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Key Points:

- A wetter climate history resulted in a multi-year legacy of accelerated nitrogen (N) cycling in tallgrass prairie
- Precipitation effects on N fluxes may be an important mechanism underlying climate legacies in carbon fluxes
- The strength and sometimes direction of N cycling legacies varied across years and topo edaphic gradients

Supporting Information:

Supporting Information may be found in the online version of this article.

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Climate Legacy Effects Shape Tallgrass Prairie Nitrogen Cycling

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Abstract Climate change is expected to shift precipitation regimes in the North American Central Plains with likely impacts on ecosystem functioning. In tallgrass prairies, water and nitrogen (N) can co-limit ecosystem processes, so changes in precipitation may have complex effects on carbon (C) and N cycling. Rates of N supply such as N mineralization and nitrification respond differently to short- and long-term patterns in water availability, and previous climate patterns may exert legacy effects on current N cycling that could alter ecosystem sensitivity to current precipitation regimes. We used a long-term precipitation manipulation at Konza Prairie (Kansas, USA) to assess how previous and current precipitation influence tallgrass prairie N cycling. Supplemental irrigation was applied across upland and lowland prairie for ~25 years to reduce water deficits; in 2017, we reversed some of these treatments and added a reduced rainfall treatment across both historic rainfall regimes, allowing us to assess how previous climate and current rainfall patterns interact to shape N cycling. In lowland prairie, previous irrigation doubled N mineralization and nitrification rates the year following cessation of irrigation. Reduced microbial C:N ratio and lower relative investment in N-acquiring enzymes in previously irrigated lowlands suggested that a wetter climate created a legacy of increased N availability for microbes. Internal plant N resorption increased under short-term irrigation but recovered to ambient levels following previous irrigation. Together, these results suggest that a history of wetter conditions can create a legacy of accelerated N cycling, with consequences for both plant and microbial functioning.

Plain Language Summary Climate change will likely alter rainfall patterns in the Central US Plains, affecting the growth and activity of plants and soil microbes. Processes that support soil fertility respond differently to short- and long-term changes in water availability, such that rainfall amounts in the past may continue to affect current nitrogen (N) cycling. Such climate legacies can affect current ecosystem processes indirectly through persistent changes in soil N availability. To test the effects of previous and current rainfall on N cycling, we used a grassland long-term experiment in which we supplemented natural rainfall with irrigation for ~25 years. We then switched control and irrigated treatments and added a simulated drought treatment to learn how past and current rainfall independently and interactively affect current N cycling. In lowland tallgrass prairie, a historically wetter climate increased soil N supply and reduced relative microbial investment in acquiring N from soil organic matter. Additionally, while short-term higher rainfall appeared to increase relative plant N limitation, a long-term history of wetter conditions did not have this effect. Together, these results suggest that a wetter past climate can result in continued elevated soil N availability, reduced plant and microbial N limitation, and potentially higher rates of plant growth.

1. Introduction

Carbon (C) and nitrogen (N) cycles are strongly shaped by climate at local to global scales (Elrys et al., 2021; Piao et al., 2020), 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 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 aboveground net primary productivity (ANPP; Seastedt & Knapp, 1993), potentially affecting ecosystem C balance and sequestration. To understand how past and future precipitation regimes will interact to 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, in many ecosystems the relationship between annual precipitation (AP) and ANPP can be partially explained by precipitation amounts in the year preceding measurement (Griffin-Nolan et al., 2018; Sala et al., 2012). 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 (Broderick et al., 2022; Hawkes et al., 2017; Sala et al., 2012). Current climate models do not account for legacy effects that alter the interactions between C and 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 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 (Jin et al., 2013; Morris et al., 2022; Wang et al., 2006). 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 climate patterns 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, thereby changing the quality and quantity of plant inputs or the capacity for microbial nutrient transformations (de Nijs et al., 2019; Veach & 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 more N-limited due to decreased soil N mobility (Manzoni et al., 2014; Marschner & Rengel, 2012). A wetter precipitation history may result in legacies of increased plant inputs, larger substrate pools, and/or higher microbial activity, leading to persistent accelerated N cycling rates (Dijkstra et al., 2018) and decreased plant reliance on N resorption (Vergutz et al., 2012; Zhao et al., 2017). 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 & 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, 1997), despite large stores of N in soil organic matter (SOM) (Risser & Parton, 1982). Relative water and N limitation may also vary with topography and with soil water availability (Turner et al., 1997). This co-limitation can result in indirect effects of climate change on prairie ecosystems via changes in N cycling responses, making climate sensitivity in these ecosystems hard to predict. For example, plant N resorption varies with soil moisture (Heckathorn & DeLucia, 1994, 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 as needed to satisfy actual evapotranspiration demand and minimize plant water stress for >25 years (Knapp et al., 2001). 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.

