Supplementary Material for

Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades

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Methods

Both the BioDIV and BioCON experiments are located at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA and were established on sandy soils (Nymore series, Typic Ustipsamments) on old fields after removing the prior vegetation. These analyses used all BioDIV plots (n=152) with uniform fire treatment and without oaks and all BioCON plots (n=74) from the main random assemblage experiment that were grown at ambient levels of CO₂ and N. Prior to planting in 1994 and 1995 the BioDIV site was bulldozed to remove the top 6–8 cm of A horizon soil to reduce the seed bank; whereas the BioCON site was treated with methyl bromide for the same purpose prior to planting in 1997; otherwise the two experiments were planted and maintained similarly.

BioDIV plots were originally each 9 x 9 m and seeded with 1, 2, 4, 8 or 16 grassland–savannah perennial species randomly selected from a pool of 18 species that included C4 and C3 grasses, legumes, forbs and trees (see 6, 12 for more info). The BioCON plots are 2 x 2 m and were seeded with 1, 4, 9 or 16 grassland species randomly selected from a pool of 16 species that included C4 and C3 grasses, legumes, and forbs (18). The species richness treatments span the range of native diversity (standardized by spatial scale) in diverse native and anthropogenic successional grasslands at Cedar Creek. Native savanna grasslands at our site average 10 plant species per 0.5 square-meter quadrat (29), and 16.3 species per 1.0 square-meter quadrat (30). Our sampling, in BioCON for instance, was made using 0.1 square-meter quadrats and shows richness in plots planted with 16 species to be 9-11 (31) due in part to local extinctions in individual plots and in part to sampling only a small fraction of each plot. In contrast, 20 former
prairie sites (32) that had been farmed and then restored to grassland through the Conservation Reserve Program had a median of 3 species per 1.0 square-meter quadrat, a mean of 3.5 species, and a range of 1 to 8 species per square-meter. Our 16 species treatments are thus roughly representative of high-diversity native vegetation, while our 2 and 4 species treatments have diversity similar to grasslands of anthropogenic origin. Thus, one of our key findings- that differences between plots planted with 8 or 9 vs. 16 species are important- is relevant to how losses of diversity from native systems might influence their function.

Each year in BioCON and in most years in BioDIV every plot was measured for species composition and richness, and above- and below-ground plant biomass (0 to 30 cm in BioDIV, 0-20 cm in BioCON) in late summer (August) and the plant C and N concentrations of those samples were assessed in most years (6, 18-21). A relatively small fraction of the plot area is harvested each year, and different areas are harvested in different years. For example, to clarify the degree of disturbance for BioCON (which has much smaller individual plots and thus would be more prone to a disturbance effect), any given biomass sampling of each plot represented 1/40th of the plot for aboveground biomass and 1/2000th for belowground biomass and soils; and such sampling was done only once or twice per year, and any given location once every fifth year. Given that burning removes most of the residual aboveground biomass in roughly two of three years, the many-fold lesser disturbance from clipping a small area likely does not influence the ecosystem processes, and in any case, is done identically in every plot.
Comparing shapes of BEF relationships

Following Cardinale et al. (1) and Schmid et al. (33), we fit multiple functions that potentially describe the shape of the relationship between biomass and richness: linear, log (natural logarithm), power function, and Michaelis-Menten (M-M). These four relationships have different biological interpretations (1, 33, 34). The M-M relationship saturates, which suggests that some species are functionally redundant, and thus early extinctions will not decrease biomass. The log relationship decelerates without saturating, which suggests that some species influence biomass more than others, and thus early extinctions could decrease biomass less than later extinctions. The power function was fit following Cardinale et al. (1). The ‘b’ exponent of this function can indicate that ecosystem functioning is independent of richness (b = 0), that biomass is a decelerating function of richness (0 < b < 1), or that biomass is a linear function of richness (b = 1). The linear relationship incrementally increases, which suggests that each species has a proportional effect, and thus early extinctions will decrease biomass the same amount as later extinctions. Distinguishing between these four relationships will help determine whether the consequences of species extinctions will likely arise in the near or distant future. We used the Akaike Information Criterion (AIC) to compare the four fits (Table S1).

