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Title: Addition of multiple limiting resources reduces grassland diversity

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59

60 **Summary:**

61 **Niche dimensionality provides a general theoretical explanation for biodiversity:**
62 **more niches, defined by more limiting factors, allow for more ways species can**
63 **coexist¹. Because plant species compete for the same set of limiting resources, theory**
64 **predicts that addition of a limiting resource eliminates potential trade-offs, reducing**
65 **the number of species that can coexist². Multiple nutrient limitation of plant**
66 **production is common and therefore fertilization may reduce diversity by reducing**
67 **the number or dimensionality of belowground limiting factors. At the same time**
68 **nutrient addition, by increasing biomass, should ultimately shift competition from**
69 **belowground nutrients towards a one-dimensional competitive tradeoff for light³.**
70 **Here we show that plant species diversity decreased when a greater number of**
71 **limiting nutrients were added across 45 grassland sites from a multi-continent**
72 **experimental network⁴. The number of added nutrients predicted diversity loss,**
73 **even after controlling for effects of plant biomass, and even where biomass**
74 **production was not nutrient-limited. We found that elevated resource supply**
75 **reduced niche dimensionality and diversity and increased both productivity⁵ and**
76 **compositional turnover. Our results point to the importance of understanding**
77 **dimensionality in ecological systems that are undergoing diversity loss in response to**
78 **multiple global change factors.**

79
80 **Text:**

81 The search for the mechanisms underlying the coexistence of multiple species was inspired by
82 Darwin's observations of the problem of the "entangled bank", or how different checks on the
83 growth of individuals underlie the number of species found together⁶. One of the most general
84 theoretical explanations for this problem is that greater dimensionality, or number of non-
85 overlapping ecological niches, allows for the coexistence of a greater number of species^{1,7}.
86 However, plant coexistence challenges this understanding: rather than occupying unique
87 resource niches, plants share and are limited by the same essential resources⁸. The coexistence of
88 plants competing for the same resources therefore requires stoichiometric and physiological
89 tradeoff differences for shared limiting resources². Furthermore, plant resources are spatially
90 separated, with elemental nutrients (e.g., nitrogen, phosphorus, potassium) and water acquired
91 belowground and light aboveground. This suggests that two, non-independent resource-based
92 mechanisms could maintain plant diversity: multi-dimensional tradeoffs for belowground
93 limiting nutrients, juxtaposed with a one-dimensional tradeoff for light aboveground.

94
95 Resource competition theory predicts that addition of a limiting resource makes that resource
96 non-limiting, thereby eliminating a competitive tradeoff contributing to coexistence². Because
97 some factor must ultimately limit growth, resource additions will lead to a reduction in the
98 number and a shift in the identity of growth-limiting factors. In the case of plants, addition of
99 multiple nutrients should reduce the dimensionality of belowground resource tradeoffs, increase
100 biomass production, and ultimately shift the prevailing form of resource competition towards a
101 single, aboveground limiting resource, light^{3,5}. Support for this hypothesis has been demonstrated
102 in four grassland experiments. All found plant biomass production was limited by multiple
103 resources, and diversity decreased as a function of the number of belowground resources made
104 non-limiting^{5,9-11}. These results are consistent with the hypothesis that multi-dimensional
105 tradeoffs for belowground resources, and light competition mediated by aboveground biomass

106 production, might jointly contribute to maintaining plant diversity in natural communities. While
107 multiple limitation of primary producer communities is common¹², a recent global study
108 demonstrated substantial site-level variation in the number and identity of co-limiting resources,
109 with ca. 25% of sites showing no evidence that biomass production was nutrient limited¹³. The
110 question remains whether the dimensionality of nutrient resources might contribute to plant
111 diversity independently of the presumed importance of indirect effects of biomass on diversity.
112

