

1 *Running Head: Increasing Nutrient Enrichment Effects*

2 INCREASING EFFECTS OF CHRONIC NUTRIENT ENRICHMENT ON PLANT DIVERSITY

3 LOSS AND ECOSYSTEM PRODUCTIVITY

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36

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

53 *Keywords: Nutrient Network, NutNet, Community Ecology, Biodiversity, Ecosystem Ecology*
54 *Grasslands*

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57 Human activities have increased the input of limiting nutrients into many ecosystems through
58 burning of fossil fuels, use of agricultural fertilizers, and other activities that create and distribute
59 biologically available nutrients (Vitousek et al. 1997a, Vitousek et al. 1997b, Steffen et al. 2015).
60 This enrichment can alter ecosystem productivity, biogeochemistry, species richness, and species
61 composition (Lawes and Gilbert 1880, Vitousek et al. 1997b, Elser et al. 2007, Hillebrand et al.
62 2007, Lewandowska et al. 2016, Simkin et al. 2016, Midolo et al. 2019). Because of the tight
63 coupling between fluxes of inorganic and organic material and the effects of biodiversity on
64 ecosystem processes (Hobbie 2008, Tilman et al. 2014, Hobbie 2015, Riggs et al. 2015), there is
65 the potential for indirect effects or feedbacks that may increase or dampen the effects of global
66 nutrient enrichment over time (Smith et al. 2009, Isbell et al. 2013a, Avolio et al. 2014, Smith et
67 al. 2015).

68 Experimental work in grassland ecosystems illustrates the potential for indirect effects and
69 feedbacks to alter the impacts of nutrient enrichment over time. In many types of grasslands
70 (e.g., alpine tundra, Africa grassveld, montane meadows, mesic grasslands, prairies, desert
71 grasslands, and old fields), addition of limiting mineral nutrients, such as nitrogen and
72 phosphorus, often rapidly leads to an increase in biomass production and a loss of plant diversity
73 (Lawes and Gilbert 1880, Elser et al. 2007, Borer et al. 2014b, Fay et al. 2015, Gasarch and
74 Seastedt 2015, Ward et al. 2017). Because of the negative effect of plant diversity loss on
75 productivity (Reich et al. 2001, Tilman et al. 2014), nutrient-induced losses of biodiversity may
76 diminish the effect of nutrient addition on productivity over time (Isbell et al. 2013a). Nutrient
77 enrichment also can alter decomposition rates and carbon cycling (Knorr et al. 2005, Hobbie
78 2008, Hobbie 2015, Riggs et al. 2015), which may change the accumulation of dead plant

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

82 Alternatively, positive feedbacks may increase the strength of nutrient effects over time. For
83 example, increased productivity can increase soil organic matter (Conant et al. 2001, Fornara and
84 Tilman 2012), which in turn may increase water holding and cation exchange capacity, thereby
85 reducing leaching and promoting nutrient retention (Hobbie 2008, Fornara and Tilman 2012,
86 Isbell et al. 2013b, Hobbie 2015), potentially leading to further increases in productivity. If
87 nutrient effects on ecosystems develop slowly over time due to long term feedbacks (Smith et al.
88 2009, Isbell et al. 2013a, Avolio et al. 2014, Smith et al. 2015), our understanding of nutrient
89 effects on ecosystems may be biased, because most experiments in ecology are relatively short-
90 term (< 5 years) (Elser et al. 2007, Silvertown et al. 2010, Pierik et al. 2011, Smith et al. 2015,
91 Hughes et al. 2017).

92 While fertilization experiments in grasslands have found that nutrient addition often initially
93 increases aboveground plant biomass and decreases plant diversity (Elser et al. 2007, Borer et al.
94 2014b, Fay et al. 2015), a few longer term (> 5 yr) experiments suggest that the positive effects
95 of nutrients on aboveground live biomass will either remain constant or decline with time
96 (Jenkinson et al. 1994, Isbell et al. 2013a, Avolio et al. 2014), while the effects on diversity or
97 richness may continue to increase (Isbell et al. 2013a, Harpole et al. 2016) (but see, Pierik et al.
98 2011). However, it is difficult to draw general inference about how nutrient effects might unfold
99 through time, because the conflicting evidence comes from experiments conducted at different
100 sites and using different treatments and sampling methodologies.

101 Here we address this knowledge gap by examining how increased nutrient supply (nitrogen,
102 phosphorus, potassium, and micronutrients) affects aboveground live biomass, dead biomass
103 (plant litter), and diversity over time using a 5-11 year nutrient-addition experiment replicated at
104 47 grassland sites in twelve countries on six continents that are part of the Nutrient Network
105 (NutNet) distributed experiment (Borer et al. 2014a, Borer et al. 2017). This experimental
106 network allows novel insights, because each site uses identical treatments and sampling
107 methodologies, in contrast to meta-analyses in which methodological and biological signals are
108 often confounded (Elser et al. 2007, Gruner et al. 2008, Midolo et al. 2019). Furthermore, these
109 treatments are replicated across a wide range of grassland ecosystems including alpine tundra,
110 montane meadows, mesic grasslands, prairies, desert grasslands, and old fields, thereby capturing
111 important environmental gradients of elevation (0-4241 m), latitude (38° S to 69° N), mean
112 annual precipitation (250 – 1900 mm yr⁻¹), soil nutrient levels (e.g., 270-1200 ppm N, 10—230
113 ppm P), species richness (3-26 g m⁻²), and aboveground live biomass (28 – 870 g m⁻²), our
114 measure of net primary production. Analyses of short-term NutNet data have shown that nutrient
115 addition generally decreases diversity and increases aboveground biomass (Elser et al. 2007,
116 Borer et al. 2014b, Fay et al. 2015).

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

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

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

132 METHODS

133 *Study System and experimental design.*

134 This work was conducted within the Nutrient Network Distributed Experiment, a globally
135 replicated nutrient and herbivore manipulation experiment (NutNet; www.nutnet.org) (Borer et
136 al. 2014a, Borer et al. 2017). For this study, we used data from 47 sites that had been receiving
137 nutrient addition for 5-11 years (Appendix S1: Appendix S1: Table S7). At each of these sites,
138 we replicated an identical experiment that factorially combined three nutrient-addition treatments
139 each at two levels (Control or Fertilized): Nitrogen Addition (+N; 10 g N m⁻² yr⁻¹ as timed-
140 release urea), Phosphorus Addition (+P; 10 g P m⁻² yr⁻¹ as triple-super phosphate), and Potassium
141 and Micronutrient Addition (+K; 10 g K m⁻² yr⁻¹ as potassium sulfate and 100 g m⁻² yr⁻¹ of a
142 micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and
143 1% Zn). N, P, and K were applied annually, and the micronutrient mix was applied once at the
144 start of the study to avoid toxicity of largely immobile micronutrients.

