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LETTERS

Biodiversity and ecosystem stability in a decade-long grassland experiment

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Human-driven ecosystem simplification has highlighted questions about how the number of species in an ecosystem influences its functioning. Although biodiversity is now known to affect ecosystem productivity[1–6], its effects on stability are debated[6–13]. Here we present a long-term experimental field test of the diversity–stability hypothesis. During a decade of data collection in an experiment that directly controlled the number of perennial prairie species[4], growing-season climate varied considerably, causing year-to-year variation in abundances of plant species and in ecosystem productivity. We found that greater numbers of plant species led to greater temporal stability of ecosystem annual aboveground plant production. In particular, the decadal temporal stability of the ecosystem, whether measured with intervals of two, five or ten years, was significantly greater at higher plant diversity and tended to increase as plots matured. Ecosystem stability was also positively dependent on root mass, which is a measure of perenniating biomass. Temporal stability of the ecosystem increased with diversity, despite a lower temporal stability of individual species, because of both portfolio (statistical averaging) and overyielding effects. However, we found no evidence of a covariance effect. Our results indicate that the reliable, efficient and sustainable supply of some foods (for example, livestock fodder), biofuels and ecosystem services can be enhanced by the use of biodiversity.

The hypothesis that greater ecological diversity leads to greater stability[7] has been a point of interest and debate for a half century[7–14]. Field observations[10,11,14,15] and laboratory experiments[16–18] have generally shown that greater diversity is associated with greater ecosystem stability but lower species stability, much as predicted by models of multispecies competition[8,19]. However, well-replicated field experiments that manipulate variables of interest have become indispensable in ecology. The only such field experiment to test for diversity–stability relations so far has been an eight-week study that had mixed results[13,20], leading a major review to conclude that the effects of diversity on stability remained unresolved[6]. We propose that diversity has consistent stabilizing effects on ecosystem processes once timescales are sufficient to incorporate the average net effects of diversity on both resistance to and recovery from perturbations.

Here we present the dependence of the temporal stability of ecosystems and species on plant diversity in a long-term grassland biodiversity experiment that established 168 plots containing 1–16 species[4]. Stability has several meanings in ecology[8,21]. We focus on temporal stability, *S*, which measures the degree of constancy in a variable relative to its mean. *S* is defined as μ/σ, where μ is the mean value for a time period and σ is its temporal standard deviation over the same interval[19,22]. Many factors can cause the abundances and primary productivity of plant species to vary, including precipitation[10,23], temperature[23], life histories, and interactions with other organisms. During the ten years of data collection that followed two years of establishment for our 12-year experiment (Methods), precipitation during the growing season varied more than twofold and average daily high temperatures during the growing season ranged from 21.5 to 24.4 °C, creating growing seasons with widely different climatic conditions. Here we determine whether observed temporal variability in plant biomass was dependent on plant community diversity. We examine both ecosystem stability (temporal stability of aboveground plant biomass summed across all species in a plot, a measure of primary productivity) and species stability (temporal stability of the aboveground biomass of individual plant species).

We determined ecosystem stability with the use of all ten years (1996–2005) of data collected annually on aboveground biomass production within each plot. Ecosystem stability was determined both without detrending and after detrending data for each plot to remove variation attributable to a ten-year trend of generally increasing plot biomass in higher-diversity treatments (Methods). To ensure normality, all temporal stabilities were log transformed before analyses.

Detrended ecosystem stability was an increasing function of the number of planted species (Fig. 1a; $F_{1,161} = 44.9, P < 0.0001$). It was similarly dependent on the 1996–2005 plot-average Shannon diversity index, H' ($F_{1,161} = 13.7, P = 0.0003$), on plot-average effective species number, $e^{H'}$ ($F_{1,161} = 20.2,\ P < 0.0001$) and on realized species number (that is, the number of abundant species contributing to 90% of total biomass in 2005; Fig. 2; $F_{1,160} = 23.7$, $P < 0.0001$). Greater ecosystem stability at higher diversity meant there was lower proportional change in the annual production of biomass in plots with greater plant diversity. On average, the treatment plots with the highest diversity were about 70% more stable than monocultures (Fig. 1a). This greater ecosystem stability at higher diversity has been called the insurance value of biodiversity[17].