This experimental design allowed us to assess how these contrasting precipitation histories affect current tallgrass prairie ecosystem processes, as well as the sensitivity of these processes to current rainfall conditions. 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, positive legacies also were apparent across both plant functional groups, with few differences in plant community composition across climate treatments. Long-term release from water stress also resulted in high microbial biomass and elevated labile C pools. Because other studies have documented strong responses of C cycling rates to N in this ecosystem (Riggs & Hobbie, 2016; Seastedt & Knapp, 1993; Turner et al., 1997), altered N cycles may underlie climate legacies in C cycling in this grassland. Specifically, accelerated N cycling as a result of long-term irrigation may be an important mechanism maintaining high ANPP and soil respiration, as well as a driver of positive legacies in these C fluxes after cessation of irrigation.

In this study, we assessed how previous and current rainfall regimes affect N cycling processes in tallgrass prairie. Prior research in this same experiment suggested higher microbial activity (i.e., greater soil respiration and labile soil C pools) under both current and historic wetter conditions, suggesting potential faster microbial release of N from litter and/or SOM (Broderick et al., 2022). We predicted that this legacy of higher microbial activity and labile substrates would lead to higher soil N availability under both long-term irrigation and current water additions, as well as a legacy of elevated N transformation rates once long-term irrigation ended. Therefore, for 2 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 previously documented C cycling processes to N availability across historic and current climate treatments, we also measured several indices of ecosystem N limitation. Because ANPP remained elevated under long-term water additions, with no evidence of exacerbated N limitation of plant growth under wetter conditions, we hypothesized that plants may be responding to a wetter climate by altering nitrogen use efficiency. 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), facilitating the continued high sensitivity of ANPP to water availability after >25 years of supplemental water (i.e., no evidence of exacerbated N limitation reducing climate sensitivity). We also predicted that soil microbial communities would be less N limited as a result of previous long-term irrigation, 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 are based, in part, on persistent differences in the cycling of a co-limiting nutrient as a function of a past wetter climate.

2. Methods

2.1. 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 AP 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 loam (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 (Allen & Palmer, 2011).

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 long-term plant community composition surveys. All soil sampling took place immediately outside these long-term plots. We used 24 plots each in the uplands and lowlands, avoiding plots on slopes (Figure S1 in Supporting Information S1).

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, 3 × 3m drought shelters with roofs made of polycarbonate slats designed to reduce ambient precipitation by 66% (Yahdjian & Sala, 2002) were erected over 24 newly established plots, six 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.

2.2. Inorganic N Pools, Net N Mineralization and Nitrification Rates

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, but had holes in 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 hr) 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 (fresh weight) 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 five replicates.

2.3. Microbial Biomass N

Microbial biomass N (MBN) was measured in the same soil samples used for initial N concentration in June–August of 2018 and 2019, using the chloroform fumigation extraction technique (Cabrera & 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. MBN was calculated as the difference in total extractable N with and without fumigation. We conducted persulfate digestions of the extracts and analyzed the digested samples on a Flow Solution Autoanalyzer. 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).

2.4. Microbial Extracellular Enzyme Assays

In August of 2019, we measured hydrolytic enzyme potential activities (Saiya et al., 2002; Sinsabaugh et al., 1999) 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 μM reference standards. The assays were incubated at a final substrate concentration of 40 μM for 2 hr (βG), 3.5 hr (NAG), and 16 hr (LAP). After the incubation interval, the assay was stopped by adding 10 μl 0.5 M 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 (Broderick et al., 2022; Wilcox et al., 2016), we did not adjust these values based on SOM 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).

2.5. 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 hr, 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).

2.6. Data Analysis

Due to 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 mineralization and nitrification rates, for which many values were negative, the Yeo-Johnson transformation was used because it corrects for positive skew while allowing for negative rates (Yeo & 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. Model main effects are included in Supporting Information S2.

