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Seed size- and density-related hidden treatments in common biodiversity experiments

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Abstract

Aims

With a few exceptions, most well-known field biodiversity experiments on ecosystem functioning have been conducted in plant communities (especially grasslands) in which different numbers of species are planted as treatments. In these experiments, investigators have either kept the total seed weight or seed number constant across treatment plots. However, although in some cases attempts have been made to randomly choose species for planting from a designated species pool, the issue of possible 'hidden treatments' remains unsolved. Particularly, the total and relative abundance among species and across treatments could still affect the results. This study aims to determine whether treatments related to planted seed abundance and seed size may contribute to observed productivity.

Methods

We re-analyzed data from four biodiversity experiments based on a common seeding design (i.e. diversity treatments).

Important Findings

We show that diversity (richness) treatments usually involve a hidden treatment related to the planted seeds (i.e. weight, number and seed size) that ultimately affect plant density. Thus, the un-intended hidden treatment of seeding more seeds on more diverse plots contributes to the productivity to some degree. Such derivative but often neglected hidden treatments are important for further improvement of experimental design and have significant implications in ecological restoration.

Keywords: experimental design • productivity • restoration • seed number • seed weight

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INTRODUCTION

A frequently adopted approach in investigating the role of species diversity on productivity is using field experiments. Results from the majority of these experiments show that annual biomass production increases with diversity (Hector *et al.* 1999; Loreau *et al.* 2002; Tilman *et al.* 2001). The enhanced productivity has been typically interpreted by species facilitation, niche complementarity, the insurance hypothesis or selection/sampling effects (Grime 2002; Huston 1997; Loreau *et al.* 2002; Palmer and Maurer 1997), among others. Huston (1997) points out several 'hidden treatments' especially those related to physical conditions, non-random species selection and sampling effects in common seeding experiments. Recent work demonstrates the importance of diversity-induced changes in plant size and density (relative abundance or evenness) of seeded species among species and across treatments (Balvanera *et al.* 2006; He *et al.* 2005; Marquard *et al.* 2009; Wilsey and Polley 2003). However, many other factors may also have changed with diversity treatments therefore contributing to the enhanced productivity. Yet, such possible treatment-related (hidden) effects and consequences may hold critical clues regarding how diversity is related to ecosystem functions and have important implications for restoration and management practices.

Most biodiversity experiments have been conducted in grasslands through seeding various numbers of species (Balvanera *et al.* 2006; Cardinale *et al.* 2009; Hooper *et al.* 2005; Schmid *et al.* 2009). To date, the most common seeding scenarios include using either the same seed weight (e.g. Fridley 2002; Guo *et al.* 2006; Tilman *et al.* 2001; Wang *et al.* 2007) or the same number of seeds across experimental treatments and/or plots (e.g. Callaway *et al.* 2003; Hector *et al.* 1999; Hooper and Vitousek 1997; Mulder *et al.* 2004; Naeem *et al.* 1996; Spaekova and Leps 2001; Troumbis *et al.* 2000; Wilsey and Polley 2003). If seed weight is kept constant across treatment plots and seed weight is divided equally among species (a substitutive design), the seed weight per species on each plot will be reversely related to the species richness (e.g. Hilles Ris Lambers *et al.* 2004) but the total number of seeds per plot (i.e. seed density) will vary with the number of species planted. Thus, this common 'seed weight constant' design clearly has a hidden treatment of non-constant plant density. If the total seed number is kept constant across treatment plots (i.e. the total seed number is divided equally among species—also a substitutive design) (Fig. 1). Other possible diversity treatment scenarios include either keeping the total seed weight constant across species (but not treatments) or keeping the total seed number constant across species (but not treatments; e.g. Zhang and Zhang 2006). In such cases, the total number (or weight) of seeds would increase with diversity, also leading to high plant density in high diversity treatments. However, these two scenarios are rarely adopted.

In addition to the effects of planted number of seeds, there is also evidence that seed size to some degree affects seed germination rate and plant performance such as survival and growth. Large-seeded species may have greater germination and growth rate than small-seeded species (e.g. Huston 1997; Shipley and Parent 1991; Silvertown 1981; Walters and Reich 2000). The variation in both species richness coupled with that in seed size among species may ultimately lead to the differences in plant density (Turnbull *et al.* 2000) and thus productivity (Marquard *et al.* 2009). For example, treatments involving diverse seed sizes might lead to the resulting community favoring larger seeded species.

