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Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity

Abstract Interactions between biotic and abiotic processes complicate the design and interpretation of ecological experiments. Separating causality from simple correlation requires distinguishing among experimental treatments, experimental responses, and the many processes and properties that are correlated with either the treatments or the responses, or both. When an experimental manipulation has multiple components, but only one of them is identified as the experimental treatment, erroneous conclusions about cause and effect relationships are likely because the actual cause of any observed response may be ignored in the interpretation of the experimental results. This unrecognized cause of an observed response can be considered a "hidden treatment." Three types of hidden treatments are potential problems in biodiversity experiments: (1) abiotic conditions, such as resource levels, or biotic conditions, such as predation, which are intentionally or unintentionally altered in order to create differences in species numbers for "diversity" treatments; (2) non-random selection of species with particular attributes that produce treatment differences that exceed those due to "diversity" alone; and (3) the increased statistical probability of including a species with a dominant negative or positive effect (e.g., dense shade, or nitrogen fixation) in randomly selected groups of species of increasing number or "diversity." In each of these cases, treatment responses that are actually the result of the "hidden treatment" may be inadvertently attributed to variation in species diversity. Case studies re-evaluating three different types of biodiversity experiments demonstrate that the increases found in such ecosystem properties as productivity, nutrient use efficiency, and stability (all of which were attributed to higher levels of species diversity) were actually caused by "hidden treatments" that altered plant biomass and productivity.

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Introduction

The field of ecology is distinguished by the complexity of the processes and interactions that are its primary focus and also the primary excuse for ecologists' failure to effectively address major environmental problems. Repeated calls for more rigorous and relevant ecology (e.g., Suter 1981; Peters 1991; Shrader-Frechette and McCoy 1994; Sarkar 1996) have led to an increasing emphasis on the need for "mechanistic models" (Schoener 1986; Tilman 1987a) and the use of an experimental approach that manipulates such factors as resources and predation rates (Hairston 1989; Underwood 1996). Rigorous experimental tests of hypotheses are essential as ecology increasingly addresses issues of political, social, and economic importance. Experimental results typically have a much greater impact than new theory, models, or observations. However, poorly designed experiments or misinterpretations of experimental results have the potential to mislead scientists and policy makers alike.

Biodiversity has recently emerged as an issue of both scientific (Wilson 1988; Ehrlich and Wilson 1991; Peters and Lovejoy 1992) and political (United Nations Environment Programme 1992; World Resources Institute 1992; Heywood and Watson 1995) concern primarily because of an increase in extinction rates caused by human activities (Myers 1979; Ehrlich and Ehrlich 1981; Lawton and May 1995; Pimm et al. 1995). Biodiversity is considered to be important for a variety of reasons (Oldfield 1989; Randall 1994; Rolston 1994), but recent attention has focused on its potential importance for the adequate functioning of the Earth's ecosystems (Schulze and Mooney 1993). This concern about the environmental consequences of biodiversity loss (and thus, a

potential justification for biodiversity conservation) has elevated biodiversity to near the top of the research agenda for ecology and environmental sciences in general (Solbrig 1991; Lubchenco et al. 1991; National Science and Technology Council 1995a, b; President's Council on Sustainable Development 1996). Strong theoretical and experimental results are needed to provide guidance for environmental policy and resource management. However, it is not always simple to "rigorously assess the ecosystem function of biodiversity in a manner that speaks plainly to the concerns of the public and policy makers" (Kareiva 1996).

Biodiversity is a quintessential ecological phenomenon because it represents the net result of a complex set of interacting ecological, evolutionary, biogeographical, and physical processes. A particular problem associated with biodiversity experiments is that the primary experimental treatment of varying the number of species is often correlated with variation in other biological or physical factors that can have a stronger effect on the experimental response than the putative primary treatment. These "hidden treatments" can produce strong biological responses that may be misinterpreted as consequences of a particular level of species diversity.

A brief history of biodiversity experimentation

Species diversity is easily manipulated, and consequently there is a long history of "biodiversity" experiments, beginning in 1843 with the Park Grass Experiments at the Rothamsted Experimental Station in Great Britain (Lawes et al. 1882). In these experiments, various fertilizer and liming treatments were evaluated for their effect on the yield of pastures for grazing and hay production. An early result that strengthened over time was a decrease in the number of species that occurred in the plots with the highest yield (resulting from the highest fertilizer application) (Silvertown 1980).

Many other experiments have produced similar results, with the number of species of terrestrial or aquatic plants almost always decreasing in response to increased productivity caused by nutrient addition (see Grime 1973a, b, 1979; Huston 1979, 1980, 1994; Austin and Austin 1980; Tilman 1987b, 1993). Similar patterns are found along natural gradients of plant productivity (Keddy and MacLellan 1990; Al-Mufti et al. 1977; Bakker 1989; Huston 1980, 1994). The well-known phenomenon of eutrophication of aquatic systems results from a dramatic increase in algal productivity and biomass (and an equally dramatic reduction in the number of algal species) caused by nutrient enrichment (Schindler 1974; Proulx et al. 1996). The mechanism generally presumed to cause this decrease in diversity is an increased intensity of competition at higher levels of productivity (Grime 1979; Huston 1979, 1994; Tilman 1988; Keddy 1989; Reader and Best 1989), although separation of the effects of competition from other effects of the physical conditions that influence productivity is not always straightforward.

Thus, in most situations, an *increase* in plant productivity (typically associated with larger plants and/or higher plant biomass) that results from addition of nutrients results in a *decrease* in the number of plant species coexisting in a given area or volume. In some situations where soil fertility or other factors limit plant productivity to extremely low levels, an increase in plant productivity from very low to moderately low levels allows more species to survive and reproduce, leading to an increase in plant diversity. Consequently, along the entire gradient from very low to very high productivity, the response of diversity to productivity is unimodal, with a maximum at intermediate levels (the "humpbacked" response, *sensu* Grime 1973a, b).

Current interest in the ecosystem functions of biodiversity (e.g., Schulze and Mooney 1993) requires experimental approaches that are able to distinguish the effect of biodiversity on ecosystem processes from the effect of ecosystem processes on biodiversity. An increasing understanding of ecological processes and improved technology encourage the hope that "the influence of biodiversity can be elegantly dissected through experimental manipulations" (Kareiva 1994).

