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Title: Community change can buffer chronic nitrogen impacts, but multiple nutrients tip the scale

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Abstract

Anthropogenic nitrogen (N) inputs are causing large changes in ecosystems worldwide. Many previous studies have examined the impact of N on terrestrial ecosystems; however, most have added N at rates that are much higher than predicted future deposition rates. Here, we present the results from a gradient of experimental N addition (0-10 g N m^{-2}) in a temperate grassland. After a decade of N addition, we found that all levels of N addition changed plant functional group composition, likely indicating altered function for plant communities exposed to ten years of N inputs. However, N addition only had weak impacts on species composition and this functional group shift was not driven by any particular species, suggesting high levels of functional redundancy among grasslands species. Adding other nutrients (P, K, and micronutrients) in combination with N caused substantially greater changes in the relative abundance of species and functional groups. Together, these results suggest that compositional change within functional groups may buffer grasslands from impacts of N deposition, but concurrent eutrophication with other elements will likely lead to substantial changes in plant composition and biomass.

Keywords: nitrogen deposition, species turnover, global change, nutrient co-limitation, plant functional group

Introduction

Human activities have increased the inputs of nitrogen (N) to ecosystems (Galloway et al. 2004), altering species composition and diversity (Simkin et al. 2016) and changing rates of primary production and turnover (Elser et al. 2007, Harpole et al. 2011, Komatsu et al. 2019, Midolo et al. 2019, Stevens 2019). Grasslands make up two-thirds of the world's terrestrial carbon (C) sink, providing a crucial function in the face of increasing atmospheric CO_2 levels (Schimel et al. 1994) and other anthropogenically-driven changes like N deposition. Grassland plant community composition and productivity are both sensitive to changing nutrient inputs; plant species diversity tends to decrease with added nutrients, whereas productivity often increases (Borer et al. 2014, Fay et al. 2015, Harpole et al. 2016). With increasing amounts of N entering ecosystems globally (Galloway et al. 2008), it is important to understand the ways in which grassland plant community composition will respond, because changes in species and functional group composition can alter ecosystem stability and function (Hillebrand et al. 2018). Increased N availability can decrease species richness

(Roem et al. 2002), and change plant functional group composition (e.g., relative abundance of grasses or forbs) (e.g. Inouye and Tilman 1988, Piper et al. 2005, Bowman et al. 2012, Lind et al. 2013) which can alter carbon flux rates (Peng et al. 2017b, 2017a). However, most experiments testing the effect of N on grassland ecosystems have applied N at rates substantially exceeding current or projected rates of N deposition, and few studies are designed to examine the effect of realistic rates of N inputs on compositional turnover (Midolo et al. 2019, Korell et al. 2020), in spite of N's key role for plant biomass, abundance, and diversity (Allan et al. 2015).

Many grassland N addition studies that examine the effects of small amounts of added N also add other commonly limiting nutrients, such as phosphorus (P), potassium (K), and micronutrients (μ) in addition to multiple levels of added N (e.g. Inouye and Tilman 1988, Clark and Tilman 2008, Bai et al. 2010, Cleland and Harpole 2010, Fornara et al. 2012, Heskel et al. 2014). With this design, single nutrient impacts on the response of the community to elemental supply cannot be distinguished from nutrient co-limitation, which is common in grasslands and other ecosystems (Elser et al. 2007, Fay et al. 2015). The addition of other resources also may alter plant biomass allocation (Chapin et al. 1987, Gleeson and Tilman 1992), and therefore also alter the overall community response. Furthermore, many N-addition studies add P, K, and μ in large quantities to ensure that the only limiting nutrient is N. Many of the studies adding N in combination with other nutrients (often P and K) have found rapid declines in species richness (Suding et al. 2005, Midolo et al. 2019), whereas studies that have added N alone have found increases, decreases, or negligible changes in species richness (Song et al. 2011, Bowman et al. 2012) highlighting the general importance of co-limitation. Changes in functional group composition can also differ when multiple nutrients are added. For example, the addition of multiple nutrients along with N can lead to significant losses of forb species at low N inputs rates (Clark and Tilman 2008), whereas addition of N alone has been found to have negligible effects on forb biomass (You et al. 2017). Though adding nutrients other than N informs the consequences of elevated N inputs when other elements are in high supply, the addition of these supplemental elements does not mimic current deposition conditions, in which N makes up the majority of anthropogenically-derived inputs to ecosystems (US Environmental Protection Agency 2018).

N deposition represents a continuous and cumulative impact to Earth's ecosystems (Smith et al. 2009); imitating the impacts of N deposition through N addition experiments can provide insight into how this perturbation may change ecosystems. In the past decade, N gradient studies have demonstrated significant species losses beginning at low levels of N addition (Song et al. 2011), or beginning at high levels of N (Prager et al. 2017), or no effect (Bowman et al. 2012). Thus, the response of plant communities to low rates of N addition remains unresolved. Importantly, recent work has demonstrated that many responses to N are nonlinear (ex. soil respiration, Peng et al. 2017a, plant diversity, Prager et al. 2017), or that there are thresholds for species loss (Bai et al. 2010), suggesting that it is not possible to predict low N addition impacts using results from higher N addition studies. For this reason, it is necessary to measure responses across a range of N inputs in order to characterize the shape (i.e. predicted non-linearity) of ecosystem responses to increasing anthropogenic N supply rate.

