

Running Head: Increasing Nutrient Enrichment Effects

INCREASING EFFECTS OF CHRONIC NUTRIENT ENRICHMENT ON PLANT DIVERSITY LOSS AND ECOSYSTEM PRODUCTIVITY

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Abstract. Human activities are enriching many of Earth's ecosystems with biologically limiting mineral nutrients such as nitrogen (N), phosphorus (P), and potassium (K). In grasslands, this enrichment generally reduces plant diversity and increases productivity. The widely demonstrated positive effect of diversity on productivity suggests a potential negative feedback, whereby nutrient-induced declines in diversity reduce the initial gains in productivity arising from nutrient enrichment. In addition, plant productivity and diversity can be inhibited by accumulations of dead biomass, ~~and which may be altered by~~ nutrient enrichment ~~may speed or slow decomposition, potentially increasing or decreasing accumulation of dead plant biomass.~~ Over longer timeframes, nutrient addition can increase soil fertility by increasing soil organic matter and nutrient pools. We examined the effects of 5-11 years of nutrient addition at 47 grasslands in twelve countries. Nutrient enrichment increased aboveground live biomass and reduced plant diversity at nearly all sites, and these effects became stronger through time. We did not find evidence that nutrient-induced losses of diversity reduced the positive effects of nutrients on biomass, however ~~nutrient-induced dead biomass accumulation reduced live biomass~~ nutrient effects on live biomass increased more slowly at sites where litter was also increasing ~~ass~~, regardless of plant diversity. This work suggests that short-term experiments underestimate the long-term effects of human-caused nutrient enrichment on global, grassland ecosystems.

Keywords: Nutrient Network, NutNet, Community Ecology, Biodiversity, Ecosystem Ecology
Grasslands

Human activities have increased the input of limiting nutrients into many ecosystems through burning of fossil fuels, use of agricultural fertilizers, and other activities that create and distribute biologically available nutrients (Vitousek et al. 1997a, Vitousek et al. 1997b, Steffen et al. 2015). This enrichment can alter ecosystem productivity, biogeochemistry, species richness, and species composition (Lawes and Gilbert 1880, Vitousek et al. 1997b, Elser et al. 2007, Hillebrand et al. 2007, Lewandowska et al. 2016, Simkin et al. 2016, Midolo et al. 2019). Because of the tight coupling between fluxes of inorganic and organic material and the effects of biodiversity on ecosystem processes (Hobbie 2008, Tilman et al. 2014, Hobbie 2015, Riggs et al. 2015), there is the potential for indirect effects or feedbacks that may increase or dampen the effects of global nutrient enrichment over time (Smith et al. 2009, Isbell et al. 2013a, Avolio et al. 2014, Smith et al. 2015).

Experimental work in grassland ecosystems illustrates the potential for indirect effects and feedbacks to alter the impacts of nutrient enrichment over time. In many types of grasslands (e.g., alpine tundra, Africa grassveld, montane meadows, mesic grasslands, prairies, desert grasslands, and old fields), addition of limiting mineral nutrients, such as nitrogen and phosphorus, often rapidly leads to an increase in biomass production and a loss of plant diversity (Lawes and Gilbert 1880, Elser et al. 2007, Borer et al. 2014b, Fay et al. 2015, Gasarch and Seastedt 2015, Ward et al. 2017). Because of the negative effect of plant diversity loss on productivity (Reich et al. 2001, Tilman et al. 2014), nutrient-induced losses of biodiversity may diminish the effect of nutrient addition on productivity over time (Isbell et al. 2013a). Nutrient enrichment also can alter decomposition rates and carbon cycling (Knorr et al. 2005, Hobbie 2008, Hobbie 2015, Riggs et al. 2015), which may change the accumulation of dead plant

biomass. This accumulated dead biomass may limit plant productivity over time by creating a physical barrier or reducing light at ground level (Seastedt et al. 1991, Foster and Gross 1998, Coleman and Levine 2007, Clark and Tilman 2010, Seabloom 2010, Hobbie 2015).

Alternatively, positive feedbacks may increase the strength of nutrient effects over time. For example, increased productivity can increase soil organic matter (Conant et al. 2001, Fornara and Tilman 2012), which in turn may increase water holding and cation exchange capacity, thereby reducing leaching and promoting nutrient retention (Hobbie 2008, Fornara and Tilman 2012, Isbell et al. 2013b, Hobbie 2015), potentially leading to further increases in productivity. If nutrient effects on ecosystems develop slowly over time due to long term feedbacks (Smith et al. 2009, Isbell et al. 2013a, Avolio et al. 2014, Smith et al. 2015), our understanding of nutrient effects on ecosystems may be biased, because most experiments in ecology are relatively short-term (< 5 years) (Elser et al. 2007, Silvertown et al. 2010, Pierik et al. 2011, Smith et al. 2015, Hughes et al. 2017).

While fertilization experiments in grasslands have found that nutrient addition often initially increases aboveground plant biomass and decreases plant diversity (Elser et al. 2007, Borer et al. 2014b, Fay et al. 2015), a few longer term (> 5 yr) experiments suggest that the positive effects of nutrients on aboveground live biomass will either remain constant or decline with time (Jenkinson et al. 1994, Isbell et al. 2013a, Avolio et al. 2014), while the effects on diversity or richness may continue to increase (Isbell et al. 2013a, Harpole et al. 2016) (but see, Pierik et al. 2011). However, it is difficult to draw general inference about how nutrient effects might unfold through time, because the conflicting evidence comes from experiments conducted at different sites and using different treatments and sampling methodologies.

