

Addition of multiple limiting resources reduces grassland diversity

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

The search for the mechanisms underlying the coexistence of multiple species was inspired by Darwin's observations of the problem of the 'entangled bank', or how different checks on the growth of individuals underlie the number of species found together⁶. One of the most general theoretical explanations for this problem is that greater dimensionality, or number of non-overlapping ecological niches, allows for the coexistence of a greater number of species^{1,7}. However, plant coexistence challenges this understanding: rather than occupying unique resource niches, plants share and are limited by the same essential resources⁸. The coexistence of plants competing for the same resources therefore requires stoichiometric and physiological trade-off

differences for shared limiting resources². Furthermore, plant resources are spatially separated, with elemental nutrients (for example, nitrogen, phosphorus, potassium) and water acquired belowground and light aboveground. This suggests that two, non-independent resource-based mechanisms could maintain plant diversity: multi-dimensional trade-offs for belowground limiting nutrients, juxtaposed with a one-dimensional trade-off for light aboveground.

Resource competition theory predicts that addition of a limiting resource makes that resource non-limiting, thereby eliminating a competitive trade-off contributing to coexistence². Because some factor must ultimately limit growth, resource additions will lead to a reduction in the number and a shift in the identity of growth-limiting factors. In the case of plants, addition of multiple nutrients should reduce the dimensionality of belowground resource trade-offs, increase biomass production, and ultimately shift the prevailing form of resource competition towards a single, aboveground limiting resource, light^{3,5}. Support for this hypothesis has been demonstrated in four grassland experiments. All of these experiments found plant biomass production was limited by multiple resources, and diversity decreased as a function of the number of belowground resources made non-limiting^{5,9–11}. These results are consistent with the hypothesis that multi-dimensional trade-offs for belowground resources, and light competition mediated by aboveground biomass production, might jointly contribute to maintaining plant diversity in natural communities. Although multiple limitation of primary producer communities is common¹², a recent global study demonstrated substantial site-level variation in the number and identity of co-limiting resources, with around 25% of sites showing no evidence that biomass production was nutrient limited¹³. The question remains whether the dimensionality of nutrient resources might contribute to plant diversity independently of the presumed importance of indirect effects of biomass on diversity.

Here we tested for loss of species diversity in response to multiple nutrient additions⁵ using the Nutrient Network, a globally-distributed,

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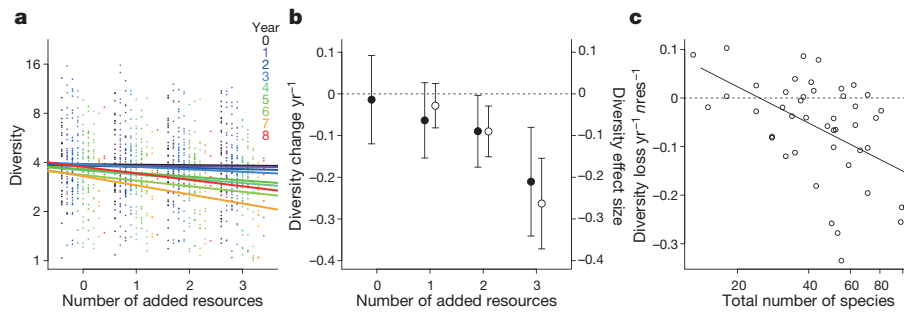


Figure 1 | Biodiversity and number of resources. **a**, Loss of species diversity with greater number of added resources (effective number of equally abundant species: ESN_{PIE}); this effect increased with years of treatment 1–8 (Extended Data Table 1); year 0 shows pre-treatment diversity. Bold lines show overall mean responses of 45 sites; y axis is log-transformed. **b**, Greater number of added resources increased

nutrient addition experiment, replicated across grassland sites on six continents (NutNet; <http://www.nutnet.org>)⁴. We added factorial combinations of phosphorus (P), nitrogen (N), and potassium (K_{+H} ; the K addition treatment included sulfur and a one-time addition of micronutrients; see Methods), with the aim of removing potential limitations from different combinations of the essential nutrient elements that most strongly affect plant growth in natural and managed systems worldwide¹³. Our treatments varied in the number of elemental resources they contained; hereafter, we use the term ‘number of added resources’ (1, 2 or 3) to represent the minimum number of potentially limiting elemental nutrients added (see Methods).