Finally, assessing the impact of N addition on ecosystems depends on the response variable in question. For example, if a species becomes more dominant as another species becomes less dominant, metrics like species richness or evenness may not detect those changes (Hillebrand et al. 2018), despite the large shift in community composition. In contrast, species turnover can capture changes in relative abundance and richness, and can better inform how communities are changing through time (Hillebrand et al. 2018). Furthermore, slight shifts in the abundance of species within the same functional group may result in large shifts at the higher, functional level of grouping; thus, grouping species by function may reveal shifts in community structure that are not evident at the species level. For example, N addition can reduce the abundance of slow-growing, nutrient efficient C_4 grasses relative to the more profligate C_3 grasses (Inouye and Tilman 1988, Isbell et al. 2013). Thus, using turnover metrics to assess the effect of N on both species and functional groups could present a more holistic understanding of how N addition is affecting grassland ecosystems.

Here, we experimentally tested the effects of long-term N addition on plant species and functional group composition in an old field temperate grassland. We added N at four rates (0, 1, 5, and 10 g N m^{-2} year^{-1}), which is comparable to the range of N deposition experienced globally (Galloway et al. 2008), including current ambient N deposition in Minnesota (~0.9 g N m^{-2} year^{1}, Ackerman *et al.* 2019). To determine whether other nutrients co-limit growth or alter community responses, an additional treatment added N at the highest rate (10 g N m^{-2} year^{-1}) with a mix of commonly limiting nutrients (P, K, and μ). We examined how N addition affected the abundance of different plant functional groups, and rates of species and functional group turnover over ten years. We tested *a priori* predictions that species and functional groups with low minimum N requirements for growth (ex. C_4 grasses) would decrease in abundance with increasing N (Reich et al. 2001, Craine et al. 2002, Lavorel and Garnier 2002, Suding et al. 2005, Harpole and Tilman 2006). However, we predicted that as N (and other nutrient) availability increased, species and functional group turnover would change (Harpole et al. 2016) as strong N competitors declined in abundance, leading to domination of the plots by a few, fast-growing species (Grime 1979, Tilman 1987). Thus, our hypotheses were that, along a gradient of increasing N supply:

H1: aboveground biomass would increase, driven by increasing C_3 grass abundance;

H2: C_4 grass and forb abundance would decrease, despite an overall increase in aboveground biomass; and

H3: species turnover rates would change over time.

We also hypothesized that the mix of N with other nutrients (PKμ), if they are co-limiting, would increase rates of species turnover compared to N-alone (H4), and that graminoid species would dominate with multi-nutrient addition while forb species would decline to local extinction (H5).

Methods

Our experiment was set up in 2007 in an old field at Cedar Creek Ecosystem Science Reserve in East Bethel, MN (mean annual temperature 6.3ºC, mean annual precipitation 750 mm year^{-1}, 45.4020° N, 93.1994° W). The field was abandoned from agriculture in 1950, fifty-seven years prior to the beginning of this experiment. Cedar Creek is located on the Anoka Sand Plain, characterized by sandy loam soils with very low total N pool relative to other grasslands (Fay et al. 2015). Background N deposition at this site is approximately 0.9 g N m^{-2} year^{-1} (equivalent to 90 kg N ha^{-1} year^{-1}; Ackerman et al. 2019). The experiment was a completely randomized block design composed of five blocks of six 25-m^2 plots each with treatments, as follows: ambient N deposition (Control), +1 g N m^{-2} year^{-1} (N1), +5 g N m^{-2} year^{-1} (N5), +10 g N m^{-2} year^{-1} (N10), and a multi-nutrient addition plot with +10 g m^{-2} year^{-1} of N, phosphorus (P), and potassium (K) (NPKμ). All plots, including the control (0 g N m^{-2} year^{-1}), were exposed to the same ambient N deposition. A 100 g mix of commonly limiting micronutrients (17% Fe, 12% S, 6% Ca, 3% Mg, 2.5% 1% Cu, 1% Zn, Mn, 0.1% B, and 0.05% Mo) was added to the NPK treatment only in Year 1 to prevent toxicity. P was added as triple super phosphate ($Ca(H_2PO_4)_2$), and K added as potassium sulfate (K_2SO_4). N was added to the plots as time-released urea (CH_4N_2O). Even though the majority of N deposition is composed of NH_x and NO_y compounds (Galloway et al. 2004), time-released urea has been found to be an effective mimic of N deposition (Jia 2020); furthermore, a study across 4 North American grasslands found no differences in grassland responses to different forms of N added (ammonium nitrate vs. urea vs. calcium nitrate; Seabloom et al. 2013). All nutrients were added at the end of each growing season, and herbivores were not excluded from any of the plots.

A 1-m^2 permanent quadrat was randomly established in one of the four 2.5 x 2.5 m quadrants of each plot and percent cover (henceforth "cover") of all species present was recorded annually. Cover was estimated independently for each species, such that cover values could sum to over 100%. Cover was visually estimated each year at peak biomass (typically mid-August). All researchers collecting cover data calibrated their estimates with each other. To help control for among-investigator error, as well as for inter-annual variation, we used log-response ratio (Treatment/Control in each year) for our turnover analyses. In our analyses of cover, we grouped plants by function: C_3 grasses (order Poales, C_3 photosynthetic pathway), C_4 grasses (order Poales, C_4 photosynthetic pathway), sedge (*Carex spp*. and *Cyperus spp.*), legume (family Fabaceae), woody (non-Fabacea woody species), forb (non-Poales, non-Fabaceae herbaceous species), fungi, or bryophytes. Biomass was clipped annually in two 10 cm x 100 cm strips, sorted to functional group, dried to a constant mass, and weighed to the nearest 0.01 g. For biomass we also separated previous year's aboveground growth that had senesced and become litter. Sedges and C_3 and C_4 grasses were grouped into a single category (graminoid) for biomass measures. The locations of these strips were moved each year, so they were always sampled in previously unclipped locations.

