topographic positions here is less pronounced than at other KPBS locations (Knapp et al. 2001).

Irrigation Transect Experiment—The Irrigation Transect Experiment, which consists of an impact sprinkler-based irrigation system that was established along a single upland-to-lowland transect in 1991, and expanded to include a replicate transect in 1993. Adjacent control transects subject to ambient precipitation were established parallel to each irrigated transect (Appendix 1, figure 1). The impact sprinklers are on vertical risers ~ 1.0 m high to deliver water in a ~15-m radius circle, and spaced to produce a relatively even application along each irrigated transect. Irrigated transects were supplied with groundwater from the site as needed to mitigate water stress during the growing season by maintaining soil water content at or above 0.25 cm^3/cm^3 at 0-30 cm depth. The amount of water added annually varied depending on ambient precipitation inputs and soil conditions. All irrigation water was applied between 05:00 and 13:00 to minimize loss from evapotranspiration and to avoid heavy winds. On average, irrigation increased annual water inputs by ~32% compared to ambient precipitation in control plots (Caplan et al. 2019). During the current study period, irrigated plots received 27% more precipitation than control plots in 2017 and 2018 (when annual precipitation inputs were 724 and 811 mm, respectively, and long water deficits developed throughout the growing season [Appendix 1, figure 2]), but only 13% more precipitation in 2019, as this was a naturally high precipitation year (1131 mm total; Appendix 1, table 1). At 10-m intervals along each irrigated and control transect 10-m^2 plots were established to allow for replicated sampling of ANPP and other response variables (Appendix 1, figure 1); all soil sampling and ANPP measurements for this study were conducted in new plots immediately adjacent to selected long-term upland and lowland long-term sampling plots to minimize disturbance effects. Plots on rocky slopes in the transitional area between uplands and lowlands were excluded from this study.

In 2017, we imposed new treatments to assess the legacies of contrasting past precipitation regimes and their influence on responses to newly imposed precipitation treatments. Half of each irrigation line was shifted to switch the long-term irrigation and control treatments in a subset of plots in both the upland and lowland topographic positions (Appendix 1, figure 1). This allowed us to assess precipitation treatment legacies and their effects on ecosystem responses to new precipitation regimes (i.e., supplemental water in former control plots and a reduction in water availability in former irrigated plots). Additionally, we erected 3-m x 3-m passive rainout shelters over areas that were previously irrigated or previously received ambient precipitation. The shelter roofs were constructed with clear polycarbonate slats spaced to exclude and redirect 66% of incoming rainfall. Gutters were used to divert water away from plots and the shelters were deployed from April-November each year. Shelters roofs were approximately 1 m off the ground and were raised periodically during the growing season to accommodate tall vegetation. The combination of long-term, ongoing and reversed irrigation and control treatments coupled with new reduced rainfall treatments resulted in six replicate plots for each combination of previous climate treatment (2 levels: irrigated (I) or ambient (A) rainfall) and current climate treatment (3 levels: irrigated (I), ambient (A), and reduced (R) rainfall) in each topographic position (upland and lowland) for a total of 72 plots. Crucially, this experimental design allowed us to compare plots experiencing the same current precipitation conditions, but having contrasting precipitation histories (e.g., (I→R vs. A→R). To document effects of irrigation and drought on soil moisture relative to ambient conditions, we measured volumetric water content from 0-15 cm depth using time domain reflectometry (TDR) probes (CS616, Campbell Scientific) in a subset of plots of each treatment (3 plots/treatment combination in each topographic location).

Aboveground primary production—Full methods for ANPP measurement can be found in Konza LTER metadata (Blair 2021). Briefly, five 0.1-m^2 subsamples were clipped at ground-level in each plot at peak biomass near the end of the growing season. Biomass was sorted into grass, forb, and woody vegetation, dried at 60 °C, and weighed. This approach provides a good estimate of ANPP (g m^{-2}) in these grasslands (Knapp et al. 1998a). Total ANPP was estimated as the sum of grass, forb and woody biomass. Subsamples (n=5) were averaged for each plot to estimate plot-level ANPP. Response variables of interest included total ANPP, grass ANPP, forb ANPP, and proportion forb ANPP of total as an index of relative forb abundance.

Plant community composition—Surveys of the plant community took place in June and August of 2017-2019. Within each plot, a 1-m^2 frame was placed in a fixed location and percent cover was estimated for each species present. The maximum cover value for each species in each plot in a given year was retained from the two growing-season sampling events and used in all subsequent analyses.

Soil CO_2 efflux—Soil respiration was measured approximately biweekly during the growing season in 2018 and 2019, between late April to mid-October. Data from outside the growing season were collected less frequently but were excluded from analyses due to very low values and minor contributions to total annual soil CO_2 flux (Knapp et al. 1998b). Polyvinyl chloride (PVC) rings (10-cm diameter) were inserted 5 cm into the ground after spring burning, leaving ~2-cm aboveground (precise heights were recorded before each measuring event, and interior collar volume was adjusted accordingly for calculations). Any vegetation growing within the rings was clipped at ground level at least 1 hour before measurements. Soil respiration was measured between 10:00 and 14:00 on sunny days using a LI-8100 infrared gas analyzer (LI-COR, Lincoln, NE, USA), with care to avoid measurements within 24 hours of a heavy precipitation event (Harper et al. 2005).

Total soil C and N—To assess potential effects of long-term precipitation treatments on soil C and N stocks, in July 2018 we collected one soil core (5 cm diameter x 10 cm depth) per plot in the 48 non-drought treatment plots. We did not take cores from under the shelters to limit destructive sampling, and because we did not anticipate short-term (1-2 year) effects of drought on the very large pool of soil C in these grasslands (Rice et al. 1998). Soil was passed through a 4-mm sieve to remove roots, rocks and debris, dried, and then ground on an 8000D mixer/mill (SPEX, Metuchen, NJ) and analyzed for total C and N on a Flash EA 1112 C/N auto analyzer (Thermo Fisher Scientific, Waltham, MA).

Microbial biomass C (MBC) and extractable organic C (EOC)—We collected soil samples from all irrigated and control plots (N=48) in 2018 and from irrigated, control, and sheltered plots (N=72) in 2019. Samples were collected with a 5-cm hammer core to 10-cm depth at the beginning of June, July and August of each sampling year. Soils were passed through a 4-mm sieve to remove rocks, roots, and debris, and then 11.5 g subsamples were extracted with 50 mL 0.5 M K_2SO_4. Extractable organic C was determined on a TOC analyzer (Shimadzu, TOC-L, USA) (Paul et al. 1999). Because these soils are carbonate-poor (Macpherson et al. 2008), we are confident in using this salt-extractable C as an index of extractable organic C across treatments without removing carbonates. To assess microbial biomass C, we subjected 11.5-g soil subsamples to chloroform fumigation (Vance et al. 1987), these were subsequently extracted with K_2SO_4 and analyzed for C content as with unfumigated samples. MBC was calculated as the difference in extractable C between fumigated and unfumigated samples; no correction factors were used (Joergenson et al. 2011).

Root standing biomass and chemistry—Root biomass was sampled using 5-cm diameter cores taken in mid-June 2019 to a 20-cm depth and divided into 0-10 cm and 10-20 cm depths. Due to shallow soils in uplands, samples were restricted to 0-10 cm depth there. Roots were retrieved by passing soil through a series of sieves (4-mm, 2-mm and 1-mm mesh) under running water. Roots were further cleaned of soil debris using water flotation and were dried for 48 hours at 60 °C and weighed. All roots were ground and analyzed for total C and N similarly to the soil samples.

Data analysis— Our analyses focused primarily on assessing the legacy effects of contrasting precipitation regimes by comparing ecosystem processes and properties in treatments experiencing the same current precipitation conditions but having contrasting precipitation histories (e.g., (I→R vs. A→R). However, we were also interested in evaluating the effects of ongoing long-term irrigation (I→I) and ambient (A→A) treatments on response variables. We evaluated the effects of past irrigation (I) and ambient (A) treatments on current responses (i.e., one type of legacy effect), the effects of current precipitation treatments (I, A, and R) on current responses, and interactions between past and current precipitation treatments (i.e., legacy effects that result in differential sensitivity to current conditions). To assess main effects and interactions of precipitation treatments on ecosystem processes and properties, we used linear mixed models

(LMMs) or general linear mixed models (GLMMs) with a log-link function, based on which best met assumptions of homogeneity of variances. For proportion forb ANPP, a beta binomial GLMM with a logit-link function was used (Warton and Hui 2011). All analyses were conducted in R 3.4.2 (R Core Team 2019) using the packages lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), emmeans (Lenth et al. 2021), gamm4 (Wood and Scheipl 2020), MuMIn (Bartoń 2019), glmmTMB (Brooks et al. 2017), and tidyverse (Wickham et al. 2019). For post-hoc model evaluation when there were significant interacting factors, we evaluated the effect of the current treatments, past treatments, and their interactions within each landscape position, and within each year if applicable, in emmeans. Significance was set at $\alpha \leq 0.05$.

For all models, current precipitation treatment, past precipitation treatment, topographic position (upland and lowland), and year and their interactions were treated as fixed effects. For one plot (1 of 216 measurements), forb ANPP was 0 g/m^2; this was replaced with 0.001 to accommodate a GLMM, which better met model assumptions of homogeneity of variances. For volumetric water content, we present the data for 2017 but did not include it in statistical models since data collection did not begin until August of that year. For root biomass, separate models were run for 0-10 cm depth in the uplands and lowlands and for 10-20 cm depth in the lowlands, since deeper sampling was only possible in lowland plots. To test the effect of landscape position, a second model was run with only the 0-10 cm depth. Similarly, for EOC and MBC, separate models were run for 2018 and 2019 samples because the sampling scheme differed between years. A second model was run including only plots sampled in both years (i.e., non-shelter plots). One outlier was removed from the root biomass samples at 10-20 cm depth, as it was more than double all other recorded responses.

We assessed whether treatments significantly affected plant community composition using multivariate analyses. We first assessed the effect of current treatment, previous treatment, landscape position, and year with an overall permutational analysis of variance (PERMANOVA). Because we were interested in knowing which treatments differed from each other, we took several steps to simplify models to allow for pairwise treatment comparisons. First, we combined previous treatment and current treatment to create one variable with six levels. We then separated data by landscape position and year and then ran a post-hoc analysis using the pairwise.adonis function (Martinez Arbizu, 2019) for each year and position subset. Because we analyzed the effects of climate variables separated by year and topography, we presented these community responses as separate non-metric multidimensional scaling (NMDS) plots.

For response variables measured multiple times within the same plot, we accounted for the repeated measures by incorporating plot nested within transect replicate as a random intercept. If plots were sampled repeatedly within a growing season (e.g., VWC, soil respiration), we also included day of year as a random intercept, scaled to have a mean of 0 and standard deviation of 1. Because current treatment was applied within a transect, for all response variables we tested models that allowed each transect a random slope for current treatment (*sensu* Barr et al. 2013). Because the inclusion of this term resulted in an overfitted model (cor = 1 or -1), we removed this complex term for all models. For a small number of analyses, the model was overfit by including even the simplest random effect; we interpret this result to mean that larger-scale spatial variability represented by sampling transect was not important in driving patterns in our data. Model outputs are included in Appendix 2.

2.3 Results

<u>*Soil water content*</u>—Annual and growing season rainfall inputs and supplemental irrigation varied considerably among years (Supplemental table 1). Although ambient precipitation inputs in 2017 and 2018 were within one standard deviation of the 30-year average preceding the study years (851 ± 235 mm), the distribution of this precipitation resulted in water deficits for much of the growing season in 2018 (Appendix 1, figure 2). Therefore, current irrigation in 2018 significantly increased mean soil water content by 19%, and the reduced rainfall treatment decreased soil water content by 17%. Data from 2017 were not included in the models since TDR probes were installed in the middle of the growing season, but trends were similar (Figure 1). In 2019, when ambient precipitation (1131 mm) was nearly 200 mm higher than average, the drought treatment decreased soil water content by 10% and irrigation treatment had no effect, likely because high ambient precipitation resulted in very wet soils in both ambient and irrigated treatments. Although the model revealed some significant three- and four-way interactions involving previous irrigation treatments, post-hoc tests did not suggest notable legacy effects on soil water content; therefore, VWC data for current treatments is presented averaged across past treatments (Figure 1). As expected, lowland soils generally had a higher water content than upland soils.

<u>*ANPP*</u>— Across all years, treatments, and topographic positions, total ANPP increased with current irrigation and decreased with the reduced rainfall treatment, regardless of treatment history. ANPP also was higher in lowlands than uplands, and was higher in years with more ambient precipitation (2019 > 2017 > 2018; all main effects $p < 0.001$). However, ANPP was also significantly increased by past irrigation (i.e., a legacy effect) regardless of current treatment

($p<0.001$; Figure 2). Two significant interactions help clarify how the effect of current and past climate treatments varied across topography and among years. First, the effect of the current precipitation treatments varied with year and natural precipitation inputs. In 2017, ANPP was 20% higher in the currently irrigated treatment compared to the reduced rainfall treatment ($p < 0.001$) regardless of treatment history, with each treatment only marginally significantly different than the intermediate ambient treatment (A vs I: $p = 0.067$; A vs R: $p = 0.053$). The differences in ANPP among current treatments became much more pronounced in the natural drought year of 2018, when ANPP in the current irrigation treatment averaged 35% higher, and ANPP in the reduced rainfall treatment averaged 37% lower, than in ambient conditions ($p<0.001$ for all comparisons). In contrast, there were no effects of current irrigation or rainfall reduction treatments in 2019, a very wet year. Second, the strength of treatment legacies driving ANPP varied with topographic position. Across all current treatments and years, the legacy of past irrigation increased ANPP by an average of 33% in the lowlands ($p < 0.001$), but had a smaller and only marginally significant effect (12% increase; $p = 0.052$) in the uplands. Finally, a three-way interaction between current treatment, past treatment, and year revealed differential sensitivity to current climate regimes among plots with different treatment histories. A legacy of irrigation conferred some resistance to the new drought treatment in 2017 and 2018, maintaining higher ANPP under rainfall reduction shelters in previously irrigated plots (I→R vs. A→R); ANPP under shelters in plots with a legacy of irrigation was 26% greater in 2017 ($p = 0.037$) and 35% greater in 2018 ($p = 0.002$) across both topographic positions compared to sheltered plots without a legacy of irrigation. A legacy of irrigation also increased ANPP in the current ambient treatment (I→A vs. A→A) by nearly 30% in 2018 ($p = 0.014$) and by nearly 40% in 2019 (p < p.001) across both topographic positions.

Grasses dominate productivity in this system, and patterns of grass ANPP were similar to total ANPP, with higher productivity in lowlands and in higher-precipitation years (Appendix 1, figure 3). Current irrigation increased grass ANPP in 2018 ($p = 0.035$), and current reduced rainfall decreased ANPP in both 2017 and 2018 (both $p < 0.001$); however, both treatment effects disappeared under higher ambient rainfall in 2019. Grass ANPP exhibited positive legacy effects of previous irrigation in all years. Across all current treatments, past irrigation increased grass ANPP by 15% in both 2017 ($p = 0.004$) and 2018 ($p = 0.023$), and by 25% in 2019 ($p < 0.001$). A three-way interaction between current treatment, past treatment, and year revealed that a legacy of irrigation increased grass ANPP in the current ambient treatment (I→A vs. A→A) in 2019 ($p = 0.003$) and marginally in 2018 ($p = 0.100$); however, unlike total ANPP there was no evidence of a significant legacy of irrigation on grass ANPP in the reduced rainfall treatment (I→R vs. R→R), perhaps due to greater variation in the proportion of grass biomass in individual plots or due to a large legacy effect on forb ANPP. Although forbs made up less than 10% of biomass on average, and never more than 25% of biomass in any treatment, forb ANPP was highly responsive to current and past precipitation treatments (Appendix 1, figure 4). Current irrigation increased forb ANPP in the lowlands by over 100% in 2017 and almost 90% in 2018 compared to currently ambient plots ($p = 0.016$ and $p = 0.015$, respectively), and the reduced rainfall treatment decreased forb ANPP by >80% in the uplands in 2018 ($p < 0.001$). However, a legacy of past irrigation more than doubled forb ANPP in the lowlands averaged across all years, and in the drought year of 2018, forb ANPP in previously irrigated lowland plots was three times higher than in comparable ambient plots (I→A vs. A→A), despite equivalent precipitation inputs.

To assess whether these changes in ANPP by different functional groups shifted the relative contributions of forbs to total ANPP, we compared the proportion of forb ANPP across treatments, years and landscape position; our analysis focused on the interactions. The current irrigation treatment had double the percent forb ANPP (10% vs. 5%) of the reduced rainfall treatment in the uplands ($p = 0.024$), while forb ANPP in the irrigated treatment in the lowlands was only marginally higher than in the ambient lowlands ($p=0.057$); no other treatment combinations differed significantly. An interaction between current treatment and year showed that these differences were limited to 2018. A legacy of past irrigation nearly doubled percent forb ANPP (14% vs 8%) in the lowlands; an interaction with year suggested this effect was marginal in 2017 ($p = 0.057$), highly significant in during the drought year of 2018 ($p < 0.001$), but disappeared in the wetter year of 2019 (Figure 3).

Community composition—An overall PERMANOVA indicated that the main effects of both current and previous treatment, as well as landscape position, affected plant community composition. There was also a significant interaction between current and previous treatment, and there was a significant interaction between previous treatment and topographic position. Within-year pairwise PERMANOVAs for the uplands revealed no differences in community composition among treatment combinations in 2017 and 2019, but that all experimentally-imposed drought treatments differed from other treatments in the naturally dry year of 2018. In the lowlands, there were similar patterns—no treatment differences in 2017, but communities in the reduced rainfall treatments differed from other treatments in 2018. In 2019, the only communities that significantly differed from each other were the long-term ambient (A→A) and the previously irrigated (I→A) treatment, providing evidence of some irrigation legacy effect (Figure 4).

Soil CO_2 efflux—Soil respiration was significantly affected by all main effects (current and past treatment, topography, and year; all $p < 0.001$), but interpretation of the results was complex due to multiple interacting factors. For brevity, our interpretation is based on the four-way interaction between current treatment, past treatment, topographic position, and year. In general, soil respiration averaged much lower across all treatments and topographic positions in 2018 than in 2019 (4.75 vs 10.04 μmol/m^2/s) as a result of much lower ambient precipitation through most of the growing season. In the shallower soil uplands, the reduced (A→R) rainfall treatment further decreased soil respiration in 2018 to an average of 2.23 μmol/m^2/s compared to the ambient treatment (4.13 μmol/m^2/s) and to both short-term current (A→I; 4.93 μmol/m^2/s) and long-term ongoing (I→I; 5.89 μmol/m^2/s) irrigation ($p<0.001$; Figure 5). In addition, while long-term ongoing irrigation (I→I) maintained higher soil respiration compared to long-term ambient conditions (A→A; $p<0.001$); respiration in the short-term current irrigation treatment (A→I) was intermediate and not significantly different from either treatment. There was no evidence that a history of irrigation affected the sensitivity of CO_2 flux to the reduced rainfall treatment in the uplands. However, in the lowlands in 2018, both short- and long-term irrigation increased respiration compared to the long-term ambient treatment (means of 6.45, 7.76, and 4.58 μmol/m^2/s, respectively; $p<0.001$), but with a much larger effect of long-term (60% increase for I→I) compared to the newly irrigated treatment (40% increase for A→I). Importantly, a legacy of past irrigation (I→A; 6.01 μmol/m^2/s) also maintained 31% higher soil respiration compared to the ambient treatment ($p=0.011$) despite identical current rainfall amounts. Although soil respiration in the previously irrigated treatment remained higher than in the ambient treatment, it was not as high as in the long-term currently irrigated treatment (I→I: p=0.019). As expected, the experimental drought treatment in plots without a history of irrigation (A→R) decreased soil respiration rates in lowlands compared to the ambient treatment (3.16 vs. 4.58 $\mu mol/m^2/s$; p=0.002); however, soil respiration was not significantly reduced by the experimental drought treatment in plots with a legacy of irrigation (4.19 $\mu mol/m^2/s$; I→R). In the lowlands in 2018, soil respiration in the experimental drought treatment was 33% higher in plots with a legacy of irrigation compared to plots without a legacy of irrigation (I→R vs. A→R; p = 0.032). In contrast to the array of treatment effects and interactions in 2018, there were no significant effects of either current or previous treatments on soil respiration in 2019, a much wetter year.

<u>Total soil C and N</u>—Soil C content was higher in the uplands (4.27 ± 0.08 %) than the lowlands (3.79 ± 0.11 %; p<0.001) across all treatments, and the effects of precipitation treatments on total soil C were relatively small (Figure 6a). An interaction between irrigation history and topographic position suggested that a legacy of long-term irrigation was higher soil C content in the lowlands (p=0.002). However, a three-way interaction indicated that this effect was driven mainly by lower soil C under the new current irrigation treatment (A→I) compared to ambient (A→A), long-term irrigated (I→I), and formerly irrigation (I→A) treatments, rather than a legacy of enhanced soil C from previous irrigation.

<u>Extractable organic C (EOC)</u>—Extractable organic C (EOC) was more responsive to both current and past treatments was than total soil C. In 2018, short-term current irrigation (A→I) nearly doubled EOC in the uplands compared to the ambient treatment, though this effect was

not significant (Figure 6b). In the lowlands in 2018, EOC in the long-term irrigated treatment was nearly 80% higher than in the ambient treatment ($p = 0.028$) and nearly triple that in short-term current irrigated plots (which had the lowest values, $p < 0.001$); while previously irrigated plots switched to ambient conditions had nearly recovered to the long-term ambient treatment levels. In 2019, short-term irrigation in the upland again nearly doubled EOC compared to ambient plots ($p = 0.004$). Long-term irrigated plots also had higher EOC than ambient plots ($p = 0.020$), and even after reversal to ambient conditions EOC remained high (46 μg C/ g soil in I→A vs. 48 μg C/ g soil in I→I). In the lowlands in 2019, long-term irrigation increased EOC by about 80% compared to ambient plots and those that were newly irrigated, and imposing drought in historically irrigated plots marginally decreased EOC by 34% ($p = 0.068$). However, despite the effects of current treatments on EOC, there was no evidence that a legacy of irrigation affected EOC pools in currently ambient or droughted plots in the lowlands in 2019.

Microbial biomass C (MBC)—Microbial biomass tended to be higher in 2018 than 2019, though statistical comparisons of year effects were not possible due to different sampling schemes in each year. In 2018, a three-way interaction between current treatment, past treatment, and landscape position showed that long-term irrigation (I→I) maintained significantly higher MBC than did the reversal to ambient treatment (I→A; $p = 0.016$) in the lowlands, suggesting that a legacy of past irrigation was increased sensitivity of MBC to the natural drought conditions of 2018. However, ambient (A→A) and short-term current irrigation (A→I) treatments had intermediate MBC levels so that no other treatment combinations were significantly different from one another. In 2019, a wet year, MBC in the I→A reversal treatment recovered to levels comparable to the long-term irrigated plots (I→I), but this long-term irrigation treatment still maintained significantly higher MBC than the irrigation to drought treatment (I→R; $p = 0.002$). Additionally, the long-term irrigation treatment supported higher MBC than the short-term current irrigation treatment (A→I; $p < 0.001$; Figure 7).

Root biomass and chemistry—Overall, root biomass did not vary with treatment, likely in part due to high spatial variability among treatments and among plots within treatments (Appendix 1, figure 5). There was some indication of an effect of current and past treatments in the uplands; however, post-hoc examinations revealed no significant differences among treatments. Root biomass did not differ between the uplands and lowlands in the top 10 cm, and most root biomass was in the top 10 cm in the lowlands.

Root C content was unaffected by treatment across all sampling depths (data not shown). Lowland roots, and especially deep lowland roots, tended to have lower N, and higher C/N ratios, than upland roots. In the uplands (where sampling was only conducted from 0-10 cm depth), short-term current irrigation increased root N content compared to drought, and it decreased root C/N compared to reduced rainfall and ambient treatments. Interestingly, unlike short-term irrigated plots, long-term irrigated plots did not differ in root chemistry from controls in the upland. In the lowland 0-10 cm roots, the overall model indicated a significant effect of current treatment (N: $p=0.044$; C/N: $p=0.020$), but post-hoc analysis indicated only marginally higher root N ($p=0.095$), and lower C/N ($p=0.060$) in the current irrigation vs. rainfall reduction treatments. The chemistry of deeper roots in the lowland (10-20 cm) did not respond to previous or current climate treatments.

2.4 Discussion

The influence of past climate conditions on current ecosystem processes, and the sensitivity of these processes to future climate variability, is a gap in our understanding of how climate and climate changes drive ecosystem functioning. Here, we document the legacy effects of long-term increased precipitation and reduced water stress on carbon cycling processes and their responses to changing precipitation inputs, including drought. For two key C fluxes, ANPP and soil respiration, there were significant legacy effects of 25 years of irrigation of comparable magnitude to contrasting current climate treatments, suggesting that the legacy effects of different precipitation regimes are important for understanding how climate and climate changes shape ecosystem processes. In some cases, such as with ANPP, legacy effects not only persisted for more than one year but were stronger in the second and third years, suggesting that the effects of climate history on carbon cycling are not necessarily short-lived and also may be contingent on current conditions. In addition, patterns of response to both current and past climate treatments often differed in direction or magnitude between upland and lowland sites, suggesting that fine-scale topoedaphic factors can contribute to a mosaic of climate sensitivity across the landscape.

Positive legacies of climate on ANPP—Though the relationship between current-year precipitation and ANPP is well established in this grassland and other ecosystems (Briggs and Knapp 1995; Huxman et al. 2004; Fay et al. 2011) and was supported by our results, we found that precipitation treatment history was also an important driver of total ANPP. For example, a positive legacy of past irrigation maintained 35% greater ANPP in the experimental drought (rainfall reduction) treatment in 2018 and increased ANPP by nearly 40% in the currently ambient treatment in 2019, despite identical current rainfall amounts. In another example of a positive legacy effect, ANPP in the long-term irrigated treatment was nearly 30% higher than in the current short-term irrigated treatment in 2019. These legacy effects are striking and appeared up to 3 years after the treatment reversals. Though upland prairie soils are shallower and generally more water limited (Knapp et al. 1993; Wilcox et al. 2016), we found positive legacies of past irrigation primarily in the lowlands, perhaps because long-term irrigation in the deeper soil lowlands shifted the plant community to a greater extent (Knapp et al. 2012; Collins et al. 2012; see below), creating a longer lasting effect on ecosystem functioning.

Previous studies in tallgrass prairie described positive ANPP legacies (higher-than-expected) following periodic droughts, perhaps due to increased N availability with the return of adequate rainfall following drought (Griffin-Nolan et al. 2018; Sala et al. 2012). Here, as we hypothesized, we observed a positive legacy of increased precipitation (*i.e.*, increased precipitation in the *past* leads to higher-than expected *current* ANPP). Importantly, this precipitation legacy on ANPP lasted for multiple years and persisted through both naturally dry (2018) and wet (2019) years, longer than many previously-documented legacy effects (Delgado-Balbuena et al. 2019; Sala et al. 2012). This highlights the importance of following the recovery of ecosystem processes for multiple years after changes in long-term manipulations, as only one year of data may substantially underestimate the occurrence and strength of legacy effects.

We suggest that the effects of the past precipitation treatments on the plant community contributed substantially to the positive ANPP legacies observed here. While we did not find strong evidence of current shifts in plant community composition as a result of irrigation history, we observed differential responses to both past and current treatments at the functional level (grass vs. forb). Prior studies in this same experiment found that long-term irrigation led to a shift in lowland plant community composition towards dominance of more productive species (*i.e.*, *Panicum virgatum*) (Knapp et al. 2012; Collins et al. 2012). While more recent data suggests that this grass has declined in historically irrigated areas (Smith *et al*., in prep), our results suggests that differential sensitivity to historical conditions between functional groups may contribute to the ANPP legacies seen here. Unlike earlier plant surveys in this experiment (Collins et al. 2012), we found that forb biomass in the lowlands averaged twice as high in plots with a history of irrigation across all study years, and that it disproportionately contributed to ANPP in previously irrigated prairie.

Other studies have suggested that a wetter climate can increase root biomass and belowground productivity in grasslands (Wu et al. 2011), perhaps maintaining greater water uptake and enhanced ANPP even after conditions become drier; however, we did not find evidence for altered belowground biomass in any treatments (see discussion below). Structural changes in the soil may also lead to legacy effects of previous climate patterns, such as changes in soil carbon and therefore water retention (Monger et al. 2012). Indeed, recent work in our experimental system found that decades of wetter conditions resulted in higher water holding capacity in irrigated soils (Caplan et al. 2019). However, these effects were most pronounced in the uplands, while we found the largest ANPP legacies in the lowlands. Moreover, we did not find evidence that previous irrigation increased current soil water content in our study plots. While it is possible previous irrigation increased water storage at deeper soil depths not captured by our measurements in the lowlands, the dominant grasses in this ecosystem take up water from surface soils (though forbs can access deeper soil water during water-limited conditions; Nippert and Knapp 2007). Additionally, even deeper soil water (i.e., 60 cm) is generally depleted by September and recharged during the dormant season and early spring in these grasslands (Craine and Nippert 2014), suggesting that carryover of deep soil water from past irrigation is unlikely to drive these ANPP legacies. Finally, wet conditions may accelerate N cycling, resulting in higher plant-available N—and consequently ANPP—in subsequent years (Sala et al. 2012). Preliminary results from ongoing research (data not presented here) suggests that previous irrigation increased N supply rates, primarily in the lowlands, so this mechanism may also contribute to the ANPP legacy effects (Broderick et al., *in prep*). Because we observed a muted, but still significant, legacy effect on grass ANPP, it is likely that both plant community changes and biogeochemical legacies contribute to the overall ANPP legacy response we observed.

Past and current precipitation drive soil CO_2 *flux*—Current irrigation and drought treatments were strong drivers of soil respiration rates, consistent with previous findings that soil respiration in these grasslands is highly responsive to soil moisture (Harper et al. 2005; Fay et al. 2011) and recovers rapidly from climate manipulations (Hoover et al. 2016). Yet, we also found that a legacy of irrigation affected soil respiration, but in the direction opposite of our predictions. Ongoing long-term irrigation increased respiration almost twice as much as current short-term irrigation compared to the ambient treatment, despite identical current rainfall and irrigation inputs. A positive legacy of irrigation also increased respiration by 24% across all current treatments in 2018, the driest year of our study. Since soil CO_2 flux is commonly understood to be highly responsive to current conditions and variable in time (Lee et al. 2004; Savage et al. 2009), the persistence of this legacy two years after treatment reversal is striking. However, our results are consistent with emerging work showing a large effect of climate history in driving even highly dynamic fluxes such as soil respiration (Hawkes et al. 2017). It is unclear whether the lack of effects after 2018 are because the legacy effects faded by the third growing season post-reversal, or whether the legacy effects are long-lasting but are more apparent under severe drought and, therefore, not detectable in a wet year such as 2019. However, the interaction between past and current treatments in the lowlands in 2018, in which a history of irrigation increased ANPP in currently ambient and droughted—but not currently irrigated—treatments, does suggest that legacy effects may be especially salient under drought stress.

Although we did not attempt to separate the plant and microbial components contributing to the soil respiration responses, other research offers some insights into the potential mechanisms driving them. We did not detect differences in root biomass among treatments, but the positive ANPP legacy we describe may contribute to higher rates of root and rhizosphere respiration with previous irrigation (Zhang et al. 2019). In a short-term grassland experiment, irrigation increased autotrophic but not heterotrophic respiration, consistent with the link between aboveground C fixation and root/rhizosphere CO_2 efflux (Moinet et al. 2016). Microbial responses can also contribute to soil respiration climate legacies, in part due to changes in the size and composition of microbial communities. This would be consistent with the finding that that long-term drought stress selects for microbial communities with lower respiration rates in tallgrass prairie (Veach and Zeglin 2020; Evans and Wallenstein 2012; Göransson et al. 2013). However, when soils collected much earlier in this irrigation experiment were exposed to laboratory-based dry-rewet cycles, microbial communities responded similarly to current water stress regardless of irrigation history (Williams 2007), and more recent studies found that the long-term irrigation treatment did not alter microbial community composition (Carter 2019). We found higher microbial biomass in long-term irrigated treatment, and reversal from irrigated to drought conditions (natural drought in 2018 and experimental drought in 2019) both caused a sharp decrease in microbial biomass (see below). This opens the possibility that mechanisms behind the positive legacy in soil respiration may vary with current conditions: a large microbial community released from water stress may underlie high CO_2 fluxes in the long-term irrigated treatment (Du et al. 2020), and mortality-induced flushes of resources may cause high CO_2 fluxes upon re-exposure to water deficits in the reversal treatment (Birch 1958). We did not assess changes in community-level microbial carbon use efficiency, which has been found to vary with climate (Manzoni et al. 2012). Previous studies found that CUE increases with decadal-scale drought (de Nijs et al. 2019), suggesting that our long-term experimental release from drought may have the opposite effect, decreasing CUE and potentially increasing microbial CO_2 efflux. However, an earlier incubation study of these soils found that substrate utilization efficiency actually increased after seven years of irrigation (Williams and Rice 2007), making it unlikely that CUE changes are a major driver of the soil respiration legacy we describe.

Belowground carbon pools—The major C fluxes in this system, ANPP and soil respiration, responded similarly to long-term irrigation; so it is not surprising that there was no long-term effect of a wetter climate on total soil carbon pools. Although net C uptake generally increases in precipitation addition experiments, this is driven by aboveground production and biomass (Wu et al. 2011; Zhou et al. 2016), since belowground biomass allocation tends to be less sensitive to increased precipitation than aboveground biomass (Song et al. 2019). In the annually burned site for this experiment, little aboveground production enters the litter and soil C pools, which may contribute to the stability of total C pools despite decades of climate alteration. Interestingly, we found a decrease in soil C during the second year of irrigation, which was surprising after such a short treatment period. Although total C pools are generally understood to be slow-changing, some studies have reported differences in these pools after just months or years of treatment

(Connell et al. 2021). In our case, wetter conditions may have caused a spike in microbial respiration of labile C, which is consistent with both the high rates of soil CO_2 flux with current irrigation and the very low extractable organic C in the lowlands in 2018 under short-term irrigation found in this study and others (Hammerl et al. 2019; Fierer and Schimel 2003).

Trends in EOC were stronger than total C but were similar in direction, particularly in the lowlands, suggesting that under some circumstances this pool may be a useful indicator of slow-changing but important total carbon pools (Toosi et al. 2012). In the uplands, EOC pools were almost identical in long-term irrigation treatment and in the irrigated to ambient reversal treatment, while in the lowlands, I→A reversals were intermediate between long-term irrigation and controls, both of which provide some support for legacy effects of irrigation. While EOC generally increases under drought stress as microbial uptake and respiration decreases and soil aggregates are disrupted (Homyak et al. 2017; Hammerl et al. 2018), we did not see this in our study, and under some conditions (e.g., uplands in 2018), short-term irrigated soils that were released from drought stress had the highest EOC of any treatment. If short-term irrigation caused a flush of osmolytes released from a drought-acclimated microbial community (Fierer and Schimel 2003), this could explain the high EOC under this treatment in the water stress-prone uplands. As noted above, microbial biomass was higher under long-term irrigation, but a history of irrigation also increased the sensitivity of MBC to natural (in 2018) and experimental (in 2019) drought. This reinforces that the effects on pools and fluxes can be distinct, and that microbial uptake of soil C and respiration can vary independently from the size of the microbial community (Hawkes et al. 2017).

Root biomass and nutrient composition did not respond to current or previous climate treatments in our study, which is consistent with studies finding larger aboveground than belowground plant responses to climate in tallgrass prairie (Wilcox et al. 2015). Although a meta-analysis suggests that wetter conditions usually increase belowground biomass (Wu et al. 2011), effects on belowground biomass and productivity tend to be smaller than their aboveground counterparts (Song et al. 2019). Overall, these results suggest that management regimes can interact with previous and current climate effects on fluxes to regulate overall changes in soil C sequestration.

Spatial and temporal variation in legacy effects—The strength and sometimes the direction of climate legacies observed in this experiment varied across space and time, offering insight into what conditions support strong climate legacy effects. First, ANPP legacy effects lasted longer than soil respiration legacies. The plant community changes in this perennial system with long-term irrigation, which took nearly a decade to develop (Knapp et al. 2001; Collins et al. 2012; Knapp et al. 2012), provides a mechanism for persistent changes in ANPP long after climate conditions reverse, across both dry and wet years. The changes we document in the relative abundance of major plant functional groups and their contributions to ANPP are likely important drivers of carbon cycling legacies in these grasslands. Potential changes in nutrient dynamics associated with long-term climate treatments can also persist and maintain long ANPP legacies (Shen et al. 2016), which is the focus of ongoing work in this system. Conversely, microbial breakdown of soil organic carbon, as well as the physiological processes of root respiration and exudation that contribute to soil CO_2 fluxes, may have fewer “storage” mechanisms for climate legacies to persist (Evans and Wallenstein 2012). This may be particularly true in our system in which frequent fire limits legacies in litter inputs, soil organic matter accumulation, etc. that have been found to contribute to soil respiration legacies in other ecosystems (Shen et al. 2016). If C flow into ecosystems is more sensitive to climate legacies than C efflux, this has implications for future projections of ecosystem C storage across climate regions. Additionally, legacy effects in most response variables were concentrated in the lowland area of our study, even though the shallow, well-drained upland soils are more prone to water stress. The community change in the lowlands likely also drove these differences in legacy effects. It is possible that in the typically wetter lowlands, the addition of more water was sufficient to cause a community shift to more mesic species. While perennial vegetation often stabilizes ecosystems and prevents short-term legacy effects (e.g., Sternberg et al. 2017), long-term changes in climate drivers can actually cause changes in community and consequently larger functional legacies, a result that would not appear in previous-year-precipitation legacy studies. Although the upland and lowland areas of the experiment received similar water inputs in our study, those water additions were likely better retained in the lowlands, which have deeper soils with a higher soil clay content (34% vs. 27% clay). While these differences in soil depth and texture were not large enough to cause legacies of higher soil water content after irrigation, they may have been large enough to control plant community responses, and therefore ANPP responses, across the topography gradient. Within each growing season, irrigation inputs may have been better retained and utilized by plants and microbes in the lowlands than in the shallower, rockier uplands, contributing to the changes in plant and microbial functioning that persisted even after re-introduction of water stress.