The linear, log, and power functions were fit with the lm function, and the M-M function was fit using the nls function, in R 2.11.1. Details of fits for several biomass response variables (total, aboveground, belowground) and richness predictor variables (planted, realized) are provided in Table 1 and Supplementary Tables 1-4. Results are similar using either the power-fits or log-fits (i.e., the relationship becomes less saturating.
and more linear over time). We use a single functional approach (power-fits) in the figures, for simplicity and because the shape of the curve is more flexible, because it clearly demonstrates the changing nature of the relationships over time without needing to focus on comparing the statistical fits across different functions, and because it was the best model on average across all years in both experiments. Additionally, the similar shifts over time in the shapes of the biodiversity response functions of the two experiments occurred despite important differences in the experimental response patterns. Over time in BioCON, biomass decreased in all but the 16 species mixtures; whereas in BioDIV, biomass generally increased, but much more in 16 species mixtures than in lower diversity treatments.

**Complementarity and selection effects**

Complementarity and selection effects were quantified by Loreau and Hector’s (22) additive partition of the net biodiversity effect (NBE):

\[ NBE = S\Delta RY + S\text{cov}(\Delta RY, M), \]

where S is species richness, \( \Delta RY \) is the difference between the observed and expected relative yield, and M is monoculture productivity. In Equation 1, the first (average) term is the complementarity effect and the second (covariance) term is the selection effect. The observed relative yield for species \( i \) was calculated as \( Y_{oi}/M_i \) where \( Y_{oi} \) and \( M_i \) are the observed mixture and monoculture yields for species \( i \), respectively. The expected relative yield was taken as the planted relative abundance of each species. These measures summarize many types of interactions. A positive complementarity effect indicates that on average interspecific interactions are more favorable than intraspecific
interactions (e.g., due to niche partitioning or interspecific facilitation). A negative complementarity effect indicates that on average intraspecific interactions are more favorable than interspecific interactions (e.g., due to interspecific interference competition). A positive or negative selection effect indicates that the most or least productive species in monocultures overyielded most in mixtures (i.e., benefits most from interspecific interactions compared to intraspecific interactions). As in Fargione et al. (12), monocultures with <2.5 g m\(^{-2}\) of biomass were excluded (i.e. they are near zero and outliers). To account for seeding issues that arose in the establishment phase for BioDIV, biomasses of *Monarda fistulosa* and *Solidago rigida* were combined into a single compound species in all plots; and biomasses of *Amorpha canescens*, *Petalostemon candida*, and *Petalostemon villosa* were combined into a single compound species in all plots.

*Estimating the size of plant diversity effects over time.* To assess diversity effects on plant biomass and plant N content for each experiment year, we calculated response ratios (35) that compared means from the highest level of planted species richness (16 species) to means from monoculture plots, as well as other diversity contrasts (such as the highest to second highest diversity levels, or 4 species plots to monocultures). As a check on whether proportional or absolute effects showed comparable results, we also compared differences between the highest and next highest diversity treatment on an absolute basis and found these results to be consistent (Fig S6) -- the amount of biomass increase per added species between the highest and second-highest diversity levels also grew with time. Soil N parameters had skewed distributions that contained outliers even after
transformation, so we calculated response ratios for these parameters using the median instead of the mean. Finally, to assess effects of planted species richness among plots comprised of equal proportions of each plant functional type, we calculated response ratios comparing the highest diversity treatment to plots that contained one species from each of the four plant functional types. In all cases, response ratios were $\log_{10}$ transformed such that positive effects of plant species richness are indicated by response ratios greater than zero. Temporal trends in response ratios were then evaluated using linear least squares regression. Results of non-linear fits, such as quadratic or logarithmic models, are reported when these yielded substantially higher coefficients of determination ($R^2$ values).

