

Nutrient addition drives declines in grassland species richness primarily via enhanced species loss

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Abstract

(1) Declines in grassland diversity in response to nutrient addition are a general consequence of global change. This decline in species richness may be driven by multiple underlying processes operating at different timescales. (2) Nutrient addition can reduce diversity by enhancing the rate of local extinction by competitive exclusion, or by reducing the rate of colonization by constraining the pool of species able to colonize under new conditions. (3) Partitioning net change into extinction and colonization rates will better delineate the long-term effect of global change in grasslands. (4) We synthesized changes in richness in response to experimental fertilization with nitrogen, phosphorus and potassium with micronutrients across 30 grasslands. We quantified changes in local richness, colonization, and extinction over 8-10 years of nutrient addition, and compared these rates against control conditions to isolate the effect of nutrient addition from background dynamics. (5) Total richness at steady state in the control plots was the sum of equal, relatively high rates of local colonization and extinction. On aggregate, 30-35% of initial species were lost and the same proportion of new species were gained at least once over a decade. (6) Absolute turnover increased with site-level richness, but was proportionately greater at lower richness sites relative to starting richness. (7) Loss of total richness with nutrient addition, especially N in combination with P or K, was driven by enhanced rates of extinction - primarily of native, perennial, and forb taxa - with a smaller contribution from reduced colonization. (8) Reduced colonization plateaued after the first few (< 5) years after nutrient addition, while enhanced extinction continued throughout the first decade.

Synthesis. Our results indicate a high rate of colonizations and extinctions underlying the richness of ambient communities, and that nutrient enhancement drives overall declines in richness primarily by exclusion of previously established species. Moreover, enhanced

extinction continues over long time scales, suggesting continuous, long-term community responses and a need for long-term study to fully realize the extinction impact of increased nutrients on grassland composition.

Introduction

Increased nutrient supply is consistently linked to declines in species richness among plant communities (Borer et al., 2014; Harpole et al., 2016; Hautier et al., 2009; Midolo et al., 2019; Rajaniemi, 2002). Although richness is a common indicator of a system's response to global change, a decline in richness can be generated by multiple processes operating at different timescales. Canonical ecological theory (e.g. MacArthur & Wilson, 1967) suggests that richness reflects a balance between rates of species gains and losses through time. Consistent turnover is a normal quality of undisturbed communities, producing a dynamic composition but steady-state equilibrium in richness (MacArthur & Wilson, 1967). Disruption of this equilibrium via alteration to either gains or losses of species by global change drivers therefore underlies declines in richness (Fig. 1).

In terrestrial plant communities, a decline in species richness associated with increased nutrient supply has been attributed to, among other factors, a reduction in belowground resource limitation or modification to soil biogeochemistry, reducing belowground niche space and subsequently shifting competition aboveground for light (Harpole et al., 2016; Hautier et al., 2009). Species may then be lost via competitive exclusion by superior competitors for light (Borer et al., 2014). Multi-nutrient limitation of plant productivity is common in grasslands, (Fay et al., 2015) and therefore fertilization with multiple nutrients may accelerate loss of richness (Figure 1b) by reducing the number or dimensionality of belowground limiting factors (Harpole et al., 2016; Harpole & Tilman, 2007). The reduction of niche-space can lead to the loss of perennial, generally slower-growing and native species in favor of faster-growing, more resource-acquisitive annual or invasive species (Suding et al., 2005; Tognetti et al., 2021).

Fertilization also can lead to the gain of new native and non-native species (Flores-Moreno et al., 2016). Specifically, non-native species often respond differently to nutrient enrichment than natives, becoming dominant with fertilization (Seabloom et al., 2015). This is likely because nutrient addition reduces invasion barriers, particularly in nutrient limited systems where native species are locally adapted, or because some exotic species have evolved locally in human-dominated higher nutrient ecosystems (Davis et al., 2000; Seabloom et al., 2015). However, even small numbers of introduced species can maintain high relative abundance, leading to greater local extinction rates without compensatory colonization rates (Seabloom et al., 2013). This is frequently the case among introduced species that are dominant in their native range, leading to homogenization in the composition of sites with the same dominant species (Firm et al., 2011). This process could ultimately reduce colonization rates by constraining the pool of species able to successfully colonize (Figure 1c). Increased nutrient supply can therefore reduce overall richness through the loss of inferior soil resource competitors while favoring a more limited subset of highly competitive resource-acquisitive (often non-native) species.

Regardless of whether increased nutrient supply predominantly affects richness via colonization or extinction, the timescale by which these mechanisms play out is uncertain. Colonization can be affected at short or long time-scales depending on propagule pressure (Poulsen et al., 2007), while rates of extinction will vary with population size, lifespan, and the strength of priority effects (Uricchio et al., 2019; Werner et al., 2016). While theory suggests that the relative importance of extinction and colonization and their sensitivity to disturbance should vary with site-level conditions (Shurin, 2007), empirical studies in plant systems are few and relegated to single locations (Kaarlejärvi et al., 2017; Olofsson & Shams, 2007). The magnitude of local turnover rates can increase with regional species richness (MacArthur & Wilson, 1967)

while the rate and magnitude of disturbance can have different consequences depending on site fertility, climate, and pre-dominance of distinct functional groups (Mouillot et al., 2013; Svensson et al., 2009), emphasizing the need to control for both local and regional diversity. Generalizing across sites and across time scales will therefore cut through these idiosyncrasies to provide broadly encompassing mechanistic explanations for the changes in species richness associated with increased nutrient supply (Magurran et al., 2010).

