**Title**

The Role of Global Environmental Changes, Land Management, and Plant Functional Traits in Regulating Grassland Ecosystem Functions

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**Publication Date**

2024

# The Role of Global Environmental Changes, Land Management, and Plant Functional Traits in Regulating Grassland Ecosystem Functions

By

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2024

## Acknowledgements

First and foremost, I would like to express my deepest gratitude to my family and friends for their unwavering support throughout this long and challenging journey. This dissertation would not have been possible without you. To my incredible sisters, Danielle, Michelle, and Gabrielle, thank you for always being there to listen and offer advice when I needed it most. I'm so grateful for each of you. To my parents, your encouragement and trust in my abilities have meant the world. I also want to extend my heartfelt thanks to all the friends who helped me push through moments of doubt and kept me grounded.

I am immensely proud to join the ranks of Black women with doctorate degrees, and I want to especially recognize my grandmother, Dr. Doretha Traylor, who achieved her PhD in Education over 50 years ago. Her perseverance and accomplishments in the face of adversity have always inspired me. To the many brilliant and ambitious women who paved the way and whom I've had the privilege to look up to—thank you for showing me what is possible.

My deepest thanks also go to my advisor, Nick Barber, for his guidance, trust, and encouragement to pursue ambitious projects and collaborations with different labs and organizations. I've learned so much under your mentorship. To my committee members, David and Valerie, I greatly appreciate your support, feedback, and insight during this process. Thanks also to my qualifying exam committee: Cristina Lazcano, Becca Lewison, Jennifer Funk, and Luke Miller—your advice was invaluable in shaping my work.

I owe a special thanks to my lab mates: Azeem, Kendall, and Liz, for your camaraderie and support, and to our amazing undergraduate lab mates: Maricela, Marieke, Sam P., Sam I., Travis, Connor, Alec, and Lydia—your help in the lab was instrumental to this work.

Finally, this dissertation is dedicated to all the people who have supported me along the way. From my earliest inspirations to my most recent accomplishments, I could not have done this alone. Thank you all.

## Abstract

Grasslands cover 40% of the world's land surface and provide a large range of ecosystem services including carbon storage, nutrient cycling, and habitat for diverse species. However, these systems and the services they provide are threatened by global environmental changes, including species invasions, drought, and atmospheric nitrogen (N) deposition. Grasslands often require management practices that support the diverse plant communities driving ecosystem functions. In many grasslands, this involves the use of disturbances such as herbivory through introducing large grazers (or mowing/clipping as a proxy) and fire through the application of prescribed burns. Overcoming biodiversity losses may also involve planting native species for restoration of degraded grasslands. However, how these grassland management and restoration practices affect ecosystem functions, particularly in the face of climate variability and rising atmospheric N deposition, remains underexplored. A large body of research now focuses on plant traits and the functional diversity of plants in a community to understand how they can affect grassland resilience to environmental changes. The goals of this research are to understand how grassland restoration practices affect grassland communities and functions under climate changes, and to explore plant trait diversity as a regulator of critical grassland ecosystem functions.

Chapter 1 uses long-term data in an invaded California grassland experiment to evaluate clipping management effects on grassland communities and ecosystem functions in an experimental drought. Results revealed that drought did not affect the relative abundances of native and invasive plants, but it increased aboveground plant biomass and decreased root biomass allocation. Clipping management increased root biomass post-drought, but this disturbance also facilitated the spread of noxious invaders, highlighting the complexity of restoration strategies and their outcomes. However, neither clipping, experimental drought, nor invasive species altered soil organic carbon.

Chapter 2 investigated the impact of rising atmospheric N deposition on grassland ecosystems using a long-term N fertilization experiment in the same California grassland. Like drought effects, long-term N fertilization did not significantly alter the relative abundance of native or invasive plant groups, but it did influence ecosystem functions like increasing soil N cycling rates and belowground biomass. Clipping management did not alter plant community or ecosystem responses to N deposition. Plant community composition was more strongly influenced by priority effects than by N deposition or clipping management.

Chapter 3 examined how prescribed fire and grazing affect soil ecosystem functions in mesic tallgrass prairies and to what extent this may be mediated by plant taxonomic or functional trait diversity. The direct impacts of these management practices on soil properties were significant, with bison presence increasing soil N and recent fires decreasing carbon mineralization rates. However, many soil properties like microbial biomass carbon, microbial biomass N, and soil organic matter, remained unaffected. Older restoration sites tended to have reduced soil nitrate levels, and this was slightly mediated by higher functional trait diversity in older plots. However, plant diversity did not mediate grazing or fire effects on soil and did not have strong impacts on any other soil functions measured.

Finally, Chapter 4 used a biodiversity-ecosystem function experiment to explore the relationship between plant diversity (functional and taxonomic) and soil extracellular enzyme activities (EEAs) in a tallgrass prairie. Plant diversity did not significantly influence most of the EEAs studied, except for the activity of N-acetyl-β-D-glucosaminidase (NAG), an enzyme that degrades chitin. This suggests other factors besides plant taxonomic or functional trait diversity may play a more crucial role in regulating these activities, but plant diversity might interact with fungal abundance in a way that affects chitin availability as a substrate for these enzymes and the microbes that produce them.

Collectively, these studies improve our understanding of plant-soil relationships and underscore the complexity of grassland restoration and the need for nuanced management strategies to enhance ecosystem functions and resilience especially in the face of global environmental changes.

## CHAPTER 1

## The role of invasive and native plants in grassland ecosystem response to long term precipitation variability and clipping management

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### Abstract

Globally, grasslands face significant that can impact biodiversity and ecosystem functions. Restoration usually includes planting native species as this can play a large part in mitigating invasive species, but less is known about how native plant establishment may shift long-term community structure and ecosystem responses under drought or how these effects may change with habitat management. Using long term plant and soil data from an experimental grassland in California's Central Valley, we assessed the recovery of the grassland communities and ecosystem functions after drought under a clipping management regime. Although drought did not significantly alter the composition of native vs invasive species, it did increase plant biomass, decrease root:shoot ratios, and decrease soil water holding capacity. This negative effect on water holding capacity was eliminated when native plants were present. Notably, clipping management increased root biomass post-drought. Although clipping also facilitated noxious invaders like *Elymus caput-medusae* and *Aegilops triuncialis,* it is not possible to determine if this caused the increase in root biomass. These findings highlight the complexity of drought impacts on grassland ecosystem function and restoration outcomes. More research is needed to optimize management practices that will support ecosystem biodiversity and stability, especially as droughts become more frequent and severe.

### Introduction

Grasslands face numerous threats, including climate change, which puts their diverse communities and the ecosystem services they provide at risk of degradation. These threats include more frequent, severe, and prolonged droughts, as well as increased storms and heavier precipitation events

(Dai 2007, Loarie et al. 2008, Prugh et al. 2018, Harrison et al. 2020). Additionally, invasive annual plants have dominated in California's Central Valley Grasslands and can have stronger responses to climate changes than native plants (Dukes et al. 2011). Invasive plants can alter biogeochemical cycles and primary productivity, decrease biodiversity, and increase fire frequency (D'Antonio & Vitousek 1992, Ehrenfeld 2003, Corbin & D'Antonio 2004, Bradley et al. 2006). By changing nutrient availability and soil structure, invasive plants can exacerbate the effects of precipitation variability, potentially hindering ecosystem recovery. Their ability to outcompete native species for water and nutrients can also disrupt the resilience of grassland ecosystems to variable precipitation patterns. Understanding community dynamics of invasive species and how these affect ecosystem function during future climate conditions will guide effective grassland management.

Drought is a disturbance that can change plant community composition by reducing dominant plant species, which may facilitate native and rare species (Prugh et al. 2018, Valliere et al. 2019). Drought can also exacerbate invasion under altered nitrogen regimes (Everard et al. 2010, Garboski et al. 2021). These potential changes in community composition are likely mediated by plant traits, and the traits of dominant plant functional groups can be strong indicators of ecosystem stability (Grman et al. 2010). Invasive Mediterranean grasses, such as *Bromus* spp., are widespread in California due in part to their ability to outcompete native species for water, space, and other resources (Davis & Mooney 1985, Corbin et al. 2007, Abraham et al. 2009). They germinate earlier in the season, have higher growth rates, and achieve higher seedling density than native plants (Jackson and Roy 1986, Holmes and Rice 1996, Abraham et al. 2009). Although these traits may allow them to outcompete natives for resources, their sensitivity to precipitation variability may put grassland ecosystem stability at risk when they account for a large portion of grassland primary productivity. Native perennial grasses, on the other hand, have deep roots to access soil moisture, increasing their stability against drought stress (Holmes and Rice 1996). However, if native species are greatly reduced by invasives, their abundance may be insufficient to compensate for low productivity of invasive plants during drought years. Furthermore, the impacts of invasive species on ecosystem functions likely varies depending on the function, with belowground biomass or soil functions potentially differing from aboveground biomass patterns. Because plant community composition influences ecosystem functions, understanding the magnitude and direction of drought-induced changes in plant community composition can inform predictions of ecosystem responses to climate variation.

When native species are planted in restorations, this can be a strong driver of long-term plant community compositions. because native perennial species are more persistent in the plant community than annuals and can suppress invasive annual plants. Planting native species can also suppress invasives due to priority effects (Chase 2003, Werner et al. 2016) where early arriving species can preempt or modify niches affecting the performance of later arriving species (Fukami 2015) such as changes in abundance, aboveground biomass, root length density, and allocation to root to shoot growth (Körner et al. 2008, Weidlich et al. 2018). These changes can affect ecosystem functions like soil biogeochemical cycling, that are dependent on certain plant for traits like high N input and fast turnover (Pérez Castro et al. 2020). Invasive species can establish successfully without priority (Mason et al. 2013) but may generate stronger priority effects than native species (Dickson et al. 2012, Wilsey et al. 2015, Stuble & Souza 2016). A longer interval between species arrival can also increase the influence of priority effects (von Gillhaussen et al. 2014, Young et al. 2017). There is uncertainty, however, surrounding the persistence of priority effects and how they are influenced by climate (Weildlich et al. 2021). One plant community study found that priority effects lasted up to 7 years (Collinge & Ray 2009), but disturbances associated with global change may disrupt priority effects and mute their impacts on communities and ecosystems. Grassland restorations are a prime location to understand the strength of native plant establishment on ecosystem functions under variable abiotic conditions. Persistent management is needed of annual communities to maintain a native community.

Clipping or mowing is a management strategy that is often used in grasslands to mimic disturbances that decrease invasive species dominance with the goal of increasing native diversity (Maron & Jeffries 2001). The removal of aboveground biomass directly affects plant communities by altering resource availability, usually providing additional space and light to species that are otherwise outcompeted by dominants like invasive grasses (Burke and Grime 1996, Hautier et al. 2009). Invasive species removal in particular can reduce buildup of a thatch layer which might inhibit other species. However, clipping plant communities can have adverse effects depending on community composition and abiotic conditions. Clipping during drought can remove thatch that would otherwise trap moisture and buffer drought conditions (Carlyle et al. 2014). In addition, clipping seasonaly likely mediates the relationship between invasion and drought recovery because the effects of fall clipping are seen within the current growing season while late spring clipping affects plant productivity of the following growing season (Prevéy et al. 2014). Together, these findings suggest that the interaction between invasive annual cover and clipping management may be a key regulator of temporal variability in biomass and other critical ecosystem functions during post-drought recovery.

The goal of this study was to understand how priority effects influence ecosystem function responses to precipitation variability, and if clipping management during drought mediates ecosystem function. In this study, we used a long-term rainfall manipulation experiment to address three questions: (1) How does precipitation variability over 14 years affect primary production and soil function? (2) Are these relationships mediated by planting native species? (3) Does clipping modify community and functional resilience to precipitation variability? Identifying the mechanisms underlying ecosystem stability will contribute to greater understanding of how ecosystem functions will respond to global environmental changes and inform habitat management.

## Methods

This study was conducted on an experimental site located in Davis, California. This area is characterized by a Mediterranean climate with hot dry summers and cool wet winters. In July, temperatures reach a maximum of 32°C, and in January temperatures reach 14°C during the day. Mean annual rainfall from 1980-2021 was 385 mm/year. Soils are primarily a coarse-loamy texture. The study site is abandoned land that had been previously cultivated for agriculture prior to 1985 (Carey et al. 2015). In 2007, experimental plots were established, 96 of which are used in this study. These plots measure 1.5 m x 1.5 m in size and received factorial combinations of three manipulations: seeding, clipping, and precipitation. Plots received seeds of equal mass of one of two community types: (1) native species, naturalized annuals, and noxious annual invaders, or (2) only naturalized annuals and noxious annuals. Native perennial grasses included *Stipa pulchra*, *Elymus glaucus*, *Elymus triticoides, Bromus carinatus, Acmispon americanus,* and *Lupinus bicolor*. Exotic naturalized annuals planted include *Avena fatua, Bromus hordeaceus, Festuca perennis*, and *Trifolium subteranneum* that have been present in California grassland communities for decades to centuries (Jackson 1985). A new wave of noxious annual invasive plants includes *Elymus caput-medusae* and *Aegilops triuncialis* that were first recorded in the late 1800s and 1914, respectively, and by the end of the 20th century began to reach high abundances in some locations (Peters et al. 1996, Bossard et al. 2000, D'Antonio et al. 2007).

Rainout shelters were installed starting in the 2011-2012 growing season and removed in spring 2019. Dry treatments consisted of a 30-50% decrease in mean annual precipitation. Wet treatments received 12-25% more precipitation than the mean annual precipitation. Control treatments consisted of ambient precipitation conditions. Plots received fall or spring clipping treatments each year to simulate intense grazing in addition to unclipped (control) plots. This project used a subset of 14 blocks to implement precipitation manipulations (3 wet, 3 dry, and 8 control). Species cover was assessed annually using percent cover classes using a modified Daubenmire approach (0–5, 5–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80, 80–90, 90–100%) during early flowering (late March-mid April) mostly to assess forb cover, and late-spring (mid-May to mid-June) to assess cover of grasses. From 2016-2021 we collected above- and belowground biomass from the 96 plots. Shoots were cut 4 cm above the soil surface from a 68 $cm^2$ area. Roots were collected using a soil corer (1.9 cm diameter x 15 cm deep). Annual peak biomass data collection started in 2007, but not all plots were measured each year, so we focus on 2016-2020 data that is complete for all seeding, water, and clipping manipulations. This period included a historic drought year in 2018 and a wet year in 2017 and 2019. Although precipitation treatments ended after 2019, data from 2020 was still included due to time lag effects that annual precipitation can have on plant communities.

For each plot, we collected a 180 mL soil sample in December 2020 from 0-15cm depth using a soil corer (1.9 cm diameter x 15 cm deep). Soil samples were sieved through a 2 mm sieve and analyzed for soil organic matter (SOM) and soil water holding capacity (WHC). SOM was analyzed by calculating the ash-free weight after burning for 500 C for 4 hours (adapted from Robertson et al. 1999). Water holding capacity was measured using a field method adapted from Robertson et al. (1999) and Romano and Santini (2002).

*Statistical analysis*

We analyzed plant cover of the three functional groups (natives, naturalized, noxious), shallow belowground and aboveground biomass, and root to shoot biomass ratio using repeated measures linear mixed models with lmer() in the lme4 package (Bates et al. 2015) of R (R Development Core Team 2018). We also examined the cover of *B. diandrus,* a naturalized species that reached high abundance in some plots. To understand how invasive priority effects influence biomass responses to drought, we set initial seeding treatment, precipitation treatment, and their interactions as fixed factors. Year was a fixed time factor, and the rainout shelter blocks were a random factor.

To determine effects of clipping during drought we analyzed plots where species arrived simultaneously (no invasive priority effects). Plant cover and belowground biomass were response variables. Precipitation treatment, clipping treatment, and their interaction were fixed treatment factors with year as a fixed time factor and block as a random factor. Log transformations of all cover and biomass data were used to meet model assumptions of residual normality and homoscedasticity.

We analyzed soil WHC and SOM using a linear mixed model. Initial seeding treatment, precipitation, clipping, and the three-way interaction were fixed treatment factors. Block was a random factor. A square root transformation was used to analyze SOM and WHC. All models were analyzed using “type 3” $X^2$ tests with Anova() in the car package (Fox & Weisberg 2011). For all analyses, post-hoc Tukey tests were carried out with emmeans() in the emmeans package (Lenth et al. 2019).

## Results

The planting of native species in 2007 successfully increased native plant cover from 27% to an average of 47% over the period from 2016 to 2020 (Table 1.3, Figure 1.4). However, this increase in native plant cover did not result in a significant change in plant biomass (Table 1.1, Figure 1.3). Native planting did not affect naturalized or noxious cover in control water plots. However, in both wet and dry plots, the introduction of native plants led to a reduction in naturalized plant cover, decreasing it from 83% to 64% and 62%, respectively (Figure 1.4). Despite these changes in plant cover, native planting did not significantly affect SOM (Figure 1.6). Additionally, while native planting reduced WHC in control plots that only received natural precipitation, this effect was negated in the wet and dry treatments (Figure 1.5).

Dry treatments had a significant impact on biomass distribution, increasing aboveground biomass by 137% (Figure 1.2) while decreasing belowground biomass by 48% (Figure 1.3), indicating a shift of biomass from belowground to aboveground. Additionally, dry treatments were effective in reducing noxious invasive plant cover (Figure 1.4). Although dry treatments did not significantly affect the cover of native or naturalized plants overall, it is noteworthy that *Bromus diandrus* cover was particularly high in dry treatments in 2017 (Figure 1.S2). This anomaly was likely due to an unusually wet year historically, making the "dry" treatments relatively wetter than anticipated. No significant effects of water treatments were observed on SOM or WHC.

Fall clipping had a notable impact on root biomass, increasing it by 60% from 44.7 g/m$^2$ to 71.5 g/m$^2$ (Figure 1.S1). Fall clipping also significantly increased naturalized plant cover from 69% to 89%. When clipping was conducted in fall or spring, noxious weed cover increased from 10% to 18% and 22%, respectively. However, clipping treatments did not have a significant effect on WHC or SOM (Figure 1.5 & 1.6). The interaction between water and clipping treatments did not produce any significant effects on plant cover, biomass, WHC, or SOM.

## Discussion

Understanding how biotic and abiotic factors regulate ecosystem function is essential to restoring grassland ecosystems. Ecosystem functions can be regulated by conditions such as drought and land management actions (de Vries et al. 2012, Craine et al. 2013, Hoffman et al. 2016, Wang et al. 2020, Williams & de Vries 2020) or biotic factors like community assembly processes (e.g., priority effects, Fukami & Morin 2003, Delory et al. 2019). This study demonstrates that drought can increase aboveground biomass. If invasive plant biomass increases, this can contribute to fire fuel loads making fires more severe and intense (D'Antonio & Vitousek 1992, Ziska et al. 2005). Drought decreased soil WHC only when invasive plants had priority. Soil WHC can affect the soil's ability to supply plants with water for growth which is the major limiting factor for plant productivity during drought conditions. SOM was not affected by treatments likely due to slow C cycling processes that take decades to detect. This study also supports the claims that clipping to simulate grazing can increase root biomass (Liu et al. 2021) even during drought, but more information is needed on how this is related to increases in noxious invaders and changes to soil functions such as nitrogen cycling.

### *Plant Productivity and Cover*

Plant productivity is an important component of the carbon cycle, and variation in productivity can lead to instability in other ecosystem functions. Because precipitation has large effects on plant growth (Maurer et al. 2020), productivity can be sensitive to interannual variation in precipitation. This sensitivity to precipitation may be pronounced in the future, because regions like California that already have a variable climate are expected to experience more droughts that are prolonged and more severe (Maurer et al. 2020, Cook et al. 2015). Variation in precipitation events can also lead to long-term changes in primary productivity even when total precipitation is constant across years (Gheradi and Sala 2019). Understanding how these changes in precipitation affect primary production can improve our understanding of how changing climates affect plant carbon inputs.

This study manipulated plant community assembly to determine how priority effects are influencing plant production with and without drought. Drought treatments affected above- and belowground biomass, and in particular reduced root-to-shoot ratios. We expected drought to decrease aboveground biomass and increase root-shoot ratios as seen in other studies (Ciais et al. 2005, Eziz et al. 2017). However other studies also have found that biomass allocation in response to drought can be species-specific and even depend on the rhizosphere microbes (Li et al. 2024, Kørup et al. 2018). One possible mechanism for the surprising decrease in root to shoot ratios is a shift in communities to greater abundances of annual plants with shallow roots, like *Bromus diandrus,* relative to deeper-rooted perennial species. Although drought can decrease aboveground primary productivity (ANPP), it can also increase performance of annual grass species such that ANPP can recover completely within the year after drought (Stampfli et al. 2018). There was high coverage of *B. diandrus* observed during the experimental drought in 2017 and 2018, but this was not consistent across all years (Fig. S2), and overall annual variation in aboveground biomass did not match *B. diandrus* variation. Therefore it is unlikely that an increase *B. diandrus* alone accounted for increases in aboveground biomass due to drought. The high coverage of *B. diandrus* in 2017 and 2018 may have been a result of the record breaking rainfall that occurred in 2017, which caused the dry treatments to be relatively wet compared to other years. Other changes in aboveground biomass may have been the result of shifts in community composition and differences in biomass allocation strategies among other species. Plants' root:shoot ratio response to water limitation can be species specific and range from positive, to neutral or negative (Bloom et al. 1985, Meier & Leuschner 2008, Guasconi et al. 2018) as plants may allocate energy and growth toward obtaining aboveground resources such as light. Shifts in belowground C allocation that affect growth have been found in other plants experiencing drought: several years of experimental drought treatments, like this study, decreased plant C uptake and allocation belowground (Fuchslueger et al. 2016). Drought also altered shoot and root N concentrations, which could have implications for ecosystem C and N cycling if it occurred here as well.

Communities in 2020 have a somewhat similar cover of noxious and naturalized plants but there is higher native cover when natives were planted in 2007. Although priority effects may be expected to be less important in harsh environments (Chase 2007), we found that initial seeding composition was an important factor in determining native and naturalized plant cover, but not noxious plants. Native plants did not respond to drought conditions but had a significantly positive response to priority effects, even during drought. invasive priority may prevent native plants from establishing either through niche preemption or niche modification (Fukami 2015). Invasive plants may also alter soil characteristics or facilitate microbial communities that are not conducive to native growth (Ridenour & Callaway 2001, Eviner & Chapin 2002, Batten et al. 2006, McLeod et al. 2016). Invasives annuals may also occupy a significant amount of space in a given year making it difficult for native plants to establish.

Total naturalized annual plant cover only responded to dry conditions when native and invasive species arrived simultaneously. When invasives had priority, they were resistant to dry treatments. This indicates that a competitive advantage like priority effects will benefit them during drought stress. Naturalized plants also responded positively to wet treatments when invasives had priority, but this treatment did not affect cover in simultaneous arrival plots. Competition may be causing these patterns, with naturalized species experiencing less competition in drought where native species are less common. When the main potential competitors are noxious annual species that grow little under drought due in part to their later phenology, naturalized species are freed from competition and can spread more rapidly. Conversely, locally adapted native species that are drought tolerant are significant competitors and reduce naturalized species cover in these same dry conditions. In other western North American grasslands, invasive *Bromus* species show a similar increase when high temperature and drought causes native plant mortality (Bradley et al. 2016), and exotic Mediterranean grass cover increases under experimental drought (Souther et al. 2020, Loesser et al. 2007).

Priority effects did not affect plant biomass or root-to-shoot ratios which, perhaps because of the overwhelming influence of water limitation on plant productivity in this semi-arid environment. Although invasive priority decreased native plant cover, total plant biomass may remain unchanged if invasive annual biomass compensates for the loss of native plants.

### *Soil Function*

Grasslands provide essential soil function due in part to high root productivity which is beneficial for soil carbon and water storage (Bardgett et al. 2021). However, geographic precipitation patterns regulate the relationship between soil function and production. Sala et al. (1988) studied 9500 sites and found that the relationship between soil water and primary production can depend on mean annual precipitation. They showed a negative relationship between soil water availability and production in dry regions, while this relationship was positive in wet regions. I measured soil water holding capacity (WHC), as well as SOM, an important C pool.