Future directions—Our observations of persistent legacies of previous climate on grassland carbon fluxes warrant further study into the mechanisms behind these climate legacy effects. Previous studies in arid systems have pointed to mechanisms such as soil moisture storage and altered vegetation density as drivers of ANPP legacies (Reichmann and Sala 2013), but in this mesic grassland plant functional composition and altered nutrient cycling appear to be more important drivers of multi-year climate legacies. Further work could test these proposed mechanisms explicitly and assess whether their strength varies systematically across precipitation gradients. The legacies we found in soil respiration are more difficult to attribute to one cause, but future work could assess how previous climate conditions affect microbial communities, sensitivity to drought, root respiration and exudation, and other processes. One particularly interesting question is whether different mechanisms (microbial mortality and nutrient release due to cessation of irrigation, and a larger microbial biomass under long-term irrigation) may contribute to positive CO_2 flux legacies across distinct current climate conditions. Finally, we propose that the lowlands in our study may have had a larger capacity for climate legacies due to its deeper, finer-textured soil; in contrast, the shallow and rocky soils of the uplands limited the potential responses in plant functional shifts, ANPP, and soil respiration. This is in contrast to findings that sensitivity to increased precipitation in grasslands tends to be higher in uplands and lowest in deep, fine-textured soils (Du et al. 2020), suggesting that there may be differences in short-term responsiveness and potential for legacies to long-term climate shifts across soils and topographic positions. It will be important to test the universality of these patterns and which topo-edaphic factors contribute to the potential for climate legacies.

Conclusion—This work represents one of few manipulations of long-term climate with imposition of multiple new climate treatments, and it allows us to uniquely assess how ecosystem responses to current climate may depend on climate legacies. Previous climate treatments affected the sensitivity of carbon cycling to drought conditions, which are expected to become more frequent and severe across much of the world's terrestrial ecosystems, and these effects persisted for several years—suggesting that true climate legacies may be more important than suggested by previous-year precipitation carryovers. Legacy effects on carbon influx (ANPP) and carbon efflux (soil respiration) were comparable in magnitude but differed in their longevity, suggesting that net C uptake may be sensitive to climate legacies. Precipitation legacy effects are not currently included in earth system models (ESMs; Anderegg et al. 2015), but our results add to evidence suggesting that climate history is an important determinant to how ecosystems will respond to novel climate regimes.

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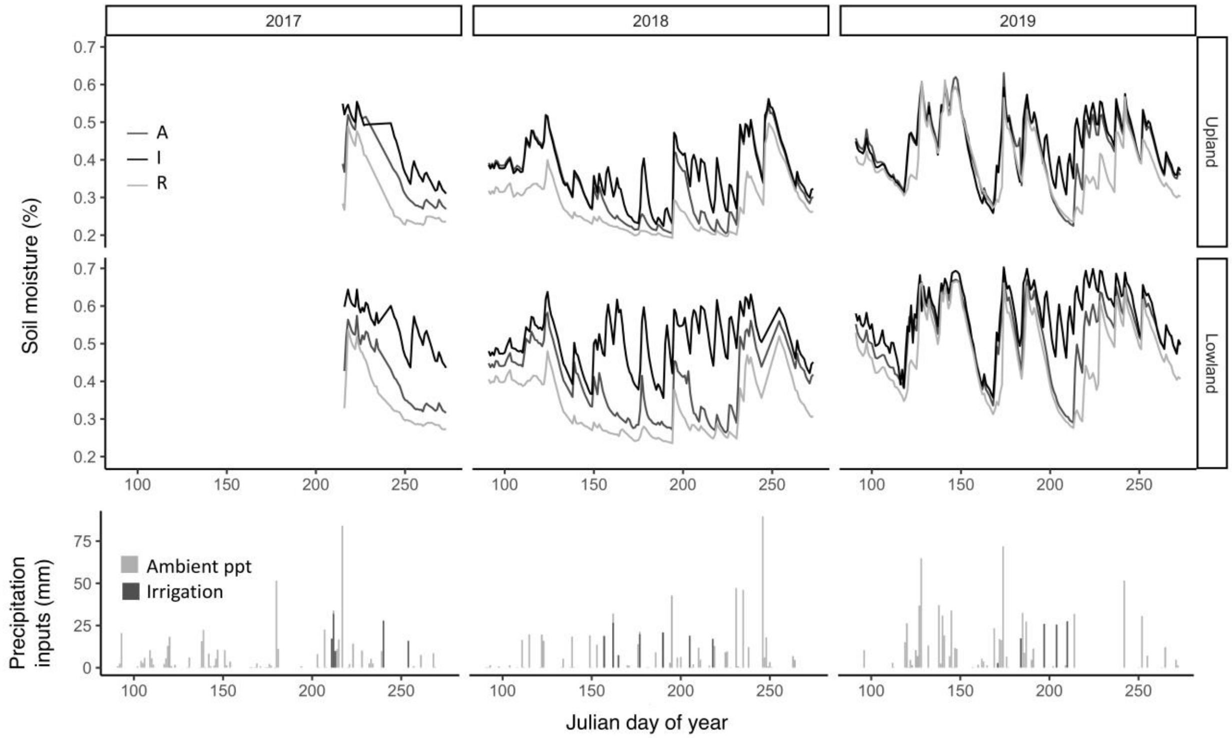

Figure 2.1. Volumetric soil water content (top) and daily precipitation inputs (bottom) from 0-15 cm during the growing seasons of 2017-2019. For soil moisture, grey lines are currently ambient (A), black lines are currently irrigated (I), and light grey lines are currently reduced (R) rainfall treatments. Past treatments did not affect VWC and are therefore not shown. Data collection began in August 2017; patterns are shown for all three years, but statistical models only include data from 2018 and 2019. For precipitation inputs, light grey bars represent ambient precipitation and dark grey bars represent supplemental water from irrigation.

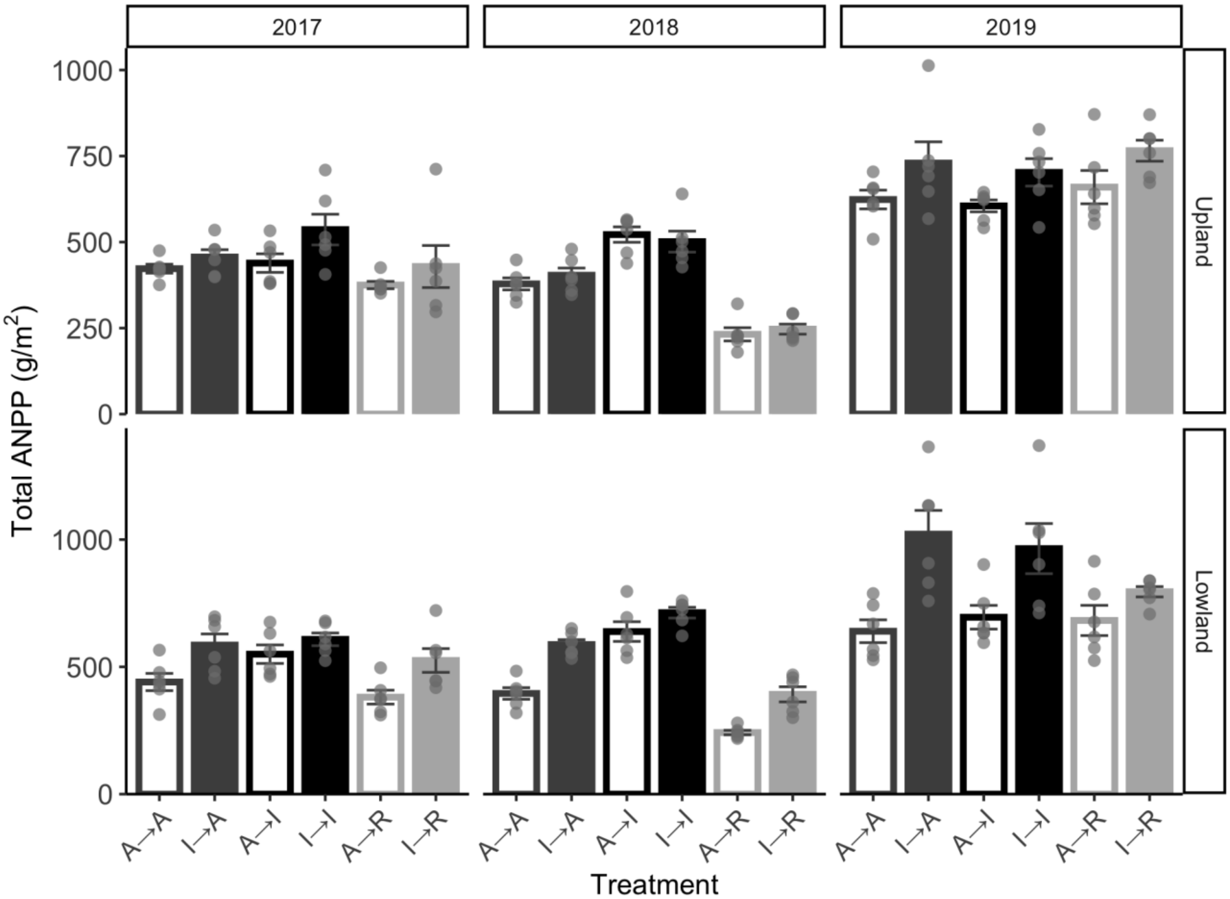

Figure 2.2. Total ANPP across treatments in the uplands and lowlands in 2017-2019. Historically irrigated (filled) treatments are paired with historic controls (open) for each current treatment by shading color. Points represent individual plot averages across subsamples. Error bars represent standard error of the mean.

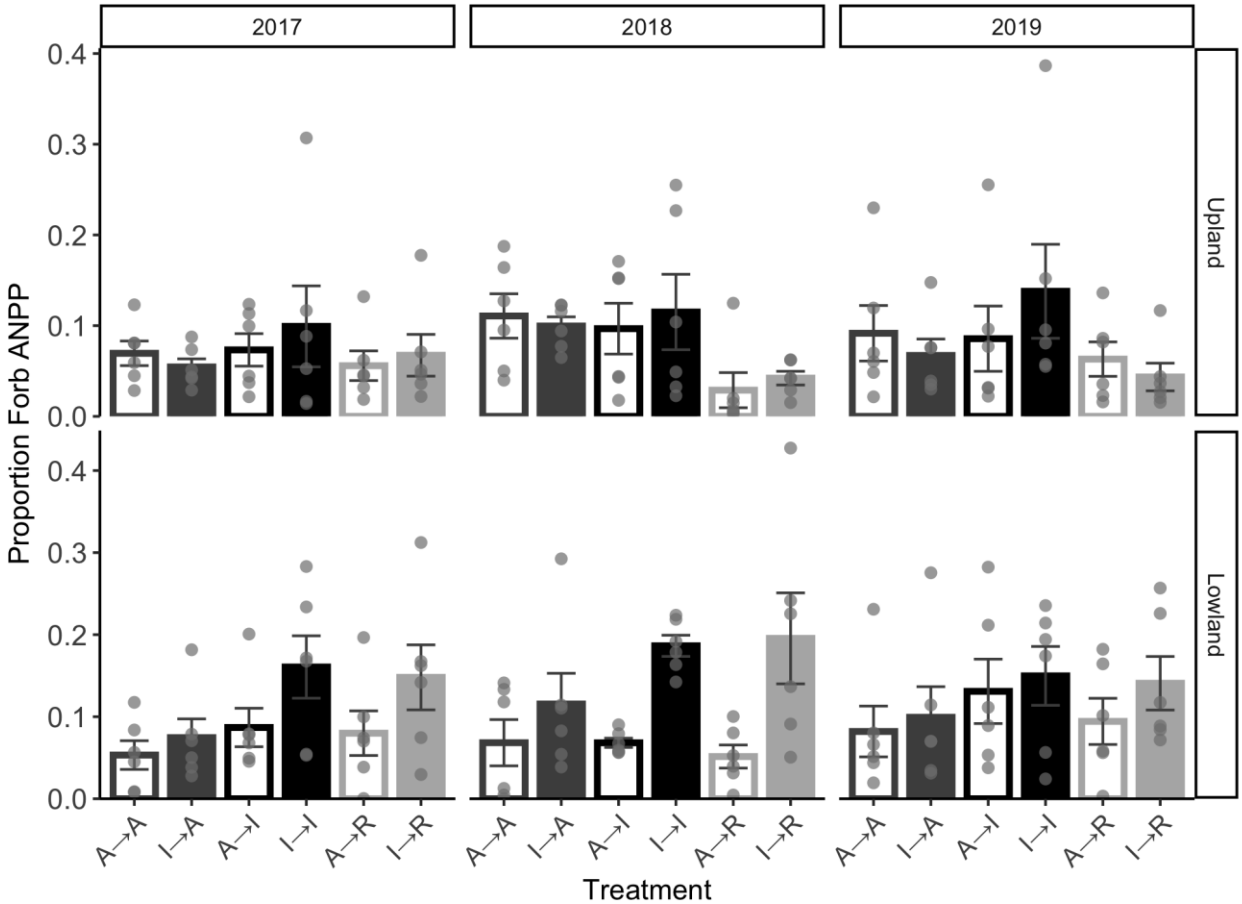

Figure 2.3. Proportion of total ANPP made up by forbs across treatments in the uplands and lowlands in 2017-2019. Historically irrigated (filled) treatments are paired with historic controls (open) for each current treatment by shading color. Points represent individual plot averages across subsamples. Error bars represent standard error of the mean.

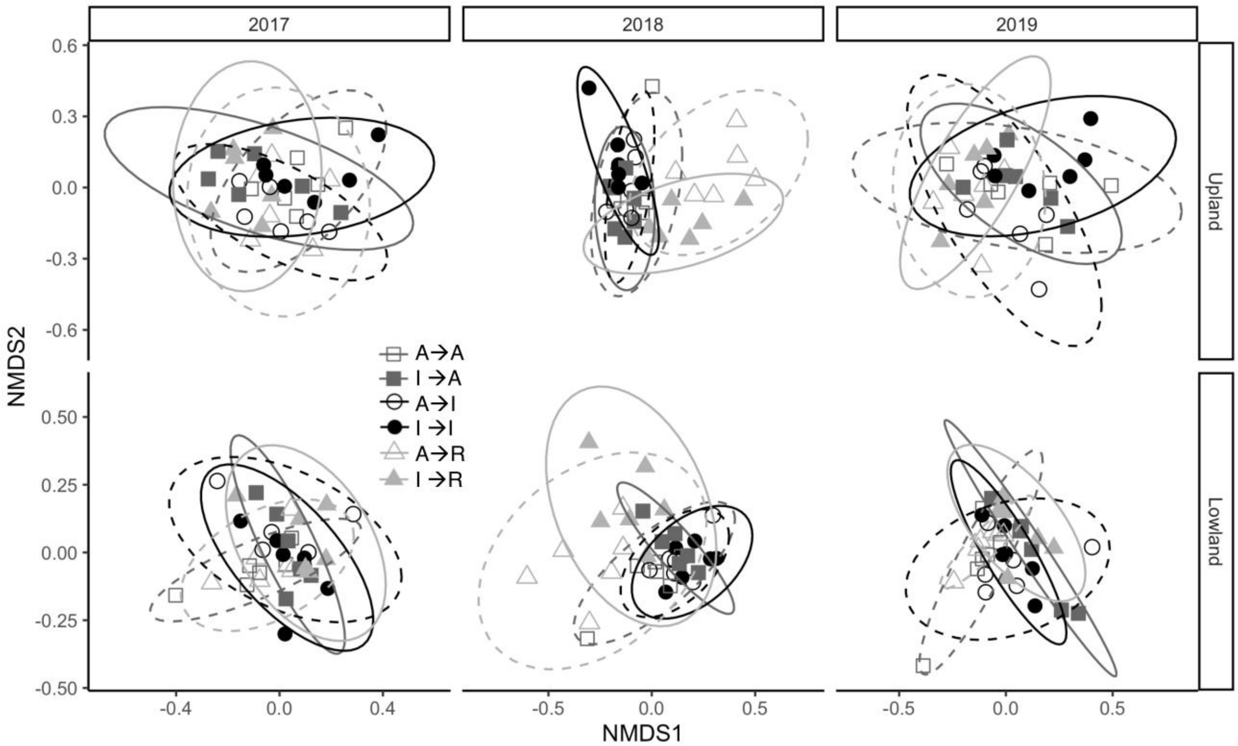

Figure 2.4. NMDS of plant community composition in 2017-2019 based on Bray-Curtis dissimilarity. All distances are calculated within each landscape position and year. Point colors and shapes represent current treatment; filled points were previously irrigated, while open points were not. Ellipses represent 95% confidence interval within groups, with solid lines representing previous irrigation and dashed lines not previously irrigated.

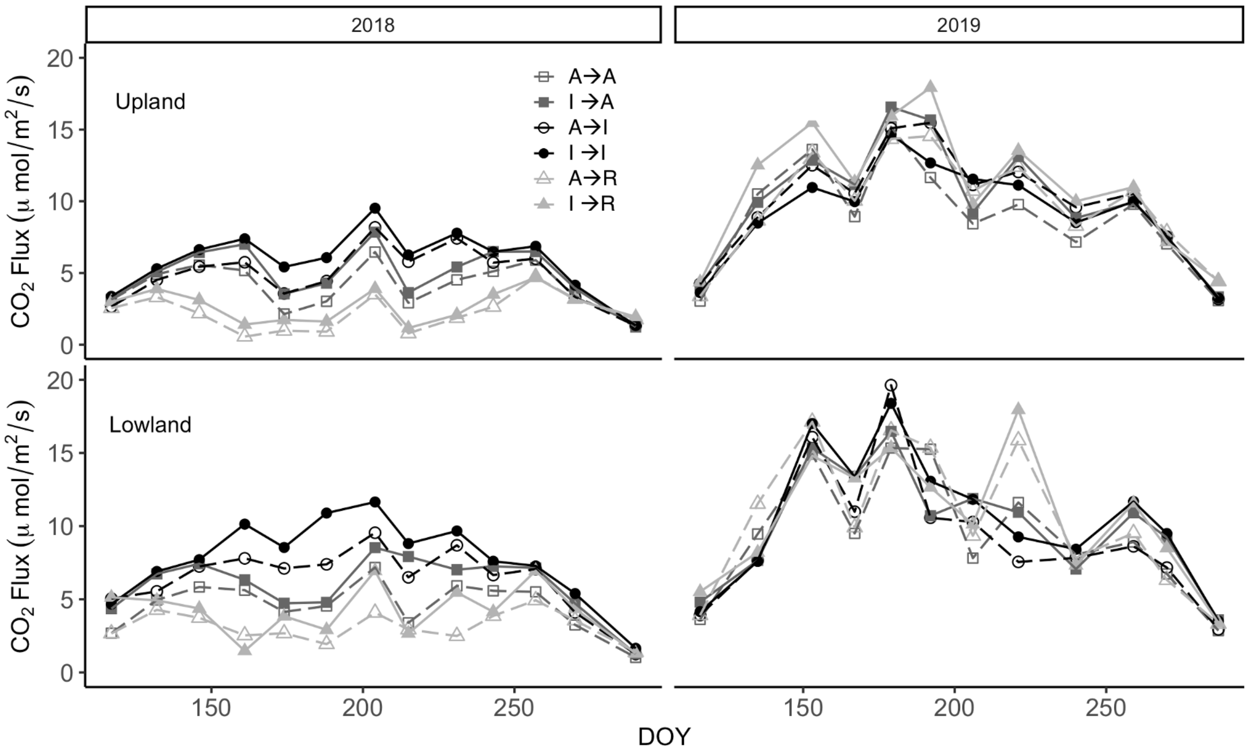

Figure 2.5. Soil respiration throughout the growing season in 2018 and 2019. Points represent mean CO_2 flux in each treatment on each sampling day of year (DOY). Shape and outline colors on points indicate current treatment. Filled shapes and solid lines indicate historically irrigated plots, while open shapes and dashed lines indicated past treatment.

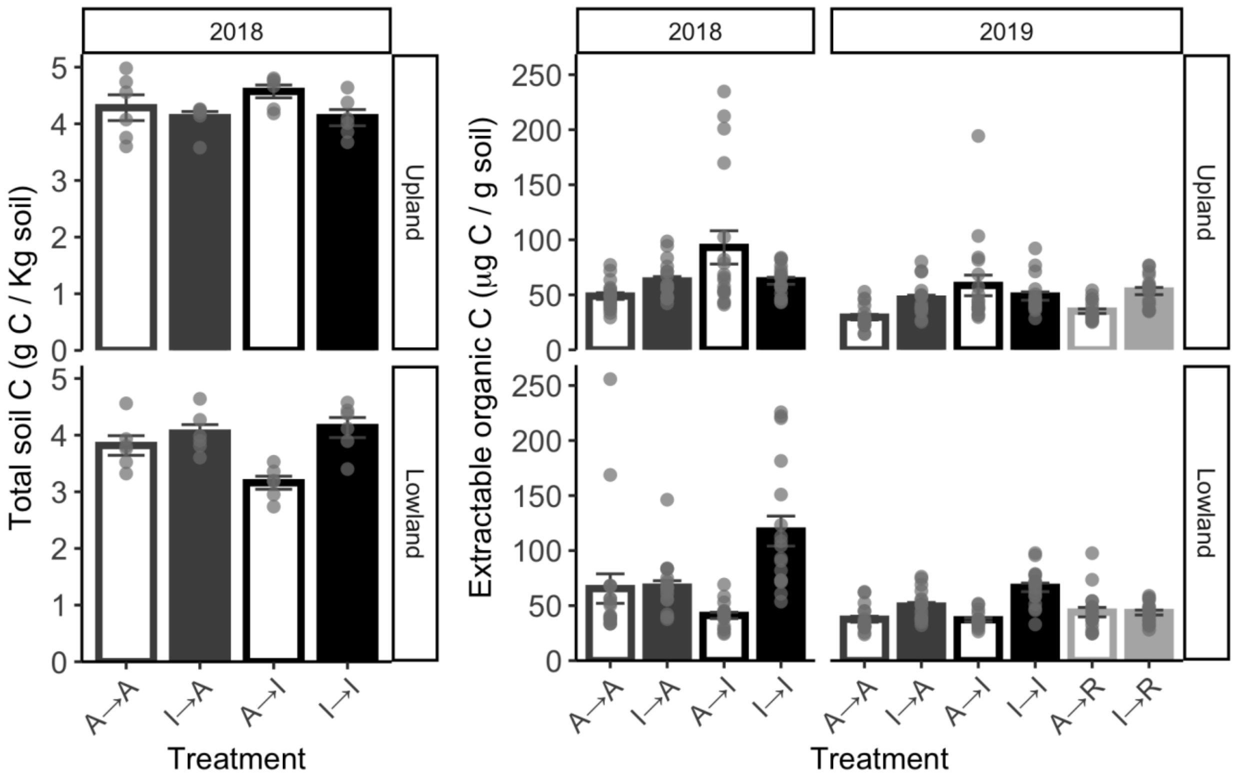

Figure 2.6. (A) Total soil C in the top 10 cm in 2018. Points represent individual plot values. (B) Extractable organic carbon (EOC) averaged across sampling months in 2018 and 2019. Points represent individual plot values for each month June-August, and error bars represent standard error. For both panels, bar outline color represents current treatment, and bar fill represents historical irrigation treatment. Error bars represent standard error.

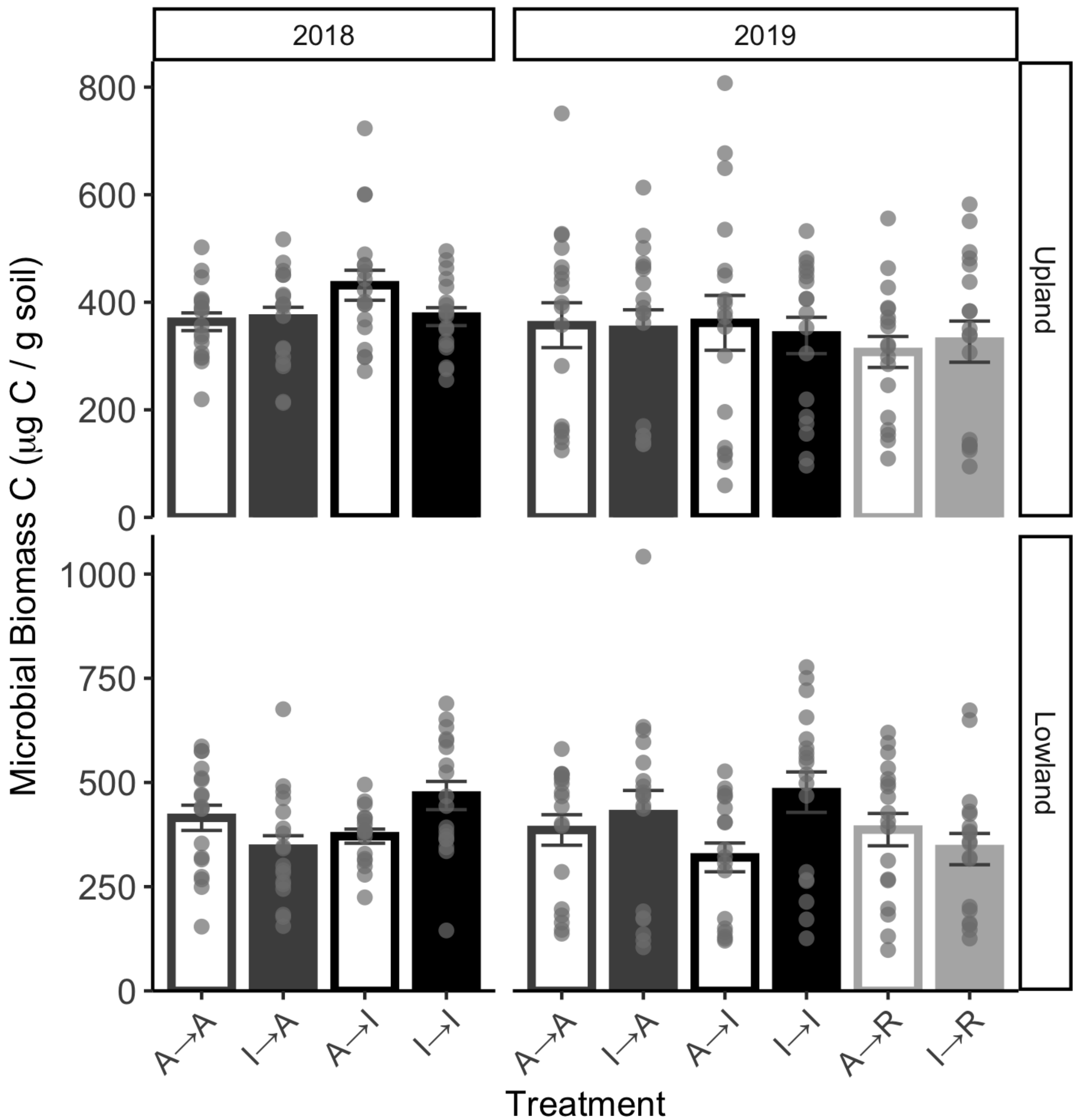

Figure 2.7. Microbial biomass carbon (MBC) averaged across sampling months in 2018 and 2019. Bar outline color represents current treatment, and bar fill represents historical irrigation treatment. Points represent individual plot values for each month, and error bars represent standard error.

Chapter 3 – Climate legacy effects shape tallgrass prairie nitrogen cycling

3.1 Introduction

Carbon and nitrogen cycles are strongly shaped by climate at local to global scales (Piao et al. 2020, Elrys et al. 2021), and future changes in precipitation will be a key determinant of ecosystem functioning under climate change (Quan et al. 2019). The size and timing of rainfall events have direct and immediate effects on plant and microbial physiological processes, such as photosynthesis and respiration, via changes in soil moisture (Savage et al. 2009). However, precipitation can also indirectly affect a multitude of ecosystem processes over longer time periods by exerting controls on the amount and quality of litter inputs (Ren et al. 2015), the release, transformation, and retention of nutrients (McCulley et al. 2009), and the protection and stabilization of soil carbon (C) (Bai et al. 2020). These indirect effects of climate can affect ecosystem processes in ways that are not easily inferred from short-term responses to precipitation variability. Moreover, in ecosystems that are co-limited by water and nutrient availability, the effect of soil water availability on the processes that determine soil fertility and nutrient supply rates can feed back to alter the relationship between precipitation and ecosystem processes, such as ANPP (Seastedt and Knapp 1993), potentially affecting ecosystem C balance and sequestration. To understand how future precipitation regimes will shape ecosystem functioning, it is critical to understand these linked responses of nutrient and C cycles to long-term changes in water availability.

The indirect effects of precipitation regimes on ecosystem processes tend to operate more slowly (months to years) than direct effects, which can result in a temporal mismatch between changes in precipitation and ecosystem responses. For example, the relationship between annual precipitation (AP) and above net primary production (ANPP) in many ecosystems can be partially explained by precipitation amounts in the year preceding measurement (Sala et al. 2012; Griffin-Nolan et al. 2018). Accordingly, there is growing evidence that the responses of ecosystems to climate change may depend on historical climate conditions, often referred to as legacy effects (Sala et al. 2012; Hawkes et al. 2017; Broderick et al. 2022). Current climate models do not account for legacy effects that alter the interactions between C and nitrogen (N) cycling (Averill et al. 2016), which may be an important driver of sensitivity to future climates.

To assess how biogeochemical legacies of past climate conditions may shape ecosystem sensitivity and drive responses to current or future climates, it is necessary to consider how key nutrient cycling processes respond to short- and long-term precipitation regimes. In many ecosystems, short-term variation in soil water availability strongly affects N supply rates, including net N mineralization and nitrification (Wang et al. 2006; Jin et al. 2013). Yet studies across natural rainfall gradients have found similar field-estimated N mineralization rates despite very different mean water availability (McCulley et al. 2009), suggesting that long-term differences in rainfall regimes affect N cycling differently than short-term fluctuations in soil moisture conditions. Rainfall regime shifts could have distinct biogeochemical consequences if long-term climate patterns alter the size, composition, or activity of plant or microbial communities, thus changing the quality and quantity of plant inputs or the capacity for microbial nutrient transformations (de Nijs et al. 2019; Veach and Zeglin 2020). Internal plant N cycling may also respond differently to short- and long-term variation in soil water availability, especially in ecosystems subject to N losses from plant residue due to volatilization by frequent fire. Under water stress, plants and microorganisms may become comparatively less N-limited if water limits productivity, or potentially more N-limited due to decreased soil N mobility

(Marschner and Rengel 2012; Manzoni et al. 2014). A wetter precipitation history may lead to persistent accelerated N cycling rates (Dijkstra et a. 2018) and decreased plant reliance on N resorption (Zhao et al. 2017; Vergutz et al. 2012). Conversely, persistent wet conditions can accelerate gaseous or leaching N losses (Groffman et al. 2009), leading plants to tighten internal N cycling (Aranibar et al. 2004) or become less responsive to water availability due to enhanced N limitation (Ren et al. 2017). Collectively, the short- and long-term responses of N cycling to water availability may be key factors in predicting how, and on what timescales, ecosystem C dynamics respond to altered precipitation regimes.

Grasslands cover 30-40% of earth's terrestrial surface (Dixon et al. 2014), provide a suite of important ecosystem goods and services (Sala and Paruelo 1997), and account for much of the stored carbon in soils (Schlesinger 1997). Future changes in precipitation associated with climate change may alter C uptake and storage in grasslands, with important implications for greenhouse gas levels (Pendall et al. 2018). Mesic grasslands subject to frequent fires, such as tallgrass prairie, can be limited by both water and N (Blair 1998), so responses to climate change in these grasslands are likely driven in part by N cycling responses to previous and current climate patterns making them hard to predict. For example, plant N resorption varies with drought stress (Heckathorn and DeLucia 1994; Heckathorn and Delucia 1995) and tallgrass prairie N mineralization varies with water availability in the short-term (Jin et al. 2013). Both previous climate patterns and current precipitation conditions may shape N cycling processes, resulting in climate legacy effects on N availability and N limitation. It is therefore important to understand to what extent N cycling rates are shaped by both previous and current precipitation in order to explain and predict responses to future climate change.

To experimentally assess the effect of precipitation legacies on current ecosystem functioning, we used a long-term irrigation experiment in an annually-burned tallgrass prairie in northeast Kansas, USA, designed to simulate a wetter climate by adding supplemental water to increase growing-season precipitation and minimize water stress for >25 years. In 2017, treatments in a subset of the long-term irrigated and ambient plots were reversed, and a new drought treatment was added across both historic precipitation treatments. A recent study using this experimental framework revealed positive legacy effects of long-term water addition treatment, with ANPP and soil respiration remaining elevated for multiple years after re-exposure to water stress (Broderick et al. 2022). While different responsiveness between grasses and forbs to previous climate treatments contributed to these positive legacy effects (Broderick et al. 2022), positive legacies also were apparent across both plant functional groups and in elevated labile C pools. Because of the strong responses of C cycling rates to N in this ecosystem (Seastedt and Knapp 1993; Turner et al. 1997, Riggs and Hobbie 2016), altered N cycles may be another strong driver of climate legacies on C cycling in this grassland.

In this study, we assessed how previous and current rainfall regimes affect N cycling processes in tallgrass prairie. We predicted that soil N availability of soils would be higher with both a history of long-term irrigation and current water additions, and we expected a potential legacy of elevated N transformation rates once long-term irrigation ended. Therefore, for two years following treatment reversal in this experiment, we measured monthly in-situ net N mineralization and net nitrification rates under continuous irrigated and ambient conditions and under a reversal of past treatments. In order to assess the relationship of C cycling processes to N availability across historic and current climate treatments, we also measured several indices of ecosystem N limitation. We expected that a history of previous irrigation would result in more conservative internal cycling of N in plants (indicated by greater N resorption during senescence), which would account for the continued high sensitivity of ANPP to water availability after > 25 years of supplemental water (that is, no evidence of exacerbated N limitation reducing climate sensitivity). Because past research in this experiment found that both previous and current irrigation led to higher microbial activity (Broderick et al. 2022), we also predicted that both previous and current water additions would increase N availability for soil microbes, as evidenced by lower microbial biomass C:N ratios and lower relative investment in N-acquiring extracellular enzymes. Support for these predictions would suggest that previously demonstrated climate legacy effects on C cycling may be based, in part, on persistent differences in the cycling of a co-limiting nutrient as a function of a past wetter climate.

3.2 Methods

Study site and experiment—To assess potential legacies of contrasting precipitation regimes, we used a long-term water addition experiment, the Irrigation Transect Experiment (Knapp et al. 2001), at Konza Prairie Biological Station (KPBS) in Manhattan, Kansas, USA. KPBS has a mean annual temperature of 12.8 °C and mean annual precipitation of 825 mm. The Irrigation Transect Experiment was established in an area of unplowed, native tallgrass prairie that spans an upland-lowland topographic gradient characteristic of prairies in the Flint Hills ecoregion. The upland site is in the Clime-Sogn complex (fine, mixed mesic Udic Haplustolls), with shallow, rocky soils (~10 cm in some sites) with a texture to that depth of 15% sand, 58% silt and 27% clay. The lowland soil is an Irwin silty clay loams (fine, mixed mesic Pachic Argiustolls), with a texture to 10 cm of 15% sand, 51% silt and 34% clay. The site is burned annually in the early spring, which is a common management practice for these grasslands.

The full experimental design has been described elsewhere (Broderick et al. 2022), but briefly, an irrigation treatment was initiated along a transect spanning the upland-to-lowland topographic gradient in 1991. Impact sprinklers were erected to deliver water in a ~15-m circle radius and spaced to deliver water evenly along the transect. A second replicate irrigation transect was added in 1993. Control transects, which receive ambient precipitation, were established adjacent to irrigated transects. Supplemental water was supplied during the growing season to maintain soil water content at 0.25 cm^3/cm^3 or higher (to 30 cm depth); on average, irrigation increased growing-season precipitation by ~32% though actual water addition varied by year based on ambient precipitation amounts and timing. Circular plots (10-m^2) were demarcated along both irrigated and control transects for sampling plant community composition. All soil sampling took place immediately outside these long-term plots. We utilized 24 plots each in the uplands and lowlands, avoiding plots on slopes (Appendix B, figure 1).

In 2017, irrigation lines were shifted so that the treatments in half of the plots in both uplands and lowlands were switched (some that used to be irrigated now experienced ambient precipitation, and vice versa). Additionally, 3x3m drought shelters with roofs made of polycarbonate slats designed to reduce ambient precipitation by 60% (Yahdjian and Sala 2002) were erected over 24 newly established plots, 6 in each of the historically irrigated and historically ambient sites in both upland and lowland locations. Together, this new treatment structure resulted in 72 plots, with six replicates of the historic (irrigated or ambient) treatments crossed with current (irrigated, ambient, or reduced) rainfall treatments in both the upland and lowlands sites.

<u>*Inorganic N pools, net N mineralization and nitrification rates*</u>—Net N mineralization and nitrification rates were measured monthly during the growing season (May-September) in 2018 and 2019 using an *in situ* incubated core technique (Hart et al. 1994). Because of the amount of disturbance involved with this method, we did not measure net mineralization and nitrification under the rainout shelters. However, in June-August 2019, soil cores were collected from sheltered plots at the same time the "initial" cores were collected for mineralization assays in non-sheltered plots so that inorganic N pools could be compared across all treatment combinations (see below). At the beginning of each month, an initial soil core (5 cm diameter, 10 cm depth) was collected, and a PVC core of the same dimensions was hammered into the soil within 20 cm of the initial core. These PVC cores were capped, and had two holes drilled into the core sides above the soil surface to allow for gas exchange during the incubation period. Incubated cores were retrieved after about 28 days. Initial core collection and incubated core placement were timed to avoid following a major rainfall (within 24 hours) to avoid inducing anaerobic conditions.

