

Neighborhoods have little effect on fungal attack or insect predation of developing seeds in a grassland biodiversity experiment

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Abstract Numerous observational studies have documented conspecific negative density-dependence that is consistent with the Janzen–Connell Hypothesis (JCH) of diversity maintenance. However, there have been few experimental tests of a central prediction of the JCH: that removing host-specific enemies should lead to greater increases in per capita recruitment in areas of higher host density or lower relative phylogenetic diversity. Using spatially randomized plots of high and low host biomass in a temperate grassland biodiversity experiment, we treated developing seedheads of six prairie perennials to factorial applications of fungicide and insecticide. We measured predispersal seed production, seed viability, and seedling biomass. Results were highly species-specific and

idiosyncratic. Effects of insect seed predators and fungal pathogens on predispersal responses varied with neither conspecific biomass nor phylogenetic diversity, suggesting that—at least at the predispersal stage and for the insect and fungal seed predators we were able to exclude—the JCH is not sufficient to contribute to negative conspecific density-dependence for these dominant prairie species.

Keywords Coexistence · Cedar Creek · Janzen–Connell · Diversity maintenance

Introduction

Although plant species directly compete for a handful of resources, they coexist in large numbers. To explain this phenomenon, ecologists have proposed numerous hypotheses to explain the maintenance of species diversity (Chesson 2000; Fargione and Tilman 2002; Tilman and Pacala 1993; Wright 2002). A necessary condition for stable coexistence is that species limit themselves more than they limit other species, resulting in decreasing per capita or per biomass growth rates of each species as its population or biomass increases and increasing growth rates as a species becomes rare. If conspecific negative density- or biomass-dependence limits each species more than it limits others, then it will promote stable coexistence and maintain plant diversity (Chesson 2000; Tilman and Pacala 1993). Here, we use “negative density-dependence” (NDD) as shorthand for this phenomenon. Strong empirical evidence exists for NDD at early stages of plant recruitment, especially during the seed to seedling stages (Comita et al. 2010; Harms et al. 2000; Webb et al. 2006). One of the leading hypotheses of species coexistence, the Janzen–Connell Hypothesis (JCH), proposes that specialized natural enemies reduce

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recruitment of plants in a density- or distance-dependent manner (Connell 1971; Janzen 1970), such that seed or seedling survival increases with decreasing conspecific density or with increasing distance from conspecific individuals.

Empirical studies suggest that natural enemies, especially insect herbivores (Hammond and Brown 1998; Norghauer et al. 2006) and fungal pathogens (Bagchi et al. 2010; Petermann et al. 2008), may play a key role in maintaining plant diversity through NDD. However, few studies have experimentally manipulated the presence of natural enemies and the densities of plant hosts to determine the contribution of natural enemies to NDD for several co-occurring plant species (Freckleton and Lewis 2006). Studies that do not manipulate natural enemy abundance are open to alternative conjectures regarding the mechanisms underlying NDD, such as intraspecific competition (Bell et al. 2006; Dobson and Crawley 1994; Freckleton and Lewis 2006; Terborgh 2012). Studies that do not simultaneously manipulate natural enemies and plant densities do not control for factors that may covary with natural density, such as soil characteristics. Many of these studies focus on the seed and seedling stages following dispersal; fewer studies determine the contribution of predispersal natural enemies to NDD (Kolb et al. 2007; Maron and Crone 2006).

Predispersal natural enemies significantly reduce plant fecundity (Crawley 1992), but the relative importance among different groups remains unknown (Maron and Crone 2006). Ecologists have long debated the relative influence of different groups of natural enemies on plant populations, focusing on the roles of vertebrates and insects (Maron and Crone 2006), but the potential for pathogens to regulate plant populations and maintain diversity is becoming more widely recognized in natural systems (Mordecai 2011). Because insects and pathogens have very different life histories, determining their relative importance can aid our understanding of the influence of predispersal natural enemies on plant populations and species coexistence (Beckman and Muller-Landau 2011). Few studies have determined the interactive influence of insects and pathogens on plant production in natural communities in which the surrounding community can mediate interactions between natural enemies and plants (but see Dickson and Mitchell 2010); instead most previous studies were conducted with isolated plants in pots or focused on a single species (Hatcher 1995; Morris et al. 2007). Besides the direct effects of these natural enemies on plant fecundity, interactions among plants, insects, and pathogens may be complex; for example, insects can facilitate plant damage by pathogens and vice versa (Hatcher 1995). Reduced plant fecundity due to natural enemies has implications for plant population dynamics and community diversity (Crawley 1997).

While the classical assumption of the JCH is that natural enemies are host specific, there is much empirical evidence suggesting that insects and pathogens can have broad host ranges. Generalist natural enemies can promote NDD if they have differential effects across species (Hersh et al. 2012) or become locally adapted (Konno et al. 2011). Differential effects of generalist natural enemies on plant survival and growth may depend on the phylogenetic distance among hosts (Gilbert and Webb 2007; Hersh et al. 2012; Novotny et al. 2006). Observational studies suggest a role of NDD in maintaining local diversity by reducing the survival and growth of those seedlings and saplings that are surrounded by closely related neighbors (Uriarte et al. 2004; Webb et al. 2006). Although the mechanisms behind these observed patterns are rarely identified, Liu et al. (2012) recently provided experimental evidence that fungal pathogens reduce seedling survival of phylogenetically related species in a manner consistent with observed patterns. The measures of phylogenetic diversity used to describe the neighborhood of a focal individual are typically calculated from the phylogenetic distance between neighboring plants and the focal species, irrespective of neighbor abundance (Liu et al. 2012; Webb et al. 2006). However, weighting phylogenetic distance by the relative abundances of species present in a community may better explain the influence of neighbors on the performance of a focal plant (Cadotte et al. 2010).