Soil WHC was affected by interactions among priority effects, drought conditions, and clipping management. Soil WHC was higher when invasive plants had priority, but this effect disappeared during drought or with a clipping treatment. Plots with increased soil WHC would be beneficial due to increases in heavy rainfall events predicted with climate change, however if these plots have high invasive cover they will also be the most negatively affected by drought due to invasive plants sensitivity to rainfall. The abiotic stress of drought and clipping may have overwhelmed these effects and erased any differences in water storage. In fact, invasive priority plots had lower soil WHC in drought treatments. Having well-established native perennials with dense, deep roots may be important in stabilizing soil WHC when abiotic stressors like drought and clipping are present. There is a relationship between soil water and species richness, but this relationship can change direction or magnitude over time (Fischer et al. 2019). There is also a relationship between the presence of certain functional groups and soil water (Leimer et al. 2014, Fischer et al. 2019). Knowing priority effects may also mediate soil water inflates this uncertainty. More research is needed to understand the fate of soil water availability during future droughts.

In contrast to differences in WHC, SOM was not affected by priority effects or drought. SOM is low in these grasslands, and changes in SOM may be resistant against drought conditions. Changes in soil functions can also take decades to appear (Ontl & Shulte 2012). Although this study started 12 years ago, SOM production is a slow C-cycle process, so it may take more time for detectable changes to occur. Drought can affect soil carbon by reducing plant C inputs, increasing dissolved organic carbon and microbial biomass carbon rather than SOM (Deng et al. 2021), so the relatively dry conditions in this experiment could cause slow SOM production even under the "wet" precipitation treatment. Other microbial and biogeochemical measures like enzymatic activity, N fluxes, and other components of the C cycle could provide more insight into drought effects and provide a more comprehensive view of soil ecosystem processes (Pérez Castro et al. 2019).

### *Clipping Management*

Clipping or mowing vegetation is a common restoration action that can regulate community composition and increase root biomass, a goal for California grassland management. Abundant aboveground biomass represents fuel that increases fire risk. High root biomass can be indicative of native grass dominance and increased plant C inputs for soil carbon storage. Here, fall clipping achieved these goals by increasing root biomass, but it also significantly increased cover of invasive plants. Clipping disturbances to native plants might reduce their competitive ability against invasive plants, particularly because of their overlapping growth and flowering phenology. Increasing invasive plant cover and abundance is especially concerning because they can contribute to fire fuel and fire intensity, while also challenging conservation goals through decreased plant diversity and native plant cover (Davies & Svejcar 2008). Spring clipping significantly increased noxious plant cover in all water treatments. However, clipping treatments did not affect native plant cover. Thus, spring clipping had more beneficial and direct implications for the naturalized plants than the native plants, a discouraging sign for its application in managed grasslands. Clipping did not interact with drought, indicating that patterns seen in clipped plots will prevail during drought conditions. This managed disturbance will need to be paired with careful monitoring and control of naturalized and noxious annual plants to avoid negative impacts on native plant cover, biomass, and diversity.

## Conclusion and Management Implications

Drought will continue to be a frequent stressor in California grasslands in the future, so it is important to know how communities and ecosystems will respond, and how we should manage them in these conditions. Supporting native perennial grasses is a common management goal partly due to their ability to suppress noxious invaders like *Elymus caput-medusae* and *Aegilops triuncialis* (Eviner & Malstrom 2018). Although this study did not show that drought affected total plant biomass, other studies have shown increased aboveground productivity of invasive grasses in response to drought. Drought can facilitate invasive species because of their fast growth and high seed production (Davis et al. 2000, Diez et al. 2012), but it may also reduce other invasives because they respond more negatively to unfavorable conditions than natives (Valliere et al. 2019). The observed increase in noxious invasive plant cover in this study supports the idea that drought can facilitate invasive species. This is likely due to their fast growth rates and high seed production, which allow them to quickly take advantage of the reduced competition and altered environmental conditions brought on by drought. Consequently, invasive species may outcompete native plants, further altering ecosystem dynamics and potentially hindering recovery from drought conditions Native plants in the Central Valley may not respond to drought because they are already adapted to extremely arid conditions. Throughout this experiment, this area and much of California experienced a severe drought from 2013-2016. Although drought did not significantly affect SOM, our study shows that it did affect plant biomass, indicating plants may be shifting biomass aboveground during drought. Priority effects did not affect plant biomass but did affect soil water and its response to drought. Further research is needed to determine if increased root biomass in response to clipping is due to changes in individual plants' C allocation or changes in community composition.

Understanding how future climates are affecting plant C allocation and root traits can guide grassland managers seeking strategies to support ecosystem C cycling.

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Table 1.1. Results of linear mixed effect model analyses of plant biomass (aboveground and belowground at 0-15cm depth) and root-to-shoot ratio in unclipped plots only.

| | aboveground biomass | | | belowground biomass | | | root-to-shoot ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | $X^2$ | df | *P* | $X^2$ | df | *P* | $X^2$ | df | *P* |
| priority | 0.47 | 1 | 0.492 | 1.56 | 1 | 0.212 | 2.36 | 1 | 0.124 |
| water | 7.20 | 2 | **0.027** | 9.77 | 2 | **0.008** | 26.68 | 2 | **<0.001** |
| Year | 5.56 | 4 | 0.235 | 21.21 | 4 | **<0.001** | 27.39 | 4 | **<0.001** |
| priority x water | 2.61 | 2 | 0.271 | 2.35 | 2 | 0.309 | 0.57 | 2 | 0.752 |

Table 1.2. Results of linear mixed effect model analyses of plant belowground biomass for (0-15cm depth) in simultaneous arrival plots only.

| Factor | $X^2$ | df | *P* |
|---|---|---|---|
| Water | 4.79 | 2 | 0.091 |
| Clipping | 11.61 | 2 | **0.003** |
| Year | 35.79 | 4 | **<0.001** |
| water x clipping | 2.65 | 4 | 0.617 |

Table 1.3. Results of linear mixed effect model analyses of plant cover for three plant functional groups (native, naturalized annual, and noxious annual plants).

| | native cover | | | naturalized cover | | | noxious cover | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | $X^2$ | df | *P* | $X^2$ | df | *P* | $X^2$ | df | *P* |
| priority | 35.84 | 1 | **<0.001** | 5.17 | 1 | 0.023 | 1.56 | 1 | 0.210 |
| water | 0.26 | 2 | 0.8777 | 3.66 | 2 | 0.161 | 57.64 | 2 | **<0.001** |
| clipping | 0.85 | 2 | 0.653 | 17.87 | 2 | **<0.001** | 39.10 | 2 | **<0.001** |
| Year | 50.03 | 4 | **<0.001** | 14.22 | 4 | **0.007** | 129.74 | 4 | **<0.001** |
| priority x water | 2.30 | 2 | 0.317 | 12.79 | 2 | **0.002** | 1.51 | 2 | 0.469 |
| priority x clipping | 0.17 | 2 | 0.917 | 0.21 | 2 | 0.899 | 2.37 | 2 | 0.306 |
| water x clipping | 8.84 | 4 | 0.065 | 6.41 | 4 | 0.189 | 2.71 | 4 | 0.608 |
| priority x clipping x water | 3.15 | 4 | 0.533 | 3.34 | 4 | 0.503 | 8.14 | 4 | 0.087 |

Table 1.4. Results of linear mixed effect model analyses of soil functions (SOM and WHC).

| | SOM | | | WHC | | |
|---|---|---|---|---|---|---|
| Factor | $X^2$ | df | *P* | $X^2$ | df | *P* |
| priority | 1.57 | 1 | 0.210 | 3.78 | 1 | **0.052** |
| water | 1.18 | 2 | 0.555 | 2.56 | 2 | 0.277 |
| clipping | 1.28 | 1 | 0.259 | 3.37 | 1 | 0.066 |
| water x priority | 2.66 | 2 | 0.265 | 11.91 | 2 | **0.003** |
| clipping x priority | 3.76 | 1 | **0.053** | 6.41 | 1 | **0.011** |
| water x clipping | 0.11 | 2 | 0.947 | 4.06 | 2 | 0.131 |
| priority x clipping x water | 2.84 | 2 | 0.242 | 19.47 | 2 | **<0.001** |

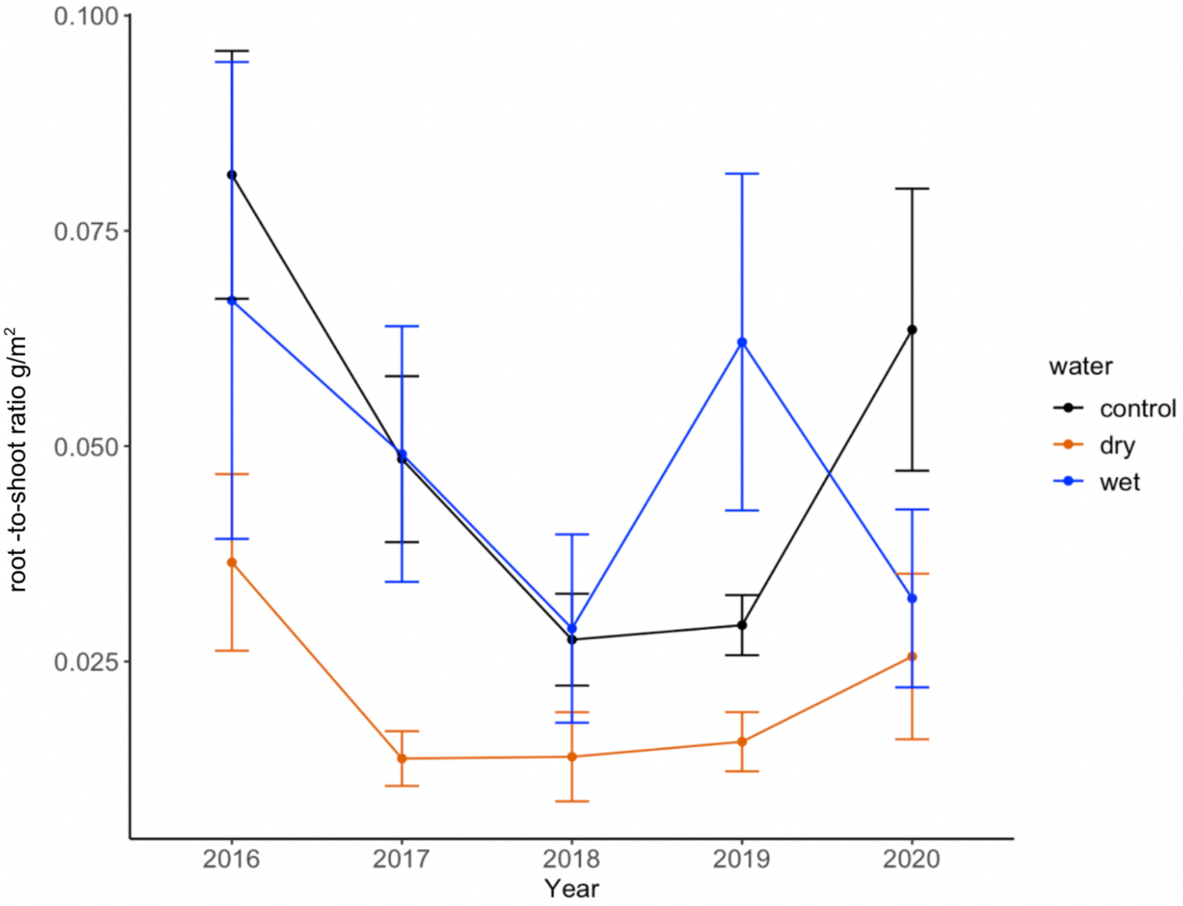

**Figure 1.1.** Root-to-shoot ratio of all plant species in unclipped plots from 2016 to 2020 for the three precipitation manipulation treatments and across plant community treatments (invasisve priority vs simultaneous arrival). Values are mean ± 1 s.e..

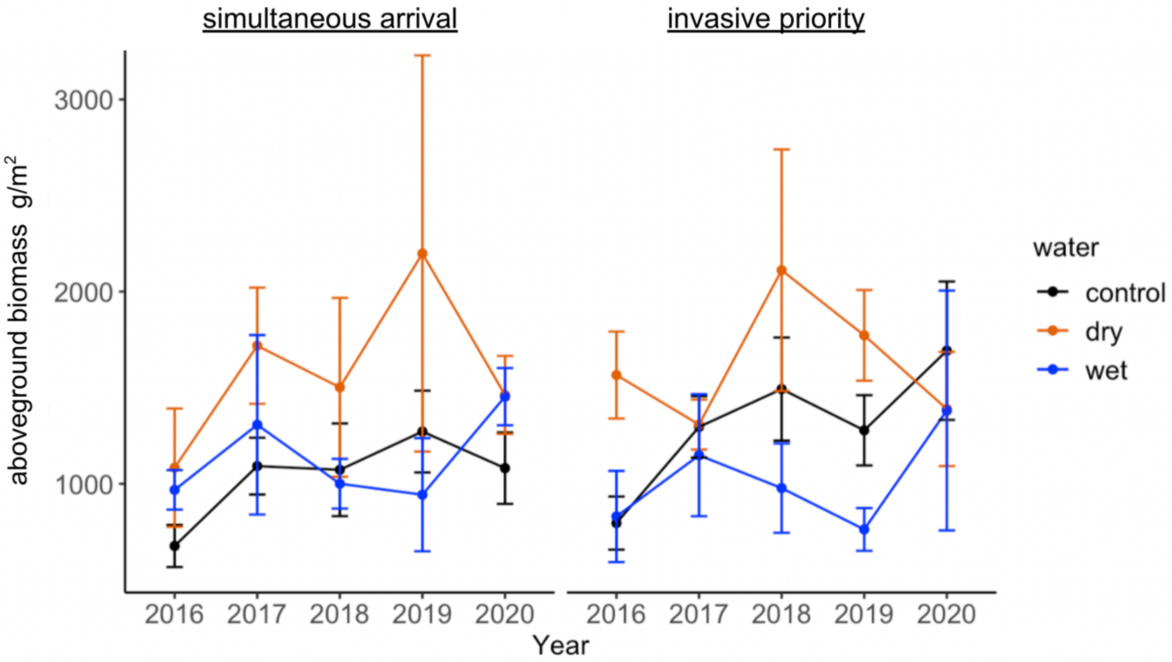

**Figure 1.2.** Aboveground biomass of all plant species from 2016 to 2020 in unclipped plots for the three precipitation manipulation treatments in two priority treatments (invasive priority, simultaneous arrival). Values are mean ± 1 s.e..

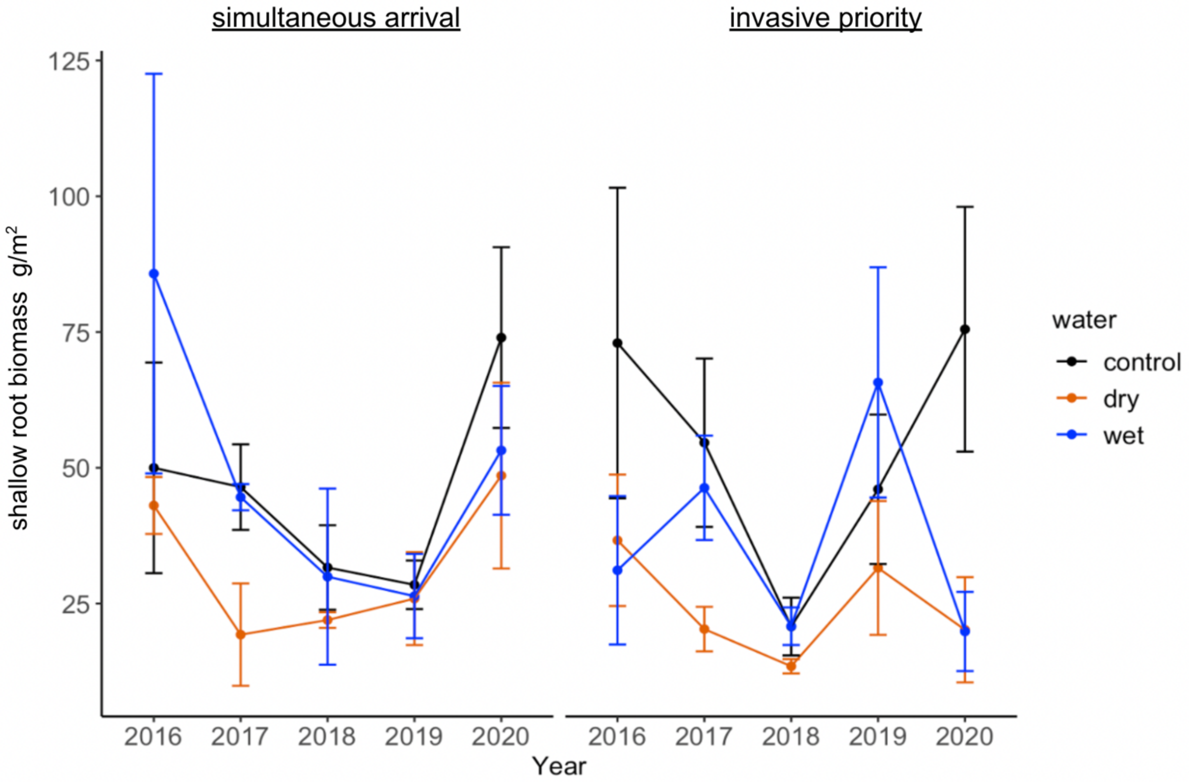

**Figure 1.3.** Shallow root biomass (0-15cm depth) of all plant species in unclipped plots from 2016 to 2020 for the three precipitation manipulation treatments in two priority treatments (simultaneous arrival, invasive priority). Values are mean ± 1 s.e.

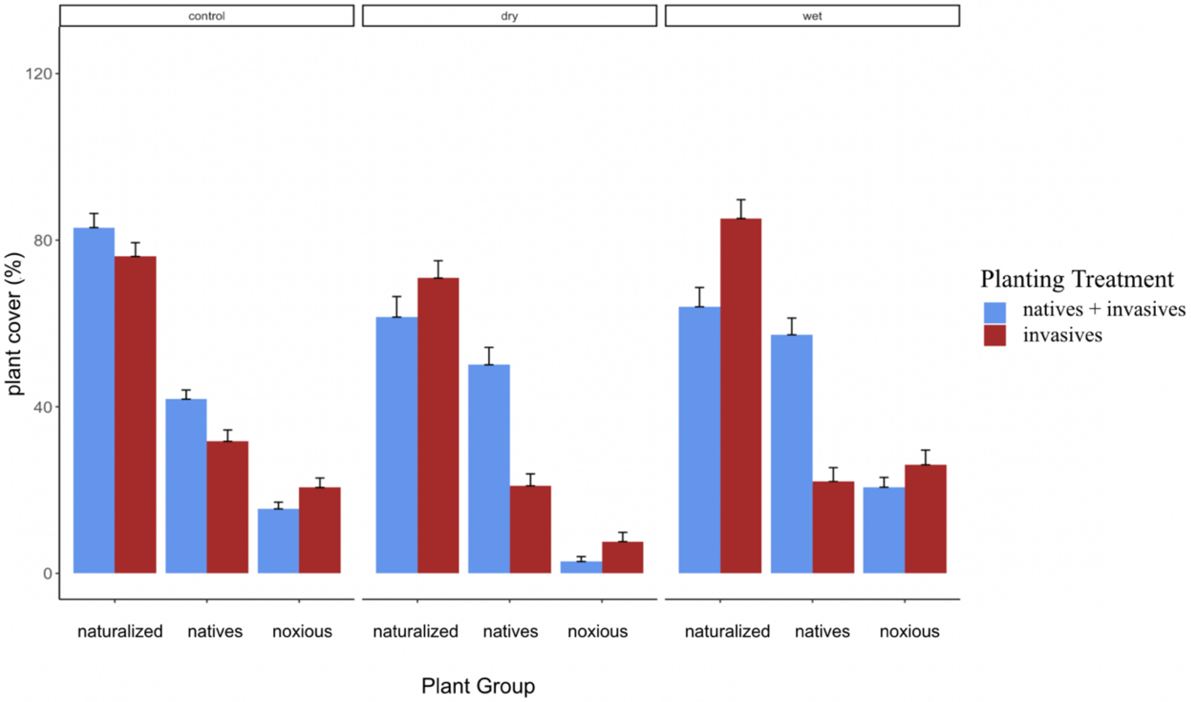

**Figure 1.4.** Plant cover for three plant functional groups (native, naturalized annual, and noxious annual plants) across all treatments: three precipitation manipulation treatments, two clipping treatments, and two priority treatments (simultaneous arrival, invasive priority).

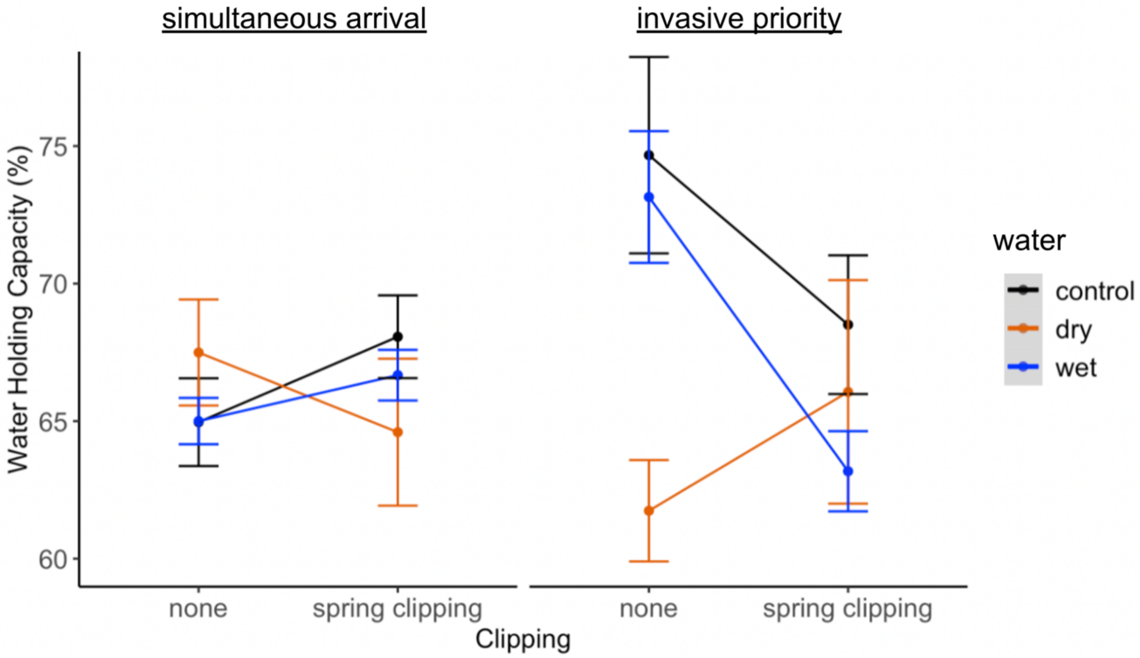

**Figure 1.5**. Water holding capacity of soil in 2020 for three precipitation manipulation (wet, dry, control) treatments in two priority treatments (simultaneous arrival, invasive priority). Values are mean ± 1 s.e.

