Grassland species loss resulting from reduced niche dimension

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Intact ecosystems contain large numbers of competing but co-existing species. Although numerous alternative theories have provided potential explanations for this high biodiversity, there have been few field experiments testing between these theories. In particular, theory predicts that higher diversity of coexisting competitors could result from greater niche dimensionality[1], for example larger numbers of limiting resources or factors. Alternatively, diversity could be independent of niche dimensionality because large numbers of species can coexist when limited by just one or two factors if species have appropriate trade-offs[2]. Here we show that plant coexistence and diversity result from the 'niche dimensionality' of a habitat. Plant species numbers decreased with increasing numbers of added limiting soil resources (soil moisture, nitrogen, phosphorus and base cations), which is consistent with theoretical predictions that an increased supply of multiple limiting resources can reduce niche dimension. An observational field study gave similar results. The niche dimension hypothesis also explained diversity changes in the classic Park Grass Experiment at Rothamsted. Our results provide an alternative mechanistic explanation for the effects of nutrient eutrophication on the diversity of terrestrial, freshwater and marine ecosystems.
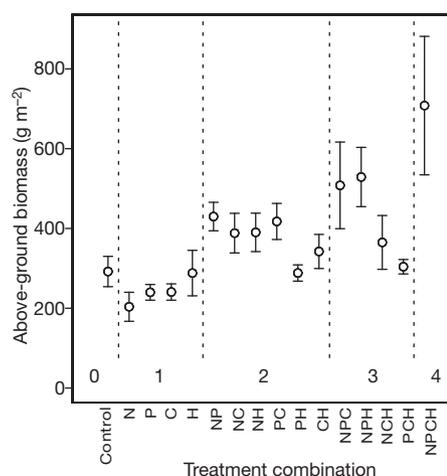
Multiple resource limitation, heterogeneity, and interspecific differences can explain the stable coexistence of numerous competing species[3]. Niche theory predicts that resource-dependent community assembly imposes a stochastic limit on the similarity of coexisting competing species and thus limits the number of species that can coexist along any particular limiting resource trade-off axis[4]. Resource addition can reduce the number of limiting resources, allowing fewer trade-off opportunities and thus fewer coexisting species. Additionally, an increased supply of limiting resources might change the stoichiometry[5,6] or decrease the heterogeneity of limiting resources, thereby decreasing the size of the region of niche space for coexistence without necessarily reducing the number of limiting resources[3,7]. Because species are evolutionarily differentiated with respect to particular limiting resources, changes in the identity of limiting resources can also reduce the effective niche dimension of existing communities because those species may lack the requisite trait differentiation and trade-offs for coexistence when limited by different resources. Environmental change can therefore reduce the niche dimension of particular ecological communities through decreases in the number or heterogeneity of limiting resources or through changes in the identity and stoichiometry of limiting resources[8].

To experimentally test the response of species number to increased supply of multiple limiting resources, we applied a factorial addition of nitrogen, phosphorus, cations (K^+, Mg^{2+} and Ca^{2+}) and water to a grassland at the University of California's Sedgwick Reserve, Santa Ynez, California, USA (see Methods and Supplementary Information). Plant productivity was limited by all four experimental

resources (Fig. 1; significant four-way interaction, Supplementary Table S1), and interactions involving N, P and water were significant for species loss. Resources were strongly co-limiting: we found no significant effect of adding any single resource, but plots that received all four resources had significantly greater productivity and fewer species than did plots that received any of the four possible combinations of three resources (Supplementary Table S2).

To test the 'niche dimension' hypothesis, which predicts that decreased numbers of limiting factors should lead to a decreased number of species, we categorized experimental resource additions as the number of limiting resources added to a plot (that is, 0 = control, 1 = addition of any single resource, 2 = addition of any combination of two resources, and so on). An increased number of resources added, with the objective of overcoming resource limitation, led to a nonlinear decrease in species number because of strong co-limitation (3.1 species were lost with four added resources; Fig. 2a). Changes in the supply of multiple limiting resources may therefore have important consequences for biodiversity in ecosystems that are strongly co-limited: diversity may show little response to increases in the supply of a single resource but show catastrophic loss of species with increases in two or more resources.