113 Here we tested for loss of species diversity in response to multiple nutrient additions⁵ using the
114 Nutrient Network, a globally-distributed, nutrient addition experiment, replicated across
115 grassland sites on six continents (NutNet; www.nutnet.org)⁴. We added factorial combinations of
116 phosphorus (P), nitrogen (N), and potassium (K+ μ ; the K addition treatment included sulphur and
117 a one-time addition of micronutrients; see Methods), with the aim of removing potential
118 limitations from different combinations of the essential nutrient elements that most strongly
119 affect plant growth in natural and managed systems worldwide¹³. Our treatments varied in the
120 number of elemental resources they contained; hereafter, we use the term “number of added
121 resources” (1, 2, or 3) to represent the *minimum* number of potentially limiting elemental
122 nutrients added (see Methods).
123

124 If competition for multiple belowground resources contributes to species coexistence, then
125 diversity should decrease as a function of the number of resources added. Species diversity
126 decreased as more resources were added, and this effect increased with duration of treatment
127 (Fig. 1a; Extended Data Table 1). Greater number of added resources increased the annual rate of
128 diversity loss, even after controlling for differences in experiment duration (Fig. 1b). We found a
129 similar proportional loss of diversity with a greater number of added resources (using the log-
130 ratio effect size of treatment divided by control diversity; Fig. 1b), meaning that in terms of the
131 number of potential species lost, relative diversity losses and annual rate of diversity loss were
132 similar. Sites differed in the size of their species pools, which ranged from 13 to 103 observed
133 species over a 3-year period, and we found that the magnitude of diversity loss rate per added
134 resource increased with local species pool size (Fig. 1c).
135

136 We found that increasing the number of added resources increased live biomass (Fig. 2a), and
137 decreased the proportion of photosynthetically active radiation (PAR) transmitted through the
138 canopy to the ground surface (Fig. 2b). Further, the amount of litter biomass, which can also
139 contribute to light limitation and diversity loss¹⁴ increased with the number of added resources
140 (Fig. 2c). Importantly, despite the complex causal effects of changes in multiple resources on the
141 relationship between diversity and biomass, the number of added resources remained a
142 significant predictor of diversity loss, even after controlling for the potential contributing effects
143 of species pool size, live biomass, total cover (a proxy for total plant abundance), light
144 transmittance, and litter mass (Extended Data Tables 2, 3). If species coexist though tradeoffs in
145 resource-ratio requirements, changes in belowground resource supply could cause changes in
146 competitive dominance and lead to species exclusion², independent of aboveground effects of
147 biomass. In a subset of sites that did not show a biomass response to multiple nutrient addition,
148 we nevertheless observed declines in diversity consistent with this theory (Fig. 3 a, b: open
149 points, n=11), similar to sites where biomass production was multiple-resource limited (Fig. 3 a,
150 b: filled points, n=34). Overall, 14 sites of 45 sites in this study showed some type of negative
151 biomass response to N, P or K+ μ addition suggesting the potential for elevated nutrient

152 concentrations supply to cause negative physiological responses in species not adapted to high
153 nutrient concentrations¹⁵ or to large stoichiometric imbalances in resource supply¹⁶.

154
155 Diversity loss increased only weakly with biomass increase in plots receiving all three resources,
156 providing some support for indirect effects of biomass as a contributing, but not a sole,
157 mechanism of diversity loss due to fertilisation (Fig. 3c). If species losses were most strongly
158 associated with biomass increases, we would expect the greatest effects on both responses to be
159 associated with the same nutrient addition treatment, but this was true for only 22 of 45 cases
160 (Chi-square, $p < 0.0001$). The loss of diversity was not driven by the addition of any single added
161 resource (e.g., N); greatest diversity loss occurred with the addition of a combination of two or
162 more resources in 31 of 45 cases. These findings further highlight that biomass production and
163 diversity can be controlled differently by multiple resources. Overall, these results support our
164 conclusion that resource niche dimensionality can contribute to species diversity independently
165 of indirect effects mediated by biomass production.