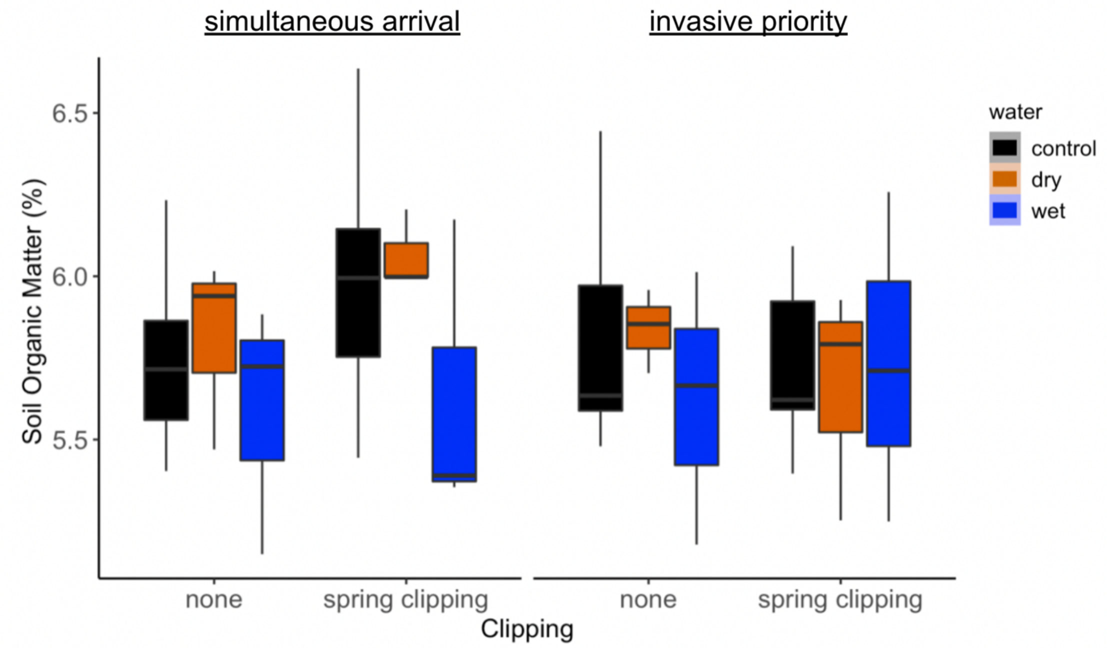

**Figure 1.6**. Soil organic matter of soil collected in 2020 for three precipitation manipulation treatments in two priority treatments (simultaneous arrival, invasive priority).

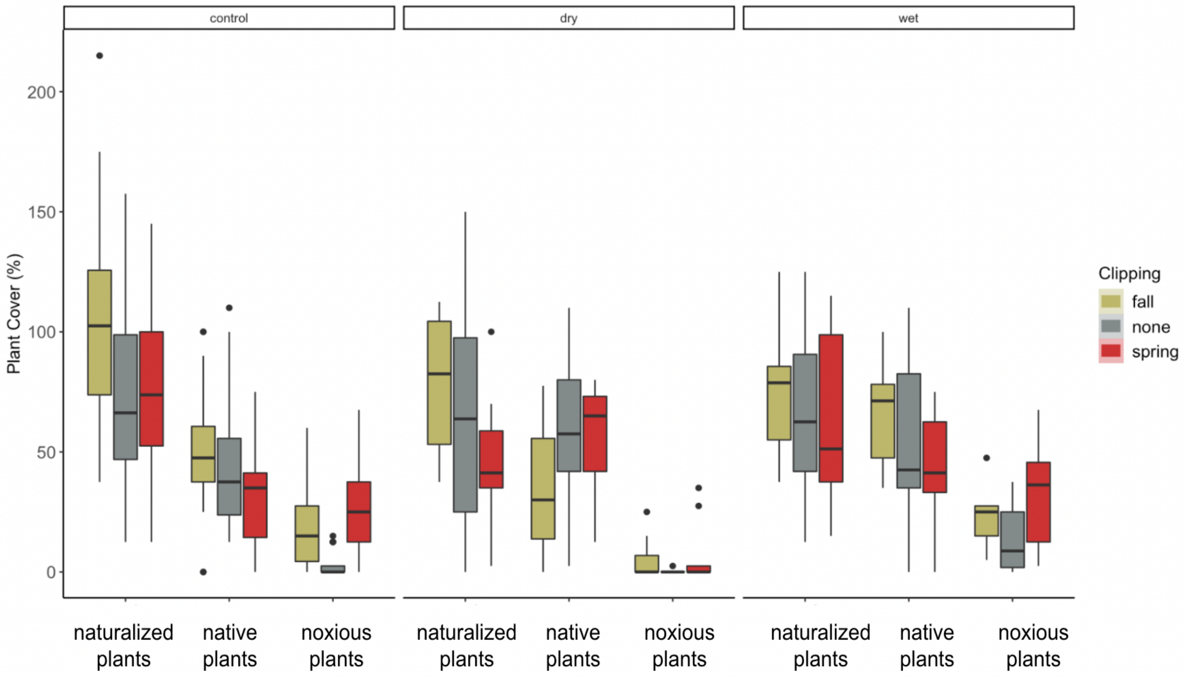

**Figure 1.S1.** Plant cover of native, noxious, and naturalized in plots with simultaneous arrival of native noxious and naturalized species (2016-2019). Plots received clipping treatments (fall, spring, or control), and water treatments (wet, dry, or none).

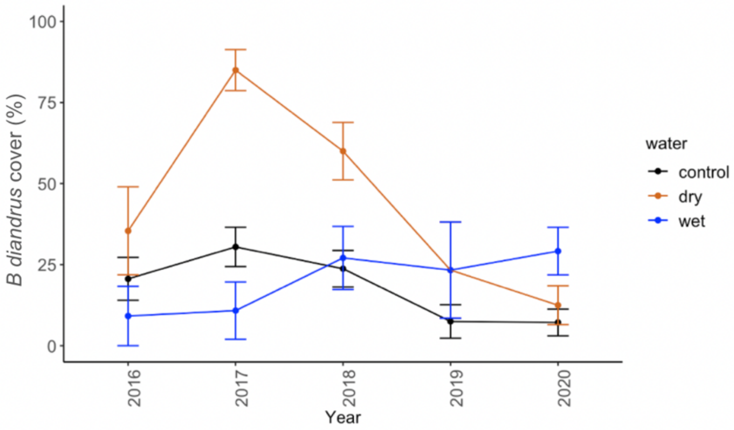

**Figure 1.S2.** *Bromus diandrus* plant cover for three precipitation manipulation treatments from 2016-2020. Values are mean ± 1 s.e.

## CHAPTER 2

# Clipping and Native Planting Do Not Modify Community and Ecosystem Response to Nitrogen Depoition

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## Abstract

Global atmospheric nitrogen (N) deposition rates have surged due to anthropogenic activities and are projected to continue rising, posing significant threats to ecosystem structure and functions such as invasive species spread and biogeochemical cycling. Many grassland ecosystems, including those in California, are heavily invaded by exotic species, and restoration efforts like clipping and native planting are employed to mitigate these invasions and other environmental disturbances. However, the effectiveness of these treatments under high N deposition remains unclear. In this study, I analyzed a 14-year N fertilization experiment in a California annual grassland to assess community and ecosystem responses to high N deposition, with and without annual spring clipping, and under two different planting treatments: (1) invasive species only and (2) native and invasive species mix (planted in 2007). Surprisingly, neither plant cover nor biomass (aboveground or belowground) responded to N fertilization, indicating that the community was already saturated with N. However, N fertilization significantly increased soil nitrification (from 1.44 mg N $gDW^{-1}$ to 20.72 mg N $gDW^{-1}$) and net mineralization (from -17.59 mg N $gDW^{-1}$ to 9.68 mg N $gDW^{-1}$). While clipping did not modify community or ecosystem responses to fertilization, it independently reduced native plant cover from 47% to 29%, increased noxious weed cover from 5% to 16%, and increased deep root biomass (15-30cm) by 20% across all fertilization treatments. Clipping had no significant impact on the cover of naturalized annuals or soil nitrogen cycling rates. The native mix planting from 2007 did not alter the community's response to fertilization but had a lasting influence on community dynamics. When natives were included in the planting mix, native plant cover increased from 32% to 44% by 2020. These results suggest that while

long-term N deposition may not directly affect plant community composition or biomass, it can significantly alter soil N fluxes. Additionally, native planting and clipping treatments may not be sufficient to mitigate the impacts of increased nitrogen deposition on N cycling rates, which could lead to reactive N losses into groundwater and the atmosphere. However, incorporating native species into planting mixes can have lasting positive effects on community structure, as invasive plants can exhibit priority effects, modifying or preempting niches that make it more difficult for native plants to establish. Further research is needed to explore alternative restoration practices that can effectively counteract the adverse effects of increased atmospheric N deposition.

## Introduction

Grasslands have been degraded by human activities and are one of the least protected biomes globally (Hoekstra et al. 2005), thus restoration is necessary to maintain or improve ecosystem functions and biodiversity. However, other environmental change drivers can make restoration difficult, especially if management efforts are not continuous. In addition, historical reference sites can no longer be used as targets for restoration projects because their abiotic environments and the species that inhabit them have changed. Grassland management often implements disturbances, such as grazing or mowing, to control non-native species in restoration, but results vary and can depend on plant community composition and abiotic factors (Harrison et al. 2003, Pavlů et al. 2007, Török et al 2021). This means that the outcomes of management disturbances might change with environmental changes such as increased N deposition. Understanding how to best manage grasslands in a changing world requires long-term studies that monitor ongoing restoration treatments because the impacts of management and environmental changes may take time to appear.

Increasing atmospheric N deposition is an important global change driver that affects grassland plant communities and restoration efforts by facilitating non-native species and changing belowground biogeochemical processes that are parts of the soil N and C cycles (Lu et al. 2014; Niu et al. 2016, Guo et al. 2017). Many non-native grasses are annual, fast-growing, and resource acquisitive species that flourish from N additions. N deposition rates increased from 34 Tg N per year in 1865 to 100 Tg N per year in 1995, and rates are expected to increase to 200 Tg N per year by 2050 (Galloway et al. 2008), largely due to anthropogenic causes like fossil fuel combustion and use of N fertilizers (Vitousek et al. 1997, Gruber and Galloway 2008). N deposition has altered community structure by decreasing species diversity, increasing dominance of non-native species (Hobbs and Huenneke 1992, Bobbnik et al. 1998, Clark & Tilman 2008), and increasing productivity (Vitousek & Howarth 1991). These non-native plants also provide fuel that can increase the severity of fires, especially in California grasslands (Brooks et al. 2004). Restoration methods to counteract non-natives, such as manipulation of priority effects to promote native species, have had varying success (Stuble & Sourza 2016, Weidlich et al. 2021), with outcomes depending on the environment and changing over time. N deposition may contribute to these inconsistent outcomes by creating conditions that overwhelm native plants and alter ecosystem function, but determining this requires long-term experiments manipulating environmental drivers like N deposition and drought.

Fertilization and disturbance can have opposing effects on plant community structure. Increased N facilitates fast growing species (Caldwell et al. 1985, Huenneke et al. 1990, Adler et al. 1998) like many of the invasive grasses dominating California grasslands. N fertilization that increases a plant's biomass and height gives a competitive advantage for other resources such as space and light (Grime 1973, Hautier et al 2009, Fang et al. 2012). However, some physical disturbances can reduce invasive species biomass to free up resources for native plants (Hobbs and Huenneke 1992, Jauni et al. 2015), so disturbance is frequently used in restoration to tackle species invasions and increase species richness. Although this favors plants with conservative traits that make them more resilient to disturbances (Lavorel et al. 1997), populations of less disturbance-resilient invasive annual plants may recover quickly due to abundant seed production. Clipping is a management technique used to reduce species dominance (Germano et al. 2001), especially in communities experiencing N deposition because it reduces the competitive pressure on native plants with conservative traits. However, clipping results depend on the plant community (Kimball & Schiffman 2003), so there is a need to understand the levels of disturbance required to combat the dominance of invasive plants that are also facilitated by global N deposition.

Because global change, and the restoration management actions carried out in response, cause shifts in plant community composition, they also likely alter belowground ecosystem functions like belowground productivity, soil N cycling, C storage, and water storage. The extent to which plants regulate soil ecosystem processes is difficult to determine because of confounding variables in experiments including environmental parameters, soil organisms, and study length. However, many studies have shown that soil processes and microorganisms are largely regulated by plants (Stone 1975, Hooper and Vitousek 1998, Windham and Ehrenfeld 2003, Corbin and D'Antonio 2004). Functional trait groupings can explain plants' roles in soil function and primary production (Fornara and Tilman 2008, Du et al. 2018, Fischer et al. 2019), but most studies categorize plants by growth form (i.e., grass, forb, legume). Other characteristics such as life history, phenology, and growth rate can affect ecosystem function, so understanding how global changes differentially affect these functional groups can help predict community structure and plant-mediated ecosystem functions.

In California grasslands, the region has been subject to multiple waves of invasion that include an early wave of Mediterranean grasses now deemed naturalized and near impossible to eradicate. These species are annual grasses with rapid growth rates and early phenology. A second wave includes *Aegilops triuncialis* and *Taeniatherum caput-medusae,* two noxious invaders that are severely altering community structure with detrimental economic and agricultural effects. These species are also particularly deleterious to species richness due to being newer in the invasion process.  In addition to native perennial plants, these functional groups respond differently to environmental stress and differ in their effects on plant diversity, soil ecosystem processes, and microbial communities (Fornara and Tilman 2008, Du et al. 2018, Fischer et al. 2019).Traits such as longevity, phenology, and growth rate can affect competition for resources: Leffler et al. (2013) showed that a common native perennial grass differed in N uptake rate compared to an annual naturalized early season grass (*Bromus tectorum)* and a noxious late-season annual grass (*Taeniatherum caput-medusae).* Fertilization may favor naturalized invasive species because their shallow roots, fast growth rates, and early phenology allow them to exploit resources sooner than other plants (Kimball et al. 2011, Wolkovich and Cleland, 2011). Noxious plants, although likely to be favored over deep-rooted perennial grasses, should be favored second to naturalized plants due to a later phenology. If the community is dominated by naturalized and noxious annuals, this may also increase N cycling rates because these plants have greater N uptake rates and tissue quality than perennial grasses (Leffler et al. 2013). The increase in plant biomass following fertilization should also change the C:N ratio in plant inputs to the soil (Liu et al 2013), which could have large implications for biogeochemical cycles. Planting native plants early in restoration is important because priority effects, the order of species arrival, can have lasting effects on plant community composition and plant biomass (Körner et al. 2008, Weidlich et al. 2018) and invasive plants may respond more favorably to priority effects (Stuble & Souza 2016).

In this study, I examined how N fertilization can affect plant productivity, N cycling rates, and C storage, and if these effects are mediated by invasive priority and clipping management. This study was part of a long-term N deposition experiment established in 2008 in a California valley grassland (Carey et al. 2017).. N fertilization and clipping treatments were used to determine effects on plant productivity, N cycling, and C storage among functional groups and test the following questions: (1) How does N deposition affect plant communities and ecosystem functions? (2) Are these effects mediated by priority effects? (3) Can clipping management mediate effects of fertilization?

## Methods

### *Experimental plot details*

This study took place in at the UC Davis Campbell Tract Experimental Site in Davis, CA (38.545751, -121.784780). Prior to plot establishment, the land was used for agricultural purposes and then lay fallow for twenty-two years. Soils were primarily the Reiff series (75%, coarse-loamy, mixed, superactive nonacid, thermic Mollic Xerofluvents) with a lesser amount of the Brentwood soil series (25%, fine, smectitic, thermic Typic Haploxerepts) (USDA Web Soil Survey, http://websoilsurvey.sc.egov.usda.gov). This is a semi-arid environment that experiences warm, dry summers (mean maximum temperature 33°C)   and wet, cold winters (mean maximum temperature 14°C) that correspond with the growing season of cool season grasses. Rainfall has high inter- and intra-annual variability, with mean annual precipitation of 445 mm (1990-2019). In the year of this study (fall 2020-spring 2021 growing season), temperature ranges were -1.7 C to 22.8 C in January and ranged from 22.8C to 41.7 C in July 2021, and rainfall was very low, at only 187 mm. Common native  plants in this area are largely perennial bunchgrasses such as *Stipa pulchra,* and *Elymus triticoides*. Some exotic annual species (*Bromus hordeaceus*, *Avena fatua*, *Festuca perennis)* invaded centuries ago and are part of the natural landscape. These species are referred to as naturalized. *Elymus caput-medusae* and *Aegilops triuncialis,* are part of a new wave of noxious plants that invaded decades ago and are some of the most invasive plant species in grasslands that are displacing native plants, reducing plant diversity, and altering nutrient cycles and fire regimes (Davies and Svejcar 2008, Kyser et al. 2014).

This experimental study was set up in fall 2007 using 48 plots that are 1.5x1.5m in size, separated from one another with a 1m buffer. Plots were laid out in a randomized block design (8 blocks with 6 plots in each block) To assess the influence of species arrival order (priority effects) on community composition, particularly in relation to invasive plants, plots were seeded with equal masses of one of two community types: (1) a mix of native species, naturalized annuals, and noxious annual invaders, or (2) only naturalized annuals and noxious annuals (hereafter referred to as invasive priority plots) (see Table 4 for species list). The naturalized annuals used in this study are exotic species that have been present in California grassland communities for decades to centuries (Jackson 1985). In contrast, noxious annual invasive plants represent a newer wave of exotics that were first recorded in the late 1800s and early 1900s, and began to reach high abundances in some locations by the late 20th century (Peters et al. 1996, Bossard et al. 2000, D'Antonio et al. 2007). Plots were exposed to factorial clipping and fertilization. Clipping treatments included clipped plots or unclipped controls. Clipping treatmets were applied each spring at the time of peak growth (roughly mid-March to early April) with electric grass clippers, which should presumably limit seeding of non-native grasses due to limited soil moisture to support regrowth.

This was meant to mimic mowing treatments for invasion control in restoration, and certain aspects of seasonal grazing. Fertilization treatment consisted of plots receiving N fertilization of 45 kg N/ha each year to mimic future rates of atmospheric N deposition, or unfertilized control plots. Nitrogen was added as aqueous ammonium nitrate solution with N delivered in 500ml of water per application. N additions began in the 2008-9 growing season (after 1 year of plant establishment) From 2008 through the 2017-18 field season, N was applied in 3 equal applications through the rainy season (generally late December, early February and mid-March, with timing adjustments based on timing of grass seedling establishment), except for the 2013-14 growing season, which was shorter due to drought, so the full rate of N addition was split across 2 applications rather than 3. Due to more frequent droughts and shorter rainy seasons, from fall 2018 on, . was administered in 2 equal applications per year (generally December and early February). .

### *Measurements*

Plots were sampled in spring 2020, towards the end of the 13th growing season after plot establishment (and 12th year of N fertilization). Vegetation composition was measured as percent cover of each species, using a modified Daubenmire method, with bins of: 0-5%, 5-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, and 90-100%. Cover assessments occurred twice per spring, in late March/early April to capture much of the forb cover (e.g. clovers, lupine), and in late May-mid-June to capture grass species cover. For species identified at both time points, the cover of the time point with the highest percent cover for that species was used. Belowground and aboveground biomass was collected in April 2020. Aboveground biomass greater than 4cm from the ground was collected using scissors. Belowground biomass was collected using a soil corer that is 1.9 cm wide to a depth of 0-15 cm for shallow root biomass, and 15-30 cm for deeper root biomass.

In December 2020, a 180 mL soil sample was collected from the to 15 cm using a soil corer with a diameter of 1.9 cm. These samples were used for analyses of N cycling rates. Net rates of N mineralization and nitrification were measured by weighing out each field moist soil sample to 5g into a labeled specimen cup twice (one for initial and one for final N concentrations after incubation). For the initial set of samples, a 25 ml KCl solution was added and samples were shaken for one hour at 175 rpm on an orbital shaker. After the sample settled, the supernatant was filtered through Whatman No. 1 filter paper and collected into sample vials. Samples were stored at -20C until analysis on a UV spectrometer. The second set of samples ("finals") were incubated in a covered container with no light exposure for 7 days, and then extracted similar to the initial samples. Analysis was done on a UV spectrometer using Doane & Horwarth (2003) procedure for determining NO3 and Forster (1995) procedure for calculating NH4. For NH4 analysis, two reagent solutions were added to sample extracts: sodium nitroprusside and sodium hypochlorite. For NO3 calculations 0.5M Vanadium Chloride solution was added to samples to reduce nitrate to nitrite. Net mineralization was determined from the difference in inorganic N between the incubated sample (finals) and the initial set of samples. Net nitrification was calculated as the difference between incubated (final) and initial NO3.

### *Statistical analysis*

All data were analyzed using a linear mixed model with block as a random factor and fertilization, clipping, planting treatment and the 2-way interactions between fertilization x planting treatment and fertilization x planting treatment as fixed factors. An interaction between clipping and planting treatment was not analyzed in this study due to insignificant effects seen in similar plots in a previous study (Traylor et al. *in prep*.. Fertilization and priority interactive effects were not analyzed for shoot biomass due to missing samples. Models were analyzed using the "type III" test with Anova() in the car package (Fox & Weisberg 2011).

## Results

Fertilization alone did not affect native, noxious, or naturalized plant cover in 2020, after 12 years of annual fertilization additions (Figure 2.1, Table 2.1). This treatment also had no effect on shallow root, deep root, or shoot biomass (Figure 2.2). Fertilization additions did increase soil net nitrification from

1.44 mg N gDW$^{-1}$ to 20.72 mg N gDW$^{-1}$ and net mineralization from -17.59 mg N gDW$^{-1}$ to 9.68mg N gDW$^{-1}$ (Figure 2.3, Table 2.3).

The interaction between clipping and fertilization did not affect plant cover, biomass, or soil nitrification or mineralization. Clipping under all treatments decreased native plant cover from 47.42%(+/-4.93) to 28.59% (+/-3.83), increased noxious plant cover from 4.84% (+/- 1.40) to 15.78% (+/- 2.83), and had no effect on naturalized annuals (Figure 2.4, Table 2.1). Clipping alone increased deep root biomass by 20% from 37.18g/m$^2$ in unclipped to 45.12 m$^2$ in clipped plots but had no effect on shallow root biomass (Figure 2.5, Table 2.2). Clipping did not influence soil net nitrification or mineralization.

The interaction between fertilization and planting treatment had a moderately significant effect on native plant cover where invasive priority decreased native plant cover from 46% (+/- 6) to 27% (+/- 7) under high fertilization (Figure 2.6). Invasive priority effects alone did decrease native plant cover from 44% (+/- 4) to 32% (+/- 5) (Table 2.1) and increased net mineralization from -9.77 mg N gDW$^{-1}$to 1.87 mg N gDW$^{-1}$ (Figure 2.7).

## Discussion

Understanding the impact of N deposition on grassland ecosystems is essential, given that humans have increased atmospheric N deposition to levels that have caused plant invasions and biodiversity loss (Vitousek et al. 1997, Galloway et al. 2004). Many grasslands require heavy management due to invasive annual grasses, many of which are highly responsive to elevated soil N (DeFalco et al. 2001, Brooks 2003), and this issue can be exacerbated under high N deposition (Clark 2019, Allen et al. 1998). Plant and ecosystem responses to N deposition will affect the success of current restoration treatments, so understanding these responses can be useful in adjusting management strategies aimed at reducing invasive species and promoting diverse native communities. This study looked at experimental communities with and without invasive priority to determine how they respond to N inputs. I predicted that fertilization would promote plant productivity and invasive cover due to the resource acquisitive nature of invasive annual grasses. I also predicted that N fertilization would increase rates of nitrification and mineralization, with more marked increases in the invasive priority plots where invasive plants were more abundant due to N uptake rates and tissue quality. I also predicted that clipping management would buffer effects of fertilization on plant communities and soil ecosystem function by increasing native plant cover and reducing nitrogen cycling rates. I expected this would be due to native plants' conservative traits that allow them to be resistant to disturbances. I found that fertilization did increase rates of soil N cycling in soils, which may have negative environmental implications if plants cannot efficiently use N at high levels and results in reactive N losses or eutrophication. Clipping did not alter plant biomass or soil nitrogen responses to fertilization but did alter plant communities. Clipping decreased native cover and increased noxious weeds. This study shows that N deposition can alter rates of biogeochemical cycling and that different land management strategies other than spring clipping treatments might be needed to mitigate these biogeochemical effects in a high-N world.

### *Plant communities*

Plant community composition, as measured by relative abundance of native, naturalized, and noxious species, was not affected by 14 years of fertilization. The plants in these communities may already be adapted to high N deposition and have reached a threshold where they are saturated by N (Niu et al. 2016, Bobnick et al. 2010, Zong et al. 2019, Aber et al. 1989). This can cause more N loss into the ecosystem than what is obtained by plants (Niu et al. 2016). Although native and invasive plants did not respond differently to N deposition, with increased N losses to the environment this may change over time. N deposition can also reduce plant N use efficiencies, which could amplify N losses to the environment (Egan et al. 2019). Other environmental factors like precipitation and herbivory could also be stronger drivers of plant community composition and productivity and override effects of N deposition disturbance. It is likely that, over time, invasive species will have a greater response to N deposition. Invasive species in this study, like *Bromus* spp. and *Avena fatua*, have significant competitive advantages over native plants in disturbed environments because of their high growth rate, N use efficiencies, seed production, and generalist traits, and these advantages may be stronger under high N conditions (Vasques et al. 2008, Carlson & Hill 1985). Precipitation is likely a stronger driver of vegetation dynamics in these grasslands which would diminish the impact of fertilization (Cleland et al. 2013), but this impact would be erased under prolonged drought.