Ultimately, the direct influence of increased nutrient supply on plant richness stems from its impact on background rates of colonization and extinction. While there are approximate expectations for changes in both components (either gains or losses) following nutrient addition, it is unclear how these components combine to drive declines in overall richness. In this study, we synthesized temporal patterns in richness from 30 grassland sites with nutrient addition treatments. We quantified the rate of species gains and losses over a period of 8-10 years and assessed their relative contribution to total species richness in control vs treatment plots. Using these data, we asked the following questions:

1. What are species turnover rates in grasslands under ambient conditions?
2. Are declines in richness with nutrient addition driven by reduced colonization rates, enhanced local extinction rates, or both?
3. Do changes to colonization and extinction rates operate synchronously or at distinct timescales?
4. Does the predominant cause of species decline change with the addition of multiple nutrients?

5. Are species lifeform, life history and provenance associated with reduced colonization or enhanced extinction rates with nutrient addition?

Materials and Methods

Experimental Design.

The Nutrient Network (NutNet) is a globally distributed experiment replicated across short-statured, primarily herbaceous ecosystems (hereafter called grasslands) on six continents (Borer et al., 2014). Most sites with experimental treatments contain three replicated treatment blocks (21 of 30 sites; range 1 - 6 blocks per site). Within each block, eight different nutrient addition treatments are applied to 5 x 5 m plots. Thus, most sites have a total of 24 experimental units (3 blocks x 8 nutrient treatments; Borer et al., 2014). These nutrient addition treatments represent the factorial combinations of nitrogen (N), phosphorus (P), and potassium and micronutrients (K μ), plus a control. Nutrient addition rates are: 10 g N m⁻² yr⁻¹ as timed-release urea, 10 g P m⁻² yr⁻¹ as triple superphosphate, 10 g K m⁻² yr⁻¹ as potassium sulfate and 100 g m⁻² of a micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn). N, P, and K are applied annually, while the micronutrient mix was applied only once at the start of the study to prevent toxicity of largely immobile micronutrients. Treatments are hereafter referred to shorthand by their nutrient components *e.g.*, ‘NK’ for Nitrogen + Potassium and micronutrients, ‘NPK’ for full nutrient additions. All present species are identified and their cover estimated visually each year to the nearest 1% in a permanent 1 m² quadrat in each 5 x 5 m plot. Measurements are taken annually at the time of peak biomass, or twice annually in highly seasonal sites, in which case maximum cover across both censuses was reported. For the current study, we only selected sites with at least eight years of treatment data

(n = 30 sites) to capture longer-term dynamics in species turnover, as well as control for otherwise uneven sample sizes at disparate time intervals.

Data manipulation

We quantified the rate of species gains and losses over a period of 8-10 years, and assessed their relative contribution to total species richness (absolute and proportional) in control vs nutrient treatment plots. Taxa that were only identified to genus were treated as a single species, representing a slightly conservative estimate of richness. Because overall richness varied across sites (Seabloom et al., 2021), the same number of species lost will likely have a larger effect on function in species-poor than species-rich communities (Symstad et al., 1998). We accounted for this in two ways: 1) by quantifying colonizations and extinctions proportionate to a plot's pre-treatment baseline richness, in addition to absolute change in richness, and 2) by examining explicitly whether patterns of species gain and loss differ once initial species richness is included as a fixed covariate.

In each year of treatment, we classified species present in an experimental unit as either “original” or “new”. “Original” species were defined as those present in a plot in the first 2 years of the time series (in the pre-treatment year or the first year of treatment), and therefore represent species that were either already established or colonized very early in nutrient addition treatments. In contrast, “new” species were any species not classified as “original” that were observed in a plot after the first year of treatment, and were therefore not originally present and arrived after plots had been subject to treatment effects for at least one full year. “Species gained” in any given year was defined as the number of “new” species present during that year (not cumulative over the time series), and “species lost” were the number of “original” species not present during that year. These definitions let us quantify the relative contribution of both

local colonization and extinction to total richness across time and treatments. Proportional richness was calculated by dividing a given year's total richness by the richness in treatment year 0, and is therefore relative to a plot's pre-treatment baseline richness.

To determine whether richness alters patterns of species gains and losses with nutrient addition, we stratified plots based on their initial richness. Categories were based on the richness in a 1m² quadrat in the pretreatment year: 1-10 species (n=394), 11-15 species (n=171), 16-20 species (n=119), and greater than 21 species (n = 65). We chose category sizes iteratively by looking at various ranges of starting richness and lumping groups with similar slope.

Analyses

To generalize trends across sites, we fit generalized additive models (GAMs) separately for change in plot-level total richness, gains, and losses through time (8-10 years of treatment). GAMs were fit to each type of richness (*i.e.* “total”, “new”, and “original” in both absolute and proportional richness). To select a best-fit smooth model, we fit three increasingly nested hierarchical GAMs: site as a random effect, site and block, and site, block and plot. We crossed these models with Restricted Maximum Likelihood (REML)-selected smoothing parameters at 3, 4 or 5 spline knots. We selected the model with the best Akaike information criterion (AIC; Sakamoto et al., 1986) and generalized cross-validation score (GCV; Table S1). These models allowed for both qualitative assessment of the broader trends in turnover dynamics through time, as well as quantitative comparisons of the magnitude of local colonizations and extinctions between treatments. For most analyses, we compared Control models to NPK models to emphasize the effect of full nutrient addition. To assess the effect of each nutrient treatment, we averaged the difference in richness between each treatment and the control in each year of treatment (Figure 3).