Inorganic N concentrations of initial and incubated soil samples were determined using KCl extraction. Soil samples were returned to the lab and stored at 4 °C until processing and were generally extracted within 3 days of collection. Soils were sieved through a 4-mm mesh to remove roots, rocks and debris, and an 11.5-g subsample was extracted with 50 ml 2 M KCl and filtered prior to analyses. NH_4^+ and NO_3^- concentration of extracts were determined by colorimetric analysis on a Flow Solution Autoanalyzer (Alpkem, Wilsonville, Oregon) by the

Kansas State Soils Testing Lab. Concentrations of 0 were assumed to be below detection levels and were replaced with 0.001 to allow for transformations for statistical analysis.

Daily net N mineralization rates for each month were calculated as the difference in total inorganic N (NH_4^+ and NO_3^-) between final and initial samples, divided by the incubation time in days. Net nitrification rates were calculated similarly as the difference between final and initial NO_3^- concentrations divided by time. Seasonal net N mineralization and nitrification rates were calculated by summing the monthly rates and gap filling the period between in situ incubations by multiplying the daily rate by the number of days until the next initial core, so that the 2-4 days between monthly incubations were assumed to have the same daily rates as the preceding month. Four plots were removed from these seasonal calculations due to a missing month time point (e.g., disturbance by animals); these plots were distributed among treatments so that each treatment combination had at least 5 replicates.

Microbial biomass N—Microbial biomass N was measured in the same soil samples used for initial N concentration in June, July, and August of 2018 and 2019, using the chloroform fumigation extraction technique (Cabrera and Beare 1993). In 2019 only, we assayed microbial biomass N under shelters in the droughted plots as well as in all non-sheltered plots. We extracted 11.5-g subsamples with 50 ml 0.5 M K_2SO_4 and filtered the extracts prior to digestion and analysis for total dissolved N. To determine microbial biomass N, we extracted both fumigated and non-fumigated subsamples of each sample. Microbial biomass N was calculated as the difference in total extractable N after and before fumigation. We conducted persulfate digestions of the extracts and analyzed the digested samples on a Flow Solution Autoanalyzer. Microbial biomass N (MBN) was calculated as the difference in total dissolved N concentration in the fumigated and unfumigated digested samples; no correction factors were used (Joergenson et al. 2011). Microbial biomass C (MBC) was measured on these same samples and presented in an earlier paper (Broderick et al. 2022). Those values were used here to calculate the microbial biomass C/N ratio (MBC/MBN).

*Microbial extracellular enzyme assays—*In August of 2019, we measured hydrolytic enzyme potential activities (Sinsabaugh et al. 1999; Saiya et al. 2002) as described in Zeglin et al. (2013) and Connell et al. (2021). Soil samples were sieved to remove plant roots, then frozen at 4 °C until assays were conducted. We focused our analyses on two key N-acquiring enzymes: leucyl aminopeptidase (LAP; EC 3.4.11.1, L-leucine-7-amido-4-MC) and β-N-acetylglucosaminidase (NAG; EC 3.2.1.14, 4-MUB-N-acetyl-β-*D*-glucosaminide), and the C-acquiring enzyme, β-glucosidase (βG; EC 3.2.1.21, 4-MUB- β-*D*-glucoside). Hydrolytic enzyme potentials were assessed using fluorometric substrates 4-methylumbelliferone (MUB) (NAG and βG) and 7-amino-4-methylcoumarin (MC)(LAP). 1 g soil samples were thawed and combined with 100 ml 50 mM sodium acetate buffer (pH 5) to form a slurry. We combined 200 μl of soil slurry with 50 μl of the corresponding substrate in 96 well plates, with six analytical replicates and triplicate quench standards per sample and replicate blanks, negative controls, and 200 uM reference standards. The assays were incubated at a final substrate concentration of 40μM for 2 hrs (βG), 3.5 hrs (NAG), and 16 hrs (LAP). After the incubation interval, the assay was stopped by adding 10 μl 0.5M NaOH, raising the pH to >8, and fluorescence (excitation of 360 nm and emission at 450 nm) was measured on a FilterMax F5 microplate reader (Molecular Devices, San Jose, California). Because total C changes slowly and is relatively stable in annually burned, ungrazed tallgrass prairie (Connell et al. 2020), even in this long-term climate experiment (Wilcox et al.

2016; Broderick et al. 2022), we did not adjust these values based on soil organic matter concentrations (Sinsabaugh et al. 2008). Instead values were standardized to nmol of substrate degraded g^{-1} of dry soil. Relative microbial C/N demand was calculated as ln(βG)/(ln(NAG)+ln(LAP)) (Sinsabaugh et al. 2008).

Plant N concentration and resorption—Three leaves from separate plants of the dominant grass *Andropogon gerardii* were collected from each plot during the growing season (July) and after senescence (November) in 2018. Leaves were dried at 60°C for 48 hours, ground using an 8000D mixer/mill (SPEX, Metuchen, NJ) and analyzed for total C and N on a Flash EA 1112 C/N autoanalyzer (Thermo Fisher Scientific, Waltham, MA). N resorption proficiency was measured as the N concentration in senesced leaves, and N resorption efficiency was calculated as the percent reduction in N concentration between green and senesced leaves (Killingbeck 1996).

Data analysis—Despite the typically high spatial variability of inorganic N concentrations and N transformation rates (Hart et al. 1994), only one outlier value (i.e., >150% of all other measured values) for ammonium concentration was removed prior to analysis. We evaluated the legacy effects of past precipitation treatment as well as the effects of current precipitation treatments on soil N availability, N transformation rates, soil MBN, and enzyme activities using mixed-effect modeling using the lme4 package (Bates et al. 2015) in R 3.4.2 (R Core Team 2019). Current precipitation treatment, previous precipitation treatment, topographic position (upland and lowland), and year and their interactions were modeled as fixed effects. If a response variable was measured only once during a growing season (i.e., seasonal mineralization and nitrification rates, N resorption), we included transect as a random effect. Since inorganic N, microbial biomass C, N and C/N had different sampling schemes for 2018 and 2019, these responses were analyzed with separate models for each year. All variables that did not need special consideration of negative values were log-transformed as needed to meet assumptions of normality and homogeneity of variances. For net N nitrification rates, where many values were negative, the Yeo-Johnson transformation was used because it allows for negative rates (Yeo and Johnson 2000). To account for repeated-measure design for response variables measures multiple times within a plot (i.e., monthly inorganic N concentrations, monthly mineralization and nitrification rates, and MBC and MBN), we added two random intercepts to these models: plot nested within transect, and scaled day of year.

3.3 Results

Climate and volumetric water content—Patterns in volumetric soil water content across treatments during the study are reported elsewhere (Broderick et al. 2022), but summarized here (Figure 1). Intra-annual rainfall variability in 2018 resulted in prolonged water deficits (Appendix B, table 1; Appendix B, figure 2), so that water content was affected by current irrigation (19% increase) and drought (17% decrease) treatments. In 2019, a wet year (1131 mm total precipitation), the drought treatment decreased soil water content but irrigation did not significantly increase soil water content compared to the ambient treatment. Previous precipitation treatments did not affect current soil water content.

Soil available N—Soil ammonium concentrations in 2018 were slightly, but significantly, higher with a history of irrigation (2.2 vs. 2.1 mg N/kg soil; $p = 0.044$; Figure 2). However, in 2019 current and previous treatments had larger effects on ammonium pools, and we focus our analysis on the three-way interaction between current treatment, previous treatment, and landscape position. In the lowlands, the long-term irrigated treatment (I→I) had higher ammonium concentrations than long-term ambient (A→A), previously irrigated (I→A), short-term irrigated (A→I), and in the reduced rainfall treatment regardless of irrigation history ($p < 0.001$ for all comparisons). In contrast, there were no significant differences among treatments in the uplands.

Across current treatments, nitrate concentrations in 2018 were marginally lower in the previously irrigated treatment ($p=0.083$; Figure 3). In the upland prairie, current ambient treatments had nitrate concentrations that were more than three times higher than currently irrigated prairie ($p=0.001$), but treatments did not differ in the lowlands. In 2019, the reduced rainfall treatment had higher nitrate concentrations than either currently ambient or irrigated prairie ($p=0.02$ and $p<0.001$, respectively), but historic irrigation had no effect on nitrate concentrations.

Net N mineralization rate—There was considerable between-month variation in net N mineralization rates, so that random effects (day of year in particular) explained more variation in the model than the fixed effects (marginal $R^2= 0.088$). Mineralization rates tended to decrease throughout the growing season (Figure 4). In May 2019, high spring rainfall amounts resulted in saturated soils and very high net mineralization rates in the lowland plots; however, excluding these plots did not qualitatively affect results, suggesting that this single sampling point is not the main driver of the patterns we outline below. Further, we present model results for seasonal as well as mean monthly mineralization rates.

We found no significant effects of current treatments on N mineralization rates (Figure 4). In contrast, previous irrigation treatments affected N mineralization rates; for brevity, we focus on the three-way interaction between previous treatment, landscape position, and year. In 2018, mineralization rates in the lowlands were twice as high in plots with a history of irrigation (0.093 vs 0.046 µg N/ g soil/ day; $p = 0.029$). Previous irrigation had the opposite effect in the wet year of 2019, lowering mineralization rates in the lowlands by 30% (0.25 (I) vs. 0.36 (A) µg N/ g soil/ day; $p=0.002$). There were no significant treatment effects on N mineralization rates in the uplands in either year.

Patterns were qualitatively similar when assessing seasonal N mineralization, and aggregating data over the growing season dramatically increased the variation explained by the model (marginal $R^2 = 0.560$; Figure 5). For aggregate seasonal N mineralization rates, we found a significant interaction between current treatment and year, with current irrigation reducing seasonal mineralization in 2018, the dry year of our study ($p=0.021$). At the seasonal level, the effect of previous irrigation on N mineralization in the lowlands in 2019 was no longer significant ($p = 0.101$), despite a similar 30% decrease.

Net nitrification rates—Similar to net N mineralization rates, net nitrification rates were quite variable and the models left much variation unexplained (marginal $r^2=0.094$; Figure 6). There was a significant three-way interaction indicating that the effects of historic treatment varied with year and topography. In 2018, previous irrigation decreased nitrification by 32% in the uplands (0.056 (I) vs. 0.082 (A) µg N/ g soil/ day, $p=0.035$); in the lowlands, the opposite pattern emerged, with nitrification rates more than twice as high in previously irrigated prairie (0.067 (I) vs. 0.032 (A) μg N/ g soil/ day; p = 0.007). Previous irrigation treatment did not affect nitrification rates in 2019, and current irrigation did not significantly affect rates in either year.

Aggregating nitrification rates over the season increased the model fit (marginal r^2 = 0.331; Figure 7) and yielded similar patterns. One difference was a significant decrease in nitrification rates with current irrigation (7.6 vs 9.5 μg N / g soil; p = 0.037). However, there was a large (~2x) increase in nitrification rates with previous irrigation in the lowlands in 2018 (p = 0.016), similar in magnitude to the daily rates.

Microbial biomass N and C/N ratio—In 2018, long-term irrigated plots (I→I) had 50% higher MBN than long-term ambient (A→A) plots (p<0.001; Figure 8), and were higher than any other treatment combination. No other treatments significantly differed from each other. The effects of treatments on the microbial biomass C/N ratio in 2018 can be understood in the context of two significant two-way interactions. First, an interaction between current and previous treatments showed that the long-term ambient treatment had a higher C/N ratio than any plots with previous or current irrigation (A→I: p=0.037; I→A: p<0.001, I→I: p<0.001). An interaction between previous treatment and landscape position showed that, in the lowland, the C/N ratio was 23% lower with a history of irrigation (p<0.001).

In 2019, MBN was higher in the lowlands (33 vs. 30 ppm; p<0.001), and the reduced rainfall treatment had a lower MBN than the current ambient or irrigated treatments (A vs R: p=0.011; I vs. R: p=0.003). Historic irrigation increased MBN (p<0.001); an interaction with landscape position suggested the strength of this effect varied across the landscape, with just a 6% higher MBN with irrigation in the uplands but a 30% higher MBN in the lowlands (U: p=0.025; L: p<0.001). The microbial biomass C/N ratio varied strongly with sampling month, with June ratios much lower than those in July or August, primarily due to temporal patterns in MBC. There was a slightly lower C/N ratio in the previously irrigated treatment (11.2 vs. 11.7; $p=0.048$); otherwise, climate treatments did not affect microbial C/N ratios in 2019. An alternate model including sampling date as a fixed effect, rather than a random effect, did not qualitatively change the effects of current or previous climate treatments.

Relative microbial nutrient demand via extracellular enzymes—We focused on C- and N-acquiring microbial extracellular enzymes to assess relative microbial investment in obtaining these resources. Activity potentials of the C-acquiring enzyme β-glucosidase were 23% lower under current irrigation compared to ambient plots in the uplands ($p=0.023$), but there was no effect in the lowlands and previous irrigation did not affect β-glucosidase activity (Figure 9). N-acetyl glucosaminidase activity potentials were 18% lower under the reduced precipitation treatment compared to ambient and irrigated treatments (vs. A: $p=0.002$; vs. I: $p<0.001$). However, this N-acquiring enzyme was also influenced by previous irrigation; in the lowlands, a history of irrigation decreased NAG activity by 34% ($p=0.041$). Similarly, across both topographic positions, Leucine-amino peptidase activity potentials decreased by 40% with previous irrigation ($p<0.001$).

When assessing relative microbial C/N demand $[\ln(\beta G)/(\ln(NAG)+\ln(LAP))]$, we focused on the three-way interaction between current treatment, previous treatment, and landscape position. While there were no effects of precipitation treatments in the uplands, in the lowlands, ambient plots with a history of irrigation (I→A) had C/N demand ratios 8% higher than long-term ambient plots (A→A; $p=0.011$). This trend was even more apparent in the rainfall reduction treatment, with previously irrigated plots (I→R) having C/N demand ratios 15% higher than plots without a history of irrigation (A→R; p=0.027), suggesting lower relative N demand in previously irrigated plots.

Plant N concentration and resorption—Green plant N concentration was lower in currently irrigated compared to ambient plots (p=0.003) and precipitation reduction increased plant N (p=0.030), as expected (Appendix B, figure 3). However, we also found that previous irrigation increased plant N concentrations in the lowlands (p=0.021). Current precipitation treatment also affected N resorption proficiency, which was lowest in the irrigated treatment (A vs. I: p=0.003; Figure 10) and highest in the reduced rainfall treatment (p=0.011). N resorption efficiency showed a similar pattern but the only significant difference was between the irrigated and reduced treatments (p=0.006). Historic irrigation did not affect either measure of N resorption (Figure 10).

3.4 Discussion

In order to predict and mitigate the effects of climate change on ecosystem functioning, it will be important to understand the ecosystem properties and processes that underlie climate sensitivity. Long-term climate patterns can produce legacies that shape C fluxes in the face of novel climate scenarios, and these climate legacies are likely to vary in their magnitude, direction, and causes across and within ecosystems (Sala et al. 2012; Kannenberg et al. 2020, Broderick et al. 2022). Here, we provide evidence that previous rainfall regimes create N cycling legacies, which may in turn be important drivers of C cycling responses to current rainfall regimes in mesic grasslands. Long-term mitigation of water stress to simulate a wetter climate via irrigation resulted in elevated N mineralization and nitrification rates that persisted for two years following cessation of irrigation treatment. Microbial communities also appeared to be less limited by N with previously wetter conditions, as evidenced by the higher microbial C/N ratio and the lower relative investment in N-acquiring extracellular enzymes. Finally, while long-term wetter conditions can lead to N losses and exacerbated plant N limitation (Ren et al. 2017), in this tallgrass prairie we found that—unlike the response to short-term changes in water availability—the dominant grasses showed no change in internal N cycling in response to long-term irrigation, and actually had higher growing-season N concentrations following a history of irrigation. On the whole, these responses were most pronounced in the deeper-soiled lowland prairie, consistent with a prior study in this experiment documenting larger carbon cycling responses to irrigation treatments in the lowlands (Broderick et al. 2022). Together, these results suggest that in grasslands that are co-limited by water and N such as this one, previous climate patterns may continue to shape N cycling and availability for years, ultimately affecting ecosystem resource limitation and C dynamics.

Soil inorganic N pools—Soil inorganic N pools in tallgrass prairie are small compared to total N, vary greatly in space and time, and turn over rapidly (Blair et al. 1998). It was therefore not surprising that, on the whole, inorganic soil N pools were not influenced by previous irrigation. One exception was ammonium, with concentrations in the long-term irrigated (I→I) treatment higher than any other treatment in the lowlands in 2019. Previous research in this experiment also reported a positive effect of irrigation on soil ammonium pools (Wilcox et al. 2016). While many studies report decreases in inorganic N with increasing precipitation as N becomes comparatively more limiting and cycles more conservatively (McCulley et al. 2009; Austin and Vitousek 1998), other studies have also found that inorganic N increases with precipitation across climate gradients (Austin and Sala 2002). Although long-term wetter conditions can lead to enhanced leaching and gaseous N losses in some ecosystems, exacerbating N limitation (Unger and Jongen 2015; Ren et al. 2017), the high immobilization potential of soils in tallgrass prairie and low rates of both leaching and denitrification in these grasslands likely prevented this in our experiment (Blair et al. 1998). Larger ammonium pools under the long-term release from water stress may be due to the accelerated growth and turnover of the microbial community (Gerschlauer et al. 2016; Meier et al. 2017). Nitrate concentrations varied with current conditions, with higher concentrations in the reduced rainfall treatment and lower concentrations in the irrigated treatment. Since nitrate is the most available form of N for grassland plants (Seastedt and Ramundo 1990; Rice and Tiedje 1989; Dell and Rice 2005), the release from water stress combined with the increased mobility of nitrate under wetter conditions may have increased plant nitrate uptake, and/or caused leaching of N deeper into the soil profile. The uptake of ammonium and nitrate by plants is not included in field assessments of net N mineralization and nitrification (Hart et al. 1994), so it is unsurprising that legacy effects in flux rates did not accompany changes in these pools.

N flux rates—We found that previous irrigation had contrasting effects on two major N transformation rates, net N mineralization and net nitrification. Net N mineralization showed opposing responses to previous irrigation depending on ambient rainfall conditions. In the very dry growing season of 2018, plots with a history of irrigation had higher N mineralization rates. This legacy effect was apparent across all current treatments. In long-term irrigated treatments, the combination of a higher microbial biomass along with increased N mobility under irrigation may support persistent high N mineralization (Hassink 1994; Marschner and Rengel 2012; Li et al. 2019). Conversely, in previously irrigated prairie now subject to natural water deficits, drought-induced mortality in the microbial community when soils experienced extended dry periods may result in a flush of labile organic N resources (Xiang et al. 2008). Previous results from this experiment revealed that long-term release from water stress resulted in greater microbial biomass C, but upon reversal to ambient conditions there was a sharp drop to biomass levels comparable to non-irrigated controls (Broderick et al. 2022). Therefore, in the I→A reversal treatment, release of nutrients from the death of drought sensitive microbes after repeated dry/wet cycles may have contributed to the increased rates of N mineralization in this treatment (Franzluebbers 1999; Xiang et al. 2008). In 2019, a very wet year, we saw the opposite effect, with a history of irrigation decreasing net N mineralization rates. By 2019 microbial biomass levels had recovered from the drop following initial treatment reversal (Broderick et al. 2022), and saturated soils coupled with high microbial biomass during this wet year may have enhanced microbial immobilization and reduced net N mineralization.

Our study falls between two common temporal scales for assessing climate effects on N cycling, and may offer unique insight into climate change responses in the coming decades. Short-term (weeks-months) increases in water availability tend to increase N mineralization (Jin et al. 2013), mostly due to increased microbial activity and N mobility (Marschner and Rengel 2012). At the same time, studies across persistent climatic gradients (*i.e.*, centuries-millennia of differences in water availability) do not find changes in N mineralization with mean annual precipitation, which has been attributed to concurrent increases in soil organic C that increase potential N immobilization (Barrett et al. 2002; McCulley et al. 2009 Feyissa et al. 2021). We did not find changes in soil C during the duration of our study (Wilcox et al. 2016; Broderick et al. 2022), and we did not find legacies of previous irrigation on soil moisture. Therefore, we suggest that different mechanisms, such as changes in the size and functional composition of the microbial community, may drive these decades-scale responses to climate patterns, and account for the differing legacy effects depending on current conditions (wet or dry year).

The effects of historic climate treatment on nitrification rates also varied by year and with topography. We found a modest decrease in nitrification rates in the uplands in 2018, but a large increase in rates in the lowlands of that year. We also found differences between responses of pools and fluxes; for example, sharp increases in nitrification with previous irrigation in the lowlands did not accompany any response in nitrate pools. This may be due to increased plant uptake in the lowlands with previous irrigation (associated with higher ANPP without changes in plant N content), especially in forbs with a higher N demand (Broderick et al. 2022; Tjoelker et al. 2005). Importantly, short- and long-term precipitation manipulations had distinct effects on net nitrification rates. Current irrigation tended to decrease nitrification rates, perhaps due to the high potential for N immobilization at this site and rapid uptake of N by plants (McCulley et al. 2009; Lü et al. 2014). In contrast, long-term irrigation increased these rates in the lowlands. Persistent increases in precipitation may increase the size of the nitrifier community, leading to this climate legacy, while short-term water availability primarily acts through physiological mechanisms. Importantly, positive responses of both N transformation rates were limited to the lowlands in our study, suggesting that this acceleration in N cycling may support the high rates of ANPP and soil respiration also documented in previously-irrigated lowland prairies (Broderick et al. 2022).

<u>*Microbial N demand*</u>—The accelerated N cycling rates in 2018 with previous irrigation suggests that microbial communities experienced increased N availability. Indeed, we found that both the

microbial C/N ratio (primarily in 2018) and the relative investment in N-acquiring enzymes (in 2019) supported this conclusion. Because microbial extracellular enzymes reflect relative investment in C and N acquisition (Sinsabaugh et al. 2008; Moorhead et al. 2012), these results are consistent with increased N mineralization relative to immobilization, as well as increased nitrate production, in the previously irrigated treatments. Interestingly, C cycling enzyme activities (βG) did not vary with previous or current irrigation treatments: rather, decreases in both N-acquiring enzymes (NAG and LAP) drove these differences in relative microbial investment. The differential responses of C-processing and N-acquiring enzymes may indicate that rates of organic matter processing may be decoupled from patterns of litter N release or immobilization (Moorhead et al. 2012; Yahdjian et al. 2006). Like other legacy effects, enzyme activity responses emerged only in the lowlands, and were largely consistent across current treatments, suggesting that, regardless of current conditions, a history of previous irrigation results in more N available for soil microbial communities.

Plant N limitation—Increased precipitation inputs can exacerbate plant N limitation, especially if wet conditions lead to N losses (Ren et al. 2017). However, we found no effect of long-term irrigation on N resorption, suggesting that plants were not increasing the efficiency of internal recycling. Instead, we actually found higher green N concentrations with previous irrigation, and this effect was in the lowlands—where most C and N legacies have been identified in this experiment. Another long-term irrigation study found a decreased responsiveness of ANPP to water additions after persistently wetter conditions (Ren et al. 2017), which was linked to increasing N limitation over time. However, our results suggest that, even after decades of irrigation in tallgrass prairie, N was more available and remained so two years following cessation of irrigation. While aboveground plant litter does not contribute significantly to soil N inputs due to frequent fire, belowground plant biomass is a key driver of the large carbon stores in prairie soils (Rice et al. 1998; Seastedt and Knapp 1993). While previous work has documented decreases in root N with short-term irrigation (Broderick et al. 2022), consistent with the finding that extreme drought increases root N (Roy et al. 2016), long-term irrigation did not affect root N content (Broderick et al. 2022), further suggesting that accelerated N cycling mitigated any nutrient limitation usually associated with shorter precipitation increases. Moreover, the combination of wetter conditions without a decrease in N content may accelerate decomposition rates, contributing to a positive feedback that may contribute to accelerated C and N cycling responses. This may be an underexplored mechanism for climate legacies to maintain higher-than-expected rates of C and N cycling.

Carbon cycling legacies via biogeochemical legacy effects—The persistent high ANPP in previously-irrigated prairie was apparent across plant functional groups, suggesting that plant community legacies alone do not explain this positive legacy. We suggest that the increased net N mineralization and nitrification rates in the lowlands with previous irrigation, combined with the lack of evidence for altered nitrate pool sizes, suggests that increased N supply and plant uptake may contribute to the previously reported positive precipitation legacy in ANPP. The strongest legacy effects in ANPP, soil respiration, and microbial biomass pools occurred in the lowland (Broderick et al. 2022), consistent with our finding that the strongest positive legacy effects on N availability were in the lowlands of our study. Although the uplands tend to be drier and putatively more water-limited, the higher responsiveness of C and N cycling in the lowlands may be due to the deeper, finer-textured soils that retain water more effectively, leading to a larger impact of additional water on ecosystem processes.

Conclusions and significance—A history of long-term irrigation resulted in a legacy of higher N mineralization and nitrification rates in lowland tallgrass prairie. These effects lasted two years following treatment reversal (through 2018). Multiple indices suggested that previous irrigation decreased microbial biomass N limitation, shown by a lower microbial C/N ratio and reduced investment in N-acquiring extracellular enzymes. This study suggests that previous climate patterns, particularly past rainfall regimes, may be an important determinant of N cycling rates in tallgrass prairie. Moreover, these legacy effects coincide with strong positive legacies in C cycling and are a putative mechanism driving persistently high ANPP and soil respiration. Further work is needed to assess whether altered N cycling is a common driver of climate legacies in other grasslands. Finally, since we document climate legacies lasting at least three years, typical short-term climate manipulation studies lasting <5 years may document responses that are buffered by climate legacy effects. Therefore, these experiments may underestimate the responses of C and N cycling to persistent climate change, highlighting the importance of decade-long climate experiments.

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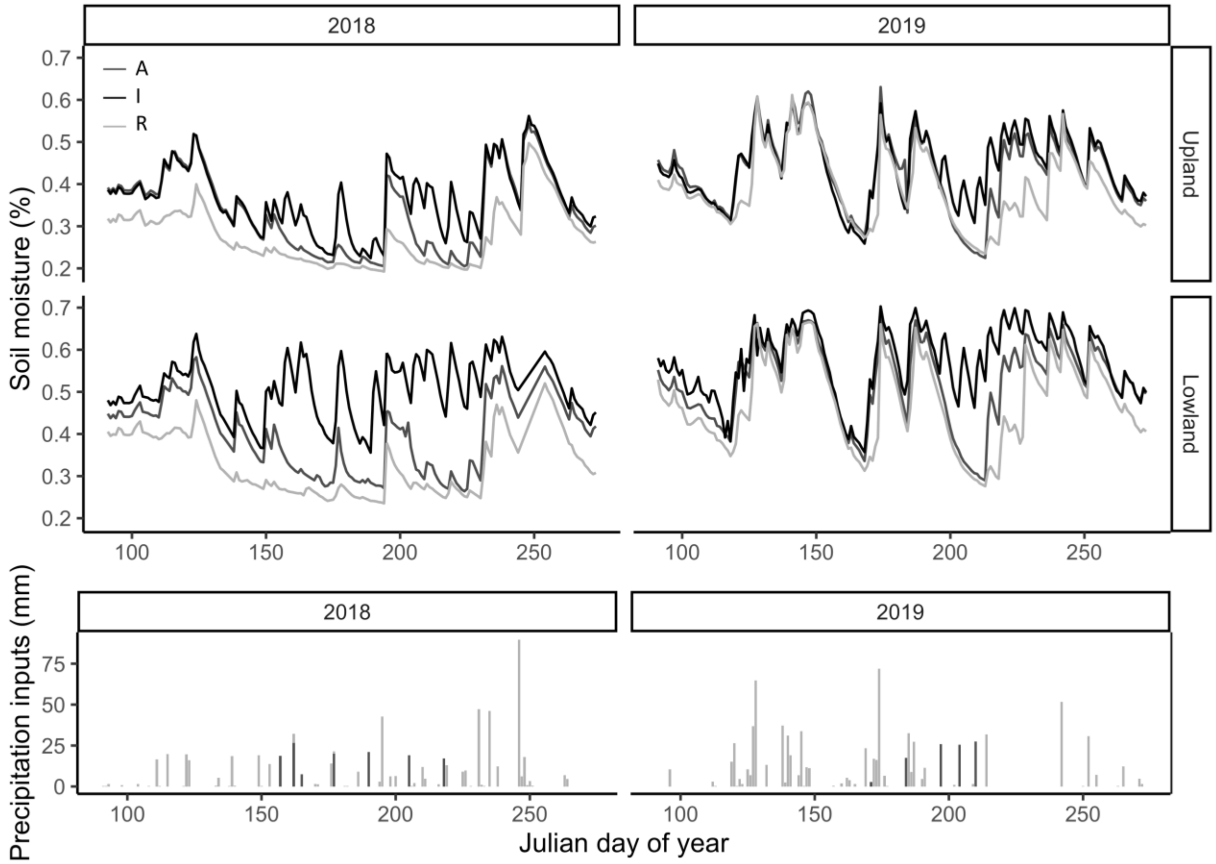

Figure 3.1 Volumetric soil water content (top) and daily precipitation inputs (bottom) from 0-15 cm during the growing seasons of 2018 and 2019. Soil moisture for current ambient (A, dark grey lines), irrigated (I, black lines), and reduced (R, light grey lines) rainfall treatments are shown; previous treatments are not shown since they did not affect VWC.

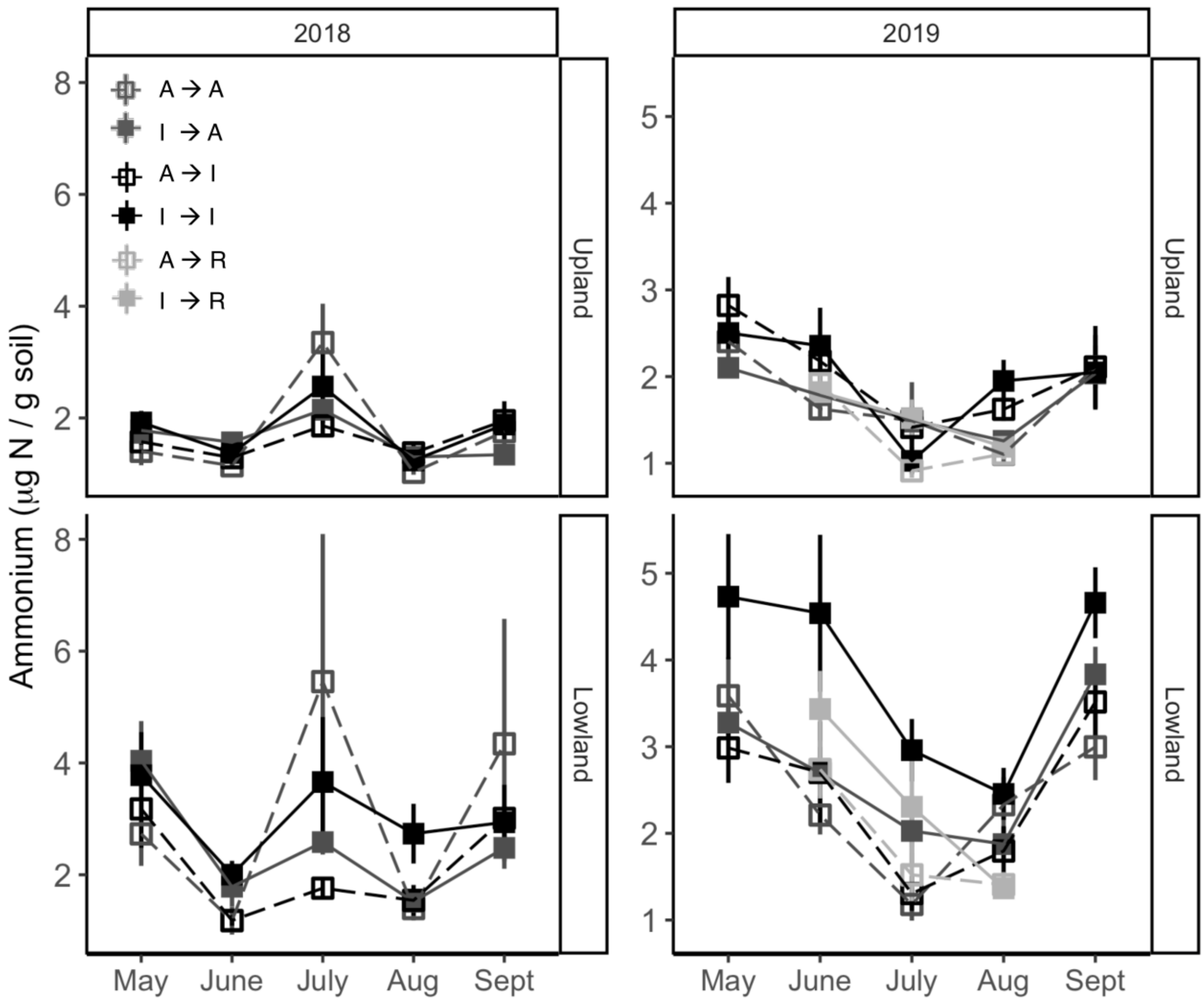

Figure 3.2 Mean soil ammonium concentrations (with standard error bars) 0-10 cm depth across sampling months in upland and lowland prairie in 2018 and 2019. Dark grey outlined points indicate current ambient treatments, black outlined points indicate current irrigated treatments, and light grey outlined points indicate current drought treatments (only June-August 2019). Filled points were previously irrigated.

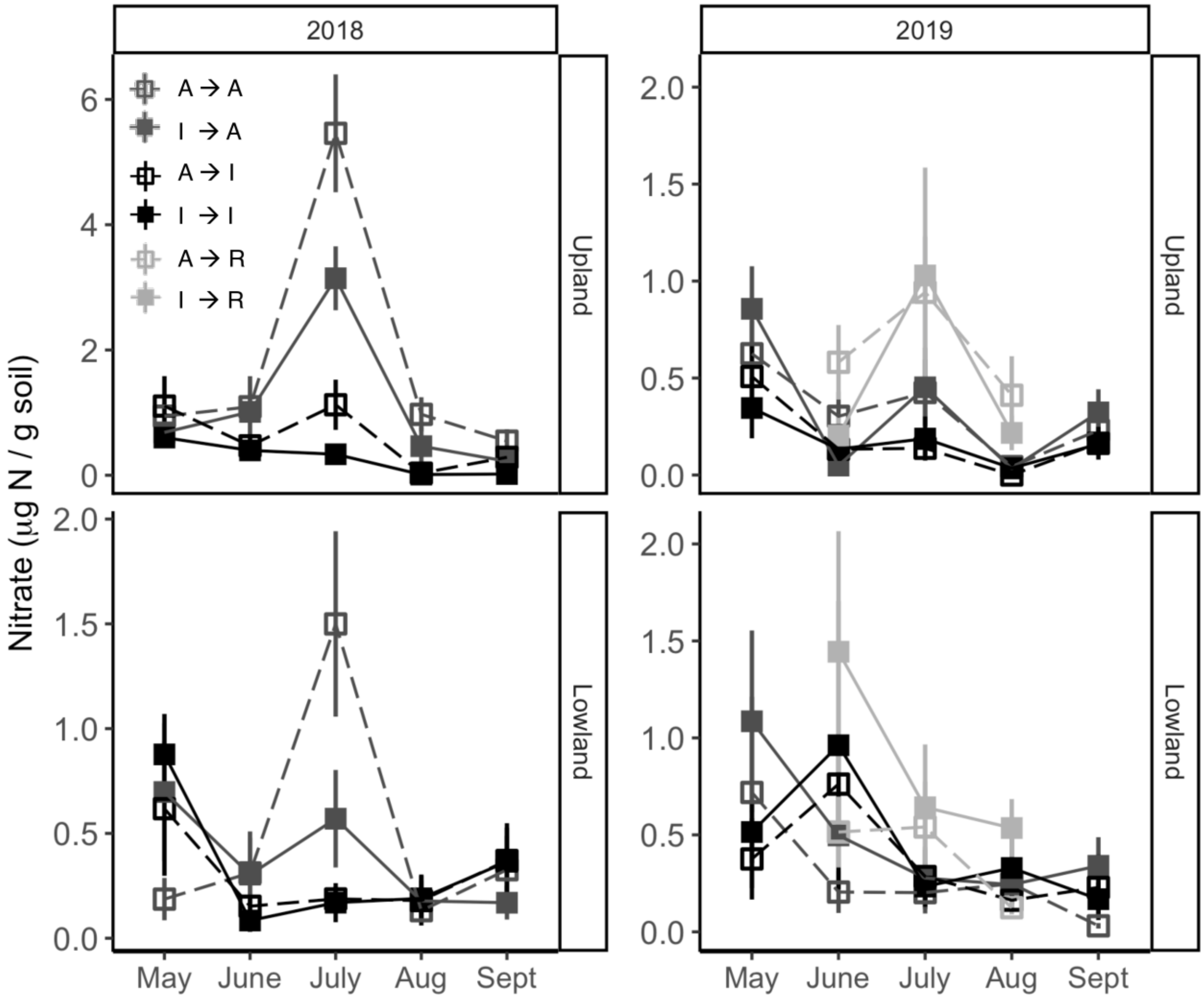

Figure 3.3 Mean soil nitrate concentrations (with standard error bars) 0-10 cm depth across sampling months in upland and lowland prairie in 2018 and 2019. Dark grey outlined points indicate current ambient treatments, black outlined points indicate current irrigated treatments, and light grey outlined points indicate current drought treatments (only June-August 2019). Filled points were previously irrigated.