We used linear mixed-effects models (R packages "lme4" and "lmerTest," Bates et al. 2015, Kuznetsova et al. 2017) to assess changes in live (non litter) biomass over time, with treatment coded as a factor and treatment year as the fixed effects and block, plot, and year as random effects, with block and plot as nested random effects. We assessed the effect of N alone (Control, N1, N5, and N10) in a separate model from the effect of N vs added nutrients (N10 and NPKμ). Pre-treatment data (Year 0) is included in the figures but was not included in the models.

We calculated species turnover (Hillebrand et al. 2018) using percent cover in each plot using the Bray-Curtis dissimilarity index (BCD). To calculate the distance between two years, we subtracted the Bray-Curtis dissimilarity index of Year$_n$ from the subsequent Year$_{n+1}$ (BCD$_{n+1}$ – BCD$_n$) for each plot, which we called temporal turnover. Bray-Curtis distance was calculated using the 'vegan' package in R (R Core Team 2017, Oksanen et al. 2018). In order to account for interannual variation during the experiment, as well as right-skewed abundance data, we calculated the log-response ratio (LRR; Treatment/Control in each year). We used linear mixed-effect models to analyze these effects of added N on temporal turnover, total biomass, and functional group biomass with treatment (Control, N1, N5, and N10 coded as factors) as the fixed effect, and block, plot (nested within block), and year as random effects. To assess the effect of additional nutrients on temporal turnover, we used a linear mixed-effect model with treatment (N10 vs. NPKμ) as the fixed effect and block, plot (nested within block), and year as random effects. Biomass was square-root transformed, and cover data was log-transformed, to meet assumptions of normality. Similar trends emerged from our analyses of percent cover and biomass of functional groups, so we present analyses of percent cover data because it allowed us to differentiate between C_3 and C_4 grasses, and sedges. To assess changes in functional group and species composition, we used non-metric multidimensional scaling (NMDS) and PERMANOVA using Bray-Curtis distances calculated from biomass and percent cover of functional groups. All statistical analysis was conducted in R version 4.0.2 (R Core Team 2017).

Results

Changes in aboveground biomass and percent cover

Nitrogen addition increased aboveground plant biomass throughout the study period (Fig. 1, $F_{3,12} = 4.69$, $p = 0.022$). All N addition treatments had significantly more biomass compared to Control (Appendix S1: Table S1; N1, $p = 0.0075$; N5, $p = 0.0078$; N10, $p = 0.02$). Through time, the multi-nutrient treatment had higher aboveground biomass than the N alone treatment (Fig. 1, $F_{4,12} = 12.72$, $p = 0.024$), indicating that growth was still limited by nutrients other than N even when N inputs were high. The cumulative amount of N added did not affect biomass at any treatment level (Appendix S2: Fig. S1, $F_{1,551} = 2.64$, $p = 0.11$). Despite a decade of N and other nutrient addition, soil pH did not change during the study period (Appendix S2: Fig. S2, $F_{4,21} = 0.78$ $p = 0.40$), and this qualitative result remained unchanged even after removing an outlier in the N10 treatment.

We then focused on the two main functional groups in our experimental plots, graminoids and non-leguminous forbs, which were the only two functional groups present in every plot throughout the experiment. After ten years of N addition, graminoid biomass significantly increased across the N gradient (Fig. 1; $F_{3,12} = 3.56$, $p = 0.047$). This trend was driven by large increases in graminoid biomass in the lowest N addition treatment (Appendix S1: Table S2; N1, $p = 0.0093$) and smaller, but still significant, increases in the medium (N5) and high (N10) N addition treatments ($p = 0.045$ and 0.037, respectively). Addition of N10 in combination with other nutrients (NPKμ) increased graminoid biomass (Fig. 1, $F_{1,4} = 7.52$, $p = 0.052$) after ten years of nutrient addition, compared to the N10 treatment, though the effect was only marginally significant. Forb biomass did not change over time in the Control or any N treatments (Appendix S1: Table S3; $F_{3,15} = 0.816$, $p = 0.50$). However, forb biomass significantly decreased in the multi-nutrient treatment compared to the N10 treatment (Fig.1, $F_{1,57} = 4.16$, $p = 0.046$).

Species-level percent cover data provided more insight into the changes in graminoid biomass, because biomass data was only divided by functional group. We examined the change in percent cover of C_3 and C_4 grasses and sedges (*Carex spp.* and *Cyperus spp.)*. The effect of N varied by photosynthetic pathway within the grasses, but not between the grasses and sedges (Appendix S1: Tables S4-S6). Although C_3 grass cover was similar across all N addition treatments and Control ($F_{3,17} = 0.709$, $p = 0.56$); over time, C_3 cover increased more rapidly in the N10 plots than in the Control plots (Fig. 2a, $p = 0.012$). The NPKμ treatment had more C_3 cover than the N10 treatment ($F_{1,4} = 12.083$, $p = 0.026$), and had significantly less C_4 grass cover than did the N alone treatment (Fig. 2b, $F_{1,27} = 4.39$, $p = 0.045$). There were no differences in C_4 cover across the N gradient ($F_{3,12} = 1.12$, $p = 0.38$), nor did sedge cover differ across the N gradient or between the multi-nutrient and N alone treatment (Appendix S1: Fig. S3).