3. Results

3.1. 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. Intra-annual rainfall variability in 2018 resulted in prolonged water deficits

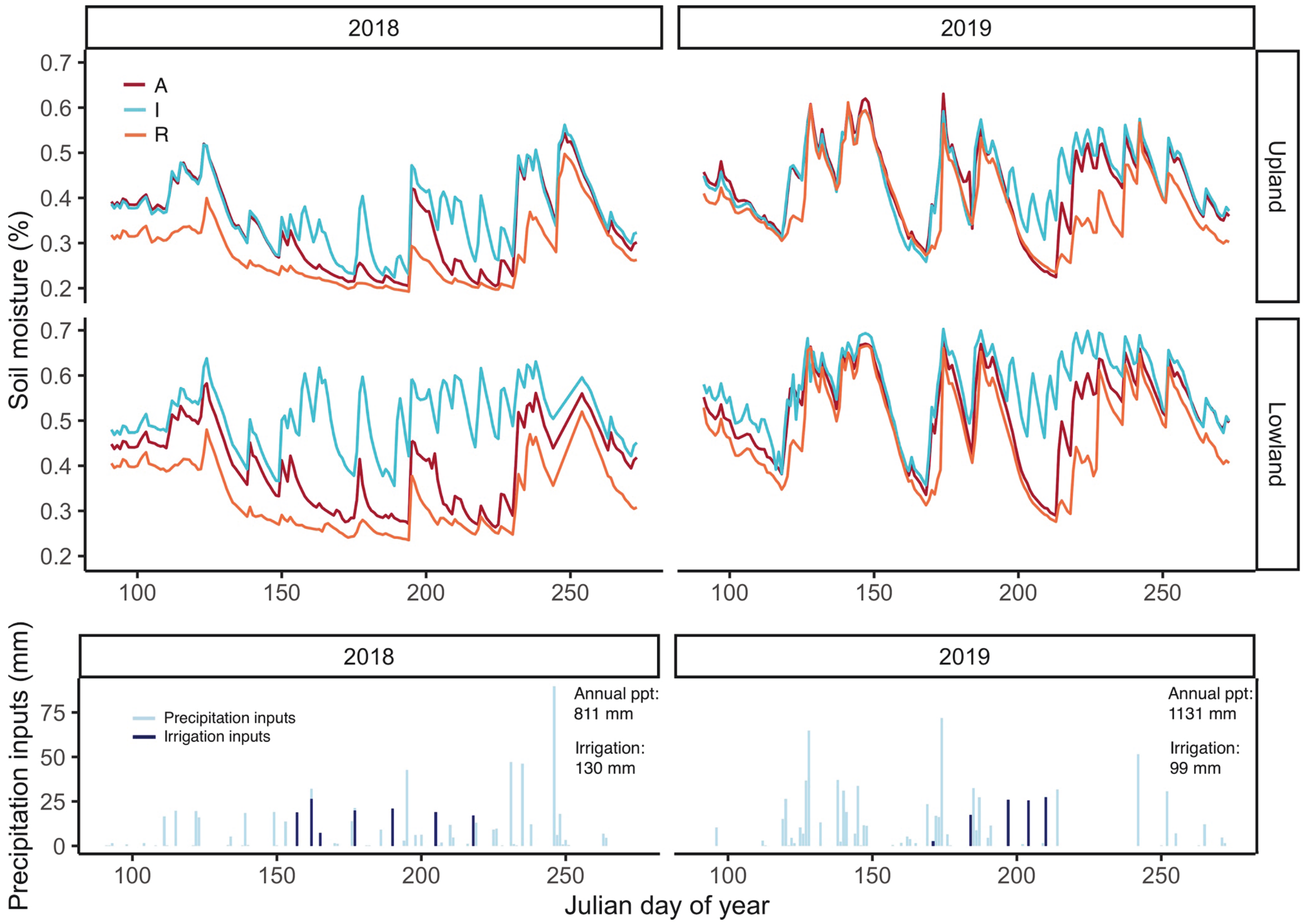

Figure 1. Volumetric soil water content (top) and daily precipitation inputs (bottom) from 0 to 15 cm during the growing seasons of 2018 and 2019. Soil moisture for current ambient (A, dark gray lines), irrigated (I, black lines), and reduced (R, light gray lines) rainfall treatments are shown; previous treatments are not shown since they did not affect VWC.

(Figure S2 and Table S1 in Supporting Information S1), so that water content was affected by current irrigation (19% increase) and drought (17% decrease) treatments (Figure 1). In 2019, a wet year (1,131 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.

3.2. Soil Available N

Soil ammonium concentrations in 2018 were slightly, but significantly, higher with a history of irrigation (2.2 vs. 2.1 μg N/g soil; $p = 0.044$; Figure 2a). 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 2b). 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.