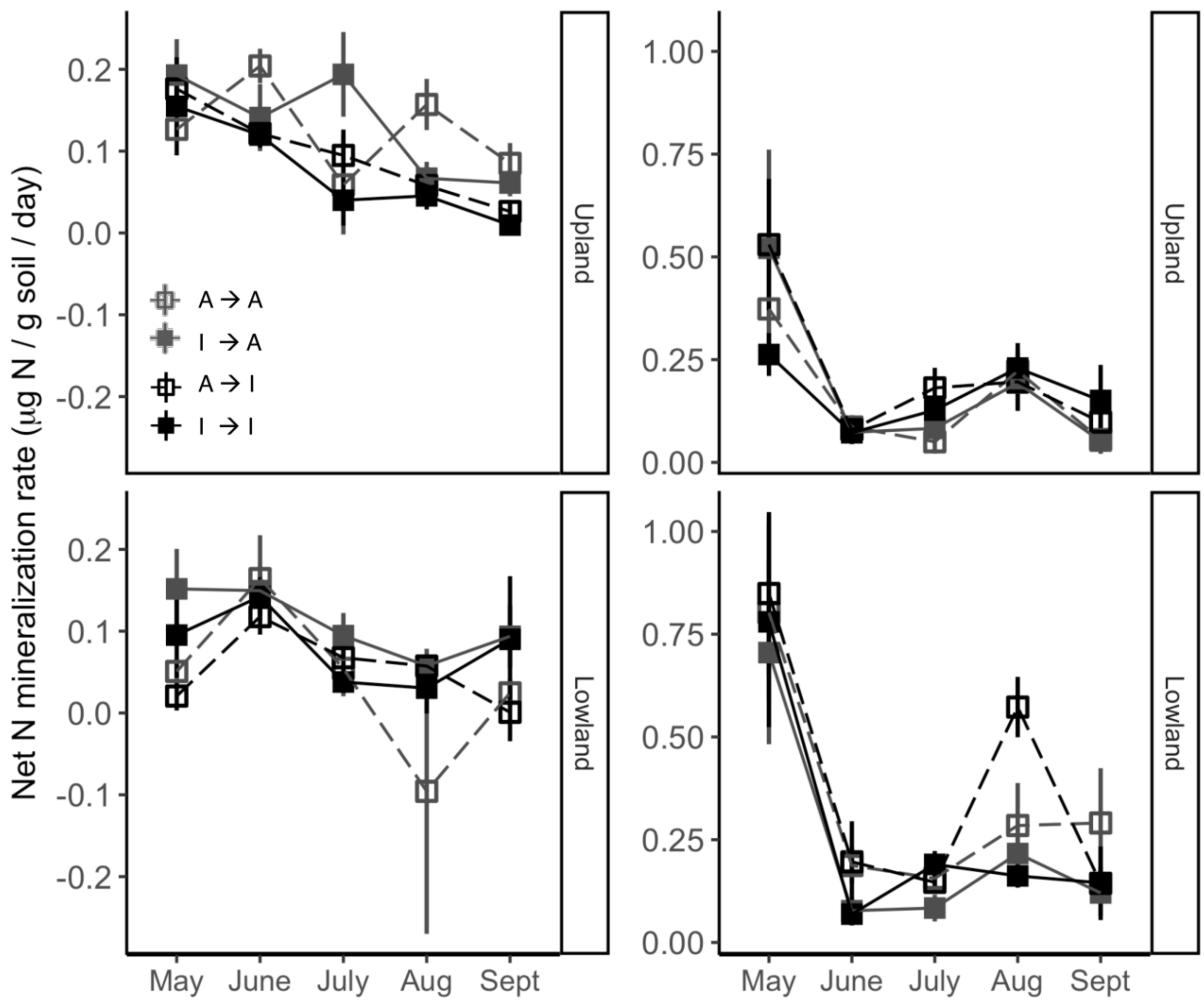

Figure 3.4 Mean monthly net N mineralization rates (with standard error bars) from 0-10 cm in upland and lowland prairie in 2018 and 2019. Dark grey outlined points indicate current ambient treatments, black outlined points indicate current irrigated treatments, and light grey outlined points indicate current drought treatments (only June-August 2019). Filled points were previously irrigated.

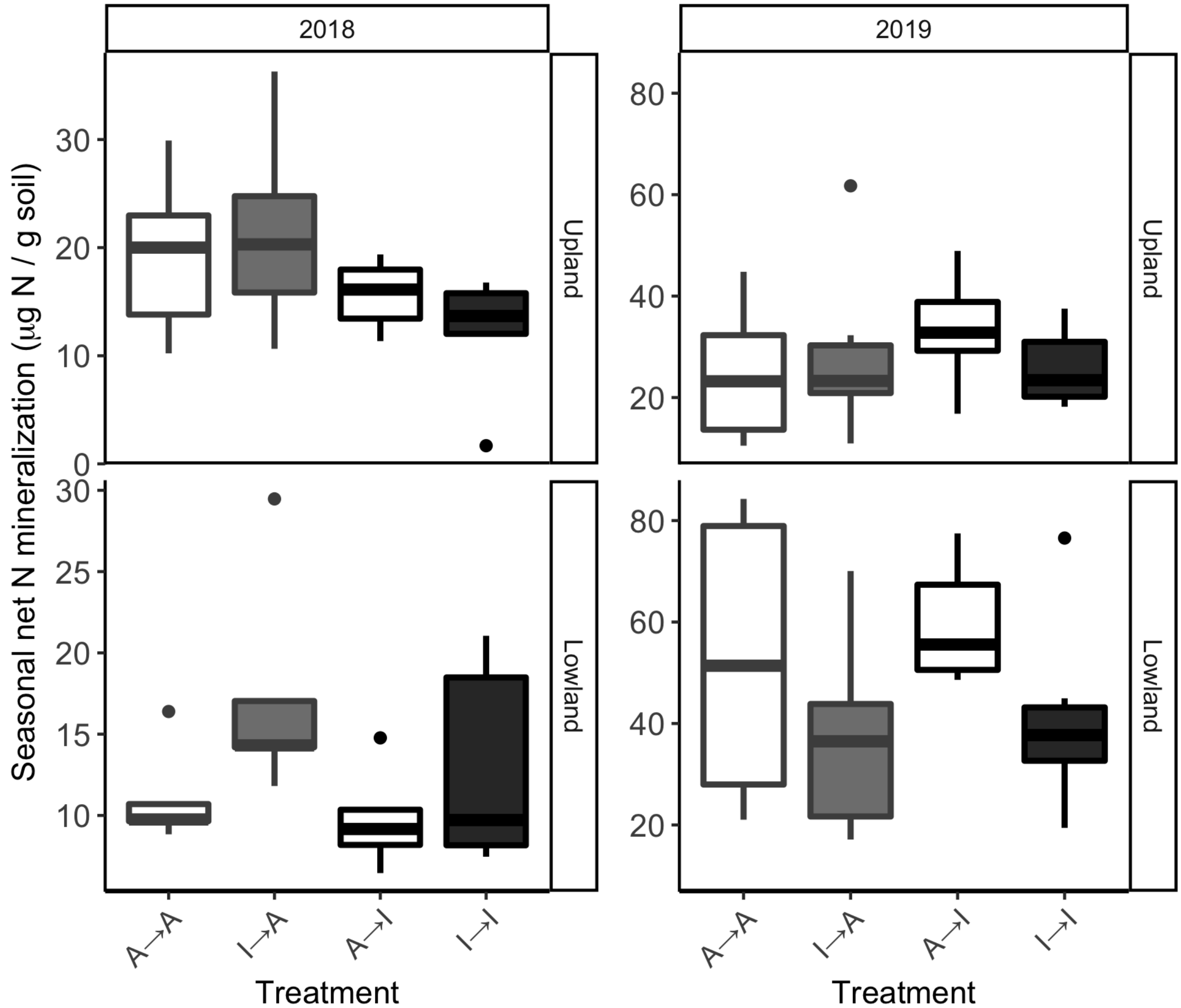

Figure 3.5 Boxplot of seasonal net N mineralization rates from 0-10 cm in upland and lowland prairie in 2018 and 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

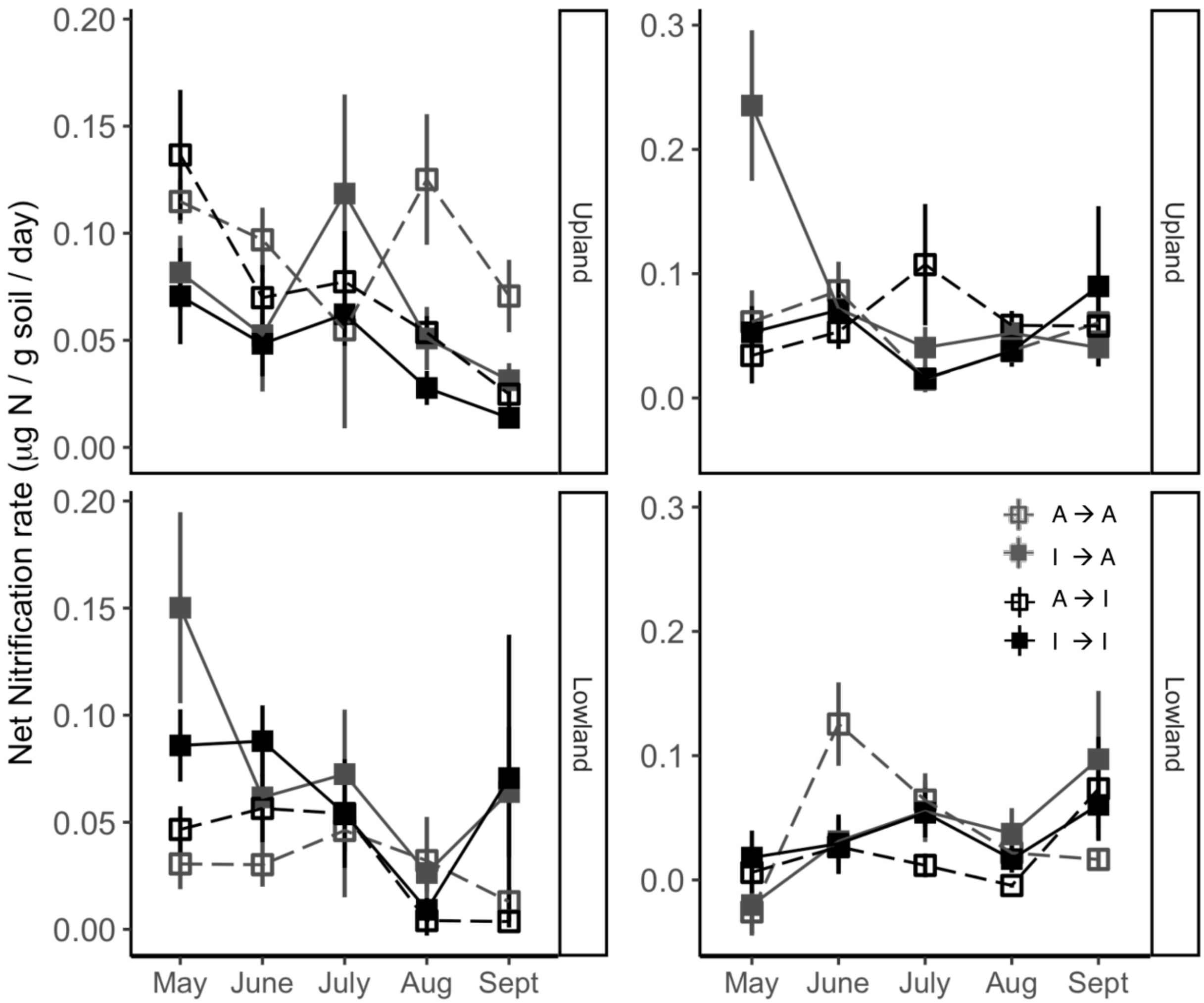

Figure 3.6 Mean monthly net nitrification rates (with standard error bars) from 0-10 cm in upland and lowland prairie in 2018 and 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

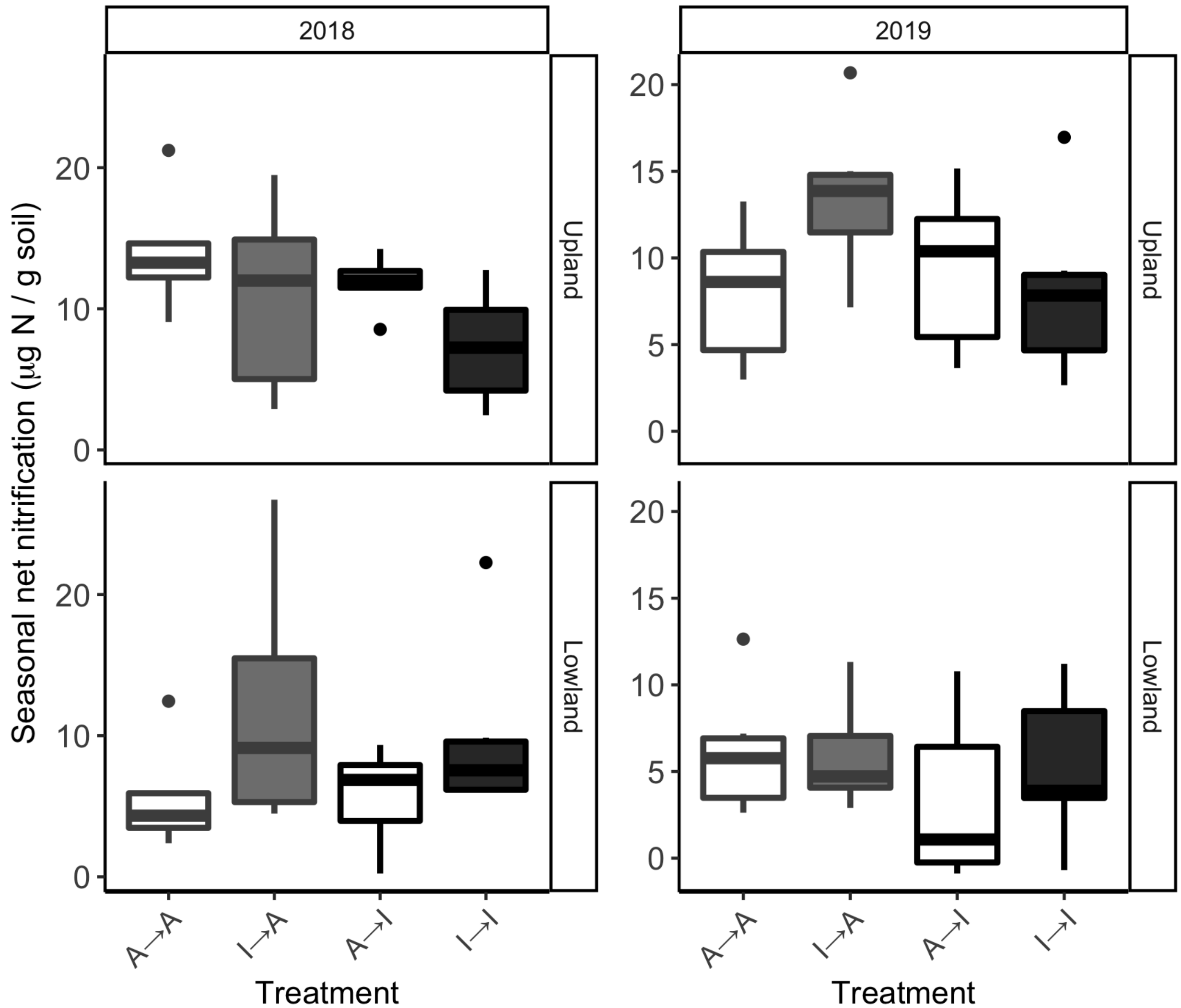

Figure 3.7 Boxplot of seasonal nitrification rates from 0-10 cm in upland and lowland prairie in 2018 and 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

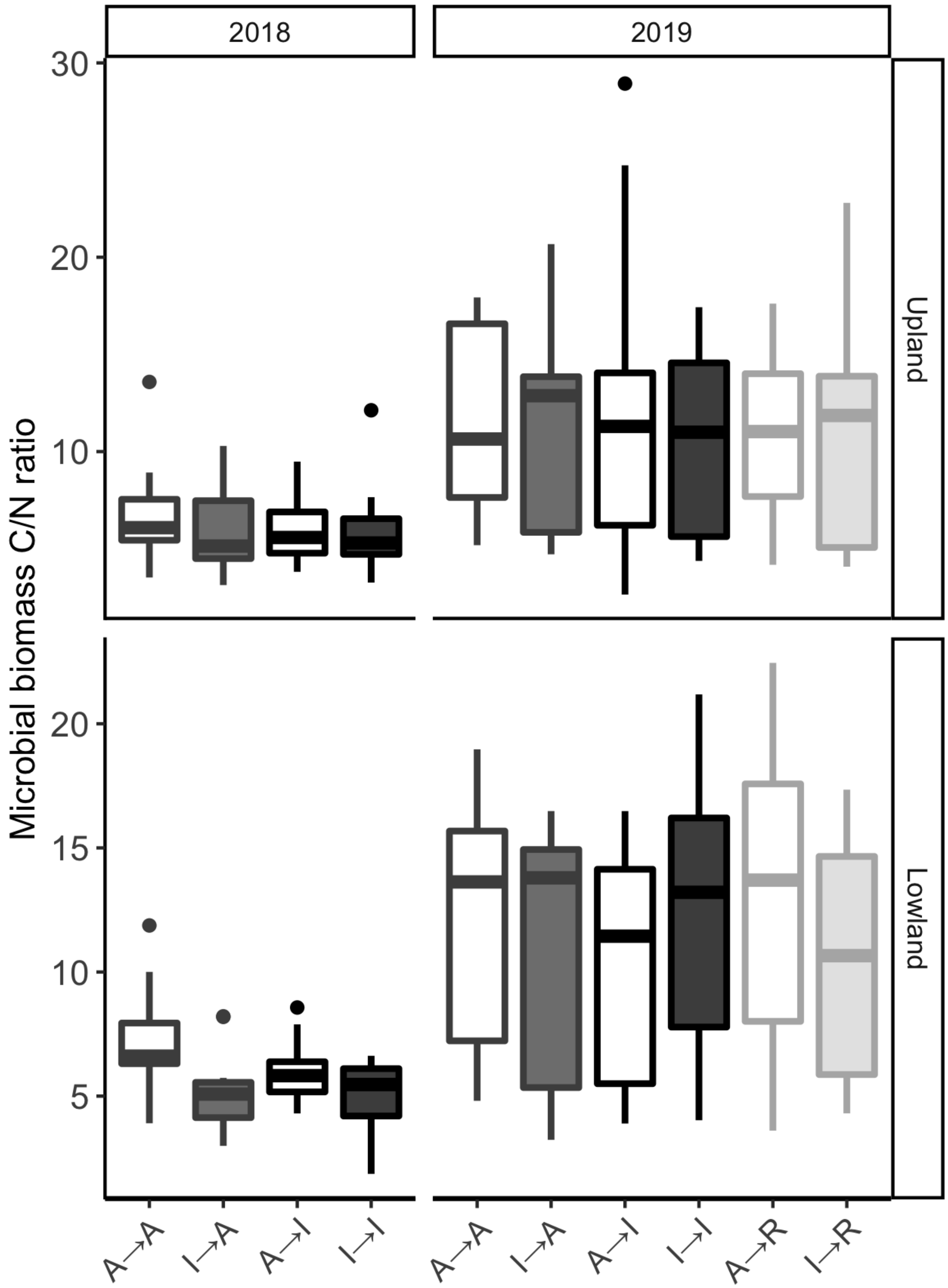

Figure 3.8 Boxplot of microbial biomass C/N ratio from 0-10 cm in upland and lowland prairie in 2018 and 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

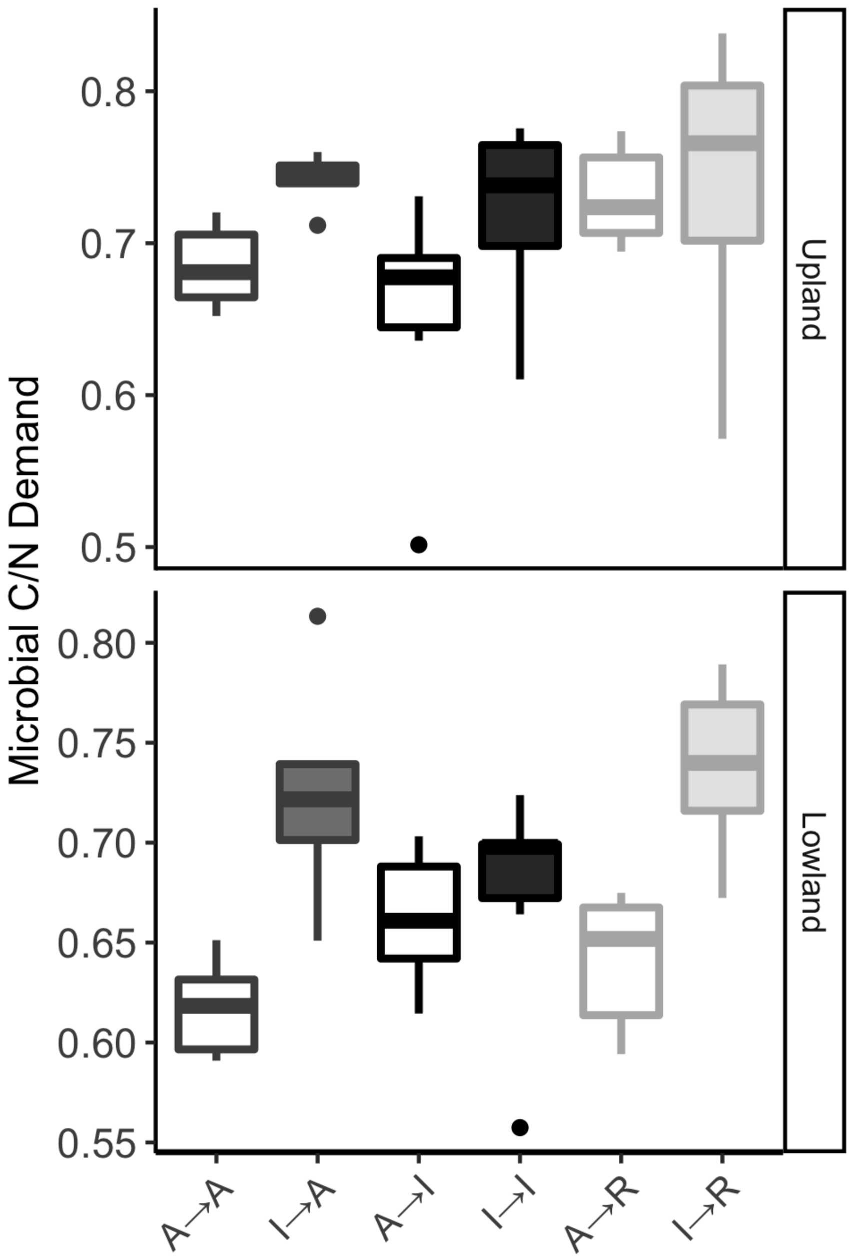

Figure 3.9 Boxplot of microbial relative N demand via investment in extracellular enzymes, from 0-10 cm in upland and lowland prairie in August 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

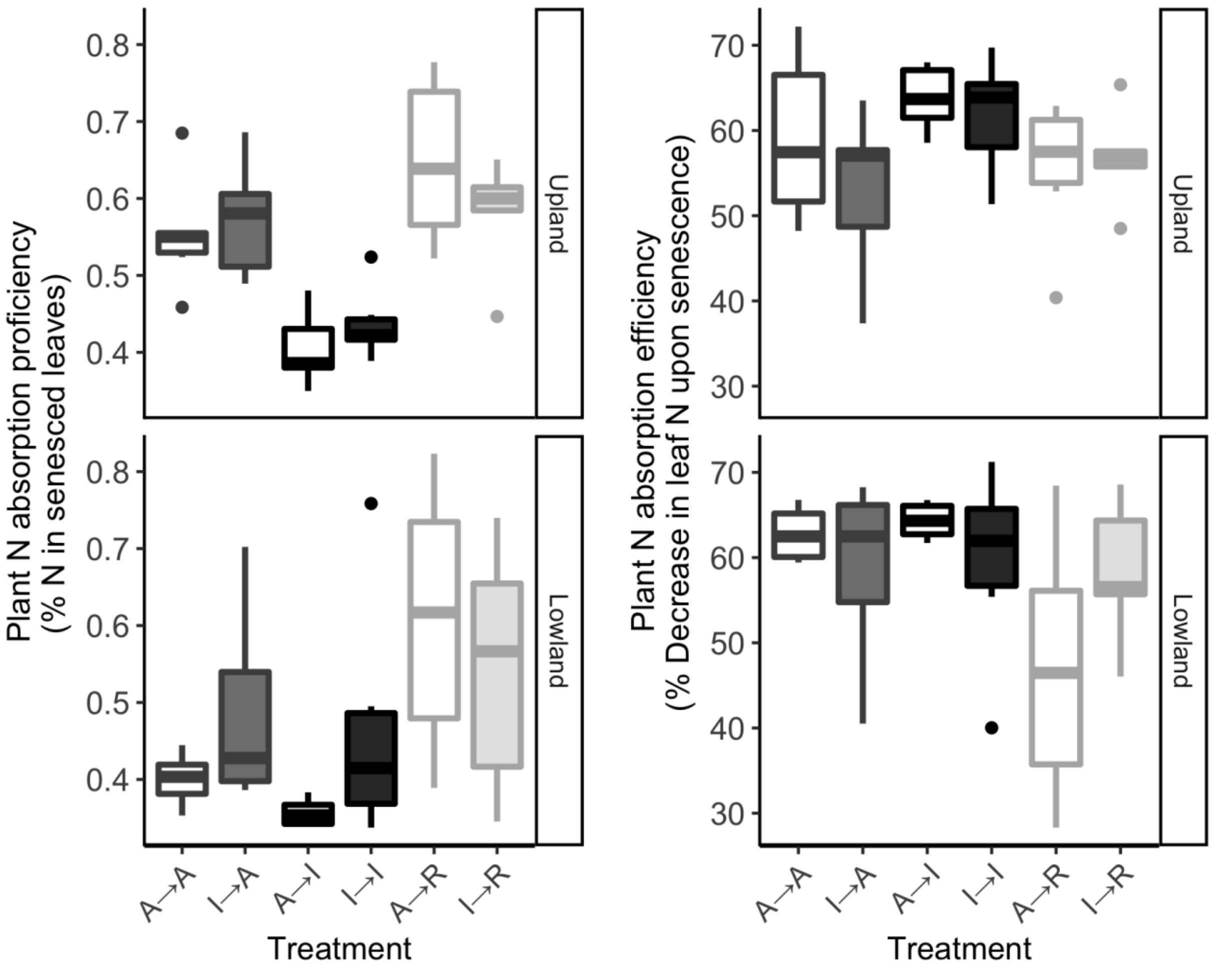

Figure 3.10 N resorption proficiency (%N in senesced leaves) (left) and N resorption efficiency (% decrease in N upon senescence) (right) of; *A. gerardii* in 2018. Box outline color indicates current treatment, and filled boxes were previously irrigated.

Chapter 4 – Relative insensitivity of root decomposition to current and historic rainfall patterns in tallgrass prairie

4.1 Introduction

The cycling of carbon (C) and nutrients in ecosystems depends on the rate at which decomposers break down litter (Chapin et al. 2009; Sokolov et al. 2008). The rate of nitrogen (N) release from litter and soil organic matter (SOM) controls N availability and net primary productivity in many ecosystems (Vitousek and Howarth 1991), and the breakdown of organic matter controls the rate of CO_2 release to the atmosphere (Raich and Schlesinger 1992). The rate of decomposition is often more sensitive than net primary productivity to climate (Parton et al. 1995), and both temperature and precipitation patterns are changing at an unprecedented rate (IPCC 2018). Therefore, it is important to understand how changes in precipitation regimes associated with climate change will affect decomposition dynamics in order to better predict ecosystem functioning in a changing climate.

Climate profoundly shapes the dynamics of both mass loss and nutrient release in decomposing litter. Under low to moderate moisture conditions, increasing soil moisture tends to increase the rate of litter mass loss, soil CO_2 efflux, and N mineralization (Jin et al. 2013; Wu et al. 2020); but at high soil moisture levels anaerobic conditions can occur, slowing decomposition (Neckles and Neill 1994). In addition to the direct effects of soil moisture, climate can also alter litter chemistry, thereby indirectly influencing decomposition rates (Suseela and Tharayil 2017; Prieto et al. 2019; Murphy et al. 2002). Wetter conditions can lead to lower plant N content through nutrient dilution and slower N release from litter, resulting in a tightened N cycle and potentially higher ecosystem N limitation (Aranibar et al. 2004; Ren et al. 2017). Short-term variation in precipitation can alter decomposition directly, for example by increasing leaching losses from litter and stimulating microbial activity (Yahdjian et al. 2006). However, effects of climate on litter quality (Yahdjian et al. 2006; Murphy et al. 2002) and the cycling of co-limiting nutrients (Wu et al. 2012) also may occur on longer timescales that are not captured by short-term studies. Moreover, initial responses of decomposition rates to climate changes may be short-lived if microbial communities can rapidly adapt to novel conditions (Bontti et al. 2009; Guo et al. 2020). Overall, there is a need to assess how decomposition rates respond to longer-term and more persistent changes in climate and the implications for C and nutrient dynamics.

There is increasing evidence that previous climate conditions can exert legacy effects on C fluxes in many ecosystems. For example, positive precipitation legacies on plant productivity can occur following wet years if wet conditions increase tiller density or the availability of co-limiting nutrients (Sala et al. 2012; Reichmann et al. 2013); they can also occur following dry years if the decrease in N demand during drought leads to enhanced N reserves to be accessed following drought (Sala et al. 2012; Hofer et al. 2017). However, the effect on soil C storage of these positive climate legacies on plant productivity is unclear, especially if previous climate patterns exert legacies on decomposition-related C efflux. Strickland and colleagues (2015) found that the climate history of decomposer communities explained as much variation in decomposition as did current climate conditions. Leizeaga et al. (2020) documented climate legacies on soil respiration and suggested that such legacies could be caused by changes in plant inputs and subsequent altered decomposition rates. However, there are few studies of the impact of past climate conditions on decomposition in grasslands, where the primary litter inputs are often belowground. In frequently burned grasslands, such as tallgrass prairie, most plant inputs to soil C are from roots and rhizomes (Rice et al. 1998) and the rates of root decomposition may be an important mechanism by which previous climate conditions can exert legacy effects on soil

CO_2 efflux and nutrient release. Therefore, climate legacy effects on root decomposition rates may modulate current C and N cycling and add to our understanding of how previous climate conditions can affect ecosystem processes (Averill et al. 2016).

Grasslands are important ecosystems that provide forage, support diversity, and store large amounts of C in soil organic matter (SOM) as a product of decomposition (Seastedt and Knapp 1998). They are also limited to varying degrees by water availability and are sensitive to variations in amounts and timing of rainfall (Knapp et al. 1998; Fay et al. 2003). In the central United States, precipitation is projected to become more variable, with longer, more frequent periods of wet and dry conditions (IPCC 2018). The tallgrass prairies in this region are shaped by climate variability and are sensitive to both wet and dry years (Hayes and Seastedt 1987; Knapp and Smith 2001; Fay et al. 2011), but they are also thought to be resilient, showing few climate legacy effects in C cycling (Sala et al. 2012; Griffin-Nolan et al. 2018; but see Moran et al. 2014). However, recent evidence suggests that long-term climate anomalies can lead to persistently altered C fluxes, including ANPP and soil respiration (Broderick et al. 2022). Because of the disproportionately high importance of belowground inputs in grasslands, and potential co-limitation by both water and N, climate effects on belowground decomposition rates have an outsized influence on rates of C fluxes and patterns of N availability (Hui and Jackson 2006). Root decomposition varies with long-term precipitation amounts (Bontti et al. 2009), suggesting that extended periods of wet or dry conditions may alter root decomposition dynamics, as well as affect the sensitivity of decomposition to future climate conditions (i.e., legacy effects). Indeed, long-term climate conditions have been shown to exert legacies on tallgrass prairie ANPP (Broderick et al. 2022) and microbial community functioning (Veach and Zeglin 2019), suggesting that both substrate availability (i.e., plant inputs) and the activity of the decomposer community may also be affected by climate legacies. Therefore, we sought to assess if historical climate conditions contribute to contemporary patterns of litter mass loss, C mineralization, and N release in tallgrass prairies.

To assess the effects of previous and current precipitation patterns on root litter decay rates and N release patterns, we conducted a two-year decomposition study within a long-term grassland water addition experiment. The Irrigation Transect Experiment at Konza Prairie Biological Station (NE KS) has supplemented ambient rainfall with irrigation water since 1991 to decrease plant water stress during the growing season. Amounts of water added varied depending on ambient conditions, but averaged a ~32% increase in growing-season precipitation over the length of the experiment. After ~25 years of treatment, the configuration of the irrigation lines was shifted in 2017 so that irrigated and ambient treatments were switched in half of the plots, with previously irrigated prairie reversing to ambient precipitation and vice versa. Additionally, we also added drought shelters across both previously irrigated and ambient prairie to reduce growing-season rainfall inputs by 66%. This unique combination of treatments allowed us to assess how both past and current climate conditions affected the dynamics of root decomposition in tallgrass prairie. To quantify decomposition, we buried litterbags of *Andropogon gerardii* (big bluestem, the dominant plant in this prairie) root tissue and analyzed patterns in mass loss and N content over two years. Because previous findings from this experiment did not indicate a difference in root chemistry with long-term irrigation treatments (Broderick et al. 2022), we used a common root litter substrate collected from an area of non-irrigated, annually burned prairie in the vicinity of the irrigation experiment. This allowed us to assess climate-induced legacies on mass loss and net N release or immobilization, a function of microbial C and N demand, from a common substrate in all treatments. We hypothesized that supplementing rainfall through irrigation would accelerate decomposition rates, and that imposing drought would decrease rates. Since the shallow, rocky upland soils tend to have greater water deficits, we expected these effects to be greatest in the upland. We hypothesized that a larger/more active microbial community as a legacy of historically wetter conditions (Broderick et al. 2022) would maintain elevated decomposition rates in plots with a history of irrigation. Finally, we also predicted that, based on a documented increase in N availability and N cycling rates in previously irrigated prairie (Broderick, unpublished data), that another legacy of a historically wetter climate would be accelerated N release from litter.

4.2 Methods

Study site—This study was conducted at Konza Prairie Biological Station, a Long-Term Ecological Research site in Manhattan, Kansas, USA. The 30-year average temperature in this region is 12.8°C, and the thirty-year average precipitation is 851mm, with most of that falling during the growing season (April-September). The experiment took place in an annually burned native tallgrass prairie from which large grazers were excluded. The experiment spanned a moderate topographic gradient typical of the region, with uplands and lowlands separated by a steep, rocky slope. The upland site is characterized by shallow (<0.5 m), rocky soils in the Clime-Sogn complex (fine, mixed mesic Udic Haplustolls; 15% sand, 58% silt and 27% clay), and are more subject to water stress. The lowland has deeper (>1 m) soils in the Irwin series (fine, mixed mesic Pachic Argiustolls; 15% sand, 51% silt and 34% clay).

The first year of the decomposition study, 2019, was an exceptionally wet year (annual precipitation 1131 mm), while the second year had closer to average precipitation (833 mm). The experiment ran through June 2021, which was a dry year (632 mm annual precipitation; Appendix C, Table 1; Appendix C, Figure 1).

Irrigation Transect Experiment—To assess the effect of previous and current precipitation regimes on decomposition dynamics, we used contrasting treatments in a long-term field experiment, the Irrigation Transect Experiment, which was designed to assess the responses of tallgrass prairie to extended release from water stress. In native annually burned prairie, a paired irrigation line and control transect was established in 1991 spanning an upland-lowland gradient; in 1993, a second replicate irrigation line and control transect was also installed (Appendix C, Figure 2). The irrigation lines were equipped with impact sprinklers ~1 m above the ground that distributed water in a ~15 m radius evenly across the transect. Groundwater was applied to irrigated transects between 05:00 and 13:00 as needed to maintain soil water content at or above 0.25 cm^3/cm^3 throughout the growing season of each year. Irrigation input varied among years based on ambient precipitation, but on average increased growing-season precipitation inputs by 32% (Caplan et al. 2019). Along these paired irrigated and control (ambient precipitation) transects, 10-m^2 circular sampling plots were established to record plant community composition; all sampling in this study was conducted immediately outside these plots to avoid disturbing long-term species composition plots.

To compare the effect of previous and current precipitation treatments on ecosystem processes, in 2017 we reversed the treatments in a subset of plots by shifting the irrigation lines on half of the upland and lowland plots (i.e., previously irrigated plots were now ambient and vice versa). We maintained existing long-term treatments in the remaining plots. We also added a new reduced rainfall treatment in both the previously irrigated and ambient treatments. We erected 24 3x3m rainout shelters with roofs made of polycarbonate slats placed to passively divert 66% of incoming rainfall (Yahdjian and Sala 2002). These shelters were deployed in April of each year and were set ~1 m off the ground; shelters were raised as needed to accommodate vegetation until they were taken down in November. This generated 72 total plots: six replicates of each treatment combination (long-term ambient, A→A; irrigated to ambient, I→A; ambient to irrigated, A→I; long-term irrigated, I→I; ambient to reduced, A→R; irrigated to reduced, I→R) in both the uplands and the lowlands (Appendix C, Figure 2). This unique experimental design allows us to compare short- and long-term effects of precipitation regimes, as well as identify legacy effects of previous climate treatments.