Here we address this knowledge gap by examining how increased nutrient supply (nitrogen, phosphorus, potassium, and micronutrients) affects aboveground live biomass, dead biomass (plant litter), and diversity over time using a 5-11 year nutrient-addition experiment replicated at 47 grassland sites in twelve countries on six continents that are part of the Nutrient Network (NutNet) distributed experiment (Borer et al. 2014a, Borer et al. 2017). This experimental network allows novel insights, because each site uses identical treatments and sampling methodologies, in contrast to meta-analyses in which methodological and biological signals are often confounded (Elser et al. 2007, Gruner et al. 2008, Midolo et al. 2019). Furthermore, these treatments are replicated across a wide range of grassland ecosystems including alpine tundra, montane meadows, mesic grasslands, prairies, desert grasslands, and old fields, thereby capturing important environmental gradients of elevation (0-4241 m), latitude (38° S to 69° N), mean annual precipitation (250 – 1900 mm yr⁻¹), soil nutrient levels (e.g., 270-1200 ppm N, 10—230 ppm P), species richness (3-26 g m⁻²), and aboveground live biomass (28 – 870 g m⁻²), our measure of net primary production. Analyses of short-term NutNet data have shown that nutrient addition generally decreases diversity and increases aboveground biomass (Elser et al. 2007, Borer et al. 2014b, Fay et al. 2015).

We focus on grasslands, because these systems have been used extensively to test the interactive effects of nutrient enrichment and biodiversity on ecosystem processes (Elser et al. 2007, Tilman et al. 2014), and because grasslands account for about a third of terrestrial productivity (Chapin et al. 2002), making them important regulators of the global carbon cycle and climate. From a conservation perspective, grasslands are among the most endangered of the terrestrial ecosystems due to extensive conversion to human-dominated land uses, biological invasions, and well-documented loss of diversity in response to nutrient enrichment (Hoekstra et al. 2005,

Ramankutty et al. 2008, Seabloom et al. 2013). Grasslands are also logistically tractable, as the small stature of the plants allows for smaller plots sizes. In addition, a few plant families of similar structure dominate grasslands worldwide, which makes it easier to compare results across worldwide.

In our analyses, We-we explicitly test (1) if the strength of nutrient effects on plant biomass and diversity changes over time, (2) if nutrient-induced diversity loss reduces the positive effects of nutrient addition on plant biomass, and (3) whether the nutrient-induced accumulation of dead biomass reduces in the positive effects of nutrient addition on plant biomass?

METHODS

Study System and experimental design.

This work was conducted within the Nutrient Network Distributed Experiment, a globally replicated nutrient and herbivore manipulation experiment (NutNet; www.nutnet.org) (Borer et al. 2014a, Borer et al. 2017). For this study, we used data from 47 sites that had been receiving nutrient addition for 5-11 years (Appendix S1: Appendix S1: Table S7). At each of these sites, we replicated an identical experiment that factorially combined three nutrient-addition treatments each at two levels (Control or Fertilized): Nitrogen Addition (+N; 10 g N m⁻² yr⁻¹ as timed-release urea), Phosphorus Addition (+P; 10 g P m⁻² yr⁻¹ as triple-super phosphate), and Potassium and Micronutrient Addition (+K; 10 g K m⁻² yr⁻¹ as potassium sulfate and 100 g m⁻² yr⁻¹ 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 were applied annually, and the micronutrient mix was applied once at the start of the study to avoid toxicity of largely immobile micronutrients.

The N addition rates ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) were chosen to overcome N limitation, and are higher than would be used if the goal were to mimic anthropogenic N deposition (Clark and Tilman 2008, Stevens et al. 2015). ~~Note that a~~Ammonium nitrate was used in 2007 instead of urea at some sites, but there were no detectable differences between these N sources on plant biomass or diversity (Seabloom et al. 2015).

Each site was set up as a completely randomized blocked design typically with 3 replicates (ranged from 1-6). The experimental unit was a 5 x 5 m plot.

Starting in the year prior to fertilization, we annually sampled aboveground plant biomass and plant community composition. Biomass was sampled by clipping all aboveground biomass (live and dead) in two 0.1 m x 1 m strips, sorting current year's biomass (live biomass) from previous year's biomass (dead biomass), drying the biomass to a constant mass at 60 °C, and weighing it to the nearest 0.01 g. The locations of the biomass strips were moved each year to avoid effect of the harvest on production estimates in subsequent years. We sampled plant community composition in a permanent 1 m x 1 m quadrat by visually estimating the areal cover of each species independently, such that the total summed cover may exceed 100% in multi-layer communities.

We collected surface soil samples in the pre-experimental duration and every 3-5 years thereafter. Several 10 cm deep cores were combined for each plot and air dried to a constant mass. All soils were then shipped to ~~Seabloom and Borer's~~ lab at University of Minnesota (USA) for storage and processing prior to analyses. A subsample (~80-100g) from each plot was homogenized by grinding the soil with two steel beads (Daisy Premium 3/8" steel slingshot ammo) with 90 minutes of vigorous shaking using a paint shaker. Approximately 20 (18-25) mg

of ground, homogenized soils were then packed into 5 x 9 mm tin capsules for carbon and nitrogen analysis using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) calibrated with the analytical standard, atropine ($C_{17}H_{23}NO_3$). 20g of the ground, homogenized soils were sent to Waypoint Analytical (Memphis, TN, USA) to measure major nutrients, micronutrients, soil pH, organic matter, cation exchange capacity, and texture of the soil (percent sand, silt and clay; only measured in the Control Plots). Phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, zinc, and sodium (in parts per million) were measured using the Mehlich-3 method. Soil pH was measured with a water pH meter on a 1:1 soil:water suspension. Cation exchange capacity (CEC), reported here as meq/100 g (milliequivalents of charge per 100 g of dry soil), is a measure of the capacity of soil surfaces to retain cations and is used as an indicator of quality and productivity of the soil. CEC was calculated using the ppm of Ca, Mg, and K reported from the Mehlich-3 method using the following relationship: $CEC = (ppm\ Ca / 200) + (ppm\ Mg / 120) + (ppm\ K / 390)$. Percent organic matter content in soil was measured using the Loss on Ignition (LOI) method (combustion for two hours at 400 °C). Values of organic matter reported here were not treated with acid prior to combustion. Texture was measured using the hydrometer method. In brief, the soil sample was shaken with Sodium Hexametaphosphate (HMP) solution, and then transferred to a settling cylinder and mixed. The percent sand, silt, and clay particles were calculated from hydrometer density readings taken at 40 seconds and two hours.