Long-term plant composition was driven more by priority effects, with invasive plant priority reducing native cover. Although priority effects can occur through niche modifications like preemptive use of soil N (Fukami 2015), it is not likely that this was the case in our study. I did not see changes in plant communities with added N, so invasive effects could have occurred through modifications of the soil environment, such as chemical changes, allelopathy, or changes in the soil microbial community that facilitate invasives (Klironomos 2002, Batten et al. 2008). Another mechanism of priority effects is niche preemption: when invasives arrive first (priority effects), they may outcompete natives for other limited resources besides N such as space or light. In California grasslands, native plant establishment is reduced by niche preemption by the high density of invasive seedlings that outcompete natives for resources like space and light (Abraham et al. 2009, Weidlich et al. 2021, D’Antonio et al. 2016).

There was a slight but nonsignificant positive effect of fertilization on noxious plants cover. These plants are new invaders (<100 years) that may not be adapted to these generally low N soils (Peters et al. 1996, Bossard et al. 2000, D’Antonio et al. 2007). If they are not adapted to low-nutrient soils like most invasives in California grasslands with low N requirement (Jackson et al. 1998), then they could respond well to fertilization even after 10 years. This trend could indicate that they may drive community shifts in the future under high atmospheric N deposition levels. Critical N deposition loads in plants can be up to 150 kg N/ha/year, which is three times more than the amount used in this study (Peng et al. 2019). Noxious plants had the lowest cover of the three groups and are likely to have difficulty competing with naturalized and native species even under high N conditions. However, because many natives and naturalized plants did not respond to long-term N fertilization, increases in atmospheric N deposition could create a new niche, allowing mainly noxious plants to thrive.

### *Soil effects*

Fertilization increased soil nitrification and mineralization rates. This could increase reactive N losses that cause negative impacts on ecosystems, including polluting waterways, acidifying the soil, affecting biodiversity, and increasing the emission of nitrous oxide, a greenhouse gas. A previous study in similar plots showed that nitrogen fertilization responses after 5 years also resulted in higher nitrate availability and nitrification potential (Carey el a. 2015). An increase in N release from the soil might be triggered by higher levels of dissolved organic N and total soil N, along with a lower C:N ratio in the soil (Chapin et al. 2009, Barrios et al. 1996). Fertilization did not affect composition of the three plant groups, indicating that the relative abundances of these groups is not driving soil nitrogen cycling. Although Caret et al. (2017) found that noxious weeds decrease nitrate and nitrification potential compared to naturalized and native plants. Increased soil nitrification and mineralization can result from increased microbial activity such as increased activity of N-acetyl-β-D-glucosaminidase (NAG) or other enzymes (Wang et al. 2018). Changes in microbial activity could also alter organic matter breakdown and reduce the soil's potential for storing C (Waldrop et al. 2004, Lange et al. 2015) which is a critical function in grassland ecosystems and important for mitigating impacts of climate change.

Clipping was expected to modify invasive plant response to fertilization, but I did not see these effects. Naturalized species showed resilience after 14 years of fertilization and 11 years of clipping treatments by maintaining high cover and remaining the dominant species. This likely contributed to the stability of plant above- and belowground biomass because invasive species can recover quickly after these disturbances (Kotanen 2004, Seabloom et al. 2003). Further, this may have suppressed any positive response of native species to clipping treatments, so clipping may not cause as great a disturbance as needed to benefit conservative native species.  This is consistent with other studies finding that clipping does not benefit native perennial grasses and is not sufficient enough to reduce invasive grasses (Hayes & Holl 2003, Stromberg et al. 2007, Lulow 2008). In this way, the resilience of these invasive species can cause challenges for management efforts aimed at increasing biodiversity. Additionally, this resilience can lead to communities dominated by invasive grasses that are vulnerable to severe fires, exacerbating the abundance of invasive grasses due to their abundant seed bank (D'Antonio et al. 2002, Menke 1992).

Therefore, increasing biodiversity in these grasslands may require additional targeted management than clipping alone.

### *Conclusions*

Given current rapidly increasing rates of N deposition (Galloway et al. 2008), it is imperative to understand how plant communities and ecosystems will respond to this disturbance. N deposition did not strongly affect plant communities long-term as they likely became saturated with nitrogen. However, there were strong effects on soil N cycling which could have damaging effects on ecosystem functions, for example by causing reactive N losses into groundwater and the atmosphere. We also found some evidence that invasive dominated plant communities may increase soil N mineralization and a slight increase of noxious invaders with fertilization. Clipping management and fertilization did not interact to affect plant communities, but clipping alone was a strong driver of plant composition. Clipping increased noxious plant cover and decreased native plant cover, suggesting that the benefits of clipping may be limited in an already heavily disturbed environment. The lack of response of native species to clipping treatments indicates that a more targeted treatment would be needed to increase native plant abundance, especially under increased N deposition rates. However, the effects of N deposition on ecosystems extend beyond scope of this study. This includes interactions with precipitation that modify N cycling in plants, changes in microbial activity and biodiversity, nutrient imbalances in plant tissues, and exacerbating the negative effects of drought (Steven et al. 2011). These complex interactions underscore the need for a comprehensive understanding of N deposition effects on ecosystems. These effects could be researched in future studies on longer time scales to determine if there is precipitation or age that modifies microbial and plant species' response to N deposition.

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Table 2.1. Results of linear mixed effect model analysis of plant cover (native, noxious, and naturalized) in 2020.

| | Native Plant Cover (2020) | | | Noxious Plant Cover (2020) | | | Naturalized Plant Cover (2020) | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | $X^2$ | df | *P* | $X^2$ | df | *P* | $X^2$ | df | *P* |
| fertilization | 0.69 | 1 | 0.405 | 3.24 | 1 | 0.072 | 0.49 | 1 | 0.484 |
| priority | 11.47 | 1 | **<0.001** | 0.13 | 1 | 0.719 | 0.14 | 1 | 0.709 |
| clipping | 9.75 | 1 | **<0.001** | 23.50 | 1 | **<0.001** | 0.03 | 1 | 0.855 |
| fertilization x priority | 2.96 | 1 | 0.085 | 0.35 | 1 | 0.557 | 2.17 | 1 | 0.141 |
| fertilization x clipping | 0.02 | 1 | 0.888 | 0.12 | 1 | 0.730 | 0.00 | 1 | 0.963 |

Table 2.2. Results of linear mixed effect model analysis of plant below- and aboveground biomass in 2020.

| | shallow root biomass (0-15 cm) (April 2020) | | | deep root biomass (15-20cm) (April 2020) | | | live shoot biomass (April 2020) | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | $X^2$ | df | *P* | $X^2$ | df | *P* | $X^2$ | df | *P* |
| fertilization | 0.15 | 1 | 0.696 | 0.18 | 1 | 0.674 | 1.87 | 1 | 0.172 |
| priority | 3.02 | 1 | 0.082 | 1.51 | 1 | 0.220 | 4.43 | 1 | **0.035** |
| clipping | 2.76 | 1 | 0.096 | 5.07 | 1 | **0.024** | 13.02 | 1 | **<0.001** |
| fertilization x priority | 0.19 | 1 | 0.661 | 0.75 | 1 | 0.387 | - | - | - |
| fertilization x clipping | 0.46 | 1 | 0.498 | 0.08 | 1 | 0.775 | 0.20 | 1 | 0.657 |

Table 2.3. Results of linear mixed effect model analysis of soil net nitrification and net mineralization from soils collected in 2020.

| | Net Nitrification (2020) | | | Net Mineralization (2020) | | |
|---|---|---|---|---|---|---|
| | $X^2$ | df | p | $X^2$ | df | p |
| fertilization | 40.33 | 1 | **<0.001** | 33.42 | 1 | **<0.001** |
| priority | 1.88 | 1 | 0.170 | 7.90 | 1 | **0.005** |
| clipping | 0.00 | 1 | 0.309 | 0.04 | 1 | 0.847 |
| fertilization x priority | 0.01 | 1 | 0.108 | 1.58 | 1 | 0.208 |
| fertilization x clipping | 0.16 | 1 | 0.395 | 2.07 | 1 | 0.150 |

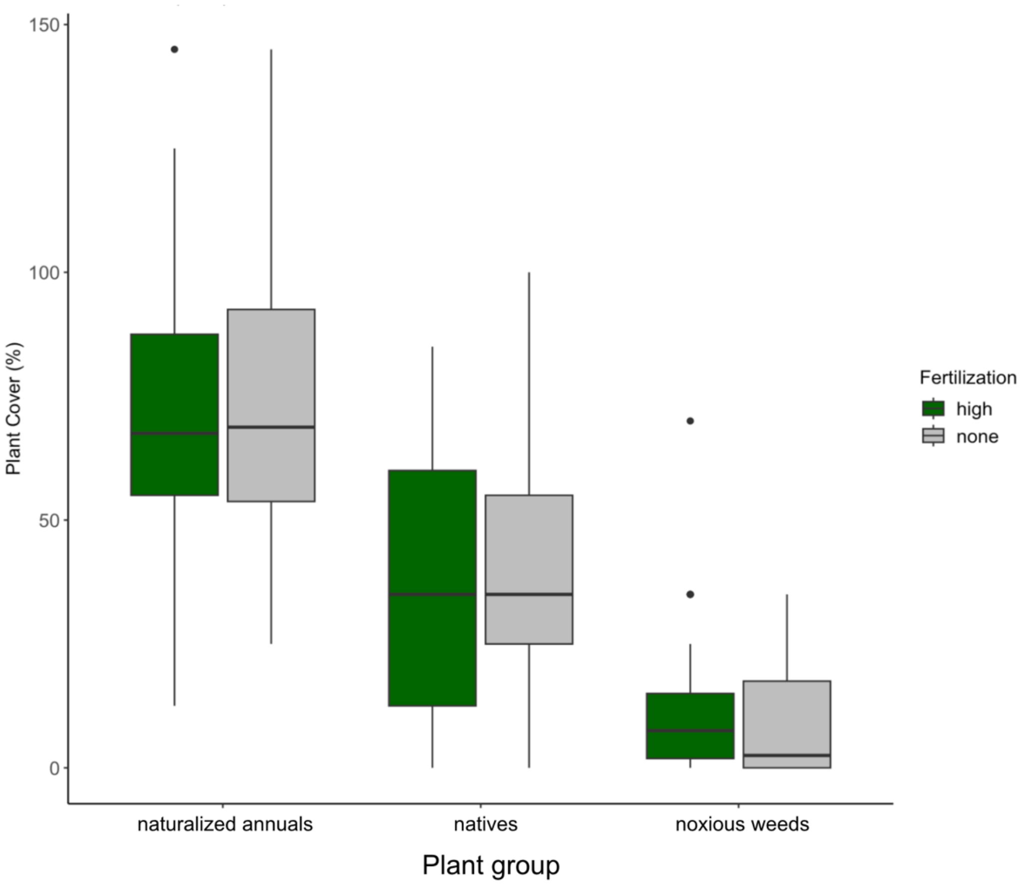

Figure 2.1. The effects of annual fertilization over 14 years on naturalized annual, native, and noxious plant cover in 2020 across all clipping and planting treatments. P>0.05 for all plant groups.

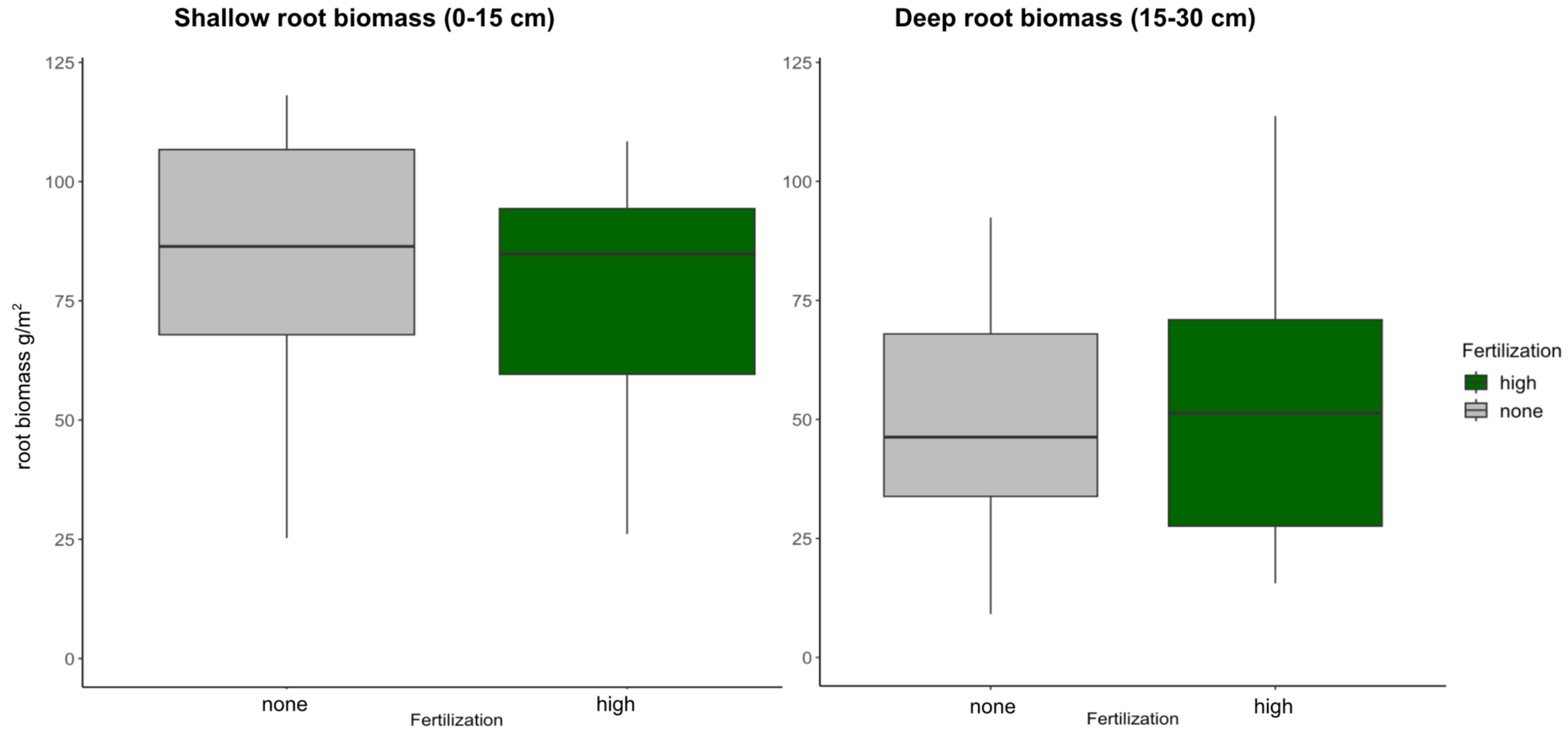

Figure 2.2. The effects of fertilization on shallow and deep root biomass across all clipping and planting treatments (p>0.05 for shallow and deep root biomass).

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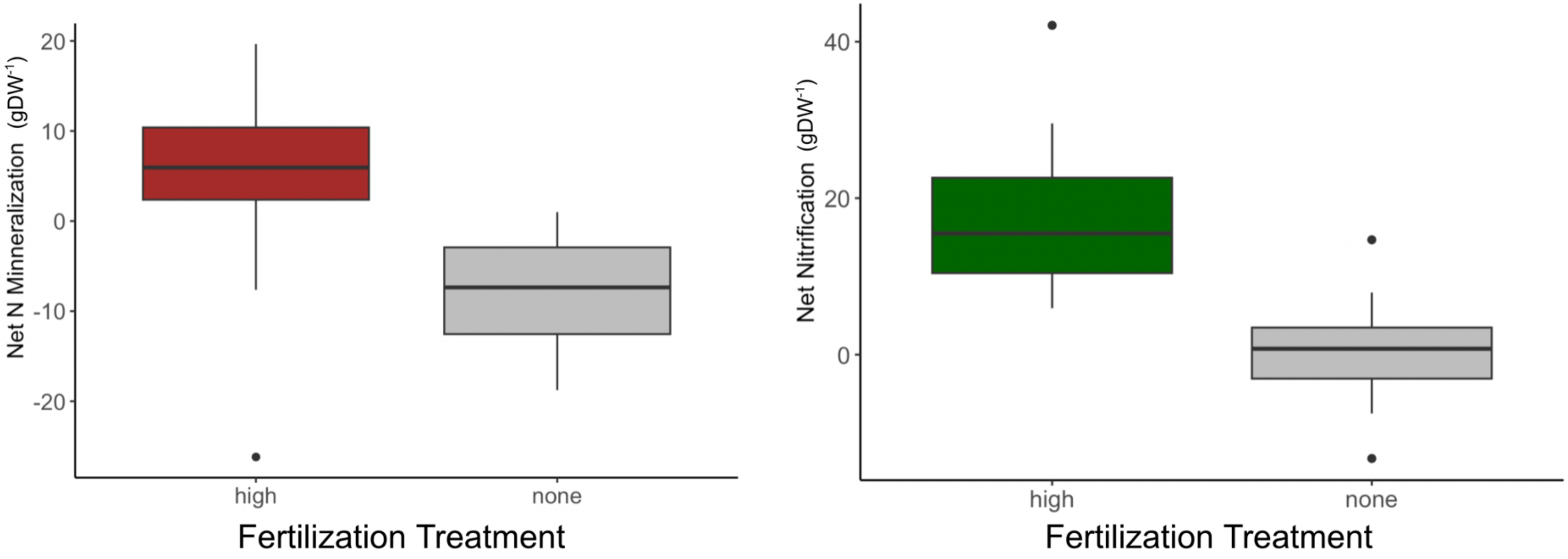

Figure 2.3. Soil net N mineralization (left) and net nitrification (right) of after 12 years of fertilization treatments.

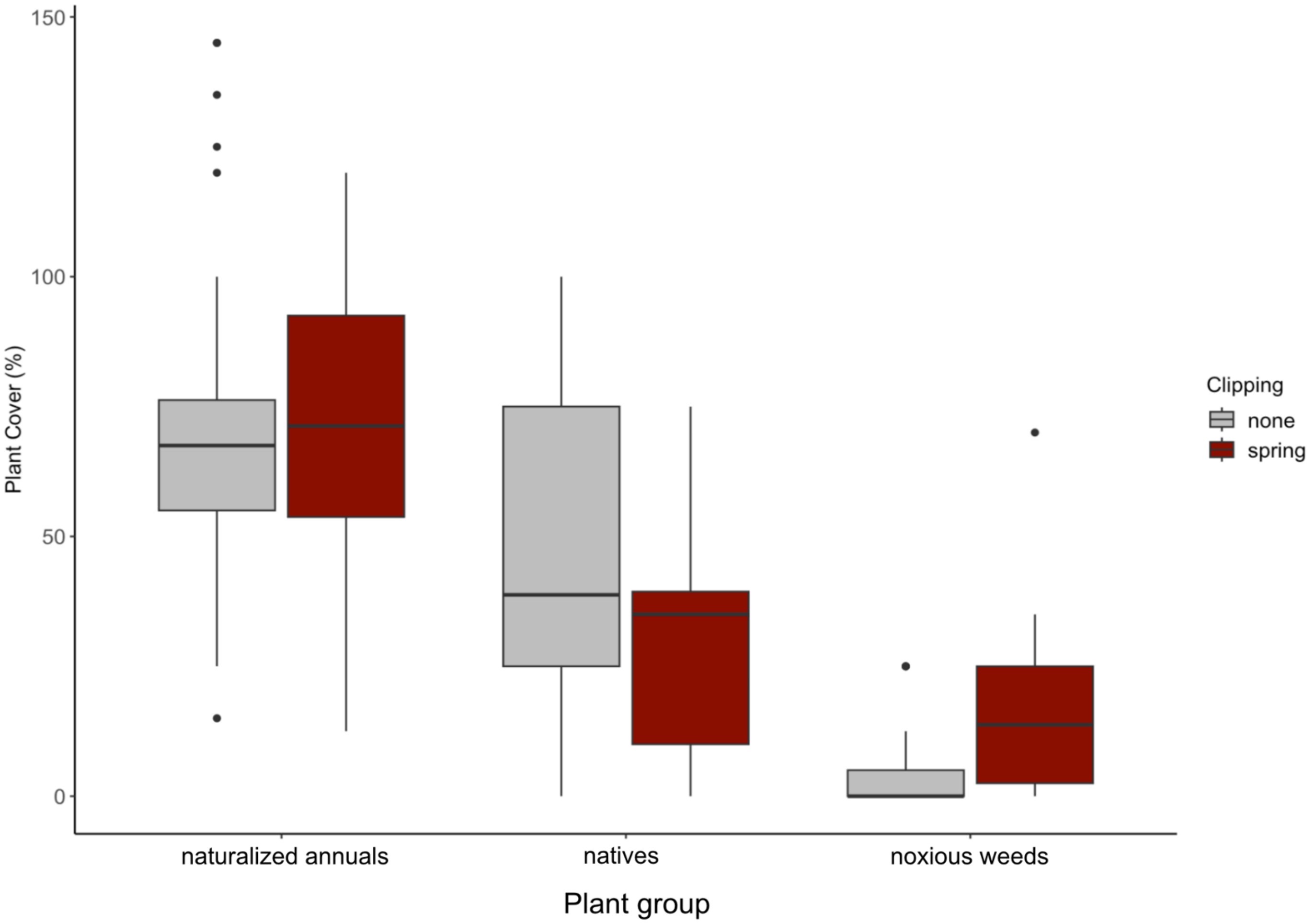

Figure 2.4. The effect of spring clipping on cover of three different plant groups across all fertilization and planting treatments ($p<0.05$ for native plants and noxious weeds, and $p>0.05$ for naturalized annuals).

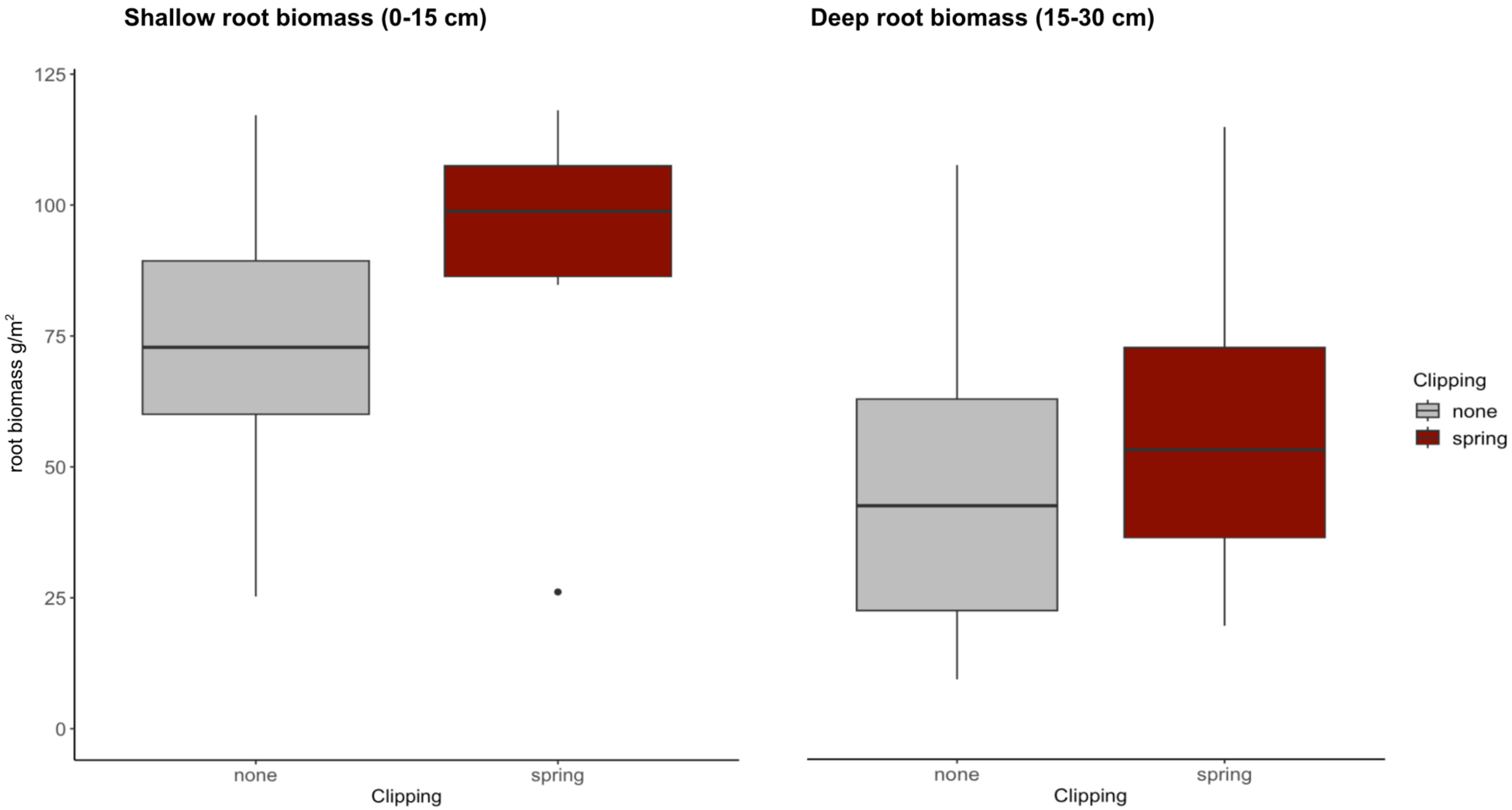

Figure 2.5. The effect of spring clipping on shallow (0-15cm) and deep (15-30cm) root biomass across all planting and fertilization treatments. P>0.05 for shallow root biomass and p<0.05 for deep root biomass.