166
167 For resource dimensionality to contribute to species coexistence, species must trade off their
168 competitive abilities for different limiting resources, and changes in resource supply ratios
169 should drive species compositional turnover². We found that a greater number of added resources
170 increased the compositional divergence from control plots (Fig. 4a). Plots receiving a single
171 resource treatment (N, P and $K_{+μ}$ treatments) diverged as much from each other as they did on
172 average from the control plots (Fig. 4b), consistent with different species trading off competitive
173 abilities for different resources². We found that greater diversity loss was weakly associated with
174 greater community dissimilarity when all three resources were added together (Fig. 4c),
175 suggesting that resource addition caused changes in community composition that were not
176 always associated with diversity loss. Both composition and diversity of communities contribute
177 to ecosystem functioning, and many of the proposed mechanisms of the effect of species
178 diversity on ecosystem function are resource-based¹⁷. Additionally, nutrient enrichment impacts
179 some groups of species more than others (e.g., a loss of natives in favor of exotic grasses¹⁸).
180 Because changes in resource supply led to communities of fewer species and of different
181 compositions, we expect changes in resources, acting through diversity loss, to have both direct
182 and indirect effects on ecosystem functions¹⁹.

183
184 While our results are consistent with predictions of the resource niche dimension hypothesis,
185 they are also likely conservative. Our experimental design, a factorial manipulation of three
186 resource treatments, represents a lower-bound estimate of the dimensionality of nutrient
187 resources because our $K_{+μ}$ treatment included sulphur and up to 10 other macro- and micro-
188 nutrients, of which more than one may have been limiting¹³. Multiple chemical forms of a
189 limiting nutrient can also contribute to species diversity²⁰, further expanding potential resource
190 dimensionality. Stronger tests of the role of multiple resource competition for structuring species
191 coexistence require physiological studies quantifying species-specific functional traits and
192 tradeoffs²¹, and testing whether species respond to resource treatments similarly in different
193 environments. Deeper mechanistic insight can also be gained by asking how resource-dependent
194 diversity patterns and mechanisms change across scales (e.g., from local to regional) in response
195 to global change drivers such as nutrient pollution²². Our results point to, but do not distinguish
196 among, the presumed resource competition mechanisms² that underlie the resource dimension
197 hypothesis.

198

199 We found that greater diversity loss was associated with sites with soil P, K, pH and % sand, but
200 not with soil N, or with latitude, or mean annual precipitation (Extended Data Table 4),
201 suggesting that variation in soil properties may influence the degree to which communities
202 respond to changes in resource availability²³. We did not test or control for other potential
203 limiting factors such as herbivory or water, which can interact with nutrients in complex ways,
204 and themselves contribute to species coexistence. For example, changes in nutrient availability
205 affect photosynthetic tissue quantity and quality, and may alter the pattern and intensity of
206 herbivory²⁴, and the level of soil water depletion through transpiration losses. Our multi-year
207 experimental results may still under-estimate nutrient effects when considering that global
208 eutrophication represents a chronic and cumulative environmental change over many decades.
209 Estimating effective upper bounds on ecologically relevant resource dimensionality will depend
210 on the degree to which multiple limiting factors covary, how they change in time and space, and
211 how multiple limiting factors interact with each other in promoting coexistence. Global change is
212 driving environmental conditions beyond multiple planetary boundaries²⁵, and changing the
213 limiting factors that structure species diversity²⁶. Understanding the mechanisms that underlie
214 diversity loss caused by multiple global change factors is necessary to develop effective
215 management strategies for restoring and preserving Earth's biodiversity.

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273 **Supplementary Information** is linked to the online version of the paper at
274 www.nature.com/nature.

275
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287
288
289 **Author Contributions:**

290 WSH analysed the data and wrote the paper with contributions and input from all authors. LS,
291 EML and JF contributed to data analysis. WSH, EWS and ETB developed and framed the
292 research questions. WSH, EWS, ETB and EML are Nutrient Network coordinators. All authors
293 collected data used in this analysis. Author contribution matrix provided as Table S2.

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296
297 The authors have not competing financial interests.