Diversity Metrics

We measured plant diversity as the Effective Number of Species based on the Probability of Interspecific Encounter (ENS_{PIE}), as this measure provides a more scale-independent measure of potential biodiversity effects than richness and is much more robust to the effect of rare species

than species richness (Chase and Knight 2013). ENS_{PIE} estimates the number of equally abundant species and is equivalent to the Inverse Simpson's index of diversity. We calculated ENS_{PIE} as $1/\sum_{i=1}^S p_i^2$ where S is the total number of species and p_i is the proportion of the community cover represented by species i (Chase and Knight 2013). Simpson's evenness (E) is directly related to ENS_{PIE} through the relationship $E = ENS_{PIE} / S$ (Smith and Wilson 1996), thus we can factor diversity directly into its richness and evenness components through the relationship:

$$ENS_{PIE} = S * E$$

ENS_{PIE} was positively correlated with richness ($r=0.742$) but only weakly correlated with evenness ($r=0.1622$) across all samples in our data. Richness and evenness were negatively correlated ($r=-0.41$).

Data Analyses

Ecosystem responses to nutrient enrichment could follow a variety of different shapes through time (e.g., no change or accelerating, decelerating, or linear change through time)(Smith et al. 2009, Smith et al. 2015), so we fit a flexible model that allowed for nutrient effects to increase or decrease in strength through time and for the shape of these curves to vary. Considering the case where nutrient effects are getting stronger through time, we might see a number of different curve shapes:

Decelerating effects through time: this would be the case where effects initially increase in strength, but this rate of change slows as the system starts equilibrating to a new level (e.g., Isbell et al. 2013a). In this case, a plot of effect size through time will be concave down.

212 *Accelerating effects through time:* in this case, we would see little response for a period of time
213 and then a rapidly increasing effect size. This could occur in a buffered system, where a
214 treatment needs to exceed a threshold before the system changes. In this case, a plot of effect
215 size through time will be concave up.

216 *Linear effects through time:* While unrealistic in the long-run, ~~there could be a case where we~~
217 ~~have not run the experiment to see any deceleration it is possible we could observe a linear~~
218 ~~change in effect size over the duration of the so the effect sizes keep increasing through time.~~
219 ~~experiment.~~

220 We used a statistical model that allows all of these shapes. Specifically, our statistical model of
221 biomass or diversity change through time was as follows:

$$222 \log(y) = \beta_0 + \beta_1 N + \beta_2 \log(t) + \beta_3 \log(t)N$$

223 Where β_i is the i th regression coefficient, N is a dummy variable that indicates whether a sample
224 is from a control plot ($N=0$) or a nutrient addition plot ($N=1$), and t is the duration of the
225 experiment (years of treatment). For control plots ($N=0$), our predictive equation in log space is:

$$226 \log(y) = \beta_0 + \beta_2 \log(t)$$

227 and for nutrient addition plots, our predictive equation was:

$$228 \log(y) = (\beta_0 + \beta_1) + (\beta_2 + \beta_3) \log(t)$$

229 In the case where we were modeling plant biomass ($y = \log(\text{biomass})$), β_0 is the mean biomass
230 ($\log \text{ g m}^{-2}$) in a control plot at time = 1, β_0 is the marginal increase in biomass ($\log \text{ g m}^{-2}$) due to

231 adding nutrients in year 1, β_2 is the change in mean biomass (log g per log yr), and β_3 is the
232 marginal effect of adding nutrients on the change in mean biomass (log g per log yr).

233 In back-transformed space, we get following relationship:

234
$$y = e^{\beta_0 + \beta_1 N} t^{\beta_2 + \beta_3 N}$$

235 For control plots ($N=0$), our predictive equation was:

236
$$y = e^{\beta_0} t^{\beta_2}$$

237 In this case, if $\beta_2 < 1$ the rate of biomass change through time is slowing (Case 1), if $\beta_2 > 1$ the
238 rate of biomass change through time is increasing (Case 2), and if $\beta_2 = 1$ there is a linear change
239 in biomass over time (Case 3). The mean biomass (g) in a control plot in year 1 ($t=1$) is e^{β_0} .

240 For nutrient addition plots, our predictive equation is:

241
$$y = e^{\beta_0 + \beta_1 t} t^{\beta_2 + \beta_3}$$

242 In this case, if $(\beta_2 + \beta_3) < 1$ the rate of biomass change through time is slowing (Case 1), if
243 $(\beta_2 + \beta_3) > 1$ the rate of biomass change through time is increasing (Case 2), and if $(\beta_2 + \beta_3) = 1$
244 there is a linear change in biomass over time (Case 3). The mean biomass (g) in a fertilized plot
245 in year 1 ($t=1$) is $e^{(\beta_0 + \beta_1)}$.