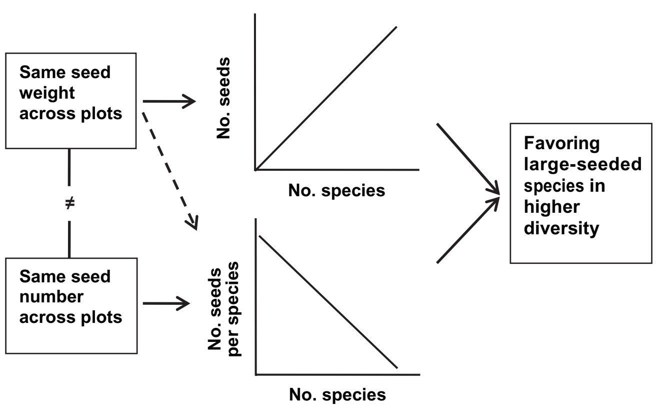

Figure 1: the commonly used two treatment scenarios (i.e. same number or weight of seeds across plots) and density-related consequences. In both cases, the amount of seeds (weight or number) per species decreases with diversity. If the same weight is the same across plots (top), the plant density will increase with diversity because most species have small seeds in nature and most likely in the experimental species pool as well. An exception would be when the species in low-diversity treatments are intentionally or incidentally selected to have small seeds. However, if the seed weight is the same across plots (bottom), the resulting density will rely on the composition of species and their seed sizes that may be the major cause of greater variation in productivity in most experiments, especially in low-diversity treatments (i.e. either large- or small-seeded species are randomly selected and the resulting number of seeds being planted can change drastically). The '≠' sign indicates that the two designs are likely to produce different quantitative results although the positive diversity–productivity relations may hold.

In biodiversity experiments, a crucial issue is how different designs might alter the results unexpectedly besides those produced by different number of species. Here, we use data from a grassland experiment based on a common seeding design, and we examine: (i) whether different seed numbers are planted for different levels of diversity treatment and therefore may be treated as a 'hidden' treatment, (ii) how plant density as a hidden consequence of different numbers of seeds planted might contribute to productivity and (iii) whether germination may be related to seed number and size thus affecting plant density and productivity.

METHODS

The experiments were conducted at four grassland sites in North Dakota, the US in 2002–03, i.e. Lostwood National Wildlife Refuge (LW), Devils Lake Wetland Management District (DL), Northern Prairie Wildlife Research Center (NP; with two additional, higher diversity treatments) and Sheyenne National Grassland (SH). Each site was relatively homogeneous in terms of slope, vegetation and soil conditions. All sites were excluded from grazing by large herbivores and chosen for having a thin humus layer to reduce the possibility of inhibition on seed germination. During site preparation, the aboveground vegetation was removed and treatment plots were evenly treated with a prescribed burn (except NP and SH) and glyphosate herbicide during the growing season.

Fifteen evenly spaced experimental plots (5×5 m) were established at LW, DL and SH sites, and 25 plots were established at the NP site in late 2002. Plots were spaced 5 m apart in all cardinal directions. Diversity treatments (the number of seeded species) were randomly assigned to plots (five plots per treatment) within a site. Plots were seeded in May 2003 at a rate of 11.2 kg ha^{-1} of pure live seeds. LW, DL and SH were seeded with 2, 8 and 16 native perennial species and NP was seeded with 2, 4, 8, 16 and 32 species. The total diversity (seeded + resident species) in some plots exceeded diversity in nearby natural grasslands and recent experiments. The seeds were mixed with equal weights of each species. Number of seeded species on each plot was divided evenly between the two major functional groups, i.e. grasses and forbs; thus, the grass-to-forb ratio in terms of seed weight was 1:1. The 32 species included 9 C4 grasses, 7 C3 grasses, 3 legume forbs and 13 non-legume forbs. The species seeded at each site were locally common, and therefore, the species mixes varied among sites. However, the same two-species mix, consisting of *Andropogon geradii* and *Linum lewisii*, was used at all sites (see online supplementary

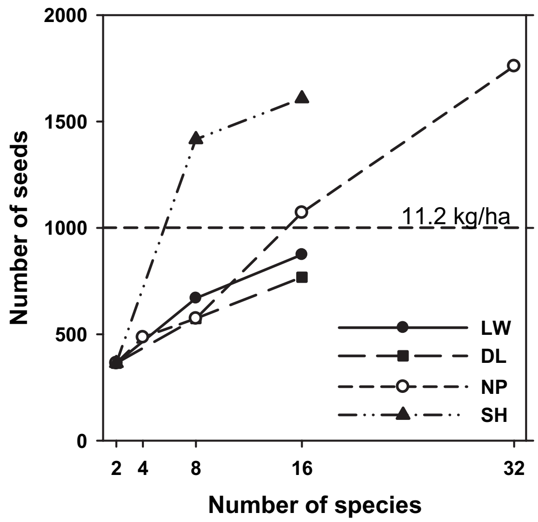

Figure 2: relationships between the number of species and total number of seeds planted at the four experimental sites. When the total weight of seeded species was kept the same (11.2 kg ha^{-1}) across all treatments and sites, the total number of seeds increased with planting diversity.

material for supplementary Table S1). The resident (preexisting, non-seeded) species were allowed to persist in all plots, and their growth was monitored along with seeded species.