298
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300

301 **Figure Legends:**

302
303

304 **Figure 1. Biodiversity and number of resources.**

305 **a**, Loss of species diversity with greater number of added resources (effective number of equally
306 abundant species: ESN_{PIE}); this effect increased with years of treatment 1-8 (Extended Data
307 Table 1); year 0 shows pre-treatment diversity. Bold lines show overall mean responses; y-axis is
308 log-transformed. **b**, Greater number of added resources increased the mean rates of diversity loss
309 per year (filled points; $F_{1,134}=24.8$, $P<0.0001$), and the proportional loss of species relative to the
310 controls, shown as the effect size (open points; $F_{1,134}=46.2$, $P<0.0001$). **c**, Rate of diversity loss
311 per added resource (nres) was associated with greater total site species number (log), $R^2=0.25$,
312 $P=0.0004$, $n=45$). (mean \pm 95% c.i.).

313
314

315 **Figure 2. Biomass and light.**

316 **a**, The rate of live biomass change per year increased with an increasing number of added
317 resources ($F_{1,1031}=55.0$, $P<0.0001$). **b**, The proportion of photosynthetically active radiation
318 (PAR) reaching the ground surface decreased with a greater number of added resources,
319 expressed as annual rate of change ($F_{1,782}=62.4$, $P<0.0001$). **c**, The mean rate of litter (dead
320 biomass) change per year increased with the number of added resources ($F_{1,783}=4.37$, $P=0.037$).
321 (mean \pm 95% c.i.).

322
323

324 **Figure 3. Multiple resource limitation.**

325 **a**, Increased number of added resources resulted in positive and increasing biomass at sites
326 showing multiple resource limitation (filled points); sites not limited by multiple resources
327 tended to show negative biomass responses with resource addition (open points). **b**, Increased
328 number of added resources drove similar diversity loss at sites where biomass production was
329 limited by multiple resources (filled points) and at sites where it was not (open points). **c**,
330 Negative relationship between the effect of addition of three resources on biomass and diversity
331 (1-tail test for negative relationship, $R^2=0.11$, $P=0.012$, $n=45$). (mean \pm s.e.)

332
333

334 **Figure 4. Community composition.**

335 **a**, Community composition diverged from control plots with greater number of added resources
336 (Bray-Curtis dissimilarity index). Resource addition caused greater dissimilarity of community
337 composition relative to mean pre-treatment dissimilarity, indicated by grey stars. **b**, Addition of
338 single nutrient additions of N, P or K_{+P} resulted in communities that diverged as much from each
339 other as they did on average from the control plots. Pre-treatment values indicated by grey stars.
340 **c**, Negative relationship between the effect of addition of three resources on community
341 dissimilarity relative to controls and diversity (1-tail test for negative relationship, $R^2=0.10$,
342 $P=0.019$, $n=45$). (mean \pm 95% c.i.).

343

Methods:

Experiment Design: The Nutrient Network (NutNet) is a collaborative, distributed experimental network⁴. Sites are located across herbaceous terrestrial systems on six continents. Vegetation types represented include grasslands, savannas and meadows and occur across a wide range of climate and environmental factors (Table S1). At the 45 sites (on five continents) with appropriate experimental data for these analyses, one year of pre-treatment (year 0) data were collected followed by at least 3 years and up to 8 years of treatment data. Individual site experiments share identical design and sampling protocols, with minor site-specific differences in terms of replication and treatment duration (Table S1). We applied factorial combinations of nitrogen (N), phosphorus (P), and potassium plus micronutrients, designated here as the “K+ μ ” treatment, giving eight treatment combinations including the control with no added resources. N was applied annually at 10 g N m⁻² yr⁻¹ as time-release urea. Ammonium nitrate was used in 2007 at some sites before switching to urea due to restricted availability of ammonium nitrate; we found no differences in the short-term effects of alternative N sources in a separate experiment at four sites¹⁸. P was applied at 10 g P m⁻² yr⁻¹ as triple-super phosphate, which also included Ca at 8.1 g Ca m⁻² yr⁻¹. The K+ μ treatment added a mix of K and S (10 g K m⁻² yr⁻¹ and 3.9 g S m⁻² yr⁻¹ as potassium sulphate) and micronutrients (100 g m⁻² yr⁻¹ of a mixture composed of 6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn). Micronutrients were only applied during the first treatment year to minimise potential for toxic metal accumulation. Plots were 5 m x 5 m and randomised within 1 to 6 blocks (Table S1), with all eight treatment combinations occurring once per block. Sampling occurred at approximately peak biomass times for each site.