Using a natural enemy removal experiment within an 11-year old biodiversity experiment, we quantified the extent of NDD due to insect seed predators and fungal pathogens on seed production, seed viability, and seedling biomass. Our design allowed us to ascribe our results to the actions of natural enemies and to experimentally isolate the effects of density (operationally measured as biomass due to the difficulty of identifying individuals in grassland systems) from other factors that may naturally covary with density in unmanipulated systems. For six temperate grassland plant species distributed across plots varying in host biomass and relative phylogenetic diversity, we collected seeds from seedheads treated with pesticides and measured the number of seeds produced per seedhead, seed mass, germination rates, and subsequent biomass of germinated seeds. With this experiment, we addressed the following questions:

1. What are the direct and interactive effects of insect seed predators and pathogens on seed production, seed viability, and seedling biomass of prairie plants?
2. Do predispersal insect seed predators, fungal seed pathogens, or their combination result in NDD across species—that is, do recruitment rates decrease with host biomass in the presence of natural enemies?

3. Does phylogenetic diversity of neighboring plants relative to a focal species influence reductions in recruitment rates due to natural enemies? If so, does weighting phylogenetic distance by species' relative abundances better explain changes in recruitment rates?

Materials and methods

Study site

Our seed predation experiment was nested inside a long-term grassland biodiversity experiment at Cedar Creek Ecosystem Science Reserve (CCESR), Bethel, Minnesota, USA. The biodiversity experiment was explicitly designed to manipulate plant species richness, with which mean plant species biomass, although not expressly maintained, was negatively correlated. Of the 324 biodiversity experiment plots seeded in 1994, 168 were maintained every year thereafter to contain one, two, four, eight, or 16 prescribed species from a pool of 18 late-successional perennial savanna species. Apart from the 39 monocultures (i.e. 1-species plots), to which the 18 species were assigned so as to span all of the species in the experiment, species for the remaining 129 plots were chosen randomly. Plots were 9×9 m and separated by at least 4 m of frequently mowed vegetation. The entire experimental area, including aisles, was approximately 265×252 m. Plots were routinely weeded to remove non-prescribed species, but the densities of prescribed species were free to naturally vary. A more detailed description can be found in Tilman et al. (2001) and at the CCESR website (<http://www.cedarcreek.umn.edu/research/exper/e120>).

Field treatments

In 2005, we selected six species based on the availability of sufficient monoculture replication (Table 1). For most species in the biodiversity experiment there were only one or two monoculture plots; we used all species for which there were three monoculture plots except for one, *Andropogon gerardii*, which had two monoculture and a two-species plot in which its woody species competitor was almost entirely absent (which made it a de facto monoculture). For each of the six species we randomly selected three 16-species plots (hereafter “polycultures”) from the 35 polycultures in the larger biodiversity experiment, with the stipulation that they contained the focal species and were not already assigned to some other focal species. In total, 36 plots (18 monocultures and 18 polycultures) were used for our natural enemy removal experiment.

In each plot, we selected eight seedheads for treatment, each belonging to a separate plant. Seedheads were defined

as complete inflorescences with all seeds connected to a single stem at the base of a plant. Along either the north or south side of the plot, we set eight temporary flags 1 m in from the edge and 1 m from each other as seedheads emerged from mid-July to August. We selected the nearest seedhead to each flag within a plot and randomly assigned it to one of four treatments, namely, insecticide, fungicide, insecticide and fungicide, or water (controls). Plots contained two replicate seedheads per treatment.

After flowering, between mid-July and mid-August, seedheads were saturated weekly with the respective treatment using spray bottles. We used a 3 % concentration of Mavrik Aquaflow (Wellmark International, Schaumburg, IL), a pyrethroid insecticide with the active ingredient tau-fluvalinate (22.3 % by volume) and a 1 % concentration of Captan (Bonide Products, Inc., Oriskany, NY), a phthalimide fungicide (50 % by volume). The insecticide Mavrik Aquaflow has a low toxicity to honey bees (Johnson et al. 2006). We were careful to postpone the insecticide treatment on the insect-pollinated species until they were finished flowering.

We based our methods on a study conducted at Cedar Creek by K. Kitajima and D. Tilman (unpublished data). Kitajima and Tilman applied insecticide to seedheads of five dominant grasses in four old-fields in which host species had high biomass and found that the insecticide treatment significantly reduced the proportion of developing insect seed predators within florets compared to controls. In untreated seedheads, these researchers found 1- to 2-mm-long orange or white larvae identified as *Diptera* (likely from the *Cecidomyiidae* family) and 2- to 4-mm-long larvae identified as *Lepidoptera* (likely from the *Microlepidoptera* group) that had grown in place of seeds with their mouthparts at the base of the floret, presumably consuming resources intended for the developing seed. Similarly, Mitchell (2003) identified several host-specific fungi that attack species in this system, including *Phyllosticta* sp., *Colletotrichum* sp., and *Puccinia andropogonis*. Kitajima and Tilman's comparison with control seedheads revealed that seed predation reduced potential seed yields by over 75 % in *Agropyron repens* and *Schizachyrium scoparium* and by 39 and 15 % in *Andropogon gerardii* and *Sorghastrum nutans*, respectively (unpublished data).