Changes in community composition and turnover

After 10 years of chronic nutrient addition, when assessed by functional group, communities differed along the N gradient. By Year 10, the functional group communities across the N gradient significantly differed from each other (Appendix S2: Fig. S4, $F_{3,216} = 3.91$, $p = 0.003$), as well as between the multi-nutrient and N alone treatment (Table 1; $F_{1,108} = 9.91$, $p = 0.002$). Despite these significant differences in functional group composition in Year 10, individual species composition did not differ among the N addition treatments ($F_{3,78} = 0.583$, $p = 0.988$) or between the N10 and NPKμ treatments ($F_{1,34} = 0.876$, $p = 0.55$) (Appendix S2: Table 1).

Over time, functional group composition in N addition plots diverged from Control plot composition (Fig. 3, $F_{1,142} = 6.635$, $p = 0.011$). However, rates of functional group turnover did not differ among the N addition treatments (Fig. 3, $F_{2,142} = 0.313$, $p = 0.73$), indicating that the rate of N addition was not driving these changes. In contrast, species turnover did not differ with N addition ($F_{2,8} = 3.19$, $p = 0.1$) and occurred at a constant rate through time ($F_{1,8} = 0.154$, $p = 0.7$), which, when taken with functional group changes, suggests that species replacement and losses differed by functional group.

We also examined the impact of additional nutrients on the rates of community turnover. Functional group turnover increased marginally between the N10 and NPKμ treatments ($F_{1,93} = 3.88$, $p = 0.051$), as well as over time (Fig. 3, $F_{1,93} = 2.906$, $p = 0.09$). There was no change in species turnover between the N10 and NPKμ treatments ($F_{1,8} = 0.44$, $p = 0.53$), and species turnover did not change through time turnover between the N10 and NPKμ treatments (Fig. 4, $F_{1,84} = 0.51$, $p = 0.48$).

Finally, we examined the potential for N impacts on the community due to chronic N addition. The cumulative amount of N added through the duration of the experiment did not impact functional group turnover (Appendix S2: Fig. S5a, $F_{1,144} = 1.99$, $p = 0.16$) or species turnover (Appendix S2: Fig. S5b, $F_{1,84} = 0.495$, $p = 0.48$).

Discussion

In this study, we hypothesized that low rates of N addition, similar to what enters ecosystems through reactive N deposition, would trigger increases in aboveground biomass (H1) and shifts in species and functional group turnover (H3). Nitrogen addition increased plant aboveground biomass, with strong effects even at very low N supply rates. Nitrogen addition also altered the composition of the plant community, with substantial impacts at our lowest N addition treatment (N1; 1 g N m^{-2} $year^{-1}$). The strongest effects on composition occurred at the functional group level. For example, N addition increased total graminoid biomass across our experimental N gradient, and the addition of N plus other nutrients reduced C_4 grass cover relative to C_3 grass cover. When assessing the turnover of the functional group communities throughout time, we found that all N addition treatments became increasingly different from Control. Despite this directional change in functional groups, we found no consistent change in species-level composition, suggesting redundancy within functionally similar sets of species.

The increase in aboveground biomass was driven by increases in graminoid biomass in all treatments, whereas forb biomass did not change through the decade of this experiment. Notably, the lack of change in forb biomass indicates the abundance of forbs is not diminishing as graminoids increase in dominance, contrary to our original hypothesis (H2) that forb abundance would decrease with increasing N addition. This is concordant with a recent meta-analysis by You and colleagues (2017) that similarly found no significant changes in forb biomass in response to N addition across 89 studies that included 67 sites. Similarly, we did not find strong evidence that C_3 grasses were increasing, (H1) or C_4 grasses were decreasing, with increasing N addition (H2). Rather, we only found significant changes in C_3 and C_4 grass abundance in our multi-nutrient treatment (H5), indicating possible co-limitation of functional group abundance in this ecosystem.

Studies in some areas of the world, however, including near our study site, have found significant species losses after multiple years of both high and low N addition (Clark and Tilman 2008, Bai et al. 2010), which are often driven by losses of individual forb species (Clark and Tilman 2008). However, many such studies add P, K, and micronutrients in addition to multiple rates of N (Clark and Tilman 2008, Bai et al. 2010). Contrary to our hypothesis that low rates of N addition would trigger changes in species turnover (H3), we found no changes in the rate of species community turnover during our study, and the rate of turnover did not differ among treatments, either. Past work from this ecosystem (Tilman 1987, Clark and Tilman 2008) and many others (Midolo et al. 2019) have found significant species losses over time with N addition. Our results suggest that while species change in relative dominance in response to the treatments, species within functional groups that increase in dominance with N addition play functionally similar roles to the species they are replacing. This could indicate that doubling of current rates of N deposition in this area (current rates are 0.9 g N m^{-2} $year^{-1}$; our lowest N treatment is similar to a doubling of ambient deposition) may not lead to local extinction of species, but rather may benefit many functionally similar species. Recent work in these same experimental plots (Widdig et al. 2020) has demonstrated significant shifts in belowground bacterial and fungal communities despite seeing no changes in aboveground species richness. Thus, it is possible that changes in soil microbial community composition precede large changes in plant species composition; for example, changes in belowground community composition can influence plant leaf traits and allocation (Sayer et al. 2017), and thus could lead to changes in plant composition as nutrient supply is altered. We propose that, in this ecosystem, changes to plants species composition provide an incomplete picture of the whole community response to altered nutrient supply.

