DISSERTATION

INTERACTIVE EFFECTS OF CHRONIC NITROGEN ADDITION AND PULSED DELUGE EVENTS ON COMMUNITY STRUCTURE AND FUNCTION OF THE SHORTGRASS STEPPE

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ABSTRACT

INTERACTIVE EFFECTS OF CHRONIC NITROGEN ADDITION AND PULSED DELUGE EVENTS ON COMMUNITY STRUCTURE AND FUNCTION OF THE SHORTGRASS STEPPE

Global change is intensifying climate extremes, leading to more intense droughts and deluges (i.e., large rainfall events). Simultaneously, mass industrialization has led to the continuous alteration of resources such as atmospheric CO_2 and reactive nitrogen. Patterns of perturbations can be evaluated through the “pulse-press” framework which describes resource alteration along a temporal continuum: from discrete, short-term “pulse” to chronic, long-term “press”. The combination of chronic press and discrete pulse resource alterations is expected to cause large and potentially unexpected changes to ecosystem structure (i.e., diversity, community composition) and function (i.e., productivity). Pulse-press dynamics have been observed and explored in theory and models, but robust experimental evidence is lacking. Therefore, this dissertation experimentally evaluated pulse-press dynamics in two grassland ecosystems: a shortgrass steppe in northeastern Colorado and a tallgrass prairie in northeastern Kansas. The first chapter of this dissertation established how the two grassland ecosystems responded to five years of nitrogen press along a gradient of addition levels. Within the shortgrass steppe, I found that nitrogen addition did not affect productivity but caused community change. In contrast, production increased within the tallgrass site without changes in community composition. These divergent responses, likely mediated by site-level differences in aridity, demonstrate that structural and functional responses may decouple under nitrogen press. The second chapter examined the interaction of pulsed deluge timing and nitrogen press at the shortgrass steppe. An extreme deluge applied mid-late growing season in 2021 had no significant effect on structure or function, while an early-mid deluge in 2022 synergistically interacted with nitrogen addition resulting in large increases to production. This synergistic interaction was driven by an increase in a weedy forbaceous species and showed that nitrogen press and pulsed deluge events can generate unexpected effects on ecosystem function. The third and final chapter of this dissertation assessed the legacy effects of deluge pulse amid ongoing nitrogen press. We monitored post-deluge plots and found unexpected production legacies that were not predicted by the initial response to deluge but were influenced by growing season precipitation. Community composition changes persisted even during the dry 2024 growing season, when production legacies were no longer evident. These results demonstrate that effects of deluge pulse amid ongoing nitrogen press may generate lagged responses, independent from initial responses. Collectively, this work establishes that interacting pulse and press resource alterations can produce large, complex, and often unforeseen effects on the structure and function of grassland ecosystems.

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INTRODUCTION

Large changes in ecosystem structure and function are often the result of multiple interacting perturbations. For example, in lakes, rising water temperatures promote cyanobacterial dominance, and when combined with nutrient runoff, can lead to algal blooms that degrade ecological structure and value (Kosten et al. 2012, Scheffer et al. 2015). In a western Tasmanian forest, a remnant paleoendemic community of pencil pine was severely reduced after lightning-ignited fires in 2016 destroyed a major refuge. These fires were preceded by years of warm, dry conditions, which increased the flammability of understory vegetation (Harris et al. 2018). These examples of interacting perturbations and their effects on ecosystems may seem disparate but can be unified and evaluated through the "pulse-press" framework.

The pulse-press framework describes patterns of resource alteration on a continuum from discrete, short-term "pulse" to chronic, long-term "press" (Smith et al. 2009, Collins et al. 2011, Ratajczak et al. 2017, Harris et al. 2018, Pellegrini and Jackson 2020). By organizing disturbances based on their temporal characteristics, this framework helps identify commonalities in how ecosystems respond to global change. Press perturbations are typically the result of anthropogenic influence such as, rising air and ocean temperatures, increased atmospheric CO_2, eutrophication, etc. In contrast, pulses cause short-term alteration in resource availability that may occur naturally but are intensified or eliminated with global change: fires, floods, drought, etc. (Smith et al. 2009). If the duration or intensity is great enough, pulse or press forces may independently cause ecological change, but their interactions can generate outcomes greater than the sum of their parts. Long-term resource press can erode ecosystem stability and resistance, then the addition of a short-term resource pulse may tip the system beyond critical thresholds, leading to large, and potentially unexpected, shifts in ecosystem structure and function (Scheffer et al. 2015, Harris et al. 2018). As both types of perturbations are expected to continue or intensify, it is essential to understand their individual and combined impacts on ecosystems.

Before the interaction of pulse and press perturbations can be assessed, the influence of press alone must be established. The increasing availability of reactive nitrogen (i.e., biologically available forms) is one such press perturbation that is occurring worldwide. Combustion of fossil fuels and nitrogen fixation for agricultural fertilization leads to widespread eutrophication through increased atmospheric deposition or leeching (Vitousek and Howarth 1991, Peters and Meybeck 2000, Galloway et al. 2003, Gruber and Galloway 2008, Fowler et al. 2013). Grasslands ecosystems are often nitrogen-limited, so inputs commonly result in altered structure and function (LeBauer and Treseder 2008, Smith et al. 2009, Bai et al. 2010, Isbell et al. 2013a, Avolio et al. 2014, Fay et al. 2015, Komatsu et al. 2019, Avolio et al. 2020, Seabloom et al. 2021). However, the degree and pattern of ecological change is influenced by the magnitude of the press, which is variable across time and space (Houseman et al. 2008, Isbell et al. 2013a, Linabury et al. *in review*) (Chapter 1). Thus, nitrogen press must be studied across a gradient to understand the full scope of functional and compositional change and for improving predictions of ecosystem behavior under ongoing eutrophication.

Once the effects of press perturbation are established, the additional influence of pulse may be assessed. Deluges are statistically extreme rainfall pulses that occur naturally but are expected to become more frequent as climate change intensifies the hydrological cycles (Knapp et al. 2008; Smith et al. 2009; Smith 2011; IPCC 2014). Like the timing of seasonal rainfall, the timing of deluge is a determinant of ecosystem structure and function (Heisler-White et al. 2008, Post and Knapp 2020, Griffin-Nolan et al. 2021, Hajek and Knapp 2022, Zhang et al. 2023, Hajek et al. 2024). Theory and simulations have predicted that pulse-press (i.e., deluge-nitrogen) forces may synergistically interact, producing effects greater than the sum of their individual impacts, but experimental evidence is lacking (Scheffer et al. 2001, 2015, Ives and Carpenter 2007, Smith et al. 2009, Ratajczak et al. 2017, Harris et al. 2018). In water-limited systems, for example, a well-timed deluge may enhance the uptake of accumulated nitrogen, thereby amplifying both productivity and community shifts (Bai et al. 2010, Peng et al. 2020, Seabloom et al. 2021, Yang et al. 2023). However, few studies explicitly replicate pulse-press perturbations, so it remains difficult to predict the specific conditions under which such synergies may emerge.

If pulse-press perturbations synergistically interact, their effects are expected to produce large initial impacts which may give rise to lingering legacy effects (Milchunas and Lauenroth 1995, Vinton and Burke 1995, Isbell et al. 2013b, Monger et al. 2015). Deluge pulses may be especially likely to lead to legacies, since the volume and timing of previous years' rainfall is a predictor of current-year productivity (Sala et al. 2012, Gong et al. 2020). However, deluge legacies may be complicated by interactions with ongoing nitrogen press. Species with acquisitive traits that promote rapid resource uptake typically benefit from both nitrogen enrichment and high rainfall (Meng et al. 2023, Ratcliffe et al. 2024). As a result, compositional shifts initiated by a rainfall pulse may be maintained or amplified under continued nitrogen addition. The magnitude of nitrogen addition affects the degree of compositional shift (Isbell et al. 2013a, Simkin et al. 2016, Ma et al. 2021), so the role of nitrogen in modifying deluge legacy is likely contingent on the magnitude of nitrogen press. Thus, to fully understand pulse-press dynamics, legacies must also be assessed across a gradient of nitrogen press.

This dissertation integrates the concepts described above through experimental tests of pulse–press interactions. In Chapter 1, I assessed the effects of a short-term (five-year) nitrogen press applied along a gradient of addition levels. In Chapter 2, I examined how the timing of pulsed deluge events interacted with the nitrogen gradient. In Chapter 3, I investigated the legacy effects of deluge events in the context of ongoing nitrogen press. Through this work, I aim to provide critical experimental data that enhances our understanding of pulse–press dynamics in a changing world.

CHAPTER 1: Contrasting effects of five years of nitrogen addition on plant community structure and function in a semi-arid vs. mesic grassland

1. Summary

Anthropogenic activities cause accumulation of biologically reactive nitrogen in ecosystems worldwide, leading to substantial changes in plant community structure and function, particularly in nitrogen-limited grasslands. Responses of plant communities and primary productivity vary depending on the magnitude of eutrophication and climate of the ecosystem, yet the exact form of these relationships is largely unknown. Here, we report results from the first five years of an experiment in which nitrogen was added at 8 levels, ranging from 0 to 30 g m^{-2} at two grassland sites bookending the broad precipitation gradient of the US Central Plains: 1) semi-arid shortgrass steppe and 2) mesic tallgrass prairie. This allowed us to examine the mediating effects of climate on short-term responses to nitrogen addition. Although nitrogen addition caused a decrease in plant species richness at both grassland sites, the two sites differed in their responses in aboveground net primary productivity (ANPP) and plant composition. At the shortgrass site, we found no effect of nitrogen addition at any level on ANPP, but compositional change occurred starting at 5 g m^{-2}. In contrast, ANPP at the tallgrass site increased at 5 g m^{-2} then saturated, but no significant compositional change was observed. Collectively, these results provide two key insights: 1) ANPP and plant community responses can be decoupled with short-term nitrogen addition, and 2) site-level water limitation can result in contrasting responses of grasslands to five years of nitrogen addition, but with these effects manifesting at the same critical load of addition.

2. Introduction

Anthropogenic activities are changing the availability of resources in ecosystems worldwide (Vitousek et al. 1997a, Peters and Meybeck 2000). Through industrialization and modern agricultural practices, human activities have fixed more nitrogen into biologically available “reactive” forms than all biological processes in marine or terrestrial systems (Vitousek and Howarth 1991, Gruber and Galloway 2008, Vitousek et al. 2010). This nitrogen is added to ecosystems through atmospheric deposition and/or runoff from agricultural fields, ultimately resulting in widespread eutrophication (Galloway et al. 2003, Fowler et al. 2013). Unlike natural disturbances that alter resource availability in a discrete pulse, modern nitrogen eutrophication is a continuous press of alleviating resource limitation and therefore could lead to large shifts in ecosystem structure and function (Stevens et al. 2015).

Grassland ecosystems may be particularly sensitive to eutrophication given that they are consistently nitrogen-limited (LeBauer and Treseder 2008, Fay et al. 2015). Nitrogen addition in grasslands generally increases plant production, alters community composition, and decreases richness (LeBauer and Treseder 2008, Smith et al. 2009, Bai et al. 2010, Isbell et al. 2013a, Avolio et al. 2014, 2020, Komatsu et al. 2019, Seabloom et al. 2021). Additionally, differing resource-use strategies among plant species result in unequal competitive abilities causing species reordering and shifts in dominance following eutrophication (Stevens et al. 2004, Funk 2008, Lebauer and Treseder 2008, Borer et al. 2014, Isbell et al. 2015, Harpole et al. 2016, Koerner et al. 2016, Tatarko and Knops 2018). Species that fall towards the “acquisition” end of the resource acquisitive-conservative trait spectrum typically gain competitive advantage (Xia and Wan 2008, Yang et al. 2011, La Pierre and Smith 2015, Feng et al. 2023), while less competitive species are locally lost resulting in richness declines (Zavaleta et al. 2003a, Harpole et al. 2007, Isbell et al. 2013b, Midolo et al. 2019, Clark et al. 2019, Ma et al. 2021, Yang et al. 2023). However, the level of N addition at which plant productivity and compositional changes occur is unclear for many ecosystems because most studies employ a single level of nitrogen addition that completely alleviates all N limitation (e.g., 10 g m^{-2}), limiting our ability to predict grassland response to eutrophication at the more commonly occurring lower levels (Galloway et al. 2003).

By observing plant community and productivity changes across a gradient of nitrogen addition, we may identify the level of nitrogen addition at which plants are impacted, which will ultimately aid our predictions of ecosystem responses to eutrophication. The few experiments that have utilized a resource gradient often identify a range of nitrogen that alters grasslands from the minimum “critical load” up to a saturation point (Clark and Tilman 2008, Bai et al. 2010, Symstad et al. 2019, Peng et al. 2020, Yang et al. 2023). The nitrogen critical load is the minimum amount of nitrogen needed to elicit change in a given functional or compositional metric, and it varies widely among plant communities. For example, species loss and increased production can occur at low nitrogen addition rates in long-term grassland experiments (Clark and Tilman 2008, Bai et al. 2010, Symstad et al. 2019). As nitrogen addition increases beyond the critical load, grassland responses often saturate, meaning that any additional nitrogen fails to stimulate further changes (Bai et al. 2010, Isbell et al. 2013b, Peng et al. 2020, Yang et al. 2023). This pattern is frequently observed for plant production but is less consistent for changes in plant community metrics—production may respond more consistently as the rate of photosynthesis is governed by stoichiometric constraints and water limitation, while community responses may be reliant on the traits of the species present (Yang et al. 2011, Avolio et al. 2014). These nitrogen gradient experiments, though powerful, are still uncommon, and it remains unknown if these patterns will hold across grasslands with varying environmental properties.

Nitrogen availability is a strong mediator of grassland structure and function, but water colimitation may constrain change under eutrophication and help explain why grassland responses saturate with increasing nitrogen. Multiple resources are needed for photosynthesis, with each resource having the potential to limit energy production and growth. Colimitation by water and nitrogen are frequently observed in grasslands, though the intensity of co-limitation appears to vary across grassland aridity (Burke et al. 1998, Lee et al. 2010, La Pierre et al. 2016). Dry grasslands (arid and semi-arid) are primarily water-limited; therefore, nitrogen addition may have little to no influence (Lauenroth et al. 1978, Ladwig et al. 2012). A more mesic grassland with higher precipitation is expected to be more equally limited by water and nitrogen, leading to greater sensitivity to the input of either resource (Burke et al. 1998, Yang et al. 2023). Colimitation is also observed across years with variable resource availability, with grasslands more responsive to nitrogen addition during wet years (Yang et al. 2011, Ladwig et al. 2012, Collins et al. 2017, Ma et al. 2019), so it's expected that the degree of nitrogen-water colimitation will vary among sites with unique climate and among years with variable precipitation.

To assess how grassland structure and function respond across a gradient of chronic nitrogen addition, we established a long-term nitrogen addition experiment - the Chronic Addition of Nitrogen Gradient Experiment (ChANGE) – in a semi-arid (shortgrass steppe) and a mesic grassland (tallgrass prairie). These grasslands bracket the broad precipitation gradient that occurs in the Central US (ranging from ~330 to 860 mm, west to east) that has been shown to strongly influence plant productivity and species richness (Sala et al. 1988, La Pierre et al. 2016, Forrestel et al. 2017). Comparing these sites allows us to examine how grasslands respond to nitrogen availability across communities with unique climates and plant diversity. Nitrogen was added along a gradient of eight concentrations from 0-30 g m^{-2} for five consecutive years while aboveground net primary productivity (ANPP) and plant species composition were monitored each year. With this approach, we assessed 1) the level of nitrogen required to elicit an initial change in ecosystem function (ANPP) and community structure (i.e., richness, species gains and losses, and community composition). Previous research from the shortgrass site found little effect of fertilization on structure and function when nitrogen was applied at 10-15 g m^{-2} per year (Lauenroth et al. 1978), which could indicate that this level of nitrogen fertilization was insufficient to meet the critical load, or changes were limited by water availability. The semi-arid shortgrass site is primarily water-limited, so we hypothesized that above-average precipitation may be necessary for nitrogen to reach a critical load that would elicit changes to ecosystem function and community structure (Lauenroth et al. 1978, Ladwig et al. 2012). Based on previous research at a mesic tallgrass prairie site, we predicted that changes to plant production and communities would occur with nitrogen levels between 4 to 10 g m^{-2} (Gibson et al. 1993, Tilman 1993, Avolio et al. 2014, Smith et al. 2016). Second, we assessed 2) the magnitude and pattern of change in ANPP and community structure as nitrogen levels increased. At both sites, we predicted that plant production and community changes would increase linearly until another resource became limiting, causing the response to saturate (Isbell et al. 2013a, Yang et al. 2023). The mean saturation point of grassland production globally has been estimated at 15 g m^{-2}, but climate and local soil properties create wide variability within this estimate (Peng et al. 2020). Thus, our research seeks to establish nitrogen's critical load and saturation points for plant production and community dynamics in both semi-arid and mesic grasslands.

3. Methods

3.1 Study Sites

This study was conducted in two grasslands: a semi-arid shortgrass steppe and a mesic tallgrass prairie (Table 1.1). The semi-arid shortgrass steppe site, hereafter referred to as "shortgrass", that we selected for our study is located within the Central Plains Experimental Range in northeastern Colorado, USA (40° 50' N, 104° 45' W). Elevation at the study site ranges from 1,600 to 1,700 m, and mean annual precipitation is 366 mm (Griffin-Nolan et al. 2018a). Yearly precipitation (measured from October 1 of the preceding year to September 30 of the focal year) during the study years ranged from 262.9 mm to 376.9 mm. The study site is located in a pasture that has been ungrazed by cattle since 2000, with minimal topographic variation and relatively homogeneous vegetation (Griffin-Nolan et al. 2018a). During the study period, the four most dominant species based on relative abundance of the control plots were all grasses. In order from most to least abundant, the dominant species were the C_4 grass, *Bouteloua gracilis*; the C_3 grasses, *Elymus elymoides* and *Vulpia octoflora*; and the sedge, *Carex Eleocharis.* Across all years of the study, average richness in the control plots was 9.2 ± 2.6 species. Perennial and annual forb species make up the bulk of diversity at the site.

The mesic tallgrass prairie site, hereafter referred to as "tallgrass", selected for this study is located within the Konza Prairie Biological Station in the Flint Hills of northeastern Kansas, USA (39° 05' N, 96° 35' W). This site has never been plowed. Elevation at Konza ranges from 320 m to 444 m and the mean annual precipitation is 835 mm (Collins et al. 2021). Yearly precipitation (measured from October 1 of the preceding year to September 30 of the focal year) during the study ranged from 637 mm to 1103 mm. The study site is located in an ungrazed, upland site within a watershed that has been annually burned since 2001 (Collins et al. 2021).

Table 1.1 Summary of climate variables and geographic properties of the shortgrass site and the tallgrass site. The most-abundant species were based on relative cover of the control plots.

	Shortgrass Semi-Arid Shortgrass Steppe	**Tallgrass** Mesic Tallgrass Prairie
Mean annual precipitation (mm)	366	835
Mean annual temperature (°C)	8.6	12
Elevation (m)	1,600 - 1,700	320 - 444
Coordinates	40° 50' N, 104° 45' W	39° 05' N, 96° 35' W
Most-abundant species (ordered)	1. *Bouteloua gracilis* (C_4 grass) 2. *Elymus elymoides* (C_3 grass) 3. *Vulpia octoflora* (C_3 grass) 4. *Carex Eleocharis* (sedge)	1. *Andropogon gerardii* (C_4 grass) 2. *Sorghastrum nutans* (C_4 grass) 3. *Amorpha canescens* (forb) 4. *Lespedeza violacea* (forb)

We assessed the most dominant species during the study period based on relative abundance of the control plots. The C_4 grass, *Andropogon gerardii*, was the dominant species in the community followed by the C_4 grass, *Sorghastrum nutans*, and two forb species, *Amorpha canescens* and *Lespedeza violacea*. Average richness across the control plots during the study duration was 13.6 ± 2.1 species.

3.2 Experimental Design

In 2013 we established a randomized complete block design at both sites, in which 8 different levels of nitrogen (0, 2.5, 5, 7.5, 10, 15, 20, and 30 g m^{-2}) were randomly assigned to one of eight 5 x 5 m plots within each of six blocks (8 treatments * 6 blocks = 48 total plots per site). Blocks were arrayed on the landscape so that there was a minimum 10 m distance between each block. Plots within each block were arrayed in two rows of four plots, with each plot separated by a 1 m buffer. Pre-treatment data were collected in 2013, and nitrogen fertilization occurred throughout the duration of the experimental period from 2014 to 2018. Nitrogen in the form of time-release urea was evenly applied by hand to each 5 x 5 m plot during late May or early June. Each 5 x 5 m plot was divided into four 2.5 x 2.5 m plots, with one 2.5 x 2.5 m plot designated for sampling for this study.

3.3 Aboveground Net Primary Productivity Measurements

ANPP was estimated at peak biomass (late August/early September) each year by clipping all vegetation at ground level within two 20 x 50 cm quadrats, located within the same subplot as the plant composition measurements. Quadrats were moved yearly to prevent resampling over time. Biomass was separated into grass, forb, and woody functional groups, dried for 48 hours at 60 ºC, and weighed to the nearest 0.01 g. Mean biomass (excluding previous year's dead) across the two quadrats in each plot was multiplied by 10 to estimate ANPP (g m^{-2}).

3.4 Plant Diversity and Community Composition

At each study site, we surveyed plant community composition within a 1 x 1 m permanent sampling area (located in a corner of the designated 2.5 x 2.5 m plot, with a 0.5 buffer from the edge of the plot) at the beginning (late May or early June) and end (mid- to late-August) of the growing season in each year of the study. We visually estimated percentage aerial cover to the nearest 1% for species under 50% abundance and to the nearest 5% for species above 50% abundance for each species rooted within the sampling area. When early and late measures of cover were estimated for a species, we used the maximum cover estimate.

3.5 Statistical Analyses

Cover estimates of species within each plot were used to calculate multiple diversity metrics using functions within R's (version 4.4.1; R Core Team 2024) Codyn package, (version

2.0.5; (Hallett et al. 2016, 2018). Species richness and species evenness (Evar; Smith and Wilson 1996) were calculated using the community_structure() function. Species losses and species gains were calculated with the RAC_change() function that calculates the proportion of species lost or gained between the pre-treatment reference year and a specific treatment year. We also calculated a complete species loss metric to qualitatively understand species loss between pre-treatment years and treatment years. If a species was present during 2013 or 2014 then not present in subsequent years, it was considered completely lost. For this single analysis, both 2013 and 2014 were considered pre-treatment years to compensate for low richness at the shortgrass site during a 2013 drought. We also used the simper() function from the vegan package, version 2.6-8 (Oksanen et al. 2024) to conduct a similarity percentages analysis, which identifies influential plant species that were responsible for existing dissimilarity between treatments.

To assess the influence of nitrogen on ANPP, species richness, evenness, and abundance of influential species at each site, we used a linear mixed-effects model. Year and nitrogen were categorized as fixed effects, while block and plot were designated as random effects with plot nested within block. This structure allowed blocking and repeated measures to be represented in the model. We used the lme() function from the nlme R package, version 3.1-164 (Pinheiro et al. 2024) so that we could include a compound symmetry covariance structure, which allows stronger correlation between years that are closer together. Model results were analyzed using the anova.lme() function then further assessed through pairwise nitrogen treatment comparisons with the emmeans() function. Growing season precipitation was not considered as a covariate in our analyses despite its correlation with ANPP at both sites (Appendix 1 Figure 1), due to a negative growing season precipitation trend over the five years of treatment (-0.75 for shortgrass,

-0.26 for tallgrass). Thus, year was considered a proxy for precipitation variation during the study. We assessed data for outliers and found one statistically significant and influential outlier in the shortgrass ANPP data (block E during 2014). We excluded this outlier from all shortgrass ANPP analyses. We accounted for this unbalanced design by running our ANOVA as a type III sums of squares. Data were visually assessed through diagnostic plots and met standards of normality without data transformations being required, unless otherwise noted.

The effect of nitrogen on multivariate species composition was evaluated for each site with permutational multivariate analysis of variance (perMANOVA) using a Bray Curtis dissimilarity matrix based on absolute species abundances with the adonis2() function with blocking. Data were analyzed separately for each year due to constraints of perMANOVA. Pairwise comparisons were conducted through pairwise.adonis2(), version 0.4.1, a wrapper function using adonis2() (Arbizu 2018). All multivariate species composition analyses were run using the vegan package, version 2.6-8 (Oksanen et al. 2024). Analyses were conducted using R, version 4.4.1 (R Core Team 2024).

4. Results

4.1 Aboveground productivity

On average, nitrogen addition at any level did not affect ANPP at the shortgrass site; conversely, nitrogen addition significantly increased ANPP in tallgrass (Table 1.2, Figure 1.1). A significant increase (+20.8% across all fertilized plots) in tallgrass ANPP was found starting at and after 5 g m^{-2}, but there was no additional increase with increasing nitrogen addition amount past 5 g m^{-2} (Figure 1.1d). Year strongly influenced ANPP at both sites, which is reflected in the

Table 1.2. ANOVA results of linear mixed-model assessing the effect of nitrogen treatment over time (year) on ANPP, species gains, species losses, species richness, and evenness. Significant differences ($p \leq 0.05$) are bolded. Block E in 2014 is excluded from shortgrass ANPP analysis due to strong influence of an outlier. The time variable for species gains and species losses is the difference between two compared years. Gains and losses are calculated as a proportional difference between a reference year and a focal year. Due to low precipitation in 2013, 2014 is used as the reference time point.

		Shortgrass			**Tallgrass**		
		d.f.	F-value	p-value	d.f.	F-value	p-value
ANPP	Nitrogen	7,187	1.82	0.082	7,195	4.35	**<.001**
	Year	4,187	27.76	**<.001**	4,195	99.30	**<.001**
	Nitrogen : Year	28,187	0.93	0.565	28,195	1.53	0.051
Gains	Nitrogen	7,155	0.94	0.479	7,155	2.27	**0.032**
	2014-Trt.Year	3,155	15.76	**<.001**	3,155	2.93	**0.036**
	Nitrogen : 2014-Trt.Year	21,155	1.52	0.076	21,155	1.08	0.378
Losses	Nitrogen	7,155	3.26	**0.003**	7,155	2.02	0.056
	2014-Trt.Year	3,155	9.60	**<.001**	3,155	2.24	0.086
	Nitrogen : 2014-Trt.Year	21,155	0.84	0.668	21,155	0.46	0.981
Richness	Nitrogen	7,195	2.7	**0.011**	7,195	2.36	**0.025**
	Year	4,195	21.21	**<.001**	4,195	6.79	**<.001**
	Nitrogen : Year	28,195	1.43	0.083	28,195	0.53	0.975
Evenness	Nitrogen	7,195	0.79	0.592	7,195	1.35	0.228
	Year	4,195	9.96	**<.001**	4,195	7.53	**<.001**
	Nitrogen : Year	28,195	1.08	0.365	28,195	0.72	0.848

significant correlation between growing season precipitation and ANPP of the control plots ($r = 0.96$ and 0.90 for shortgrass and tallgrass, respectively; Figure 1.1a,c; Appendix 1 Figure 1). However, nitrogen treatment and year did not significantly interact, indicating no change in the effect of nitrogen addition over time or in response to interannual precipitation. We separated ANPP by functional group (forb and grass), but found that only year, not nitrogen, influenced functional group biomass (Appendix 1 Table 1, Appendix 1 Figure 2).