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

We found that increasing the number of added resources increased live biomass (Fig. 2a), and decreased the proportion of photosynthetically active radiation (PAR) transmitted through the canopy to the ground surface (Fig. 2b). Further, the amount of litter biomass, which can also contribute to light limitation and diversity loss¹⁴ increased with the number of added resources (Fig. 2c). Importantly, despite the complex causal effects of changes in multiple resources on the

the mean rates of diversity loss per year (filled points; $F_{1,134} = 24.8$, $P < 0.0001$), and the proportional loss of species relative to the controls, shown as the effect size (open points; $F_{1,134} = 46.2$, $P < 0.0001$). **c**, Rate of diversity loss per added resource ($nres$) was associated with greater total site species number (log), $R^2 = 0.25$, $P = 0.0004$, $n = 45$). Error bars show mean $\pm$ 95% confidence intervals.

relationship between diversity and biomass, the number of added resources remained a significant predictor of diversity loss, even after controlling for the potential contributing effects of species pool size, live biomass, total cover (a proxy for total plant abundance), light transmittance, and litter mass (Extended Data Tables 2 and 3). If species coexist though trade-offs in resource-ratio requirements, changes in belowground resource supply could cause changes in competitive dominance and lead to species exclusion², independent of aboveground effects of biomass. In a subset of sites that did not show a biomass response to multiple nutrient addition, we nevertheless observed declines in diversity consistent with this theory (Fig. 3a, b: open points, $n = 11$), similar to sites where biomass production was multiple-resource limited (Fig. 3a, b: filled points, $n = 34$). Overall, 14 sites of 45 sites in this study showed some type of negative biomass response to N, P or K_{+H} addition suggesting the potential for elevated nutrient concentrations supply to cause negative physiological responses in species not adapted to high nutrient concentrations¹⁵ or to large stoichiometric imbalances in resource supply¹⁶.

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

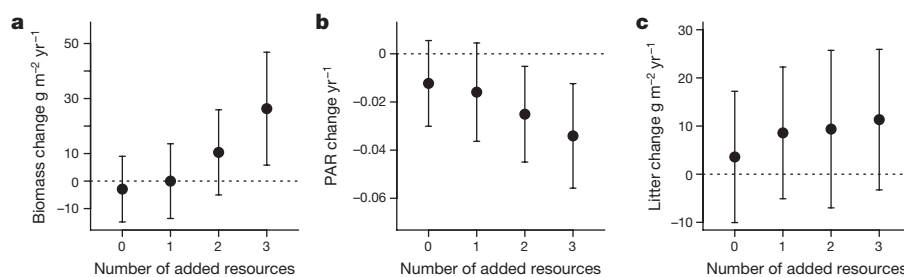


Figure 2 | Biomass and light. **a**, The rate of live biomass change per year increased with an increasing number of added resources ($F_{1,1031} = 55.0$, $P < 0.0001$). **b**, The proportion of photosynthetically active radiation (PAR) reaching the ground surface decreased with a greater number

of added resources, expressed as annual rate of change ($F_{1,782} = 62.4$, $P < 0.0001$). **c**, The mean rate of litter (dead biomass) change per year increased with the number of added resources ($F_{1,783} = 4.37$, $P = 0.037$). Error bars show mean $\pm$ 95% confidence intervals.

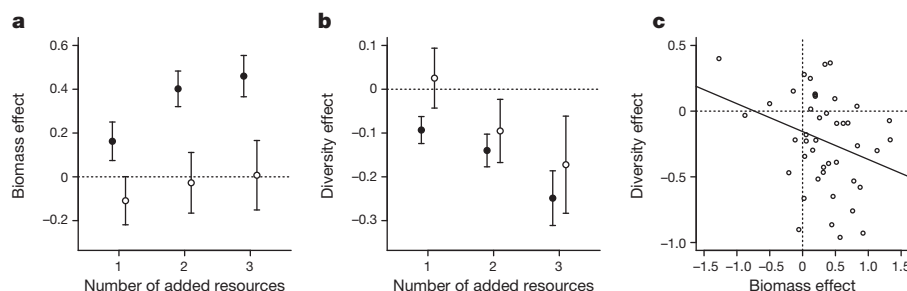


Figure 3 | Multiple resource limitation. **a**, Increased number of added resources resulted in positive and increasing biomass at sites showing multiple resource limitation (filled points); sites not limited by multiple resources tended to show negative biomass responses with resource addition (open points). **b**, Increased number of added resources drove

similar diversity loss at sites where biomass production was limited by multiple resources (filled points) and at sites where it was not (open points). **c**, Negative relationship between the effect of addition of three resources on biomass and diversity (one-tailed test for negative relationship, $R^2 = 0.11$, $P = 0.012$, $n = 45$). Error bars show mean $\pm$ s.e.

