The plant ecology of soil nutrient supply from species to ecosystems

A Dissertation

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Dedication

To my wife Tonje for her unwavering support

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Introduction

The supply of soil nutrients is a fundamental process structuring plant communities and the functioning of ecosystems. As such, the manipulation of soil nutrients has been a key tool for the ecologist to understand which nutrients are important in a given ecosystem and how soil nutrients impact plant community composition (Tilman 1982, Chapin et al. 1986, Vitousek and Howarth 1991, Vitousek and Farrington 1997, Schlesinger and Bernhardt 2013, Borer et al. 2014a). The experimental creation of a soil nutrient gradient informs how plants are differentiated in their competitive ability (Lawes et al. 1882, Tilman 1987, Silvertown et al. 2006). However, plants do not only passively respond to soil nutrient gradients, but also themselves modify soil nutrient supply (Tansley 1935, Crocker and Major 1955, Jenny 1958, Zinke 1962, Wedin and Tilman 1990, Hobbie 1992, 2015, Berendse 1998, Aerts and Chapin III 2000, Jobbágy and Jackson 2004, Ehrenfeld et al. 2005, Reich et al. 2005, Bardgett 2010, Waring et al. 2015). Exposition of the plant ecology of soil nutrient supply therefore requires a dual approach that considers experimental manipulation of both soil nutrients and species composition. In this dissertation, I use two long term experiments to explore the ecology of soil nutrient supply. I leverage two core experimental designs available in plant ecology through: (1) the addition of limiting soil nutrients as fertilizer to an existing plant community and (2) the manipulation of plant community composition through seeded permanent plots and composition control through manual removal.

The addition of fertilizer to a plant community offers an experimental design of great value to test theory in plant ecology but constrained within a set of assumptions. Resource competition theory posits that species compete for limiting nutrients leading to

competitive exclusion under the condition that there is only one limiting nutrient (MacArthur 1958, Titman 1976). Consequently, a prediction of resource competition theory is that as nutrients are added to an ecosystem and they become no longer limiting, a diverse community will be reduced to a sole species that is a poor competitor for soil nutrients but a strong competitor for light or whatever is most limiting (Tilman 1982). This prediction has largely drawn out across many experiments where the addition of soil nutrients has caused the decrease of plant biodiversity (Clark and Tilman 2008, Isbell et al. 2013, Harpole et al. 2016). However, our interpretation of the design of nutrient addition experiments assumes that plots are spatially independent and often exclude herbivory to focus solely on competition amongst plants. I test these two assumptions in this dissertation.

In two chapters, I use an existing experiment to expand our understanding of the manipulation of soil nutrient supply through the study of two additional covariates: metacommunity processes and herbivory. There is considerable theory that both metacommunity processes and herbivory are crucial factors in plant ecology, but these theories have had few long-term experimental tests (Holt et al. 1994, Leibold et al. 2004, Borer et al. 2014b, Grainger and Gilbert 2016, Holt and Bonsall 2017, Thompson et al. 2020). Long-term permanent plots are required to study plant community dynamics as the outcome of competition may require several generations and short term experiments are subject to transient dynamics (Tilman 1989). I therefore used the experiment "e001 Long-Term Nitrogen Deposition: Population, Community, and Ecosystem Consequences" at the Cedar Creek Ecosystem Science Reserve (CCESR) (Tilman 1987). Within this experiment, I focused my thesis on Field C. Field C is one of the oldest fields at the

CCESR with a plant community that is diverse and representative of a late-successional tallgrass ecosystem (Inouye et al. 1987).

In my first chapter, "Might field experiments also be inadvertent metacommunities?", I challenged the assumption of spatial independence of the experimental design (Legendre 1993, Legendre et al. 2002, 2004). While the use of gridded plots has been effective in annual production agriculture where composition is controlled and reset through seeding and tillage, plant ecology studies the long-term dynamics of species composition. As such, the arrival of plant propagules, namely seeds, into a plot may impact its community composition (Hanski 1998, 2001). In this chapter, I demonstrate that the effect of nitrogen addition on plant species richness was spatially interdependent with the species richness of neighboring plots. Through a combination of longitudinal analyses and visualization from 1982-2004, I demonstrate that 1) Control plots appear to have lost diversity in proportion to their neighborhood diversity and 2) That species appear to have dispersed across the experimental field into plots with a nitrogen addition rate that matches their competitive ability. I use this case study to raise the possibility that field experiments may be inadvertent metacommunities.

In my third chapter, "Plant community responses to experimental nitrogen addition crossed with herbivore exclusion", I explore the plant community dynamics following the removal of the fences that shielded all plots in e001 Field C from herbivory from 1982-2004. Using the post fencing removal data from 2005-2019, I explore the joint effects of nitrogen addition crossed with plant herbivory on aboveground biomass, plant species richness and the composition of the plant community. The experimental design offers the opportunity to test resource competition theory (Holt et al. 1994), under the

assumption that there is only one sole limiting nutrient, nitrogen. I assumed that white-tailed deer, *Odocoileus virginianus*, was the main mammalian herbivore (Ritchie et al. 1998, Knops et al. 2000), and therefore interpret the experimental design as a deer x nitrogen addition factorial. My analyses demonstrated that deer presence was associated with a mild decrease in total aboveground biomass regardless of the addition of N. Deer presence was associated with increasing species richness, but only in plots that received no added N. However, the effect of deer on biomass and species richness was weak and largely inconsequential compared to N addition. Deer impacted the plant community most strongly in changing the composition of forb species. Using both field observations and clipped and sorted biomass, I demonstrate that deer appear to be florivores structuring the community of herbaceous forbs. Forbs appear to display tradeoffs in their ability to compete for nitrogen and their resistance to deer herbivory and or florivory.

While the addition of fertilizer offers great insights into the competitive ability of plants for limiting soil nutrients, treating plant composition as the experimental variable is essential to understand the plant ecology of soil nutrient supply. In my remaining chapter 2, "Plant biodiversity and the regeneration of soil fertility", I explore the effects of plant biodiversity on soil fertility. Building on theoretical and empirical work conducted at CCESR (Tilman 1987, Tilman et al. 1996, Zak et al. 2003, Harpole and Tilman 2006, Fornara and Tilman 2008, Dybzinski et al. 2008, Fornara et al. 2009), I measured nutrients commonly thought to limit plant productivity within the soil and those same nutrients in plant biomass. I used these data to test the hypothesis "that the sustainability of soil nutrient cycles and thus of soil fertility depends on biodiversity" (Tilman et al. 1996).

The work in this chapter consolidates several lines of evidence to improve our understanding of how biodiversity-induced changes in soil nutrient supply impacts ecosystem functioning. The chapter was inspired by Wedin (1990), who argued plant species impacts on soil nutrient supply may be as important as competition for those soil nutrients. Within the Biodiversity II experiment, the chapter builds on work demonstrating that the rate of soil N-mineralization (Zak et al. 2003), that the growth of a phytometer (Dybzinski et al. 2008) and that the accumulation of soil carbon and nitrogen all increased with plant biodiversity (Fornara and Tilman 2008, Fornara et al. 2009, Yang et al. 2019). The chapter draws from models of ecosystem development (Vitousek and Reiners 1975, Walker 2003, Peltzer et al. 2010) and theory relating to positive feedbacks within natural systems (DeAngelis et al. 1986, Ehrenfeld et al. 2005). I additionally leverage a classification technique, that of plant functional groups (Tilman 2001), to explore the possibility that plant functional diversity underpins increases in soil fertility within diverse plant assemblages.

In Chapter 2, I found evidence to support the hypothesis that experimentally increasing plant biodiversity was associated with increases in soil fertility (Tilman et al. 1996). I found that plant biodiversity increased soil C and N, soil cation exchange capacity, soil calcium, magnesium, potassium, and soil pH. I additionally found that the pool of N, K, Ca and Mg in plant biomass increased as a function of plant biodiversity in both roots and shoots. To explore why diversity may underpin the increase in ecosystem function, I used the presence of distinct plant functional groups, grasses, legumes and forbs to test for the impact of functional biodiversity on the accumulation of ecosystem pools of nutrients. I found that no functional group on its own could increase N, K, Ca

and Mg as much as did plots containing all three functional groups. Furthermore, we found that the species in these three functional groups had tradeoffs in their tissue N-content, K-content and root mass. I argue that these traits may reflect a link between their presence in a plot and the ecosystem accumulation of nutrients. In combination, the evidence in this chapter suggests that the joint accumulation of limiting macronutrients in both plant biomass and the soil suggest a possible mechanism for the strengthening effect of plant biodiversity on productivity through time (Reich et al. 2012, Eisenhauer et al. 2012, Guerrero-Ramírez et al. 2017, Thakur et al. 2021).

In summary, this dissertation adds to the body of knowledge in several areas of plant ecology. We have new evidence that metacommunity dynamics may be lurking in our long-term nutrient addition experiments in plant communities. We have new evidence that plant biodiversity may be essential for the accumulation of soil fertility and several plausible mechanisms for why plant biodiversity may be causing these patterns. We have new evidence showing that deer have notable impacts on the community composition of forb species. The knowledge gained in these three chapters highlights the value of long-term ecological research where the inherent feedbacks within an ecosystem can be measured through time. In all cases, we see that understanding soil nutrient supply is fundamental to understanding plant ecology.

Chapter 1. Might field experiments also be inadvertent metacommunities?

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George N. Furey, Peter L. Hawthorne, David Tilman. 2022. Might field experiments also be inadvertent metacommunities? Ecology.

Introduction

Ecological field experiments traditionally use a grid of permanent plots, randomization, replication, and repeated measurements to infer causation and to minimize the effects of all other processes (Lawes et al. 1882, Tilman 1989, Resetarits and Bernardo 1998, Borer et al. 2014a). However, statistical analyses and interpretation of such experiments have often overlooked the possibility that plots may be spatially interdependent (Legendre 1993, Legendre et al. 2002, 2004). While there are numerous possible causes of spatial autocorrelation (Koenig 1999), the ability or inability of species to disperse among plots may be particularly important in plant ecology (Leibold et al. 2004, Thompson et al. 2020). Such metacommunity processes can be broadly defined as the factors controlling the dispersal of species from point to point within a community and the joint impacts of dispersal and of interspecific interactions on the composition and diversity of the community (Leibold et al. 2004, Thompson et al. 2020). If metacommunity processes are operating within an ecological field experiment, then the response observed in each plot may depend both on its treatment and on its neighbors' treatments.

Metacommunity dynamics might arise within long-term experiments if plots are akin to habitat patches subject to the dynamics of propagule flow, persistence, and extinction. In a metacommunity, establishment and persistence of a species in a patch is a function of the local patch properties, competitive interactions, and the rate of propagule input from neighboring patches (Thompson et al. 2020). Taken together, the balance between colonization and extinction events, mediated by local competition and metacommunity dispersal, determine local species richness (Thompson et al. 2020). In an

experimental setting, treatments that alter species' abundances or the biodiversity of a plot through competitive mechanisms could impose second-order impacts on neighboring plots by altering the flow of propagules, shifting the balance between colonization and extinction rates (Hanski 1998, 2001).

Metacommunity effects between plots might additionally arise where there are interspecific tradeoffs between colonization rates and a second variable, such as competitive ability (Tilman 1994, Catford et al. 2018). For example, if species are subject to a competition-colonization tradeoff, inferior competitors could coexist in space by dispersing more rapidly to transiently open patches until displaced by the arrival of a species that is a superior competitor (Tilman 1994, Kerr et al. 2002). In contrast, highly competitive species may be more sensitive, at the metacommunity scale, to experimental treatments that reduce the number of viable patches. The loss of propagules that had been dispersing into a plot before treatments were imposed on its neighbors could impose an 'extinction debt' on those species that are poor colonizers and could eventually cause such species to be lost (Tilman et al. 1994).

Existing long-term field experiments on plant communities may offer unique opportunities to test metacommunity theory predictions (Mouquet and Loreau 2003, Leibold et al. 2004, Cadotte 2006, Thompson et al. 2020, Record et al. 2021) relating to the effects of plot-to-plot dispersal on plant species' abundances and diversity. For example, theoretical predictions that dispersal might impact local species diversity must be tested empirically (Brudvig et al. 2009, Haddad et al. 2015, Grainger and Gilbert 2016, Germain et al. 2017). In the case of a gridded-experiment, a plot may be impacted by dispersal dynamics that move propagules either away from a plot or into a plot

(Hanski 1998). Metacommunity theory may therefore be most relevant when the design of a field experiment places treatments in close proximity such that the distance between plots falls within the dispersal kernels of the species (Sullivan et al. 2018).

We re-analyzed results from a gridded nitrogen (N) addition grassland field experiment that began in 1982 (Tilman 1987) to test if N-dependent shifts in plant species composition and richness had impacts on species composition and richness of neighboring plots. Given the potential for immigration and emigration to impact local diversity (Cadotte 2006), we sought to determine if there was a positive relationship between neighborhood species richness and the species richness of a focal plot. Such neighborhood effects may help answer a curious and previously unexplained result observed in this experimental field: control plots that received no added nutrients had, on average, lost species richness through time (Clark and Tilman 2008). In contrast, long-term studies at our site using permanent plots in successional grasslands and native savannah that were not fertilized did not lose species richness through time (Isbell et al. 2019).

We used three lines of evidence to determine if metacommunity processes may have influenced the results of this N-addition experiment. (1) If, after controlling for the observed direct effects of treatments, was a focal plot's plant species richness significantly dependent on neighboring plots? (2) Was the change in species richness of control plots dependent on the species richness of neighboring plots? (3) Did the abundances of the three most common species reflect the neighborhood abundances of each species?

Methods

Site description: The experiment was conducted at the Cedar Creek Ecosystem Science Reserve in east-central Minnesota, USA. The site has a low organic matter, nutrient poor, sandy soil with a particle size distribution of ~90% sand (Grigal 1974). The vegetation of this field when the experiment was implemented had been undergoing secondary succession following its abandonment from agriculture in 1934. The field is located at 45.397334°, -93.191648° and referred to as "Field C" within the experiment e001: Long-Term Nitrogen Deposition: Population, Community, and Ecosystem Consequences. In 1982, the vegetation consisted mainly of perennial tall-grass prairie species common to the central United States. Following twice-annual fertilization that began in 1982, high N plots became dominated by the perennial grasses *Elymus repens* and *Poa pratensis* and annuals such as *Chenopodium album* and *Polygonium convovulus*.

Experimental Design: The experiment consists of fifty-four plots laid out in a 6-plot-wide by 9-plot-long rectangular grid. A diagram to scale is presented in Appendix S1: Fig. S1. Each plot is 4 by 4 m with 1 m buffers between them. There are nine treatments replicated six times each in an un-blocked fully randomized design. The main experimental treatment is denoted by letters A-I, with the addition of N as ammonium nitrate at 7 rates of B-1.02, C-2.04, D-3.4, E-5.44, F-9.52, G-17.0, or H-27.2 g m^{-2} yr^{-1} of N. All 7 of these treatments also receive other major plant nutrients (P, K, Ca, Mg and trace metals) to assure that N is the limiting nutrient (Tilman 1987). There are two additional treatments that receive no N. The six control plots (Treatment I-0.00) receive no nutrients of any kind. The other, Treatment A, receives the same amounts of the other non-N major plant nutrients as do the N addition plots (A-0.00). Nutrient addition was added as half of the annual nutrient addition rate applied in early May and half in late

June of each year. Sheet metal was installed to a depth of 30 cm in the middle of all buffer strips to prevent fertilizer movement and root foraging for nutrients between plots. All plots were fenced in 1982 to exclude large herbivores. Because fencing was removed for half of the plots in 2005, we focus on results from 1982 to 2004.

Sampling: Vegetation was sampled in each plot by clipping a 10 cm by 3 m strip, sorting this vegetation to species, then drying to constant mass and weighing each species. Species were identified using an on-site herbarium. All clipped strips were at least 50 cm from plot edges. The same area was never clipped twice, and areas to be clipped were never immediately adjacent to an area that had been clipped during the past four years. Data is freely accessible via the Environmental Data Initiative (Tilman 2020). The computer code used in the analyses of these data is archived via Zenodo https://doi.org/10.5281/zenodo.3908538.

Data Analysis: Our analyses and data manipulation used R (R version 4.1.1). We conducted analyses at two levels of the plant community: the number of species observed in each plot, henceforth referred to as species richness, and the aboveground biomass of the three most abundant species (as grams m^{-2}): *Elymus repens, Poa pratensis* and *Schizachyrium scoparium.* These two response variables were used to explore the three questions listed below.

Question 1: Was a focal plot's plant species richness dependent on neighboring plots*?*

We analyzed species richness in two ways using either the entire time series with one observation per plot in each year or using the time-series average of species richness for each plot. To analyze species richness using the entire time series, we used a linear mixed-effects model (nlme; Pinheiro and Bates 2000), testing the dependence of plot

species richness on N, year and neighborhood species richness. A random intercept was included for each plot. The sample size was fifty-four plots across twenty-three years. N was treated as a continuous variable with the natural log of N + 1, referred to in-text as ln[N+D], to account for non-linearity (based on patterns in the model residuals). We added a constant to allow the log transformation of zero values and to approximate the 1 gram of background N deposition (Clark and Tilman 2008). Year was treated as a continuous variable with 1982 coded as year zero to allow direct interpretation of the intercept. Neighborhood species richness was treated as an autocovariate regressor (Dormann et al. 2007), calculated as the mean of species richness in the four plots immediately adjacent to a plot, except for plots on an edge or corner where three or two plots were used, respectively. We used the time-series mean of this variable for each plot as a more conservative test. A temporal autocorrelation function was used (*nlme::corARMA*). A separate variance term was included for each level of nutrient addition (*nlme*::*varIdent*) to account for unequal variance across experimental treatments.

The second model used generalized least squares to test the dependence of the average plot species richness (1982-2004) on N (ln[N+D]) and the average neighborhood species richness. The sample size was fifty-four plots. A separate variance term was included for each level of nutrient addition (*nlme*::*varIdent*) to account for unequal variance across experimental treatments. Standard error of fitted values was estimated using the delta method with R package *AICcmodavg* (Mazerolle 2020).

Spatial autocorrelation was tested using R package *spdep* treating the experiment as a 6x9 grid (Bivand 2013). The spatial weights to test for autocorrelation using the Moran's I statistic were calculated using weights coding to avoid over-representation of

edges, coded as “S” in *spdep* (Tiefelsdorf et al. 1999). The qualitative result of the Moran’s I test did not change using row standardized “W” or globally standardized “C” (Bivand 2013). Spatial autocorrelation was tested on the residuals for the generalized least squares model testing the dependance of the average of species richness on ln[N+D] both with and without a covariate of neighborhood species richness. Moran's I was tested to a lag of three, half the shortest length of plots in a row of six, and *P*-values were adjusted using the False Discovery Rate correction (Benjamini and Hochberg 1995). The same Moran's I test was similarly tested on the nutrient addition treatment to check that the spatial dependance occurred at the level of the response variable and not the explanatory variable (Fortin 2005).

Question 2: Was the change in species richness of control plots dependent on the species richness of neighboring plots*?*

We tested the dependence of species richness in the six control plots that received no nutrient addition (Treatment I-0.00) on an interaction between neighborhood species richness and year using a linear mixed-effects model. Year was treated as a continuous variable with 1982 coded as year zero to improve interpretation of the intercept. A random intercept was included for each plot. The sample size was six plots across twenty-three years. 95% confidence intervals were calculated with R package *emmeans* (Lenth 2020) using five degrees of freedom. We additionally determined the time trend of species richness for each control plot. We tested a separate ordinary least squares regression for each control plot with species richness as the response variable and year as the explanatory variable. *P*-values were adjusted using the False Discovery Rate correction (Benjamini and Hochberg 1995). We then tested for a correlation between the

average neighborhood species richness for each plot and the six separate time slopes using a major axis regression. This second analysis serves as an additional robustness check to test if the trend through time is correlated with the effect of neighborhood species richness.

Question 3: Did the abundances of the three most common species reflect the neighborhood abundances of each species?

We used a separate linear mixed effects model for each of three dominant species in this experiment – *Schizachyrium scoparium, Poa pratensis* and *Elymus repens* (Tilman 1987, Clark and Tilman 2008, Isbell et al. 2013). The sample size was fifty-four plots across twenty-three years. Each model had, as the response variable, the square root (to address heteroscedasticity) of the biomass of a species in each plot in each year (referred to as focal plot abundance). For each species, we determined how its focal plot abundance depended on the nutrient addition treatment as an unordered, categorical variable (9 levels, Treatments A-I), year as a continuous variable, and each species' neighborhood abundance as a continuous variable using a full-factorial that included all two-way and three-way interactions. Year was treated as a continuous variable with 1982 coded as year zero to improve interpretation of the intercept. Neighborhood abundance was calculated similarly to neighborhood species richness. For each species and each year, we calculated its mean neighborhood biomass in the plots adjacent to each focal plot. For all models, plot was included as a random intercept, a temporal autocorrelation function was used (*nlme::corLin*), and a separate variance term for each level of nutrient addition (*nlme*::*varIdent*) was included to account for unequal variance across experimental treatments. *P*-values were adjusted using the False Discovery Rate

correction (Benjamini and Hochberg 1995). 95% confidence intervals were calculated with R package *emmeans* using five degrees of freedom.

We assume that higher neighborhood biomass of a given species would lead to a greater source of propagules of that species to a focal plot and consider neighborhood biomass to be a *post hoc* proxy for species neighborhood dispersal. Animated visuals were designed to demonstrate the abundance of each species through time in space (Video S1-S6). These videos were created using *ggplot2*, animated using R package *gganimate* (1.0.7) (Pedersen and Robinson 2020) and encoded to .mov format using software *ffmpeg* (4.2.2). For a given species, the significance of neighborhood biomass on focal plot biomass provides an indication of how much the local abundance of a species positively depended on its abundance in neighboring plots *i.e.* metacommunity 'patches' (Hawthorne 2012). The maps displayed in Fig. 2 were designed using R graphical package *ggplot2* (3.3.5) (Wickham 2016) and colored using *viridis* (0.6.1) (Garnier et al. 2021). The diagram in Appendix S1: Fig. S1 was designed using SketchUp 2021 (Tribble).

Results

Was a focal plot's plant species richness dependent on neighboring plots?

Our analyses show that plots with greater (or lower) species richness in their neighborhood had greater (or lower) species richness than otherwise expected for their nutrient treatment. In particular, we first tested the effects of the natural log of N addition (ln[N+D]), of the time-series mean of neighborhood species richness, and of year on focal plot species richness using a linear mixed effects model (Appendix S1: Table S1). For each additional species in a focal plot's neighborhood, the species richness of focal

plots increased by an average of 0.30 ± 0.11 SE species ($P = 0.011$). On average, focal plot species richness decreased through time (slope of -0.20 species yr^{-1}) and as reported previously (Clark and Tilman 2008), focal plot species richness decreased with ln[N+D]. When using a single average value for each plot (averaged 1982-2004), ln[N+D] had a negative effect (a loss of -2.21 species per 1 log unit of added N; $P < 0.001$) and neighborhood species richness had a positive effect on species richness, with an increase of 0.40 ± 0.08 (SE) species on average for each species in a focal plot's neighborhood; $P < 0.001$) (Figure 1-1).

We then tested for spatial autocorrelation in the effect of ln[N+D] on focal plot species richness. The observed effect of neighborhood species richness corresponds with spatial autocorrelation in the regression residuals of the effect of ln[N+D] on focal plot species richness (Figure 1-1). A global Moran's I test indicated that high and low residuals were aggregated in space to a range that included the four plots immediately adjacent to each focal plot, *i.e.*, a radius of about 5 m from the center of a focal plot to the center of adjacent plots (Moran's I = 0.32, Z-statistic = 3.34, $P = 0.005$) (Figure 1-1). The addition of neighborhood species richness as a variable in the model accounted for the spatial autocorrelation in the response variable and the Moran's test no longer indicated aggregation (I = -0.024, Z = -0.05, $P = 0.96$) (Figure 1-1). While the effect of N on species richness was spatially autocorrelated, the experimental nutrient addition variable itself was not (I = 0.024, Z = 0.43, $P = 0.67$).

Was the change in species richness of control plots dependent on the species richness of neighboring plots?

Maps of the nutrient addition rate for each plot and their species richness visually suggest that control plots (Treatment I-0.00, those that received no nutrients) lost more species when surrounded by plots that lost species from high rates of N addition (Figure 1-2). To quantitatively explore this possibility, we tested for a two-way interaction between year and neighborhood species richness on focal plot species richness using a linear mixed effects model with only the six control plots (Treatment I-0.00). The statistical model had a positive year by neighborhood species richness interaction ($P < 0.001$, Appendix S1: Table S2-S3), with the effect of neighborhood species richness becoming stronger through time (Figure 1-3). An interaction plot (Figure 1-3) across a range of possible neighborhood species richness values (-2 standard deviations (SD) to + 2 SD) demonstrates the trend in focal plot species richness through time modulated from positive to negative as neighborhood species richness declined.

The data underlying this significant interaction (Figure 1-4) show that plot species richness had year-to-year variability, but that only those control plots with the greatest declines in their neighborhood species richness had significant ($P < 0.05$) declines in species richness. These results are summarized in Figure 1-4g where we see that three plots had significant declines in species richness through time whereas three had a slope that did not differ from zero ($P > 0.05$). Using major axis regression, we found a significant positive correlation between the average neighborhood species richness for each plot and each plot's slope for species richness through time ($R^2 = 0.77$, $P = 0.021$, (Figure 1-4g). This correlation shows that control plots lost species through time in proportion to their neighborhood species richness.

Did the abundances of the three most common species reflect the neighborhood abundances of each species?

The North American perennial bunchgrass *Schizachyrium scoparium* was dominant in control and low N treatments, the Eurasian perennial grass *Poa pratensis* came to dominate in mid-level N treatments and the Eurasian perennial grass *Elymus repens* came to dominant in high N treatments (Appendix S1: Fig. S2) (Tilman 1987, Clark and Tilman 2008, Isbell et al. 2013). For each of these three species, their abundances were correlated with a three-way interaction among the nutrient addition treatments, each species' neighborhood abundance and year (Appendix S1: Table S4-S9). The model interactions demonstrate that high neighborhood abundance of a species had a positive effect on the abundance of that species and that the effect was contingent on the rate of N addition and varied through time. For the low N dominant native grass *Schizachyrium scoparium*, values from the fitted model demonstrate a decline in its abundance in the control plots (Treatment I-0.00) fit with a neighborhood abundance of *Schizachyrium scoparium* of zero, and an increase in its abundance in control plots fit with a high neighborhood abundance (mean + 1 SD) (Figure 1-5). These trends can be contrasted with a decline through time in *Schizachyrium scoparium* in high N plots 9.52 g m^{-2} N yr^{-1} (Figure 1-5) regardless of its neighborhood abundance. For *Poa pratensis*, the mid-range N dominant grass, its abundance in control and mid nitrogen plots (3.40 g m^{-2} N yr^{-1}) increased faster when it had higher abundance in neighborhood plots (Figure 1-5). While *Poa pratensis* was initially abundant in plots receiving 9.52 g m^{-2} N yr^{-1}, it declined in abundance as *Elymus repens* increased, but persisted longer when it had high neighborhood abundance (Figure 1-5). For the high-N dominant, *Elymus repens,* the

interaction shows that high N plots (9.52 g m^{-2} N yr^{-1}) that were in a neighborhood with high abundances of *E. repens* were colonized by it (Figure 1-5), but high neighborhood abundance did not lead to high abundance in control plots (Figure 1-5). Thus, for each of these three dominant species, greater (or lower) neighborhood abundance tended to lead to greater (or lower) abundance than otherwise associated with the nutrient addition treatments.

To help visualize the effect of neighborhood abundance on the abundance of each species in a focal plot, a time-lapse visual for each species is presented both with and without the nutrient addition treatments colored. In the videos without the treatments colored, one can observe that species' abundances appear related to their abundance in adjacent plots, particularly *E. repens* (Video S1). *E. repens* spread across the field to dominate the high N plots, but remained rare in control plots (Video S2). In contrast, *S. scoparium,* which was initially abundant in all plots, was extirpated from high N plots to remain only in control and low nitrogen plots (Video S3, Video S4). *P. pratensis,* which was initially present in almost all plots, decreased in abundance in high N plots and remained in mid-range N addition plots and was present, but in lower abundance in control and low N addition plots (Video S5, Video S6).

Discussion

We found that even in a well-controlled field experiment, metacommunity processes appear to be impacting both plant species richness and species' abundances. The negative effect of high rates of N on focal plot species richness was increased or decreased when neighboring plots had lower or higher species richness than a focal plot. The effect of neighborhood species richness on focal plot species richness appears most

important in the control plots. Control plots neighbored by high N treatments and their associated low biodiversity lost species through time whereas those neighbored by plots with high neighborhood species richness had no detectable loss in species richness. Finally, we found that changes in the abundance of the three common grass species through time depended not only on N addition, but also on their neighborhood abundance. In total these analyses suggest that both the experimental N treatments and metacommunity processes co-determined plant species richness and plant species' abundances observed in the plots.

Our analyses suggest that species spillover, (Brudvig et al. 2009), from one plot to another violated the frequently-made assumption of spatial independence of experimental treatments. The original experimental design had implicitly assumed that a 1-meter buffer around 4 m x 4 m plots, metal flashing buried to a depth of 30 cm, and always sampling a plot at least 0.5 m from its edges would be sufficient to prevent the treatment imposed on a neighboring plot from influencing nearby plots (Tilman 1987). However, long-term experiments often generate unexpected results (Brown 1998). In our case, spatial processes became unexpectedly important. These results add credence to the hypothesis that spatial patterns in ecology may not be solely random error (Legendre 1993, Wiegand et al. 2017), but rather represent meaningful ecological processes, in our case perhaps the interplay of resource competition and dispersal.