The addition of multiple soil resources led to the dominance of a single species in our experiment and to strong decreases in light levels, consistent with a decrease in niche dimensionality towards

Figure 1 | **Multiple resource limitation of plant productivity.** The greatest effect of factorial addition of resources occurred with the addition of all four resources: N, P, C and H represent the addition of nitrogen, phosphorus, cations and water. Vertical dotted lines group treatment combinations by the number of added resources, shown at the bottom of each group. Results are shown as means ± s.e.m.

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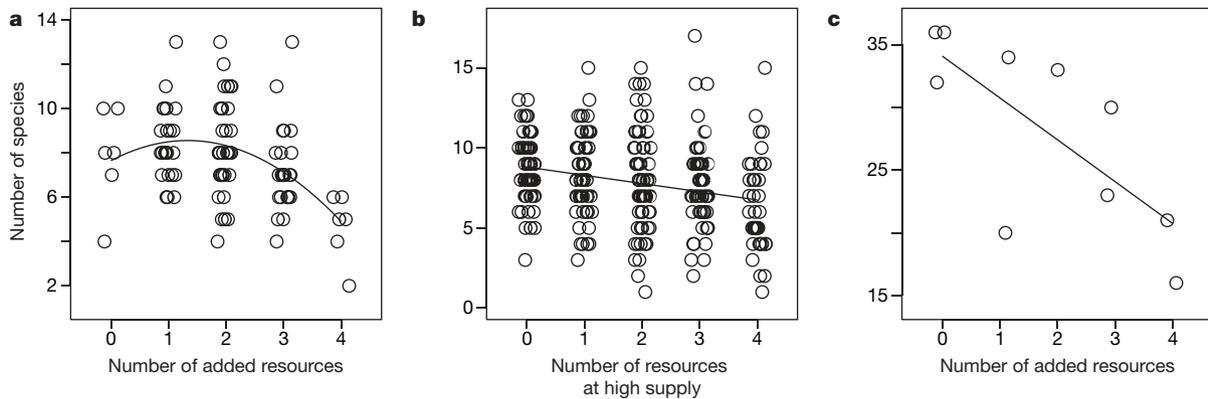
Figure 2 | Species loss with reduction of niche dimension. a, Species number plotted against number of added resources ($R^2 = 0.196$, $P < 0.0001$, significant quadratic term, $n = 96$) in the Sedgwick experiment. **b**, Species number plotted against number of resources at high supply ($r = -0.23$, $P = 0.0008$, $n = 215$) in the Sedgwick observational study. **c**, Species number plotted against number of added resources ($R^2 = 0.55$, $P = 0.016$, $n = 10$) in the Park Grass Experiment. Points are jittered for clarity.

single-factor limitation by light and exclusion of all except a single species best adapted to low light but high nutrient concentrations[3]. *Bromus diandrus* increased from 24% average relative biomass in the control plots to 56% in plots receiving all resources. All other species decreased with increasing numbers of added resources (for example *Nassella pulchra* decreased from 57% to 36% relative biomass, and *Bromus hordeaceus* from 13% to 4%).

To compare our experimental results with patterns of species number and nutrient supply in unmanipulated vegetation, we established 215 permanent observational plots in natural grasslands at Sedgwick Reserve. We used buried ion-exchange resins to estimate resource supply for multiple potentially limiting nutrients (N, P, K and Mg; see Methods and Supplementary Table S3). For comparative purposes we categorized the supply of N, P, K and Mg in each observational plot as either high (above the median) or low (below the median) and scored plots by the number (0–4) of these four resources that were at high supply and were therefore less likely to be limiting. Species number decreased with greater numbers of resources at high supply (0.5 species were lost per resource at high supply; Fig. 2b). A loss of less than one species per resource may be because 'high' supply rates reflected a decrease in resource limitation rather than its complete elimination. Although significant, this relationship was weak ($R^2 = 0.053$), indicating the possible importance of other limiting factors besides these particular nutrients as determinants of diversity in these plots.