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

Although our results are consistent with predictions of the resource niche dimension hypothesis, they are also probably conservative. Our experimental design, a factorial manipulation of three resource treatments, represents a lower-bound estimate of the dimensionality of nutrient resources because our K_{+M} treatment included sulfur and up to 10 other macro- and micro-nutrients, of which more than one may have been limiting¹³. Multiple chemical forms of a limiting nutrient can also contribute to species diversity²⁰, further expanding potential resource dimensionality. Stronger tests of the role of multiple resource competition for structuring species coexistence require

physiological studies quantifying species-specific functional traits and trade-offs²¹, and testing whether species respond to resource treatments similarly in different environments. Deeper mechanistic insight can also be gained by asking how resource-dependent diversity patterns and mechanisms change across scales (for example, from local to regional) in response to global change drivers such as nutrient pollution²². Our results point to, but do not distinguish among, the presumed resource competition mechanisms² that underlie the resource dimension hypothesis.

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

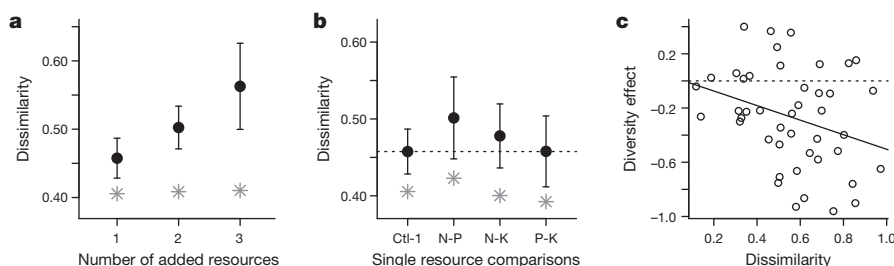


Figure 4 | Community composition. **a**, Community composition diverged from control plots with greater number of added resources (Bray–Curtis dissimilarity index). Resource addition caused greater dissimilarity of community composition relative to mean pre-treatment dissimilarity, indicated by grey stars. **b**, Addition of single nutrient additions of N, P or K_{+M} resulted in communities that diverged as much from each other as

they did on average from the control plots. Pre-treatment values indicated by grey stars. **c**, Negative relationship between the effect of addition of three resources on community dissimilarity relative to controls and diversity (one-tailed test for negative relationship, $R^2 = 0.10$, $P = 0.019$, $n = 45$). Error bars indicate mean $\pm$ 95% confidence intervals.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to W.S.H. (stan.harpole@idiv.de).

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment.

Experimental 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 (Supplementary Table 1). 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 (Supplementary Table 1). 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 $10\text{ g N m}^{-2}\text{ yr}^{-1}$ 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 $10\text{ g P m}^{-2}\text{ yr}^{-1}$ as triple-super phosphate, which also included Ca at $8.1\text{ g Ca m}^{-2}\text{ yr}^{-1}$. The $K_{+μ}$ treatment added a mix of K and S ($10\text{ g K m}^{-2}\text{ yr}^{-1}$ and $3.9\text{ g S m}^{-2}\text{ yr}^{-1}$ as potassium sulphate) and micronutrients ($100\text{ g m}^{-2}\text{ yr}^{-1}$ 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 $5\text{ m} \times 5\text{ m}$ and randomized within 1 to 6 blocks (Supplementary Table 1), 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 (that is, 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 (that is, 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 (that is, richness) and found similar results as those using ESN_{PIE} (Extended Data Table 1).

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

$$ESN_{PIE} = \frac{1}{\sum_i^s p_i^2} \quad (1)$$

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

We measured aboveground live biomass by clipping two $1\text{ m} \times 10\text{ cm}$ strips of vegetation in each plot, sorting the sampled tissue to live (current year's production) and dead (previous years' production) fractions, drying at 60°C for 48 h and weighing. At most sites, photosynthetically active radiation PAR was measured above the plant canopy and at the ground surface and the proportion of transmitted light calculated.

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

Statistical analysis. All analyses used R version 3.2.2. We used linear mixed-effects models (R package lme) to test the interaction of number of added resources and the number of treatment years, on diversity (ESN_{PIE}) and richness. Site and block were modelled as nested random effects. We included in the model an autocorrelation structure, a first-order autoregressive model (AR(1)), where observations are expected to be correlated from one year to the next, and found a substantial improvement in model fit when we compared this model to a model with no autocorrelation structure (lower AIC = $\Delta 608$ and likelihood ratio tests, $L.Ratio = 610$, $P < 0.0001$)²⁹. Treatment effects increased in magnitude with time (significantly negative interaction between number of added resources and year; Supplementary Table 2). To allow standardized comparison of sites that differed in the year they were established and in duration of nutrient addition, we used two approaches to quantify the changes in species diversity. First, we calculated the annual rate of change of our response variables to standardise site responses. Second, for analyses that required an effect size, calculated as the log ratio of the treatment response divided by the control, we used the most recent year of treatment data, which ranged from 3 to 8 years of annual nutrient application duration (Supplementary Table 1). Log ratio effect size estimates would not have been possible using the rate of change estimates, which can take zero or negative values. Log ratio effect sizes tend to be normally distributed, centre zero effects (control levels) at zero log ratios, and scale responses to make proportional effects directly comparable between sites³⁰.