Response Measurements:

Biodiversity estimates are scale-dependent²⁷, and increased resource availability can alter diversity-scaling relationships by changing the size of species pools and thus introduce differences in the coverage of sampling between treatments, due to larger and fewer individuals per area sampled²⁸, and contribute to the loss of rarer species. We calculated species diversity as the effective species number, which estimates the probability of interspecific-encounter if all species are equally abundant (ESN_{PIE}). ESN_{PIE} has been shown to be less sensitive to scaling issues than other metrics²², and is representative of the maximum slope of the species-area accumulation function. We used ESN_{PIE} because NutNet sites vary in their species pools and therefore their species accumulation curves will differ, which creates a challenge to compare species diversity when sampled at a fixed area²². ESN_{PIE} has been shown to be relatively insensitive to such sampling area issues because it essentially measures the maximum change in species number as a function of sampling area (i.e., the slope at the x-intercept of the species accumulation curve). Because the resource dimension hypothesis and underlying resource ratio theory assume that species trade off for different limiting factors, predictions for diversity change describe changes in competitive dominance; ESN_{PIE} captures these predicted changes in dominance better than simple measurements of local species extinction (i.e. richness loss). We used the aggregate number of species observed at a site as an estimate of the asymptote of the species accumulation function, and of the regional species pool. We also used simply the number of species (i.e., richness) and found similar results as those using ESN_{PIE} (Extended Data Table 1).

390 We measured species diversity annually by estimating the % cover of each plant species within a
 391 1 m x 1 m fixed location in each plot; the total cover typically summed to greater than 100% due
 392 to multiple canopy layers. We quantified species diversity as the probability of interspecific
 393 encounter (PIE), or effective species number (ESN_{PIE}), assuming species relative abundances are
 394 equal:
 395

$$ESN_{PIE} = \frac{1}{\sum_i^s p_i^2} \quad \text{eq. 1}$$

396
 397 where p_i is the proportion of species i in a community of size s ; ESN_{PIE} is derived from the
 398 inverse of Simpson's diversity index²².
 399

400
 401 We measured aboveground live biomass by clipping two 1 m x 10 cm strip of vegetation in each
 402 plot, sorting the sampled tissue to live (current year's production) and dead (previous years'
 403 production) fractions, drying at 60 C for 48 hours and weighing. At most sites,
 404 photosynthetically active radiation PAR was measured above the plant canopy and at the ground
 405 surface and the proportion of transmitted light calculated.
 406

407 We categorised plant communities at sites as multiple-resource limited if biomass responded
 408 positively to fertilisation with combinations of different nutrients. Specifically, we designated
 409 sites as "multiple-resource limited" if biomass increased with the independent addition of
 410 different resources or if biomass responded synergistically to two or more added resources (i.e.,
 411 the response to one nutrient was dependent on the level of another and their combined effect was
 412 super-additive)¹¹. Sites that showed no response or negative biomass response or responded
 413 positively to only one resource we categorised as not multiple-resource limited. Thirty-four of
 414 the 45 sites showed increased biomass in response to multiple added resources; eight did not
 415 respond positively to resource addition, and three responded positively to a single resource (i.e.,
 416 single resource limited¹¹).
 417