As seeds ripened from August to September, we visited plots three times a week and harvested seedheads, along with >10 cm of stem, once they were fully mature but before seeds dispersed (on a few occasions, we noticed that some seeds appeared to have dispersed before collection, but these never constituted more than a small fraction of the total seeds on the seedhead, as estimated from the remaining structures). However, nine seedheads along with their supporting structures were entirely missing before collection (1 *Andropogon gerardii*, 1 *Schizachyrium scoparium*, 1

Table 1 Seed trait data determined from field-collected seedheads, one per individual. Seedling mass determined from growth of field-collected seeds in the greenhouse. Values are counts or means with standard deviation in parentheses. Relevant sample sizes given in table. See text for details

Species ^a	Functional type	No. seedheads sampled	Seeds per seed-head ^b	Seed mass (mg) ^b	No. seeds germinated in greenhouse trial	Germination per seedhead ^b	Seedling mass (mg) ^b	No. seedlings grown in greenhouse trial	Conspecific biomass in the biodiversity experiment (g m ⁻²) ^b	
									Low diversity	High diversity
<i>Andropogon gerardii</i>	C4	47	61 (34)	2.5 (0.5)	1,366	0.38 (0.23)	39.0 (13.4)	391	57 (17)	41 (34)
<i>Sorghastrum nutans</i>	C4	47	102 (69)	1.4 (0.5)	1,318	0.15 (0.21)	38.5 (18)	188	77 (45)	40 (17)
<i>Schizachyrium scoparium</i>	C4	47	44 (21)	1.3 (0.2)	1,249	0.30 (0.27)	19.3 (8.2)	202	42 (9)	47 (13)
<i>Liatris aspera</i>	Forb	38	213 (133)	2.3 (0.6)	1,068	0.13 (0.13)	11.6 (6.0)	45	59 (4)	13 (4)
<i>Lespedeza capitata</i>	Legume	48	163 (141)	2.8 (0.43)	1,229	0.89 (0.14)	24.4 (5.4)	652	98 (13)	18 (22)
<i>Petalostemum purpureum</i>	Legume	44	43 (46)	1.5 (4.1)	808	0.96 (0.10)	11.0 (5.2)	241	50 (24)	18 (23)

^a Names follow Gleason and Cronquist (1963)

^b Standard deviation is given in parenthesis

Sorghastrum nutans, 2 *Liatis aspera*, and 4 *Petalostemum purpureum*) and were thus not included in analyses.

Seeds per seedhead, greenhouse germination, and greenhouse seedling biomass

Collected seedheads were stored dry at room temperature and ambient humidity for several weeks to after-ripen. We separated seeds from stems and vegetative materials and hand-counted the apparent number of seeds per seedhead. We then weighed seeds collected from each seedhead and divided this value by the total number of seeds to calculate mean mass per seed for each seedhead. The number was “apparent” because we did not hull seeds to visually quantify seed predation by insects or fungi. Thus, some seed hulls likely contained insects or seeds consumed by fungi.

Next, to assess differences in seed viability due to natural enemy treatments, we tested germination on 30 apparent seeds per seedhead wherever possible. Apparent seeds that contained insects or seeds consumed by fungi would not germinate, providing a means to indirectly measure seeds consumed by predators. For approximately 80 % of our seedheads, we were able to allocate 30 seeds to the germination assay. For those seedheads with fewer seeds, we allocated as many seeds as we could to the germination assay while reserving three seeds in the event that we needed them for subsequent assays. For approximately 95 % of our seedheads, we were able to allocate at least 15 seeds to the germination assay. In total, we tested germination on 7,038 seeds.

All germination procedures followed the guidelines of the Association of Official Seed Analysts (AOSA 1999). To test germination, we first scarified seeds of our two legume species by blowing them twice against coarse (240D) sandpaper using compressed air (25 PSI; D. Vellekson, Dept. of Agronomy & Plant Genetics, University of Minnesota). We then evenly distributed seeds from a single seedhead on the top of two sheets of Anchor Steel Blue Seed Germination Blotter (Anchor Paper Company, St. Paul, MN) in a petri dish (diameter 8.5 cm) and saturated the paper with a 0.2 % (concentration) KNO_3 solution, following which the petri dishes were frozen for 1 day and then stored at 5 °C for 3 weeks to break dormancy. The petri dishes were placed in a greenhouse in blocks by species and randomly within species, all of which were randomly moved at least once a week. We monitored the seeds daily for 2 weeks, maintaining the blotter paper at saturation, removing fungally attacked seeds (there were few), and planting germinated seeds in seedling pots (4 × 4 cm; depth 5 cm) by seedhead (i.e., seedlings that germinated from the same seedhead were planted together). In total, 2,905 seeds germinated.

We grew these seedlings for 4.5 weeks in unsterilized soil collected from CCSR in the greenhouse with ample light (16 h day⁻¹) and water. We harvested all of the

seedlings of all of the species at the same time and before roots outgrew their pots. In total, 1,719 seedlings survived. We separated shoots from roots, washed roots, and dried all material to constant mass. For seedlings from a given seedhead, we weighed shoots together and roots together and then divided our measured value by the number of seedlings to arrive at mean individual seedling biomass. Here, we report total (i.e., root + shoot) seedling biomass.