The otherwise paradoxical loss of species richness from unmanipulated control plots in this experimental field becomes understandable when the metacommunity impacts of the N treatments are formally included in analyses. Incorporating a given plot's compositional neighborhood may account for the impacts of changes in propagule

loss and arrival. When neighborhood effects are significant, they suggest that plots are spatially interdependent (Legendre 1993, Legendre et al. 2004, Fortin 2005). As shown in Fig. 2, control plots 18, 28 and 30 are all adjacent to high N plots that became low in species richness whereas plots 33, 53 and 54 are adjacent to other no added N or low added N plots that retained relatively higher species richness. Consistent with this, plots 18, 28 and 30 all significantly lost species through time whereas the other three maintained their initial species richness (Figure 1-4).

Our results do not qualitatively change previously reported results demonstrating that N addition leads to the loss of plant species richness (Tilman 1987, Clark and Tilman 2008). However, the reported quantitative effects of N addition on plant species richness are clarified by considering metacommunity effects. Our results suggest that spatial processes related to dispersal within an experiment may amplify the effect of treatments that reduce biodiversity due to the concomitant decrease in source propagules. If there exists a background rate of dispersal within a field that helps maintain biodiversity (Shmida and Wilson 1985), reducing the diversity of some plots via a treatment might cause an overall decline in the biodiversity of that field. Interestingly, the gamma diversity of this experiment, which is the total number of species observed across all fifty-four plots, declined through time ($F_{1,21} = 13.2$, $p = 0.002$) (Appendix S1: Fig. S3) even though the various N treatments could be viewed as increasing spatial resource heterogeneity (Tilman 1982).

The subject of experimental design and analysis is a long standing issue in ecology (Hurlbert 1984, Tilman 1989, Resetarits and Bernardo 1998, Oksanen 2001, Legendre et al. 2004, Borer et al. 2014a). In our experiment, we suggest that treatments

are sufficiently close to each other in space such that treatments applied in a plot's neighborhood became an added variable (Brudvig et al. 2009). Our results raise several questions. Might the reduction or elimination of metacommunity effects require much larger plots, large buffers between plots, and sampling only the interior of each plot? Might the use of smaller plots with large buffers cause treatments with small effect sizes to be obscured by metacommunity effects? Our results suggest that it may be worthwhile to test for metacommunity effects in field experiments, and to consider the possibility that factors other than dispersal, such as the spatial dynamics of disease or predation/herbivory (Holt and Kotler 1987), may also impact interpretation of observed results.

We suggest that our results are consistent with metacommunity models of habitat destruction, modified by the perspective that high rates of N addition may be operationally equivalent for native plant species to the destruction of a plot (Tilman et al. 1994). The low focal plot diversity and almost complete absence of native grassland species in plots receiving >9 g m^{-2} yr^{-1} of N in our experiment suggests that such plots could be viewed from the metacommunity perspective as having had their native grassland habitat ecologically “destroyed.” For example, the spatial patterns of the abundance of *S. scoparium* appear similar to simulations of a metacommunity model with in which its patches of its habitat were destroyed (Video S4, Tilman et al. 1994). From this viewpoint, high rates of N addition functionally fragmented the habitat (Tilman et al. 1994, Hanski 1998, 2001, Hawthorne 2012). The observed net effect of this fragmentation on diversity is revealed by the control plots, which lost plant species when neighboring plots had few species because of high rates of N addition. Empirically

derived dispersal kernels for many species in this plant community suggest that neighborhood plots are within a range that could be colonized by most of these species (Sullivan et al. 2018). However, if experimental treatments reduce or eliminate the ability of propagules of native grassland species to reach an undisturbed remnant grassland site, our results suggest that unmanipulated sites will lose native species through time and thus exhibit a biodiversity extinction debt (Tilman et al. 1994). As such, our analyses support the possibility that metacommunity processes may be important for the conservation of biodiversity (Chase et al. 2020).

The abundances of each of the three dominant species depended both on the experimentally-imposed N gradient and on their neighborhood abundance, suggesting a link between their competitive and dispersal ability. These species may exhibit a tradeoff between competition and colonization (Tilman 1994). For instance, *E. repens,* an invasive grass and a weak N competitor, but strong colonizer (Tilman 1994), became more abundant with higher rates of N addition, especially when surrounded by plots in which it was abundant (Figure 1-5; Video S1; Video S2). In contrast, *S. scoparium* is a strong N competitor but a weak colonizer (Tilman 1994). Its neighborhood abundance, when high, may have slightly slowed its decline in high N plots (Figure 1-5, Video S4), but it could not persist via mass-effects, *i.e.,* dispersal of propagules, into high N plots (Shmida and Wilson 1985, Mouquet and Loreau 2003). Similarly, *E. repens* did not effectively colonize and dominate control plots in which it was a weak competitor (Figure 1-5; Video S2). If one considers high N plots as sinks for *S. scoparium* and control plots as sinks for *E. repens,* then we did not find evidence that sources of propagules from neighborhood biomass could maintain their abundance in those

experimental treatments for which they are poorly adapted. Rather *E. repens* appears to have invaded into high N plots in proportion to its neighborhood abundance starting from a few patches at the start of the experiment (Video S2).

Our analyses do not prove *sensu stricto* that our experimental plots are subject to metacommunity effects since our analyses are based on correlational patterns. Rather these analyses suggest that experiments like ours might be inadvertent metacommunities: where within-experiment dispersal and local competition combine to violate the spatial independence of experimental treatments. Our three distinct types of analyses are all consistent with this possibility. In the hope that our results might encourage similar analyses so that the generality of our results can be determined, we offer a few cautionary thoughts and suggestions. Like most studies with relatively small sample sizes, it is always possible that some unconsidered spurious factor may have contributed to the observed results. Addressing the issue more directly may benefit from methodological adjustments in future experiments. First, whole-plot presence/absence data would improve the ability to track plot diversity and composition, particularly of less common species. Additional types of analyses, such as analyzing the temporal lags or spatial distance-decay for each species (Palmer 1988), might better track dispersal among plots. Finally, the collection of seed rain or other quantitative measures of dispersal, and data on soil seed banks, could better test how neighborhood abundance influences propagule density in nearby plots.

Many ecological experiments have plots laid out in a grids like this one, and could provide added insights into if or how metacommunity processes operate in those experiments and for their types of species (Brudvig et al. 2009, Record et al. 2021).

Future experiments might be designed to either minimize the potential for metacommunity effects through use of appropriately large plots, or to simultaneously explore treatment effects, metacommunity effects, and their interactions. Starting a field experiment with permanent plots may well represent the simultaneous creation of an inadvertent metacommunity arena. In our case, metacommunity processes appear to impact the spatial independence of plots. We suggest that both metacommunity processes and resource addition jointly determined plant species richness and species abundances in our experiment.

Figures

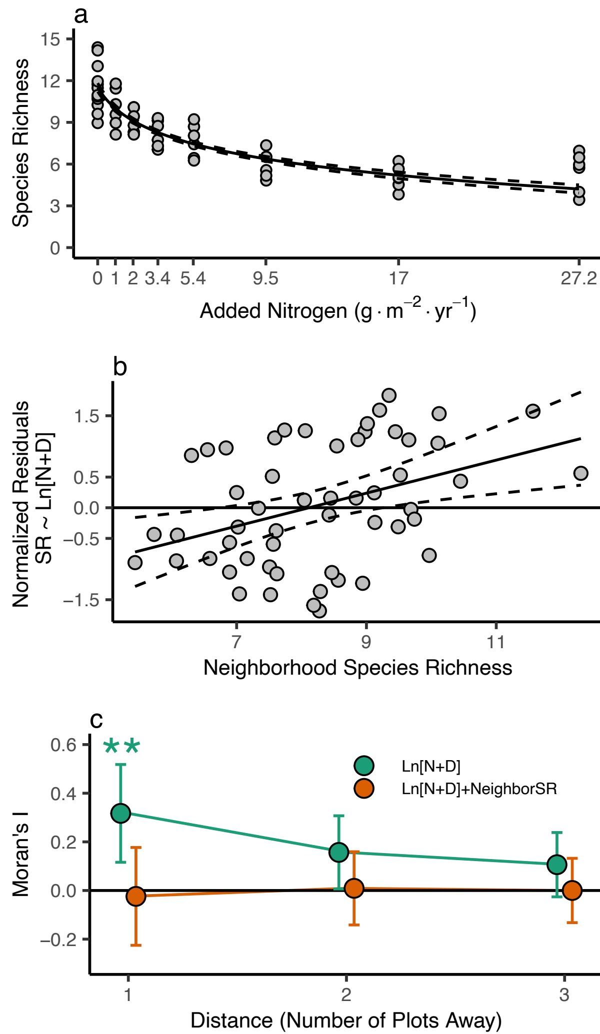

Figure 1-1: The dependance of focal plot species richness on nitrogen and neighborhood species richness. All parts of this figure use observed responses averaged across 1982 through 2004, with a single average value per plot. (a) Focal plot averaged species richness regressed on the log[added nitrogen + 1], termed (ln[N+D]) (n = 54). The black line represents the fitted relationship ± a standard error. (b) The normalized residuals from part A (residuals of a regression of focal plot averaged number of species (SR) on ln[N+D]) are regressed on averaged neighborhood number of species (n = 54). The black line represents the fitted relationship ± a 95% C.I. (c) Global Moran's I statistic ± two

times the square root of its variance for regression models with only ln[N+D] and ln[N+D] plus averaged neighborhood species richness. ** indicates the result of the hypothesis test rejecting that Moran's I = 0 was $P < 0.01$.

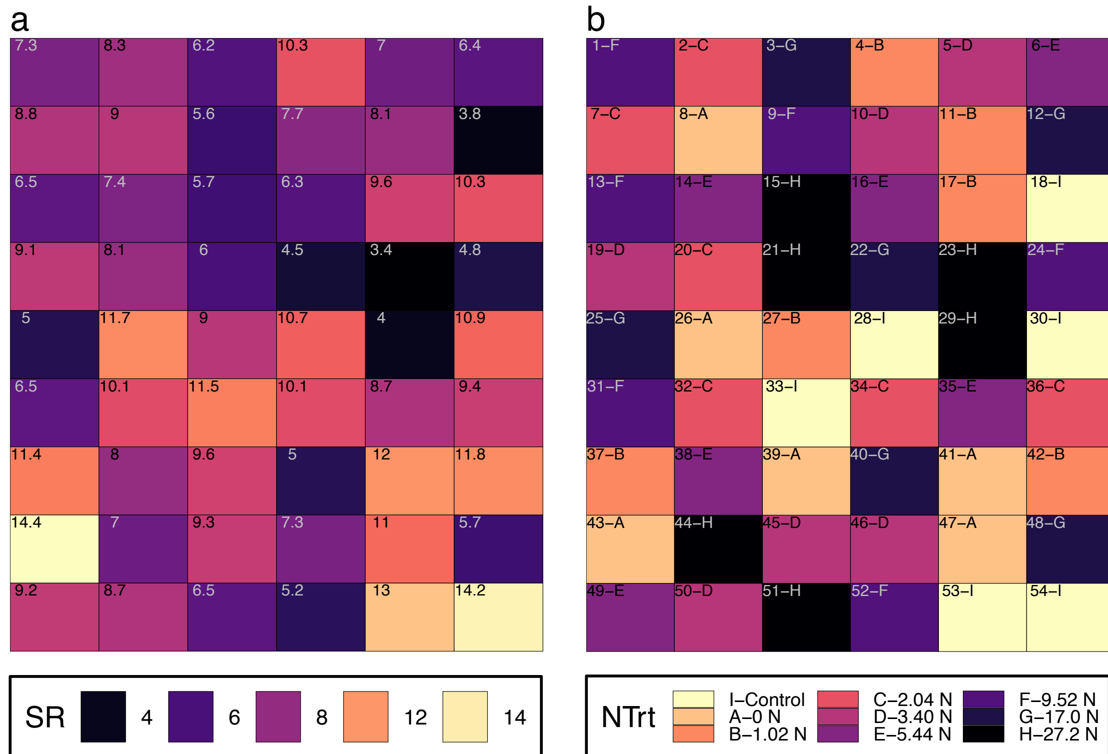

Figure 1-2: Maps of the experimental field and spatial patterns of species richness. (a) The average species richness (1982-2004) for each plot labeled in the top left corner where cooler colors indicate low species richness and hotter colors indicate high species richness (range = (3.4, 14.4)). (b) The experimental design with plot number labeled in the top left corner and its nutrient addition letter designation. Each tile represents one plot colored with its nutrient addition treatment where cooler colors indicate high added nitrogen and hotter colors indicate low or no added nitrogen (g m^{-2} N yr^{-1}).

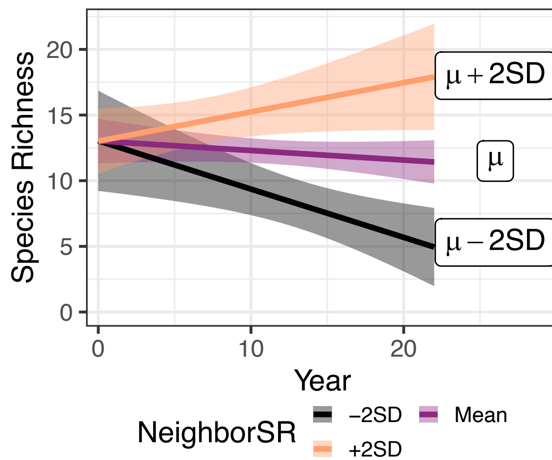

Figure 1-3: Interaction plot testing the dependance of focal plot species richness on an interaction between year and neighborhood species richness in I-0.00 control plots (n = 6). Years included are 1982-2004. Here 1982 is denoted as year zero, 1992 as year ten and 2002 as year twenty. The three lines show fitted relationships (± 95% confidence interval) based on a linear mixed effects models testing the dependance of focal plot species richness on an interaction between year and neighborhood species richness. The purple line labeled μ shows the fitted relationship when using the mean neighborhood species richness of 8.0 species. The orange line labeled μ+2SD uses a neighborhood richness that is 2 standard deviations greater than the mean (13.7 species). The black line labeled μ-2SD uses a neighborhood richness that is 2 standard deviations less than the mean (2.4 species).

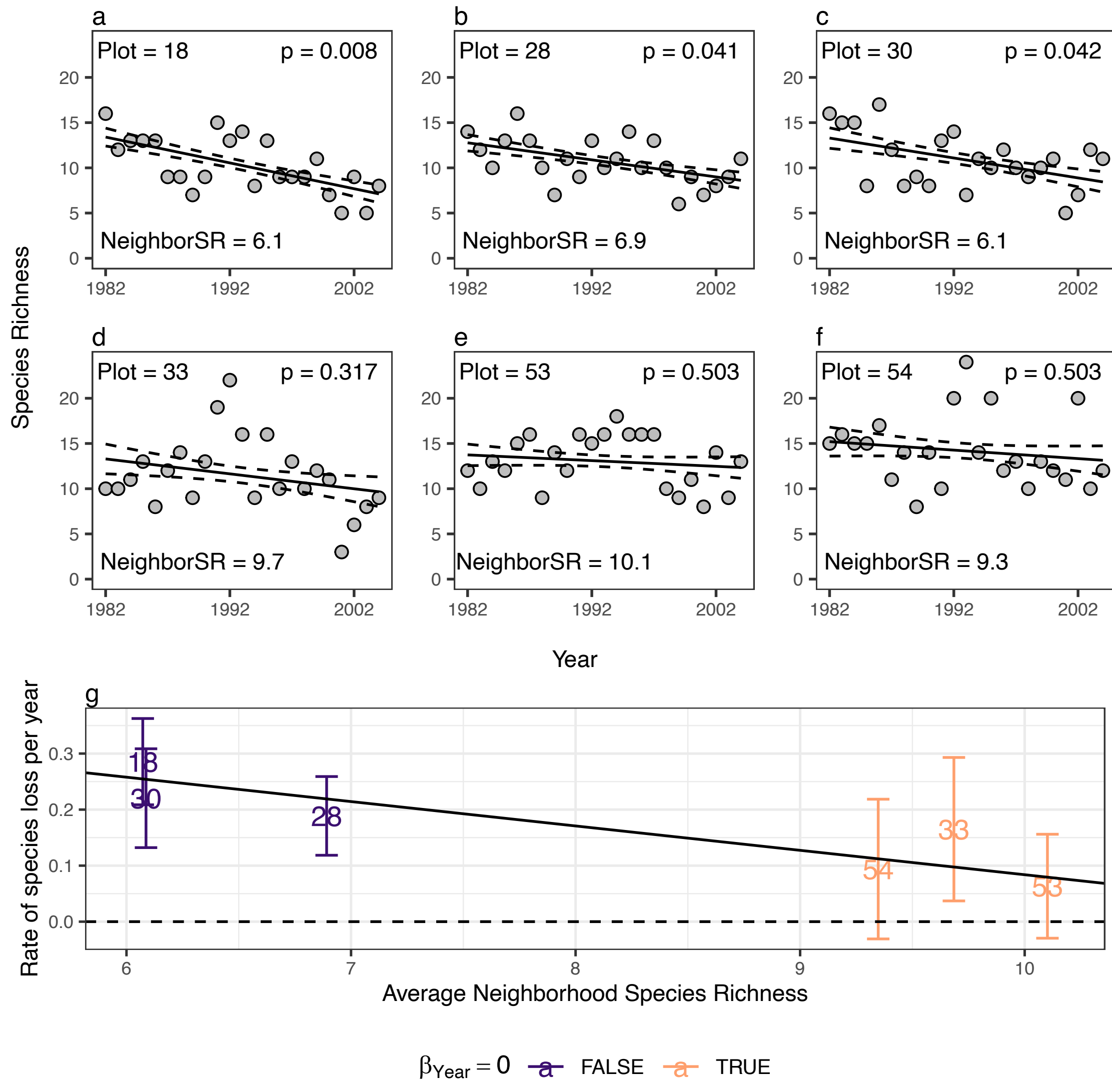

Figure 1-4: Correlations among neighborhood species richness and focal plot species richness in six control plots (I-0.00).(a)-(f) Trends in species richness through time (1982-2004). Each point represents the species richness in one plot in one year. The lines represent a linear regression ± 1 SE. The average neighborhood species is denoted in each panel along with the plot number and the *P*-value of the regression. (g) Testing the dependance of species loss on neighborhood species richness. Y-variable: The inverse of the regression slope ± 1 SE from regressions of focal plot number of species on year from a separate regression for each of six control plots. X-variable: Mean across all years of

neighborhood number of species [1982-2004]. Each effect size is labeled with its plot number and colored based on the *P*-value for a test determining if an effect size differs from 0, with purple indicating significant difference ($P < 0.05$) and orange indicating that the null hypothesis was not rejected $P > 0.05$. The line indicates a fit from a major axis regression.

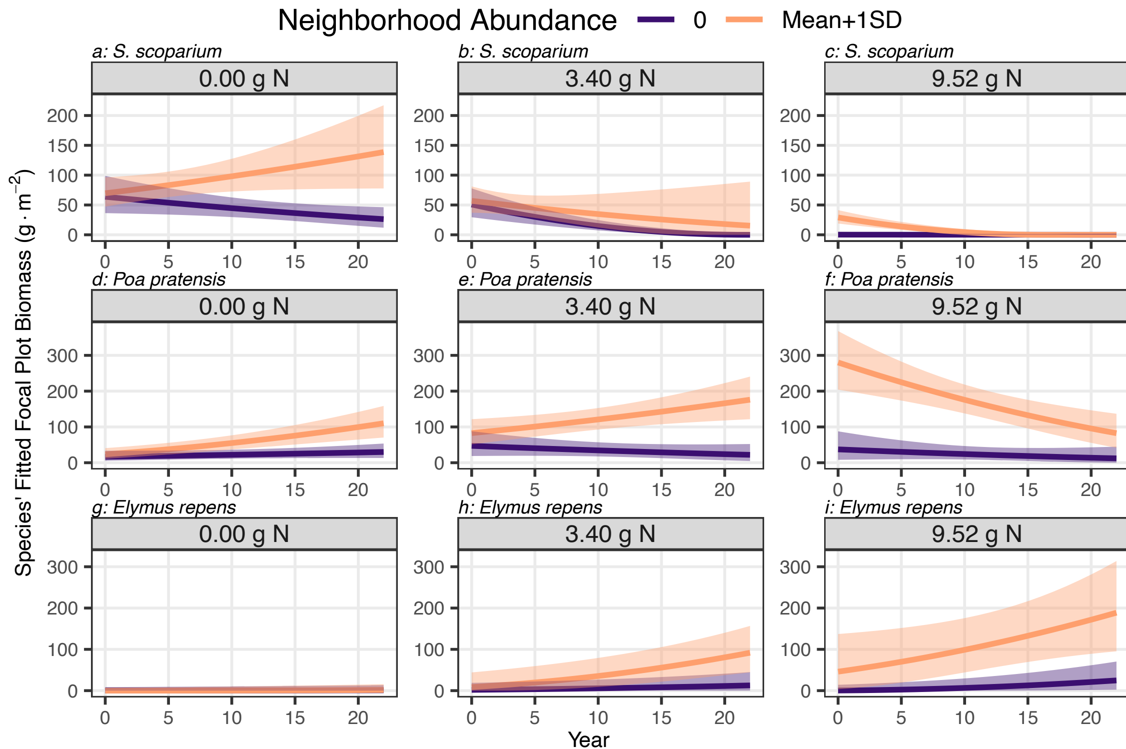

Figure 1-5: Testing the dependance of individual species' abundances on the experimental nutrient addition treatments and each species' neighborhood biomass across years 1982-2004. 1982 is denoted as year zero and 2002 as year twenty. Each panel displays the fitted values ± a 95% confidence interval from a linear mixed effects model testing a three-way interaction between experimental nutrient addition treatments, each species' neighborhood biomass and year. Fitted values are shown for the control plots (I-0.00 g m^{-2} yr^{-1} of N), the nitrogen addition treatment that received 3.40 g m^{-2} yr^{-1} of N and the nitrogen addition treatment that received 9.52 g m^{-2} yr^{-1} of N. Fitted values are shown with zero neighborhood abundance (purple) and high neighborhood abundance (orange) (mean + 1 SD of neighborhood abundance). (a) *Schizachyrium scoparium* (Treatment I-0.00); (b) *S. scoparium* (Treatment D-3.40) (c) *S. scoparium* (Treatment F-9.52); (d) *Poa pratensis* (Treatment I-0.00); (e) *P. pratensis* (Treatment D-3.40); (f) *P. pratensis*

(Treatment F-9.52); (g) *Elymus repens* (Treatment I-0.00); (h) *E. repens* (Treatment D-3.40); (i) *E. repens* (Treatment F-9.52).

Chapter 2. Plant biodiversity and the regeneration of soil fertility

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Introduction

For a soil to be fertile, it must supply sufficient amounts of the multiple nutrients that may limit plant growth, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), and have sufficient organic matter to retain water and nutrients (Karlen et al. 2001, Hartemink 2005). Low levels of one or more of these factors would reduce plant productivity. In natural ecosystems, plants contribute to the creation of fertile soils through the fixation of carbon (C) and N, through root chemical liberation of unavailable forms of soil minerals and their movement from deep to surface soils, and through the uptake and retention of nutrients (Jenny 1958, Zinke 1962, Berendse 1998, Aerts and Chapin III 2000, Jobbágy and Jackson 2004, Ehrenfeld et al. 2005, Reich et al. 2005, Bardgett 2010, Hobbie 2015, Waring et al. 2015). However, if plant species differ in their capacity to liberate, capture or retain particular limiting soil nutrients (Aerts and Chapin III 2000), then any one species growing alone might lead to the creation of a soil relatively deficient in those nutrients that it has difficulty obtaining. If plants species have tradeoffs between their abilities to acquire different nutrients, with each species being better at acquiring some nutrients but poorer for others (Tilman 1982), then a diversity of plant species may be essential for the long-term accrual of the multiple elements that are required for a soil to be fertile.

Here we use a long-term grassland biodiversity field experiment to explore the potential role that different perennial grassland plant species, plant traits, and plant biodiversity may play in generating and restoring soil fertility. While greater plant biodiversity is associated with greater primary productivity and soil C accumulation (Fornara and Tilman 2008, Cong et al. 2014, Lange et al. 2015, Yang et al. 2019),

increased soil C alone does not make a soil more fertile. Greater fertility also requires increases in all potentially limiting nutrients such as N, P, K, Ca and Mg, as well as optimal soil pH and adequate soil cation exchange capacity (Karlen et al. 2001, Hartemink 2005). Here we test the hypothesis "that the sustainability of soil nutrient cycles and thus of soil fertility depends on biodiversity" (Tilman et al. 1996).

Because greater plant species richness has been associated with greater uptake of available soil nutrients and greater plant biomass production (Tilman et al. 1996), higher plant biodiversity might increase soil fertility if the increased nutrients in plant biomass are returned to the soil as plant tissue decomposes (Jenny 1958, Zinke 1962, Vitousek and Reiners 1975, Berendse 1998, Walker 2003, Jobbágy and Jackson 2004, Ehrenfeld et al. 2005, Dybzinski et al. 2008) and if greater plant diversity leads to lower leaching losses of these nutrients (Dijkstra et al. 2007). This increase in soil fertility could then increase biomass production, creating a positive feedback as even more nutrients were added to the soil from greater biomass inputs (DeAngelis et al. 1986, Walker 2003, Ehrenfeld et al. 2005). In particular, as roots, leaves and other plant parts are shed, soil bacteria, fungi and invertebrates modify and stabilize these organic matter inputs and release nutrients as they decompose plant tissue (Zak et al. 2003, Dybzinski et al. 2008, Bardgett 2010, Eisenhauer et al. 2012, Putten et al. 2013, Bardgett and Putten 2014, Hobbie 2015, Lange et al. 2019, Kravchenko et al. 2019). Nutrients released by decomposition can increase plant growth and thus the amount of plant biomass that subsequently gets returned to the soil (Ehrenfeld et al. 2005). On a nutrient poor soil, greater plant diversity may lead to greater accumulation of soil nutrients and organic matter and therefore may cause plant productivity to increase more through time than in

low diversity ecosystems (Dybzinski et al. 2008, Reich et al. 2012, Guerrero-Ramírez et al. 2017).

Our experiment, planted in the spring of 1994, manipulated the composition and diversity of perennial grassland plant species growing on a sandy, degraded soil. In August 1993 the upper 6-8 cm of topsoil was removed from an abandoned agricultural field to eliminate a weedy soil seed bank. The field was then plowed and disked multiple times, and had bare soil from August 1993 until planted in spring 1994. The 154, 9 x 9 m plots established for this experiment were seeded to have 1, 2, 4, 8 or 16 perennial grassland species randomly chosen from a pool of 18 species. Here, 'plant diversity' refers to the number of species planted in a plot. We additionally calculated plant 'functional group diversity' based on a functional grouping commonly applied to grasslands, classifying plant species as grasses, legumes, or forbs (Tilman 2001). Plots were never fertilized, were annually burned in early spring before green-up, and were fenced to exclude large vertebrate herbivores. The glacial outwash sandplain soils of our site in east-central Minnesota, USA, are agronomically classified as “very low” in organic matter, N, and K, but “very high” in P (*SI Appendix,* Table S1). Using archived soil samples collected from each plot before planting in 1994 and samples collected after 23 years of growth in 2017, we measured soil total N and C, exchangeable K, Ca, and Mg, soil cation exchange capacity, soil pH and extractable Bray P in the upper 0-20 cm of the soil profile. In August 2017 both aboveground and belowground (root; 0-30 cm depth) plant biomass were measured as were N, P, K, Ca, and Mg in both aboveground and root biomass. We additionally measured aboveground plant tissue chemistry for each individual plant species.

Methods

Experimental design. The experimental field had been abandoned from agriculture for more than fifteen years when, in August of 1993, the herbicide glyphosate was applied and surface vegetation, once dead and dried, was burned. The top 6–8 cm of soil was scraped off to reduce the presence of weedy annual plant seeds in the soil seed bank. This also reduced soil carbon and soil nutrient levels. The site was then plowed twice and harrowed multiple times that year, and again in May 1994 before planting. The 168 plots, initially 13 m by 13 m but subsequently reduced to the central 9 m by 9 m portion, were planted with 1, 2, 4, 8 or 16 perennial plant species randomly chosen from a species pool of eighteen. The species pool consisted of common perennial grassland species of regional tallgrass prairie and two oaks common in nearby oak savannas. Herbaceous species were functionally categorized as C4 grasses, C3 grasses, legumes and non-leguminous forbs, with four species in each functional group.

Because of poor establishment, 14 plots were dropped from the experiment, leaving 154 plots. In particular, the two oak species failed to survive because of annual burning. Two of the four C3 grasses, *Agropyron smithii* and *Elymus canadensis*, initially germinated but failed to survive long-term. The final experimental design thus consisted of 154 plots seeded with 1, 2, 4, 8, or 16 randomly selected perennial grassland species, and with 32, 28, 29, 30 and 35 replicates of each diversity level, respectively. Monocultures were not by design replicated rather the monoculture treatment was based on random draws of single species from the species pool. Most species were randomly assigned to two monocultures. However, *Poa pratensis* and *Panicum virgatum* have one monoculture; *Liatris aspera*, *Lespedeza capitata*, *Dalea purpureum* and *Schizachyrium*

scoparium have three monocultures; and *Sorghastrum nutans* has four. In addition, one forb species, *Solidago rigida*, failed to germinate during the first year and was planted with another forb, *Monarda fistulosa* in spring 1995. In the third year, *S. rigida* germinated and eventually became well established and dominated its monocultures.