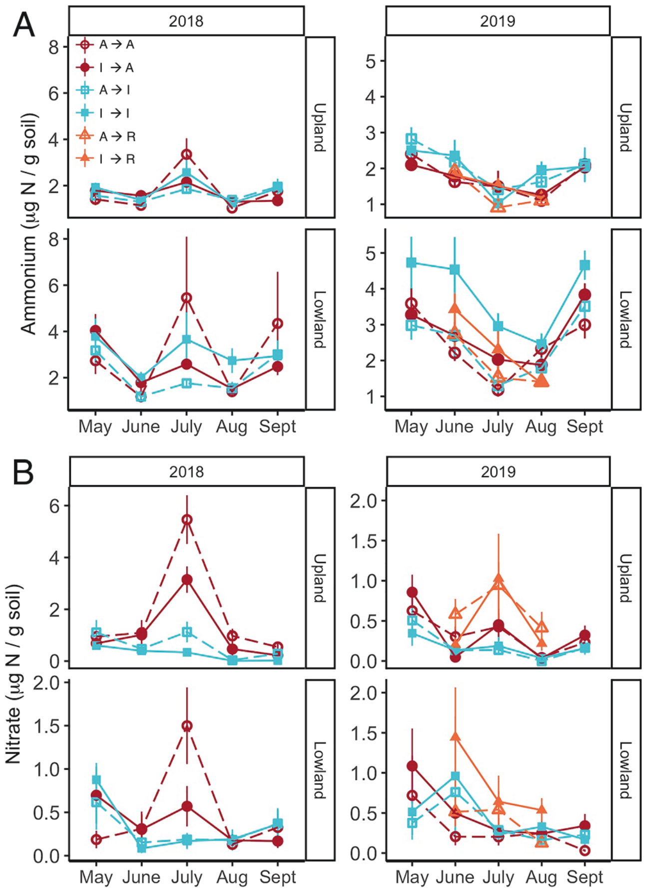

Figure 2. (a) Mean soil ammonium and (b) mean nitrate concentrations (with standard error bars) 0 to 10 cm depth across sampling months in upland and lowland prairie in 2018 and 2019. Legend shows historic treatment (ambient (A) or irrigated (I), followed by an arrow and the current treatment (A, I, and reduced rainfall). Red outlined circles indicate current ambient treatments, blue outlined squares indicate current irrigated treatments, and orange outlined triangles indicate current drought treatments (only June–August 2019). Filled points were previously irrigated.

3.3. 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 3a). 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 3a). 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 current or previous 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 $R^2 = 0.610$; Figure 3b). 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 only marginally significant ($p = 0.080$), despite a similar 30% decrease.

3.4. Net Nitrification Rates

Similar to net N mineralization rates, net nitrification rates were quite variable and the fixed effects left much variation unexplained (marginal $r^2 = 0.094$; Figure 4a). 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 4b) 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 (~2×) increase in nitrification rates with previous irrigation in the lowlands in 2018 ($p = 0.016$), similar in magnitude to the daily rates.

3.5. Microbial Biomass N and C:N Ratio

In 2018, long-term irrigated plots (I→I) in the lowlands had much higher MBN than any treatments (all $p < 0.01$; Figure 5a). 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 ($p < 0.001$).