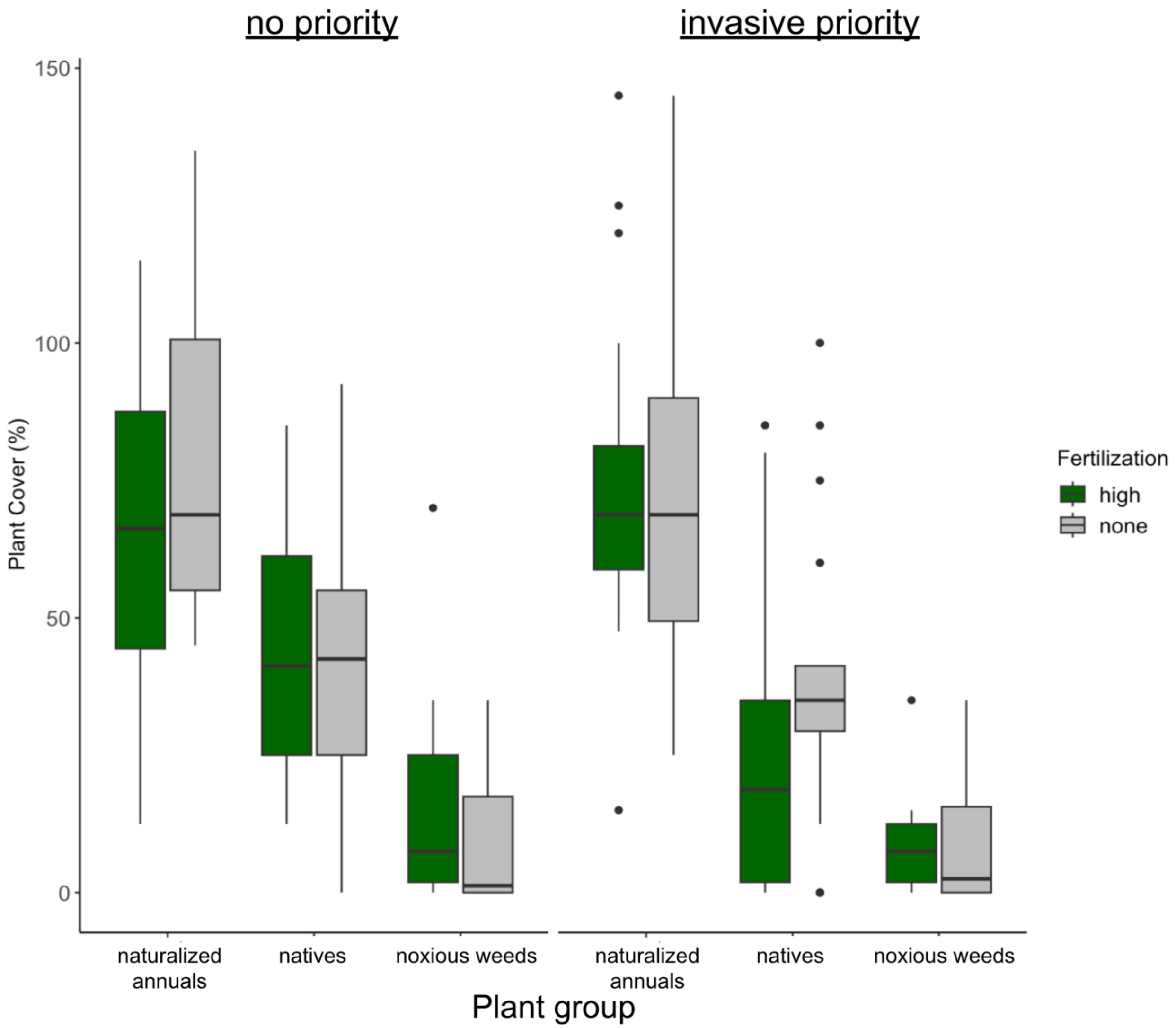

Figure 2.6. The effects of fertilization (high, none) and planting treatment (no priority and invasive priority effects) on naturalized annual, native, and noxious plant cover (across all clipping treatments). The interaction between fertilization and planting treatment on all plant groups has a p-value greater than 0.05.

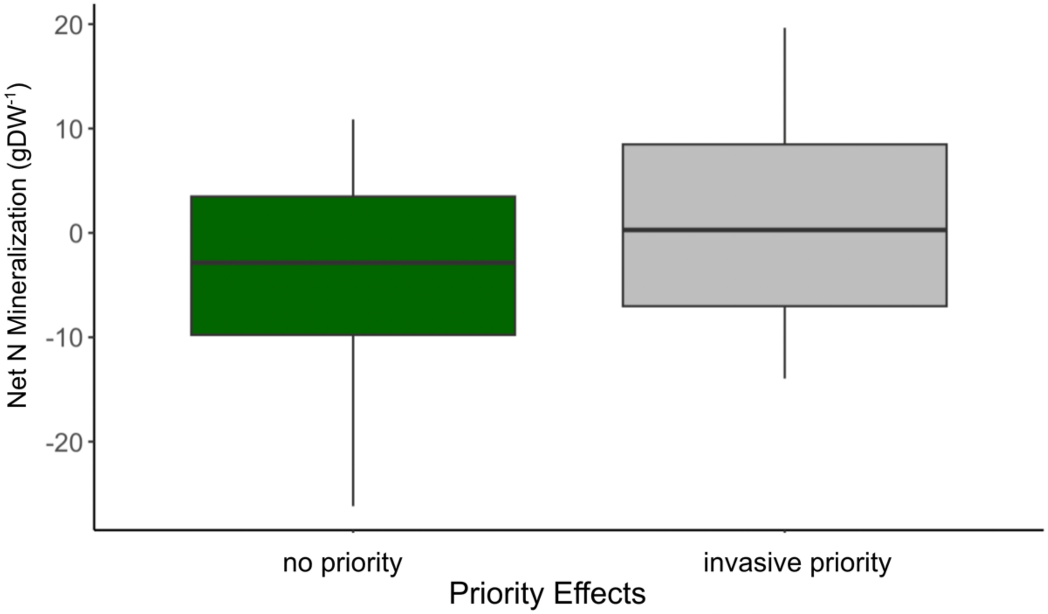

Figure 2.7. Soil net N mineralization increased from invasive priority effects.

**Natives**

*Acmispon americanus*
*Bromus carinatus*
*Elymus glaucus*
*Elymus triticoides*
*Lupinus bicolor*
*Festuca microstachys*
*Stipa pulchra*

**Exotic naturalized annuals**

*Avena fatua*
*Bromus hordeaceus*
*Festuca perennis*
*Trifolium subterraneum*

**Invasive noxious weeds**

*Aegilops triuncialis*
*Elymus caput-medusa*

Table 2.4. Plant species used in planting treatments in 2007.

# CHAPTER 3

## Bison and fire impacts on soil carbon and nitrogen are not mediated by plant diversity

Camille Traylor, Nick Barber, Meghan Midgley

### Abstract

As ecosystems are degraded worldwide, restoration is necessary to maintain biodiversity and support ecosystem functions. Grasslands often undergo management interventions like grazing and prescribed fire to enhance biodiversity and control invasive species, but connections between these restoration activities and soil ecosystem functions remain underexplored. This study examines the effects of restoration practices—prescribed fire, grazing, and time since restoration began (restoration age)—on soil ecosystem functions in a tallgrass prairie, focusing on the roles of plant functional trait diversity and species richness in mediating these effects. Using piecewise structural equation models, I found direct impacts of management practices on soil properties outweighed those mediated by changes in plant functional trait diversity or species richness. Specifically, bison presence increased soil nitrogen while recent fires decreased carbon mineralization rates. Restoration age correlates with reduced soil nitrogen and nitrate, except in remnant prairies where nitrogen levels are notably higher. Despite a slight decrease in nitrate with increased functional dispersion, other soil attributes remain unchanged by plant diversity. Restoration-induced changes in plant community composition, namely invasive species dominance, are likely stronger drivers of soil ecosystem function in this system. This study highlights the complexity of restoration effects on soil ecosystem function and suggests a need for further research into how management influences soil functions and plant community composition.

### Introduction

Soil biogeochemical cycling is a fundamental part of grassland ecosystem function and a key to mitigating impacts of greenhouse gas emissions such as carbon dioxide, methane, and nitrous oxide. However, grasslands are degraded worldwide, altering these critical functions (Gibson 2009, Török et al.

2021). Restoration often aims to manage plant communities to increase or sustain biodiversity (Brudvig 2011, Suding 2011, Bullock et al. 2011), but less is known about how restoration activities and ongoing management affect carbon and nitrogen cycles. Additionally, because plant diversity affects soil ecosystem functions, restoration may indirectly affect soil through changes in the plant community, but the magnitude of these indirect effects is not fully understood. Grasslands' potential for supporting carbon and nitrogen pools and fluxes (Lal 2004, Lehmann & Kleber 2015) make supporting soil ecosystem functions an important goal for the restoration and management of degraded grasslands (Gang et al. 2014, Török et al. 2021).

Knowledge of functional trait diversity can be used to predict how communities will respond to different environmental changes and to target specific management practices aimed at supporting ecosystem resilience (Lavorel & Garnier 2002, Funk et al. 2008). Grassland management strategies to increase biodiversity include grazing and prescribed fire (Fuhlendorf et al. 2009, Spasojevic 2010). By reducing dominant plant species like invasive grasses and encroaching woody plants, grazing and fire increase biodiversity and may support nutrient cycling and resilience against these and other natural disturbances, although this can depend on burn intensity and frequency (Hartnett et al. 1996, Harrison et al. 2003, Collins & Calabrese 2012). However, it is unclear to what extent grazing and fire directly affect nutrient cycling as opposed to indirect effects through changes in plant diversity. Because grazing effects on soil carbon and nitrogen depend on a range of environmental factors including soil texture, precipitation, grazing intensity, and plant community composition (Derner & Schuman 2007, McSherry and Ritchie 2013, Souther et al. 2020), measuring direct versus indirect effects in a controlled restoration over an extended period can help tease apart their relative contributions to soil ecosystem functions. Restoration that selects for diverse plant traits either through grazing management, prescribed fire, or native planting could be conducive to a plant community that supports belowground ecosystem functions in grasslands.

Biodiversity–ecosystem function (BEF) relationships, although sometimes positive, are complex and depend on a variety of factors including plant functional diversity (Naeem et al. 1994, Tilman et al.

1997, Diaz & Cabido 2001, Wu et al. 2015). Although positive BEF relationships are regularly found in carefully controlled experiments, the relationships in field settings are less consistent (van der Plas 2019). Plant diversity can affect resource use through selection effects—increasing the likelihood that a species is included that makes a large contribution to a function— and through niche complementarity—the idea that the presence of multiple species will ensure more niches are filled spatially or temporally (Tilman et al. 1997, Loreau et al 2001, Roscher et al 2012). For this reason, both high species richness and high functional trait diversity may be hypothesized to positively correlate with function. Although some positive BEF studies in grasslands show that plant diversity affects ecosystem functions like inorganic N pools, soil microbial biomass, and soil water retention (Diaz & Cabido 2001, Bach et al. 2012), the majority of studies have focused on aboveground productivity. The studies on the relationship between plants and soil functioning show positive, negative, and neutral relationships (Carvalho et al., 2014; Ratcliffe et al., 2017, Lange 2015, Chen et al. 2020, Knops et al. 2007, Dawud et al. 2017) and are often difficult to capture due to the decadal time scales needed to understand changes in soil ecosystem functions.

The goal of this project is to understand how soil ecosystem functions respond to restoration management and if these responses are mediated by plant diversity. Restoration management includes the application of prescribed fire, the introduction of large grazers, and the time since land was removed from crop rotation and replanted with native species. I measured soil nutrient pools and fluxes in a landscape-scale tallgrass prairie restoration project where restored prairies received native planting between a range of 10-35 years ago, and experience frequent prescribed fire. Some restorations are also grazed by American Bison (*Bison bison*). Given evidence for management activities like fire and grazing to shape plant diversity, and for plant diversity to regulate soil processes, I predicted that plant trait diversity would mediate management effects on carbon storage and nitrogen cycling. I also expected direct management impacts on soil to be weaker when plant trait diversity is higher because diverse communities have a greater flexibility in responding to disturbances, and because their increased plant trait diversity increases soil carbon and nitrogen levels, which enhances recovery after disturbances. I used structural equation modeling to determine the relative strength of this indirect pathway compared to direct impacts of management on soil carbon and nitrogen pools and fluxes and specifically answer two questions: (1) How do grazing, recent fire, and length of restoration time affect soil carbon and nitrogen functions? (2) To what extent are these soil responses mediated by changes in plant functional trait diversity and species richness?

## Methods

### *Study Site*

The study area is Nachusa Grasslands, a 1400 ha reserve owned and managed by The Nature Conservancy in northern Illinois, consisting of restored and remnant tallgrass prairie, oak savanna, and woodland. Much of the prairie in the reserve was restored from previous row crop agriculture over the past four decades by seeding with native plant mixes and annual weed control with herbicides. Restorations received initial planting of a wide variety of species 8-39 years before this study. Remnant and restoration management also includes frequent burning and grazing by bison introduced to a portion of the reserve in 2014. Approximately 130 bison roam freely year round over 800 ha of the reserve. All sites are managed with prescribed burns, about every two years in spring or late fall, although exact fire return intervals vary. Since burns are staggered across different sites, the landscape in any given year features a mix of areas that have been burned in the previous dormant season or not (Rowland-Schaefer et al. 2022). For more details on management and plant communities in Nachusa, see Barber et al. (2017, 2019) and Guiden et al. (2023). Mean annual precipitation is 975 mm with 640 mm occurring during the growing season (April-September). Average minimum and maximum temperatures are 3.6˚C and 14.7˚C, respectively (NOAA 2012). Prairie plant communities are a mix of C3 and C4 grasses and C3 forbs. Dominant C4 grasses include *Andropogon gerardii*, *Sorghastrum nutans*, and *Schzachyrium scoparium*, and common C3 grasses include *Elymus canadensis* and non-native *Bromus inermis*. C3 forbs include numerous Asteraceae, Fabaceae, and other families. Soil across Nachusa comprise loams, silt loams, and sandy loams (Bach & Kleiman 2021).

### *Sampling Design*

To evaluate the mechanisms regulating soil carbon, we sampled from 18 restored (8-39 years old) and 2 remnant (never plowed) prairies, including 7 restorations and 1 remnant to which bison have free access (Hosler et al. 2021) (Table 3.S1). Because prescribed fire locations vary each year, this sampling design provides variation among sites in age (years since restoration, or original remnant) bison presence (present or absent), and recent fire (burned in the previous dormant season or not). However, all sites are regularly burned. In 2015, ten 0.5m x 0.5m permanent quadrats were established at each site across a 60m x 60m grid for annual vegetation surveys (Blackburn et al. 2020). We collected 150mL sample of soil by aggregating 6 soil cores  taken from the top 10 cm directly adjacent to each vegetation monitoring quadrat in June 2022 for analyses. Vegetation surveys of each quadrat were performed in July 2022, recording basal percent cover of each species per quadrat to the nearest percent. These survey data were used to calculate functional diversity, measured as functional dispersion (FDis) (Laliberte & Legendre 2010). Traits used were root type (rhizomatous, fibrous, primary), functional group (C3 grass, C4 grass, forb, legume), percent leaf C, percent leaf N, leaf dry matter content, specific leaf area, leaf area, leaf toughness, and life history (perennial, annual). Trait data were collected in 2017 as part of a vegetation survey measuring prairie restorations (Farrell 2018), and abundance-weighted functional dispersion was calculated for each plot in the current study using the dbFD() function in the R package FD (Laliberté et al. 2014).

### *Soil Analysis*

Soil was analyzed at the Morton Arboretum in Lisle, IL. Samples from each quadrat were passed through a 2 mm sieve and used to measure soil parameters to describe soil function:  pH, organic matter, carbon mineralization, total organic C , total soluble nitrogen, ammonium, soil nitrate, and bacterial biomass carbon and nitrogen. Soils were stored at 4 ˚C until analysis. Soil pH was measured using a 1:1 soil water ratio using a pH electrode. Soil organic carbon was measured by calculating the ash-free weight after burning in a furnace at 450˚C for 16 hours. To measure ammonium, we used the salicylate-nitroprusside method (Sims et al. 1995). To quantify nitrate, the $VCl_3$/Griess method was employed and measured at 540 nm absorbance on a microtiter plate reader (Hood-Nowotny et al. 2010). Carbon and Nitrogen were extracted using 0.5M $K_2SO_4$ and analyzed using high-temperature oxidation and chemiluminescence detection (TOC-L and TNM-L TOC-TN analyzer, Shimadzu, Kyoto, Japan). C mineralization rates were analyzed by incubated samples for 3 hours at 23°C and measuring the change in CO2 concentrations (LI-6200, Li-Cor Incorporated, Lincoln, Nebraska). Soil microbial biomass carbon and nitrogen concentrations were measured by assessing changes in organic carbon and total nitrogen extracted with 0.5M $K_2SO_4$ from 10 g subsamples following a 4-day chloroform fumigation (Brookes et al. 1985, Vance et al. 1987). Total organic carbon and nitrogen in the extracts were quantified using high-temperature oxidation on a Shimadzu TIC/TOC analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). The microbial biomass carbon values were adjusted for a 45% extractability rate (Beck et al. 1997), and the microbial biomass nitrogen values for a 54% extractability rate (Brookes et al. 1985).

### *Statistical Analysis*

Statistical analyses were performed to evaluate the effects of land management practices—grazing, recent fire, and restoration age—on soil ecosystem functions and plant diversity and the effects of plant diversity on soil functions within a landscape-scale tallgrass prairie restoration project. Using Structural Equation Modeling (SEM) was performed through the piecewiseSEM package in R (Lefcheck 2016). We used piecewiseSEM because of its ability to manage complex models with multiple dependent variables and incorporate various types of regressions into a cohesive model. We explored both direct and indirect pathways by which management practices impact soil parameters: carbon and nitrogen pools and fluxes (total organic carbon, soil organic matter, carbon mineralization, microbial biomass carbon, ammonium, nitrate, total nitrogen, microbial biomass nitrogen), pH, and moisture content. Indirect pathways included potential mediation through plant functional trait diversity and species richness. Our SEMs included linear mixed models with site as a random variable. Models adhered to normality and heteroscedasticity assumptions.

The piecewise SEM was designed based on hypothesized causal pathways, drawing from prior research on restoration effects on soil ecosystem functions and plant communities (Yang et al. 2019, McNaughton 1994), and the influence of plant diversity on soil functioning (Van Der Heijden et al. 2008, Yang et al. 2019). The model treated management age, bison grazing, and fire history as independent variables. Plant diversity metrics, specifically species richness and functional trait diversity, were integrated as both outcomes and predictors in various regressions, highlighting their potential mediating role in linking management practices with soil ecosystem functions. Each SEM consisted of three linear mixed models: (1) management effects (bison, fire, age) as independent variables and species richness as the response variable, (2) management effects as independent variables and plant functional dispersion (FDis) as the response variable, and (3) plant diversity (species richness and FDis) as the independent variables and the soil function as the response variable. In each of the three mixed models, site was used as a random factor. The model uses grazing as a categorical variable, age was numerical, and fire history (whether or not the site was burned in the preceding 2021–2022 dormant season) was also categorical. A correlated error term was included for plant species richness and functional trait diversity to account for their correlation with each other. This process was repeated to create an SEM for each soil response variable.

Directed separation tests were used to identify any missing direct paths from management to soil ecosystem functions, therefore assessing conditional independencies. Significant tests ($p<0.05$) indicated that additional pathways needed to be incorporated into the SEM, in which case a management variable was added into the third linear mixed model of the SEM. The overall model fit was evaluated using Fisher's C, with relationships between variables expressed through path coefficients. These coefficients, along with calculated $R^2$ values from the piecewiseSEM package, helped quantify the direct and indirect effects of management practices. Specifically, we calculated plant-mediated pathways as the product of coefficients from the management effect on plants and the subsequent plant effect on soil paths.

## Results

### *Plant Diversity Impacts*

Remnants have the lowest FDis (0.123 +/- 0.009), but restored sites show a trend where FDis increases over time from 0.157 (+/- 0.004) and 0.160 (+/- 0.006) in 10 and 12 year old plots, to 0.183 (+/- 0.10) and 0.199 (+/- 0.006) in 31 and 35 year old plots, respectively (Figure 3.1, Figure 3.3). Species richness was also higher in younger restoration sites. Species richness was 15.8 (+/-0.9) and 16.5 (+/- 1.0) in 10 and 12 year old plots, while older plots have 12.5 (+/- 0.9) and 14.9 (+/- 0.7) species in 31 and 35 year old plots (Figure 3.5). Remnant plots had the lowest species richness (12.2 +/- 0.06).

Functional dispersion (FDis) plays a small role in mediating effects of restoration age on soil nitrate (Figure 3.1, Table 3.1). As restoration age increases, FDis increases, which leads to a small decrease in soil nitrate. However, the direct impact of restoration age had a stronger influence on soil nitrate compared to the effects mediated by FDis. Soil nitrate decreased from 0.38 (+/- 0.1) and 0.22 (+/- 0.06) in 10 and 12 year plots to amounts less than half of this in older plots (21-35 years old). However, nitrate was highest in remnant plots (1.1 +/- 0.37) and values varied widely across remnant plots. In restoration sites, soil nitrate was generally higher in sites with lower FDis. When FDis was 0.199 and 0.183, soil nitrate was low (0.02 and 0.03). When FDis ranged between 0.157 and 0.166, soil nitrate was between 0.10 and 0.43. Although FDis slightly mediates an age-soil nitrate relationship, it does not regulate any of the other measured nitrogen properties. For example, nitrate slightly decreased as FDis increased, but ammonium, total soluble nitrogen, and microbial biomass nitrogen were unaffected by plant diversity (Figure 3.4). Soil pH and carbon measurements were also unaffected by FDis. Species richness did not regulate any soil functions measured.

*Management Impacts*

Restoration age is associated with a decrease in both total soil nitrogen and soil nitrate (Figure 3.3). Bison presence had a positive effect on ammonium resulting in an increase in total nitrogen (Figure 3.6). Bison presence had a negative effect on soil pH (Figure 3.7). Recent fire events (occurring within the last two years) decreased carbon mineralization rates (Figure 3.9). Microbial nitrogen and carbon biomass were unaffected by management and plant diversity (Figure 3.8).