246 To test whether the effects of nutrient addition increased over time, we used a mixed-effects
247 model with +N, +P, and +Kμ, and experimental duration. Experimental duration is the number of
248 years the nutrient treatments had been applied; experimental duration was log₁₀ transformed. We
249 included experimental duration and site as random effects. Because of the large number of

parameters, the model with effects of all 8 nutrient treatments (+N \times +P \times +K μ) and their interactions with experimental duration with random slope and intercepts for each site did not converge. For this reason, we estimated treatment differences among sites (random slopes and intercept) over time using only the Control and All Nutrient (+N, +P, and +K μ). Model specifications in R are included with each table of regression results.

We tested for interactions between nutrient addition effects on live biomass, dead biomass, and diversity over time, to determine if nutrient effects became weaker over time at sites where nutrients lead to rapid declines in diversity or increases in dead biomass. To do this, we tested for correlations between the slopes of the nutrient effect on each of these metrics and experimental duration, as estimated by the nutrient effect by experimental duration interaction. For example, we tested if sites with increasing losses of diversity through time would also eventually have weakening nutrient effects on live biomass through time. Similarly, if nutrient enrichment induced increases in dead biomass that reduced productivity, we would expect a negative correlation between the nutrient by experimental duration interaction for live and dead biomass, indicating that nutrient effects on dead biomass were increasing and nutrient effects on live biomass were decreasing over time. We tested this relationship using standardized major axis (SMA) estimation (Warton et al. 2006). SMA summarizes the relationship between two variables, as opposed to predicting the value of one variable (i.e., the dependent variable in regression) using a second variable (the independent variable in regression). We used the `sma` function in the `smatr` R package.

We also used regressions to examine whether nutrient effects on live biomass, dead biomass, and plant diversity increased or decreased through time. At the site level, we included a water

availability index (Mean Annual Precipitation/Potential Evapotranspiration), mean annual precipitation, mean annual temperature, mean diurnal temperature range, mean annual temperature range, and temperature in the wettest quarter. The plot-level soil predictors were pre-treatment soil pH, total base cations (sum of Ca, Mg, and K), % soil N, and the soil C:N ratio. Note that % soil N, % soil organic matter, and % soil organic C are all highly correlated ($r > 0.92$ for all comparisons), so we only included % soil N in the regression models. Similarly, cation exchange capacity and base cations are highly correlated ($r = 0.93$), so we only include base cations in the regression models. The plant community measures included were pre-treatment live biomass, dead biomass, and plant diversity. Finally, we included the change in nutrient effect over time as described in the correlation analysis above. For example, we included the change in nutrient effect over time on dead biomass and plant diversity as explanatory variables of the change in nutrient effect over time on live biomass. In our regression of site level characteristics that could predict the change in nutrient effects over time, many of our predictor variables co-varied (e.g., pre-treatment live biomass, soil N, soil C:N, and ~~aridity~~water availability index). To account for the potential for multiple models that had similar explanatory power due to this correlation, we used a multi-model approach, as described in Grueber et al. (2011) using the dredge and model.avg functions in the MuMIn library. We standardized the input variables using the arm library. In the model averaging, we included all models within 4 AIC_c units of the best model. We ran these models with and without soils data, as we were missing soils data at some sites.

All analyses were conducted in R (v. 3.4.1; R Foundation for Statistical Computing, Vienna, Austria). Mixed effects models were fit using the lmer function in the lme4 R library.

295 ~~We tested for declining effects of nutrient addition on live biomass, associated with loss of~~
 296 ~~diversity, however this was not the case. Instead~~In contrast to our expectations, we found
 297 increasing effects of nutrient addition on live biomass through time at ~~almost all~~most sites
 298 despite increasing ~~losses of diversity~~ (Figs. 1 & 2), and there was an overall significant
 299 interaction between treatment duration and fertilization (Table S2). While the nutrient effects
 300 grew stronger, the rate of change was decelerating in most cases. Nutrient enrichment,
 301 especially the addition of N, increased live biomass but did not have a consistent effect on dead
 302 biomass and there was not an interaction between fertilization and treatment duration (Figs. 1 &
 303 3; Appendix S1: Tables S1 & S2). The effects of nutrient enrichment on live biomass increased
 304 at ~~nearly all~~most sites for up to 11 years (Fig. 1; Appendix S1: Tables S1 & S2). In contrast to
 305 the consistent effects of nutrient addition on live biomass, nutrient effects on dead biomass
 306 became stronger at some sites and weaker at others (Fig. 1; Appendix S1: Tables S1 & S2).
 307 Nutrient addition caused increasingly strong reductions in plant diversity (ENS_{PIE}) at nearly all
 308 sites (Figs. 2 & 3; Appendix S1: Table S1 & S2), primarily due to the effects of N addition (Fig.
 309 3, Appendix S1: Table S1). This loss in diversity was primarily caused by increasing losses of
 310 richness at all sites (Figs. 2 & 3). In contrast to richness, nutrient ~~addition~~-effects on evenness did
 311 not show a consistent increase over time (Fig. 2). The change in ~~Nutrient-nutrient~~ effects on
 312 diversity over time were not correlated with change in nutrient effects on live biomass over time
 313 (Fig. 4), however there was a negative correlation between the trajectory of nutrient effects on
 314 live and dead biomass over time (Fig. 4).

In addition to the negative effects of dead biomass on live biomass, we found evidence that nutrient effects on live biomass were stronger at more productive sites with higher pre-treatment biomass (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). Similarly, we found that nutrients effects on diversity loss increased in strength faster at sites that had high diversity at the start of the experiment (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). Nutrient effects on dead biomass were weaker at sites with high levels of pre-treatment dead biomass when we included soil chemistry in the models which include 37 of 47 sites, but this effect was not significant for the full set of sites (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). A potential determinant of dead biomass is domestic grazing, as the size sites with domestic grazers present had lower levels of dead biomass in control plots ($p=0.011$). There was no evidence that estimates of the change in nutrient effects over time were affected by among-site differences in experiment duration, which ranged from 5-11 years (Appendix S1: Tables S4 & S5). Furthermore, the change in nutrient effects over time was not dependent on a site's climate or soil chemistry (Appendix S1: Tables S4 & S5).