We also measured ecosystem stability without detrending by dividing the ten years of data into non-overlapping intervals of shorter duration (two or five years) for which biomass had small or no temporal trends, and then calculating plot stability for each interval as $S = \mu/\sigma$. A repeated-measures multiple analysis of variance (MANOVA) that had the two five-year stabilities as dependent variables showed that greater numbers of planted species led to greater ecosystem stability ($F_{1,161} = 21.1,\ P < 0.0001$) and that stability was greater for the second five-year period (time effect: $F_{1,161} = 12.2,\ P = 0.0006$). A species-number × time interaction ($F_{1,161} = 6.29,\ P = 0.013$) indicated a stronger positive effect of greater species numbers on ecosystem stability in the second time period. Another repeated-measures MANOVA that had the five two-year measures of ecosystem stability as dependent variables showed that greater species numbers led to greater ecosystem stability

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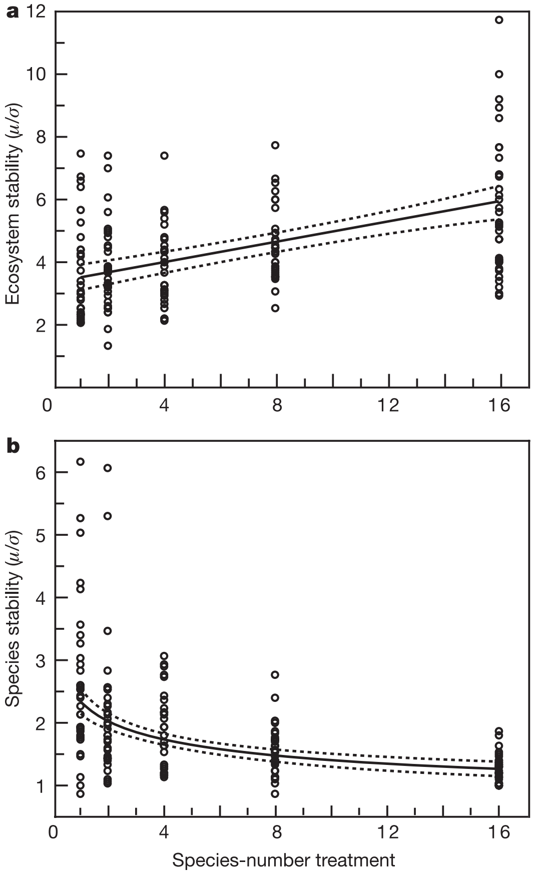

Figure 1 | Dependence of temporal stability of each plot on experimentally imposed species-number treatment. **a**, Ecosystem temporal stability for the decade from 1996 to 2005 was an increasing function of the number of planted species. Ecosystem stability is the ratio of mean plot total biomass to its temporal standard deviation, determined after detrending. The regression line and its 95% confidence interval are shown (untransformed data: $F_{1,159} = 43.7$, $P < 0.0001$). To reduce the difference in y axis scale between the two parts of this figure, a single data point (species number of 16, ecosystem stability of 15.76) is not shown but was included in all analyses. **b**, Plot-average species temporal stability, determined with species biomass data for 2001–2005, was a declining function of the number of planted species. The regression curve and 95% confidence intervals are based on a fit of log(species stability) on log(species number), with $F_{1,159} = 72.3$, $P < 0.0001$.

($F_{1,155} = 16.5$, $P < 0.0001$), stability had a weak tendency to increase through time ($F_{4,152} = 2.24$, $P = 0.067$) and there was no species-number × time interaction ($F_{4,152} = 0.60$, $P = 0.66$). Similar repeated-measures MANOVAs, of both two-year and five-year stabilities, that used realized species number as the independent variable yielded similar results. The greater ecosystem stability of higher-diversity plots resulted from their having lower temporal standard deviations, for a given mean plot biomass, than plots with lower diversity (Fig. 3). In total, on average across the decade of measurement, ecosystem stability was significantly positively dependent on plant diversity, and this result was robust with respect to data detrending and the intervals over which stability was determined.

In contrast to ecosystem stability, stabilities of individual species (log transformed), determined with our five-year record of abundances of each species planted in each plot, were a declining function of the number of planted species ($F_{1,988} = 134.3$, $P < 0.0001$) and, similarly, of effective species number, $e^{H'}$ ($F_{1,988} = 83.6$, $P < 0.0001$). We also calculated the average, for each plot, of the species stabilities of all species planted in the plot, and found that the plot-average