## Discussion

In ecological restoration, management practices are fundamentally aimed at rehabilitating degraded systems. Today, this work also often focuses on enhancing plant trait diversity and supporting ecosystem functions. My study used piecewise structural equation models to investigate the direct and indirect effects of key prairie land management factors – bison presence, fire history, and the length of time the site has been managed – on soil ecosystem function. We found that these factors had significant direct impacts on soil properties, rather than indirect effects mediated through changes in plant diversity. Although bison presence and prescribed fires influenced soil nitrogen pools and carbon fluxes respectively, these site characteristics did not have consistent effects on plant diversity. Prescribed fire reduced carbon mineralization rates, yet it had no substantial effect on total organic carbon or microbial biomass carbon. Additionally, plant diversity metrics such as species richness and functional trait diversity were influenced by the age of restored sites, but they followed opposite patterns. This study highlights the need for further research to better understand the ways in which restoration treatments and management alter plant community compositions and how individual plant traits are linked to soil ecosystem functioning.

### *Plant community relationships*

Plant diversity (species richness and functional diversity) was not directly affected by bison or fire but was influenced by the age of sites: older restorations had lower species richness but a greater diversity of plant traits. A decline in species richness over time is commonly observed in prairie restorations and is often associated with increased dominance by fire tolerant C4 grasses (Sluis 2002, Grman et al. 2013, Hansen & Gibson 2014, Barber et al. 2017). This same shift to C4 grasses and forbs over time was detected in this study. The most abundant species in young sites (10-17 years old) were invasive C3 grasses like *Bromus inermis* and *Poa pratensis,* and native C3 graminoid *Carex* spp. Communities in older sites (21-35) had more forbs and C4 grasses like *Sorghastrum nutans* and *Andropogon gerardii*. C3 grasses were more abundant where FDis was low, but C3 grass abundance did not have a significant relationship with soil nitrate. Thus, although FDis was negative correlated with soil nitrate, C3 abundance does not seem to be a strong driver of this effect. I also observed an increase in functional trait diversity over time, suggesting that the plant community is shifting toward a greater variety of traits. Older sites have a greater diversity of forbs that vary in traits such as leaf dry matter content, specific leaf area, and leaf C and N. Increasing diversity in plant traits could indicate complementarity and increased resource use efficiency as indicated by less soil nitrogen in these sites. Interestingly, there was little to no relationship between plant diversity and all other soil ecosystem functions. This aligns with the understanding that biodiversity-ecosystem function relationships are complex and can vary, ranging from positive to negative to neutral and depending on many environmental factors (Oliver et al. 2015).

### *Nitrogen dynamics*

Grassland management not only directly altered soil nitrogen but also indirectly influenced soil nitrogen though management induced changes in plant communities. Soil nitrate decreased with site age, but also via changes in plant trait diversity. However, this indirect pathway was much weaker than the direct effects of age on soil nitrate, indicating that a different age-associated mechanism unrelated to plant diversity contributes to the decrease in soil nitrate. Declines in soil nitrate over time could indicate a trajectory towards pre-agricultural levels. Changes in plant community composition, like shifts to C4 grasses, may also be a driving factor (Tilman & Wedin 1991), however this plant response could also be a result of low nitrate levels if C4 grass dominance is more likely under low-N conditions. Decreases in nitrate over time can also be caused from increased biomass and N uptake, with N present in plant tissues rather than in soil. Although we did not investigate associations between plant biomass and soil nitrate, grassland species allocate a significant portion of their biomass belowground. Root dynamics are major contributors to soil processes (Prieto et al., 2012, Bardgett et al. 2014, Vives-Peris et al. 2020), and an inverse relationship between nitrate levels and root biomass has been indicated in other studies (Baer 2002, Scherer-Lorenzen et al. 2003). Although microbial biomass carbon and nitrogen were unaffected by management in our study, microbes can still regulate nitrogen cycling through fluxes in immobilization rates driven by factors such as changes in community composition and microbial relationships with root exudates (Zhalnina et al. 2018, de Vries et al 2012).

Increases in plant trait diversity only slightly modified the age-soil nitrate relationship, and this effect may be due to niche partitioning among species (Hooper et al. 2005, Cardinale 2007), but this effect is not consistent (Jung et al., 2010). Because older sites had higher trait diversity, post-restoration succession in these communities may shift composition to species with traits that can act complementarily and partition resources to maximize nitrate use efficiency. However, remnant sites had at least 30% lower plant trait diversity than any of the restoration sites, indicating there may be a non-linear age-functional diversity relationship similar to microbial diversity studies in these same soils (Barber et al. 2023). As sites age, soil nitrate may peak followed by a drop off in much older sites (>35 years). The oldest restoration analyzed was 35 years old which is relatively young compared to remnant sites, which may be centuries old. Remnant sites also differ from restoration sites in that remnants have not experienced the same anthropogenic disturbances and were left out of cultivation due to having a rocky topography compared to restoration sites which could result in species and trait compositions very different from those that exist in restorations where a high diversity of plants, including many forbs, was purposely introduced in recent decades. In particular, grasses are more abundant in remnants than restorations (average 51% vs. 41% cover), while forbs in general (39% vs. 50%) and legumes specifically (<4% vs. 8%) are more abundant in restorations.

Total soil nitrogen decreased slightly with age, which was unexpected as other chronosequence studies have shown that total soil nitrogen generally increases in post-agricultural grassland restorations (Baer 2002, Rosenzweig et al. 2016). We may have seen the opposite effect because the range of restoration site ages was 10-35 years old and did not include a younger restoration site in an earlier successional stage that likely has higher N accumulation rates than a site that has been managed for 10 or 35 years. Another reason we may not have seen an increase in nitrate over time is because soil C and N stocks get built up over centuries thus requiring longer datasets to detect age effects on soil nutrient pools. Studies have also predicted that N and C recovery could take as many as 350 years to get back to levels of a native prairie (Rosenweig et al 2016). This age range used in this study may not be large enough to detect N patterns post-agriculture or long-term trends in soil nitrogen (Knops & Tilman 2000, Matamala et al. 2008, Rosenzweig et al. 2016). Other studies have indicated rapid decreases in nitrogen accumulation following restoration from cultivated land due to turnover of annual and biennial species (Hernandez et al 2013). We did not detect changes in ammonium or microbial biomass nitrogen over time, however microbes could be affecting nitrogen fluxes like denitrification and immobilization. Similarly to nitrate, the amount of total nitrogen in remnant plots was similar to that of soils in young sites.

Bison presence was also a significant factor contributing to increases in the total nitrogen pool. This was expected due to fecal and urine inputs from bison that increase plant productivity and nitrogen cycling. Grazing studies have shown both increases and decreases in soil nitrogen pools through several mechanisms. Grazing can promotes greater root biomass allocation, particularly in high precipitation environments, which helps to reduce nitrogen losses (Piñeiro et al. 2010). Grazing can also enhance microbial activity, leading to increased nitrogen mineralization and a larger total nitrogen pool, especially early in the growing season when soil moisture is higher (Shan et al. 2011). However, grazing can also reduce soil nitrogen pools through different processes: soil nitrate and ammonium levels (Zhou et al. 2017) may decline because of reduced legume cover, which plays a key role in nitrogen fixation. Grazing effects on soil nitrogen can depend on many factors like precipitation and soil nutrient levels (Frank and McNaughton 1993, McNaughton et al. 1997, Piñeiro et al. 2010). Thus, while bison grazing impacts soil nitrogen through various mechanisms, its overall effect on the nitrogen cycle is complex, necessitating further research to fully understand the ecological consequences of reintroducing bison to managed grasslands.

### *Carbon dynamics*

Prescribed fire is used to reduce species dominance and can also affect grazer behavior (Fuhlendorf & Engle 2004), but less is known on how fire can directly alter soil biogeochemical cycling, especially due to carbon processes occurring over long timescales. In the study, recent prescribed fires (burned in the past year did decrease soil carbon mineralization, but other carbon measurements (soil organic carbon and microbial biomass carbon) were unaffected compared to sites burned more than two years ago. Microbial activity can be resilient to fire in grasslands (Docherty et al. 2012, Dooley & Treseder 2011). Frequent burning may decrease carbon stocks because of a shorter recovery period between fires. Frequent fire in a longleaf pine ecosystem decreased carbon storage (Wright et al. 2021), and Pelligrini et al. (2008) found that frequent burning can reduce soil carbon by 21% in savanna grasslands, but this effect appeared only after 18 years. We did not see evidence of decreased soil carbon pools in our study as a result of varying times since prescribed fire, but times since fire in this study only differed by 1-2 years between most sites, so all sites could be considered frequently burned. Carbon dynamics are important to understand because carbon sequestration is a key factor for mitigating climate changes, so long term studies are needed to fully understand the role of fire frequency and intensity in soil carbon dynamics.

Contrary to my expectations, carbon dynamics were not significantly affected by grazing, which may be due to the other abiotic factors like precipitation and soil type. Grazing can increase soil organic carbon in subtropical and upland grasslands when precipitation is high and when soils are coarse textured. However, grazing may lead to soil carbon loss when soil texture is fine, such as those with high clay content, depending on grazing regime (Derner & Schuman 2007, McSherry and Ritchie 2013). Although high clay content in soils has been linked to increases in soil organic carbon due to their role in clay particles in forming soil aggregates that can protect organic carbon from decomposition (Plante et al. 2006, Schweizer et al. 2019), the soils in this study are predominantly sandy and silty loams, with very little clay. This may explain the lack of impact of grazing on carbon dynamics observed here. Additionally, the shorter timescale of this study may not be sufficient to capture changes in soil carbon, which can take decades to centuries to become noticeable.

Understanding these age-soil nitrate relationships, bison-soil nitrogen interactions, and the impact of fire on carbon mineralization relationships can inform restoration efforts focused on increasing biodiversity to promote ecosystem functioning. C4 grasses like *S. scoparium* and *A. gerardii* increased over time, indicating that shifts to native C4 grasses, and away from invasive C3 grasses, may be related to the decrease in soil nitrate in older restorations. Strategic selection of plant species for prairie restorations could favor species that are better suited for changing climates over time. Management impacts on nutrient cycling and plant communities underscores their importance in mitigating impacts of climate change.

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Table 3.1. Summary of structural equation model results. df=degrees of freedom, FDis = Functional Dispersion, TOC= total organic carbon, SOM = soil organic matter, CMin=carbon mineralization, TN=total nitrogen, MBC=microbial biomass carbon, MBN=microbial biomass nitrogen.

| Response | Predictor | Estimate | Standard error | df | Critical value | P | Standardized estimate | Marginal $R^2$ |
|---|---|---|---|---|---|---|---|---|
| FDis | age | 0.0011 | 5e-04 | 12.5361 | 2.3293 | **0.0373** | 0.2565 | 0.11 |
| FDis | bison | 0.0091 | 0.0063 | 12.4351 | 1.4415 | 0.1742 | 0.1556 | |
| FDis | fire | 2e-04 | 0.0061 | 12.7529 | 0.0287 | 0.9775 | 0.0031 | |
| spprich | age | -0.1361 | 0.0726 | 12.5076 | -1.8741 | **0.0845** | -0.2403 | 0.08 |
| spprich | bison | 0.9219 | 0.942 | 12.4322 | 0.9786 | 0.3464 | 0.1231 | |
| spprich | fire | -0.8539 | 0.9054 | 12.669 | -0.9432 | 0.3632 | -0.1164 | |
| TOC | FDis | -31.1555 | 30.6565 | 155.6382 | -1.0163 | 0.3111 | -0.08 | |
| TOC | spprich | -0.0842 | 0.241 | 155.883 | -0.3496 | 0.7271 | -0.0277 | |
| SOM | FDis | 0.3033 | 2.301 | 151.1027 | 0.1318 | 0.8953 | 0.0095 | |
| SOM | spprich | -0.0147 | 0.0181 | 151.7246 | -0.8124 | 0.4178 | -0.0587 | |
| CMin | FDis | -0.3795 | 0.3275 | 154.9329 | -1.1587 | 0.2483 | -0.0882 | |
| CMin | spprich | -0.0018 | 0.0026 | 154.9402 | -0.7063 | 0.481 | -0.0542 | |
| CMin | fire | -0.0852 | 0.0306 | 15.3517 | -2.7835 | **0.0137** | -0.3452 | 0.12 |
| MBC | FDis | 373.7167 | 297.2251 | 148.762 | 1.2574 | 0.2106 | 0.0844 | |
| MBC | spprich | 0.1881 | 2.3416 | 149.3345 | 0.0803 | 0.9361 | 0.0054 | |
| $NH^4$ | FDis | -0.4602 | 1.1027 | 151.906 | -0.4174 | 0.677 | -0.0314 | |
| $NH^4$ | spprich | 0.0018 | 0.0086 | 153.9459 | 0.214 | 0.8308 | 0.0161 | |
| $NH^4$ | bison | 0.2315 | 0.1251 | 15.2475 | 1.8509 | **0.0837** | 0.2694 | 0.07 |
| NO3 | FDis | -1.3839 | 0.7421 | 152.8428 | -1.8648 | **0.0641** | -0.1475 | |
| NO3 | spprich | -0.0087 | 0.0058 | 154.7416 | -1.5089 | 0.1334 | -0.1189 | |
| NO3 | age | -0.0114 | 0.005 | 16.9078 | -2.2926 | **0.035** | -0.2736 | 0.12 |
| TN | spprich | -0.0312 | 0.0292 | 148.6652 | -1.0702 | 0.2863 | -0.0701 | |
| TN | FDis | -3.9066 | 3.7239 | 146.0474 | -1.0491 | 0.2959 | -0.0685 | |

|  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
| TN | age | -0.0917 | 0.0389 | 14.6733 | -2.3581 | **0.0327** | -0.3633 | 0.22 |
| TN | bison | 1.4658 | 0.5098 | 13.9954 | 2.8754 | **0.0122** | 0.4394 |  |
| MBN | FDis | 42.4791 | 44.0194 | 150.4818 | 0.965 | 0.3361 | 0.0685 |  |
| MBN | spprich | -0.1499 | 0.3467 | 151.1013 | -0.4323 | 0.6662 | -0.0309 |  |
| pH | FDis | 0.9166 | 0.6776 | 144.7311 | 1.3526 | 0.1783 | 0.0734 |  |
| pH | spprich | -0.007 | 0.0053 | 146.2246 | -1.3227 | 0.188 | -0.0722 |  |
| pH | bison | -0.3353 | 0.1334 | 15.1118 | -2.5131 | **0.0238** | -0.4588 | 0.12 |
| ~~FDis | spprich | 0.1588 | - | 159 | 2.0094 | **0.0231** | 0.1588 |  |

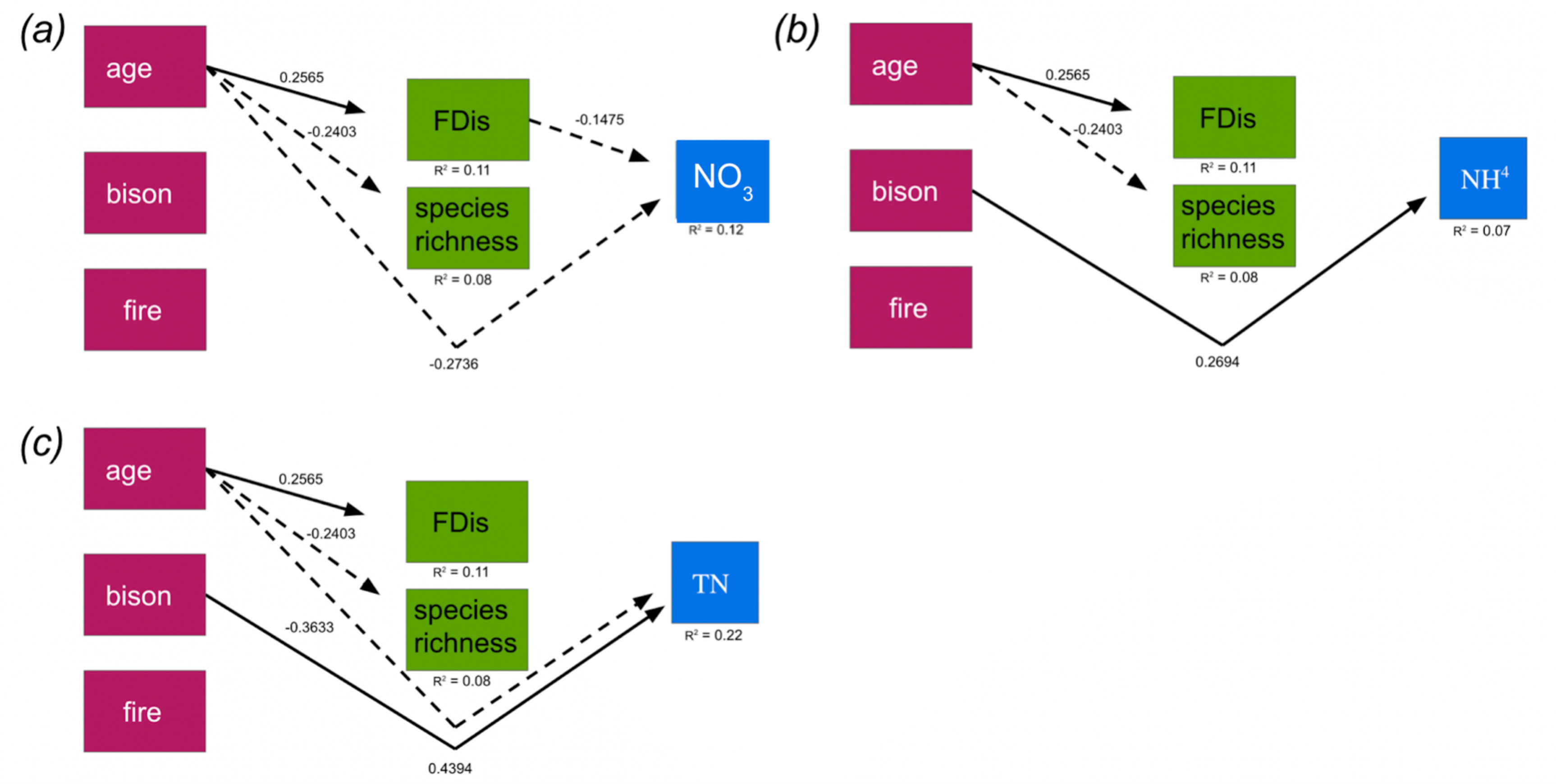

Figure. 3.1. Visualization of the paths linking restoration (magenta), plant diversity (green), and soil nitrogen functions (blue), (a) soil nitrate ($NO_3$), (b) ammonium ($NH^4$), and (c) total soil nitrogen (TN) in restored tallgrass prairie ecosystems. FDis=functional dispersion. The path diagrams depict how restoration affected soil functions, taxonomic diversity, and functional diversity of plants. Only paths where $P < 0.10$ are shown here (see Table 3.1 for all paths). Arrows represent the standardized path coefficient with the associated coefficient value listed. Dotted arrows show negative path coefficients while solid arrows show positive path coefficients. $R^2$ values for each linear mixed model are also shown.

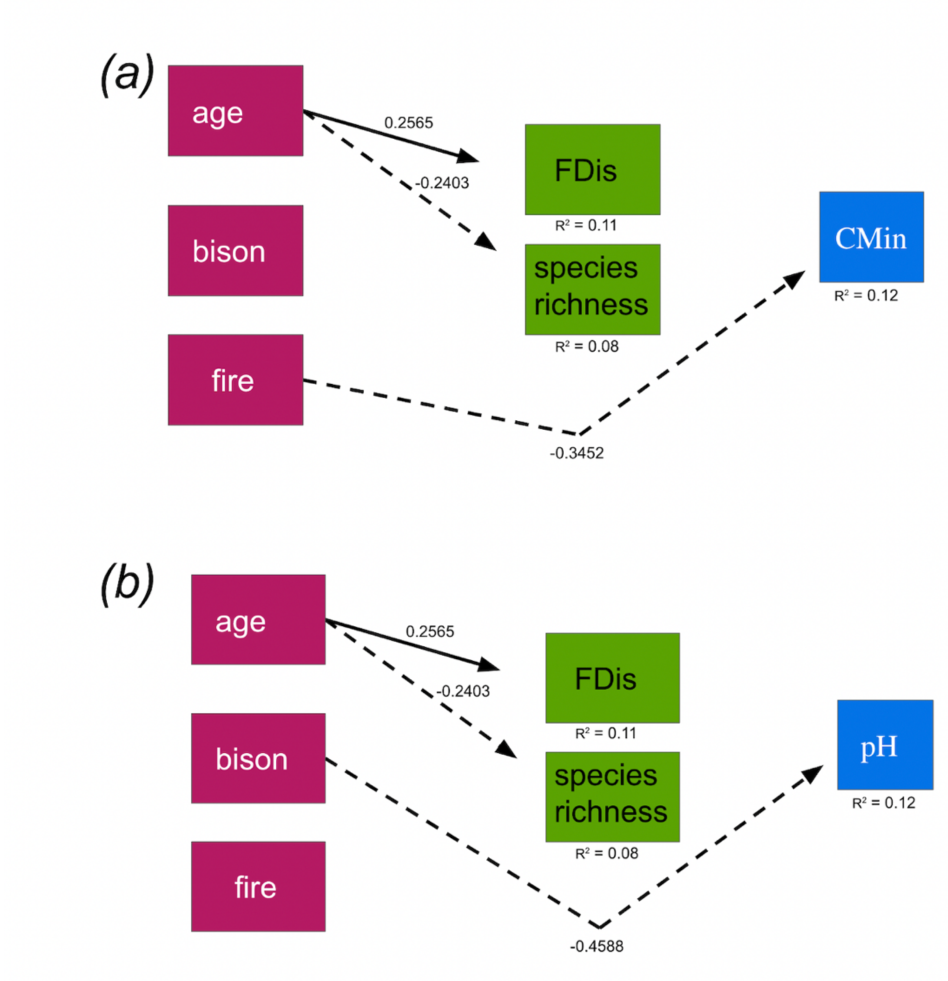

Figure. 3.2. Path diagram showing linkages between restoration (magenta) plant diversity (green) and soil functions (blue) (a) carbon mineralization (CMin), and (b) soil pH. FDis=functional dispersion. Only paths where $P < 0.10$ are shown here (see Table 3.1 for all paths). Arrows represent the standardized path coefficient with the associated coefficient value listed. Dotted arrows show negative path coefficients while solid arrows show positive path coefficients. $R^2$ values for each linear mixed model are also shown.