Decomposition experiment—In June 2019, we began a two-year experiment assessing the effect of previous and current climate treatments on belowground decomposition rates. We harvested living roots of *A. gerardii* from a nearby (<0.5 km) site and air-dried them for 2 days. We constructed 5 cm x 10 cm litterbags from 16 x 18 mesh fiberglass window screen (1.1 mm x 1.3 mm openings), and filled each bag with 1.5 g of air-dried root litter. This mesh size allows most micro- and meso-fauna to access litter (Robertson and Paul 2000). In June of 2019, six bags were buried near each long-term sampling plot (~1 m outside to minimize disturbance), approximately 5 cm deep and within 1 foot of each other. Five "traveller" bags were taken into the field, immediately returned, then oven dried at 60°C for 2 days and weighed to get an adjusted initial mass. These bags were used to account for (1) litter lost in transport and (2) the difference between air-dried initial masses and oven-dried final masses. One randomly chosen litterbag from each plot was collected at 72, 148, 275, 375, 630, and 735 days, so that the total length of the study was ~2 years. A few bags were unearthed by animals; in this case we skipped collection at those plots during one of the middle collection dates (most often at 630 days) to maximize sample size for the final collection date. Litter in retrieved bags was gently washed to remove soil, dried, and weighed. Material from litterbags were then ground on an 8000D mixer/mill (SPEX, Metuchen, NJ) and analyzed for total C and N content on a Flash EA 1112 C/N auto analyzer (Thermo Fisher Scientific, Waltham, MA). Litterbag masses were corrected for soil contamination using the following equation (Harmon et al. 1999; Norris et al. 2001):

$$LF = (SaC - SlC)/(LiC - SlC)$$

where LF is the fraction of the bag that is actually litter, SaC is the %C of the sample, SlC is the %C of the soil, and LiC is the %C of the litter. The N concentration of buried litter were similarly corrected for soil contamination using the following equation (Harmon et al. 1999; Norris et al. 2001):

$$LN = (SaN - (FSl \times SlN))/LF$$

where LN is %N of the litter, SaN is the %N of the sample, FSI is the fraction of sample that is soil (i.e. 1- LF), and SlN is the %N of the soil. C and N soil values for corrections were determined separately for the upland and lowland areas, and averaged across climate treatment since these pools are large and have not changed with climate treatments (Broderick et al. 2022)

Data analysis—. We calculated decomposition (decay) rates using a single exponential decay model with the equation:

$$X_t / X_0 = e^{-kt}$$

where X_t / X_0 is the proportion of mass remaining at time t, k is the annual decay rate, and t is time elapsed in years. We compared mass loss and net N dynamics of litter in each treatment during the two-year experiment using linear mixed models in R (R core team) with the packages lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), emmeans (Length et al. 2021), MuMIn (Bartoń 2019), and tidyverse (Wickham et al. 2019). Current treatment, previous treatment, and landscape position were main effects in all models (except when separate models were run for uplands and lowlands to determine decay rates as influenced by topographic position); plot nested within replicate transect was a random effect for response variables sampled repeatedly within a plot. When assessing differences in response variables among sampling time points, time point was treated as a categorical variable, except when calculating decay rates. Data were log-transformed as necessary to meet assumptions of homogeneity of variances.

4.3 Results

Mass loss and decay rates—At the end of the two-year study, a greater percentage of litter mass remained in the lowlands than in the uplands (43% vs. 35%; $p<0.001$; Figure 2), indicating slower decomposition in the lowlands. Across topographic positions, mass loss also varied with current treatment but not as expected, with the irrigated treatment having a higher percentage mass remaining (44%) than the reduced rainfall treatment (35%; $p=0.027$). There was no significant effect of previous rainfall treatment on the percent mass remaining at the end of the study.

A model assessing mass across all sampling dates revealed that previous irrigation resulted in greater mass remaining in the lowlands ($p=0.043$), contrary to our hypothesis; the opposite pattern (greater mass in the previously ambient treatment) emerged in the uplands but was not significant. An interaction between previous and current treatments showed that, across sampling dates, mass remaining in the long-term continuous irrigation (I→I) treatment tended to be higher than that in the drought treatments, regardless of treatment history (A→R: p=0.029; I→R: p=0.089). Finally, there was a significant four-way interaction between time, current treatment, previous treatment, and landscape position. At day 149 in the uplands, the percent mass remaining in the previously irrigated (I→A; 55%) was lower than the control (A→A; 74%; p=0.026) and marginally lower than the long-term irrigated treatment (I→I; 72%; p=0.075). At the final time point in the lowlands, the long-term irrigated treatment had a higher mass remaining (57%) than the drought treatment (A→R; 35%; p-0.003), and a marginally higher mass remaining than the previously irrigated treatment (I→A; 40%; 0.077). No other treatments differed at any sampling time point.

Decomposition rate (k)—An initial ANCOVA over all data suggested that the litter decay rate was higher in the uplands (k=0.486) than the lowlands (k=0.419; p<0.001); however, due to complex interactions, we subsequently ran separate ANCOVAs within each topographic position to assess precipitation treatment effects. Treatments did not affect decay rates in the uplands. In the lowlands, the long-term irrigated treatment (I→I) had a lower k than both previously irrigated treatments (I→A and I→R; p=0.012 and p-0.006, respectively; Table 1), consistent with higher mass remaining across dates in the lowland previously irrigated treatment.

Nitrogen concentration of litter over time— Nitrogen concentration increased over the course of the study period across all treatment combinations, from 0.42% initially to an average of 0.66% at the final time point (735 days). Averaged across the entire study, there was a lower litter N concentration in the currently irrigated treatment than in the current ambient (p=0.004) or reduced (p<0.001) rainfall treatments. Similar to mass loss patterns, there were also several significant interactions; we focus on the most notable here. An interaction between current and previous treatment showed that litter in the long-term continuously irrigated treatment (I→I) maintained a lower N concentration than the irrigation reversal treatment (I→A; p=0.003), irrigation-to-drought treatment (I→R, p<0.001), and (marginally) the long-term ambient treatment (A→A; p=0.067). Litter in the I→R treatment maintained the highest average N concentration of all treatment combinations, and differed from that in the short-term irrigated treatment (A→I; p=0.018) and marginally from that in the long-term ambient treatment (A→A; p=0.051). Finally, a four-way interaction between time point, current treatment, previous treatment, and landscape position suggested that these treatment differences were most pronounced later in the study. At 275 days, average litter N concentration in the long-term irrigated treatment was significantly lower (0.44%) than the irrigation reversal (I→A; 0.60%; p=0.040) in the uplands. At 630 days in the lowlands, litter in the long-term irrigated treatment had significantly less nitrogen (0.49%) than the irrigation-to-drought treatment (I→R; 0.72%, p<0.001), and marginally less nitrogen than the irrigation reversal treatment (I→A; 0.64%; p=0.062). Additionally, at 630 days in the lowlands the two drought treatments differed, with a history of irrigation resulting in the I→R treatment having a higher N concentration than the A→R treatment (0.54%; p=0.016). Consistent with the end-of-experiment data [previous paragraph], at the final time point (735 days) in the lowlands, the N concentration in the I→I treatment (0.58%) was lower than that in the I→R treatment (0.80%, p=0.009), and marginally lower than the I→A treatment (0.75%; p=0.074). At the final time point in the uplands, litter N concentration in the long-term ambient treatment was higher than that of the short-term irrigated treatment (p=0.003) and the long-term irrigated treatment (p=0.010), and marginally higher than that of the drought treatment with no irrigation history (A→R; 0.61%; p=0.056).

When we analyzed the final litter N concentrations, N concentration in the remaining litter was lower in the currently irrigated treatment than in ambient (p=0.001) or reduced (p=0.018) rainfall treatments (Figure 3). A three-way interaction showed a higher N concentration in the long-term ambient treatment in the uplands (0.77%), that exceeded that of the short-term irrigated (A→I) treatment (0.55%; p=0.013) and marginally exceeded that of the long-term irrigated (I→I) treatment (0.56% = 0.072). In the lowlands, the long-term irrigated treatment had a lower N concentration (0.58%) than the irrigated-to-reduced treatment (I→R; 0.80%; p=0.019).

Nitrogen release or immobilization—Net release or immobilization of N, often expressed as % change in the absolute amount of N in the litter, is a function of both mass loss and changes in N concentration of the remaining litter. The percent N remaining in the root litter generally decreased during the study, with much of that decline occurring after day 375—dropping to an average of 51% at 630 days, before rising to 57% at the end of the study (735 days; Figure 4). The average percent N remaining was greater in the lowlands than the uplands throughout the study (p<0.001), consistent with slower mass loss in the lowlands. In the lowlands, litter in the previously irrigated treatments had a higher %N remaining than that in previously ambient treatments (p=0.004). An interaction between previous treatment, landscape position, and sampling point indicated that historic irrigation affected N release at specific dates. At 149 days, previous irrigation resulted in lower %N remaining in the uplands (69% vs 78% in ambient; p=0.070), but higher %N remaining in the lowlands (93% vs 80% in ambient; p=0.070). Similarly, at 375 days litterbags in the previous irrigation treatment in the lowlands had marginally higher %N remaining (95% vs. 85% in ambient, p=0.056)

N immobilization potential of litter—The relationship between mass loss and changes in N concentration in the remaining litter can provide insights into the immobilization potential of decomposing litter (Aber and Melillo 1982; Seastedt et al. 1992. However, when we assessed how percent mass remaining varied as a function of N concentration, there was no effect of current treatment, previous treatment, or landscape position on the slope of this relationship (Appendix 4, Figure 2), indicating similar immobilization potential as a function of mass loss among all treatment combinations.

4.4 Discussion

In grassland ecosystems, the sensitivity of belowground decomposition to precipitation is thought to be an important component of the response of ecosystem C and N cycling to climate change (Bontti et al. 2009; Penner and Frank 2019). However, it is difficult to separate short- and long-term responses to climate changes, and there have been few, if any, studies of whether root decomposition rates are affected by past as well as current climate conditions. Here, we showed that tallgrass prairie root decomposition is surprisingly resistant to short-term, current (3-5 year) increases in precipitation. However, a longer-term history of release from water stress (nearly 30 years of wetter conditions) in lowland prairie slowed mass loss rates. This was contrary to our expectations and the common observation that decomposition rates increase along grassland precipitation gradients (McCulley et al. 2005; Bontti et al. 2009). Moreover, root litter decomposed the fastest in treatments where drought stress was recently exacerbated (i.e. A→R and I→A treatments), suggesting that decomposition is more sensitive to short-term decreases in precipitation than increases in precipitation. While we did find differences in N release patterns that were consistent with patterns of mass loss among treatments, most notably lower litter N concentrations across dates in the long-term irrigated treatment in the lowlands, we did not find differences in the N immobilization potential of litter, suggesting that differences in N concentrations among treatments are driven by differences in mass loss rates. Our results are consistent with previous work suggesting that litter mass loss and ecosystem N cycling responses to climate can be decoupled (Yahdjian et al. 2006). Overall, climate legacy effects in tallgrass prairie were not as pronounced in litter decomposition as they were for other ecosystem C fluxes and N transformations (Broderick et al. 2022; Broderick, unpublished data). These results further suggest that previously reported positive legacies of irrigation on soil respiration and N mineralization are most likely derived from the SOM pool in these grasslands, rather than from fresh root litter.

Mass loss responses to climate treatments—Surprisingly, rates of root litter mass loss tended to be slower with long-term irrigation. Slower rates of mass loss in plots with a history of long-term irrigation is in contrast to our hypothesis and to the generally observed increase in both leaf (McCulley et al. 2005) and root (Bontti et al, 2009) decomposition rates with increasing precipitation across the Central Plains. However, root decomposition does not vary as strongly with precipitation as leaf decomposition (Silver and Miya 2001; See et al. 2019) and may be more sensitive to saturated soil conditions. While precipitation is especially important for physical breakdown and leaching in surface litter (Austin and Vitousek 2000; McCulley et al. 2005), very wet soils can result in anaerobic conditions and slow decomposition of buried roots (Neckles and Neill 1994). Slower decomposition in wetter soils has been previously documented in tallgrass prairies (von Haden and Dornbush 2014). The start of our decomposition experiment coincided with a naturally wet year (2019), during which lowland soils were periodically waterlogged. However, the greatest divergence in mass loss among treatments occurred in the second year of the study. In addition, only long-term (I→I), and not short-term (A→I), water addition led to decreased decomposition, suggesting that factors other than current soil water content played a role in affecting decomposition rates. Climate legacy effects on decomposition have been linked to changes in microbial community composition (Allison et al. 2013; Haugwitz et al. 2016), so responses of decomposer communities to wetter conditions may have developed in the long-term treatment. Indeed, research conducted earlier in this experiment found that soil microfaunal decomposer communities changed with extended irrigation, with decreases in microbial-feeding mites and nematodes (O'Lear et al. 1999; Todd et al. 1999). Additionally, a recent short-term (2-year) precipitation addition experiment at Konza prairie also documented reductions in microbial-feeding nematodes, and less C respired by nematodes, under wetter conditions (Franco et al. 2021). Both of these results are consistent with the slower decomposition seen with persistent water inputs. Regardless of the specific mechanism, these results emphasize that long-term responses of decomposition to changes in precipitation patterns may not be revealed by short-term studies.

Some of the fastest decomposition rates in the lowlands were in prairie subject to exacerbated water stress compared to past conditions (i.e., the I→A, A→R and I→R treatments). This was unexpected, as previous studies have suggested that root decomposition decreases under experimental drought in tallgrass prairies (Reed et al. 2009; Harper 2002). However, another recent study in this same experiment documented a decrease in microbial biomass coupled with an increase in soil CO_2 flux upon re-introducing water stress (Broderick et al. 2022), which would be consistent with accelerated decomposition in these treatments. It is possible that the physical disruption associated with more frequent dry-wet cycles accelerated physical fragmentation of litter and/or microbial activity, and therefore root decomposition, in prairies with increased drought stress (Birch 1958). Alternatively, since these treatments only showed the fastest decomposition in the lowlands, it is possible that this effect is due to the comparative release from saturated soil conditions relative to persistently wet soils for portions of the study period. This explanation is supported by the higher decomposition rates across all treatments in the uplands of our study. Further research is needed to assess whether this drought effect varies consistently with topoedaphic factors (e.g., consistent increases in fine-textured, lowland soils) to tease out these potential explanations.

Nitrogen dynamics of litter—Litter in the long-term irrigated treatment generally maintained lower N concentrations throughout the study, especially in the lowlands. This is most likely due to slower decomposition with irrigation in the lowlands, since there was no difference among treatments in the relationship between mass loss and nutrient concentration (i.e., immobilization potential). This is consistent with research in arid grasslands that found differences in mass loss, but not nutrient immobilization potential, along grassland precipitation treatments (Yahdjian et al. 2006).

While N concentration of the litter increased throughout the study, the mass of N present in litter almost never exceeded initial amounts and we did not see a significant net immobilization phase. This is consistent with previous findings at Konza Prairie that grassland roots do not readily immobilize nitrogen despite a relatively high initial C/N ratio (Seastedt et al. 1992; Harper 2002; Parton et al. 2007). The one exception was the I→R treatment after about 1 year of decomposition. Drought tends to create high concentrations of available N in this grassland by reducing mobilization and plant uptake (Seastedt and Knapp 1993); this may account for the higher N content and periods of net immobilization in this treatment.

Because previous research in this experiment found accelerated N cycling with a history of irrigation, the lack of responsiveness of root litter N dynamics was unexpected. We hypothesized that faster net N release from litter in previously irrigated prairie would have occurred and contributed to a previously reported increased N pool for plant and microbial growth; yet N was released more slowly from litter under long-term irrigation due to a slower decay rate. It is possible that the rapid turnover of fine roots (Stewart and Frank 2008), not measured in this study, contributed to patterns of N availability with previous and current precipitation regimes in these soils. Additionally, the lack of response of root litter N release to treatments may reflect that the largest substrate for N mineralization (and subsequently nitrification) in grasslands is SOM rather than litter, so decomposition dynamics can vary independently of rates of soil N cycling (Yahdjian et al. 2006). Moreover, the soils in these grasslands have a very high N immobilization potential so that decaying roots do not immobilize N in the litter, regardless of the degree of N limitation (Seastedt et al. 1992). Therefore, the documented increase in N supply rates and alleviation of plant and microbial N limitation with a legacy of previous irrigation appear to be less tied to litter N release than N release from SOM. Other work has also found that, while increased N availability can accelerate aboveground decomposition, it can lead to slower belowground decomposition in savannas, perhaps due to changes in the decomposer community (Norris et al. 2013). We previously documented that previous irrigation increased rates of N supply in this system (Broderick et al. 2022), offering further evidence that patterns in available N can operate independently of rates of litter N release.

<u>Spatial and temporal trends in decomposition dynamics</u>—Litter mass loss responses varied with topodaphic conditions and across sampling timepoints. Other C cycling processes also vary across upland and lowland positions in this grassland, with higher ANPP in lowlands particularly in frequently burned prairie (Briggs and Knapp 1995). While there has been less work on belowground C fluxes across topography in this system, decomposition rates have been found to vary across topographic gradients in other hilly grasslands (Gong et al. 2016). The high clay content in the lowland soils may better protect belowground litter from decomposition (Lane and BassirRad 2005), and the high water holding capacity of these fine-textured lowland soils may have contributed to periodic saturated, anaerobic conditions in the irrigated treatment. Differences in decomposition rates among treatments were also most pronounced in the later stages of decomposition. The first growing season of this study was 2019, a naturally wet year, so it is possible that nearly all soils were similarly saturated until later in the study during which irrigation treatments substantially altered water availability. Although the higher responsiveness of the wetter lowlands to previous climate treatments was unexpected, other studies suggest that wetter grassland sites—in this case, lowland soils—are more likely to experience persistent changes in soil moisture with long-term irrigation, leading to altered ecosystem functioning (Moran et al. 2014; Smith et al. 2009). The responses to climate treatments in this fine-textured, mesic grassland differ substantially from findings in more drought-prone grasslands (McCulley et al. 2005). Since tallgrass prairies are uniquely high-producing, carbon-storing ecosystems, these findings help differentiate an important distinction in how decomposition dynamics in these grasslands will respond to short- and long-term climate changes.

Conclusions—To predict how changes in climate will alter C and N cycling in grasslands, a better understanding how decomposition responds to short- and long-term changes in precipitation is needed. A history of release from water stress with irrigation slowed root decomposition, while drought stress led to faster decomposition rates. Therefore, unlike in more arid grasslands, an increased frequency of dry years may increase C loss from tallgrass prairie ecosystems. Moreover, rates of N release from decomposing litter did not vary with previous or current climate treatments, despite changes in N cycling across previous climate treatments, highlighting how patterns in N availability can operate independently of decomposition rates in these grasslands. Finally, our work stresses the importance of topoedaphic factors in determining climate sensitivity of decomposition rates and raises new questions about the generality of mechanisms controlling decomposition rates across topographic gradients.

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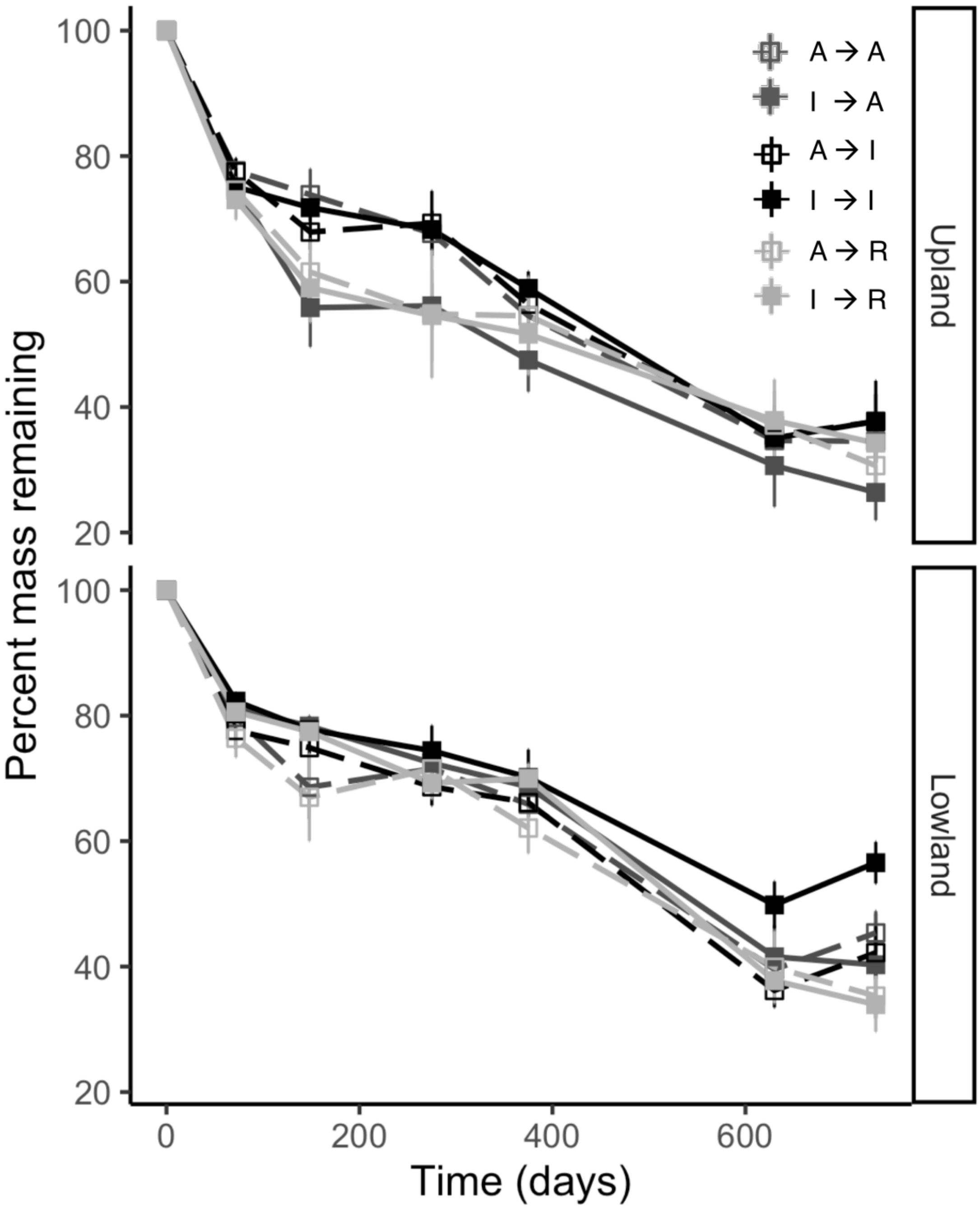

Figure 4.1. Percent mass remaining in litterbags across the study period. Points represent mean (+SE) mass in each treatment at each sampling date. Shape and outline colors on points indicate current treatment. The first letter shows previous treatment (ambient (A) or irrigated (I), followed by current treatment (A, I or reduced (R) rainfall treatment). Filled shapes and solid lines indicate historically irrigated plots, while open shapes and dashed lines indicated previous treatment.

Table 4.1. Annual decay rates for each treatment combination in upland and lowland tallgrass prairie, as determined by analysis of covariance. The first letter shows previous treatment (ambient (A) or irrigated (I), followed by current treatment (A, I or reduced (R) rainfall treatment). Marginal R^2 values were obtained from a linear mixed model for each treatment combination.

		k	R^2			k	R^2
Upland	A→A	0.519	0.776	Lowland	A→A	0.354	0.667
	I→A	0.591	0.616		A→I	0.519	0.492
	A→I	0.484	0.586		I→A	0.405	0.744
	I→I	0.477	0.668		I→I	0.257	0.615
	A→R	0.440	0.321		A→R	0.446	0.651
	I→R	0.403	0.481		I→R	0.534	0.770

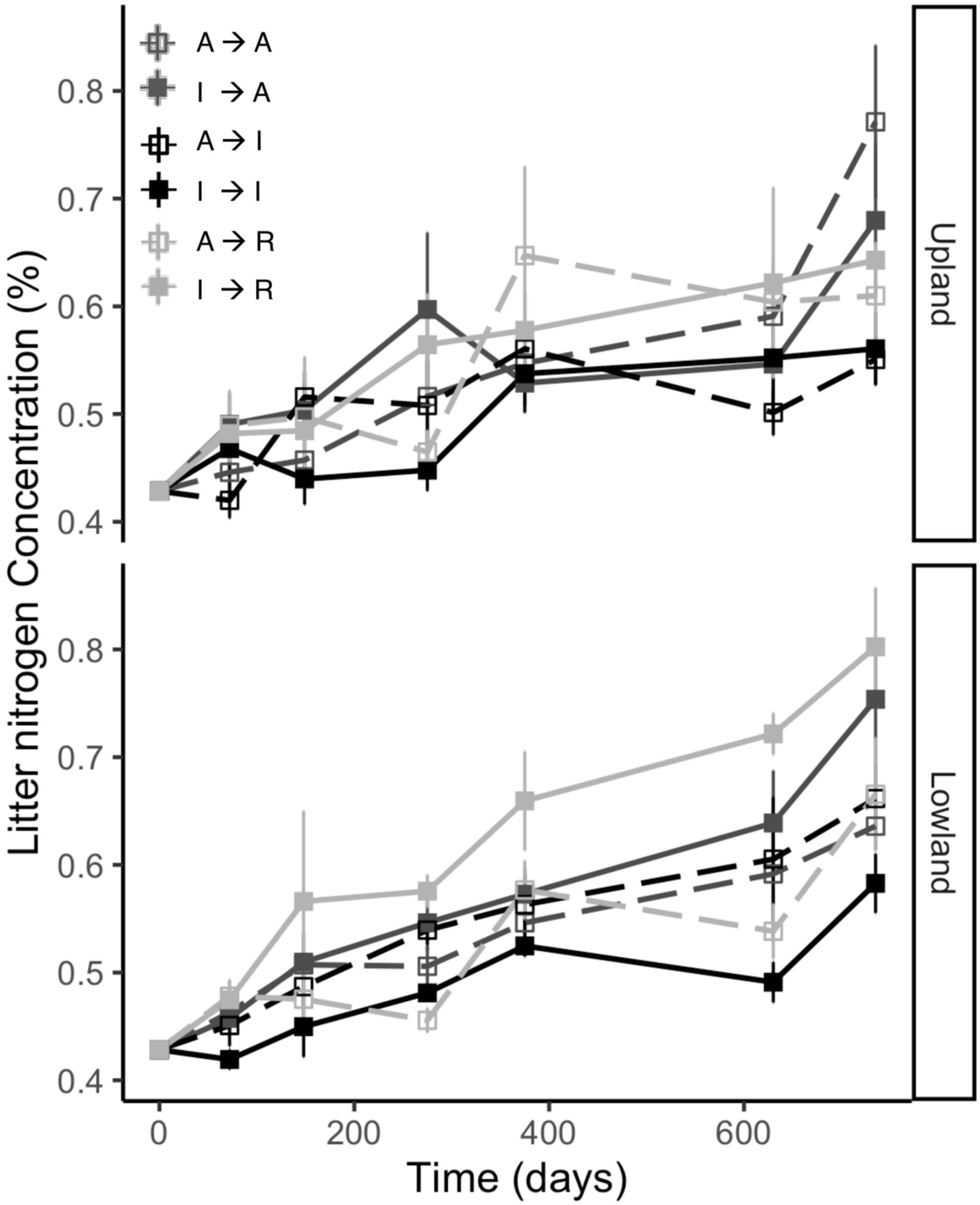

Figure 4.2. Nitrogen concentration in litterbags across the study period. Points represent mean %N in each treatment (+SE) at each sampling date. Shape and outline colors on points indicate current treatment. The first letter shows previous treatment (ambient (A) or irrigated (I), followed by current treatment (A, I or reduced (R) rainfall treatment). Filled shapes and solid lines indicate historically irrigated plots, while open shapes and dashed lines indicated previous treatment.

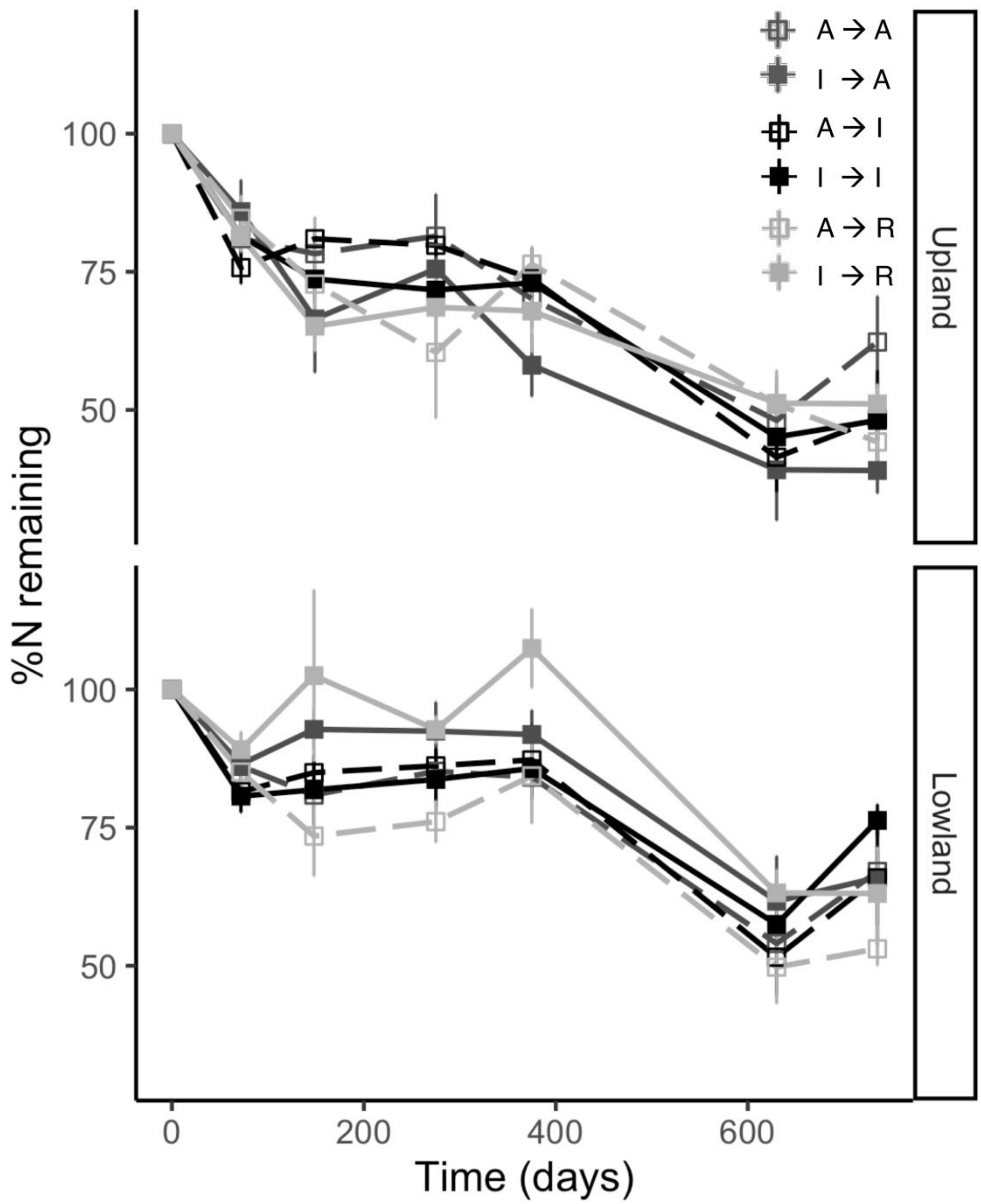

Figure 4.3. Percent N remaining in litterbags across the study period. Points represent mean N (as percent of initial) in each treatment (+SE) at each sampling date. Shape and outline colors on points indicate current treatment. The first letter shows previous treatment (ambient (A) or irrigated (I), followed by current treatment (A, I or reduced (R) rainfall treatment). Filled shapes and solid lines indicate historically irrigated plots, while open shapes and dashed lines indicated previous treatment.

Chapter 5 – Drought resistance in restored tallgrass prairie does not vary with restoration age

5.1 Introduction

Grasslands cover 30-40% of the earth's terrestrial surface (Dixon et al. 2014) and provide important ecosystem services such as soil carbon (C) storage (Schlesinger 1997), forage production (Barnes and Nelson 2003), and maintenance of biodiversity (Collins et al. 1998). Temperate grasslands are one of the most endangered ecosystems globally due to extensive conversion to urban and agricultural uses and low protection of remaining areas of intact grassland (Hoekstra et al. 2005). This is especially true for the North American tallgrass prairies, which have been reduced to less than 5% of their historic range (Samson and Knopf 1994). The large C storage potential in prairie soils (DeLuca and Zabinski et al. 2011) combined with their characteristic resilience to climate extremes such as droughts (Hoover et al. 2014) make tallgrass prairies a potentially important ecosystem for C sequestration under a less hospitable future climate. Accordingly, there has been increasing effort to restore and protect these prairies to support the survival of endemic species and restore valuable ecosystem services (Blair et al. 2014; DeLuca and Zabinski et al. 2011; Carter and Blair 2012a).

Active restoration of grasslands in former agricultural lands can potentially triple rates of C sequestration compared to passive succession (Yang et al. 2019), helping to mitigate the negative effects of climate change. Re-establishment of prairie plants also mitigates erosion by building soil structure and stability (Bach et al. 2010; Scott et al. 2017), and active restoration of prairies can minimize nitrogen (N) leaching and gaseous losses (Baer et al. 2002; Tomer et al. 2010). However, restoration efforts are resource-intensive and often fail to restore ecosystem structure and/or functioning to targeted levels (Herrick et al. 2006; Norland et al. 2018), threatening the large-scale feasibility of restored grasslands as a C sink (DeLuca and Zabinski et al. 2011). While native tallgrass prairies are generally considered to be resilient to climate variability and climate extremes (Hoover et al. 2014), the vulnerability of restored prairies to climate extremes remains unclear, as does the sensitivity and predictability of responses to climate variability as these prairies mature. Given the predicted increases in climate variability as a result of climate change, it is important to assess how ecosystem-level sensitivity to climate develops in restored grasslands.

Studies that document how prairie functioning changes with restoration age suggest that aboveground net primary productivity (ANPP), belowground root biomass, soil microbial biomass, and soil structure can recover relatively quickly (within a few decades) in restored prairies (Baer et al. 2002; Scott et al. 2017). Yet, some attributes such as plant diversity appear to be more variable in their recovery (McLauchlan and Knispel 2005; Carter and Blair 2012a), and others such as soil organic matter (SOM) may take a century or more to recover to levels characteristic of native prairie (Baer et al. 2002; McLauchlan et al. 2006; DeLuca and Zabinski 2011; Rosenzweig et al. 2016). While a study of the oldest restored tallgrass prairie (~65 years old) found that ANPP and soil respiration responded similarly to water stress (Kucharik et al. 2006), it remains unclear how climate sensitivity changes as a restored prairie matures. With drought projected to increase in frequency and severity throughout the historic range of tallgrass prairie (Dai 2011), it may be especially challenging to restore functioning ecosystems under these novel climate conditions (Manning and Baer 2018). It is therefore important to investigate not only how restored prairie ecosystem processes change through time but also how those changes affect its sensitivity to perturbations such as drought.

As restored prairies age, changes in vegetation may affect drought resistance. Several studies have found increases in C_4 grass cover with restoration age (Baer et al. 2002; McLachlan and Knispel 2005), and this functional group can buffer prairies from drought through their high water use efficiency and drought tolerance (Knapp and Medina 1999), as well as their potential for building SOM and increasing soil water holding capacity (O'Brien et al. 2010). However, other studies have found that highly productive grasslands are more sensitive to drought than low-productivity grasslands, potentially due to lower drought tolerance in taller grass species (Wang et al. 2007). Since C_4 grasses are the largest contributors to ANPP in frequently burned tallgrass prairies (Tieszen et al. 1997; Knapp and Medina 1999; Nippert et al. 2007), they may play an important role in determining how patterns in grass ANPP influence sensitivity and resilience of prairie ecosystems. However, plant diversity also can influence ecosystem attributes such as ANPP, root biomass, and soil carbon that affect climate sensitivity (Yang et al. 2019; Upton et al. 2018). Multiple patterns have been documented for changes in plant diversity across restoration age; while some find decreased diversity with restoration age (Baer et al. 2002), others found richness and floristic diversity to reach levels in native prairies within a decade (Carter and Blair 2012a). More diverse grasslands can exhibit higher drought resistance and resilience through a "portfolio effect" or due to compensatory dynamics (Tilman and Downing 1994; Tilman et al. 1998; Hallett et al. 2014). However, negative (Xu et al. 2018) or neutral (Díaz et al. 2007) biodiversity and ecosystem function (BEF) relationships have also been documented in grasslands (van der Plas 2019), complicating our ability to predict how ecosystem sensitivity will change with communities as restored prairies develop. It is therefore important to assess how contrasting restoration outcomes, such as cover of native warm season grass vs. high forb diversity, may influence climate sensitivity

Soil respiration is a major C flux from prairies (Raich and Schlesinger 1992), and the response of this flux to changes in precipitation patterns can be complex due to respiration responses by plants, microbes, and their interactions (Bardgett et al. 2008). As restored prairies mature, increases in microbial biomass and root biomass likely contribute to higher rates of soil CO_2 flux with increasing prairie age (Rosenzweig et al. 2016; Maher et al. 2010; Baer et al. 2002). Improved structure soil in older prairies (Scott et al. 2017) may increase water retention, buffering respiration from short-term drought. However, newly planted prairies converted from row-crop agriculture tend to have high amounts of soil inorganic N (iN) (Rosenzweig et al. 2016), which also may support high rates of soil CO_2 flux under drought conditions in recent restorations (Han et al. 2012). To understand how restoring these grasslands will affect, and in turn be affected by, climate change, it is crucial to investigate how climate sensitivity changes with plant biomass, soil properties, and microbial attributes as restorations age.

In this study, we utilized a chronosequence of restored tallgrass prairies spanning nearly 20 years, including prairies from 4 to >20 years since planting, at a single location in northeast Kansas to explore pattens in ecosystem structure, function, and climate sensitivity as restored prairies mature. We measured plant community composition, ANPP, and selected soil C and N pools over two years with contrasting precipitation inputs. Along this chronosequence we also imposed a 2-year field drought experiment to reduce incoming precipitation by 66% and assessed the response of ANPP, soil respiration, plant community composition, soil nutrient pools, and microbial biomass to drought stress. We hypothesized that older prairie restorations would have higher rates of ANPP and soil CO_2 efflux, which would be associated with lower plant diversity, higher grass contributions to ANPP, and larger microbial biomass pools. Moreover, due to the effects of more established plant and microbial communities, we expected that the sensitivity of C fluxes to experimental drought would be highest in the most recent restorations.