Three types of hidden treatments

In any experiment to investigate the effect of diversity on ecosystem properties, the primary treatment is logically the number of species, with different treatment levels being different number of species. One or more ecosystem properties or processes would be measured to look for a response to the treatment, with the specific processes determined by the hypotheses that motivated the experiment. Since productivity is one of the most basic and important ecosystem processes, there is considerable interest in the effect of species diversity on productivity, particularly the primary productivity of plants. However, the strong effect of productivity (as manipulated by addition of nutrients or other resources) on species diversity makes evaluating the effect of species diversity on productivity particularly complex.

The following three examples illustrate different situations in which experimental responses attributed to variation in species diversity are much more likely to be the result of "hidden treatments" that altered plant productivity or biomass within the experimental design. Three different types of hidden treatments are illustrated, each representing a different pitfall for ecological experiments. As a consequence, properties that are attributed to species diversity may have nothing to do with the diversity levels intended to be the experimental treatments, but rather are caused by other factors intentionally or unintentionally manipulated in the course of the experiments, that is, the "hidden treatments."

Use of nutrient additions
to create "diversity treatments"

A recently published long-term experiment (Tilman and Downing 1994; Tilman 1996) used a combination of experimental nutrient additions and natural climatic variability to evaluate the effect of species diversity on the temporal stability of primary productivity. Theories about the relationship between diversity and the stability of community and ecosystem processes have a long history in theoretical ecology (Elton 1958; MacArthur 1972; May 1973; DeAngelis 1975; McNaughton 1977; Pimm 1979, 1984) and direct relevance to such current issues as biodiversity loss and environmental sustainability (Schulze and Mooney 1993; Vitousek and Hooper 1993; Kareiva 1994, 1996; Moffat 1996).

In an analysis of 13 years of biomass production of over 200 plots (4×4 m; 4 fields $\times$ 9 nutrient treatments $\times$ 6 replicates per field) Tilman (1996, p. 361) concluded that "greater plant species diversity led to greater stability of plant community biomass after a perturbation. This supports the hypothesis that diversity stabilizes community and ecosystem processes." In this experiment, the only measured response variable was the maximum annual aboveground biomass in the experimental plots, which in herbaceous plant communities is strongly correlated with net primary productivity. The stability of primary productivity in plots with different numbers of species was evaluated in response to the natural variability in precipitation, in particular the effect of a severe drought in 1988.

The conclusion that diversity increased stability was based on the statistical significance of ANOVAs and regression analyses, with the following derived statistics used to characterize different aspects of the stability of biomass production: (1) coefficient of variation o/f biomass for each plot across all years ($CV = 100 \times 1SD/mean$) as an indication of year-to-year variation in biomass (i.e., the inverse of stability); (2) rate of biomass change from two years before the drought (1986) to the drought (1988), expressed as a yearly rate, $\log_e (\text{biomass}_{1988}/\text{biomass}_{1986})/2$ as a measure of "drought resistance"; and (3) relative biomass deviation for post-drought years [(mean pre-drought biomass − biomass of a particular post-drought year)/(mean pre-drought biomass − drought, 1988, biomass)] as a normalized measure of the rate of return toward "equilibrium" following a perturbation, also called "resilience" (Pimm 1984). All three of these measures of stability were positively correlated with species richness in the experimental plots.

The experimental treatment is presumed to be number of plant species per plot, subdivided into eight categories for ANOVA (Fig. 4 in Tilman 1996) or used as a continuous variable in regression analysis. However, on closer examination it becomes apparent that the experimental treatments are actually varying levels of nitrogen addition, which caused variation in both net primary productivity and species richness. The primary response variable in this experiment is the stability of net primary productivity, and not simply the rate of net primary productivity. Consequently, the difficulty of separating the strong effects of fertilization on the magnitude of the response variable, from the effects of other factors, such as species richness, on the variability of the response variable, is much greater than it would have been if species richness had been manipulated independently of productivity. The following points must be kept in mind to understand the experimental results:

1. The variation in species diversity among the plots was primarily a result of nitrogen addition, which produced a gradient from low biomass-high diversity plots to high biomass-low diversity plots. Based on the published figures, there was a strong negative correlation between species number and both the nitrogen addition rate and the actual biomass in the treatment plots (Fig. 1A, B). Added to the changes in species diversity that resulted from nitrogen addition, there was also variation in diversity between the four experimental fields (ranging from an average of 8.6 to 14.9 species /0.3 m^2), as a result of differences in time since abandonment and perhaps other factors (Tilman 1993). This between-field variation in the number of available species may have partially obscured the relationship between individual plot biomass and plot species number, since field identity was a significant factor in the multiple regressions that were presented (Tables 1 and 2 in Tilman 1996). In addition to causing a reduction in species richness, the fertilizer addition may also have selected for species with lower root:shoot ratios, as well as caused a shift to lower root:shoot ratios within species. A lower root:shoot ratio would make plants more susceptible to drought, and is yet another factor confounding the analysis of these experiments (Givnish 1994).

2. The nitrogen addition treatment was constant through time for each plot. Each set of replicates received the same nitrogen addition (ranging from 1 to 28 g/m^2) each year. The rainfall was highly variable between years, with all plots receiving the same rainfall each year regardless of their nitrogen level. Consequently, biomass production in the low-nitrogen plots was chronically limited by nitrogen and never achieved high levels, even in years of abundant rainfall. Total biomass in these plots was low, and interannual variability in biomass production was low because biomass was limited by a resource (nitrogen) that was applied at a constant rate through time. In contrast, biomass production in the high-nitrogen plots was limited primarily by water availability rather than nitrogen. Because precipitation varied greatly from year to year, biomass production in the high-nitrogen plots varied greatly through time, reaching high levels during wet years when net primary productivity (NPP) was unlimited by either water or nitrogen and dropping to low levels during dry years. During the extreme drought of 1988, biomass production in all plots was similarly low, regardless of nitrogen treatment (Fig. 2A in Tilman 1996). The consequences of interactions between limiting resources are

well known (e.g., Liebig's "law of the minimum") and variation in limiting resources is the basis of many of the current theories and models of species diversity (Grime 1979; Huston 1979; Tilman 1982; Pastor and Post 1986; O'Neill et al. 1989; Keddy 1989; DeAngelis 1992; Huston and DeAngelis 1994). A similar interactive effect of nitrogen and rainfall on herbaceous biomass has been reported for the long-term Park Grass Experiments in Great Britain (Cashen 1947).