Plots were annually burned early each spring before green-up but received no fertilizer. Each plot was annually weeded by hand to remove non-planted species. The experiment was fenced to exclude white-tailed deer. Additional details can be found on the Long-Term Ecological Research program website for the Cedar Creek Ecosystem Science Reserve under experiment name "e120: Biodiversity II: Effects of Plant Biodiversity on Population and Ecosystem Processes" (https://www.cedarcreek.umn.edu).

Calculation of plant functional groups. For each plot in the experiment, we categorized planted species as grasses (*Poaceae*), legumes (*Fabaceae*) and forbs (*Asteraceae*, *Lamiaceae* and *Apocynaceae).* We then used these binary variables to create an additional categorical variable that measured the presence of each functional group and combinations of those functional groups. This grouping gave 7 functional group compositions: G, L, F, G+F, L+F, G+L, and G+L+F. This experiment was not designed with balanced functional group composition and the sample size was as follows: G = grasses only, n = 22; F = forb only, n =10; L = legumes only, n = 11; FL = at least 1 forb and 1 legume, n = 5; GL = at least 1 grass and 1 legume, n = 23; GF = at least 1 grass and 1 forb, n = 14; GFL = at least 1 grass, 1 legume and 1 forb, n = 69.

Field collection of soil samples. Nine soil cores per plot were collected in an evenly spaced 3 x 3 sampling grid pattern in September of 2017 to a depth of 60 cm in 20 cm

increments using a 1.9 cm diameter soil corer. The 9 cores from a plot were then combined, dried at 60 ^oC, sieved to a 2-mm fraction, then well mixed. Soil samples were similarly taken and processed in 1994 prior to planting. Those samples remained in glass archived vials until analysis. The 2017 soil samples were similarly archived.

Plant biomass sampling. In August, near the time of peak aboveground biomass, two parallel 0.10 m by 6 m strips were clipped at the soil surface in each plot each year to sample plant biomass. Strips were located in the middle half of each plot, separated by about 1 to 2 m, and located so as to not clip an area that had previously been clipped within the past decade. One strip per plot was sorted to species; the other was unsorted. Root biomass was subsequently sampled in the clipped area, with a 5.1 cm diameter soil probe used to collect three cores per strip (six per plot) to a depth of 30 cm. Roots were washed over a mesh screen to remove soil. Roots and aboveground biomass were dried in a dehumidified drying room at 60 ^oC until achieving constant mass. Aboveground biomass was sampled annually starting in 1996. Belowground biomass has been sampled in years 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2006, 2010, 2015 and 2017.

Laboratory analysis of soil samples. The University of Minnesota Research Analytical Laboratory (Saint Paul, MN, USA) analyzed soil samples that we collected from depth increments of 0-20 cm, 20-40 cm and 40-60 cm in 2017 and 0-20 cm in 1994 for exchangeable cations (calcium, magnesium, potassium, sodium) using a pH 7 ammonium acetate extraction and for aluminum using a 1M KCl extraction followed by analysis using an Inductively Coupled Argon Plasma Optical Emission Spectrometer (iCap 7600 Duo ICP-OES Analyzer, Thermo Fisher Scientific, Waltham, MA, USA) (Soil Survey Staff 2017). Effective cation exchange capacity ("CEC") was measured by the

summation method of exchangeable Ca, Mg, K, Na and Al (Soil Survey Staff 2017) and referred to in-text as CEC. Extractable P was measured using a standard Bray-1 extract (Eliason et al. 2015) (0.025 M HCl and 0.03 M NH_4F) and analyzed colorimetrically on a Brinkmann PC 900 probe colorimeter (Thermo Fisher Scientific, Waltham, MA, USA). A commercial laboratory, Waypoint Analytical (Memphis, TN, USA), analyzed soil samples at depths 0-20 cm, 20-40 cm and 40-60 cm in 2017 and 0-20 cm, 20-40 cm and 40-60 cm in 1994 for soil pH in a 1:1 soil : deionized water slurry. Soil total carbon and nitrogen were analyzed in soil samples from depth increments of 0-20 cm, 20-40 cm and 40-60 cm in 1994, 2015 and 2017. The average of 2015 and 2017 values was used to reduce sampling noise. Ground soil was analyzed using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA). Because of the lack of carbonate minerals in these soils (Grigal 1974) total C represents total organic C. In order to evaluate the agronomic status of our starting soil (*SI Appendix,* Table S1), Waypoint Analytical measured total soil organic matter using loss on ignition for 1994 soil.

Laboratory analysis of plant samples. The dried aboveground and belowground biomass sampled in August of 2017 were analyzed for their chemical composition. The homogenate unsorted clipped strip of dried biomass for each of 154 plots was ground completely and then subsampled. Additionally, for all monoculture plots and five, 16-species plots, the total sorted quantity, including leaves, stems and inflorescences if present, of each of the fifteen plant species was additionally ground to provide estimates of their individual traits. Because the legume, *Lupinus perennis*, has a spring growth and seed shedding pattern that required a separate spring biomass sampling to determine its

tissue nutrient contents, samples of *L.* perennis from June in 2019 were also analyzed and used instead of the 2017 sample. Plant samples were ground using a Model 4 Wiley Mill (Thomas Scientific, Swedesboro, New Jersey, USA) and analyzed at a commercial laboratory, Waypoint Analytical (Atlantic, Iowa, USA), for tissue chemistry using method 3050B of the Environmental Protection Agency Manual SW-846. Specifically, each plant sample was digested in concentrated nitric acid followed by heating for 15 minutes at 95 °C. 30% hydrogen peroxide was then added until effervescence was no longer observed followed by 5 ml of concentrated HCl and continued heating for 30 minutes. 5-10 ml of water was finally added to the sample following filtration using Whatman #2 and analyses using Inductively Coupled Plasma Optical Emission Spectrometry (Perkin Elmer Optima 8300, Waltham, MA, USA).

Estimation of ecosystem nutrient pools. The concentration of each measured soil variable was adjusted to an area density quantity (g m^{-2}) with soil bulk density. Bulk density was measured in 2018 to a depth of 60 cm in 20 cm increments using an AMS Inc. (American Falls, ID, USA) split soil core sampler with a removal jack (part numbers 400.99, 403.41, 403.73, 211.05, and 211.06) in a subset of plots (87) with a sample size of 26, 16, 15, 15, 15 at 1, 2, 4, 8, and 16 number of species respectively randomly chosen while including two replicates for each species in monoculture where available. For unmeasured plots, we estimated unmeasured values using a linear regression of the dependency of bulk density (0-20 cm) on % soil C (0-20 cm) measured in 2017. Bulk density was not measured in 1994. We estimated bulk density (0-20 cm) values in each plot in 1994 using the measured % soil C values in 1994 (0-20 cm) and the regression fit with % soil C in 2017 (0-20 cm). The predicted mean bulk density of 1.45 g cm^{-3} (0-20

cm) in 1994 approximates measured soil bulk density at the site's soil survey of the Nymore series of 1.4 g cm^{-3} (0-23 cm) (pp 22. Table 5, Grigal 1974). Estimated bulk density in 1994 at 0-20 cm had treatment means ± 1 SE g cm^{-3} of 1.45 ± 0.01, 1.44 ± 0.00, 1.45 ± 0.01, 1.44 ± 0.01 and 1.44 ± 0.01 at 1, 2, 4, 8 and 16 number of species respectively. Bulk density measured in 2018 at 0-20 cm had treatment means ± 1 SE g cm^{-3} of 1.46 g cm^{-3} ± 0.015, 1.43 g cm^{-3} ± 0.015, 1.42 g cm^{-3} ± 0.019, 1.36 g cm^{-3} ± 0.019, and 1.37 g cm^{-3} ± 0.018 at 1, 2, 4, 8, and 16 number of species respectively. We then used the equivalent soil mass approach to adjust for sampling 20 cm deep across all plots despite an assumed change in density by adding mass from the 20-40 cm depth increment or subtracting mass at 0-20 cm relative to the change in bulk density from the reference value in 1994 (Ellert and Bettany 1995). The pool of nutrients in aboveground biomass was calculated using the % of each element in the biomass from the unsorted clipped strip multiplied by the dry biomass in each plot. Aboveground biomass was calculated as the dry-weight (g m^{-2}) average of both sorted and unsorted strips as has been done historically in this experiment. Plant litter, dead biomass on the soil surface, was not included in this measurement, but there were negligible quantities of litter given that the field is annually burned. Belowground biomass was calculated as the dry weight (g m^{-2}) (0-30 cm) for each plot. To reduce sampling noise from interannual variability, the average of aboveground and belowground biomass measured in 2015 and 2017 was used for all statistical analysis and calculations. To improve readability, we refer to these as 2017 within the text as the year when the plant tissue was chemically analyzed. The change in ecosystem nutrient pools for N, P, K, Ca and Mg was estimated using the sum

of the change of each element in the soil (2017-1994; 0-20 cm) and the sum of its quantity in aboveground and belowground biomass (0-30 cm).

Statistical analysis. Analyses were performed using R version 4.1.1 and JMP 14 Pro. Linear regressions were used to test the dependence of soil and plant variables on experimental plant biodiversity using the natural log of plant species number (1, 2, 4, 8, 16). For analysis of the plant biomass pools, the percent increase from the monoculture mean was used as the response variable. For each set of analysis, a false discovery rate (Benjamini and Hochberg 1995) correction (FDR) was applied to the *P*-value for each regression. The regression results were robust to a variety of transformations of the y-variable and are presented on the untransformed y-scale. The dependance of the sum of aboveground and belowground biomass (total biomass) on the log of plant biodiversity and soil variables (C, N, Ca, Mg, K, P, pH) was tested using a generalized least squares model with a power variance structure (varPower) on the fitted values (R package *nlme*). Multimodel inference (R package *MuMIn*) was used as a model selection approach with each soil variable and the natural log of plant biodiversity with the conditional average using the Bayesian Information Criterion. The dependance of ecosystem nutrient pools (N, K, Ca and Mg) on the presence of different plant functional group sets was tested using a generalized least squares model with a variance structure for the factor to account for unequal variance (varIdent, *nlme*). Differences among means were compared using least squares means (R package *emmeans*) followed by a Tukey correction using the Satterthwaite estimation of the degrees of freedom. As supplemental analyses, we ran these same tests of the effects of functional group presence on the change in soil C, N, K,

Ca and Mg, on aboveground and belowground biomass (belowground log transformed) and on the pools of N, K, Ca and Mg in aboveground and belowground biomass.

A tradeoff surface among plant species in their traits was tested by analyzing the dependence of belowground biomass (0-30 cm) in monoculture on % aboveground N and % aboveground K using a linear regression. The time-series mean of belowground biomass for each species' in monoculture was the response variable and the average tissue chemistry (%N and %K) for each species measured in its monocultures and five, 16-species plots, were the explanatory variables. R graphical package *rgl* was used to generate a regression plane in Fig. 4 with an aspect ratio of 1:1:1 (x:y:z).

Results

Higher levels of plant diversity led to increases in numerous factors that contribute to soil fertility. Comparison of pre-treatment 1994 soils to 2017 soils shows that plots with higher plant diversity had significantly greater increases in soil N, K, Ca, Mg, and C, in cation exchange capacity and in soil pH (Figure 2-1; *SI Appendix,* Fig. S1; *SI Appendix,* Fig. S2; *SI Appendix,* Table S2). Soil P levels, which were very high before planting, remained very high in 2017 with no detectable effect of plant diversity (Figure 2-1h; *SI Appendix,* Table S2). Although no plots were ever fertilized, by 2017 the soils of the 16-species treatment had 29% more total soil N (0-20 cm depth) than the monoculture mean of these same species, 95% more soil K, 30% more soil Ca, 29% more soil Mg, 35% more total soil C, 34% greater cation exchange capacity and had a less acidic soil (0.2 pH increase from monocultures) (Figure 2-1). Although soil bulk density declined with plant diversity from a mean ± SE of 1.46 g cm^{-3} ± 0.015 in the monocultures to 1.37 g cm^{-3} ± 0.018 in 16 species plots ($F_{1,85} = 23$, $R^2 = 0.21$, $p<0.001$), expressing soil elemental levels

on either a concentration basis or area density basis were qualitatively similar (*SI Appendix,* Fig S3; *SI Appendix,* Fig S4; *SI Appendix,* Fig S5; *SI Appendix,* Table S3).

The greater accumulation of N, K, Ca and Mg in surface soils (0-20 cm depth) at higher plant diversity was accompanied by even greater percent increases relative to monocultures in the pool size of these nutrients in both aboveground and belowground plant biomass in 2017 (Figure 2-2). Linear regressions show that tissue pools of N, K, Ca, and Mg in above and in below-ground biomass were, relative to average levels across all monocultures, positively dependent on the log of plant diversity (all $p<0.001$, Figure 2-2; *SI Appendix,* Fig S6; *SI Appendix,* Fig S7; *SI Appendix,* Table S4).

Why though might the production of greater plant biomass and the accumulation of soil nutrients depend on plant diversity? Diversity is thought to impact ecosystem processes because of functional differences between species (Tilman 2001). Because the herbaceous perennial species of tallgrass prairie are often functionally classified as grasses (*Poaceae*), legumes (*Fabaceae*) and forbs (not including legumes; *Asteraceae*, *Lamiaceae* and *Apocynaceae),* we tested if the rate of ecosystem accumulation of particular nutrients was related to the presence of these plant functional groups. To do this, we classified each plot by its presence of grass (G), or legume (L) or forb (F) species. This gave seven functional group compositions: G, L, F, G+F, L+F, G+L, and G+L+F. Ecosystem pools of accumulated N, K, Ca and Mg (Fig. 3) were calculated as the change in each plot of each element in the soil from 1994 to 2017 (as g m^{-2} of each element in the 0-20 cm soil depth increment) plus the total amount of each element accumulated in shoots and roots by 2017 (as g m^{-2}), since all plant biomass had been removed the year before planting.

The presence of all three functional groups, the G+F+L plots, was associated with the largest increases in ecosystem pools of N, K, Ca and Mg compared to when just a single functional group was present (Figure 2-3). In particular, plots containing all three functional groups (G+L+F) had significantly greater accumulation in soils plus plant biomass of each of the four nutrients, N, K, Ca and Mg, than did the plots with just a single functional group (the F, or G, or L plots; Fig. 3; *SI Appendix,* Table S5). This was not the case for any combination of just two functional groups (Figure 2-3). Neither the G+F nor the F+L plots accumulated significantly greater ecosystem pools of N, or K, or Ca or Mg than did the G or F or L plots (Figure 2-3). Results were intermediate for the G+L plots, which were not significantly different from F or L in Mg accumulation or from L in Ca accumulation, but had greater N accumulation than plots planted with a single functional group or with G+F. Finally, although G+F+L and G+L did not differ in ecosystem pools of N, Ca or Mg, G+F+L had significantly greater K pools than all other functional compositions except F+L, suggesting that the presence of forbs was an important cause of the observed large increases in K at high plant biodiversity.

In total, plots planted with a single functional group accumulated significantly lower ecosystem pools of most nutrients than did G+F+L plots, and those planted with two functional groups only significantly exceeded single functional groups in one-fourth of the pairwise comparisons (Figure 2-3). Separate analyses for each of the plant, root and soil nutrient pools demonstrates that, when compared to plots planted with a single functional group, G+F+L produced more aboveground and belowground biomass and accumulated more C, N, K, Ca and Mg in 87% of the comparisons (39 out of 45 comparisons) (*SI Appendix,* Fig S8).

On an even finer scale, for amounts of each of the four nutrients in aboveground biomass, G+F+L was significantly greater than G+L, but was never significantly greater than F+L (*SI Appendix,* Figure S8). For root nutrients, G+F+L was significantly higher in root K than both F+L and G+L. The only functional group with root K levels as high as those of G+F+L was F, the forb only plots. For root Mg, F+L did not differ from G+F+L, but G+F+L had significantly more Mg than G+L. For root Ca the opposite occurred: G+F+L did not differ from G+L but had significantly more Ca than F+L. In total, these results suggest that forbs, and the joint presence of forbs and legumes, are important contributors of K and Mg to ecosystem pools and that legumes and the joint presence of legumes and grasses, are more important contributors of N and Ca.

Since not all of these nutrients may be limiting to the production of plant biomass, we determined which soil variables were more strongly correlated with observed diversity-dependent changes in productivity while accounting for the effect of plant diversity. We used linear multiple regressions and multimodel inference, finding that total plant biomass depended positively on the $\log_e$ of the number of species (156 ± 25.6 g m^{-2} biomass per 1 $\log_e$(Number of Plant Species), $p<0.001$), soil exchangeable K (51.3 ± 8.8 g m^{-2} biomass per g m^{-2} of K, $p<0.001$), total soil N (2.88 ± 0.67 g m^{-2} biomass per g m^{-2} of N, $p < 0.001$), and total soil C (0.23 ± 0.05 g m^{-2} biomass per g m^{-2} of C, $p < 0.001$) (*SI Appendix,* Table S6). This analysis suggests that total soil C, total soil N and exchangeable soil K are the soil variables most strongly associated with the amount of plant biomass produced in this field experiment which is consistent with our soil analyses that indicated agronomically low soil levels of N, K, and organic matter at the start of our experiment (*SI Appendix,* Table S1).

Finally, we determined how the plant species in the three functional groups might differ in traits relevant to the accumulation of soil K, N and C. For K and N, we used average measured aboveground tissue concentrations of K and N for each species in monoculture and 16-species plots. For soil C accumulation, we used average monoculture root mass for each species because prior results of this experiment showed that greater root biomass (as g m^{-2}) was the variable most strongly associated with greater increases in soil C (Fornara and Tilman 2008, Yang et al. 2019).

These three measured traits of the species (root mass, tissue %N, tissue %K) defined a regression plane (Figure 2-4, *SI Appendix*) ($F_{2,12} = 6.3$, $R^2 = 0.51$, $p = 0.014$). The species within each functional group tended to be similar to each other, as evident by their tendency to cluster (Figure 2-4). On this trade-off surface, perennial C4 grasses were low in both tissue %N and %K but had the highest root biomass. Forbs and legumes, which had less root biomass than C4 grasses, were further differentiated: legumes had higher %N, but markedly lower %K. Forbs, in contrast, had higher %K, but lower %N (Figure 2-4). Forbs and legumes had similar %Ca and %Mg levels, and their levels were greater than for grasses (*SI Appendix,* Fig S10).

Discussion

Early in this experiment, greater plant diversity was associated with greater capture of soil nitrate (Tilman et al. 1996) and with 16 species plots being ~100% more productive than the average of these species in monocultures. After 23 years, we find that greater plant diversity was associated with higher levels of soil C, N, K, Ca, Mg and with 16 species plots being ~200% more productive than monocultures. The progressively greater primary productivity observed through time at higher diversity in this and other

long-term biodiversity experiments (Reich et al. 2012, Guerrero-Ramírez et al. 2017, but see Kardol et al. 2018) and the greater accumulation of multiple nutrients and C in soil and in plant biomass (Figure 2-1 and Figure 2-2), suggest the existence of a positive feedback effect (DeAngelis et al. 1986, Ehrenfeld et al. 2005) of plant diversity on soil fertility that increased primary productivity through time.

We hypothesize that high plant diversity, and especially the joint presence of grass, legume, and forb species, leads to greater liberation and capture of limiting soil nutrients (Tilman et al. 1996). This, in turn, allows greater production of plant biomass. We suggest that the nutrient and C contents of the greater biomass (roots and shoots) produced by diverse mixtures of grass, legume and forb species is then recycled when it senesces, helping create a more fertile soil. This more fertile soil would then further increase plant biomass production and biomass nutrient pools, in a positive feedback loop that would persist until an equilibrium is reached (Vitousek and Reiners 1975, Walker 2003, Ehrenfeld et al. 2005).

We note, though, that the annual early spring burning in our experiment likely volatilizes some of the C and N that had been in litter from senesced aboveground biomass, but also likely deposits biologically available forms of other elements in ash (*e.g.* Ca, Mg and K). Because root mass in our prairie-like high-diversity plots is about four times the aboveground biomass, senesced biomass from root turnover may add C, N and other nutrients to soil (Dijkstra and Smits 2002, Walker 2003, Jobbágy and Jackson 2004, Ehrenfeld et al. 2005, Reich et al. 2005, Hobbie 2015, Waring et al. 2015).

The 3-way tradeoff shown in Fig. 4 suggests why plant functional diversity may have been essential for increasing soil fertility in our experiment, which was unfertilized.

It suggests that no single species and no functional group could, by itself, span the full space of the root biomass-N-K tradeoff surface and thus cause soil C, N, and K, which seem to limit productivity, to all increase. Increases in all three of these were associated with greater productivity in our experiment, and thus with the hypothesized feedback effect of greater productivity and its nutrient contents on soil fertility. In contrast, increased N, P, K fertilization is often required to increase the productivity of an agricultural monoculture crop, and such fertilization can also lead to increased soil C (Bundy et al. 2011). Our monocultures, though, were never as productive as our high diversity plots (Yang et al. 2019). At our site, soil P was at agronomically very high levels both prior to planting and twenty-three years later. While root biomass, N, and K differed among functional groups, and while soil C, soil N and soil K increased with diversity, we found that soil P was not significantly dependent on plant diversity or functional groups, and tissue P levels did not differ among functional groups when growing on this P rich soil (Figure 2-1h; *SI Appendix,* Fig. S9; *SI Appendix,* Fig. S10).

The trait differences (Figure 2-4) between grasses, legumes, and forbs suggest a mechanistic link from plant traits to the effect of functional diversity on primary productivity and the accumulation of soil nutrients. The tradeoff surface shows that each functional group should contribute to the soil more of one of root biomass, N or K, but less of the other two. Because legumes were high in %N and %Ca while forbs were high in %K, %Ca and %Mg, the presence of each of these functional groups should have particularly enriched soil for those elements that were in higher relative concentration in its biomass (Figure 2-3; *SI Appendix,* Fig S8; *SI Appendix,* Fig S10). The high root biomass of C4 grasses may have decreased nutrient losses via leaching and helped

increase soil organic C (Tilman et al. 1996, Dijkstra et al. 2007, Fornara and Tilman 2008, Yang et al. 2019). Moreover, no functional group growing alone increased soil C and nutrients as much as occurred when all three groups were present (Figure 2-3; *SI Appendix,* Figure S8).

The increases in surface soil exchangeable K, Ca and Mg may have come from root uptake of these elements in deeper soils that was concentrated at the surface as aboveground tissues and shallow roots died and decomposed or as elements were deposited as ash from litter during early spring burns (Jobbágy and Jackson 2004). Ca, for example, tends to move unidirectionally from roots to shoots with limited resorption when tissues senesce (Hanger 1979, Dijkstra and Smits 2002). If greater root uptake and recycling of nutrients from ash or root turnover is the mechanism for the accumulation of soil fertility in high-diversity plots, then one would expect a coupling of the accumulation of plant and soil pools (Amundson et al. 2007). For example, soil K was highly dependent on plant diversity ($R^2 = 0.47$) and K was the nutrient with the largest % increase (370%) in its aboveground plant pool when comparing 16-species plots to the mean of all monocultures (Figure 2-2). For K, Ca and Mg, 16 species plots had ~150-370% greater aboveground pools and ~90-150% greater root pools than monocultures, indicating that higher plant diversity led to greater ecosystem capture and retention in biomass of these cations (Figure 2-2). Moreover, in these sandy soils, increases in soil organic C were correlated with increases in cation exchange capacity (1994: $R^2 = 0.20$, $p<0.001$; 2017: $R^2 = 0.52$, $p<0.001$; *SI Appendix,* Fig S11), which should increase K, Ca and Mg retention in these soils.

Our long-term experiment revealed surprisingly large diversity-dependent increases in soil fertility. This magnitude, however, might depend on our initial soil characteristics. The soils of our site, which formed on a glacially deposited sand plain, are classified taxonomically as entisols, which have limited horizon development (Grigal 1974). At the beginning of this experiment some of the topsoil and its organic matter were removed, and soils were plowed and disked, which tended to homogenize the remaining topsoil with deeper soil layers. The low initial levels of soil C and N and high levels of P in our starting soils are characteristic of geologically young soils undergoing progressive development (Peltzer et al. 2010). Thus, if degraded and abandoned agricultural soils at our site accumulate C and nutrients in a logistic manner (Knops and Tilman 2000), the rates of increase in soil C, N, K, Ca and Mg that we observed at high plant diversity may be greater than if our soil had initially been higher in these elements.

Our results, and their likely mechanistic basis, may provide insights into methods to restore soil C and increase limiting soil macronutrients in agroecosystems and managed forests. For instance, incorporating greater plant functional diversity via appropriate choice of the plant species used in crop rotations, intercropping or cover crops may lead to long-term increases in soil fertility and subsequent reductions in the amount of fertilizer needed (McDaniel et al. 2014, Yu et al. 2015, Finney and Kaye 2017, Li et al. 2020). Because our results suggest it is not simply the number of plant species that matters, but rather the appropriate suite of complementary plant traits, it would be interesting to determine if as few as perhaps three such plant species might offer notable soil benefits relative to monocultures.

In our study, the increased inputs of senesced plant biomass that occurred at higher diversity had to be transformed and mineralized by the soil microbial and invertebrate communities, suggesting that soil microbial biodiversity may also help explain the results in Fig. 1 (Heijden et al. 2008, Bennett et al. 2020), which is an intriguing possibility (Bardgett 2010, Eisenhauer et al. 2012, Bardgett and Putten 2014, Lange et al. 2015, 2019). Greater accumulation of plant or soil pathogens in monocultures, or increases in soil mutualists or decreases in soil pathogens at high plant diversity are other possible ways that microbial biodiversity might impact ecosystem functioning through time (Eisenhauer et al. 2012, Thakur et al. 2021).

In total, our results show that plant diversity, including plant functional diversity, can play a significant role in the generation of soil fertility, likely via positive feedback effects of diversity-dependent increases in nutrient capture and productivity on soil fertility. Our results raise the interesting possibility that the high plant diversity of most natural ecosystems may have been an important factor leading to the creation of fertile soils around the world. Efforts to increase soil C stores and fertility of degraded soils may be aided by creative uses of plant diversity.

Figures

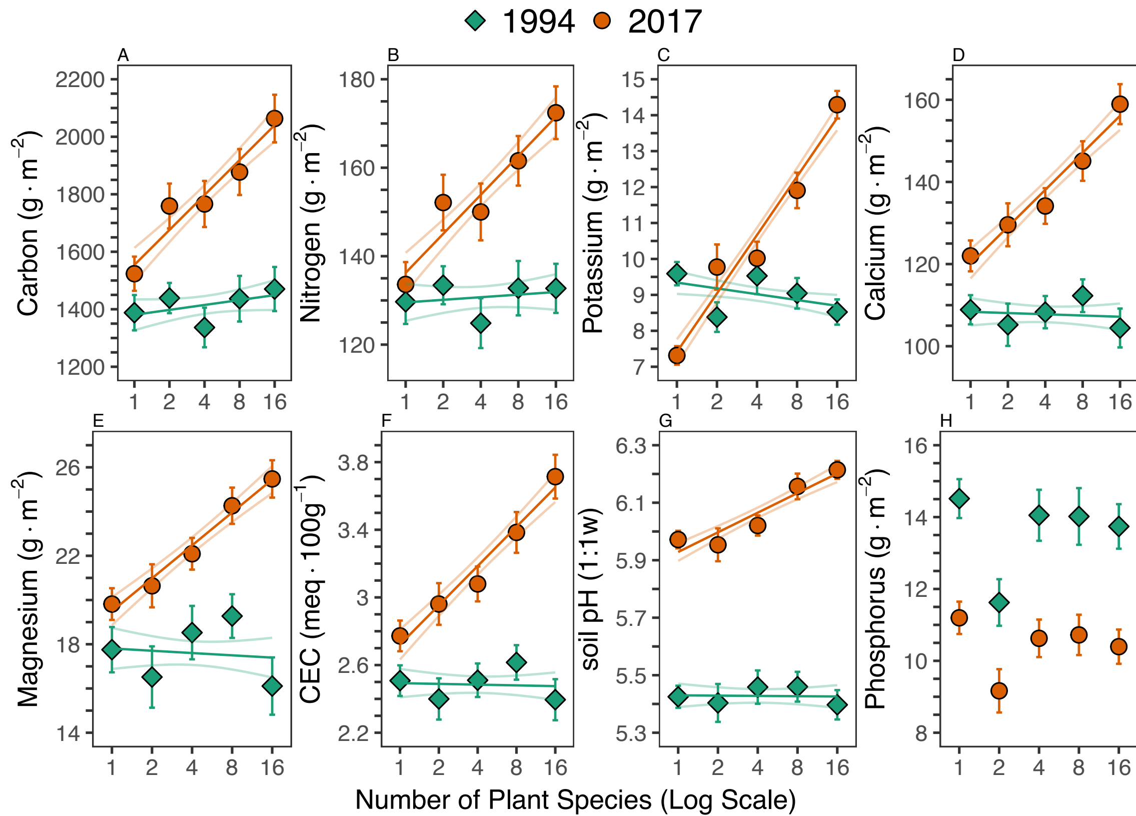

Figure 2-1: Soil chemistry vs. plant diversity. Mean ± 1 S.E. of soil chemistry (0-20 cm depth) before planting in 1994 in green (diamond) and in 2017 in orange (circle) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** cation exchange capacity (CEC), **g** soil pH, and **h** extractable Bray phosphorus versus number of planted species (1, 2, 4, 8, or 16; log scale). Lines are linear regressions ± 1 S.E. (n = 154 plots). The quantity (g m^{-2}) for C, N, P, K, Ca and Mg were calculated using soil bulk density. Sample sizes for each diversity level (1 to 16 species) are 1 species = 32 plots; 2 = 28; 4 = 29; 8 = 30; 16 = 35.