To evaluate the functional makeup of species gained and lost, we further partitioned the data along three functional axes that characterize below- and above-ground competitive tradeoffs: lifeform (forbs vs. grasses), lifespan (annuals vs. perennials), and provenance (native vs. introduced). Each of these six functional subgroups was modeled separately with GAMs in the same manner described above. Finally, we subtracted Control model predictions from NPK model predictions to focus on the full-treatment effect on gains or losses of each functional group (Figure 4). We did not model changes in proportional richness by functional subgroup because highly uneven sample sizes of some subgroups in many plots misrepresented the magnitudes of change in those plots.

Because nutrient addition treatments favor specific functional groups, they could further impact diversity by constraining the pool of species able to compete and persist. To quantify this, we first created community matrices from each site representing the presence/absence of each potential species gained (“new” species as defined above) after the first full year of treatment or “original” species that persisted through the final year of measurement for each 1m² plot. We then computed the pairwise Jaccard dissimilarity index in species gained/retained after years 0 and 1 among plots within each treatment within each site (Figure S3), and averaged these indices across sites (Figure S4). Higher values indicate that species gained or retained within treatments were highly dissimilar, while lower values indicate greater similarity.

Finally, we identified all species at each site that were gained, lost, or persisted during the length of each site’s study period in Control and NPK treatments only. We then quantified the proportion of these species in each category that were unique to either treatment, and what proportion of each category was identified in both treatments (Figure 7). Any new species as defined above were classified as a species “gained”, any original species that were not present in

the final two years of treatment were classified as “lost”, and original species that were present in the final two years of treatment were classified as “persisted”.

All work was done in R version 4.1.0 (R Core Team, 2021). GAMs were fit and compared using the ‘mgcv’ package (Wood, 2017). Jaccard indices were calculated using the ‘vegan’ package (Oksanen et al., 2013).

Results

Aggregated across 30 sites, control plots exhibited approximately balanced rates of local colonization and extinction resulting in consistent richness through time (Figure 2). However, this consistent richness was maintained by highly dynamic turnover processes. Over a decade, 30-35% of original species were lost in at least one year, while 30-35% of initial richness was gained as new species (Figure 2). In both control and NPK plots, the greatest colonization and extinction rates occurred within approximately the first 5 years of the experiment. While the rate of local extinction leveled off in control plots, enhanced local extinction in NPK plots continued throughout the time series. Thus, full nutrient addition plots (NPK) exhibited overall declines in richness. These declines were primarily driven by local extinction of the original species and secondarily by reduced colonization by new species (in NPK relative to control, 46% more original species were lost vs. 27% fewer new species gained). This relationship was the same using both absolute and proportional metrics of richness. After a decade of treatment, NPK plots contained 3.5 fewer species on average; proportionally, richness declined by 20% in these plots.

Comparing years 0-1 to years 8-10 across all nutrient enrichment treatments, enhanced extinction was generally greater than reduced colonization (as much as 4 times greater, Figure 3). When measured as proportional richness, reduced colonization contributed to richness loss only

in NPK and NK treatments, while enhanced extinction contributed to proportional richness loss in all treatments. The strongest changes in enhanced extinction and reduced colonization for both absolute and proportional metrics were observed in N addition treatments, especially when N was added in tandem with other nutrients. Partitioned along functional axes, forbs contributed disproportionately to enhanced losses in NPK treatments compared to control (Figure 4; 1.76 ± 0.52 SE additional forb losses, 0.22 ± 0.21 SE grass losses, $\Delta 1.99$), as did perennials (1.63 ± 0.46 SE additional perennial losses, 0.46 ± 0.35 SE annual losses, $\Delta 1.17$), and natives (1.80 ± 0.51 SE additional native losses, 0.21 ± 0.30 SE introduced losses, $\Delta 1.58$). There was similar differentiation in functional characteristics of reduced gains under NPK treatment, though the magnitude was muted reflecting fewer reduced gains overall (Figure 4; 0.56 ± 0.18 SE fewer forb gains, 0.09 ± 0.09 SE fewer grass gains, $\Delta 0.47$; 0.65 ± 0.18 SE fewer perennial gains, 0.19 ± 0.18 SE fewer annual losses, $\Delta 0.45$: 63%; 0.74 ± 0.23 SE fewer native gains, 0.18 ± 0.14 SE fewer introduced gains, $\Delta 0.56$).

Nitrogen addition plots had slightly higher Jaccard dissimilarity among replicates and were therefore more variable than non-N treatments in species gained or retained after a decade, contrary to our expectation that nitrogen addition would homogenize plant composition (Figure S4; +3% in N treatments; $t = 4.07$, $p \ll 0.001$). However, analyzing the proportion of species uniquely gained, lost, or persisting in either control or NPK indicates that NPK plots gained slightly fewer overall unique species relative to control plots (-12.0%; Figure S1). NPK plots similarly lost more unique species after a decade of treatment (+14.6%), and fewer unique species persisted relative to control plots (-17.1%; Figure S1).