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

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

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

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

167 homogenized soils were then packed into 5 x 9 mm tin capsules for carbon and nitrogen analysis
168 using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010
169 CHNSO Analyzer, Valencia, California, USA) calibrated with the analytical standard, atropine
170 ($C_{17}H_{23}NO_3$). 20g of the ground, homogenized soils were sent to Waypoint Analytical
171 (Memphis, TN, USA) to measure major nutrients, micronutrients, soil pH, organic matter, cation
172 exchange capacity, and texture of the soil (percent sand, silt and clay; only measured in the
173 Control Plots). Phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron,
174 manganese, zinc, and sodium (in parts per million) were measured using the Mehlich-3 method.
175 Soil pH was measured with a water pH meter on a 1:1 soil:water suspension. Cation exchange
176 capacity (CEC), reported here as meq/100 g (milliequivalents of charge per 100 g of dry soil), is
177 a measure of the capacity of soil surfaces to retain cations and is used as an indicator of quality
178 and productivity of the soil. CEC was calculated using the ppm of Ca, Mg, and K reported from
179 the Mehlich-3 method using the following relationship: $CEC = (\text{ppm Ca} / 200) + (\text{ppm Mg} / 120)$
180 $+ (\text{ppm K} / 390)$. Percent organic matter content in soil was measured using the Loss on Ignition
181 (LOI) method (combustion for two hours at 400 °C). Values of organic matter reported here were
182 not treated with acid prior to combustion. Texture was measured using the hydrometer method.
183 In brief, the soil sample was shaken with Sodium Hexametaphosphate (HMP) solution, and then
184 transferred to a settling cylinder and mixed. The percent sand, silt, and clay particles were
185 calculated from hydrometer density readings taken at 40 seconds and two hours.

186 *Diversity Metrics*

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

190 than species richness (Chase and Knight 2013). ENS_{PIE} estimates the number of equally abundant
191 species and is equivalent to the Inverse Simpson's index of diversity. We calculated ENS_{PIE} as
192 $1/\sum_{i=1}^S p_i^2$ where S is the total number of species and p_i is the proportion of the community
193 cover represented by species i (Chase and Knight 2013). Simpson's evenness (E) is directly
194 related to ENS_{PIE} through the relationship $E = ENS_{PIE}/S$ (Smith and Wilson 1996), thus we can
195 factor diversity directly into its richness and evenness components through the relationship:

$$196 \quad ENS_{PIE} = S * E$$

197 ENS_{PIE} was positively correlated with richness ($r=0.74$) but only weakly correlated with
198 evenness ($r=0.16$) across all samples in our data. Richness and evenness were negatively
199 correlated ($r=-0.41$).

200 *Data Analyses*

201 Ecosystem responses to nutrient enrichment could follow a variety of different shapes through
202 time (e.g., no change or accelerating, decelerating, or linear change through time)(Smith et al.
203 2009, Smith et al. 2015), so we fit a flexible model that allowed for nutrient effects to increase or
204 decrease in strength through time and for the shape of these curves to vary. Considering the case
205 where nutrient effects are getting stronger through time, we might see a number of different
206 curve shapes:

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

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

214 *Linear effects through time:* While unrealistic in the long-run, it is possible we could observe a
215 linear change in effect size over the duration of the experiment.

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

$$218 \log(y) = \beta_0 + \beta_1 N + \beta_2 \log(t) + \beta_3 \log(t)N$$

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

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

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

$$224 \log(y) = (\beta_0 + \beta_1) + (\beta_2 + \beta_3) \log(t)$$

225 In the case where we were modeling plant biomass ($y = \log(\text{biomass})$), β_0 is the mean biomass
226 ($\log \text{ g m}^{-2}$) in a control plot at time = 1, β_1 is the marginal increase in biomass ($\log \text{ g m}^{-2}$) due to
227 adding nutrients in year 1, β_2 is the change in mean biomass ($\log \text{ g per log yr}$), and β_3 is the
228 marginal effect of adding nutrients on the change in mean biomass ($\log \text{ g per log yr}$).

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

$$230 \quad y = e^{\beta_0 + \beta_1 N} t^{\beta_2 + \beta_3 N}$$

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

$$232 \quad y = e^{\beta_0 t} \beta_2$$

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

236 For nutrient addition plots, our predictive equation is:

$$237 \quad y = e^{\beta_0 + \beta_1 t} \beta_2 + \beta_3$$

238 In this case, if $(\beta_2 + \beta_3) < 1$ the rate of biomass change through time is slowing (Case 1), if
239 $(\beta_2 + \beta_3) > 1$ the rate of biomass change through time is increasing (Case 2), and if $(\beta_2 + \beta_3) = 1$
240 there is a linear change in biomass over time (Case 3). The mean biomass (g) in a fertilized plot
241 in year 1 ($t=1$) is $e^{(\beta_0 + \beta_1)}$.

242 To test whether the effects of nutrient addition increased over time, we used a mixed-effects
243 model with +N, +P, and +K μ , and experimental duration. Experimental duration is the number of
244 years the nutrient treatments had been applied; experimental duration was \log_{10} transformed. We
245 included experimental duration and site as random effects. Because of the large number of
246 parameters, the model with effects of all 8 nutrient treatments (+N \times +P \times +K μ) and their
247 interactions with experimental duration with random slope and intercepts for each site did not

248 converge. For this reason, we estimated treatment differences among sites (random slopes and
249 intercept) over time using only the Control and All Nutrient (+N, +P, and +K μ). Model
250 specifications in R are included with each table of regression results.

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

266 We also used regressions to examine whether nutrient effects on live biomass, dead biomass, and
267 plant diversity increased or decreased through time. At the site level, we included a water
268 availability index (Mean Annual Precipitation/Potential Evapotranspiration), mean annual
269 precipitation, mean annual temperature, mean diurnal temperature range, mean annual

270 temperature range, and temperature in the wettest quarter. The plot-level soil predictors were
271 pre-treatment soil pH, total base cations (sum of Ca, Mg, and K), % soil N, and the soil C:N
272 ratio. Note that % soil N, % soil organic matter, and % soil organic C are all highly correlated (r
273 > 0.92 for all comparisons), so we only included % soil N in the regression models. Similarly,
274 cation exchange capacity and base cations are highly correlated ($r = 0.93$), so we only include
275 base cations in the regression models. The plant community measures included were pre-
276 treatment live biomass, dead biomass, and plant diversity. Finally, we included the change in
277 nutrient effect over time as described in the correlation analysis above. For example, we included
278 the change in nutrient effect over time on dead biomass and plant diversity as explanatory
279 variables of the change in nutrient effect over time on live biomass. In our regression of site level
280 characteristics that could predict the change in nutrient effects over time, many of our predictor
281 variables co-varied (e.g., pre-treatment live biomass, soil N, soil C:N, and water availability
282 index). To account for the potential for multiple models that had similar explanatory power due
283 to this correlation, we used a multi-model approach, as described in Grueber et al. (2011) using
284 the dredge and model.avg functions in the MuMIn library. We standardized the input variables
285 using the arm library. In the model averaging, we included all models within 4 AIC_c units of the
286 best model. We ran these models with and without soils data, as we were missing soils data at
287 some sites.