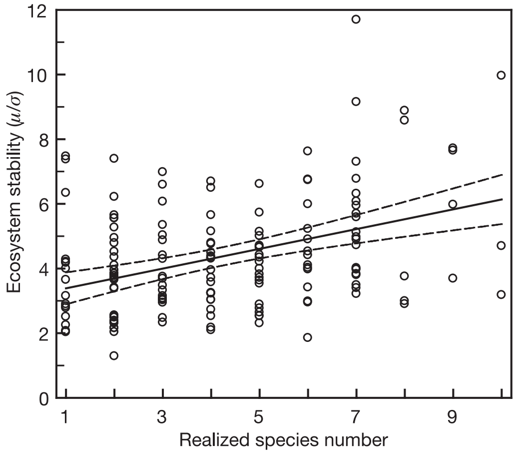

Figure 2 | Dependence of ecosystem temporal stability from 1996 to 2005 on realized species number. All species, whether planted or weedy, were ranked by proportional abundance in the sorted 0.6 m^2 clipped strip of 2005. Proportional abundances were summed, in order from the most abundant species, to determine the realized species number, which is the number of more abundant species comprising 90% of the total aboveground biomass of a plot. Ecosystem stability was also significantly dependent on realized species number determined with cutoffs of 75% ($P < 0.0001$) and 99% ($P = 0.002$). As in Fig. 1a, one data point (realized species number of 7.2, ecosystem stability of 15.76) is not shown but was included in all analyses.

species stability (log transformed) was a declining function of planted species number ($F_{1,159} = 63.5$, $P < 0.0001$; Fig. 1b) and of $e^{H'}$ ($F_{1,159} = 57.1$, $P < 0.0001$). Species stabilities were not detrended, but results were similar if detrended.

We used stepwise regression to evaluate the influence of root mass, functional group composition (presence or absence of C_3 grasses, C_4 grasses, legumes or non-legume forbs), weedy species biomass, initial soil fertility (initial total soil nitrogen) and species number on ecosystem stability. In both forward addition and backward elimination analyses, the same three variables were retained, with ten-year detrended ecosystem stability remaining positively dependent on species number ($F_{1,159} = 16.2$, $P < 0.0001$) and also being positively dependent on root mass ($F_{1,159} = 23.0$, $P < 0.0001$) but negatively dependent on the presence of legumes ($F_{1,159} = 4.42$, $P = 0.037$). The positive effect of root mass probably occurred because roots are the perenniating structure of these herbaceous perennial species, and higher root mass should provide a larger store of nutrients and energy to buffer growth in response to environmental variation. Weedy biomass had no significant ($P > 0.05$) effects on stability and was neither added nor retained in the forward or backward stepwise regressions, respectively. Similarly, in repeated measures analyses using two-year or five-year ecosystem stabilities, weed biomass had no significant effects ($P > 0.1$) but ecosystem stability remained an increasing function of numbers of species planted ($P < 0.001$). This indicates that any disturbance that might have been associated with differences between treatments in weeding intensity did not influence results. Diversity did affect invading weedy species. After cessation of weeding in subplots, total numbers of plant species and total biomass increased more at lower diversity than at higher diversity[24].

The strength and consistency of the long-term stabilizing effects of diversity on ecosystem productivity that we observed contrast with mixed effects observed when a short-term drought was imposed on a biodiversity experiment[13]. In that study, the proportion of aboveground plant biomass lost after an 8-week drought was independent of diversity[13], indicating, by a metric analogous to ours, no effect of diversity on short-term proportional resistance stability. Because more diverse plots had greater biomass, the absolute biomass loss was greater at greater diversity, which was interpreted as showing lower absolute resistance stability at higher diversity[13]. However, during the

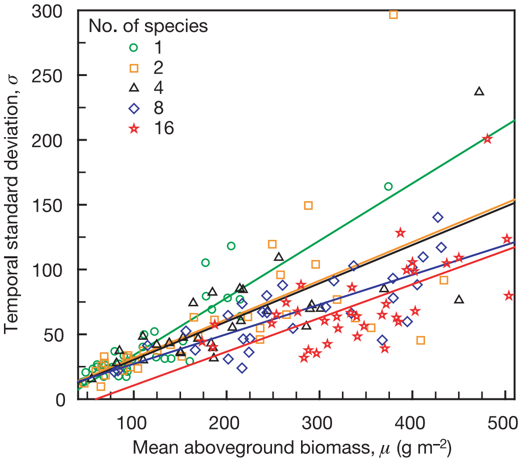

Figure 3 | Effects of plant diversity on the relationship between mean biomass and its temporal standard deviation. Each point shows the mean biomass of a plot and its temporal standard deviation based on the five annual samples collected from 2001 to 2005. Data for each species-number treatment were fitted by a separate regression line. Data were not detrended. The results show that high-diversity treatments have lower temporal standard deviations (lower risks) for a given mean biomass (return).

four years before imposition of drought, more diverse plots had greater temporal stability[25]. In combination, these two studies indicate that there might be short-term variability in effects of diversity on ecosystem stability but that, in the long term, higher plant diversity causes greater ecosystem stability.