Absolute gain and loss increased in magnitude with starting initial richness (Figures S2, S3). Higher-richness control plots (20+ species) gained approximately three times as many new

species over the course of the experiment as plots with lower starting richness (< 16 species; Figure S3). The greatest level of extinctions occurred when all nutrients were added together (NPK), and starting richness had the strongest interaction with nutrient addition in these NPK plots (Figure S2). For proportional richness, the opposite trend was observed, albeit weaker in magnitude; despite low richness plots exhibiting the smallest absolute changes, proportional gain and loss decreased with increased total richness. In both the control and NPK treatment, plots with a starting richness less than 11 had the greatest proportional gains (Figures S2, S3).

Discussion

Species richness dynamics are driven by local colonization (gains) and extinction (losses) of species through time (MacArthur & Wilson, 1967). Declines in overall richness may be a consequence of anthropogenic disturbance (Butchart et al., 2010), as is the case in grasslands in response to increased nutrient supply (Harpole et al., 2016; Hautier et al., 2009; Rajaniemi, 2002). Alteration to either the rate of local colonization or extinction can underlie directional responses of species richness to grassland perturbation, but the relative contribution of each is unresolved. Using data from 30 sites across the global Nutrient Network experiment, we found that declines in grassland plant species richness with nutrient addition (N, P and K plus micronutrients) up to a decade were predominantly driven by enhanced rates of local extinction, while reduced rates of colonization played a relevant but secondary role, particularly at shorter time scales.

In aggregate, total richness in control plots remained constant through time despite substantial changes in species composition due to persistent occurrence of colonization and extinction. As a result, the largely stable richness was underlain by a continuous churning of

species. N addition caused declines in richness due to both reduced rates of local colonization and enhanced extinction. However, species loss from enhanced local extinction was 2-5 times greater than from reduced colonization. These treatment effects did not therefore appear to have influenced richness dynamics in control plots despite their proximity (Furey et al., 2022). Similar to our findings, changes to the richness of alpine plant communities as a result of fertilization and soil disturbance were driven primarily by extinctions, rather than colonizations, regardless of whether total richness increased or decreased (Olofsson & Shams, 2007). Likewise, fertilization in a tundra plant community led to more species lost than gained, resulting in an overall decline in richness (Kaarlejärvi et al., 2017). Consistency between our results, aggregated across many sites, and results from these individual sites suggest that fertilization-induced changes in total richness through time are more sensitive to enhanced extinction rates than to constrained colonization in plant communities.

Colonization and extinction over time

We found that species richness responses to nutrient addition were cumulative across years, consistent with classic studies of Nutrient Network or other sites (Bobbink et al., 2010; Borer, Seabloom, et al., 2014; Harpole et al., 2016; Seabloom, Adler, et al., 2021). These changes are not necessarily constant over time, and deconstructing responses into extinction and colonization processes can clarify the timescale-dependence of mechanisms underlying cumulative responses to nutrient addition. Colonization could be amplified early if nutrient-adapted species establish soon after nutrient addition, especially in otherwise nutrient-poor plots, or depressed on longer time scales if fewer random arrivals from the regional species pool are able to successfully colonize (Smith et al., 2009). Extinction, meanwhile, could happen quickly if some existing species are outcompeted by fast-growing competitive dominants. Colonization leveled off in

both control and fertilized plots by the end of 10 years; however, the curve flattened more quickly in fertilized plots, suggesting that fertilized communities quickly constrain the colonization rate of new species. The rate of local extinction of original species, by contrast, continued to increase without leveling off by the end of 10 years. Equilibrium was therefore never reached in plots with chronic NPK addition due to sustained local extinction, implying competitive exclusion at immediate time scales, as well as ongoing extinction that can play out over a decade or longer. Mechanistically, this sustained extinction could reflect an “extinction debt”, where higher abundances in some species slow their eventual extirpation (Cousins & Vanhoenacker, 2011; Kuussaari et al., 2009; Tilman et al., 1994), drawing out the ultimate effect of nutrient enhancement over longer time scales. Similarly, eventual losses could be drawn out in plots or sites with a greater proportion of longer-lived perennials due to slower turnover of pre-established species. If population sizes or life spans are mediating extinction rates, then disturbances such as climate extremes could accelerate losses, suggesting a potentially harmful interaction between global change drivers.

Altogether, nutrient-induced declines in total richness were initially a product of higher extinction rates, which are likely to lead to continued decline in richness beyond our 8-10 year window of observation. Shrinking niche-space is a potential explanation for this decline. Though we do not quantify this explicitly, responses of different functional groups to nutrient addition align with expectations for which types of plants are typically lost when niches shrink. With nutrient addition, forbs, perennials and native species disproportionately contributed to enhanced losses and reduced gains relative to grasses, annuals and introduced species. A functional shift toward faster growth with nutrient addition suggests an enhanced importance of light competition above-ground and, at the same time, a reduced role for below-ground competition as

limiting factors are ameliorated (Harpole et al., 2016; Hautier et al., 2009). These results recapitulate strong theoretical and empirical bedrock predicting these findings, especially in grasslands: enhanced nutrients favor faster-growing, more resource-acquisitive species (Chapin, 1980; Suding et al., 2005; Tilman & Wedin, 1991; Tognetti et al., 2021), which tend to characterize introduced species (Flores-Moreno et al., 2016; Van Kleunen et al., 2010), and consequently disfavor poorer light-competitors such as forbs, and more resource-conservative perennials and natives (Stevens et al., 2006; Suding et al., 2005).