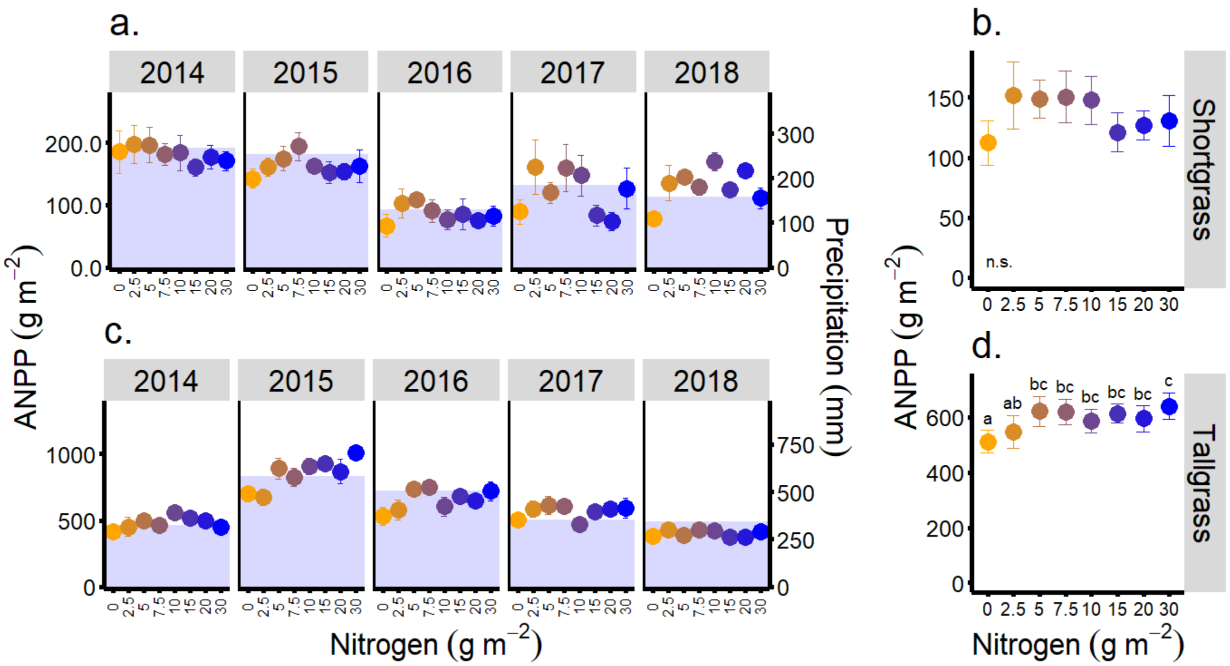

Figure 1.1. Effect of nitrogen addition treatments on ANPP at shortgrass (a, b) and tallgrass (c, d) sites over time (a, c) and averaged across treatment years (b, d). The shaded blue areas in plots a and c indicate the total growing season precipitation (mm) from May 1st to August 31st and correspond to the secondary y axis. All error bars represent mean ±SE. Letters within plots b. and d. indicate significant differences ($p \leq 0.05$) between pairwise comparisons of nitrogen treatments.

4.2 Species losses and gains, species richness, and species evenness

At the shortgrass site, there was no significant effect of nitrogen on species gains, but species losses significantly increased with increasing nitrogen addition, with significantly higher losses with both 20 and 30 g m^{-2} compared with 0 g m^{-2} (Table 1.2, Figure 1.2a). Species richness at the shortgrass site was strongly influenced by year and declined with increasing nitrogen addition (Table 1.2; Figure 1.2b). Across all plots treated with nitrogen, there were 1.36 fewer species, or a 15% decrease in richness. The 10 and 30 g m^{-2} treatments had significantly lower richness than the control treatment, with on average 1.92 fewer species (Figure 1.2b). Unlike at the shortgrass site, the tallgrass site demonstrated a significant gain in species with nitrogen addition, though significant differences only occurred between 2.5 and 15 g m^{-2} (Table

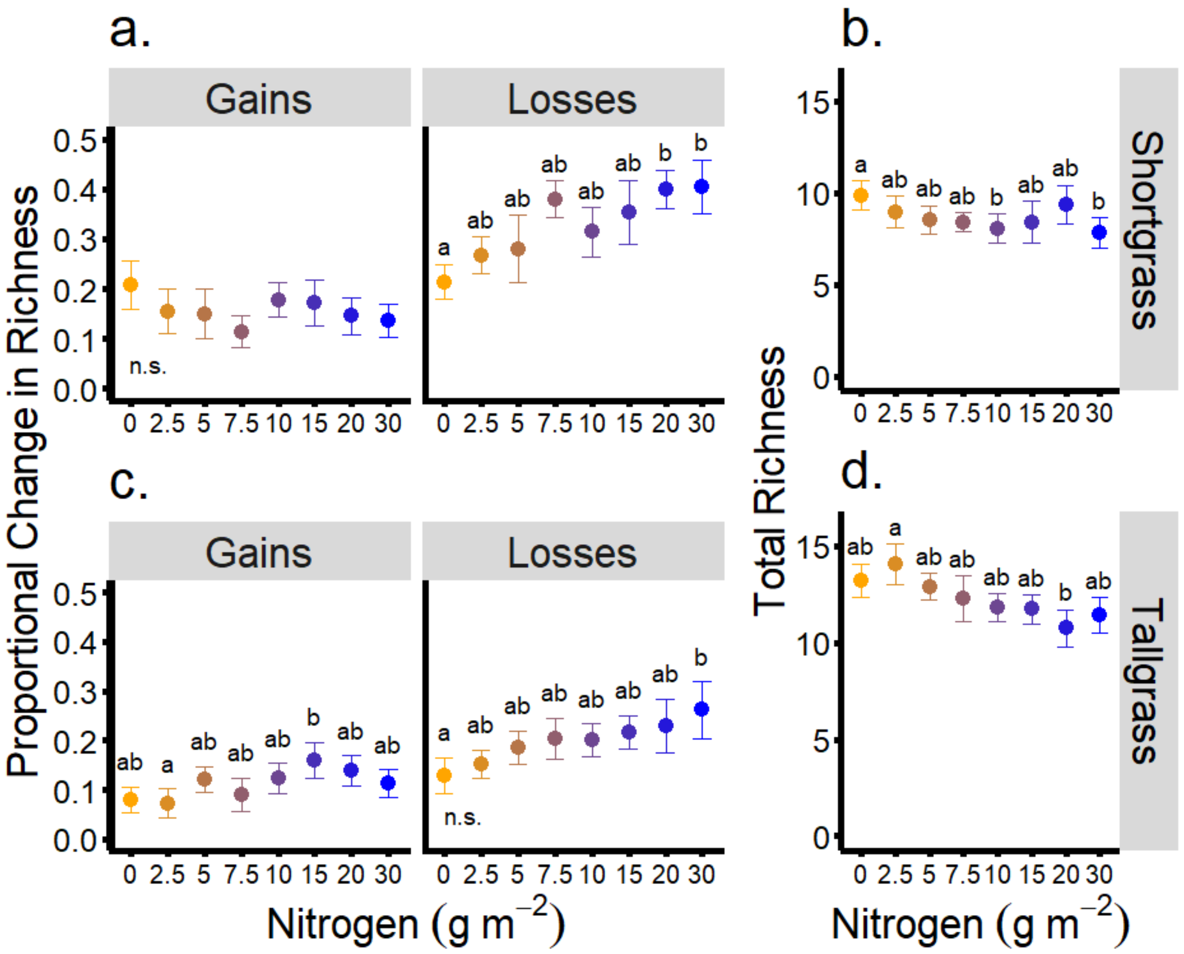

Figure 1.2. Effect of nitrogen treatment on species gains and losses (a, c) and on total richness (both averaged across treatment years; b, d) at the shortgrass (a, b) and tallgrass sites (c, d). All error bars represent mean ±SE. Letters within plots indicate significant differences ($p \leq 0.05$) between pairwise comparisons of nitrogen treatments. Species gains are calculated as the number of species present at the focal time point and not present at the reference time point (the opposite for species losses), divided by the total number of species shared between each time point. Note that species losses at the tallgrass site was only marginally significant ($p = 0.056$), but a significant pairwise difference was present.

1.2; Figure 1.2c). Species losses was only marginally significant ($p = 0.056$), yet there was a significant pairwise difference between the 0 g m^{-2} control and the 30 g m^{-2} treatment. Year and nitrogen also significantly affected species richness, but the effect was more muted than at the shortgrass site (Table 1.2; Figure 1.2d). When averaged across the nitrogen addition treatments, tallgrass plots lost 1.09 species, or an 8% decrease in richness, compared to the control treatment. The 2.5 and 20 g m^{-2} treatments were significantly different, with the 20 g m^{-2} having 3.3 fewer species than the 2.5 g m^{-2} plots (Figure 1.2d). As with ANPP, we observed no interaction between nitrogen treatment and year for either site. Evenness was only influenced by year, with nitrogen addition having no significant effect (Table 1.2; Appendix 1 Figure 3).

4.3 Community composition

Plant community composition within the shortgrass site significantly responded to nitrogen addition starting in the second year of treatment (2015; Appendix 1 Table 2; Figure 1.3). In contrast, plant composition was not affected by nitrogen in any year during the experimental period for the tallgrass site. Post-hoc pairwise comparisons revealed that community composition at the shortgrass site was altered at nitrogen addition rates as low as 5 g m^{-2} in 2018 but were most-consistently altered at rates between 10-30 g m^{-2} (Appendix 1 Table 3). During the fourth year of treatment (2017), significant pairwise comparisons primarily occurred between control plots and treatments plots after 7.5 g m^{-2}. During the fifth year of treatment (2018), significant pairwise comparisons occurred exclusively between control and treatments plots beginning as low as 5 g m^{-2}. We identified three shortgrass species that contributed most to differences in composition, all of which were considered among the most abundant during our study period: *Bouteloua gracilis* (C_4 perennial grass), *Vulpia octoflora* (C_3 annual grass), and *Elymus elymoides* (C_3 perennial grass; Figure 1.4). The abundances of all three influential species were shown to be significantly affected by nitrogen treatment and year (Appendix 1 Table 4). Across all years, *Bouteloua gracilis* and *Vulpia octoflora* declined with nitrogen addition, while *Elymus elymoides* increased with nitrogen addition (Figure 1.4). These differences in abundances with nitrogen addition were consistent and likely drove the significant change in species composition changes observed at the shortgrass site.

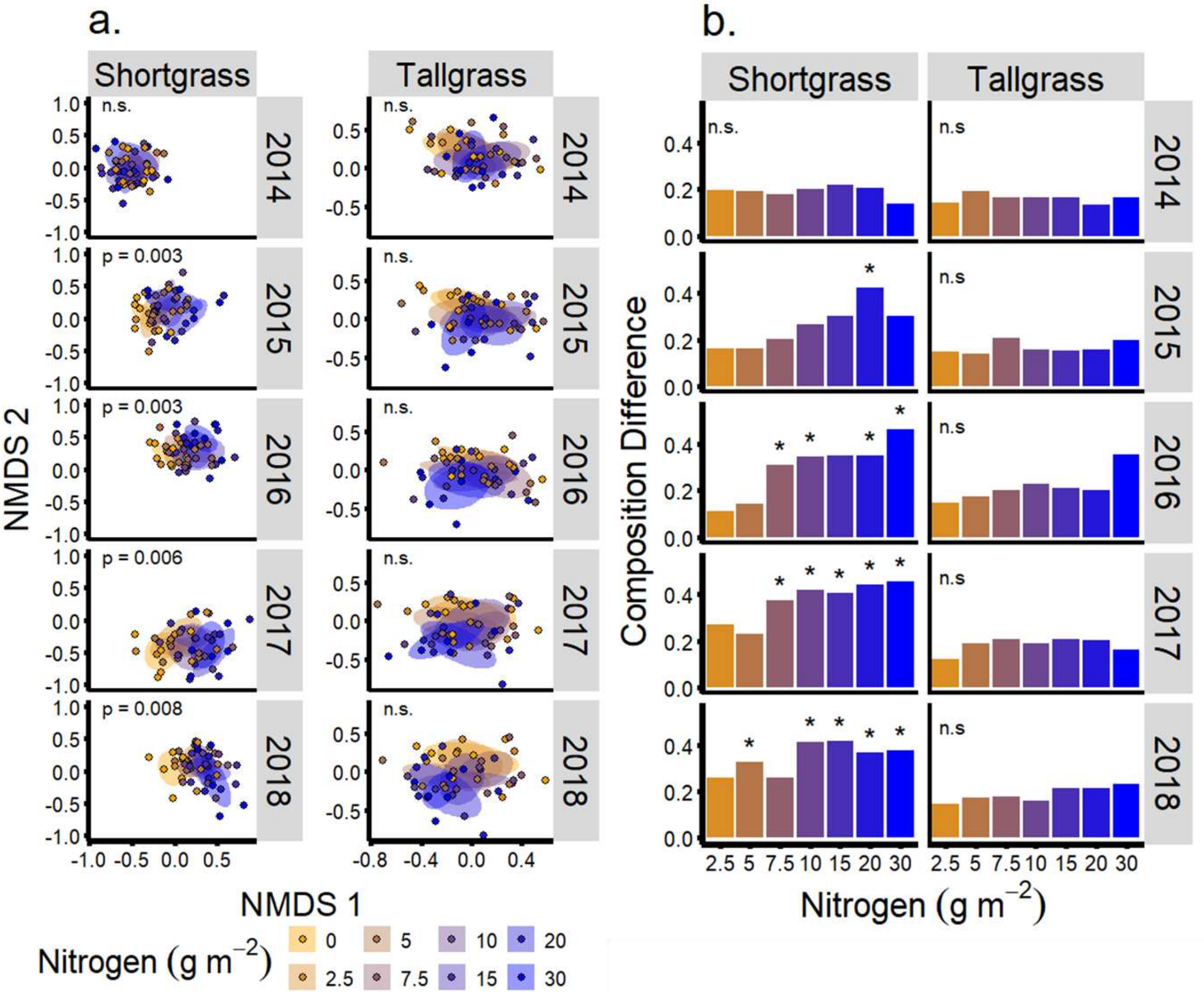

Figure 1.3. Nonmetric Multidimensional Scaling (NMDS) plot of plant communities (a) and contributing multivariate compositional differences (b) by nitrogen treatment across treatment years in the shortgrass and tallgrass sites. In plot a, points indicate ordination of individual plots and ellipses represent the 95% confidence interval. Years without significant nitrogen effects on composition are indicated with a “n.s.” in the upper left corner. Years with significant nitrogen effects are indicated with the associated p-value in the upper left corner. In plot b, multivariate composition differences were calculated between the controls and nitrogen plots. Years with no significant effect of nitrogen on overall community composition change are indicated with “n.s.” in the upper left corner of the plot. Significant pairwise differences between the control and nitrogen treatment are indicated with a “*” above the treatment level (Appendix 1 Table 3).

5. Discussion

In this study we assessed how plant community structure and function respond to five years of nitrogen addition at eight different levels across two grassland ecosystems with unique

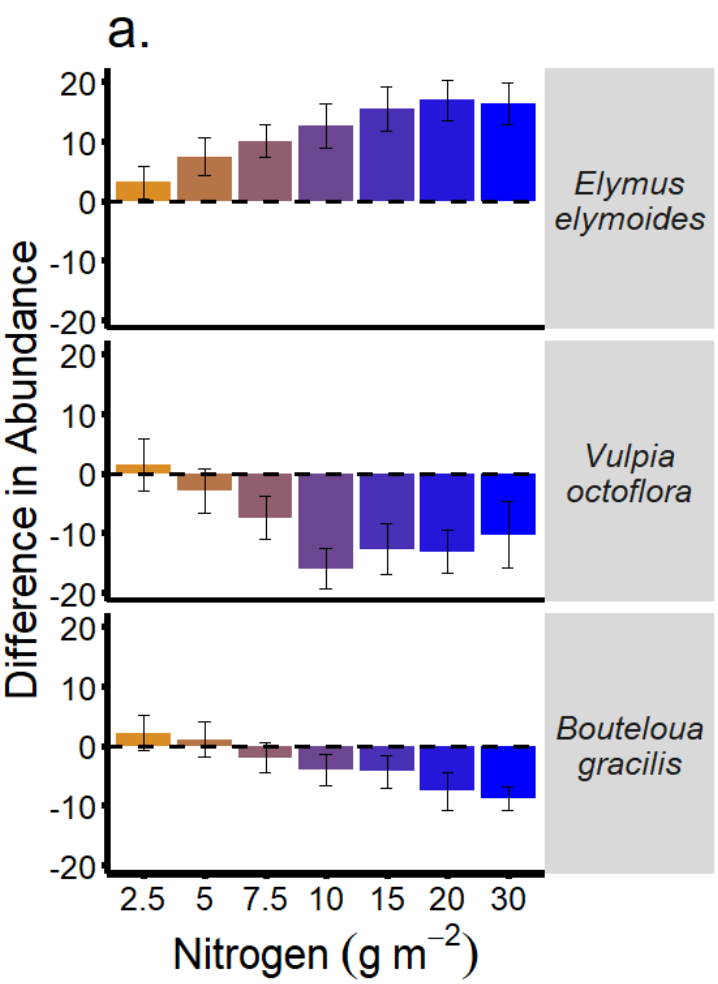

Figure 1.4. Mean difference in abundance from the 0 g m^{-2} control to the nitrogen treatments of the three species that contributed most to compositional dissimilarity between nitrogen treatments using control as the reference at the shortgrass site. Data is averaged across treatment years. Species are organized by decreasing contribution to average dissimilarity, so that the top plot represents the species that most contributed to differences among treatments. All three species responded significantly to nitrogen treatments. Error bars represent mean ±SE.

climates and plant community compositions. We first identified the level of nitrogen required to elicit initial change (the critical load) in ecosystem function and community structure at both sites. At the shortgrass site, production was not affected by nitrogen addition at any level, likely due to water-limitation; however, community change occurred at levels as low as 5 g m^{-2}. Productivity at the tallgrass site increased at 5 g m^{-2} without any significant changes in community composition. This result demonstrates that community function and composition can decouple under nitrogen addition. Second, we described the magnitude and pattern of change as nitrogen levels increased. We expected a saturating pattern of change as nitrogen increased, but when change occurred, the true pattern ranged from linear to saturating. Community change at the shortgrass steppe was best described as saturating after 5 g m^{-2} during the fifth year of fertilization, and production at the tallgrass site saturated after 5 g m^{-2}. Other community metrics, such as richness, species gains and losses, and influential-species abundance responded more linearly to increasing nitrogen addition. Thus, the pattern of change along a gradient of increasing nitrogen addition, can vary from linear to saturating.

Production at the shortgrass site did not respond to nitrogen during any year or at any level despite five consecutive years of fertilization. The semi-arid shortgrass steppe is a primarily water-limited system, so we predicted that above-average precipitation may be necessary to observe a nitrogen effect that will allow us to estimate the critical load of ecosystem function (Lauenroth et al. 1978, Ladwig et al. 2012). The average yearly precipitation for this site is 366 mm, while the highest amount received during the experimental period was 377 mm in 2017, so there was likely never a year when precipitation was high enough that nitrogen would become the primary limiting resource (Lauenroth et al. 1978, Burke et al. 1998, Ladwig et al. 2012). However, nitrogen could be building up in the system, so a sufficiently wet growing season may cause a large response in future years (Noy-Meir 1973, Yahdjian and Sala 2010, Brown et al. 2022).

In contrast, production at the tallgrass site responded to nitrogen addition starting at 5 g m^{-2}, indicating a critical load between 2.5 and 5 g m^{-2}, consistent with our predictions and previous work (Seastedt and Ramundo 1990). Beyond 5 g m^{-2}, the ANPP response saturated with increasing nitrogen, which may represent the point at which water replaces nitrogen as a limiting resource (Burke et al. 1998, Yang et al. 2023). The point where we identified an increase in ANPP is also the point at which saturation begins, perhaps suggesting that this system is similarly limited by water and nitrogen. The overall saturating pattern was noticeably more linear during the wettest year of 2015 (and to a lesser degree in 2016) (Figure 1.1c). Indeed, there was a significant increase in overall ANPP observed at 30 g m^{-2}, which was driven by the more linear response of 2015. This suggests that the saturating response we observed is not due to constraints of plant growth, but due to the increasing limitation of precipitation. Thus, it may be that in years with above average growing season rainfall the limits of productivity under a nitrogen gradient will be observed given sufficient time.

As in many previous studies (Zavaleta et al. 2003a, Stevens et al. 2004, Harpole et al. 2007, Isbell et al. 2013b, Ma et al. 2019, Midolo et al. 2019, Yang et al. 2023), nitrogen addition resulted in reduced species richness at both sites. Patterns of species losses, gains, and richness were more linear than saturating, but differences between treatments were modest with significant differences only appearing between treatments at each end of the nitrogen addition gradient. Species that were completely lost with fertilization were rare or subordinate species and frequently annuals. The species most lost under fertilization were all forbs: *Ipomopsis laxiflora* and *Machaeranthera tanacetifolia* at the shortgrass site, and *Aster ericoides* and *Sisyrinchium campestre* at the tallgrass site. These and other commonly lost species did not strongly contribute to either production or relative cover (Smith and Knapp 2003, Smith et al. 2020), so in this way, species richness was significantly altered, without significant effects to production. A higher critical load (10g m^{-2} for shortgrass, 20 g m^{-2} for tallgrass) is necessary to reduce richness (compared with production), but this finding may not be predictive of long-term effects. Previous research has shown that richness decreases at relatively low rates of addition (Ma et al. 2019, Symstad et al. 2019, Yang et al. 2023). In particular, one tallgrass site showed significant species loss occurred with addition rates as low as 1 g m^{-2} when applied for over two decades, with the actual critical load being estimated at 0.5 g m^{-2} (Clark and Tilman 2008). The critical load for species richness may change over time as nitrogen effects accumulate.

In contrast to our productivity findings, community composition was only affected by nitrogen addition at the shortgrass site. The critical load was as low as 5 g m^{-2}, but this was affected by the duration of fertilization. In the second year of fertilization (2015), significant community change appeared at 10 g m^{-2}, and in subsequent years, the critical load lowered to 7.5 g m^{-2} in 2016 then to 5 g m^{-2} in 2018. This pattern demonstrates that the critical load for community composition gradually decreased with consecutive years of fertilization and may continue to decrease in future years. Differences in composition between the control plots and nitrogen plots indicate a similar saturating pattern, as with tallgrass ANPP. During the fourth and fifth year of fertilization, compositional differences appear consistently saturating after 5-7.5 g m^{-2}. These differences were driven by the three influential species: *Vulpia octoflora*, *Elymus elymoides,* and *Bouteloua gracilis*. *Vulpia octoflora* declined under nitrogen addition, which is expected given that it is one of the more conservative grass species (Blumenthal et al. 2020) in the shortgrass prairie and is likely to be less competitive under fertilization (Xia and Wan 2008, Yang et al. 2011, Avolio et al. 2014, Feng et al. 2023). Also, *Vulpia octoflora* is an early-season species that senesces in early June at our site, so it may not benefit from the nitrogen that is added in late May or early June. *Elymus elymoides* and *Bouteloua gracilis* have similar leaf economic traits (Blumenthal et al. 2020), but their opposing response to nitrogen may be explained by differences in phenology. *Elymus elymoides* is a cool-season C_3 and may have been able to capitalize on increased resources earlier in the season, thereby outcompeting *Bouteloua gracilis*, a warm-season C_4. Other studies have also shown a decline of *Bouteloua gracilis* under nitrogen addition (Milchunas and Lauenroth 1995, La Pierre et al. 2016, Tatarko and Knops 2018), perhaps due to a similar lowered ability to compete (Lowe et al. 2003).

Community composition in the tallgrass site was not significantly affected by nitrogen addition at any level, despite significant changes in species richness. This may be partially due to annual burning at the tallgrass site, which has a strong, stabilizing effect on community composition (Collins et al. 2021). Tallgrass prairie communities are also highly competitive and limited by multiple resources (Callaway and Walker 1997, Knapp et al. 1998, Avolio et al. 2014). Any changes to competition dynamics that may have accompanied nitrogen addition were likely insufficient to alter the already highly competitive interactions between species, the limitations of other nutrients, and the stabilizing forces of fire. Previous research has shown community change over time with nitrogen addition in tallgrass sites (Avolio et al. 2014), but five years of fertilization was not sufficient to cause change at our site.

Overall, we found no evidence that nitrogen effects changed over time in any recorded metric. For nitrogen effects to change over time (i.e., accumulate), this may require more than five years of fertilization or may have required years with above average precipitation. We are assuming that nitrogen will take time to build up in the system, but we also expect that nitrogen will be lost to biotic and abiotic processes such as ground water leaching, surface runoff, or ammonium immobilization (Galloway et al. 2003, McClain et al. 2003, Austin et al. 2004, Greaver et al. 2016). Some ecosystems, especially arid and semi-arid lands, have low nitrogen retention. For example, a nitrogen-addition study in a desert grassland found no accumulation of nitrogen after 9-10 years of fertilization (Ladwig et al. 2012). It is important to state that we did not measure nitrogen availability during the study period, so we do not know how much nitrogen was retained after consecutive years of fertilization.

In summary, we examined the effects of five years of experimental nitrogen addition on semi-arid and mesic grassland productivity and plant composition and diversity. Our findings lead to two major conclusions. First, plant production and community composition change can decouple under nitrogen addition in a semi-arid and mesic grassland ecosystem. Nitrogen addition increased production only at the mesic tallgrass site and altered community composition only at the semi-arid shortgrass site, while decreasing species richness at both. This decoupling is contrary to the expectation that production changes with chronic resource alteration are driven by shifts in plant composition (Smith et al. 2009). Second, historical precipitation regime and annual water limitation can result in contrasting responses of a semi-arid and mesic grassland to short-term nitrogen addition. Colimitation by water and nitrogen on plant growth likely played a strong role in influencing the responses we observed. The semi-arid shortgrass site is primarily water-limited, so nitrogen addition on average had no effect on production. In contrast, the tallgrass site experiences less water limitation when compared to nitrogen limitation, and therefore was more responsive to nitrogen addition, especially during wetter years. Thus, our study found that changes in plant composition or aboveground production both manifest at 5 g m^{-2} but can separately occur with nitrogen addition in semi-arid and mesic grassland ecosystems, and that these responses appear to be governed by water limitation in both interannual precipitation variability and long-term climate.

CHAPTER 2: Timing governs synergistic interaction of deluge pulse and nitrogen press on productivity within a semi-arid grassland

1. Summary

Ecosystems are being altered by increasing nitrogen fertilization and deposition globally, representing chronic resource “press.” In tandem, climate change is intensifying weather events, leading to more intense deluges (i.e., large rainfall events), which represent resource “pulses.” Nitrogen and water addition can independently increase aboveground net primary productivity (ANPP) in grasslands. When combined, their effects may be simply additive, or synergy may emerge, whereby chronic nitrogen addition and a pulsed deluge may cause an increase in ANPP that is greater than the sum of their separate effects. Furthermore, the magnitude of nitrogen addition and the timing of deluge events (i.e., early vs. late growing season) have been shown to differentially affect ANPP, so these factors may also influence the interaction of nitrogen and deluge. To test the role of nitrogen magnitude and deluge timing in moderating pulse-press interactions, we leveraged a long-term nitrogen addition study in a shortgrass steppe of northeastern Colorado in which eight levels of nitrogen addition, ranging from 0 to 30 g m^{-2} yr^{-1}, have been added since 2014. Deluge events representing the 95th percentile of historic precipitation events were applied mid-late season (late July-early August) in 2021 and early-mid season (late June) in 2022 to separate subplots within each nitrogen addition plot. The 2021 mid-late deluge did not significantly increase ANPP or interact with nitrogen addition, whereas the 2022 early-mid deluge increased average ANPP by 300% compared to nitrogen-only treatments. We determined that the combined effects of nitrogen addition and early-mid deluge were synergistic, with nitrogen rates of 10, 15, 20 g m^{-2} being most responsive. The synergistic response appeared driven by fast-growing, weedy forbs. These results highlight how increases in pulsed events, such as deluges, may generate unexpected impacts on a key ecosystem function in

grasslands undergoing chronic alterations in nitrogen availability.