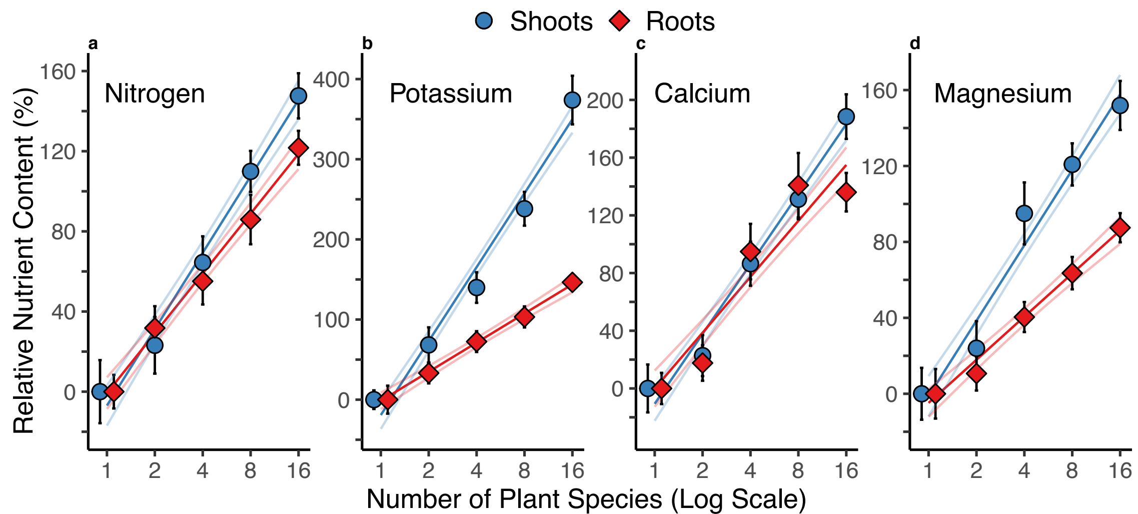

Figure 2-2: Nutrient contents relative to monoculture levels. The 2017 relative shoot (blue; circle) and root (red; diamond) nutrient content of biomass for each diversity treatment expressed as the percent of the 2017 mean nutrient content of all monocultures combined (mean ± 1 S.E.). Percent change relative to the mean of all monocultures for **a** nitrogen, **b** potassium, **c** calcium and **d** magnesium contained in aboveground shoot biomass and belowground (0-30 cm) root biomass. Lines are linear regressions ± 1 S.E. (n = 154 plots). Shoots are dried aboveground biomass and roots are dried belowground biomass (0-30 cm). Biomass was multiplied by the concentration of each element.

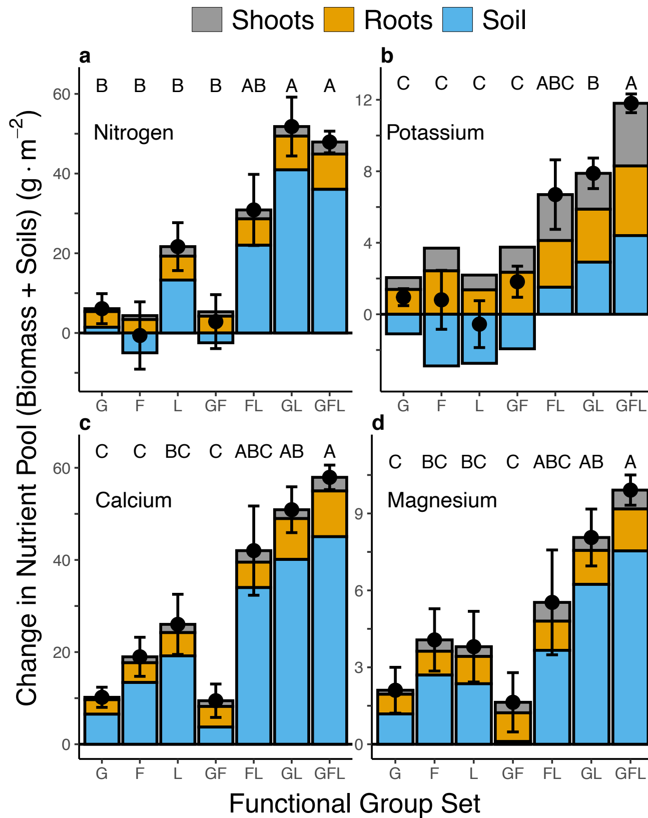

Figure 2-3: Change in ecosystem total nutrient pools (black points) for each functional group composition for **a** nitrogen, **b** potassium, **c** calcium and **d** magnesium. Each black point shows the mean of the total ecosystem pool ± 1 SE. Pools were defined as the change from 1994 to 2017 in soil levels of a nutrient (0-20 cm depth increment) plus amounts of that nutrient in aboveground biomass and in roots (0-30 cm) in 2017; sum expressed as g of nutrient m^{-2}. Bars show the value for each nutrient in aboveground biomass (grey), in belowground biomass (yellow) and in soil (blue). Bars with negative values, shown below the zero line, indicate a reduction from 1994 to 2017 for an element. Functional group compositions: G = grasses only, n = 22; F = non-legume forb only, n =10; L = legumes only, n = 11; FL = at least 1 forb and 1 legume, n = 5; GL = at least 1 grass and 1 legume, n = 23; GF = at least 1 grass and 1 forb, n = 14; GFL = at least 1

grass, 1 legume and 1 forb, n = 69. Letters indicate if means for a particular nutrient differ (*P*<0.05) following a Tukey correction.

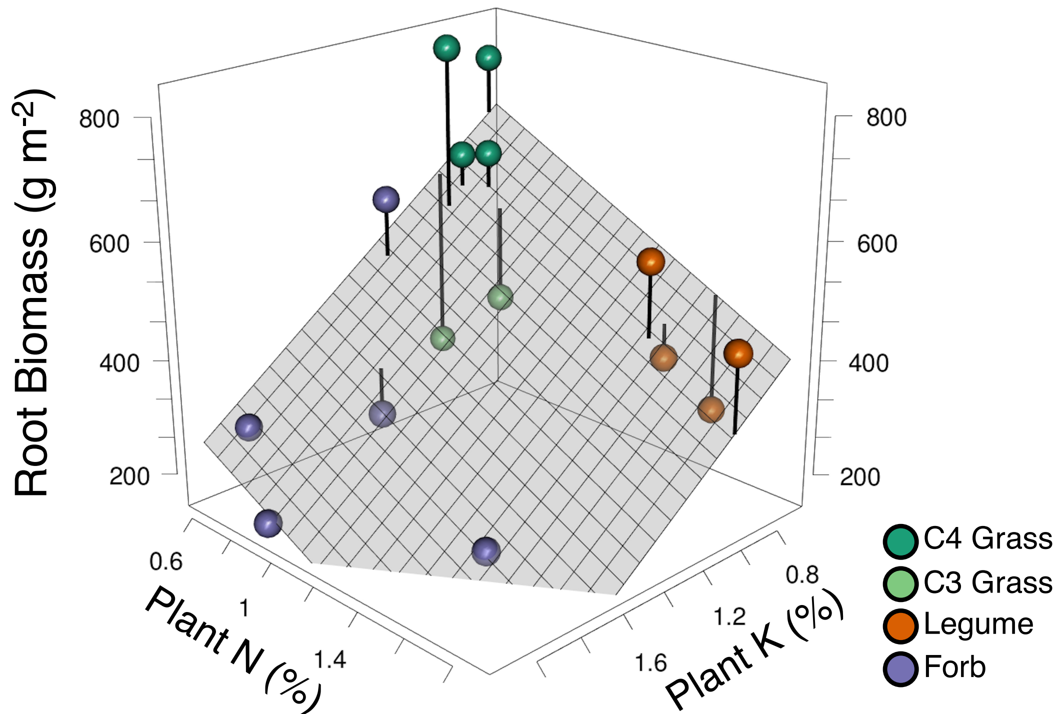

Figure 2-4: Empirical tradeoff surface among plant traits for the fifteen herbaceous perennial plant species that persisted in the experiment. A regression plane ($F_{2,12} = 6.3$, $R^2 = 0.51$, p = 0.014) is fitted to species-specific measured values of % aboveground tissue potassium (K) (x-axis), % aboveground tissue nitrogen (N) (y-axis), and mean monoculture root biomass (g m^{-2}; 0-30 cm depth; z-axis). Each point represents the three measured traits of each of 15 species (*SI Appendix,* Table S7) classified as grasses (C4 grasses in dark green and C3 grasses in light green), forbs (purple) and legumes (orange). %N and %K represent the mean across each species' monocultures and the biomass of each species in five 16-species plots. Root biomass represents the mean root mass (0-30 cm depth) of each species' monocultures. Removing the two C3 grasses (lighter green;

below the plane), which are subdominant species in this ecosystem and grew poorly in monoculture, increased the fit of the plane to $F_{2,10} = 15$, $R^2 = 0.75$, $p = 0.001$ (not shown). The point for *Andropogon gerardii* (C4 grass) was slightly jittered in the x and y axis to avoid overplotting with *Sorghastrum nutans* (C4 grass).

Chapter 3. Plant community responses to experimental nitrogen addition crossed with herbivore exclusion

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Introduction

Herbivory and resource competition are fundamental processes that jointly structure plant communities (Huntly 1991, Chase et al. 2002, Aschehoug et al. 2016) and that can determine whether interspecific interactions among plants lead to competitive exclusion or coexistence (Holt et al. 1994). Ecologists have long sought to understand the mechanisms underpinning exploitative resource competition (Hutchinson 1959, Tilman 1982, 2011, Chesson 2000, Grace and Tilman 2003). Other theory has explored how herbivory could impact coexistence in the absence of direct competition, but indirectly via apparent competition (Holt and Kotler 1987, Holt and Bonsall 2017). A combination of both exploitative and apparent competition suggests that an inferior competitor can coexist with a competitively dominant species in the presence of a herbivore (Holt et al. 1994). Because plants simultaneously experience both herbivory and exploitative competition in natural food webs, it can be difficult to assess both their interactive and independent impacts on the outcome of plant competition. Understanding the joint effects of exploitative competition and herbivory in a plant community necessitate factorial experimental designs that cross the addition of limiting nutrients and the presence/absence of an herbivore. Here we report results of a long-term experiment in a tall-grass prairie ecosystem in which addition rates of the limiting nutrient, nitrogen (N) combined with all other limiting nutrients, were fully crossed with a fencing treatment that excluded the only large mammalian herbivore remaining in the ecosystem, white-tailed deer, *Odocoileus virginianus*.

Herbivory has been shown to impact plant diversity, but the results often depend on both the type of herbivory and which plants are susceptible. Herbivory may increase

plant diversity when an herbivore consumes the dominant species (Paine 1966, Hillebrand et al. 2007, Koerner et al. 2018). The decrease in abundance of a dominate competitor can act as a coexistence mechanism if plants have interspecific tradeoffs in competitive ability versus resistance to herbivory where species either have resistance to or are suspectable to herbivory depressing their abundance (Levin et al. 1977, Holt et al. 1994, Viola et al. 2010). For instance, if a tradeoff were to exist between competitive ability for soil N and resistance to herbivory, species that are most abundant in plots with no added N, and thus are likely stronger competitors for N, those that draw resources to a lower level (R*), should be more helped by fencing. Conversely those species that increase with added N (which should be poorer N competitors) should increase in the absence of fencing, those with a higher P* that either resist herbivory or sustain a higher abundance of herbivores (Holt et al. 1994). It is not clear however, if these theoretical predictions hold empirically where a more common outcome has been a tradeoff between growth and defense (Coley et al. 1985, Fine et al. 2004, 2006, Viola et al. 2010, Lind et al. 2013).

Herbivory is often selective with the herbivore preferentially consuming different plant species depending on their traits. A simple yet useful heuristic is that grazers focus on grasses whereas browsers focus on herbaceous non-grasses, hereafter forbs (Gordon and Herbert 2019). Numerous studies have shown that grazers do have large impacts on grasses in family *Poaceae* (McNaughton 1985, Augustine and McNaughton 1998, Knapp et al. 1999, Towne et al. 2005). In contrast, browsing such as by white-tailed deer, the focal herbivore of this study, appears to be selective on forbs which benefits their grass competitors (Anderson et al. 2005, Wiegmann and Waller 2006, Rooney 2009). Such

browsing preferences may have cascading ecosystem consequences when deer selectively target species or plant tissues with higher N concentrations, such as N-fixing species, and thus lower total system N availability and influence species' competitive interactions for N (Ritchie et al. 1998).

Mammalian herbivores may specialize on specific types of plant tissues, (Gordon and Herbert 2019), and therefore each form of herbivory might uniquely impact competitive interactions. Florivory represents the direct consumption of flowers (McCall and Irwin 2006). Several studies suggest that white-tailed deer is a florivore of herbaceous plant species (Augustine and Frelich 1998, Anderson et al. 2001, 2007, Geddes and Mopper 2006, Flaherty et al. 2018, Palagi and Ashley 2019). However, there is less information on how florivory may interact with resource competition in structuring the composition of herbaceous plant species. It is possible that florivory may be an important component to interspecific trade-offs related to competition, growth and defense against herbivores (Coley et al. 1985, Holt et al. 1994, Viola et al. 2010, Lind et al. 2013). Such tradeoffs may only be exposed under certain soil resource conditions, such as under N limitation, and dependent on the type of herbivory in the ecosystem such as florivory.

Our objective was to test how the removal of fences in a long-term nutrient addition experiment impacted total plant biomass, plant biodiversity and species composition. We used permanent plots in a long-term grassland experiment to measure the joint and independent impacts of herbivory and resource addition on a plant community. The design included an experimental perturbation, with all plots first being fenced to exclude white-tailed deer for twenty-two years (1982 to 2004) and then with

half of them being changed to be unfenced (2005 to 2019). We use the resultant data to test if the presence of white-tailed deer, following their absence for two decades, decreased or increased total aboveground plant biomass and changed plant biodiversity both contingent on added N fertilizer with all other limiting nutrients. Based on how abundances of individual plant species responded to various combinations of N addition and the presence/absence of fences, we determined if the dominant plant species exhibited tradeoffs like those assumed for an R* vs. P* tradeoff or growth-defense tradeoff (Lind et al. 2013). Lastly, we present some opportunistic observations of deer browsing to gain an insight into the specific form of herbivory on the plant community. Our research questions were therefore:

1) How do (a) total aboveground plant biomass and (b) plant species richness depend on N addition, the presence/absence of a deer exclosure, and/or their interaction?

Our expectation for the main effect of each treatment was that the removal of fences would lower total aboveground biomass and increase plant species richness. We expected that the addition of N combined with the removal of fences would increase the production of plant biomass through a compensation mechanism. We expected that the removal of fences would counter-act the decrease in species richness associated with increased added N.

2) Is the abundance response of individual species to N addition and fencing removal consistent with a tradeoff between N-dependent competitive ability and benefitting from the removal of fences?

Our expectation was that there would be a tradeoff amongst plant species such that those that decreased with added N would be those species that most benefitted from fencing whereas those that increased with added N would be more abundant outside the fence.
3) Which species had detectable incidences of florivory, and on a species-by-species basis does a greater proportion of florivory correspond with a lower abundance of a species in the presence of deer (unfenced plots)?
We did not have *a priori* expectations for the impact of deer florivory on the plant community rather we report observations following the failure of one section of fencing that motivated counting incidences of deer browsing.

Methods

Site description: The experiment was conducted at the Cedar Creek Ecosystem Science Reserve, Minnesota, United States of America. The site has a nutrient-poor, sandy soil with a particle size distribution of ~90% sand (Udipsamments) (Grigal 1974). The field is located at 45.397334°, -93.191648° and referred to as "Field C" within the experiment "e001: Long-Term Nitrogen Deposition: Population, Community, and Ecosystem Consequences". The field was abandoned from maize row crop agriculture in 1934. The field was dominated by the native prairie perennials that invaded during secondary succession (Tilman 1987).

Experimental Design: The experiment consists of 54 plots sized 4 by 4 m with 1 m buffers between them arranged in a 9 x 6 rectangular grid. In 1982, each of 54 plots in an area were fenced to exclude white-tailed deer and were randomly assigned to receive nutrient treatment of either no added nutrients of any kind, or of one of 8 levels of added nitrogen (0.0, 1.02, 2.04, 3.4, 5.44, 9.52, 17.0, 27.2 g N m^{-2} yr^{-1}) plus nutrients P, K, Ca,

Mg, S and trace metals (Tilman 1987). There are two sets of plots that receive no added N. Treatment I which received no nutrients of any kind and Treatment A which received no N, but all other nutrients. Treatment A serves as the control to test solely for the effect of N addition whereas Treatment I serves as the control for nutrient addition of any kind.

The fertilizer addition was conducted as follows. Each plot received N as ammonium nitrate with a N content of 34%. In early May and again in late June of 1982 and each subsequent year, one-half of the annual amounts of all nutrients for each plot were mixed together and then manually broadcast on each plot. Each treatment received the following amounts of ammonium nitrate twice each year (0.0 = 0 g m^{-2}; 1.02 = 1.5 g m^{-2}; 2.04 = 3 g m^{-2}; 3.4 = 5 g m^{-2}; 5.44 = 8 g m^{-2}; 9.52 = 14 g m^{-2}; 17.0 = 25 g m^{-2}; 27.2 = 40 g m^{-2}). Additionally, all treatments except for the true control (Treatment I) received twice annually 10 g m^{-2} yr^{-1} P_2O_5; 10 g m^{-2} yr^{-1} K_2O; 15.0 g m^{-2} yr^{-1} $MgSO_4$; 20 g m^{-2} yr^{-1} $CaCO_3$; 18.85 μg m^{-2} yr^{-1} $ZnSO_4$; 9 μg m^{-2} yr^{-1} $CuSO_4$; 7.65 μg m^{-2} yr^{-1} 161 μg m^{-2} yr^{-1} $MnCl_2$; $CoCO_2$; 7.55 μg m^{-2} yr^{-1} $NaMoO_4$. Sheet metal was installed in between all plots to a depth of 30 cm to prevent root foraging for nutrients between plots and fertilizer contamination.

A deer herbivory by nutrient addition experiment was imposed in 2005 on the existing nutrient addition experiment that had been established in 1982. All plots were fenced from 1982 - 2004. The experimental design is a full factorial with nine nutrient treatments, two levels of fencing, and three replicates of each of the eighteen treatment combinations (though a subset of nutrient treatments were considered, as described below). The experiment was initiated in fall of 2004 by removing the fence that had surrounded the full experiment. After fencing removal, three of the six replicates for each

of the nine nutrient addition treatments were randomly chosen to be re-fenced. The other three replicates of each nutrient treatment were unfenced. Randomization was repeated a few times until the resulting spatial arrangement of fencing assured that deer could freely enter all unfenced plots. The mesh size of the fence was large enough to not exclude small mammals, but whenever a plains pocket gopher, *Geomys bursarius*, entered a plot it was trapped and removed. The removal of fencing coincided with the initiation of an annual burning of all plots of this experiment in the fall of 2004. Note that only the southern portion of this field consisting of e001 is burned. The layout of the experiment is presented in Appendix S1: Fig. S1.

Sampling: Vegetation in each plot was annually sampled by clipping, then sorting to species, drying and weighing, the aboveground plant biomass in a 10 cm x 3 m strip. Plant abundances in all plots were sampled in August of 2005, the first growing season for this herbivory x nutrient addition experiment, and in 10 of the subsequent 14 years from 2006 through 2019 (not clipped in 2012, 2013, 2016, and 2017). Vegetation sampling used the same protocol as had begun in 1982.

We conducted a separate estimate of deer herbivory across all plots in August of 2016. This sampling was initiated following an observation that a white-tailed deer, identified through the presence of its tracks and feces, broke through fences enclosing plots 25, 26, 31, 32 and 37 sometime between July 3rd and 4th, 2016. We observed that the deer preferentially consumed the flowers of forb species, many of which were rare or absent outside the fence enclosure. Following an informal visual survey for signs of vegetation consumption, the fence was repaired. A separate survey was conducted across all experimental plots in August during peak biomass in this ecosystem when the plots

are normally clipped. To formally estimate the extent that deer florivory (flower consumption) could be occurring across all plots in the experiment we used a 4 m x 0.5 m quadrat in each plot randomly assigned to the east or west side of each plot. To allow comparison across different inflorescence structures, each stem with a visible inflorescence in any stage of anthesis was counted. We recorded deer florivory as the removal of a whole inflorescence below the peduncle that could be visually compared to an intact version within the fencing treatment where possible. In each transect, the total number of stems per each species was estimated and we recorded how many of these stems had a removed inflorescence.

Data analyses: Analyses used R version 4.1.1, with experimental variables being the crossed fencing and nutrient addition treatments. We refer to the fencing treatment as "UnFenced" and "Fenced". We report analyses on a subset of experimental treatments. We used only those treatments that receive all potentially limiting nutrients along the gradient of N addition excluding the true controls (Treatment I). We did this for two reasons: 1) Theoretical models of resource competition assume there is only one sole limiting nutrient (Tilman 1982, Holt et al. 1994), which is only achieved experimentally by adding all potentially limiting nutrients along the N addition gradient. Tests of a R* vs. P* tradeoff (Holt et al. 1994) must use experimental conditions that match the theoretical assumptions. We therefore compare the N addition plots to the N addition control plots that did not receive N but did receive the other nutrients as do all plots that received N (Treatment A). 2) Historically in this experiment, the two treatments that received no added N have not shown any difference. However, there is a change in soil chemistry (higher soil pH in Treatment A) and composition that promoted the abundance

of forbs in the plots that receive the PKCaMg+ fertilizer mix. The compositional differences, irrespective of fencing, between A and I must be discussed in their own manuscript. We identify treatments by their annual N addition rates of 0.0, 1.02, 2.04, 3.4, 5.44, 9.52 g m^{-2} yr^{-1} of N. The maximum N treatment in this subset then approximates a doubling of the background soil net N mineralization rate. The sample size for our analyses is thirty-six plots (36 plots = 6 nutrient treatments x 2 fence treatments x 3 replicates). We did not consider the two highest levels of N addition (as explained by Clark and Tilman (2008)) because these rates are biologically unrealistic (270% and 490% above *in situ* N mineralization rates; Pastor et al 1987), causing plant die-offs, invasions by exotic annuals and extreme biomass oscillations.

Question 1a: Does total aboveground plant biomass depend on N addition, deer exclosure or their interaction?

Aboveground plant biomass was calculated as the sum of live aboveground herbaceous biomass (g m^{-2}). The dependance of aboveground biomass on a fully crossed interaction with the natural log of year as a linear continuous variable (2005-2019), N addition as an unordered categorical variable (6 levels), and fencing as a categorical variable (2 levels) was determined using a linear mixed effects model (*nlme*) (Pinheiro and Bates 2000). Plot was included as a random intercept. A separate variance term was included for both the effect of deer and N treatments to account for unequal variance, to address heteroscedasticity and to improve the model fit to the data (*nlme::varIdent*). We additionally tested a log transformed y-variable, but it reduced the fit to the data (Appendix S1, Figure S2). Plot was included as a random intercept. A compound symmetry temporal autocorrelation structure was included. We tested the significance of

each parameter using a nested likelihood ratio test. We did not retain any interactions at $P > 0.05$.

Question 1b: Does plant species richness depend on N addition, deer exclosure, or their interaction?

Species richness was calculated as the number of observed herbaceous species in each plot removing mosses and lichens and species with a woody growth-form. The same model selection procedure was used as for the model for aboveground biomass and the same specification except with a linear term for year that improved the model fit. For the N addition treatments where species richness depended on the presence of fences, an additional analysis was run. A t-test was used to determine a difference in species richness between pre- and post-fencing removal using the two years prior to fencing removal and the last two years of data post-fencing removal.

Question 2: Is the abundance response of individual species to N addition and fencing removal consistent with a tradeoff between N-dependent competitive ability and benefitting from the removal of fences?

The dependance of each individual plant species' abundance (aboveground biomass g m^{-2}) on the natural log of year as a linear continuous variable, the effect of N as a linear continuous variable and the fencing exclosure as a categorical variable (two levels) were tested using a linear mixed effects model. We used N as a continuous linear variable to report effect size of biomass per g of added N and it often gave a more parsimonious fit than the natural log of added N. Plot was included as a random intercept. A separate variance term was included for both experimental variables to account for unequal variance, to address heteroscedasticity and to improve the model fit to the data

(*nlme::varIdent*). *P*-values were adjusted using the false discovery rate correction to retain power while controlling for type 1 errors (Benjamini and Hochberg 1995). The top 10 most abundant grass, legume and forb species were tested representing ~88% of the total aboveground biomass. Models for the species less abundant than these frequently failed to converge. We dropped the first two years after the fences were removed. Species abundance patterns strongly displayed transient dynamics, such as when: "[a] system can undergo complex dynamics during the transition from its original state to the new experimentally imposed state (Tilman 1989)." This exclusion was necessary at the level of individual species as there was carry-over effects from pre-treatment fencing conditions that took several years to realize post fencing removal. Additionally, to simplify the statistical models for each species and the effect size we report, we did not include year x fencing interactions for each species as we did for both total biomass and species richness. Where we used the mean response to fencing removal across the years included, we seek to report the long-term outcome of the fencing removal perturbation. Years included are therefore 2007-2019 (not clipped in 2012, 2013, 2016, and 2017).

The effect size for N and the effect size for fencing on each individual species' biomass were tested for correlation using major axis regression (Lind et al. 2013). In this manuscript, we denote species in family *Poaceae* as grasses (n = 4), species within the base and core eudicots as forbs (Families: *Euphorbiaceae* n =1; *Asteraceae* n = 4) and species in family *Fabaceae* as legumes (n = 1).

Question 3: Which species had detectable incidences of the consumption of their flowers outside the fencing exclosure?

The proportion of inflorescences removed per each species is presented as supplemental data to support the statistical models of each species' abundance. We used the total number of browsed inflorescences divided by the total number of counted inflorescences to calculate a proportion. The hypothesis that the proportion of browsed inflorescences was equal to zero was tested using a two-proportion Z-test (*prop.test*).

Results

There was no interaction between fencing and added N in the statistical model for total live aboveground biomass (P = 0.27, Appendix S1: Table S1). Biomass depended on main effects for added N, fencing and the natural log of year (Appendix S1: Table S1). Plots outside the fence had less biomass (44.6 ± 19.4 SE, df = 29, P = 0.0292) on average across the N treatments of 0.0 g N – 9.52 g N m^{-2} yr^{-1} (Figure 3-1). Biomass increased with added N (Appendix S1: Table S2). The biomass for each treatment when averaged across time is presented in Supplemental Appendix S1: Fig. S3. The average biomass for each treatment in each year is presented in Supplemental Appendix S1: Fig. S4.

The removal of fences increased plant species richness, but only in plots where N was not added. Species richness depended on a significant three-way N by fencing by year interaction (P = 0.002) (Appendix S1: Supplemental Table S3). The interaction reveals a gain of ~0.6 species per 0.3 m^2 per year outside the fence with no added N (Figure 3-2). Relative to the two years prior to fencing removal (~15 years, average of 2003 and 2004 to the average of 2018 and 2019), in the treatment without added N (n = 6), plots outside the fence gained 6.3 species (95% C.I., [1.94, 10.73], df = 10, P = 0.0093, n = 12) whereas plots inside the fence did not change in species richness (mean of 1.83 species; 95% C.I., [-2.09, 5.75], df = 10, P = 0.32, n = 12).

Of the 10 most abundant grass, legumes and forb species, the removal of fencing changed the abundances of five forb species, but only of one grass species. Forbs *Solidago rigida, Symphyotrichum oolentangiense* (formerly *Aster azureus*) and *Euphorbia corollata* decreased by 32 ± 13 SE g m^{-2}, 5.8 ± 2.5 SE g m^{-2}, and 6.9 ± 1.9 SE g m^{-2} of biomass respectively outside the fence ($P = 0.045$, $P = 0.045$, $P < 0.001$, with effect sizes representing 14%, 2.6% and 3% of the mean total live aboveground biomass in plots receiving no added N) whereas forbs *Artemisia ludoviciana* and *Ambrosia coronopifolia* increased by 34 ± 12 SE g m^{-2} and 7.9 ± 2.6 SE g m^{-2} of biomass, respectively, outside the fence ($P = 0.027$, $P = 0.023$, with effect sizes representing 15% and 3.5% of the mean total live aboveground biomass in plots receiving no added N) (Appendix S1: Supplemental Table S4). Legume *Lathyrus venosus* decreased, but not significantly, by 6.4 ± 3.3 SE g m^{-2} biomass ($P = 0.086$). The fencing treatment had a significant effect on the abundance of C3 grass *Panicum oligosanthes* of 3.7 ± 1.6 SE g m^{-2} more biomass outside the fence ($P = 0.045$, with an effect size representing 1.6% of the mean total live aboveground biomass in plots receiving no added N) (Appendix S1: Supplemental Table S4).

As to N addition, the forb *S. rigida* decreased by 4.7 ± 1.9 SE g m^{-2} per g m^{-2} of annual N addition ($P = 0.039$) whereas *A. ludoviciana* increased by 13.9 ± 2.4 SE g m^{-2} per g N m^{-2} yr^{-1} ($P < 0.001$) (Appendix S1: Supplemental Table S4). The grass *Sorghastrum nutans* decreased by 0.44 ± 0.18 g m^{-2} per g N m^{-2} yr^{-1} ($P = 0.039$), whereas grasses *Poa pratensis* and *Elymus repens* increased by 7.1 ± 1.5 SE and 11 ± 3.6 SE g m^{-2} per g N m^{-2} yr^{-1} (Both $P < 0.001$) (Appendix S1: Supplemental Table S4).