Differentiation in reduced gains among functional groups mirrored losses, suggesting an environmental filter on initial colonization. Interestingly, reduced gains only became functionally differentiated 4-5 years after treatments started. Speculatively, this could reflect a weaker initial environmental filter on colonization that becomes more apparent through time, perhaps reflecting growing dominance of more resource-acquisitive species. Greater habitat connectivity and dispersal could therefore dampen losses under nutrient addition, particularly because grasslands are capable of supporting greater diversity than their steady-state richness suggests (Foster & Tilman, 2003; Shackelford et al., 2021). Accordingly, dispersal limitation may enhance declines in both absolute and functional richness. In aggregate, these results imply that functionally diverse species can colonize nutrient-enriched environments, at least early in treatment, while only a more limited subset can persist. Going forward, closer examination of shifts in the demography of different functional groups could provide a more direct understanding of these consequences for diversity. Furthermore, consideration of shifts in species rank-abundance could better explain the differences in time-scale we observe in altered turnover dynamics (Avolio et al., 2019). Ultimately, absolute richness is only a rough measure of biodiversity change (Hillebrand et al., 2018), and consideration of more comprehensive metrics could refine our

understanding of the role of colonization in maintaining or failing to maintain diversity under nutrient-enriched conditions.

Starting richness and magnitude of response

When accounting for the starting richness of each plot, magnitudes of both absolute species colonization and extinction increased with greater richness, though lower richness plots experienced the greatest proportional change over time. However, these initial proportionate changes in colonization rates leveled off more quickly in lower richness plots, suggesting rapid colonization and dominance by more competitive species at lower richness sites. The consequences of nutrient addition may therefore take longer to manifest in more species rich communities, even if the proportionate endpoints are comparable.

More broadly, the background fluxes in a plot's richness appear to be in proportion to its initial richness. This is not necessarily surprising. Higher richness plots may be associated with greater environmental heterogeneity in limiting factors, resulting in higher turnover (Hodapp et al., 2018; Richardson & Pyšek, 2012). Alternatively, a larger species pool, coupled with variation in dispersal among years, could drive higher colonization rates simply by demographic stochasticity (assuming a relationship between site- and plot-level diversity; (Hubbell, 2005; MacArthur & Wilson, 1967). It is probable that both of these explanations contribute to the relationship observed, though in a highly scale-dependent manner (Chisholm & Lichstein, 2009; Seabloom, Batzer, et al., 2021). Notably, this is contrary to expectations of other frameworks like the diversity-resistance hypothesis (Kennedy et al., 2002). However, the expectation that more speciose communities should be more resistant to invasion (i.e., any species currently absent from a location, native or nonnative) does not pan out in NPK treatments, though we did not control for the prior presence of non-native species (see Firn et al., 2011).

Finally, the rate of colonization only begins to saturate toward the end of our time series in high richness plots. In contrast, lower richness plots reach total saturation in colonization around 5 years from treatment start. This could reflect a larger site-level species pool; in higher richness sites, new species can presumably accumulate for longer by whatever mechanism. This does not account however for earlier saturation in extinction rates in the same high richness plots. It is possible that higher richness plots in our study are, on average, sensitive to and responding to directional environment change even in control plots, especially if this encompassed different pre-treatment management histories (Lindholm et al., 2020), leading to asymmetry between colonization and extinction. The high-richness category was also our smallest category (n = 76 plots). Higher richness plots could therefore be more sensitive to outliers, particularly if those plots are (for example) recovering from disturbance.

Similarity and variation across nutrient treatments

We found that patterns of total richness, extinction (loss of original species), and colonization (gain of new species) were directionally consistent across nutrient addition treatments, where all combinations of nutrients had reduced total absolute richness. Treatments containing N had the greatest effect on original species loss, aligning with many previous studies that have shown that N enrichment leads to species losses across scales (Bobbink et al., 1998, 2010; De Schrijver et al., 2011; Field et al., 2014; Stevens et al., 2004). Limitation of grassland production by N and P (Fay et al., 2015) may alter the competitive growth environment, determining grassland richness. We find mixed evidence for the impact of P enrichment on grassland richness; P-addition alone showed little effect on richness, but P in synergy with either N or K enhanced richness loss over the effect of either nutrient alone. Unsurprisingly, K (plus micronutrients) addition had no significant effect until added with N or K because the

aboveground biomass production of few grasslands is limited by micronutrients, alone (Fay et al., 2015).

Overall, treatments with increasing numbers of nutrients added, especially in combination with N, increasingly magnified the loss of total richness. Similarly, we did not find a decrease in colonization rates until multiple nutrients were added. Indeed, N alone can *increase* proportional colonization, suggesting that these effects may depend on the site's initial diversity and fertility. Collectively, these results suggest that a loss of local diversity through both enhanced extinction and reduced colonization may be driven by enhanced production with the addition of a greater number of nutrients (Harpole et al., 2016; Tilman et al., 1982). As more nutrients are available belowground, competition transitions aboveground for space and light (Hautier et al., 2009), or at least increases in importance (Grime, 1973; Newman, 1973). As competition shifts aboveground, new species may have difficulty establishing or increasing when rare (Hastings, 1980), thus reducing colonization or enhancing local extinction. We see a disproportionate loss of forbs, perennials and native species in alignment with this interpretation. These effects are likely scale-dependent however, preventing some generalization. Fertilized treatment plots were connected to the metacommunity of a site, allowing storage of propagules in the regional species pool. In contrast, atmospheric nitrogen deposition takes place at broader scales, potentially reducing the possibility for recolonization of original species facing local extinction.