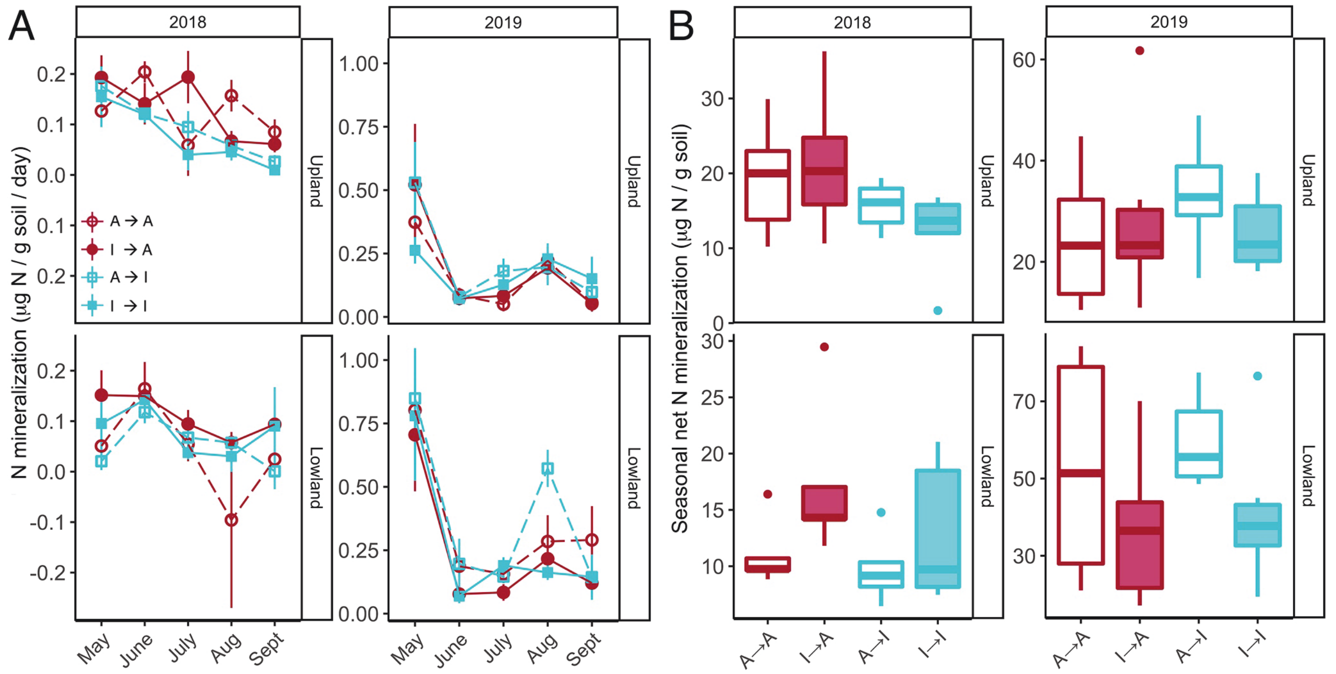

Figure 3. (a) Daily net nitrogen mineralization rate for each month (with standard error bars) and (b) seasonal mineralization rate. In situ rates were calculated from soils 0 to 10 cm deep in upland and lowland prairie in 2018 and 2019. In (a), red outlined circles indicate current ambient treatments, and blue outlined squares indicate current irrigated treatments. Filled points were previously irrigated. In (b), box outline color indicates current treatment, and filled boxes were previously irrigated.

In 2019, MBN was higher in the lowlands (33 vs. 30 µg N/g soil; $p < 0.001$), 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.

3.6. 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 5b). 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% ($p = 0.004$). 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(βG)/(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

