Positive interactions between herbivores and plant diversity shape forest regeneration

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The effects of herbivores and diversity on plant communities have been studied separately but rarely in combination. We conducted two concurrent experiments over 3 years to examine how tree seedling diversity, density and herbivory affected forest regeneration. One experimentfactorially manipulated plant diversity (one versus 15 species) and the presence/absence of deer (Odocoileus virginianus). We found that mixtures outperformed monocultures only in the presence of deer. Selective browsing on competitive dominants and associational protection from less palatable species appear responsible for this herbivore-driven diversity effect. The other experiment manipulated monospecific plant density and found little evidence for negative density dependence. Combined, these experiments suggest that the higher performance in mixture was owing to the acquisition of positive interspecific interactions rather than the loss of negative intraspecific interactions. Overall, we emphasize that realistic predictions about the consequences of changing biodiversity will require a deeper understanding of the interaction between plant diversity and higher trophic levels. If we had manipulated only plant diversity, we would have missed an important positive interaction across trophic levels: diverse seedling communities better resist herbivores, and herbivores help to maintain seedling diversity.

1. Introduction

Anthropogenic activities are dramatically altering biodiversity around the globe [1,2], with potentially serious consequences for ecosystem function [3]. Although our current understanding of terrestrial biodiversity–ecosystem function relationships derives primarily from manipulations of primary producers [3], this trophically limited scope is problematic for several reasons. Owing to small population sizes and attractiveness to human hunters [4], animals at higher trophic levels are often more vulnerable to extinction than lower trophic levels. Moreover, there is ample evidence that herbivores are integral to ecosystem functioning [4–7]. Indeed, some experimental and theoretical work shows that the sign, magnitude and mechanisms of plant diversity effects can be altered by other trophic levels [7–15], although these experiments are still relatively rare.

Our understanding of the relationship between terrestrial biodiversity and ecosystem functioning is not only trophically limited, but also constrained primarily to grassland ecosystems [3]. Forests cover vast swaths of the land and can be exceptionally diverse, yet only a few published studies have manipulated tree diversity [16–19], probably because trees are slow-growing, large organisms. However, tree seedlings are easily manipulated and ecological dynamics at this stage can dictate the structure of mature forests [6,20]. Additionally, limited evidence suggests that diversity has strong effects in tropical seedling communities [17], but to our knowledge no study has tested how herbivores might moderate these diversity effects even though herbivores are known to strongly influence forest systems, especially in the temperate zone [21].

Here, we present a factorial manipulation of tree seedling diversity (one versus 15 species) and the presence/absence of a key herbivore (white-tailed deer, Odocoileus virginianus). Because plants may perform better in diverse assemblages than in monocultures owing to the acquisition of positive interspecific interactions.
[22,23] and/or owing to the loss of negative intraspecific dynamics [13,24], we conducted a concurrent density manipulation (three versus 15 individuals) to measure the strength of negative density-dependent dynamics in our system. We tested two primary hypotheses: first, that seedling diversity and deer would interact to affect plant survival and growth, and second, that intraspecific density would influence negative density-dependent dynamics. Note that while the first hypothesis asks about patterns of plant performance in monocultures versus mixtures, the latter seeks to illuminate underlying mechanisms. Based on the literature, we made several predictions. First, that plant performance would increase with diversity [3] and decrease with browsing [21,25,26], but that this diversity–performance link would be altered by deer. In particular, we predicted that palatable species might show the greatest performance increase in polycultures because unpalatable species might buffer palatable neighbours from attack [23,27,28]. We also predicted that decreasing intraspecific plant density would reduce both herbivore attack rates and intraspecific competition [20]. We tested each of these hypotheses and predictions by following plant performance and herbivore attack in our experimental plots for 3 years.

2. Material and methods

(a) Site and species selection
The experiment occurred in an approximately 70-year-old secondary forest at the Smithsonian Environmental Research Center (38°53’ N, 76°33’ W) where deer densities are low to moderate (approx. 3–8 individuals km⁻²; J. D. Parker 2011–2013, unpublished data). The species pool included 15 common, co-occurring tree species: Acer rubrum (L.), Carpinus caroliniana (Walter), Carya alba (L.), Carya glabra (Mill.), Cornus florida (L.), Fagus grandifolia (Ehrh.), Fraxinus pennsylvanica (Marsh.), Lindera benzoin (L.), Liquidambar styraciflua (L.), Liriodendron tulipifera (L.), Nyssa sylvatica (Marsh.), Platanus occidentalis (L.), Quercus alba (L.), Quercus falcata (Walter), Carya (L.), and Quercus rubra (L.) (electronic supplementary material, appendix 1, table A1).