Neighbor densities and phylogenetic distances

We based conspecific density on aboveground biomass data obtained from the biodiversity experiment (Table 1). Specifically, a 0.1 × 6-m section of vegetation was clipped in 2005 from all of our 36 plots, sorted to species, dried, and weighed. Based on the aboveground biomass from the biodiversity experiment, we excluded one entire plot from our analyses because the biomass of the focal species was more than 11 standard deviations above the mean of the other 35 plots (excluded plot: *Liatis*, 387 g m⁻²; mean of remaining plots: 46 g m⁻²; maximum of remaining plots: 128 g m⁻²).

Using the phylogeny available for Cedar Creek (Cadotte et al. 2009), we constructed two measures of relative phylogenetic diversity: (1) average phylogenetic diversity and (2) abundance-weighted phylogenetic diversity for our focal species. For individuals of focal species t in plot j , average phylogenetic diversity was calculated as $mPD_{t,j} = \sum_{s=1}^n d_{s,t} / n$, where n is the number of all

species planted in plot j and $d_{s,t}$ is the phylogenetic distance between species s and the focal species t . Abundance-weighted phylogenetic diversity was calculated as $aPD_{t,j} = \sum_{s=1}^n A_{s,j} d_{s,t} / \sum_{s=1}^n A_{s,j}$, where $A_{s,j}$ is the above-

ground biomass of species s in plot j . For monocultures, $mPD_{t,j}$ and $aPD_{t,j}$ equal zero; as plots become more diverse, phylogenetic diversity increases either relative to the number of species ($mPD_{t,j}$) or weighted by abundance ($aPD_{t,j}$). Phylogenetic distances ($d_{s,t}$) were calculated from the phylogeny available in Cadotte et al. (2009) using the picante package in R (Kembel et al. 2010).

Statistical analyses

Using generalized linear mixed models, we analyzed the influence of plant density and relative phylogenetic diversity on reductions in early plant recruitment due to natural enemies. Response variables included the number of apparent seeds per seedhead, the number of seeds germinated, and seed and seedling biomass (root + shoot, mg). The number of seeds germinated was analyzed with binomial

errors and the number of apparent seeds per seedhead was analyzed with Poisson errors; in each case, individual-level random effects were included to account for overdispersion (Maindonald and Braun 2010). Seed and seedling biomass were square-root transformed and analyzed with normal errors. Pesticide treatments, species, covariates, and relevant interactions were included as fixed effects with plot-level random effects. Covariates describe the surrounding neighborhood of the focal species and include conspecific density and a measure of relative phylogenetic diversity (either average or abundance-weighted). We standardized covariates by subtracting the mean across species and dividing by one standard deviation to make coefficient estimates easier to interpret (Gelman and Hill 2007).

Model selection was performed using Akaike Information Criterion (AIC) on a set of candidate models varying in covariates describing the focal species' neighborhood [Models 1–6 in Electronic Supplementary Material (ESM) Table S1; Burnham and Anderson 2002]. We compared the selected model to models with two- and three-way interactions removed to determine whether model complexity could be reduced (Models 7, 8 in ESM Table S1). For the best-fit model, we provide the coefficient of determination (R^2), which is the square of the correlation coefficient between fitted and observed values. To compare models, linear mixed models differing in fixed effects were fit by maximum likelihood estimation, and the most parsimonious model was refit with the restricted maximum likelihood method to estimate parameters. We used the Laplace approximation of likelihoods to estimate parameters of fixed- and random-effects (Bolker et al. 2009) and the lme4 package in R (Bates et al. 2011; R Development Core Team 2011).

Results

All measures of plant performance varied among species and were differentially affected by pesticide treatments and neighborhood indices. The most parsimonious models of all plant responses analyzed included a measure of phylogenetic diversity. Variation in germination was the only response best explained by both phylogenetic diversity, specifically standardized abundance-weighted phylogenetic diversity (aPD), and conspecific density ($R^2 = 0.98$, ESM Table S2). For the other plant responses, the most parsimonious models included standardized aPD for seed mass ($R^2 = 0.77$, ESM Table S3) and standardized average phylogenetic diversity (mPD) for apparent seed number ($R^2 = 1.00$, ESM Table S4) and seedling biomass ($R^2 = 0.72$, ESM Table S5). Although the best models did not include two-way interactions between pesticide treatments and neighborhood indices nor three-way interactions among species, pesticide treatments, and neighborhood

indices, there was some support for including these two-way interactions in models of all plant responses ($1.7 < \Delta AIC < 5.8$) as well as the three-way interactions in models of seedling biomass ($\Delta AIC = 2.1$). This result suggests that each response was affected by local phylogenetic diversity but that the influence of pesticide treatments on plant performance was less affected by variation in local phylogenetic diversity and plant density.

Direct and interactive effects of natural enemies on plant performance

Pesticide treatments increased at least one measured response for five of the six species. Insecticide treatment, either alone and in combination with fungicide, increased the germination of *Andropogon gerardii* seeds (Fig. 1). Fungicide treatment increased seed germination of *Sorghastrum nutans* at low aPD , while the combination of fungicide and insecticide had less of an effect (Fig. 1). Fungicide treatment, both alone and when applied with insecticide, increased germination of *Liatris aspera* seeds, while the application of insecticide alone slightly decreased germination of *Liatris aspera* at high aPD (Fig. 1). The application of insecticide alone increased germination of *Lespedeza capitata*, but had less of an effect when applied with fungicide (Fig. 1). The combination of insecticide and fungicide slightly increased germination of *Petalostemum purpureum* seeds compared to controls (Fig. 1).