Nutrient addition increased soil nutrient pools, carbon, and organic matter. Specifically, N addition led to increased soil C and N (Appendix S1: Fig. S7; Appendix S1: Table S6), and P addition increased soil P, cation exchange capacity, and base cations, possibly due to the calcium (Ca) in the P source (triple-super-phosphate; Appendix S1: Fig. S7; Appendix S1: Table S6).

DISCUSSION

Short- and long-term studies have identified differing responses to chronic nutrient enrichment. Although an analysis of nutrient responses at 42 grassland sites in the NutNet experiment found

increasing effects of fertilization on live biomass over the short-term (3 years) (Fay et al. 2015), we expected, based on previous long-term fertilization experiments, that the positive effects of nutrients on aboveground live biomass would either plateau and remain constant or decline with time (Jenkinson et al. 1994, Isbell et al. 2013a, Avolio et al. 2014). In contrast, we found that the effect of nutrient enrichment on live biomass continued to increase at nearly all sites for 11 years (Fig. 1; Appendix S1: Table S1 & S2), despite increasing losses of plant diversity and increasing dead biomass accumulation at some sites. Accumulation of dead biomass was the strongest predictor of the trajectory of nutrient effects over time, with increased biomass accumulation reducing the effects of nutrient on live biomass. We did not find evidence that increasing losses of diversity could cause nutrient effects on biomass to decline over time.

A possible mechanism explaining the increasingly strong effects of fertilization on biomass is a concomitant increase in soil nutrient pools and buildup of soil C and organic matter (Fornara and Tilman 2012, Crowther et al. 2019), which could lead to increased water holding and cation exchange capacity and greater nutrient retention (Fornara and Tilman 2012, Isbell et al. 2013b, Hobbie 2015). We found that N addition led to increased soil C and N (Appendix S1: Fig. S7; Appendix S1: Table S4), and P addition increased soil P, cation exchange capacity, and base cations, possibly due to the calcium (Ca) in the P source (triple-super-phosphate; Appendix S1: Fig. S7; Appendix S1: Table S6). These nutrient-induced alterations to soil chemistry could act to further increase productivity.

While many studies have examined nutrient effects on plant productivity and biomass (Elser et al. 2007), there is little basis for *a priori* expectations of how nutrient effects on dead biomass accumulation would change through time, although we did expect a general coupling between live and dead biomass (Hobbie 2015, Grace et al. 2016). Dead biomass accumulation can

directly reduce plant growth by acting as a physical barrier and reducing light to young plants (Foster and Gross 1998, Coleman and Levine 2007, Clark and Tilman 2010, Seabloom 2010). In the longer term, dead biomass can affect productivity through the influence of senesced plant material on nutrient cycling, either facilitating or delaying nutrient release to plants (Hobbie 2015). In contrast to the consistently positive effects of nutrients on live biomass, nutrients effects on the accumulation of dead biomass increased at some sites and decreased at others (Fig. 1; Appendix S1: Table S1 & S2). In meta-analysis spanning grasslands, forests, and tundra ecosystems, N effects on decomposition has been shown to vary depending on N addition rates, ambient N deposition rates, an litter quality (e.g., lignin content) (Knorr et al. 2005). Based on this work, the N addition rate we used ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) would be expected to increase decomposition rates.

While we did not measure decomposition rates directly, our results suggest that nutrient addition may be increasing the rate of turnover of dead biomass, because fertilization increased biomass production and soil carbon, but it did not consistently increase total standing dead biomass. The effects of nutrients on decomposition are governed by complex feedbacks between primary productivity, plant tissue chemistry, and soil nutrient cycling (Hobbie 2008, Hobbie 2015), and the effects can vary among different locations (Hobbie 2008). Given the potential for increased nutrient supply rates to alter decomposition and ultimately carbon storage, developing a general understanding of nutrient supply on decomposition remains an important unresolved challenge in ecosystem ecology (Hobbie 2008).

Based on past work, we expected nutrients to continuously reduce diversity over time (Isbell et al. 2013a, Harpole et al. 2016), and we found this to be the case (Fig. 2; Appendix S1: Table S1 & S2), primarily due to the increasingly strong effects of N addition (Appendix S1: Table S1).

Declines in diversity could arise either through reduced richness, due to ~~local~~ increased extinction or reduced colonization rates, or reduced evenness, reflecting an increasingly skewed abundance distribution of species. We found that loss in diversity in response to nutrients was driven primarily by continuing richness loss at all sites (Fig. 2). In contrast to richness, nutrient addition did not have a consistent effect on evenness (Tables S1 & S2), and nutrient effects on evenness did not show a consistent increase over time (Fig. 2), although evenness varied widely among sites and across years and nutrient addition did change evenness at individual sites (Figure S5).