2. Introduction

Increasing industrialization has led to alteration of resources worldwide (Vitousek et al. 1997b). The combustion of fossil fuels and increased synthetic fixation for agricultural fertilization have caused reactive nitrogen (i.e., biologically active forms) to accumulate in environmental pools (Galloway et al. 2003, Fowler et al. 2013). While disturbances often alter resource availability for a discrete period, directional and chronic resource alteration, such as the accumulation of reactive nitrogen, represents a novel facet of the Anthropocene that is defined as a resource "press" (Smith et al. 2009). Chronic nitrogen addition may be particularly impactful, given that many terrestrial ecosystems, including grasslands, are nitrogen-limited (Fay et al. 2015). Resources may accumulate over time, leading to productivity increases, followed by changes in competition dynamics, dominant species turnover, and eventual local species loss (Smith et al. 2009). As the magnitude of nitrogen press increases, plant production often saturates when another resource becomes limiting (Bai et al. 2010, Peng et al. 2020, Seabloom et al. 2021, Yang et al. 2023), but competition dynamics are not governed by the same stoichiometric constraints and may decouple from production (Linabury et al. *in review*) (Chapter 1). The degree of this ecological change is influenced by the magnitude of the press (Houseman et al. 2008, Isbell et al. 2013a, Linabury et al. *in review*). Thus, nitrogen presses must be studied across a gradient to understand the full scope of functional and compositional changes.

While resource presses alone are an important driver of ecological change, these chronic resource alterations are expected to interact with other concurrent forces of anthropogenic change, particularly those that result in pulsed changes in resources. Deluges are statistically extreme rainfall events that occur naturally but are expected to become more frequent as climate change intensifies the hydrological cycles (Knapp et al. 2008; Smith et al. 2009; Smith 2011; IPCC 2014). Deluges result in large, but short-term alteration to water resources, so unlike continuous nitrogen press, deluge “pulses” are discrete in nature (Smith et al. 2009). Arid and semi-arid grasslands may be particularly sensitive to deluges, as they are primarily water-limited, with biological activity driven by episodic rainfall (Sala and Lauenroth 1982, Austin et al. 2004). Deluges recharge deep soil water reserves and result in lower relative evaporation, which could strongly influence semi-arid grasslands (Knapp et al. 2008). The timing of seasonal rainfall is a critical determinant of semi-arid grassland structure and function (Heisler-White et al. 2008, Griffin-Nolan et al. 2021, Hajek and Knapp 2022, Zhang et al. 2023, Hajek et al. 2024), and Post and Knapp (2020) found that a mid-season deluge stimulated production more than early- or late-season deluges, likely by facilitating production during a period when water was limiting, and capable of increased plant growth. As climate change intensifies, the likelihood of deluge pulses overlapping with chronic nitrogen deposition is expected to increase, requiring a framework to predict the outcomes of these co-occurring events, particularly in water-limited ecosystems.

The temporal pattern of anthropogenic nitrogen input and deluge makes these events well-suited to evaluation through the “pulse-press” framework which describes resource alteration along a continuum from short-term to chronic long-term (Ives and Carpenter 2007, Smith et al. 2009, Collins et al. 2011, Ratajczak et al. 2017, Harris et al. 2018, Pellegrini and Jackson 2020). Long-term resource press can erode ecosystem stability and resistance, then the addition of a short-term resource pulse may tip the system beyond critical thresholds, leading to large, and potentially unexpected, shifts in ecosystem function and structure (Scheffer et al.

2015, Harris et al. 2018).

The combination of water pulses and nitrogen press may trigger a particularly strong response in semi-arid grasslands, where water and nitrogen are the most limiting resources (Fay et al. 2015, Collins et al. 2017, Brown et al. 2022). Many experiments have combined water and nitrogen in grasslands and often find additive effects (Lauenroth et al. 1978, Zavaleta et al. 2003b, Harpole et al. 2007, Xu et al. 2018, Lü et al. 2018, Meng et al. 2023), meaning that the individual effects of resources can predict the combined response. However, these studies do not directly test pulse-press dynamics as described above. Alternatively, theory and simulations have predicted that pulse-press forces may synergistically interact, producing effects greater than the sum of their individual impacts (Figure 2.1) (Scheffer et al. 2001, 2015, Ives and Carpenter 2007, Smith et al. 2009, Ratajczak et al. 2017, Harris et al. 2018). Given semi-arid grasslands are primarily water-limited, the effect of nitrogen press on productivity can be minimal (Lauenroth et al. 1978; Collins et al. 2017, Linabury et al. *in review*) (Chapter 1). If a well-timed deluge coincides with accumulated nitrogen, resource limitation would be temporarily alleviated, so plant growth would be maximized. These conditions are the most likely to lead to synergistic interaction of pulse-press resource alterations, but the rarity of experimental evidence makes it difficult to predict the conditions under which synergy will occur.

In this study, we leveraged a long-term nitrogen addition experiment to test for the first time the interaction of nitrogen press and deluge pulse within a semi-arid shortgrass steppe in northeastern Colorado. Nitrogen fertilization has been ongoing since 2014 in a gradient of eight levels: 0, 2.5, 5, 7.5, 10, 15, 20, 30 g m^{-2}. After eight to nine years of nitrogen addition, we applied a 95th percentile deluge event during mid-late season in 2021 and early-mid season in 2022. This multi-year experiment allowed us to assess how the level of chronic nitrogen addition

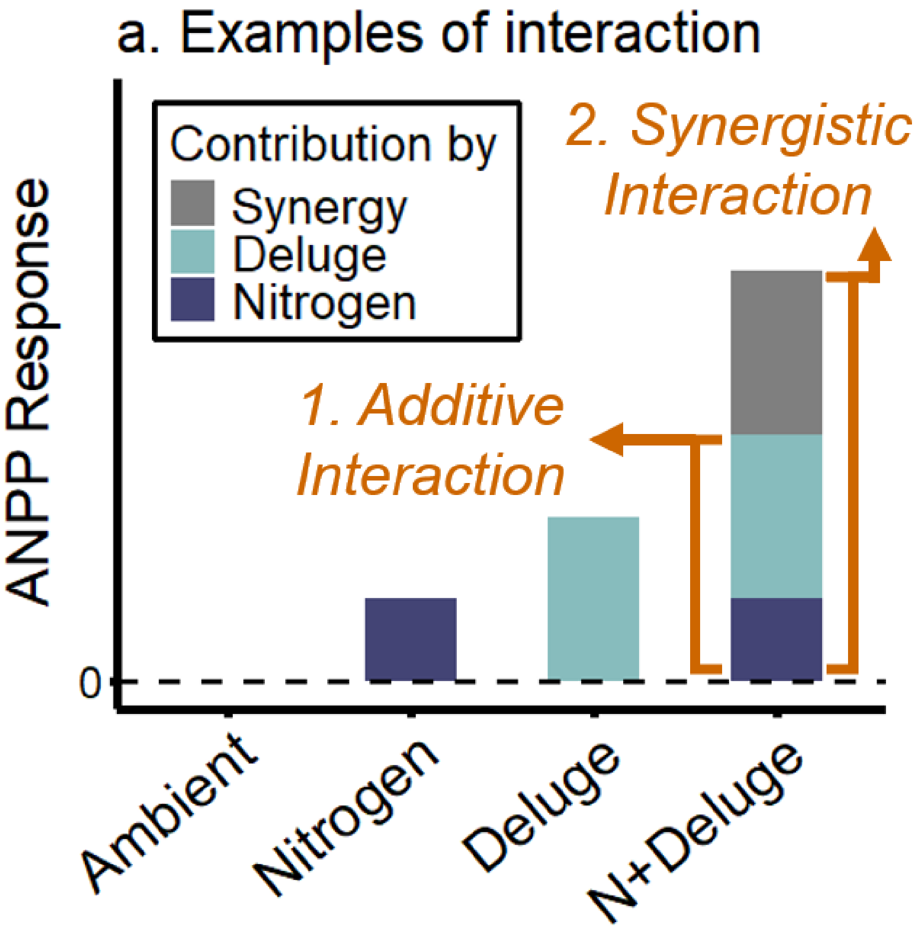

Figure 2.1. Conceptual figure of the response of plant production to nitrogen and deluge with examples of potential interaction. The addition of nitrogen or deluge is expected to increase production, while the combination of nitrogen and deluge may result in additive or synergistic interaction. Additive interactions (arrow 1) result in production increases that are equal to the sum of their parts (i.e., sum of the nitrogen and deluge production response), while synergistic interactions (arrow 2) result in a production increase that is greater than the sum of their parts.

interacts with deluge timing and how environmental context alters the structure (plant community composition and diversity) and function (aboveground net primary productivity, ANPP) of a semi-arid grassland. We addressed two key questions. First, does the magnitude of nitrogen addition govern the interaction between a discrete pulse and a press gradient? We predicted that synergistic interactions would be most likely when plant growth is not nitrogen-limited, so synergy with deluge was expected at higher (non-limiting) levels of nitrogen addition.

This would result in saturating effects of the deluge, where there would be a deluge effect until the point that nitrogen is no longer limiting (Bai et al. 2010, Peng et al. 2020, Seabloom et al. 2021, Yang et al. 2023). Second, does the timing of deluge determine the interaction between a discrete pulse and a press gradient? We predicted that an early-mid season deluge is more likely to result in synergistic effects than a mid-late deluge. An early-mid season deluge may provide precipitation during a period when water is limiting and plants are rapidly growing, whereas a mid-late deluge would not coincide with the most active portion of the growing season (Post and Knapp 2020, Post et al. 2021).

3. Methods

3.1 Site Description

This research was conducted within the semi-arid shortgrass steppe ecosystem of the central US. The study site was established at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Central Plains Experimental Range (CPER), which is located in northeastern Colorado, USA (40° 50' N, 104° 45' W). The mean elevation is 1650 m and the mean annual temperature is 8.6 °C. Mean annual precipitation is 321mm, with most precipitation falling as rain during the growing season from May to September (Lauenroth 2008). Our experiment was established in a pasture with minimal topographical variation and relatively homogeneous vegetation. The site was undisturbed and had been ungrazed by cattle since 2000 (Griffin-Nolan et al. 2018b). During the focal period of this experiment (2020-2022), the most dominant species based on relative abundance of the control plots were the C_3 grasses *Vulpia octoflora* and *Elymus elymoides*, the sedge *Carex Eleocharis*, and the forb species *Sphaeralcea coccinea* (listed from most to least abundant).

3.2 Experimental design: Nitrogen press

This project occurred within a long-term nitrogen-addition experiment where fertilization has been ongoing since 2014 as a gradient of eight levels: 0, 2.5, 5, 7.5, 10, 15, 20, 30 g m^{-2}. Nitrogen was applied annually during early June by hand-scattering time-release urea, which enters the soil as ammonium (Perin et al. 2020). This study employs a randomized complete block design with six blocks arrayed across the landscape with minimum separation of 10 m. Blocks are composed of eight 5 x 5 m plots (4 plots x 2 plots) separated by a 1 m walkway. Each nitrogen treatment is replicated once per block and was randomly assigned at the beginning of the experiment (8 nitrogen levels * 6 blocks = 48 total plots, with each nitrogen treatment replicated six times). Plots are further divided into 4 2.5 x 2.5 m subplots, one designated for permanent sampling and two designated for the two experimental deluge events.

3.3 Experimental design: Deluge pulse

We defined deluge as a 95th percentile rainfall event using long-term (1980-2018) growing season (May 1 to August 31) rainfall data sourced from the CPER headquarters (Hoover 2020). Small rainfall events (>3 mm) may trigger physiological plant responses but are too small to meaningfully contribute to plant production, so we conservatively excluded rainfall events of less than 3 mm (Sala and Lauenroth 1982, Heisler-White et al. 2008, Knapp et al. 2015, Post and Knapp 2021). We then summed precipitation that fell on consecutive days, since many natural deluge events occur over several days (Noy-Meir 1973, Loik et al. 2004, Post and Knapp 2021). The 95th percentile for the long-term record was then calculated, which was a 42 mm rainfall event. In our long-term dataset, there were 28 historic rainfall events that fell at or above the 95th percentile. The duration of these deluge events ranged from one to five days and were distributed across the growing season. This site has a high degree of spatial variability in precipitation (Post et al. 2021), therefore rainfall data for the study period were obtained from a USDA-ARS rain gauge (referred to as USDA_nunn_scan_2017) located in an adjacent pasture.

We applied the 42 mm deluge in 2021 and 2022 to the 2.5 x 2.5 m subplots designated for these treatments. The deluge subplots will be referred to as "N+Deluge" or "N+D" subplots. Non-deluge plots are referred to as "core" (nitrogen addition only) subplots. Prior to deluges, we buried 15 cm wide aluminum flashing to a depth of 8 cm in the soil around each 2 x 2 m deluge subplots (with a 0.5 m buffer on all sides) to minimize surface runoff of water. Deluges were applied for two consecutive days (21 mm for two days) to allow water to penetrate the soil and minimize pooling on the soil surface. Water was sourced from the CPER headquarters as non-potable well water that contained nitrate but was below drinking water guidelines of 10 mg l^{-1}. Water was delivered to on-site tanks, then pumped with gas-powered pumps and applied manually using a flowmeter to designated subplots as simulated rainfall. Deluges were applied during periods without forecasted precipitation. Some natural precipitation fell, but it was <3 mm, and there were no significant differences between soil moisture before and after natural rainfall events. The 2021 deluge was applied between 27 July 2021 and 7 August 2021, and the 2022 deluge was applied between 23 June 2022 and 28 June 2022 (Appendix 2 Table 1). To align with the convention of Post and Knapp (2020), we refer to these events as the 2021 "mid-late" season deluge and the 2022 "early-mid" season deluge. Deluge treatments could not be applied in a single two-day period to all blocks, so application date varied by block (Appendix 2 Table 1).

We recorded soil moisture 2 days before deluges then monitored until the end of August after soil moisture between deluge and non-deluge subplots no longer significantly differed. Soil moisture was measured as volumetric water content (% VWC) using a HydroSense II Handheld

Soil Moisture Sensor (Campbell Scientific, Inc., Logan, UT), equipped with 20 cm soil moisture probes.

3.4 Soil nitrogen availability

We estimated biologically available nitrate and ammonium using mixed bed ion exchange resin bags to simulate roots. Bags were created with a tablespoon of mixed-bed resin, which was zip-tied within a square of spandex swimsuit lining fabric, following the Nutrient Network methodology (Riggs and Hobbie 2016). All resin bags were soaked in a 10% HCL acid bath to remove contaminants, washed in deionized water, then refrigerated until deployment. Before the deluges, two replicate resin bags were buried to a depth of 10 cm into randomly selected locations within the core nitrogen plots, and a second pair was buried in randomly selected locations within the active deluge plot. Bags were retrieved after 32 days, once soil moisture differences between deluge and core nitrogen subplots were no longer significant. Resin bags were rinsed with DI water then replicates were extracted together with 80ml 2M KCL. Samples were shaken at 200 rpm for 2 hours then filtered through 40 grade Whatman filter paper. Extracts were neutralized with NaOH then run on an O.I. Analytical 3700 Automated Chemistry Analyzer to assess ammonium and nitrate content. One outlier nitrate sample and the sample from the paired deluge subplot were removed from the dataset. We calculate total nitrogen as nitrate plus ammonium (mg l^{-1}).

3.5 Aboveground net primary productivity

Aboveground net primary productivity (ANPP g m^{-2}) was estimated by sampling two 20 x 50 cm quadrats at the end of the growing season (early to mid-August) to capture maximum growth from core nitrogen plots and deluge subplots. Sampled areas were mapped to prevent resampling. Biomass was clipped at ground level then separated into grass, forb, and woody functional groups. Samples were dried for 48 hours at 60ºC in a drying oven, sorted again for accuracy and to separate out previous year's dead biomass, then weighed. Woody biomass was only found in one sample across 2021 and 2022, so it was not representative of the site and was excluded from analysis. Grass and forb biomass were summed for each quadrat and then quadrats were averaged within each plot and multiplied by ten to estimate ANPP g m^{-2}.

3.6 Plant community richness, evenness and composition

We assessed plant community composition in two surveys at the beginning (early-to-mid June) and end (late August) of each growing season of the study. Core nitrogen plots were surveyed using a 1 x 1 m permanent sampling area, while N+Deluge subplots were surveyed using 1 x 1 m sampling areas established within the center of each deluge subplot. We estimated aerial cover to the nearest 1% or 5% for each species rooted within the sampling area. Across early and late surveys, we recorded the maximum cover value for each species. These data were used to calculate richness and evenness (Evar) with the community_structure() function within the Codyn R package, version 2.0.5 (Hallett et al. 2020), and in analyses described below.

3.7 Canopy greenness

Canopy greenness is a measure of plant phenology that captures the amount of photosynthetically active tissue across the growing season (Post and Knapp 2020, Zhang et al. 2024). Greenness was measured using digital photographs of the 1 x 1 m community composition sampling areas (Post and Knapp 2020). Photographs were taken of all plots before the deluge application and until the end of the growing season each year of the study. The camera was positioned directly above the sampling area at a height of 1.4 m. Images were cropped to include only the 1 x 1 m sampling area. Greenness was calculated as the green chromatic coordinate (GCC), or the ratio of green to red and blue light within each pixel in an image: green

/ (blue + red + green) (Post and Knapp 2020, Zhang et al. 2024). All pixel values were averaged to obtain one value per image. As a color ratio, GCC is robust against natural light variation. The EBImage R package, version 4.46.0 was used to extract GCC data (Pau et al. 2010).

3.8 Statistical analyses

To analyze ANPP, richness and evenness, and nitrogen data, we used a replicated regression mixed-effect model with a split-plot design and blocking, followed by type III ANOVA for mixed models. Nitrogen level and deluge treatment were considered fixed effects represented as factors, while block and plot were random effects with plot nested in block to account for the split-plot design. Analyses were run separately for each year to account for differences in timing of the deluge. Our model utilized the R lmer() function within the lme4 package, version 1.1-35.5 (Bates et al. 2015). Canopy greenness was a repeated measure, so we added “day of the year” as a fixed factor and a compound symmetry correlation structure to account for repeated measures. This model used the lme() function from the nlme package, version 3.1-166 (Pinheiro et al. 2023). Our split-plot design was not conducive to analysis of traditional community composition data, so we simplified our multivariate data into univariate data by quantifying the difference in plant community composition as the distance between centroids of the nitrogen-only plots and the corresponding N+Deluge subplots within each block. This was done using the multivariate_change() function from Codyn (Hallett et al. 2020). We assessed this data with a version of our model that removed plot as a random factor, since the split-plot design was already being accounted for in our initial calculations. We identified influential species that contributed the most to community composition differences between nitrogen treatments for each year using the simper() function from the Vegan package, 2.7-0 (Oksanen et al. 2024).

To identify the mechanisms and factors that determined the ANPP response to deluge, we used AIC model selection and a multiple regressions model. We first identified predictors that were expected to contribute to deluge response. If predictors were correlated to each other, we selected the predictor with the lower AIC. The model selection function dredge() from the MuMIn package, version 1.48.4 (Bartoń 2024), was used to assess which predictors should be included in our additive linear model with blocking. We followed with an analysis of variance to assess the R^2 of each selected predictor to assess how much variation that each variable explained. This was conducted with the partR2() function from the partR2 package, version 0.9.2 (Stoffel et al. 2021).

All analyses were conducted in R (version 4.2.2) (R Core Team 2024) with a marginal significance threshold of $p \leq 0.1$ and significance threshold of $p \leq 0.05$.

4. Results

4.1 Precipitation and deluge treatments

Precipitation during the 2021 treatment year (Jan1-Dec31) was 312 mm, just under the long-term mean annual precipitation of 321 mm (Lauenroth 2008), while 2022 was a drought year with only 210 mm (Appendix 2 Table 2). Precipitation from January 1 to the end of the growing season (August 31) was 241.6 mm in 2021 (deluge plots receiving 283.6 mm) and was 169.2 mm in 2022 (deluge plots receiving 211.2 mm) (Figure 2.2a).

The effect of deluge on soil moisture was similar between the 2021 mid-late and the 2022 early-mid deluges (Figure 2.2b). In both years, soil moisture peaked the day after deluge application. The 2021 mid-late deluge increased soil moisture by 247% compared to core nitrogen plots, and soil moisture remained significantly elevated for 15 days. The 2022 early-mid

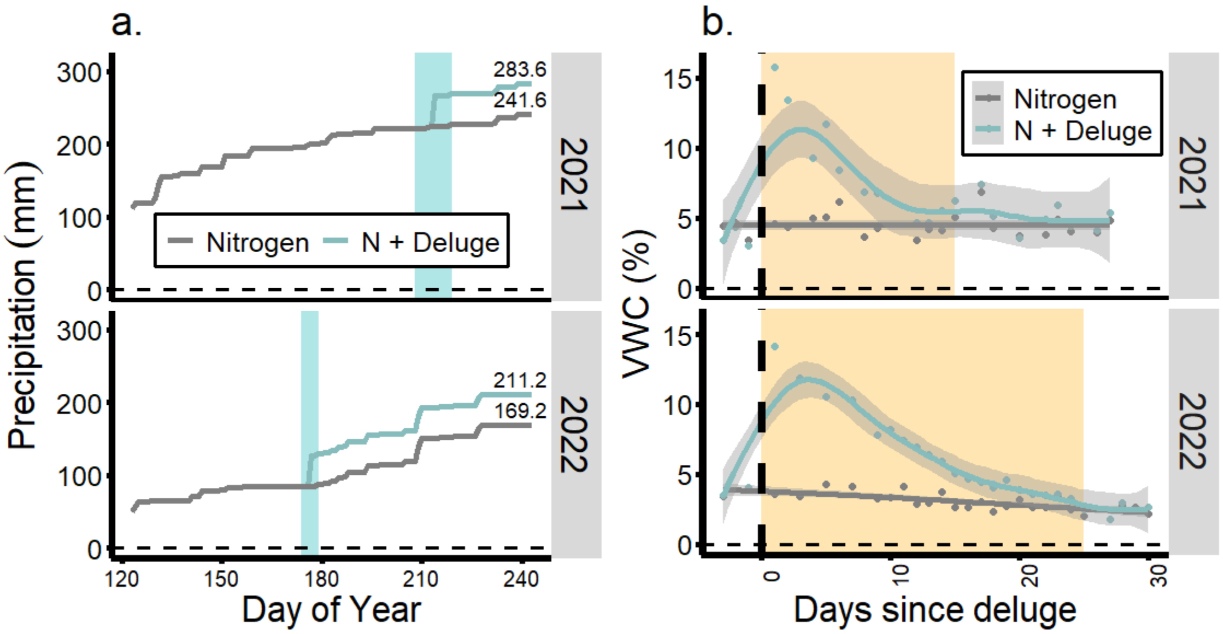

Figure 2.2. Precipitation (a) and soil moisture (b) for ambient conditions (gray lines) and with deluge treatment (blue lines) in 2021 and 2022. Plot a shows accumulated precipitation across the growing season for ambient conditions and deluge treatments. Text inside the plot represents precipitation totals from January 1 to August 31 for ambient conditions and deluge treatments. Blue shaded areas represent the period when the deluges were applied (Appendix 2 Table 1). Plot b shows soil moisture as volumetric water content (VWC%) after the mid-late 2021 deluge and the early-mid 2022 deluge. The yellow shaded areas indicate the duration of significant treatment differences. Treatment differences were found for 15 days after the 2021 deluge was applied, and 25 days after the 2022 deluge. Soil moisture in deluge subplots peaked the day after deluge, with a 247% increase in 2021 and a 292% increase in 2022.

deluge increased soil moisture by 292% and differences remained elevated for 25 days (Figure 2.2b).

4.2 Soil nitrogen availability

Availability of soil nitrate and total nitrogen mg l^{-1} was significantly increased by nitrogen addition, deluge, and their interaction in 2021 and 2022 (Appendix 2 Table 3; Appendix 2 Figure 1). In both experimental years, nitrate availibility did not respond to nitrogen addition alone, but significantly increased with the deluge treatment. When averaged across nitrogen addition levels, deluge increased availible nitrate by 376% in 2021 and 216% in 2022. Total nitrogen availibility was significantly increased by nitrogen alone. When compared to the 0 g m^{-2} control, significant treatment differences occurred at 30 g m^{-2} in 2021, and 20 and 30 g m^{-2} in 2022. The addition of deluge caused nitrogen treatment differences to become more prominent. Significant differences in total nitrogen occurred between the 0 g m^{-2} control and 7.5, 15, 20, and 30 g m^{-2} in 2021 and 15, 20, 30 g m^{-2} in 2022. Overall, the 2021 mid-late deluge had a much greater influence on total nitrogen compared to the 2022 early-mid deluge, increasing total nitrogen avaibility on average by 314% and 29%, respectively.

4.3 Aboveground net primary productivity

In 2021, nitrogen addition significantly increased ANPP, grass production, and forb production on average by 61%, 19%, and 353% compared to the control, respectively (Appendix 2 Table 4, Figure 2.3, Appendix 2 Figure 2). However, the mid-late deluge had no significant influence on ANPP, nor was there interaction between nitrogen and deluge (Appendix 2 Table 4; Figure 2.3). In contrast, nitrogen addition alone had no significant effect on ANPP or grass production in 2022, but forb biomass significantly increased in response to nitrogen addition, deluge treatment, and their interaction. On average, forb biomass increased by 164.7% across nitrogen levels, 206.4% in response to deluge, and 1087.9% in response to N+Deluge. In 2022, the early-mid deluge also significantly increased productivity by an average of 312% across all deluge plots compared to corresponding nitrogen-only plots. Nitrogen and deluge addition significantly interacted, with significant pairwise differences between the 0 g m^{-2} control plots and the 10 and 15 g m^{-2} plots for the N+Deluge treatment (Figure 2.3b). Grass biomass was not significantly affected.