Our results, comparing N with and without other nutrients, demonstrate that co-limitation by multiple nutrients, not single-nutrient limitation, determined the response of the community. A study conducted in a field adjacent to the current study (Clark and Tilman 2008) found rapid species losses at N addition rates similar to our lowest treatment level (N1); however, N was added in combination with a mix of other nutrients, including P, K, and micronutrients in this study (Clark and Tilman 2008). The substantial differences in composition and aboveground biomass between the N10 and NPKμ treatments of the current study suggest that nutrient co-limitation likely played a key role in the species losses found in the earlier work (Clark & Tilman 2008). After a decade of chronic nutrient addition, the NPKμ plots were dominated by C_3 grasses. C_4 grasses tend to have higher nitrogen use efficiencies (NUE) than C_3 grasses (Wedin and Tilman 2010), thus the addition of nearly ten times the amount of N to the system could have given C_3 grasses a competitive advantage over C_4 grasses. However, we found that C_3 grasses only became dominant when other nutrients were supplied in addition to N, indicating co-limitation of growth. Thus, it is possible that conclusions made from earlier multiple nutrient addition studies about the negative impacts of low N addition—and applied to inference about N deposition—are overstated, and instead provide insight into the impacts of nutrient co-limitation. Our study may, however, underestimate the effects of N deposition given that we are not manipulating other commonly deposited nutrients at rates similar to their deposition.

Our findings highlight that N addition studies that also add other elements need to be interpreted within the context of nutrient co-limitation, as N addition effects clearly depend on the addition of other nutrients (Harpole et al. 2011, 2016). For this reason, studies adding multiple nutrients generate strong tests of co-limitation but weaker tests of N limitation *per se*. To fully test the rates of N at which other resources become limiting in this ecosystem, treatments of low N (at similar rates to our N1 and N5 treatments) should be paired with other nutrients. It is possible most species in this old field grassland are primarily N limited, and thus may not react to the addition of other nutrients (Tilman 1982) until enough N is added. However, given the minimal impact across a large gradient of added N observed in this study, it seems that species composition in grasslands similar to our study site are likely to be resistant to change by realistic N deposition input (Bowman et al. 2012), whereas enrichment with multiple nutrients, such as through agricultural runoff, may substantially impact grassland functional group composition.

Our work points to the importance of the entire N supply of a system when considering the effects of N addition experiments. Many current comparable N addition experiments (ex. Bai et al. 2010, Song et al. 2011) are located in Inner Mongolia, China, which receives nearly twice as much ambient N deposition as our study site (Ackerman et al. 2019). Work at these high deposition sites has shown rapid species losses at N addition levels similar to our manipulations. Furthermore, N deposition rates in these regions have doubled since the 1980s, whereas N deposition rates have only increased by 20% in Minnesota (Ackerman et al. 2019). Thus, even though rates of supplemental N addition between our study and the studies in Inner Mongolia are comparable, the lowest amount of N added is actually much higher in the studies based in Inner Mongolia. In contrast, our study finds similar trends to a low N addition gradient in the Rocky Mountains of Colorado, USA (Bowman et al. 2012), which receives about half as much N deposition as our site (Ackerman et al. 2019). Thus, grassland responses appear to be contingent on the rate and identity of nutrients added, but our work demonstrates that these responses must be interpreted and compared in the context of the stoichiometry of ambient nutrient deposition rates.

The results of this study shed new light on the interpretation of past work. It has long been thought that added amounts of N, even in small amounts, cause significant shifts in plant species diversity (Clark and Tilman 2008); indeed, similar results emerged after half a decade of low N supply, alone, in various experiments in China (Song et al. 2011, Zeng et al. 2016). The idea that very low amounts of N addition can trigger large species losses, however, has often been applied across many ecosystems, with many recent studies based on the findings of Clark and Tilman (2008), which was conducted in a field adjacent to the one used in our study. Our work demonstrates that rates of N addition similar to ambient deposition alone are unlikely to cause the observed species losses in this system that have been attributed to N supply rate (Clark and Tilman 2008), but rather that the rates of site-level or regionally limiting nutrients should be a key consideration when estimating the effects of

N enrichment. Recent work (Komatsu et al. 2019), however, indicates that plant community richness and turnover can take longer than a decade to respond to experimental manipulation, and we may thus expect to see greater changes across our experimental N gradient in the future.

Taken together, the results of our work demonstrate that compositional and functional changes respond differently to the supply of nitrogen alone. This provides important new insights into the interpretation of past work (e.g. Clark and Tilman 2008), suggesting that in grassland environments that are primarily N-limited, N supply at similar rates as anthropogenic deposition (0 - 5 N m^{-2} $year^{-1}$) will have large impacts on species diversity (Song et al. 2011, Zeng et al. 2016) and functional group composition. Instead, we find the addition of multiple nutrients (N, P, and μ) have a stronger effect on functional group composition and turnover than N alone. Although we found weak species level compositional shifts, higher taxonomic groupings (i.e. functional groups) show consistent and predictable change in our study and in others (Midolo et al. 2019). Based on our work, we expect to find more predictable plant community responses to N deposition at the functional, rather than taxonomic, level (Laughlin et al. 2017), and small, if any, changes to plant communities with increasing rates of N deposition. This work builds from past work to demonstrate that compositional turnover within functional groups may buffer grasslands from impacts of N deposition, but concurrent eutrophication with other elements will likely lead to rapid compositional and functional changes.

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data, MEW performed the analyses, MEW and ETB wrote the initial draft, and all authors contributed edits to the final draft.

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Table 1. PERMANOVA of functional group composition in year 10 using Bray-Curtis distances (permutations = 999). Treatment, coded as a factor, was used as the fixed effect in the model, and separate models were run for the N addition treatments (Control, N1, N5, and N10) and the added nutrients comparison (N10 and NPKµ). Functional group composition (determined using biomass data) differed across treatments for both the N addition comparison (p = 0.003) and N10 vs. NPKµ comparison (p = 0.002).