When the interannual variation in biomass production is considered in terms of limiting resources, it becomes clear why species diversity was positively correlated with the various measures of stability. The high nitrogen-high biomass plots had variable biomass production because of the high variability of their primary limiting resource (water). These high biomass plots also had low diversity, presumably because of the intense competition associated with their higher productivity. The lower variability in biomass production of the low nutrient-low biomass (high diversity) plots is a consequence of the relative insensitivity of these plots to variation in precipitation because their productivity was limited primarily by continually low nitrogen availability. Because diversity was *reduced* by the same experimental treatments (nitrogen addition) that shifted resource limitation from nitrogen to water and *increased* variability in net primary production, there appeared to be a positive relationship between species diversity and stability. However, this was a spurious correlation, not a causal relationship. The treatment levels of species diversity were simply another response to the hidden

Fig. 1A–F Relationships of the stability of plant biomass production to plant diversity and plant biomass in the Cedar Creek long-term diversity experiments. **A** Average number of plant species in each nitrogen treatment after 4 years of nutrient addition. **B** Average number of plant species per plot in relation to average biomass for each level of nitrogen addition. Note that species diversity is reduced in direct proportion to the increase in biomass caused by fertilization. **C** Decreasing drought-induced relative reduction of annual biomass production at higher levels of species richness (lower biomass and nitrogen addition). **D** Increasing drought-induced reduction of annual biomass production at higher levels of plant biomass (higher biomass and nitrogen addition). **E** Decreasing recovery of annual biomass production at higher levels of species richness (lower nitrogen addition) following 1988 drought. **F** Increasing recovery of annual biomass production at higher levels of plant biomass (higher nitrogen addition) during recovery from drought. Note that both the relative decrease in biomass production caused by the 1988 drought (**D**) and the relative increase in biomass production following the drought (**F**) are positively correlated with biomass (fertilization level) which is consistent with the resource limitation hypothesis. However, the negative correlation of species richness with recovery from the drought (**E**) is inconsistent with the species diversity hypothesis (see text) (based on Fig. 2 from Tilman 1996)
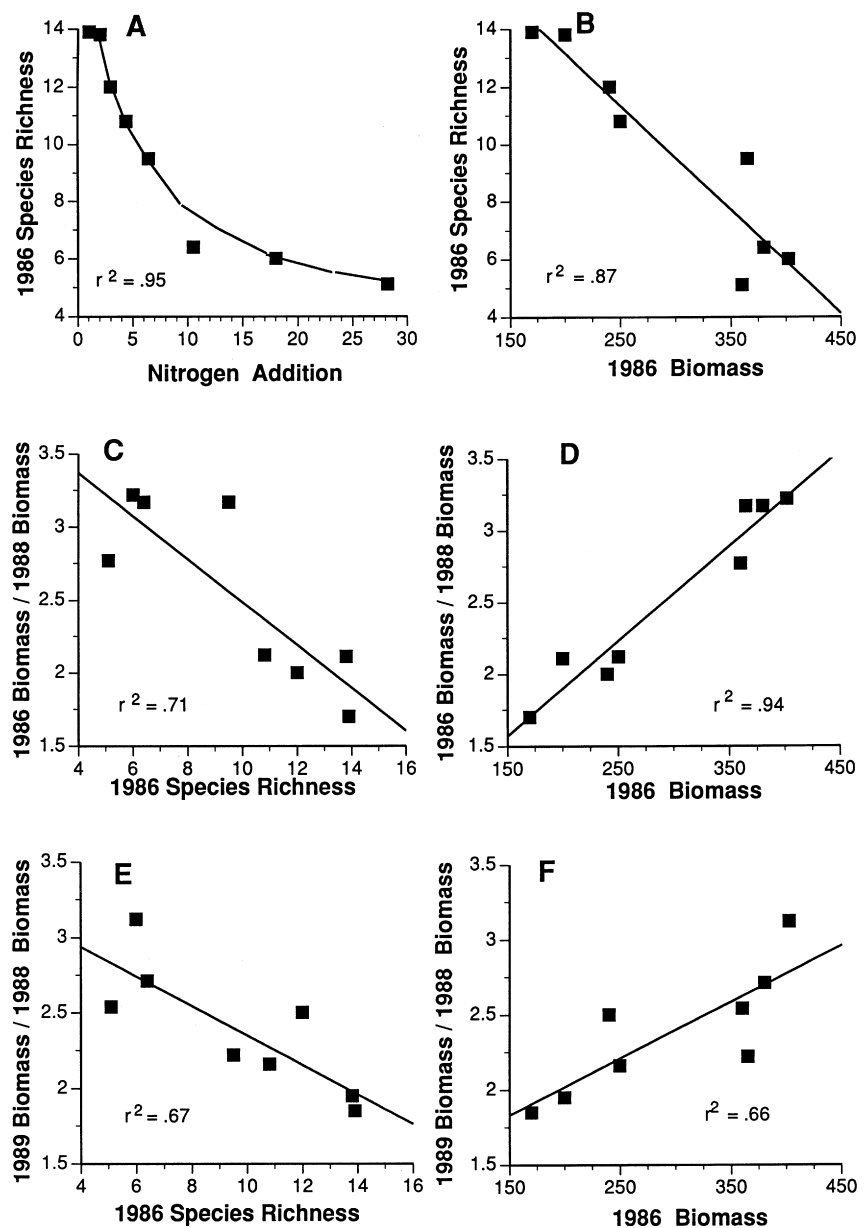
treatment (nitrogen addition), not the cause of the observed biomass responses.