Past work suggested that the positive effects of nutrient enrichment on live biomass would decline over time at sites where there was a concurrent loss in plant diversity (Isbell et al. 2013a) or increase in dead biomass. We tested for this relationship by examining the correlations between the change over time in the nutrient effects on live biomass, dead biomass, and plant diversity. We did not find evidence of a negative feedback between fertilization induced diversity loss and live biomass; live biomass continued to increase in response to nutrients for up to a decade at nearly all sites. Furthermore, sites experiencing ongoing diversity loss did not have decelerating nutrient effects on live biomass through time (Fig. 4), as would be expected if the nutrient induced diversity loss were counteracting the effects of nutrients on productivity (Isbell et al. 2013a). It is possible that diversity loss could eventually start to counteract the positive effects of nutrient on plant biomass; the experiment that found this effect had been adding nutrients for more than 25 years (Isbell et al. 2013a). However, the longest running nutrient addition experiment has not shown large shifts after more than a century (Jenkinson et al. 1994). Ultimately, standardized, long-term experiments replicated at sites representing a range of conditions are necessary to distinguish biological from methodological effects of nutrient

addition among sites and studies (Borer et al. 2017). For example, Avolio et al. (2014) found that a decade of N and P addition did not alter the richness in a tallgrass prairie community, while Isbell et al. (2013a) found that the effects of nutrient addition on richness strengthened through time, but in a different tallgrass prairie using different methods. In contrast, our standardized experiment collocated at with these previous studies showed a consistent response of increasingly strong declines in richness over time (konz.us & cdc.us; Figures S1, S3, & S4), suggesting that methodological differences, such nutrient addition rates or sampling protocols, may explain differences in the inference between these two long-term experiments (Isbell et al. 2013a, Avolio et al. 2014).

~~However, d~~Dead biomass accumulation has been shown to suppress plant recruitment in a variety of herbaceous ecosystems (van der Valk 1986, Foster and Gross 1998, Coleman and Levine 2007, Clark and Tilman 2010, Seabloom 2010), and our results supported the generality of this relationship. The smallest change in nutrient effects on live biomass over time occurred at sites where nutrient addition led to increased dead biomass accumulation (Fig. 4).

Our experiment was replicated across a wide range of conditions globally, underscoring the generality of the strengthening of nutrient effects over time. At nearly all sites, nutrient effects on live biomass and diversity ~~continued to increase~~became stronger over time. This suggests that short-term experiments (< 5 years) will underestimate the effects of nutrient enrichment on ecosystems and communities. Experiments spanning multiple decades have shown that short-term experiments may generate biased estimates of long-term treatment effects (Reich et al. 2012, Isbell et al. 2013a), highlighting the value of long-term ecological data (Hughes et al. 2017).

Over multiple decades, nutrient addition can lead to soil carbon accumulation (Fornara and Tilman 2012), which can increase soil moisture and nutrient holding capacity (Hobbie 2015). Via this pathway, plant growth may increase ecosystem productivity, rather than simply depleting limiting nutrients. In our experiment, nitrogen addition consistently increased both soil nitrogen and soil carbon across sites suggesting that nutrient enrichment could increase soil fertility over time, and potentially inducing the continued increase in live biomass in response to nutrients.

Many experiments and models have demonstrated that nutrient addition in grassland ecosystems can induce plant diversity declines (Lawes and Gilbert 1880, Miller et al. 2005, Clark et al. 2007, Harpole and Tilman 2007, Hautier et al. 2009, Borer et al. 2014c, Harpole et al. 2016, Ward et al. 2017), and the few long-term experiments have shown that these effects can continue for decades (Lawes and Gilbert 1880, Jenkinson et al. 1994, Isbell et al. 2013a, Ward et al. 2017). Here we have shown that an increasing effect of nutrients on diversity loss through time is a general phenomenon in many grassland ecosystems. This result suggests that current understanding, which is largely based on short-term experiments (< 5 years) (Elser et al. 2007), may be underestimating the severity of effect of nutrient enrichment on biodiversity. The mismatch between short experimental duration and the time for ecosystems to fully respond to nutrient enrichment is particularly problematic given that human activities are continuing to increase the supplies of limiting nutrients into many of Earth's ecosystems (Vitousek et al. 1997a, Galloway et al. 2008, Steffen et al. 2015).

In interpreting our results, it is important to note that the N addition rates ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) we use were chosen to overcome N limitation, and are higher than would be used if the goal were to mimic anthropogenic N deposition (Clark and Tilman 2008, Stevens et al. 2015). Lower rates of

N addition would likely lead to weaker effects, though still positive, positive effects on live biomass and richness and could potentially inhibit decomposition rates (Knorr et al. 2005, Isbell et al. 2013a, Midolo et al. 2019).

Human activities are concurrently altering a variety of interacting environmental factors that drive ecosystem processes and ultimately ecosystem services that are necessary for human wellbeing (Vitousek et al. 1997a, Vitousek et al. 1997b, Steffen et al. 2015), and the ecosystem-level effects of many of these factors may not be fully evident for a decade or more (Reich et al. 2012, Isbell et al. 2013a). For this reason, long-term experiments are critical for predicting the effects of humans on ecological systems (Silvertown et al. 2010, Hughes et al. 2017). Nevertheless, long-term experiments in ecology remain relatively rare, and funding such experiments is increasing difficult (Silvertown et al. 2010, Hughes et al. 2017). Human impacts on ecosystems also vary spatially, and ecologists have recently started replicating experiments at global scales (Borer et al. 2014a, Borer et al. 2017). Now that some distributed experiments have been in place for more than a decade, we can ask novel questions about the factors that determine the trajectory of ecosystem response to global change (Borer et al. 2017). Here we have shown a remarkably consistent increasing effect of nutrient addition on plant production and concomitant decline in diversity in wide array of grassland ecosystems including deserts, prairies, and alpine tundra. However, these responses were not fully coupled; nutrient-induced declines in diversity did not reduce the effects of nutrients on live biomass. These results suggest that, due to their short duration, many experiments to date have likely underestimated the effects of human driven eutrophication on biodiversity loss and ecosystem productivity.

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Data accessibility statement:

Data supporting the results will be archived at the Environmental Data Initiative (<https://environmentaldatainitiative.org/>). The data DOI will be included at the end of the article upon acceptance.