We used the effect sizes from regressions of the dependence of each species' biomass on N and fencing to determine if there might be a tradeoff between competition for N and benefitting from the removal of fences. Using the effect size of all six forbs and legumes, our results suggest the possibility of a R* vs. P* tradeoff (Figure 3-3a), based on a major axis regression with a positive slope of 3.38 grams of biomass gained outside the fence for each gram of added N ($R^2 = 0.85$, $P = 0.009$) (Figure 3-3a). In contrast, only one grass species, *P. oligothanses*, changed in abundance with the fencing treatment (Figure 3-3b).

A visual survey of florivory on individuals of these species indicated that several forb species had been browsed, but no grasses had any evidence of deer florivory (Figure 3-4). A Z-test on the percentage of browsed inflorescences indicated that four species had detectable levels of florivory: *S. oolentangiense, E. corollata, S. rigida*, and *L. venosus* (all $P < 0.001$). Although these counts of florivory events came from one season's field observations, all the species with detectable deer florivory also had less biomass outside the fence: *S. oolentangiense* 5.8 ± 2.5 SE g m^{-2}, *E. corollata* 6.9 ± 1.9 SE g m^{-2}, *S. rigida* 32 ± 13 SE g m^{-2}. *L. venosus* did not significantly change in biomass from the fencing treatment (6.4 ± 3.3 SE g m^{-2} less biomass outside the fence) given the sample size and variance (but see, Ritchie and Tilman 1995, Ritchie et al. 1998, Knops et al. 2000)

Discussion

The removal of fences, which allowed white-tailed deer access to half the plots, caused declines in total live aboveground biomass and increases in plant species richness. The most interesting findings though, were changes in the abundance of several forb species. We found that the forb species in this experiment appear to have interspecific

tradeoffs between their competitive ability for soil N and benefitting from the removal of fences. *S. rigida* was highly abundant when N was the sole limiting nutrient inside the fence. In contrast, *A. ludoviciana* was highly abundant when N and all other nutrients were highly available outside the fence. Unfenced plots had a mild ~16% (45 g m^{-2}) decrease in plant biomass, comparable in magnitude to the increase in biomass associated with the addition of ~1-2 g N m^{-2} yr^{-1} with all other nutrients. The increase in biomass of the forbs *A. ludoviciana* and *Ambrosia coronopifolia* after the removal of fencing appears to have mostly offset the decrease in biomass of *Solidago rigida, Euphorbia corollata* and *Symphyotrichum oolentangiense*. Additionally, if the fencing treatment protects plants from deer browsing and deer are florivores of grassland forbs, then the removal of flowers is a small proportion of the total biomass of these plots, but, in the long term, could decrease the abundances of species subject to florivory by decreasing recruitment.

It is interesting that the increased abundance of white-tailed deer in the 20th century has been associated with reductions of forb abundance in the understory of northern forests of North America (Côté et al. 2004, Wiegmann and Waller 2006). White-tailed deer have also been shown to consume flowers of *Polemonium vanbruntiae* in bogs (Flaherty et al. 2018), to consume flowers of *Iris hexagona* in a salt marsh (Geddes and Mopper 2006), and to reduce the abundance of *Trillium* in forest understory (Augustine and Frelich 1998). A comparison of islands with and without deer found that the forb *Clintonia borealis* had fewer genets per ramet when deer were present (Palagi and Ashley 2019). In a prairie restoration when deer were hunted, the amount of browsed stems of *S. rigida*, which we also report as being deer browsed, decreased (Anderson et al. 2007).

In contrasts to forbs, we found positive effects on the abundance outside the fence of one C3 grass species (*P. oligosanthes*) and no effect of fencing on the abundance of the native C4 grass (*S. nutans*), consistent with deer being browsers with minimal impact on grasses with intercalary meristems and low-nutrient tissues (Gordon and Herbert 2019). A similar long term study in a forest ecosystem in Wisconsin found that eighteen years of deer exclosure caused increased abundance of forbs inside the fence, whereas grass abundance increased outside the fence (Rooney 2009). A follow-up study found that graminoids had low palatability to white-tailed deer (Begley-Miller et al. 2014). In our experiment, grasses were differentiated along an axis of N addition rather than fencing (Wedin and Tilman 1993). Rather than large increases in grass abundance when fences were removed, we found an increase in the forb *A. ludoviciana* outside the fence. *A. ludoviciana* has purported physical and chemical defenses that are tolerated by its specialist insect herbivore *Hypochlora alba* (Smith and Kreitner 1983), but presumably not by many other herbivores.

An interesting comparison can be drawn to a study at the Konza Prairie, where *Bison bison*, which preferentially graze on grasses such as *S. scoparium*, caused an increase in the cover of two forbs, Missouri goldenrod *Solidago Missourensis* and heath aster *Aster ericoides* (now *Symphyotrichum ericoides*) (Towne et al. 2005). In our study, deer reduced the abundance and flowers of two congeners of these species: *S. rigida* and *A. azureus* (now *S. oolentangiense*). Deer florivory might cause grassland communities to be depauperate of poorly defended forbs, whereas grazers such as *B. bison* consuming grasses might increase forb abundance. A more diverse community of mammalian herbivores, such as existed historically in the great plains of North America (Hartnett et

al. 1997), might have maintained high plant diversity because of the differing effects of different types of herbivores.

The response of individual plant species to the experimental treatments demonstrated that forb species varied along an axis of N addition and fencing (Figure 3-3), but mostly contingent on two dominant species. The observed tradeoff among forb species shows that *S. rigida* was most abundant in plots with no added N, but with all nutrients, inside the fence, and became much rarer outside the fence and when N with all other nutrients were added at higher rates. *A. ludoviciana* had exactly the opposite responses to these treatment combinations, being most abundant outside the fence and when N with all other nutrients were added at high rates. This species was rare inside the fence and N was not added or added at a low rate with all other nutrients. The markedly different responses of these two species were clearly visible in the field (Figure 3-5): *S. rigida* is the species with yellow flowers and *A. ludoviciana* is the species with silver-grey foliage and flowers. In this photo, a 0.0 g N m^{-2} yr^{-1} with all other nutrients inside a fence treatment is on the right and *S. rigida* is the dominant species. On the left is a 9.52 g N m^{-2} yr^{-1} with all other nutrients outside the fence treatment in which *A. ludoviciana* is the dominant species. The combination of a fence and fertilizer within only a distance of ~ 10 m shifted the dominant plant species of this community between two species that had opposite responses to N addition and fencing.

Our results suggest that florivory, and by extension other causes of seed predation, may be an important factor influencing the composition and diversity of grasslands, which is conceptually similar to the Janzen-Connell hypothesis for tropical forests (Janzen 1970). Theory suggests that an R* vs. P* tradeoff can act as a coexistence

mechanism in plant communities via a combination of apparent and exploitative competition (Holt et al. 1994). The consumption of seeds has also revealed an competition-defense tradeoff in an annual plant community when granivorous ants preferentially consumed the seeds of species that had both larger seeds and were competitively dominant, albeit with mixed effects on coexistence (Petry et al. 2018). Considering Petry et al. (2018) and the present study suggests a testable hypothesis that the consumption of flowers or seeds may be a form of herbivory that invokes an R* vs. P* tradeoff.

The data presented appears consistent with previous results at our site showing that deer are the main mammalian herbivore (Ritchie and Tilman 1995, Ritchie et al. 1998, Knops et al. 2000), however we cannot state for certain what drives the effects of fencing on species' abundances. Fencing can cause interesting dynamics such as when the exclusion of small granivorous mammals dramatically increased grass abundance in a desert plant community (Brown and Heske 1990, Brown 1998). We have no data on the role of small mammals in this experiment. Species that became more abundant outside the fence may be those that are susceptible to herbivory and display compensatory growth that is not captured by permanent structures (Augustine and McNaughton 1998). Furthermore, our observations of deer florivory could be biased and must be treated with skepticism. Nevertheless, our analyses and observation suggest that *A. ludoviciana* is the key species that benefitted from the fencing removal. Future studies could be conducted to understand if this response is one of tolerance or resistance to deer herbivory including characterization of potential chemical and physical defenses (Smith and Kreitner 1983).

Conclusions

We suggest that white-tailed deer mainly impacted a tall-grass prairie ecosystem by modifying its community composition with small percentage effects on aboveground biomass. Our observations suggest that deer florivory may be a major factor impacting abundances of dominant forb species (Augustine and Frelich 1998, Anderson et al. 2001, 2007, Geddes and Mopper 2006, Flaherty et al. 2018, Palagi and Ashley 2019). There may be consequential impacts on the species that pollinate angiosperms and require floral rewards for survival (Sakata and Yamasaki 2015, Nakahama et al. 2020). We hope our study will inspire ecologists to consider the specific impact of white-tailed deer on grassland forbs and the reproducibility of florivory as an impactful ecological force.

Figures

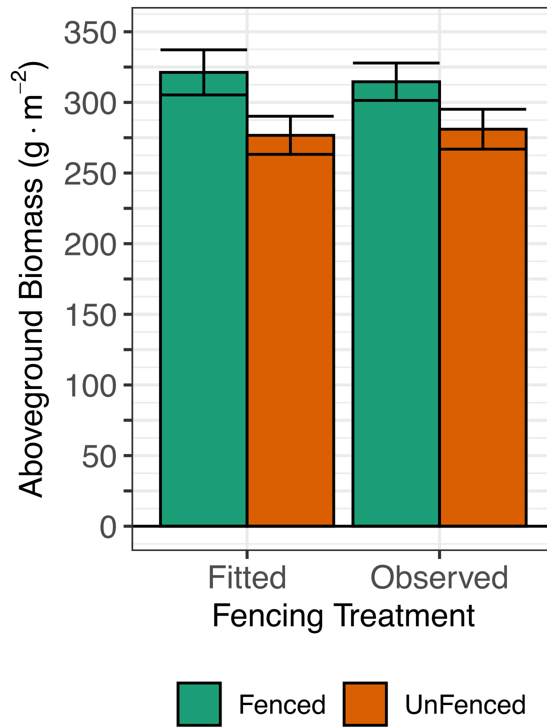

Figure 3-1: Plant total live aboveground biomass across fencing exclosure treatments. Observed and fitted mean total aboveground biomass (g m^{-2}) ± 1 SE averaged across N addition treatments 0.0 – 9.52 g N m^{-2} yr^{-1} for each fencing treatment (UnFenced = orange and Fenced = green) (n = 18). Years included 2005 through 2019 (not clipped in 2012, 2013, 2016, and 2017).

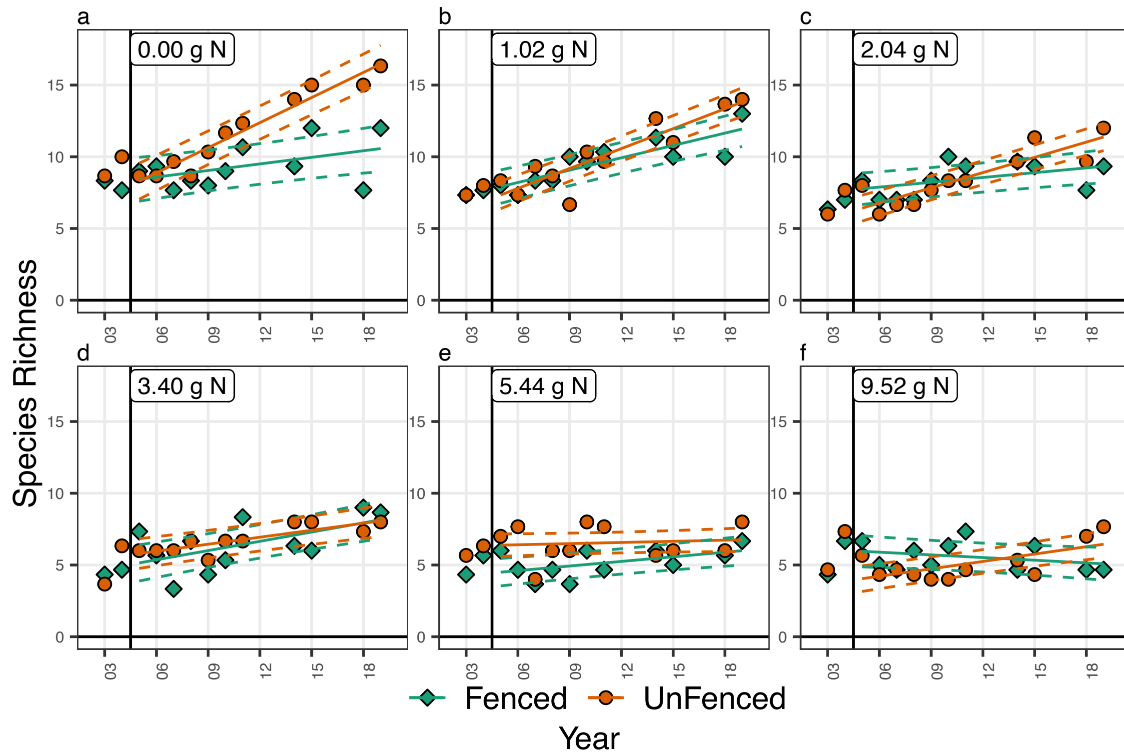

Figure 3-2: Trends in plant species richness (2003-2019), for each nitrogen addition treatment (g m^{-2} N yr^{-1}), showing deer excluded plots (green; diamond) and unfenced plots (orange; circles). Each point represents the observed mean of the number of species for each nitrogen treatment at each fencing treatment (n = 3 within each level of added N). The lines represent fitted values ± 1 SE from a linear mixed effects model testing the dependance of the number of species on a three-way interaction between the categorical fencing treatment, the added nitrogen treatment as a categorical variable and year as a linear continuous variable (>2004). The vertical bar denotes when the fences were removed in the fall of 2004 and deer could enter half the plots freely. Two pre-treatment years are shown in 2003 and 2004 (but not included in the model) followed by the years with fences removed 2005-2019 excluding 2012, 2013, 2016, and 2017. Nitrogen addition treatments: (a) 0.00 g N m^{-2} yr^{-1} (b) 1.02 N g m^{-2} yr^{-1} (c) 2.04 g N m^{-2} yr^{-1} (d)

3.40 g N m^{-2} yr^{-1} (e) 5.44 g N m^{-2} yr^{-1} (f) 9.52 g N m^{-2} yr^{-1}. *n.b.* all treatments also received all other limiting nutrients as fertilizer.

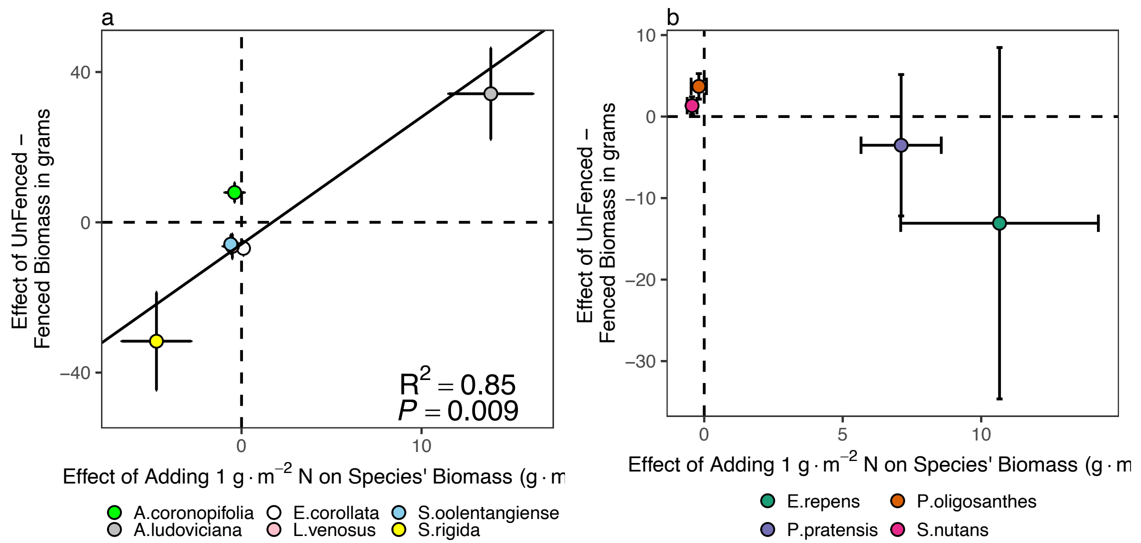

Figure 3-3: The effect size of a separate linear mixed effects model for each of (a) forb and legumes and (b) grasses species testing the dependence of an individual species' biomass on nitrogen (linear variable) and fencing (categorical variable). The y-axis represents the difference in biomass for each species when the biomass of a species in unfenced plots has subtracted from it the biomass of that species inside the fence. The x-axis represents the slope of a continuous linear variable of added nitrogen (g N m^{-2} yr^{-1}) on each species' biomass. Each point represents the coefficient in each species' statistical model ± 1 SE from Appendix S1: Supplemental Table 4. The fitted line represents a major axis regression displaying the relationship between both variables for forbs and legume species.

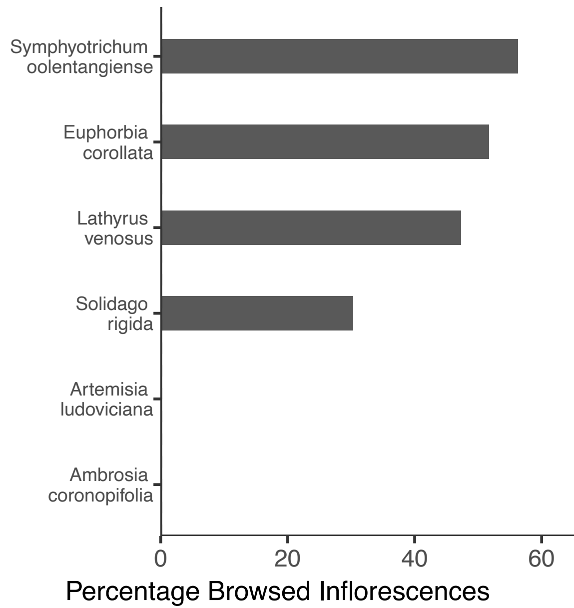

Figure 3-4: Percentage of deer-browsed inflorescences of abundant species for forbs (including legumes) species observed in unfenced plots. Each point represents the number of browsed inflorescences for each species out of the total number of stems for each species counted across plots (0 g N m^{-2} yr^{-1} - 9.52 g N m^{-2} yr^{-1} (n=18)).

Figure 3-5: Picture of treatment plots 9 (9.52 g N m^{-2} yr^{-1} unfenced; left) and plot 8 (0 g N m^{-2} yr^{-1}; right). Note the silver-grey foliage and flowers of *Artemisia ludoviciana* and the yellow flowers of *Solidago rigida*. Photo from August 8th, 2016 illustrates dominance by *S. rigida* inside the fence in plots with no added nitrogen contrasted with dominance by *A. ludoviciana* outside the fence at high added nitrogen.

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Appendix for Chapter 1

Might field experiments also be inadvertent metacommunities?

Supplemental Table S1: Summary table for a linear mixed effects model testing for the dependence of focal plot species richness on Ln[N+D] (the natural log of added nitrogen + deposition of ~1), year (as a linear variable 1982-2004) coded as 0-22, and the time series mean of the neighborhood species richness. n = 54 plots across 23 years.

Parameter	Coefficient	SE	DF	t-value	*P*-value
Intercept	11.02	1.00	1,187	11.03	<0.01
Ln[N+D]	-1.84	0.15	51	-12.57	<0.01
Year	-0.20	0.02	1,187	-10.84	<0.01
Neighborhood Species Richness	0.30	0.11	51	2.65	0.011

Supplemental Table S2: Summary ANOVA table for a linear mixed effects model of the dependence of focal plot species richness in control plots on neighborhood species richness, year (1982-2004) coded as 0-22, and their interaction. n = 6 plots across 23 years.

	numDF	denDF	F-value	*P*-value
Intercept	1	129	46.66	<0.01
Neighborhood Species Richness	1	129	13.69	<0.01
Year	1	129	0.00	0.99
Neighborhood Species Richness*Year	1	129	12.09	<0.01

Supplemental Table S3: Summary table displaying the coefficients for a linear mixed effects model of the dependence of focal plot species richness in control plots on neighborhood species richness, year (1982-2004) coded as 0-22, and their interaction. n = 6 plots across 23 years.

Term	Coefficient	SE	DF	t-value	*P*-value
Intercept	13.043	1.91	129	6.83	<0.01
Year	-0.494	0.13	129	-3.70	<0.01
Neighborhood Species Richness	-0.002	0.19	129	-0.01	0.99
Year * Neighborhood Species Richness	0.052	0.02	129	3.48	<0.01

Supplemental Table S4: Summary ANOVA table for the linear mixed effects model testing the abundance of *Schizachyrium scoparium*. NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Schiscop.neighbor is the neighborhood biomass of *Schizachyrium scoparium* in adjacent plots. n = 54 plots across 23 years.

	numDF	denDF	F-value	*P*-value
(Intercept)	1	1,161	110.25	<0.001
NTrt	8	45	25.45	<0.01
Year	1	1,161	7.25	0.012
Schiscop.neighbor	1	1,161	0.29	0.616
NTrt:Year	8	1,161	9.98	<0.01
NTrt:Schiscop.neighbor	8	1,161	18.49	<0.001
Year:Schiscop.neighbor	1	1,161	14.90	<0.01
NTrt:Year:Schiscop.neighbor	8	1,161	22.71	<0.001

Supplemental Table S5: Summary table displaying the coefficients of linear mixed effects model testing the abundance of *S. scoparium*. NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Schiscop.neighbor is the neighborhood biomass of *S. scoparium* in adjacent plots.

Term	Coefficient	SE	DF	t-value	*P*-value
(Intercept)	7.986	0.761	1,161	10.500	<0.01
NTrtA-0.00	0.949	1.159	45	0.819	0.417
NTrtB-1.02	-1.786	1.094	45	-1.632	0.11
NTrtC-2.04	-2.838	1.182	45	-2.401	0.021
NTrtD-3.40	-0.859	1.007	45	-0.853	0.398
NTrtE-5.44	-4.412	0.951	45	-4.640	<0.01
NTrtF-9.52	-7.470	0.919	45	-8.125	<0.01
NTrtG-17.0	-8.509	1.130	45	-7.529	<0.01
NTrtH-27.2	-7.466	0.941	45	-7.930	<0.01
Year	-0.130	0.048	1,161	-2.692	<0.01
Schiscop.neighbor	0.005	0.009	1,161	0.538	0.59
NTrtA-0.00:Year	-0.185	0.072	1,161	-2.568	0.01
NTrtB-1.02:Year	-0.036	0.069	1,161	-0.527	0.598
NTrtC-2.04:Year	-0.046	0.076	1,161	-0.608	0.543
NTrtD-3.40:Year	-0.198	0.063	1,161	-3.142	<0.01
NTrtE-5.44:Year	-0.036	0.059	1,161	-0.613	0.54
NTrtF-9.52:Year	0.117	0.057	1,161	2.058	0.04
NTrtG-17.0:Year	0.203	0.071	1,161	2.862	<0.01
NTrtH-27.2:Year	0.114	0.058	1,161	1.953	0.051
NTrtA-0.00:Schiscop.neighbor	0.002	0.013	1,161	0.155	0.877
NTrtB-1.02:Schiscop.neighbor	0.030	0.014	1,161	2.080	0.038
NTrtC-2.04:Schiscop.neighbor	0.049	0.015	1,161	3.358	<0.01
NTrtD-3.40:Schiscop.neighbor	0.001	0.011	1,161	0.074	0.941
NTrtE-5.44:Schiscop.neighbor	0.045	0.011	1,161	4.051	<0.01
NTrtF-9.52:Schiscop.neighbor	0.063	0.011	1,161	5.794	<0.01
NTrtG-17.0:Schiscop.neighbor	0.073	0.014	1,161	5.098	<0.01
NTrtH-27.2:Schiscop.neighbor	0.075	0.011	1,161	6.824	<0.01
Year:Schiscop.neighbor	0.004	0.001	1,161	3.860	<0.01
NTrtA-0.00:Year:Schiscop.neighbor	0.002	0.002	1,161	1.308	0.191
NTrtB-1.02:Year:Schiscop.neighbor	-0.002	0.002	1,161	-1.147	0.251
NTrtC-2.04:Year:Schiscop.neighbor	-0.004	0.002	1,161	-2.336	0.02
NTrtD-3.40:Year:Schiscop.neighbor	-0.002	0.002	1,161	-0.957	0.339
NTrtE-5.44:Year:Schiscop.neighbor	-0.006	0.001	1,161	-4.734	<0.01
NTrtF-9.52:Year:Schiscop.neighbor	-0.008	0.001	1,161	-6.973	<0.01
NTrtG-17.0:Year:Schiscop.neighbor	-0.011	0.001	1,161	-7.805	<0.01
NTrtH-27.2:Year:Schiscop.neighbor	-0.011	0.001	1,161	-7.954	<0.01

Supplemental Table S6: Summary ANOVA table for the linear mixed effects model testing the abundance of *Elymus repens.* NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Elymrepe.neighbor is the neighborhood biomass of *Elymus repens* in adjacent plots. n = 54 plots across 23 years.

	numDF	denDF	F-value	*P*-value
Intercept	1	1161	0.04	0.847
NTrt	8	45	0.84	0.616
Year	1	1161	4.18	0.058
Elymrepe.neighbor	1	1161	3.25	0.091
NTrt*Year	8	1161	4.57	<0.01
NTrt*Elymrepe.neighbor	8	1161	17.20	<0.001
Year*Elymrepe.neighbor	1	1161	3.72	0.072
NTrt*Year*Elymrepe.neighbor	8	1161	10.70	<0.01

Supplemental Table S7: Summary table displaying the coefficients of linear mixed effects model testing the abundance of *E. repens*. NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Elymrepe.neighbor is the neighborhood biomass of *E. repens* in adjacent plots.