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

290 RESULTS

291 In contrast to our expectations, we found increasing effects of nutrient addition on live biomass
292 through time at most sites despite increasing losses of diversity (Figs. 1 & 2), and there was an
293 overall significant interaction between treatment duration and fertilization (Table S2). While the
294 nutrient effects grew stronger, the rate of change was decelerating in most cases. Nutrient
295 enrichment, especially the addition of N, increased live biomass but did not have a consistent
296 effect on dead biomass and there was not an interaction between fertilization and treatment
297 duration (Figs. 1 & 3; Appendix S1: Tables S1 & S2). The effects of nutrient enrichment on live
298 biomass increased at most sites for up to 11 years (Fig. 1; Appendix S1: Tables S1 & S2). In
299 contrast to the consistent effects of nutrient addition on live biomass, nutrient effects on dead
300 biomass became stronger at some sites and weaker at others (Fig. 1; Appendix S1: Tables S1 &
301 S2).

302 Nutrient addition caused increasingly strong reductions in plant diversity (ENS_{PIE}) at nearly all
303 sites (Figs. 2 & 3; Appendix S1: Table S1 & S2), primarily due to the effects of N addition (Fig.
304 3, Appendix S1: Table S1). This loss in diversity was primarily caused by increasing losses of
305 richness at all sites (Figs. 2 & 3). In contrast to richness, nutrient effects on evenness did not
306 show a consistent increase over time (Fig. 2). The change in nutrient effects on diversity over
307 time were not correlated with change in nutrient effects on live biomass over time (Fig. 4),
308 however there was a negative correlation between the trajectory of nutrient effects on live and
309 dead biomass over time (Fig. 4).

310 In addition to the negative effects of dead biomass on live biomass, we found evidence that
311 nutrient effects on live biomass were stronger at more productive sites with higher pre-treatment
312 biomass (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). Similarly, we found that
313 nutrients effects on diversity loss increased in strength faster at sites that had high diversity at the

314 start of the experiment (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). Nutrient effects
315 on dead biomass were weaker at sites with high levels of pre-treatment dead biomass when we
316 included soil chemistry in the models which include 37 of 47 sites, but this effect was not
317 significant for the full set of sites (Appendix S1: Fig. S6; Appendix S1: Tables S4 & S5). A
318 potential determinant of dead biomass is domestic grazing, as the size sites with domestic grazers
319 present had lower levels of dead biomass in control plots ($p = 0.011$). There was no evidence that
320 estimates of the change in nutrient effects over time were affected by among-site differences in
321 experiment duration, which ranged from 5-11 years (Appendix S1: Tables S4 & S5).
322 Furthermore, the change in nutrient effects over time was not dependent on a site's climate or
323 soil chemistry (Appendix S1: Tables S4 & S5).

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

328 DISCUSSION

329 Short- and long-term studies have identified differing responses to chronic nutrient enrichment.
330 Although an analysis of nutrient responses at 42 grassland sites in the NutNet experiment found
331 increasing effects of fertilization on live biomass over the short-term (3 years) (Fay et al. 2015),
332 we expected, based on previous long-term fertilization experiments, that the positive effects of
333 nutrients on aboveground live biomass would either plateau and remain constant or decline with
334 time (Jenkinson et al. 1994, Isbell et al. 2013a, Avolio et al. 2014). In contrast, we found that the
335 effect of nutrient enrichment on live biomass continued to increase at nearly all sites for 11 years

336 (Fig. 1; Appendix S1: Table S1 & S2), despite increasing losses of plant diversity and increasing
337 dead biomass accumulation at some sites. Accumulation of dead biomass was the strongest
338 predictor of the trajectory of nutrient effects over time, with increased biomass accumulation
339 reducing the effects of nutrient on live biomass. We did not find evidence that increasing losses
340 of diversity could cause nutrient effects on biomass to decline over time.

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

350 While many studies have examined nutrient effects on plant productivity and biomass (Elser et
351 al. 2007), there is little basis for *a priori* expectations of how nutrient effects on dead biomass
352 accumulation would change through time, although we did expect a general coupling between
353 live and dead biomass (Hobbie 2015, Grace et al. 2016). Dead biomass accumulation can
354 directly reduce plant growth by acting as a physical barrier and reducing light to young plants
355 (Foster and Gross 1998, Coleman and Levine 2007, Clark and Tilman 2010, Seabloom 2010). In
356 the longer term, dead biomass can affect productivity through the influence of senesced plant
357 material on nutrient cycling, either facilitating or delaying nutrient release to plants (Hobbie
358 2015). In contrast to the consistently positive effects of nutrients on live biomass, nutrients

359 effects on the accumulation of dead biomass increased at some sites and decreased at others (Fig.
360 1; Appendix S1: Table S1 & S2). In meta-analysis spanning grasslands, forests, and tundra
361 ecosystems, N effects on decomposition has been shown to vary depending on N addition rates,
362 ambient N deposition rates, an litter quality (e.g., lignin content) (Knorr et al. 2005). Based on
363 this work, the N addition rate we used ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) would be expected to increase
364 decomposition rates.

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

374 Based on past work, we expected nutrients to continuously reduce diversity over time (Isbell et
375 al. 2013a, Harpole et al. 2016), and we found this to be the case (Fig. 2; Appendix S1: Table S1
376 & S2), primarily due to the increasingly strong effects of N addition (Appendix S1: Table S1).
377 Declines in diversity could arise either through reduced richness, due to increased extinction or
378 reduced colonization rates, or reduced evenness, reflecting an increasingly skewed abundance
379 distribution of species. We found that loss in diversity in response to nutrients was driven
380 primarily by continuing richness loss at all sites (Fig. 2). In contrast to richness, nutrient addition
381 did not have a consistent effect on evenness (Tables S1 & S2), and nutrient effects on evenness

382 did not show a consistent increase over time (Fig. 2), although evenness varied widely among
383 sites and across years and nutrient addition did change evenness at individual sites (Figure S5).

384 Past work suggested that the positive effects of nutrient enrichment on live biomass would
385 decline over time at sites where there was a concurrent loss in plant diversity (Isbell et al. 2013a)
386 or increase in dead biomass. We tested for this relationship by examining the correlations
387 between the change over time in the nutrient effects on live biomass, dead biomass, and plant
388 diversity. We did not find evidence of a negative feedback between fertilization induced
389 diversity loss and live biomass; live biomass continued to increase in response to nutrients for up
390 to a decade at nearly all sites. Furthermore, sites experiencing ongoing diversity loss did not have
391 decelerating nutrient effects on live biomass through time (Fig. 4), as would be expected if the
392 nutrient induced diversity loss were counteracting the effects of nutrients on productivity (Isbell
393 et al. 2013a). It is possible that diversity loss could eventually start to counteract the positive
394 effects of nutrient on plant biomass; the experiment that found this effect had been adding
395 nutrients for more than 25 years (Isbell et al. 2013a). However, the longest running nutrient
396 addition experiment has not shown large shifts after more than a century (Jenkinson et al. 1994).