Conclusions

We found that plant richness in grasslands arises from a balance of colonization and extinction, but with considerable species turnover. Thus, conceptions of stable richness in

grasslands through time must also appreciate that species composition and potentially associated functional characteristics may be fluid. Declines in richness associated with nutrient enhancement resulted from a disruption of this equilibrium. The loss of diversity with nutrient enhancement can therefore be understood as a product of two unequal processes. The failure of pre-established species to persist under enriched conditions generally accounts for the greatest loss in richness, particularly with addition of N, though this may only be evident with long-term assessment. Some loss in richness may also stem from a constraint in the number of species able to initially colonize in new conditions. These losses are predominantly characterized by slower-growing, more resource-conservative species, suggesting a shift in competition from below-ground resources to light. Furthermore, baseline community richness can mediate the relative rate of these processes. Lower-richness communities are more immediately sensitive to nutrient enhancement, changing the most within five years of fertilization. Conversely, the extinction impacts of fertilization may take longer to manifest in higher richness sites. Altogether, appreciation for the proximate impacts of nutrient enhancement on transient dynamics in grasslands draws the most relevant ecological mechanisms and their timescales into sharper relief.

Author Contributions

All contributions can be found as supplementary material. Briefly, AJM, CREW, GRA, EAS, MFC, LA, AB, PBR, ML, LMH conceived and conceptualized questions. AJM, CREW and GRA wrote the manuscript. All authors contributed feedback, sections and edits to the manuscript. AJM, CREW and GRA implemented data analysis. All authors provided feedback on methods. LMH, AM, ETB, EWS, SH, JAH, KJK, JMHK, SAP, NH, LB, TO, CAA, DDB, EEC, PAF, and JLM contributed site data. ETB, EWS, SH and PW coordinated and managed standardized experimental protocols and site data.

Data Availability

All data are archived and distributed via the Nutrient Network (NutNet).

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Figures

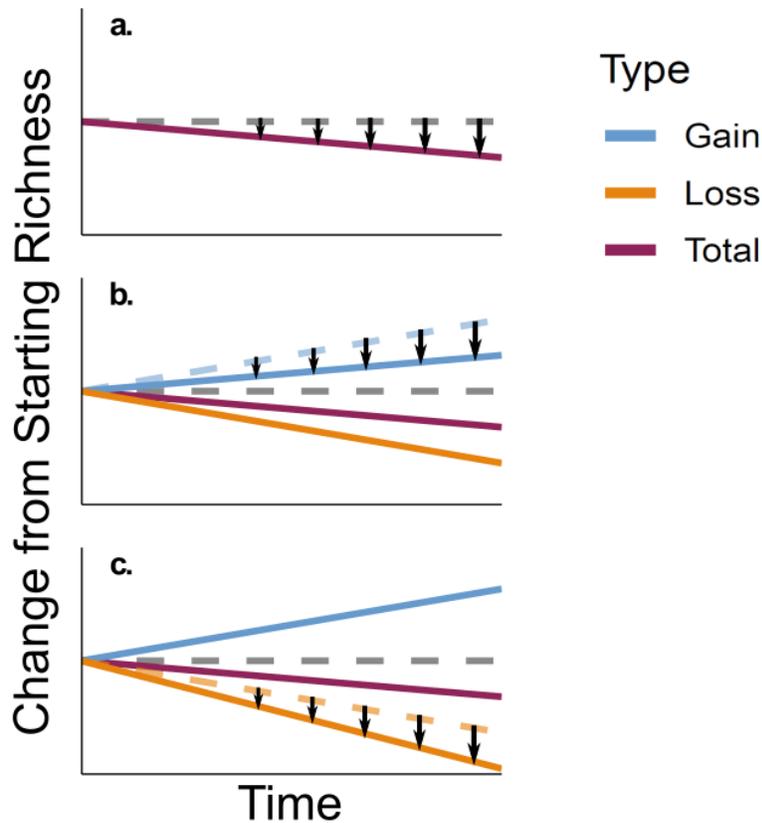


Figure 1: Conceptual figure describing how a change in total richness can arise from different alterations to turnover dynamics. Arrows indicate a change from control conditions. (a) Total richness declines relative to the control with nutrient addition. This phenomenon could result from a reduced rate of gain of new species through time (b), or from an enhanced rate of loss of species originally present pre-treatment through time (c), or some combination of these two processes. Purple lines are the change in total richness, blue lines are the gain of species not already present in treatment year 0, and orange lines are the loss of species from treatment year 0. Dotted lines represent rates in control treatments, and solid lines rates under nutrient addition.