The relationship of resilience and resistance to the experimental nitrogen additions confirms that species diversity per se has little direct effect on the stability of biomass production in these experiments. While the proportional reduction in biomass during the drought year was greater in the low diversity plots than in the high diversity plots (Fig. 1C), the correlation is even stronger with pre-drought biomass (Fig. 1D). The high biomass (low diversity) plots experienced the greatest absolute and proportional reduction in biomass in response to the drought, as would be expected on the basis of the limiting resource interactions described above. Recovery from the drought (i.e., resilience, expressed as the proportional increase in biomass from 1988 to 1989) is actually negatively correlated with species richness (Fig. 1E) and positively correlated with pre-drought biomass (Fig. 1F). This pattern is inconsistent with the hypothesized positive effect of diversity on stability and resilience, but fits perfectly with the limiting resource hypothesis proposed above. When the effects of biomass are factored out by dividing the post-drought biomass recovery by the pre-drought biomass decrease (called the "relative biomass deviation"), the significance of the diversity-resilience correlation is greatly reduced (Tilman 1996, p. 354).

A shift between two limiting resources with contrasting variability is the most probable explanation for the patterns of variation in productivity in this experiment. While some species in these experiments apparently demonstrated compensatory responses that reduced variability in productivity (Fig. 8 in Tilman 1996), these effects were small in comparison to the effects of variation in the two primary resources, nitrogen and water. Thus, the conclusion that diversity increases ecosystem stability is not supported by these experimental results. Species diversity was not the true treatment variable, and consequently the only legitimate conclusion is that, in this experiment, temporal variability in primary productivity is a simple consequence of variability in the limiting resources.

Published analyses of the long-term fertilization responses at the British Park Grass Experiment indicate that, as at Cedar Creek, plot biomass (manipulated by fertilization) has a much stronger correlation with the stability of biomass production than does species richness (Dodd et al. 1994). Out of 42 years sampled between 1862 and 1991, plot biomass was significantly correlated with the coefficient of variation (CV) of biomass production in 30 cases, while species richness was significantly correlated only in 3 cases. In contrast to the Cedar Creek results, in the Park Grass Experiments the high biomass plots were most stable, reflected in a negative correlation between plot biomass and the CV of biomass production. Differences in the treatment types and duration between the two experiments may help explain this discrepancy. In the Park Grass Experiments the long-term effects of ammonification and leaching by sulfate and nitrate resulted in extreme acidification of soils that did not receive supplemental lime (Johnston et al. 1986). The stunted vegetation of these extremely acidic soils is much more sensitive to variation in precipitation than vegetation on the limed soils (Dodd et al. 1994; Silvertown et al. 1994). Because all plots in the Cedar Creek fertilization experiment receive lime to prevent acidification, this phenomenon has not occurred there.

Creating "diversity treatments" using non-random selections of species

Artificially constructed and highly controlled experimental ecosystems have great potential for evaluating the mechanisms responsible for observed changes in ecosystem function in response to variation in species number or in such physical conditions as temperature or carbon dioxide concentration (Kareiva 1994). In the Ecotron experiment (Naeem et al. 1994a, 1995), the investigators carefully constructed replicate ecosystems in a total of 14 climate-controlled growth chambers (8 m^3). Three levels of diversity were created using a nested grouping of plants and animals (including bacteria, nematodes, Collembola, earthworms, and insects) in which the low diversity treatment (9 species – of which 2 were plants, 4 identical replicates) was a subset of the species in the intermediate diversity treatment (15 species – of which 5 were plants, 4 identical replicates), which was a subset of the species in the high diversity treatment (31 species – of which 16 were plants, six identical replicates).

These treatments were envisioned as representing "increasingly depauperate versions of the high diversity mesocosms." Response variables included "community properties": (1) percentage of the ground surface area covered by plants; (2) height distribution of the volume occupied by plants; (3) number of individuals of snails, insects, earthworms, Collembola, and insect larvae parasitized by wasps; as well as "ecosystem process" rates: (1) carbon dioxide flux rates; (2) percentage of photosynthetically active radiation (PAR) intercepted by the vegetation, which was used as a surrogate for productivity; (3) decomposition of surface grass litter and buried birch sticks; (4) nutrient "retention" in soil, based on tri-weekly chemical analyses of soil samples; and (5) water retention, based on the amount of water that drained out of the chambers.

The complexity and cost of this type of highly controlled experiment impose constraints on the number of treatments and replicates in the experimental design. Adequate and appropriate replication is a concern in any experimental design, but a type of false replication, called pseudo-replication, was identified as a particular problem in ecological experiments (Hurlbert 1984). This problem arises when multiple samples from a single experimental unit (e.g., plot, chemostat) are counted as replicates for statistical purposes. Multiple instances of

the same treatment (e.g., plots in different locations in a field that all receive the same amount of fertilizer) are the replicates with regard to the experimental treatment, not multiple samples from a single unit.

For any experiment, treatments must be carefully defined (presumably in the context of specific hypotheses), before the issue of appropriate replication can be resolved. When species diversity is the experimental treatment, with treatment levels being different numbers of species, the definition of replicates is particularly critical. Species number is a simple concept that is distinct from the identities or properties of the species involved. For example, a set of replicates of the treatment "three species", could include three species of bacteria, three species of plants, and three species of birds. The near impossibility of identifying any dependent variables that could be used to compare the responses of different levels of this type of treatment confirms the absurdity of this example.

However, the degree of taxonomic or functional constraint that must be imposed on comparisons of different levels of species diversity is not a trivial issue. For plants, one must ask whether the effect of species diversity (i.e., species number) can be distinguished from the effect of species identity by restricting the eligible species to angiosperms (ignoring gymnosperms), or to trees (ignoring shrubs and herbs), or to shade intolerant trees (ignoring other tolerance classes), or to shade intolerant trees of similar maximum height, etc. The importance of these distinctions is revealed by the possibility of such pointless experiments as evaluating the effect of species number on biomass production with treatments of three species of trees versus six species of grass.

Once the experimental organisms have been appropriately selected, one can ask how the specific treatments can be replicated. Even among such a constrained subset as non-nitrogen fixing summer annuals with a maximum height between 25 and 50 cm, many different sets of three species could be selected. If the treatment is to be species number, then the replicates of each level of species number must be sets that differ in the identity of the component species. Multiple sets of the same species are not replicates with regard to species number, but are replicates for an experiment investigating the properties of that specific set of species.