Pesticides had little effect on species seed mass; however, application of the insecticide and fungicide combination increased seed mass of *Liatris aspera* compared to controls while each of these pesticides applied separately had no effect (Fig. 2). Application of insecticide and the combination of insecticide and fungicide increased seed number of *Sorghastrum nutans*, and the application of fungicide alone and in combination with insecticide increased seed number of *Lespedeza capitata*, especially at lower mPD (Fig. 3). The application of both insecticide and fungicide in combination increased seedling biomass of *Andropogon gerardii* but not when each pesticide was applied separately, while fungicide treatment decreased seedling biomass of *Sorghastrum nutans* compared to controls, with less of an effect when applied with insecticide (Fig. 4).

Influence of density-dependence on plant performance

The probability of germination increased with standardized conspecific density for *Andropogon gerardii* and *Liatris aspera* seeds and decreased with standardized conspecific density for *Schizachyrium scoparium* seeds (Fig. 1). Standardized conspecific density had little effect on the germination of *Sorghastrum nutans*, *Lespedeza capitata*, and *Petalostemum purpureum* seeds.

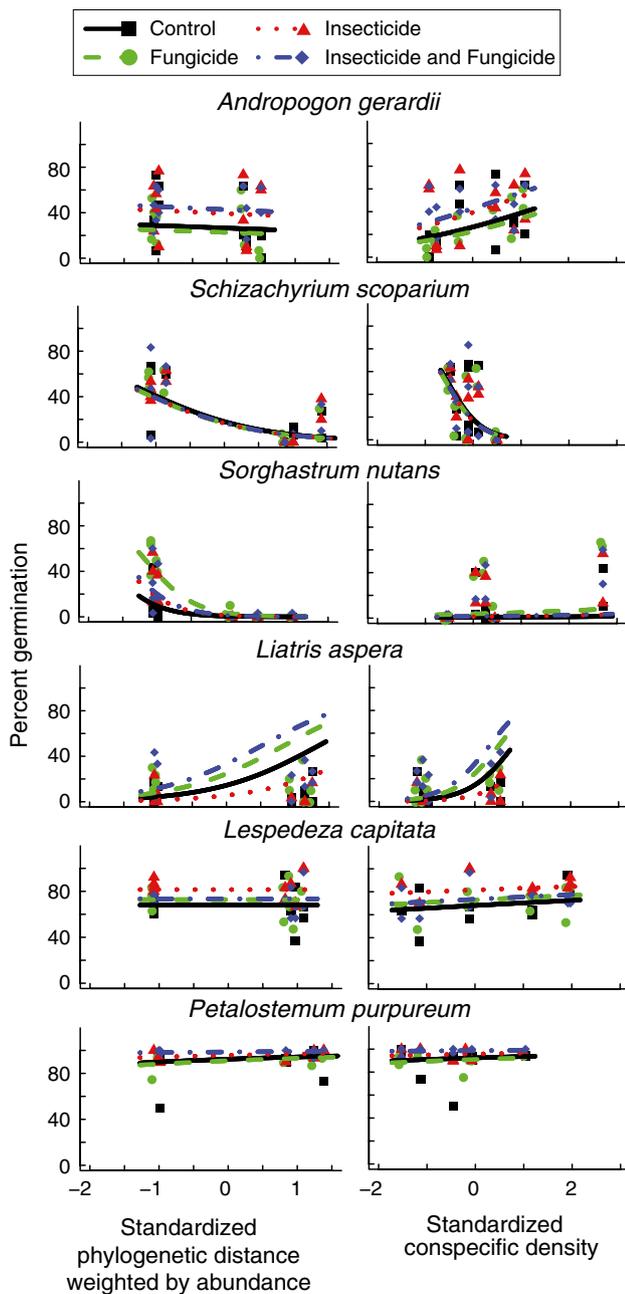


Fig. 1 Effect of enemy removal treatments on germination of six grassland species in Minnesota in relation to standardized phylogenetic distance weighted by abundance ($aPD_{i,j}$) and standardized conspecific density. Lines are best fits from the most parsimonious generalized linear mixed model (for full analyses, see ESM Table S2)

Influence of phylogenetic diversity on plant performance

Standardized phylogenetic diversity weighted by abundance (aPD) explained variation in percentage germination (Fig. 1) and seed mass (Fig. 2), while standardized average