To test whether the interaction between deluge and nitrogen addition in 2022 was additive vs. synergistic, the effects of nitrogen (core plots only) and deluge (0 g^{-2} N+Deluge

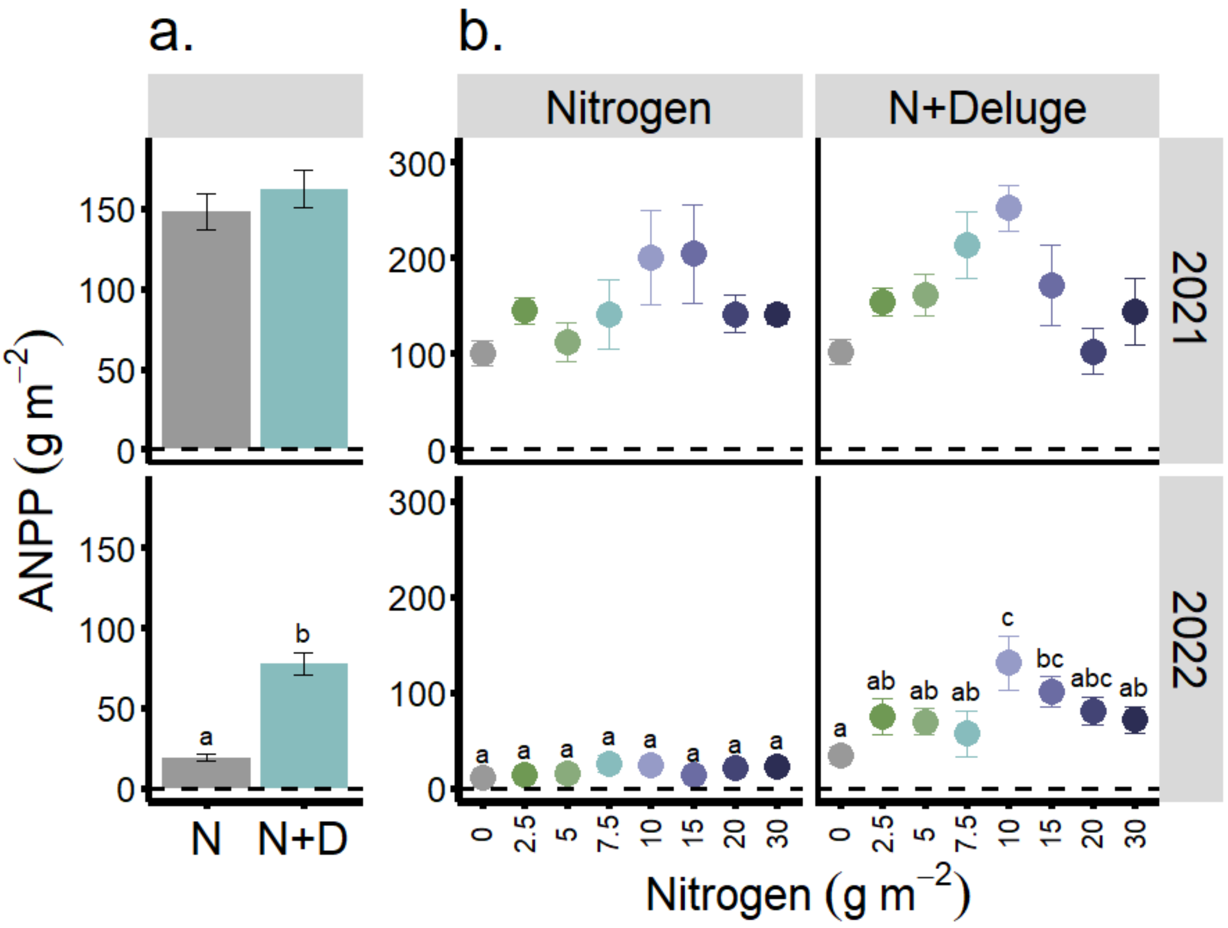

Figure 2.3. The effect of deluge on plant production across nitrogen treatments (a) and the effect of deluge treatments by nitrogen addition level (b) in 2021 and 2022. In 2021, there was a significant effect of nitrogen, but no deluge effect or nitrogen-deluge interaction. In 2022, deluge significantly affected productivity with a nitrogen-deluge interaction. All error bars represent mean ±SE. Letters indicate significant differences ($p \leq 0.05$) between pairwise comparisons of nitrogen treatments.

treatment only) on ANPP were separately calculated then summed together to predict the expected additive interaction (Figure 2.4a). If effects were additive, nitrogen and deluge were predicted to increase productivity by an average of 31 g m^{-2} (8 g m^{-2} being attributed to the effect of nitrogen addition, 23 g m^{-2} being attributed to the effect of deluge), but productivity was actually increased by 84 g m^{-2} on average (Figure 2.4c). The 10, 15, and 20 g m^{-2} N+Deluge treatments were significantly higher than the corresponding predicted additive effect. Therefore,

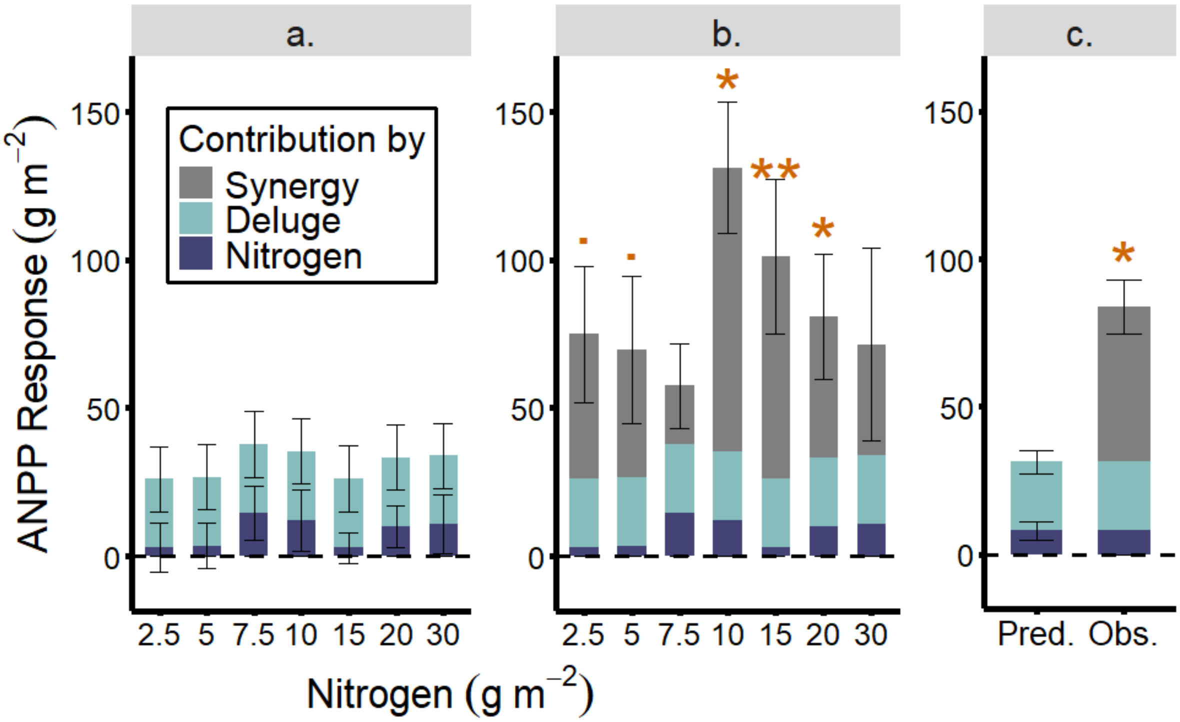

Figure 2.4. The predicted additive effect (a, c) compared to observed synergistic effect (b, c) on production (as increase in ANPP g m^{-2}) of nitrogen and early-mid deluge for 2022. Plot a demonstrates the predicted effect of nitrogen and early-mid deluge if effects were additive. Predicted effects were calculated based on the single-factor response compared to the control. Plot b shows the observed effect of nitrogen and deluge treatments. Any production increase that exceeds the additive predicted amount is attributed to a synergistic effect (gray). Plot c represents data averaged across nitrogen levels of plots a and b to show differences in deluge effects. The orange asterisks in plots b and c indicate treatments with production increases that were significantly greater ($p \leq 0.05$) than the predicted additive effect, while the orange dots indicate marginal significance ($p \leq 0.1$). All error bars represent mean ±SE.

this interaction between nitrogen and deluge was synergistic.

4.4 Plant community richness, evenness, and composition

Species richness was significantly affected by nitrogen in both 2021 and 2022 (Appendix 2 Table 4; Appendix 2 Figure 3a). In 2021, average richness decreased from 7.8 in unfertilized control plots to 7.0 species in nitrogen plots, with significant pairwise differences between the 2.5 and 20 g m^{-2}. In 2022, nitrogen addition decreased average richness from 8.0 in unfertilized controls to 5.8 species, and significant pairwise differences were found between the 0 g m^{-2}

control and the 5, 15, and 30 g m^{-2} treatments. Deluge significantly affected richness in 2022, increasing average richness from 5.8 in nitrogen plots to 6.4 in the N+Deluge subplots (Appendix 2 Figure 3b). In contrast, species evenness was not significantly affected by nitrogen addition in either year of the study but was significantly affected by the 2022 deluge that decreased evenness in the N+Deluge plots by 31.6% (from 0.55 to 0.37) (Appendix 2 Table 4, Appendix 2 Figure 4).

Overall, community composition (centroid difference between each core nitrogen treatment and corresponding N+Deluge treatment) was not significantly affected by the deluge treatment in either year of the study (Appendix 2 Figure 5), suggesting that there was not an interactive effect of the nitrogen and deluge treatments.

4.5 Canopy greenness

In both 2021 and 2022, canopy greenness was not affected by nitrogen but was significantly increased by deluge (Appendix 2 Table 5; Appendix 2 Figure 6). During 2021, the deluge treatment increased canopy greenness, and this effect did not vary over time. During 2022, deluge resulted in increased canopy greenness from day 182 to 201. Differences between the core nitrogen plots and N+Deluge subplots reappeared on day 210 following a large natural rainfall. There was also a nitrogen and deluge interaction in 2022. Without deluge, increasing nitrogen caused a decrease in greenness, but the addition of deluge countered the reduction in greenness with nitrogen addition (Appendix 2 Figure 6).

5. Discussion

In this study, we combined a gradient of chronic nitrogen addition with short-term deluge pulses at two different times during the growing season to test how the magnitude of nitrogen and the timing of deluge governs the interaction between a discrete pulse and a press resource alteration. In 2021, ANPP and plant richness were influenced by nitrogen addition alone, but not by mid-late deluge. In contrast, the early-mid 2022 deluge interacted with nitrogen to produce synergistic effects on ANPP; however, interactive effects of nitrogen and deluge treatments on plant species richness and evenness were not observed.

We predicted that synergistic interactions would occur at higher, non-limiting levels of nitrogen addition. While effects of the deluge were greatest at mid-high levels of nitrogen, we found an overall synergistic interaction between nitrogen and deluge (Figure 2.4c). The actual synergistic effect exceeded the predicted additive increase at every level of nitrogen (Figure 2.4b). We also expected that synergistic increases in production would saturate once nitrogen was no longer limiting, but the response was unimodal, with synergistic effects significantly exceeding additive effects at 10, 15, and 20 g m^{-2} nitrogen (Figure 2.4b). This unimodal pattern was also present for ANPP in the N+Deluge plots, with greatest production at 10 g m^{-2}. Nitrogen may cease to limit productivity at 10 g m^{-2}, but higher levels (e.g., 30 g m^{-2}) could have negative effects, particularly if nitrogen accumulates in the system. Similar unimodal production responses have been observed across nitrogen gradients (Ma et al. 2021, Spohn et al. 2025) and several mechanisms may explain why higher rates of nitrogen suppress production, such as soil acidification (Kimmel et al. 2020, Liu et al. 2022), increased soil magnesium (Tian et al. 2016, 2022), and reduced functional stability (Zhang et al. 2016, Ma et al. 2021). Unfortunately, we could not examine these mechanisms in the present study.

A second prediction was that an early-mid deluge would be more likely to result in synergistic effects than a mid-late deluge, which was confirmed. Plant phenology and interannual precipitation variability likely contributed to differences in response to the 2021 and 2022 deluges. Both deluges increased canopy greenness, but the magnitude of green-up was much greater in response to the 2022 early-mid deluge (Appendix 2 Figure 6). The 2021 mid-late deluge may have been applied when plants had begun senescing or were becoming photosynthetically dormant, so production effects were limited. In contrast, the early-mid deluge may have provided critical water resources to vulnerable emerging plants to establish actively growing plants, leading to greater survival and higher production overall (Post and Knapp 2020, Post et al. 2021). The 2022 deluge may have also "primed" the system to take advantage of natural rainfall events later in the season. A large, natural rainstorm mid-season in 2022 caused deluge treatment differences to reemerge. Deluge effects on soil moisture had ceased being significant, so this response represents a "within-season" deluge legacy. The natural rainfall likely magnified productivity gains that were attributed to the early-mid deluge. Additionally, there were notable precipitation differences between 2021 and 2022 that may have mediated the relative effect of nitrogen addition. From the beginning of the year to the end of the growing season (Jan 1 to Aug 31), 242 mm of precipitation fell in 2021 and 169 mm fell in 2022 (Figure 2.2a). The 2022 early-mid deluge may have eased water limitation which allowed greater relative response to nitrogen compared to the less water-limited growing season of 2021. In summary, timing of deluge may contribute to synergistic effects on ANPP by promoting growth during periods of high potential photosynthetic activity, "priming" a system to respond to natural inputs of precipitation and easing water-limitation during naturally dry conditions.

To better understand what may have led to the synergistic effects of nitrogen press and deluge pulse observed in the second year of the study, we used model selection to assess several potential mechanisms: total nitrogen, soil moisture, GCC greenness index, and the proportion of forb to grass ANPP (Appendix 2 Table 6). Only one predictor was selected for inclusion in the multiple regression model - the proportion of forb to grass ANPP, which explained 28% of the variation in productivity response. This suggests that the response of fast-growing forb species was a key determinant of the pulse-press synergistic effect on productivity, which can be further illustrated through three lines of evidence (Figure 2.5). First, in both years of the study, there was a significant, positive correlation between the proportion of forb to grass production and ANPP (Figure 2.5a), indicating that forbs were a key contributor to variability in ANPP. When early-mid deluge was added in 2022, this slope increased, demonstrating that forbs were also primarily responsible for production increase under deluge. Second, the relative contribution of forbs to ANPP increased from 2021 to 2022 due to an invasion of weedy or non-native forb species (Figure 2.5b). This shift in dominance likely resulted in plant communities with greater capacity to rapidly respond to resource addition through increased aboveground plant growth. Third, nitrogen addition decreased the abundance of native grasses and increased the abundance of highly productive weedy species, particularly in 2022 (Figure 2.5c; Appendix 2 Table 7). In 2022, nitrogen decreased the abundance of the native, perennial C_3 grass, *Elymus elymoides*, and increased the abundance of *Chenopodium sp.*, a weedy forb. The overall production response to deluge was likely driven by the 170% increase in *Chenopodium sp.* abundance, as plot biomass was significantly correlated to *Chenopodium sp.* abundance ($p = 0.025$). The *Chenopodium* species within the site have acquisitive traits, such as tall stature and high leaf nitrogen, that allow for greater exploitation of resources, compared to a more conservative, perennial species like *Elymus elymoides* (Xia and Wan 2008, Blumenthal et al. 2020, Feng et al. 2023). An experiment within the same site in 1978 found similar invasion of weedy and non-native species into plots that received both nitrogen and water addition for four years (Lauenroth et al. 1978), with these community differences being maintained for years after cessation of the resource

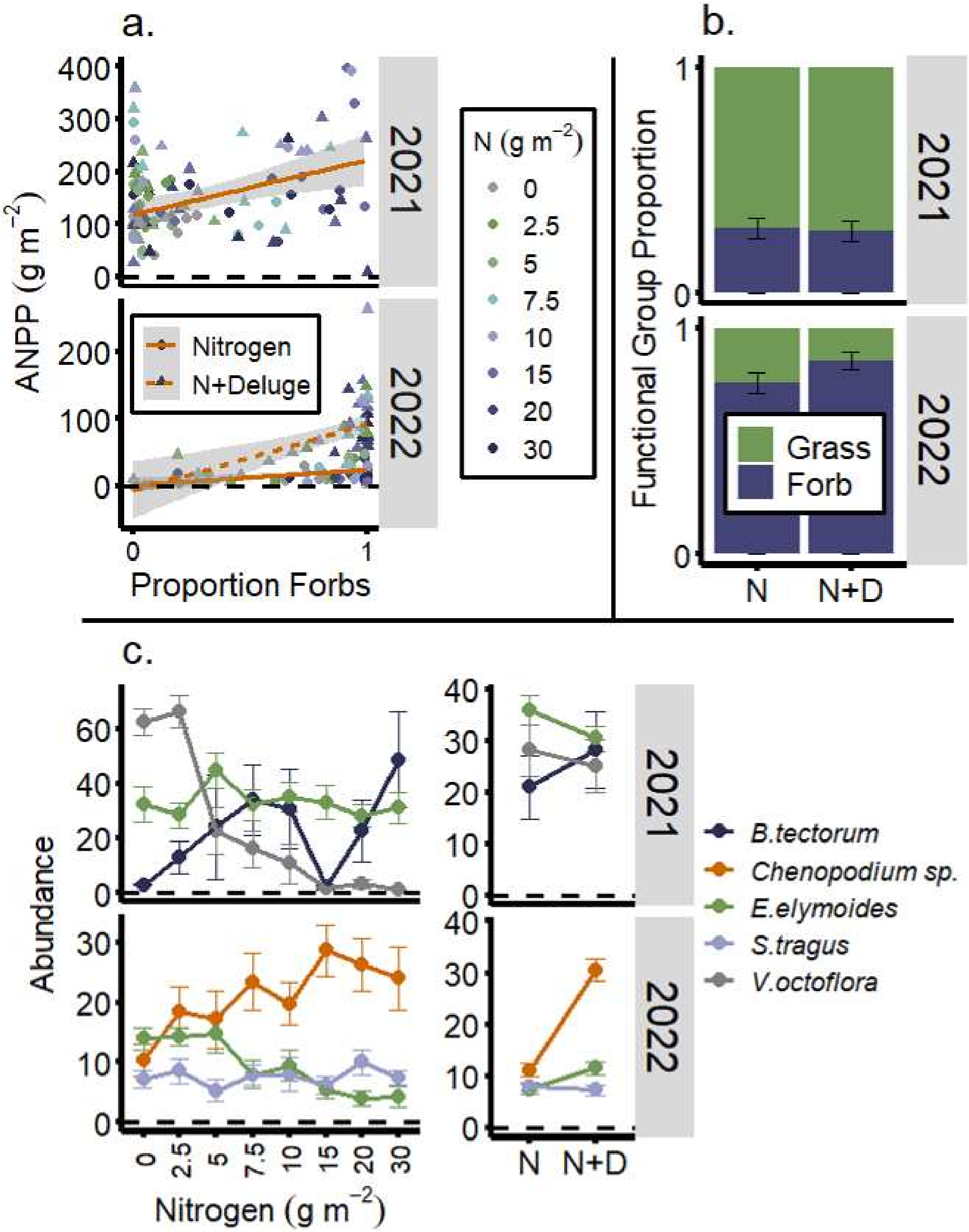

Figure 2.5. The influence of increasingly abundant high-productivity forb species in driving synergistic interaction of deluge and nitrogen. Total ANPP increases with the increasing proportion of forb to grass ANPP in each plot by nitrogen (color) and deluge treatment (shape, linetype) (a). Regression lines indicate a significant effect of the proportion of forbs to grass on total ANPP. The dominance of forbs also increased from 2021 to 2022 at the site level (b). Native grasses dominated in 2021, while non-native and weedy forbs become dominant in 2022. Nitrogen overall decreased the abundance of native grasses (*Elymus elymoides*, *Vulpia octoflora*) and increased the abundance of weedy or non-native species (*Bromus tectorum*, *Chenopodium sp.*, *Salsola tragus*) (c). The 2022 deluge significantly increased the abundance of native *E. elymoides* by 54.8% while increasing the weedy *Chenopodium sp.* by 172.8%.

addition treatments (Milchunas and Lauenroth 1995). Furthermore, community diversity (as evenness) decreased in 2022 (Appendix 2 Table 4, Appendix 2 Figure 4), which demonstrates that a single deluge can homogenize plant communities by increasing the abundance of non-native and weedy species. Thus, it appears that the ability by the invading forbs to rapidly respond to increased resource availability is what led to the synergistic response we observed in ANPP.

Several factors may be expected to control nitrogen and deluge interaction, but there is evidence to suggest that these were unimportant in our experiment. The 2021 deluge occurred during the eighth year of nitrogen addition while the 2022 deluge occurred during the ninth year of addition, but deluge had a similar effect on nitrate availability in 2021 and 2022. In fact, total nitrogen availability was stimulated more by the 2021 mid-late deluge than the 2022 early-mid deluge (Appendix 2 Table 3, Appendix 2 Figure 1). Thus, differences in nitrogen availability likely did not influence the synergistic interaction of nitrogen and deluge in 2022. Species richness is another factor that can influence community response to nutrient addition (Isbell et al. 2013a). Like many other nitrogen-addition studies, richness decreased in fertilized plots (Zavaleta et al. 2003b, Isbell et al. 2013a, Seabloom et al. 2021). In contrast, deluge increased species richness (Appendix 2 Figure 3b), but the effect was small (5.8 to 6.4 species) and many of these species were rare and did not persist to late season. Indeed, within N+Deluge plots, there was no correlation between species richness and ANPP in either 2021 or 2022 ($p = 0.74$, $p = 0.92$, respectively) (Seabloom et al. 2021). Therefore, richness was unlikely to be a mediator of the nitrogen-deluge synergistic interaction.

In conclusion, the interaction of nitrogen and deluge on grassland productivity is primarily mediated by the timing of deluge. We propose that this interaction results from multiple mechanisms, which together lead to a synergistic ANPP response. The early-mid deluge occurred during a time of high photosynthetic potential and primed plants to respond to natural rainfall events later in the growing season. In tandem, nitrogen addition facilitated the replacement of native grasses by weedy, non-native species with acquisitive traits, which further contributed to growth and dominance under both nitrogen and deluge treatments. This study provides the first experimental evidence showing how long-term nitrogen press can impact plant composition over time (weedy forb dominance), leading to synergistic responses when deluge pulses are applied. We demonstrate that long-term nitrogen addition and extreme rainfall can produce synergistic effects that cannot be predicted by the independent addition of nitrogen or deluge, nor by previous nitrogen- and water-addition experiments that lack either the long-term nature of press or the extremity of an intense pulse (Lauenroth et al. 1978, Zavaleta et al. 2003b, Harpole et al. 2007, Xu et al. 2018, Lü et al. 2018, Meng et al. 2023). More experimental studies are needed to better understand the conditions under which these interactions occur, and the infrastructure to study pulse-press dynamics is already in place. Indeed, long-term, nutrient-addition studies exist across the globe in unique ecosystems (Seabloom et al. 2021). Adding a single extreme pulse into these long-term studies would allow for the experimental study of pulse-press dynamics. It will also be essential to monitor pulse-press experiments for legacy effects that may indicate the crossing of a critical threshold, leading to a new sustained state in the community (Scheffer et al. 2015; Harris et al. 2018). As anthropogenic changes continue, synergistic responses in ecosystem functioning may become more common, calling for improved understanding of the conditions under which long-term presses interact with extreme resource pulses to produce novel ecosystem responses.

CHAPTER 3: Legacy effects of deluge pulse-nitrogen press interactions in semi-arid grassland

1. Summary

Global change is altering the frequency and intensity of environmental perturbations, resulting in novel combinations of chronic (press) and acute (pulse) resource alteration. The overlap of sustained press and discrete pulse is expected to cause large and potentially surprising changes to ecosystem structure and function and may generate lingering effects in subsequent years (i.e., legacies). In a previous research experiment, two pulsed deluge (i.e., extreme rainfall) events were applied across a gradient of chronic nitrogen addition (eight levels from 0-30 g m^{-2}) in a semi-arid shortgrass steppe. The deluge applied mid-late growing season in 2021 had no significant effect on structure or function, while the deluge applied early-mid growing season in 2022 had a synergistic effect on production and altered community structure. In this manuscript, the legacy effects from those deluge events were evaluated in the context of ongoing nitrogen addition. We found that deluge legacies significantly affected productivity, species richness, evenness, and community composition differences in one or more legacy years. Surprisingly, the initial response to deluge was not predictive of the legacy response. The 2021 deluge had no initial effect on production, but production legacies appeared in 2022 and 2023. The largest production legacy occurred in 2023 during a wet season and was driven by increases in weedy, invasive forbs. This 2023 legacy effect was consistent across nitrogen addition levels and deluge treatment timing. Production legacies did not occur in 2024, yet significant legacy effects on richness, evenness, and community composition differences persisted. We also found that nitrogen addition and deluge legacy interacted to influence evenness and homogenize community composition in deluge legacy plots. These findings demonstrate that a single deluge

pulse, amid on-going nitrogen press may generate unexpected legacy effects that alter the structure and function of a grassland ecosystem for at least three years after extreme deluge.

2. Introduction

Anthropogenic forces alter both the intensity and temporal pattern of environmental perturbations that result in changes to resource availability. Global change is intensifying naturally-occurring perturbations such as wildfires, droughts, and extreme rainfall events (deluges) (Knapp et al. 2008, 2015, Easterling et al. 2017, Harris et al. 2018). In tandem, the rise of industrialization results in the continuous and on-going alteration of resources (Vitousek et al. 1997b, Galloway et al. 2003). These patterns of perturbations may be evaluated through the "pulse-press" framework which describes resource alteration along a temporal continuum: from discrete, short-term "pulse" to chronic, long-term "press" (Smith et al. 2009, Collins et al. 2011). By categorizing resource alteration through temporal patterns, researchers may identify shared ecological responses to perturbations and develop predictions for how multiple perturbations may interact to produce unique ecological changes. The combination of chronic press and discrete pulse resource alterations are expected to cause large and potentially surprising changes to ecosystem structure and function. Persistent press may erode ecosystem resistance, so the addition of a sufficient pulse may trigger abrupt change or tip the system into a distinct state (Scheffer et al. 2001). Pulse-press dynamics have been observed and explored in theory and models, but robust experimental evidence is lacking (Scheffer and Carpenter 2003, Ratajczak et al. 2017, Harris et al. 2018). Only recently have these dynamics begun to be tested experimentally (Collins et al. 2017), Linabury and Smith *submitted*) (Chapter 2), and the ecological legacies of such events are still largely unknown. In this study, we evaluated the ecological legacies of the first explicit test of pulse-press dynamics in a semi-arid grassland ecosystem.

In a recent study, we experimentally tested how the timing of deluge events interacts with a gradient of chronic nitrogen addition in a shortgrass steppe ecosystem (Linabury and Smith *submitted*) (Chapter 2). Two deluge events were applied to a gradient of nitrogen addition treatments (eight levels from 0-30 g m^{-2}) that had been on-going for eight to nine years at the time of study. Deluge events representing the 95th percentile of historic precipitation events were applied mid-late growing season in 2021 and early-mid season in 2022 to different subplots within each nitrogen press plot. Despite both deluge events resulting in similar increases in soil moisture and nitrate availability, we found that the timing of deluge determined community response to pulse-press perturbations. The mid-late 2021 deluge had little effect on productivity and showed no interaction with nitrogen addition. In contrast, the early-mid 2022 deluge led to a 300% increase in average ANPP compared to nitrogen addition treatments alone. This effect was synergistic, meaning that the combined effect of nitrogen press and deluge pulse exceeded the sum of their independent effects. The synergistic effect was driven by fast-growing, weedy forb species. These findings suggest that an increase in pulsed precipitation events could lead to large and unexpected shifts in a key ecosystem function within grasslands already experiencing chronic nitrogen enrichment. While this research fills a critical gap in knowledge, theory predicts that pulse-press interactions may also produce persistent legacy effects that could impact future ecosystem responses to pulse or press perturbations. It is therefore essential to continue monitoring these ecosystems to assess potential long-term impacts.

Pulse perturbations that trigger large changes to community structure and function are likely to produce lingering legacy effects. Here, we define "legacy" as a response that persists after the direct effect of perturbation (i.e., increased soil moisture) is no longer present (Vilonen et al. 2022). Legacies may persist for years or even decades after the initial perturbation (Milchunas and Lauenroth 1995, Vinton and Burke 1995, Isbell et al. 2013b, Monger et al. 2015) and occur through multiple possible mechanisms. Deluge pulses may create legacies through storage of belowground resources in perennial species, shifts in litter, altered nutrient availability, or shifts in community composition (Milchunas and Lauenroth 1995, De Boeck et al. 2018, Gong et al. 2020, Broderick et al. 2022, Reynaert et al. 2022, Meng et al. 2023, Hajek and Knapp 2024). A study in the shortgrass steppe found large changes in production and community composition during a 4-year period of water and nitrogen addition, with persistent legacies observed even 20 years after the cessation resource alteration (Milchunas and Lauenroth 1995). Precipitation pulses may be especially likely to lead to legacies, since the volume and number of rain events from previous years' precipitation is a predictor of current-year productivity (Sala et al. 2012, Gong et al. 2020). Indeed, single deluge pulses can produce persistent legacies (Concilio et al. 2015, Hajek and Knapp 2024, Ning et al. 2024), though these effects are variable and warrant further research.

Legacy effects may also be complicated by interaction with press perturbations. Species with acquisitive traits that enable rapid resource uptake are most likely to benefit from both deluge pulses and nitrogen press (Meng et al. 2023, Ratcliffe et al. 2024). As a result, changes initiated by a pulse event may be maintained or amplified under continued nitrogen addition. Indeed, experiments that add both nitrogen and water typically see the greatest changes when both nitrogen and water are added together (Lauenroth et al. 1978, Zavaleta et al. 2003a, Harpole et al. 2007, Xu et al. 2018, Lü et al. 2018, Meng et al. 2023). However, the effects of nitrogen on community composition can vary depending on the level of addition (Isbell et al. 2013a, Simkin et al. 2016, Ma et al. 2021, Linabury et al. *in review*) (Chapter 1), so we expect that the role of nitrogen addition in modifying deluge legacy is likely contingent on the magnitude of nitrogen press. Thus, to fully understand pulse-press legacy dynamics, our goal was to examine how pulse legacies vary across a gradient of press intensities.