5.2 Methods

<u>*Study site*</u>—This study was done at the Konza Prairie Biological Station near Manhattan, Kansas, USA, a site which is mostly native tallgrass prairie with some row-crop agriculture and prairie restored from cultivation. Mean annual temperature at this site is 12.8 °C, and mean annual precipitation is 835 mm, with most of the precipitation falling during the growing season (April-September). At this site, we utilize a restoration chronosequence consisting of four sites that were restored to prairie from row-crop agriculture. These prairies were restored in 1998, 2006, 2010, and 2016 using similar methods. Cultivated fields were subject to a rotational cropping system, with cultivation of wheat and soybean being most common. Restored fields were seeded with a mixture of 60-70% grass seed, and the rest forb seed (See Appendix E for planted species; Baer et al. 2003; Klopf et al. 2014; Scott and Baer 2019). Seeds were applied at a rate of 600 seeds m^2 by hand broadcast (2006, 2010, 2016 restorations; forbs in 1998 restoration) or with a seed drill (grass in 1998 restoration) (Rosenzweig et al. 2016). We also chose a nearby remnant prairie site on Konza Prairie as a reference for native conditions. All four restored prairies and the remnant prairie are on deep Reading silty clay loam soils and are within 1 km^2, minimizing environmental variation between prairies not due to restoration year. All prairies are burned every year in late spring, consistent with regional management practices.

During the first year of our study (2020), total annual precipitation was 833 mm (621 mm during the growing season, April-September). In 2021, the second year of our study, total and growing-season precipitation inputs were 632 and 424, respectively (Appendix D, figure 1).

Experimental design—To assess the response of native and restored prairies to drought, we established twelve 2 × 2-m plots in each restoration sequence and native prairie (Appendix D, figure 2). These plots were divided into three blocks (with four plots within close proximity being grouped together in one block) to account for potential similarity among plots due to spatial location. In the 1998 prairie, these plots were placed within one long strip of restored prairie and avoided uncharacteristic patches of invasive species. The 2006 prairies are located within a large experiment that assesses the effects of different seeding rates; thus, plots for our study were arranged in groups of two within six 5×5-m larger prairie to match the seeding rates of the other restorations. The 2010 and 2016 plots were within three large replicate restorations, with four plots each. Climate treatments were assigned to plots depending on restoration design: in the 1996, 2010, 2016, and remnant prairies, two drought and two control plots were randomly assigned in each block. Due to the small size of the 2006 restoration prairies, one drought and one control were assigned within each restored prairie strip, with the northeast plot always assigned as the drought plot to avoid runoff from drought shelters into ambient plots [see below]. Each plot was divided into four 1-m^2 subplots for designated sampling in each

In June 2020, we erected 3×3-m shelters with 30-cm clear polycarbonate slats over drought plots designed to divert ~66% of incoming rainfall (Yahdjian and Sala 2002). These shelters were about ~1 m off the ground and oriented at a slight angle to aid precipitation runoff, with a gutter to deflect rainfall away from plots. The shelters were deployed again in April 2021, and were removed in the fall of each year.

ANPP—Standing plant biomass was clipped in September 2020 and 2021 to estimate ANPP. At each sampling, two 0.1-m^2 subsamples were collected in a 1-m^2 subplot. Biomass was clipped to the ground and sorted into grass, forb, and woody species, excluding any biomass from the previous year (identified by fire markings). Samples were dried at 60 °C and weighed as a measure of ANPP. Because annual fires limit woody species to short-stature grassland forbs (primarily *Amorpha canescens*), woody biomass was combined with herbaceous forbs to assess "forb" ANPP.

Plant species composition—Plant community composition was assessed in mid-July 2020 and 2021. In a fixed 1-m^2 frame, we estimated cover of each species present to the nearest percent. Sedges were grouped as one *Carex* spp. taxon due to most being post-flowering and difficult to identify to species at this period. Due to the layered canopy in this grassland, total cover values could exceed 100%.

Soil CO_2 efflux—We measured soil respiration approximately biweekly throughout the growing season of May-October in 2021 only. Soil respiration outside the growing season generally contributes very little to annual soil CO_2 flux (Knapp et al. 1998) and was therefore not measured. We inserted polyvinyl chloride (PVC) rings (10-cm diameter) into the ground 5 cm deep in the spring. We collected soil respiration measurements between 10:00 and 14:00 on sunny days using a LI-8100 infrared gas analyzer (LI-COR, Lincoln, NE, USA). We avoided measuring immediately after a precipitation event (Harper et al. 2005), and we clipped any vegetation within the collar >1 hour before measuring.

Soil extractable C and N pools—In early August 2020 and 2021, we collected soil to 15 cm depth in three 2-cm composite cores. Fresh samples were sieved through a 4 mm sieve and roots were removed. We assessed inorganic N pools by extracting 11.5 g subsamples of fresh soil with 50 ml 2M KCl. Concentrations of NH_4^+-N and NO_3^--N were determined by colorimetric analysis on a Flow Solution Autoanalyzer (Alpkem, Wilsonville, Oregon) by the Kansas State Soils Testing Lab. Microbial biomass carbon was determined using the chloroform fumigation extraction technique on 11.5 g soil subsamples (Vance et al. 1987), followed by extraction with 50 ml 0.5M K_2SO_4. Total organic C was determined on a TOC analyzer (Shimadzu, TOC-L, USA) (Paul et al. 1999), and microbial biomass was calculated as the difference in TOC concentrations in fumigated and unfumigated samples; no correction factors were used (Joergenson et al. 2011). Extractable organic carbon (EOC) from the unfumigated samples was used as an index of labile C. Due to laboratory limitations in summer 2020 related to the COVID-19 pandemic, microbial biomass and EOC were only assessed in 2021.

Data analysis—We used linear mixed models to assess the effect of restoration year, rainfall treatment, and year of data collection on the ecosystem responses using the lme4 package in R (Bates et al. 2015), along with lmerTest (Kuznetsova et al. 2017), emmeans (Lenth et al. 2021), gamm4 (Wood and Scheipl 2020), MuMIn (Bartoń 2019), and tidyverse (Wickham et al. 2019). Block was used as a random effect, as was day of year for soil respiration data. Data were natural log-transformed as necessary to meet assumptions of linear models. For forb ANPP data and nitrate concentrations, both of which were zero-inflated, we used a tweedie model with a log link in the glmmTMB package (Brooks et al. 2017). For proportion forb data, we used a zero-inflated beta model from the same package. Restoration year was treated as a categorical variable to aid comparisons across years; alternate models using restoration age as a numeric predictor with "age" of native prairie set to 50, 100 and 200 years yielded similar results and did not change the effects of restoration treatment.

Differences in plant community composition among restoration years were visualized with an NMDS in the vegan package (Oksanen et al. 2019), and we analyzed changes in community composition using a PERMANOVA with the pairwise.adonis function in the pairwiseAdonis R package (Martinez Arbizu, 2019). We assessed the species contributing most to differences in community composition by running an indicator species analysis using the multipatt function in the indicspecies package (De Caceres and Legendre 2009). Significance for all analyses was set at $\alpha = 0.05$.

5.3 Results

ANPP—Total ANPP was marginally higher in 2020 than 2021 ($p=0.053$), but neither restoration year nor drought treatment affected total ANPP (Figure 1). Grass ANPP patterns were similar to total ANPP, with 13% higher grass ANPP in 2021 ($p <0.001$), but no effects of drought treatment or restoration age (Appendix D, figure 3). However, forb ANPP did vary with restoration year; the newest restoration (2016) had the lowest forb ANPP, which was significantly lower than both the 2006 and 2010 restorations (Appendix D, figure 4). Overall, the mid-age prairies (2006 and 2010 restorations) had the highest forb ANPP (both >200 g/m^2; other restorations <100 g/m^2; native prairie 104 g/m^2). A marginally significant interaction between restoration year and sampling year ($p=0.061$) suggested these differences in forb ANPP among restorations were most pronounced in 2020, the drier year. Differences among prairies of different restoration age were even more pronounced in the proportion of ANPP composed of forbs; the 2006 and 2010 restorations had a higher proportion of total ANPP made up of forbs than the native prairie, and the 1998 and 2016 restored prairies (2006: all $p<0.05$; 2010: all $p<0.01$). The reduced rainfall treatment did not affect the percent of ANPP composed by forbs (Figure 2).

Plant community composition and diversity—Plant species richness varied with restoration age. The newest (2016) restoration had the highest richness and differed significantly from the 1998 ($p=0.007$) and 2006 ($p=0.004$) restorations. The reduced rainfall treatment marginally increased average richness by about 1.7 species in the 2016 restoration ($p=0.081$; Figure 3a). Plant species evenness also varied with restoration year; the native prairie, 2010 and 2016 restorations all had significantly higher evenness than the 1998 and 2006 restorations (all $p<0.05$; Appendix D, Figure 5). When we investigated a marginally significant interaction between rainfall treatment and restoration year, results suggested that evenness decreased with drought in the 2006 restoration. Shannon's diversity (H) showed similar patterns as evenness, with H higher in the native prairie, 2010 and 2016 restorations than the 1998 and 2006 restorations (all $p<0.05$). Reduced rainfall decreased H in the 2006 restoration ($p=0.022$; Figure 3b).

The overall PERMANOVA showed a strong effect of restoration year on community composition ($p<0.001$). To avoid counting the two sampling events (2020 and 2021) as separate replicates, we then ran a pairwise PERMANOVA separately on each year to investigate differences between restorations. Each showed that, in both years, all prairies significantly differed from each other ($p<0.01$). An NMDS suggested that prairie community composition tends to become more similar to that of native prairie through time (Figure 4), but that the 2006 restoration had a distinct community. Reduced rainfall did not affect the composition of plant communities.

An indicator species analysis showed that the most recently restored prairie was associated with some annual grasses and ruderal/invasive species (Table 1). The older restorations were associated with various forbs, and sedge species and several perennial grasses were strongly associated with the native prairie. However, despite variation in species compositions between restorations, cover of C_4 grasses averaged more than 60% and made up the majority of plant cover in every prairie in both years (Appendix D, Figure 6)

Soil respiration—Soil respiration was strongly affected by restoration age and generally increased with restoration age. The 2006 and 2010 restorations had nearly identical average respiration rates (~7.5 μmol/m^2/s) and the 1998 and 2006 differed only marginally ($p=0.065$); otherwise all restorations differed significantly from each other, increasing from 5.6 μmol/m^2/s in the 2016 restoration to 8.9 μmol/m^2/s in the 1998 restoration and 11.7 μmol/m^2/s in the native prairie (Figure 5). However, average respiration rates were not affected by the drought treatment.

Soil inorganic N—Total inorganic N (iN) varied with restoration; an interaction with sampling year showed these differences were most pronounced in 2021, the drier year. In this year, native prairie and the 2010 restoration had high iN (2.9 and 3.0 mg N/kg soil, respectively), and the 1998 and 2006 prairies had the lowest iN (2.0 and 1.8 mg N/kg soil, respectively). These prairies differed significantly from each other (all $p<0.05$; Figure 6), but other restorations did not. Moreover, a marginally significant interaction between rainfall treatment and year suggested that reduced rainfall did not affect iN in the first year of treatment, but it decreased total inorganic N by 12% in 2021 ($p=0.059$). Since ammonium concentrations were far higher than nitrate concentrations, ammonium trends largely tracked those of total iN. Unlike with total iN, both the native prairie and the 2010 restoration had significantly higher ammonium levels than the 2016 restoration (all $p<0.05$; Appendix D, Figure 7), and the difference between the 1998 and 2010 restorations was not as pronounced and not significant. There was no effect of rainfall treatment on ammonium levels. Nitrate concentrations were very low, particularly in the restored prairies in 2020 (Appendix D, Figure 8).

Microbial biomass and extractable organic carbon—Microbial biomass in 2021 was far lower in every restoration than in native prairie ($p<0.001$; Figure 7a), and no restorations significantly differed from each other. In the 2010 restoration, microbial biomass was 46% higher under the reduced rainfall conditions, a difference that was marginally significant ($p=0.052$).

Extractable organic carbon (EOC) concentrations in the native prairie were marginally higher than those in the 2010 ($p=0.0064$; Figure 7b). In the native prairie, the reduced rainfall treatment resulted in ~30% lower EOC concentrations than in the ambient rainfall treatment ($p=0.027$). No other restoration or rainfall treatment combinations differed.

5.4 Discussion

North American tallgrass prairies are highly threatened and much native prairie has been lost to land-use change (Samson and Knopf 1994). Given the increased occurrence of more frequent and severe droughts (IPCC 2018), successful restoration efforts will be increasingly crucial to this ecosystem’s persistence. As more grassland, either abandoned cropland or degraded land, is restored to tallgrass prairie, the importance of understanding how quickly this ecosystem properties recovers to native prairie increases. These recovery trajectories may also influence how the sensitivity of restored prairie ecosystems mature in the context of increased climate variability. Overall, restored prairies were surprisingly resistant to experimental drought across restorations ranging from 4 to 22 years old. Despite changes in plant community structure and composition across the restoration sequence, plant community composition and several metrics of ecosystem function were remarkably robust to two years of experimental drought. This may be due to the rapid establishment (<5 years) and inherent drought tolerance of the dominant C_4 grasses seeded in these restored prairies (Baer et al. 2002). Our results suggest that, even in the first decade of conversion from agriculture, restored prairies can potentially withstand a multi-year extreme drought, an optimistic finding for land managers and restoration practitioners.

Plant community composition and productivity—Despite differences in community composition across the chronosequence, productivity in native and restored prairies was insensitive to experimental drought. Total ANPP was similar across all restorations and the native prairie, as well as in ambient and droughted prairie. This is consistent with previous work finding that ANPP does not change directionally through time in restored prairies (Baer et al. 2002). Moreover, drought sensitivity has been found to vary as a function of plant biomass more so than community attributes such as richness in experimental grasslands (Wang et al. 2007), so it is not surprising that these restored grasslands with similar ANPP all were resistant to experimental drought. The lack of response of ANPP to both natural and experimental severe drought, as well as to water additions, has also been documented in recently restored prairies (Carter and Blair 2012b; Manning and Baer 2018; Camill et al. 2004), suggesting that the rapid recovery of ANPP resistance to drought in restored prairies may be a general phenomenon of tallgrass prairies. However, the lack of sensitivity to experimentally-imposed drought contrasts with the fact that, across treatments and restorations, ANPP was marginally higher in the wetter year (2020) than the drier year (2021) of our study. Several possible explanations could explain this differential sensitivity to natural climate variability and experimental drought. While this shelter design has proven effective in native prairie (Broderick et al. 2022), they may be less effective in restored prairies; while overland runoff of rainfall is low in native prairie soils (Gray et al. 1998), more compacted soils and reduced soil structure and soil water holding capacity associated with recent agricultural land use could have reduced water infiltration and increased horizontal runoff from ambient treatments into drought treatments (O'Brien et al. 2010; Scott et al. 2017). Alternatively, low-rainfall years in this system are more often associated with very high temperatures (Hayden 1998), which would exacerbate water stress in a naturally dry year compared to the rainfall exclusion experiment used in our study.

The proportion of total ANPP composed by grasses was highest in the oldest and youngest prairies, with the proportion of forbs peaking in the mid-age (~10-14 years old) prairies. These mid-aged restorations were associated with high-producing prairie forb species, largely from the *Helianthus, Solidago* and *Silphium* genera. This coincided with a higher evenness and Shannon's diversity in these mid-age prairies. While other studies suggest that native grasses dominate ANPP within a decade of restoration (Baer et al. 2002; Carter and Blair 2012a), we found an initial peak (<10 years old) in proportional grass ANPP, followed by a high spike of forb ANPP before reaching native prairie levels of high grass dominance at <20 years old. High forb ANPP in recently planted prairies has previously been associated with early successional plants (Baer et al. 2002). While we did document ruderal species in the 2016 restoration, they were primarily annual grasses and short-statured forbs, and consequently the proportion of ANPP composed by forbs was low. Therefore, similar to other results (Carter and Blair 2012a; Camill et al. 2004; Baer et al. 2002), forb dynamics drove changes in the grass/forb composition of prairies as restorations matured in our study, while grass ANPP remains relatively stable.

While the 2-year experimentally-imposed drought did not have large effects on plant functioning overall, there was some evidence that drought affected some species. In the 16-year-old prairie (2006 restoration), drought decreased species evenness and diversity. Because this difference did not result in changes in ANPP, it is possible that compensatory dynamics also helped maintain functioning under drought stress (Catano et al. 2022). However, it is perhaps more likely that the dominant C_4 grasses largely drove ecosystem responses to drought, buffering functional consequences of species loss (Grime 1998; Smith et al. 2020)

Soil respiration, microbial biomass and labile C—Soil respiration generally increased with restoration age, consistent with previous findings across a similar tallgrass prairie chronosequence (Rosenzweig et al. 2016). The native prairie had very large microbial biomass pools compared to the restorations, which likely contributed to high soil CO_2 efflux. Microbial biomass pools tended to be larger in older restorations but was still much lower than in native prairie after 22 years, similar to other findings across restored prairie chronosequences (Baer et al. 2002; Matamala et al. 2008; Rosenzweig et al. 2016). While we did not measure root biomass or belowground productivity, and these properties tend to be highly spatially variable, root biomass in tallgrass prairies generally increases as restorations age (Baer and Blair 2008; Maher et al. 2010). In fact, previous work using this chronosequence suggests a positive trend of root biomass with age (Scott et al. 2017). Therefore, higher root respiration in older prairies may also contribute to this increase in soil respiration across the chronosequence. Labile organic C was highest in native prairies but was highly variable in the ambient treatment. Since EOC pools are influenced by plant exudation and microbial uptake, these pools are highly spatially variable. It is therefore not surprising that EOC levels did not vary consistently across restoration years, consistent with previous work suggesting that potential C mineralization—which is in part a function of both labile C and microbial biomass pools—recovers quickly in restored prairies (Baer et al. 2002, 2010). The smaller EOC pool in the drought treatment in the native prairie may be due to reduced plant C exudates and reduced microbial activity under drought stress. This rainfall treatment effect did not appear in the restored prairies, perhaps due to the lower microbial biomass and consequently smaller fluxes of labile C in and out of microbial pools.

Soil inorganic N—Concentrations of inorganic N varied idiosyncratically with prairie age, being high in restored prairied and in one of the newer prairies (i.e., 2010 restoration) in 2021. Soil inorganic N is a highly dynamic pool influenced by several processes, including N mineralization, immobilization, plant N uptake, and nitrification, so it is not surprising that these pools did not change predictably through the chronosequence. Native prairies have large stores of SOM and a large microbial biomass pool (Zak et al. 1994; Rice et al. 1998), resulting in a high potential for iN to accumulate, especially under water-stressed conditions when plant uptake is inhibited (Seastedt and Knapp 1993). While we did not find responses to the experimental drought treatment, the iN pool in the native prairie was significantly larger than in some restorations only in the drier year of 2021. Inorganic N concentrations were also high in one of the recent restorations (2010, 10 years old). This prairie had one of lowest proportions of grass

ANPP, and the low cover of native C_4 grasses—and their associated N uptake—has been linked to lower soil iN levels in grasslands recovering from agriculture (Mahaney et al. 208). It is therefore possible that differences in the plant community as restorations age contributed to changes in N availability, consistent with recent findings that soil N cycling is shaped by plant functional composition in restored grasslands (Fry et al. 2018). Previous work has found that inorganic N drops sharply early in restoration as plants establish and take up N and organic inputs with high C:N ratios promote greater immobilization in the soil (Baer et al. 2003; Rosenzweig et al. 2016). Since the most recently planted prairie was four years old in this study, we likely did not document the initial drop typical of the first 1-3 years after planting. The two oldest restorations had the lowest iN levels. This may reflect rapid establishment of native grasses and subsequent high iN uptake and immobilization by these grasses (Carter and Blair 2012), but the much slower accumulation of soil organic N, the substrate for N mineralization, and therefore slower iN supply rates (Baer et al. 2002; McLauchlan et al. 2006; DeLuca and Zabinski 2011; Rosenzweig et al. 2016).

Conclusions—Grassland ecosystem services may be threatened by climate change (Zhao et al. 2020), and it is important to understand how climate sensitivity varies in restored prairies. Overall, we documented striking resistance to a multi-year experimental drought across prairies of different restoration age. While soil respiration and plant community composition varied across the restoration chronosequence, ANPP recovered rapidly to levels similar to native prairie in all restorations, consistent with some previous studies (e.g., Baer et al. 2002). Prairie structure and function were not sensitive to the drought treatment, adding to evidence that even very recently planted prairies can withstand climate variability (Carter and Blair 2012b; Manning and

Baer 2018; Camill et al. 2004). This may be linked to the rapid establishment of belowground root networks that confer drought tolerance to native grasses (Nippert et al. 2012), which that constituted the majority of biomass across all restoration ages. Future prairie restoration efforts are likely to contend with a more unpredictable climate, but our results suggest that restored prairies can be quite buffered from drought conditions and may continue to provide ecosystem services under more frequent and severe water stress.

5.5 References

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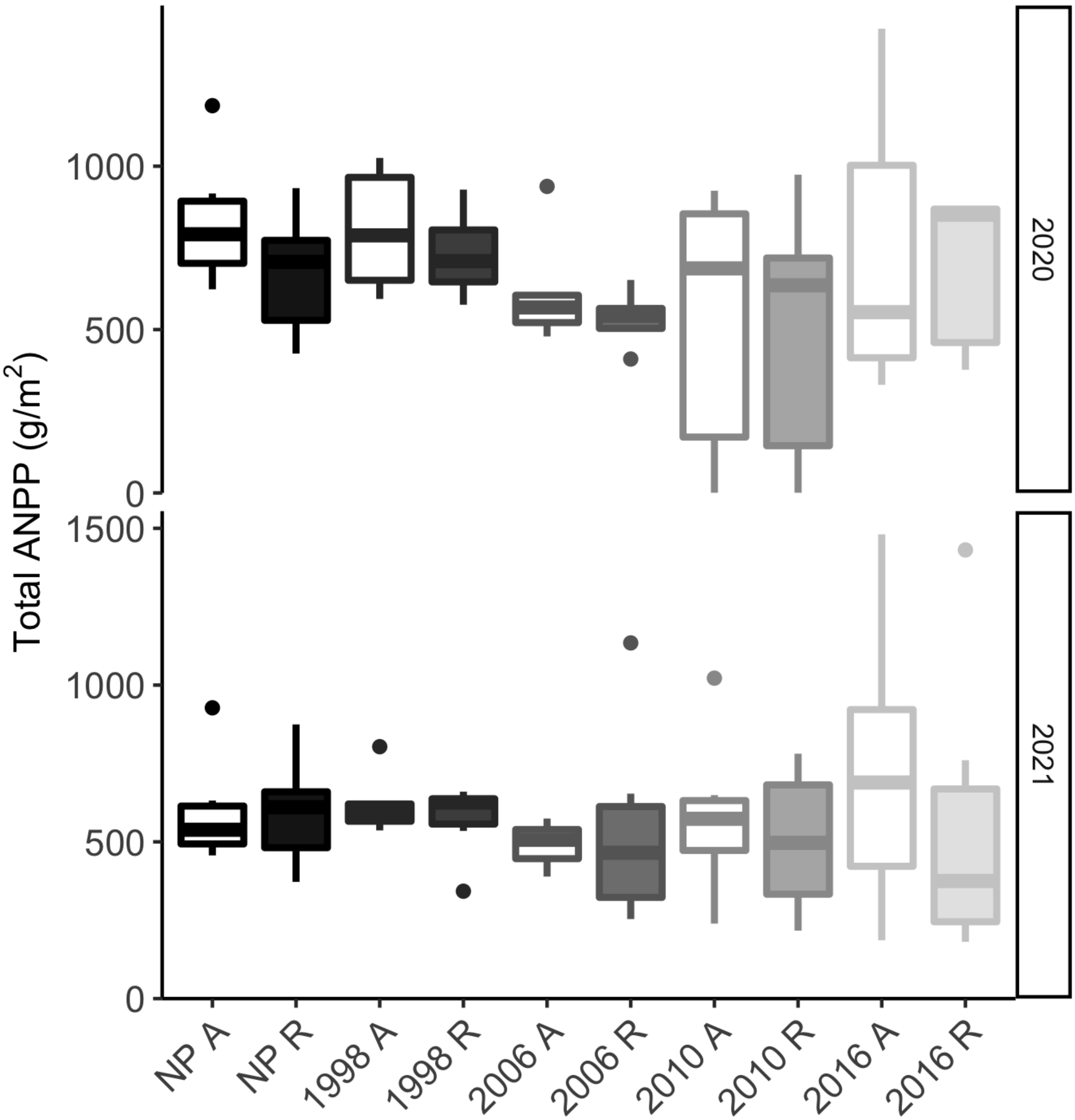

Figure 5.1 Total ANPP (g/m^2) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

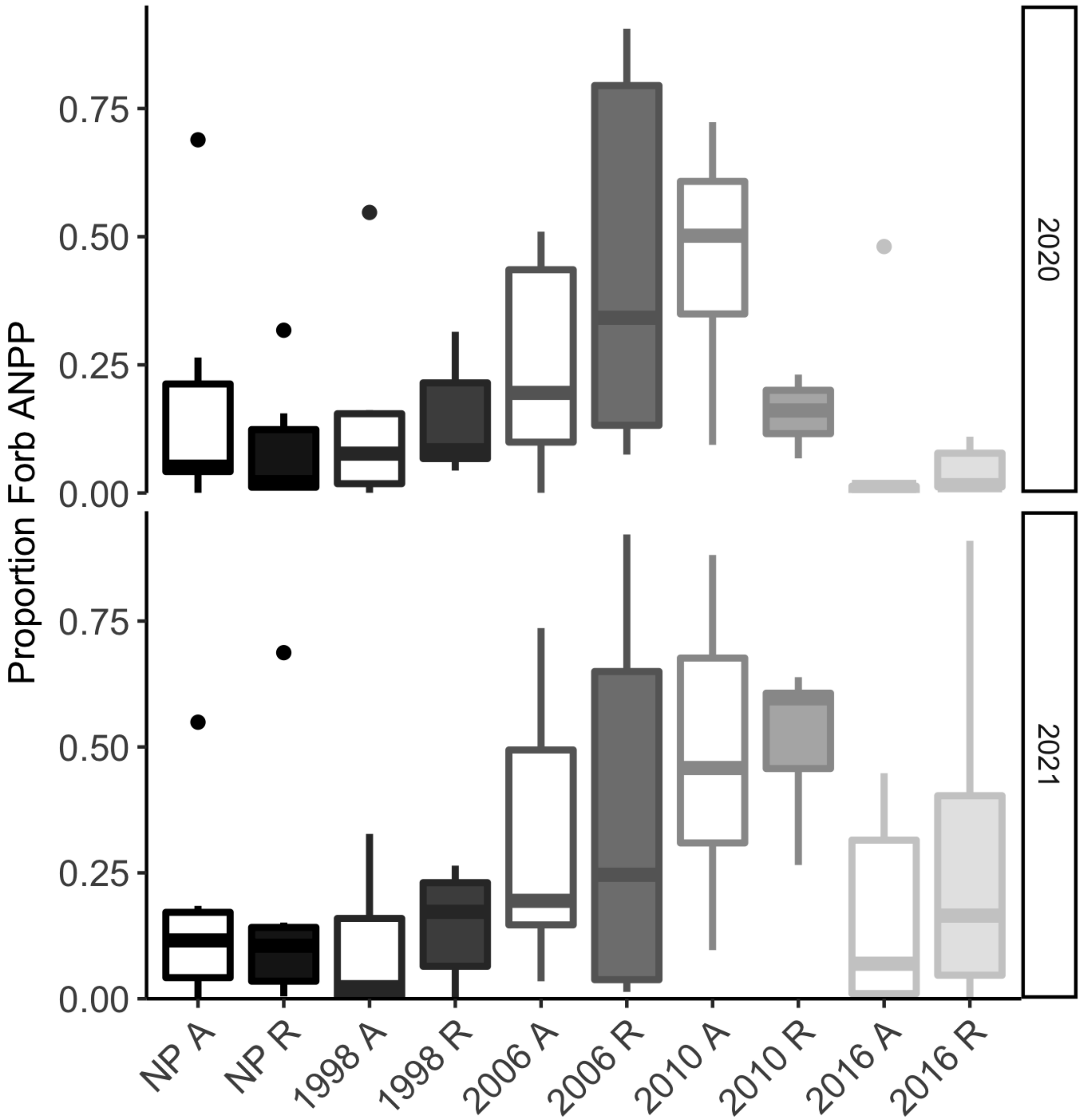

Figure 5.2 Proportion of total ANPP composed by forbs in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

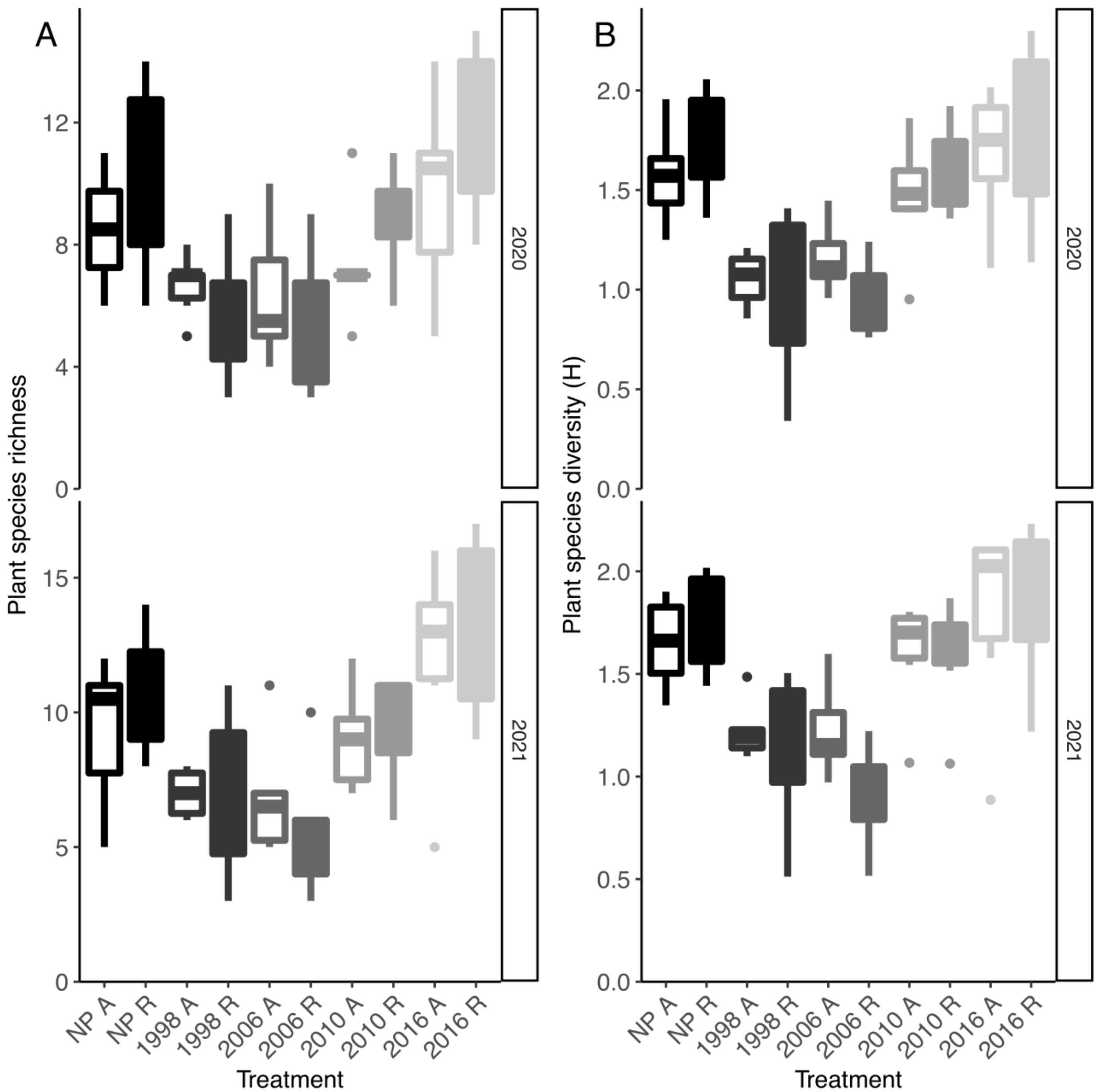

Figure 5.3 (A) Plant species richness (/m^2) and (B) Shannon's Diversity (H') in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

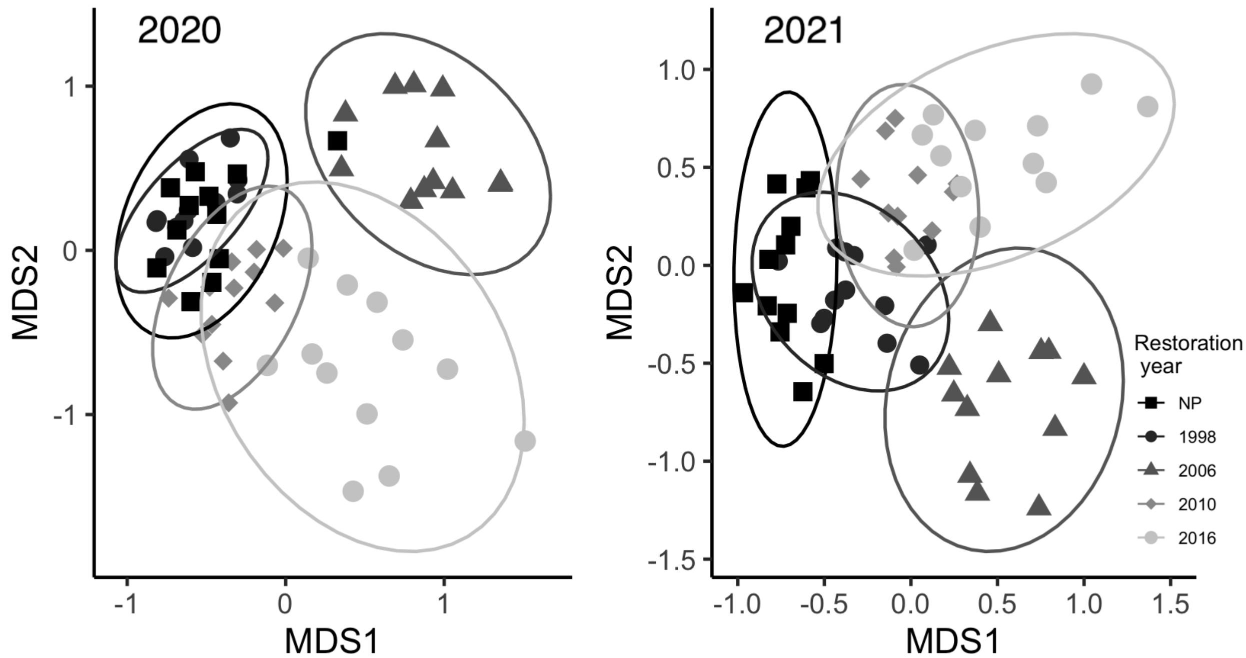

Figure 5.4 NMDS of plant communities in 2020 and 2021. Each point is a plot, and its color and shape indicate restoration year. Because the PERMANOVA did not indicate a significant effect of rainfall treatment in either year, data are aggregated across ambient and reduced plots within each prairie.

Table 5.1 Plant species associated with each restoration treatment, as determined by indicator species analysis. All results are significant at $p<0.05$.