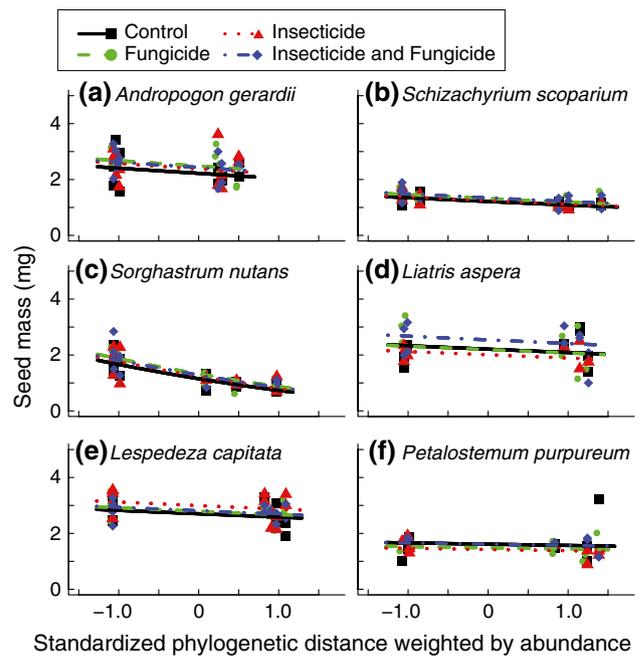


Fig. 2 Effect of enemy removal treatments on seed mass of six grassland species in Minnesota in relation to standardized phylogenetic distance weighted by abundance ($aPD_{i,j}$). Lines are best fits from the most parsimonious linear mixed model (for full analyses, see ESM Table S3)

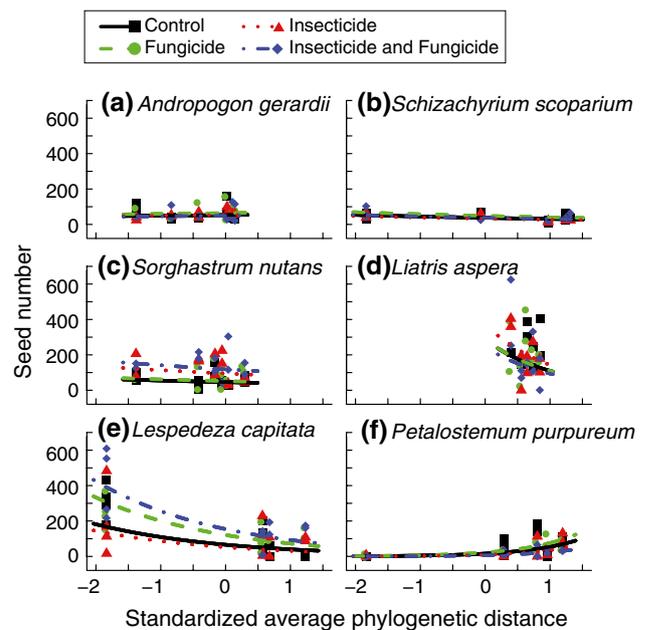


Fig. 3 Effect of enemy removal treatments on seed number of six grassland species in Minnesota in relation to standardized average phylogenetic distance ($mPD_{i,j}$). Lines are best fits from the most parsimonious generalized linear mixed model (for full analyses, see ESM Table S4)

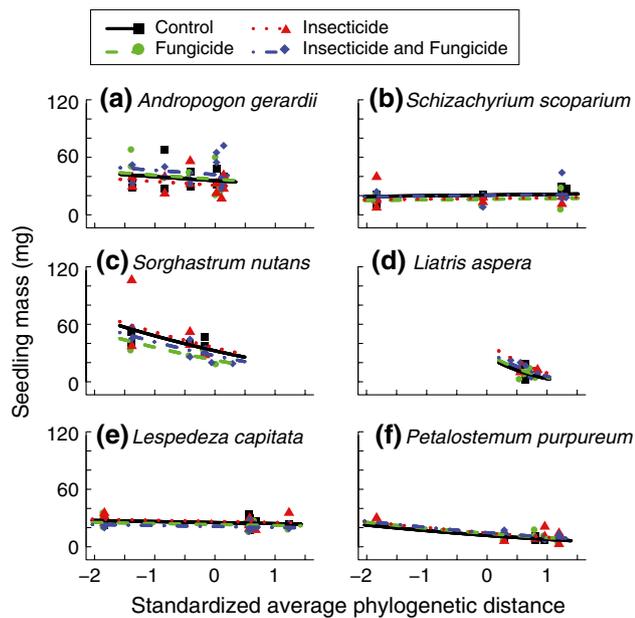


Fig. 4 Effect of enemy removal treatments on seedling biomass of six grassland species in Minnesota in relation to standardized average phylogenetic distance ($mPD_{i,j}$). Lines are best fits from the most parsimonious linear mixed model (For full analyses, see ESM Table S5)

phylogenetic diversity (mPD) explained variation in seed number (Fig. 3) and seedling mass (Fig. 4). The probability of germination of *Schizachyrium scoparium* and *Sorghastrum nutans* seeds decreased with standardized aPD , whereas that of *Liatris aspera* seeds increased as neighborhoods became more diverse, as reflected by the positive relationship between germination and standardized aPD (Fig. 1). Standardized abundance-weighted phylogenetic diversity had little effect on the germination of *Andropogon gerardii*, *Lespedeza capitata*, and *Petalostemum purpureum* seeds. Seed mass decreased with standardized aPD for *Sorghastrum nutans* and otherwise had little effect on the seed mass of other species (Fig. 2). Seed number decreased with standardized mPD for *Lespedeza capitata* and *Liatris aspera* and increased with standardized mPD for *Petalostemum purpureum*, with little relationship for other species (Fig. 3). Seedling biomass decreased with standardized mPD for *Sorghastrum nutans* and *Liatris aspera* and was little affected by standardized mPD for *Andropogon gerardii*, *Schizachyrium scoparium*, *Lespedeza capitata*, and *Petalostemum purpureum* (Fig. 4).