In the Ecotron experiments (Naeem et al. 1994a, 1995), the replicates of the diversity treatments were identical chambers with exactly the same species composition in each. Thus, there was no replication of species number, the putative treatment, and the actual treatments were simply different sets of species, which were replicated. As a result, the effect of the number of species present in each treatment cannot be distinguished from the effect of the particular species chosen for that treatment, and the results of this experiment cannot be generalized beyond the group of species that was used. The failure to replicate the species diversity treatment was compounded by a strong size bias in the groupings of species used for implementing the experimental treatments. Specifically, the species chosen for the low diversity treatment were plants that only grow to a small maximum size, while the intermediate and higher diversity treatments included species of greater maximum size, with the highest number of large species in the high diversity treatment (Table 1, Fig. 2A). All plants in this experiment were herbaceous annuals, so the large species also tended to be fast-growing species.

The size bias in species selection dominates the results of the Ecotron experiment. All of the measurements that were significantly correlated with species diversity are simple properties of plant tissue. Consequently, the amount or rate of these properties inevitably increase with increasing plant biomass, including (1) plant cover (larger plants cover more ground area than small plants); (2) height distribution of vegetation volume (taller plants have more leaves high above the ground than short plants); (3) insect numbers (more plant-feeding insects can be supported on large plants than on small plants: Gilbert and Smiley 1978; Bach 1980; Cytrynowicz 1991); (4) carbon dioxide flux (plants with large total leaf area can take up more carbon dioxide than plants with small total leaf area: Fields et al. 1992); and (5) light interception (more light is intercepted by large plants with a high total leaf area than by small plants with lower total leaf area).

The issue of size bias in this experiment has been raised previously with regard to three of the species (André et al. 1994; Naeem et al. 1994b). However, the problem is more extensive than suggested in the previous discussion, which presented results of glasshouse experiments to support the validity of the Ecotron experiments (Naeem et al. 1994b, 1995). The results of the glasshouse pot experiments cannot legitimately be compared to the results of the Ecotron growth chambers for two primary reasons. First, plant species are extremely plastic in terms of size, with most species reaching much larger sizes under conditions of high resource availability (e.g., large pots or fertilized soil) than under low resource availability (e.g., small pots or low nutrient soil). Since the glasshouse experiments were conducted in 20-cm pots, which have much less total soil volume than the $1 \times 1 \times 0.3$ m soil volume of the Ecotron experiments, plants in the Ecotron experiments had much higher availability of soil resources, which would have allowed species with large maximum sizes (e.g., *Sonchus oleraceus*) to achieve sizes much closer to their maximum in the Ecotron than in the 20-cm pots. Second, the spatial density of individual plants in the pots was 6 times higher than in the Ecotron experiment, which further invalidates any comparison of either total productivity or individual species responses between the pot studies and the Ecotron experiments. The pattern of species performance in the pot experiments does not disprove the presence of size bias in the main Ecotron experiment. This size bias is confirmed by the height profiles of the three treatments (Fig. 2A), which clearly demonstrate the smaller size of the species in the low-diversity treatment.

Table 1 Species used in two experiments on the effect of biodiversity on ecosystem processes. The maximum and minimum heights (cm) are from the species descriptions in Stace (1991) for the Ecotron Experiment and from Gleason and Cronquist (1963) for the Cedar Creek Experiment. An *asterisk* indicates that the typical minumum size was estimated as 33% of the maximum from Gleason and Cronquist (1963)

Ecotron Experiment		Cedar Creek Constructed Grassland Experiment		
Species	Typical maximum	Species	Minimum	Maximum
I. (Low diversity)		Andropogon gerardii	100	300
Senecio vulgaris	30	Achillea millefolium	20	100
Stellaria media	50	Bouteloua gracilis	15	60
		Lespedeza capitata	60	150
II. (Med. diversity) + I.		Rudbeckia hirta	30	100
Chenopodium album	150	Agropyron smithii	40	90
Spergula arvensis	40	Anemone cylindrica	30	100
Cardamine hirsuta	60	Asclepias tuberosa	30	70
		Aster azureus	20	150
III. (High diversity) + I + II		Astragalus canadensis*	50	150
Aphanes arvensis	10	Buchloe dactyloides	30	100
Arabidopsis thaliana	30	Coreopsis palmata	50	90
Capsella bursa-pastoris	40	Elymus canadensis	100	
Conyza canadensis	100	Euphorbia corollata	30	100
Lamium purpureum	45	Koeleria cristata	30	60
Poa annua	20	Liatris aspera	40	120
Sinapsis arvensis	100	Panicum virgatum*	67	200
Sonchus oleraceus	150	Petalostemum purpureum	30	100
Tripleurospermum inodorum	60	Poa pratensis	30	100
Veronica arvensis	30	Schizachyrium scoparium	50	120
Veronica persica	50	Solidago nemoralis	10	100
		Sorghastrum nutans	100	200
		Sporobolis cryptandrus	30	100
		Vivia villosa*	33	100

Thus, none of the conclusions of the experiment (Naeem et al. 1994a, 1995; Kareiva 1994, 1996) with regard to the effects of loss of biodiversity on ecosystem function are valid. The only legitimate conclusions are that certain ecosystem processes occur at higher rates in groups of large plants than in groups of small plants under the particular conditions of this experiment.

Creating "diversity treatments" using randomly selected sets of species

The constructed grassland experiments at Cedar Creek, Minnesota, United States (Tilman et al. 1996) avoided both the confounding effect of fertilization and the problem of pseudoreplication and non-random or biased species groupings. However, a third type of sampling problem affected these experiments, illustrating one of the most subtle hidden treatments in experimental ecology: the increasing probability of selecting species with a specific property (e.g., large maximum height, stress tolerance, nitrogen-fixation ability, high seed germination rate) in samples of increasing number that are randomly selected from any group of species. This phenomenon can be called the "selection probability effect." A related phenomenon is the "variance reduction effect," which is the higher probability of similarity or overlap in composition and/or performance between samples of many species drawn from a given pool than between samples of few species drawn from the same pool. This effect reduces the variance among replicate large samples of a population in comparision with replicate small samples. In addition, it increases the probability that large "natural" samples will be similar to large statistical samples from the same population (e.g., certain types of null models).