To accomplish this goal, we monitored the experiment described in Linabury and Smith (*submitted*) (Chapter 2) to assess potential legacy effects of two pulsed deluge events within the context of an on-going gradient of nitrogen press in a semi-arid shortgrass steppe ecosystem in northeastern Colorado. We addressed two questions: First, does the initial response to a deluge pulse predict the magnitude of short-term legacy effects? We predicted that the magnitude of response to deluge would be correlated to the magnitude of legacy effect. The 2021 mid-late season deluge had no effect on productivity, richness, or evenness, so we did not expect legacy effects. In contrast, the 2022 early-mid deluge caused large changes to productivity, richness, and evenness, so we expected these changes to persist in subsequent years. Second, we asked: How does an on-going press gradient govern deluge legacy effects? The level of nitrogen addition influenced the magnitude of production response to the 2022 mid-late deluge, with the greatest synergistic effect observed for 10, 15, and 20 g m^{-2} of nitrogen addition when deluge was added (Linabury and Smith *submitted*). Therefore, we expected that legacy effects would be most prominent at these mid-to-high ranges of nitrogen addition. Because there was no interactive effect of nitrogen and deluge on richness or evenness, the nitrogen gradient was not expected to influence richness or evenness in years after the deluge treatments.

3. Methods

3.1 Site Description

This research was conducted within a semi-arid shortgrass steppe ecosystem at United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Central Plains Experimental Range (CPER) located in northeastern Colorado, USA (40° 50' N, 104° 45' W, mean elevation 1650 m). Mean annual temperature is 8.6 °C and the mean annual precipitation is 321 mm, with most water falling as rain during the growing season from May to September (Lauenroth and Burke 2008). This experiment was conducted within an undisturbed pasture that has been ungrazed since 2000 (Griffin-Nolan et al. 2018). During the focal years of the study (2022-2024), weedy invasive species became abundant over previously dominant native grasses. The most dominant species in the control plots, in order of highest relative abundance, were the invasive tumbleweed *Salsola tragus*, the weedy forb *Chenopodium sp*, the native C_3 grass *Elymus elymoides*, the native C_4 grass *Bouteloua gracilis*, and the native forb *Sphaeralcea coccinea*.

3.2 Experimental design: Nitrogen press

This study occurred within a long-term nitrogen-addition gradient experiment. Nitrogen has been applied annually since 2014 as an eight-level gradient: 0, 2.5, 5, 7.5, 10, 15, 20, 30 g m^{-2}. The experiment uses a randomized complete block design with six blocks distributed across the landscape, each separated by at least 10 m. Each block contains eight 5 × 5 m plots (arranged in a 4 × 2 grid), with 1 m walkways between plots. Nitrogen addition levels were randomly assigned at the beginning of the experiment and are replicated once per block, with each nitrogen addition rate replicated six times (8 nitrogen levels × 6 blocks = 48 plots). Each plot is subdivided into four subplots: one designated for permanent sampling and two designated for the deluge experiment. Nitrogen is applied in early June using time-release urea scattered by hand across the soil surface. Subplots that only receive nitrogen will be referred to "core" nitrogen plots, or nitrogen-only plots.

3.3 Experimental design: Deluge pulse

Using local, long-term (1980-2018) precipitation records, we calculated the 95th percentile deluge during the growing season, as a 42 mm rainfall event. Two separate deluge events were applied mid-late growing season (late-July to early-August) 2021 and early-mid growing season (early-June) 2022 into two separate subplots (Figure 3.1). Deluges were applied as simulated rainfall over two consecutive days into 2 x 2 m subplots framed by aluminum flashing embedded 8 cm into the soil to minimize surface runoff. Soil moisture data was collected 2 days before deluge treatments and through the end of August. Soil moisture remained significantly different between core nitrogen plots and deluge plots for 15 days in 2021 and 25 days in 2022 down to 20cm, so there were no lingering differences in soil moisture in legacy years. See Linabury and Smith (*submitted*) (Chapter 2) for complete detail.

Nitrogen plus deluge subplots will be referred to as N+Deluge subplots or specifically as "nitrogen+mid-late 2021" (N+M-L 2021) deluge subplots and "nitrogen+early-mid 2022" (N+E-M 2022) deluge subplots. Data was collected within the N+Deluge subplots from the year of deluge through 2024, so the mid-late 2021 deluge has three years of legacy data and the early-mid 2022 deluge has two years of legacy data.

3.4 Soil nitrogen availability

Biologically available nitrate and ammonium was estimated using mixed bed ion exchange resin bags. Bags were created following the NutNet methodology (Riggs and Hobbie 2016) and extraction methods are detailed in Linabury and Smith (*submitted*) (Chapter 2).

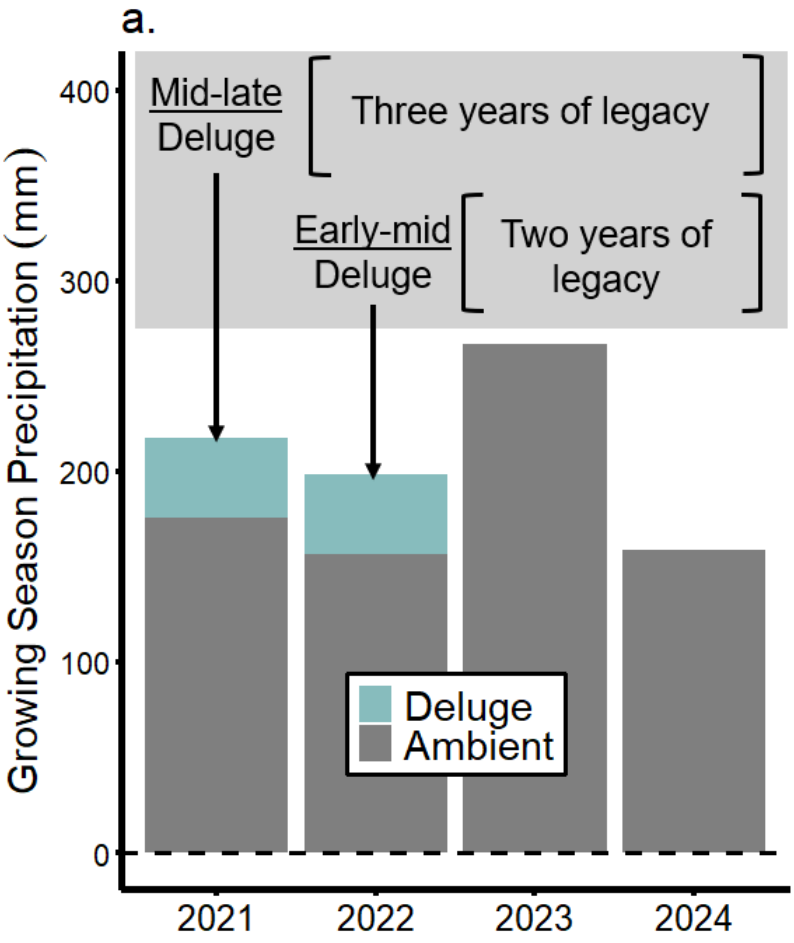

Figure 3.1. Ambient growing season precipitation across focal years with deluge treatments and legacy duration. Growing season precipitation is measured as accumulated precipitation from April 1 to August 31. Deluge treatments added an additional 42mm of percipitaiton. The mid-late growing season deluge was applied in late-July to early-August 2021, and legacies were monitored from 2022-2024. The early-mid deluge was applied in early-June 2022, and legacies were monitored from 2023-2024.

Extracts were neutralized with NaOH then run on a O.I. Analytical 3700 Automated Chemistry Analyzer to assess ammonium and nitrate content. Total nitrogen was calculated as ammonium plus nitrate (mg l^{-1}).

3.5 Aboveground net primary productivity

Plant productivity was measured annually as aboveground net primary productivity (ANPP g m^{-2}) by sampling two 20 × 50 cm quadrats per plot during peak biomass (late August to early September). In core nitrogen plots, quadrats were located within the same 2.5 × 2.5 m subplot used for plant composition surveys. In N+Deluge subplots, quadrats were placed adjacent to community composition areas within the flashed subplot during years when deluge was applied and in subsequent legacy years. Quadrat sampling locations were mapped and never resampled. Vegetation was clipped to ground level and sorted into grass and forb functional groups. Woody biomass was rare and not representative of the site, so woody data was not included in this analysis. Samples were dried at 60°C for 48 hours in a drying oven, then re-sorted to ensure accuracy and to remove previous year's dead biomass before weighing. The mass from each quadrat was averaged together and multiplied by 10 to estimate ANPP g m^{-2}.

3.6 Plant community richness, evenness and composition

We surveyed plant community composition at the beginning (mid-June) and end (late August) of each growing season and recorded maximum plant cover for each species recorded. In core nitrogen plots, surveys were conducted within a permanent 1 × 1 m sampling area, while sampling areas were established in the middle of each N+Deluge subplots. We estimated aerial cover to the nearest 1% (or nearest 5% when cover exceeded 50%) for each species rooted within the sampling area. These data were used to calculate species richness and evenness (Evar) using the community_structure() function in the codyn R package (version 2.0.5; Hallett et al. 2020), as described in the analyses below.

3.7 Statistical Analyses

All statistical analyses were performed in R (version 4.2.2; R Core Team 2024), using a marginal significance threshold of $p \leq 0.1$ and a primary significance threshold of $p \leq 0.05$.

We analyzed legacy ANPP, species richness and evenness, species abundance, and nitrogen data using replicated regression mixed-effects models with a split-plot design and blocking followed by Type III ANOVA for mixed models. Nitrogen level and deluge treatment were treated as factorial fixed effects, while block and plot were treated as random effects, with plot nested within block to account for the split-plot design. Analyses were conducted separately within each year to accommodate high interannual variability. Additionally, to account for deluge treatments being applied in different years, two separate analyses were performed: core nitrogen data were assessed with the 2021 deluge legacy data then core nitrogen data were assessed with the 2022 deluge legacy data. Models were implemented using the lmer() function from the lme4 package (version 1.1-35.5; Bates et al. 2015). Due to limitations of the split-plot design for traditional multivariate community composition analysis, we quantified plant community change as the distance between group centroids within multidimensional space from core nitrogen-only plots to corresponding N+Deluge subplots within each block, using the multivariate_change() function from the codyn package (Hallett et al. 2020). Centroid distance was then assessed with a modified version of the original model that dropped "plot," because the split-plot structure was already accounted for in the centroid distance calculation. Last, we used the simper() function from the *vegan* package (version 2.7-0; Oksanen et al. 2024) to identify influential species that drove differences between treatments. For correlation analysis, we calculated the Log Response Ratio (LRR) between the core nitrogen plots and the corresponding N+Deluge subplots to accommodate high interannual variability.

4. Results

4.1 Ambient productivity and precipitation

During legacy years (2022-2024), growing season precipitation (April 1 to August 31) was 156.5 mm in 2022, 267.0 in 2023, and 158.496 in 2024 (Figure 3.1; Appendix 3 Table 1).

Within the 0 g m^{-2} nitrogen control plots, average ANPP was highly variable: 11.8 g m^{-2} in 2022, 336.0 in 2023, and 33.3 in 2024. High production variability was likely due to differences in precipitation seasonality and dominant species composition. The most abundant species in 2022 was the native grass *Elymus elymoides*, and in 2023 and 2024 the invasive tumbleweed *Salsola tragus* was the most abundant.

4.2 Aboveground net primary productivity

During legacy years, ANPP and forb production (the forb component of ANPP) were not significantly affected by nitrogen, though grass production (the grass component of ANPP) was consistently and significantly decreased by nitrogen addition (Figure 3.2a; Appendix 3 Table 2; Appendix 3 Figure 1). Because ANPP was unaffected by nitrogen in legacy years, legacy data may be pooled across nitrogen levels (Figure 3.2b).

In 2022, there was a significant ANPP legacy resulting from the 2021 mid-late deluge (Figure 3.2b; Appendix 3 Table 2). Production increased by 70.5% in deluge legacy plots, which was driven by a significant increase in grass production. In 2023, significant legacy effects were observed from both deluge treatments, driven by a significant increase in forb production. Compared to the core nitrogen plots, ANPP increased by 121.5% in the 2021 deluge legacy plots and by 127.0% in the 2022 deluge legacy plots. No significant legacies were present in 2024. There was no interaction between nitrogen addition and deluge treatment during any legacy year for ANPP, forb production, or grass production

To investigate potential drivers of ANPP deluge legacy effects, we correlated the initial deluge response to the legacy response (as LRR) but found no significant correlation ($p > 0.05$) in any legacy year or with either deluge treatment (Figure 3.3). In contrast, we found strong correlation ($R = 0.60$, $p < 0.001$) between the magnitude of ANPP legacy (the absolute change in

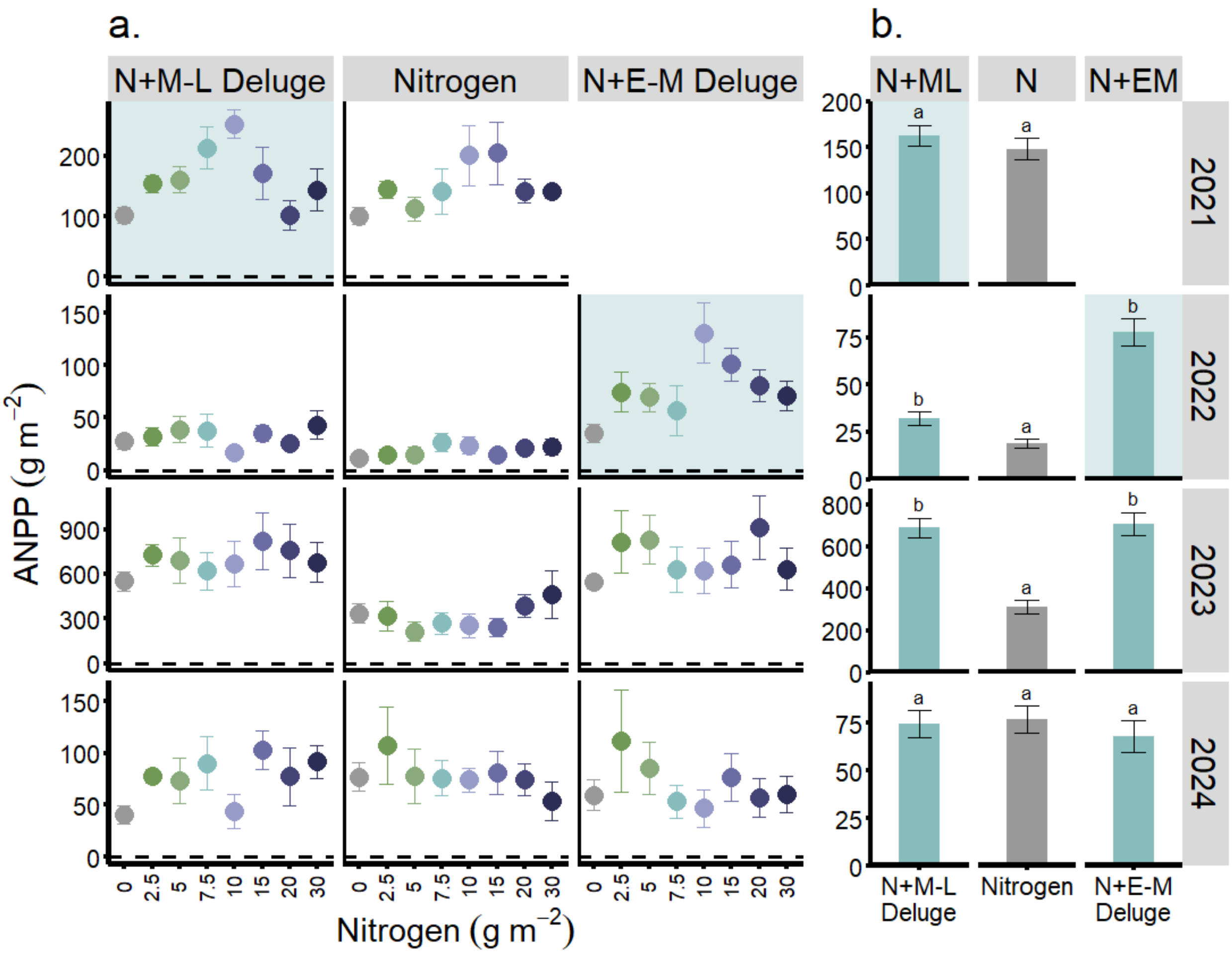

Figure 3.2. The effect of deluge on plant production by nitrogen addition level (a) and averaged across nitrogen addition level (b). In legacy years, there was no significant effect of nitrogen on ANPP, so legacy data can be pooled across nitrogen levels (b). Blue shaded plots indicate years in which the deluge treatments were applied. The y axis scale varies by year to account for high innerannual variabiltiy and reflect the model which assessed the within-year response. All error bars represent mean ±SE. Letters in plot b indicate significant differences ($p \leq 0.05$) between pairwise comparisons of deluge treatments.

ANPP from core nitrogen plots to deluge legacy plots) and yearly growing season precipitation (defined as April 1 to August 31; Appendix 3 Figure 2).

4.3 Plant community richness, evenness, and composition

Nitrogen addition significantly reduced species richness in every legacy year (Appendix 3 Table 3; Appendix 3 Figure 3). Across all deluge and non-deluge plots, richness decreased by 27.5% (2.2 species) in 2022, 27.9% (3.2 species) in 2023, and 33.6% (2.9 species) in 2024.

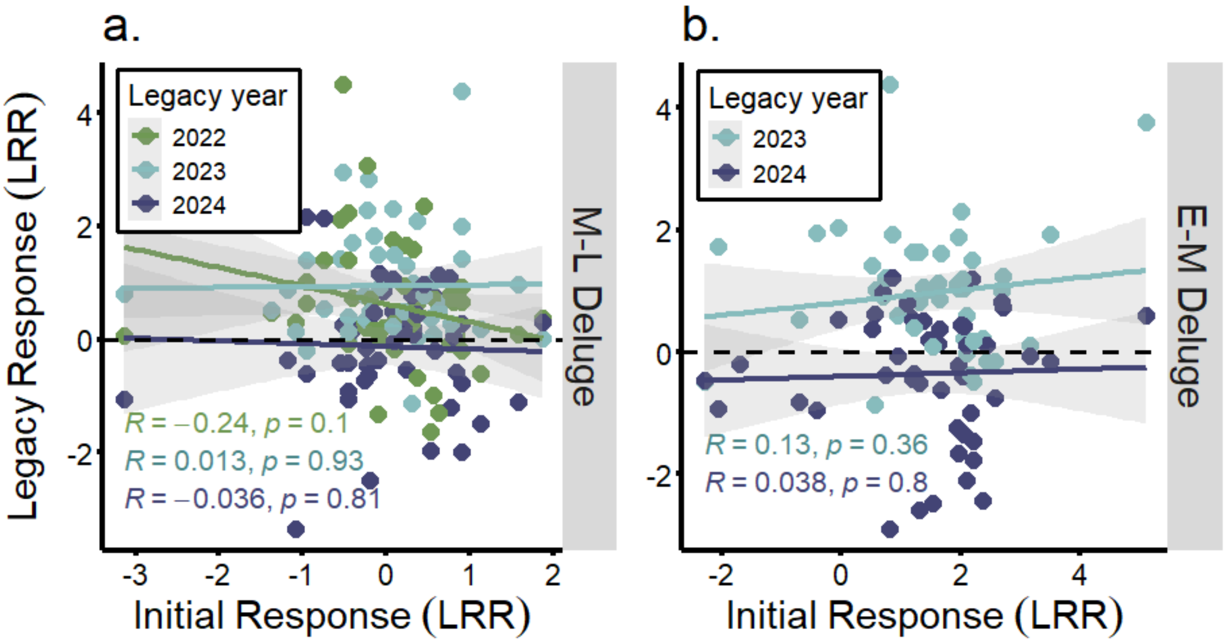

Figure 3.3. The correlation between initial ANPP response to the ANPP response in legacy years for the 2021 mid-late deluge (a) and the 2022 early-mid deluge (b). Response is caclulated as the log response ratio. In legacy years, there was no significant effect of nitrogen, so legacy data is pooled across nitrogen addition levels. Statistics in the lower left of each plot reflect the correlation coefficient and pearson's correlation p-value asssessed for each legacy year.

Deluge effects on richness were only significant in 2024: the 2021 mid-late deluge legacy increased species richness by 23.3% (1.3 species) and the 2022 early-mid deluge legacy increased richness by 10.2% (0.6 species). Nitrogen addition and deluge did not interact in any legacy year.

The response of evenness was variable across treatments and legacy years (Appendix 3 Table 3; Appendix 3 Figure 4). In 2022, the mid-late 2021 deluge legacy caused a modest, but significant 17.6% decrease in evenness. In 2023, nitrogen significantly reduced evenness, but only in the analyses that assessed core nitrogen data and the 2021 mid-late deluge legacy data. In 2024, deluge had no significant effect on evenness, but nitrogen significantly decreased evenness in both deluge treatment legacies, and significantly interacted with deluge. In the core nitrogen-only plots, evenness decreased by 36.4%, but in deluge plots, the impact of nitrogen was reduced: evenness decreased by 12.0% in the 2021 mid-late deluge plots and by 8.6% in the 2022 early-mid deluge legacy plots. This interaction was likely driven by the 0 g m^{-2} nitrogen addition treatment without deluge which had greater evenness than any other nitrogen or deluge treatments.

Community composition differences, measured as the distance between group centroids, was significantly reduced by increasing nitrogen addition for post-deluge plots in 2022 and 2023 (Appendix 3 Table 4; Appendix 3 Figure 5). This convergence in community composition during legacy years suggests that nitrogen interacts with deluge legacy to homogenize plant communities. Across all years and treatments where deluge legacy effects were significant, the smallest centroid distances (indicating greatest similarity between core nitrogen plots and deluge legacy plots) occurred under high nitrogen addition (15, 20, and 30 g m^{-2}).

Three species were identified as influential: *Elymus elymoides*, *Chenopodium sp*., and *Salsola tragus* (Figure 3.4; Appendix 3 Table 5). The effect of nitrogen and deluge legacy on these species is discussed below.

5. Discussion

In this research, we investigated the legacy effects of two deluge events across a nitrogen gradient in a semi-arid shortgrass steppe. We found that deluge legacies significantly affected ANPP, species richness, evenness, and community composition differences in one or more years following deluge events. Surprisingly, the initial production response to deluge did not predict responses during legacy years. Unexpected production legacies emerged even after communities showed no immediate response to the 2021 deluge. Deluge legacy effects on ANPP were independent of ongoing nitrogen addition, but nitrogen and deluge legacy interacted to influence

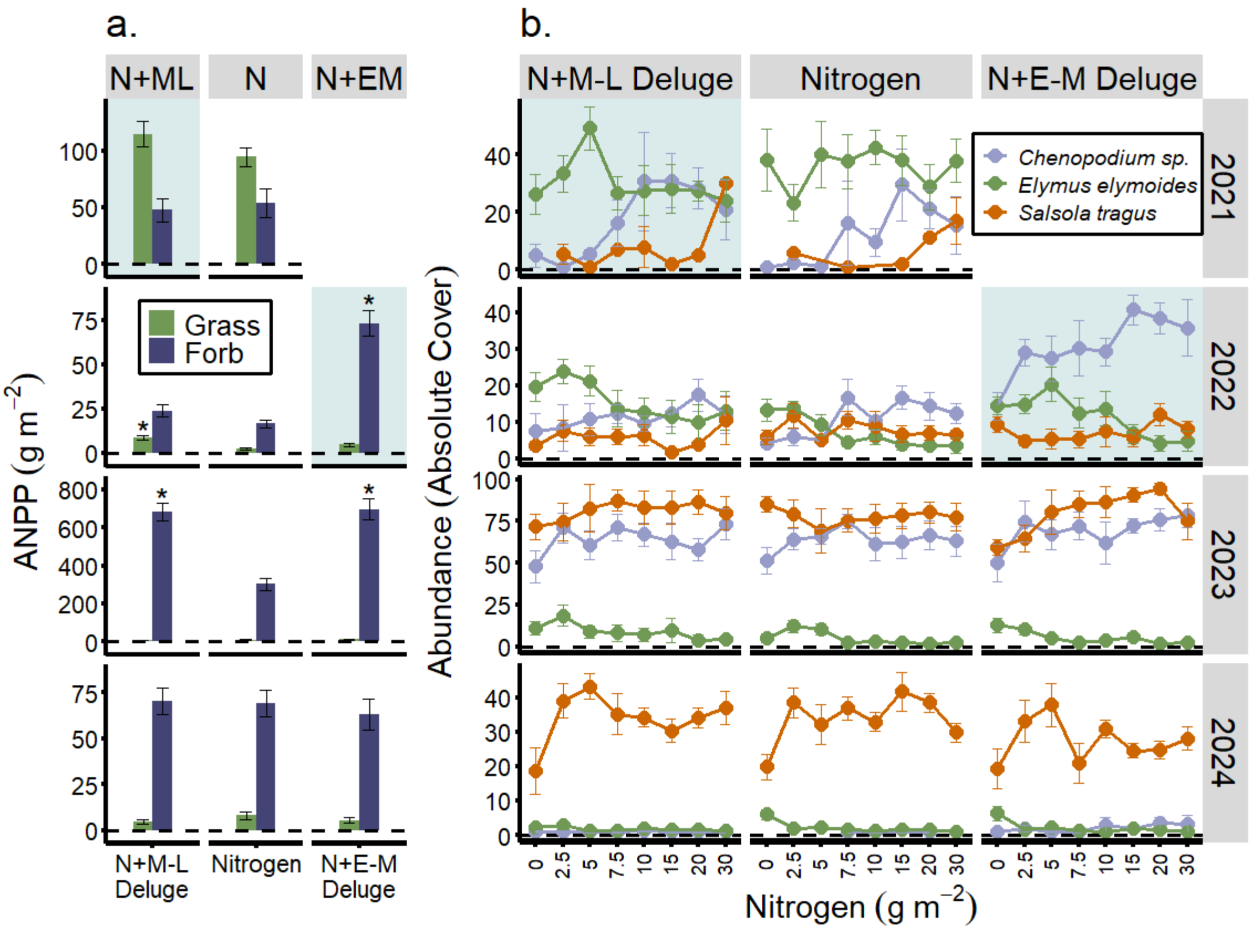

Figure 3.4. The effect of deluge on forb and grass production (a) and the effect of nitrogen and deluge on influential species abundance (b). Blue shaded plots indicate years in which the deluge treatments were applied. The "*" in graph a indicates significant production differences between the core nitrogen plots and the N+Deluge treatment. The y axis scale varies by year to account for high innerannual variabiltiy and reflect the model which assessed the within-year response. All error bars represent mean ±SE.

evenness and community composition. Production legacy effects were strongest in 2023, a year with high precipitation. In contrast, there was no production legacy during the dry 2024 season, though significant legacy effects on richness, evenness, and community composition differences persisted. These findings demonstrate that a single deluge pulse amid on-going nitrogen press may generate unexpected legacy effects that alter the structure and function of a grassland ecosystem for at least three years after deluge.

It is reasonable to assume that a large initial response to perturbation will lead to lingering legacy effects, so we predicted that the magnitude of initial response to deluge would predict the magnitude of legacy response. However, our results did not support this prediction. The initial response to deluge was uncorrelated to the legacy response for both deluge treatments and in all legacy years (Figure 3.3). The 2021 mid-late season deluge had no initial effect on productivity, richness, evenness, or community composition differences, yet significant deluge effects appeared in legacy years. This 2021 deluge led to increased ANPP in both 2022 and 2023, followed by notable changes in community structure: richness increased in 2023, a nitrogen-deluge interaction affected evenness in 2024, and community composition differences were significantly reduced in 2023 and 2024. In contrast, the 2022 early-mid deluge caused substantial changes to productivity, richness, and evenness, but these initial effects still did not predict the magnitude of legacy responses in productivity. These findings indicate that a single pulsed deluge event can lead to substantial and unexpected legacy effects, and that the initial response to deluge does not reliably predict the trajectory or magnitude of those effects.