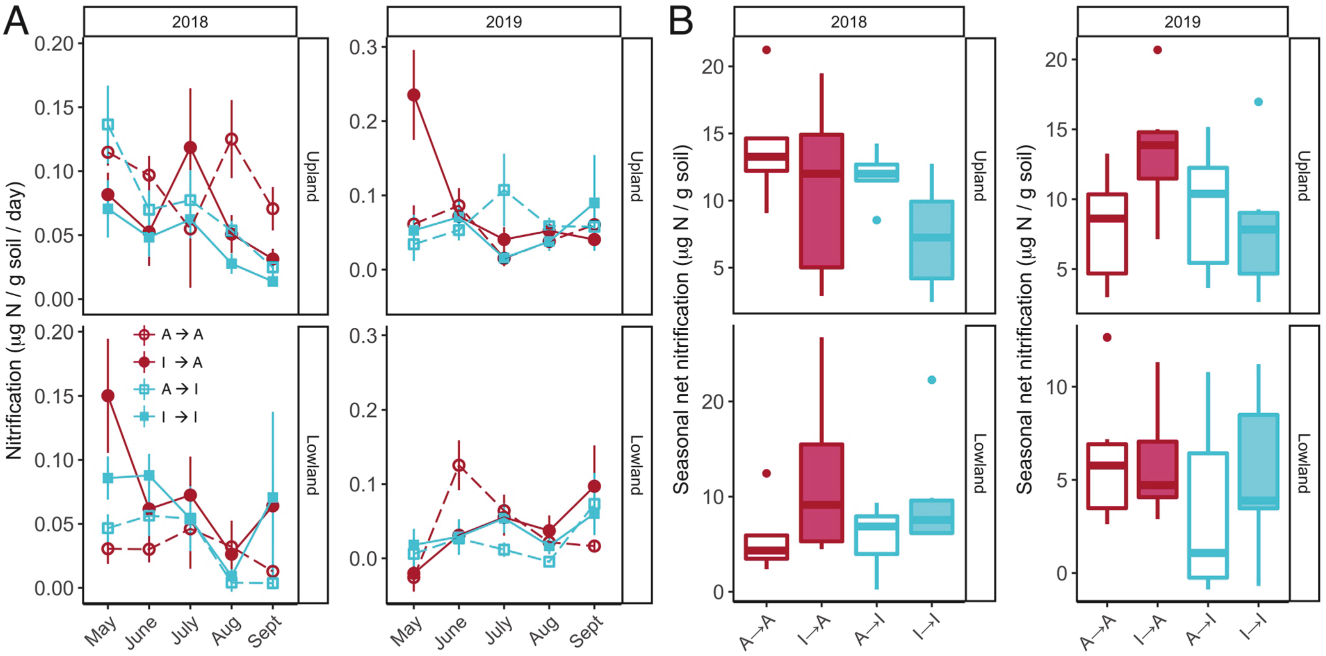

Figure 4. (a) Daily net nitrification rate for each month (with standard error bars) and (b) seasonal nitrification rate. In situ rates were calculated from soils 0 to 10 cm deep in upland and lowland prairie in 2018 and 2019. In (a), red outlined circles indicate current ambient treatments, and blue outlined squares indicate current irrigated treatments. Filled points were previously irrigated. In (b), box outline color indicates current treatment, and filled boxes were previously irrigated.

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.

3.7. Plant N Concentration and Resorption

Green plant N concentration was lower in currently irrigated compared to ambient plots ($p = 0.002$) and the drought treatment increased plant N ($p = 0.027$), as expected (Figure S3 in Supporting Information S1). However, we also found that previous irrigation increased plant N concentrations in the lowlands ($p = 0.006$). In the uplands, current irrigation increased N resorption proficiency (i.e., plant tissue reached a lower concentration upon senescence) compared to ambient and reduced rainfall treatments ($p < 0.001$; Figure 6a); similarly, the drought treatment had the lowest resorption proficiency in the lowlands (A vs. R: $p = 0.006$; I vs. R: $p < 0.001$). N resorption efficiency (percent decrease in N concentration from green to senesced) showed a similar pattern, with higher efficiency in the currently irrigated treatments than the reduced rainfall treatment ($p = 0.005$). Historic irrigation did not affect either measure of internal N recycling (Figure 6b).

4. Discussion

In order to predict and potentially 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 (Broderick et al., 2022; Kannenberg et al., 2020; Sala et al., 2012). In the Central Plains, precipitation is expected to become more variable within and between years, raising questions about how sensitive these ecosystems will be to altered precipitation patterns as well as how previous rainfall history across the region will moderate this sensitivity. 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 irrigation to simulate a wetter climate resulted in elevated N mineralization and nitrification rates that persisted for

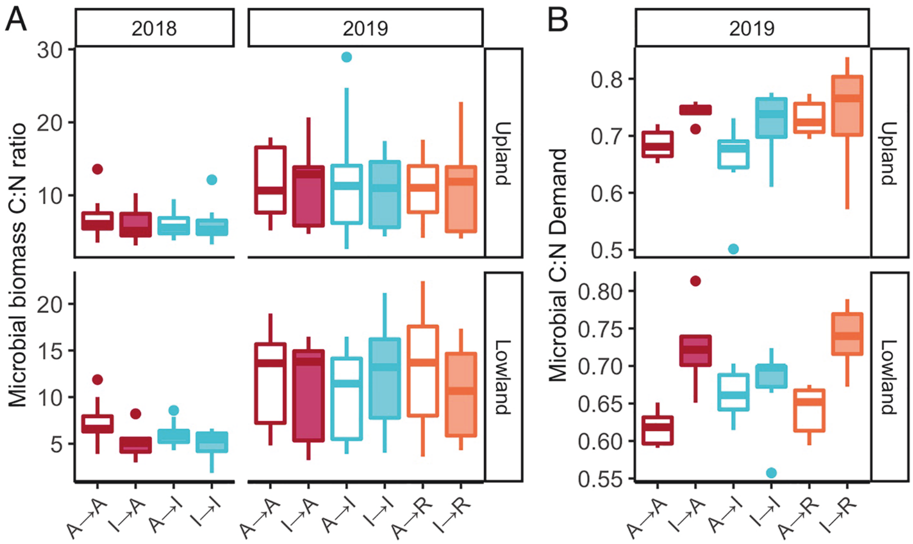

Figure 5. (a) Boxplot of microbial biomass (c) nitrogen (N) ratio from 0 to 10 cm in upland and lowland prairie in 2018 and 2019, and (b) boxplot of microbial relative N demand via investment in extracellular enzymes, from 0 to 10 cm in upland and lowland prairie in August 2019. Box outline color indicates current treatment, and filled boxes were previously irrigated.