Our results support the predictions of competition theory that greater diversity leads to greater ecosystem stability and lower species stability[8,19,22].This theory predicts that greater ecosystem stability at higher diversity can result from increasingly negative covariance in the abundances of competing species at higher diversity[19,22] (covariance effect), from the manner in which temporal variance in species abundances scales with abundance[19,22,26,27] (statistical averaging or portfolio effect), and/or from the manner in which species abundances scale with diversity[19,22] (overyielding effect; greater ecosystem total biomass at higher diversity).

The covariance effect requires that total covariance (temporal covariance in abundances for each pair of species, summed across all possible pairs of species) decline as diversity increases. However, regression showed no dependence of total covariance for 2001–05 on species number ($F_{1,161} = 0.83$, $P = 0.36$). Our results thus do not support the covariance effect, a conclusion also reached in an earlier analysis of non-experimental data[22]. The portfolio effect[22,26] requires temporal variance, s^2, in the abundance of a species to scale with its biomass, m, as $s^2 = c_1 m^z$, with $1 < z < 2$, which it did, with $z = 1.60$. This supports the role of the portfolio effect in stabilizing higher-diversity plots. In multiple regression, summed variances were a declining function of species number ($F_{1,160} = 15.7$, $P < 0.0001$) and an increasing function of total biomass ($F_{1,160} = 127$, $P < 0.0001$), which also supports the portfolio effect. The overyielding effect requires[22] that total plot biomass, B, increase with diversity, which it did each year of the experiment. For 2001–05, plots containing 16 species had, on average, 180% more biomass than monocultures. In total, these analyses strongly indicate that greater ecosystem stability at higher diversity resulted from portfolio and overyielding effects.

Rapidly increasing human population and consumption, and concomitant demand for food and energy, are making society increasingly dependent on services provided by remaining natural and managed ecosystems[28,29]. Biodiversity experiments have shown that greater numbers of plant species lead to a greater production of biomass[1–6]. Our results show that the long-term stability of an ecosystem service—the annual production of biomass and thus of potential biofuels and livestock fodder[1–6]—also depends on biodiversity. Biodiversity can therefore be an important element for the reliable and sustainable provisioning of ecosystem services.

METHODS

Experimental design. In a 7-ha field at Cedar Creek Natural History Area, Minnesota, USA, we controlled the number of plant species in 168 plots, each 9 m × 9 m. Plots were randomly assigned to be seeded with 1, 2, 4, 8 or 16 perennial grassland species, with 39, 35, 29, 30 and 35 replicates, respectively, of the diversity levels. The composition of each plot was randomly chosen from a set of 18 perennials (four C_4 grasses, four C_3 grasses, four legumes, four non-legume forbs and two woody species). All plots received $10\,\mathrm{g\,m^{-2}}$ of seed in May 1994 and $5\,\mathrm{g\,m^{-2}}$ in May 1995, with seed mass divided equally between species. Treatments were maintained by weeding three or four times each year. Weeds were removed while still small, with care being taken to minimize any disturbance. Plots were burned annually in spring before growth began. Five woody monocultures are not included in analyses because burning effectively eliminated woody species from multispecies plots. Plots were annually sampled in mid-August for aboveground living plant biomass by clipping, drying and weighing four parallel and evenly spaced 0.1 m × 3.0 m vegetation strips per plot from 1996 to 1999 and four 0.1 m × 6.0 m strips per plot from 2000 to 2005. Different locations were clipped each year. Biomass from one strip per plot was sorted to species from 2001 to 2005. The Shannon diversity index, H', used abundances of each species, planted or weedy, in each plot by means of estimates of percentage cover for 1996–2000 (four $0.5\,\mathrm{m}^2$ subplots per plot) and sorted biomass for 2001–2005. See ref. 4 for further details.

Sampling effort. To eliminate potential bias from different sampling efforts for the first four in comparison with the last six years, for each of the last six years two clipped strips per plot were randomly chosen for an analysis of ecosystem stability. The full data gave similarly significant and positive effects of diversity on all three measures of ecosystem stability.

Detrending and other analyses. Detrending was done, for each plot, by means of linear regression of annually measured plot biomass on the logarithm of year and used all ten years of plot data. The logarithm of year provided a generally better fit than year; both gave similar results. The standard deviation, σ_d, of residuals for each regression measures detrended variation. The detrended temporal stability, S_d, of a plot was $S_d = \mu/\sigma_d$. Each plot had a single detrended stability value for the ten-year period. In contrast, when data were divided into shorter intervals that did not require detrending, there were multiple values of S, calculated as $S = \mu/\sigma$, per plot. We divided the data either into two subsets, each five years in duration (1996–2000 and 2001–05) or into five subsets, each two years in duration (1996–97, 1998–99, 2000–01, 2002–03 and 2004–05). These temporal sequences of S values for each plot were analysed with the use of repeated-measures MANOVA.

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