Term	Coefficient	SE	DF	t-value	*P*-value
Intercept	0.2036	1.0568	1,161	0.1927	0.847
NTrtA-0.00	0.1918	1.5247	45	0.1258	0.9
NTrtB-1.02	0.5815	1.5118	45	0.3847	0.702
NTrtC-2.04	0.3034	1.5475	45	0.1960	0.845
NTrtD-3.40	1.1756	1.5655	45	0.7509	0.457
NTrtE-5.44	0.2583	1.6329	45	0.1582	0.875
NTrtF-9.52	0.3583	1.6326	45	0.2195	0.827
NTrtG-17.0	4.2290	1.8384	45	2.3003	0.026
NTrtH-27.2	0.3498	1.7793	45	0.1966	0.845
Year	0.0305	0.0149	1,161	2.0451	0.041
Elymrepe.neighbor	-0.0032	0.0018	1,161	-1.8030	0.072
NTrtA-0.00*Year	-0.0029	0.0332	1,161	-0.0874	0.93
NTrtB-1.02*Year	0.0612	0.0296	1,161	2.0653	0.039
NTrtC-2.04*Year	0.0190	0.0403	1,161	0.4714	0.637
NTrtD-3.40*Year	0.0683	0.0456	1,161	1.4978	0.134
NTrtE-5.44*Year	0.2410	0.0629	1,161	3.8294	<0.01
NTrtF-9.52*Year	0.1700	0.0597	1,161	2.8486	<0.01
NTrtG-17.0*Year	0.0872	0.0991	1,161	0.8801	0.379
NTrtH-27.2*Year	0.3161	0.0879	1,161	3.5943	<0.01
NTrtA-0.00*Elymrepe.neighbor	-0.0362	0.0083	1,161	-4.3847	<0.01
NTrtB-1.02*Elymrepe.neighbor	-0.0138	0.0051	1,161	-2.7247	<0.01
NTrtC-2.04*Elymrepe.neighbor	0.0134	0.0160	1,161	0.8389	0.402
NTrtD-3.40*Elymrepe.neighbor	0.0158	0.0092	1,161	1.7080	0.088
NTrtE-5.44*Elymrepe.neighbor	0.0366	0.0092	1,161	3.9674	<0.01
NTrtF-9.52*Elymrepe.neighbor	0.0535	0.0143	1,161	3.7482	<0.01
NTrtG-17.0*Elymrepe.neighbor	0.1278	0.0251	1,161	5.0965	<0.01
NTrtH-27.2*Elymrepe.neighbor	0.0826	0.0119	1,161	6.9494	<0.01
Year*Elymrepe.neighbor	0.0003	0.0001	1,161	1.9279	0.054
NTrtA-0.00*Year*Elymrepe.neighbor	0.0029	0.0005	1,161	5.7258	<0.01
NTrtB-1.02*Year*Elymrepe.neighbor	0.0021	0.0004	1,161	5.5778	<0.01
NTrtC-2.04*Year*Elymrepe.neighbor	0.0004	0.0010	1,161	0.3674	0.713
NTrtD-3.40*Year*Elymrepe.neighbor	0.0014	0.0005	1,161	2.6417	<0.01
NTrtE-5.44*Year*Elymrepe.neighbor	-0.0008	0.0007	1,161	-1.1906	0.234
NTrtF-9.52*Year*Elymrepe.neighbor	0.0007	0.0010	1,161	0.6387	0.523
NTrtG-17.0*Year*Elymrepe.neighbor	-0.0034	0.0017	1,161	-2.0021	0.046
NTrtH-27.2*Year*Elymrepe.neighbor	-0.0028	0.0008	1,161	-3.3761	<0.01

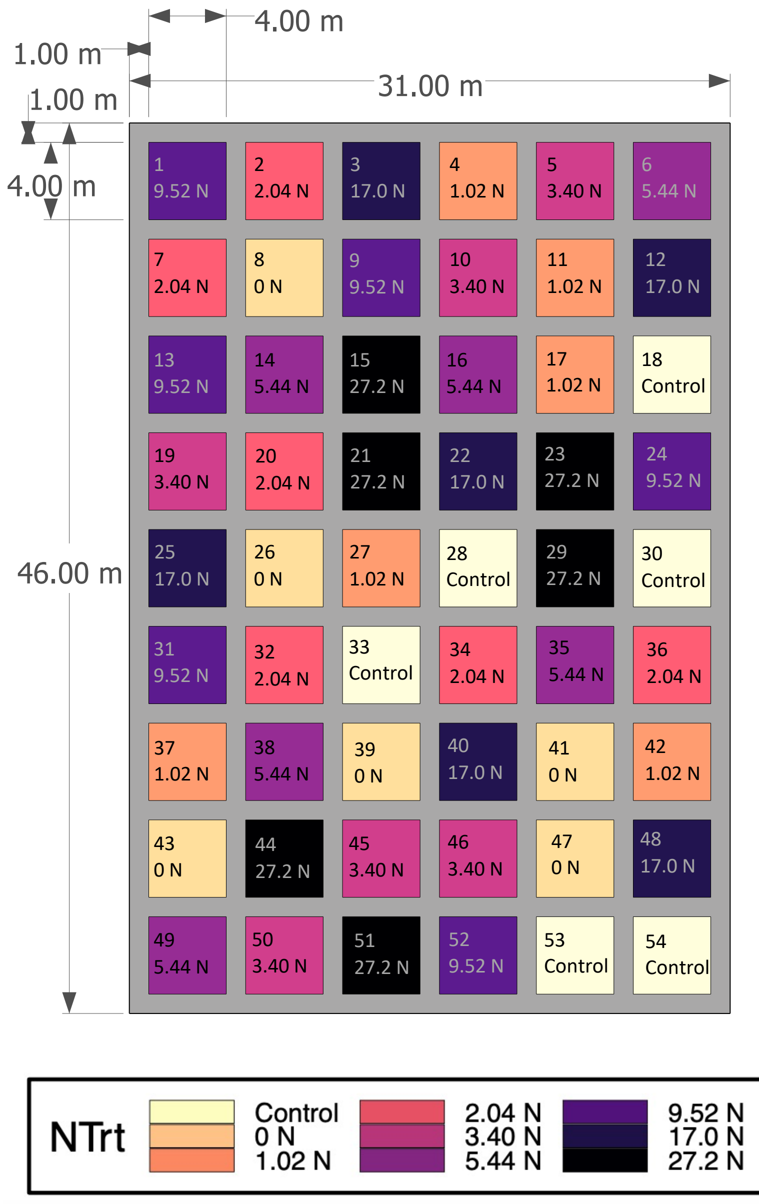
Supplemental Table S8: Summary ANOVA table for the linear mixed effects model testing the abundance of *Poa pratensis*. NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Poaprate.neighbor is the neighborhood biomass of *Poa pratensis* in adjacent plots. n = 54 plots across 23 years.

	numDF	denDF	F-value	pval
Intercept	1	1,161	36.66	<0.01
NTrt	8	45	2.97	0.015
Year	1	1,161	2.30	0.155
Poaprate.neighbor	1	1,161	1.29	0.292
NTrt*Year	8	1,161	4.78	<0.01
NTrt*Poaprate.neighbor	8	1,161	6.17	<0.01
Year*Poaprate.neighbor	1	1,161	7.51	0.012
NTrt*Year*Poaprate.neighbor	8	1,161	2.17	0.041

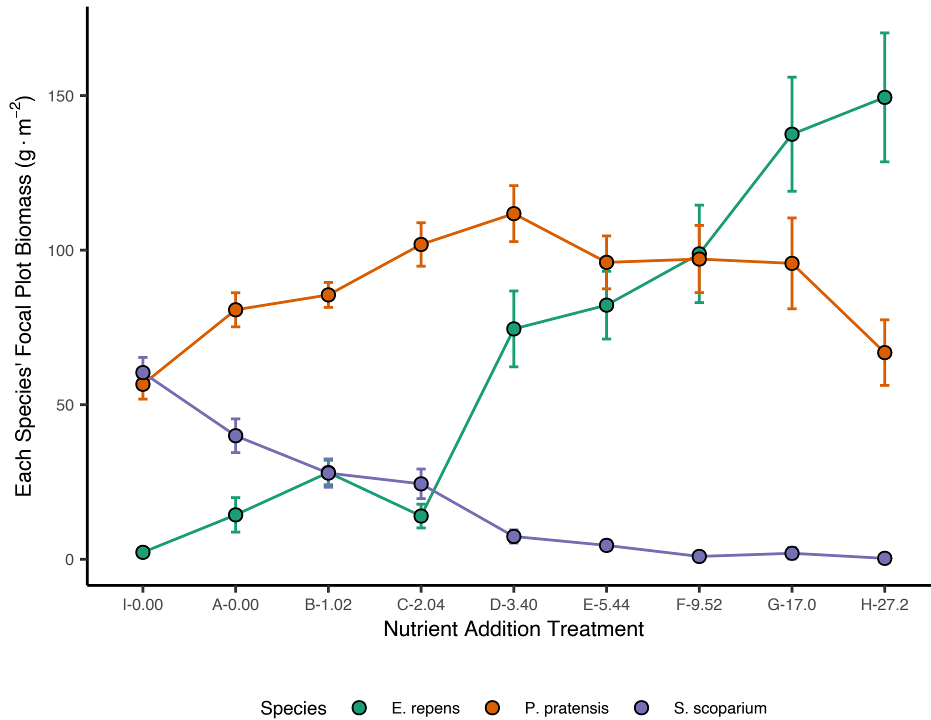
Supplemental Table S9: Summary table displaying the coefficients of linear mixed effects model testing the abundance of *P. pratensis*. NTrt = nutrient addition treatment as a factor with nine levels. Year is a continuous variable (1982-2004) coded as 0-22. Poaprate.neighbor is the neighborhood biomass of *P. pratensis* in adjacent plots.

Term	Coefficient	SE	DF	t-value	*P*-value
Intercept	4.065	0.671	1,161	6.055	<0.01
NTrtA-0.00	-0.973	1.038	45	-0.938	0.353
NTrtB-1.02	2.118	1.016	45	2.085	0.043
NTrtC-2.04	1.340	1.043	45	1.285	0.205
NTrtD-3.40	2.796	1.190	45	2.349	0.023
NTrtE-5.44	3.659	1.144	45	3.198	<0.01
NTrtF-9.52	2.041	1.435	45	1.423	0.162
NTrtG-17.0	3.282	1.852	45	1.772	0.083
NTrtH-27.2	0.663	1.617	45	0.410	0.684
Year	0.064	0.042	1,161	1.518	0.129
Poaprate.neighbor	0.006	0.005	1,161	1.137	0.256
NTrtA-0.00*Year	0.125	0.069	1,161	1.801	0.072
NTrtB-1.02*Year	-0.050	0.064	1,161	-0.791	0.429
NTrtC-2.04*Year	0.001	0.068	1,161	0.013	0.989
NTrtD-3.40*Year	-0.163	0.079	1,161	-2.048	0.041
NTrtE-5.44*Year	-0.288	0.080	1,161	-3.607	<0.01
NTrtF-9.52*Year	-0.183	0.102	1,161	-1.797	0.073
NTrtG-17.0*Year	-0.414	0.145	1,161	-2.850	<0.01
NTrtH-27.2*Year	-0.204	0.117	1,161	-1.747	0.081
NTrtA-0.00*Poaprate.neighbor	0.013	0.009	1,161	1.485	0.138
NTrtB-1.02*Poaprate.neighbor	0.001	0.008	1,161	0.113	0.91
NTrtC-2.04*Poaprate.neighbor	0.019	0.008	1,161	2.323	0.02
NTrtD-3.40*Poaprate.neighbor	0.010	0.009	1,161	1.118	0.264
NTrtE-5.44*Poaprate.neighbor	0.028	0.009	1,161	2.962	<0.01
NTrtF-9.52*Poaprate.neighbor	0.070	0.012	1,161	5.645	<0.01
NTrtG-17.0*Poaprate.neighbor	0.041	0.019	1,161	2.199	0.028
NTrtH-27.2*Poaprate.neighbor	0.044	0.013	1,161	3.424	<0.01
Year*Poaprate.neighbor	0.001	0.001	1,161	2.740	<0.01
NTrtA-0.00*Year*Poaprate.neighbor	-0.001	0.001	1,161	-1.609	0.108
NTrtB-1.02*Year*Poaprate.neighbor	0.000	0.001	1,161	0.192	0.848
NTrtC-2.04*Year*Poaprate.neighbor	-0.001	0.001	1,161	-1.317	0.188
NTrtD-3.40*Year*Poaprate.neighbor	0.001	0.001	1,161	0.805	0.421
NTrtE-5.44*Year*Poaprate.neighbor	0.001	0.001	1,161	0.674	0.5
NTrtF-9.52*Year*Poaprate.neighbor	-0.003	0.001	1,161	-2.815	<0.01
NTrtG-17.0*Year*Poaprate.neighbor	0.000	0.002	1,161	0.115	0.908
NTrtH-27.2*Year*Poaprate.neighbor	-0.001	0.001	1,161	-1.016	0.31

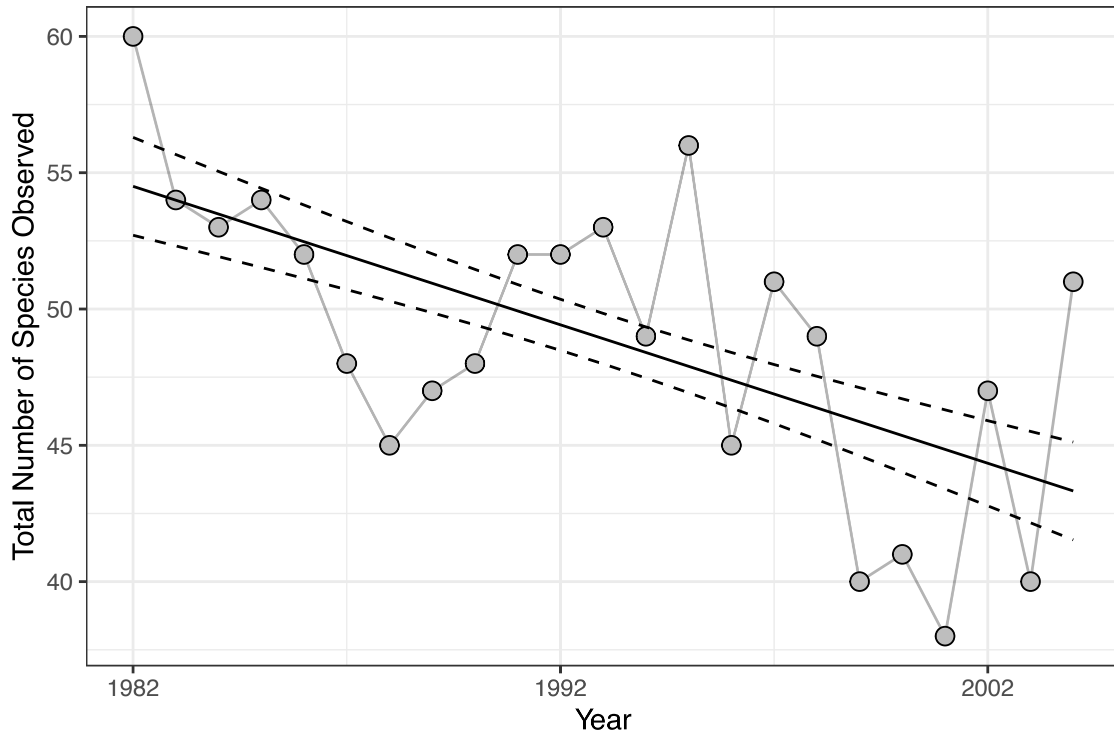
Supplemental Figures



Supplemental Figure S1: The experimental design. Each tile represents one plot colored with its nutrient addition treatment. The control recieves no nutrients and the rest recieve added N + all other nutrients from 0 - 27.2 g m^{-2} N $year^{-1}$. Each number represents the assigned plot number. Arrows denote the length in meters of each aspect of the experimental design. Each plot is 4 m x 4 m with a 1 m buffer.



Supplemental Fig. S2: Abundance of dominant plant species across the nutrient addition treatments. Each point represents the mean abundance (g m^{-2}) ± 1 standard error for each species for years 1995-2004. The last 10 years is shown to approximate the equilibrium conditions of each species. *Elymus repens* = green; *Poa pratensis* = orange; *Schizachyrium scoparium* = purple. Each mean represents 6 plots. n = 54 plots across 10 years.



Supplemental Figure S3: Trend in the total number of species at the field level (gamma diversity) through time (1982-2004). Each point represents the total number of unique species observed across all plots in each year. The fitted line represents a linear regression ± 1 standard error.

Appendix for Chapter 2

Plant biodiversity and the regeneration of soil fertility

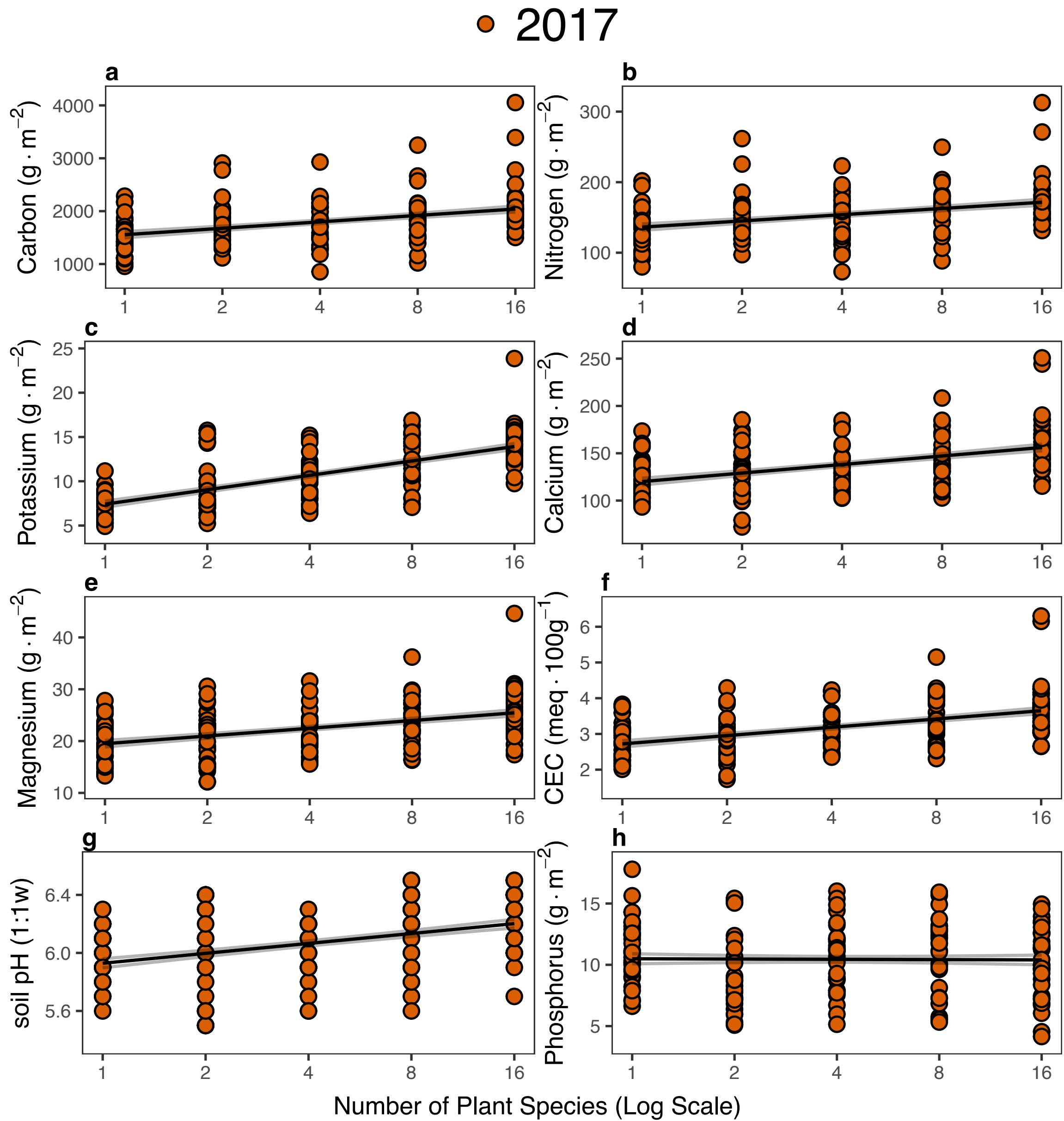


Figure S1: Soil chemistry (area density g m^{-2}) of each plot vs. plant diversity in 2017, the 23rd year of the experiment. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable Bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions ± 1 S.E. (n = 154 plots).

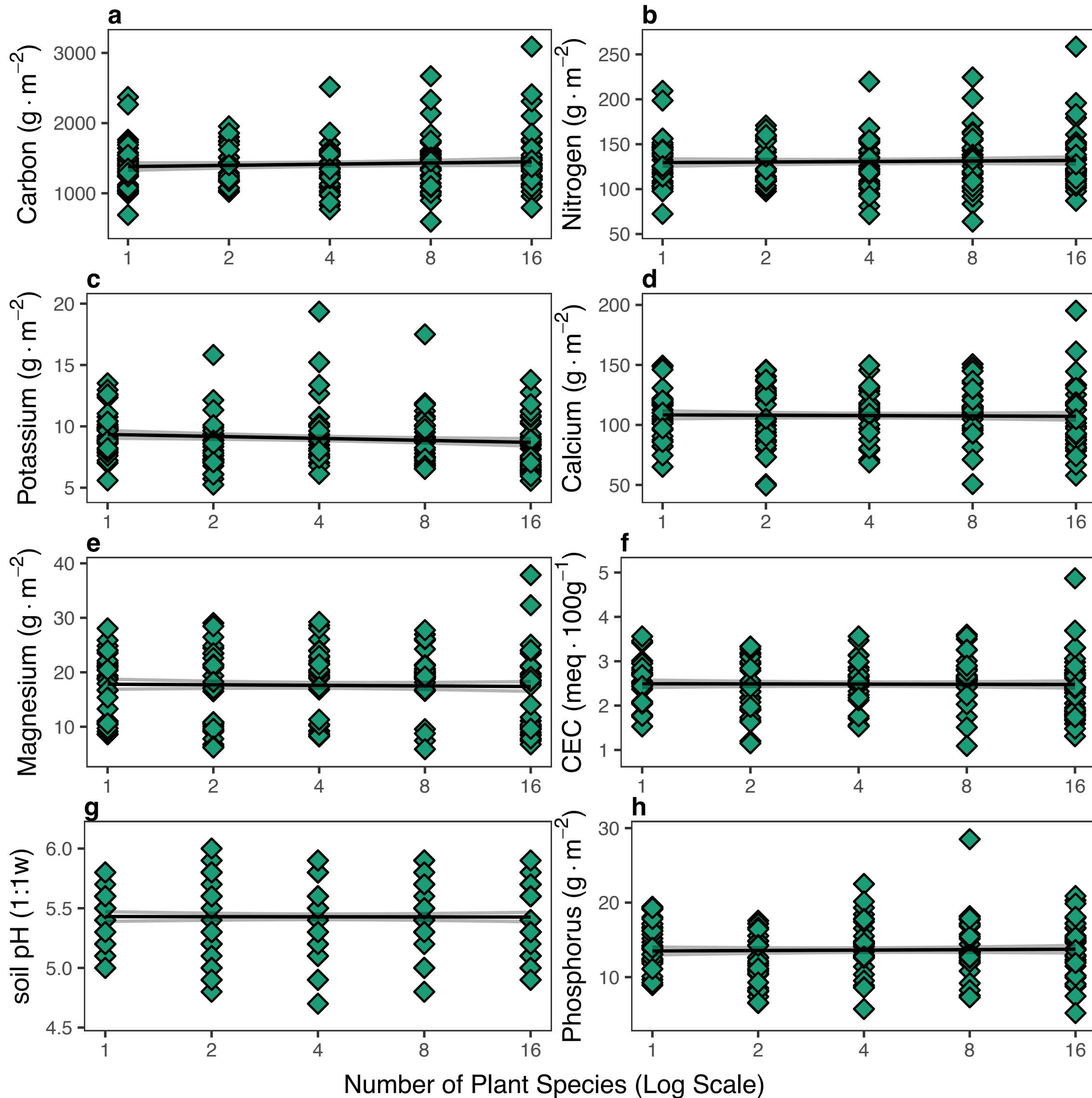


Figure S2: Soil chemistry (area density g m^{-2}) of each plot vs. plant diversity before planting in 1994. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable Bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions ± 1 S.E. (n = 154 plots).

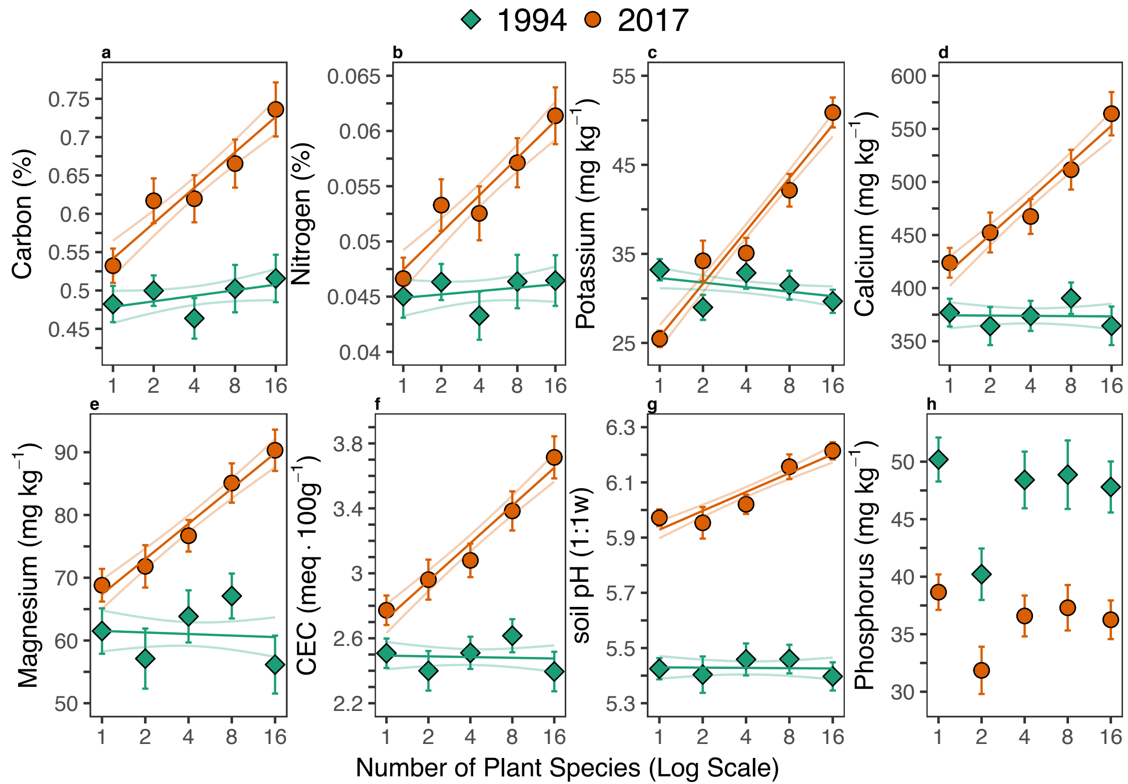


Figure S3: Mean soil chemistry (concentration) vs. plant diversity. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; before planting in 1994 in green (diamond) and in 2017 in orange (circle) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity **g** soil pH and **h** extractable bray phosphorus versus number of planted species (1, 2, 4, 8, or 16; log scale). Lines are linear regressions ± 1 S.E. (n = 154 plots).

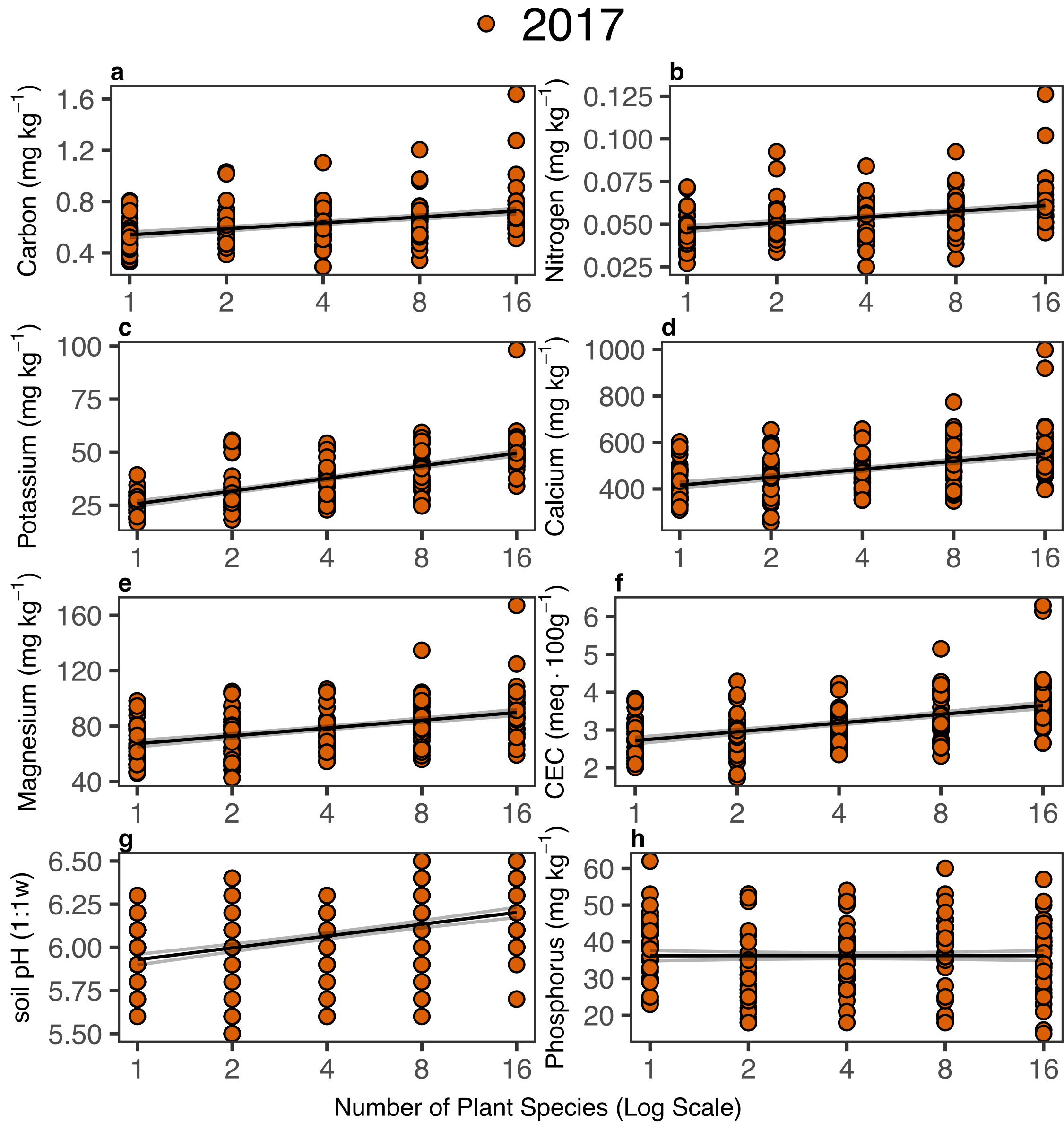


Figure S4: Soil chemistry (concentration) of each plot vs. plant diversity in 2017, the 23rd year of the experiment. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions ± 1 S.E. (n = 154 plots).

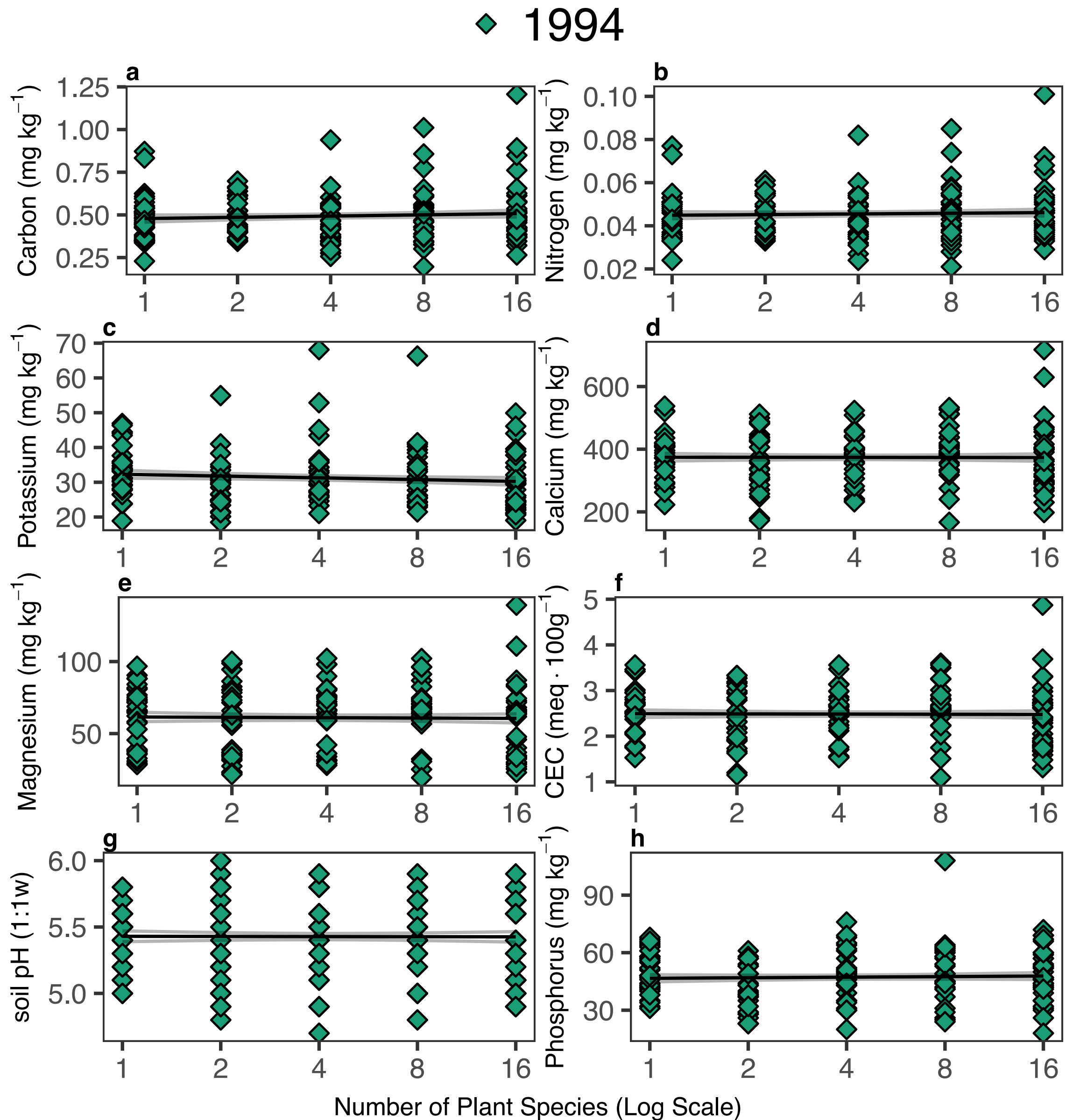


Figure S5: Soil chemistry (concentration) of each plot vs. plant diversity before planting in 1994. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions ± 1 S.E. (n = 154 plots).