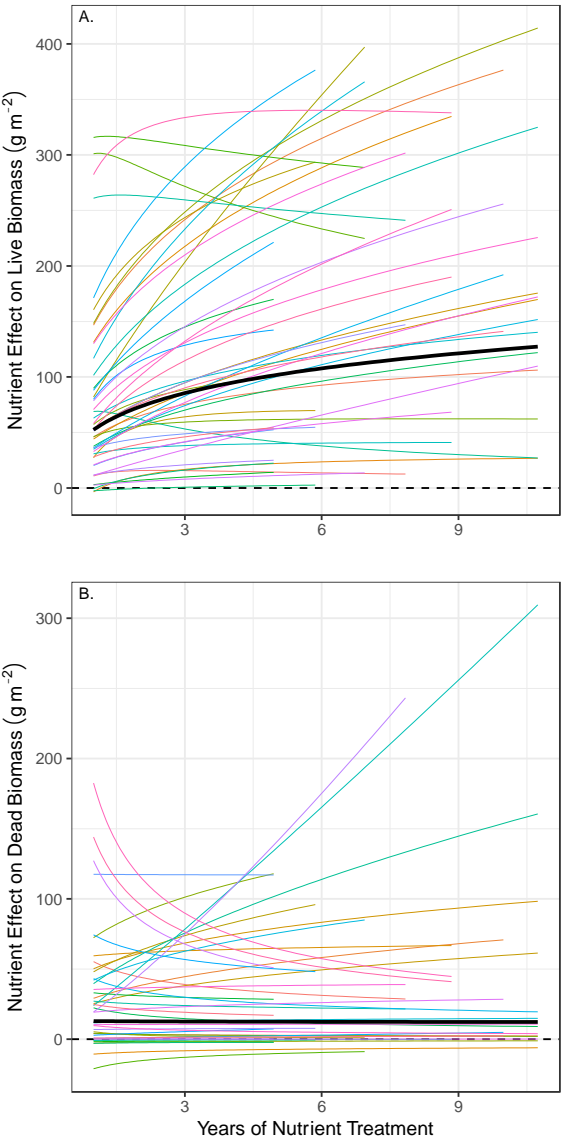
FIGURE LEGENDS

Figure 1. Effect of nutrient enrichment on live and dead aboveground plant biomass in grassland ecosystems. Colored lines indicate individual sites, and the solid black line shows the mean response across sites. Models were fit using $\log_{10}(\text{Treatment/Control})$ versus $\log_{10}(\text{Number of Years of Treatment})$, and back-transformed for plotting as the difference between Treatment (i.e., all nutrients added) and Control plots. Standard errors of all parameter estimates are presented in Appendix S1: Table S2, and site species models with raw data are presented in Figures S1 and S2.

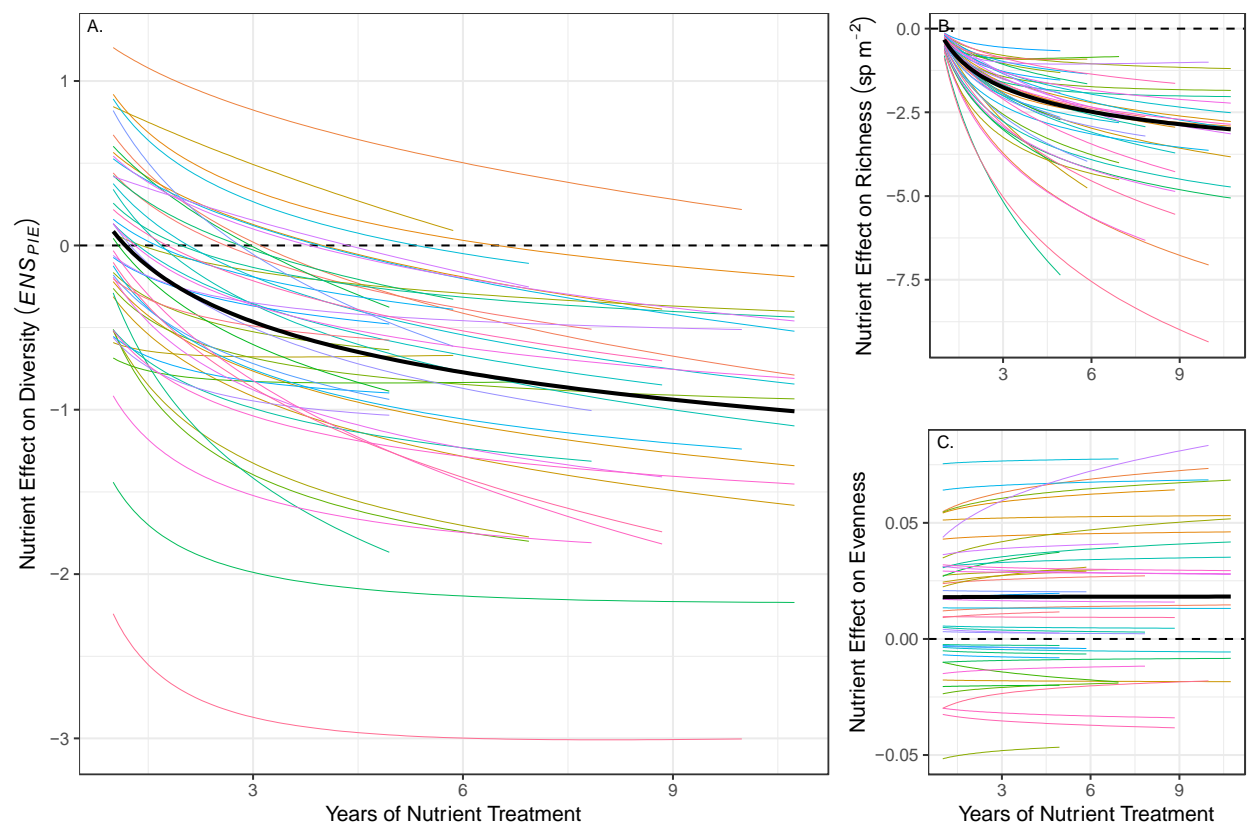
Figure 2. Effects of nutrient enrichment on diversity (ENS_{PIE}), richness (S , species m^{-2}), and evenness ($ENS_{PIE} S^{-1}$) in grassland ecosystems. Colored lines indicate individual sites, and the solid black line shows the mean response across sites. Models were fit using $\log_{10}(\text{Treatment/Control})$ versus $\log_{10}(\text{Number of Years of Treatment})$, and back-transformed for plotting as the difference between Treatment (i.e., all nutrients added) and Control plots. Standard errors of all parameter estimates are presented in Appendix S1: Table S2, and site species models with raw data are presented in Figures S3, S4, and S5.