Source	Factor	df	Sum of squares	R^2	*F*	*P*
Control, N1, N5, N10	Treatment	3	1.134	0.051	3.906	**0.003**
	Residuals	216	20.911	0.949	-	-
	Total	219	22.045	1.000	-	-
N10 vs. NPKµ	Treatment	1	1.115	0.0840	9.906	**0.002**
	Residuals	108	12.155	0.916	-	-
	Total	109	13.269	1.000	-	-

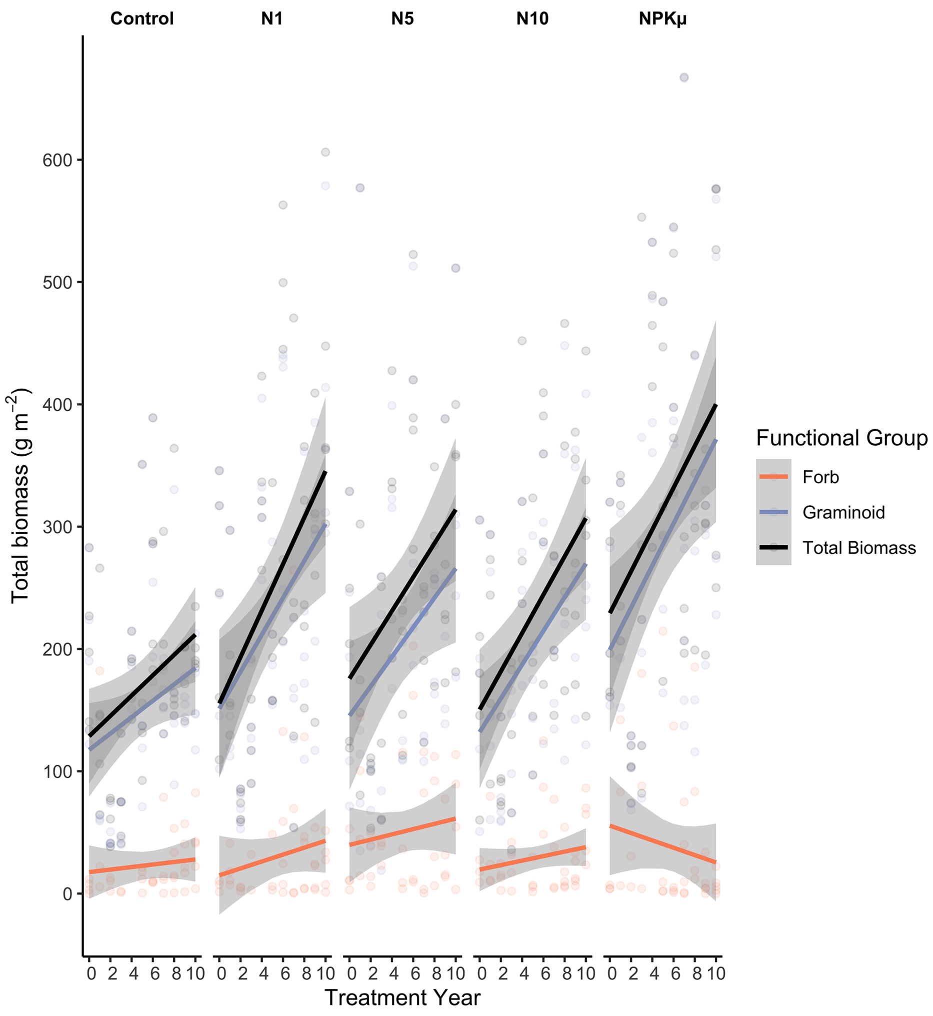
Figure Captions

Figure 1. Total biomass of forb species (orange), graminoid species (purple), and total plot biomass (black) from Year 0 (pre-treatment) through Year 10. Total biomass was significantly (p = 0.022) higher in the N addition treatments than in the Control treatment. Through time, the NPKμ treatment had more biomass than N10 (p = 0.024). Graminoid biomass was higher in the N addition treatments compared to Control (p = 0.047), and graminoid biomass was marginally higher in the NPKμ treatment compared to the N10 treatment (0.052). Forb biomass did not change over time in any of the N addition treatments (p = 0.5) but did decrease in the NPKμ treatment (p = 0.046). There were no interactions between year and treatment for any treatment. The shaded regions represent 95% confidence intervals.

Figure 2. Change in percent cover of C_3 grasses (a) and C_4 grasses (b) from Year 0 (pre-treatment) through Year 10. There were gains in C_3 cover over time in the N10 treatment as compared to Control (p = 0.012); however, there were no other changes in C_3 or C_4 cover across the N gradient (p = 0.56 for C_3; p = 0.38 for C_4). The NPKμ treatment had more C_3 cover than did the N10 treatment (p = 0.026), and the NPKμ treatment lost more C_4 grass cover over time (p = 0.045) compared to the N10 treatment. The shaded regions represent 95% confidence intervals.

Figure 3. Functional group turnover generally increased in all treatments throughout the study period. Relative to Control, functional group turnover increased over time in the N addition treatments (p = 0.011) and between the NPKμ and N10 treatments (p = 0.09). Functional group turnover did not differ between the N addition treatments and Control (p = 0.73) but was marginally higher in the NPKμ treatment compared to the N10 treatment (p = 0.051). The shaded regions represent 95% confidence intervals.

Figure 4. Species turnover did not change over time or in any treatment (p = 0.10 for N addition treatments, p = 0.53 for N10 vs. NPKμ comparison), nor did it change over time (p = 0.7 for N addition treatments, p = 0.48 for N10 vs. NPKμ comparison). The shaded regions represent 95% confidence intervals.