(b) Design
In April 2011, we planted 1-year-old bare-root seedlings (Carolina Wetland Services, Charlotte, NC, USA) into 1 m² plots that were separated from other plots by at least 5 m. For the factorial manipulation of deer and diversity, we planted 15 seedlings into each monoculture plot (n = 90 plots; six plots per species) and polyculture plot (n = 50 plots, each plot with one individual per species). We then excluded deer from half of the monocultures and polycultures with 1.5 m high, 2 cm wire mesh. While polyculture compositions were initially uniform, differential mortality led to unique species compositions in 35 of 50 polycultures in 2011, 45 of 50 in 2012 and 48 of 50 in 2013. To examine the strength of density-dependent interactions, we established additional uncaged monocultures with only three seedlings (‘low-density monocultures’; n = 2 plots per species, except for Carpinus caroliniana and both Carya species, which had one plot each). After planting all plots, we removed naturally occurring seedlings, trimmed the remaining vegetation below seedling height and then allowed plots to grow undisturbed.

(c) Data
We assessed plant survival and stem height in August 2011, September 2012 and July 2013. If a seedling had no leaves but a pliable stem, we counted it as alive as these plants generally leafed out in subsequent years. We counted missing seedlings or those with no leaves and a brittle stem as dead. We also measured stem height from the ground to the tip of the highest live apical bud. In August 2013, we harvested all above-ground biomass and weighed it to the nearest 0.001 g to convert height data to biomass using allometric equations (electronic supplementary material, appendix 2). In August 2011, September 2012 and July 2013, we noted which plots had received deer damage or naturally occurring insect herbivory. In September 2012 and July 2013, we also quantified the magnitude of damage in each plot by counting the number of leaves that were damaged versus undamaged by each herbivore type. If deer had consumed all leaves, we assumed that a single node had been present and recorded the number of leaves typically found at a single node for that species (i.e. for species with opposite leaves, we conservatively assumed that only two leaves had been eaten).

3. Data analyses
We conducted analyses in R (v. 3.0.1, R Foundation for Statistical Computing; packages lmer (R Core Team) and lme4 [29]). We excluded plots with complete mortality, because this resulted primarily from canopy trees falling and smashing our plots, or owing to a fungal outbreak in 2011 that killed both experimental and naturally occurring L. tulipifera. Remaining replication for each year and treatment can be found in the electronic supplementary material, appendix 1, table A1). Because we did not employ a fully factorial design with all levels of density, diversity and deer exclusion, we subset the overall dataset for each analysis to avoid confounding variables. To address the first hypothesis about the interaction between deer and diversity, we excluded the uncaged monocultures with three individuals and used only monoculture and polyculture plots planted with 15 individuals to examine patterns of plant performance. We further subset these data to only uncaged monocultures and polycultures to examine patterns of herbivory, because deer were excluded from caged plots and we wanted to make consistent comparisons across the two herbivore types. To address the second hypothesis about plant density, we excluded all caged plots and polycultures, focusing only on uncaged monocultures planted with three or 15 individuals.