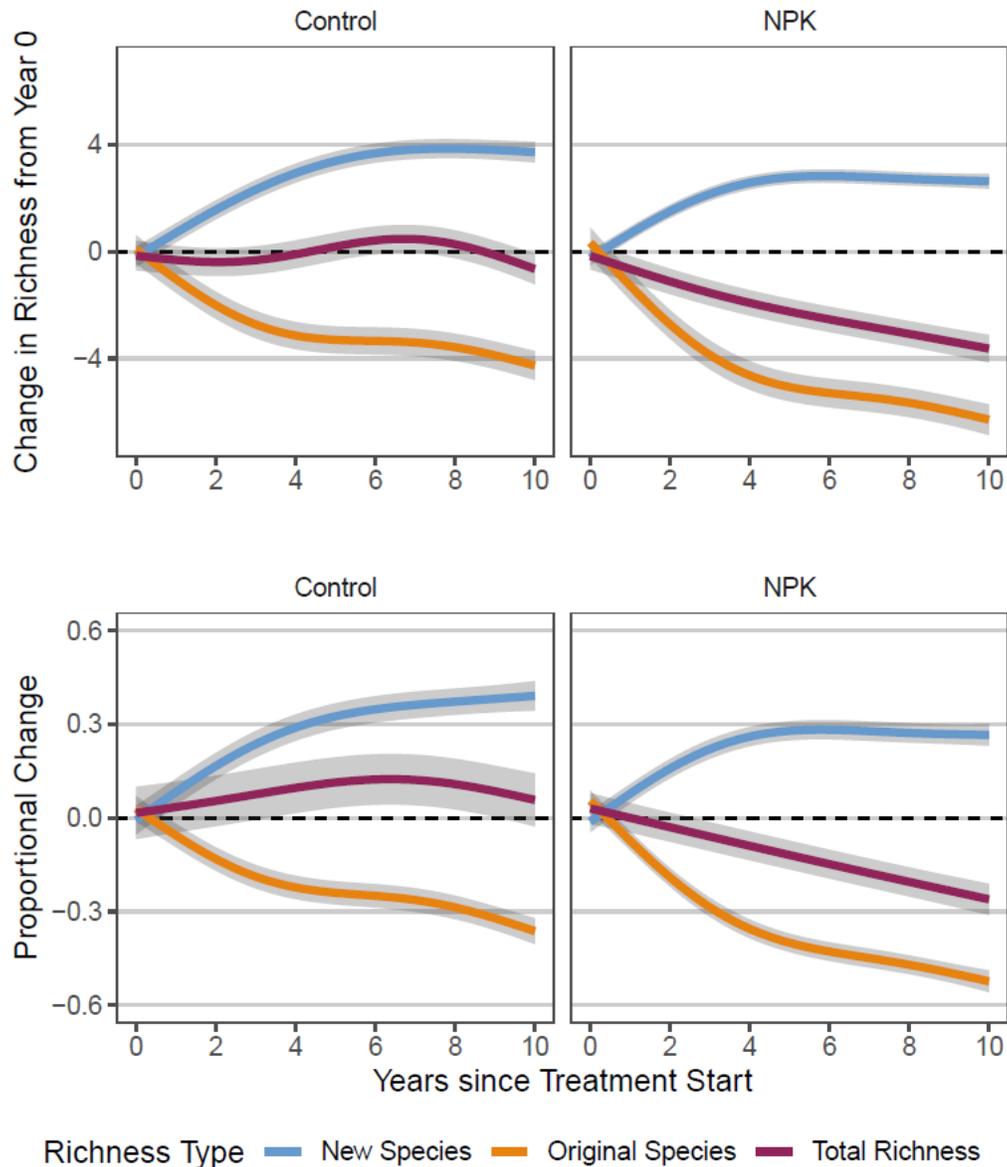


Figure 2: The change in absolute (top) and proportional (bottom) richness from baseline during the first 10 years of measurement in the control (left) vs NPK addition treatments (right), aggregated across 30 NutNet sites. Lines are fit from generalized additive models with site, block and plot as nested random effects. Proportional richness is the change in richness relative to plot richness in year 0. Confidence intervals are one standard error from the estimate. Purple lines are

the change in total richness, orange lines are the loss of species present in treatment year 0 and 1, and blue lines are the gain of species after treatment year 1.