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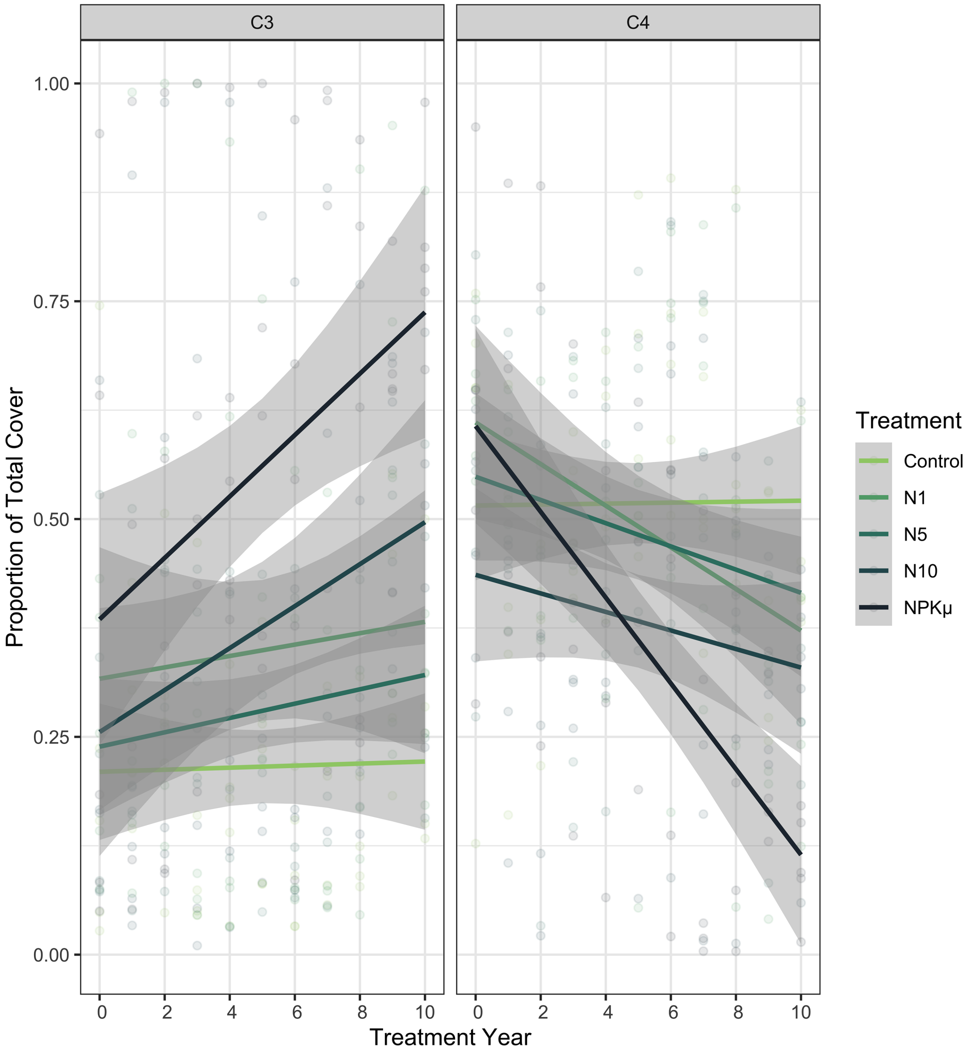

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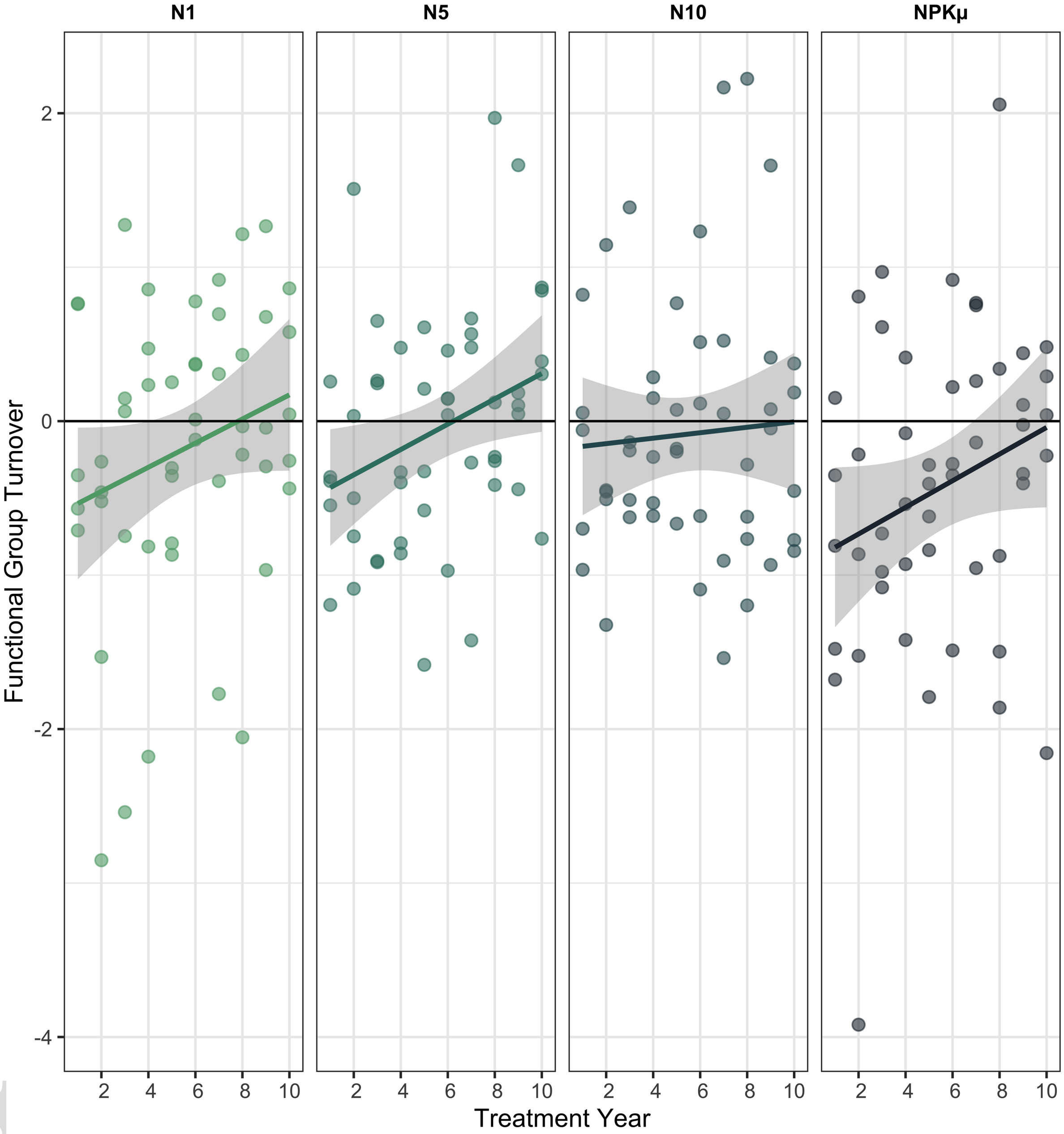