Our extension of the niche dimension hypothesis is essentially multivariate: species number and productivity both respond directly to the experimental addition of resources (Fig. 3a). The joint response of

species number and productivity to the number of resources at high supply was highly significant for the Sedgwick resource addition experiment and observational study (Fig. 3b; multivariate analysis of variance linear contrasts: squared canonical correlations 0.28, 0.086; $F_{2,85} = 16.88$, $F_{2,209} = 9.89$; $P < 0.0001$ and $P < 0.0001$, respectively). Because both species number and evenness determine species diversity, we calculated the 'effective' species number e^H (H is Shannon's diversity index); its value is equivalent to the number of equally abundant species. The diversity (e^H) pattern across the combined natural and augmented productivity gradient at Sedgwick was strikingly monotonic (Fig. 3c), indicating that an increased supply of multiple resources decreased both species number and evenness.

Species loss resulting from fertilization could potentially be a transient response reflecting a local lack of species adapted to a 'vacant niche' represented by the new resource environment[9]. We tested this by using data from the longest-running resource addition experiment of which we are aware[10] (see Methods). The Park Grass Experiment was established at Rothamsted Experimental Station (Harpenden, Hertfordshire, UK) in 1856 in grassland that previously had been mown and grazed for hundreds of years[11]. Since 1856, plots have received annual application of fertilizers[10]. Productivity at this site is limited by multiple resources including N, P and K (ref. 12). Consistent with our experiment at Sedgwick is the observation that increased numbers of added limiting resources led to decreased species number (3.3 species lost per added resource; Fig. 2c). Fertilization has led to species loss that has persisted for 150 years[12], which is consistent with reduced niche dimensionality.
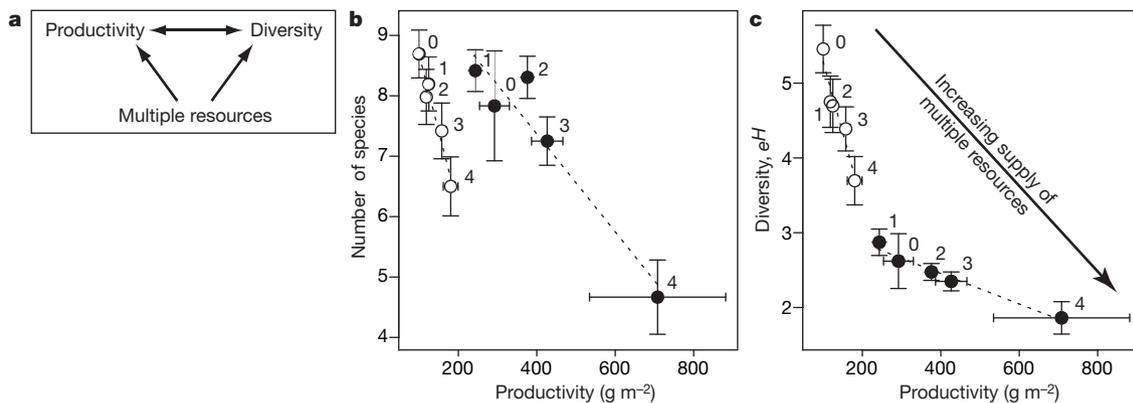
Figure 3 | Effects of multiple resource supply on diversity and productivity across natural and experimental productivity gradients. a, Increased supply of multiple resources decreases diversity through reduced niche dimension, and increases productivity; diversity and productivity are correlated. **b**, Joint response of species number and productivity to increased number (labelled numerals) of resources added or at high supply (that is, reduced niche dimension). **c**, Joint response of diversity and productivity to increased number of resources added or at high supply. Open circles in **b** and **c** are observational plots; filled circles are experimental plots. Results are shown as means ± s.e.m.

Although plant species number typically decreases with experimentally increased productivity[10,13–16], it is unclear how productivity could in itself cause species loss. The proposed causal mechanisms of the negative effects of resource addition on species number have been essentially indirect ones mediated through effects of productivity on light and litter[17–19], or through consumers[20,21]. In our experiment, resource addition increased production, and consequently increased litter ($R^2 = 0.13$, $P = 0.0003$) and exponentially decreased light levels ($R^2 = 0.31$, $P < 0.0001$), suggesting possible indirect effects of resource limitation on species number.