(a) Hypothesis 1: tree diversity and deer presence/absence will interact to affect seedling performance
We examined survival with mixed effects logistic regression (glmer in R package lme4) with year as a random factor, and diversity (monoculture/polyculture), deer (cage/no cage) and diversity × deer as fixed effects (n = 399 plots across 3 years). We examined ln-transformed biomass per individual with a mixed effects linear model (lmer in R package lme4), with year as a random factor, and deer, diversity and deer × diversity as fixed effects (n = 392 plots with height data across 3 years). Because diversity changed through time owing to mortality, we also analysed biomass patterns with observed diversity per plot and found that variation in observed diversity among polycultures had little effect on biomass per individual, so we retained diversity as a binary variable. Finally, with total plot biomass, we used Loreau & Hector’s [30] equations to calculate complementarity and selection, replacing zero values with a marginal non-zero number (0.001 g) to facilitate these calculations, which cannot be computed with zero values.
We examined herbivory separately for deer and insects \((n = 203\) plots across 3 years), using mixed effects logistic regression to determine the likelihood that a plot would be attacked (‘likelihood of damage’) with year as a random effect and diversity as the fixed effect. We also included realized density as a covariate to account for any differences in patch size that might impact herbivore recruitment \([31]\). For the plots that had received at least some damage in 2012 and 2013 (deer: \(n = 85\) plots, 43 monocultures and 42 polycultures; insects: \(n = 134\), 86 monocultures and 48 polycultures), we used the same predictors to examine the number of damaged versus undamaged leaves in each plot (‘intensity of damage’). Finally, we asked whether damage in mixture could be predicted from damage in monoculture by calculating the mean proportion of leaves damaged in uncaged monocultures and uncaged polycultures for each species. We analysed these condensed data \((n = 28; 14\) species \(\times 2\) years) with a mixed effects linear model with year as a random factor, monoculture damage as a fixed effect and polyculture damage as the response variable. \(Liriodendron\dagger\) was excluded from this analysis because plants died before insect or deer browse occurred. If species received the same damage in monoculture and polyculture, then the linear model should estimate a slope equal to one. If the confidence intervals surrounding the estimate were less than one, we concluded that polyculture damage was lower than monoculture damage across species.

(b) Hypothesis 2: increasing density will increase negative density-dependent dynamics

To assess the strength of intraspecific interactions, we compared survival, growth and herbivore attack in uncaged monocultures with three versus 15 individuals. We examined survival with mixed effects logistic regression \((\text{glmer} \text{ in } R\text{ package } \text{lme4})\) with density as the fixed effect, and species identity and year as random factors \((n = 190\) plots across three years). Note that for survival, density equalled the initial planting density (to avoid circularity), but for the other analyses (e.g. damage, biomass) density equalled planted density in 2011 and realized density in 2012 and 2013. With the same predictors as survival, we examined ln-transformed biomass/individual \((n = 185\) plots with height data) and the likelihood that a plot would receive at least some damage \((n = 185)\). Finally, we examined monocultures that had received at least some damage in 2012 and 2013 (deer: \(n = 60\) and insects: \(n = 119\)) and used the same model as above to examine the intensity of damage in these plots.

4. Results

(a) Tree diversity and deer presence/absence will interact to affect seedling performance

By the end of the third year, caged monocultures had the highest survival with 11.7 individuals on average \((\text{figure 1a})\). Although deer generally reduced survival, seedling diversity helped to buffer against the negative impacts of deer browsing with monocultures suffering greater mortality than polycultures in the presence of deer \((\text{deer } \times \text{ diversity}: \chi^2 = 15.2, p < 0.0001; \text{ figure 1a})\). Deer also reduced plant biomass by 20% \((\text{deer}: \chi^2 = 11.6, p = 0.003; \text{ figure 1b})\). However, polycultures consistently produced larger individuals than monocultures regardless of exposure to deer \((\text{diversity}: \chi^2 = 32.3, p < 0.0001; \text{deer } \times \text{ diversity}: \chi^2 = 0.3, p = 0.573)\).

Across years, all polycultures displayed positive complementarity \((\text{figure 2})\), indicating that species on average produced more biomass in mixture than expected from monocultures. In exposed plots in 2012 and 2013, significant negative selection also occurred \((\text{figure 2})\), indicating that subdominant species benefited the most from a diverse community. That subdominant species was probably \(A.\ rubrum\), which was very small in exposed monocultures but 245% larger in exposed mixtures by 2013 \((\text{electronic supplementary material, appendix 1, table A1})\).

Plant diversity also altered patterns of browsing. Polycultures were more likely to be browsed than monocultures \((\chi^2 = 21.3, p < 0.0001)\), with 81% of polycultures browsed across years, compared to only 46% of monocultures \((\text{figure 3a})\). However, among the plots that were visited by deer, the intensity of damage was 53% higher in monoculture than in polyculture \((\chi^2 = 72.9, p < 0.0001)\). In addition, the relationship between damage in mixture and damage in monoculture across species \((\text{figure 3c})\) was significantly lower than one \((0.80 \pm 0.14, \text{mean} \pm 95\% \text{ confidence interval (CI)})\).