Second, we sought to understand how nitrogen press would govern pulse legacy effects, and predicted that lingering effects from deluge would interact with ongoing nitrogen addition. However, no interactive effects of nitrogen and deluge legacy on ANPP were observed in any legacy year or with either deluge treatment, meaning that the effect of deluge legacy was consistent, regardless of the level of nitrogen addition (Figure 3.3). While production was unaffected by interaction, nitrogen addition and deluge legacy interacted to effect evenness and community composition. Deluge legacy alone did not affect evenness, but when combined with nitrogen, deluge legacy dampened the effect of nitrogen (Appendix 3 Figure 4). In the core nitrogen plots, evenness sharply declined with increasing nitrogen addition. In N+Deluge plots, this effect was muted, largely because evenness in N+deluge plots was lower across all nitrogen addition levels, so evenness in N+Deluge plots was lower and less variables. There was also significant interaction between nitrogen and deluge legacy for community composition differences (Appendix 3 Figure 5). When deluge was added, high nitrogen plots (15, 20, 30 g m^{-2}) became more similar. Combined, these data suggest that nitrogen with deluge legacy caused communities to become less diverse and more homogeneous. This is likely because nitrogen addition and deluge treatment both benefit fast-growing, acquisitive species that capitalize on resource influx, making them more dominant (Xia and Wan 2008, Blumenthal et al. 2020, Feng et al. 2023). In summary, nitrogen addition and deluge legacy do not interact to effect production, but do significantly affect evenness and community composition, which, in turn, may effect productivity in future years if community differences remain (Milchunas and Lauenroth 1995, De Boeck et al. 2018, Gong et al. 2020, Broderick et al. 2022, Reynaert et al. 2022, Meng et al. 2023, Hajek and Knapp 2024, Ratcliffe et al. 2024).

Neither the initial response to deluge nor nitrogen press predicted the production legacy response to deluge, but some potential mechanisms underlying these legacies can be considered. The mid-late 2021 deluge caused an increase in production during the first legacy year, despite 2022 being a dry, low-productivity year. This legacy effect was due to a significant increase in grass production (Figure 3.4a), accompanied by a significant increase in *Elymus elymoides* cover (Figure 3.4b). *Elymus elymoides* did not initially respond to the 2021 mid-late deluge, but this cool-season C_3 perennial may have been entering dormancy (Winslow et al. 2003, Hajek et al. 2024). Instead, access to lingering deep-soil moisture may have supported increased growth the following spring (Nippert and Holdo 2015, Ning et al. 2022). Previous work has demonstrated that deluge events recharge soil moisture which can remain elevated into the following year, especially in soil depths below 20 cm, which would not have been captured by the 20cm soil moisture probes used in this study (Knapp et al. 2008, He et al. 2012, Hajek and Knapp 2024). Supporting this idea, Hajek and Knapp (2024) applied two late-season (September and October) deluges in the same shortgrass steppe and found increased productivity the following year. This was driven primarily by an increase in a dominant perennial grass that was attributed to increases in deep soil moisture. Species responses, especially those of perennials like *Elymus elymoides* with access to elevated soil moisture, may be delayed, manifesting in subsequent years as legacy effects.

Drivers of the 2023 production legacy are more cryptic, but varied recruitment of weedy forbs is a possible mechanism. Despite the difference in seasonal and yearly timing of deluge treatments, the 2023 legacy effects were similar across both post-deluge treatments (Figure 3.4a; Appendix 3 Table 2). In deluge legacy plots, production more than doubled, primarily driven by increased forb biomass, primarily the weedy *Chenopodium sp.* and the invasive *Salsola tragus*. However, the cover of these species was unaffected by nitrogen addition or deluge treatment and consistent across plots (Appendix 3 Table 5), suggesting that differences in biomass were due to increases in the mass of individual species that were not reflected in cover. This may demonstrate differences in weedy species recruitment. The year prior, core nitrogen plots had significantly less plant cover than both deluge treatment plots ($p < 0.001$). Greater light availability may have increased germination of weedy forb seeds within core nitrogen plots. Counterintuitively, greater recruitment of weedy species can lead to less production due to greater competition (Zeiter et al. 2006). Therefore, the deluge treatment plots with more plant cover had lower germination of weedy forbs, leading to less competition among individuals and greater production. This is a possible, but under-studied mechanism of production legacies, so future studies should investigate the link between light availability, weedy species recruitment, and the ultimate impacts on production.

Another possible explanation of the 2023 deluge production legacy is the utilization of increased soil nitrogen following mobilization by deluge (Linabury and Smith *submitted*) (Chapter 2); however, several lines of evidence challenge this explanation. If elevated nitrogen availability increased production in 2023 N+Deluge legacy plots, a similar increase in production would be expected in the fertilized core nitrogen plots. Yet, nitrogen addition had no effect on production in 2023. It is possible that core nitrogen plots had greater levels of ammonium from urea fertilization (Perin et al. 2020), while previously-deluged plots retained nitrate that was mobilized during the year of deluge (Linabury and Smith *submitted*). However, nitrogen data was only collected during years in which deluge was applied and not monitored in legacy years. Research at other dry grassland sites has shown low soil nitrogen retention, with elevated nitrogen returning to ambient levels with a season to a few years after treatment has ended (Meng et al. 2023, Hajek and Knapp 2024). Last, we found no significant correlation between the initial nitrogen response to deluge and production response in legacy years for nitrate, ammonium, or total N (Appendix 3 Figure 6). The utilization of elevated nitrogen in years following deluge is a possible explanation of production legacy effects in sites with high soil nitrogen retention, but it is unlikely to be a mechanism of legacy within our experiment.

Mechanisms of deluge production legacy may vary, but the magnitude of deluge legacy (i.e., the increase of ANPP from core nitrogen plots to corresponding N+Deluge plots) was strongly correlated to growing season precipitation (Appendix 3 Figure 2). These findings align with those from Meng et al. (Meng et al. 2023) who found production legacies were strongly influenced by interannual precipitation after cessation of a 13-year period of nitrogen and water addition within a dry grassland ecosystem. This indicates that dry years may mask legacy effects, while wet years cause these effects to reemerge. These results show that the magnitude of the deluge legacy is at least partially explained by variation in growing season precipitation.

Our findings underscore the complex and unpredictable nature of pulsed deluge legacies in a semi-arid grassland undergoing long-term nitrogen press. Deluge legacy effects on productivity were not predicted by the initial response to deluge, nor were they consistently governed by nitrogen press. Instead, legacies were shaped by dominant species response, initial deluge timing, and interannual precipitation variability. Even during years when production legacies were absent, legacy effects on evenness and community composition differences remained. While this research only monitored legacies for two to three years after deluge, legacy effects may persists, especially because legacies are often generated by long-lasting differences in community composition (Milchunas and Lauenroth 1995, De Boeck et al. 2018, Broderick et al. 2022, Meng et al. 2023). Future research should explicitly test possible mechanisms of deluge legacy, including: nitrogen retention, soil microbe influence, plant litter build-up, and seed bank utilization. Additionally, the unexpected production legacies observed here highlight the importance of incorporating deluge events into models predicting ecosystem productivity based on interannual precipitation (Sala et al. 1988, Knapp and Smith 2001, Knapp et al. 2017). As extreme weather events become more frequent and interannual variability intensifies, understanding the delayed and interacting effects of resource pulses under continued press will be essential for predicting the trajectory of grassland ecosystems under global change.

CONCLUSIONS

During this dissertation, I described the effect of nitrogen press on grassland communities, investigated the interaction of nitrogen press and dulse pulse timing, then assessed the legacy effects of pulse amid ongoing nitrogen press. Several findings stand out as key contributions to our understanding of pulse–press dynamics. First, I demonstrated that five years of nitrogen press altered community composition and production, but that these responses decoupled between a semi-arid shortgrass steppe and a mesic tallgrass prairie. Next, I found that timing mediates the synergistic interaction between deluge pulse and nitrogen press, likely facilitated by plant phenology, interannual precipitation, and invasion. Last, I found that the initial response to deluge pulse does not predict legacy effects in subsequent years, and that effects of deluge may be delayed, manifesting as legacies.

I am honored to add these conclusions to our collective scientific knowledge, but pulse-press dynamics are a timely research focus that require continued investigation. The mechanisms of deluge legacy remain partially unresolved and given their impact on ecosystem structure and function, future work should explicitly investigate the drivers of these delayed effects. Possible avenues for investigation include understanding patterns of soil nitrogen mobilization and retention after deluge, possible shifts in soil microbial composition and associated traits, and differences in seed production or seed bank utilization that would influence recruitment. Additionally, I remain loyal to the pulse-press framework as a method to understand shared ecological responses to unique patterns and combinations of perturbation. However, pulse-press studies require a platform of experimental long-term press, so I will seek collaboration with established projects within the LTER network or pursue opportunities with networks of coordinated experiments. Through these efforts I hope to explore new combinations of pulse-press perturbations and to understand the role of aridity, interannual variability, and species traits in mediating interaction.

In conclusion, this work underscores the nuanced and often unexpected ways that pulse and press disturbances interact across space and time. As climate extremes intensify, understanding the timing, intensity, and legacy of ecological responses is essential. By deepening our grasp of pulse–press dynamics, we improve our ability to forecast ecosystem change and to steward resilience in an increasingly variable world.

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APPENDIX 1

Appendix 1 Table 1. ANOVA results of the linear mixed-model assessing the effect of nitrogen treatment over time (year) on aboveground net primary production (ANPP) of grass and forb functional groups. Significant differences ($p \leq 0.05$) are bolded. Block E in 2014 is excluded from shortgrass data due to an influential outlier. A square root transformation was applied on forb data to meet assumptions of normality.

		Shortgrass			**Tallgrass**		
		d.f.	F-value	p-value	d.f.	F-value	p-value
Forb sqrt()	Nitrogen	7,187	1.64	0.127	7,195	1.74	0.101
	Year	4,187	18.91	**<.001**	4,195	3.89	**0.005**
	Nitrogen : Year	28,187	0.85	0.679	28,195	0.98	0.502
Grass	Nitrogen	7,187	1.21	0.299	7,195	1.71	0.108
	Year	4,187	52.03	**<.001**	4,195	90.86	**<.001**
	Nitrogen : Year	28,187	0.95	0.542	28,195	1.26	0.182

Appendix 1 Table 2. Results of perMANOVA analysis on the effect of nitrogen treatment on community composition using absolute species abundance (as a Bray Curtis dissimilarity matrix). Significant differences (p ≤ 0.05) are bolded. No significant differences existed in the 2013 pre-treatment year for both sites.

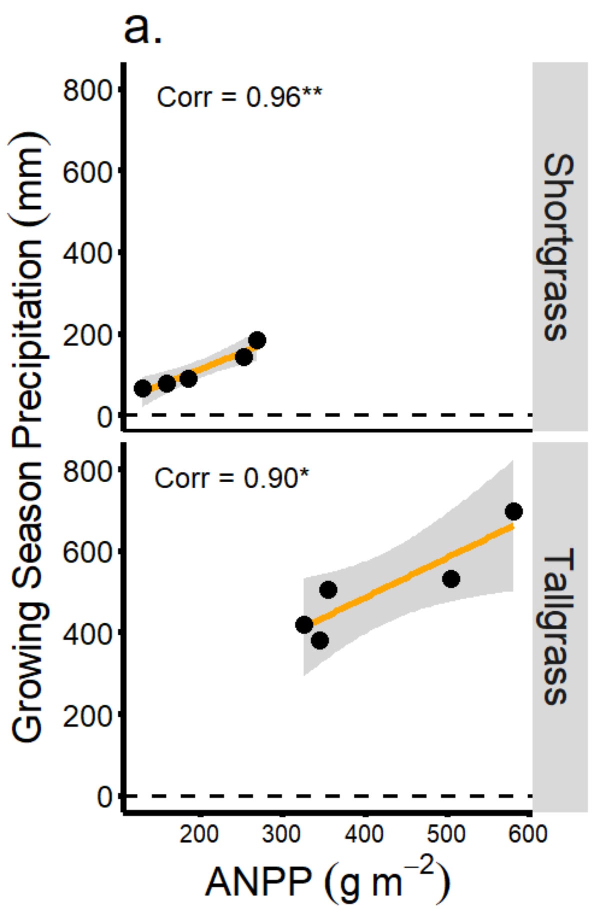
	Shortgrass				**Tallgrass**			
	d.f.	SS	Pseudo-F	p-value	d.f.	SS	Pseudo-F	p-value
2014	7	0.788	0.823	0.490	7	0.486	0.799	0.733
2015	7	1.370	1.564	**0.003**	7	0.471	0.755	0.777
2016	7	1.492	2.057	**0.003**	7	0.803	0.971	0.416
2017	7	1.776	1.474	**0.006**	7	0.700	0.843	0.705
2018	7	1.063	1.459	**0.008**	7	0.775	0.819	0.499

Appendix 1 Table 3. Pairwise comparisons of the perMANOVA analysis on the effect of nitrogen treatment on community composition using absolute species abundance (as a Bray Curtis dissimilarity matrix) at the shortgrass site. This analysis was only performed on shortgrass site, since tallgrass composition did not significantly respond to nitrogen during any year of the study. Significant differences ($p \leq 0.05$) are bolded, marginally significant differences ($0.1 \leq p > 0.05$) are in black text. No significant differences were found during the 2013 pre-treatment year at either site.

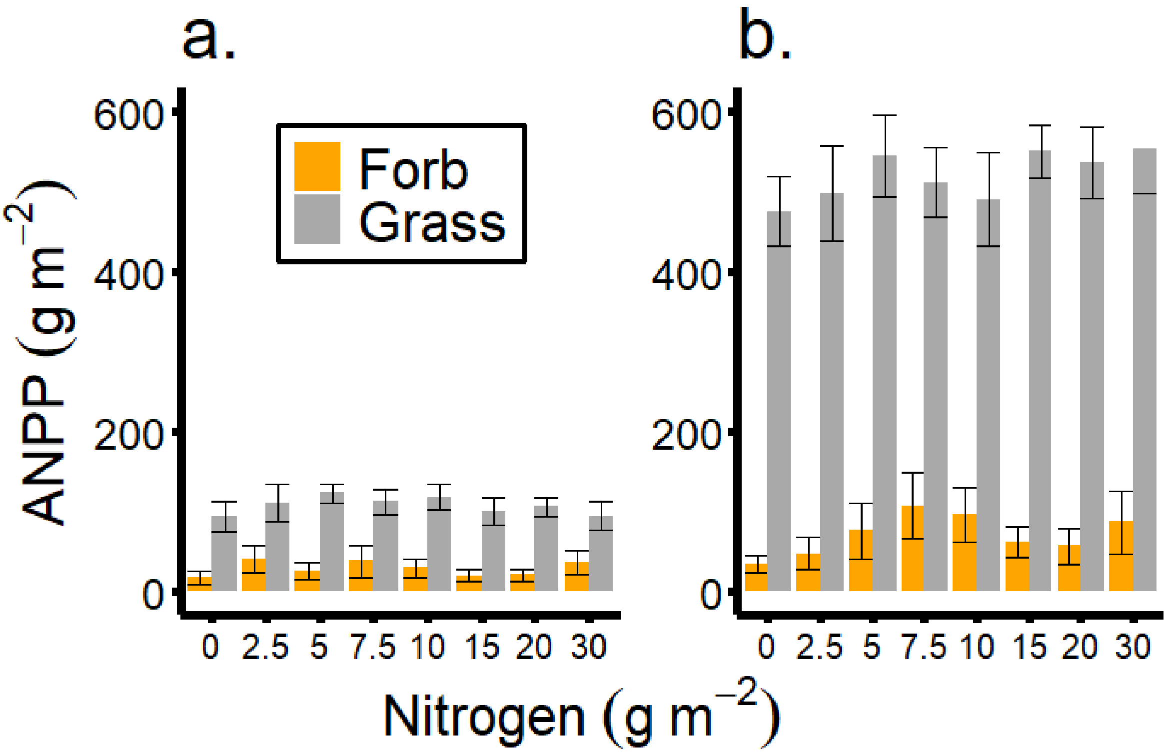
	Shortgrass							
2014	0.0	2.5	5.0	7.5	10.0	15.0	20.0	30.0
0.0								
2.5	n.s.							
5.0	n.s.	n.s.						
7.5	n.s.	n.s.	n.s.					
10.0	n.s.	n.s.	0.058	n.s.				
15.0	n.s.	n.s.	n.s.	n.s.	n.s.			
20.0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
30.0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
2015	0.0	2.5	5.0	7.5	10.0	15.0	20.0	30.0
0.0								
2.5	n.s.							
5.0	n.s.	n.s.						
7.5	n.s.	n.s.	n.s.					
10.0	n.s.	**0.037**	n.s.	n.s.				
15.0	n.s.	**0.040**	n.s.	n.s.	n.s.			
20.0	**0.014**	**0.005**	**0.023**	**0.025**	**0.033**	n.s.		
30.0	n.s.	0.070	n.s.	n.s.	n.s.	n.s.	n.s.	
2016	0.0	2.5	5.0	7.5	10.0	15.0	20.0	30.0
0.0								
2.5	n.s.							
5.0	n.s.	n.s.						
7.5	**0.039**	0.078	0.090					
10.0	**0.037**	0.052	0.086	n.s.				
15.0	0.051	0.065	0.086	n.s.	n.s.			
20.0	**0.024**	**0.017**	**0.042**	n.s.	n.s.	n.s.		
30.0	**0.012**	**0.008**	**0.009**	n.s.	n.s.	n.s.	n.s.	
2017	0.0	2.5	5.0	7.5	10.0	15.0	20.0	30.0
0.0								
2.5	n.s.							
5.0	n.s.	n.s.						
7.5	**0.012**	n.s.	n.s.					
10.0	**0.008**	n.s.	n.s.	n.s.				
15.0	**0.001**	n.s.	n.s.	n.s.	n.s.			
20.0	**0.004**	n.s.	n.s.	n.s.	n.s.	n.s.		
30.0	**0.003**	**0.036**	0.066	n.s.	n.s.	n.s.	n.s.	
2018	0.0	2.5	5.0	7.5	10.0	15.0	20.0	30.0
0.0								
2.5	n.s.							
5.0	**0.020**	n.s.						
7.5	n.s.	n.s.	n.s.					
10.0	**0.007**	n.s.	n.s.	n.s.				
15.0	**0.011**	n.s.	n.s.	n.s.	n.s.			
20.0	**0.002**	n.s.	n.s.	n.s.	n.s.	n.s.		
30.0	**0.016**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

Appendix 1 Table 4. ANOVA results of linear mixed-model assessing the effect of nitrogen treatment and time (year) on abundance differences between control and nitrogen treatments of influential species. Species were identified as "influential" through simper analysis. This analysis was only performed on influential shortgrass species, since tallgrass composition did not significantly respond to nitrogen. Significant differences ($p \leq 0.05$) are bolded.

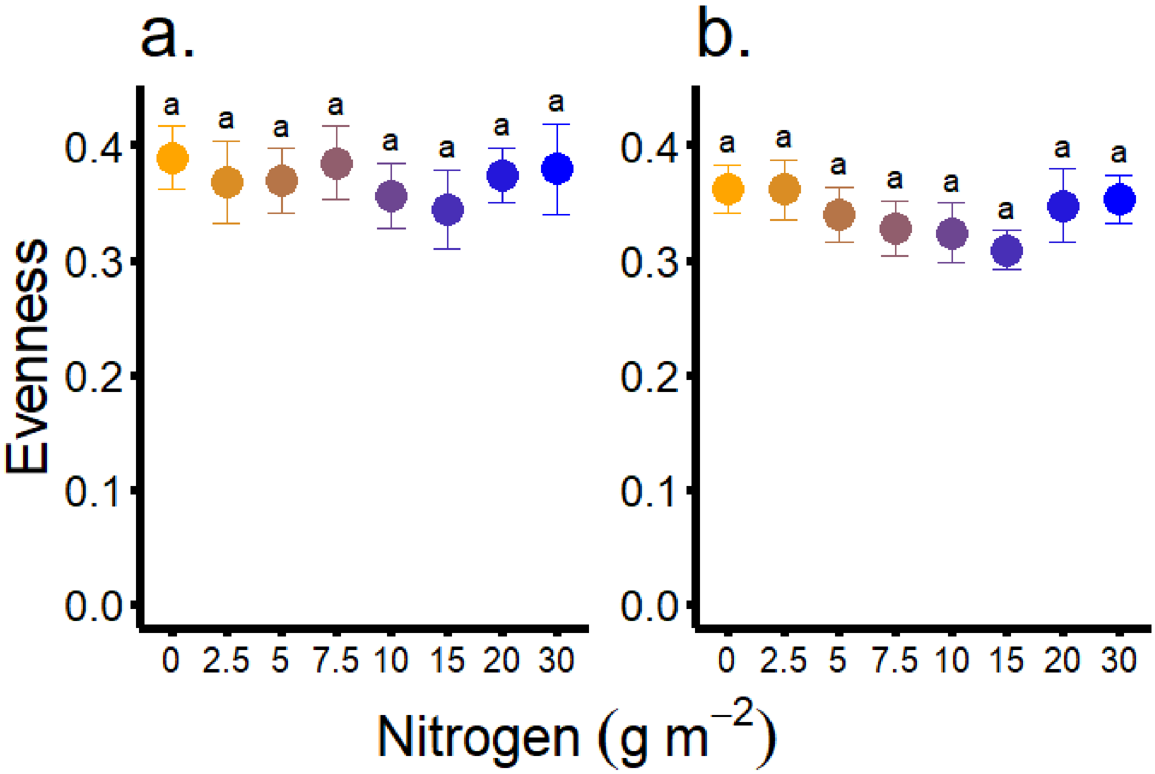
		Shortgrass		
		d.f.	F-value	p-value
Elymus elymoides	N-N2	6,170	3.24	**0.005**
	Year	4,170	12.07	**<.001**
	N-N2 : Year	24,170	1.02	0.438
Vulpia octoflora	N-N2	6,146	2.88	**0.011**
	Year	4,146	11.32	**<.001**
	N-N2 : Year	24,146	0.75	0.792
Bouteloua gracilis	N-N2	6,170	3.41	**0.003**
	Year	4,170	22.84	**<.001**
	N-N2 : Year	24,170	0.92	0.580



Appendix 1 Figure 1. Correlation of ANPP with growing season precipitation (May to September) by site. The correlation is indicated in the upper left corner of the plot. Pearson's correlation tests were used to test significance which is indicated by a "*."



Appendix 1 Figure 2. Effect of nitrogen treatment on functional group ANPP at shortgrass (a) and tallgrass (b) sites across treatment years. Nitrogen did not affect functional group ANPP during experimental years. All error bars represent mean ±SE. Block E is excluded from shortgrass ANPP data due to strong influence of an outlier.



Appendix 1 Figure 3. The effect of nitrogen treatment on evenness at shortgrass (a) and tallgrass (b) sites across treatment years. All error bars represent mean ±SE.

APPENDIX 2

Appendix 2 Table 1. Dates of application for the 2021 late-mid season deluge and the 2022 early-mid deluge events. Deluge application was split between two consecutive days and varied by block.

Year	Block	Deluge Date 1 (day/month/year)	Deluge Date 2 (day/month/year)
2021	A	7/27/2021	7/28/2021
2021	B	7/27/2021	7/28/2021
2021	C	8/4/2021	8/5/2021
2021	D	8/3/2021	8/4/2021
2021	E	8/6/2021	8/7/2021
2021	F	8/6/2021	8/7/2021
2022	A	6/23/2022	6/24/2022
2022	B	6/23/2022	6/24/2022
2022	C	6/25/2022	6/26/2022
2022	D	6/25/2022	6/26/2022
2022	E	6/27/2022	6/28/2022
2022	F	6/27/2022	6/28/2022

Appendix 2 Table 2. Precipitation (mm) during 2021 and 2022 for ambient conditions and N+Deluge subplots (ambient + 42 mm deluge). Rainfall data for the study period were obtained from a USDA-ARS rain gauge (referred to as USDA_nunn_scan_2017) located in a pasture adjacent to the study site.

		Precipitation (mm)			
		Growing season	Water year	Jan1-Aug31	Calendar year
2021	Ambient	134.1	278.9	241.6	311.9
	Ambient+Deluge	176.1	320.9	283.6	353.9
2022	Ambient	128.8	252.5	169.2	210.3
	Ambient+Deluge	170.8	294.5	211.2	252.3

Appendix 2 Table 3. ANOVA results of the linear mixed-model assessing the effect of nitrogen addition and deluge treatment on availibility of nitrate and total nitrogen during 2021 and 2022. Total nitrogen is calculated as nitrate plus ammonium (mg l^{-1}). Significant differences ($p \leq 0.05$) are bolded.

			Nitrogen (mg l^{-1})		
Year	Form		d.f.	F-value	p-value
2021	Nitrate	Nitrogen	7,31.31	7.25	**<0.001**
		N+Deluge	1,36	150.90	**<0.001**
		Nitrogen : N+Deluge	7,36	3.97	**0.003**
	Total Nitrogen	Nitrogen	7,31.31	15.37	**<0.001**
		N+Deluge	1,36	120.30	**<0.001**
		Nitrogen : N+Deluge	7,36	5.44	**<0.001**
2022	Nitrate	Nitrogen	7,35	8.16	**<0.001**
		N+Deluge	1,40	163.82	**<0.001**
		Nitrogen : N+Deluge	7,40	4.48	**0.001**
	Total Nitrogen	Nitrogen	7,35	24.14	**<0.001**
		N+Deluge	1,40	6.80	**0.013**
		Nitrogen : N+Deluge	7,40	2.74	**0.020**

Appendix 2 Table 4. ANOVA results of the linear mixed-model assessing the effect of nitrogen addition and deluge treatment in 2021 and 2022 on overall plant production (ANPP g m^{-2}), grass production, forb production, species richnes, and species evenness. Significant differences ($p \leq 0.05$) are bolded.

		2021			**2022**		
		d.f.	F-value	p-value	d.f.	F-value	p-value
Production	Nitrogen	7,35	3.66	**0.005**	7,35	2.18	0.061
	Deluge	1,40	0.91	0.345	1,40	87.62	**<.0001**
	Nitrogen : Deluge	7,40	0.93	0.494	7,40	2.37	**0.040**
Production Grass	Nitrogen	7,35	2.77	**0.021**	7,35	1.75	0.129
	Deluge	1,40	2.24	0.142	1,40	3.55	0.067
	Nitrogen : Deluge	7,40	0.20	0.983	7,40	0.35	0.926
Production Forb	Nitrogen	7,35	4.17	**0.002**	7,35	2.40	**0.041**
	Deluge	1,40	0.19	0.663	1,40	78.92	**<.0001**
	Nitrogen : Deluge	7,40	0.52	0.813	7,40	2.33	**0.043**
Richness	Nitrogen	7,35	3.25	**0.009**	7,35	7.52	**<.0001**
	Deluge	1,40	3.00	0.091	1,40	4.26	**0.045**
	Nitrogen : Deluge	7,40	0.33	0.934	7,40	1.58	0.171
Evenness	Nitrogen	7,35	0.59	0.7595	7,35	0.51	0.8225
	Deluge	1,40	1.07	0.3075	1,40	46.77	**<.0001**
	Nitrogen : Deluge	7,40	0.94	0.4839	7,40	1.17	0.3403

Appendix 2 Table 5. ANOVA results of the linear mixed-model assessing the effect of nitrogen and deluge on greenness (GCC) across the growing season after deluge in 2021 and 2022. This model included “day of year” as a main effect and a compound symmetry correlation structure to account for repeated measures across the growing season. Significant differences ($p \leq 0.05$) are bolded.