Native prairie	1998	2006	2010	2016
Carex sp.	*Salvia azurea*	*Helianthus mollis*	*Silphium integrifolium*	*Bouteloua curtipendula*
Symphyotrichum oblongifolium	*Asclepias verticillata**	*Solidago canadensis*	*Echinacea augustifolia*	*Eragrostis cilianensis*
Sporobolus compositus	*Symphiocarpus orbicularis†*	*Ambrosia psilostachya*	*Ulnus pumila†*	*Panicum capillare*
Solidago missouriensis				*Solanum carolinense*
Dichanthelium oligosanthes				*Cirsium altissimum*
Tripsacum dactyloides				*Euphorbia dentata**
Silphium laciniatum				*Elymus canadensis*
Amorpha canescens				*Kummerowia stipulacea**
				Setaria viridis
				*Paspalum setaceum**

**Only significant in 2020*
†Only significant in 2020

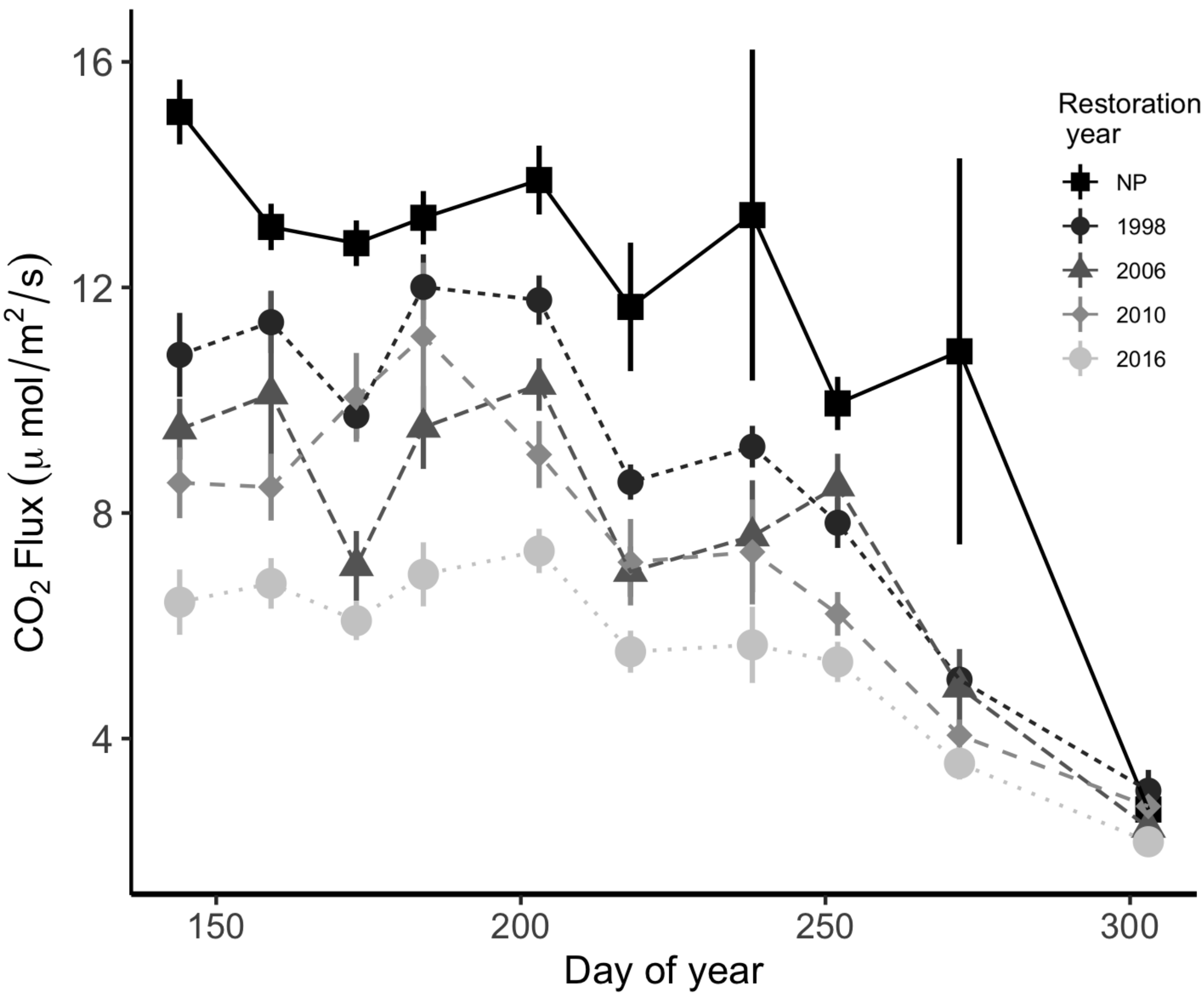

Figure 5.5 Soil CO_2 efflux (mean +SE) across the growing season in 2021. Point shape and line type indicate restoration year; values are averaged across precipitation treatment as these groups did not differ in efflux rates

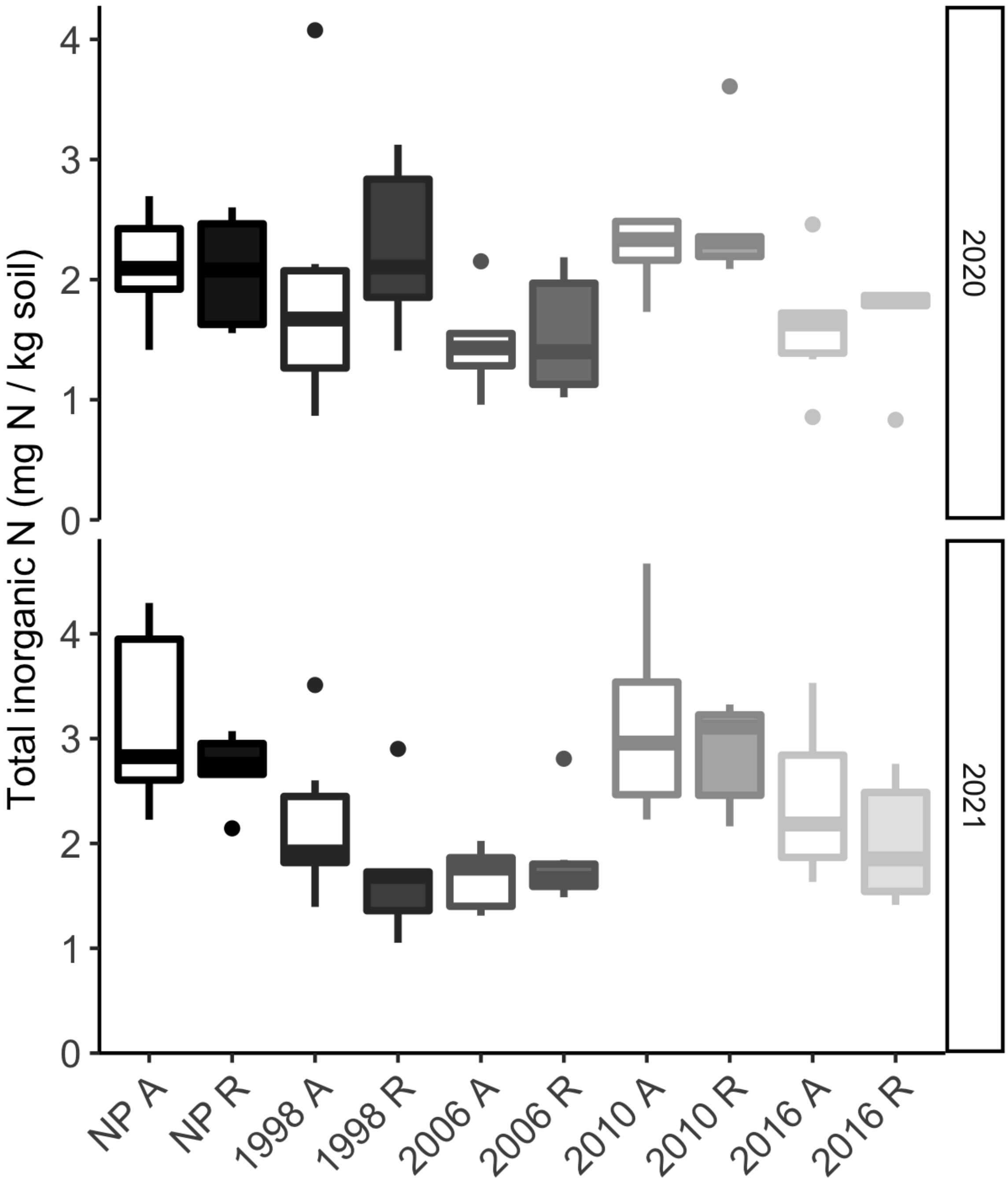

Figure 5.6 Total inorganic N (mg N/ kg soil) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

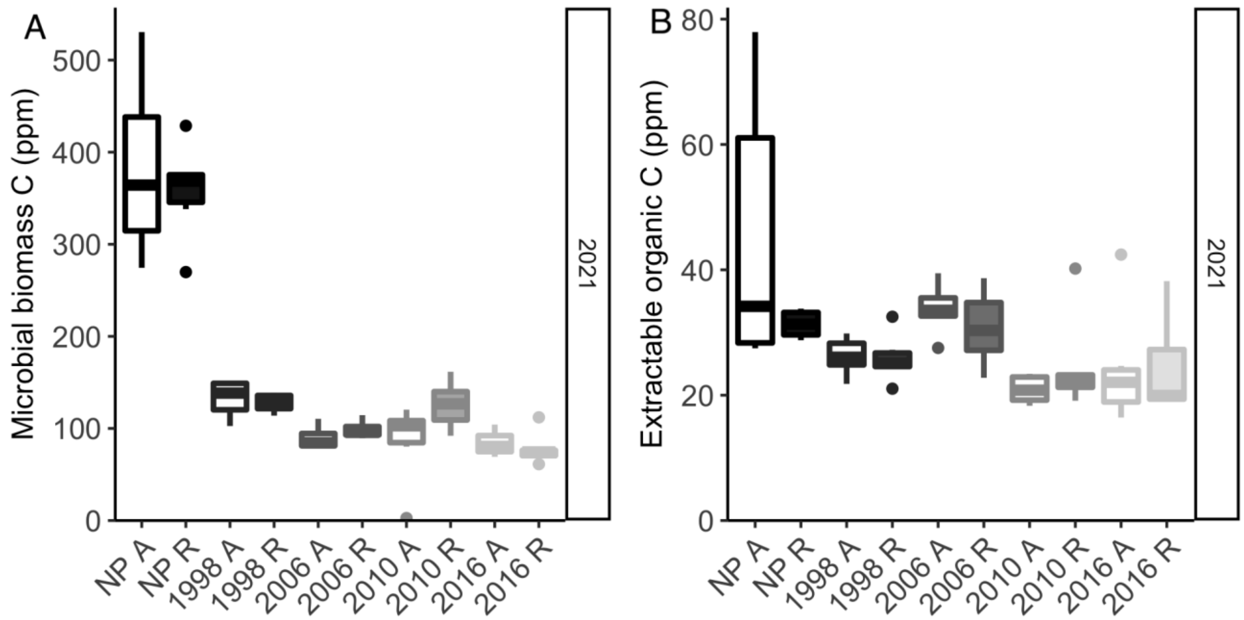

Figure 5.7 Boxplot of (A) microbial biomass C (ppm) and (B) extractable organic C (ppm) in 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

Chapter 6 – Conclusions

Climate change is expected to alter the amounts and timing of precipitation across the North American Central Plains (IPCC 2018), with implications for ecosystem functioning for grasslands in this region. The tallgrass prairies at the eastern, wetter end of this region are exceptionally productive grasslands that store more soil carbon (C) per unit volume than comparable forests (Blair et al. 1998). In these ecosystems, water and nitrogen (N) can co-limit plant and microbial functioning (Seastedt and Knapp 1993). Rates of N cycling processes such as N mineralization and nitrification, two important processes that determine N availability for plants, also vary with water availability (Wang et al. 2006; Risch et al. 2019). Therefore, precipitation regimes can have both direct effects on C cycling and indirect, complex effects on C fluxes via changes in N cycling, making the effects of long-term climate change on ecosystem functioning in tallgrass prairies hard to predict.

The effects of contrasting precipitation regimes on tallgrass prairie C and N cycling occur on several timescales; initial physiological responses by plant and microbial communities may lead to smaller changes in functioning, while long-term changes in community composition or ecosystem structure can result in larger effects (Smith et al. 2009). The long-term effects of climate in tallgrass prairies may lead to climate legacy effects when the climate changes, if the responses of historic climate conditions continue to shape current ecosystem processes and potentially alter their sensitivity to current climate regimes or to extreme events (e.g., droughts) (Sala et al. 2012). The role of precipitation history in shaping how ecosystems will respond to future climate change are not well understood, making it hard to incorporate these effects into ecosystem models (Averill et al. 2016). Therefore, I set out to identify the presence, direction, and magnitude of climate legacy effects on tallgrass prairie C and N cycling to better understand the timescales and mechanisms by which precipitation patterns shape ecosystems. I also set out to see how the development of restored tallgrass prairies (i.e., time since restoration from agricultural use) affects sensitivity to precipitation variability and how this compares to an intact native prairie. This work aimed to improve our understanding of the role of climate in driving ecosystem processes in both native and restored prairies.

Through this research, I found that historic climate conditions exerted legacies on current tallgrass prairie ecosystem functioning and altered ecosystem sensitivity to drought (Chapter 2). A history of increased precipitation over more than two decades resulted in higher rates ANPP and soil respiration than prairie with a history of ambient conditions when both were exposed to the same current precipitation regime. Further, the increase in these rates due to climate history were on a magnitude comparable to responses to current increases in precipitation. In some cases, these legacy effects lasted up to three years. I also found that previously irrigated prairie maintained higher rates of ANPP and soil CO_2 flux than controls when exposed to experimental drought. These climate legacies were associated with an increase in forbs in this system. This is one of the first studies to experimentally demonstrate how contrasting long-term precipitation histories can influence current ecosystem functioning for years into new climate regimes. Moreover, these effects were most pronounced in the lowlands of our study site, showing that the prevalence and strength of climate legacies can vary both within and across ecosystems.

I also found that long-term irrigation accelerated N supply rates (i.e., net N mineralization and nitrification), and shifts in nutrient ratios and extracellular enzyme investment suggested reduced N limitation of plants and microbes (Chapter 3). This contrasts with findings in drier grasslands in which extended increases in precipitation led to higher N losses and exacerbated N limitation (Ren et al. 2017), likely because tallgrass prairies have a higher immobilization potential and tighter N cycling than semiarid grasslands. Again, these effects were concentrated in the lowlands, suggesting that increases in water availability in deeper, fine-textured soils had the potential to increase N cycling rates, likely contributing to the higher rates of plant and microbial C fluxes outlined in Chapter 2. Therefore, effects on N cycling may be an important mechanism contributing to climate legacies in C cycling in ecosystems co-limited by water and N.

I assessed how previous and current precipitation regimes affect mass loss and N release in belowground litter in tallgrass prairie (Chapter 4). Mass loss during decomposition was slowed somewhat in the long-term irrigated treatments, which contrasts with previous findings that long-term irrigation increased C flux rates in this system (Broderick et al. 2022). In mesic systems, belowground decomposition may be prone to saturated, anaerobic soils, inhibiting decomposer activity (von Haden and Dornbush 2014) and supplemental water may reduce the abundance or activities of decomposer organisms (O'Lear et al. 1999, Todd et al. 1999; Franco et al. 2021). These results suggest that the previously-documented acceleration of C and N mineralization as a result of historically wetter conditions, detailed in Chapter 2 and Chapter 3 of this dissertation, are driven by microbial processing of SOM pools, rather than fresh plant litter (Yahdjian et al. 2006). Because these SOM pools are important for long-term C sequestration, it will be important that future studies assess how climate legacies may affect stable C pools across ecosystems.

Finally, I expanded my focus on climate effects in prairie ecosystems to include restored prairies, an increasingly important land use for conserving prairie species and ecosystem services. I found that C fluxes in prairies ranging from 4 to 22 years old were similar across restoration ages and surprisingly resistant to experimental drought. This may be due to the rapid establishment of drought-tolerant dominant warm-season grasses, supporting the idea that helping establish the dominant species that drive ecosystem processes may help restore the rates of C cycling to levels characteristic of remnant ecosystems, as well as their characteristic resistance to climate variability (Smith et al. 2020).

Together, this research highlights some important mechanisms by which climate shapes ecosystem processes. Plant community composition plays a role in driving both climate legacy effects, with the high responsiveness of forbs to previous treatment in native prairie, and the stability of ANPP in restored prairie, with the rapid establishment of drought-tolerant native grasses maintaining ANPP under drought. N cycling may also drive ecosystem sensitivity to climate variability, with higher N availability with following a history of wetter conditions associated with maintaining higher ANPP and soil CO_2 flux under water stress. Because previous climate conditions were shown to buffer prairie functioning from drought, my results also suggest that short-term precipitation experiments may underestimate the long-term responses of ecosystems to climate change. Finally, my research offers an optimistic prognosis that even recently established prairies can maintain functioning under more stressful precipitation regimes, and that restoration efforts can succeed even in a changing climate.

6.1 References

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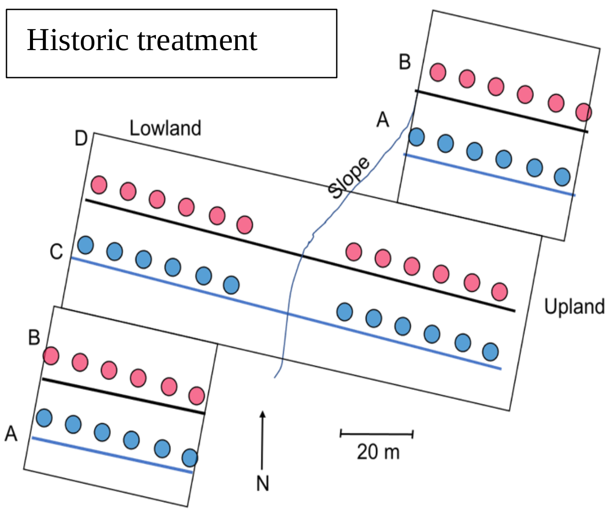
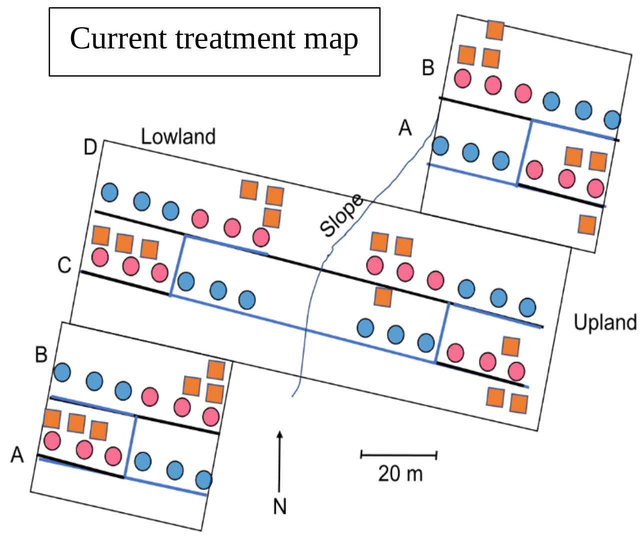
Appendix A Supplementary figures and tables for Chapter 2

Appendix A, Table 1: Annual and growing-season precipitation, plus water added in irrigated treatments during study period.

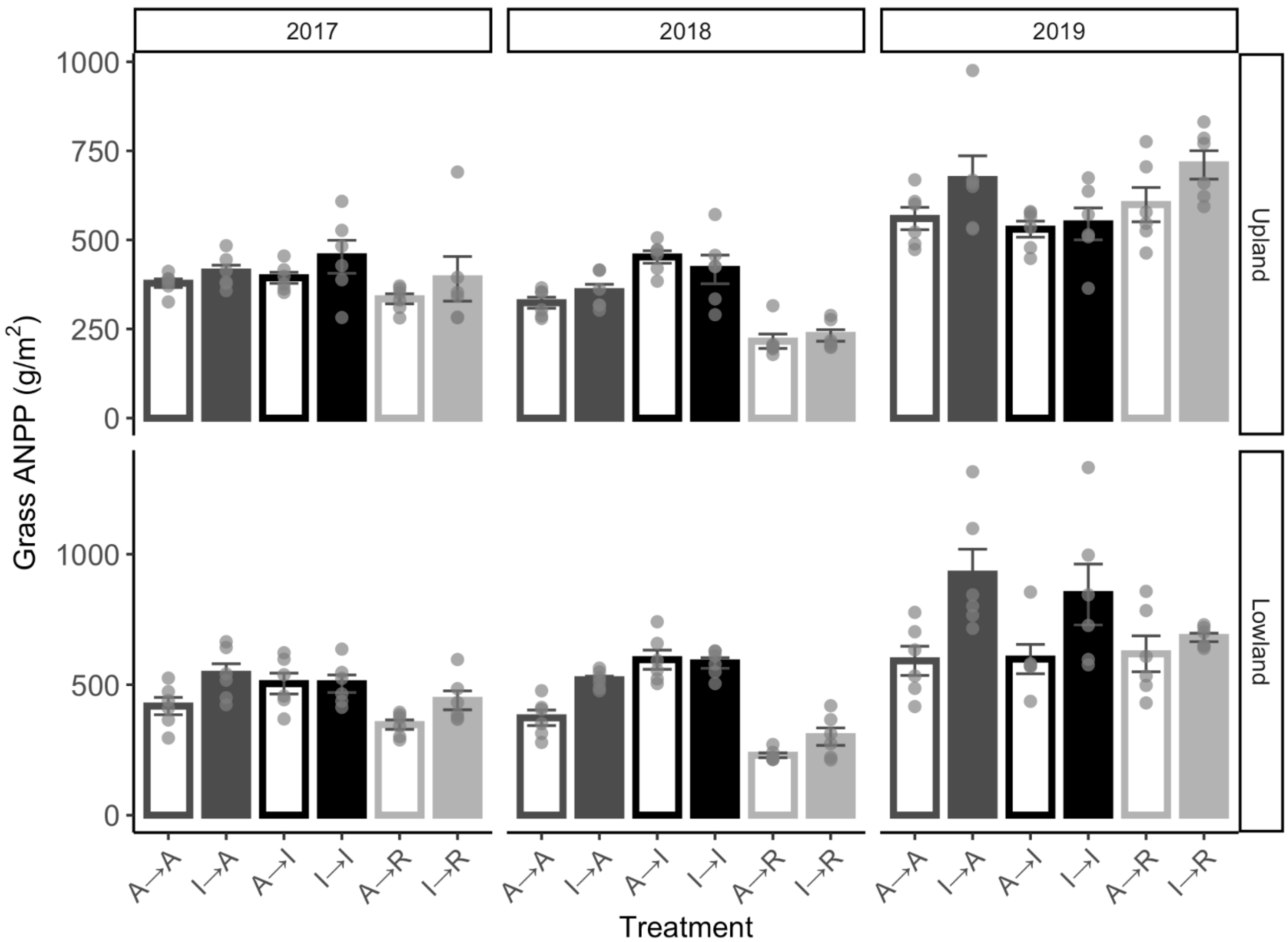
	2017	2018	2019
Annual precipitation (mm)	724	811	1131
Growing-season precipitation (mm)	467	516	885
Water addition (mm)	105 (upland) 101 (lowland)	140 (upland) 119 (lowland)	97 (upland) 101 (lowland)

Appendix A, Figure 1: Maps of study area. Irrigated plots are blue circles, and ambient plots are red. In 2017, irrigation lines were shifted so that half of historically irrigated plots ceased being irrigated, and half of historic controls began irrigation treatment. Additionally, plots under drought shelters (orange squares) were added along transects in areas that were historically either irrigated or ambient. The study area is topographically divided into an upland and lowland area; plots on the sloped areas were excluded from this study and are not included in this map.

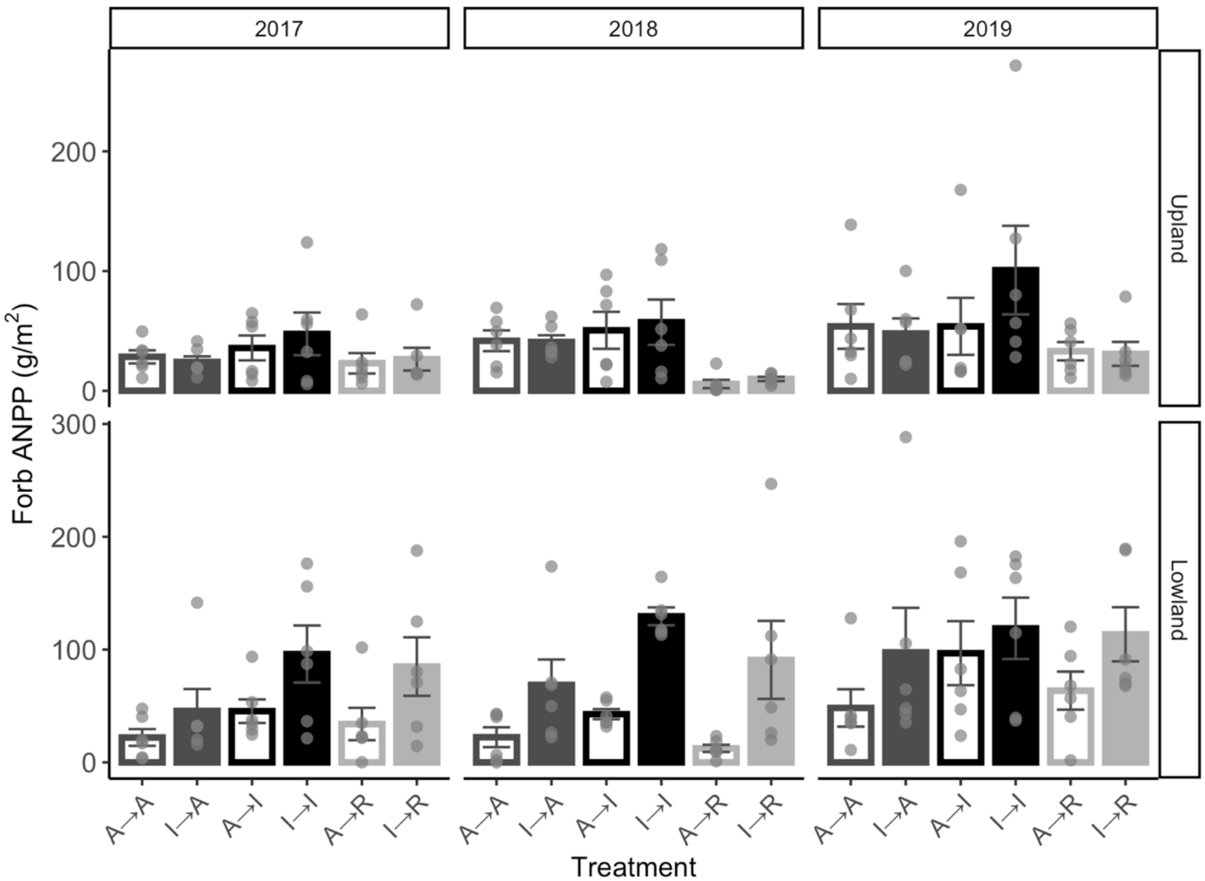




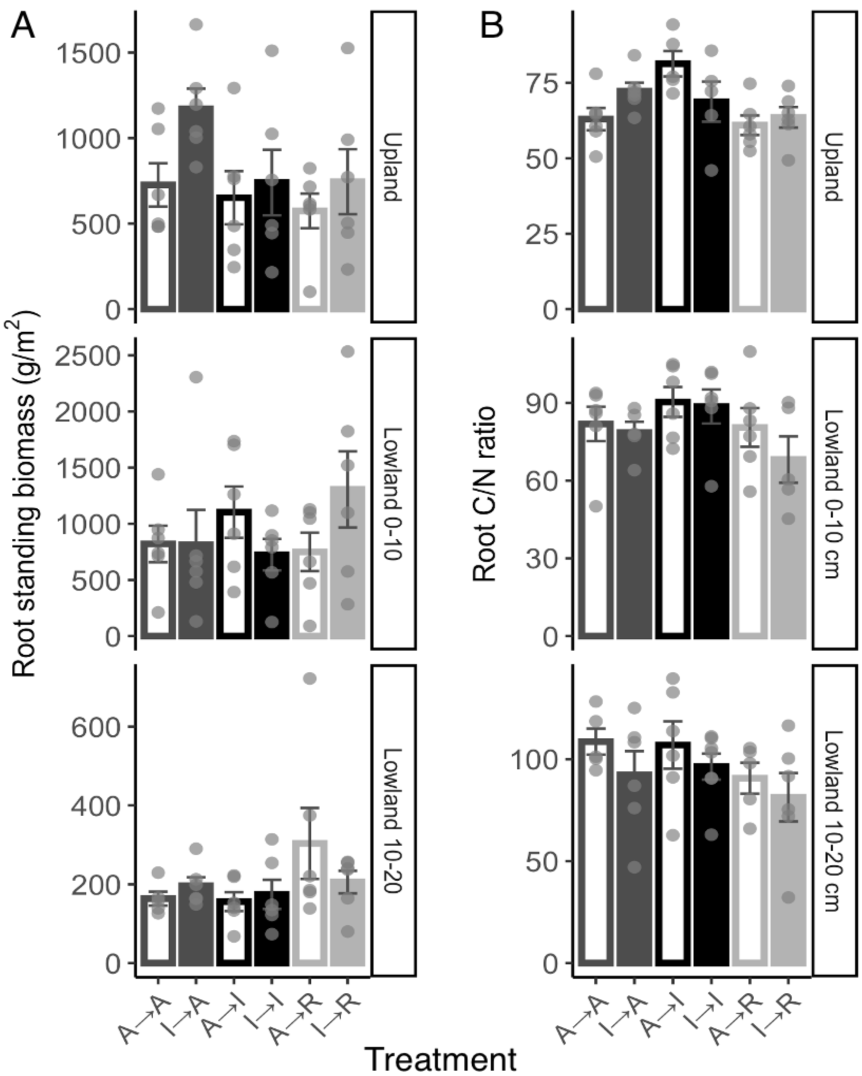
Appendix A, Figure 2: Grass ANPP across treatments in the uplands and lowlands in 2017-2019. Historically irrigated (filled) treatments are paired with historic controls (open) for each current treatment by shading color. Points represent individual plot averages across subsamples. Error bars represent standard error of the mean.



Appendix A, Figure 3: Forb ANPP across treatments in the uplands and lowlands in 2017-2019. Historically irrigated (filled) treatments are paired with historic controls (open) for each current treatment by shading color. Points represent individual plot averages across subsamples. Error bars represent standard error of the mean.

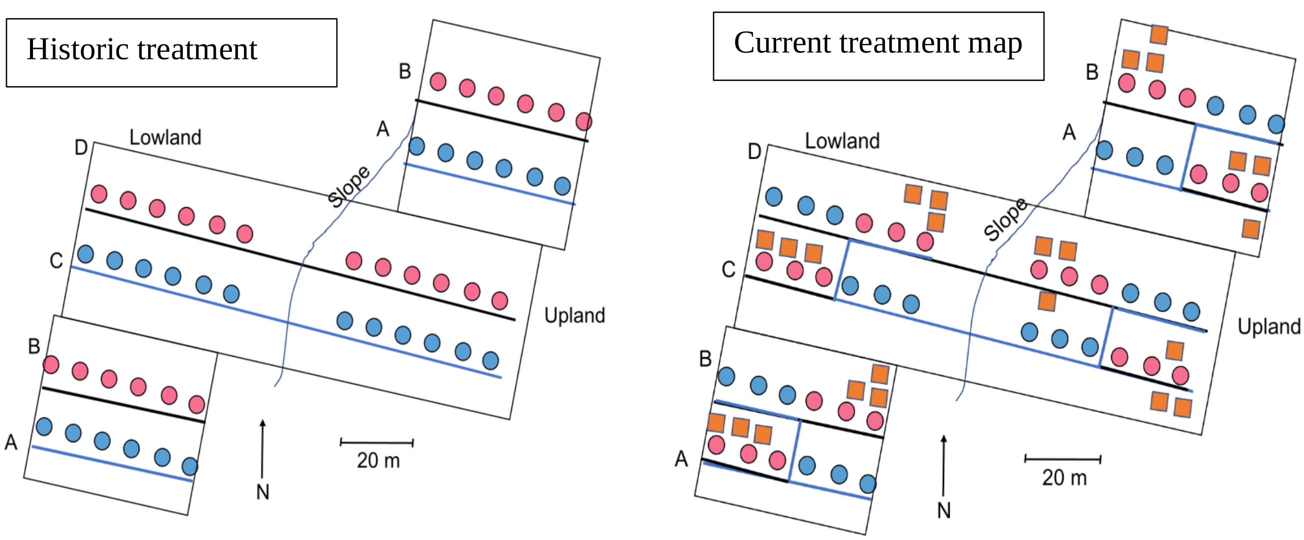


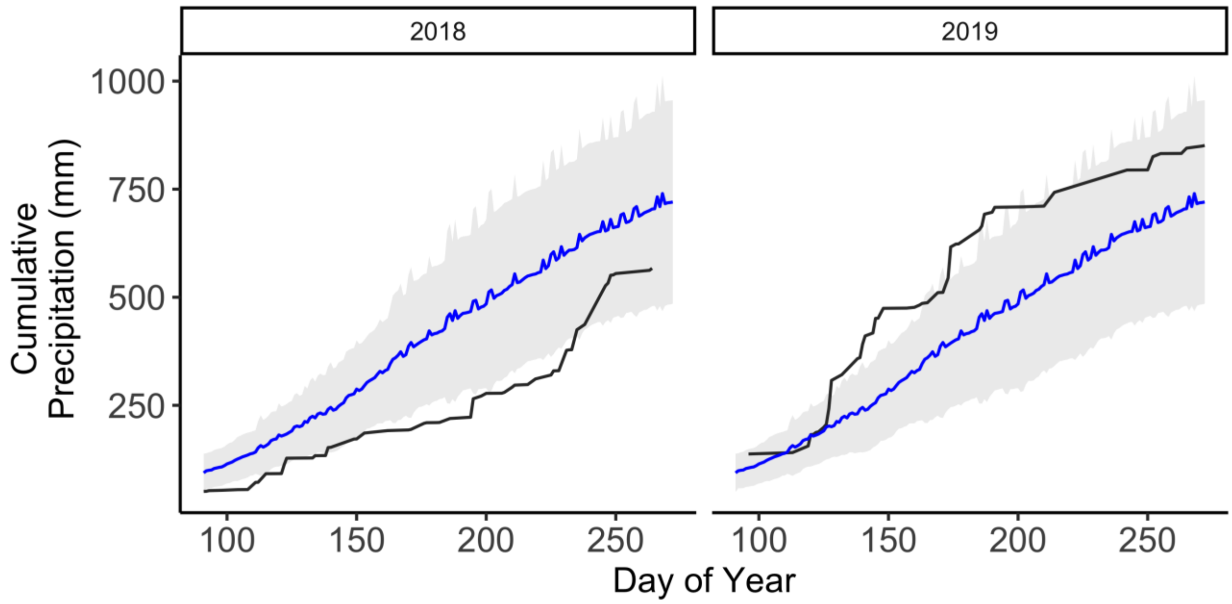
Appendix A, Figure 4: (A) Mean root biomass and (B) C/N ratio in 2019. In both figures, top panels show upland 0-10 cm depth, middle show lowlands 0-10 cm depth, and bottom show lowlands 10-20 cm depth. Bar outline color represents current treatment, and bar fill represents historical irrigation treatment. Points represent individual plot values for each month, and error bars represent standard error.



Appendix B Supplementary figures and tables for Chapter 3

Appendix B, Figure 1: Maps of study area. Irrigated plots are blue circles, and ambient plots are red. In 2017, irrigation lines were shifted so that half of historically irrigated plots ceased being irrigated, and half of historic controls began irrigation treatment. Additionally, plots under drought shelters (orange squares) were added along transects in areas that were historically either irrigated or ambient. The study area is topographically divided into an upland and lowland area; plots on the sloped areas were excluded from this study and are not included in this map.

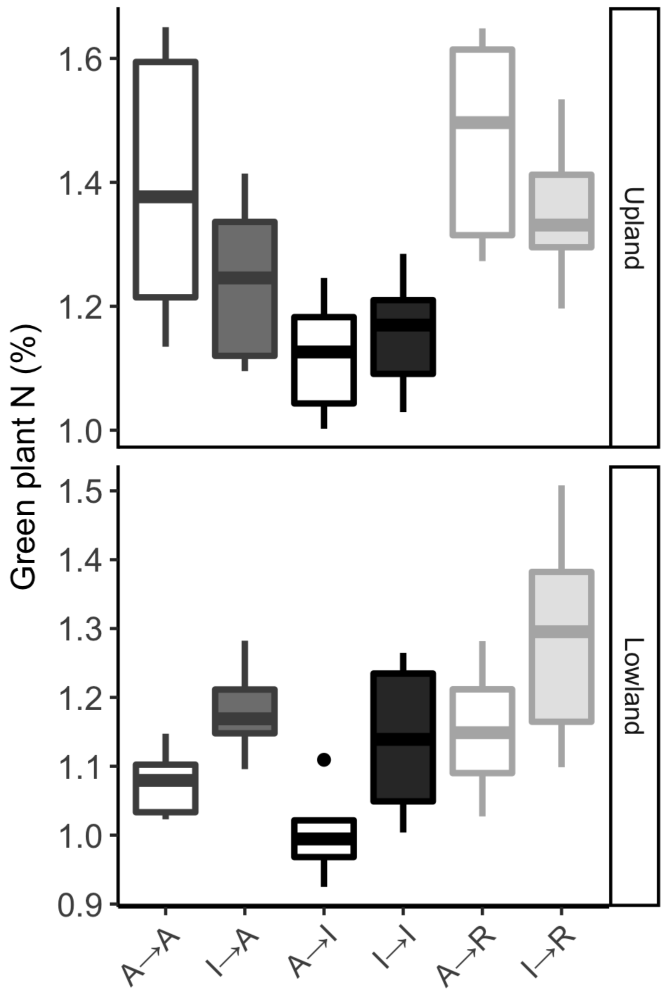




Appendix B, Figure 2: Cumulative precipitation during the growing season (May-September) during the study period (black line), presented against the mean (blue line) and standard deviation (grey shading) of the 30-year average.

Appendix B, Table 1: Annual and growing-season precipitation, plus water added in irrigated treatments during study period.

	2018	2019
Annual precipitation (mm)	811	1131
Growing-season precipitation (mm)	516	885
Water addition (mm)	140 (upland) 119 (lowland)	97 (upland) 101 (lowland)

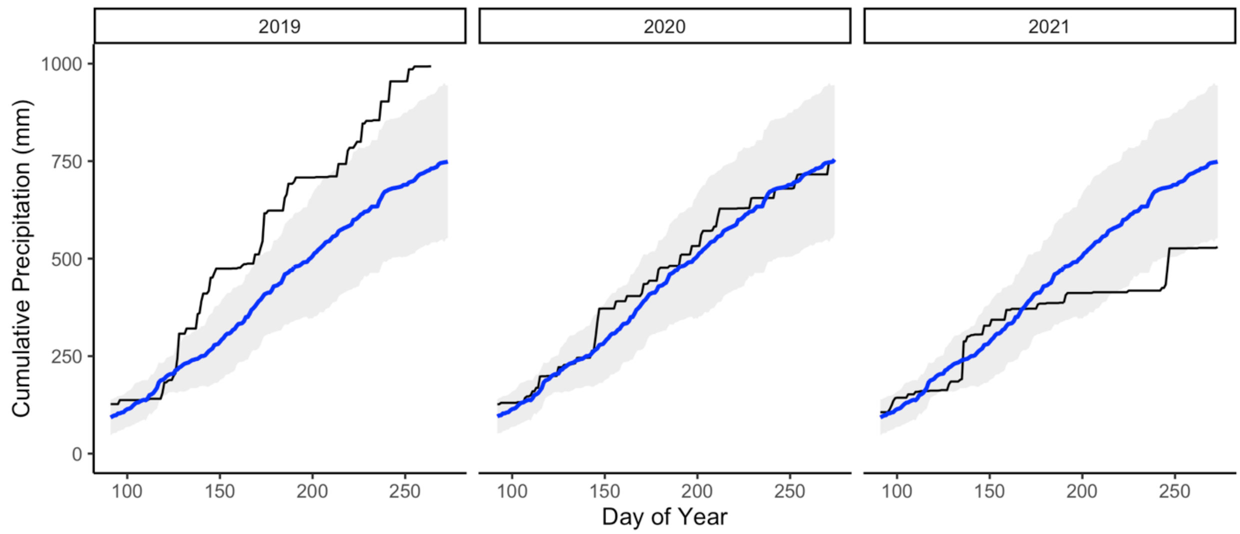


Appendix B, Figure 3: Green N content (%) of *A. gerardii* in 2018. Box outline indicates current treatment, and filled boxes were previously irrigated.