Unlike the Ecotron experiments, the grassland experiments at Cedar Creek focused on the effect of variation in the number of plant species alone. In the experimental design, 147 plots (3 × 3 m) were randomly assigned to one of seven species-richness treatments that were planted as seeds on bare ground, and allowed to grow under ambient climate with supplemental watering and weeding to maintain species composition. The species-richness treatments were 1, 2, 4, 6, or 8 species (20 replicates of each), 12 species (23 replicates), and 24 species (24 replicates). Each replicate was created by randomly drawing (with replacement) from the total pool of 24 species, so each replicate at a given level of species diversity was potentially composed of a different subset of the available species, with the inevitable increase in similarity between the replicates in the higher species richness treatments. The plants were grown for 2 years, after which time measurements were made. Although no ecosystem processes were actually measured, the properties that were measured are assumed to represent the net result of ecosystem processes operating at different rates over the same time period: (1) percentage of ground surface covered by plants, which was used as a surrogate for productivity; and (2) soil nitrate concentration in the plant rooting zone (0–20 cm depth) and below the rooting zone (40–60 cm depth), used as a surrogate for efficiency of nutrient uptake.
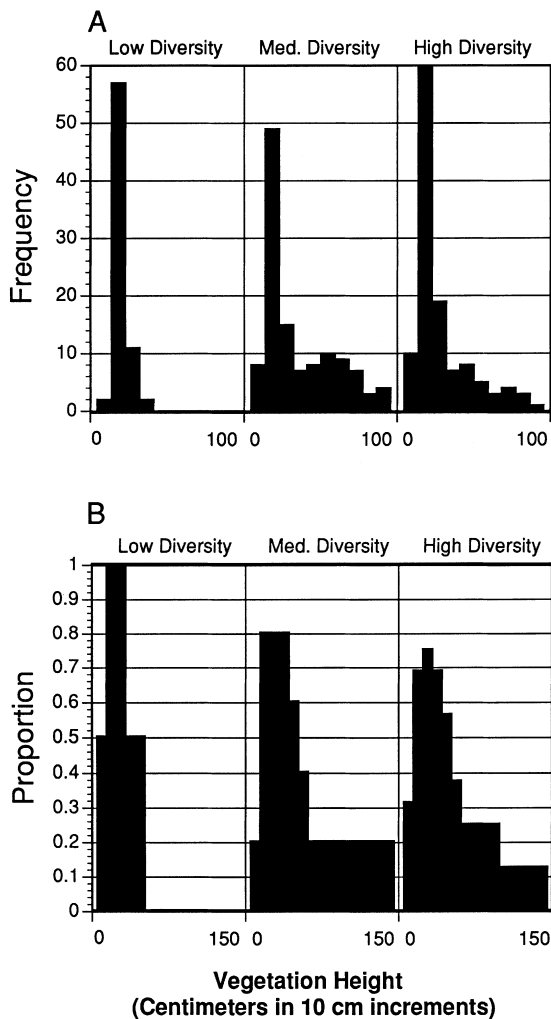
Fig. 2A, B Qualitative similarity between height distribution of plant volume observed at three diversity levels and simple calculations based on the sizes of plant species used in the Ecotron experiment. **A** Observed vertical distribution of volume occupied by plant leaves in the Ecotron growth chambers, in 10 cm increments (from Naeem et al. 1995). Note the low maximum height of leaf distribution in the low-diversity treatment. **B** Calculated proportion of the total number of species in each diversity level that would be present in each 10-cm height increment, assuming that each species has leaf biomass in the upper three-quarters of the typical maximum height reported for that species in a standard flora (Stace 1991). Note the small maximum size of species used in the low-diversity treatment

In these experiments, the various diversity treatments were appropriately replicated. However, the interaction of the "selection probability effect" with the particular set of species chosen for implementing the design confounds analysis of the effects of species diversity by introducing a correlation between the number of plant species in each treatment and the maximum possible amount of plant biomass in that treatment. The 24 species used in the experiment vary greatly in size (Table 1), with the result that the probability of a replicate containing a species that can grow to a large size increases with the number of species in the replicate.

This bias is particularly significant because multi-species groups of plant are typically dominated by individuals of the largest species (Grime 1979; Austin and Austin 1980; Huston and Smith 1987). Consequently, most of the biomass in each treatment was contributed by one or a few of the dominant species in that treatment (with dominance based on height, seed production, or other properties that confer a competitive advantage), and reflects the effects of those few species rather than some average response of the total number of species originally planted in the plot. This inference is supported by: (1) the authors' observation that five of the species "had significantly greater abundance *in the higher diversity treatments* than expected on the basis of their proportion in the seed mixture" [italics added] (Tilman et al. 1996, p. 719); (2) a published photograph of the experiment (Kareiva 1996) that showed a large proportion of the heavily vegetated plots dominated by a single species (apparently the yellow-flowered *Rudbeckia hirta*, a fast-growing invasive species of roadsides and disturbed prairies), while many of the other plots were mostly bare ground; and (3) published data (Fig. 1A in Tilman et al. 1996) that reveal that "effective species richness" (the number of equally abundant species needed to produce the observed value of the Shannon-Weaver index, H'; effective species richness is calculated as $e^{H'}$, where $H' = \sum p_i \times \ln p_i$, and p_i is the proportion of the total biomass composed of species i) is half or less of the number of species originally planted per treatment, particularly *in the higher diversity treatments*, where the "selection probability effect" for large dominant species is greatest.