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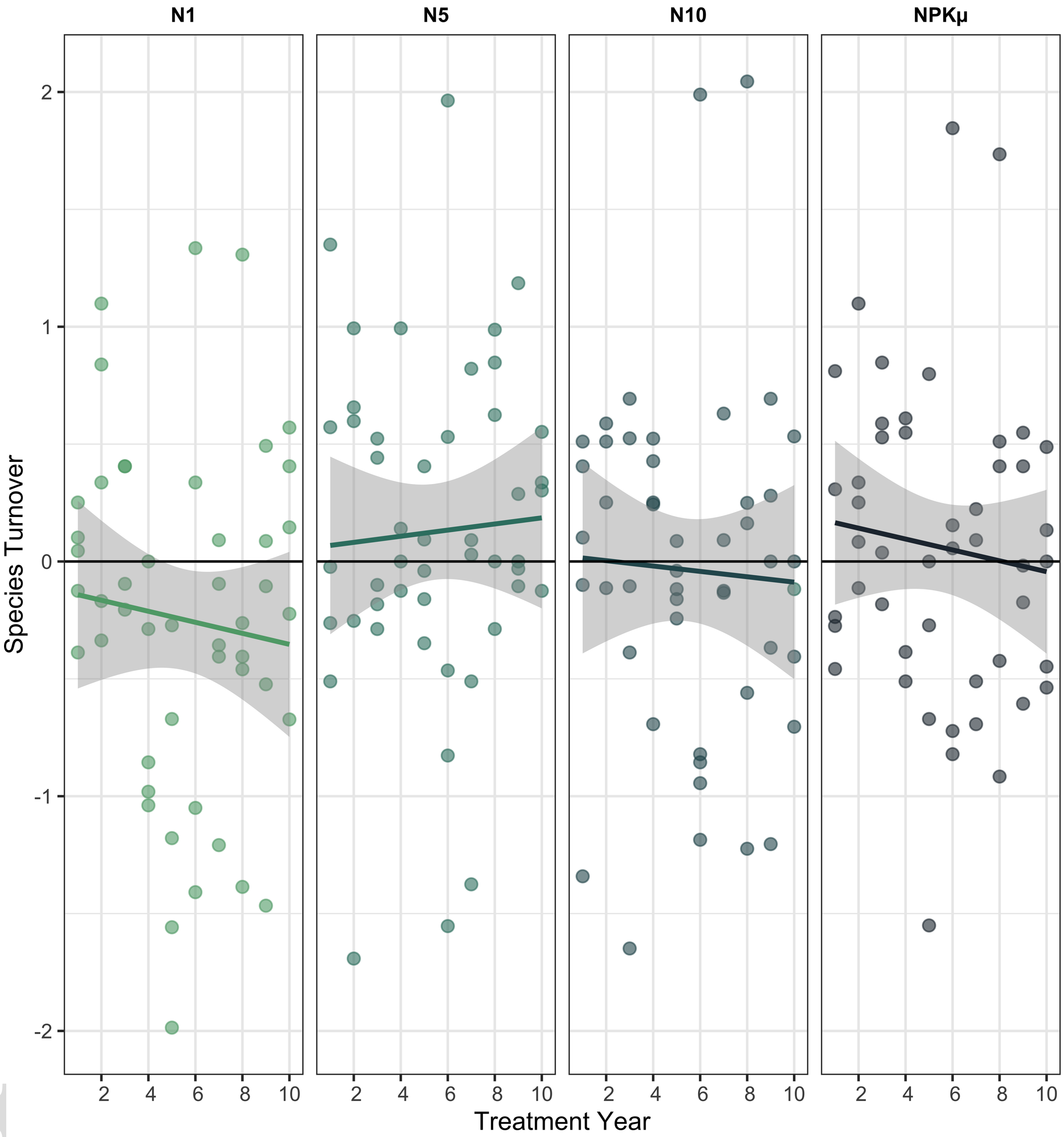

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