We used linear mixed-effects models (R package lme) to test the effects of number of treatment years, site richness, log live biomass, log dead biomass, PAR, total species cover, and the number of added resources on diversity (ESN_{PIE}), with plot nested in block nested in site as random effects. Models using dead biomass and PAR used the subset of 32 sites for which we had data for these variables. We calculated mean values at each site for the annual rate of diversity loss and diversity effect size, and tested for linear relationships between these variables and the number of added resources using regression with site as a block term. We used step-wise linear regression and AIC criteria to test for relationships of loss of diversity (from addition of three resources) with latitude, longitude, and environmental covariates of mean annual precipitation, and soil N, P, K, pH, percentage clay, and percentage sand. Plant community composition changes were quantified using Bray-Curtis multivariate distances (R package vegan).

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Extended Data Table 1 | The effects of nutrient addition on diversity loss and richness loss increase with time

ESN_{pie}	Num. DF	Den. DF	F	P
intercept	1	6555	370.3	<0.0001
year	1	6555	39.3	<0.0001
nres	1	1049	32.2	<0.0001
year x nres	1	6555	26.1	<0.0001

Richness	Num. DF	value	SE	P
intercept	1	6555	146.4	<0.0001
year	1	6555	209.1	<0.0001
res	1	1049	91.8	<0.0001
year x nres	1	6555	33.5	<0.0001

Linear mixed-effects model of the effects of number of treatment years (ARIMA type-1 autocorrelation) and the number of added resources on diversity (log ESN_{pie}) and richness, with plot nested in block, nested in year, nested in site, as random effects, using all 45 sites. There was a significant, negative interaction between the number of added resources (*nres*) and year of treatment (*year*).

Extended Data Table 2 | The number of added resources predicts diversity loss after controlling for other variables

	Num. DF	Den. DF	F	P
intercept	1	1029	329.9	<0.0001
years of treatment	1	42	12.1	0.0012
site richness	1	42	21.5	<0.0001
log live biomass	1	1029	17.4	<0.0001
total cover	1	1029	4.8	0.029
number of added resources	1	1029	35.4	<0.0001

Linear mixed-effects model of the effects of number of treatment years, site richness, log live biomass, total species cover, and the number of added resources on diversity (ESN_{pie}), with plot nested in block nested in site as random effects, using all 45 sites and data from the maximum treatment year for each site. Δ AIC between model with number of added resources and model without was 33, log-likelihood ratio 35.0, $P < 0.0001$.

Extended Data Table 3 | The number of added resources is an important predictor even after controlling for other variables, for sites that had light and litter data

	Num. DF	Den. DF	F	P
intercept	1	643	285.3	<0.0001
years of treatment	1	29	14.1	0.0008
site richness	1	29	25.7	<0.0001
log live biomass	1	643	7.9	0.0052
total cover	1	643	4.5	0.034
log dead biomass	1	643	0.34	0.56
PAR	1	643	18.2	<0.0001
number of added resources	1	643	15.6	0.0001

Linear mixed-effects model of the effects of number of treatment years, site richness, log live biomass, log dead biomass, PAR, total species cover, and the number of added resources on diversity (ESN_{plu}), with plot nested in block nested in site as random effects, using data from the maximum treatment year for each site, and the subset of 32 sites for which there was dead biomass and PAR data. Δ AIC between model with number of added resources and model without was 15, log-likelihood ratio 15.6, $P < 0.0001$.

Extended Data Table 4 | Diversity loss due to addition of nutrients associated with soil properties

	DF	SS	F	P
soil P	1	0.16	1.72	0.20
soil K	1	0.018	0.20	0.66
pH	1	0.46	5.03	0.034
% sand	1	0.73	8.05	0.0089
residuals	25	2.28		

Stepwise multiple regression (backward with AIC criteria for model comparisons) retained soil P, K, pH, and percentage sand as predictors of diversity loss from the addition of three resources, for the 30 sites with soil analysis data (excluding one site for extreme value of P). The variables latitude, longitude, mean annual precipitation, and soil percentage N were not retained. Overall model is significant ($r^2 = 0.375$, $F_{4,25} = 3.75$, $P = 0.016$).