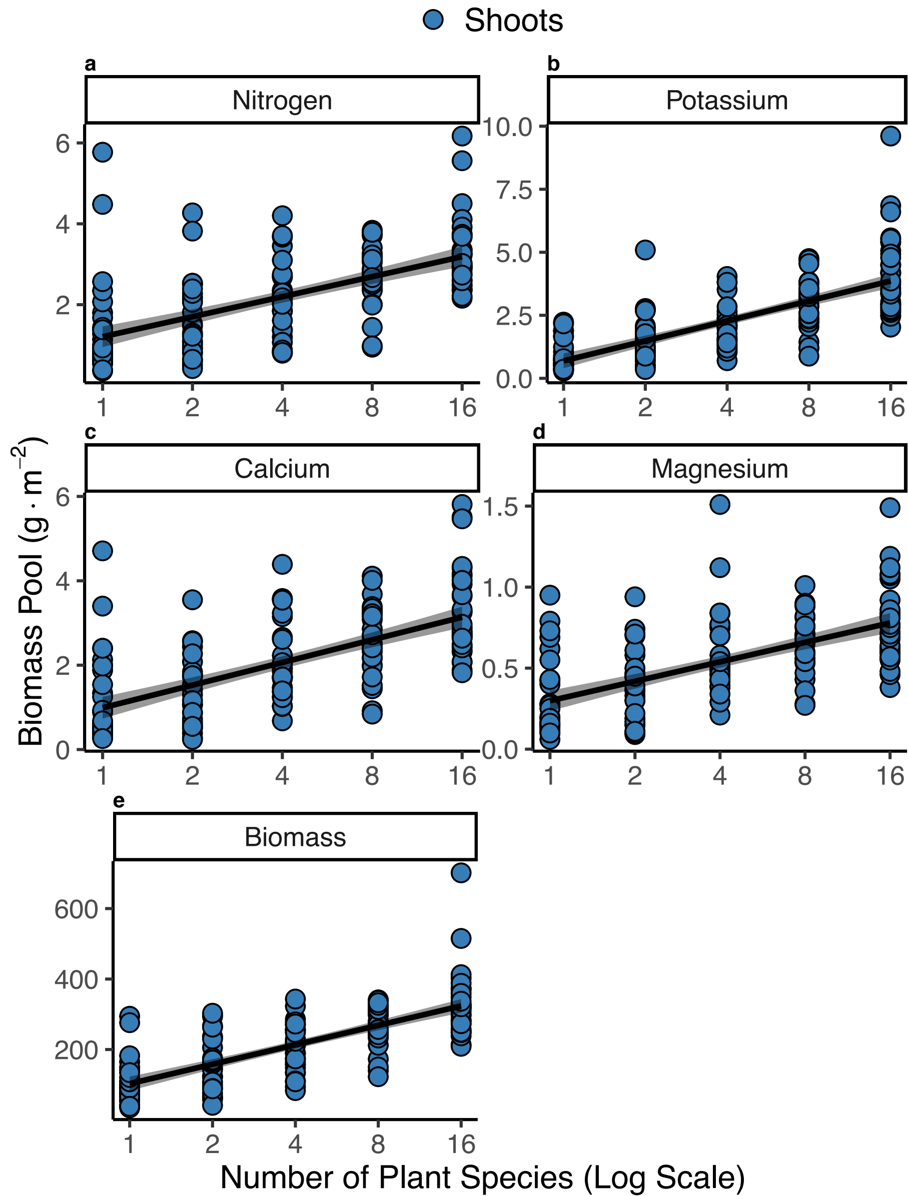


Figure S6: **a-d** Tissue nutrient content in aboveground biomass of each plot (concentration of element * biomass g m^{-2}) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** potassium, **c** calcium and **d** magnesium contained in aboveground plant biomass of each plot (blue; circle), showing the total mass of each element in biomass measured in 2017 (g m^{-2}). **e** Total aboveground dry plant biomass in each plot (g m^{-2}) versus plant diversity. Regression lines show dependence of each variable on the natural log of the number of species ± 1 S.E. (n = 154).

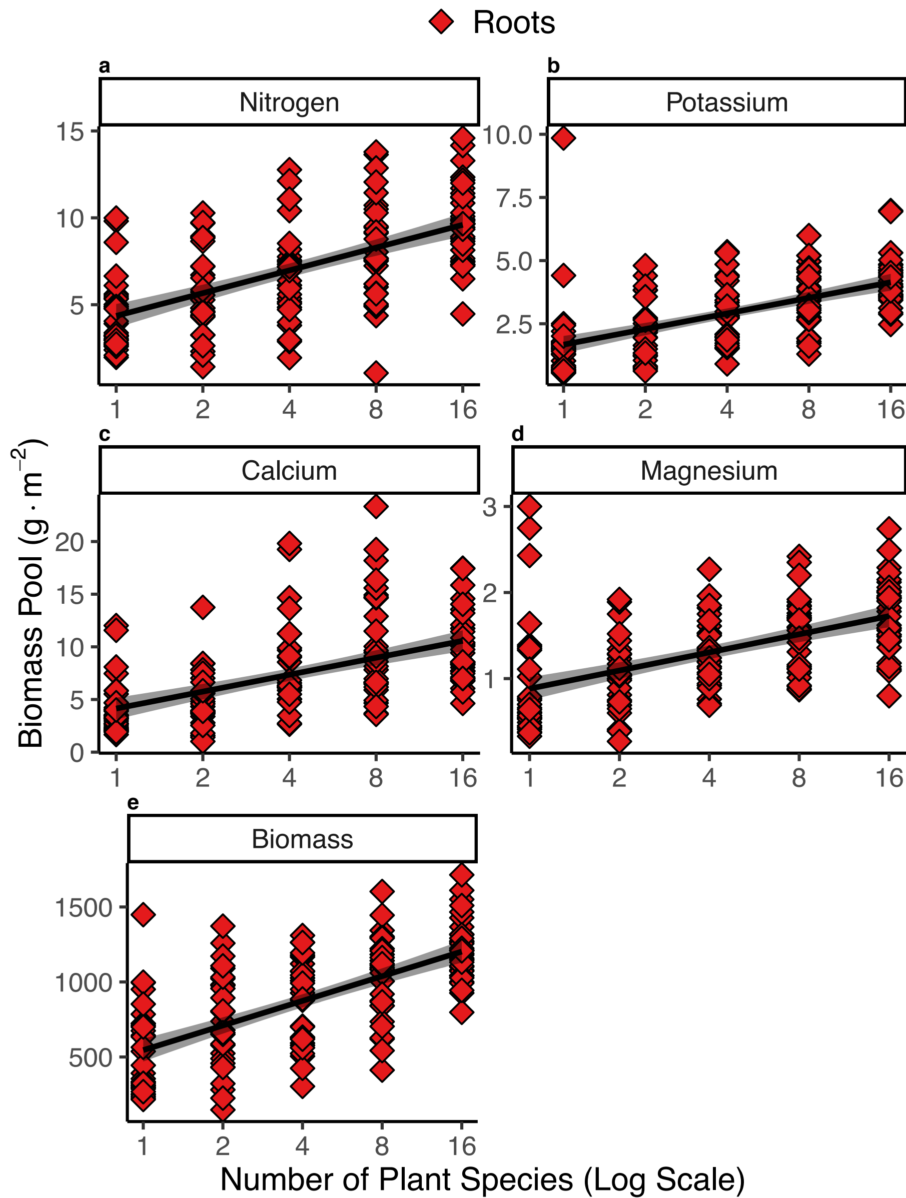


Figure S7: **a-d** Tissue nutrient content in belowground biomass of each plot (concentration of element * biomass g m^{-2}) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** potassium, **c** calcium and **d** magnesium contained in belowground plant biomass of each plot (red; diamond), showing the total mass of each element in biomass (g m^{-2}). **e** Total belowground dry plant biomass in each plot (g m^{-2}) versus plant diversity. Regression lines show dependence of each variable on the natural log of the number of species ± 1 S.E. (n = 154).

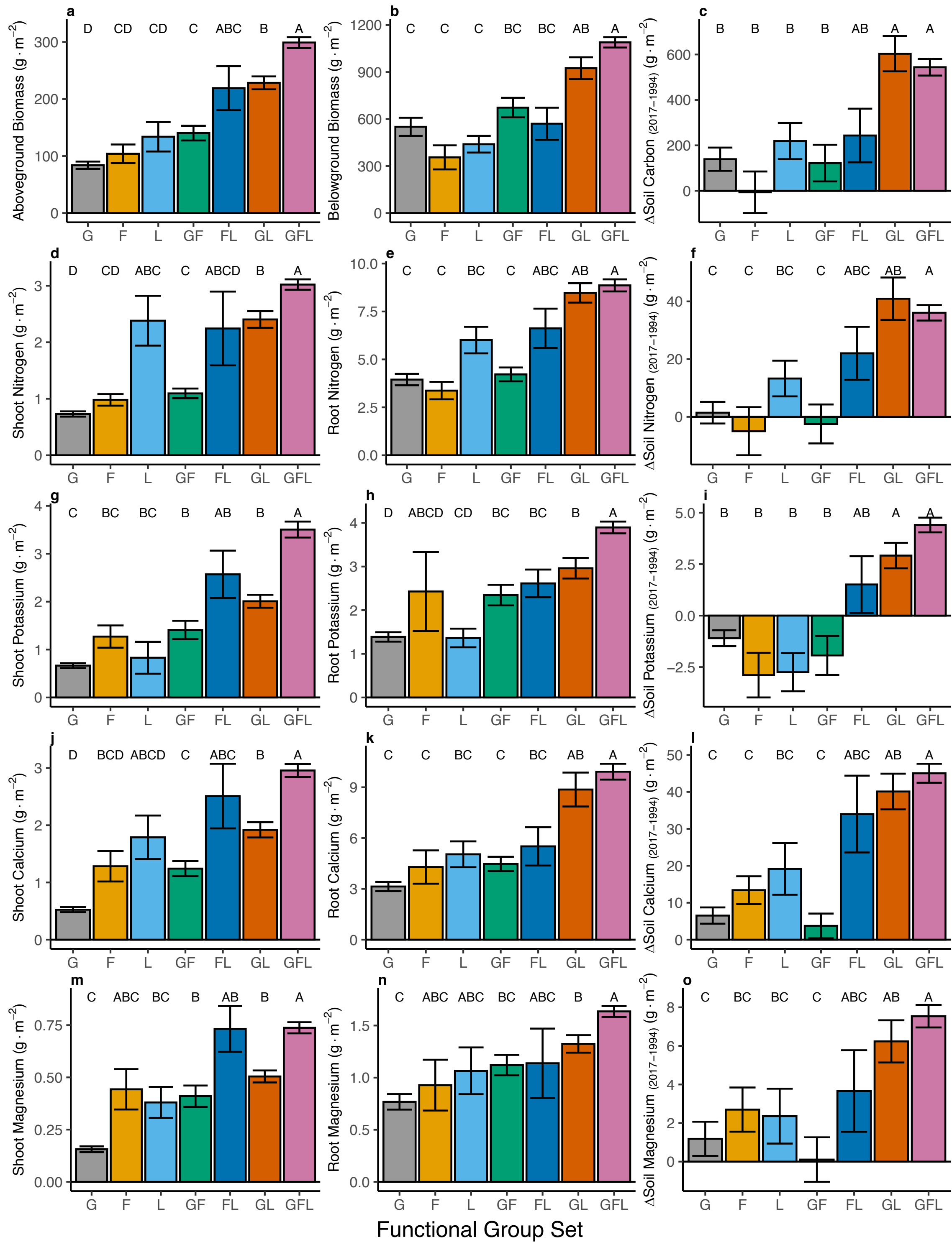


Figure S8: Biomass, nutrient pools and changes in soil nutrient pools from 1994 to 2017 by functional group composition. These panels display the mean ± 1 S.E. for each functional group composition for aboveground and belowground biomass (2017, g m^{-2};

roots 0-30 cm), the quantity of nitrogen, potassium, calcium, and magnesium in those plant tissues (2017, g m^{-2}), and the change in soil carbon, nitrogen, potassium, calcium and magnesium (g m^{-2}, 0-20 cm, 2017 - 1994). **a** aboveground biomass**, b** belowground biomass (0-30 cm), **c** change in soil carbon, **d** quantity of nitrogen in aboveground biomass, **e** quantity of nitrogen in belowground biomass, **f** change in soil nitrogen, **g** quantity of potassium in aboveground biomass, **h** quantity of potassium in root biomass, **i** change in soil potassium, **j** quantity of calcium in aboveground biomass, **k** quantity of calcium in belowground biomass, **l** change in soil calcium, **m** quantity of magnesium in aboveground biomass, **n** quantity of magnesium in belowground biomass, **o** change in soil magnesium. Functional group compositions: G = grasses only, n = 22; F = forb only, n =10; L = legumes only, n = 11; FL = at least 1 forb and 1 legume, n = 5; GL = at least 1 grass and 1 legume, n = 23; GF = at least 1 grass and 1 forb, n = 14; GFL = at least 1 grass, 1 legume and 1 forb, n = 69. Letters indicate if means differ ($P<0.05$) following a Tukey correction.

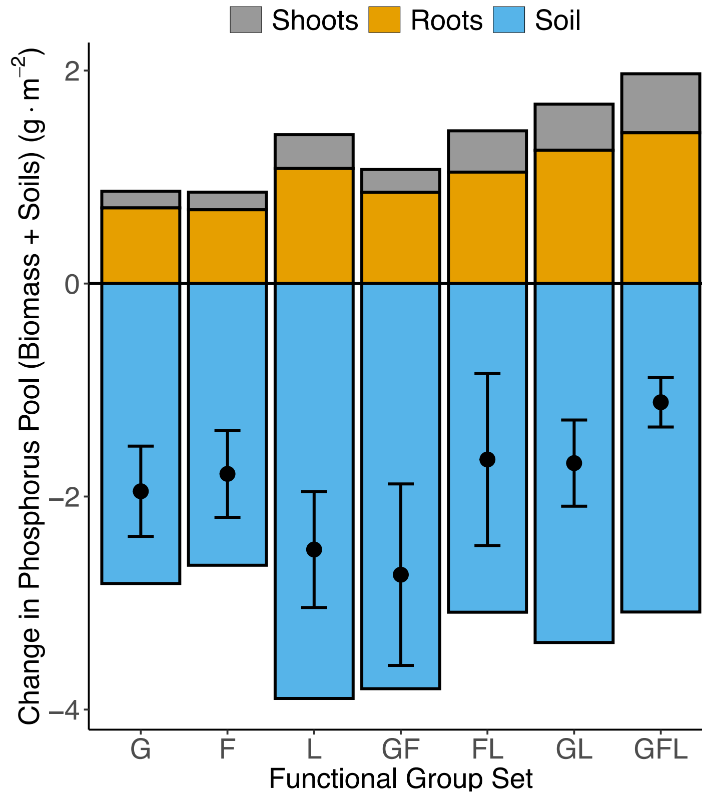


Figure S9: Change in ecosystem nutrient pools for each functional group composition for phosphorus. Pools defined as change from 1994 to 2017 in soil levels (0-20 cm depth increment) plus amounts in aboveground biomass and in roots (0-30 cm) in 2017; sum expressed as g of nutrient m^{-2}. Each point shows the mean ± 1 SE. Bars show the relative value for phosphorus in aboveground biomass (grey), phosphorus in belowground biomass (yellow) and soil (blue). Functional group compositions: G = grasses only n = 22; F = forb only n =10; L = legumes only n = 11; FL = at least 1 forb and 1 legume n= 5; GL = at least 1 grass and 1 legume n = 23; GF = at least 1 grass and 1 forb n = 14; GFL = at least 1 grass, 1 legume and 1 forb n = 69. Means did not differ (all $P > 0.05$).

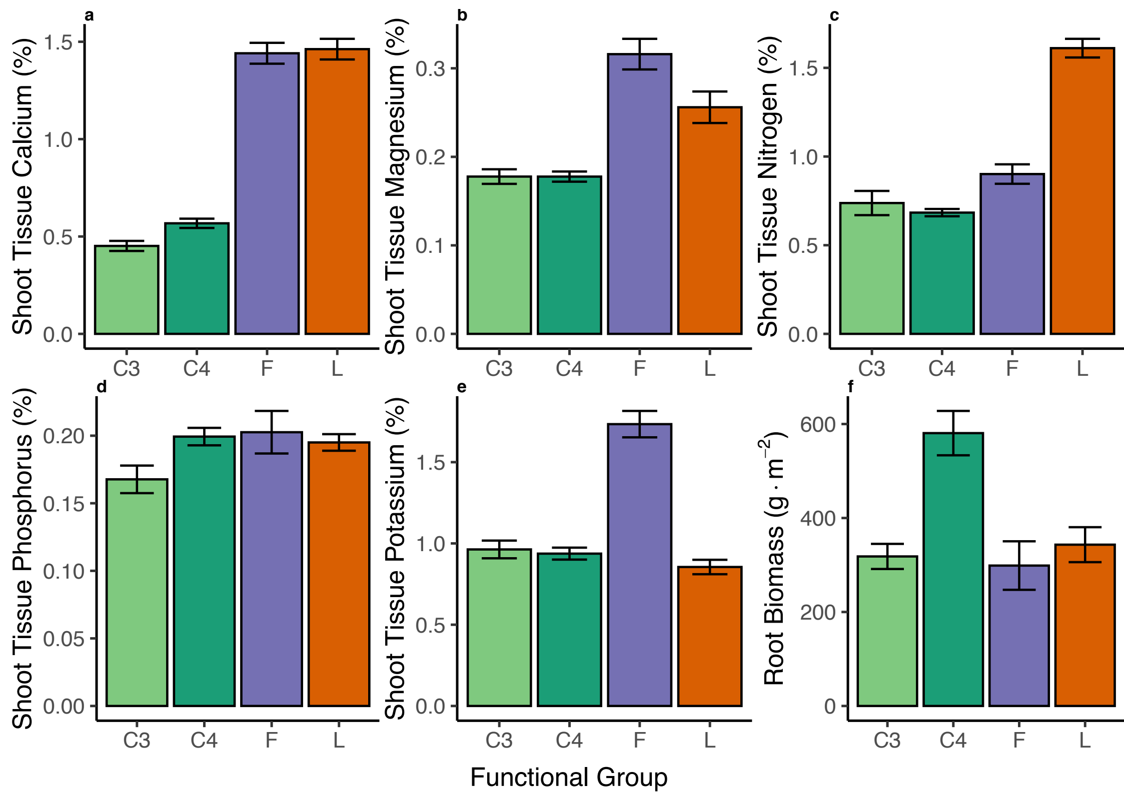


Figure S10: Tissue trait values and root mass by functional group composition. Each bar represents the mean ± 1 SE of each trait for each functional group type. Functional group composition is defined as C4 grass (4 species), C3 grass (2 species), forb (5 species) and legume (4 species) (See Table S7). Shoot chemistry represents the whole plant percentage of each element from samples taken from monocultures and 16-species plots (C3 n = 13; C4 n = 30; forb n = 35; legume n = 30). Root biomass represents the measured values from monoculture plots to a depth of 30 cm (C3 n = 3; C4 n = 10; forb n = 9; legume n = 10).

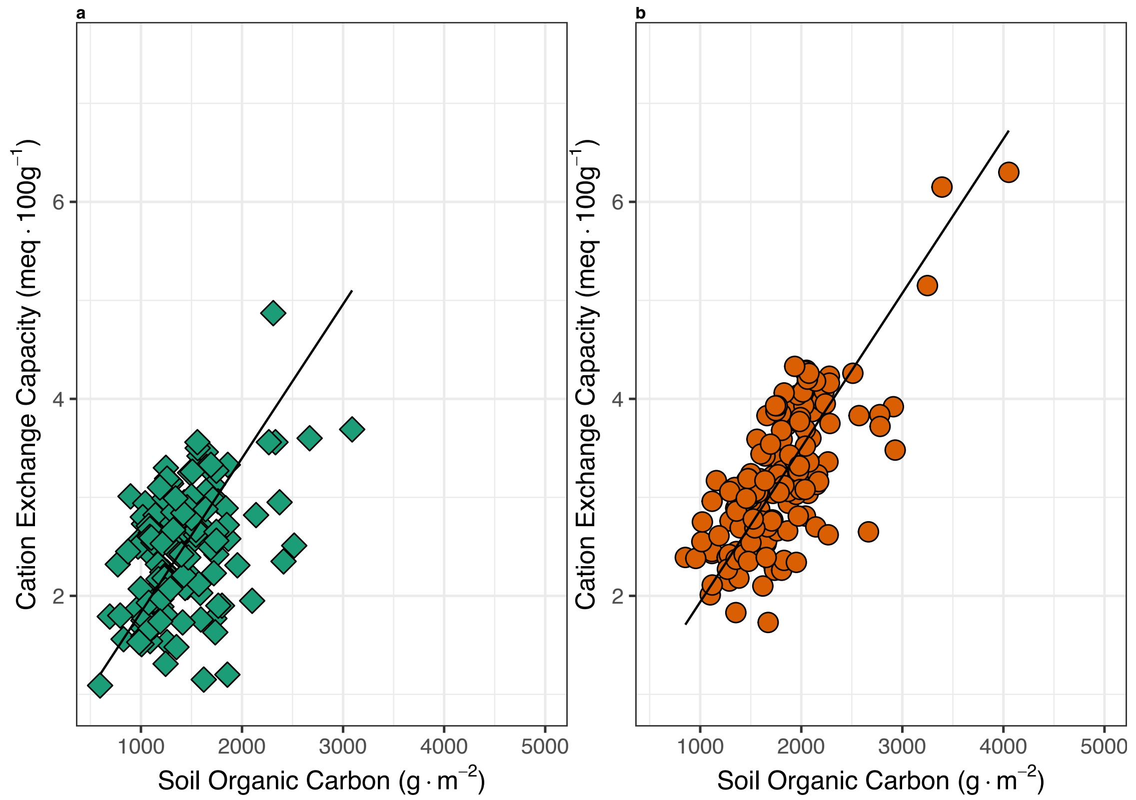


Figure S11: Bivariate relationship between soil organic carbon and soil cation exchange capacity. Baseline values from 1994 **a** are shown as green diamonds and values from 2017 **b** are shown as orange circles (n=154). The black line represents a fit from a major axis regression (1994: R^2=0.20, p<0.001, 2017: R^2=0.52 p<0.001)

Supplemental Tables.

Table S1: Soil characteristics measured in 1994 and classification of relative levels based on guidelines of the University of Minnesota Agricultural Extension Service

Nutrient	Test	Value	Relative Levels
Organic matter	Loss on Ignition (400 C)	1.04%	Very Low
Total nitrogen	Combustion	0.046%	Very Low
Available phosphorus	Bray-1 P	47 (mg/kg)	Very High
Available potassium	Ammonium Acetate pH 7	31 (mg/kg)	Very Low

Table S2: Linear regressions testing the dependence of each soil variable (area density g m^{-2}; except for CEC and pH) on the natural log of the number of planted species. A separate regression is shown for each variable in 1994 (pre-treatment) and in 2017 (n=154). Note that all 1994 regressions P values have $P>0.25$, and all 2017 regressions except for phosphorus have $P<0.0001$.

Soil Variable	Year	F-Statistic [1,152]	R^2	P-value
Calcium	1994	0.051	0.00	0.82111
Carbon	1994	0.646	0.00	0.42268
CEC	1994	0.017	0.00	0.89523
Magnesium	1994	0.079	0.00	0.77967
Nitrogen	1994	0.126	0.00	0.72318
pH	1994	0.004	0.00	0.95127
Phosphorus	1994	0.077	0.00	0.78137
Potassium	1994	1.666	0.01	0.19873
Calcium	2017	40.496	0.21	<0.0001
Carbon	2017	26.690	0.15	<0.0001
CEC	2017	43.372	0.22	<0.0001
Magnesium	2017	35.440	0.19	<0.0001
Nitrogen	2017	23.999	0.14	<0.0001
pH	2017	30.938	0.17	<0.0001
Phosphorus	2017	0.020	0.00	0.88636
Potassium	2017	136.373	0.47	<0.0001

Table S3: Linear regressions testing the dependence of each soil variable (concentration mg kg^{-1}) on the natural log of the number of plant species. A separate regression is shown for each variable in 1994 (pre-treatment) and in 2017 (n=154). Note that all 1994 regressions P values have *P*>0.25, and all 2017 regressions except for phosphorus have *P*<0.0001.

Soil Variable	Year	F-Statistic [1,152]	R^2	P-value
Calcium	1994	0.003	0.00	0.95768
Carbon	1994	0.760	0.00	0.38484
Magnesium	1994	0.036	0.00	0.85036
Nitrogen	1994	0.220	0.00	0.64002
Phosphorus	1994	0.156	0.00	0.69372
Potassium	1994	1.266	0.01	0.26229
Calcium	2017	38.836	0.20	<0.0001
Carbon	2017	24.691	0.14	<0.0001
Magnesium	2017	37.007	0.20	<0.0001
Nitrogen	2017	22.574	0.13	<0.0001
Phosphorus	2017	0.000	0.00	0.99463
Potassium	2017	126.390	0.45	<0.0001

Table S4: Linear regressions testing the dependence on $\log_e$(number of plant species) of the 2017 % change relative to the 2017 monoculture mean for aboveground (shoots) and belowground (roots) biomass (0-30 cm) and the quantity of nitrogen, calcium, magnesium, and potassium within those tissues. See Figure 2 for graphs of regressions for all variables except biomass. A separate regression is shown for each variable (n=154).

Nutrient	Shoots|Roots	F-Statistic [1,152]	R^2	P-value
Biomass	Shoots	185.605	0.55	<0.0001
Calcium	Shoots	107.760	0.41	<0.0001
Magnesium	Shoots	89.289	0.37	<0.0001
Nitrogen	Shoots	92.032	0.38	<0.0001
Potassium	Shoots	180.298	0.54	<0.0001
Biomass	Roots	114.936	0.43	<0.0001
Calcium	Roots	60.450	0.28	<0.0001
Magnesium	Roots	61.385	0.29	<0.0001
Nitrogen	Roots	90.581	0.37	<0.0001
Potassium	Roots	79.612	0.34	<0.0001

Table S5: Ecosystem nutrient pools by functional group composition. Pools defined as change from 1994 to 2017 in soil levels (0-20 cm depth increment) plus amounts in aboveground biomass and in roots (0-30 cm) in 2017; sum expressed as g of nutrient m^{-2}. Functional group compositions: G = grasses only n = 22; F = forb only n =10; L = legumes only n = 11; FL = at least 1 forb and 1 legume n = 5; GL = at least 1 grass and 1 legume n = 23; GF = at least 1 grass and 1 forb n = 14; GFL = at least 1 grass, 1 legume and 1 forb n = 69. Group letters indicate if means differ ($P<0.05$) following a Tukey correction.