To determine the germination rate of seeded species, in early summer 2003, density of each seeded species was recorded after germination on a portion of each plot (1.5 × 1.5 m). Densities of each seeded and resident species in a 1 × 1 m portion of the opposite corner of each plot were recorded in late-summer 2003; and all plants were clipped at the ground level for determination of biomass in the laboratory. The fresh plant material was collected species by species and was later oven-dried to determine dry weight (for more details, see Guo *et al.* 2006). As the aboveground biomass of resident species was totally removed during site treatments, similar to that of seeded species, the annual biomass production (aboveground) of these species can be used as the estimate of productivity of preexisting vegetation. Seed size (mass) data were collected from a variety of published sources and the seed number under each diversity treatment was calibrated based on the seed mass of individual species and total seed weight planted on each treatment plot.

Both linear and non-linear (quadratic) regressions were used for each site. Plant density and annual biomass

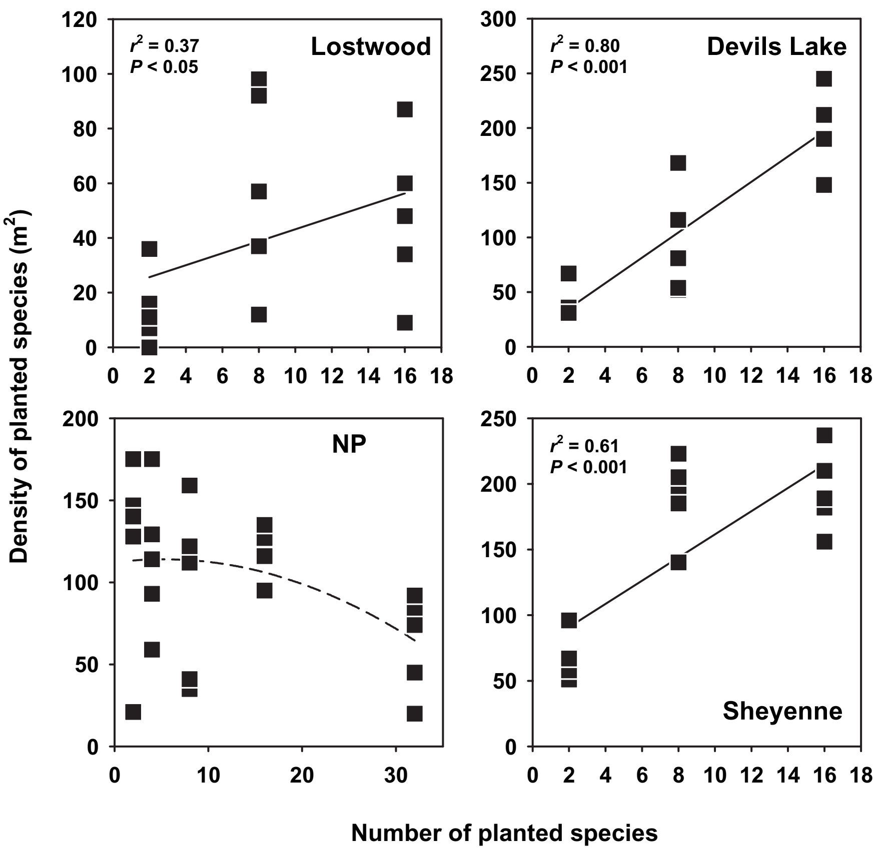

Figure 3: the relationships between the number of seeds planted and resulting plant density at the four experimental sites. Very high seed density might have had adverse effects on germination thus plant density due to phytochemical or allelopathic inhibition in the case at the NP site.

production were used as response variables. Because the aboveground biomass and litter of both seeded and resident species was totally removed during seed-bed preparation, the annual biomass production (aboveground) of these species provided an estimate of productivity for each group and for all species.