Functional diversity calculation. Functional diversity was calculated using an abundance-weighted version of the dendrogram-based diversity metric FD (36), using the traits leaf N concentration and root mass fraction. Trait data were collected from the ambient monocultures of the BioCON experiment for all years from 1998-2010. Other trait data were available only as species means or for only a subset of years, and were not used here. As similar trait data over time were not available for the majority of species or years in BioDIV, here we present results for BioCON only. Full details on this calculation are as follows:

First, we compiled a list of candidate traits based on previous work in BioDIV, BioCON, and elsewhere, and on availability of trait data, which included specific leaf area, leaf nitrogen concentration (by mass), specific root length, height, N-fixation ability, seed mass, and root mass fraction. We selected leaf N concentration (leaf N) and
root mass fraction (RMF) because these capture plant strategies for resource consumption and biomass production, and much prior research at this and other sites have found these traits to be good predictors of functions associated with aboveground productivity (37, 38). These traits were not highly correlated with one another in our dataset. Over the 13 years of the experiment, in one year the two traits were significantly positively correlated (Spearman’s rho 0.64), while in three the traits were significantly negatively correlated (rho -0.78 to -0.56). In addition, these traits were measured for each of the 16 BioCON species in replicate ambient monoculture plots in each year of the experiment, presenting an opportunity to examine temporal variation in functional diversity.

FD is calculated using a normalized species x trait matrix (columns are by trait and have mean zero, standard deviation unity), by calculating multivariate distances between species based on their traits, clustering those distances into a dendrogram, and summing the branch lengths in a given community (36). Here we use an abundance-weighted version of Petchey & Gaston’s FD, termed FDA, where the standardized trait values are scaled by the relative abundance of species within a plot, and the FD value is then calculated as described above on the resulting dendrogram based on abundance-weighted trait values. Similar patterns as presented in Fig. 3E were found when using Rao’s quadratic entropy, Q, (39), a measure which emphasizes evenness of relative abundances more strongly than it emphasizes trait distinctiveness (40). For comparison, we present the results using Rao’s Q (Fig. S9).

References

Contrasting scenarios how plant diversity effects on productivity can increase over time. In scenario I increasing plant diversity effects are mainly due to a growing difference between low (LD) and intermediate diversity (ID) plant communities, whereas plant productivity varies little between ID and high diversity (HD) plant communities ($\Delta y_{ID-LD} \gg \Delta y_{HD-ID}$). This results in a saturating response curve and suggests minor changes in ecosystem functioning due to the loss of some species. In scenario II increasing plant diversity effects are also due to a growing difference between ID and HD plant communities ($\Delta y_{ID-LD} \approx \Delta y_{HD-ID}$). This results in a non-saturating response curve and suggests that even a modest decrease in diversity has significant negative effects on ecosystem functioning. It should be noted that changes in the plant diversity effect on productivity are given qualitatively, i.e., they can be either due to stabilizing ID (scenario I) or HD (scenario II) and deteriorating LD communities, due to stabilizing LD and ameliorating ID (scenario I) or HD (scenario II), or both deteriorating LD (scenarios I) or ID (scenario II) and ameliorating ID (scenario I) or HD (scenario II) communities, respectively.

Planted SR needed to yield total biomass predicted for 16 sp. 

Experiment year

**Fig S1**
**Fig S2** Panel A shows temporal trends in estimates of the planted species richness (SR) required to generate 90% of the relative yield for plots planted with 16 species. Each estimate is based on a simple two-step calculation. First, the equations for each power function shown in Fig. 1 (i.e. for the power fits of the biodiversity-productivity relationship in each year) were used to predict the relative yield for a 16 species plot. Then the same equation was used to solve for the number of planted species needed to generate a relative yield that was 90% of that predicted for 16 species plots. Thus, the Y-axis in Panel A is a standardized version of the original power fit exponents shown in Fig. 1. Panel B shows temporal trends in the coefficient of determination ($R^2$) for power fits of the biodiversity-productivity relationship in each year. In this case, the $R^2$ values are presented for the fits of total biomass expressed as relative yield (Fig. 1 and Table 1). Curved lines from log functions are shown when they gave substantially higher $R^2$ values than linear fits.