Discussion

Direct and interactive effects of natural enemies on plant performance

There is evidence that species respond differentially to natural enemies within natural communities (Beckman

and Muller-Landau 2011; Dickson and Mitchell 2010). In our study, application of insecticide, fungicide, or their combination increased one of the measures of seed production, seed viability, or seedling biomass in five of the six species studied. For two species, pesticides reduced seed viability or seedling biomass. In a previous study of the four most common grasses at the same site, pesticide treatments applied to whole plots had negative to positive effects on seed production depending on the species (Dickson and Mitchell 2010). Similar species-specific responses in seed production to pesticide treatments have been found in other systems, such as a Neotropical forest (Beckman and Muller-Landau 2011). In contrast to a previous study at the same site (Dickson and Mitchell 2010), we found interactive effects between insects and pathogens for several species, including *Liatris aspera*, *Sorghastrum nutans*, *Lespedeza capitata*, and *Petalostemum purpureum*. This variation in the direction and magnitude of interactive effects among species is consistent with results reported in a recent meta-analysis determining interactive effects between types of enemies which included studies with a range of plant life histories and environments and measured plant responses (Morris et al. 2007). Interactive effects of insects and pathogens on plant fecundity may furthermore depend on interannual variation in the effects of insect seed predators and pathogens, which has been documented for several species (Dickson and Mitchell 2010; Kolb et al. 2007).

Given the current wide-spectrum activity of the chemicals that must be used, enemy removal experiments of insects and microbes are blunt instruments (Crawley and Pacala 1991) that may have unintentional effects. Pesticides may have phytotoxic effects on plants or remove non-target organisms, such as mutualists (Paul et al. 1989; Siemann et al. 2004). Although in our study the fungicide treatment increased germination, *Sorghastrum nutans* produced less individual seedling biomass under the fungicide treatment, perhaps because the fungicide removed beneficial endophytes (Clay and Schardl 2002) that would have helped the seedlings acquire nutrients or defend against soil-borne enemies or due to the potential of pesticides to be phytotoxic (Paul et al. 1989; Siemann et al. 2004).

Influence of density-dependence on plant performance

In general, reductions in plant performance due to natural enemies did not increase with host biomass as would be expected for NDD. For most species, pesticide treatments increased plant performance consistently across neighborhoods varying in conspecific plant densities. For *Lespedeza capitata* and *Sorghastrum nutans*, the fungicide increased seed production or viability in less diverse neighborhoods, but models including interactions between pesticides and

neighborhood had less support than models excluding these two-way interactions. The severity of foliar disease at the same location increased with lower plant species richness, which was partly due to increased host biomass (Mitchell et al. 2002). Our results indicate that the JCH, at least at the predispersal stage for the species examined here and for the enemies that we were able to exclude, is not sufficient to maintain diversity in our system by itself.

We found species-specific responses in germination to densities of conspecifics. Observational studies of NDD in tropical forests (Comita et al. 2010) and experimental studies investigating negative feedbacks by pathogens in temperate grasslands (Klironomos 2002) and tropical forests (Mangan et al. 2010) have shown that NDD at the postdispersal stage is stronger for rarer species than abundant species, whereas the most common species experience positive feedbacks with mutualistic microorganisms (Klironomos 2002; Reynolds et al. 2003). This might also be true for our prairie system at the predispersal stage as most of the species included in this study are dominant. Only one species, *Schizachyrium scoparium*, experienced decreased germination with increasing conspecific density. Because conspecific density did not interact with pesticide treatments, this result suggests another mechanism other than JCH is contributing to NDD at the predispersal stage in this species. For example, Castelli and Casper (2003) previously found that an arbuscular mycorrhizal (AM) fungus specific to *S. scoparium* reduced its biomass after inoculation. A negative feedback between *S. scoparium* and its specific AM fungi could contribute to the pattern observed here, reducing the viability of mature seeds as densities of *S. scoparium* increase.

For *Andropogon gerardii* and *Liatris aspera*, germination increased with conspecific densities, suggesting a positive density-dependence. Various mechanisms can result in positive density-dependence, such as predator satiation (Bonal et al. 2007), increasing pollen availability (Wagenius et al. 2007), or mutualistic interactions with microorganisms (Bever et al. 2010). Insect seed predators can become satiated due to limited egg production or a constrained growing season. However, because pesticide treatments did not interact with plant densities, it is unlikely that predator satiation contributed to positive density-dependence. *Andropogon gerardii*, a self-incompatible, wind-dispersed plant, is unlikely to be pollen limited because of its high abundance, as was shown by a pollen addition study in Northfield, Minnesota (McKone et al. 1998). In contrast, *Liatris aspera* is a self-incompatible plant, and its bee pollinators move among nearest neighbors such that plant density may affect pollen dispersal distances (Levin 1968; Levin and Kerster 1969). In general, increasing local conspecific density of plants is expected to reduce pollen limitation (Knight et al. 2005), and the benefit of increasing

pollen availability may outweigh potential reductions in fecundity due to natural enemies (Jones and Comita 2010). Positive feedbacks between mutualistic microorganisms may contribute to this positive density-dependence (Reynolds et al. 2003). However, Hartnett et al. (1993) found that the increased benefit of mycorrhizal fungi on *Andropogon gerardii* biomass (compared to soil with no mycorrhizae) decreased with neighborhood density. If positive density-dependence outweighs negative density-dependence at high densities, species coexistence will be destabilized.