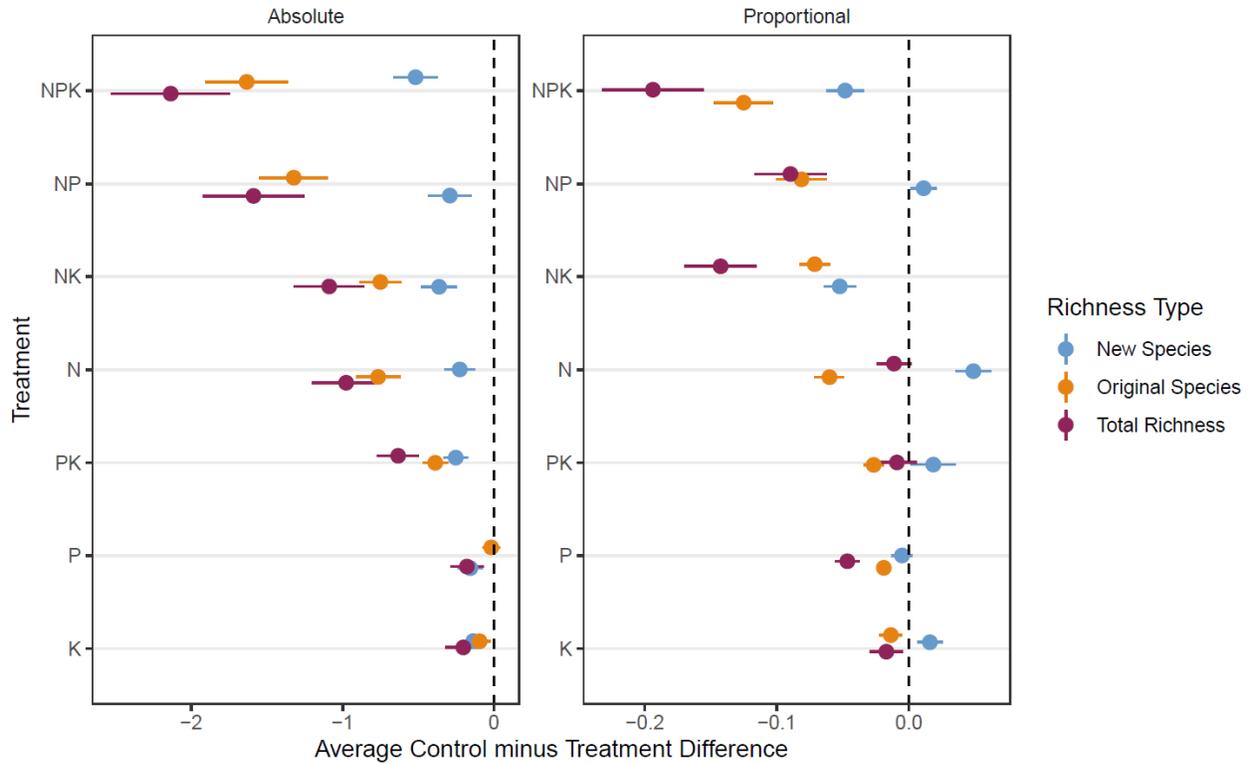


Figure 3. The change in mean annual richness of each nutrient treatment relative to the control (dashed vertical lines) calculated for each treatment year and averaged across all years for (a) absolute and (b) proportional richness. Error bars are one standard error of the mean. Purple points are the change in total richness relative to the control, blue points are the partition of total richness change associated with new species gain, and orange points are the partition associated with loss of original species.

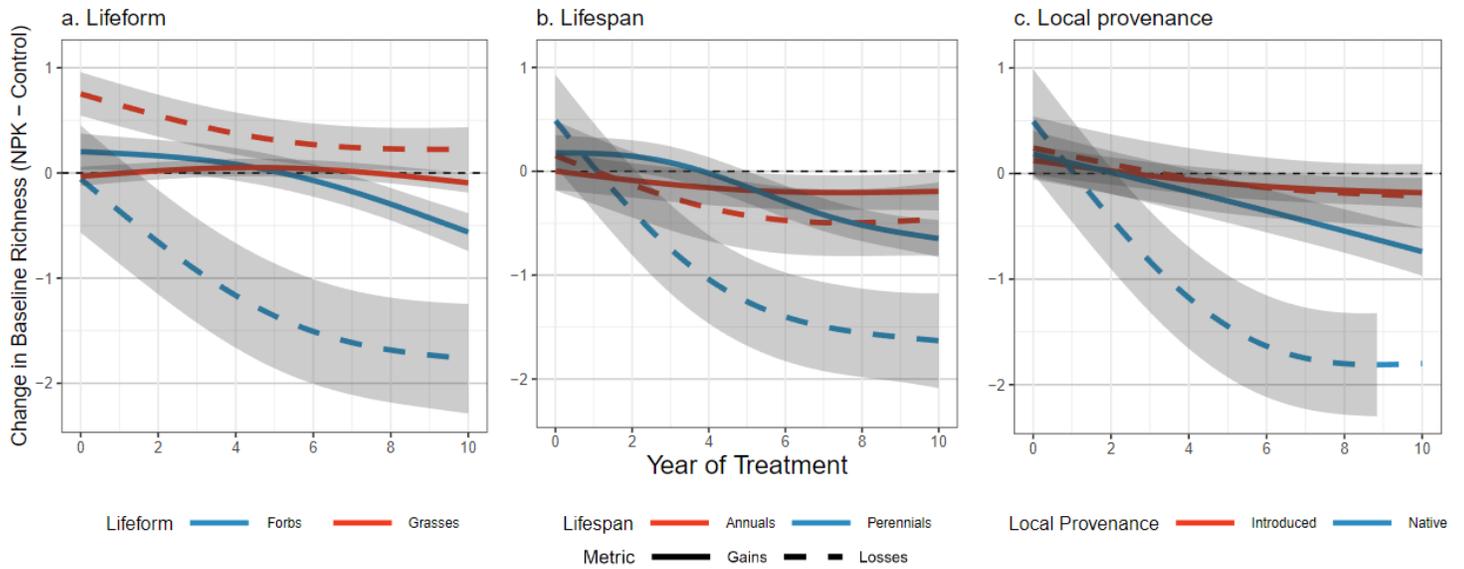


Figure 4: The difference between modeled “Control” change in richness through time and “NPK” change in richness through time (NPK – Control), partitioned by species gained (solid line) or species lost (dotted line) and by functional subgroup: (a) lifeform, (b) lifespan and (c) local provenance. This difference represents the “effect size” of NPK treatment on the Control treatment gains or losses through time, aggregated across 30 NutNet sites. Negative values indicate fewer species gained or more species lost in NPK compared to Control treatments respectively.