By contrast, plant diversity had little effect on foliar insect damage \((\chi^2 = 0.8, p = 0.364)\). All of the polycultures and 99% of the monocultures had at least some insect damage, and although diversity significantly impacted the intensity of damage \((\chi^2 = 5.1, p = 0.023)\), effect sizes were small with less than 2% differences in leaf consumption between treatments. In addition, the relationship between damage in mixture and monoculture did not differ from 1 \((0.97 \pm 0.27, \text{mean} \pm 95\% \text{CI})\).

(b) Increasing density will increase negative density-dependent dynamics

We found limited evidence to suggest that negative density-dependent dynamics increased with plant density. Neither
survival nor growth declined as intraspecific density increased (survival: \( \chi^2 = 2.1, p = 0.144 \); biomass: \( \chi^2 = 0.3, p = 0.555 \)). Increasing plant density also had no effect on the likelihood that a deer would visit a plot (\( \chi^2 = 2.5, p = 0.110 \), although it did increase the probability of insect visitation (\( \chi^2 = 14.0, p < 0.001 \)). If a plot was attacked, then the intensity of damage was significantly affected by density. However, the effect sizes were small and in different directions depending on the herbivore, with the odds of deer browse increasing by 3% and the odds of insect damage decreasing by 4% with density (deer: \( \chi^2 = 14.4, p < 0.001 \); insects: \( \chi^2 = 49.4, p < 0.0001 \)).

5. Discussion

Manipulations of diversity in forested systems are rare [32], as are those that simultaneously control more than one trophic level [7]. Here, we factorially manipulated both diversity of tree seedlings and the presence/absence of an important vertebrate herbivore to explore how forested systems responded to simultaneous changes in diversity and herbivory. In the absence of deer, seedlings had higher survival in monospecific stands, whereas in the presence of deer, diversity enhanced both seedling survival and growth, apparently owing to selective browsing on competitive dominants and associational protection of susceptible species by less palatable species. By contrast, we found little evidence for strong negative density-dependent effects in monocultures. Taken together, these results suggest that higher seedling performance in diverse patches is owing to the acquisition of positive interspecific interactions and not owing to the loss of negative intraspecific interactions.

Changes in plant diversity are known to have cascading consequences for higher trophic levels [33,34]. We examined how changes in diversity impacted herbivory by both a large vertebrate and by small invertebrates. Only deer showed a strong response to changes in plant diversity. Deer were more likely to visit polycultures (figure 3a), probably because a highly preferred species was always present in polycultures [35], but they did less damage when they were there (figure 3b), especially to highly preferred species like A. rubrum, F. pennsylvanica and Q. rubra (figure 3c). We hypothesize that less palatable species discouraged deer from lingering within a polyculture [36], buffering highly preferred species from heavy damage [23]. Similar results have been observed in other systems, where increasing plant diversity reduced consumption [10,28,37]. However, it is possible to observe the opposite pattern, especially among generalists, where increasing diversity increases consumption owing to the beneficial effects of diet-mixing [38,39]. Functional trait diversity probably determines which of these scenarios will occur [28]. Species with particularly repellant chemistry, for example, can provide associational resistance for more palatable neighbours [23,40], leading to reduced consumption as diversity increases.
Mortality at the seedling stage is thought to structure the composition of mature forests [6,20]. In our experiment, while seedling diversity altered patterns of deer herbivory, browsing also interacted with diversity to influence seedling survival (figure 1a). The highest overall survival occurred in caged monocultures. This is consistent with a long literature showing that enemies are important for maintaining diversity, and that without enemies we would expect a greater preponderance of monospecific stands [6,41,42]. High survival in caged monocultures may be transient, however, because as the seedlings mature they are likely to accumulate other enemies (i.e. soil pathogens, [43]) and compete more intensely for the same niche space [44]. In the presence of deer, we observed greater mortality in monocultures than in mixtures. We hypothesize that this pattern resulted both from associational protection of palatable species by unpalatable neighbours and from shifts in competitive dynamics. Similar to Paine’s classic work showing that consumers enhance diversity by preferentially consuming dominant species [45], we found that deer consumed the seven most productive species, but avoided five of the seven least productive species (electronic supplementary material, appendix 1, table A1). Because deer browse in polyculture was not very intense (figure 3b), we propose that deer consumed enough of the competitive dominants to favour the survival of subdominant species but not enough to kill the highly preferred species, leading to higher survival overall. Although there can be extremely high seedling mortality at high deer densities (e.g. more than 25 individuals km\(^{-2}\) [21,25]), deer densities in our system (approx. 3–8 individuals km\(^{-2}\)) are more on a par with their historical norms [26]. This suggests that moderate densities of deer may play an important role in maintaining seedling diversity in temperate forests, as consumers do in other ecosystems [6,41,42].