397 Ultimately, standardized, long-term experiments replicated at sites representing a range of
398 conditions are necessary to distinguish biological from methodological effects of nutrient
399 addition among sites and studies (Borer et al. 2017). For example, Avolio et al. (2014) found that
400 a decade of N and P addition did not alter the richness in a tallgrass prairie community, while
401 Isbell et al. (2013a) found that the effects of nutrient addition on richness strengthened through
402 time, but in a different tallgrass prairie using different methods. In contrast, our standardized
403 experiment collocated at with these previous studies showed a consistent response of
404 increasingly strong declines in richness over time (konz.us & cdcr.us; Figures S1, S3, & S4),

405 suggesting that methodological differences, such nutrient addition rates or sampling protocols,
406 may explain differences in the inference between these two long-term experiments (Isbell et al.
407 2013a, Avolio et al. 2014).

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

413 Our experiment was replicated across a wide range of conditions globally, underscoring the
414 generality of the strengthening of nutrient effects over time. At nearly all sites, nutrient effects on
415 live biomass and diversity became stronger over time. This suggests that short-term experiments
416 (< 5 years) will underestimate the effects of nutrient enrichment on ecosystems and communities.
417 Experiments spanning multiple decades have shown that short-term experiments may generate
418 biased estimates of long-term treatment effects (Reich et al. 2012, Isbell et al. 2013a),
419 highlighting the value of long-term ecological data (Hughes et al. 2017).

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

427 Many experiments and models have demonstrated that nutrient addition in grassland ecosystems
428 can induce plant diversity declines (Lawes and Gilbert 1880, Miller et al. 2005, Clark et al. 2007,
429 Harpole and Tilman 2007, Hautier et al. 2009, Borer et al. 2014c, Harpole et al. 2016, Ward et al.
430 2017), and the few long-term experiments have shown that these effects can continue for decades
431 (Lawes and Gilbert 1880, Jenkinson et al. 1994, Isbell et al. 2013a, Ward et al. 2017). Here we
432 have shown that an increasing effect of nutrients on diversity loss through time is a general
433 phenomenon in many grassland ecosystems. This result suggests that current understanding,
434 which is largely based on short-term experiments (< 5 years) (Elser et al. 2007), may be
435 underestimating the severity of effect of nutrient enrichment on biodiversity. The mismatch
436 between short experimental duration and the time for ecosystems to fully respond to nutrient
437 enrichment is particularly problematic given that human activities are continuing to increase the
438 supplies of limiting nutrients into many of Earth's ecosystems (Vitousek et al. 1997a, Galloway
439 et al. 2008, Steffen et al. 2015).

440 In interpreting our results, it is important to note that the N addition rates ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) we
441 use were chosen to overcome N limitation, and are higher than would be used if the goal were to
442 mimic anthropogenic N deposition (Clark and Tilman 2008, Stevens et al. 2015). Lower rates of
443 N addition would likely lead to weaker effects, though still positive, positive effects on live
444 biomass and richness and could potentially inhibit decomposition rates (Knorr et al. 2005, Isbell
445 et al. 2013a, Midolo et al. 2019).

446 Human activities are concurrently altering a variety of interacting environmental factors that
447 drive ecosystem processes and ultimately ecosystem services that are necessary for human
448 wellbeing (Vitousek et al. 1997a, Vitousek et al. 1997b, Steffen et al. 2015), and the ecosystem-
449 level effects of many of these factors may not be fully evident for a decade or more (Reich et al.

450 2012, Isbell et al. 2013a). For this reason, long-term experiments are critical for predicting the
451 effects of humans on ecological systems (Silvertown et al. 2010, Hughes et al. 2017).
452 Nevertheless, long-term experiments in ecology remain relatively rare, and funding such
453 experiments is increasing difficult (Silvertown et al. 2010, Hughes et al. 2017). Human impacts
454 on ecosystems also vary spatially, and ecologists have recently started replicating experiments at
455 global scales (Borer et al. 2014a, Borer et al. 2017). Now that some distributed experiments have
456 been in place for more than a decade, we can ask novel questions about the factors that determine
457 the trajectory of ecosystem response to global change (Borer et al. 2017). Here we have shown a
458 remarkably consistent increasing effect of nutrient addition on plant production and concomitant
459 decline in diversity in wide array of grassland ecosystems including deserts, prairies, and alpine
460 tundra. However, these responses were not fully coupled; nutrient-induced declines in diversity
461 did not reduce the effects of nutrients on live biomass. These results suggest that, due to their
462 short duration, many experiments to date have likely underestimated the effects of human driven
463 eutrophication on biodiversity loss and ecosystem productivity.

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- 474 Avolio, M. L., S. E. Koerner, K. J. La Pierre, K. R. Wilcox, G. W. T. Wilson, M. D. Smith, and
 475 S. L. Collins. 2014. Changes in plant community composition, not diversity, during a
 476 decade of nitrogen and phosphorus additions drive above-ground productivity in a
 477 tallgrass prairie. *Journal of Ecology* **102**:1649-1660.
- 478 Borer, E. T., J. B. Grace, W. S. Harpole, A. S. MacDougall, and E. W. Seabloom. 2017. A
 479 decade of insights into grassland ecosystem responses to global environmental change.
 480 *Nature Ecology & Evolution* **1**:0118.
- 481 Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D.
 482 Smith. 2014a. Finding generality in ecology: a model for globally distributed
 483 experiments. *Methods in Ecology and Evolution* **5**:65-+.
- 484 Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B.
 485 Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S.
 486 Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. J. Chu, E. E. Cleland, M. J.
 487 Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Z. Du, J. Firn, Y.
 488 Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M.
 489 H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B.
 490 A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock,
 491 J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J.
 492 Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang.
 493 2014b. Herbivores and nutrients control grassland plant diversity via light limitation.
 494 *Nature* **508**:517-+.
- 495 Borer, E. T., E. W. Seabloom, C. E. Mitchell, and J. P. Cronin. 2014c. Multiple nutrients and
 496 herbivores interact to govern diversity, productivity, composition, and infection in a
 497 successional grassland. *Oikos* **123**:214-224.
- 498 Chapin, F. S., M. P. A., and H. A. Mooney. 2002. *Principles of terrestrial ecosystem ecology*
 499 New York : Springer
- 500 Chase, J. M., and T. M. Knight. 2013. Scale-dependent effect sizes of ecological drivers on
 501 biodiversity: why standardised sampling is not enough. *Ecology Letters* **16**:17-26.
- 502 Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Fargione, L. Gough, K. L. Gross, S. C. Pennings,
 503 K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants
 504 of species loss following nitrogen enrichment. *Ecology Letters* **10**:596-607.
- 505 Clark, C. M., and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen
 506 deposition to prairie grasslands. *Nature* **451**:712-715.
- 507 Clark, C. M., and D. Tilman. 2010. Recovery of plant diversity following N cessation: effects of
 508 recruitment, litter, and elevated N cycling. *Ecology* **91**:3620-3630.
- 509 Coleman, H. M., and J. M. Levine. 2007. Mechanisms underlying the impacts of exotic annual
 510 grasses in a coastal California meadow. *Biological Invasions* **9**:65-71.
- 511 Conant, R. T., K. Paustian, and E. T. Elliott. 2001. Grassland management and conversion into
 512 grassland: Effects on soil carbon. *Ecological Applications* **11**:343-355.
- 513 Crowther, T. W., C. Riggs, E. M. Lind, E. T. Borer, E. W. Seabloom, S. E. Hobbie, J. Wubs, P.
 514 B. Adler, J. Firn, L. Gherardi, N. Hagenah, K. S. Hofmockel, J. M. H. Knops, R. L.
 515 McCulley, A. S. MacDougall, P. L. Peri, S. M. Prober, C. J. Stevens, and D. Routh. 2019.
 516 Sensitivity of global soil carbon stocks to combined nutrient enrichment. *Ecology Letters*
 517 **22**:936-945.