RESULTS AND DISCUSSION

The results reveal two fundamental 'hidden' issues associated with previous diversity treatments that have been largely ignored, i.e. the seed density and seed size. Using one of the common seeding methods in biodiversity experiments, i.e. the total weight of seeds was kept constant across treatment plots (all plots received the same weight of seeds), the total number of seeds apparently increased with planting species diversity (Fig. 2). The increased seed density with diversity then led to higher plant density in high diversity treatments (Fig. 3) that in turn contributed to the higher productivity on more diverse plots (Fig. 4). The apparent increase of planted seeds with diversity thus adds support to Marquard *et al.* (2009; see also He *et al.* 2005) that diversity-induced density contributes to productivity.

The unintended increase in the number of seeds with diversity intuitively led to a comparison with the other common design where the seed number was kept constant across treatment plots and seeds are evenly distributed among species (i.e. every species has the same number of seeds). In this latter design, the high diversity treatments would contain more species with larger seeds if the species were randomly selected from a reasonably large species pool similar to natural communities in which few species had large seeds while most have small seeds (e.g. Guo *et al.* 2000).

Using the most commonly used seeding scenarios mentioned above, the amount (weight or number) of seeds per species decreases with diversity, diversity treatments unintentionally alter plant density that affects productivity. In some rare cases in which the same number or weight of seeds is planted for each species (e.g. Zhang and Zhang 2006), the more diverse plots also contain even more seeds. The two common adopted designs in previous and ongoing seeding experiments are likely to yield different results unless all species have similar seed sizes. Accumulating evidence links productivity to seed size (and plant size) composition in the planted community because seed germination and plant performance are often related to seed size. For example, species with large seeds also showed higher germination rate (Fig. 5; see also online supplementary material for supplementary Fig. S1). When the same weight of seeds was planted across plots, the number of seeds increased with diversity. If the seed number was kept constant across species, the more species, the more seeds were

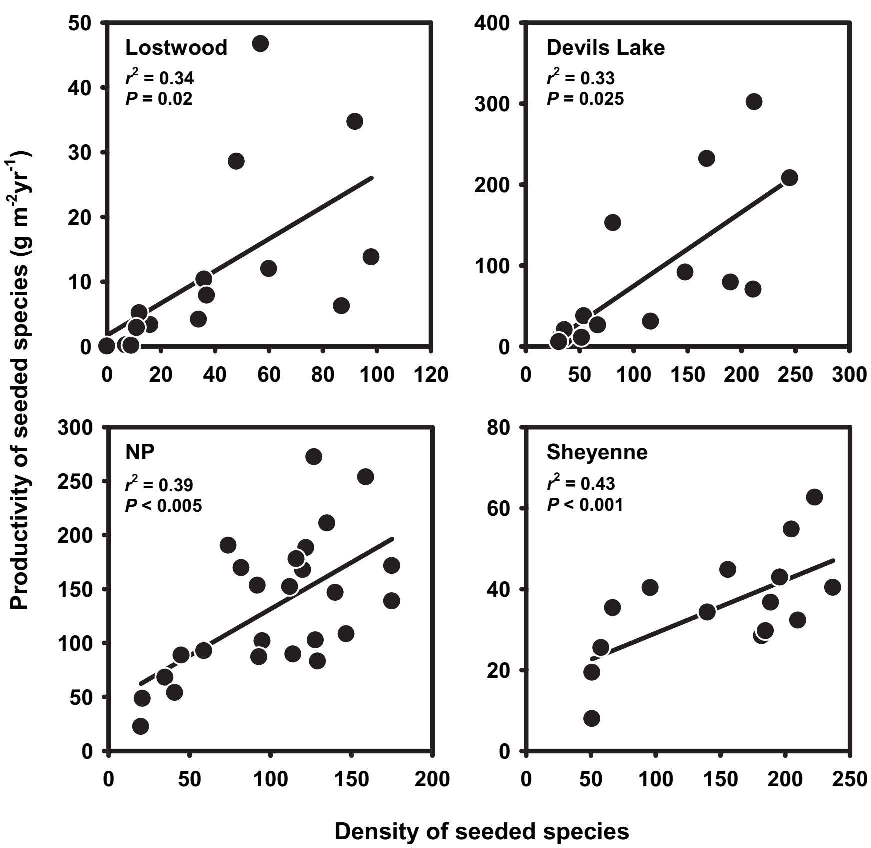

Figure 4: the relationships between plant density and productivity at the four experimental sites.