Figure 3. Effects of nutrient addition treatments on live and dead aboveground plant biomass and diversity (ENS_{PIE}), richness (S , species m^{-2}), and evenness ($ENS_{PIE} S^{-1}$) in grassland ecosystems. Error bars indicate 1 standard error (SE) among site means for each treatment. Parameter estimates and standard errors for all treatment effects are shown in Appendix S1: Table S1.

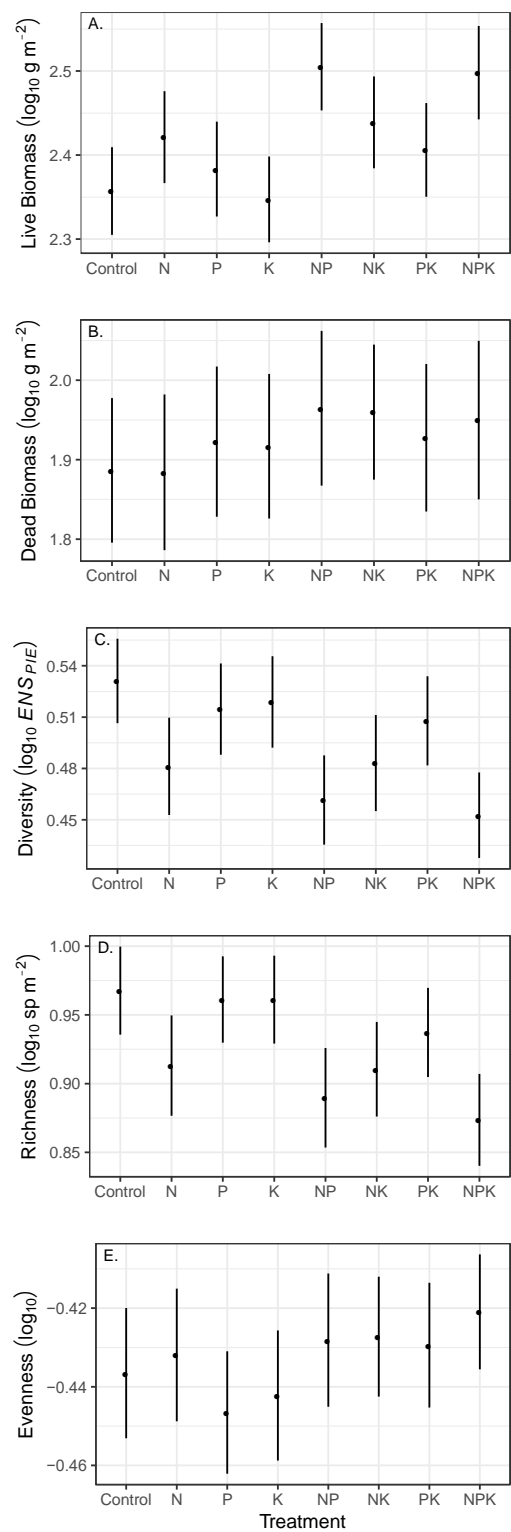
Figure 4. Change over time of the effects of nutrient enrichment on live biomass, dead biomass, and diversity at individual sites (slopes in Figure 1). Values of zero (dashed lined) indicate effects that are constant over time, positive values indicate increasing effects through time, and negative values indicate decreasing effects through time. All slopes are from \log_{10} transformed variables versus \log_{10} transformed number of years of treatment. Diversity is measured as ENS_{PIE} . Correlations and significant tests are based on standardized major axis (SMA) estimation and are detailed in Appendix S1: Table S3. Large open circles represent the mean value across all sites with error bars representing two standard errors of the mean (SEM).



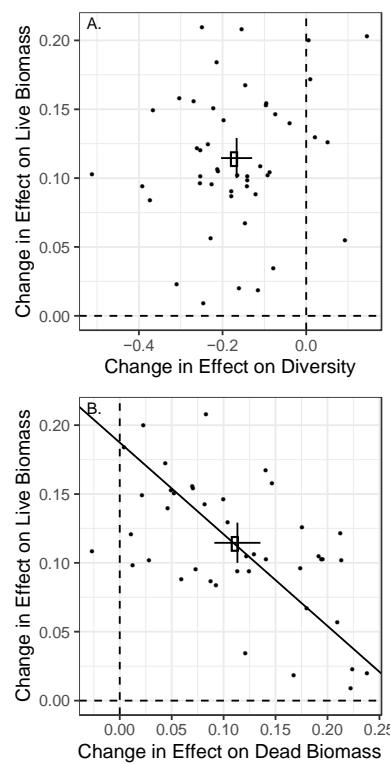
738 **Figure 2.**



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742 **Figure 4.**



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