518 Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T.
519 Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen
520 and phosphorus limitation of primary producers in freshwater, marine and terrestrial
521 ecosystems. *Ecology Letters* **10**:1135-1142.

522 Fay, P. A., S. M. Prober, W. S. Harpole, J. M. H. Knops, J. D. Bakker, E. T. Borer, E. M. Lind,
523 A. S. MacDougall, E. W. Seabloom, P. D. Wragg, P. B. Adler, D. M. Blumenthal, Y.
524 Buckley, C. J. Chu, E. E. Cleland, S. L. Collins, K. F. Davies, G. Z. Du, X. H. Feng, J.
525 Firn, D. S. Gruner, N. Hagenah, Y. Hautier, R. W. Heckman, V. L. Jin, K. P. Kirkman, J.
526 Klein, L. M. Ladwig, Q. Li, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L.
527 Moore, J. W. Morgan, A. C. Risch, M. Schutz, C. J. Stevens, D. A. Wedin, and L. H.
528 Yang. 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* **1**:5.

529 Fornara, D. A., and D. Tilman. 2012. Soil carbon sequestration in prairie grasslands increased by
530 chronic nitrogen addition. *Ecology* **93**:2030-2036.

531 Foster, B. L., and K. L. Gross. 1998. Species richness in a successional grassland: Effects of
532 nitrogen enrichment and plant litter. *Ecology* **79**:2593-2602.

533 Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. C. Cai, J. R. Freney, L. A.
534 Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen
535 cycle: Recent trends, questions, and potential solutions. *Science* **320**:889-892.

536 Gasarch, E. I., and T. R. Seastedt. 2015. Plant community response to nitrogen and phosphorus
537 enrichment varies across an alpine tundra moisture gradient. *Plant Ecology & Diversity*
538 **8**:739-749.

539 Grace, J. B., T. M. Anderson, E. W. Seabloom, E. T. Borer, P. B. Adler, W. S. Harpole, Y.
540 Hautier, H. Hillebrand, E. M. Lind, M. Pärtel, J. D. Bakker, Y. M. Buckley, M. J.
541 Crawley, E. I. Damschen, K. F. Davies, P. A. Fay, J. Firn, D. S. Gruner, A. Hector, J. M.
542 H. Knops, A. S. MacDougall, B. A. Melbourne, J. W. Morgan, J. L. Orrock, S. M.
543 Prober, and M. D. Smith. 2016. Integrative modelling reveals mechanisms linking
544 productivity and plant species richness. *Nature* **529**:390-393.

545 Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S.
546 Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008.
547 A cross-system synthesis of consumer and nutrient resource control on producer biomass.
548 *Ecology Letters* **11**:740-755.

549 Harpole, W. S., L. L. Sullivan, E. M. Lind, J. Firn, P. B. Adler, E. T. Borer, J. Chase, P. A. Fay,
550 Y. Hautier, H. Hillebrand, A. S. MacDougall, E. W. Seabloom, R. Williams, J. D.
551 Bakker, M. W. Cadotte, E. J. Chaneton, C. J. Chu, E. E. Cleland, C. D'Antonio, K. F.
552 Davies, D. S. Gruner, N. Hagenah, K. Kirkman, J. M. H. Knops, K. J. La Pierre, R. L.
553 McCulley, J. L. Moore, J. W. Morgan, S. M. Prober, A. C. Risch, M. Schuetz, C. J.
554 Stevens, and P. D. Wragg. 2016. Addition of multiple limiting resources reduces
555 grassland diversity. *Nature* **537**:93-96.

556 Harpole, W. S., and D. Tilman. 2007. Grassland species loss resulting from reduced niche
557 dimension. *Nature* **446**:791-793.

558 Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for Light Causes Plant Biodiversity
559 Loss After Eutrophication. *Science* **324**:636-638.

560 Hillebrand, H., D. S. Gruner, E. T. Borer, M. E. S. Bracken, E. E. Cleland, J. J. Elser, W. S.
561 Harpole, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Consumer
562 versus resource control of producer diversity depends on ecosystem type and producer

563 community structure. *Proceedings of the National Academy of Sciences of the United*
564 *States of America* **104**:10904-10909.

565 Hobbie, S. E. 2008. Nitrogen effects on litter decomposition: a five-year experiment in eight
566 temperate grassland and forest sites. *Ecology* **89**:2633-2644.

567 Hobbie, S. E. 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends*
568 *in Ecology & Evolution* **30**:357-363.

569 Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2005. Confronting a biome
570 crisis: global disparities of habitat loss and protection. *Ecology Letters* **8**:23-29.

571 Hughes, B. B., R. Beas-Luna, A. K. Barner, K. Brewitt, D. R. Brumbaugh, E. B. Cerny-
572 Chipman, S. L. Close, K. E. Coblenz, K. L. De Nesnera, S. T. Drobniitch, J. D. Figurski,
573 B. Focht, M. Friedman, J. Freiwald, K. K. Heady, W. N. Heady, A. Hettinger, A.
574 Johnson, K. A. Karr, B. Mahoney, M. M. Moritsch, A. M. K. Osterback, J. Reimer, J.
575 Robinson, T. Rohrer, J. M. Rose, M. Sabal, L. M. Segui, C. C. Shen, J. Sullivan, R.
576 Zuercher, P. T. Raimondi, B. A. Menge, K. Grorud-Colvert, M. Novak, and M. H. Carr.
577 2017. Long-Term Studies Contribute Disproportionately to Ecology and Policy.
578 *Bioscience* **67**:271-281.

579 Isbell, F., P. B. Reich, D. Tilman, S. E. Hobbie, S. Polasky, and S. Binder. 2013a. Nutrient
580 enrichment, biodiversity loss, and consequent declines in ecosystem productivity.
581 *Proceedings of the National Academy of Sciences of the United States of America*
582 **110**:11911-11916.

583 Isbell, F., D. Tilman, S. Polasky, S. Binder, and P. Hawthorne. 2013b. Low biodiversity state
584 persists two decades after cessation of nutrient enrichment. *Ecology Letters* **16**:454-460.

585 Jenkinson, D. S., J. M. Potts, J. N. Perry, V. Barnett, K. Coleman, and A. E. Johnston. 1994.
586 Trends in herbage yields over the last century on the Rothamsted Long-term Continuous
587 Hay Experiment. *Journal of Agricultural Science* **122**:365-374.

588 Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: A
589 meta-analysis. *Ecology* **86**:3252-3257.

590 Lawes, J. B., and J. H. Gilbert. 1880. Agricultural, botanical, and chemical results of experiments
591 on the mixed herbage of permanent meadow, conducted for more than twenty years in
592 succession on the same lands. Part 1. *Philosophical Transactions of the Royal Society of*
593 *London, Series B* **171**:289-416.