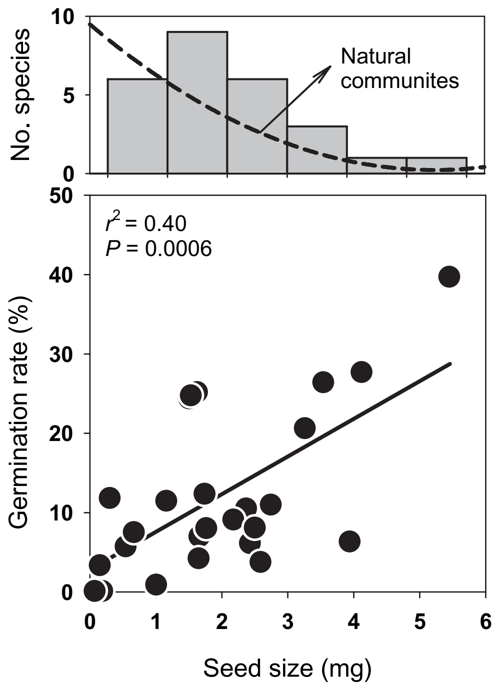

Figure 5: the histogram of seed size distribution among seeded species (top) and the relationships between seed size and germination rate at the NP site (bottom). The frequency distribution of seed size among the seeded species (shaded bars) does not fully reflect the frequently reported form in natural communities (the dashed line; see Guo *et al.* 2000) due to the under-representation of small-seeded species. The large-seeded species appeared to have greater germination rates (see online supplementary material for Supplementary Fig. S1).

planted. However, experimental designs that keep the same seed weight across species or keep the same number seeds across plots seemed not widely adopted.

Although very high seed density could also have adverse effects on resulting plant density through phytochemical or allelopathic inhibition (such as the case at the NP site where surrounding habitat of the same area usually supports much less species based on field observations, see Brown and Fridley 2003; Guo *et al.* 2006; Jiang *et al.* 2005), in most cases, high seed density usually leads to high density (Turnbull *et al.* 2000). To date, however, the fact of diversity-treatment induced changes in the number of seeds has not received the deserved attention because the focus has always been the role of species diversity. Unfortunately, our experimental design and available data do not allow statistical separations between the effects of diversity and those from seed density (particularly for each diversity treatment, the same species thus same amount of seeds were planted). To fully evaluate the relative contribution of each factor, a more sophisticated design and much more replicates are needed (Spaekova and Leps 2001). For example, to tackle such problems, future design could simultaneously control both diversity and seed density in one experiment, i.e. controlling diversity but allowing seed density to vary, and vice versa.

In controlled homogeneous physical environment, low seed density could limit productivity and high seed density generally leads to increased productivity unless it reaches the level that very high seed density and diversity result in adverse effects through phytochemical or allelopathic inhibition (see Brown and Fridley 2003, Hooper *et al.* 2005) or increased competition among seeds/seedlings (e.g. Mouquet *et al.* 2002).

Variation in seed size among species could affect the evenness in both biomass and density of the species in the community as larger seeds generally show greater germination rates that affect the overall and individual species' density (Easton and Kleindorfer 2009; Silvertown 1981; but see Shipley and Parent 1991). If total seed weight is the same across plots, the total planted seed number increases with the number of species seeded, although the high diversity treatments always result in less seeds for each species unless high diversity treatments only include species with smaller seeds. It is unlikely for species in high diversity treatments to have small seeds given the 'random' selection of species from the designated species pool required to avoid the species 'selection' or 'sampling' effect. Therefore, when the same weight of seeds are distributed across plots, more seeds are planted in more diverse plots (Fig. 2).

The effects of plant density may also have been seen in some theoretical studies that yield no general diversity–productivity relationship when the relation between species richness and total abundance of species is not fixed (i.e. density can either increase or decrease or does not vary with diversity; see also Loreau *et al.* 2002). The positive effects of density on productivity may be more pronounced in early stages when density and biomass are either positively related or not related (Marquard *et al.* 2009). As succession progresses into late stages, density and biomass become negatively related (e.g. through self-thinning) and it is the large individuals that contribute most to the productivity and biomass.

Separating the effects of diversity and density in seeding experiments remains a challenge as it requires more treatments and replicates. In natural communities, diversity and abundance (density) go hand in hand and may be always positively related (May 1975). For biodiversity to effectively perform in ecosystem functions (e.g. carbon sequestration, nitrogen fixing), component species must have enough individuals. In both experiments and restoration in the future, how many species should be planted and how many individuals should be assigned to each species in a given area should be considered jointly (Guo 2007). Different from experiments that are usually conducted in relatively homogeneous habitats, restoration that involves heterogeneous environments must consider specific habitat features (Grace *et al.* 2007). For example, Menges (1991) also shows that, in fragmented habitats, germination rate increases with population size. While similar hidden treatments in other experiments remain to be explored, the hidden treatments related to seed number and size revealed here offer insights for future biodiversity experiment design and may have significant implications for restoration. If, in natural settings, seed abundance (number or weight) is always positively related to diversity, restoration efforts should reflect this ecosystem property.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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