Because the higher diversity treatments in this experiment inevitably have more large species, the primary ecosystem responses of the experiment are simply the result of higher plant biomass, rather than higher species diversity. Plant cover (the percentage of the ground surface covered by plant tissue when viewed from above) has a near linear positive correlation with increasing plant biomass up to 100% cover, above which biomass can continue to increase with no further increase in plant cover. A simple simulation of the experimental design, in which the height of each replicate was determined by the tallest of the randomly selected species (Table 1) reproduces the observed pattern of an increasing amount of vegetation (total plant cover) with an increasing number of species per treatment (compare Fig. 3A and B). The pot experiments discussed previously, that were done in conjunction with the Ecotron experiments (Naeem et al. 1994a, 1995), were similar in number of replicates and treatments to the Cedar Creek experiments and demonstrate that this "hidden treatment" (the selection probability effect) also applies to total biomass (Fig. 3C). Note that the maximum biomass in the single-species pots was as great or greater than the biomass of the high-diversity pots. This demonstrates that the amount of biomass in the high-diversity pots is set by the size of the largest species, and is not some aggregate property of high diversity.
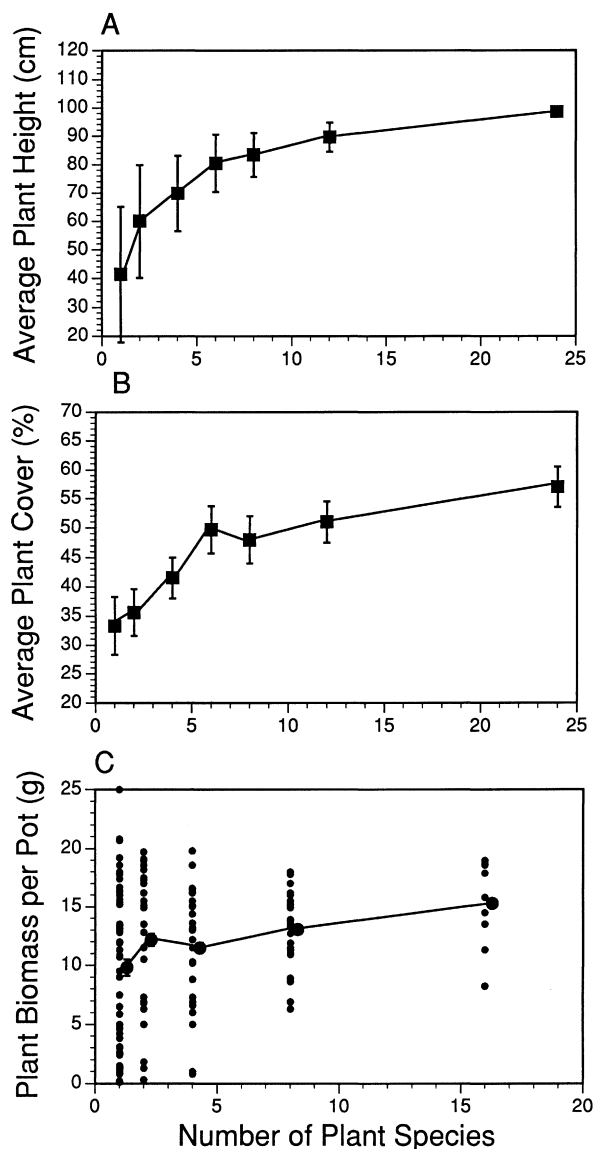
Fig. 3A–C Illustration of the "selection probability effect" in random draws of species from a common pool. A larger number of randomly drawn species has a higher probability of containing species with a specific property (e.g., large size, stress tolerance) than a lower number of species. Note reduction in variance (SEM) and assymptotic increase in mean with larger numbers of randomly drawn species. **A** Simulated values for average plant height at different levels of species diversity, based on randomly selecting species from the pool of 24 species used in the Cedar Creek Experiment and assuming that the plant height in each treatment is the height of the tallest of the randomly selected species (using typical minimum heights reported in a standard flora, Gleason and Cronquist 1963, see Table 1). Mean and standard error of the mean are the averages of ten separate simulations of the full experimental design. **B** Observed vegetative cover at each level of species richness in the Cedar Creek constructed grassland experiment (mean and SEM from Tilman et al. 1996). **C** Total biomass per pot produced in a glasshouse experiment with pots planted with different numbers of species randomly selected from the pool of species used in the Ecotron experiment (see Table 1). *Small dots* indicate the various replicates of different species combinations at each level of species richness. *Large offset dots* indicate mean with SEM (based on Fig. 13 in Naeem et al. 1995)

The other significant treatment response in the Cedar Creek constructed grassland experiments, variation in soil nitrate, is also a consequence of plant biomass rather than plant species diversity. Nitrogen in the soil is often inversely correlated with root biomass because more root biomass can take up more nitrogen, thus depleting this element in the soil (Barber 1984). Previous work at the Cedar Creek (Tilman and Wedin 1991) has demonstrated that soil nitrate is negatively correlated with root biomass ($r^2 = 0.72$). These results were obtained using monocultures of five grass species, clearly demonstrating that the effect of root biomass on soil nitrate at Cedar Creek is independent of both the number of species involved (i.e., monocultures reduce nitrate to levels as low or lower than the high-diversity treatments in the present study) and species identity (i.e., at the same level of root biomass, the effects of the species did not differ significantly).

The above problems with the design of the constructed grassland experiment would not be expected to apply to the results obtained from the natural grassland at Cedar Creek, which had relationships of plant cover and soil nitrate with species richness that were very similar to those found in the experiment. If the experimental results are interpreted improperly, how can the similar results from the natural grassland be explained? The answer may be found in the extremely poor soils of the Cedar Creek site, which is located on an ancient sand plain.

The common feature between the experiment and the natural grassland is the extremely low productivity and the large amount of open ground that was not covered by plants (Fig. 2A in Tilman 1996). In the experiment, low productivity and low plant cover (27–56% of the ground surface area) were maintained by a combination of poor soil, disturbance due to hand weeding and the resulting limitation of many of the lower diversity plots to a species composition that only includes small-statured species. In the natural grassland, the low plant cover (26–88%) is apparently caused by the poor soils (low nitrogen, low water-holding capacity), and perhaps the effect of gopher disturbances (Inouye et al. 1987). Under natural conditions a gradient in soil fertility ranging from extremely low to moderately low would typically be associated with a gradient of increasing plant cover and species richness. Under such conditions, species richness generally increases up to a productivity level at which 100% plant cover is reached, above which richness declines due to competitive interactions.