594 Lewandowska, A. M., A. Biermann, E. T. Borer, M. A. Cebrian-Piqueras, S. A. J. Declerck, L.
595 De Meester, E. Van Donk, L. Gamfeldt, D. S. Gruner, N. Hagenah, W. S. Harpole, K. P.
596 Kirkman, C. A. Klausmeier, M. Kleyer, J. M. H. Knops, P. Lemmens, E. M. Lind, E.
597 Litchman, J. Mantilla-Contreras, K. Martens, S. Meier, V. Minden, J. L. Moore, H. O.
598 Venterink, E. W. Seabloom, U. Sommer, M. Striebel, A. Trenkamp, J. Trinogga, J.
599 Urabe, W. Vyverman, D. B. Van de Waal, C. E. Widdicombe, and H. Hillebrand. 2016.
600 The influence of balanced and imbalanced resource supply on biodiversity-functioning
601 relationship across ecosystems. *Philosophical Transactions of the Royal Society B-*
602 *Biological Sciences* **371**.

603 Midolo, G., R. Alkemade, A. M. Schipper, A. Benitez-Lopez, M. P. Perring, and W. De Vries.
604 2019. Impacts of nitrogen addition on plant species richness and abundance: A global
605 meta-analysis. *Global Ecology and Biogeography* **28**:398-413.

606 Miller, T. E., J. H. Burns, P. Munguia, E. L. Walters, J. M. Kneitel, P. M. Richards, N. Mouquet,
607 and H. L. Buckley. 2005. A critical review of twenty years' use of the resource-ratio
608 theory. *American Naturalist* **165**:439-448.

- 609 Pierik, M., J. van Ruijven, T. M. Bezemer, R. Geerts, and F. Berendse. 2011. Recovery of plant
610 species richness during long-term fertilization of a species-rich grassland. *Ecology*
611 **92**:1393-1398.
- 612 Ramankutty, N., A. T. Evan, C. Monfreda, and J. A. Foley. 2008. Farming the planet: 1.
613 Geographic distribution of global agricultural lands in the year 2000. *Global*
614 *Biogeochemical Cycles* **22**:1-19.
- 615 Reich, P. B., J. Knops, D. Tilman, J. Craine, D. Ellsworth, M. Tjoelker, T. Lee, D. Wedin, S.
616 Naem, D. Bahauddin, G. Hendrey, S. Jose, K. Wrage, J. Goth, and W. Bengston. 2001.
617 Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition.
618 *Nature* **410**:809-812.
- 619 Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer.
620 2012. Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades.
621 *Science* **336**:589-592.
- 622 Riggs, C. E., S. E. Hobbie, E. M. Bach, K. S. Hofmockel, and C. E. Kazanski. 2015. Nitrogen
623 addition changes grassland soil organic matter decomposition. *Biogeochemistry* **125**:203-
624 219.
- 625 Seabloom, E. W. 2010. Spatial and Temporal Variability in Propagule limitation of California
626 Native Grasses. *Oikos* **120**:291–301.
- 627 Seabloom, E. W., E. T. Borer, Y. Buckley, E. E. Cleland, K. Davies, J. Firn, W. S. Harpole, Y.
628 Hautier, E. Lind, A. Macdougall, J. L. Orrock, S. M. Prober, P. Adler, J. Alberti, T. M.
629 Anderson, J. D. Bakker, L. A. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig,
630 M. Caldeira, C. J. Chu, M. J. Crawley, P. Daleo, E. I. Damschen, C. M. D'Antonio, N. M.
631 Decrappeo, C. R. Dickman, G. Z. Du, P. A. Fay, P. Frater, D. S. Gruner, N. Hagenah, A.
632 Hector, A. Helm, H. Hillebrand, K. S. Hofmockel, H. C. Humphries, O. Iribarne, V. L.
633 Jin, A. Kay, K. P. Kirkman, J. A. Klein, J. M. H. Knops, K. J. La Pierre, L. M. Ladwig, J.
634 G. Lambrinos, A. D. B. Leakey, Q. Li, W. Li, R. McCulley, B. Melbourne, C. E.
635 Mitchell, J. L. Moore, J. Morgan, B. Mortensen, L. R. O'Halloran, M. Partel, J. Pascual,
636 D. A. Pyke, A. C. Risch, R. Salguero-Gomez, M. Sankaran, M. Schuetz, A. Simonsen,
637 M. Smith, C. Stevens, L. Sullivan, G. M. Wardle, E. M. Wolkovich, P. D. Wragg, J.
638 Wright, and L. Yang. 2013. Predicting invasion in grassland ecosystems: is exotic
639 dominance the real embarrassment of richness? *Global Change Biology* **19**:3677-3687.
- 640 Seabloom, E. W., E. T. Borer, Y. M. Buckley, E. E. Cleland, K. F. Davies, J. Firn, W. S.
641 Harpole, Y. Hautier, E. M. Lind, A. S. MacDougall, J. L. Orrock, S. M. Prober, P. B.
642 Adler, T. M. Anderson, J. D. Bakker, L. A. Biederman, D. M. Blumenthal, C. S. Brown,
643 L. A. Brudvig, M. Cadotte, C. J. Chu, K. L. Cottingham, M. J. Crawley, E. I. Damschen,
644 C. M. Dantonio, N. M. DeCrappeo, G. Z. Du, P. A. Fay, P. Frater, D. S. Gruner, N.
645 Hagenah, A. Hector, H. Hillebrand, K. S. Hofmockel, H. C. Humphries, V. L. Jin, A.
646 Kay, K. P. Kirkman, J. A. Klein, J. M. H. Knops, K. J. La Pierre, L. Ladwig, J. G.
647 Lambrinos, Q. Li, W. Li, R. Marushia, R. L. McCulley, B. A. Melbourne, C. E. Mitchell,
648 J. L. Moore, J. Morgan, B. Mortensen, L. R. O'Halloran, D. A. Pyke, A. C. Risch, M.
649 Sankaran, M. Schuetz, A. Simonsen, M. D. Smith, C. J. Stevens, L. Sullivan, E.
650 Wolkovich, P. D. Wragg, J. Wright, and L. Yang. 2015. Plant species' origin predicts
651 dominance and response to nutrient enrichment and herbivores in global grasslands.
652 *Nature Communications* **6**:8.
- 653 Seastedt, T. R., J. M. Briggs, and D. J. Gibson. 1991. CONTROLS OF NITROGEN
654 LIMITATION IN TALLGRASS PRAIRIE. *Oecologia* **87**:72-79.