2 years following cessation of irrigation treatment. Microbial communities also appeared to be less limited by N following 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. This suggests persistent increases in N availability following a wetter climate period in these grasslands. 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.

4.1. 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 (Austin & Vitousek, 1998; McCulley et al., 2009), other studies have also found that inorganic N increases with precipitation across climate gradients (Austin & Sala, 2002). Although long-term wetter conditions can lead to enhanced leaching and gaseous N losses in some ecosystems, exacerbating N limitation (Ren et al., 2017; Unger & Jongen, 2015), 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 (Dell & Rice, 2005; Rice & Tiedje, 1989; Seastedt & Ramundo, 1990), the release from water stress combined with the increased mobility of nitrate under wetter conditions may have increased plant nitrate uptake, and/or caused

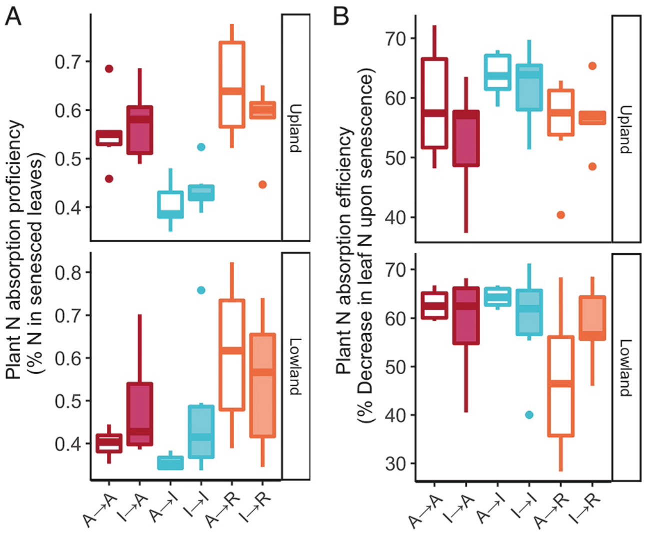

Figure 6. (a) Nitrogen (N) resorption proficiency (%N in senesced leaves) and (b) N resorption efficiency (% decrease in N upon senescence) of *A. gerardii* in 2018. Box outline color indicates current treatment, and filled boxes were previously irrigated.

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.

4.2. 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 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; Li et al., 2019; Marschner & Rengel, 2012). 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 MBC, 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 & 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 AP, which has been attributed to concurrent increases in soil organic C that increase potential N immobilization (Barrett et al., 2002; Feyissa et al., 2021; McCulley et al., 2009). We did not find changes in soil C during the duration of our study (Broderick et al., 2022; Wilcox et al., 2016) or legacies of previous irrigation on soil moisture. Moreover, while historic rainfall patterns may have altered N mineralization via effects on litter chemistry (i.e., belowground plant inputs in this annually burned system), the lack of response of root biomass and chemistry to rainfall treatments (Broderick et al., 2022) suggests that changes in plant C inputs were not the driver of N cycling legacies. 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). In these soils that have large stores of organic N (Risser & Parton, 1982), changes in the rate of mineralization can potentially have large influences on available plant N and therefore C cycling.

The effects of historic climate treatment on nitrification rates also varied by year and with topography. Nitrification rates were lower with previous irrigation in the uplands in 2018, but much higher 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; Broderick et al., 2022), especially in forbs which tend to have a higher N demand (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 increased microbial N uptake under non-drought conditions (Blair et al., 1998; Lü et al., 2014). In contrast, long-term irrigation increased these rates in the lowlands. Persistent increases in precipitation may increase the size of nitrifier populations, leading to this climate legacy, while short-term water availability primarily acts through physiological mechanisms. Importantly, significant positive legacy effects 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).

4.3. Microbial N Demand

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 (Moorhead et al., 2012; Sinsabaugh et al., 2008), 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 are 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 microorganisms.