Published information and the details of the present experiment make it clear that the Cedar Creek experimental site and prairies are at the lower end of the productivity gradient, and that consequently results from this site reflect that portion of the natural range of plant productivity over which plant diversity typically increases with increasing productivity (Grime 1973a, 1979; Huston 1994). A comparison of primary productivity at 13 grassland and forest ecosystem sites across North America revealed that only a single site had productivity lower than that at Cedar Creek, and that

the productivity at Cedar Creek was less than 20% of that recorded at the other tall-grass prairie site (Zak et al. 1994). The levels of plant biomass reported from un-fertilized plots at Cedar Creek (100–200 g/m^2) place these plots at the low end of the productivity gradient reported for 13 natural herbaceous sites studied in Great Britain (100–2000 g/m^2, Al-Mufti et al. 1977). Among the British sites, the number of plant species increased from a minimum at a biomass of 100 g/m^2 to a maxi-mum at 500 g/m^2 and then declined sharply with in-creasing biomass. Past fertilization experiments at Cedar Creek have demonstrated that plant species diversity at this site also decreases with increasing plant biomass (live and dead) above a level of 400–500 g/m^2 (Tilman 1993, 1996), which is consistent with data from the natural prairies at Cedar Creek suggesting that species richness begins to decrease at levels of plant cover greater than 90% (Fig. 2A in Tilman et al. 1996).

In the analysis of the Cedar Creek experimental data, the effect of the dominant role of above and below-ground plant biomass in causing the treatment effects is obscured by inappropriate interpretation of multiple regression analysis (Sokal and Rohlf 1981, pp. 642–656). The published analysis shows that both species richness and root mass are significantly and positively correlated with total plant cover, which is itself negatively corre-lated with soil nitrate (Table 1 in Tilman et al. 1996). Failure to consider the correlation between the three properties (root mass, species richness, and total plant cover) that were treated as independent variables in the regression on soil nitrate (Table 2 in Tilman et al. 1996) leads to the misleading inference that the effect of root mass on soil nitrate is insignificant, and to the erroneous conclusion that the effect of species richness is the explanation for the observed results. The primary conclusions of the paper, that the results "support the diversity-productivity and diversity-sustainability hypotheses" and that "the loss of species threatens ecosystem functioning and sustainability" are not sup-ported by the data.

Thus, in the previous three "biodiversity" experi-ments, the true experimental treatments were actually variation in plant biomass or plant productivity rather than variation in the number of plant species. Because all of the ecosystem response variables that were mea-sured or estimated are consequences of the amount of active plant biomass, the experiments simply demon-strate that large plants produce more biomass (and take up more CO_2 and nutrients) than small plants. To separate the effect of plant species diversity from the effect of plant biomass (or other species-specific pro-perties such as nitrogen fixation), the experimental de-sign must include at least one treatment in addition to the properly replicated species richness treatment. At least two levels of plant size (e.g., large species versus small species, or fertilized versus unfertilized) must be crossed with the number of species in order to determine whether the ecosystem function of many different species of plants differs from that of an equivalent biomass composed of a few species. As in any ecological experi-ment, all physical conditions that might affect the treatment response (e.g., soil water potential, soil nu-trient availability, temperature, solar radiation, herbi-vory) should be carefully monitored.

Discussion

If the above experiments provide no evidence that increasing biodiversity improves ecosystem function, what, if any, is the relationship between biodiversity and ecosystem processes? It is certainly easy to envision sit-uations in which addition of more species might increase the net primary productivity (or other process rate) of a plant community. The addition of species that increase nutrient availability, such as nitrogen fixers or my-corrhizal fungi, would likely increase the net primary productivity of an ecosystem, as would the addition of shade tolerant plant species under an overstory of shade intolerant species. Processes such as these underlie the phenomenon known as "overyielding," (de Wit et al. 1966) which is the basis for intercropping and related agricultural practices (Vandermeer 1989). The increase in productivity associated with intercropping results not from adding many different species, but from adding one or a few species selected for very specific properties, such as growth form or shade tolerance (Swift and Anderson 1993; Anderson 1994; for examples from natural systems, see Ewel and Bigelow 1996; Denslow 1996). Likewise, compensatory growth among species with different physiological optima or tolerances for stressful conditions could reduce the temporal variability in net primary production (i.e., increase stability) as apparently happened in the Cedar Creek long-term experiments. This type of yield compensation is a highly desirable property in agricultural systems as well.

Nonetheless, the biological interactions associated with higher species diversity apparently have only a marginal effect on the level or stability of productivity in comparison with variation in the availability of resources such as nitrogen, phosphorus, or water. The 300% increase in biomass that resulted from nitrogen addition in the Cedar Creek Experiments (Fig. 2A in Tilman 1996) vastly exceeds the possibly 10–20% biomass increase between the low and high diversity plots during the drought year (Fig. 4 in Tilman 1996).

The tremendous variation in net primary productivity across the globe, or even along a single hillslope, is not a result of variation in species diversity, but simply the consequence of variation in resources that results from the interaction of climate, geology, and soil processes (Huston 1994). The simple observation that the Earth's most productive ecosystems generally have low plant diversity while high plant diversity is found under much less productive conditions demonstrates that the number of plant species has relatively little effect on productivity. The number of plant species in a local area is best un-derstood as a response to the level of plant productivity

allowed by local conditions of soil and climate, rather than being the cause of that level of productivity.

The three experiments discussed above provide strong support for the conclusion that both local species diversity and the rates of ecosysem processes such as productivity are determined by the amount and variability of the fundamental environmental resources that regulate plant growth and ecosystem productivity. Although the investigators who conducted each of the experiments came to the conclusion that higher diversity increased productivity, stability, sustainability, and other ecosystem functions, careful evaluation of the experiments reveals that the experimental responses in each case actually resulted from a "hidden treatment" that was ignored while attributing the responses to the putative treatment. In reality, the putative "diversity" treatments were either one of many responses to the factors that produced variation in plant productivity and/or biomass (experiment 1), or an irrelevant correlate of the particular groupings of species used as treatments (experiments 2 and 3). The potential for such "hidden treatments" should be carefully evaluated in all ecological experiments that investigate complex phenomena.

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