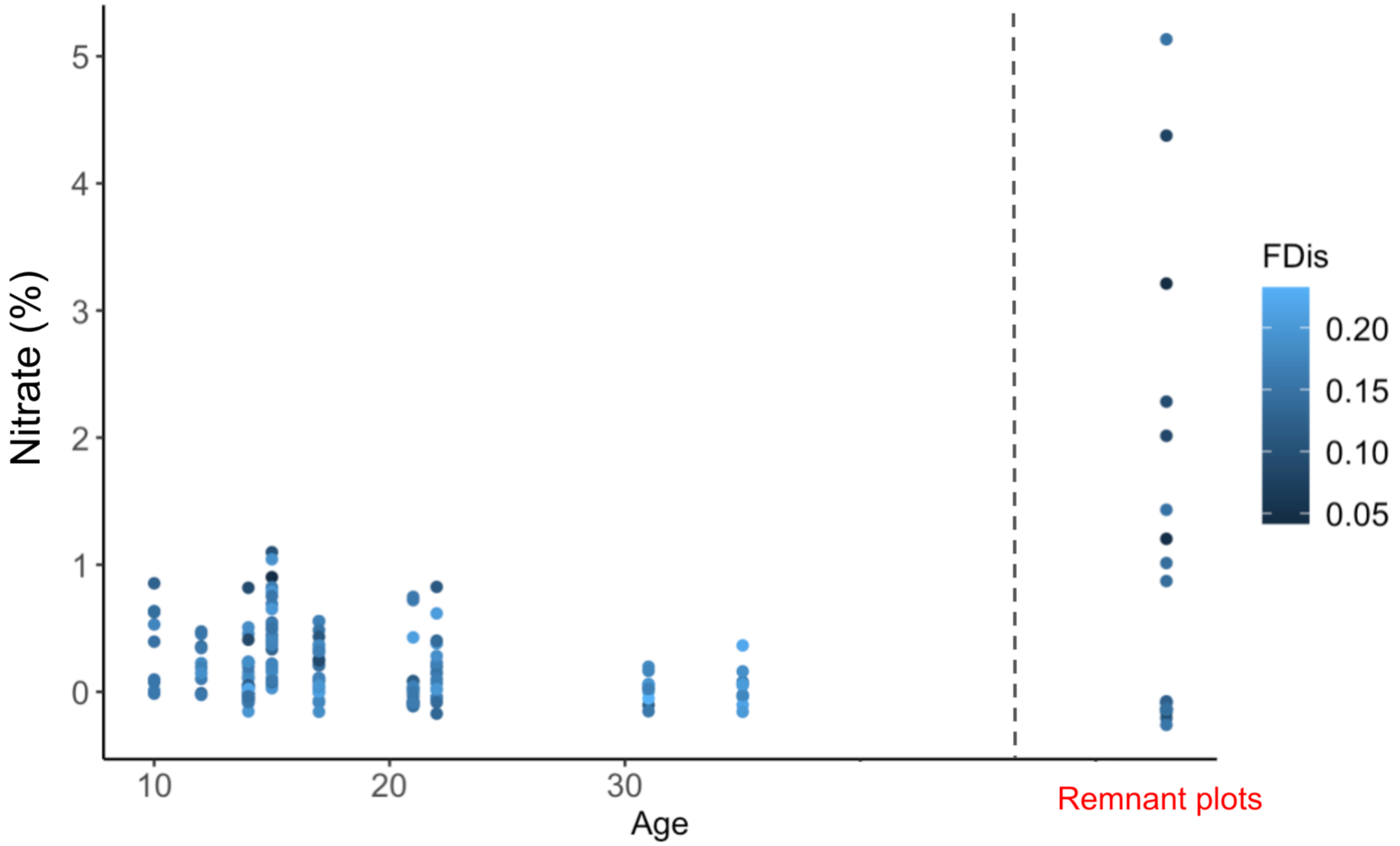

Figure. 3.3. The effects of restoration age and functional dispersion on soil nitrate across all plots. Each dot represents a plot within a restoration site. Remnant plots represent original prairie that was never disturbed from plowing.

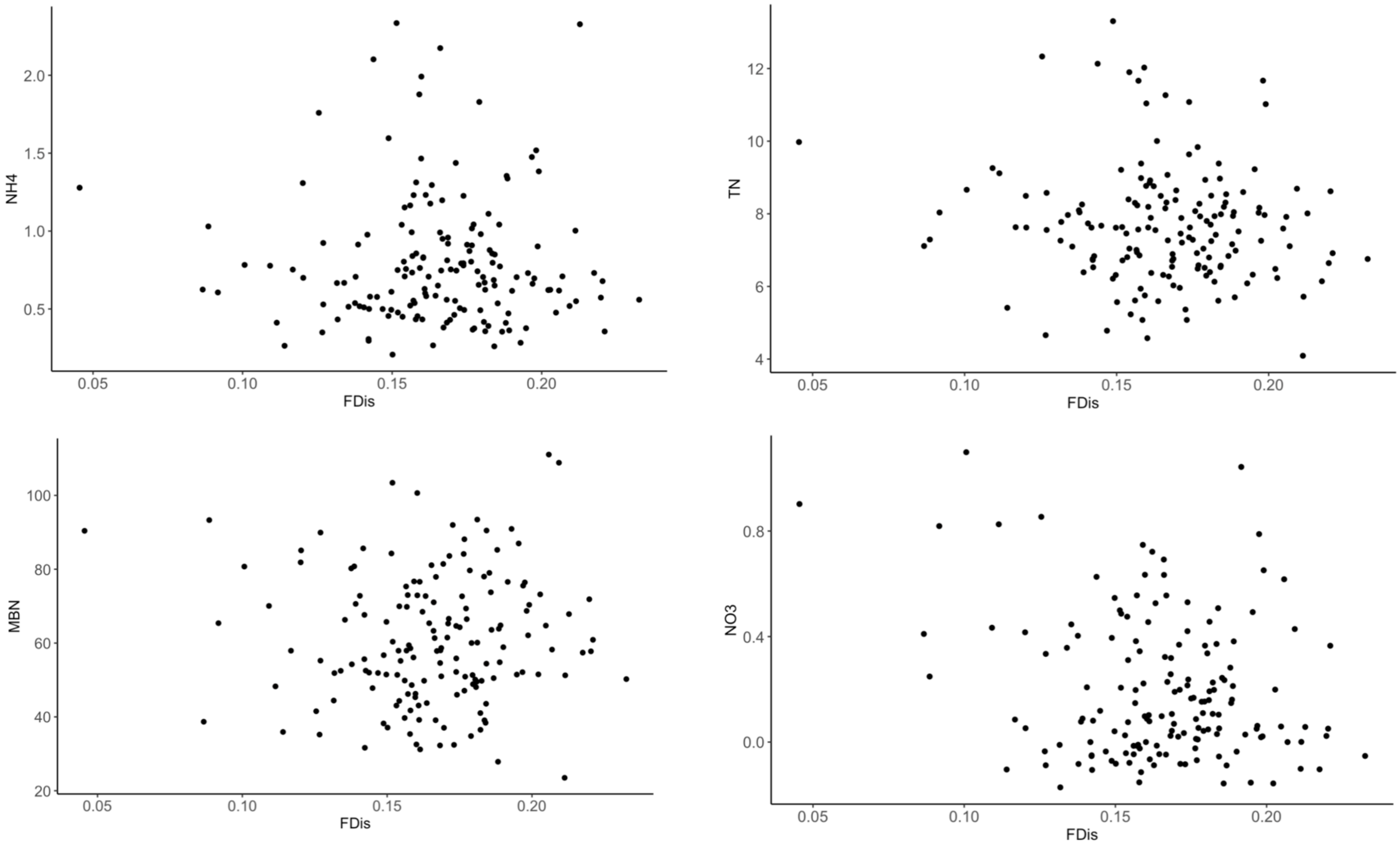

Figure 3.4. Effects of functional dispersion (FDis) on soil ammonium ($NH^4$), total soluble nitrogen (TN), microbial biomass nitrogen (MBN), and nitrate ($NO_3$) across all quadrats.

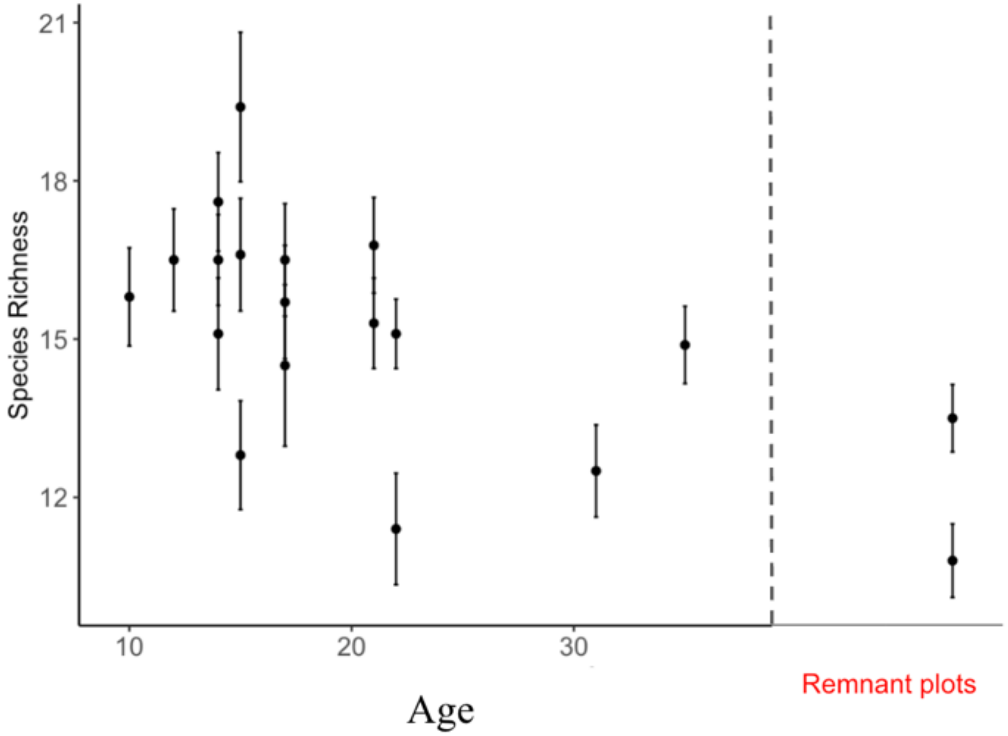

Figure 3.5. Species richness across sites of different restoration ages including remnant plots that have not received restoration. Dots represent averages across each unit +/- the standard error.

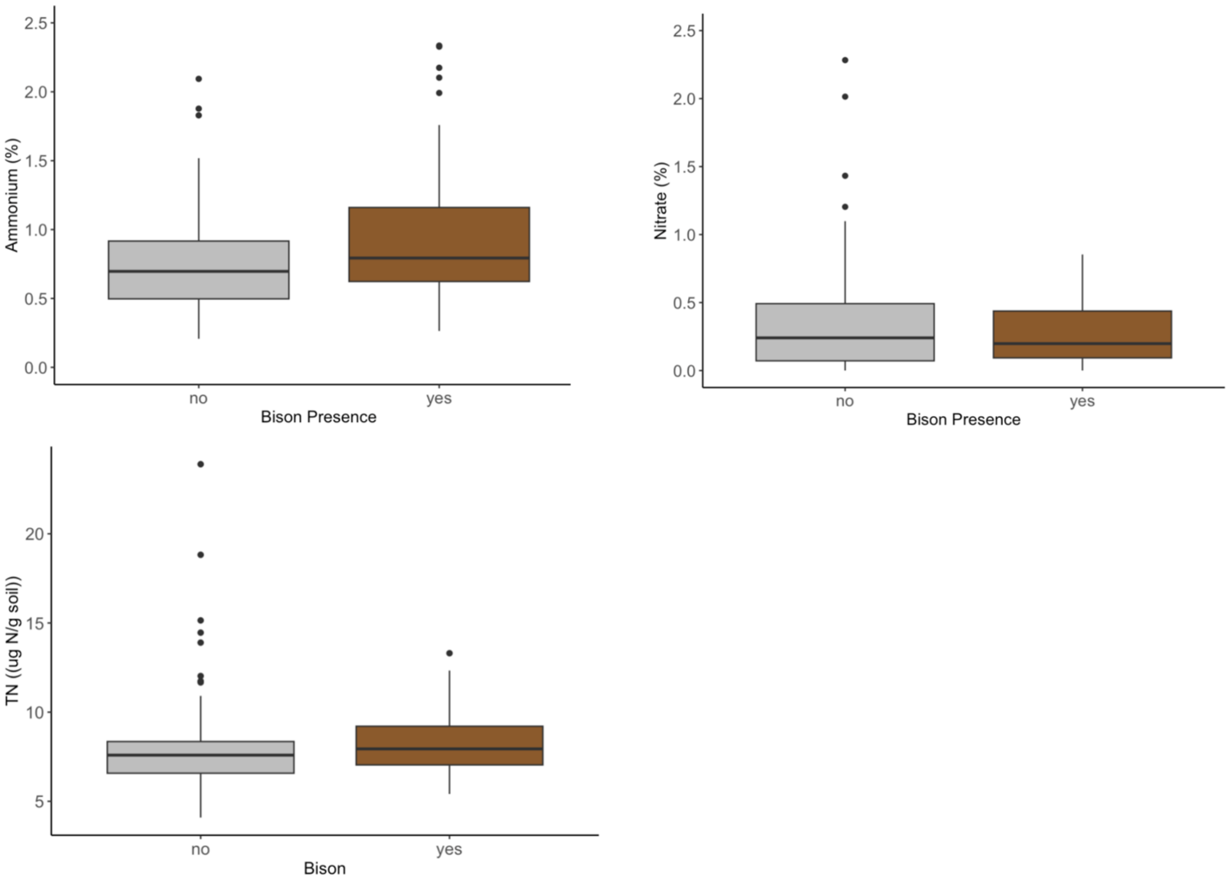

Figure 3.6. Effects of bison grazing on soil nitrogen (ammonium, nitrate, and total soluble nitrogen)

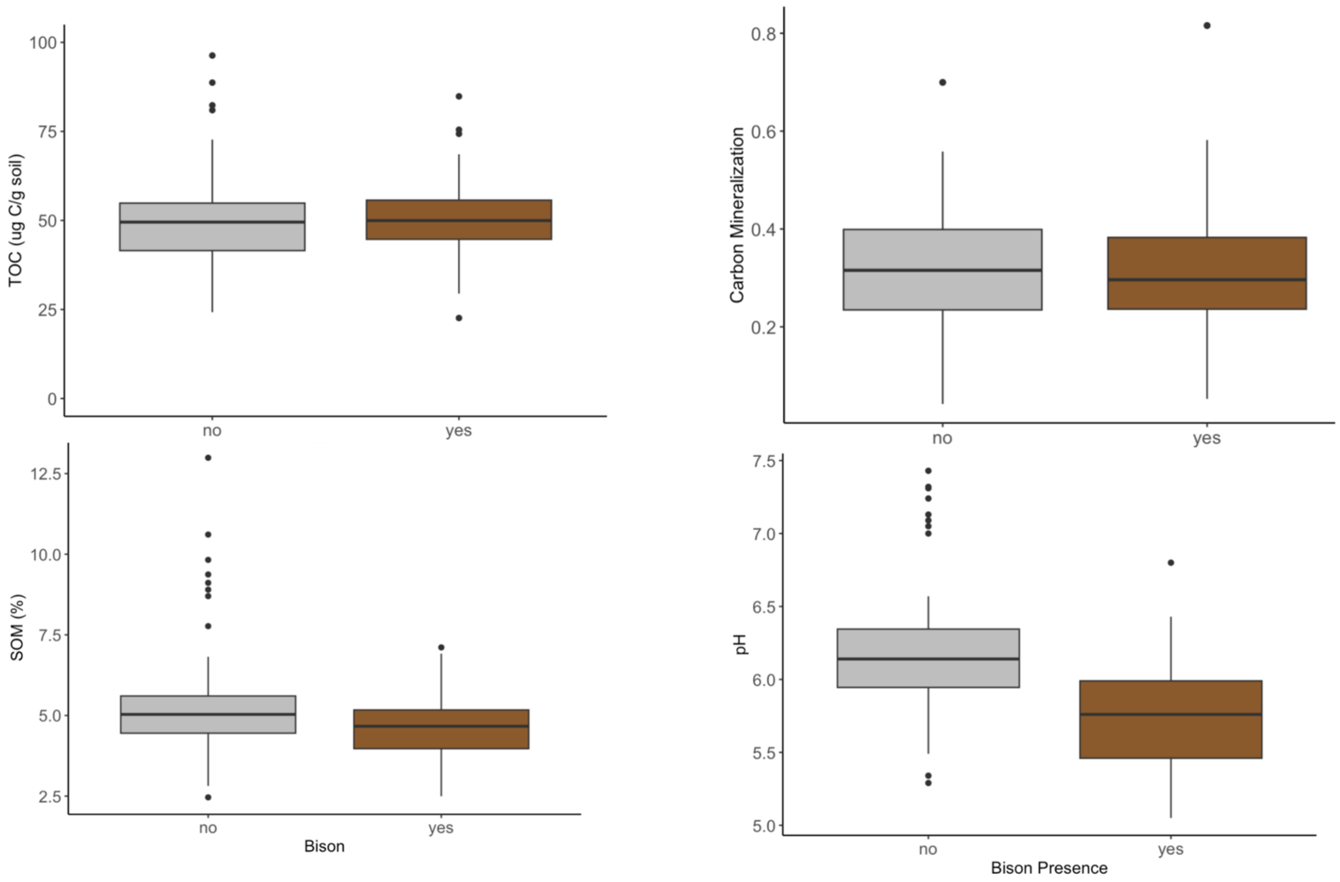

Figure 3.7. Effects of bison grazing on TOC, Carbon mineralization, SOM, and soil pH.

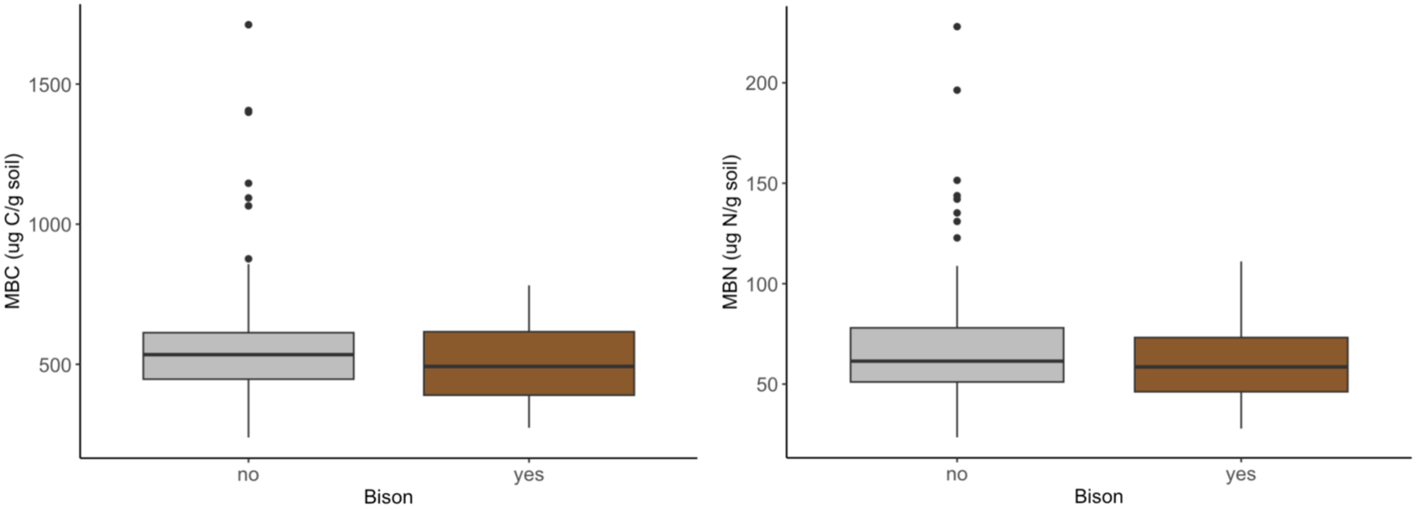

Figure 3.8. Effects of bison grazing on microbial biomass carbon and microbial biomass nitrogen.

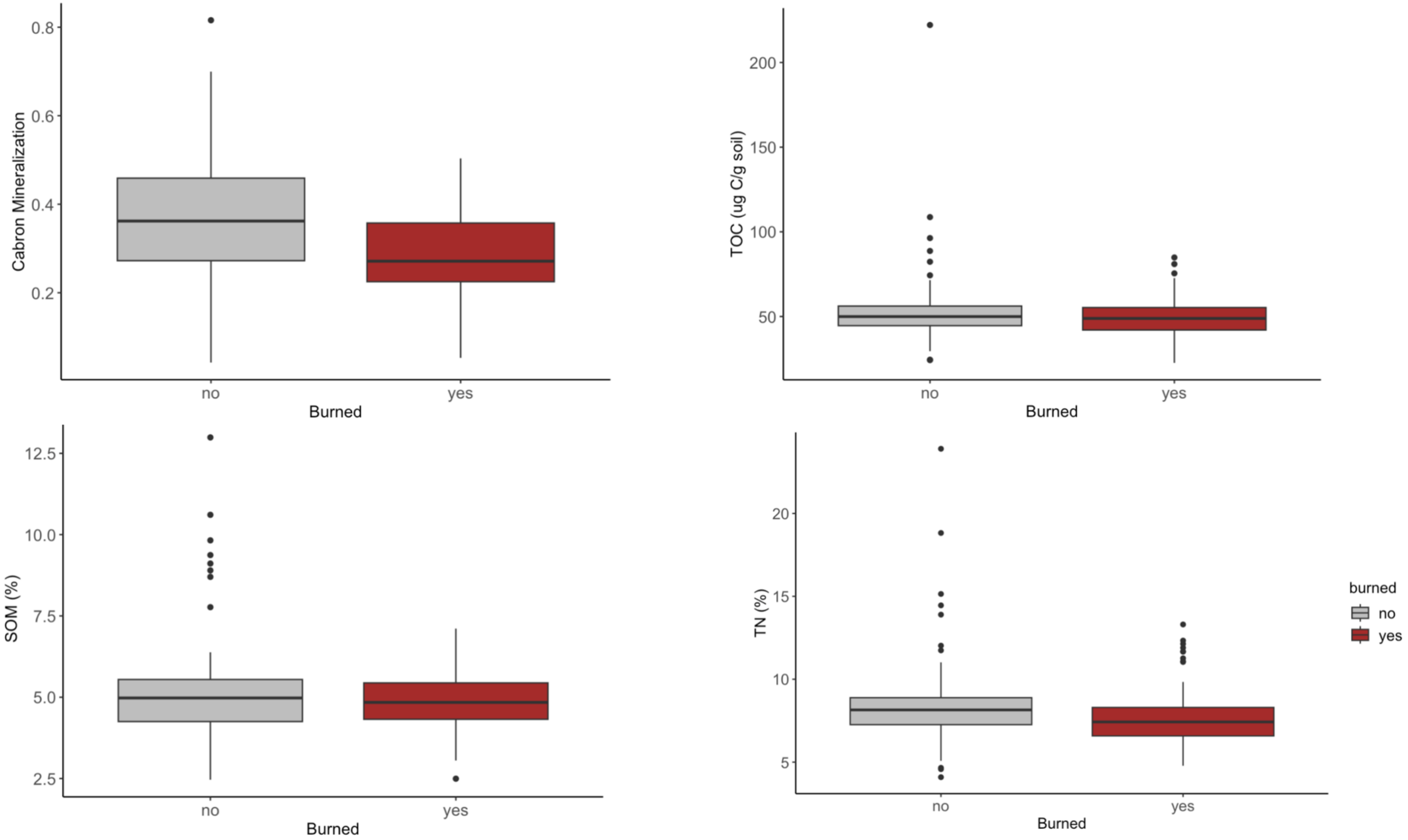

Figure 3.9. Effects of recent prescribed burns on carbon mineralization, TOC, SOM, and TN.

Table 3.S1. Sites used in this study and the land management treatment they received. Burned indicates whether a site received prescribed fire in the 2021–2022 dormant season. Age is number of growing seasons since planting during the year in which this study took place (2022).

| Unit | Bison | Burned | Age |
|---|---|---|---:|
| CCE | no | yes | 17 |
| CCEE | no | yes | 17 |
| CCW | no | no | 15 |
| CCWE | no | no | 15 |
| FC | no | no | 17 |
| HF | yes | yes | 10 |
| HL | yes | yes | 22 |
| HPW | yes | no | 15 |
| L | yes | no | 12 |
| MU | yes | yes | 35 |
| MUR | yes | yes | 53 |
| SB | no | yes | 14 |
| SBEE | no | yes | 14 |
| SBEW | no | yes | 14 |
| SF | no | yes | 22 |
| TC | no | no | 21 |
| TCE | no | no | 21 |
| TCR | no | no | 53 |
| WH | yes | yes | 31 |

# CHAPTER 4

## Influences of plant taxonomic and functional trait diversity on soil enzymatic activity

Camille A. Traylor, Nicholas A. Barber

### Abstract

Grasslands are heavily degraded ecosystems due to anthropogenic disturbances that decrease biodiversity and alter biogeochemical cycles. As biodiversity declines at an unprecedented rate, it becomes increasingly urgent to predict the effects of these declines on ecosystem functions. Grassland soils store a significant amount of terrestrial carbon, making the restoration of these systems key to mitigating the impacts of climate change. While restoration efforts often focus on enhancing taxonomic diversity to support ecosystem functions, understanding the role of functional trait diversity in influencing soil ecosystem processes is also critical. In this study, I evaluated the effects of manipulations of plant taxonomic and functional diversity on activity of extracellular enzymes in a tallgrass prairie biodiversity experiment. The enzymes analyzed are essential for the breakdown of complex organic molecules (starch, glucose, cellulose, hemicellulose, and chitin) and carry out important steps in carbon cycling. The activity of the soil enzyme N-acetyl-β-D-glucosaminidase (NAG), which breaks down chitin, significantly increased with increasing plant species richness, indicating that taxonomic diversity may regulate this aspect of soil microbial functioning. However plant species richness did not affect any other enzymes and functional trait diversity did not influence activity of any of the soil enzymes measured. These results suggest that taxonomic and functional diversity can have different, albeit weak, effects on soil ecosystem functions, and that other factors besides plant diversity like plant community composition may be a stronger driver of carbon and nutrient dynamics in tallgrass prairies.