418 **Statistical Analysis:** All analyses used R version 3.2.2. We used linear mixed-effects models (R
 419 package lme) to test the interaction of number of added resources and the number of treatment
 420 years, on diversity (ESN_{PIE}) and richness. Site and block were modeled as nested random effects.
 421 We included in the model an autocorrelation structure, a first-order autoregressive model
 422 (AR(1)), where observations are expected to be correlated from one year to the next, and found a
 423 substantial improvement in model fit when we compared this model to a model with no
 424 autocorrelation structure (lower AIC = Δ 608 and likelihood ratio tests, L.Ratio = 610, $p <$
 425 0.0001)²⁹. Treatment effects increased in magnitude with time (significantly negative interaction
 426 between number of added resources and year; SI Table 2). To allow standardised comparison of
 427 sites that differed in the year they were established and in duration of nutrient addition, we used
 428 two approaches to quantify the changes in species diversity. First, we calculated the annual rate
 429 of change of our response variables to standardise site responses. Second, for analyses that
 430 required an effect size, calculated as the log ratio of the treatment response divided by the
 431 control, we used the most recent year of treatment data, which ranged from 3 to 7 years of annual
 432 nutrient application duration (SI Table 1). Log ratio effect size estimates would not have been
 433 possible using the rate of change estimates, which can take zero or negative values. Log ratio

434 effect sizes tend to be normally distributed, centre zero effects (control levels) at zero log ratios,
435 and scale responses to make proportional effects directly comparable between sites³⁰.

436
437 We used linear mixed-effects models (R package lme) to test the effects of number of treatment
438 years, site richness, log live biomass, log dead biomass, PAR, total species cover, and the
439 number of added resources on diversity (ESN_{pie}), with plot nested in block nested in site as
440 random effects. Models using dead biomass and PAR used the subset of 30 sites for which we
441 had data for these variables. We calculated mean values at each site for the annual rate of
442 diversity loss and diversity effect size, and tested for linear relationships between these variables
443 and the number of added resources using regression with site as a block term. We used step-wise
444 linear regression and AIC criteria to test for relationships of loss of diversity (from addition of
445 three resources) with latitude, longitude, and environmental covariates of mean annual
446 precipitation, and soil N, P, K, pH, % clay, and % sand. Plant community composition changes
447 were quantified using Bray-Curtis multivariate distances (R package vegan).

448
449

450 **Methods' References:**

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459
460

461 **Extended Data Table Legends:**

462

463 **Extended Data Table 1. The effects of nutrient addition on diversity loss and richness loss**
464 **increase with time.** Linear mixed-effects model of the effects of number of treatment years
465 (ARIMA type-1 autocorrelation) and the number of added resources on diversity ($\log ESN_{pie}$)
466 and richness, with plot nested in block, nested in year, nested in site, as random effects, using all
467 45 sites. There was a significant, negative interaction between the number of added resources
468 (nres) and year of treatment (year).

469

470 **Extended Data Table 2. The number of added resources predicts diversity loss after**
471 **controlling for other variables.** Linear mixed-effects model of the effects of number of
472 treatment years, site richness, log live biomass, total species cover, and the number of added
473 resources on diversity (ESN_{pie}), with plot nested in block nested in site as random effects, using
474 all 45 sites and data from the maximum treatment year for each site. Delta AIC between model
475 with number of added resources and model without was 33, log-likelihood ratio 35.0, $p < 0.0001$.

476

477 **Extended Data Table 3. The number of added resources is an important predictor even**
478 **after controlling for other variables, for sites that had light and litter data.** Linear mixed-
479 effects model of the effects of number of treatment years, site richness, log live biomass, log

480 dead biomass, PAR, total species cover, and the number of added resources on diversity
481 (ESN_{pie}), with plot nested in block nested in site as random effects, using data from the
482 maximum treatment year for each site, and the subset of 32 sites for which there was dead
483 biomass and PAR data. Delta AIC between model with number of added resources and model
484 without was 15, log-likelihood ratio 15.6, $p < 0.0001$.

485

486 **Extended Data Table 4. Diversity loss due to addition of nutrients associated with soil**
487 **properties.** Stepwise multiple regression (backward with AIC criteria for model comparisons)
488 retained soil P, K, pH, and % sand as predictors of diversity loss from the addition of three
489 resources, for the 30 sites with soil analysis data (excluding one site for extreme value of P). The
490 variables latitude, longitude, mean annual precipitation, and soil % N were not retained. Overall
491 model is significant ($r^2 = 0.375$, $F_{4, 25} = 3.75$, $p = 0.016$).