Nutrient	Functional Group	Mean	SE	degrees of freedom	Lower confidence interval (95%)	Upper confidence interval (95%)	Group
Calcium	G	10.19	2.19	21	5.64	14.74	C
Calcium	F	18.98	4.24	9	9.39	28.57	C
Calcium	L	26.02	6.53	12	11.83	40.20	BC
Calcium	GF	9.44	3.63	13	1.61	17.28	C
Calcium	FL	42.02	9.69	12	20.98	63.06	ABC
Calcium	GL	50.90	4.97	22	40.57	61.22	AB
Calcium	GFL	57.93	2.66	65	52.61	63.24	A
Magnesium	G	2.11	0.89	21	0.24	3.97	C
Magnesium	F	4.07	1.21	9	1.32	6.82	BC
Magnesium	L	3.80	1.38	9	0.69	6.91	BC
Magnesium	GF	1.64	1.16	14	-0.85	4.12	C
Magnesium	FL	5.53	2.04	9	0.92	10.14	ABC
Magnesium	GL	8.06	1.11	22	5.76	10.36	AB
Magnesium	GFL	9.91	0.59	68	8.73	11.09	A
Nitrogen	G	6.10	3.77	21	-1.75	13.95	B
Nitrogen	F	-0.63	8.44	9	-19.76	18.49	B
Nitrogen	L	21.67	6.01	15	8.86	34.48	B
Nitrogen	GF	2.85	6.77	13	-11.78	17.47	B
Nitrogen	FL	30.88	8.92	15	11.88	49.87	AB
Nitrogen	GL	51.80	7.38	22	36.50	67.11	A
Nitrogen	GFL	47.93	2.72	68	42.50	53.35	A
Phosphorus	G	-1.95	0.42	21	-2.83	-1.07	A
Phosphorus	F	-1.79	0.41	9	-2.71	-0.86	A
Phosphorus	L	-2.50	0.54	16	-3.65	-1.34	A
Phosphorus	GF	-2.73	0.85	13	-4.57	-0.89	A
Phosphorus	FL	-1.65	0.81	16	-3.37	0.06	A
Phosphorus	GL	-1.69	0.40	22	-2.52	-0.85	A
Phosphorus	GFL	-1.11	0.23	68	-1.58	-0.65	A
Potassium	G	0.96	0.47	21	-0.03	1.94	C
Potassium	F	0.81	1.65	9	-2.93	4.54	C
Potassium	L	-0.55	1.31	12	-3.40	2.30	C
Potassium	GF	1.82	0.87	13	-0.06	3.70	C
Potassium	FL	6.70	1.94	12	2.47	10.92	ABC
Potassium	GL	7.89	0.85	21	6.11	9.66	B
Potassium	GFL	11.80	0.52	66	10.76	12.85	A

Table S6: Summary table displaying model-averaged coefficients for linear regressions testing the dependency of the sum of aboveground plus belowground biomass (Root 0-30 cm) (2015 and 2017) on the natural log of the number of planted species and on soil variables (total N, total C, Bray-P, exchangeable Ca, Mg, K and soil pH). The conditional average is presented for each coefficient.

Nutrient	Coefficient	Std. Error	Adjusted SE	z value	Pr(>|z|)
K	51.254	8.790	8.860	5.785	<0.001
logNumSp	156.232	25.602	25.810	6.053	<0.001
C	0.227	0.050	0.050	4.536	<0.001
N	2.871	0.667	0.672	4.273	<0.001
P	-7.980	6.400	6.453	1.237	0.216
Mg	-4.929	4.612	4.649	1.060	0.289
(Intercept)	-65.922	91.138	91.759	0.718	0.472

Table S7: List of the fifteen herbaceous perennial plant species that persisted in monocultures and mixtures. For each species, its functional group and plant family is shown. Each species is represented by one point in Figure 4.

Species	Functional Group	Family
Achillea millefolium	Forb	Asteraceae
Amorpha canescens	Legume	Fabaceae
Andropogon gerardii	C4 Grass	Poaceae
Asclepias tuberosa	Forb	Apocynaceae
Koeleria macrantha	C3 Grass	Poaceae
Lespedeza capitata	Legume	Fabaceae
Liatris aspera	Forb	Asteraceae
Lupinus perennis	Legume	Fabaceae
Monarda fistulosa	Forb	Lamiaceae
Panicum virgatum	C4 Grass	Poaceae
Dalea purpureum	Legume	Fabaceae
Poa pratensis	C3 Grass	Poaceae
Schizachyrium scoparium	C4 Grass	Poaceae
Solidago rigida	Forb	Asteraceae
Sorghastrum nutans	C4 Grass	Poaceae

Supplemental discussion of empirical tradeoff surface shown in Fig. 4.

We conducted additional analyses to test the robustness of the tradeoff surface shown in Fig 4. Because of poor establishment or poor survival in monoculture or the presence of a second species in a monoculture, there were difficulties in accurately determining root mass for *Poa pratensis* and *Monarda fistulosa.* We therefore tested two subsets of the available data and found that all had a fitted planar surface defining tradeoffs just like those of Fig. 4. Removing monoculture data for *P. pratensis,* which survived in only one monoculture and was rare in it, improved the fit ($F_{2,11} = 11.3$, $R^2 = 0.67$, $p = 0.0021$). Removing both *M. fistulosa* and *P. pratensis* gave a similar fit ($F_{2,10} = 10.28$, $R^2 = 0.67$, $p = 0.0038$).

To better estimate aboveground tissue chemistry for each species, Fig. 4 uses the species-specific average of tissue chemistry measurements in monoculture and in five 16-species plots. When instead we use only species-specific chemistry measured values from monoculture plots, the resulting tradeoff surface was similar, with $F_{2,30} = 8.26$, $R^2 = 0.36$, $p = 0.00139$. Removing *M. fistulosa* and *P. pratensis* increased the fit to $F_{2,27} = 13.87$, $R^2 = 0.51$, $p < 0.0001$.

Area density calculations

Formula to calculate the amount in $\frac{\mathrm{g}}{\mathrm{m}^2}$ of a nutrient in a soil core of length T.

where:
x = element in soil

C = concentration of element x ($\frac{\mathrm{mg}_x}{\mathrm{Kg}_{\mathrm{soil}}}$)

ρ = bulk density ($\frac{\mathrm{g}_{\mathrm{soil}}}{\mathrm{cm}^3}$)

T = depth or thickness of soil layer (cm)

$$\text{Area density quantity of nutrient } x_{\frac{\mathrm{g}}{\mathrm{m}^2}}$$
$$= C_{\frac{\mathrm{mg}_x}{\mathrm{Kg}_{\mathrm{soil}}}} * \frac{1_{g_x}}{1000_{\mathrm{mg}_x}} * \rho_{\frac{\mathrm{g}_{\mathrm{soil}}}{\mathrm{cm}^3}} * \frac{1_{\mathrm{Kg}_{\mathrm{soil}}}}{1000_{\mathrm{g}_{\mathrm{soil}}}} * T_{\mathrm{cm}} * \frac{100_{\mathrm{cm}}}{1_{\mathrm{m}}} * \frac{100_{\mathrm{cm}}}{1_{\mathrm{m}}}$$

Formula to determine the mass of soil per m^{-2} of surface area in a block of soil with a thickness of T. Reference (1).

Where:

M_{soil} is the mass of soil

$$M_{\mathrm{soil}} = \rho_{\frac{\mathrm{g}_{\mathrm{soil}}}{\mathrm{cm}^3}} * T_{\mathrm{cm}} * \frac{100_{\mathrm{cm}}}{1_{\mathrm{m}}} * \frac{100_{\mathrm{cm}}}{1_{\mathrm{m}}}$$
$$M_{\mathrm{soil}} = \frac{\mathrm{g}}{\mathrm{m}^2}$$

If a soil is sampled a second time and its bulk density has changed, we must calculate the added or subtracted thickness required to sample the same dry mass of soil.

Formula to calculate added or subtracted thickness:

T_{add} is the amount of extra thickness to add from deeper depths for equivalent mass. If T_{add} is negative, the soil became more dense and it is the amount to subtract to give equivalent mass, called T_{subtract} below.

If the soil bulk density decreased through time i.e. it has expanded and is less dense, then we must add soil mass from the subsoil to give a mass equivalent to the original.

$$T_{\mathrm{add}} = \frac{\left(M_{(\text{0-20cm}),1994}\,\frac{\mathrm{g}}{m^2} - M_{(\text{0-20cm}),2017}\,\frac{\mathrm{g}}{m^2}\right) * \frac{1_{\mathrm{m}}}{100_{\mathrm{cm}}} * \frac{1_{\mathrm{m}}}{100_{\mathrm{cm}}}}{\rho_{(\text{20-40cm}),2017}\,\frac{\mathrm{g}}{\mathrm{cm}^3}}$$
$$T_{\mathrm{add}} = \mathrm{cm}$$

T_{add} is the depth of added soil required to keep the total soil mass the same as the original.

If the soil bulk density increased through time i.e. it has contracted and is now more dense. We must subtract soil mass from the target soil to give equivalent mass.

$$T_{\text{subtract}} = \frac{(M_{(\text{0-20cm}),1994}\frac{\text{g}}{\text{m}^2} - M_{(\text{0-20cm}),2017}\frac{\text{g}}{\text{m}^2}) * \frac{1_{\text{m}}}{100_{\text{cm}}} * \frac{1_{\text{m}}}{100_{\text{cm}}}}{\rho_{(\text{0-20cm}),2017}\frac{\text{g}}{\text{cm}^3}}$$

$$T_{\text{subtract}} = \text{cm}$$

T_{subtract} is the depth of soil removed required to keep the total soil mass the same as the original.

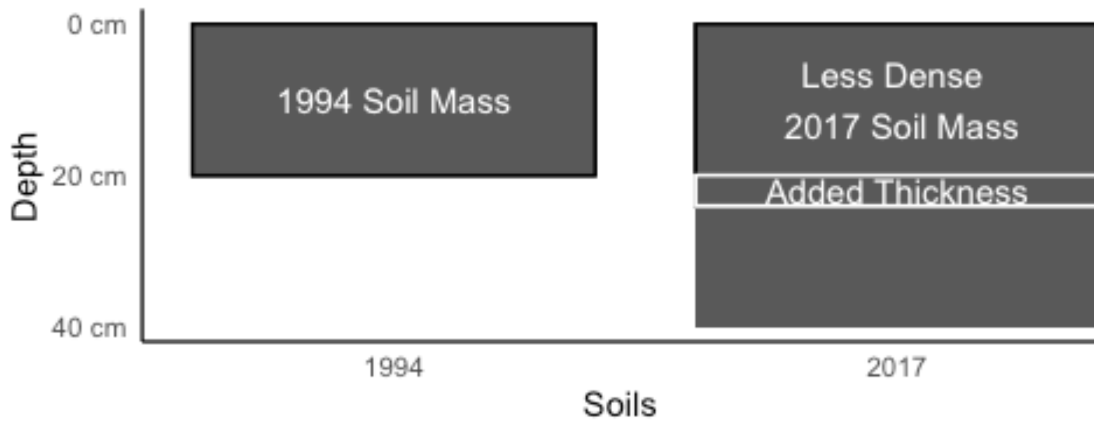
The soil bulk density was estimated in 1994 (0-20 cm) based on a regression of the dependence of soil bulk density in 2018 on % soil C in 2017 (0-20 cm). The regression was used with measured values of % soil C in 1994 (0-20 cm) to estimate soil bulk density in 1994 (0-20 cm). Estimated values in 1994 had a mean of ~ 1.45 $\frac{g}{\text{cm}^3}$ which aligns with 1.4 $\frac{g}{\text{cm}^3}$ from a soil survey of this Nymore series 0-23 cm (2).

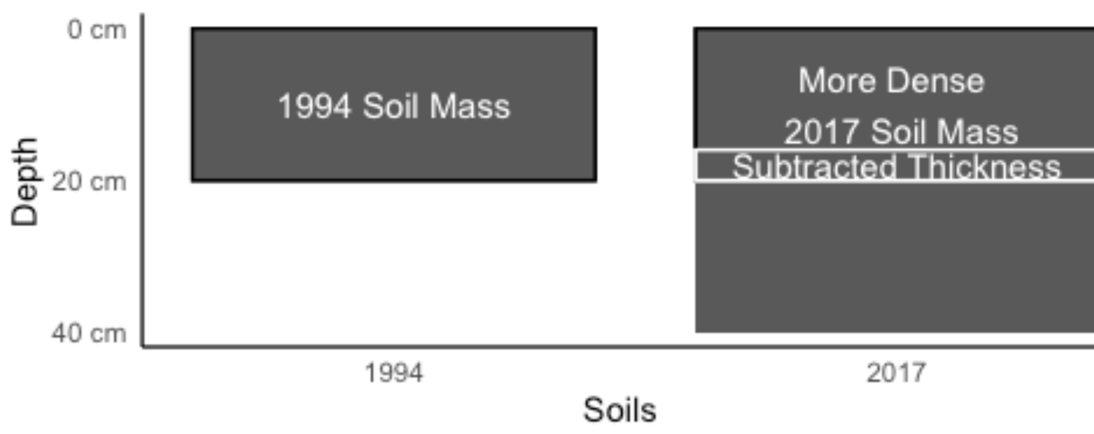
To estimate the area density quantity $\frac{\text{g}}{\text{m}^2}$ of a given soil nutrient (0-20 cm) in 2017 when the soil bulk density has decreased from the value in 1994 (0-20 cm):

$$\text{Area density } x_{\frac{\text{g}}{\text{m}^2}} = [\rho_{\frac{\text{g}_{\text{soil}}(\text{0-20cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{\text{g}_x}{\text{g}_{\text{soil}}}}(\text{0-20cm}) + [\rho_{\frac{\text{g}_{\text{soil}}(\text{20-40cm})}{\text{cm}^3}} * T_{\text{add}_{\text{cm}}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{\text{g}_x}{\text{g}_{\text{soil}}}}(\text{20-40cm})$$

To estimate the area density quantity $\frac{\text{g}}{\text{m}^2}$ of a given soil nutrient (0-20 cm) in 2017 when the soil bulk density has increased from the value in 1994 (0-20 cm):

$$\text{Area density } x_{\frac{\text{g}}{\text{m}^2}} = [\rho_{\frac{\text{g}_{\text{soil}}(\text{0-20cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{\text{g}_x}{\text{g}_{\text{soil}}}}(\text{0-20cm}) + [\rho_{\frac{g_{\text{soil}}(\text{0-20cm})}{\text{cm}^3}} * T_{\text{subtract}_{\text{cm}}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{\text{g}_x}{\text{g}_{\text{soil}}}}(\text{0-20cm})$$

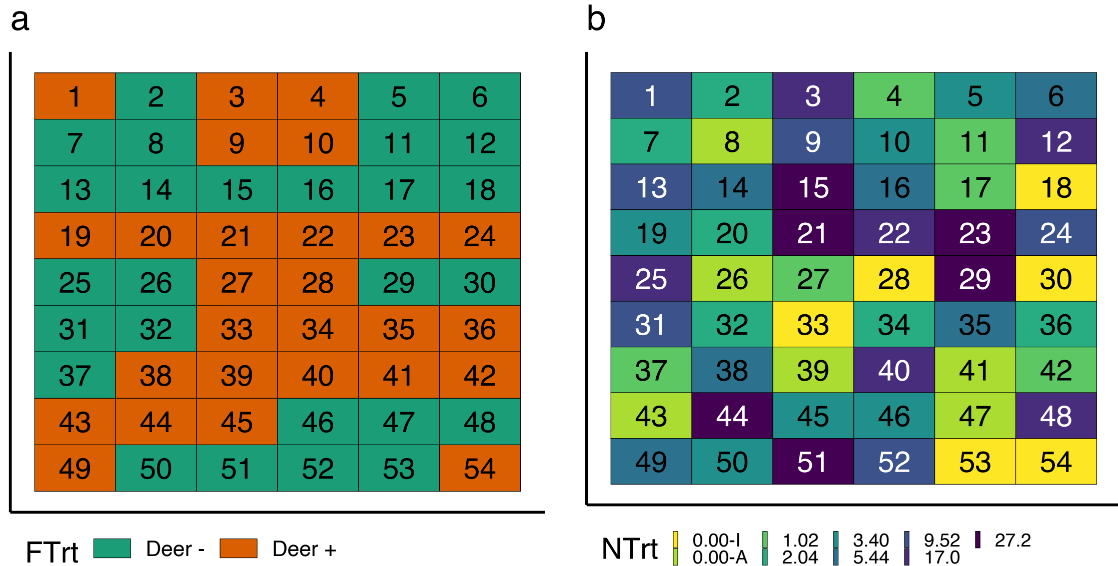

0 cm
20 cm
40 cm
Depth
1994 Soil Mass
Less Dense
2017 Soil Mass
Added Thickness
1994
2017
Soils


0 cm
20 cm
40 cm
Depth
1994 Soil Mass
More Dense
2017 Soil Mass
Subtracted Thickness
1994
2017
Soils

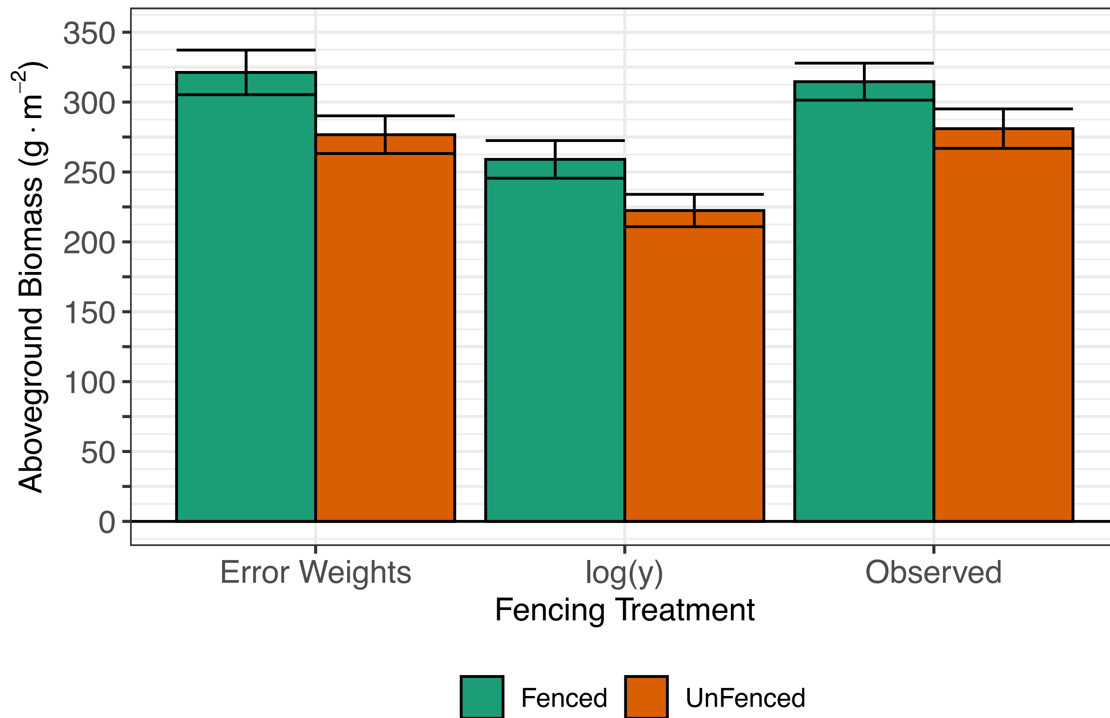
Appendix for Chapter 3

Plant community responses to an herbivore exclosure crossed with resource addition

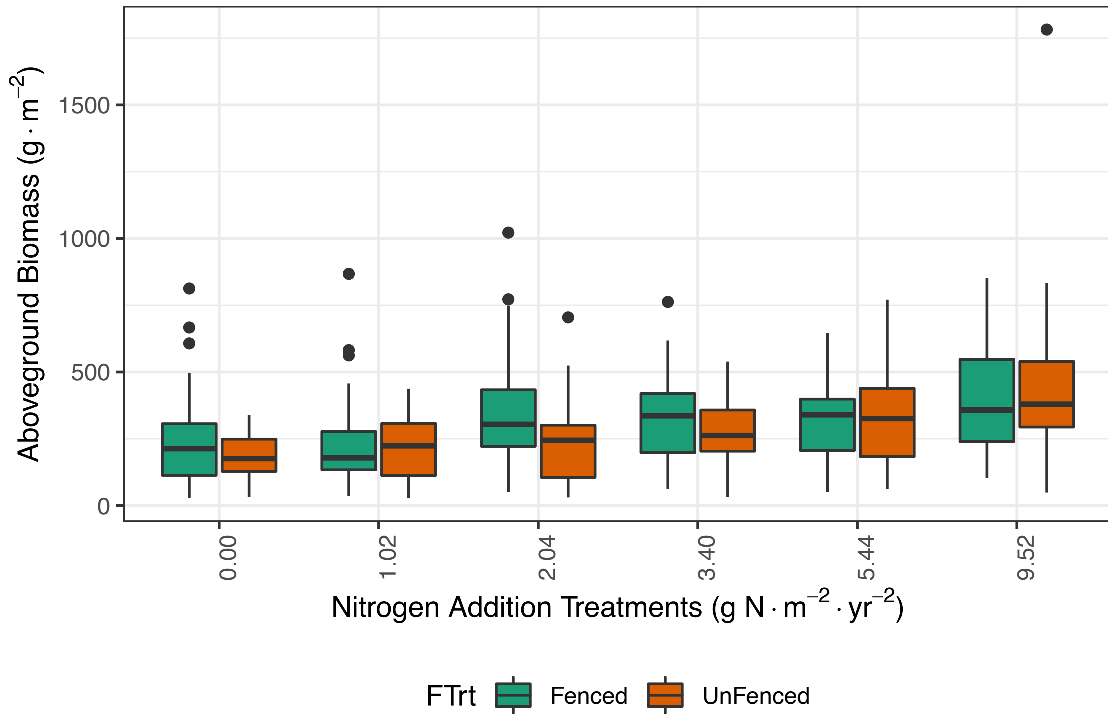
Supplemental Figures



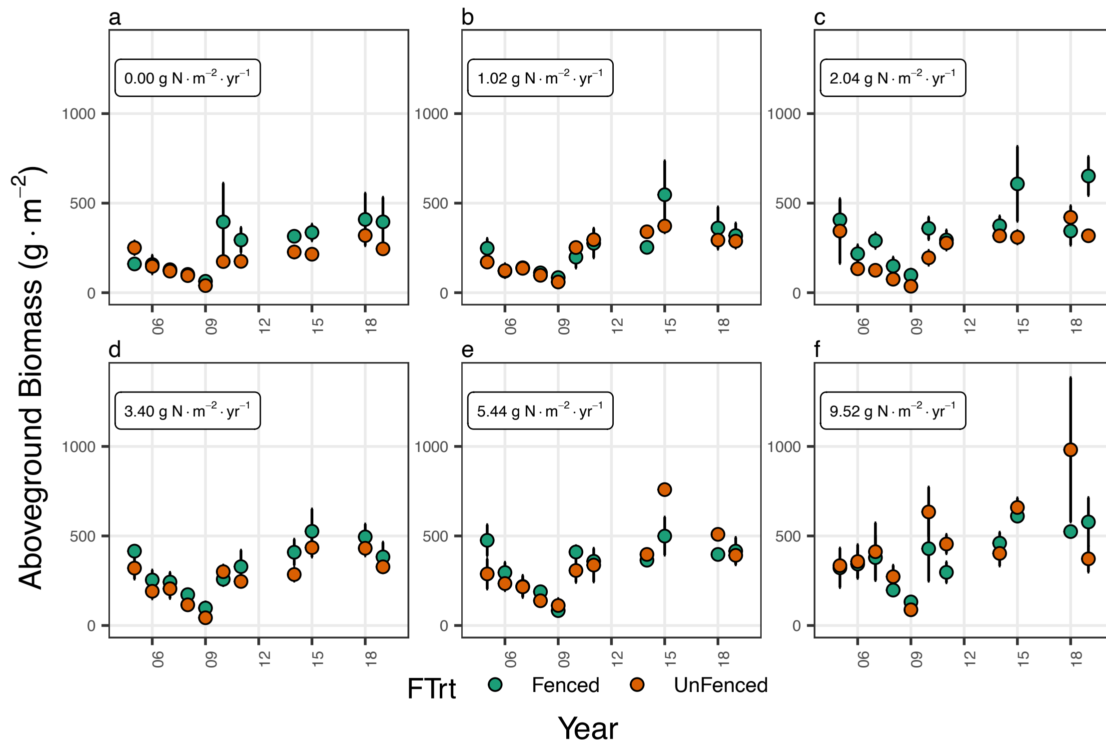
Supplemental Fig. S1: Spatial layout of the experimental design. Each tile represents one plot labelled with its assigned plot number. (a) The layout of the fencing treatment: "Deer -" Fenced plots are shown in green. "Deer +" Unfenced plots are shown in orange. (b) The layout of the nitrogen addition treatment. There are 8 levels of added nitrogen (0.0, 1.02, 2.04, 3.4, 5.4, 9.5, 17.0, 27.2 g N m^{-2} yr^{-1}) plus nutrients P, K, Ca, Mg, S and trace metals (Tilman 1987). There are two sets of control plots. Treatment I which received no nutrients of any kind and Treatment A which received no N, but all other nutrients. Treatment A serves as the control to test solely for the effect of N addition whereas Treatment I serves as the control for nutrient addition of any kind.



Supplemental Fig. S2: Plant total live abovebround biomass across fencing exclosure treatments. Bar represents: Observed mean total live abovebround biomass ± 1 SE, fitted mean total live abovebround biomass from a model with error weights, fitted geometric mean total live abovebround biomass from a model with a log-transformed response variable averaged across N addition treatments 0.0 – 9.52 g N m^{-2} yr^{-1} for each fencing treatment (n = 18). Years included 2005 through 2019 (not clipped in 2012, 2013, 2016, and 2017).



Supplemental Fig. S3: Aboveground biomass across nitrogen addition treatments. Boxplots show the range of data in each nitrogen addition treatments 0.0 – 9.52 g N m^{-2} yr^{-1} for each fencing treatment. The median is shown as a horizontal line. Each box edge is the 25% and 75% percentile. The whiskers show 1.5 * the interquartile range with outliers shown as points above or below those values. Years included 2005 through 2019 (not clipped in 2012, 2013, 2016, and 2017). Each boxplot has a sample size of 33 (11 years x 3 plots). Nitrogen addition treatments: (a) 0.00 g N m^{-2} yr^{-1} (b) 1.02 N g m^{-2} yr^{-1} (c) 2.04 g N m^{-2} yr^{-1} (d) 3.40 g N m^{-2} yr^{-1} (e) 5.44 g N m^{-2} yr^{-1} (f) 9.52 g N m^{-2} yr^{-1}



Supplemental Fig. S4: Aboveground biomass across nitrogen addition treatments in each year. Each point represents the mean ± 1 SE for each experimental contrast. Each mean has a sample size of 3. Years included 2005 through 2019 (not clipped in 2012, 2013, 2016, and 2017). Nitrogen addition treatments: (a) 0.00 g N m^{-2} yr^{-1} (b) 1.02 N g m^{-2} yr^{-1} (c) 2.04 g N m^{-2} yr^{-1} (d) 3.40 g N m^{-2} yr^{-1} (e) 5.44 g N m^{-2} yr^{-1} (f) 9.52 g N m^{-2} yr^{-1}

Supplemental Tables

Supplemental Table S1: Summary ANOVA table testing the dependance of the total live aboveground herbaceous biomass on the fencing treatment as a categorical variable, the nitrogen addition treatment as an unordered categorical variable and the natural log of year as a continuous variable for 11 years of data (2005-2019, not including 2012, 2013, 2016, 2017). n = 36.

Term	numDF	denDF	F-value	*P*-value
Fencing Treatment	1	29	5.3	0.029
Nitrogen Treatment	5	29	6.9	<0.01
log(Year)	1	359	142.2	<0.001

Supplemental Table S2: Least square means of total live aboveground biomass (g m^{-2}) across nitrogen addition treatments (g N m^{-2} yr^{-1}). Means are averaged across fencing treatments.

Nitrogen Treatment	Least Square Mean	SE	Lower CL	Upper CL
0.00	214.4	20.4	168.8	259.9
1.02	233.6	19.1	191.1	276.1
2.04	281.8	26.9	221.9	341.8
3.40	293.0	20.6	247.0	339.0
5.44	340.4	25.7	283.1	397.8
9.52	430.6	42.1	336.8	524.4

Supplemental Table S3: Summary ANOVA table testing the dependance of the number of herbaceous species on the fencing treatment as a categorical variable, the nitrogen addition treatment as an unordered categorical variable and year as a continuous variable for 11 years of data (2005-2019, not including 2012, 2013, 2016, 2017). n = 36.

Term	numDF	denDF	F-value	*P*-value
Year	1	348	19.4	<0.001
Fencing Treatment	1	24	15.8	0.0006
Nitrogen Treatment	5	24	2.8	0.042
Year*Fencing Treatment	1	348	15.9	0.0001
Year*Nitrogen Treatment	5	348	2.8	0.018
Fencing Treatment*Nitrogen Treatment	5	24	3.9	0.010
Year*Fencing Treatment*Nitrogen Treatment	5	348	3.9	0.002

Supplemental Table S4: The effect size of separate linear mixed effects model testing the dependence of individual species biomass' on nitrogen as a linear variable and the fencing treatment as a categorical variable. Using years of data 2006-2019, not including years 2012, 2013, 2016, 2017. *P*-values were corrected using the False Discovery Rate correction (FDR). SE = standard error. The variable "Fencing" is coded such that a positive value means a gain in abundance outside the fence.

Species	Effect of Nitrogen	Nitrogen SE	Nitrogen *P*-value	Nitrogen *P*-value (FDR)	Effect of Fencing	Fencing SE	Fencing *P*-value	Fencing *P*-value (FDR)
Ambrosia coronopifolia	-0.39	0.55	0.485	0.5389	7.93	2.61	0.00460	0.0232
Artemisia ludoviciana	13.85	2.35	1.3E-06	1.3E-05	34.22	12.16	0.00820	0.0273
Elymus repens	10.66	3.57	5.3E-03	0.0175	-13.09	21.56	0.54800	0.6089
Euphorbia corollata	0.11	0.20	0.585	0.585	-6.93	1.89	0.00085	8.5E-03
Lathyrus venosus	-0.51	0.52	0.3353	0.4789	-6.40	3.29	0.06030	0.0861
Panicum oligosanthes	-0.20	0.28	0.4847	0.5389	3.70	1.57	0.02410	0.0449
Poa pratensis	7.11	1.45	2.4E-05	1.2E-04	-3.52	8.67	0.68780	0.6878
Solidago rigida	-4.73	1.92	0.0194	0.0388	-31.65	13.02	0.02070	0.0449
Sorghastrum nutans	-0.44	0.18	0.0189	0.0388	1.34	1.09	0.22570	0.2821
Symphyotrichum oolentangiense	-0.60	0.33	0.0759	0.1265	-5.82	2.52	0.02700	0.0449