To quantify the relative importance of niche dimensionality (the direct effects of the number of limiting resources) as opposed to potential indirect effects of resource addition on species number mediated by productivity, we compared regression models of the number of added resources, light and litter with the use of Akaike's information criteria. We also included productivity as a predictor because it may proximally affect diversity through undetermined mechanisms. Although productivity, but not litter or light, predicted species number, the most parsimonious model (lowest Akaike's information criterion) included only the number of added resources (Supplementary Table S4a). The number of added resources was significant in every model predicting species number when it appeared last, supporting the hypothesis that niche dimensionality contributed to species number even after controlling for potential indirect effects of productivity. Comparing models of e^H, we found that both niche dimension and litter may have been important in determining diversity in this experiment (Supplementary Table S4b).

Our results suggest that a combination of a decreased number of limiting resources and changes in the identity of limiting factors resulting from indirect effects of productivity led to decreased niche dimension and diversity. Decreased resource heterogeneity may also have contributed to decreased niche dimension. Although we did not measure resource heterogeneity in this experiment, in a parallel experiment at Sedgwick the addition of N at $4 \, \mathrm{g} \, \mathrm{m}^{-2} \, \mathrm{yr}^{-1}$ increased mean inorganic soil N from 2.02 to $7.03 \, \mathrm{mg} \, \mathrm{kg}^{-1}$ ($P = 0.001$, $n = 16$) and decreased its heterogeneity, expressed as the coefficient of variation, from 0.60 to 0.45. Although the relative importance of these mechanisms is not known, results from the Park Grass Experiment suggest that recovery of diversity after eutrophication may be a slow process. Invasion by preadapted species or evolutionary niche differentiation could eventually restore species diversity, but such consequences are likely to be deemed undesirable or impracticable from a conservation perspective.

Our results suggest that human actions, such as eutrophication, that simplify habitats by decreasing their niche dimensionality can lead to long-term biodiversity loss. Loss of biodiversity could in turn further decrease ecosystem function[22]. Our experimental and observational results from terrestrial grasslands are consistent with those recently found in aquatic systems[23,24]. How species diversity scales with resource diversity in other systems will depend on the degree to which they are co-limited by multiple resources. Experiments are needed that manipulate multiple resources across important environmental gradients such as productivity and latitude in aquatic and terrestrial ecosystems. If niche axes are independent and approximately equal in importance, diversity should decrease linearly with the loss of niche dimension, but the consequences of niche loss may be even greater if species loss responds multiplicatively as suggested[8].

METHODS

We applied N, P, cations (K^+, Mg^{2+} and Ca^{2+}) and water to 96 plots ($2 \, \mathrm{m} \times 2 \, \mathrm{m}$) in a grassland at Sedgwick Reserve, beginning in February 2000. Nutrients were applied twice annually in dry form and water was applied by drip irrigation at roughly one-week intervals throughout the growing season (see Supplementary Information for details and rates). We measured above-ground plant productivity and species composition at peak biomass of the second year of the experiment in May 2001 by clipping, drying and weighing a 0.3-m^2 biomass sample from each plot. The observational plots at Sedgwick consisted of 215 plots ($2 \, \mathrm{m} \times 2 \, \mathrm{m}$) scattered across ungrazed grasslands in the 2,382-ha reserve and encompassed a wide range of topographic variability. We estimated species composition visually and

measured total live-plant above-ground biomass by clipping, drying and weighing a 0.2-m^2 sample from each plot. We used inductively coupled plasma chromatography after NaCl extraction of mixed-bed ion-exchange resins that we had buried in the soil in each plot to estimate soil nutrient supply rates over the growing season (see Supplementary Information for details). We used Park Grass species number data from 1946 to 1949 and productivity data from 1949 as reported[10]. Because plant diversity decreased strongly with lower pH caused by the addition of ammonium, we used data from the ten unlimed subplots (plots 2, 3, 4.1, 7, 8, 12, 14, 15, 16 and 17) that did not receive ammonium or manure. Statistical tests are described in more detail in Supplementary Information.

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