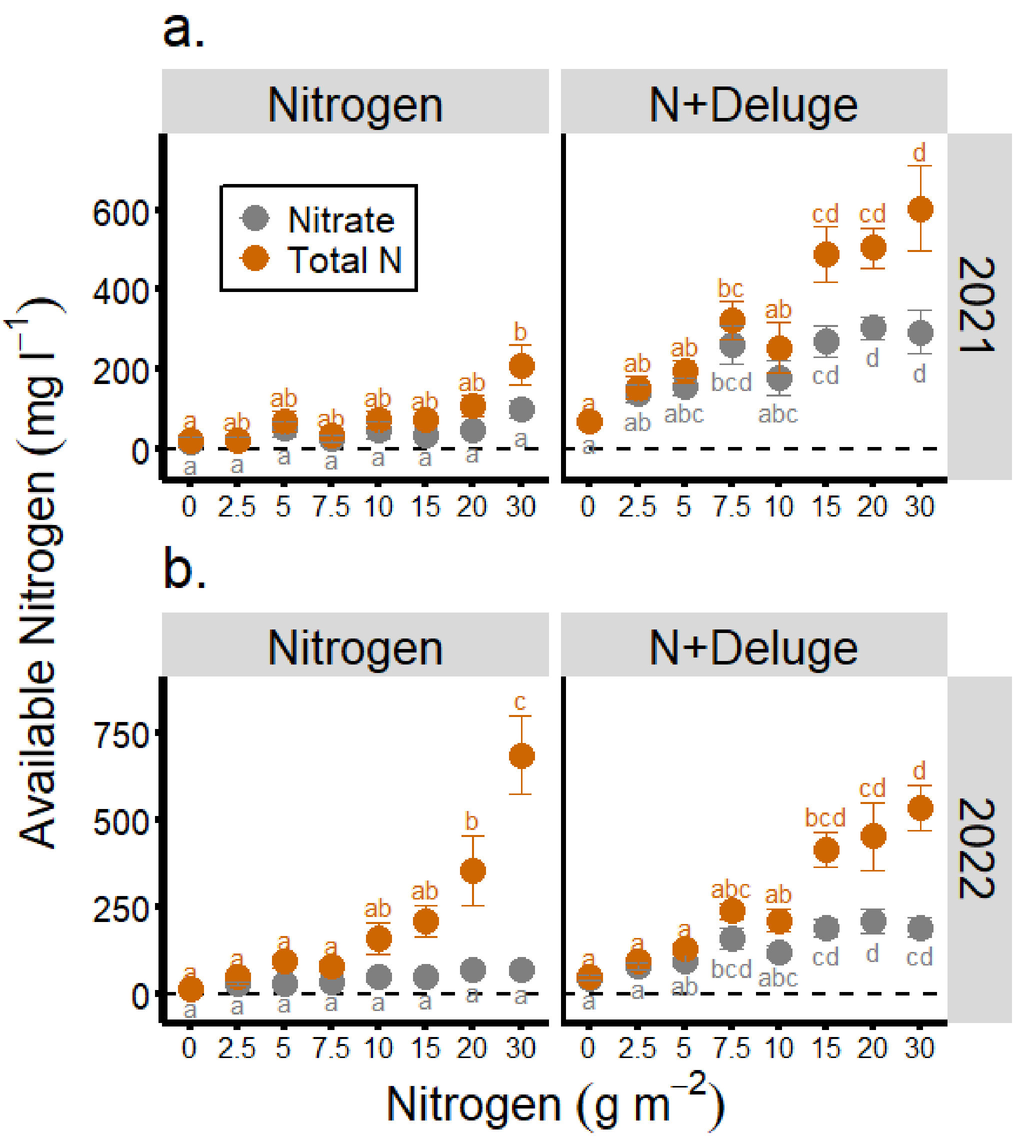
		GCC — Greenness		
		d.f.	F-value	p-value
2021	Nitrogen	7,27	1.56	0.190
	Deluge	1,243	21.06	**<.001**
	Day of Year	6,243	22.75	**<.001**
	Nitrogen : N+Deluge	7,243	1.72	0.105
	Nitrogen : Day of Year	42,243	0.89	0.664
	Deluge : Day of Year	6,243	0.81	0.563
	Nitrogen : N+Deluge : Day of Year	42,243	0.48	0.997
2022	Nitrogen	7,35	1.00	0.429
	Deluge	1,1057	319.00	**<.001**
	Day of Year	17,1058	35.00	**<.001**
	Nitrogen : N+Deluge	7,1059	5.00	**<.001**
	Nitrogen : Day of Year	119,1060	1.00	0.999
	Deluge : Day of Year	17,1061	36.00	**<.0001**
	Nitrogen : N+Deluge : Day of Year	119,1062	1.00	0.604

Appendix 2 Table 6. Model selection table with all combinations of fixed effects in an additive linear model. Predictors included: the proportion of forb to grass biomass, GCC mean, total N, and soil moisture as VWC. Models are listed in order from lowest to highest AIC. The best model with the lowest AIC score (1) only included forb proportion and explained 28% of the variation in productivity data.

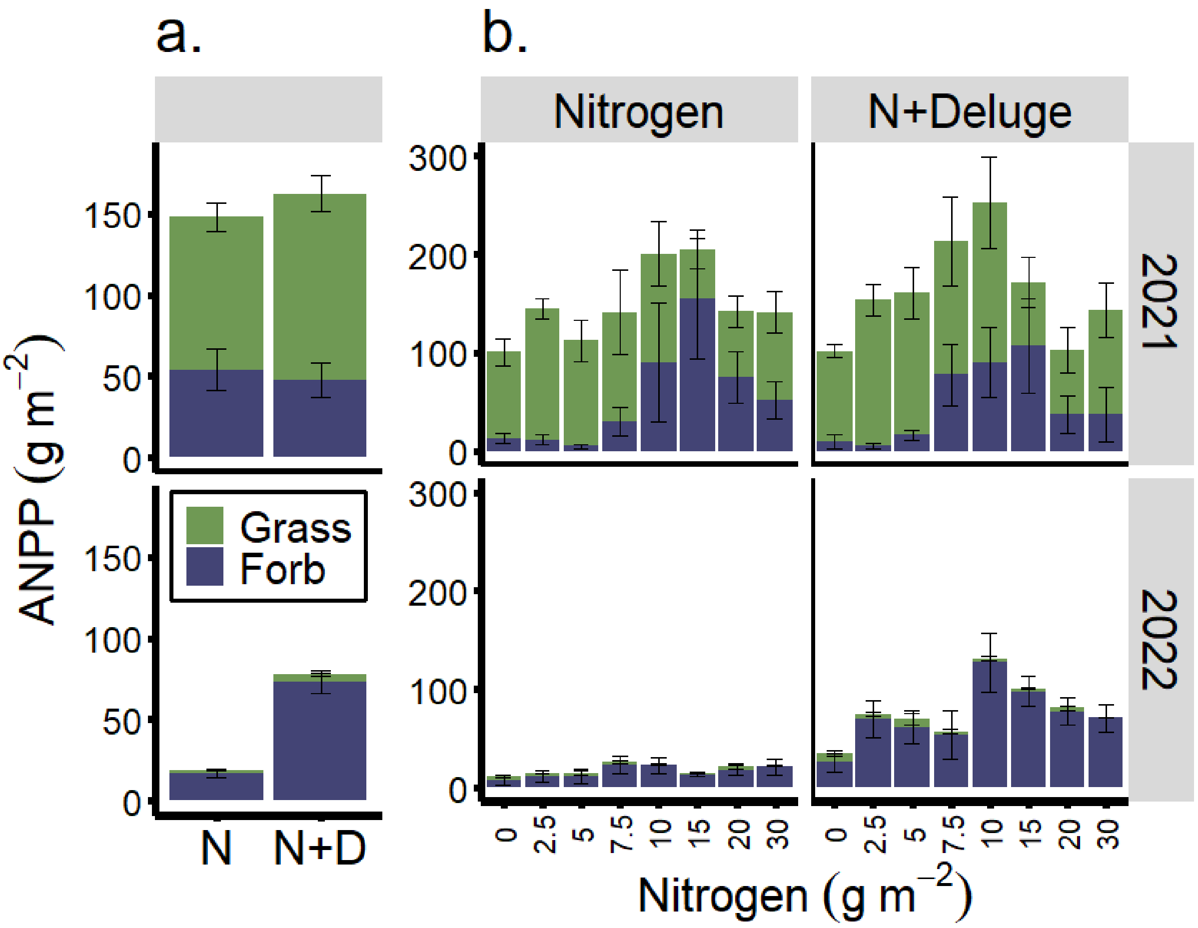
		Predictors					
Model	Intercept	Forb.Prop	GCC	Total N	VWC	df	**AIC**
1	-6.11	97.90				3	500.3
2	30.59	94.68			-4.41	4	500.8
3	-7.40	93.38		0.02		4	501.9
4	-50.37	97.66	131.30			4	502.3
5	755.70	97.42	-2115.00		-5.82	5	502.3
6	28.17	91.00		0.02	-4.25	5	502.5
7	-139.60	92.53	391.80	0.02		5	503.9
8	658.10	94.27	-1836.00	0.01	-5.51	6	504.2
9	62.63			0.06		3	513
10	105.80			0.05	-5.42	4	513.3
11	124.90				-6.13	3	513.6
12	77.65					2	513.7
13	-938.50		2953.00	0.06		4	514.1
14	791.00		2564.00			3	515
15	283.00		1128.00	0.05	-4.62	5	515.2
16	35.61		259.50		-5.95	4	515.6

Appendix 2 Table 7. ANOVA results of the linear mixed-model assessing the effect of nitrogen and deluge on the cover of the three most influential species within each year of the experiment. Species are listed in order of average contribution to dissimilarity between treatments, listed from most to least. Significant differences (p ≤ 0.05) are bolded.

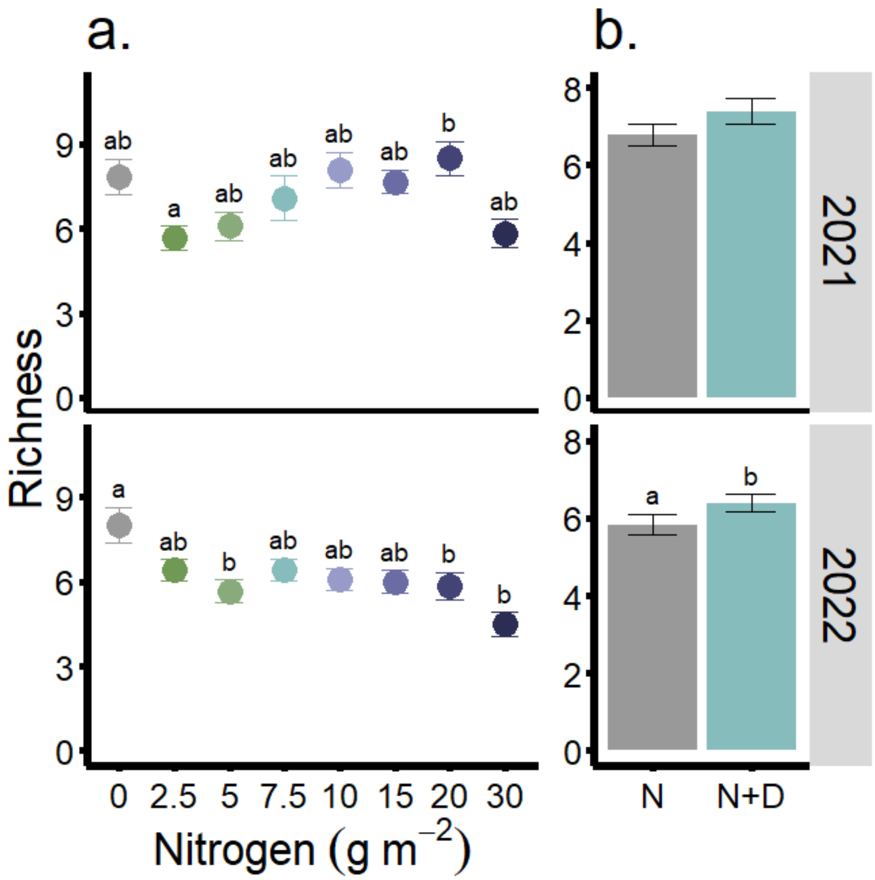
			Species Cover		
Year	Species		d.f.	F-value	p-value
2021	*1.Vulpia octoflora*	Nitrogen	1,43.02	36.10	**<0.001**
		N+Deluge	1,36.91	0.01	0.909
		Nitrogen : N+Deluge	1,45.00	0.24	0.624
	2.Elymus elymoides	Nitrogen	1,41	0.52	0.477
		N+Deluge	1,46	0.06	0.815
		Nitrogen : N+Deluge	1,46	0.83	0.368
	3.Bromus tectorum	Nitrogen	1,26.32	4.62	**0.041**
		N+Deluge	1,23.73	0.40	0.531
		Nitrogen : N+Deluge	1,26.76	3.08	0.091
2022	*1.Chenopodium sp.*	Nitrogen	1,41.010	10.20	**0.003**
		N+Deluge	1,46.265	24.76	**<0.001**
		Nitrogen : N+Deluge	1,44.550	2.14	0.150
	2.Elymus elymoides	Nitrogen	1,42.32	30.42	**<0.001**
		N+Deluge	1,41.39	8.76	**0.005**
		Nitrogen : N+Deluge	1,44.01	0.28	0.600
	3.Salsola tragus	Nitrogen	1,33.265	0.04	0.851
		N+Deluge	1,40.530	1.92	0.173
		Nitrogen : N+Deluge	1,37.217	1.87	0.179



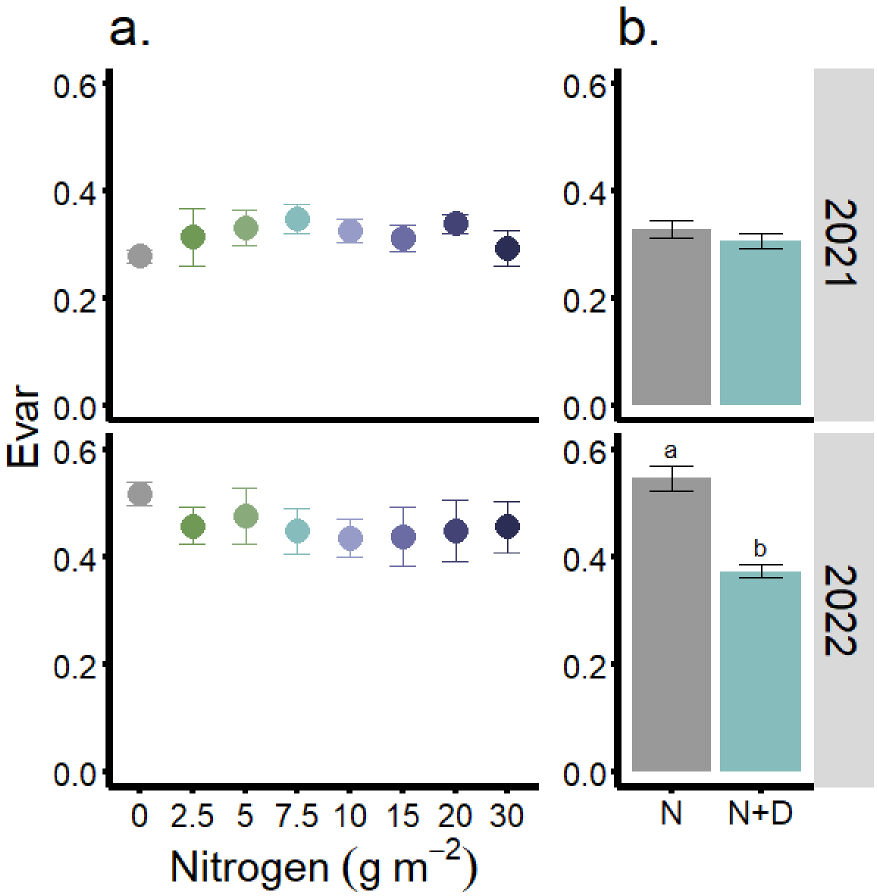
Appendix 2 Figure 1. Nitrate and total nitrogen (nitrate + ammonium, mg l^{-1}) across nitrogen addition rates and deluge treatment between 2021 and 2022. Methodology used to assess nitrogen availibility produces relative values that are only comparable between plots within the same year, so plot a and b should be assessed separately. All error bars represent mean ±SE. Letters indicate significant differences ($p \leq 0.05$) between pairwise comparisons of nitrogen treatments.



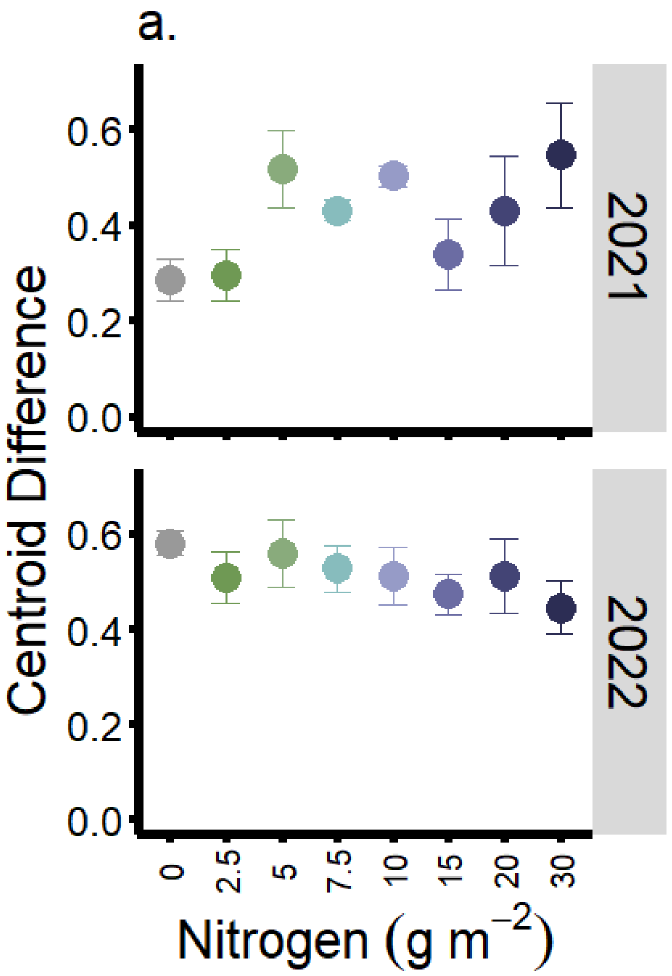
Appendix 2 Figure 2. Functional group contribution (forb and grass) to ANPP g m^{-2} by deluge treatment (a) nitrogen addition (b). In 2021, grass and forb biomass were significantly affected by nitrogen addition, but not deluge. In 2022, grass biomass was not significantly affected by nitrogen or deluge, but forb biomass significantly increased in response to nitrogen, deluge, and their interaction. All error bars represent mean ±SE.



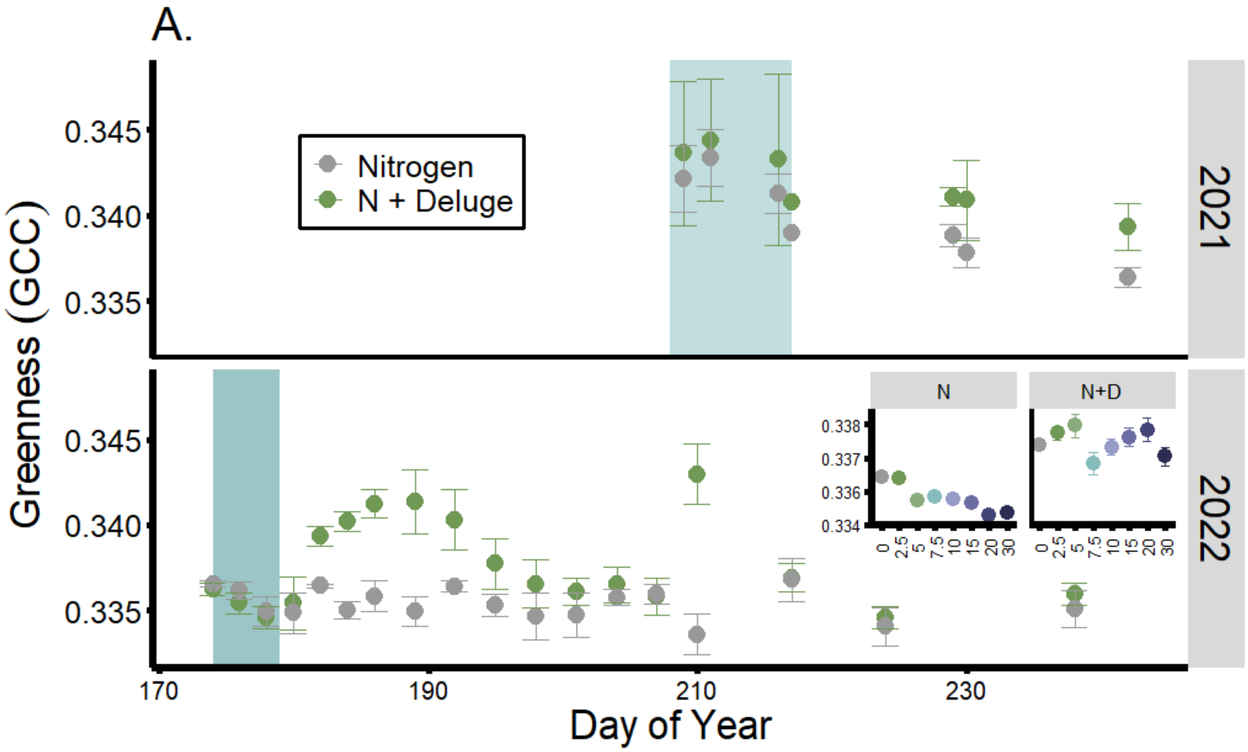
Appendix 2 Figure 3. Species richness in 2021 and 2022 by nitrogen addition and deluge treatment. In both 2021 and 2022 there is a significant affect of nitrogen, but deluge significantly increased richness only in 2022. All error bars represent mean ±SE.



Appendix 2 Figure 4. Evenness in 2021 and 2022 by nitrogen addition and deluge treatment. Evenness was not effected by nitrogen addition, but was significantly decreased by deluge treatment in 2022. All error bars represent mean ±SE.



Appendix 2 Figure 5. Plant community composition differences calculated as difference between centroids of the nitrogen-only plots and the corresponding N+Deluge plots. Nitrogen did not affect how community composition responds to deluge in 2021 (*p* = 0.086) or 2022 (*p* = 0.755). All error bars represent mean ±SE.



Appendix 2 Figure 6. The effect of deluge on canopy greenness (GCC) in 2021 and 2022. Nitrogen did not significantly affect greenness, so data is averaged across nitrogen addition rates. Blue shaded areas represent the period when the deluges were applied. Error bars represent mean ±SD. The inset plot demonstrates a significant nitrogen and deluge interaction in 2022.

APPENDIX 3

Appendix 3 Table 1. Summary of environmental variables during legacy years. Growing season precipitation is from April 1 to August 31 and water year precipitation is from October 1 of the previous year to September 30 of the current year. Control ANPP is averaged across the 0 g m^{-2} nitrogen, non-deluge control plots. Dominant species are listed from most abundant based on relative species cover.

	Growing Season Precipitation (mm)	Water Year Precipitation (mm)	Control ANPP (g m^{-2})	Dominant species
2022	156.464	252.476	11.8	1. *Elymus Elymoides* 2. *Salsola tragus* 3. *Carex eleocharis*
2023	266.954	322.58	336.0	1. *Salsola tragus* 2. *Chenopodium sp.* 3. *Sphaeralcea coccinea*
2024	158.496	228.092	33.3	1. *Salsola tragus* 2. *Bouteloua gracilis* 3. *Vulpia octoflora*

Appendix 3 Table 2. ANOVA results of the linear regression mixed-model assessing the effect of nitrogen and deluge on total ANPP, grass ANPP, and forb ANPP (g m^{-2}). Results from years when deluge treatments were applied are shaded. Analyses were conducted within each year to accommodate high interannual variability, and legacy data from the 2021 deluge and the 2022 deluge were independently assessed with core nitrogen data due to deluges being applied in different years. Data that required square root transformation to meet assumptions of normality and homogeneity are indicated under the year in which transformation was needed. Significant differences ($p \leq 0.05$) are bolded.

			Nitrogen and 2021 M-L Deluge			Nitrogen and 2022 E-M Deluge		
			d.f.	F-value	p-value	d.f.	F-value	p-value
ANPP	2021	Nitrogen	7,35	3.663	**0.005**			
		Deluge	1,40	0.913	0.345			
		Nitrogen : Deluge	7,40	0.931	0.494			
	2022	Nitrogen	7,35	0.571	0.775	7,35	2.178	0.061
		Deluge	1,40	12.994	**0.001**	1,40	87.624	**<0.001**
		Nitrogen : Deluge	7,40	0.965	0.470	7,40	2.372	**0.040**
	2023	Nitrogen	7,35	0.469	0.850	7,35	0.800	0.593
		Deluge	1,40	46.961	**<0.001**	1,40	42.448	**<0.001**
		Nitrogen : Deluge	7,40	0.622	0.735	7,40	0.795	0.596
	2024	Nitrogen	7,35	0.791	0.600	7,35	1.144	0.360
		Deluge	1,40	0.102	0.751	1,40	0.873	0.356
		Nitrogen : Deluge	7,40	1.141	0.358	7,40	0.298	0.951
Forb	2021 sqrt()	Nitrogen	7,35	3.540	**0.006**			
		Deluge	1,40	0.062	0.805			
		Nitrogen : Deluge	7,40	0.832	0.568			
	2022 sqrt()	Nitrogen	7,35	1.563	0.179	7,35	2.607	**0.028**
		Deluge	1,40	5.615	0.114	1,40	84.725	**<0.001**
		Nitrogen : Deluge	7,40	0.882	0.529	7,40	1.902	0.095
	2023	Nitrogen	7,35	0.557	0.785	7,35	0.904	0.515
		Deluge	1,40	46.639	**<0.001**	1,40	41.534	**<0.001**
		Nitrogen : Deluge	7,40	0.594	0.757	7,40	0.816	0.580
	2024	Nitrogen	7,35	1.697	0.143	7,35	1.454	0.217
		Deluge	1,40	0.005	0.943	1,40	0.370	0.547
		Nitrogen : Deluge	7,40	0.823	0.574	7,40	0.356	0.922
Grass	2021	Nitrogen	7,35	2.770	**0.021**			
		Deluge	1,40	2.240	0.142			
		Nitrogen : Deluge	7,40	0.202	0.983			
	2022	Nitrogen	7,35	2.940	**0.016**	7,35	1.753	0.129
		Deluge	1,40	24.892	**<0.001**	1,40	3.553	0.067
		Nitrogen : Deluge	7,40	2.003	0.079	7,40	0.349	0.926
	2023 sqrt()	Nitrogen	7,35	2.421	**0.039**	7,35	4.462	**0.001**
		Deluge	1,40	2.008	0.164	1,40	0.029	0.866
		Nitrogen : Deluge	7,40	1.274	0.288	7,40	0.734	0.644
	2024 sqrt()	Nitrogen	7,35	12.276	**<0.001**	7,35	12.982	**<0.001**
		Deluge	1,40	2.651	0.112	1,40	2.372	0.132
		Nitrogen : Deluge	7,40	2.223	0.053	7,40	1.586	0.169

Appendix 3 Table 3. ANOVA results of the linear regression mixed-model assessing the effect of nitrogen and deluge on richness (Rich.) and evenness (Even.). Results from years when deluge treatments were applied are shaded. Analyses were conducted within each year to accommodate high interannual variability, and legacy data from the 2021 deluge and the 2022 deluge were independently assessed with core nitrogen plots due to deluges being applied in different years. Significant differences ($p \leq 0.05$) are bolded.