Tree seedling diversity also enhanced plant growth. Diversity has been shown repeatedly to elevate biomass production in grassland and other non-forested systems [3], but less frequently in forested ecosystems. We followed our seedling communities for only 3 years so we do not know whether this trend will persist through time, but 3-year-old mixed-species plots in Panama also had higher growth (though not survival) than monocultures [17]. In addition, surveys across naturally occurring forest stands often find that various ecosystem functions, including productivity and carbon storage, are positively associated with species diversity, particularly at smaller spatial scales [46–48]. Although these observational studies cannot show that species diversity is causally responsible for productivity gains, there are now multiple long-term and large-scale experimental manipulations of tree diversity (http://www.treedivnet.ugent.be) in both temperate and tropical forests that are poised to test the hypothesis that diverse forest stands are more productive than monocultures over time spans relevant to mature forests.

Plants might achieve higher performance in diverse assemblages than in monocultures via two principal mechanisms. First, they might gain positive interactions with interspecific neighbours, or second, they might lose the negative effects of intraspecific neighbours. Positive interspecific interactions include, for example, complementary plant architectures that increase water retention [22] or repellent chemistry from one species that protects a palatable neighbour [40]. Negative intraspecific effects include competition for the same niche space or the attraction of specialist enemies [24,41,49]. Adding a simultaneous density manipulation allows one to distinguish between these two mechanisms, but is rarely done because it requires expanding on already large biodiversity experiments. Although not mutually exclusive, distinguishing between the relative importance of these two mechanisms may help us to better anticipate the consequences of biodiversity change. If facilitative interactions are more important, then the exact combinations of species may be more important (i.e. those plants with the right architectural combinations or those plants with the right repellent chemistry), and the consequence of changing biodiversity will depend on whether or not a key species newly appears or goes extinct. If escape from negative density-dependent dynamics is more important, however, then the identity of interspecific neighbours may be less important as long as intraspecific density is diluted. In this case, the addition or loss of any given species may have a small impact.

While we did not conduct a full-factorial manipulation of deer, diversity and density, our concurrent manipulation of monoculture density in plots exposed to deer found little evidence for negative density dependence. Density-dependent mechanisms may become more critical in our system as the seedlings transition to adults, outgrow deer, compete more intensely for light and water, and accumulate pathogens. At this early stage of forest regeneration, however, our results suggest that interspecific interactions (i.e. associative protection from less palatable neighbours) contributed to improved plant performance in mixture. This suggests that species composition is important, and the loss or gain of a particular species could have far-reaching impacts on forest function. Indeed, the loss of other formerly dominant tree species owing to introduced disease and insects have had profound impacts on North American forests [50].

Ultimately, our data highlight the role of herbivores in structuring forest communities [6,41] and more generally contribute to a growing literature about how consumers alter and often enhance diversity effects [7–10,13,14]. These studies derive from widely varying systems. For example, in old fields, deer enhanced the positive effects of genotypic diversity on seed production in an herbaceous forb [10], while in eelgrass beds, the richness of aquatic grazers increased both grazer and eelgrass biomass only in the presence of a crab predator [15]. Even the smell of a predaceous fish was enough to increase the effect of detritivore richness on decomposition rates [11]. Because single-trophic diversity manipulations do not typically exclude other trophic groups, these studies risk attributing too much importance to richness at a single-trophic level when dynamics at other trophic levels may be shaping the overall diversity effect. This was well demonstrated by Schnitzer et al. [13], who found that the classic relationship between plant diversity and ecosystem function disappeared when soil microbes were eliminated. Thus, if we are to truly anticipate the consequences of diversity loss on land and in the sea, we must deliberately consider interactions among multiple trophic levels.

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