- 655 Silvertown, J., J. Tallowin, C. Stevens, S. A. Power, V. Morgan, B. Emmett, A. Hester, P. J.
656 Grime, M. Morecroft, R. Buxton, P. Poulton, R. Jinks, and R. Bardgett. 2010.
657 Environmental myopia: a diagnosis and a remedy. *Trends in Ecology & Evolution*
658 **25**:556-561.
- 659 Simkin, S. M., E. B. Allen, W. D. Bowman, C. M. Clark, J. Belnap, M. L. Brooks, B. S. Cade, S.
660 L. Collins, L. H. Geiser, F. S. Gilliam, S. E. Jovan, L. H. Pardo, B. K. Schulz, C. J.
661 Stevens, K. N. Suding, H. L. Throop, and D. M. Waller. 2016. Conditional vulnerability
662 of plant diversity to atmospheric nitrogen deposition across the United States.
663 *Proceedings of the National Academy of Sciences of the United States of America*
664 **113**:4086-4091.
- 665 Smith, B., and J. B. Wilson. 1996. A consumer's guide to evenness indices. *Oikos* **76**:70-82.
- 666 Smith, M. D., A. K. Knapp, and S. L. Collins. 2009. A framework for assessing ecosystem
667 dynamics in response to chronic resource alterations induced by global change. *Ecology*
668 **90**:3279-3289.
- 669 Smith, M. D., K. J. La Pierre, S. L. Collins, A. K. Knapp, K. L. Gross, J. E. Barrett, S. D. Frey,
670 L. Gough, R. J. Miller, J. T. Morris, L. E. Rustad, and J. Yarie. 2015. Global
671 environmental change and the nature of aboveground net primary productivity responses:
672 insights from long-term experiments. *Oecologia* **177**:935-947.
- 673 Steffen, W., K. Richardson, J. Rockström, S. E. Cornell, I. Fetzer, E. M. Bennett, R. Biggs, S. R.
674 Carpenter, W. de Vries, C. A. de Wit, C. Folke, D. Gerten, J. Heinke, G. M. Mace, L. M.
675 Persson, V. Ramanathan, B. Reyers, and S. Sörlin. 2015. Planetary boundaries: Guiding
676 human development on a changing planet. *Science*.
- 677 Stevens, C. J., E. M. Lind, Y. Hautier, W. S. Harpole, E. T. Borer, S. Hobbie, E. W. Seabloom,
678 L. Ladwig, J. D. Bakker, C. J. Chu, S. Collins, K. F. Davies, J. Firn, H. Hillebrand, K. J.
679 La Pierre, A. MacDougall, B. Melbourne, R. L. McCulley, J. Morgan, J. L. Orrock, S. M.
680 Prober, A. C. Risch, M. Schuetz, and P. D. Wragg. 2015. Anthropogenic nitrogen
681 deposition predicts local grassland primary production worldwide. *Ecology* **96**:1459-
682 1465.
- 683 Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and Ecosystem Functioning. Pages
684 471-493 *in* D. J. Futuyma, editor. *Annual Review of Ecology, Evolution, and*
685 *Systematics*, Vol 45. Annual Reviews, Palo Alto.
- 686 van der Valk, A. G. 1986. The impact of litter and annual plants on recruitment from the seed
687 bank of a lacustrine wetland. *Aquatic Botany* **24**:13-26.
- 688 Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H.
689 Schlesinger, and D. G. Tilman. 1997a. Human alteration of the global nitrogen cycle:
690 Sources and consequences. *Ecological Applications* **7**:737-750.
- 691 Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997b. Human domination of
692 Earth's ecosystems. *Science* **277**:494-499.
- 693 Ward, D., K. Kirkman, and Z. Tsvuura. 2017. An African grassland responds similarly to long-
694 term fertilization to the Park Grass experiment. *Plos One* **12**.

695 **Data accessibility statement:**

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

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FIGURE LEGENDS

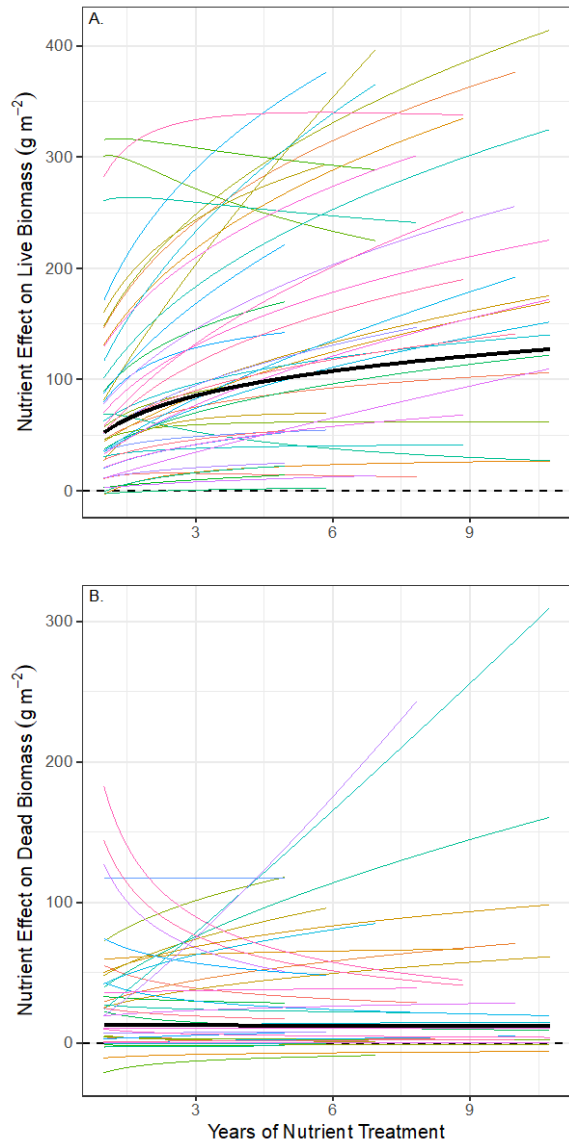
701 **Figure 1.** Effect of nutrient enrichment on live and dead aboveground plant biomass in grassland
702 ecosystems. Colored lines indicate individual sites, and the solid black line shows the mean
703 response across sites. Models were fit using $\log_{10}(\text{Treatment/Control})$ versus $\log_{10}(\text{Number of}$
704 $\text{Years of Treatment})$, and back-transformed for plotting as the difference between Treatment (i.e.,
705 all nutrients added) and Control plots. Standard errors of all parameter estimates are presented in
706 Appendix S1: Table S2, and site species models with raw data are presented in Figures S1 and
707 S2.

708 **Figure 2.** Effects of nutrient enrichment on diversity (ENS_{PIE}), richness (S , species m^{-2}), and
709 evenness ($ENS_{PIE} S^{-1}$) in grassland ecosystems. Colored lines indicate individual sites, and the
710 solid black line shows the mean response across sites. Models were fit using
711 $\log_{10}(\text{Treatment/Control})$ versus $\log_{10}(\text{Number of Years of Treatment})$, and back-transformed for
712 plotting as the difference between Treatment (i.e., all nutrients added) and Control plots.
713 Standard errors of all parameter estimates are presented in Appendix S1: Table S2, and site
714 species models with raw data are presented in Figures S3, S4, and S5.