### Introduction

Grasslands are biodiverse ecosystems essential for critical soil processes like carbon cycling. They face threats from anthropogenic-induced changes to biodiversity and biogeochemical cycles, contributing to greenhouse gas emissions (Hoekstra et al. 2005). Restoration efforts aim to understand how global biodiversity losses will influence ecosystem functions (Bullock et al., 2011; Suding, 2011), but impacts of diversity on soil carbon processes are less understood due to long timescales needed to properly analyze carbon changes (Amundson 2001). Carbon accumulation in soils happens over thousands of years, and monitoring carbon pools typically requires chronosequences, but soil enzymes, vital to many carbon cycling processes, serve as good indicators of overall soil function and can be analyzed early on because they respond sensitively to environmental changes (Alkorta et al., 2003; Dick, 1994). Plant biodiversity can influence some soil carbon processes like carbon storage (Chen et al., 2018; Steinbeiss et al., 2008), but results can vary depending on many abiotic and biotic factors (Amundson, 2001). Understanding relationships between biodiversity and soil processes is crucial for restoration efforts aimed at supporting functions that support climate change mitigation.

Restoration ecologists concerned about biodiversity-ecosystem function relationships have increasingly focused over the last two decades on using functional traits to predict how biodiversity loss will affect ecosystem function at a range of spatial scales (De Deyn et al., 2008; Funk et al., 2008; Faucon et al., 2017). Compared to processes like aboveground productivity, less research has focused on the effects of functional trait diversity on soil ecosystem functions, such as decomposition and organic matter storage. Higher plant functional diversity could enhance decomposition by facilitating a diverse carbon-degrading microbial community or by increasing the chances of including a dominant plant species that produces abundant carbon rich biomass or exudates (Naeem et al., 1994; Tilman et al., 1997, Panchal et al. 2022). Functional trait diversity can also lead to variation in litter quality that promotes resource partitioning by microbes (Wagg et al., 2017). While taxonomic diversity captures a range of unmeasured traits that may influence ecosystem functioning (Naeem et al. 1994, Loreau et al 2001, Roscher et al 2012), it does not always correlate with functional diversity. Functional diversity provides additional information by directly reflecting the traits that affect ecosystem processes. Therefore, comparing the influences of both functional trait diversity and taxonomic diversity on soil microbial functioning could offer deeper insights into the specific mechanisms driving biodiversity–soil ecosystem function relationships.

Extracellular enzymes are produced largely by bacteria, fungi, and archaea, as well as other soil organisms, and are responsible for breaking down macromolecules for assimilation of carbon and nutrients. These enzymes are critical to the processing and stabilization of several soil properties like organic matter decomposition and nutrient cycling in terrestrial ecosystems (Burns & Dick 2002). For decades, studies have used laboratory assays to measure enzyme activity to better understand soil ecosystem function dynamics (Burns & Dick 2002, Sinsabaugh et al. 2008). Enzymes are strongly regulated by the amount of substrate present in soils, which can be driven by plant litter, microbial biomass, and climate (Mendez-Millan et al., 2010, Wallenstein et al. 2011, Steinweg et al. 2012). Plant species richness may also influence microbial functioning (Steinauer et al., 2015), but it is not known if plant functional trait diversity alters microbial enzymes or if these effects vary with plant species richness. Insights into these relationships will aid in research efforts devoted to using biodiversity to support critical soil processes such as decomposition, carbon mineralization, and carbon stabilization in grasslands.

The goal of this study is to understand how soil carbon degrading enzymes respond to plant taxonomic and functional diversity. I measured enzymatic activity in a biodiversity experiment that used field data to create realistic and nonrandom plant communities that vary in richness, composition, and functional diversity. I used a fluorescence enzyme assay to bind synthetic carbon substrates to soil samples to measure rates of degradation. I address the following questions: (1) How do plant taxonomic and functional trait diversity affect soil microbial enzyme activity? (2) Do plant trait diversity and taxonomic diversity interact to influence microbial activity?

## Methods

### *Experimental Design*

This study took place in a tallgrass prairie experimental site established in DeKalb, Illinois, in 2021. Mean temperature maximums and minimums during the growing season (June-September) were 26.4°C and 15.3°C respectively in 2023 when this study took place. The mean annual precipitation was 732mm, with 320mm occurring during the growing season. Soils at the site are characterized as silt loam and silty clay loam. Experimental plots 1.5 m x 1.5 m in size were planted in 2021 with different plant communities spanning different combinations of taxonomic and functional diversity. The grassland species used (Table S1) were the 24 most frequent species occurring in restored and remnant upland prairies in the Nachusa Grasslands reserve, based on sampling of 200 quadrats in 2018. These 24 species represented 74% of total plant cover in these quadrats. Six different reference communities were created by randomly selecting 12 species from the 24 species pool. All reference communities consisted of at least one Poaceae and no more than six Asteraceae. From each reference community, species were removed and added to create combinations of 8, 12, or 16 species communities. Eight species plots have six of each species, 12 species plots have four of each species, and 16 species plots have three of each species for a total of 48 plants in each plot. There are 48 eight-species plots, 60 twelve-specie plots, and 48 sixteen-species plots. Functional diversity was calculated using functional dispersion (FDis), the mean distance of each species in multivariate trait space from the centroid of all species (Laliberté & Legendre 2010). The following traits were used to calculate FDis: functional group (C3 graminoid, C4 graminoid, legume, and forb), root morphology, specific root length, root diameter, root density, plant height, specific leaf area, leaf nitrogen content, leaf dry matter content, and seed mass.

### *Soil Analysis*

Soil samples were taken in each of the plots in July 2023 for soil enzyme assays. From each plot, six soil samples were taken to a depth of 10 cm using a metal corer (2 cm diameter) and pooled per plot into a plastic bag. The samples were stored at -20°C and shipped on dry ice to San Diego, California, for measurement of soil enzyme activity.

The activities of five hydrolytic carbon-degrading enzymes were measured to gain insights into microbial function. The enzymes were α-glucosidase (AG; degrades starch), β-glucosidase (BG; degrades cellulose-derived oligosaccharides to release glucose), β-xylosidase (BX; degrades hemicellulose), cellobiohydrolase (CBH; degrades cellulose), and N-acetyl-β-D-glucosaminidase (NAG; degrades chitin). Following the protocol of Bell et al. (2013), each sample was thawed, and 2.75 g was blended with a 50 mM sodium acetate buffer (pH ~7) and stirred for 3 minutes. While stirring, 800 μl of soil slurry was transferred to a 96-well plates containing 200 μl of 4-methylumbelliferone (MUB) with varying concentrations by row (0 μM, 2.5 μM, 5 μM, 10 μM, 25 μM, 50 μM, 100 μM). 800 μl of soil slurry was transferred to a separate empty 96-well plate followed by 200 μl of each of the five substrates. Each well was mixed thoroughly to ensure soil microbes interacted with MUB and the substrates. Plates were sealed with ThinSeal™ film and incubated in a drying oven at 35ºC for 1.5 hours. After incubation, 250 μl of solution from each well was transferred to black 96-well plates, and 10 μl of NaOH was added to each well to stop the reaction. Fluorescence was measured on a SpectraMax iD5 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA) using an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Soils were dried at 50ºC for 48 hours to quantify dry weight, and extracellular enzyme activity rates were expressed as nmol of activity per gram of dry soil per hour (nmol/g/hr).

### *Statistical analysis*

I analyzed extracellular enzyme activities using general linear mixed models with plant species richness and functional diversity and their interaction as independent variables. Initial reference community was a random factor. Log transformations of the data were used in cases where the model residuals were non-normal. The models were analyzed using "type 3" $\chi^2$-tests using Anova() in the car package (Fox & Weisberg 2011). These analyses were performed using the lme4 package (Bates et al. 2010) in R (R Development Core Team 2018).

## Results

Species richness affected activity of the enzyme NAG, which is mainly responsible for chitin degradation. NAG activity increased as species richness increased (Figure 4.1, Table 4.1). On average, NAG activity in 16-species plots was 17 and 15% higher than in 8- and 12-species plots, respectively (8 species plots = 101.0 nmols activity/hr/g soil, 12 species plots =102.4 nmols activity/hr/g soil, 16 species plots =117.7 nmols activity/hr/g soil). No other enzymes were affected by plant diversity. LAP activity was 9.0 nmols activity/hr/g soil in 8 species plots, increased to 11.3 nmols activity/hr/g soil in 12 species plots, and 17.0 nmols activity/hr/g soil in 16 species plots. BG activity was 295 nmols activity/hr/g soil in 8 species plots, decreased to 275 nmols activity/hr/g soil in 12 species plots, and increased to 323 nmols activity/hr/g soil in 16 species plots. BX activity was 23.1 nmols activity/hr/g soil in 8 species plots and 23.8 nmols activity/hr/g soil in 12 species plots. BX increased slightly to 25.8 nmols activity/hr/g soil in 16 species plots. AG activity also remained low across all plots (8 species plots = 6.4 nmols activity/hr/g soil, 12 species plots = 7.7 nmols activity/hr/g soil, 16 species plots = 9.6 nmols activity/hr/g soil). CBH activity was 96.8 nmols activity/hr/g soil in 8 species plots, 97.0 nmols activity/hr/g soil in 12 species plots, and 108.4 nmols activity/hr/g soil in 16 species plots. Functional dispersion did not affect enzymes, however, increases in functional dispersion marginally decreased NAG activity in species rich plots (Figure 4.2). Although NAG activity was highest in 16-species plots, activity was somewhat reduced in these plots at the highest levels of trait diversity.

## Discussion

Restoration ecology seeks to understand how biodiversity influences biogeochemical cycles to predict how extinctions in tall grass prairies will affect them and to design restored sites that support ecosystem functions. In plant communities, taxonomic diversity can alter biogeochemical cycles, alter microbial communities, and increase microbial activity and biomass (Tilman et al. 2014). Restoration that enhances taxonomic diversity may increase these functions, especially in early ecosystem stages or when soil health is poor. However, the species in a diverse community will differ in their contributions to ecosystem processes due to their functional traits. Functional trait-based frameworks can be used to predict how plant communities respond to environmental changes and management, as well as how plant functional diversity regulates soil ecosystem functions including carbon sequestration and nutrient cycling (De Deyn 2008, Funk et al. 2008, Drenovsky et al. 2012). However, trait and taxonomic diversity could have synergistic (or antagonistic) interactive effects. To determine if plant functional trait and taxonomic diversity support carbon degradation by soil enzymes, I analyzed extracellular enzyme activity in an experimental tall-grass prairie restoration to gain insights into their potential to degrade substrates containing various forms of carbon (chitin, starch, cellulose, hemicellulose). I predicted that taxonomic and trait diversity would increase enzyme activity due to increased selection effects or resource partitioning among plant species. My hypothesis was only partially correct, as plant diversity significantly increased the activity of only one soil enzyme. N-acetlyglucosaminidase (NAG) degradation was high in plots containing high species richness, indicating that plant diversity influences microbial rates of chitin degradation. This is partially consistent with previous research that shows increased extracellular enzyme activity with increased plant diversity (Steinauer et al., 2015). However, functional trait diversity did not influence enzyme activity in this study. A larger effect of diversity may have been detected if species richness varied more broadly (rather than from 8 to 16 species), especially if low-richness plant communities result in microbial communities that are taxonomically and functionally depauperate. This study supports the large body of research that states that restoration aimed at increasing biodiversity can support soil ecosystem functioning, including carbon processes.

Although plant diversity did not have an affect on most enzymes, species richness increased the rate of N-acetlyglucosaminidase (NAG) activity within two years of planting, which can indicate changes in soil chitin degradation. This suggests that plant diversity may create conditions conducive to enhanced breakdown of chitin by facilitating microbial communities that specialize in chitin hydrolysis. Soil enzyme activity can depend on the amount of substrate that is contained in the soil which can also be driven by plant diversity. Soil enzymes can be driven by microbial community compositions, particularly bacteria that regulate microbial biomass production (Hu et al., 2020). Fungi contain chitin in their cell walls, and their dead organic matter can also contribute to chitin substrate in soil. Thus relative abundances of fungal and bacterial necromass in the microbial community may regulate soil nutrient pools and fluxes. Plant diversity can increase microbial activity and fungal associations (Zak et al., 2003; Carney & Matson, 2006) that may facilitate a chitin-degrading microbial community. However, chitin-degrading enzymes are widespread among various microorganisms as well as in some animal digestive tracts, making correlations between microbial composition and chitin degradation hard to assess (Beier & Bertilsson 2013). Although enzymes that degrade starch, hemicellulose, and cellulose did not respond to plant diversity in this study, testing lower levels of species richness (e.g., 1-4 species) may reveal more patterns between biodiversity and enzyme activity because limited variation in litter or other plant inputs might constrain microbial communities and reduce other enzymes' activities. This would align with predictions that even intermediate grassland species extinctions (21-40%) show negative effects on grassland ecosystem function (Hooper et al. 2012).

Plant diversity can contribute to increased plant biomass through mass ratio effects that could potentially lead to increased C-inputs to the soil. Studies using isotopic and biomarker analyses have shown plant roots are a primary source of carbon substrates in the soil (Mendez-Millan et al., 2010). Functional trait diversity, which included root trait diversity, did not affect enzymes possibly because certain traits that affect C degradation, for example traits that can affect plant-mycorrhiza or plant-pathogen interactions (e.g., root exudate content and production, or allelopathic chemicals), were not included in this analysis. Characterization of these specific traits in plant communities might better predict soil enzyme activity and C degradation than the multi-trait index of functional diversity used here. By breaking down chitin-rich cell walls of pests and pathogens like insects and pathogenic fungi, these enzymes also contribute to the plant's defense mechanism. Relationships between plant diversity and soil microbial functioning can also depend on soil pH, temperature, and moisture (Wallenstein et al. 2011, Steinweg et al. 2012) that may be masking functional trait diversity influences on soil enzymes in this study, although all plots were contained in a single homogenous field and experienced similar abiotic conditions.

There was a moderate but statistically non-significant interaction between species richness and functional dispersion (*p*=0.053), such that NAG activity decreased as functional trait diversity increased, but this effect was only seen in 16-species plots. This may be due to fewer competitive interactions and more plant specialization in plots that are functionally diverse. Nutrient dynamics could be influenced by interspecific plant interactions where more efficient uptake by plants would reduce the amount of nutrient left in the soil for microbes to breakdown. As a result, the demand for nitrogen-releasing enzymes, like NAG, could decrease. This indicates that functional trait diversity does not always increase ecosystem function and likely depends on other biotic factors. Fungi, which are chitin-rich organisms, may produce more biomass when a particular set of plant traits are dominant, thus supporting more chitin-degrading microbes that produce these enzymes. Certain plant traits that facilitate fungal communities are root diameter, root nitrogen, and specific root length (Sweeney et al. 2021), and plant root traits are drivers of root-associated ectomycorrhizal and endophytic fungi (Odriozola et al. 2021). The presence of a wide range of traits in high-functional diversity plots may not be ideal for supporting high fungal abundance if plants with these fungi-facilitating trait combinations are less common.

## Conclusions

Extracellular enzymes produced by soil microbes are key for breaking down organic macromolecules into soluble forms for microbial assimilation and thus are a critical part of carbon cycling (Steinauer et al., 2015). Species richness increased activity of a chitin-degrading enzyme, perhaps because plant diversity increased litter quality or influenced the microbial community to facilitate chitin degradation. The results from this study highlight biodiversity's role in facilitating nutrient cycling processes through changes in enzyme activity because enzymes can increase soil organic matter mineralization (Hu et al., 2020). These findings also support the idea that restoration aimed at increasing plant diversity can affect certain soil ecosystem functions, particularly carbon processes. High species richness, by increasing chitin degradation, could actually reduce C storage, undercutting a common goal of restoration activities. Functional diversity may not be conducive to certain ecosystem functions in species rich plots if it increases interspecific competition. However, further research is needed to fully understand how functional trait diversity interacts with taxonomic plant diversity and with abiotic variables. This research provides insight into how biodiversity declines may affect soil ecosystem function in grasslands, especially as climate changes cause extinctions and changes to biogeochemical cycles.

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Table 4.1. Results of linear mixed effects models analyzing enzyme activity as rate of degradation of the following substrates as an effect of plant diversity (species richness, functional dispersion, and their interaction): α-glucosidase (AG), β-glucosidase (BG), β-xylosidase (BX), cellobiohydrolase (CBH), and N-acetyl-β-D-glucosaminidase (NAG). fd=functional dispersion; sr = species richness)

| Enzyme | Factor | $\chi^2$ | df | *P* |
|---|---|---|---|---|
| BG | functional dispersion | 2.71 | 1 | 0.099 |
| | species richness | 0.23 | 2 | 0.889 |
| | fd x sr | 0.40 | 2 | 0.818 |
| CBH | functional dispersion | 2.32 | 1 | 0.128 |
| | species richness | 3.50 | 2 | 0.174 |
| | fd x sr | 3.11 | 2 | 0.211 |
| NAG | functional dispersion | 0 | 1 | 0.994 |
| | species richness | 7.07 | 2 | **0.029** |
| | fd x sr | 5.87 | 2 | 0.053 |
| BX | functional dispersion | 0.21 | 1 | 0.650 |
| | species richness | 3.45 | 2 | 0.178 |
| | fd x sr | 3.35 | 2 | 0.188 |
| AG | functional dispersion | 0.11 | 1 | 0.731 |
| | species richness | 1.91 | 2 | 0.385 |
| | fd x sr | 1.73 | 2 | 0.422 |
| LAP | functional dispersion | 0.54 | 1 | 0.465 |
| | species richness | 2.82 | 2 | 0.245 |
| | fd x sr | 2.98 | 2 | 0.226 |

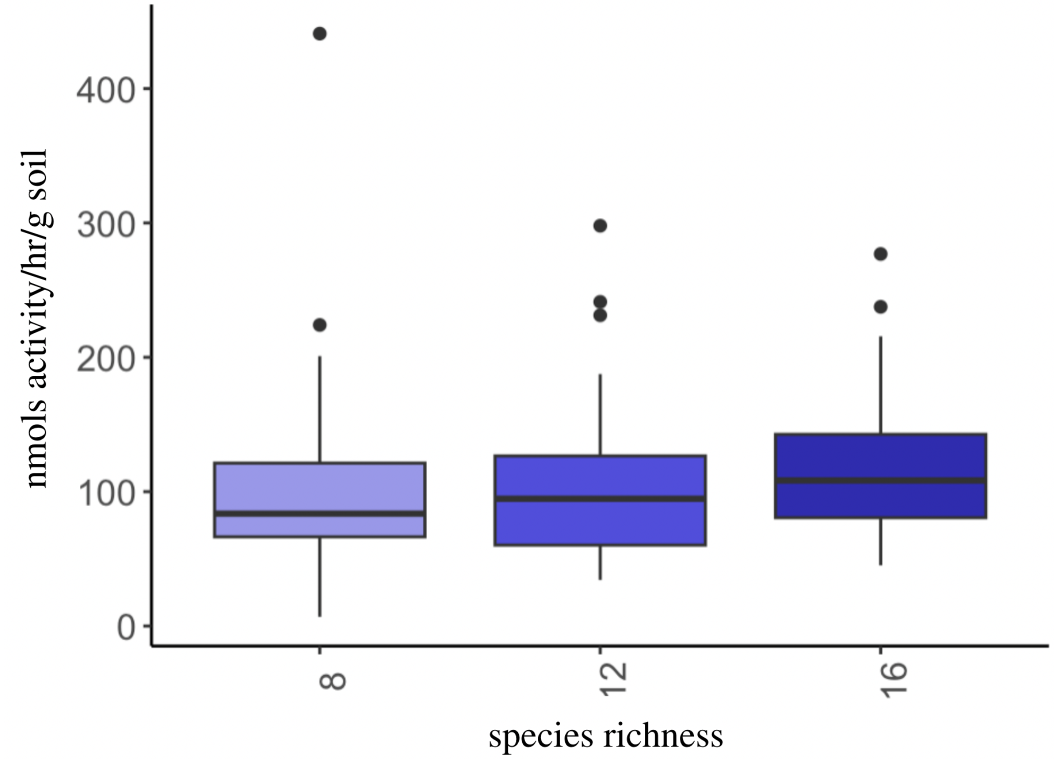

Figure 4.1. Enzymatic activities of N-acetyl-β-D-glucosaminidase (NAG) in plots with 8, 12, and 16 species. $p<0.05$ for species richness effect on NAG enzyme activity.

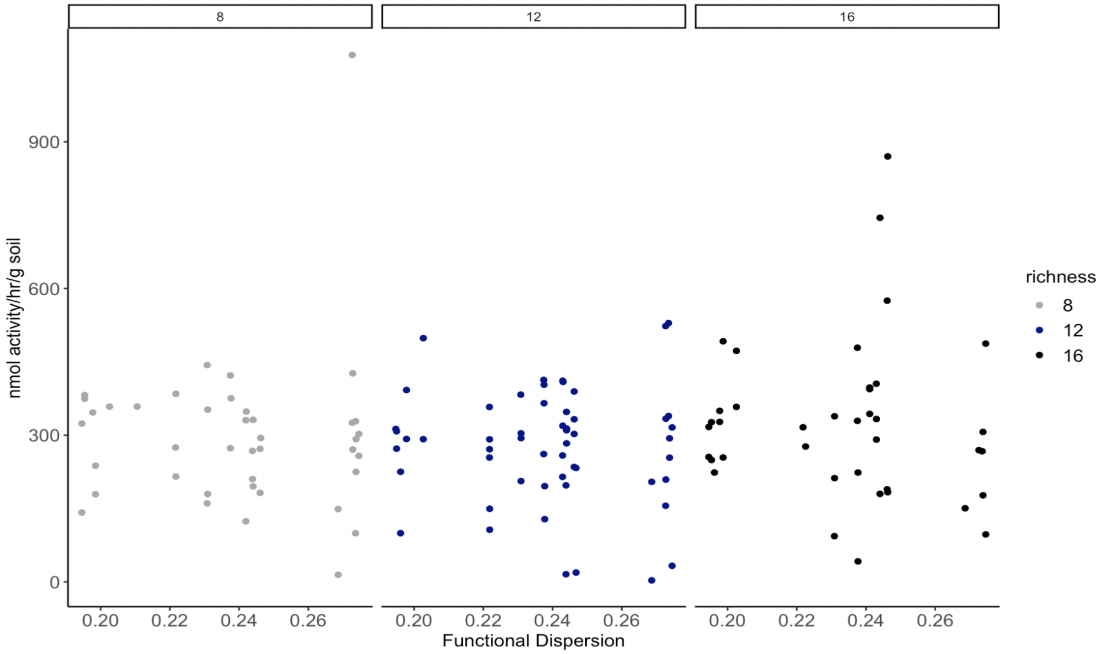

Figure 4.2. Enzymatic activity of NAG in plots with 8, 12, or 16 plant species along a functional trait diversity (calculated as functional dispersion) gradient. Enzyme activities are reported as nmol activity/hr/g dry soil. Richness=species richness. At 16-species richness, there was a weak negative relationship between NAG activity and functional dispersion.

*Achillea millefolium*
*Andropogon gerardii*
*Anemone cylindrica*
*Baptisia alba*
*Bromus inermis*
*Carex bicknellii*
*Echinacea pallida*
*Elymus canadensis*
*Helianthus occidentalis*
*Lespedeza capitata*
*Monarda fistulosa*
*Penstemon digitalis*
*Poa compressa*
*Ratibida pinnata*
*Schizachyrium scoparium*
*Silphium integrifolium*
*Solidago canadensis*
*Solidago rigida*
*Sorghastrum nutans*
*Symphyotrichum ericoides*
*Symphyotrichum laeve*
*Symphyotrichum oolentangiense*
*Trifolium pratense*
*Zizia aurea*

4.S1. Selected species used in experimental plantings based on common species found in vegetation analyses at nearby prairies. Plots contained 24 individual plants and had 8, 12, or 16 species.