**Fig S3** Power function fits for aboveground and belowground (0-20 or 0-30 cm depth, respectively) relative yield in relation to planted species richness, across years in the BioCON and BioDIV experiments. Details of all fits are provided in Table S2.
Fig S4 Power function fits for total, aboveground and belowground (0-20 or 0-30 cm depth, respectively) absolute biomass in relation to planted species richness, across years in the BioCON and BioDIV experiments. The shapes and fits of these relationships are similar to those for relative yield, for which details are provided in Tables S2 and S3.
Fig S5 Power function fits for total, aboveground and belowground (0-20 or 0-30 cm depth, respectively) relative yield in relation to observed species richness, across years in the BioCON and BioDIV experiments. Details of all fits are provided in Table S3.
Fig S6 Log ratios comparing net nitrogen mineralization rates (net N min.), net nitrification rates (net nitrif.), and ammonium concentrations (NH₄⁺) in soil of BioCON plots planted with 16 species to that of monocultures. Here, log ratios were calculated using median values instead of means due to the presence of outliers. For net N mineralization, log ratios were calculated using the sum of the median and one (because some of the median values were negative). Curved lines from log functions are shown when they gave substantially higher $R^2$ values than linear fits. Similar interannual data not available for BioDIV.
Fig S7 Responses of plots with 16 vs. 4 species, all of which were initially planted with 25% legumes. Log_{10} ratios for a subset of BioCON plots, comparing various attributes of the highest species richness level (16 species.), which was planted with four species from each of the four functional groups, to plots planted with one species from each functional group (4 species total).
For each year, there were five replicate plots planted with four species and 12 replicate plots planted with 16 species. *For soil N parameters, log ratios were calculated using median values instead of means due to the presence of outliers. In the case of net N mineralization, log ratios were calculated using the sum of the median and one (because some of the median values were negative). Lines of fit are only shown when significant temporal trends were observed (P<0.05). Curved lines from log functions are shown when they gave substantially higher $R^2$ values than linear fits. Similar interannual data not available for BioDIV.

**Fig S8** Temporal trends in the change in biomass per addition of one species in diverse plots. This was quantified as the difference between the mean total biomass of the most diverse mixtures (planted with 16 species), and that of the second most diverse mixtures (planted with 9 or 8 species for BioCON or BioDIV, respectively), divided by the difference between the number of planted species (16-9 = 7 or 16-8 = 8 for BioCON or BioDIV, respectively). In other words, this is the slope of the line between the two most diverse mixtures on the plot of total biomass vs. number of planted species. Note that adding a species to a diverse mixture had essentially no effect on total biomass during the early years of these studies, but had a substantial positive effect during later years. Thus, it would appear from the short-term results that loss of species from a diverse mixture would have no effect on total biomass, even though the longer-term results clearly indicate that these study species were not redundant with respect to their influences on total biomass.
Fig S9 Functional diversity as measured by Rao's Q over time within the three planted species richness levels of BioCON, in the ambient treatment plots. Fits are from generalized additive models ($R^2$ for the three species richness levels are 0.685, 0.667, and 0.602, for the 4, 9 and 16 species treatments, respectively). Trait and abundance data are as described in the SOM.
References and Notes


4. F. Isbell et al., High plant diversity is needed to maintain ecosystem services. Nature 477, 199 (2011). doi:10.1038/nature10282 Medline


11. A. Hector et al., BUGS in the analysis of biodiversity experiments: Species richness and composition are of similar importance for grassland productivity. PLoS ONE 6, e17434 (2011). doi:10.1371/journal.pone.0017434 Medline


17. Materials and methods are available as supplementary material on *Science* Online.


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