4.4. Plant N Limitation

Increased precipitation inputs can exacerbate plant N limitation, especially if wet conditions lead to N losses (Ren et al., 2017). Indeed, in our study plant internal N recycling was higher with current irrigation and lower with current drought, consistent with other findings (Lü & Han, 2010; Ren et al., 2015). Yet we found no effect of long-term irrigation on N resorption, contrary to our hypothesis, suggesting that plants were not increasing the efficiency of internal N recycling. Instead, we actually found higher green N concentrations in the dominant grass with previous irrigation 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. Yet our results suggest that, even after decades of irrigation in tallgrass prairie, N was more available and remained so 2 years following cessation of irrigation. These results highlight a key difference between short- and long-term effects of precipitation patterns on plant chemistry in this ecosystem. An extended wetter climate history accelerated microbial net N mineralization and nitrification, supporting higher plant available N as well as faster cycling of C via ANPP and soil respiration even after the cessation of irrigation. These effects may feed back on each other; since historically wet conditions increased plant productivity without dilution of N (with even some evidence of increased plant N), which may accelerate litter decomposition with previous irrigation and contribute to a positive feedback of accelerated C and N cycling responses. Overall, a legacy of higher precipitation and resulting accelerated N cycling mitigated any potential increase in nutrient limitation associated with higher precipitation, likely due to the high C and N content of soils in these grasslands. Our study supports other findings that, in ecosystems limited by both water and N, wetter conditions may influence C cycling in large part through its effects on N availability (Lü et al., 2014; Ren et al., 2017).

4.5. Spatiotemporal Differences in Climate Responses

Responses of N cycling to historic climate treatments, particularly N fluxes and patterns in microbial N demand, were largely concentrated in the lowlands. This was surprising, as the well-drained upland soils tend to be drier and more water limited, but was consistent with spatial variation in climate legacies in C cycling in this ecosystem (Broderick et al., 2022). Nitrification actually showed opposing trends across the topographic divide, with positive legacies in the lowlands and a slight negative legacy in the uplands, perhaps because the exceptional water deficits in upland soils during the dry year of 2018 negatively impacted the nitrifiers, especially in these previously irrigated soils (Nguyen et al., 2018). The differences in the strength and even direction in climate legacies across this modest topographic gradient indicates the need for more studies across landscapes and regions to help generalize patterns in climate legacies. Moreover, the assumption that drier ecosystems would be more sensitive to persistent increases in water availability across larger spatial scales (Huxman et al., 2004) was not supported by the stronger climate legacies in the lowlands in our study, which is consistent with other results from water additions in grasslands (Wilcox et al., 2015). Our results emphasize the role of smaller-scale topoedaphic factors in driving patterns in climate sensitivity at landscape scales, in particular the potential role of soil depth, and highlight the challenges in predicting climate legacies even within ecosystems. Legacy effects also varied between the two sampling years for several responses. N mineralization, for example, showed a positive irrigation legacy in the dry year of 2018 but a negative legacy in the wet year of 2019. This is not surprising, as gross N mineralization, immobilization, and nitrification may differ in soil moisture sensitivity (Homyak et al., 2017; Zhang et al., 2016); indeed, one recent study found that gross N mineralization and nitrification decreased under water stress, but N immobilization increased (Morris et al., 2022). While responses such as investment in N-acquiring enzymes showed climate legacies in 2019, overall legacies were stronger under natural drought (in 2018), perhaps because the legacy effects had faded after three years. Alternatively, legacies of a wetter climate may be particularly important under conditions of water stress, where increased N availability can ameliorate the effects of drought (Hofer et al., 2017). In this case, future N cycling in grasslands under a more variable climate, in which ecosystems are subject to more frequent and severe water deficits, may be increasingly shaped by previous climate conditions. A key challenge going forward will be generalizing the influence of climate history across landscapes, ecosystems, and current conditions.

4.6. Carbon Cycling Legacies via Biogeochemical Legacy Effects

The persistent high ANPP in previously irrigated prairie was apparent across plant functional groups, indicating that plant community legacies alone do not explain this positive legacy. 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.

4.7. 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 2 years following treatment reversal (through 2018). Multiple indices suggested that previous irrigation decreased microbial biomass N limitation, including 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, this study documents climate legacies lasting at least 3 years; however, most field-scale rainfall manipulation studies designed to test climate change effects also last <5 years. Short-term experiments may measure responses that are buffered by legacy effects, thus underestimating the changes in C and N cycling due to persistent climate change, highlighting the importance of decadal-scale climate experiments.

Data Availability Statement

Data are available at http://lter.konza.ksu.edu/content/wat03-climate-legacy-effects-shape-tallgrass-prairie-nitrogen-cycling.

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