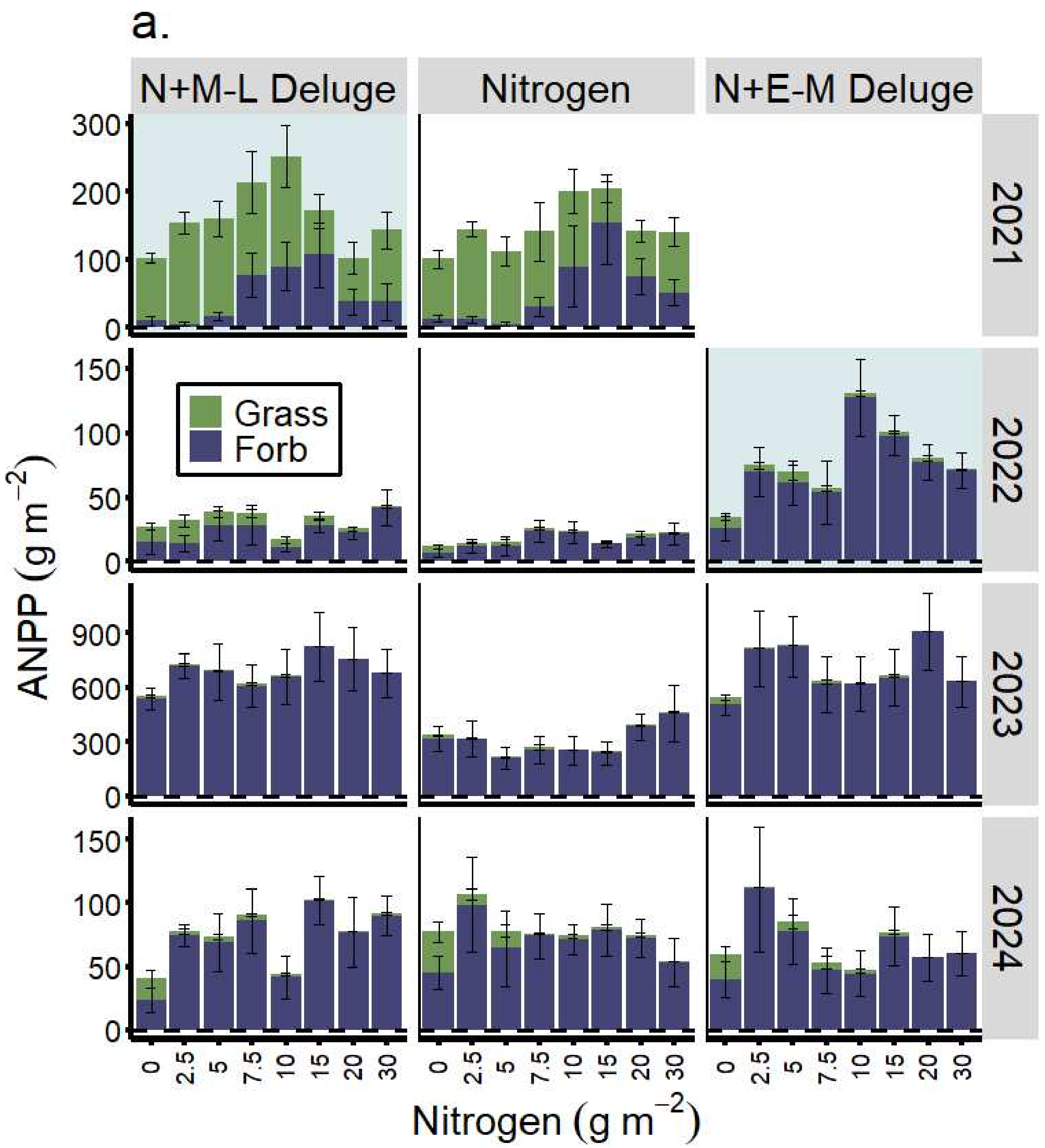
			Nitrogen and 2021 M-L Deluge			Nitrogen and 2022 E-M Deluge		
			d.f.	F-value	p-value	d.f.	F-value	p-value
Rich.	2021	Nitrogen	7,35	3.254	**0.009**			
		Deluge	1,40	2.997	0.091			
		Nitrogen : Deluge	7,40	0.334	0.934			
	2022	Nitrogen	7,35	7.106	**<0.001**	7,35	4.863	**<0.001**
		Deluge	1,40	1.476	0.232	1,40	4.263	**0.045**
		Nitrogen : Deluge	7,40	0.581	0.767	7,40	1.576	0.171
	2023	Nitrogen	7,35	5.402	**<0.001**	7,35	4.704	**0.001**
		Deluge	1,40	0.029	0.865	1,40	3.597	0.065
		Nitrogen : Deluge	7,40	0.185	0.987	7,40	0.154	0.992
	2024	Nitrogen	7,35	9.230	**<0.001**	7,35	7.927	**<0.001**
		Deluge	1,40	22.881	**<0.001**	1,40	4.082	**0.050**
		Nitrogen : Deluge	7,40	2.201	0.055	7,40	1.497	0.196
Even.	2021	Nitrogen	7,35	0.590	0.760			
		Deluge	1,40	1.069	0.308			
		Nitrogen : Deluge	7,40	0.945	0.484			
	2022	Nitrogen	7,35	0.580	0.768	7,35	0.508	0.823
		Deluge	1,40	11.890	**0.001**	1,40	46.768	**<0.001**
		Nitrogen : Deluge	7,40	0.281	0.958	7,40	1.172	0.340
	2023	Nitrogen	7,35	3.827	**0.003**	7,35	1.999	0.083
		Deluge	1,40	0.004	0.949	1,40	0.024	0.879
		Nitrogen : Deluge	7,40	0.719	0.656	7,40	0.742	0.638
	2024	Nitrogen	7,35	7.246	**<0.001**	7,35	5.289	**<0.001**
		Deluge	1,40	0.145	0.705	1,40	0.163	0.689
		Nitrogen : Deluge	7,40	4.827	**<0.001**	7,40	5.608	**<0.001**

Appendix 3 Table 4. ANOVA results of the linear regression mixed-model assessing the effect of nitrogen on community composition differences, measured as the distance between centroids of core nitrogen plots and corresponding N+Deluge plots. Results from years when deluge treatments were applied are shaded. Analyses were conducted within each year to accommodate high interannual variability, and legacy data from the 2021 deluge and the 2022 deluge were independently assessed with core nitrogen plots due to deluges being applied in different years. Significant differences (*p* ≤ 0.05) are bolded. An "*" indicates that a log transformation was needed to meet assumptions.

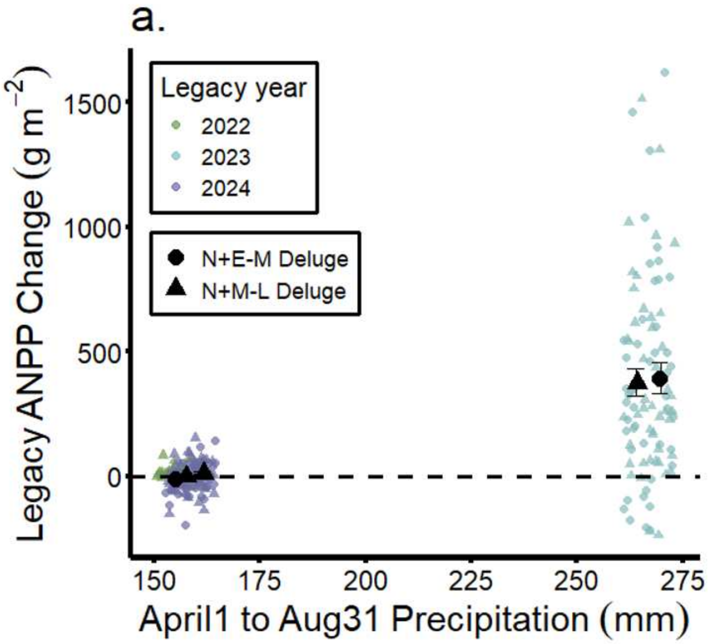
		Community Composition					
		Nitrogen and 2021 M-L Deluge			Nitrogen and 2022 E-M Deluge		
		d.f.	F-value	p-value	d.f.	F-value	p-value
2021	Nitrogen	7,35	1.981	0.086			
2022	Nitrogen	7,35	0.360	0.925	7,35	0.596	0.755
2023	Nitrogen	7,35	2.317	**0.047**	7,35	4.612	**<0.001***
2024	Nitrogen	7,35	2.874	**0.018**	7,35	2.358	**0.0439***

Appendix 3 Table 5. ANOVA results of the linear regression mixed-model assessing the effect of nitrogen and deluge on influential species cover. Results from years when deluge treatments were applied are shaded. Analyses were conducted within each year to accommodate high interannual variability, and legacy data from the 2021 deluge and the 2022 deluge were independently assessed with core nitrogen plots due to deluges being applied in different years. Significant differences ($p \leq 0.05$) are bolded.

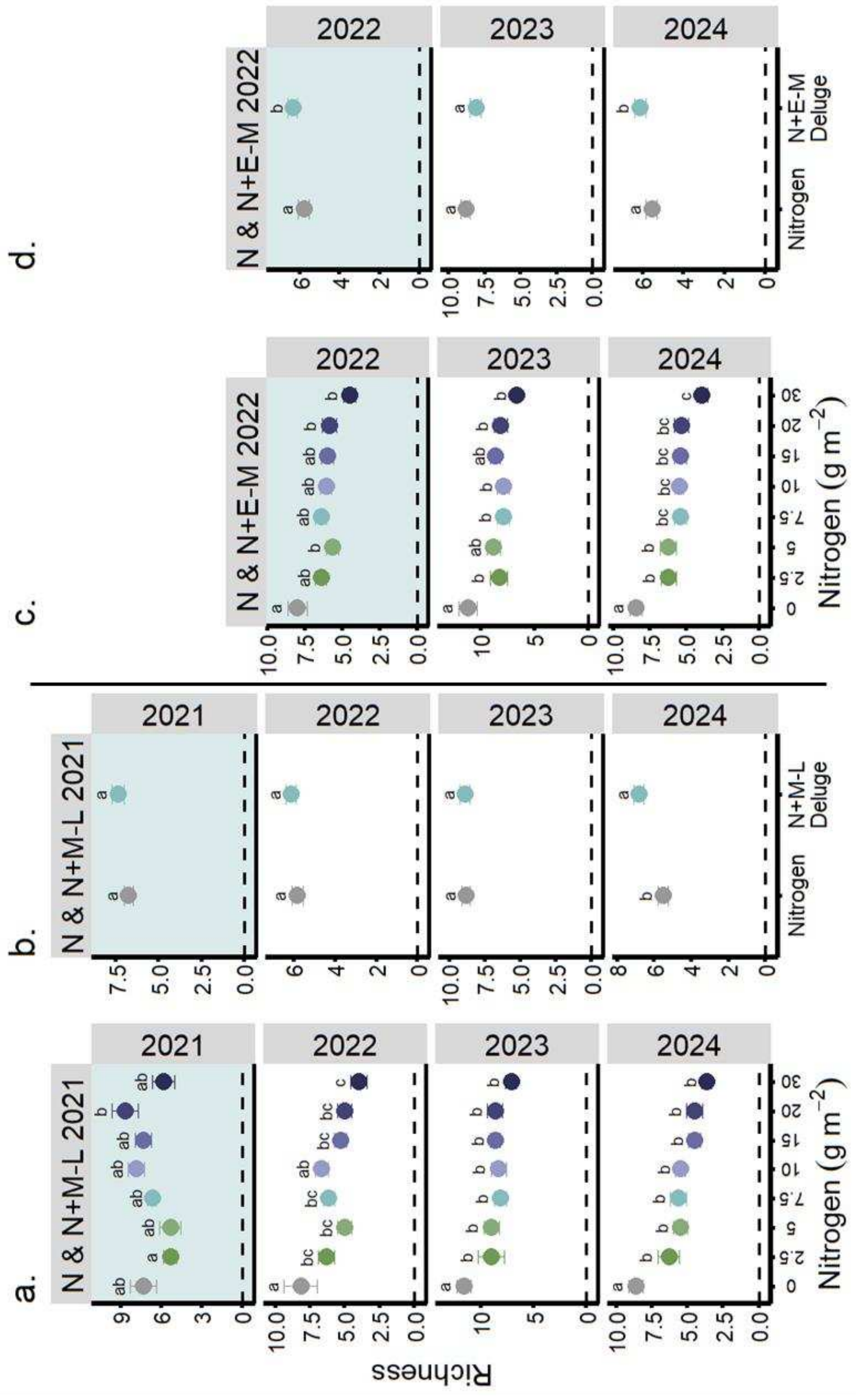
			Nitrogen and 2021 M-L Deluge			Nitrogen and 2022 E-M Deluge		
			d.f.	F-value	p-value	d.f.	F-value	p-value
Chenopodium sp.	2021	Nitrogen	7,26.05	2.426	**0.047**			
		Deluge	1,25.20	0.905	0.351			
		Nitrogen : Deluge	7,24.68	0.533	0.801			
	2022	Nitrogen	7,33.62	1.633	0.160	7,34.42	2.980	**0.015**
		Deluge	1,37.92	0.102	0.752	1,38.49	96.100	**<0.001**
		Nitrogen : Deluge	7,37.76	0.592	0.758	7,38.27	0.877	0.534
	2023	Nitrogen	7,35	1.292	0.283	7,35	1.461	0.213
		Deluge	1,40	0.006	0.938	1,40	2.024	0.163
		Nitrogen : Deluge	7,40	0.467	0.853	7,40	0.405	0.894
	2024	Nitrogen	too few data for assessment			too few data for assessment		
		Deluge						
		Nitrogen : Deluge						
Elymus elymoides	2021	Nitrogen	7,41	0.515	0.477			
		Deluge	1,46	0.056	0.815			
		Nitrogen : Deluge	7,46	0.827	0.368			
	2022	Nitrogen	7,32.96	5.648	**<0.001**	7,42.32	30.421	**<0.001**
		Deluge	1,37.89	35.849	**<0.001**	1,41.39	8.759	**0.005**
		Nitrogen : Deluge	7.37.44	0.214	0.980	7,44.01	0.280	0.600
	2023	Nitrogen	7,31.95	3.716	**0.005**	7,31.81	4.054	**0.003**
		Deluge	1,36.78	5.151	**0.029**	1,34.19	0.180	0.674
		Nitrogen : Deluge	7,36.11	0.420	0.883	7,32.58	1.769	0.128
	2024	Nitrogen	7,27.75	5.380	**<0.001**	7,23.97	6.268	**<0.001**
		Deluge	1,31.92	0.828	0.370	1,28.23	0.002	0.963
		Nitrogen : Deluge	7,31.11	2.953	**0.017**	7,26.46	0.047	0.999
Salsola tragus	2021	Nitrogen	6,3.76	1.901	0.290			
		Deluge	1,3.69	0.135	0.734			
		Nitrogen : Deluge	4,13.94	0.364	0.824			
	2022	Nitrogen	7,31.64	1.055	0.414	7,33.265	0.036	0.851
		Deluge	1,38.30	1.952	0.171	1,40.530	1.921	0.173
		Nitrogen : Deluge	7,35.87	0.051	0.818	7,37.217	1.874	0.179
	2023	Nitrogen	7,35	0.168	0.990	7,35	1.168	0.346
		Deluge	1,40	0.907	0.347	1,40	0.235	0.630
		Nitrogen : Deluge	7,40	0.746	0.635	7,40	1.956	0.086
	2024	Nitrogen	7,35	2.958	**0.015**	7,35	2.702	**0.024**
		Deluge	1,40	0.012	0.914	1,40	8.527	**0.006**
		Nitrogen : Deluge	7,40	1.937	0.089	7,40	1.820	0.110



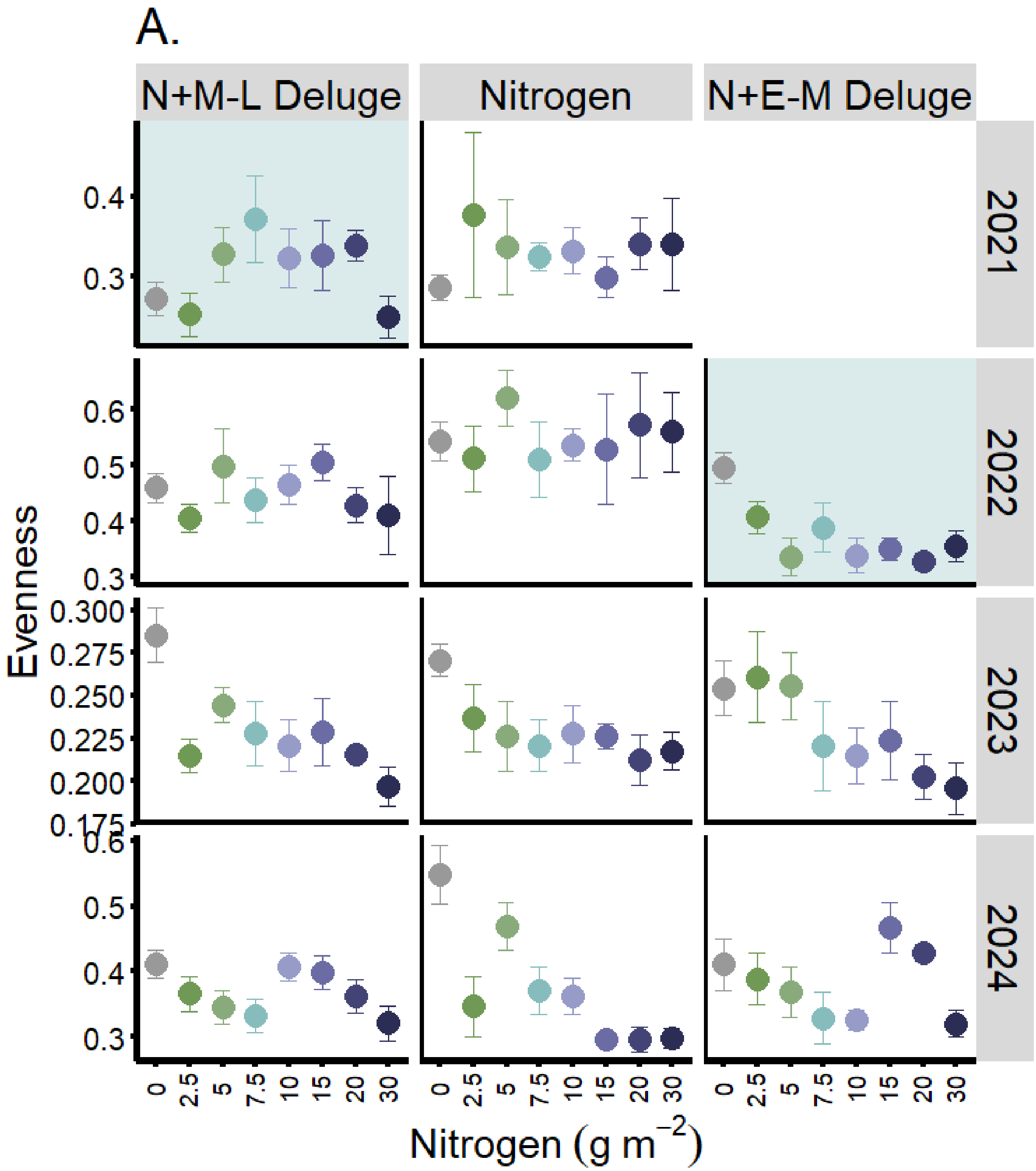
Appendix 3 Figure 1. The effect of nitrogen addition and deluge treatment legacy on forb and grass production. In legacy years, there was no significant effect of nitrogen on forb prodcution, but grass production was significantly decreased by nitrogen addition. Blue shaded plots indicate years in which the deluge treatments were applied. The y axis scale varies by year to account for high innerannual variabiltiy and reflect the model which assessed the within-year response. All error bars represent mean ±SE.



Appendix 3 Figure 2. The correlation between precipitation (cumulative from April 1 to August 31) and the absolute change in ANPP from core nitrogen plots to corresponding N+Deluge legacy plots. In legacy years, there was no significant effect of nitrogen, so data is pooled across nitrogen addition levels.

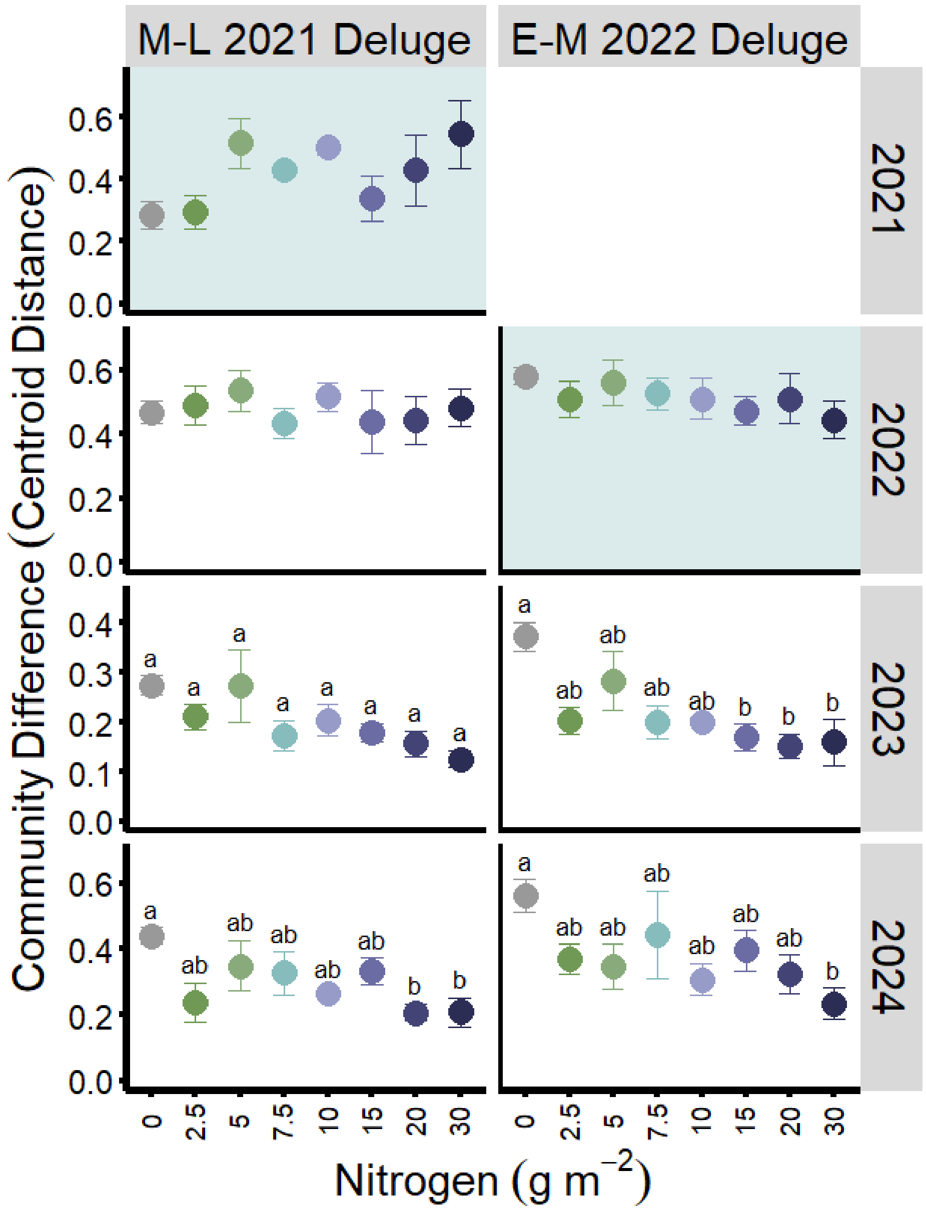

a.
N & N+M-L 2021
b.
N & N+M-L 2021
c.
N & N+E-M 2022
d.
N & N+E-M 2022
2021
2022
2023
2024
Richness
Nitrogen (g m^{-2})
0
2.5
5
7.5
10
15
20
30
Nitrogen
N+M-L Deluge
N+E-M Deluge
a
ab
b
bc
c

Appendix 3 Figure 3. The effect of nitrogen addition and deluge treatment legacy on species richness. Blue shaded plots indicate years in which the deluge treatments were applied. The y axis scale varies by year to account for high innerannual variabiltiy and reflect the model which assessed the wtihin-year response. Graphs are organized to reflect analyses between "N+M-L 2021 Deluge" and "Nitrogen" and the sepatate analysis of "N+E-M 2022 Deluge" and "Nitrogen" data. During leagacy years, there was a significant effect of nitrogen addition and deluge treatment, but no interaction. All error bars represent mean ±SE.

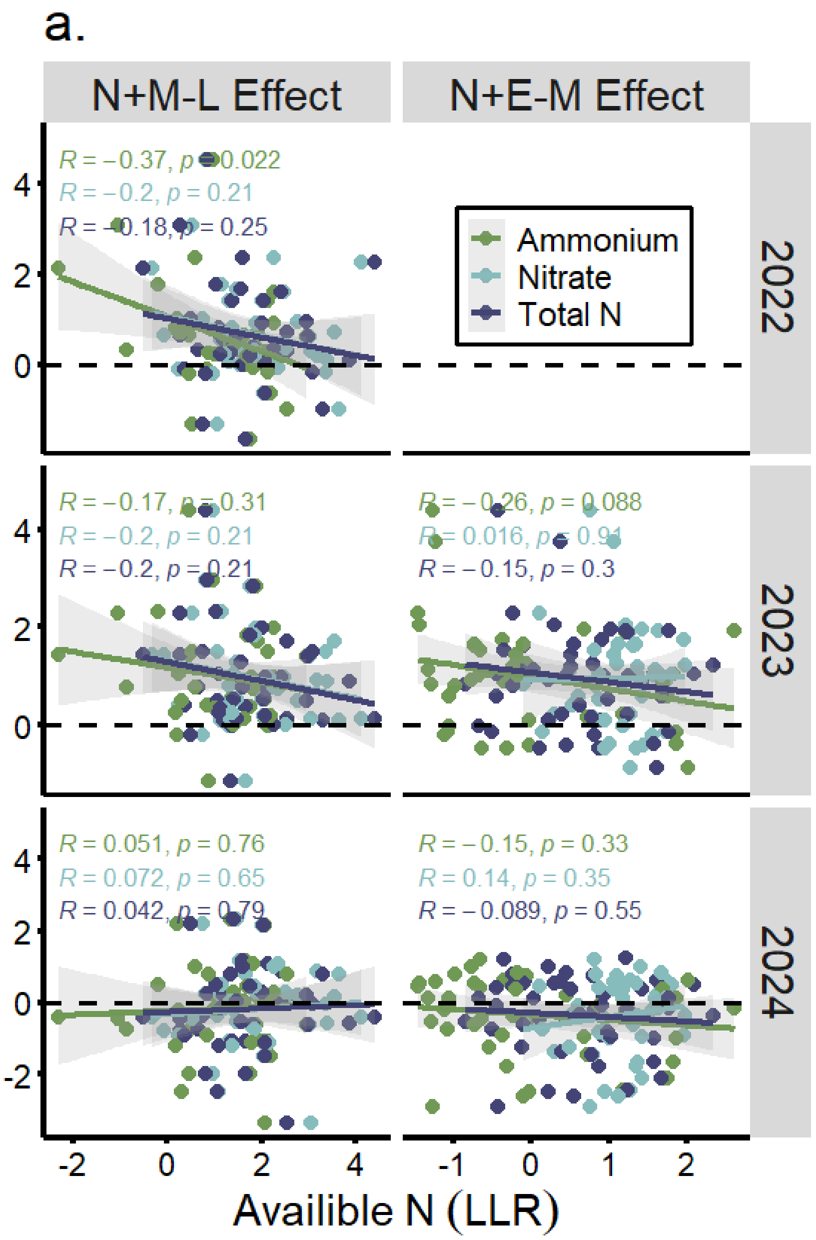


Appendix 3 Figure 4. The effect of nitrogen addition and deluge treatment legacy on species Evenness. Blue shaded plots indicate years in which the deluge treatments were applied. The y axis scale varies by year to account for high innerannual variabiltiy and reflect the model which assessed the within-year response. All error bars represent mean ±SE.

a.



Appendix 3 Figure 5. The differences in community composition measured by centroid distance between the core nitrogen plots and the corresponding N+Deluge legacy subplots across nitrogen addition levels. Mid-late 2021 deluge legacy data in 2023 had an overall significant effect of nitrogen, but this was driven by two marginally significant pairwise comparisons. Blue shaded plots indicate years in which the deluge treatments were applied. Letters indicate significant differences ($p \leq 0.05$) between pairwise comparisons.



Appendix 3 Figure 6. The correlation between initial nitrogen response to deluge and to the ANPP response in legacy years for the 2021 mid-late deluge and the 2022 early-mid deluge. Responses are caclulated as the log response ratio. In legacy years, there was no significant effect of nitrogen on ANPP, so legacy data is pooled across nitrogen addition levels. Statistics in the upper left show the correlation coefficient and pearson’s correlation p-value asssessed for each legacy year and deluge treatment.