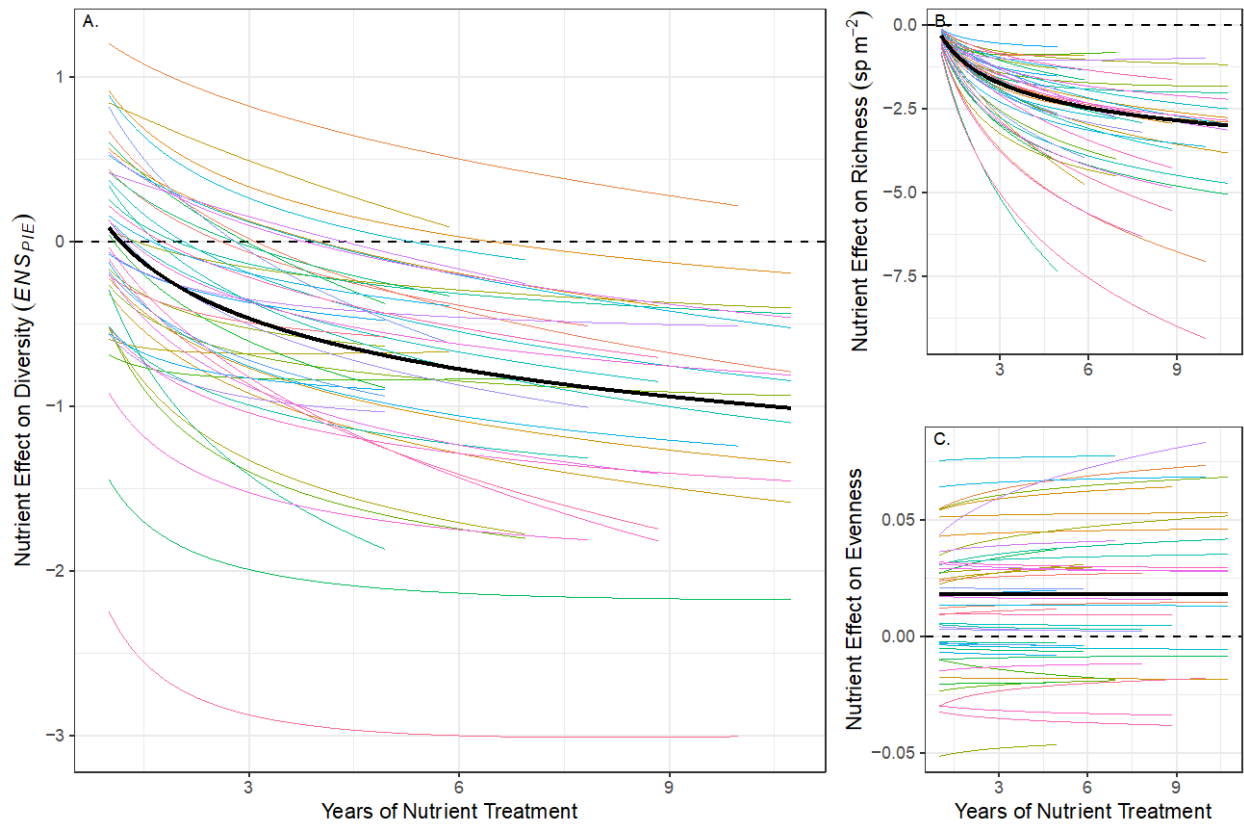
715 **Figure 3.** Effects of nutrient addition treatments on live and dead aboveground plant biomass
716 and diversity (ENS_{PIE}), richness (S , species m^{-2}), and evenness ($ENS_{PIE} S^{-1}$) in grassland
717 ecosystems. Error bars indicate 1 standard error (SE) among site means for each treatment.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

720

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

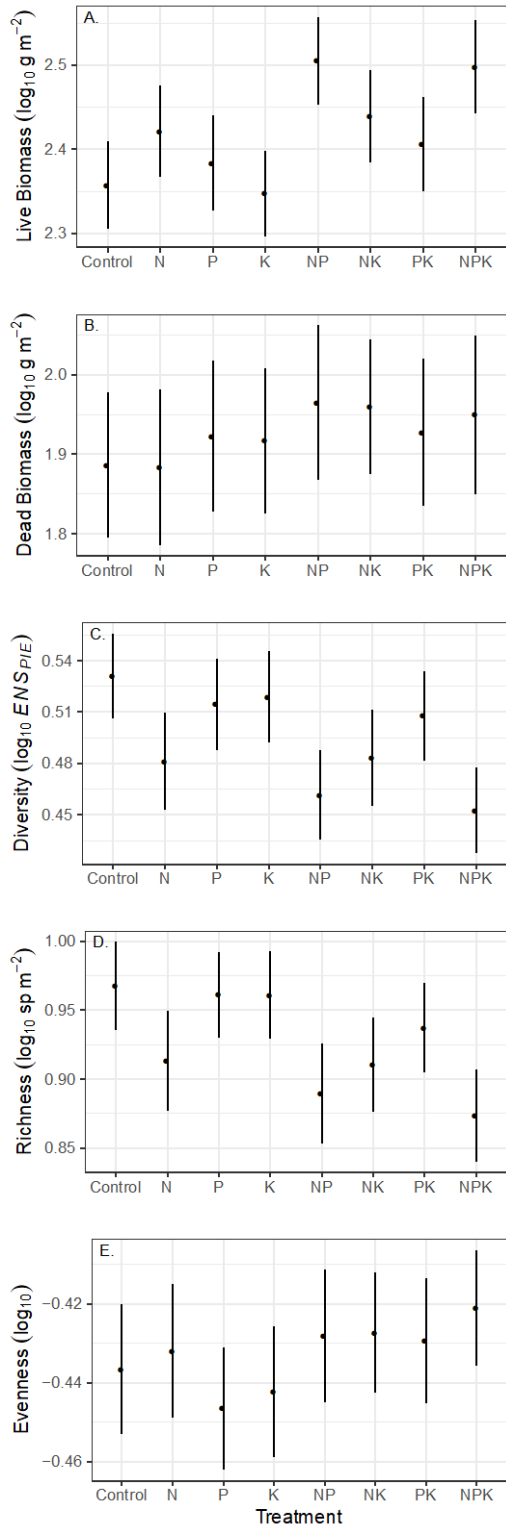


730 **Figure 2.**



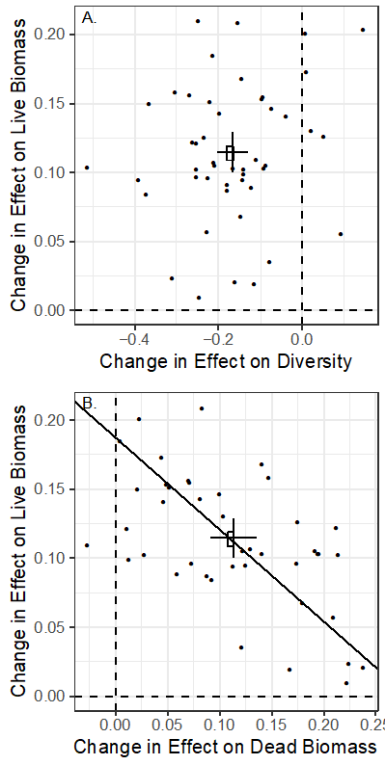
731

732 **Figure 3.**



733

734 **Figure 4.**



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736