Influence of phylogenetic diversity on plant performance

Our analyses showed that all measures of plant performance were affected by relative phylogenetic diversity but that there was less support for models that included interactions between pesticide treatments and phylogenetic diversity. Because natural enemies in many communities are not specialized on one host and instead have a phylogenetic signal in their host plants (Gilbert and Webb 2007; Novotny et al. 2006), we expected more support for models that included interactions between phylogenetic diversity and pesticides. Therefore, although natural enemies may partly explain variation in plant performance based on phylogenetic relatedness among plants, phylogenetic diversity may be capturing other mechanisms that affect plant performance.

We found that predispersal responses of certain species were reduced as neighborhoods became more diverse, independent of the relative abundance of neighboring species. Seed number decreased with standardized average phylogenetic distance *mPD* for *Lespedeza capitata* and *Liatris aspera*, and seedling biomass decreased for *Sorghastrum nutans* and *Liatris aspera*. The positive effects of less diverse neighborhoods may suggest positive feedbacks between mutualistic microorganisms (Reynolds et al. 2003), the production of higher quality seed in low diversity plots, or other such causes. For seed mass and germination the most parsimonious models included standardized phylogenetic distance weighted by plant abundance *aPD*. *Sorghastrum nutans* seed mass and percentage germination of *Schizachyrium scoparium* and *Sorghastrum nutans* seeds decreased with *aPD*. If the dispersal abilities of mutualists on *Schizachyrium scoparium* or *Sorghastrum nutans* are short, relative abundance would be more important for contributing to positive feedback between mutualists and these two species than for plant species harboring microorganisms with better dispersal abilities, although this is very speculative. For two species, more diverse phylogenetic neighborhoods increased plant performance based on the presence of species, *mPD*, for seed number of *Petalostemum purpureum* and based on the abundance of species, *aPD*, for germination of *Liatris aspera*. For both these

species, the severity of foliar fungal disease decreased with species richness, but only for *P. purpureum* did disease severity decrease with species richness of plots after accounting for increased disease severity due to higher conspecific abundance with lower species richness (Mitchell et al. 2002). Our results suggest that taking into account not only the diversity of the neighbors present, but also the abundance and evolutionary relatedness of neighbors can account for variation in plant performance.

Implications for plant populations and communities

There is no evidence that any of the populations in our biodiversity experiment, regardless of biomass, had been growing exponentially in the 11 years since the experiment was established, suggesting that there are mechanisms other than mortality due to predispersal natural enemies that suppress the per capita rates of change in areas of high conspecific biomass. If our experimental communities are “propagule saturated,” such that no amount of additional seed or seedlings can increase the biomass of a given species (Cornell 1999), then the negative effect of predispersal seed predation might ultimately be of no consequence for population dynamics. Just a relatively small number of healthy seeds might be sufficient to establish new adults in place of dead ones. However, several studies at our site (Fargione and Tilman 2005; Foster and Tilman 2003; Tilman 1997) and in grasslands in general (Clark et al. 2007; Turnbull et al. 2000; Zobel and Kalamees 2005) have demonstrated that many communities are “unsaturated”: adding seed or seedlings tends to increase the cover or biomass of resident and non-resident species. In an unsaturated community, the negative effect of predispersal seed predation will contribute to population dynamics.

A suite of mechanisms are likely structuring communities and maintaining diversity in concert (Adler et al. 2007; Chesson 2000; Fargione and Tilman 2002), although no one has, to date, clearly demonstrated their relative magnitudes and identities in any given system. The role of phylogenetic relatedness among taxa in structuring communities has received an increasing amount of attention in recent years (Cavender-Bares et al. 2009; Webb et al. 2002). From patterns of phylogenetic community structure, we can infer processes important for community assembly, but because several mechanisms can result in similar patterns, additional information is required to distinguish competing hypotheses. Manipulative studies of plant diversity can help elucidate mechanisms underlying coexistence and community assembly in plants. The manner in which natural enemies contribute to phylogenetic structure within a plant community depends on their host range and the evolution of plant defensive traits—i.e., whether traits are phylogenetically conserved or convergent (Cavender-Bares et al. 2009). In this system, we found that predispersal natural

enemies reduce recruitment rates from seed production to seedling establishment and therefore influence population dynamics of these prairie species. However, although phylogenetic relatedness between focal plants and neighbors affected plant performance, we found little support that natural enemies contribute to NDD and diversity maintenance at the predispersal stage.

Summary

In this study we tested whether the removal of natural enemies had greater effects on per capita recruitment in areas of higher host biomass, one of the crucial predictions of the JCH of diversity maintenance (Freckleton and Lewis 2006). Unlike observational studies, where unmeasured correlated variables may influence results, we had the advantage of using areas of high and low host biomass which, although not explicitly maintained, were experimentally created and randomly distributed in space (Freckleton and Lewis 2006). We found species-specific responses at the predispersal stage to insect seed predators and pathogens and to their interactive effects. Plant species also had differential responses to neighborhood phylogenetic diversity and densities of conspecifics. However, the effects of predispersal natural enemies were not sufficiently negative to cause a decrease in per capita recruitment with host biomass, a necessary requirement for stable coexistence, suggesting that, at least at the predispersal stage and for the insect and fungal seed predators which we were able to exclude, the JCH is not sufficient to maintain diversity for these six dominant prairie species by itself.

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