Appendix C Supplementary figures and tables for Chapter 4

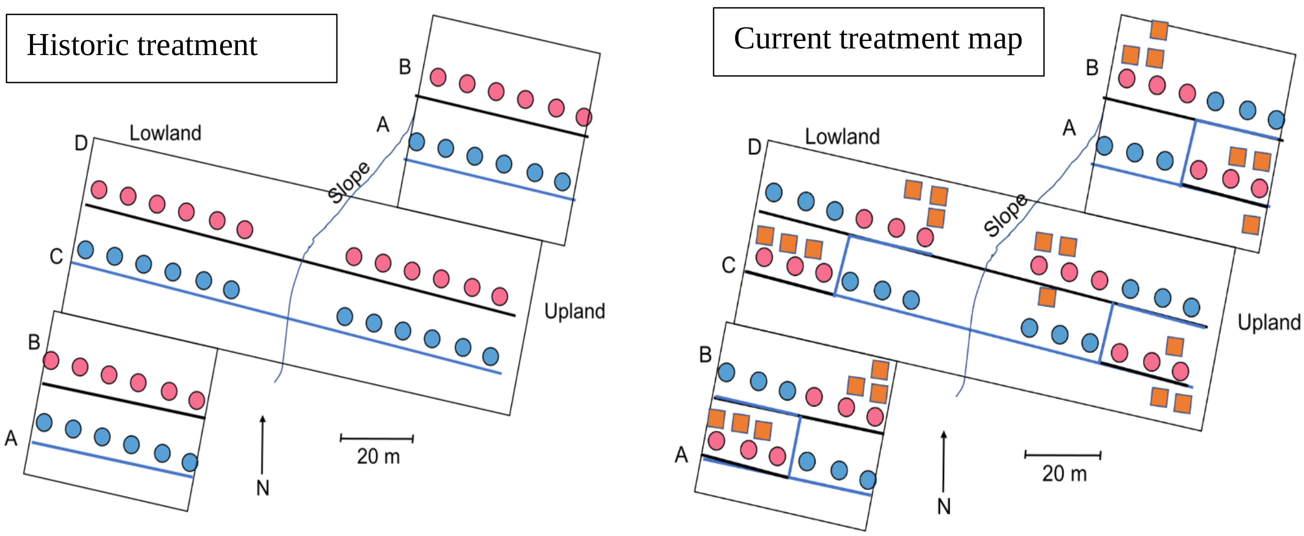
Appendix C, Table 1: Annual and growing-season precipitation, plus water added in irrigated treatments during study period.

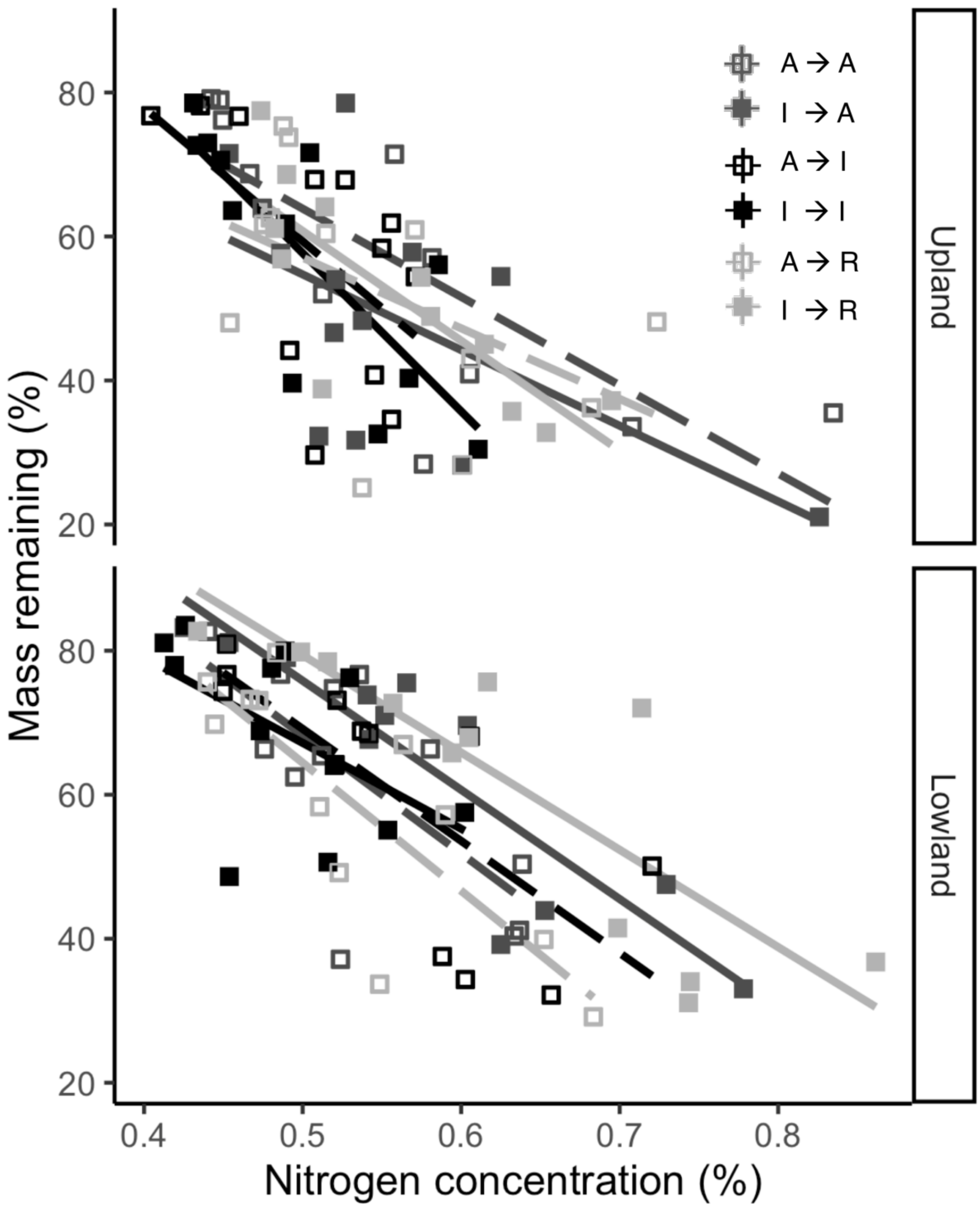
	2019	2020	2021
Annual precipitation (mm)	1131	833	632
Growing-season precipitation (mm)	885	621	424
Water addition (mm)	97 (upland) 101 (lowland)	61 (upland) 61 (lowland)	161 (upland) 161 (lowland)



Appendix C, Figure 1: Cumulative precipitation during the growing season (May-September) during the study period (black line), presented against the mean (blue line) and standard deviation (grey shading) of the 30-year average.

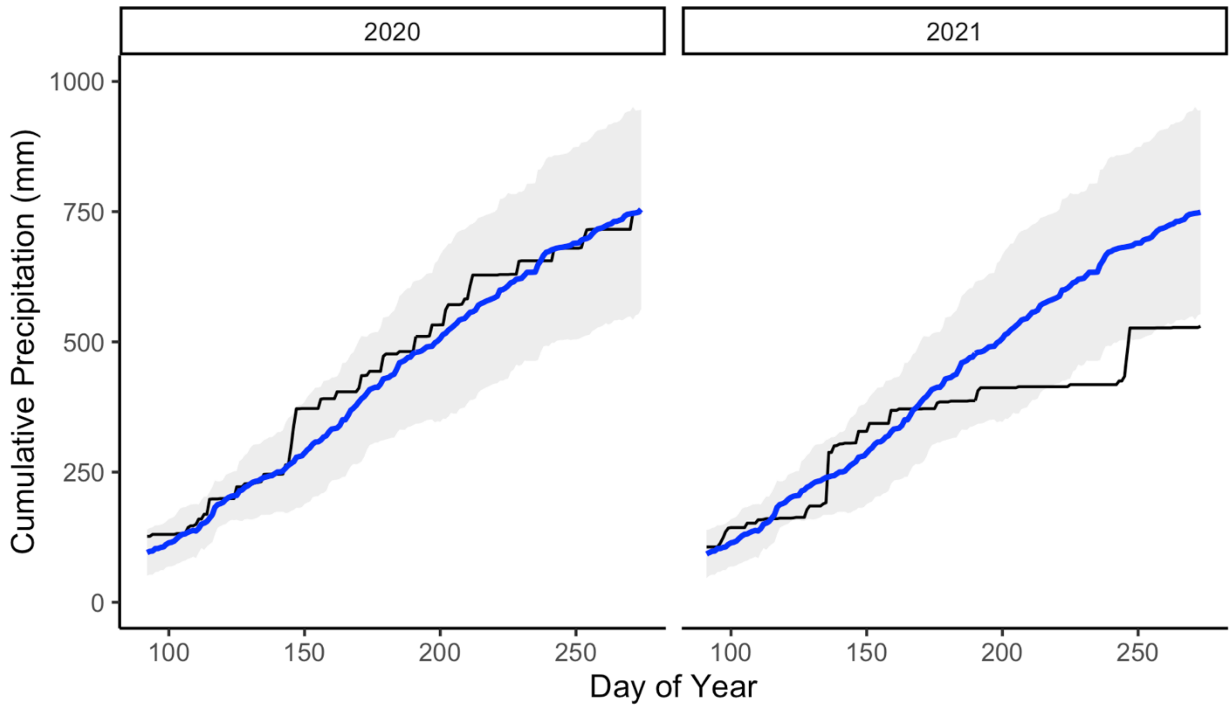
Appendix C, Figure 2: Maps of study area. Irrigated plots are blue circles, and ambient plots are red. In 2017, irrigation lines were shifted so that half of historically irrigated plots ceased being irrigated, and half of historic controls began irrigation treatment. Additionally, plots under drought shelters (orange squares) were added along transects in areas that were historically either irrigated or ambient. The study area is topographically divided into an upland and lowland area; plots on the sloped areas were excluded from this study and are not included in this map.





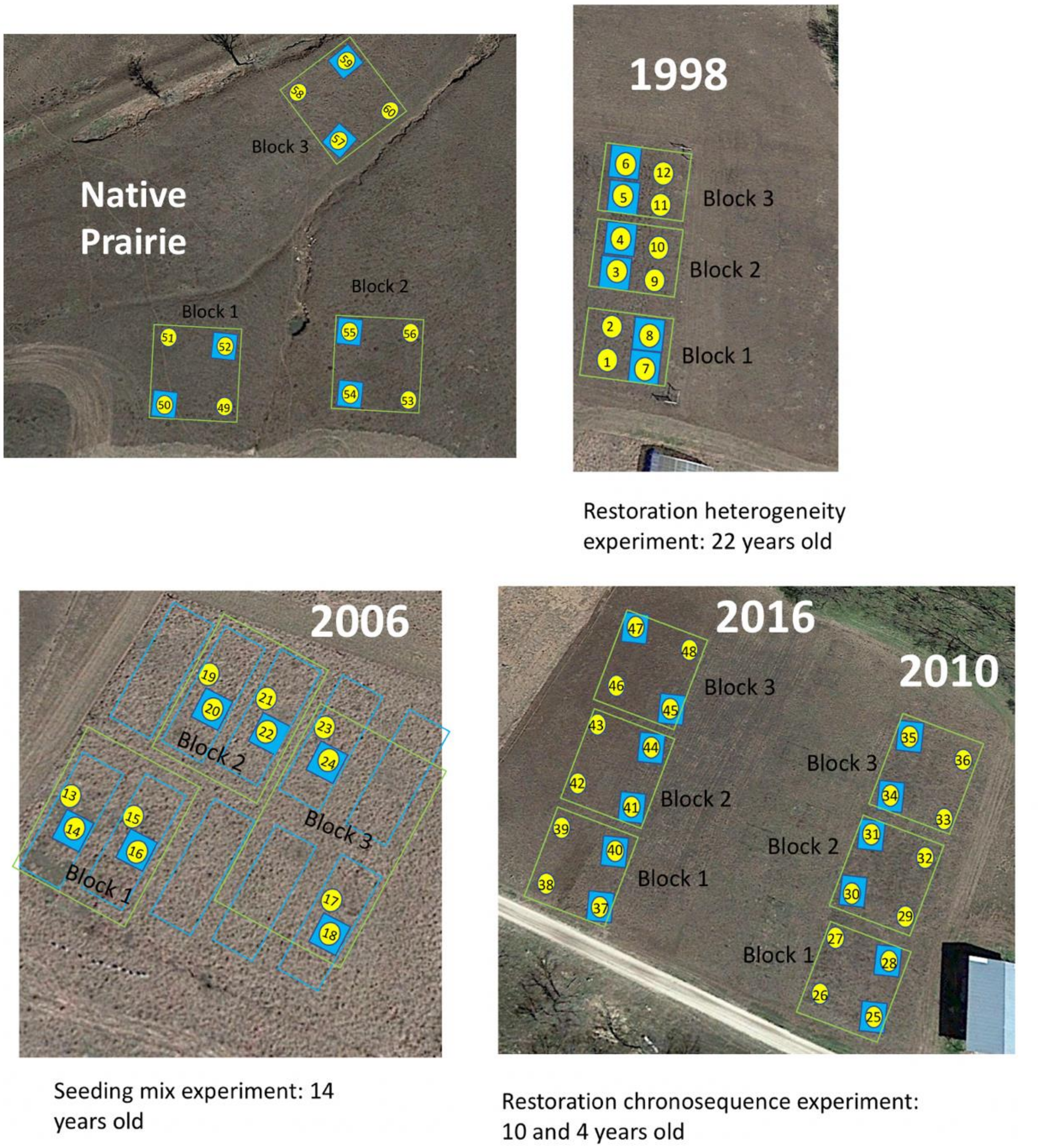
Appendix C, Figure 3: Litter mass remaining as function of litter N concentration, a measure of litter N immobilization potential. Points represent treatment means at each time point. There were no significant differences in the slope of this relationship among treatments.

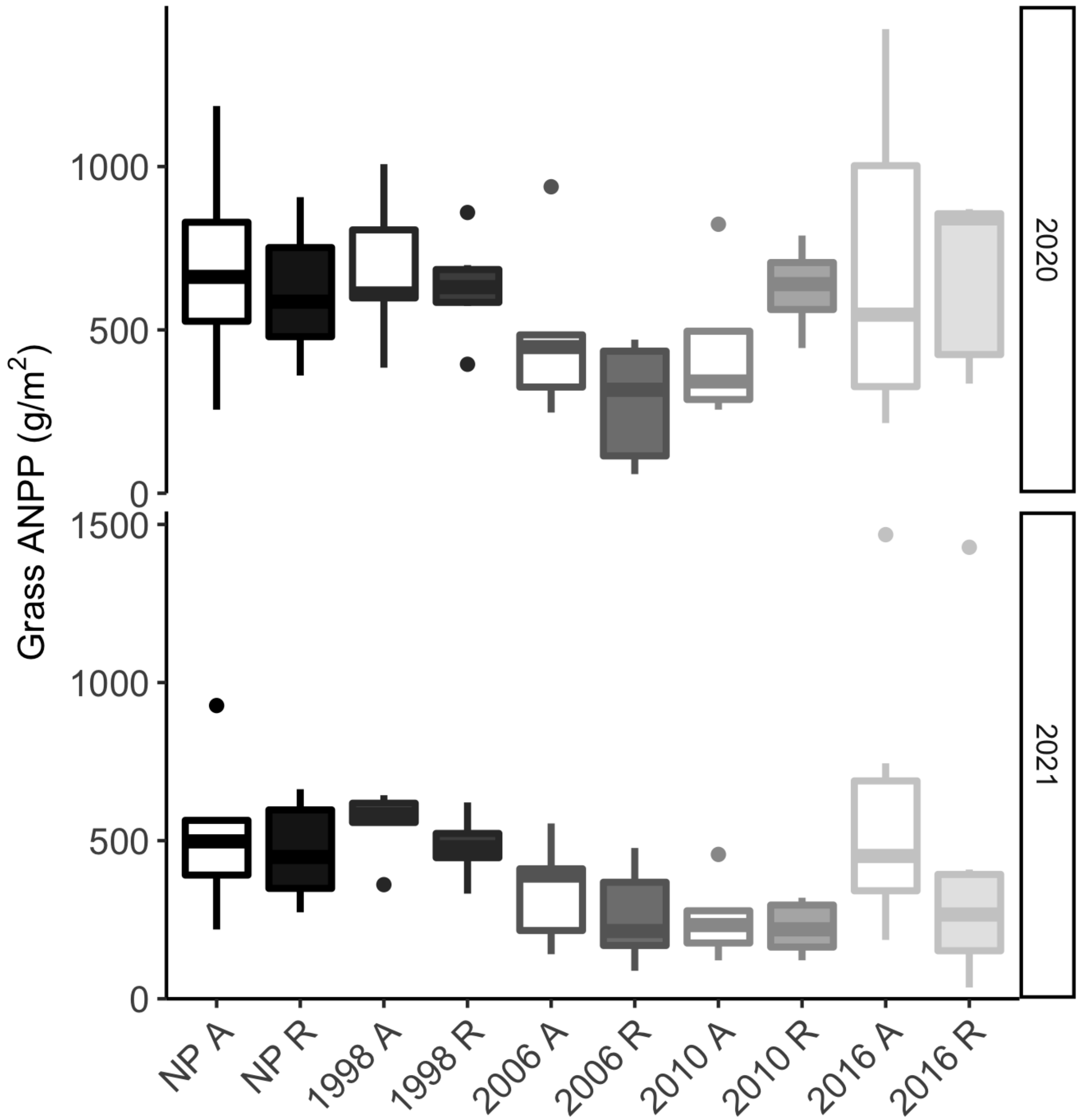
Appendix D Supplementary figures and tables for Chapter 5



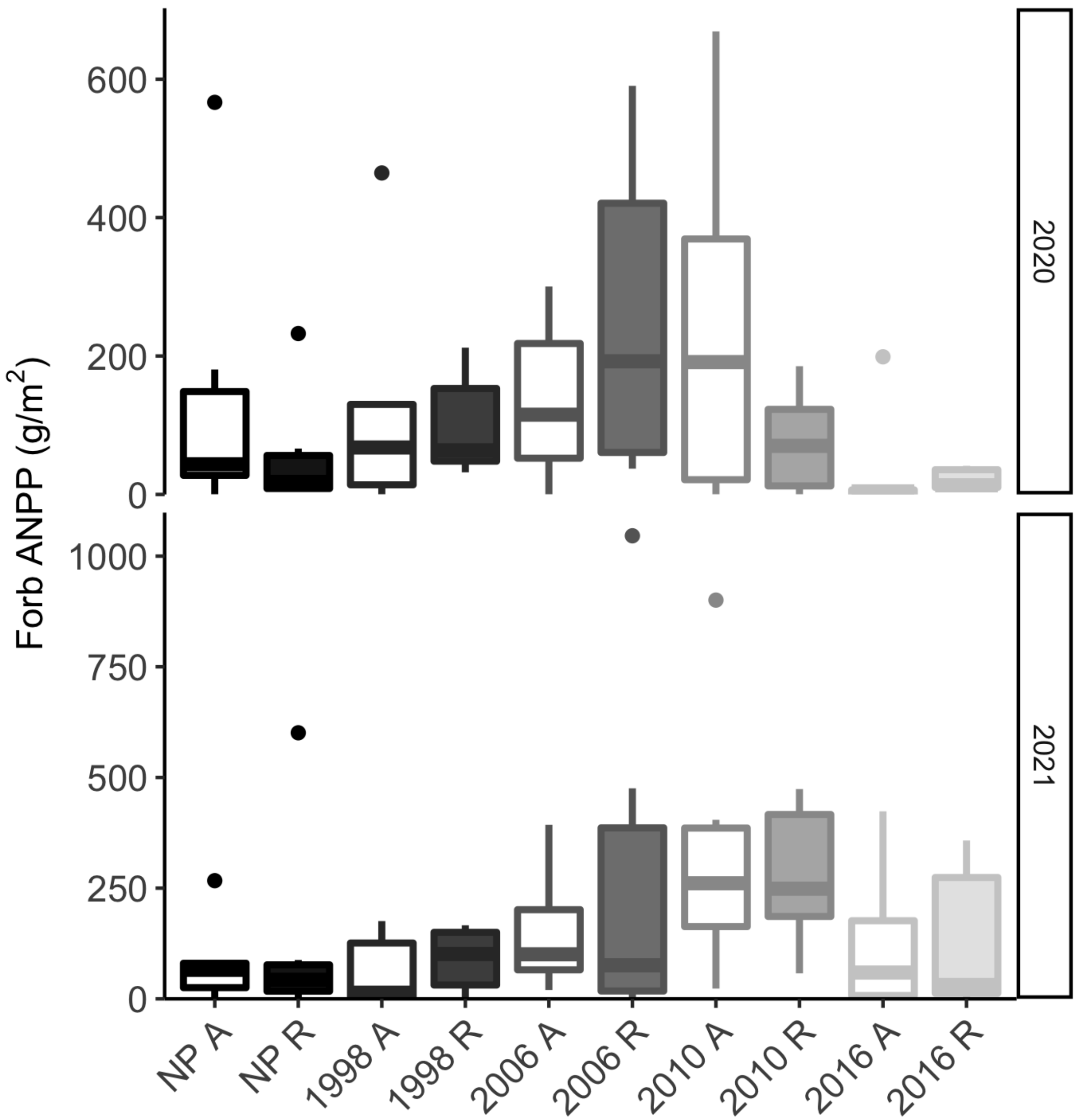
Appendix D, Figure 1: Cumulative precipitation during the growing season (May-September) during the study period (black line), presented against the mean (blue line) and standard deviation (grey shading) of the 30-year average.

Appendix D, figure 2: Map of the restorations at Konza Prairie Biological Station, as well as the reference prairie site.

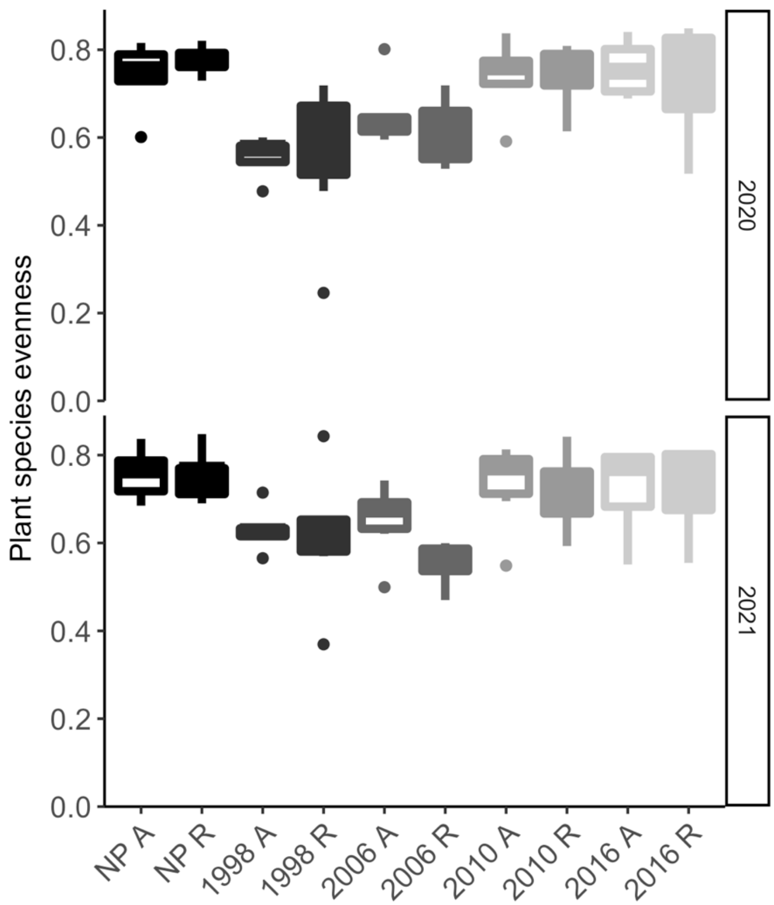




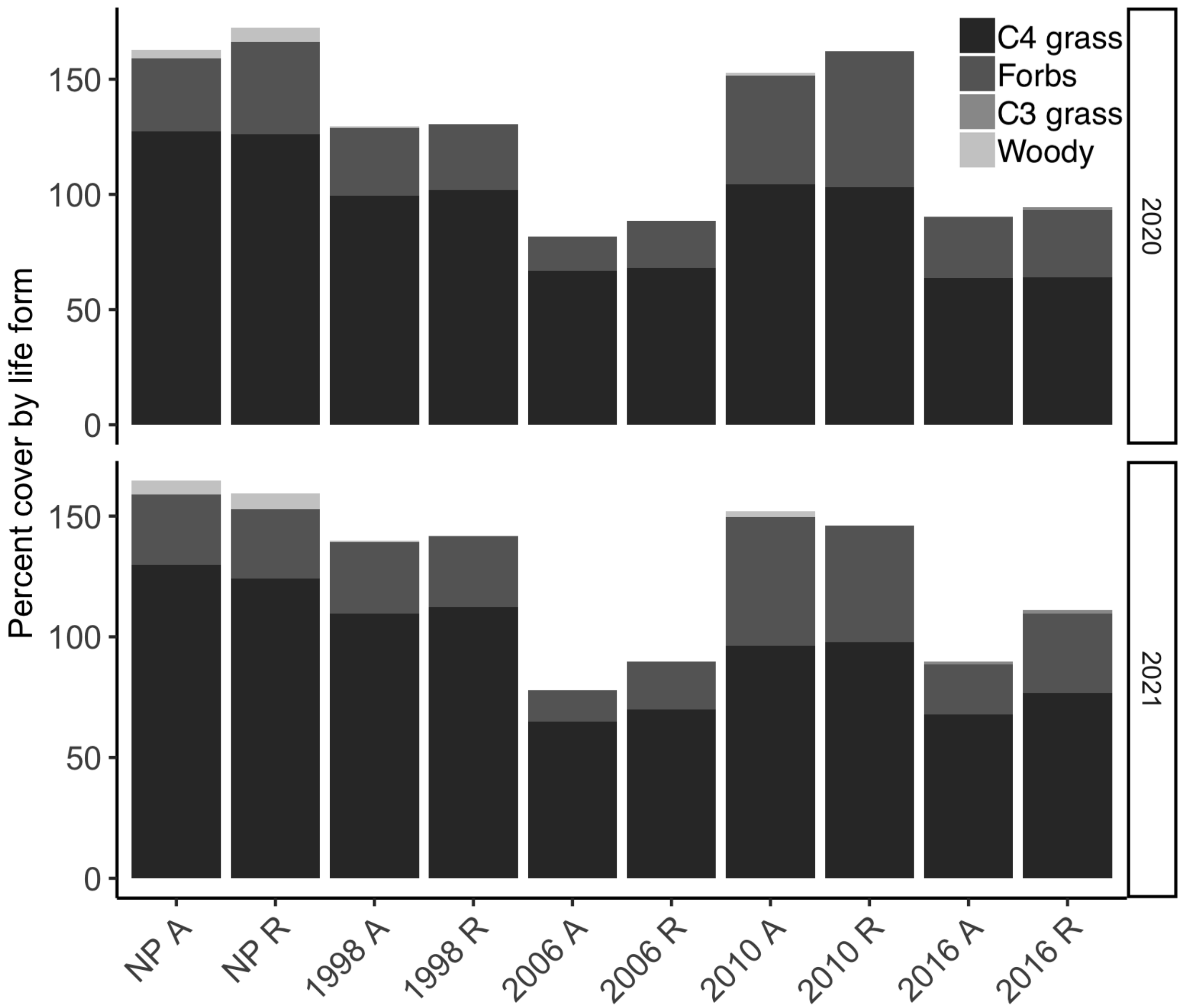
Appendix D, Figure 3: Grass ANPP (g/m^2) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.



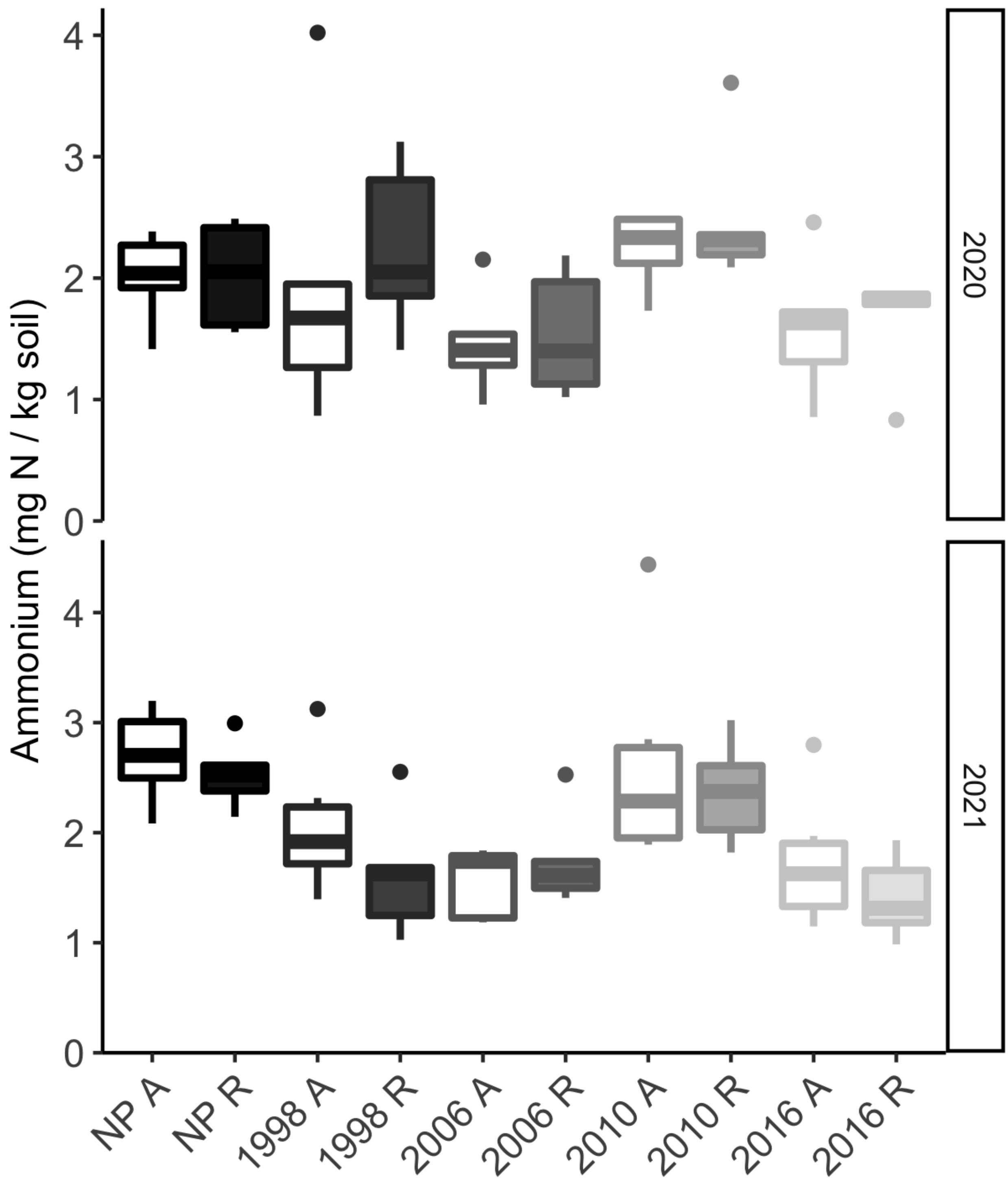
Appendix D, Figure 4: Forb ANPP (g/m^2) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.



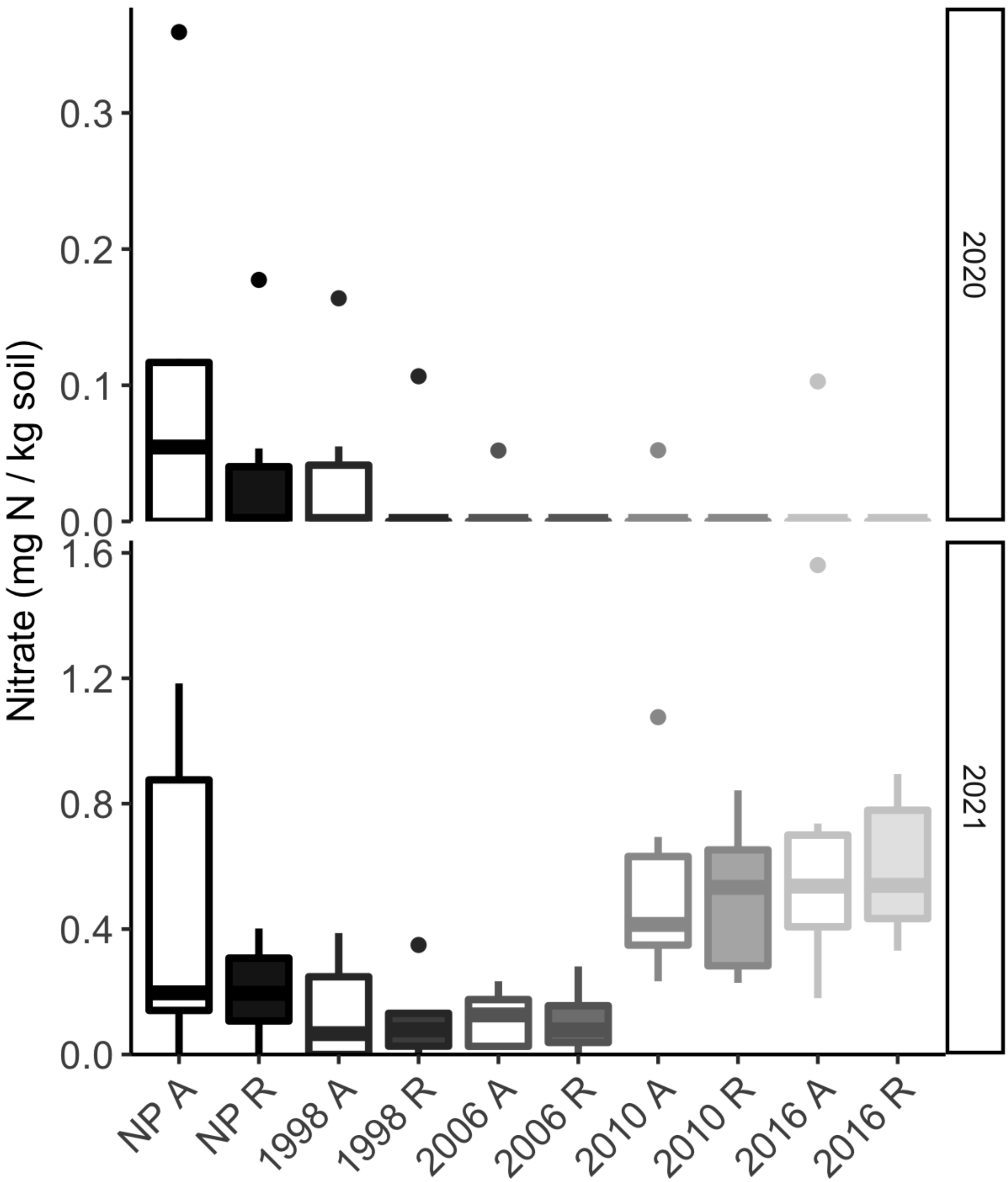
Appendix D, Figure 5: Plant species evenness in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.



Appendix D, Figure 6: Plant cover by functional group.



Appendix D, Figure 7: Soil ammonium concentrations (mg N/ kg soil) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.



Appendix D, Figure 8: Soil nitrate concentrations (mg N/ kg soil) in 2020 and 2021 in ambient (unfilled) and reduced (filled) rainfall treatments across restorations and native prairie.

Appendix E Sown species in prairie restorations in Chapter 5

1998 restoration:

Andropogon gerardii, Andropogon scoparius, Panicum virgatum, Sorghastrum nutans, Artemisia ludoviciana, Aster ericoides, Bouteloua curtipendula, Salvia azurea, Solidago canadensis, Amorpha canescens, Asclepias verticillate, Aster oblongifolius, Ceanothus herbaceus , Dalea purpurea, Asclepias viridis, Aster sericeus , Baptisia australis, Baptisia bracteata , Callirhoe involucrate, Koeleria pyramidata, Kuhnia eupatorioides, Lespediza capitata , Schrankia nuttallii, Solidago missouriensis, Sporobolus asper, Sporobolus heterolepis, Vernonia fasciculata, Desmanthus illinoensis, Echinacea angustifolia, Liatris punctata, Lomatium foeniculaceum, Oenothera macrocarpa, Penstemon cobaea, Penstemon grandifloras, Petalostemon candidus, Psoralea tenuiflora, Ratibida columnifera, Rosa arkansana, Ruellia humilis, Senecio plattensis, Sisyrinchium campestre, Triodanis perfoliata

Baer, S. G., Blair, J. M., Collins, S. L., & Knapp, A. K. (2003). Soil resources regulate productivity and diversity in newly established tallgrass prairie. *Ecology*, 84(3), 724-735.

2006 restoration:

Andropogon gerardii, Sorghastrum nutans, Schizachyrium scoparium, Bouteloua curtipendula, Elymus Canadensis, Achillea millefolium, Asclepias tuberosa, Aster oblongifolius, Baptisia australis, Dalea candidum, Delphinium virescens, Desmanthus illinoisensis, Lespedeza capitata, Liatris pycnostachya, Monarda fistulosa, Oenothera macrocarpa, Rudbeckia hirta, Solidago speciosa

Klopf, R. P., Baer, S. G., & Gibson, D. J. (2014). Convergent and contingent community responses to grass source and dominance during prairie restoration across a longitudinal gradient. *Environmental Management*, *53*(2), 252-265.

2010 and 2016 restorations:

Andropogon gerardii, *Sorghastrum nutans*, *Schizachyrium scoparium*, *Bouteloua curtipendula*, *Panicum virgatum* and *Elymus canadensis, Amorpha canescens*, *Baptisia autralis*, *Dalea purpurea*, *Dalea candida*, *Dalea multiflora*, *Desmanthus illinoiensis*, *Echinacea angustifolia, Helianthus pauciflorus, Lespedeza capitata*, *Liatris punctata*, *Oenothera macrocarpa*, *Rosa arkansana*, *Silphium integrifolium*, *Oligoneuron rigidum*

Scott, D. A., & Baer, S. G. (2019). Diversity patterns from sequentially restored grasslands support the 'environmental heterogeneity hypothesis'. *Oikos*, *128*(8), 1116-1122.